Birds as Bioindicators of Pollution in Aquatic and Terrestrial Environments

Javier Cotín Martínez

ADVERTIMENT. La consulta d'aquesta tesi queda condicionada a l'acceptació de les següents condicions d'ús: La difusió d'aquesta tesi per mitjà del servei TDX (**www.tdx.cat**) ha estat autoritzada pels titulars dels drets de propietat intel·lectual únicament per a usos privats emmarcats en activitats d'investigació i docència. No s'autoritza la seva reproducció amb finalitats de lucre ni la seva difusió i posada a disposició des d'un lloc aliè al servei TDX. No s'autoritza la presentació del seu contingut en una finestra o marc aliè a TDX (framing). Aquesta reserva de drets afecta tant al resum de presentació de la tesi com als seus continguts. En la utilització o cita de parts de la tesi és obligat indicar el nom de la persona autora.

ADVERTENCIA. La consulta de esta tesis queda condicionada a la aceptación de las siguientes condiciones de uso: La difusión de esta tesis por medio del servicio TDR (**www.tdx.cat**) ha sido autorizada por los titulares de los derechos de propiedad intelectual únicamente para usos privados enmarcados en actividades de investigación y docencia. No se autoriza su reproducción con finalidades de lucro ni su difusión y puesta a disposición desde un sitio ajeno al servicio TDR. No se autoriza la presentación de su contenido en una ventana o marco ajeno a TDR (framing). Esta reserva de derechos afecta tanto al resumen de presentación de la tesis como a sus contenidos. En la utilización o cita de partes de la tesis es obligado indicar el nombre de la persona autora.

WARNING. On having consulted this thesis you're accepting the following use conditions: Spreading this thesis by the TDX (**www.tdx.cat**) service has been authorized by the titular of the intellectual property rights only for private uses placed in investigation and teaching activities. Reproduction with lucrative aims is not authorized neither its spreading and availability from a site foreign to the TDX service. Introducing its content in a window or frame foreign to the TDX service is not authorized (framing). This rights affect to the presentation summary of the thesis as well as to its contents. In the using or citation of parts of the thesis it's obliged to indicate the name of the author.

Birds

as bioindicators of pollution in aquatic and terrestrial environments

Javier Cotín Martínez

PhD Thesis 2012

Birds as bioindicators of pollution in aquatic and terrestrial environments

Javier Cotín Martínez

Chapter I

Are the toxic sediments deposited at Flix reservoir
affecting the Ebro river biota?
Purple Heron eggs and nestlings as indicators

Chapter II

Blood biomarkers and contaminant levels
in feathers and eggs to assess environmental hazards in heron nestlings
from impacted sites in Ebro basin (NE Spain)

Chapter III

Sea, freshwater or saltpans?
Foraging ecology of terns to assess mercury inputs in a wetland landscape: The Ebro Delta

Chapter IV

Who eats where?
Insights into freshwater and marine bird communities of the Ebro Delta (NE Spain)
through Stable Isotopes and Trace Elements

Chapter V

Home range extension and overlapping
of the Ornated Tinamou (Nothoprocta Ornata) in an Andean Agro-ecosystem

Chapter VI

Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia
and their potential as environmental sentinels

Chapter VII

Calamus as an appropriate feather section
for the assessment of endogenous lead pollution

Ilustraciones capítulos: *Martí Rodríguez*

Birds

as bioindicators of pollution in aquatic and terrestrial environments

Javier Cotín Martínez

PhD Thesis

Barcelona 2012

UNIVERSITAT DE BARCELONA

Birds as bioindicators of pollution in aquatic and terrestrial environments

Las aves como bioindicadoras de contaminación en ambientes acuáticos y terrestres

Memoria presentada por el licenciado en Biología
Javier Cotín Martínez para optar al grado de
Doctor por la **Universidad de Barcelona**

Departamento de Biología Animal
Programa de doctorado en Zoología
Bienio 2005-2007

Javier Cotín Martínez
Barcelona, 2012

Directores

Dra. Carolina Sanpera Trigueros	**Dr. Lluis de Jover Armengol**
Departamento de Biología Animal	Departamento de Salud Pública
Facultad de Biología	Facultad de Medicina
Universidad de Barcelona	Universidad de Barcelona

To the birds,
who taught me how to soar

Agradecimientos

Espero no dejarme a nadie en el tintero al escribir estas líneas. No dejan de representar los personajes principales, que aunque con diferentes papeles, han formado parte de esta historia. A todos ellos, que han jugado un papel en esta aventura que ha sido mi tesis, les tengo que agradecer algo, ya sea bueno o malo, puesto que sin ellos, esta historia no sería la que es. Así que allá voy.

Primero de todos, a Xavier Ruiz, mi director de tesis, que lamentablemente falleció en la primavera del 2008. Xavier era ese tipo de personas que te hacer amar la ciencia, y que muestra pasión y energía con todo lo que hace (a veces a raudales, ¡vaya broncas nos echaba!). Fue un duro golpe tu pérdida, y a ti va dirigida la tesis principalmente, ya que aún cuando no vas a estar presente en su lectura, espero que sepas apreciarla y que te enorgullezcas, allá donde estés.

En el número dos de la pole position de agradecidos, mis directores actuales, Carolina Sanpera y Lluis Jover, que ya formando parte del grupo inicial de investigación, recibieron completamente el peso de dirigir mi tesis (el cual lo han llevado con paciencia!), incluso en momentos muy difíciles. ¡Gracias a los dos por aguantarme estos años y enseñarme tantas cosas!

Y por supuesto, al Doctor Jacinto Nadal, el cual, aunque de un modo "no oficial", siempre ha estado allí, ya no solo guiándome en el mundo académico, sino siendo un amigo para mí y todo un ejemplo a seguir.

También, asociados al proyecto de Flix, hay mucha gente a la que agradecer: Carlos Barata, Benjamín Piña, Luis Carrasco (gracias por la paciencia con el mercurio, entre otras muchas cosas!), gente de l'Aiguabarreig, Flix (Pere Josep) y del Delta del Ebro (aquí especialmente, David Bigas, que nos ha sacado de muchos apuros).

No puedo olvidarme de los Serveis Científico-Tècnics de la UB. La de tiempo y los buenos momentos que he pasado allí. Cabe destacar a Paco (de verdad, muchas gracias por todo, no sé que habría hecho sin tu ayuda!), Pili (tantos momentos con los isótopos!), Maite R, Maite, Toni (gracias por tanta ayuda con los metales!) y tanta otra gente con la que compartía el día a día de la ardua tarea de los análisis… gracias a todos!

Also thanks to Michael Griesser and Vitor Paiva for taking the time to read this thesis. Thanks to them I'll be (hopefully!) a European Doctor ;)

Toda esa aventura en Barcelona no habría empezado si no hubiera estudiado biología en la Universidad de Navarra… me siento especialmente agradecido a Rafael Miranda, mi tutor durante la carrera, y a Miriam Hernández, con la que pude disfrutar de varias jornadas de anillamiento por los Pirineos!

Ahora le toca el turno a la gente del departamento… siguiendo el orden cronológico… empieza la "Vieja Guardia" (que ahora sería más bien la "guardia prehistórica"): Jose, Rocío, Raúl, Fran, Joan, María, Sergi (que buenos momentos muestreando!), a todos vosotros, gracias por haber formado parte de esta historia y haberme enseñado tantas cosas. La “Nueva Guardia”, que ya ha pasado a ser más bien “Vieja”, estaba formada por Laura (espero nunca salgan nuestras memorias a la luz…), Manolo (ese arroz negro del Figueras!), Eloy (muchas gracias, vaya aventuras), Francesc, Isa (siempre lista para aconsejar en la tormenta!), Gemma (siempre lista para animarte), Alberto, Inés, Irene, Marisa, Debs, Blanca (y su Facebook), Toni, Albert, Teresa… y también a aquellos que están en la UB, colándose en los pica pica del departamento (Víctor), los del despacho tortuguero (Marcel) y los que pintan, ven pájaros y decoran esta tesis con dibujos! Gracias Martí! Todavía hay que ir a por los cormoranes! Ah! También a Pachi, por sus cambios en la portada y retoques de maquetación.

Tantos años en Barcelona no habrían sido lo mismo sin Maddie, Jorge (vuelve de Costa Rica!), Marc y sus buitres, Julito, Cristian y el Delta, Joanillo y los Mallos de Riglos, Rubén y sus tan terribles (a la par que “adorables”) locuras, Mieta, Marquitos y Cedric, Ainhoa y Sanse, y, por supuesto, María, Roger, Mau, Sonia y Sergio. Cuantas aventuras vividas en estos últimos dos años, y las que faltan por recorrer, ya sea en Inglaterra, en Granada, en una casa rural perdida de la mano de Dios o en Valencia….

Also I’m really thankful to my Swedish friends, Stefan, and the Öhlund’s, which are my second family. The Pygmy Owl and those lasagnes are unforgettable memories!

While I was in Germany, apart from not learning German, I met great people. Special thanks to Nadine, Anna, Little Bean, Iacun, Chocodrink, Heike, Vera (witch power!), Erik, Tobi… I have great memories of those cliffs, Krebs and those amazing birds that we all could enjoy.

Two springs ago, although not 100% related to the PhD, I could enjoy a great group of people in Granada with whom I have been able, not only to learn a lot about birds, but about life and friendship. Thanks to all of you, Gretchen, Emeline, Sharon, Marta, Patrick. This same spring we repeated the adventure, with new people (Tania, Carlota, Jonhy!), and although different, we could enjoy a really intense experience too!

Por supuesto, a mis amigos de toda la vida, que han formado parte de esta etapa: Gracias Ana (por fin la tesis!!), Ron, Santi, Leo, Regina, Pau, Antonio, Adri…

Y por último, mi familia: padres, hermanos, sobrinas, cuñada… Siempre habéis estado ahí y a vosotros en concreto va dedicada la culminación de esta etapa de mi vida, o tesis, vamos. Y a mi prima Maite, que gracias a vivir en Barcelona, se ha convertido en una amiga.

No puedo terminar estas líneas sin acordarme de Neo y Phoenix, esos compañeros de talante negro, uno de mirada plateada, otra de pico carmesí, con los que he vivido grandes momentos y me premiaron con su amistad.

Finalmente y para concluir, remarcar a unas personas que me han ayudado a mantener la cordura (Sergio), perderla (Gretchen) y a ser fuerte (Ana). Gracias de corazón a los tres.

Thanks to all of you!

Index

1 Introduction

17 Objectives of the Thesis

21 Supervisor's Report

25 Discussion

69 Conclusions

75 References

93 Chapter I
Are the toxic sediments deposited at Flix reservoir affecting the Ebro river biota? Purple Heron eggs and nestlings as indicators

113 Chapter II
Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain)

125 Chapter III
Sea, freshwater or saltpans? Foraging ecology of terns to assess mercury inputs in a wetland landscape: The Ebro Delta

137 Chapter IV
Who eats where? Insights into freshwater and marine bird communities of the Ebro Delta (NE Spain) through Stable Isotopes and Trace Elements

165 Chapter V
Home range extension and overlapping of the Ornated Tinamou (Nothoprocta ornata) in an Andean Agro-Ecosystem

187 Chapter VI
Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia and their potential as environmental sentinels

209 Chapter VII
Calamus as an appropriate feather section for the assessment of endogenous lead pollution

221 Introducción

237 Objetivos de la tesis

241 Discusión

287 Conclusiones

291 Referencias

Introduction

Biological indicators are species used to monitor the health of an environment or ecosystem. They are any biological species or group of species whose function, population, or status can be used to determine ecosystem or environmental integrity. When talking about bioindicators of contamination, the potentially used species should reflect the pollution levels of the environment and vary accordingly with them.

Birds (class Aves) are feathered, winged, bipedal, endothermic, egg-laying, vertebrate animals. There are around 10,000 living species, inhabiting ecosystems across the globe, from the Arctic to the Antarctic. Birds can be found in your backyard, in the hottest desert, in the depths of a rainforest or even in the middle of a storm in the open ocean. According to their movement patterns, birds can spend their entire life in the same area (resident species) or cross the globe twice a year (long distance migrants like the Arctic tern, *Sterna paradisaea*), with many options in between. They even can just "wander" through the oceans, as is the practice of several pelagic seabird species. Their feeding spectrum is also wide, including examples such as grass, seeds, honey, nectar, insects, fish, mammals, other birds, carrion…

So, whenever trying to monitor an environmental hazard using a bioindicator, there is a great chance of finding a bird species that may satisfy the requirements. Indeed, birds have been used as bioindicators in several pollution events, such as the decline of the raptor populations produced by the use of DDT that took place some decades ago (Helander et al., 2008, Henny et al., 2010, Sonne et al., 2010), the oil spills produced by the tankers Exxon Valdez and Prestige (Golet et al., 2002, Sanpera et al., 2008, Moreno et al., 2011), spillages of contaminants like the one that occurred at Doñana National Park, Spain (Benito et al., 1999, Gomez et al., 2004, Baos et al., 2006), pollution derived from mining activities (Wayland et al., 2006) or even radioactivity events as the one case in Chernobyl (Ruiz et al., 1988, Moller et al., 2011). Birds have also been used to study contamination in ecosystems such as the Antarctic (Corsolini et al., 2011, Jerez et al., 2011, Metcheva et al., 2011) or to evaluate the health of wetlands (Heath and Frederick, 2005, Abdennadher et al., 2011, Aliakbari et al., 2011).

Using birds as bioindicators of pollution has several advantages, but also some constraints. Birds are easy to identify and their classification and systematic are well established. They are particularly well known organisms, with much research carried out into their ecology and behaviour, and this background knowledge of biology enhances their usefulness as biomonitors. Birds are often high in the food chain; thus they may be particularly suitable as monitors of any signal that accumulates through the chain. Their long life-span means that birds integrate the effects of environmental stresses over time, providing the possibility of measuring, for example, pollution over a year or more, but show a low sensibility for short-term, low intensity perturbations. Similarly, the mobility of birds can allow monitoring over a broad spatial scale, the breadth depending on the species chosen, but migratory habits can render birds much less suitable as biomonitors because individuals may differ in their migrations to an uncertain extent and make it difficult to determine the spatial scale they represent.

Normally the key point consists of using the adequate species and samples, for instance, as each of the tissue reflects the contaminants in a different way. Organochlorides distribute among body tissues in relation to their lipid content. Heavy metals tend to be held in one particular tissue at much higher levels than others. Feathers are metabolically inert after formation, so for those avian species with well-known moult schedules, the analyses of specific individual feathers provide unique chemical information of a very discrete time, and potentially place, regardless of the sampling date (Cherel et al., 2005, Inger and Bearhop, 2008). Feathers do not only present mercury, but many other heavy metals, as they incorporate to the keratin structure. Eggs have also been used in many studies to monitor contaminants, and they have several advantages over internal tissues (Barrett et al., 1985, Becker, 1989, Burger and Gochfeld, 1989). Although females can excrete mercury into eggs, the amount that they shed in this way is usually small compared to the amount put into feathers during moult (Honda et al., 1986). About internal tissues, metals distribute differently from tissue to tissue: cadmium is concentrated in kidney, lead in bone, mercury in liver and kidney, zinc, copper and iron in liver. It is interesting to remark that blood samples provide a picture of metal levels that reflect short-term exposure (immediate dietary intake).

So, when facing the problem of monitoring or evaluating the pollution of an ecosystem, wetland or area, choosing the adequate species and sample type is of crucial importance, and should be based on knowledge of the species.

In this study we face the use of birds as bioindicators of metal pollution in two different scenarios of contamination: one that takes place in an aquatic environment, the Ebro river basin, and a second that occurs in a terrestrial environment, the Bolivian Andes. Background, pollution threats and sources are completely different, as has been the approach in each study case.

Heavy metals, environment and biological sample

Heavy metals are member of the loosely-defined subset of elements that exhibit metallic properties. It mainly includes the transition metals, some metalloids, lanthanides and actinides. Particularly mercury, cadmium and lead have prompted many investigations, mainly because they are extremely toxic (Bryan, 1979). Most have no known biological function and inputs into the environment, particularly of lead and mercury, largely result from human activities (Lantzy and Mackenzie, 1979).

Depending on the ecosystem, either terrestrial or aquatic, pollutant dynamics are rather different. In terrestrial ecosystems, the ambient environment is usually the soil, while in an aquatic one is usually the water or the sediment. Once metals get into soils or sediments, they have long residence times before they are eluted to other compartments. Acidity has a marked effect on the solubility of metals in soils and water. The mobility of metals in soils is dictated largely by the clay content, amount of organic matter and pH. In general, the higher the clay and/or organic matter content and pH, the more firmly bound are the metals and the longer is their residence time in soil. In water, the solubility of metals is strongly pH dependent. In aquatic systems, metals may become 'locked up' in bottom sediments, where they may remain for many years. However, if the pH falls, metal solubility increases and they become more mobile and hence more bioavailable. As the acidity changes, the oxidation state changes accordingly, and as a result, heavy metals can generate or break bounds with organic compounds generating specific compounds such as methyl mercury. Methylated compounds are taken up more readily than the unmethylated form, due to its high lipoaffinity.

Unlike organochlorides (another known family of pollutants), which are distributed widely among body tissues, largely in relation to their lipid content, heavy metals tend to be held in one particular tissue at much higher levels than others. The site-specificity of metals has an important influence on the choice of tissue for monitoring studies. For example, although females can excrete mercury into eggs, the amount that they shed in this way is usually small compared to the amount put into feathers during moult (Honda et al., 1986). Feathers do not only present mercury, but many other heavy metals, as they incorporate to the keratin structure. About internal tissues, cadmium is concentrated in the kidney, lead in bone, mercury in liver and kidney, zinc, copper and iron in liver. Blood samples provide a picture of metal levels that reflect short-term exposure (immediate dietary intake).

Study case A: Toxic muds at Flix. ¿Do they affect the Ebro River and its Delta?

The Ebro catchment (NE Spain) is the largest river basin in Spain and discharges into the Mediterranean Sea creating a delta of more than 30,000 ha. This river basin covers an area of 85,362 km^2 (http://www.chebro.es), potentially supplies 3 million people and it contains some heavily industrialized areas. One of these areas is the Flix site (41°14'N, 00°31'E), where a chemical industry has been in operation since the early 20th century. During all those decades, the company Ercros has developed several activities, such as producing DDT from 1945 to 1971 or PCBs from 1959 to 1987. Furthermore, HCB is a well known product resulting from the manufacture of organochlorine solvents. Mercury discharges are related to the production of Sodium hypochlorite, commonly named as bleach. Bleach hypochlorite is produced from the electrolysis of a saline solution, using a mercury cathode (Cl_3Hg formation) and a graphite anode (obtaining Na^+). Bicalcic phosphate was produced from 1973 onwards from rock phosphate, being their residues rich in ^{238}U, and thus, in the radionuclides derived from it, among them ^{226}Ra and ^{210}Pb.

These various activities and long operational period, along with the construction of a dam next to the plant around 1960, resulted in the accumulation of 200,000–360,000 tons of industrial wastes in the riverbed, occupying an area of 700 m of length and 60 m of width. The mixture of heavily polluted sediments is composed of high concentrations of heavy metals (cadmium, arsenic, copper, chromium, lead and especially mercury), selenium, organochlorines (hexachlorobenzene, pentachlorobenzene, DDTs, polychlorobiphenyls, polychloronaphthalenes and polychlorostyrenes) and radioactive ^{210}Pb (Fernandez et al., 1999, Bosch et al., 2009). In addition, the Ebro River is currently affected by different agricultural and industrial activities, with significant impact on the existing biota (Mañosa et al., 2001, Navarro et al., 2010a). The pollutants originated at the Flix site are carried

Figure 1: Two views of the Ebro Delta the Sandwich Tern colony at 'Punta del Fangar" and a heron colony at Clot.

Figure 2: Views of the two riverine sampling sites: l'Aiguabarreig and Flix.

downstream by the Ebro River to its delta 90 km away (Llorente et al., 1987, Pastor et al., 2004, Navarro et al., 2009), especially during floods (Vericat and Batalla, 2006).

As a consequence, a global study on the environmental impact of such toxic muds was undertaken to evaluate their effects prior to an ongoing project aimed at removing the polluted sediments; this last initiative implies a high risk of pollutant dispersal.

Some of the data obtained by this and other previous studies on the Flix reservoir and the Ebro River have shown high levels of PCBs in sediments (Fernandez et al., 1999) and the effects of pollutants on different sentinel species. Earthworms presented high levels of mercury (Ramos et al., 1999). Zebra mussels (*Dreissena polymorpha*) with the highest levels of Hg (THg) and methylmercury (MeHg) ever reported were found at this site, with mean values 20 times greater than the local background levels (Carrasco et al., 2008). Crayfish (*Procambarus clarkii*) and Zebra mussels exhibited high toxic stress levels (high activities and levels of antioxidant enzymes, metallothioneins, lipid peroxidation and DNA strand breaks and decreased levels of glutathione) close to the waste dumps (Faria et al., 2010), even Crayfish presented levels of mercury exceeding legal values established by European Union legislation (Suarez-Serrano et al., 2010). Mercury concentrations in tissues of Feral Carp (*Cyprinus carpio*) sampled downstream from Flix were one to two orders of magnitude higher than those from carp sampled upstream from Flix (Navarro et al., 2009) and Catfish (*Silurus ganis*) exceeded the maximum mercury level recommended for human consumption (Carrasco et al., 2011). Deformities, eroded fins, lesion and tumour (DELT) anomalies and ectoparasites were clearly more frequent at the impacted area for several fish species (Feral Carp, Roach -*Rutilus rutilus*- and Pumpkinseed Sunfish -*Lepornis gibbosus*-); also a significant lower body condition was detected for these species and Bleak (*Alburnus alburnus*), while there was a negative impact on reproductive traits for carp and pumpkinseed (Benejam et al., 2010). The responses to the pollutants were species-specific, and feral carp had the clearest effects on fitness-related traits at the impacted area, despite also being among the most tolerant to pollution (Benejam et al., 2010).

All those described adverse effects of pollution in these diverse sentinel species, together with the movement of sediments from Flix into the deltaic ecosystem, pose a threat to the Ebro Delta ecosystem and biodiversity.

The Ebro Delta (40°42'N, 00°50'E) is one of the largest wetlands in the western Mediterranean region and is home to extensive bird colonies (c.a. 12000 pairs of Audouin's Gull, 3000 of Flamingo, 400 of Purple Heron, 350 of Little Tern, 5000 of Common Tern, 500 of Gull-billed Tern, 2000 of Sandwich Tern and 1200 of Whiskered Tern among others). Occupies an area of 320 km^2: 75% of this surface corresponds to ricefields (c.a. 20.000 ha) and farmland, 20% are natural preserved areas and the last 5% urban areas (see fig. 1 and 3). This wetland presents a wide variety of habitats: at the inner part of the delta and at both river beds, the ricefields and farmland, connected to the river through channels; at the coastline, abandoned fields, lagoons, salt marshes and beaches; on each side of the Ebro Delta, surrounded by sea, there are two arrow-shaped sandy areas, known as the 'Punta del Fangar' and 'Punta de la Banya' (with an extension of 500 and 2500 ha respectively), the latter hosting a saltpan industry. Finally, the open sea and the northern and southern bays, molded by the two 'Puntas', create the patchwork of habitats. The coastal lagoons, although connected to the sea and thus expected to hold brackish water, receive considerable freshwater input from the rice fields from spring to autumn, thereby lowering their salinity, which almost reaches that of freshwater during those seasons.

To assess the environmental exposure of the toxic muds deposited at the Flix reservoir, not only the river has to be taken into account, but also the whole patchwork of habitats of the Ebro Delta, and even the sea, as sediments are washed away by the river. Preserving the biota of this ecosystem, especially the birdlife of the Ebro Delta, is a main concern. Thus, we focused in top predatory species that when feeding rely directly on the aquatic habitats associated to the river. Among bird species assumed to be most adequate bioindicators are piscivorous species, such as the ones belonging to the family Ardeidae (herons) and the family Sternidae (terns).

Herons and terns show a predictable breeding phenology and strong nest site fidelity, allowing intensive monitoring of a variety of breeding or population parameters, and have been previously and successfully used as bioindicators of environmental pollution in aquatic systems (Becker et al., 1993, De Luca-Abbott et al., 2001, Nisbet et al., 2002, Connell et al., 2003, Guitart et al., 2003, Champoux et al., 2006, Sakellarides et al., 2006, Paiva et al., 2008b). Since they feed in the upper trophic levels of these ecosystems they biomagnify and bioaccumulate some of the pollutants presents there (Baker and Sepulveda, 2009).

Herons are freshwater and coastal birds in the family Ardeidae. There are 64 recognised species in this family. The herons are medium to large sized birds with long legs and necks. The necks are able to kink in an s-shape, due to the modified shape of the sixth vertebrae. As such, the neck is able to retract and extend, and is retracted during flight, unlike most other long-necked birds. The herons are a widespread family with a cosmopolitan distribution, existing on all continents except Antarctica. Included among the main breeding heron populations at the Ebro River and Delta are Purple Heron (*Ardea purpurea*), Squacco Heron (*Ardeola ralloides*), Night Heron (*Nycticorax nycticorax*), Little Egret (*Egretta garzetta*) and Little Bittern (*Ixobrychus minutus*). These species are mainly fish predatory species, although can feed on other prey, such as crayfish in the case of the Little Bittern (Pardo-Cervera et al., 2010) or insects in the case of the Squacco Heron (Delord et al., 2004). They are mainly associated with freshwater habitats, but can also feed in different habitats such as brackish waters or even saltpans, as does the Little Egret (Abdennadher et al., 2011).

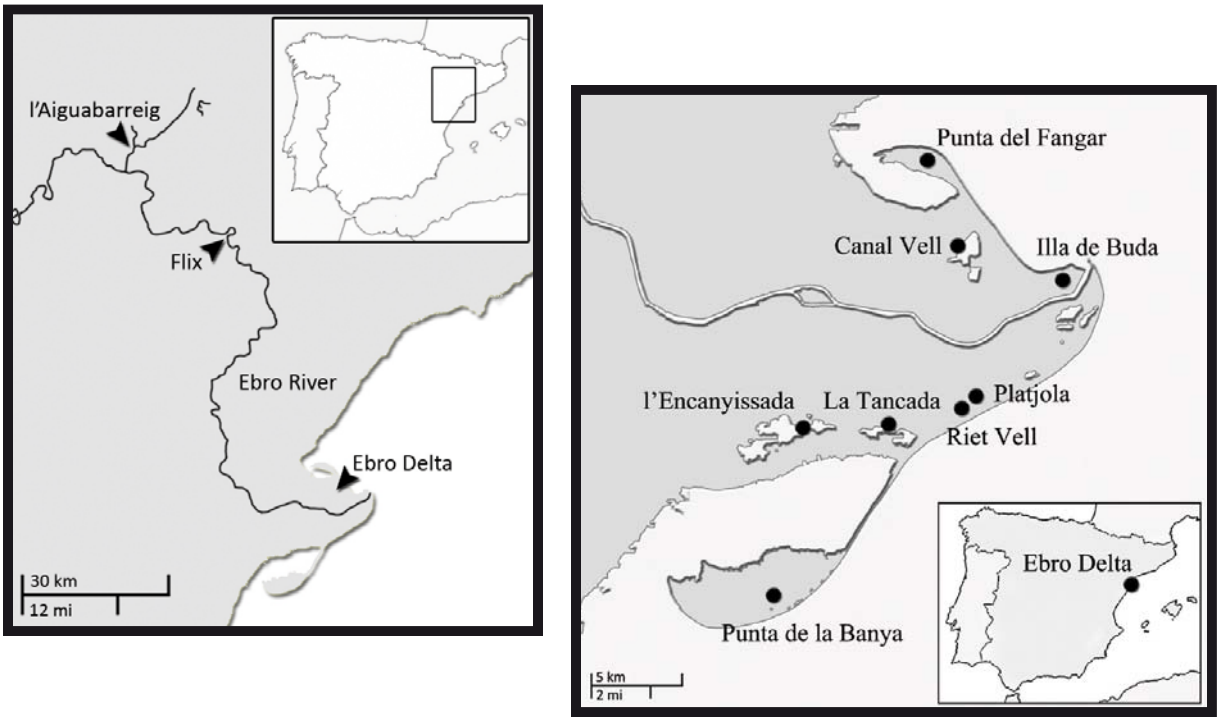


Figure 3: Maps of the localization of the three sampling sites and, in detail, the sampled bird colonies at the Ebro Delta

Terns are gull-related seabirds, which comprise 45 species (most of them migratory) grouped in 12 genera (Bridge et al., 2005) found worldwide. Five tern species breed in the Ebro Delta: Common Tern (*Sterna hirundo*), Sandwich Tern (*Sterna sandvicensis*), Little Tern (*Sternula albifrons*), Gull-billed Tern (*Gelochelidon nilotica*) and Whiskered Tern (*Chlydonias hybrida*). A wide variety of foraging habitats has been reported for these species: Freshwater habitats for the Whiskered Tern (Latraube et al., 2005, Paillisson et al., 2007); marine for the Sandwich Tern (Stienen et al., 2000) and both marine and freshwater for the Common Tern (Becker et al., 1997, Neubauer, 1998). The last two species have been described as generalist foragers. The Little Tern uses several foraging habitats in Portugal (Paiva et al., 2008a), and in the Ebro Delta it has been observed foraging in channels, lagoons and lagoon mouths (Bertolero et al., 2005). The Gull-billed Tern feeds in terrestrial (Cabo and Sanchez, 1986), freshwater (Dies et al., 2005), and even marine habitats (Stienen et al., 2008).

Additionally we sampled four additional species to complete the spectrum of bird families and habitats: two species from the family Laridae (Gulls) and two from the Rallidae (Rails, Moorhens…). There are several species of gulls breeding at the Ebro Delta, being the most common species the Yellow-legged Gull (*Larus michahellis*), Audouin's Gull (*Larus audouinii*), Black-headed Gull (*Larus ridibundus*), Slender-billed Gull (*Larus genei*) and the Lesser Black-backed Gull (*Larus fuscus*), among others. For this study we selected the Audouin's and Black-headed Gull, while the Yellow-legged Gull was not considered due to its use of crops and refuse tips (Ramos et al., 2009). The Audouin's Gull is a nocturnal predator on epipelagic fish, but can take advantage of fishery activities; especially trawler discards (Oro and Ruiz, 1997, Sanpera et al., 2007a). Black-headed Gull is a species that can exploit marine, freshwater and terrestrial habitats (Schwemmer et al., 2011), being an opportunistic forager. The two rallidae species included in this study are the Moorhen and the Purple Swamphen. These two species are, although omnivorous, mainly herbivorous, with the Purple Swamphen feeding on seeds, bulbs, stem and leaves from reeds (*Phragmites australis*), cattail (*Thypa latifolia*) and rice (*Oryza sativa*) (Cramp, 1980). Moorhen also feed mainly on plant material (Amat and Soriguer, 1984).

Given their diverse feeding strategies, these heron and tern species, together with gulls and rallids, have the capacity to provide a picture over the whole habitats of the Ebro River and Delta. Thus, they were considered suitable candidates as bioindicators of environmental pollution for the whole area.

Three sampling sites were chosen according to the breeding colonies of these species: one reference and two potentially affected by the toxic muds, which have been previously described. The focal area, Flix, despite industrial activity, has surprisingly become a valuable wetland with a profusion of nesting birds, including herons, storks and marsh harriers. The distal area, the Ebro Delta, is situated 90 km downstream. The reference area, situated 25 kilometres upstream of the polluted sediments is l'Aiguabarreig site (41°23'N, 00°19'E), a riverine island called "Illa de los Martinets", located at the confluence of two Ebro tributaries, the Cinca and Segre Rivers. This highly valuable ecological spot and sanctuary for aquatic birds is located upstream from the Flix site and therefore unaffected by the Flix factory and its toxic sediments.

Several bird samples have been proposed for the development of effective long-term monitoring programs (Pol and Becker, 2007), including samples as eggs or tissues as feathers or blood. This study was performed on eggs and nestling feathers and their blood. Data obtained from egg samples integrate the adult diet prior to egg laying because herons and terns, as income breeders, obtain most of the materials used in clutch formation from diet (Ruiz et al., 1998, Hobson et al., 2000). Moreover, tern eggs also reflect male foraging habitat as males provide females with prey during clutch formation (Nisbet, 1973, Wiggins and Morris, 1986, Gonzalez-Solis et al., 2001). On the other hand, nestling feathers and blood integrate the diet obtained by the adults and consumed by the nestlings during growth. The use of nestlings has two advantages. First, the effect of pollutant bioaccumulation is avoided, because the time of exposure has been low and similar for all individuals. Second, since all the chicks are provisioned with prey caught by parents in the surroundings of the colony, the results should reflect only the impact of local pollution.

Heavy metals, when present at high concentrations, are of special concern, as they mainly enter organisms through diet (Burger et al., 1992). However, the exposure of individuals varies according to their trophic habits. Therefore, simultaneous to the assessment of pollution levels, Stable Isotopes Analysis (SIA) was used to get a proper understanding of the species trophic ecology their relationships and habitat use (Nisbet et al., 2002, Sanpera et al., 2007b, Tavares et al., 2007, Abdennadher et al., 2011, Ramirez et al., 2011).

During the last two decades, biogeochemical markers such as stable isotopes have been used increasingly besides conventional methods in diet studies, in order to provide new perspectives on the feeding ecology of birds. Although traditional methods of diet analysis such as stomach sampling and pellet analysis can yield valuable data on general composition (e.g. identification of specific prey taxa), sampling is often heavily biased because of differential digestion rates of prey and the accumulation in the gizzard of hard items such as fish otoliths (Barrett et al., 2007). Stable isotopes have been successfully applied to yield valuable insights into feeding ecology, particularly as they provide a potential means to quantify assimilated, rather than simply ingested, diet. Stable isotope signatures of nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$) and sulphur ($\delta^{34}S$) have been extensively used in studies of bird communities, focusing on their trophic ecology and relationships (Hobson et al., 1994, Forero and Hobson, 2003, Cherel et al., 2008, Koiadinovic et al., 2008, Moreno et al., 2010). $\delta^{15}N$ of tissues reflects the trophic level, with consumer signatures being higher than in their prey (Forero et al., 2005). Information about the source of carbon entering a food web can be obtained from $\delta^{13}C$ (Hobson, 1999), providing insight about the foraging habitat. Isotopic sulphur signature ($\delta^{34}S$) is useful for determining the food sources of consumers (marine vs. terrestrial), because the essential sulphur-bearing compounds are incorporated into tissues without significant fractionation (Connolly et al., 2004) and this isotope is particularly useful for detecting or distinguishing heterogeneity among habitat uses (Peterson et al., 1985).

In addition to this, stable isotopes ratios can be used to define an 'isotopic niche'. The ecological niche concept has undergone a renaissance in recent years (Newsome et al., 2007). Although trophic niche and isotopic niche differ, both are tightly related, and data presented as bi-plots, where the isotopic values of animal tissues are represented in a δ-space, delineate a species isotopic niche. Jackson et al. (2011) proposed recently a novel multivariate ellipse-based niche (or community) metric, reformulating the previous Convex Hull Total Area (TA) defined by Layman et al. (2007) in a Bayesian framework. This new metric, the corrected area of the standard ellipse (SEA_c), gives an indication of niche width using summary information of the spread and extent of the isotopic data points and is the one used in this present work. Also, the use of biomarkers combined with chemical analyses has allowed in recent years the assessment and identification of adverse health effects of different pollutants on several waterbirds species (Fossi et al., 1996, Cordi et al., 1997, Muir et al., 1999, Vos et al., 2000, Strum et al., 2008). More recently the use of non-lethal biological techniques such as the analyses of biomarkers in blood, and contaminants in feathers and eggs have been applied when studying endangered species (Muir et al., 1999, Vos et al., 2000, Champoux et al., 2002, Franson et al., 2002, Champoux et al., 2006, Murvoll et al., 2006, Quiros et al., 2008) and their use may provide valuable data on the health status of the Ebro River waterbird populations.

Antioxidant defences represent the detoxification pathway of reactive oxygen species (ROS) in aerobic organisms (Livingstone, 2001). When ROS exceed antioxidant defences, oxidative damage to lipids within cell membranes, DNA molecules and proteins may occur, leading to a pathological condition, generally termed oxidative stress (Livingstone, 2001). In this way, knowledge about the responses of organisms to oxidative stress may provide valuable information about their health status. Recently, some authors (Winston et al., 1998, Regoli, 2000), developed an analytical method to evaluate the total oxyradical scavenging capacity (TOSC) of any given tissue, defined as the capacity of the biological sample to neutralize ROS. For a given tissue lower TOSC values are likely to be related to a lower capacity to detoxify ROS and hence a greater susceptibility to oxidative stress. The method was originally developed for rat liver (Winston et al., 1998), but in recent times it has been applied to the blood of birds in environmental surveillance programs (Corsolini et al., 2001). Plasmatic enzymes, such as the activity of lactate deshydrogenase and of B esterases such as butyrylcholinesterase and carboxylesterases may also provide valuable information on liver damage and the neurotoxic effects of pesticides in birds, respectively (Fossi et al., 1996, Cordi et al., 1997, Champoux et al., 2006, Strum et al., 2008).

Stable Isotopes as an ecological tool

Elements in nature occur in more than one stable form due to varying numbers of neutrons in the nucleus. These various forms are known as stable isotopes (as they do not decay over time) of an element and have identical chemical but varying kinetic properties due to their mass differences.

Mass spectrometers are instruments that provide an extremely accurate estimate of the ratio of the heavier to lighter isotope in an unknown sample relative to an international standard. Isotope ratios are expressed conventionally as δ values in parts per thousand (‰) according to the following delta notation:

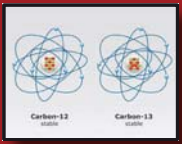
$$X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

Where X (‰) is ^{13}C, ^{15}N, ^{34}S and R are the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$. R standard for C is Pee Dee Belemnite (PDB), for N is atmospheric nitrogen (AIR) and for S is troilite from the Canyon Diablo Meteorite (CDT).

Although the properties of isotopic forms do not change dramatically amongst themselves, they behave differently in various biogeochemical processes that ultimately results in changes in the relative abundance of the heavier to the lighter isotope (isotopic fractionation). Enrichment happens when the heavier stable isotope is accumulated in the product (relative to the substrate), while the lighter isotope is preferably eliminated. On the other hand, depletion occurs when the lighter isotope is favoured. Changes in the isotopic signal between diet and consumer tissues are as a consequence of three main aspects: fractioning, discrimination and routing. The metabolic processes of enrichment and depletion are known in general as isotopic fractionation. Discrimination refers to the difference in isotopic composition between a tissue and diet while the differential allocation of isotopically distinct dietary components to different tissues is called 'isotopic routing'. Hobson and Clark (1992) described the following function:

$$Dt = Dd + \Delta dt$$

Where Dt is the isotopic signature of consumer tissue, Dd is the isotopic signature of the diet, and Δdt is the discrimination factor between diet and consumer tissue. From the ecological point of view, as energy transfer happens throughout ecosystems isotopic fractionation takes place, resulting in alterations of the consumer's stable isotope signatures relative to its diet. That is, this isotopic fractionation occurs in each trophic level and it sums throughout trophic chains. Therefore, once the appropriate assumptions are considered, stable isotope analysis is a powerful tool for reconstructing quantitatively elementary flows throughout food webs, which can help answer relevant questions in trophic ecology (Gannes et al., 1997, Bearhop et al., 2004).



Study case B: Mining at the Bolivian Andes

Anthropogenic mining has taken place in the Bolivian Andes since at least the fifteenth century (Sanabria, 2000). Particularly in the East of Oruro Department in the Eastern Andean Cordillera, there are extensive polymetallic deposits (SERGEOMIN, 1999). In the past, extraction of gold and silver (associated with sulphurs of iron, copper, zinc, lead, arsenic, antimony, etc.) was the main activity, while at present, extraction of tin and the zinc-silver-lead complex predominates (Rios, 2002). These mining activities were characterized by the deposition of large tailing piles, which accumulated in abandoned and active mines, where trace metals such as lead, arsenic, cadmium, and antimony could reach surface waters and soils, and so the biota. One example are the high cadmium levels that were found in potato tubers cultivated in agrosystems irrigated by the Chayanta River, which receives mineral residuals from the principal tin Bolivian mines, situated 60 Km away (Oporto et al., 2007, Rojas and Vandecasteele, 2007).

Pollution of this region through the centuries has affected the principal water body, Poopó Lake. Not only does its water have higher metal concentrations than the permissible values for human consumption, but the fish that live in the lake do too (Beveridge et al., 1985).

For studying the pollution spread at this area, we focused mainly on two sites, located in the Eastern Andean Cordillera, Southeast of Oruro city and East of the Poopó Lake. These sites present trace metal contamination reported in soils and water. They are called Antequera (4,000 m, 18°28’S, 66°52’W), and Poopó (3,800 m, 18°23’S, 66°58’W), with 13 kilometres between them, whose waters drain in two distinct hydrographic sub-basins, which finally drain into Lake Poopó (see fig. 4).

At Antequera, the mining is limited to the zinc–silver–lead complex and all the residuals are deposited in a storage pond that closes a little valley near the Antequera river course (Rios, 2002), where there are several old tailing piles scattered. All this industrial activity has polluted the river soils, with concentrations several times above permissible values for arsenic, antimony, cadmium, copper, chromium, tin, iron, mercury, lead and zinc (MEDMIN, 2001). On the other hand, the tin and secondarily the zinc–silver–lead complex are exploited at Poopó, where numerous tailing piles are scattered across the landscape (Rios, 2002). Metals and other elements at this site are easily dispersed by water and wind to soils and water bodies, especially to the Poopó River that drains into Lake Poopó. The soils at bends on the Poopó River have high concentrations of Fe, Zn, Cd, Cu, Pb, and Hg (MEDMIN, 2001).

For comparison purposes, another two sites without any mining activity were selected (reference sites). They are called San Pedro de Ulloma (3,850 m, 17°30’S, 68°32W), located to the West of the Desaguadero River, and Chuñavi (4,100 m, 16°17S; 68°20W), to the northwest of the city of La Paz and East of Lake Titicaca (see fig. 4). The former is 200 kilometres from the mining localities; and the latter, 280 kilometres.

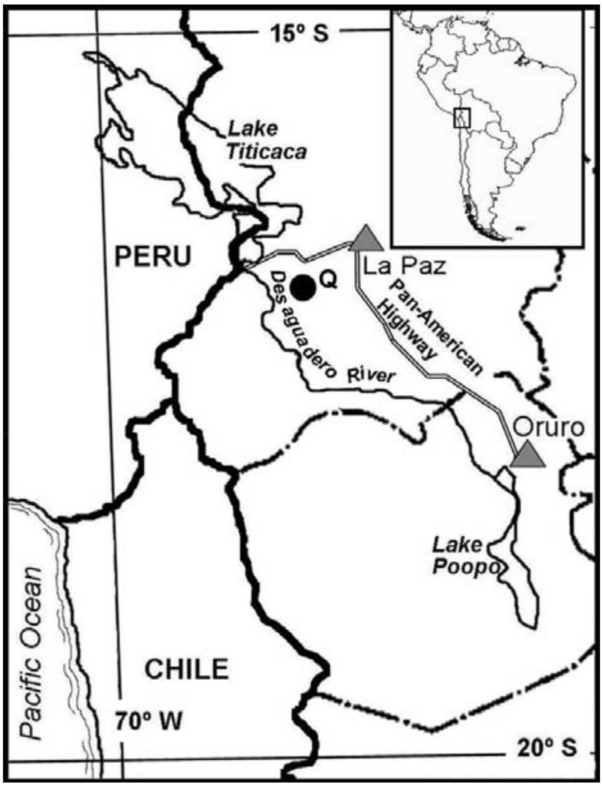


Figure 4. Study sites on the Bolivian high plateau of the Central Andes. The reference sites are called San Pedro de Ulloma or nonpolluted 1 (NP1) and Chuñavi or nonpolluted 2 (NP2); the mining sites are Antequera or Polluted 1 (P1) and Poopó or Polluted 2 (P2).

The sampling sites are dominated by vegetation formed basically by spiny and resinous bushes (i.e., *Baccharis spp., Parastrephia spp.,* and *Adesmia spp.*) and bunch grasses (i.e., *Festuca spp.* and *Stipa spp.*). Agricultural and livestock activities take place at the four sites, with the principal crops being potato tubers and barley, and extensive sheep, cattle, and llama breeding.

With the aim to assess the environmental exposure of these mining activities and the pollutants originated by them, we selected Tinamou species to evaluate their usefulness as bioindicators.

Tinamous are birds that have been studied little when compared to other bird species (Cabot, 1992, Davies, 2002). The family Tinamidae consists of about 47 species in 9 genus. Although they look similar to other ground-dwelling birds like quail and grouse, the characters they share are the result of convergence and plesiomorphy rather than shared evolutionary innovations. Tinamous have no closer living relatives than the flightless ratites, and thus are placed in their own order, Tinamiformes. All 47 species of tinamous live in South and Central America. Tinamous prefer to walk or run but will fly to avoid predation and other danger. When they have exhausted all other evasion techniques, including hiding in burrows, they may fly. Their technique is a flutter of wing-beats followed by a long glide, followed by another burst of wing-beats. Tinamous are rarely seen but often heard within their range. Although some species are quite common, they are shy and secretive. A small number of species live in more open, grassy country, but even these are wary of humans. Tinamous mainly eat small fruits and seeds off the ground or off of plants that are near the ground.

The two species that were selected are the Ornate Tinamou (*Nothoprocta ornata*) and the Darwin's Nothura (*N. darwinii*). Both species live in the Bolivian highlands between 3,700 and 4,200 m asl (Cabot, 1992, Davies, 2002, Garitano-Zavala et al., 2003) and are relatively common and major hunting targets. Highland tinamou species present a sedentary lifestyle, with small home ranges, as Pearson and Pearson (1955) determined for the Ornate Tinamou, indicating individual home ranges of on average 2.43 ha. Moreover, the feeding habits of the Ornate Tinamou and Darwin's Nothura are generalist and opportunist, consisting of a wide variety of seeds, leaves, and fruits from crops and weeds as well as invertebrates (Garitano-Zavala et al., 2003). These characteristics indicate that they could be appropriate bioindicators of the contamination derived from the mining activities.

Concerning their movements, we consider radio tracking some tinamous in order to check their home ranges, as in the field these species have very reserved behavior, with the aim to test, among others, if they are sedentary and their potential as bioindicator species of local exposure of contamination. Tracking an animal by radio involves two devices. A transmitter attached to the animal sends out a signal in the form of radio waves, just as a radio station does. A scientist might place the transmitter around an animal's ankle, wing, carapace, dorsal fin, or as in our case, the neck. A VHF receiver picks up the signal, just like a home radio picks up a station's signal. The receiver is usually in a truck, an ATV or an airplane. To keep track of the signal, animal is followed using the receiver. This approach of using radio tracking can be used to track the animal manually but is also used when animals are equipped with other payloads. The receiver is used to home in on the animal to get the payload back.

Also, as these species are one of the major hunting targets and the access to full specimens is relatively easy, we decided not only to monitor pollutants on samples of feathers, but also in internal organs, such as the liver and kidney. The histopathological status of liver and kidney was also studied, to check for adverse reactions to pollution exposure.

Pollutants analysed in feathers (which are exposed to the elements and so to exogenous contamination) have, besides the endogenous route of accumulation (diet), an exogenous route. In feathers, just after their formation, the blood supply atrophies and the feather becomes metabolically isolated from the rest of the body (Burger, 1993). Consequently, further heavy metals cannot accumulate from the bloodstream into the feathers. Thus, feathers contain information about circulating heavy metal concentrations in the blood at the time of their development. But once the feather is completed, the feather-metal levels may remain stable with time, as is the case with mercury (Appelquist et al., 1984, Veerle et al., 2004), or they may increase, since feathers are highly prone to exogenous surface contamination. Such contamination from atmospheric deposition, preening and environmental contact (with soil, dust or water) may then give a misleading indication of biologically incorporated contamination at the time of feather growth (Pilastro et al., 1993, Dauwe et al., 2002, Jaspers et al., 2004).

The measurement of lead is a good example of this circumstance, as it has been demonstrated that its concentrations increase after the feather has fully grown, so feathers reflect endogenous lead levels as well as exogenous contamination and, commonly, atmospheric pollution levels. Thus, only newly grown or nestling feathers accurately reflect the endogenous deposition, as exogenous accumulation of pollutants onto the feather surface is an important route of contamination (Veerle et al., 2004), mainly affecting the vane, which becomes more contaminated over time due to its large, structurally complex surface area (Goede and Debruin, 1984, Cardiel et al., 2011).

So, a study was also designed to test this matter, in order to assess which feather section provides the most accurate data on the original endogenous lead levels, using feathers of one of the species sampled, the Ornate Tinamou.

Objectives

Taking advantage of the widespread use of birds as bioindicators, the major objective of this thesis was to assess the usefulness of selected bird species as bioindicators of contamination in areas which have suffered episodes of intense contamination, leading to pollution hazards, in order to get a better knowledge that aids environmental restoration, monitoring and conservation.

So, to develop this major aim, we took into account two study cases, being the main objectives of this thesis:

Part A: Toxic muds at Flix. Do they affect the ornithofauna at the Ebro River and its Delta?

In the case of aquatic environments like the Ebro River, affected by the toxic muds deposited at Flix reservoir, the main objectives were:

- To evaluate whether aquatic birds such as the Purple Heron reflect the potential pollutant exposure from Flix Reservoir among different riverine and deltaic areas, and assess their usefulness as bioindicators.
- To examine if the polluted wastes of Flix reservoir affect the levels of pollution of the habitats where waterbird populations of the Ebro Delta (situated 90 km downstream) forage and feed.

In order to achieve these two main objectives, the specific aims were:

- To determine the trophic ecology and habitat partitioning of the waterbird populations, in order to appropriately assess the pollutant exposure.
- To determine if pollutant levels in eggs and nestling feathers merit conservational concern.
- To evaluate which sample type, either eggs or feathers, more accurately reflects impacts of, or exposure to, pollution.
- To determine a set of non-destructive blood markers to monitor environmental hazards of pollution.

Part B: Mining at the Bolivian Andes

In the case of the Bolivian Andes, affected by a long term history of mining, the main objectives were:

- To evaluate the potential of Tinamou species as sentinels of exposure to local trace metal pollution
- To study different routes of lead accumulation into the sentinel species, in order to assess the usefulness of feather levels as a measure of the exposure to this pollutant.

In order to achieve these two main objectives, the specific aims were:

- To study the movement habits of the tinamou species in order to evaluate their potential as bioindicators of local contamination sources.
- To compare the use of different samples types (feather, kidney, liver) when evaluating the impact of, or exposure to, local pollution.
- To evaluate the histopathological status of liver and kidneys in relation to pollution levels.
- To determine if concentrations of trace elements (arsenic, cadmium, lead and antimony) of Tinamou species warrant special conservational concern.
- To examine the importance of endogenous vs. exogenous sources of pollution in relation to feather lead levels.

Informe del Director

Supervisor's Report

El doctorando Javier Cotín Martínez presenta en su tesis doctoral titulada "Birds as bioindicators of pollution in aquatic and terrestrial environments" una serie de trabajos publicados o en revisión en revistas científicas internacionales incluidas en el *Science Citation Index*. Paso a detallar a continuación la contribución científica que ha realizado el doctorando en cada uno de los artículos, así como su factor de impacto (*Thompson Institute for Scientific Information*):

Are the toxic sediments deposited at Flix reservoir affecting the Ebro river biota? Purple Heron eggs and nestlings as indicators
Javier Cotín, Manuel García-Tarrasón, Lluis Jover, Carolina Sanpera.
Ecotoxicology (2012) 21, 1391-1402
Factor de impacto (2012): 3.051
Diseño del trabajo: **J.C.**, C.S., L.J.
Muestreo y análisis de muestras: **J.C.**, M.GT.
Análisis de resultados y redacción científica: **J.C.**, C.S., L.J.

Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain)
Carlos Barata, María del Carme Fabregat, **Javier Cotín**, David Huertas, Montserrat Solé, Laia Quirós, Carolina Sanpera, Lluis Jover, Xavier Ruiz, Joan O Grimalt, Benjamín Piña.
Environmental Pollution (2011) 158, 704-710
Factor de impacto (2012): 3.746
Diseño del trabajo: JO.G., C.B., X.R., **J.C.**, L.J., B.P., C.S.
Muestreo y análisis de muestras: **J.C.**, M.GT., MC.F., D.H., M.S., L.Q.
Análisis de resultados y redacción científica: C.B., B.P., **J.C.**

Sea, freshwater or saltpans? Foraging ecology of terns to assess mercury inputs in a wetland landscape: The Ebro Delta
Javier Cotín, Manuel García-Tarrasón, Carolina Sanpera, Lluis Jover, Xavier Ruiz
Estuarine, Coastal and Shelf Science (2011) 92, 188-194
Factor de impacto (2012): 2.247
Diseño del trabajo: **J.C.**, C.S., L.J., X.R.
Muestreo y análisis de muestras: **J.C.**, M.GT.
Análisis de resultados y redacción científica: **J.C.**, C.S., L.J.

Who eats where? Insights into freshwater and marine bird communities of the Ebro Delta (Spain) through Stable Isotopes and Trace Elements
Javier Cotín, Manuel García-Tarrasón, Lluis Jover, Carolina Sanpera.
En revisión en *The Auk*
Factor de impacto (2012): 2.156
Diseño del trabajo: **J.C.**, C.S., L.J.
Muestreo y análisis de muestras: **J.C.**, M.GT.
Análisis de resultados y redacción científica: **J.C.**, C.S., L.J.

Home range extension and overlapping of the Ornated Tinamou (*Nothoprocta Ornata*) in an Andean Agro-ecosystem
Álvaro Garitano-Zavala, Zulma Chura, **Javier Cotín**, Jacint Nadal, Xavier Ferrer
En revisión en *Journal of Field Ornithology*
Factor de impacto (2012): 1.196
Diseño del trabajo: A.GZ., J.N., **J.C.**
Muestreo y análisis de muestras: A.GZ., Z.C.
Análisis de resultados y redacción científica: A.GZ., **J.C.**

Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia and their potential as environmental sentinels
Álvaro Garitano-Zavala, **Javier Cotín**, Miquel Borrás, Jacint Nadal.
Environmental Monitoring and Assessment (2010) 168, 629–644
Factor de impacto (2012): 1.436
Diseño del trabajo: A.GZ., **J.C.**, J.N., M.B.
Muestreo y análisis de muestras: **J.C.**, A.GZ.
Análisis de resultados y redacción científica: A.GZ., **J.C.**

Calamus as an appropriate feather section for the assessment of endogenous lead pollution
Javier Cotín, Álvaro Garitano-Zavala, Carolina Sanpera, Lluis Jover, Jacint Nadal.
En revisión en *Environmental Monitoring and Assessment*
Factor de impacto (2012): 1.436
Diseño del trabajo: **J.C.**, C.S., L.J., J.N., A.GZ.
Muestreo y análisis de muestras: **J.C.**, A.GZ.
Análisis de resultados y redacción científica: **J.C.**, C.S., L.J.

Del mismo modo, informo que ninguno de los coautores participantes en los artículos que componen esta tesis han utilizado, implícita o explícitamente ninguno de estos trabajos para la elaboración de su propia tesis doctoral.

Barcelona, a 28 de Septiembre del 2012

Firmas:

Directores

Dra. Carolina Sanpera Trigueros	**Dr. Lluis de Jover Armengol**
Departamento de Biología Animal	Departamento de Salud Pública
Facultad de Biología	Facultad de Medicina
Universidad de Barcelona	Universidad de Barcelona

Discussion

Part A: Toxic muds at Flix.

Within this first part of the discussion, we will focus on the potential of aquatic birds such as the Purple Heron as sentinels of the pollutants from Flix Reservoir among different riverine and deltaic areas, assessing their usefulness as bioindicators.

Additionally other species of the family Ardeidae and species from the family Sternidae (among others) will be used to examine if the pollution derived from Flix reservoir affects the waterbird populations inhabiting the Ebro Delta.

So, the main issues are the following:

A1. Trophic ecology and habitat partitioning of the aquatic bird population of the Ebro River and its Delta

In order to study the trophic ecology and habitat partitioning of the rich bird community of the Ebro River and its Delta, we first focused on the aquatic habitats of the river itself, using the Purple Heron as target species, and secondly we looked at the whole community of the Ebro Delta (which includes terrestrial and marine habitats), selecting certain bird families.

For the first case, eggs and nestling of Purple Heron were used from both riverine sites (l'Aiguabarreig and Flix) and the deltaic site, the Ebro Delta (descriptive in Table 1). Nitrogen signatures are used to estimate trophic level, although the ratios can be affected by several factors, such as the food-chain length in a given ecosystem or by environmental differences in the baseline (Cabana and Rasmussen, 1994). The latter seems to be the case of the river Ebro, as the outstanding differences found between the riverine localities and the Ebro Delta apparently rely on baseline differences (see fig.5), likely caused by the eutrophication of the river. Both riverine sites are located at reservoirs, with marked eutrophication indicated also by the higher $\delta^{15}N$ of the main Purple Heron prey (see fig. 6). A similar situation was observed in Little Egret (Egretta garzetta) from Chikly island (Abdennadher et al., 2011), exposed to eutrophication and food-web enrichment in nitrogen rich sewage.

On the other hand, Purple Heron $\delta^{15}N$ signatures from the Ebro Delta resemble those of the freshwater tern species that inhabit this area (Cotin et al., 2011), where the continuous waterflow into ricefields and lakes probably prevents excesive eutrophication. The possibility that the $\delta^{15}N$ differences are caused by changes in diet is ruled out as Purple Heron regurgitates from all localities presented the same prey species, mainly Crayfish

	Site	Year	Sample									
			Feather					Egg				
			N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
δ¹⁵N (‰)	Aiguabarreig	2006	7	18.5	.9	16.7	19.6					
		2007	7	18.9	1.6	15.8	20.5	12	19.0	2.3	14.7	21.5
		2008	4	19.2	1.9	16.4	20.3					
	Flix	2006	10	22.3	0.7	20.8	23.2	12	20.5	1.3	18.1	22.7
		2007	11	21.6	1.2	19.0	22.8	15	19.8	2.1	16.1	22.3
		2008	9	21.6	0.5	20.6	22.2					
	Ebro Delta	2006	4	13.9	1.4	12.3	15.5	14	13.9	1.5	11.8	17.1
		2007	16	13.9	0.7	12.8	15.6	25	13.3	1.0	11.6	15.7
		2008	12	14.4	1.0	13.0	15.7					
δ¹³C (‰)	Aiguabarreig	2006	7	-25.6	0.9	-27.0	-24.3					
		2007	7	-27.5	0.5	-28.3	-27.0	12	-27.2	1.5	-29.9	-25.2
		2008	4	-27.4	0.9	-28.7	-26.8					
	Flix	2006	10	-25.8	0.8	-27.1	-24.4	12	-27.5	0.9	-28.9	-25.9
		2007	11	-27.0	0.7	-28.1	-25.9	16	-28.3	0.9	-29.2	-25.8
		2008	9	-28.6	0.4	-29.3	-28.1					
	Ebro Delta	2006	4	-24.0	1.8	-26.2	-22.2	14	-24.9	1.0	-26.3	-23.2
		2007	16	-24.1	0.9	-25.0	-22.1	25	-26.3	1.5	-30.4	-23.1
		2008	12	-22.6	3.2	-25.6	-16.9					

Table 1. Descriptive statistics of stable isotopes signatures in feathers and eggs of Purple Heron nestlings (*Ardea purpurea*) from the three sites and sampling years.

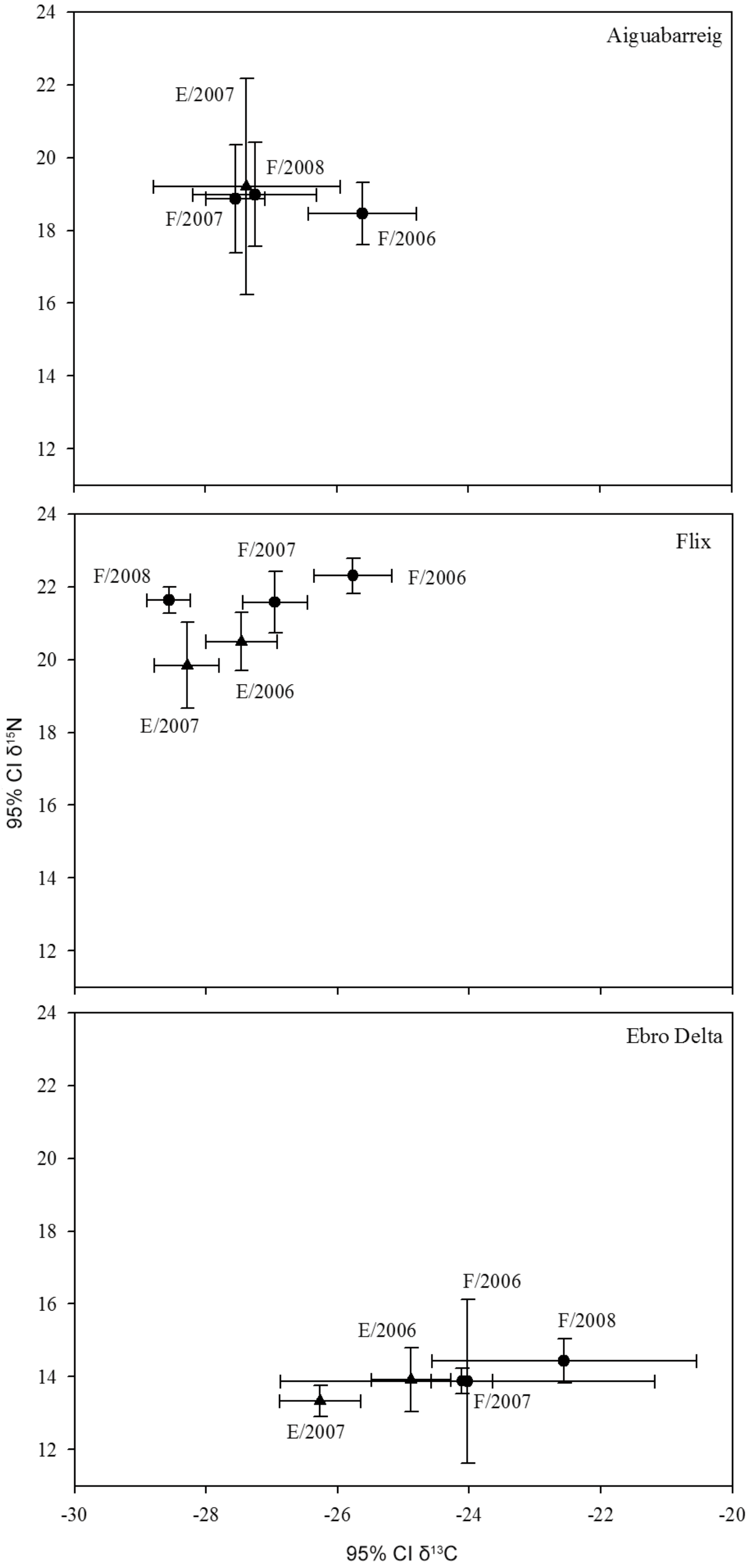


Figure 5. Plots of $\delta^{15}N$ and $\delta^{13}C$ signatures indicating the mean position of the Purple Heron nestlings and eggs of the three sampled sites in each year. Circles (feathers = F) and triangles (eggs = E) indicate mean value, and lines their corresponding 95% confidence intervals.

and Carp (J. Cotin, pers. obs.). Therefore, although δ^{15}N signatures reflect trophic level, the changes observed among the different localities are based on differences in the baseline.

Carbon signatures maintain the same pattern between sites each year, although slight differences can be observed between years (see fig. 5). The values reflect riverine and deltaic habitats, being around -24‰ for the Ebro Delta freshwater habitats (Cotin et al., 2011). Observed changes between years in riverine localities are probably due to the highly dynamic nature of the Ebro River. Annual changes at the Ebro Delta are probably related to a partial shift to more brackish feeding areas, such as the coastal lagoons, as habitats influenced by the sea present a higher carbon isotopic ratio than freshwater habitats (Michener and Schell, 1994). This fact is reflected in the higher variability of carbon signatures found in the Ebro Delta. The combined use of nitrogen and carbon signatures helps to discriminate well enough riverine and deltaic ecosystems (high nitrification and lower carbon signatures in the river).

When studying the second case, the Ebro Delta, eggs of the selected species were sampled and analyzed (see table 2). At this site carbon and sulphur stable isotopes (sulphur only analyzed in tern species) showed three distinct patterns (see table 3 and 4 for descriptives), allowing us to divide the foraging habitat structure of this area in three main scenarios: freshwater habitats (ricefields and lagoons) with low isotopic values (δ^{13}C around -24.5‰, δ^{34}S around 2-3‰), marine habitats (the sea and the bays) with δ^{13}C values around -18‰ and δ^{34}S around 18‰ and finally the saltpans (δ^{13}C around -14.5‰, δ^{34}S around 9.5‰). See figures 7 and 8.

About marine habitats, two tern and one gull species forage in these areas, the Sandwhich Tern, both colonies of Common Tern and the Audouin's Gull. The Sandwich Tern is a marine

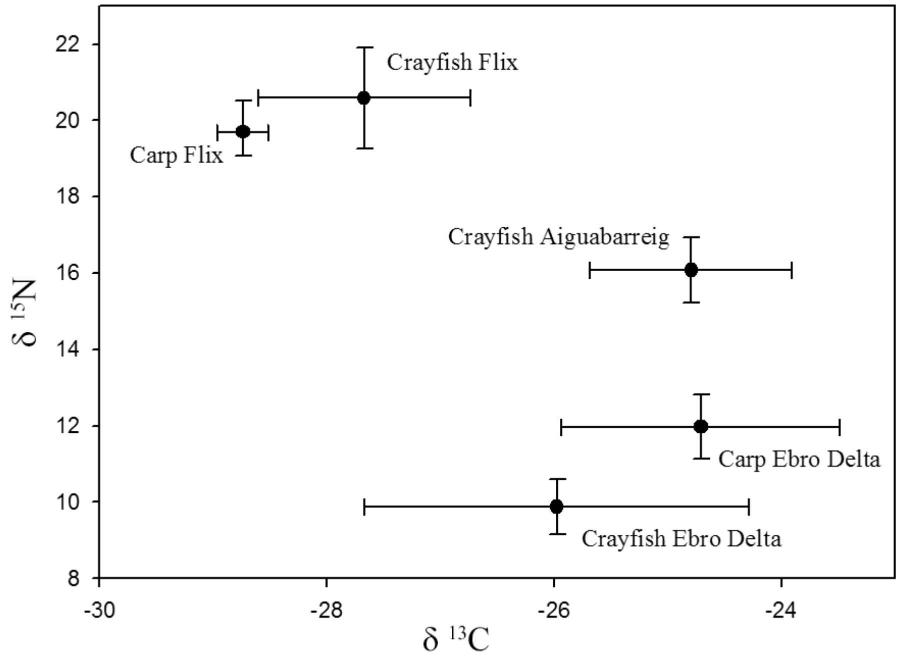


Figure 6. Plots of δ^{15}N and δ^{13}C signatures indicating the mean position of the Crayfish and Carp sampled of the three sampled sites (two for carp). Circles indicate mean value, lines their corresponding 95% confidence intervals.

Northern Hemidelta	Canal Vell	Ardea purpurea	3
		Egretta garzetta	55
		Ardeola ralloides	20
		Larus ridibundus	20
	Illa de Buda	Nycticorax nycticorax	21
		Ardeola ralloides	20
	Punta del Fangar	Sterna hirundo	18
		Gelochelidon nilotica	20
Southern Hemidelta	l'Encanyissada	Ardea purpurea	28
	La Tancada	Chlidonias hybrida	11
	Platjola	Ardea purpurea	8
	Riet Vell	Ixobrychus minutus	20
		Gallinula chloropus	20
		Porphyrio porphyrio	10
	Punta de la Banya	Sterna hirundo	10
		Sternula albifrons	24
		Sterna sandvicensis	20
		Larus audouinii	15

Table 2. Number of eggs sampled by colony at the Ebro Delta.

feeder (Stienen et al., 2000) and no other foraging habitat has ever been reported for this species. Although the Common Tern also feeds over freshwater in some areas (Becker et al., 1997, Neubauer, 1998), it is reported mainly as a marine forager (Becker et al., 1993, Granadeiro et al., 2002) and isotopic signatures indicate this is the feeding strategy that this species follows at the Ebro Delta. This observation is consistent with the findings of other studies (Arcos et al., 2002, Guitart et al., 2003). However, although the slight differences in the isotopic values of $\delta^{13}C$ and $\delta^{34}S$ between the two Common Tern colonies pointed to local differences, the huge differences in $\delta^{15}N$ indicate that the colonies used different resources. In fact, while Arcos et al. (2002) reported that the Common Tern in the Ebro Delta fed mainly on epipelagic fish (Clupeiformes), Oro and Ruiz (1997) found that this species also fed on discarded fish. Both observations are consistent with the feeding strategies described by Guitart et al. (2003) and Mateo et al. (2004) for the colonies at "Punta del Fangar" (which rely mainly on epipelagic fish) and "Punta de la Banya" (where demersal resources were obtained from fish discards). The Audouin's Gull is considered a nocturnal predator on epipelagic fish (mainly clupeiformes), but feeding is also opportunistic, using the fishery activities in neighbouring waters, especially trawler discards (Oro and Ruiz, 1997, Sanpera et al., 2007a). So, although $\delta^{13}C$ signatures of this species should point only marine habitats, the recently reported use of freshwater resources by this species, in particular Crayfish (Navarro et al., 2010b), probably lowers the carbon signature values (fig. 8).

The Little Tern showed high $\delta^{13}C$ and intermediate $\delta^{34}S$ signatures, being the only species of the study that presumably mainly feeds on the saltpans (see fig. 7 and 8). Previous studies carried out at Ria Formosa (Portugal) report on some of the factors that influence the selection of foraging habitats by this species, such as prey abundance, channels with strong currents, proximity to saltpans and conspecific information (Paiva et al., 2008a). Many studies have identified saltpans as a breeding habitat (Catry et al., 2004, Oro et al.,

	Species	N	Mean	SD	Min	Max															
$\delta^{15}N$ (‰)	Little Tern	24	14.6	0.9	12.6	16.4	○														
	Night Heron	21	13.9	1.1	12.0	15.4	●	○													
	Little Egret	55	13.6	1.2	11.2	17.4		●	○												
	Purple Heron	39	13.5	1.2	11.6	17.1		●	●	○											
	Gull-billed Tern	20	13.4	0.7	12.6	14.8		●	●	●	○										
	Squacco Heron	40	13.3	0.8	12.1	14.9		●	●	●	●	○									
	Common Tern 'Banya'	10	13.3	0.5	12.6	14.0		●	●	●	●	●	○								
	Little Bittern	20	12.8	1.1	10.6	14.3		●	●	●	●	●	●	○							
	Black-headed Gull	20	12.7	0.7	11.7	13.8				●	●	●	●	●	○						
	Whiskered Tern	11	12.6	0.9	11.5	14.9		●	●	●	●	●	●	●	●	○					
	Audouin's Gull	15	12.2	0.5	11.5	13.3								●	●	●	○				
	Sandwich Tern	20	12.1	0.3	11.3	12.7								●	●	●	●	○			
	Common Tern 'Fangar'	18	10.9	0.3	10.2	11.4													○		
	Moorhen	20	10.4	1.7	6.9	13.9													●	○	
	Purple Swamphen	10	10.0	1.2	7.8	11.8													●	●	○
$\delta^{13}C$ (‰)	Little Tern	24	-14.6	2.1	-18.3	-11.8	○														
	Common Tern 'Fangar'	18	-17.5	0.2	-17.9	-17.1		○													
	Common Tern 'Banya'	10	-17.9	0.2	-18.2	-17.6			○												
	Sandwich Tern	20	-18.0	0.2	-18.3	-17.7			●	○											
	Audouin's Gull	15	-20.1	1.8	-23.8	-18.3					○										
	Squacco Heron	40	-23.4	0.8	-24.8	-20.6						○									
	Moorhen	20	-24.4	1.1	-26.4	-21.7						●	○								
	Whiskered Tern	11	-24.6	0.7	-26.2	-23.6							●	○							
	Little Egret	55	-24.7	2.1	-28.7	-16.7							●	●	○						
	Little Bittern	20	-24.8	0.9	-26.4	-22.8							●	●	●	○					
	Gull-billed Tern	20	-24.8	0.5	-25.5	-23.4							●	●	●	●	○				
	Night Heron	21	-24.9	1.5	-27.2	-22.2							●	●	●	●	●	○			
	Purple Swamphen	10	-24.9	0.8	-26.1	-23.6							●	●	●	●	●	●	○		
	Black-headed Gull	20	-25.0	0.5	-25.9	-23.9							●	●	●	●	●	●	●	○	
	Purple Heron	39	-25.8	1.5	-30.4	-23.1								●	●	●	●	●	●	●	○

Table 3. Descriptive statistics of stable isotope signatures in eggs from the species included in the study, sorted from higher to lower values. Post hoc paired comparisons were denoted by a dot matrix. Filled circles indicate that there is not significant difference between row and columns species.

2004, Peste et al., 2004, Paiva et al., 2006a) and remark that this species is an opportunistic feeder and thus may use a wide variety of foraging grounds. The only limitation would be its small foraging range of 4-6 km when breeding (Fasola and Bogliani, 1990, Perrow et al., 2006). Observational data from transects in the Ebro Delta show that these birds forage in channels, lagoons and lagoon mouths, while salt marshes are avoided and rice fields are rarely visited (Bertolero et al., 2005); however, that study did not include transects in the saltpans (located at "Punta de la Banya" and where our samples were taken). δ^{13}C isotopic values for this species reflect a foraging habitat that differs to that of sea/freshwater/ terrestrial habitats and that is very similar to those reported by Tavares et al. (2007) in Little Terns feeding in Portuguese saltpans. In coastal hypersaline habitats, changes in primary producers raise the δ^{13}C signature (Michener and Schell, 1994). This finding indicates that Little Terns at "Punta de la Banya" feed mainly in the saltpans, which is the main habitat around the colony. Moreover, the high variance in their isotopic values also indicates the use of other habitats to a lesser extent and thus pointing to individual foraging strategies. Furthermore, the δ^{15}N values of this species were the highest of the tern species examined. These high δ^{15}N signatures would be related to the higher basal signature in the saltpans rather than true differences in the trophic levels occupied, as the diet of the Little Tern is based mainly on small fish and prawns (Norman, 1992, Bogliani et al., 1994, Paiva et al., 2006)

And finally, the freshwater habitats, with low δ^{13}C and δ^{34}S signatures, where all the heron, two tern, one gull and the two rallidae species gather together. Moorhen and the Purple Swamphen are described as mainly herbivorous, with the Purple Swamphen feeding on seeds, bulbs, stem and leaves from reeds (Phragmites australis), cattail (Thypa latifolia) and rice (Oryza sativa) (Cramp, 1980). Moorhen also feeds mainly on plant material (Amat and Soriguer, 1984) but during the chick rearing period may increase the percentage of insects. Their low δ^{15}N signatures are in agreement with the described diet and clearly differentiate these species from others. Black-headed Gull is a species that can exploit marine, freshwater and terrestrial habitats (Schwemmer et al., 2011), being an opportunistic forager. However, at the Ebro Delta, δ^{13}C signatures indicate that they rely mostly on freshwater habitats, and their relatively low δ^{15}N indicate that they feed probably insects and earthworms, which are a very common prey (Schwemmer et al., 2011). The two freshwater terns are the Whiskered

		N	Mean	SD	Min	Max
δ^{34}S (‰)	Little Tern	24	9.4	3.3	4.3	15.9
	Gull-billed Tern	20	1.6	0.6	0.5	2.5
	Whiskered Tern	11	3.4	1.5	1.2	5.5
	Sandwhich Tern	20	18.5	0.3	17.8	18.8
	Common Tern 'Fangar'	18	17.0	0.6	16.0	17.8
	Common Tern 'Banya'	10	19.0	0.4	18.4	19.6

Table 4. Descriptive statistics of sulphur stable isotope signatures (δ^{34}S) in eggs from the five tern species analyzed (Little, Gull-billed, Whiskered, Sandwich and Common Tern).

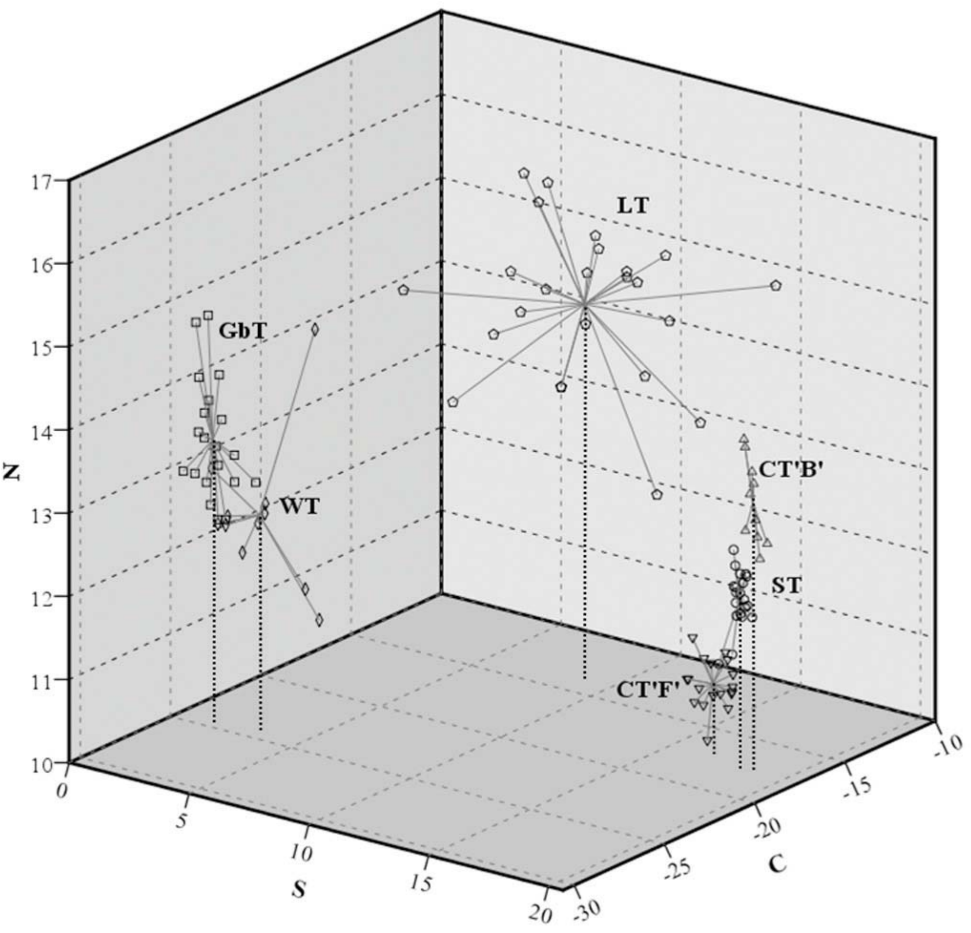


Figure 7. 3D-scatterplot showing the distribution of $\delta^{15}N$, $\delta^{34}S$ and $\delta^{13}C$ signatures of the five tern species: Little Tern (pentagons), Sandwich Tern (circles), Common Tern "Banya" (upwards triangles), Common Tern "Fangar" (downwards triangles), Gull-billed Tern (squares) and Whiskered Tern (rhombus).

and the Gull-billed Tern. The former feeds mainly on aquatic prey in wetlands (Latraube et al., 2005, Paillisson et al., 2007), rice fields, marshes and lagoons of the Ebro Delta are suitable foraging habitats for this species. Gull-billed tern is known to be a generalist forager on a range of prey (Goutner, 1991), such as grasshoppers and caterpillars (Mathew et al., 1998), amphibians and beetles (Cabo and Sanchez, 1986) or otherwise crayfish, insects and fish (Dies et al., 2005), but at the Ebro Delta, this species feeds mainly in the freshwater, as shown by its low $\delta^{13}C$ range. The slight differences found in the $\delta^{34}S$ signatures between the two freshwater terns would indicate that while the Gull-billed Tern also exploits terrestrial habitats (with lower $\delta^{34}S$ values), in agreement with previous studies (Andersen, 1945, Bogliani et al., 1990, Sanchez et al., 1991), the Whiskered Tern may use brackish waters (increasing its S isotopic values). It's interesting to notice that the Gull-billed Tern presents higher nitrogen signatures than the Whiskered Tern and the Black-headed Gull, maybe as a result of preying over bigger prey. It is of interest to note that the Common Tern egg collected on "Punta del Fangar" that was excluded from the analysis presented isotopic values very similar to the freshwater foragers. This observation indicates individual foraging strategies, even when this foraging behaviour has not been described previously for this tern population (Arcos et al., 2002, Mateo et al., 2004).

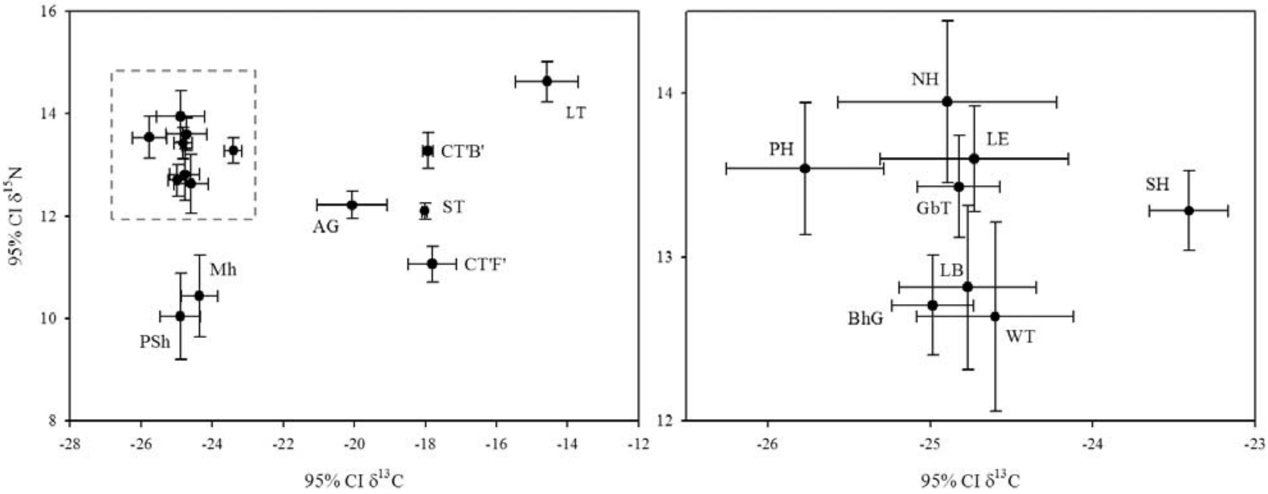


Figure 8. Plots of $\delta^{15}N$ and $\delta^{13}C$ signatures indicating the mean position of the species included in the study: Purple Heron (PH), Little Egret (LE), Night Heron (NH), Squacco Heron (SH), Little Bittern (LB), Sandwich Tern (ST), Common Tern 'Banya' (CT'B'), Common Tern 'Fangar' (CT'F'), Little Tern (LT), Gull-billed Tern (GbT), Whiskered Tern (WT), Black-headed Gull (BhG), Audouin's Gull (AG), Moorhen (Mh) and Purple Swamphen (PSh). Dot indicates mean value, and lines their corresponding 95% confidence intervals. Squared area in the left plot is enlarged at right.

The five heron species all feed on freshwater habitats. Purple Heron, Little Egret, Night Heron and Little Bittern are mainly aquatic predatory species. Differences in δ^{15}N indicate that Little Bittern feeds on lower trophic level prey, such as the crayfish (Pardo-Cervera et al., 2010). The greater variability in δ^{13}C signatures shown by the Little Egret indicate that they also might be feeding at some extent in other habitats at a lower extent, such as brackish waters or even saltpans (Abdennadher et al., 2011), thus pointing to some degree of individual heterogeneity in diet. Squacco Heron, although having the relative size of a Little Bittern, feeds mainly on insects (Orthotopera and Coleoptera) (Delord et al., 2004). It is interesting to remark the high percentage of European mole cricket (Gryllotalpa gryllotalpa) found in the pellets of this species at the Ebro Delta (unpublished data). This waterbird forages normally in the edge of ricefields and the contribution of terrestrial prey raises its δ^{13}C signatures.

According to the niche width metrics (fig. 9), which are based on the isotopic data, we can conclude that species such as the Common and Sandwich Tern are strict specialists at the Ebro Delta, with a narrow niche width. On the other hand, we have the Little Tern, some heron species (Little Egret, Purple Heron and Night Heron) and the moorhen, for which opportunistic feeding together with higher variability of δ^{13}C signatures in feeding habitats expands their trophic niche. This is especially true in the case of the freshwater species, as their, on average, higher niche width when compared to the marine species probably indicates a higher isotopic heterogeneity in those habitats.

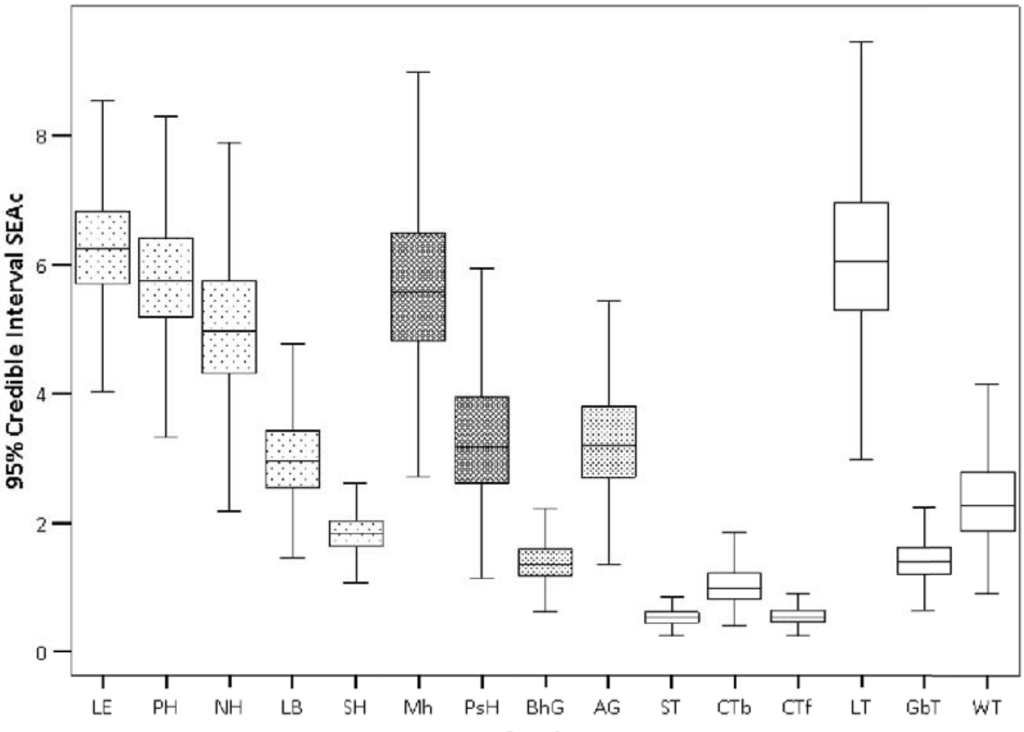


Figure 9. Corrected standard ellipse area (SEAc) of the study species sorted by family. Ardeidae: Purple Heron (PH), Little Egret (LE), Night Heron (NH), Squacco Heron (SH), Little Bittern (LB). Rallidae: Moorhen (Mh) and Purple Swamphen (PsH). Laridae: Black-headed Gull (BhG), Audouin's Gull (AG). Sternidae: Sandwich Tern (ST), Common Tern 'Banya' (CTb), Common Tern 'Fangar' (CTf), Little Tern (LT), Gull-billed Tern (GbT), Whiskered Tern (WT).

So to sum up both the river and the Ebro Delta aquatic bird communities, we could conclude that nitrogen signatures reflect trophic level, but differences among localities are greatly influenced by baseline values, being higher in the riverine sites than in the delta. Nitrogen together with carbon signatures adequately distinguish riverine and deltaic ecosystems (high nitrification and lower carbon signatures in the river). Also, we would like to highlight that the use of stable isotopes and ellipse-based niche metrics (SEA_c), applied on eggs samples, have helped unravelling the trophic ecology, niche and habitat partitioning of the aquatic bird community of the Ebro Delta: Common and Sandwich Tern behave as strict specialists at the Ebro Delta, with narrow niche widths, while in the opposite way, Little Tern, Little Egret, Purple Heron, Night Heron and the Moorhen behave like generalists foragers, with broad niche widths.

A2. Pollutant levels in eggs and nestling feathers. Conservational concern?

As with the previous section, to gain a better understanding of the pollutant levels in birds at the Ebro River, first we will compare among the selected sites (the two riverine and the deltaic one) using Purple heron eggs and nestlings and, secondly, we will focus on the Ebro Delta, using the eggs of the families Sternidae, Ardeidae, Laridae and Rallidae. In all samples we analyzed mercury, selenium, copper, lead, chromium and arsenic (chromium and arsenic only 2007-2008).

With regard to chromium and arsenic, although levels at the Ebro River showed some differences for arsenic (see table 5 and 6), levels are very similar in all sites. Chromium levels detected at Flix could be slightly influenced by the toxic muds and arsenic probably is higher at the Ebro Delta due to a partial use of foraging habitats influenced by the sea, as it has been seen in other species inhabiting this area. Arsenic and chromium levels are below toxic levels and within those reported for several bird species, including herons (Burger and Gochfeld, 2009, Padula et al., 2010), and lead levels are below those causing adverse reproductive effects (around 4000 ng/g in feathers reported by Burger and Gochfeld (2000a)).

Regarding copper, shifts in concentrations among years could reflect the dynamics of the river. This metal seems to be equally available in all the habitats, and levels could be explained by the use of copper sulphate (Cu_2SO_4) in agriculture. Levels reported are far below toxic levels, which are considered around 60 ppm (Attia et al., 2011).

Eggs are good bioindicators of selenium (Ohlendorf et al., 2011) and levels are higher at riverine sites than at the Ebro Delta (see fig. 10). Therefore, the river is probably affected by other sources of selenium rather than the polluted sediments. Although feathers are poor indicators of this pollutant (Ohlendorf and Heinz, 2011), the higher levels found at Flix seems to indicate a slight effect from this site on herons.

Avian embryos are very sensitive to the toxic effects of selenium, which is reflected by reduced hatchability of fertile eggs and teratogenic development of embryos (Janz et al.,

			Sites													
			l'Aiguabarreig					Flix					Ebro Delta			
	Year	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max
[Hg]	2006	7/7	1580.7	637.4	902.7	2684.3	10/10	7819.5	2995.3	2980.8	13616.7	4/4	2390.8	427.1	2057.5	2854.4
	2007	7/7	1638.4	543.0	863.9	2345.2	11/11	6911.5	4447.9	2718.5	10313.2	16/16	1381.6	705.3	748.5	3387.0
	2008	4/4	1512.8	151.9	1291.9	1575.7	9/9	2986.4	1084.1	2407.6	9435.4	12/12	1792.3	449.9	1098.8	2595.1
[Se]	2006	7/7	1795.9	449.6	1502.9	2481.4	10/10	2578.0	818.6	2054.8	3370.6	3/4	1709.1	347.7	1399.9	1747.6
	2007	6/7	1853.0	434.8	1650.5	2286.2	11/11	2284.6	742.8	1745.8	3534.0	13/16	1516.4	154.5	1090.2	2130.3
	2008	4/4	2202.3	160.1	2126.9	2333.4	9/9	1924.7	888.4	1643.6	3961.2	12/12	1709.4	747.8	1255.5	2200.7
[Cu]	2006	7/7	8831.0	1979.0	6979.4	9986.9	10/10	8058.6	1018.6	6510.0	10224.5	4/4	11279.8	1911.6	10188.8	12552.4
	2007	7/7	6001.1	2796.9	4113.9	7696.1	11/11	6085.2	1461.5	4431.9	7467.1	16/16	6015.8	1099.4	4073.8	7152.5
	2008	4/4	5936.2	538.0	5521.6	6324.1	9/9	5301.1	1478.4	4521.0	9712.1	12/12	7876.5	2355.9	4863.0	23391.0
[Pb]	2006	7/7	1312.9	829.0	951.7	2073.2	10/10	1692.1	367.5	1121.4	2168.4	4/4	2023.0	536.5	1603.0	2631.0
	2007	0/7					1/11	116.3	0.0	116.3	116.3	0/16				
	2008	1/4	59.9	0.0	59.9	59.9	5/9	41.2	112.6	34.6	191.1	9/12	74.3	62.6	54.0	337.1
[Cr]	2007	7/7	1622.2	224.5	1471.1	1738.8	11/11	1759.0	398.0	1508.2	2045.8	16/16	1601.7	167.6	1203.7	1792.0
	2008	4/4	1946.3	101.2	1891.9	2064.3	9/9	2087.8	183.1	1859.0	2259.8	12/12	2102.0	172.0	1781.3	2389.9
[As]	2007	2/7	111.3	19.6	101.5	121.1	3/11	122.3	13.7	119.3	132.9	14/16	159.2	34.4	125.2	268.0
	2008	4/4	68.5	26.9	55.5	92.6	9/9	60.6	37.5	48.8	117.5	12/12	156.2	76.9	77.3	215.3

Table 5. Descriptive statistics of trace elements expressed in ng/g in feathers of Purple Heron nestlings (*Ardea purpurea*) from the three sites and years. Only samples with detected values are given. For mercury and copper this represents 100% of samples.

		Sites															
		l'Aiguabarreig					Flix					Ebro Delta					
	Year	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max	
[Hg]	2006						12/12	825.3	778.3	481.7	3467.8	14/14	645.9	216.7	297.8	1124.1	
	2007	12/12	115.5	66.8	93.2	827.1	16/16	663.1	827.0	92.8	1727.5	25/25	302.0	323.7	91.0	626.5	
[Se]	2006			0.0			12/12	3933.2	920.6	2317.5	5523.6	14/14	3143.7	1081.5	1599.1	3939.6	
	2007	12/12	3545.9	1019.6	2594.6	4424.0	16/16	3477.1	621.3	2110.3	4181.4	25/25	2561.8	818.5	1471.4	3655.1	
[Cu]	2006			0.0			12/12	4950.5	1424.6	4431.1	6607.1	14/14	4704.8	595.8	3735.6	6208.4	
	2007	12/12	3677.6	808.0	2976.5	5909.0	16/16	4031.6	937.7	3030.6	5177.6	25/25	4351.8	1003.2	3350.4	5861.6	
[Cr]	2007	12/12	2500.8	164.9	2406.1	2784.8	16/16	2527.9	233.9	1478.5	2726.8	25/25	2570.0	181.5	2314.5	3026.9	
[As]	2007	10/12	127.1	26.5	111.8	173.7	11/16	140.1	29.3	123.0	159.2	25/25	167.9	52.8	100.2	303.0	

Table 6. Descriptive statistics of trace elements expressed in ng/g in eggs of Purple Heron (*Ardea purpurea*) from the three sites and years. Only samples with detected values are given. For mercury and copper this represents 100% of samples.

2010.). Although the threshold at which negative impacts occur in birds is widely disputed, Ohlendorf and Heinz (2011) described levels higher than 12 mg/kg dry weight as a concentration associated with elevated probability for reduced egg hatchability in sensitive and moderately sensitive species and levels lower than 3.0 mg/kg as a mean concentration for background conditions. Our values don't reach the first threshold, but are in all cases above the range of those reported in Little Egret eggs and feathers in Pakistan (Boncompagni et al., 2003), feathers of Black-crowned Night Heron (Nycticorax nycticorax) in USA, (Golden et al., 2003) breast feathers from several heron species from Hong Kong and Szechuan (Burger and Gochfeld, 1993) and among the highest ever reported for a heron species, although a negative impact cannot be certain, as tresholds levels in feathers have not been precisely defined (Ohlendorf and Heinz, 2011).

Concerning mercury, Flix presented the highest concentrations among the studied sites, meaning that the amount of mercury leaching from the toxic muds into the Ebro River ecosystem is high and continous at Flix Reservoir, as the concentrations are stable through the years even in a highly dynamic ecosystem as the Ebro River, as also shown in a study conducted with Zebra mussel (Carrasco et al., 2008). The only change in this pattern was during 2008, in which some of the individuals from Flix site may have been feeding upstream, away from the main mercury input, as reflected by the lower levels in nestling feathers. Levels detected at l'Aiguabarreig are much higher than the ones expected for a 'reference' site (see fig. 10), so this area must also be affected by other sources of mercury than Flix residues. Even the Ebro Delta, situated 90 km from the Flix site, shows intermediate levels that may be explained by the sediments being carried downstream. Purple Heron show relatively high concentrations at this site, as other bird species foraging at the same area have already shown (Cotin et al., 2011).

The high mercury levels detected at Flix are of special concern, Purple Heron nestlings having values as high as 13,600 ng/g in feathers, which is within the alerting range, as concentrations of mercury between 5000 and 15000 ng/g in feathers have been related to adverse effects on growth and reproduction in birds (Eisler, 1987). In fact, adverse effects have already been pointed out in previous studies for this breeding population, which in part may be due to the fact that heron embryos are highly sensitive to mercury (Heinz et al., 2009). The elevated levels of micronuclei in peripheral erythrocytes (Quiros et al., 2008) and the reduced blood antioxidant defenses (Barata et al., 2010) are some of the physiological stress responses that these birds have developed against pollution. Also, other species in this ecosystem, including Zebra mussels, Crayfish and several fish species, have shown effects from this pollutant (Navarro et al., 2009, Benejam et al., 2010, Faria et al., 2010, Suarez-Serrano et al., 2010).

Finally, it should be noted that mercury levels found in nestling feathers at Flix are far higher than the ones found in other wetlands or rivers threatened by pollution elsewhere. Mercury levels at Flix are higher then the ones found in Little Egret in Pakistan (Boncompagni et al., 2003) and Hong Kong (Connell et al., 2002) or several heron species from China (Burger and Gochfeld, 1993) and even higher than those reported for Little Egret and Night Heron feathers in the Axios Delta, Greece (Goutner and Furness, 1997) and northern Italy (Fasola et al., 1998). Higher levels have been found in heron species in the Everglades, USA (Frederick et al., 2004), but feathers were taken from adult birds conserved in museums,

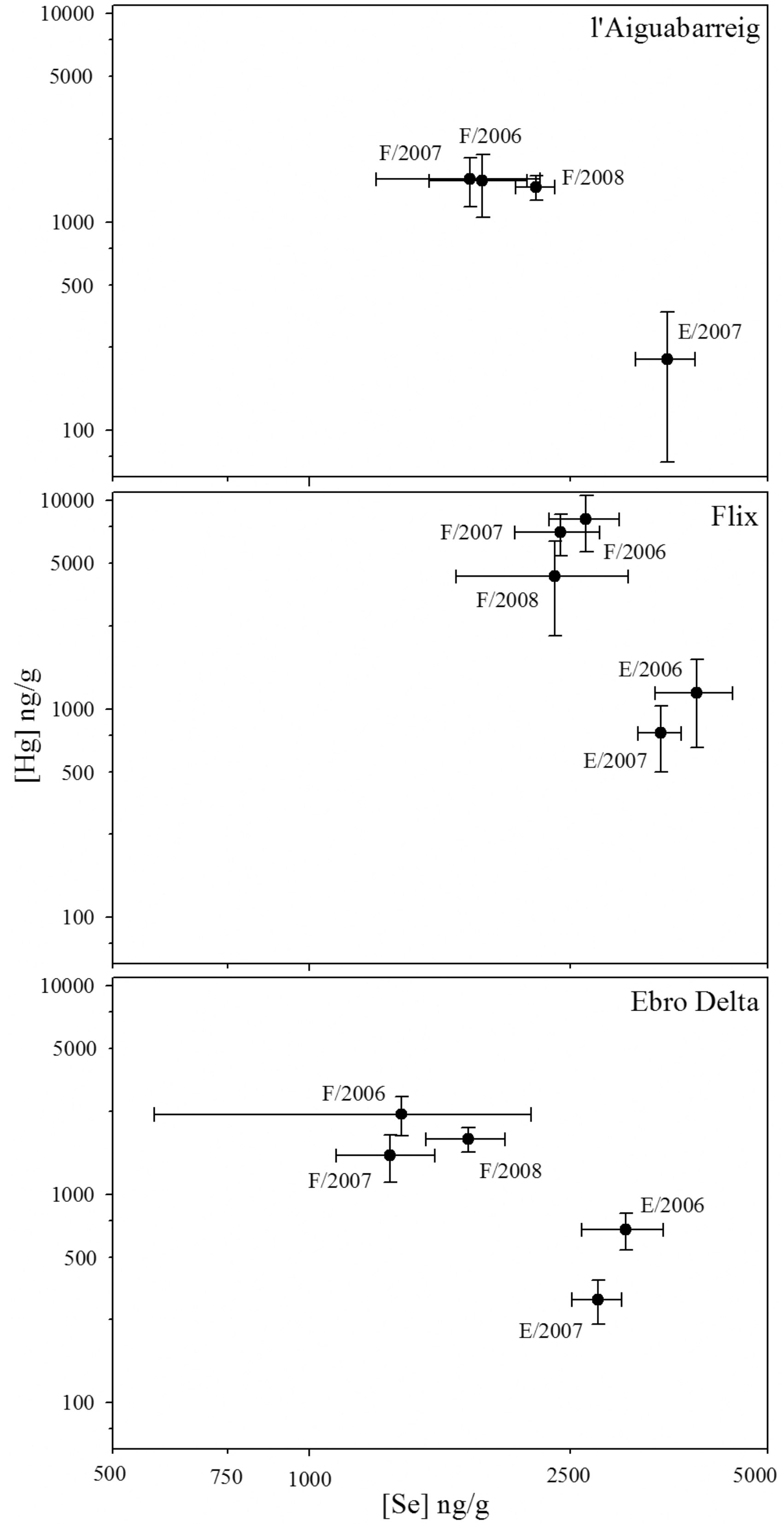


Figure 10. Plots of mercury and selenium concentrations (given on a logarithmic scale) indicating the mean position of the Purple Heron nestlings and eggs of the three sampled sites in each year. Circles (feathers = F) and triangles (eggs = E) indicate mean value, and lines their corresponding 95% confidence intervals.

	Species	N	Mean	G Mean	SD	Min	Max															
[Hg]	Audouin's Gull	15/15	5903.5	5321.9	2713.0	2299.9	11883.3	○														
	Little Tern	24/24	4150.1	3694.5	1973.7	1375.7	8621.9	●	○													
	Common Tern 'Banya'	10/10	4148.9	3688.9	1940.9	1138.5	7631.7	●	●	○												
	Sandwich Tern	20/20	2727.2	2454.7	1264.3	1030.7	4999.8		●	●	○											
	Whiskered Tern	11/11	2576.6	2309.2	1319.9	1119.6	5591.5		●	●	●	○										
	Gull-billed Tern	20/20	1770.1	1659.8	699.9	879.2	3686.6			●	●	●	○									
	Common Tern 'Fangar'	18/18	1139.6	1106.1	264.3	588.1	1663.8							○								
	Little Egret	55/55	1064.3	981.5	491.7	467.1	3450.6							●	○							
	Night Heron	21/21	1115.1	964.1	870.7	446.0	4688.9							●	●	○						
	Little Bittern	19/20	660.9	545.0	449.8	106.1	2047.6								●	●	○					
	Purple Heron	30/39	442.8	356.4	265.7	91.0	1124.1										●	○				
	Squacco Heron	35/40	535.9	351.3	639.2	52.7	3429.5										●	●	○			
	Black-headed Gull	15/20	369.1	207.0	463.5	49.1	1929.2										●	●	●	○		
	Moorhen	3/20				223.0	888.5															
	Purple Swamphen	0/10																				
[Se]	Sandwich Tern	20/20	4141.8	4116.4	477.1	3410.9	5349.2	○														
	Little Egret	55/55	3931.7	3820.5	961.5	2392.3	6518.2	●	○													
	Black-headed Gull	20/20	3585.3	3497.6	848.5	2319.9	5503.7	●	●	○												
	Audouin's Gull	15/15	3508.4	3457.8	601.5	2350.3	4255.5	●	●	●	○											
	Common Tern 'Banya'	10/10	3464.7	3445.4	384.8	2954.4	3973.3	●	●	●	●	○										
	Whiskered Tern	11/11	3148.5	3134.0	317.6	2677.7	3646.9			●	●	●	○									
	Night Heron	21/21	3023.0	2917.2	854.1	1805.3	4965.9			●	●	●	●	○								
	Little Bittern	20/20	2965.2	2915.0	566.2	2159.0	4112.7			●	●	●	●	●	○							
	Common Tern 'Fangar'	18/18	2854.5	2833.4	366.3	2394.2	3614.5			●	●		●	●	●	○						
	Little Tern	24/24	2839.4	2788.3	545.4	1851.6	3997.7			●	●		●	●	●	●	○					
	Purple Heron	39/39	2856.1	2776.8	652.2	1471.4	3939.6			●	●		●	●	●	●	●	○				
	Squacco Heron	40/40	2852.9	2771.3	767.1	1581.8	6249.1			●	●		●	●	●	●	●	●	○			
	Gull-billed Tern	20/20	2740.1	2701.9	473.0	1960.7	3905.8						●	●	●	●	●	●	●	○		
	Moorhen	19/20	1609.1	1545.8	410.6	534.0	2326.0														○	
	Purple Swamphen	2/10				1217.5	1381.1															
[Cu]	Little Egret	55/55	5750.2	5698.7	755.1	3752.1	7476.7	○														
	Night Heron	21/21	5031.6	4967.9	805.8	3365.9	6606.2	●	○													
	Purple Heron	39/39	4518.7	4471.9	667.9	3350.4	6208.4		●	○												
	Squacco Heron	40/40	4197.0	4137.5	727.3	2924.7	6600.9			●	○											
	Little Bittern	20/20	4143.7	4076.8	820.0	3080.0	6608.0		●	●	●	○										
	Common Tern 'Banya'	10/10	4134.1	3972.9	1332.9	2947.8	7234.5	●	●	●	●	●	○									
	Whiskered Tern	11/11	3688.5	3619.4	752.6	2695.1	5068.1			●	●	●	●	○								
	Little Tern	24/24	3518.9	3377.0	1199.5	1989.6	8123.2				●	●	●	●	○							
	Black-headed Gull	20/20	4932.2	3372.2	5939.8	1836.6	24019.2	●	●	●	●	●	●	●	●	○						
	Common Tern 'Fangar'	18/18	3070.0	3049.9	369.2	2617.3	3799.9						●	●	●	●	○					
	Audouin's Gull	15/15	3010.2	2967.1	522.1	2135.4	3941.9						●	●	●	●	●	○				
	Gull-billed Tern	20/20	2973.7	2932.4	530.1	2141.4	4512.8						●	●	●	●	●	●	○			
	Sandwich Tern	20/20	3058.2	2930.8	1075.9	2298.5	6027.3						●	●	●	●	●	●	●	○		
	Purple Swamphen	10/10	2989.4	2872.1	760.7	1232.2	4173.3			●	●	●	●	●	●	●	●	●	●	●	○	
	Moorhen	20/20	2818.7	2735.3	672.8	1288.4	4167.0						●	●	●	●	●	●	●	●	●	○

Table 7. Descriptive statistics of mercury, selenium and copper in eggs from the species included in the study sorted by the geometric mean. Post hoc paired comparisons were denoted by a dot matrix. Filled circles indicate that there is not significant difference between row and columns species. Concentrations are expressed in ppb (ng/g).

which probably reflect bioaccumulation. High values of mercury in blood were found for some years in the Carson River (Nevada) for two heron species, Snowy Egret (Egretta thula) and Black-crowned Night Heron (Henny et al., 2007), but to our knowledge, mercury levels detected in Purple Heron at the Flix site are the highest ever reported in nestlings feathers of a heron species.

Coming back to the Ebro Delta and focusing on the pollutant levels of the whole aquatic bird community (see table 7 and 8), but continuing with mercury levels we observe that levels are higher in terns than in herons, even for those species foraging in the same habitat, indicating phylogenetic differences in metal bioaccumulation between the family Ardeidae and Sternidae that will be discussed hereafter. Mercury levels tend to be higher in

	Species	N	Mean	G Mean	SD	Min	Max									
[Cr]	Whiskered Tern	11/11	2885.8	2880.2	189.6	2579.4	3195.0	○								
	Little Egret	35/35	2720.7	2717.9	127.3	2503.9	3172.5	●	○							
	Little Tern	14/14	2644.0	2642.1	103.3	2522.8	2842.0	●	●	○						
	Common Tern 'Banya'	10/10	2605.8	2605.4	47.7	2514.4	2678.6		●	●	○					
	Purple Heron	25/25	2605.1	2599.3	179.1	2314.5	3026.9		●	●	●	○				
	Sandwich Tern	20/20	2584.4	2579.6	161.1	2309.9	2855.8		●	●	●	●	○			
	Black-headed Gull	20/20	2574.8	2568.1	184.5	2086.8	2795.1		●	●	●	●	●	○		
	Squacco Heron	20/20	2466.8	2457.1	224.8	2077.1	2916.3			●	●	●	●	●	○	
	Audouin's Gull	15/15	2413.5	2404.2	221.3	2033.2	2921.8			●	●	●	●	●	●	○
[As]	Common Tern 'Banya'	10/10	1217.7	1179.8	293.6	633.8	1606.5	○								
	Sandwich Tern	20/20	857.0	850.4	106.0	617.4	1046.5		○							
	Little Tern	14/14	850.6	811.9	264.1	448.7	1274.1		●	○						
	Audouin's Gull	15/15	685.3	655.8	212.3	336.1	1169.1		●	●	○					
	Little Egret	35/35	185.2	178.1	52.9	94.0	318.4					○				
	Purple Heron	25/25	172.7	167.8	43.2	100.2	303.0					●	○			
	Whiskered Tern	11/11	104.7	101.2	27.2	58.9	154.5							○		
	Squacco Heron	17/20	109.6	95.7	60.7	32.5	316.9							●	○	
	Black-headed Gull	16/20	90.9	79.9	44.2	25.9	195.6							●	●	○
[Pb]	Purple Swamphen	1/10				789.1	789.1									
	Sandwich Tern	3/20				69.7	935.9									
	Whiskered Tern	1/11				176.4	176.4									
	Black-headed Gull	5/20				90.4	362.4									
	Gull-billed Tern	1/20				131.9	131.9									
	Little Tern	2/24				111.6	120.2									
	Squacco Heron	5/40				61.4	189.2									
	Little Egret	2/55				84.3	131.6									

Table 8. Descriptive statistics of chromium, arsenic and lead in eggs from the species included in the study sorted by the geometric mean. Chromium and arsenic were analysed only in samples from 2007 and 2008. Species without detectable lead levels are not shown in the table. Post hoc paired comparisons were denoted by a dot matrix. Filled circles indicate that there is not significant difference between row and columns species. Concentrations are expressed in ppb (ng/g).

marine-related species than in freshwater ones, but on top of that, mercury derived from the industry along the riverside, especially from the Flix reservoir (Cotin et al., 2012), has probably been accumulating in the sand banks of the Ebro Delta over the years, particularly in the southern hemidelta, as materials carried by the Ebro river are mainly deposited there by the Liguro-Provençal-Catalan oceanic current flowing south (Guitart et al., 2003). This fact has probably boosted the mercury concentrations of the species at the southern hemidelta, especially those feeding on fish discards (based on demersal fish) which are, among others, the Audouin's Gull (Morera et al., 1997) and the Common Tern 'Banya'. Higher mercury levels have been reported for the Audouin's Gull colony in the Ebro Delta, when compared to other colonies elsewhere in the Mediterranean (Sanpera et al., 2007a). The freshwater terns, Whiskered and Gull-billed Tern, are also exposed to mercury inputs from the river, although in lower amounts, and reach smaller concentrations than the marine terns, except for the Common Tern 'Fangar'. This common tern colony shows much lower mercury concentrations as it's situated in the Northern hemidelta, where is apart from the main mercury input of the river. Also its diet is based on epipelagic fish, which has lower mercury loads (Arcos et al., 2002) than the demersal fish. The values of this colony are similar to other Common Tern colonies that follow the same feeding habits (Monteiro et al., 1999, Guitart et al., 2003, Becker and Munoz Cifuentes, 2004, Bond and Diamond, 2009), with most values around 1000 ppb.

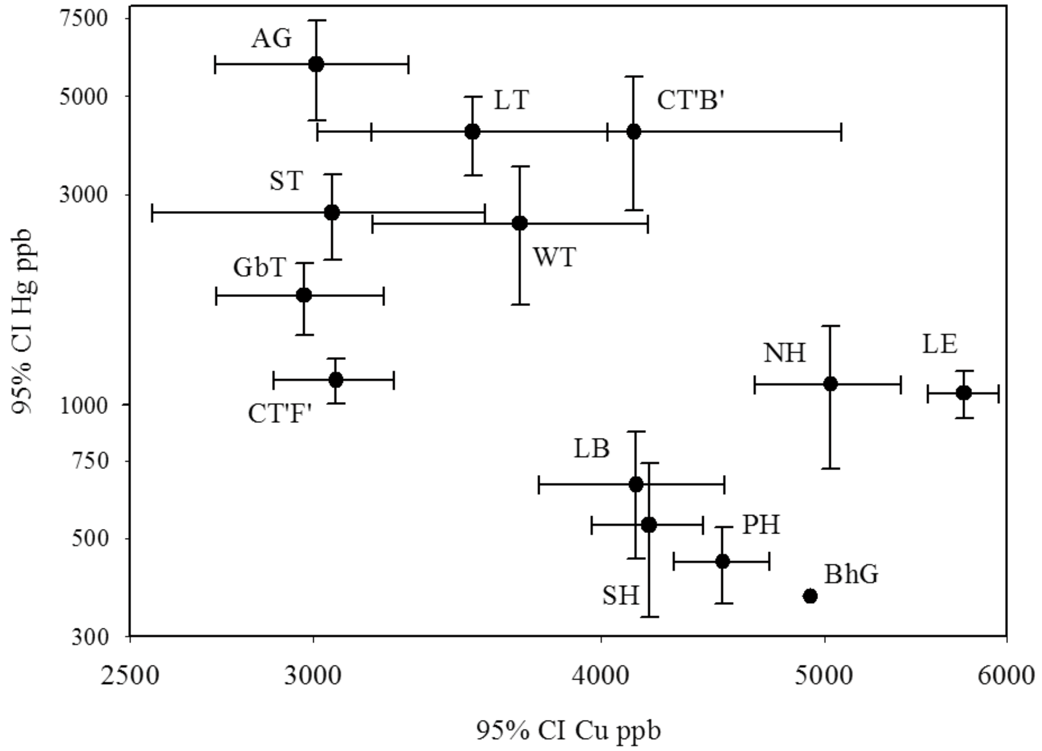


Figure 11. Plots of Hg and Cu concentrations (given on a logarithmic scale) of the species included in the study: Purple Heron (PH), Little Egret (LE), Night Heron (NH), Squacco Heron (SH), Little Bittern (LB), Sandwich Tern (ST), Common Tern 'Banya' (CT'B'), Common Tern 'Fangar' (CT'F'), Little Tern (LT), Gull-billed Tern (GbT), Whiskered Tern (WT), Black-headed Gull (BhG) and Audouin's Gull (AG). Moorhen and Purple Swamphen are not included and Black-headed Gull 95% CI is omitted due to its high variability. Dot indicates mean value, and lines their corresponding 95% confidence intervals.

The Little Tern presents the second highest mercury concentrations of all the studied species. This tern feeds mainly at saltpans, and the mercury baseline in these food webs is relatively high. Similar mercury values have been found in saltpans at Portugal (Tavares et al., 2007, Paiva et al., 2008b) and birds feeding mainly in marine habitats at the Baltic Sea presented lower values (Thyen et al., 2000).

Among herons, Little Egret shows the highest mercury levels, probably due to its partial use of saltpans and brackish waters, while the Squacco Heron presents the lowest, as this species rely mainly on insects. The Black-headed Gull also shows very low mercury concentrations, in agreement with the invertebrate-based diet indicated by the stable isotopes. Finally, the two herbivorous species, Moorhen and Purple Swamphen, showed the lowest mercury values.

About copper, no clear differences were found among species, although it is known that the agricultural use of copper sulphate (Cu_2SO_4) is widespread in the freshwater habitats, making this element more available. Nevertheless, a pattern can be delineated among groups of species: herons > terns + gulls > rallids. If we recall the pattern observed in the mercury concentrations, we can see that both metals behave oppositely at species level, so, confronting them in a plot, we observe the family pattern clearly (see figure 11): Terns (family Sternidae) have, independently of the feeding habitat, high concentrations of mercury and low of copper, while Herons (family Ardeidae) have just the opposite. These phylogenetic differences in metal bioaccumulation between both families (Ardeidae and Sternidae) had never been described previously. It is worth noting in figures 11-12 the differences between the two gull species, in which the habitat and diet differences come out clearly.

Selenium is appreciably transferred to eggs (Sell, 1977, Focardi et al., 1988), making of eggs good bioindicators for this element (Ohlendorf et al., 2011). Levels at the Ebro Delta, as with copper, did not differ among species. Only herbivorous species showed lower concentrations. Neither chromium levels show any standing difference among species or habitats. About lead, although the Ebro Delta is a frequent hunting area and the ingestion of lead pellets could be a possible threat, the study species presented very low concentrations.

For arsenic, in the four groups of species stands the pattern of higher levels in the marine species when compared to the freshwater ones. Arsenic concentrations tend to be higher in seawater than in freshwater (Kunito et al., 2008, Lucia et al., 2010) and also much higher than in terrestrial organisms or environments (Lunde, 1977). When displaying arsenic levels with carbon signatures (see fig. 13), there is an outstanding discrimination between marine and freshwater species, marine related species presenting higher levels than the freshwater species.

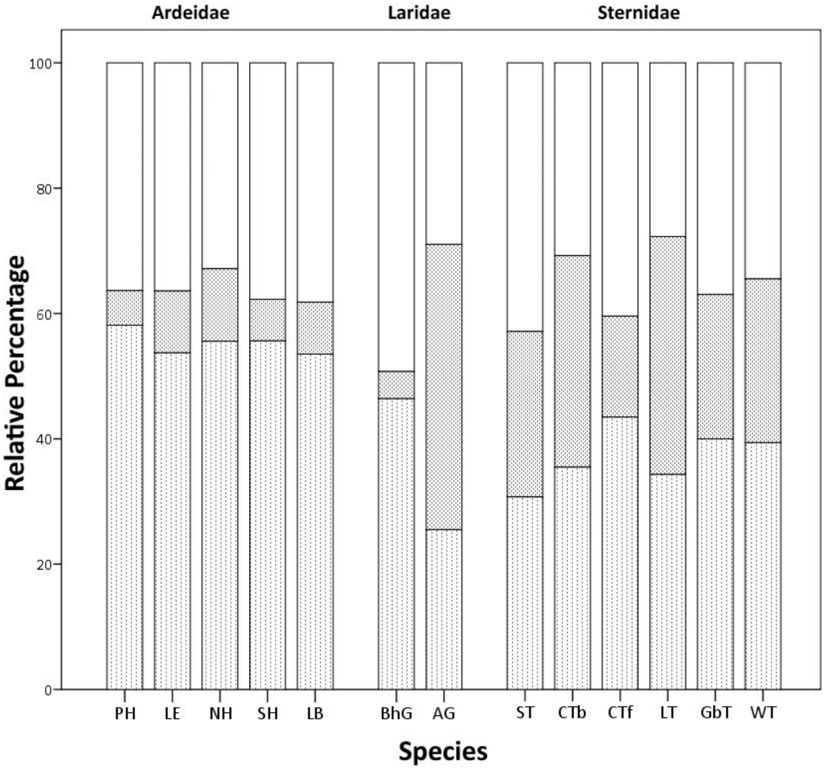


Figure 12. Relative percentages of the Copper (white) Mercury (grey) and Selenium levels (pale grey) sorted by family: Purple Heron (PH), Little Egret (LE), Night Heron (NH), Squacco Heron (SH), Little Bittern (LB), Sandwich Tern (ST), Common Tern 'Banya' (CTb), Common Tern 'Fangar' (CTf), Little Tern (LT), Gull-billed Tern (GbT), Whiskered Tern (WT), Black-headed Gull (BhG), and Audouin's Gull (AG). Moorhen (Mh) and Purple Swamphen (PsH) are not represented due to the undetectable levels of Mercury and Selenium.

If we compare the pollutant levels of the Ebro Delta aquatic bird community with other similar areas and communities, we find that, for example, at the saltworks of Thyna (Tunisia), mercury and selenium levels in Slender-billed Gull, Common Tern and Little Egret (Ramirez et al., 2011) ranged similarly, but concentrations were relatively higher at the Ebro Delta. At three different wetlands in China, levels of As, Cd, Cr, Cr, Pb, Hg and Se in Little Egret (Zhang et al., 2006) also ranged similarly, with the exception of chromium, which was much higher at the Ebro Delta, although within those reported for several bird species (Burger and Gochfeld, 2009, Padula et al., 2010). Great Egret (*Ardea albus*) mercury levels from the Florida everglades are within the range of herons at the Ebro Delta (Rumbold et al., 2001). Levels of the same elements analyzed in this study in herons and terns of the Pearl River Delta (southern China) (Lam et al., 2005) were similar, with the exception of chromium in herons (which is higher at the Ebro Delta), mercury in terns (also higher at the Ebro Delta) and copper in both families (lower at the Ebro Delta). At three different wetlands in Pakistan, levels of As, Cd, Cr, Pb, Hg and Se in three species of herons (Boncompagni et al., 2003) also ranged similarly, with once more the exception of chromium, being its levels higher at the Ebro Delta. Little Terns feeding at the Baltic Sea presented lower levels (Thyen et al., 2000), while Common Terns from the Elbe Estuary (a heavily polluted area) showed higher levels (Becker et al., 1993).

Assembling the riverine and deltaic perspective, we can conclude that all analyzed elements are out of conservational concern (excluding mercury) and within range values of other bird communities (with the exception of chromium and mercury). Mercury levels in nestlings of Purple Heron at Flix site and in eggs of Audouin's Gull, Little Tern and Common

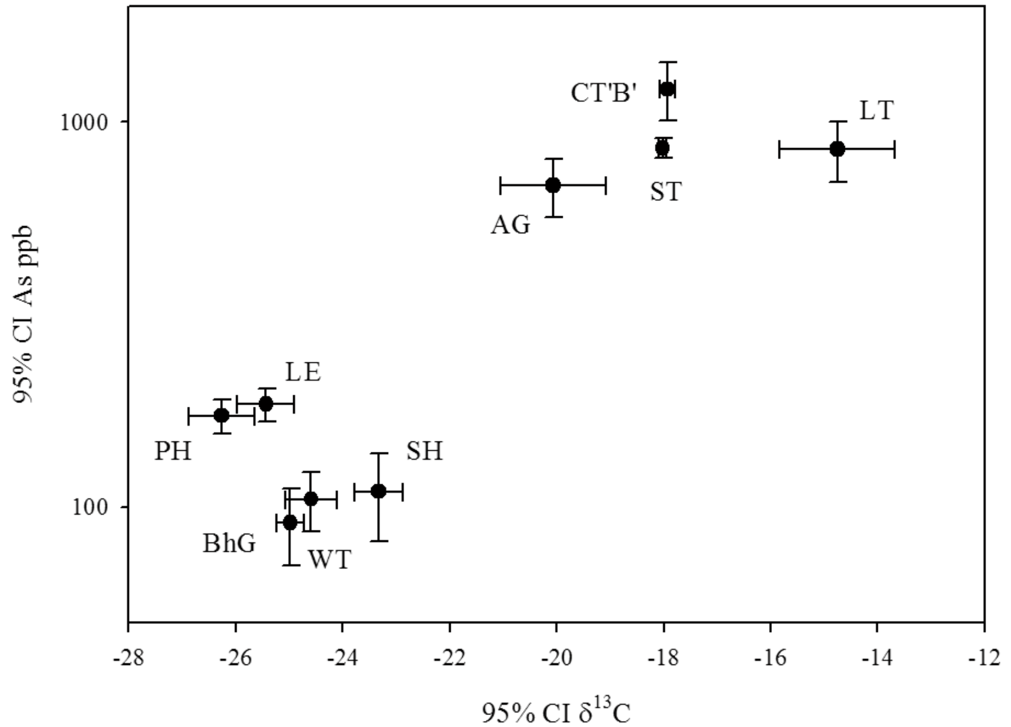


Figure 13. Plots of Arsenic concentrations (given on a logarithmic scale) and $\delta^{13}C$ signatures of Purple Heron (PH), Little Egret (LE), Squacco Heron (SH), Sandwich Tern (ST), Common Tern 'Banya' (CT'B'), Little Tern (LT), Whiskered Tern (WT), Black-headed Gull (BhG) and Audouin's Gull (AG). Dot indicates mean values, and lines their corresponding 95% confidence intervals.

Tern 'Banya' at the Ebro Delta are high enough to be of special concern; further studies are of particular relevance in order to monitor whether these values are endangering the breeding success of these species. In the case of Mercury in Purple Heron at Flix is among the highest ever reported, being their levels highly influenced by the polluted sediments at Flix reservoir. Arsenic (specially used in combination with carbon signatures) discriminates outstandingly between marine and freshwater species. Finally, and surprisingly, Terns (family Sternidae) and Herons (family Ardeidae) showed great differences in levels of mercury and copper: high mercury and low copper loads for Terns and opposite in Herons, being these differences not explained clearly by diet or habitat, so presumably there is a phylogenetical base worth to study.

A3. Feather or eggs? Which is best when it comes to pollution?

Eggs and nestling feather samples were compared using the Purple Heron as model species, due to the higher number of comparable localities (l'Aiguabarreig, Flix, Ebro Delta) and sample sizes (see tables 2, 5 and 6).

In previous studies, Burger et al. (2009) found no differences in the usefulness of egg and nestling feather samples as bioindicators of contamination in herons, although other studies found nestling feathers more representative of regional pollution, as egg samples may be influenced to a different extent by the female body burden accumulated during the wintering or migration period (Hughes et al., 1997, DesGranges et al., 2009).

Nevertheless, as the Purple Heron is considered an income breeder (Ruiz et al., 1998, Hobson et al., 2000) differences between egg and feather samples are probably due to routing. Moreover, feathers reflect the narrower diet offered to the chicks by their parents and, in a small and unknown percentage, the transfer of some of the female's burden into the egg. Accordingly, both samples would be reliable bioindicators, but feathers may be a more accurate bioindicator as they present lower variability in the isotopic data and higher levels in most of the trace elements analysed (except for chromium, but especially selenium, as this element is appreciably transferred to eggs (Sell, 1977, Focardi et al., 1988). Also, from a conservational perspective, sampling nestling feathers is more respectful to heron populations, provided that sampling protocols follow certain conditions while entering heron colonies, which are highly sensitive to human disturbance.

So we can conclude that although eggs and nestling feathers of Purple Herons are adequate bioindicators for trace element pollution in the river biota, nestling feathers present certain advantages. Nestling feathers are grown from dietary items provided by parents in the surroundings of the colony therefore show pollutant impacts on a local basis. Moreover, since chicks belong to a homogeneous age-class, the effects of age-related bioaccumulation on pollutant levels are expected to be negligible.

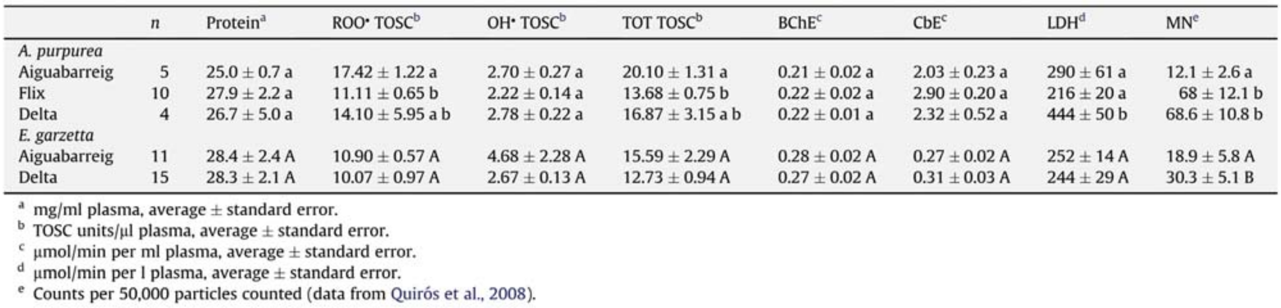
A4. Blood markers as monitors of environmental hazards of pollution

For the determination of biochemical parameters, blood samples were obtained from Little Egret and Purple Heron nestlings at l'Aiguabarreig, Flix (only Purple Heron at this site) and the Ebro Delta. Six were the biochemical parameters chosen and analyzed in the plasma of chicks (see Table 9): total oxigen scavenging capacity (TOSC) for peroxyl and hydroxyl radicals (ROO*, OH*), their sum (TOT), the plasmatic enzymatic activity measuring the buthirylcholinesterase (BhCE), carboxylesterase (CbE) and lacatate deshydrogenase (LDH) activities and number of micronucleus (MN).

From those parameters, only levels of ROO* TOSC and the activities of BChE and CbE differed significantly between herons nestlings at l'Aiguabarreig and the Ebro Delta (Table 9). The l'Aiguabarreig population showed a lower response than the other two populations in the biochemical parameters, whereas the Flix population showed a significantly low oxigen scavenging capacity (ROO* TOSC and Total TOSC). In addition, the Delta population showed a significant increase on LDH activity, indicating a specific pollution source downstream Flix. None of the studied biochemical parameters showed significant differences between the l'Aiguabarreig and the Ebro Delta populations of Little Egret. Table 9 also includes the data on MN abundance in nestlings blood (Quiros et al., 2008), which agrees with the general picture of a low impact in the l'Aiguabarreig site compared to the other two sites. In this case, the difference between sites was observed for both species.

Biochemical parameters and the frequency of micronuclei were compared to concentrations of mercury and copper. To summing up, only the sum of TOSC radicals (TOT TOSC) was considered. Several biochemical parameters in plasma showed significant correlation with trace metals in feathers, but only for Purple Heron. In this species, levels of mercury were inversely related with TOT TOSC values and both Hg and Cu levels were positively related with MN indexes and LDH activities, respectively (Fig. 14). Conversely, no significant correlations were observed between metal levels and any of the studied biomarkers for Little Egret samples (see Fig. 14).

The studied biochemical response in plasma and of micronuclei frequency in blood of chicks varied across species and nesting sites. TOT TOSC ranks in both species (12.16–20.48 U/µl plasma) were of similar magnitude to those reported by Corsolini et al. (2001) in Adélie and Emperor penguins from Wood Bay, North Victoria (10–25 U/µl plasma). Activity levels of BChE varied little in both species (0.21–0.28 µmol/min per ml), but plasmatic CbE activities in Little Egret (0.27–0.31 µmol/min per ml) were ten fold lower than those of Purple Heron (2–2.9 µmol/min per ml). Reported values for plasmatic B esterase activities were highly variable across bird species ranging from 0.7 to 5 µmol/min per ml for BChE and from 0.2 to 11 µmol/min per ml for CbE (Fossi et al., 1996). According to Fossi et al. (1996), B esterase activities tend to change depending on specimen size or species diet. In our study the observed differences in CbE activities between Little Egret and Purple Heron could be explained by differences in weight (a factor of 2) since both are piscivorous. Measured



	n	Protein[a]	ROO• TOSC[b]	OH• TOSC[b]	TOT TOSC[b]	BChE[c]	CbE[c]	LDH[d]	MN[e]
A. purpurea									
Aiguabarreig	5	25.0 ± 0.7 a	17.42 ± 1.22 a	2.70 ± 0.27 a	20.10 ± 1.31 a	0.21 ± 0.02 a	2.03 ± 0.23 a	290 ± 61 a	12.1 ± 2.6 a
Flix	10	27.9 ± 2.2 a	11.11 ± 0.65 b	2.22 ± 0.14 a	13.68 ± 0.75 b	0.22 ± 0.02 a	2.90 ± 0.20 a	216 ± 20 a	68 ± 12.1 b
Delta	4	26.7 ± 5.0 a	14.10 ± 5.95 a b	2.78 ± 0.22 a	16.87 ± 3.15 a b	0.22 ± 0.01 a	2.32 ± 0.52 a	444 ± 50 b	68.6 ± 10.8 b
E. garzetta									
Aiguabarreig	11	28.4 ± 2.4 A	10.90 ± 0.57 A	4.68 ± 2.28 A	15.59 ± 2.29 A	0.28 ± 0.02 A	0.27 ± 0.02 A	252 ± 14 A	18.9 ± 5.8 A
Delta	15	28.3 ± 2.1 A	10.07 ± 0.97 A	2.67 ± 0.13 A	12.73 ± 0.94 A	0.27 ± 0.02 A	0.31 ± 0.03 A	244 ± 29 A	30.3 ± 5.1 B

[a] mg/ml plasma, average ± standard error.

[b] TOSC units/µl plasma, average ± standard error.

[c] µmol/min per ml plasma, average ± standard error.

[d] µmol/min per l plasma, average ± standard error.

[e] Counts per 50,000 particles counted (data from Quirós et al., 2008).

Table 9. Sample size (n) and descriptive statistics for protein levels, total scavenging capacity (TOSC) for peroxyl and hydroxyl radicals (ROO*, OH*), their sum (TOT), buthirylcholinesterase (BhCE), carboxylesterase (CbE) and lacatate deshydrogenase (LDH) activities and number of micronucleus (MN). Within each species different letters indicate significant ($P < 0.05$) differences following ANOVA and post hoc Tukey's tests for Purple Heron and Student's t-tests for Little Egret data. Differences in sample size are due to missing values.

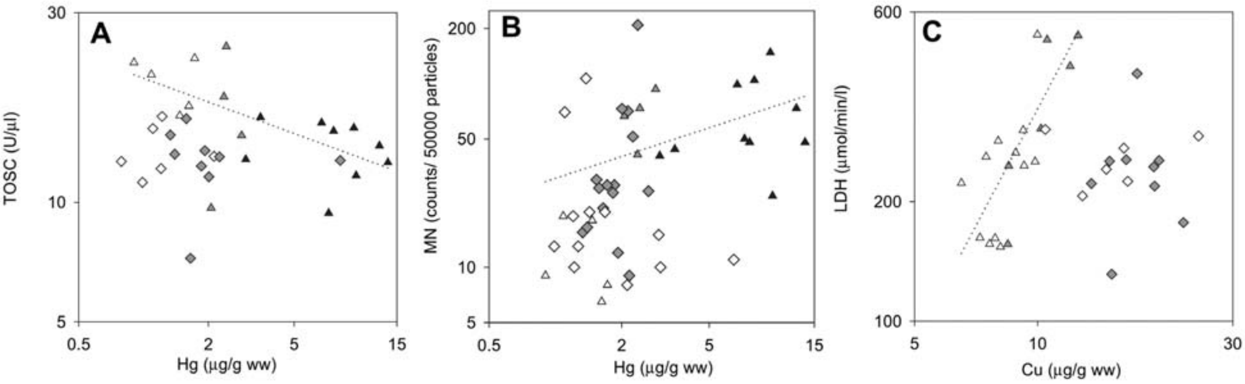


Figure 14. Bi-plots of mercury levels in feathers versus total scavenging capacity – TOT TOSC (A), micronucleated erythrocytes MN (B) and of levels of Cu versus lactate deshydrogenase – LDH (C) in Purple Heron and Little Egret. Each symbol corresponds to a single chick. Dotted lines indicate significant linear relationships, which only occurred in Purple Heron ($P < 0.05$). White, black and grey symbols correspond to values from l'Aiguabarreig, Flix and Delta sites, respectively. Data are depicted in log scale.

plasmatic LDH activities in the studied chick species (216–444 U/l) were comparable to those reported in nestlings and fledglings of Great egret and Great Blue herons (200–1700 U/l) (Hoffman et al., 2005, Champoux et al., 2006).

Reported nesting site effects on ROO*/TOT TOSC and micronuclei frequency provided the first evidence of detrimental effects of pollutants released by a chlor-alkali plant in nestlings from heron species. In particular, Purple Heron nestlings near the Flix chlor-alkali plant showed the highest frequency and levels of micronuclei (and Hg), and a reduced buffering capacity detoxify ROS (lowest values for ROO* or TOT TOSC). Moreover TOT TOSC was inversely related with mercury levels in feathers and micronuclei frequency. The observed lack of biological responses in Little Egret could be related to the absence of nesting sites at the most contaminated location (Flix). Exposure to various organic and inorganic contaminants is known to reduce TOT TOSC in bivalves and fish species and increase oxidative stress (Regoli, 2000, Regoli et al., 2005). More specifically, lab exposures showed that Cd, Cu, PCBs and PAHs caused oxidative stress, diminishing TOSC in bivalves (Lehmann et al., 2007, Company et al., 2008). Although there are no reported studies on effects of mercury in TOT TOSC, there is substantial information indicating that mercury decreases antioxidant defenses causing oxidative stress in adults and nestlings of egrets and herons (Henny et al., 2002, Hoffman et al., 2005). Therefore the observed site differences in ROO* or TOT TOSC in Purple heron nestlings agrees with previous studies and could be related to exposure to contaminants.

Plasmatic B esterases like those in liver are known to act as alternative target sites for anticholinergic compounds, thus protecting brain and muscle cholinesterases from inhibitory substances (Chambers et al., 1994). Activity levels of B esterases were similar across sites in both species, thus indicating low or no exposure to anticholinergic compounds. This finding is environmentally relevant since the use of large quantities of organophosphate and carbamate insecticides in Ebro's Delta in the past decades had dramatic effects in wildlife (Mañosa et al., 2001).

Like the other parameters, only in Purple Heron there were significant differences across sites in LDH activity with birds at the Ebro Delta showing the greatest activities. LDH activities were also positively related with levels of Cu in feathers. Exposure to mercury chloride has been observed to increase plasmatic LDH of the quail Coturnix coturnix (Dieter, 1974). In contrast, methylmercury inhibited plasmatic LDH activities of Great Egret nestlings (Hoffman et al., 2005). In the present study, total mercury levels measured in feathers were likely to be mainly methylmercury given that this is the preferred bioaccumulative form through the aquatic food web and for piscivorous birds that are at the top of the food chain (Henny et al., 2002). Under this point of view, the observed inverse relationship between LDH activity and Hg in nestlings agrees with previous studies. In contrast, the observed higher LDH activities in the Ebro Delta chicks and its direct correlation with Cu levels in feathers are difficult to explain. The widespread use of herbicides such as propanil and endosulfan in the Ebro Delta (Mañosa et al., 2001) with known oxidative stress and LDH effects in mammals (Omurtag et al., 2008, Rankin et al., 2008) may have affected Purple Heron plasmatic parameters. Indeed, increased oxidative stress levels in chicks nesting at Ebro Delta may also explain the quite high frequency of micronuclei reported by Quirós et al. (2008) in both species.

In summary, the results obtained from the selected six parameters (TOSC ROO*, TOSC OH*, BhCE, CbE, LDH and MN) provide the first evidence of an association of biological responses with pollutants released by a chlor-alkali plant in nestlings heron species, making of these parameters an adequate tool to monitor pollution environmental hazards. The identified biological responses on nestling blood parameters are the following: Purple heron nestlings from Flix (the impacted area) showed high frequency of micronuclei and a reduced buffering capacity to detoxify reactive oxygen species. At the Ebro Delta, biological response changes included high levels of plasmatic LDH in Purple Heron and high frequency of micronuclei in blood of both Purple Heron and Little Egret. These alterations might be related to the intensive agricultural activities taking place at the Ebro Delta besides Flix influence. The lack of differences found in the activity levels of B esterases in both species indicates low or no exposure to anticholinergic compounds.

Part B: Mining at the Bolivian Andes

Within this second part we focused on the potential of Tinamou species as sentinels to trace local metal pollution caused by the mining activities at the Bolivian Andes. The main issues studied were:

B1. Movement habits of the tinamou species

A key point for potential use of a species as a bioindicator of local contamination is the knowledge of its movements and home range (the area in which an animal lives and travels). If a species lives tightly associated with an area, it will reflect the pollution there as the outcome of the interaction with the environment (air, food, water). Unfortunately, little is known about the movements of the Tinamou species despite their ecological and cynegetic importance in Latin American countries. Previous studies conducted some decades ago were based on field observations of only a few individuals (Schafer, 1954, Lancaster, 1964a) and the home ranges reported varied from 0.1 ha in the Highland Tinamou (*Nothocercus bonapartei*) (Schafer, 1954) to 24 ha in the Brushland Tinamou (*Nothoprocta cinerascens*) (Lancaster, 1964a). Only for the Spotted Tinamou (*Nothura maculosa*) has the home range been studied by radio-tracking, reporting mean home ranges extensions of 16 and 19 ha (Thompson and Carroll, 2009).

About the Ornate Tinamou (*Nothoprocta ornata*), our study species, Pearson and Pearson (1955) reported an average feeding territory of 2.43 ha in the Peruvian High plateau, and on the basis of their field observations, the authors also suggest that this species is territorial and resident.

To study the movements and home range extension of the Ornate Tinamou we monitored year-round several radio-tagged sympatric individuals in a typical Andean agro-ecosystem on the Bolivian high plateau. Twelve individuals (six males and six females) were captured and fitted with necklace tags and were tracked by two methods: triangulation and homing. Because homing locations were autocorrelated, we did not use them for home range estimation, but for evaluating the accuracy of the positions calculated by the triangulation method. We calculated the home ranges with 100% MCP (Mohr, 1947) and density contours of 95% with fixed-kernel estimator with ad-hoc choice of h (Worton, 1989), because both permitted direct comparisons with other studies. We also estimated kernel 50% contours as core areas. Kernel estimators are sensitive to sample size, so we calculated home ranges and core areas only for birds with 40 or more locations, as recommended by Seaman et al. (Seaman et al., 1999).

Only 5 of the 12 tagged birds preserved the radio tag until the end of the study. The tags of the other seven birds were found in the field or the signal was lost, which occurred during a period of between 22 days and 10 months. Five of the individuals tagged were probably

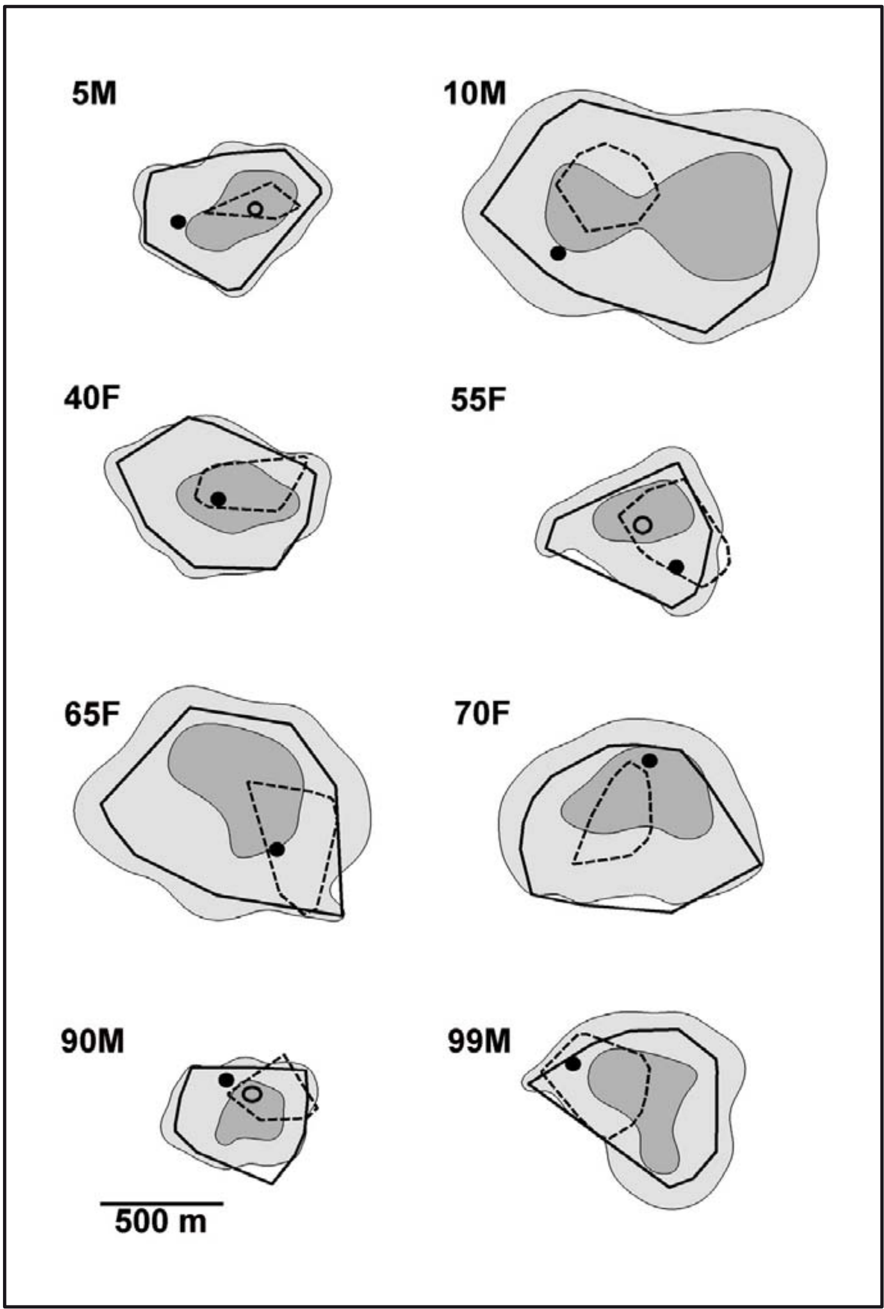


Figure 15. Home range shapes of the eight birds for which calculation was possible. Continuous lines represent 100% MCP by the triangulation method; pointed lines, the MCP obtained by homing locations; soft grey areas show kernel 95% home range; and dark grey areas, kernel 50% core areas. The closed circle shows capture point and the open circle shows tag recovery location.

Individual	Valid locs.	100% MCP (ha)	95% kernel (ha)	50% kernel (ha)	t^2/r^2
05M	178	30	37	10	1.81
10M	113	85	118	33	1.50
15M	31	NC			
20F	55	NC			
40F	192	37	45	11	1.64
55F	100	26	34	8	1.72
60F	33	NC			
65F	86	63	91	22	1.63
70F	124	53	69	18	1.69
75M	21	NC			
90M	163	22	25	6	1.76
99M	71	34	51	14	2.07
Mean ± SD		43.8 ± 21.6	58.8 ± 31.9	15.3 ± 8.9	

Table 10. Valid locations, home range (100% MCP and 95% kernel) and core area (50% kernel) size for each Ornate Tinamou (F: female or M: male) obtained using the triangulation method. t^2/r^2 is Schoener's ratio. NC = not calculated data.

predated (as the transmitter was still emitting when it was found and there were feathers nearby) and of the other two, one transmitter was found in the field without any predation signs around, while the other was never found.

Two individuals died within a month of having the tag placed and no conclusions about their movements can be assured (15M and 75) as no home range was calculated. Two individuals showed non-residential habits: Bird 20F moved 1522 m northwest from the capture point in March 2007 to the point of tag recovery in July 2007 and during the four months of triangulation technique it was located within the capture and death point, suggesting a gradual process of movement instead of residence. The monthly track pattern for bird 60F suggests it was also a non-resident bird.

For eight individuals we observed that the monthly and daily locations were equally distributed without any pattern, indicating resident habits. Because of this, we calculated the home range and core area from all-year triangulation locations. The locations of five of the eight birds were observed over a period of 11 months and the total radio-tracking days varied between 28 and 44. For those birds we calculated home range and core area, homing 100% MCP polygons were fully or mostly included in the triangulation polygons of 100% MCP and 95% kernel, which confirm the accuracy of the positions calculated by triangulation (Fig. 15).

Table 10 shows home ranges calculated by 100% MCP and 95% kernel together with the 50% kernel core area for each bird and the general mean values. Home ranges calculated by 100% MCP never exceeded 1 Km^2 and ranged from 85 to 22 ha. Core areas ranged from 33 to 6 ha. The eight tagged birds formed two clusters in the study area, both comprised of four birds (two males and two females) separated in two parallel valleys. Figure 16 shows the position of the individuals in each cluster.

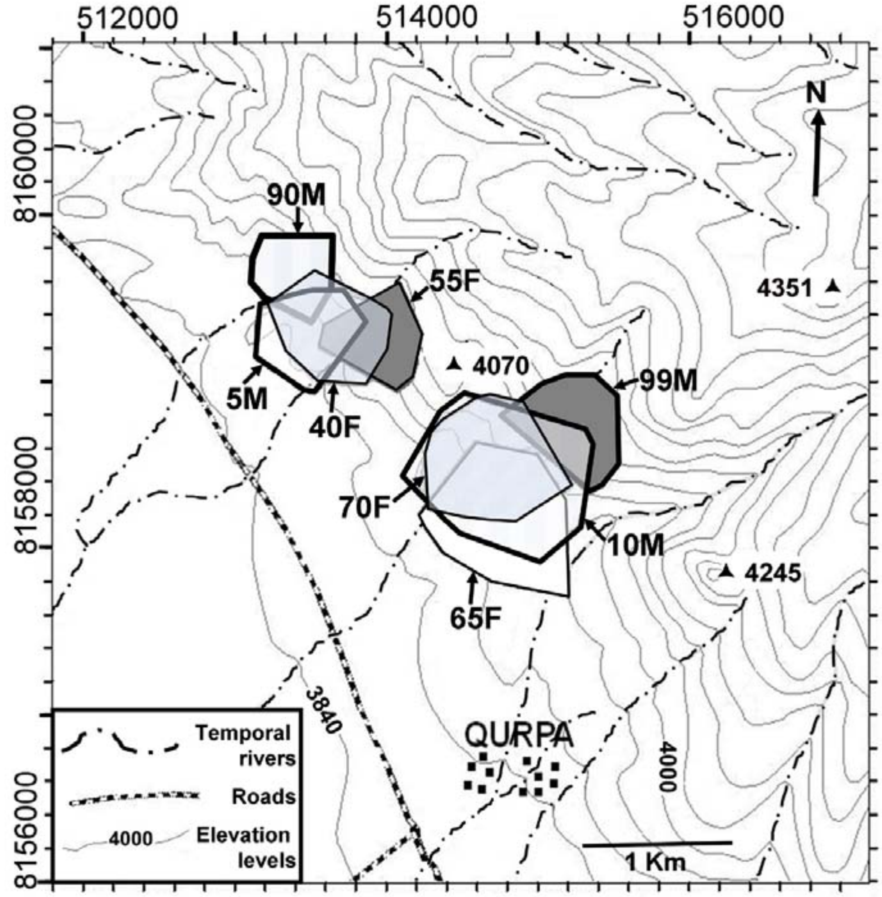


Figure 16. Position in the study area and overlapping of home ranges (MCP 100%) of the eight birds for which home range calculation was possible (each polygon identified with the bird code). Bold-lined polygons are males; light-lined polygons are females.

Those individuals showing great movements and premature death could be interpreted as sub-adult non-resident not established in a stable home range. In the case of bird 20, which had a sub-adult weight, the movement pattern was clearly directional, as it may have been looking for its own territory before a predator killed it. Bird 60 showed a skewed movement pattern over a large area (at least 111 ha) before the signal was lost. Probably this bird was searching for a territory to establish, which could explain its movement pattern.

The mean home range extensions reported for the Ornate Tinamou in this study are approximately twenty times greater than those reported by Pearson and Pearson (1955). Anyway, minimum home range values obtained agree with those for other tinamou species in the literature, while the rest of values reported are actually higher than reported previously. Earlier works, however, were made by direct or signal observations. For example, Schäfer (1954), using a hunting dog to assess home ranges, reported a very small territory of 0.1 ha for males of the Highland Tinamou (*Nothocercus bonapartei*) and territories of 20 ha for the Gray Tinamou (*Tinamus tao*). Lancaster (1964a) reported, by detecting the individual songs of four males, home ranges between 17 and 24 ha for the Brushland Tinamou (*Nothoprocta cinerascens*). The same author using the same technique reported home ranges between 11 and 19 ha for the Slaty-breasted Tinamou (*Crypturellus boucardi*) (Lancaster, 1964b). In the one other study with radio-tracking technology, Thompson and Carroll (2009) reported mean home range extensions of 16 and 19 ha for the Spotted Tinamou. Although they did not include the ranges, they highlighted the great inter-individual variation, similar to our results for the Ornate Tinamou.

Like all other tinamou species, except the Tinamotis genus (Cabot, 1992, Davies, 2002), Ornate Tinamous are sedentary, remaining in the same home range all year round where they forage, taking advantage of both spatial (different habitat types on a small scale) and temporal (annual crop management) heterogeneity, being advantageous to the generalist and opportunistic feeding behavior of this species (Garitano-Zavala et al., 2003).

The relatively small home ranges and sedentary habits of the Ornate Tinamou make of it a suitable bioindicator for local pollution exposure.

B2. Do feather, kidney and liver samples equally reflect exposure to local pollution?

Unlike organochlorides (another known family of pollutants), which are distributed widely among body tissues, largely in relation to their lipid content, heavy metals tend to be held in one particular tissue at much higher levels than others. Differences among tissues can also be indicative of different ways of pollutant entry to the organism. We compared the use of different samples types (feather, kidney, liver) when evaluating the exposure to local trace metal pollution, and we determined the concentrations of arsenic (As), cadmium (Cd), lead (Pb) and antimony (Sb) in feathers, liver, and kidney of tinamous collected at mining and reference sites. Captive birds (bred in captivity) were used for comparison reasons, in addition to the birds sampled at the reference sites.

In general terms, arsenic concentrations were higher in feathers and kidney, nearly doubling the liver concentrations (Fig. 17). Cadmium, as expected, had higher concentrations in the

	Tissues	As	Cd	Pb
Cd	Liver	0.71 (<0.001)		
	Kidney	0.51 (0.001)		
	Feather	0.68 (<0.001)		
Pb	Liver	0.71 (<0.001)	0.67 (<0.001)	
	Kidney	0.62 (<0.001)	0.55 (<0.001)	
	Feather	0.53 (0.001)	0.56 (<0.001)	
Sb	Liver	0.75 (<0.001)	0.71 (<0.001)	0.84 (<0.001)
	Kidney	0.65 (<0.001)	0.50 (0.001)	0.76 (<0.001)
	Feather	0.79 (<0.001)	0.70 (<0.001)	0.85 (<0.001)

Table 11. Nonparametric correlations between trace metal concentrations in each tissue type of the Ornate Tinamou (Nothoprocta ornata). The Spearman's Rho value is given in each box and the p value in brackets. N = 37 for feather and N = 41 for liver and kidney

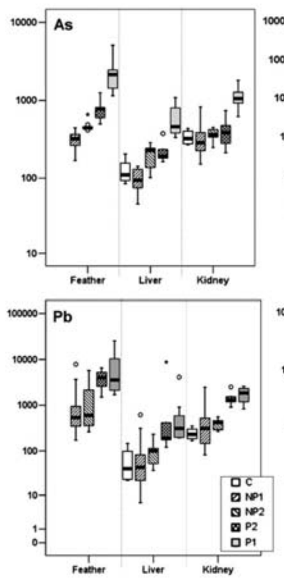


Figure 17. Box plots for each trace metal concentration in feather, liver, and kidney of Ornate Tinamou (*N. ornata*), from captivity (C), reference sites San Pedro de Ulloma (NP1) and Chuñavi (NP2) and mining sites Antequera (P1) and Poopó (P2).

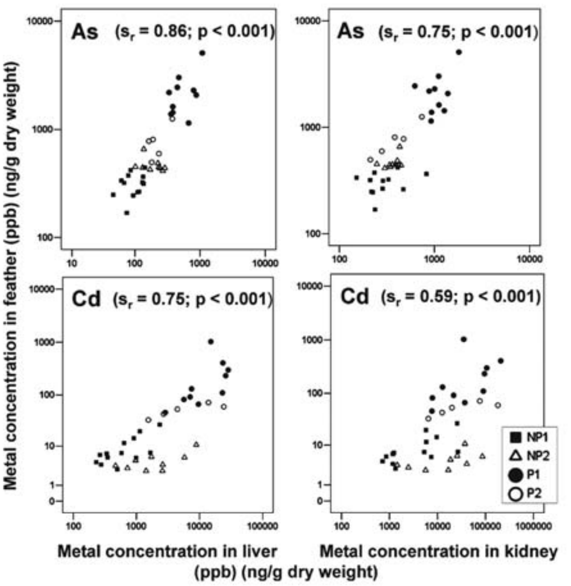


Figure 18. Scatter plots of arsenic and cadmium concentrations in feathers versus liver (left) and kidney (right) in Ornate Tinamou (*N. ornata*) from reference sites San Pedro de Ulloma (NP1) and Chuñavi (NP2), and mining sites Antequera (P1) and Poopó (P2). The Spearman correlation value (s_r) and *p* value are included.

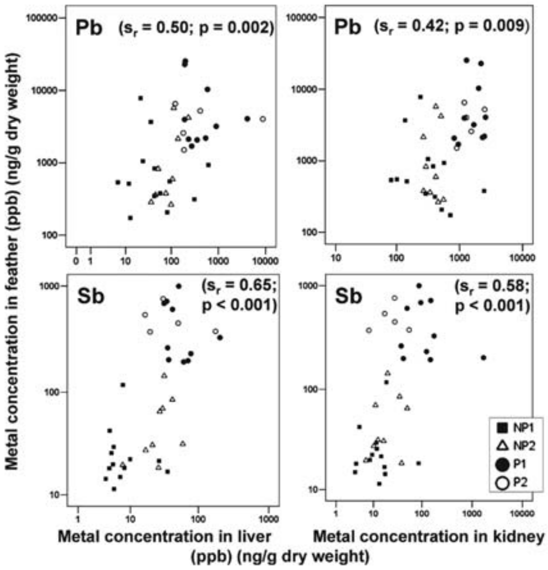


Figure 19. Scatter plots of lead and antimony concentrations in feathers versus liver (left) and kidney (right) in Ornate Tinamou (*N. ornata*) from reference sites San Pedro de Ulloma (NP1) and Chuñavi (NP2), and mining sites Antequera (P1) and Poopó (P2). The Spearman correlation value (s_r) and *p* value are included.

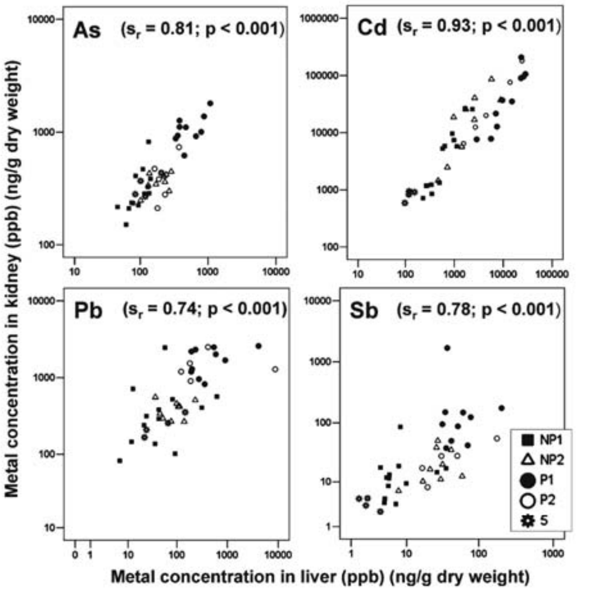


Figure 20. Scatter plots of all trace metal concentrations in kidney versus liver in Ornate Tinamou (*N. ornata*) from reference sites San Pedro de Ulloma (NP1) and Chuñavi (NP2), mining sites Antequera (P1) and Poopó (P2), and captivity (C). The Spearman correlation value (s_r) and *p* value are included.

kidney than in the other tissues. Levels of this metal were five to ten times higher in kidney than in liver and 250 to 5,000 times higher than in feathers (Fig. 17). Lead was clearly higher in feathers (around three times higher concentrations than in kidney and ten times higher than in liver). Feathers accumulated relatively more antimony than the other tissues (Fig. 17).

Concentrations of the four trace metals analyzed correlated significantly among tissues (Figs. 18, 19 and 20), and as significant correlations were also observed between the four trace metal concentrations within each tissue (Table 11), we can conclude that although the pollution level of each tissue is different, metal tissue concentrations always ranked the same.

As metal concentrations in feathers reflect the values of internal tissues (as well as potential adverse effects) in several bird species (Burger et al., 1993, Pilastro et al., 1993, Burger and Gochfeld, 2000b), the high correlation values obtained between feather, liver and kidney in both tinamou species allowed us to generalize that situation to arsenic and antimony. Also, the concentrations found in feathers are relatively constant for each metal, and probably are a significant part of the body burden, as a high proportion of the body burden of certain metals is stored in the feathers due to their affinity to the sulfhydryl-rich keratin protein and melanin pigments (Burger et al., 2008, Burger and Gochfeld, 2009). Thus, we recommend future monitoring programs use highland tinamou species based on feathers, because of several advantages. For instance, they are easy to collect noninvasively and store indefinitely. Also, the analysis of feathers from museum specimens obtained at currently polluted sites could generate historical or background data about pollution levels (Lounsbury-Billie et al., 2008).

B3. Histopathological status of liver and kidneys in relation to pollution levels.

In order to evaluate the histopathological status of liver and kidneys in relation to pollution levels, samples (around 2g) were taken from the freshly dead specimens of Ornate Tinamou and Darwin's Nothura, and examined with a microscope after adequate preparation. Readings were performed blind.

Results in liver histopathological slides revealed that some birds trapped at the four wild sites had inflammatory cells: 7% at Non Polluted 1, 22% at Non Polluted 2, 20% at Polluted 1, and 27% at Polluted 2 (at this last site, both species were taken into account), so no clear effect of pollution is reflected.

Kidney histopathological slides showed proportionally greater interstitial nephritis in birds from polluted sites (30% at Polluted 1 and 27% at Polluted 2) than in those from nonpolluted sites (15% at Non Polluted 1 and 0% at Non Polluted 2), so we found an effect of pollution in kidney samples. Moreover, thesaurismosis (a storage disease) in proximal convoluted tubules was observed in 30% of the samples only at the Polluted 1 site. Development of thesaurismosis is probably related to mineral concretions, like a phenomenon observed in a cadmium-controlled dose-dependent study of Japanese Quail (*Coturnix japonica*) (Richardson et al., 1974).

As cadmium is a trace element that accumulates preferably in the kidney, we looked for the relationship between Cd concentrations and histological damage in this organ. Although at polluted sites interstitial nephritis was more frequent and thesaurismosis was reported only at Polluted Site 1 (where this metal reaches higher concentrations), tissue effects were not related directly to individual level, as might be expected. For example, the birds with the highest cadmium concentration in kidney (over 100,000 and 200,000 ppb) had no tissue effects, while these were found in individuals with lower concentrations (95,200 ppb; 35,200 ppb, and 7,800 ppb).

Interstitial nephritis appears to be nonspecific or not directly associated with Cd concentrations, given that it was observed in individuals with Cd concentration values in a range from 850 to 105,700 ppb.

B4. Concentrations of heavy metals in Tinamou species – conservational hazard?

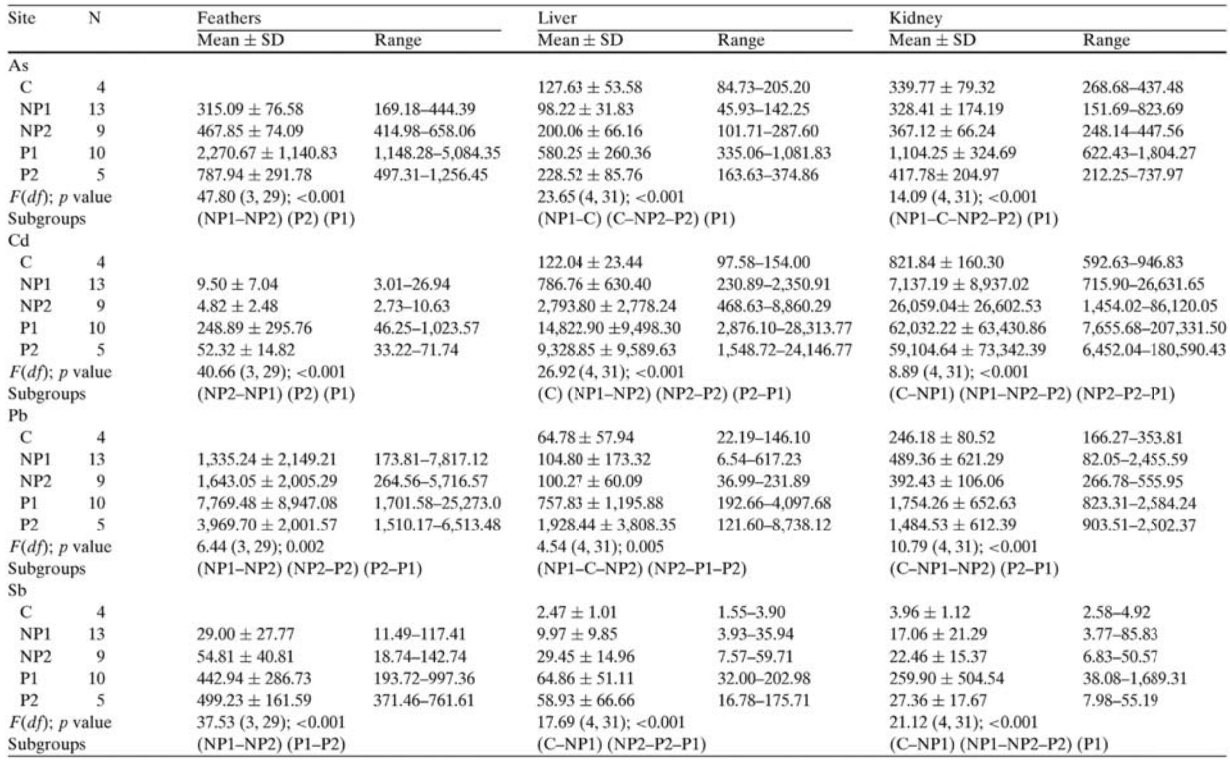
The relationship between tissue metal concentrations and adverse effects on bird health are difficult to glimpse. Several factors as interaction between elements, species-specific tolerance, as well as variables such as sex, age, body condition… make difficult to draw conclusions from metal concentrations solely.

Laboratory studies are needed to identify the metal levels that result in death or adverse impacts on behaviour, physiology or reproductive success of birds, but with the exception of mercury, lead and cadmium there are few controlled laboratory studies (Burger and Gochfeld, 2009).

Nevertheless, in order to determine concentrations of trace elements in Tinamou species and assess if they warrant special conservational concern, arsenic, cadmium, lead and antimony concentrations were determined. Captive birds (bred in captivity) were used for comparison reasons, besides the ones sampled at the reference sites.

Trace metals means, SD and ranges of those trace elements analysed for Ornate Tinamou are given in table 12 Values for Ornate Tinamou and Darwin's Nothura from the P2 site are shown in table 13 It's interesting to note that no significant interactions or sex effects were found in the analysis. All concentrations are expressed on a dry weight basis.

Results showed that the Ornate Tinamou presented site effects for all trace metals in all tissues. At the P2 site, the tissues of Darwin's Nothura had concentrations of the four trace metals analyzed that were similar to the Ornate Tinamou with, in most cases, no significant differences between the two species (Table 13). Exceptions were observed for all trace metals; in the case of lead and antimony in feathers, Darwin's Nothura concentrations doubled Ornate Tinamou ones.



Site	N	Feathers		Liver		Kidney	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
As							
C	4			127.63 ± 53.58	84.73–205.20	339.77 ± 79.32	268.68–437.48
NP1	13	315.09 ± 76.58	169.18–444.39	98.22 ± 31.83	45.93–142.25	328.41 ± 174.19	151.69–823.69
NP2	9	467.85 ± 74.09	414.98–658.06	200.06 ± 66.16	101.71–287.60	367.12 ± 66.24	248.14–447.56
P1	10	2,270.67 ± 1,140.83	1,148.28–5,084.35	580.25 ± 260.36	335.06–1,081.83	1,104.25 ± 324.69	622.43–1,804.27
P2	5	787.94 ± 291.78	497.31–1,256.45	228.52 ± 85.76	163.63–374.86	417.78± 204.97	212.25–737.97
F(*df*); *p* value		47.80 (3, 29); <0.001		23.65 (4, 31); <0.001		14.09 (4, 31); <0.001	
Subgroups		(NP1–NP2) (P2) (P1)		(NP1-C) (C–NP2–P2) (P1)		(NP1–C–NP2–P2) (P1)	
Cd							
C	4			122.04 ± 23.44	97.58–154.00	821.84 ± 160.30	592.63–946.83
NP1	13	9.50 ± 7.04	3.01–26.94	786.76 ± 630.40	230.89–2,350.91	7,137.19 ± 8,937.02	715.90–26,631.65
NP2	9	4.82 ± 2.48	2.73–10.63	2,793.80 ± 2,778.24	468.63–8,860.29	26,059.04± 26,602.53	1,454.02–86,120.05
P1	10	248.89 ± 295.76	46.25–1,023.57	14,822.90 ±9,498.30	2,876.10–28,313.77	62,032.22 ± 63,430.86	7,655.68–207,331.50
P2	5	52.32 ± 14.82	33.22–71.74	9,328.85 ± 9,589.63	1,548.72–24,146.77	59,104.64 ± 73,342.39	6,452.04–180,590.43
F(*df*); *p* value		40.66 (3, 29); <0.001		26.92 (4, 31); <0.001		8.89 (4, 31); <0.001	
Subgroups		(NP2–NP1) (P2) (P1)		(C) (NP1–NP2) (NP2–P2) (P2–P1)		(C–NP1) (NP1–NP2–P2) (NP2–P2–P1)	
Pb							
C	4			64.78 ± 57.94	22.19–146.10	246.18 ± 80.52	166.27–353.81
NP1	13	1,335.24 ± 2,149.21	173.81–7,817.12	104.80 ± 173.32	6.54–617.23	489.36 ± 621.29	82.05–2,455.59
NP2	9	1,643.05 ± 2,005.29	264.56–5,716.57	100.27 ± 60.09	36.99–231.89	392.43 ± 106.06	266.78–555.95
P1	10	7,769.48 ± 8,947.08	1,701.58–25,273.0	757.83 ± 1,195.88	192.66–4,097.68	1,754.26 ± 652.63	823.31–2,584.24
P2	5	3,969.70 ± 2,001.57	1,510.17–6,513.48	1,928.44 ± 3,808.35	121.60–8,738.12	1,484.53 ± 612.39	903.51–2,502.37
F(*df*); *p* value		6.44 (3, 29); 0.002		4.54 (4, 31); 0.005		10.79 (4, 31); <0.001	
Subgroups		(NP1–NP2) (NP2–P2) (P2–P1)		(NP1–C–NP2) (NP2–P1–P2)		(C–NP1–NP2) (P2–P1)	
Sb							
C	4			2.47 ± 1.01	1.55–3.90	3.96 ± 1.12	2.58–4.92
NP1	13	29.00 ± 27.77	11.49–117.41	9.97 ± 9.85	3.93–35.94	17.06 ± 21.29	3.77–85.83
NP2	9	54.81 ± 40.81	18.74–142.74	29.45 ± 14.96	7.57–59.71	22.46 ± 15.37	6.83–50.57
P1	10	442.94 ± 286.73	193.72–997.36	64.86 ± 51.11	32.00–202.98	259.90 ± 504.54	38.08–1,689.31
P2	5	499.23 ± 161.59	371.46–761.61	58.93 ± 66.66	16.78–175.71	27.36 ± 17.67	7.98–55.19
F(*df*); *p* value		37.53 (3, 29); <0.001		17.69 (4, 31); <0.001		21.12 (4, 31); <0.001	
Subgroups		(NP1–NP2) (P1–P2)		(C–NP1) (NP2–P2–P1)		(C–NP1) (NP1–NP2–P2) (P1)	

Table 12. Mean and Standard Deviation (SD) of trace metal levels (ppb d-w) (ng/g dry weight), in Ornate Tinamou feathers, liver, and kidney from the wild at nonpolluted sites (NP1 and NP2) and polluted sites (P1 and P2) and from captivity (C). The F value, degree of freedom (df), and p value from the ANOVA analysis are only shown for the principal factor "site"; the subgroups were formed with the Schefeé post hoc test.

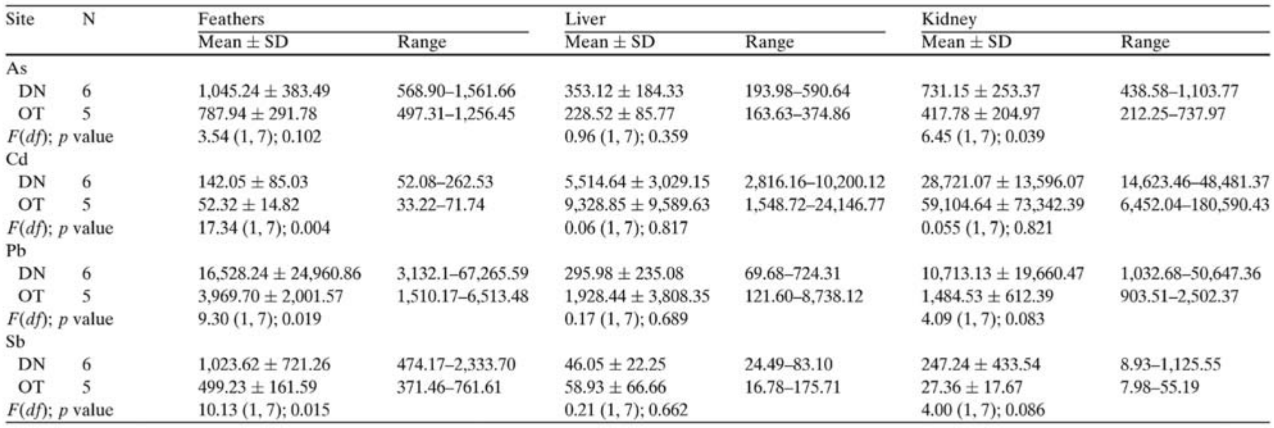
Arsenic concentrations in feathers, liver, and kidney were significantly higher at the P1 site and feathers from the polluted sites formed two distinct subgroups. As arsenic is a micronutrient for some vertebrates (Eisler, 1988a), and as it is rapidly metabolized by birds (Pendleton et al., 1995), it may not accumulate to toxic levels in tissues unless exposure is extreme (Custer et al., 2009). However, Burger and Gochfeld (2009) found that it biomagnifies in nature, with the highest values at the top of the trophic web. Feather values for arsenic reported for several bird species were between 13 to 604 ppb (Burger et al., 2008, Burger and Gochfeld, 2009). The values we found in feathers are within this range for Ornate Tinamou at all sites, except for P1, with more than 2,200 ppb; and except for Darwin's Nothura at P2, with values higher than 1,000 ppb. Thus, tinamous feather values at mining sites are among the highest ever reported. Comparison with the reference sites means that these values are related to exposure.

In liver, mean arsenic values for Ornate Tinamou have a maximum of 580 ppb at P1 and a maximum individual value of 1,081 ppb. These values are lower than the 15,000 ppb threshold reported by Goede (1985) as the upper limit for safety levels in waders. Fedynich et al. (2007) reported values of 18 to 660 ppb in migratory ducks. Although hepatic values may be considered within reported levels, they suggest that arsenic exposure influences the hepatic levels of this metal. For kidney, no previous reports are available.

Cadmium concentrations in feathers and liver were significantly higher at the P1 site, but in kidney, the difference with the unpolluted NP2 site was not clear; as with arsenic, differences between polluted and nonpolluted sites were more noticeable in feathers (Fig. 17). Liver and kidney cadmium concentrations of the captive birds were around seven times lower than the minimal media in the wild.

Cadmium is a nonessential element ubiquitous in natural environments and one of the trace metals whose accumulation and toxic effects in terrestrial, freshwater, and marine birds has been studied most. Cadmium toxicity is more common among natural vertebrate populations than was previously known (Larison et al., 2000). In particular, cadmium damages kidneys (Furness, 1996, Larison et al., 2000). Burger (1993) suggested that cadmium feather levels associated with adverse effects range from 100 ppb (shearwaters) to 2,000 ppb (terns). Mean feather values in tinamous ranged between 5 to 9.5 ppb at reference sites, which are comparatively very low, while at polluted sites, the values are several times higher for both tinamou species: at P1 (for Ornate Tinamou) and P2 (for Darwin's Nothura), values are within the toxicity range proposed by Burger (1993).

Scheuhammer (1987) suggested that cadmium threshold levels in liver are less than 3,000 ppb and Furness (1996) concluded that threshold concentrations for cadmium poisoning in birds might be expected at around 40 ppm (wet weight), but with a wide toxicity range between species and with age. In the case of Willow Grouse (*Lagopus lagopus*), Cd liver concentrations of approximately 10,000 ppb were related to high metallothionein levels, indicating a physiological response to Cd exposure (Pedersen and Hylland, 2007). Mean Cd values in liver from captive Ornate Tinamou showed very low concentrations, values from both reference sites situated within Scheuhammer's threshold levels, but at NP2, an individual reached 8,800 ppb. At P1, the mean value for Ornate Tinamou was higher than 10,000 ppb, and at both sites, several Ornate Tinamou individuals reached values higher than 20,000 ppb, indicating a high cadmium exposure in the field.



Site	N	Feathers		Liver		Kidney	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
As							
DN	6	1,045.24 ± 383.49	568.90–1,561.66	353.12 ± 184.33	193.98–590.64	731.15 ± 253.37	438.58–1,103.77
OT	5	787.94 ± 291.78	497.31–1,256.45	228.52 ± 85.77	163.63–374.86	417.78 ± 204.97	212.25–737.97
F(*df*); *p* value		3.54 (1, 7); 0.102		0.96 (1, 7); 0.359		6.45 (1, 7); 0.039	
Cd							
DN	6	142.05 ± 85.03	52.08–262.53	5,514.64 ± 3,029.15	2,816.16–10,200.12	28,721.07 ± 13,596.07	14,623.46–48,481.37
OT	5	52.32 ± 14.82	33.22–71.74	9,328.85 ± 9,589.63	1,548.72–24,146.77	59,104.64 ± 73,342.39	6,452.04–180,590.43
F(*df*); *p* value		17.34 (1, 7); 0.004		0.06 (1, 7); 0.817		0.055 (1, 7); 0.821	
Pb							
DN	6	16,528.24 ± 24,960.86	3,132.1–67,265.59	295.98 ± 235.08	69.68–724.31	10,713.13 ± 19,660.47	1,032.68–50,647.36
OT	5	3,969.70 ± 2,001.57	1,510.17–6,513.48	1,928.44 ± 3,808.35	121.60–8,738.12	1,484.53 ± 612.39	903.51–2,502.37
F(*df*); *p* value		9.30 (1, 7); 0.019		0.17 (1, 7); 0.689		4.09 (1, 7); 0.083	
Sb							
DN	6	1,023.62 ± 721.26	474.17–2,333.70	46.05 ± 22.25	24.49–83.10	247.24 ± 433.54	8.93–1,125.55
OT	5	499.23 ± 161.59	371.46–761.61	58.93 ± 66.66	16.78–175.71	27.36 ± 17.67	7.98–55.19
F(*df*); *p* value		10.13 (1, 7); 0.015		0.21 (1, 7); 0.662		4.00 (1, 7); 0.086	

Table 13. Mean and Standard Deviation (SD) of trace metal levels (ppb d-w) (ng/g dry weight) in Ornate Tinamou (*Nothoprocta ornata*) and Darwin's Nothura (*Nothura darwinii*) feathers, liver, and kidney from polluted site P2. The F value, degree of freedom (df), and p value from the ANOVA analysis are only shown for the principal factor "species".

Cadmium accumulates preferentially in kidney (Kim et al., 2007). Background cadmium levels in kidney are suggested as less than 8,000 ppb by Scheuhammer (1987), and Furness (1996) concluded that kidney threshold concentrations for Cd poisoning in birds might be expected at around 100 ppm (wet weight). Renal damage in water birds was found at over 300,000 ppb in kidney (Mateo and Guitart, 2003). Larison et al. (2000) demonstrated that 57% of the adult Willow Grouse individuals at a polluted mining site (with values of cadmium over 100,000 ppb in kidney) showed irreversible renal tubular damage. In our results, only Ornate Tinamou individuals from captivity had Cd values in kidney below Scheuhammer's background level. In the field, individual higher values were found at all sites for both tinamou species. One possible explanation is that cadmium is a trace metal naturally common in the Andean soils. No site had higher mean values than 100,000 ppb, except in some Ornate Tinamou individuals from polluted sites. At polluted sites, interstitial nephritis was more frequent, and thesaurismosis was reported only at P1. This site has the highest mean and individual Cd values for all tissues.

Concerning lead, polluted sites showed the highest concentrations, especially for kidney and at least for the P1 site in feathers (see table 12 and figure 17). Liver and kidney lead concentrations in captive birds were within the range of unpolluted sites. Lead toxicity and poisoning effects, widely studied in field and laboratory experiments, include several physiological and somatic effects and even death if exposure is high enough (Eisler, 1988b, Franson, 1996). Lead levels may increase during a bird's lifetime, depending on exposure (Grue et al., 1984). Muscular exercise, excretion, elimination, and high protein consumption are factors that reduce the physiological effects of high lead concentrations in wild birds (Burger et al., 1997, Roux and Marra, 2007). Burger and Gochfeld (2000a) considered that adverse reproductive effects in birds occur at levels of 4,000 ppb in feathers; Burger and Gochfeld (1994) found that experimentally lead injected 40-day-old Herring Gull chicks (*Larus argentatus*) had Pb concentrations in feathers of 4,790 ± 1,693 ppb and showed several motor problems, while untreated chicks had lead values from 853 to 1,205 ppb. In our study, Ornate Tinamou from reference sites had mean Pb concentrations in feathers lower than 2,000 ppb, but five individuals from both reference sites had values over 4,000 ppb. Ornate Tinamou and Darwin's Nothura from both polluted sites had mean values of 4,000 ppb or higher, with the highest values over 25,000 ppb (for Ornate Tinamou, P1 site) and 67,000 ppb (for Darwin's Nothura, P2 site). With these values, many tinamous from the polluted sites would be considered reaching toxic lead levels.

When lead concentrations in liver are 18,000 to 45,000 ppb, Pain (1996) concluded that waterfowl's biological functions may be disabled and external signs of poisoning may be observed. Franson (1996) found that 10 to 20 ppm liver Pb concentrations cause clinical signs of lead poisoning in other bird species. Clark and Scheuhammer (2003) considered that raptors have as "background" Pb exposure levels concentrations lower than 6,000 ppb in liver and/or kidney, and so raptors with concentrations over 6,000 ppb in liver or kidney have been lead exposed. They are poisoned with concentrations over 20,000 ppb. Following these criteria, all our individuals from captivity, reference sites, and perhaps from the P1 site would be considered at the background level. Only at the P2 site were higher lead concentrations found, with remarkable differences between species: one Ornate Tinamou had 8,700 ppb in liver, but lower concentrations in kidney; whereas two Darwin's Nothura individuals showed higher concentrations in kidney, but lower ones in liver (6,500 and

50,600 ppb, respectively). The last individual reaches the poisoning level cited above. Kim et al. (2007) also found great variation of Pb concentrations between five shorebird species (individual liver concentration values from n.d. to 23,250 ppb approximately and from n.d. to 141,600 ppb in kidney). The tissue with the highest lead concentration varied greatly between species.

Antimony concentrations were higher at the polluted sites, with this clearer in feathers, and for the P1 site in kidney. Feathers accumulated Sb relatively more than the other tissues (table 12, figure 17). Liver and kidney Sb concentrations of captive birds were at least four times lower than the minimum mean value in the wild birds.

Antimony is only potentially toxic, as it is not an essential trace element for plants and animals and has no known biological function (Fowler and Geering, 1991). However, as there have been much fewer studies of antimony than of other potentially toxic metals, its environmental significance may have been underestimated (Shotyk et al., 2005). In birds, antimony toxicity is cited because of the implications of the administration of antimony in the form of a potassium tartrate emetic (Carlisle and Holberton, 2006), but very few studies have reported their bioaccumulation in tissues. Lounsbury-Billie et al. (2008) reported mean values of 4 to 66 ppb in Osprey feathers, which they considered low. Our values from reference sites were in that range, but at polluted sites, values became approximately ten times higher for Ornate Tinamou and 20 times higher for Darwin's Nothura. Although our liver and kidney values cannot be compared due to the lack of other studies, concentration increases at the polluted sites (particularly P1 for Ornate Tinamou and P2 for Darwin's Nothura) over the figures for captivity and reference sites, which shows that birds exposed in the field to Sb probably accumulate this trace element in their tissues in direct proportion to their exposure, although nothing definite can be said about the possible risks of these concentrations. It is known that vertebrates excrete absorbed antimony rapidly via urine and feces, and only with exposure are high concentrations in thyroid, adrenals, liver, and kidney sometimes found (Hayes and Laws, 1991).

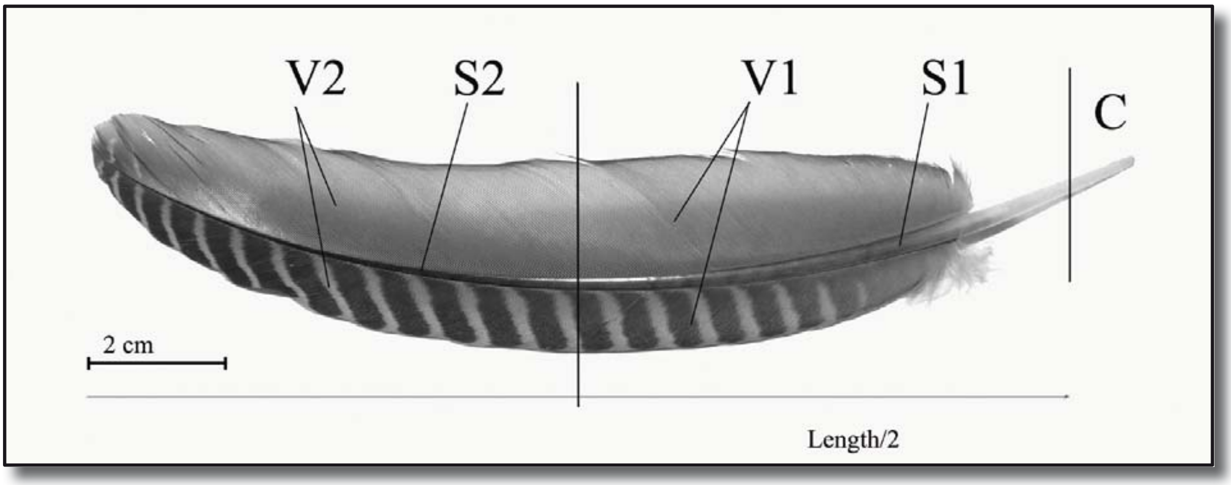


Figure 21. Feather sections (C calamus, S1 lower shaft, S2 upper shaft, V1 lower vane, V2 upper vane).

B5. Calamus and the endogenous lead pollution

Feathers have been used extensively to assess environmental pollution by certain heavy metals (Burger, 1993, Sanpera et al., 2007a, Sanpera et al., 2008), as birds excrete heavy metals into growing feathers when molting. Once the feather is formed, the blood supply atrophies and the feather becomes completely isolated from the rest of the body (Burger, 1993); consequently, no further heavy metals can accumulate from the bloodstream into the feathers. But feather-metal levels may remain stable with time, as is the case with mercury (Appelquist et al., 1984, Veerle et al., 2004), or they may increase, since feathers are highly prone to exogenous surface contamination.

Especially in the case of lead, it has been demonstrated that concentrations increase after the feather has fully grown, so feathers reflect endogenous lead levels as well as exogenous contamination. Such exogenous contamination from atmospheric deposition, preening and environmental contact (with soil, dust or water) may then give a misleading indication of biologically incorporated contamination at the time of feather growth (Pilastro et al., 1993, Dauwe et al., 2002, Jaspers et al., 2004). Exogenous contamination mainly affects the vane, which becomes more contaminated over time due to its large, structurally complex surface area (Goede and Debruin, 1984, Cardiel et al., 2011).

As exogenous surface contamination is difficult to clean from feathers (Weyers et al., 1988, Cardiel et al., 2011) and different cleansing treatments have shown similar results (Valladares et al., 2010), the amount of exogenous contamination deposited onto the feather that is removed by a cleansing process is unknown. Therefore, unless newly grown or nestling feathers are used, the lead levels in analyzed feathers will present an undetermined percentage of exogenous contamination.

Therefore, to assess which feather section provides the most accurate data of the original endogenous lead levels in order to interpret correctly the exposure to lead pollution, primary feather samples of Ornate Tinamou were divided into five sections (calamus, upper/lower

	Feather sections	N	Mean	SD	Min	Max
Lead (ppb)	Calamus	9	453.9	560.7	36.5	1587.2
	Shaft	9	2250.7	3815.8	58.8	12090.3
	Shaft (upper)	9	2911.7	4521.3	106.7	13288.2
	Shaft (lower)	9	2071.7	3706.3	44.8	11740.2
	Vane	9	11643.5	15562.3	1011.8	51981.5
	Vane (upper)	9	11484.6	19533.4	971.2	62656.2
	Vane (lower)	9	12197.3	10660.8	1092.2	37461.7
	Feather	9	14348.1	17255.3	1107.1	56294.5

Table 14. Descriptive statistics for lead concentrations in the feather sections expressed on a dry weight basis (ng/g).

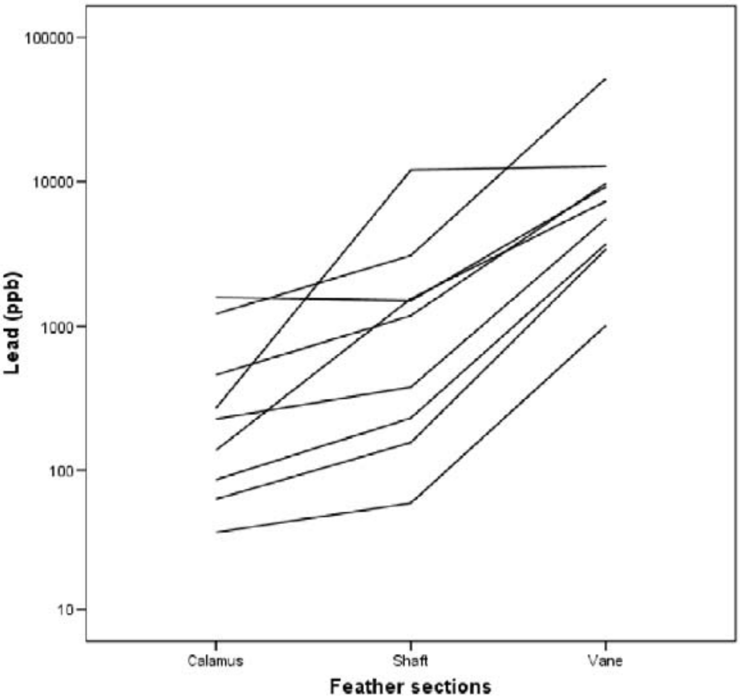


Figure 22. Individual lead concentrations profile by bird.

shaft and upper/lower vane) to compare the section unexposed to exogenous contamination with the sections exposed to it (figure 21). Calamus was considered the first 10 millimeters of the feather, which is the section embedded within the skin follicle in Ornate Tinamou.

Results (descriptive statistics showed in table 14) did not reveal any significant differences between the shaft and vane subdivisions, so analysis were carried out again joining vane and shaft subdivisions. The new analysis showed that the calamus had the lower lead concentrations, followed by the shaft, while the vane presented the highest concentrations. The individual lead concentration profiles of the feather sections are presented in figure 22.

The higher lead levels found in the shaft when compared with the calamus, together with the correlation found between the shaft and the vane, indicate that a percentage of the lead present in the shaft is exogenous. Therefore, our results show that the calamus is the feather section that likely reflects the original feather lead concentrations most accurately due to its protection against exogenous contamination, while the vane may represent the exogenous surface contamination more reliably (atmospheric deposition, sand baths and/ or preening).

The differences we found in the lead concentrations among the different feather sections suggest that the feather vane, with its net-like structure, is the most prone to retain particles of exogenous contamination even after a cleansing process with sodium hydroxide solution. Even the shaft, which presents a smoother structure, seems to be affected by exogenous contamination. It is worth mentioning that the total lead concentrations of the whole feather give similar information as the vane, that is, the part most exposed to exogenous contamination.

Conclusions

Part A: Aquatic environments – Toxic muds & Flix

1 **Stable isotopes and Ebro & River Delta bird communities trophic ecology.** $\delta^{15}N$ together with $\delta^{13}C$ adequately distinguishes riverine and deltaic ecosystems (high nitrification and lower carbon signatures in the river) and differences among sites in nitrogen signatures are greatly influenced by baseline values, being higher in the riverine sites than in the delta. SIA and SEA_c have helped unravelling the trophic ecology, niche and habitat partitioning of the aquatic bird community of the Ebro Delta: Common and Sandwich Tern behave as strict specialists at the Ebro Delta, with narrow niche widths, while in the opposite way, Little Tern, Little Egret, Purple Heron, Night Heron and the Moorhen behave like generalists foragers, with broad niche widths.

2 **Flix reservoir pollution at the River and Ebro Delta.** All analyzed elements are out of conservational concern (excluding mercury) and within range values of other bird communities (with the exception of chromium and mercury). Mercury levels in nestlings of Purple Heron at Flix site and eggs of Audouin's Gull, Little Tern and Common Tern 'Banya' at the Ebro Delta are high enough to be of special concern; further studies are of particular relevance in order to monitor whether these values are endangering the breeding success of these species.

Arsenic (specially used in combination with carbon signatures) discriminates outstandingly between marine and freshwater species. Finally, and surprisingly, Terns (family Sternidae) and Herons (family Ardeidae) show a very characteristic load pattern of mercury and copper, presumably phylogenetic, as is independent of habitat: high concentrations of mercury and low of copper for Terns and the opposite for Herons.

3 **Egg or nestling feather?** Strengths and weaknesses. Both eggs and nestling feathers of Purple Herons are adequate bioindicators for trace element pollution, but nestling feathers present certain advantages. Nestling feathers are grown from dietary items provided by parents in the surroundings of the colony therefore show pollutant impacts on a local basis. Moreover, since chicks belong to a homogeneous age-class, the effects of age-related bioaccumulation on pollutant levels are expected to be negligible.

4 **Blood biomarkers.** Results obtained from the selected blood six parameters (TOSC ROO*, TOSC OH*, BhCE, CbE, LDH and MN) provide the first evidence of an association of biological responses with pollutants released by a chlor-alkali plant in nestlings heron species, making of these parameters an adequate method to monitor environmental hazards of pollution. Purple heron nestlings from Flix (the impacted area) showed high frequency of micronuclei and a reduced buffering capacity to detoxificate reactive oxygen species. At the Ebro Delta, biological response changes included high levels of plasmatic LDH in Purple Heron and high frequency of micronuclei in blood of both Purple Heron and Little Egret. These alterations might be related to the intensive agricultural activities taking place at the Ebro Delta. The lack of differences found in the activity levels of B esterases in both species indicates low or no exposure to anticholinergic compounds.

5 **Waterbirds as bioindicators.** Waterbirds such as herons, terns and gulls species of the Ebro River & Delta have been used successfully as bioindicators of pollution.

Part B: Terrestrial environments – Mining & the Bolivian Andes

6 **Movements of Tinamou.** Thanks to the radiotracking technique, movements of the Tinamou species have been unravelled. The detected small home ranges (lower than 1 km2) and their sedentary habits make of this species an adequate bioindicator of local pollution.

7 **Feather, kidney or liver samples and trace elements.** Cadmium was mainly present in kidney and lead and antimony in feathers. Arsenic was mainly present in feathers and kidney. Nevertheless, concentrations of those four trace elements correlated among and within tissues, so qualitatively all the studied tissues showed the same response. We recommend feathers for future monitoring programs as they are a non invasive sample.

8 **Histopathological damage in liver and kidney samples.** Higher rates of histopathological damage were found in kidney at the polluted sites. Thesaurismosis was also found at one of the polluted sites. On the other hand, liver tissue did not show any effect from pollution.

9 **Mining in Bolivia. Pollution hazards?** Mining pollution has become clear due to the high levels of trace elements detected at the polluted sites in the two studied species of Tinamou, many of them trespassing hazard levels.

10 **Feather sections, exogenous & endogenous lead contamination.** Different feather sections reflected different degrees of exogenous contamination, being the calamus the most suitable section for assessing the original endogenous lead levels.

11 **Tinamou species as bioindicators.** Tinamou species at the Bolivian Andes have demonstrated their usefulness as bioindicators of local pollution.

References

Abdennadher, A., Ramirez, F., Romdhane, M.S., Ruiz, X., Jover, L. and Sanpera, C., 2011. Little Egret (*Egretta garzetta*) as a bioindicator of trace element pollution in Tunisian aquatic ecosystems. *Environmental Monitoring and Assessment* 175, 677-684.

Aliakbari, A., Savabieasfahani, M. and Ghasempouri, S.M., 2011. Mercury in egg and eggshell of Whiskered Tern (*Chlidonias hybrida)* from Anzali Wetlands of the Caspian Sea, Iran. *Bulletin of Environmental Contamination and Toxicology* 86, 175-179.

Amat, J.A. and Soriguer, R.C., 1984. Alimentacion invernal de la polla de agua (Gallinula chloropus) en las marismas del Guadalquivir. *Ardeola* 31, 136-140.

Andersen, J., 1945. The food of the Gull-billed Tern, *Gelochelidon nilotica* (Gm.). *Dansk Ornithologisk Forenings Tidsskrift* 39, 198-205.

Appelquist, H., Asbirk, S. and Drabaek, I., 1984. Mercury monitoring - Mercury stability in bird feathers. *Marine Pollution Bulletin* 15, 22-24.

Arcos, J.M., Ruiz, X., Bearhop, S. and Furness, R.W., 2002. Mercury levels in seabirds and their fish prey at the Ebro Delta (NW Mediterranean): the role of trawler discards as a source of contamination. *Marine Ecology Progress Series* 232, 281-290.

Attia, Y.A., Abdalah, A.A., Zeweil, H.S., Bovera, F., El-Din, A.A.T. and Araft, M.A., 2011. Effect of inorganic or organic copper additions on reproductive performance, lipid metabolism and morphology of organs of dual-purpose breeding hens. *Archiv Fur Geflugelkunde* 75, 169-178.

Baker, S.D. and Sepulveda, M.S., 2009. An evaluation of the effects of persistent environmental contaminants on the reproductive success of Great Blue Herons (*Ardea herodias*) in Indiana. *Ecotoxicology* 18, 271-280.

Baos, R., Jovani, R., Pastor, N., Tella, J., Jimenez, B. and Gomez, G., 2006. Evaluation of genotoxic effects of heavy metals and arsenic in wild nestling White Storks (Ciconia ciconia) and Black Kites (*Milvus migrans*) from southwestern Spain after a mining accident. *Environmental Toxicology and Chemistry* 25, 2794-2803.

Barata, C., Fabregat, M.C., Cotin, J., Huertas, D., Sole, M., Quiros, L., Sanpera, C., Jover, L., Ruiz, X., Grimalt, J.O. and Piña, B., 2010. Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain). *Environmental Pollution* 158, 704-710.

Barrett, R., Skaare, J., Norheim, G., Vader, W. and Froslie, A., 1985. Persistent organochlorines and mercury in eggs of norwegian seabirds 1983. *Environmental Pollution Series A-Ecological and Biological* 39, 79-93.

Barrett, R.T., Camphuysen, K.(J.)., Anker-Nilssen, T., Chardine, J.W., Furness, R.W., Garthe, S., Hueppop, O., Leopold, M.F., Montevecchi, W.A. and Veit, R.R., 2007. Diet studies of seabirds: a review and recommendations. *ICES Journal of Marine Science* 64, 1675-1691.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. and Macleod, H., 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology* 73, 1007-1012.

Becker, P.H., Frank, D. and Sudmann, S.R., 1993. Temporal and spatial pattern of Common Tern (*Sterna hirundo*) foraging in the Wadden Sea. Oecologia 93, 389-393.

Becker, P.H., Frank, D. and Wagener, M., 1997. Luxury in freshwater and stress at sea? The foraging of the Common Terra *Sterna hirundo* . *Ibis* 139, 264-269.

Becker, P.H., Furness, R.W. and Henning, D., 1993. Mercury dynamics in young Common Tern (*Sterna hirundo*) chicks from a polluted environment. *Ecotoxicology* 2, 33-40.

Becker, P.H. and Munoz Cifuentes, J., 2004. Contaminants in bird eggs. Recent spatial and temporal trends. *Wadden Sea Ecosystem* 18, 5-24.

Becker, P., 1989. Seabirds as monitor organisms of contaminants along the german North Sea coast. *Helgolander Meeresuntersuchungen* 43, 395-403.

Benejam, L., Benito, J. and Garcia-Berthou, E., 2010. Decreases in condition and fecundity of freshwater fishes in a highly polluted reservoir. *Water, Air and Soil Pollution* 210, 231-242.

Benito, V., Devesa, V., Munoz, O., Suner, M., Montoro, R., Baos, R., Hiraldob, F., Ferrer, M., Fernandez, M. and Gonzalez, M.J., 1999. Trace elements in blood collected from birds feeding in the area around Donana National Park affected by the toxic spill from the Aznalcollar mine. *Science of the Total Environment* 242, 309-323.

Bertolero, A., Oro, D., Martinez Vilalta, A. and Lopez, M.A., 2005. Selection of foraging habitats by Little Terns *Sterna albifrons* at the Ebro Delta (NE Spain). *Revista Catalana d'Ornitologia* 21, 37-42.

Beveridge, M.C.M., Stafford, E. and Coutts, R., 1985. Metal concentrations in the commercially exploited fishes of an endorheic saline lake in the tin-silver province of Bolivia. *Aquaculture Research* 16, 41-53.

Bogliani, G., Fasola, M., Canova, L. and Saino, N., 1990. Food and foraging rhythm of a specialized Gull-Billed Tern population *Gelochelidon nilotica* . *Ethology Ecology & Evolution* 2, 175-181.

Bogliani, G., Fasola, M., Canova, L. and Saino, N., 1994. Prey selection by parents and chicks of the Little Tern *Sterna albifrons.* . *Avocetta* 18, 9-11.

Boncompagni, E., Muhammad, A., Jabeen, R., Orvini, E., Gandini, C., Sanpera, C., Ruix, X. and Fasola, M., 2003. Egrets as monitors of trace-metal contamination in wetlands of Pakistan. *Archives of Environmental Contamination and Toxicology* 45, 399-406.

Bond, A.L. and Diamond, A.W., 2009. Mercury concentrations in seabird tissues from Machias Seal Island, New Brunswick, Canada. *Science of the Total Environment* 407, 4340-4347.

Bosch, C., Olivares, A., Faria, M., Navas, J.M., del Olmo, I., Grimalt, J.O., Pina, B. and Barata, C., 2009. Identification of water soluble and particle bound compounds causing sublethal toxic effects. A field study on sediments affected by a chlor-alkali industry. *Aquatic Toxicology* 94, 16-27.

Bridge, E.S., Jones, A.W. and Baker, A.J., 2005. A phylogenetic framework for the terns (Sternini) inferred from mtDNA sequences: implications for taxonomy and plumage evolution. *Molecular Phylogenetics and Evolution* 35, 459-469.

Bryan, G.W., 1979. Bioaccumulation of marine pollutants. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 286, 483-505.

Burger, J., 1993. Metals in avian feathers: bioindicators of environmental pollution. *Reviews in Environmental Toxicology* 5, 203-311.

Burger, J. and Gochfeld, M., 2000a. Effects of lead on birds (Laridae): A review of laboratory and field studies. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews* 3, 59-78.

Burger, J. and Gochfeld, M., 2000b. Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. *Science of the Total Environment* 257, 37-52.

Burger, J. and Gochfeld, M., 1994. Behavioral impairments of lead-injected young herring-gulls in nature. *Fundamental and Applied Toxicology* 23, 553-561.

Burger, J. and Gochfeld, M., 1993. Heavy-metal and selenium levels in feathers of young egrets and herons from Hong-Kong and Szechuan, China. *Archives of Environmental Contamination and Toxicology* 25, 322-327.

Burger, J. and Gochfeld, M., 1989. Metals in Tern Eggs in a New-Jersey Estuary - a Decade of Change. *Environmental Monitoring and Assessment* 11, 127-135.

Burger, J., Nisbet, I.C.T. and Gochfeld, M., 1992. Metal levels in regrown feathers - Assessment of contamination on the wintering and breeding grounds in the same individuals. *Journal of Toxicology and Environmental Health* 37, 363-374.

Burger, J., Rodgers, J.A. and Gochfeld, M., 1993. Heavy-metal and selenium levels in endangered Wood Storks *Mycteria Americana* from nesting colonies in Florida and Costa Rica. *Archives of Environmental Contamination and Toxicology* 24, 417-420.

Burger, J., Shukla, T., Benson, T. and Gochfeld, M., 1997. Lead levels in exposed herring gulls: Differences in the field and laboratory. *Toxicology and Industrial Health* 13, 193-202.

Burger, J. and Gochfeld, M., 2009. Comparison of arsenic, cadmium, chromium, lead, manganese, mercury and selenium in feathers in Bald Eagle (*Haliaeetus leucocephalus*), and comparison with Common Eider (*Somateria mollissima*), Glaucous-winged Gull (*Larus glaucescens*), Pigeon Guillemot (*Cepphus columba*), and Tufted Puffin (*Fratercula cirrhata*) from the Aleutian Chain of Alaska. *Environmental Monitoring and Assessment* 152, 357-367.

Burger, J., Gochfeld, M., Jeitner, C., Burke, S., Volz, C.D., Snigaroff, R., Snigaroff, D., Shukla, T. and Shukla, S., 2009. Mercury and other metals in eggs and feathers of Glaucous-winged Gulls (*Larus glaucescens*) in the Aleutians. *Environmental Monitoring and Assessment* 152, 179-194.

Burger, J., Gochfeld, M., Jeitner, C., Snigaroff, D., Snigaroff, R., Stamm, T. and Volz, C., 2008. Assessment of metals in down feathers of female common eiders and their eggs from the Aleutians: arsenic, cadmium, chromium, lead, manganese, mercury, and selenium. E*nvironmental Monitoring and Assessment* 143, 247-256.

Cabana, G. and Rasmussen, J., 1994. Modeling food-chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* 372, 255-257.

Cabo, J.M. and Sanchez, J.M., 1986. Recent Information on *Gelochelidon nilotica* in Morocco and its diet. *Alauda* 54, 207-212.

Cabot, J., 1992. Family tinamidae (tinamous). In: del Hoyo, J., Elliot, A. and Sargatal, J. (Eds.), *Handbook of the Birds of the World,* pp. 112–138.

Cardiel, I.E., Taggart, M.A. and Mateo, R., 2011. Using Pb–Al ratios to discriminate between internal and external deposition of Pb in feathers. *Ecotoxicology and Environmental Safety.*

Carlisle, J.D. and Holberton, R.L., 2006. Relative efficiency of fecal versus regurgitated samples for assessing diet and the deleterious effects of a tartar emetic on migratory birds. *Journal of Field Ornithology* 77, 126-135.

Carrasco, L., Diez, S., Soto, D., Catalan, J. and Bayona, J., 2008. Assessment of mercury and methylmercury pollution with zebra mussel (*Dreissena polymorpha*) in the Ebro River (NE Spain) impacted by industrial hazardous dumps. *Science of the Total Environment* 407, 178-184.

Carrasco, L., Benejam, L., Benito, J., Bayona, J.M. and Diez, S., 2011. Methylmercury levels and bioaccumulation in the aquatic food web of a highly mercury-contaminated reservoir. *Environment International* 37, 1213-1218.

Catry, T., Ramos, J.A., Catry, I., Allen-Revez, M. and Grade, N., 2004. Are salinas a suitable alternative breeding habitat for Little Terns Sterna albifrons?. *Ibis* 146, 247-257.

Catry, T., Ramos, J.A., Le Corre, M., Kojadinovic, J. and Bustamante, P., 2008. The role of stable isotopes and mercury concentrations to describe seabird foraging ecology in tropical environments. *Marine Biology* 155, 637-647.

Chambers, J.E., Ma, T.G., Boone, J.S. and Chambers, H.W., 1994. Role of Detoxication Pathways in Acute Toxicity Levels of Phosphorothionate Insecticides in the Rat. *Life Sciences* 54.

Champoux, L., Rodrigue, J., DesGranges, J.L., Trudeau, S., Hontela, A., Boily, M. and Spear, P., 2002. Assessment of contamination and biomarker responses in two species of herons on the St. Lawrence River. *Environmental Monitoring and Assessment* 79, 193-215.

Champoux, L., Rodrigue, J., Trudeau, S., Boily, M.H., Spear, P.A. and Hontela, A., 2006. Contamination and biomarkers in the Great Blue Heron, an indicator of the state of the St. Lawrence River. *Ecotoxicology* 15, 83-96.

Cherel, Y., Hobson, K.A. and Weimerskirch, H., 2005. Using stable isotopes to study resource acquisition and allocation in procellariiform seabirds. *Oecologia* 145, 533-540.

Cherel, Y., Le Corre, M., Jaquemet, S., Menard, F., Richard, P. and Weimerskirch, H., 2008. Resource partitioning within a tropical seabird community: new information from stable isotopes. *Marine Ecology Progress Series* 366, 281-291.

Clark, A.J. and Scheuhammer, A.M., 2003. Lead poisoning in upland-foraging birds of prey in Canada. *Ecotoxicology* 12, 23-30.

Company, R., Serafim, A., Cosson, R.P., Fiala-Medioni, A., Camus, L., Colaco, A., Serrao-Santos, R. and Bebianno, M.J., 2008. Antioxidant biochemical responses to long-term copper exposure in Bathymodiolus azoricus from Menez-Gwen hydrothermal vent. *Science of the Total Environment* 389.

Connell, D., Fung, C., Minh, T., Tanabe, S., Lam, P. and Wong, B., 2003. Risk to breeding success of fish-eating Ardeids due to persistent organic contaminants in Hong Kong: evidence from organochlorine compounds in eggs. *Water research* 37, 459-467.

Connell, D., Wong, B., Lam, P., Poon, K., Lam, M. and Wu, R., 2002. Risk to breeding success of ardeids by contaminants in Hong Kong: Evidence from trace metals in feathers. *Ecotoxicology* 11, 49-59.

Connolly, R.M., Guest, M.A., Melville, A.J. and Oakes, J.M., 2004. Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia* 138, 161-167.

Cordi, B., Fossi, C. and Depledge, M., 1997. Temporal biomarker responses in wild passerine birds exposed to pesticide spray drift. *Environmental Toxicology and Chemistry* 16, 2118-2124.

Corsolini, S., Nigro, M., Olmastroni, S., Focardi, S. and Regoli, F., 2001. Susceptibility to oxidative stress in Adelie and emperor penguin. *Polar Biology* 24, 365-368.

Corsolini, S., Borghesi, N., Ademollo, N. and Focardi, S., 2011. Chlorinated biphenyls and pesticides in migrating and resident seabirds from East and West Antarctica. *Environment International* 37, 1329-1335.

Cotin, J., Garcia-Tarrason, M., Jover, L. and Sanpera, C., 2012. Are the toxic sediments deposited at Flix reservoir affecting the Ebro river biota? Purple heron eggs and nestlings as indicators. *Ecotoxicology* 21, 1391-1402.

Cotin, J., García-Tarrasón, M., Sanpera, C., Jover, L. and Ruiz, X., 2011. Sea, freshwater or saltpans? Foraging ecology of terns to assess mercury inputs in a wetland landscape: The Ebro Delta. *Estuarine, Coastal and Shelf Science* 92, 188-194.

Cramp, S., 1980. Hand book of the birds of Europe, the Middle-East and North Africa. T*he birds of the Western Palearctic* Vol. 2. Hawks to Bustards.

Custer, C.M., Yang, C., Crock, J.G., Shearn-Bochsler, V., Smith, K.S. and Hageman, P.L., 2009. Exposure of insects and insectivorous birds to metals and other elements from abandoned mine tailings in three Summit County drainages, Colorado. *Environmental Monitoring and Assessment* 153, 161-177.

Dauwe, T., Bervoets, L., Blust, R. and Eens, M., 2002. Tissue levels of lead in experimentally exposed zebra finches (*Taeniopygia guttata*) with particular attention on the use of feathers as biomonitors. *Archives of Environmental Contamination and Toxicology* 42, 88-92.

Dauwe, T., Bervoets, L., Pinxten, R., Blust, R. and Eens, M., 2003. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. *Environmental Pollution* 124, 429-436.

Davies, S.J.J.F., 2002. *Ratites and tinamous.* Oxford University Press., New York.

De Luca-Abbott, S., Wong, B., Peakall, D., Lam, P., Young, L. and Lam, M., 2001. Review of effects of water pollution on the breeding success of waterbirds, with particular reference to ardeids in Hong Kong. *Ecotoxicology* 10, 327-349.

Delord, K., Kayser, Y., Cohez, D., Befeld, S. and Hafner, H., 2004. Fluctuations in chick diet of the Squacco Heron *Ardeola ralloides* in southern France: changes over the last 30 years. *Bird Study* 51, 69-75.

DesGranges, J., Rodrigue, J. and Champoux, L., 2009. Geographic distribution of selected contaminants in Great Blue Herons from the St. Lawrence River system, Quebec (1989-1994). *Canadian Wildlife Service Occasional Paper* 116, 1-18.

Dies, J.I., Marin, J. and Perez, C., 2005. Diet of nesting Gull-billed Terns in eastern Spain. *Waterbirds* 28, 106-109.

Dieter, M.P., 1974. Plasma enzyme-activities in coturnix Quail fed graded doses of DDE, polychlorinated biphenyl, malathion and mercuric-chloride. *Toxicology and applied pharmacology* 27.

Eisler, R., 1988a. Arsenic hazards to fish, wildlife and invertebrates: A synoptic review. *Biological Report* 85, 1.12.

Eisler, R., 1988b. Lead hazards to fish, wildlife and invertebrates: A synoptic review. *Biological Report* 85, 1.14.

Eisler, R., 1987. Mercury hazards to fish, wildlife, and invertebrates: A synoptic review. *Biological Report* 85.

Evers, D.C., Burgess, N.M., Champoux, L., Hoskins, B., Major, A., Goodale, W.M., Taylor, R.J., Poppenga, R. and Daigle, T., 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. *Ecotoxicology* 14, 193-221.

Faria, M., Huertas, D., Soto, D., Grimalt, J., Catalan, J. and Riva, M., 2010. Contaminant accumulation and multi-biomarker responses in field collected Zebra Mussels (*Dreissena polymorpha*) and Crayfish (*Procambarus clarkii*), to evaluate toxicological effects of industrial hazardous dumps in the Ebro river (NE Spain). *Chemosphere* 78, 232-240.

Fasola, M. and Bogliani, G., 1990. Foraging ranges of an assemblage of Mediterranean seabirds. *Colonial Waterbirds* 13, 72-74.

Fasola, M., Movalli, P.A. and Gandini, C., 1998. Heavy metal, organochlorine pesticide, and PCB residues in eggs and feathers of herons breeding in northern Italy. *Archives of Environmental Contamination and Toxicology* 34, 87-93.

Fasola, M., Rubolini, D., Merli, E., Boncompagni, E. and Bressan, U., 2010. Long-term trends of heron and egret populations in Italy, and the effects of climate, human-induced mortality, and habitat on population dynamics. *Population Ecology* 52, 59-72.

Fedynich, A.M., Ballard, B.M., McBride, T.J., Estrella, J.A., Garvon, J.M. and Hooper, M.J., 2007. Arsenic, cadmium, copper, lead, and selenium in migrating Blue-winged Teal (*Anas discors L.*). *Archives of Environmental Contamination and Toxicology* 53, 662-666.

Fernandez, M., Alonso, C., Gonzalez, M. and Hernandez, L., 1999. Occurrence of organochlorine insecticides, PCBs and PCB congeners in waters and sediments of the Ebro River (Spain). *Chemosphere* 38, 33-43.

Focardi, S., Fossi, C., Lambertini, M., Leonzio, C. and Massi, A., 1988. Long-term monitoring of pollutants in eggs of Yellow-legged Herring Gull from Capraia Island (Tuscan Archipelago). *Environmental Monitoring and Assessment* 10, 43-50.

Folch, J., Lees, M. and Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* 226, 497-509.

Forero, M.G., Bortolotti, G.R., Hobson, K.A., Donazar, J.A., Bertelloti, M. and Blanco, G., 2004. High trophic overlap within the seabird community of Argentinean Patagonia: a multiscale approach. *Journal of Animal Ecology* 73, 789-801.

Forero, M.G., Gonzalez-Solis, J., Hobson, K.A., Doncazar, J.A., Bertellotti, M., Blanco, G. and Bortolotti, G.R., 2005. Stable isotopes reveal trophic segregation by sex and age in the Southern Giant Petrel in two different food webs. *Marine Ecology-Progress Series* 296, 107-113.

Forero, M.G. and Hobson, K.A., 2003. Using stable isotopes of nitrogen and carbon to study seabird ecology: applications in the Mediterranean seabird community. *Scientia Marina* 67, 23-32.

Fossi, M.C., Lari, L. and Casini, S., 1996. Interspecies variation of "B" esterases in birds: The influence of size and feeding habits. *Archives of Environmental Contamination and Toxicology* 31, 525-532.

Fowler, B.A. and Geering, P.L., 1991. Metals and their compounds in the environment occurrence analysis and biological relevance, New York: VHC.

Franson, J.C., Hoffman, D.J. and Schmutz, J.A., 2002. Blood selenium concentrations and enzyme activities related to glutathione metabolism in wild emperor geese.

Environmental Toxicology and Chemistry 21, 2179-2184.

Franson, J.C., 1996. Interpretation of tissue lead residues in birds other than waterfowl. In: Beyer, W.N., Heinz, G.H. and Redmon-Norwood, A.W. (Eds.), *Environmental contaminants in wildlife: Interpreting tissue concentrations,* Boca Raton: Lewis, pp. 279.

Frederick, P.C., Hylton, B., Heath, J.A. and Spalding, M.G., 2004. A historical record of mercury contamination in southern Florida (USA) as inferred from avian feather tissue. *Environmental Toxicology and Chemistry* 23, 1474-1478.

Furness, R.W., 1996. Cadmium in birds. In: Beyer, W.N., Heinz, G.H. and Redmon-Norwood, A.W. (Eds.), *Environmental contaminants in wildlife: Interpreting tissue concentrations, Boca Raton*: Lewis, pp. 404.

Gannes, L.Z., OBrien, D.M. and delRio, C.M., 1997. Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78, 1271-1276.

Garitano-Zavala, A., Cotin, J., Borras, M. and Nadal, J., 2010. Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia and their potential as environmental sentinels. *Environmental Monitoring and Assessment* 168, 629-644.

Garitano-Zavala, A., Nadal, J. and Avila, P., 2003. The feeding ecology and digestive tract morphometry of two sympatric tinamous of the high plateau of the Bolivian Andes: The Ornate Tinamou (*Nothoprocta Ornata*) and the Darwin's Nothura (*Nothura darwinii*). *Ornitologia Neotropical* 14, 173-194.

Goede, A.A., 1985. Mercury, selenium, arsenic and zinc in waders from the Dutch Wadden Sea. *Environmental Pollution Series A-Ecological and Biological* 37, 287-309.

Goede, A.A. and Debruin, M., 1984. The use of bird feather parts as a monitor for metal pollution. *Environmental Pollution Series B-Chemical and Physical* 8, 281-298.

Goede, A.A. and Devoogt, P., 1985. Lead and cadmium in waders from the Dutch Wadden Sea. *Environmental Pollution Series A-Ecological and Biological* 37, 311-322.

Golden, N.H., Rattner, B.A., McGowan, P.C., Parsons, K.C. and Ottinger, M.A., 2003. Concentrations of metals in feathers and blood of nestling black-crowned night-herons (*Nycticorax nycticorax*) in Chesapeake and Delaware Bays. *Bulletin of Environmental Contamination and Toxicology* 70, 385-393.

Golet, G.H., Seiser, P.E., McGuire, A.D., Roby, D.D., Fischer, J.B., Kuletz, K.J., Irons, D.B., Dean, T.A., Jewett, S.C. and Newman, S.H., 2002. Long-term direct and indirect effects of the 'Exxon Valdez' oil spill on pigeon guillemots in Prince William Sound, Alaska. *Marine Ecology-Progress Series* 241, 287-304.

Gomez, G., Baos, R., Gomara, B., Jimenez, B., Benito, V. and Montoro, R., 2004. Influence of a mine tailing accident near Donana National Park (Spain) on heavy metals and arsenic accumulation in 14 species of waterfowl (1998 to 2000). *Archives of Environmental Contamination and Toxicology* 47, 521-529.

Gonzalez-Solis, J., Sokolov, E. and Becker, P.H., 2001. Courtship feedings, copulations and paternity in common terns, Sterna hirundo . *Animal Behaviour* 61, 1125-1132.

Goutner, V., 1991. Food and feeding ecology of Gull-Billed terns (*Gelochelidon nilotica*) in Greece. Revue d'Ecologie (La Terre Et La Vie) 46, 373-384.

Goutner, V. and Furness, R.W., 1997. Mercury in feathers of Little Egret *Egretta garzetta* and Night Heron *Nycticorax nycticorax* chicks and in their prey in the Axios Delta, Greece. *Archives of Environmental Contamination and Toxicology* 32, 211-216.

Granadeiro, J.P., Monteiro, L.R., Silva, M.C. and Furness, R.W., 2002. Diet of Common Terns in the Azores, Northeast Atlantic. *Waterbirds.*

Grue, C.E., Oshea, T.J. and Hoffman, D.J., 1984. Lead concentrations and reproduction in highway-nesting Barn Swallows. *Condor* 86, 383-389.

Guitart, R., Mateo, R., Sanpera, C., Hernandez-Matias, A. and Ruiz, X., 2003. Mercury and selenium levels in eggs of Common Terns (Sterna hirundo) from two breeding colonies in the Ebro Delta, Spain. *Bulletin of Environmental Contamination and Toxicology* 70, 71-77.

Hahn, E., Hahn, K. and Stoeppler, M., 1993. Bird feathers as bioindicators in areas of the German Environmental Specimen Bank: Bioaccumulation of mercury in food chains and exogenous deposition of atmospheric pollution with lead and cadmium. *Science of the Total Environment* 139-140, 259-270.

Hayes, W.J. and Laws, E.R., 1991. *Handbook of pesticide toxicology.* Volume 2. Classes of Pesticides., New York: Academic.

Heath, J.A. and Frederick, P.C., 2005. Relationships among mercury concentrations, hormones, and nesting effort of White Ibises (*Eudocimus albus*) in the Florida Everglades. *Auk* 122, 255-267.

Heinz, G.H., Hoffman, D.J., Klimstra, J.D., Stebbins, K.R., Kondrad, S.L. and Erwin, C.A., 2009. Species differences in the sensitivity of avian embryos to methylmercury. Archives of *Environmental Contamination and Toxicology* 56, 129-138.

Helander, B., Bignert, A. and Asplund, L., 2008. Using raptors as environmental sentinels: Monitoring the white-tailed sea eagle *Haliaeetus albicilla* in Sweden. *Ambio* 37, 425-431.

Henny, C.J., Hill, E.F., Grove, R.A. and Kaiser, J.L., 2007. Mercury and drought along the lower Carson River, Nevada: I. Snowy egret and Black-crowned Night-heron annual exposure to mercury, 1997-2006. *Archives of Environmental Contamination and Toxicology* 53, 269-280.

Henny, C.J., Hill, E.F., Hoffman, D.J., Spalding, M.G. and Grove, R.A., 2002. Nineteenth century mercury: Hazard to wading birds and cormorants of the Carson River, Nevada. *Ecotoxicology* 11.

Henny, C.J., Grove, R.A., Kaiser, J.L. and Johnson, B.L., 2010. North american Osprey populations and contaminants: Historic and contemporary perspectives. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews* 13, 579-603.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120, 314-326.

Hobson, K.A. and Clark, R.G., 1992. Assessing avian diets using Stable Isotopes .2. Factors influencing diet-tissue fractionation. *Condor* 94, 189-197.

Hobson, K.A., Piatt, J.F. and Pitocchelli, J., 1994. Using stable isotopes to determine seabird trophic relationships. *Journal of Animal Ecology* 63, 786-798.

Hobson, K.A., Sirois, J. and Gloutney, M.L., 2000. Tracing nutrient allocation to reproduction with stable isotopes: A preliminary investigation using colonial waterbirds of Great Slave Lake. *Auk* 117, 760-774.

Hoffman, D.J., Spalding, M.G. and Frederick, P.C., 2005. Subchronic effects of methylmercury on plasma and organ biochemistries in great egret nestlings. *Environmental Toxicology and Chemistry* 24.

Honda, K., Nasu, T. and Tatsukawa, R., 1986. Seasonal-changes in mercury accumulation in the Black-eared Kite, Milvus migrans Lineatus. *Environmental Pollution Series A-Ecological and Biological* 42, 325-334.

Hughes, K., Ewins, P. and Clark, K., 1997. A comparison of mercury levels in feathers and eggs of Osprey (*Pandion haliaetus*) in the North American Great Lakes. *Archives of Environmental Contamination and Toxicology* 33, 441-452.

Inger, R. and Bearhop, S., 2008. Applications of stable isotope analyses to avian ecology. *Ibis* 150, 447-461.

Jackson, A.L., Inger, R., Parnell, A.C. and Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80, 595-602.

Janz, D., DeForest, D., Brooks, M., Chapman, P., Gilron, G., Hoff, D., Hopkins, W., McIntyre, D., Mebane, C., Palace, V., Skorupa, J. and Wayland, M., 2010. Selenium toxicity to aquatic organisms. In: Chapman PM, Adams WJ, Brooks ML, DelosCG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP, editors. 2010. *Ecological assessment of selenium in the aquatic environment.* Pensacola (FL): SETAC., 141–231.

Jaspers, V., Dauwe, T., Pinxten, R., Bervoets, L., Blust, R. and Eens, M., 2004. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, *Parus major. Journal of Environmental Monitoring* 6, 356-360.

Jerez, S., Motas, M., Jose Palacios, M., Valera, F., Javier Cuervo, J. and Barbosa, A., 2011. Concentration of trace elements in feathers of three Antarctic penguins: Geographical and interspecific differences. E*nvironmental Pollution* 159, 2412-2419.

Kim, J., Park, S. and Koo, T., 2007. Lead and cadmium concentrations in shorebirds from the Yeongjong Island, Korea. *Environmental Monitoring and Assessment* 134, 355-361.

Koiadinovic, J., Menard, F., Bustamante, P., Cosscon, R.P. and Le Corre, M., 2008. Trophic ecology of marine birds and pelagic fishes from Reunion Island as determined by stable isotope analysis. *Marine Ecology-Progress Series* 361, 239-251.

Kunito, T., Kubota, R., Fujihara, J., Agusa, T. and Tanabe, S., 2008. Arsenic in marine mammals, seabirds, and sea turtles. *Reviews of Environmental Contamination and Toxicology,* Vol 195 195, 31-69.

Lam, J., Tanabe, S., Lam, M. and Lam, P., 2005. Risk to breeding success of waterbirds by contaminants in Hong Kong: evidence from trace elements in eggs. *Environmental pollution* 135, 481-490.

Lancaster, D.A., 1964a. Biology of the Brushland Tinamou, *Nothoprocta cinerascens. Bulletin of the American Museum of Natural History* 127, 269-314.

Lancaster, D.A., 1964b. Life history of the Boucard tinamou in British Honduras. II. Breeding biology. *Condor* 66, 253-276.

Lantzy, R.J. and Mackenzie, F.T., 1979. Atmospheric trace-metals - Global cycles and assessment of mans impact. *Geochimica et Cosmochimica Acta* 43, 511-525.

Larison, J.R., Likens, G.E., Fitzpatrick, J.W. and Crock, J.G., 2000. Cadmium toxicity among wildlife in the Colorado Rocky Mountains. *Nature* 406, 181-183.

Latraube, F., Trotignon, J. and Bretagnolle, V., 2005. Breeding biology of Whiskered Tern Chlidonias hybridus in Brenne (France). *Alauda* 73, 425-429.

Layman, C.A., Arrington, D.A., Montana, C.G. and Post, D.M., 2007. Can stable isotope ratios provide for community-wide measures of trophic structure?. *Ecology* 88, 42-48.

Lehmann, D.W., Levine, J.F. and Law, J.M., 2007. Polychlorinated biphenyl exposure causes gonadal atrophy and oxidative stress in Corbicula fluminea clams. *Toxicologic pathology* 35.

Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine pollution bulletin* 42, 656-666.

Llorente, G., Farran, A., Ruiz, X. and Albaiges, J., 1987. Accumulation and distribution of hydrocarbons, polychlorobyphenyls, and DDT in tissues of 3 species of Anatidae from the Ebro Delta (Spain). *Archives of Environmental Contamination and Toxicology* 16, 563-572.

Lounsbury-Billie, M.J., Rand, G.M., Cai, Y. and Bass, O.L.Jr., 2008. Metal concentrations in osprey (*Pandion haliaetus*) populations in the Florida Bay estuary. *Ecotoxicology* 17, 616-622.

Lucia, M., Andre, J., Gontier, K., Diot, N., Veiga, J. and Davail, S., 2010. Trace element concentrations (mercury, cadmium, copper, zinc, lead, aluminium, nickel, arsenic, and selenium) in some aquatic birds of the southwest atlantic coast of France. *Archives of Environmental Contamination and Toxicology* 58, 844-853.

Lunde, G., 1977. Occurrence and transformation of arsenic in marine-environment. *Environmental health perspectives* 19, 47-52.

Mañosa, S., Mateo, R. and Guitart, R., 2001. A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. *Environmental Monitoring and Assessment* 71, 187-205.

Mateo, R., Gil, C., Badia-Vila, M., Guitart, R., Hernandez-Matias, A., Sanpera, C. and Ruiz, X., 2004. Use of fatty acids to explain variability of organochlorine concentrations in eggs and plasma of Common Terns (*Sterna hirundo*). *Ecotoxicology* 13, 545-554.

Mateo, R. and Guitart, R., 2003. Heavy metals in livers of waterbirds from Spain. *Archives of Environmental Contamination and Toxicology* 44, 398-404.

Mathew, K.L., Lukose, C., Pethani, K.V. and Dave, H.R., 1998. Foraging behaviour of the Gull-billed Tern, *Sterna nilotica* . *Pavo* 36, 15-18.

MEDMIN, 2001. Estudio para el manejo ambiental en microcuencas de 12 zonas mineras: Matilde, Viloco, Caracoles, Colquiri, Poopó, Antequera, Cañadón Antequera, La Lava, Chorolque, Uncía, Llallagua, Colquechaca. *Technical Report. La Paz*: MEDMIN (Medio Ambiente, Minería e Industria) / Viceministerio de Minería y Metalurgia.

Metcheva, R., Yurukova, L. and Teodorova, S.E., 2011. Biogenic and toxic elements in feathers, eggs, and excreta of Gentoo penguin (*Pygoscelis papua ellsworthii*) in the Antarctic. *Environmental Monitoring and Assessment* 182, 571-585.

Michener, R.H. and Schell, D.M., 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha, K. and Michener, R.H. (Eds.), *Stable isotopes in ecology and environmental science.* Blackwell Scientific Publications, London, pp. 138-157.

Mohr, C.O., 1947. Table of equivalent populations of North American small mammals. *American Midland Naturalist* 37, 223-249.

Moller, A.P., Bonisoli-Alquati, A., Rudolfsen, G. and Mousseau, T.A., 2011. Chernobyl birds have smaller brains. *PLoS ONE* 6, 1-7.

Monteiro, L.R., Granadeiro, J.P., Furness, R.W. and Oliveria, P., 1999. Contemporary patterns of mercury contamination in the Portuguese Atlantic inferred from mercury concentrations in seabird tissues. *Marine Environmental Research* 47, 137-156.

Montesinos, A., Santoul, F. and Green, A.J., 2008. The Diet of the Night Heron and Purple Heron in the Guadalquivir Marshes. Ardeola 55, 161-167.

Moreno, R., Jover, L., Diez, C. and Sanpera, C., 2011. Seabird feathers as monitors of the levels and persistence of heavy metal pollution after the Prestige oil spill. *Environmental Pollution* 159, 2454-2460.

Moreno, R., Jover, L., Munilla, I., Velando, A. and Sanpera, C., 2010. A three-isotope approach to disentangling the diet of a generalist consumer: the Yellow-legged Gull in northwest Spain. *Marine Biology* 157, 545-553.

Morera, M., Sanpera, C., Crespo, S., Jover, L. and Ruiz, X., 1997. Inter and intraclutch variability in heavy metals and selenium levels in Audouin's gull eggs from the Ebro Delta, Spain. *Archives of Environmental Contamination and Toxicology* 33, 71-75.

Muir, D., Braune, B., DeMarch, B., Norstrom, R., Wagemann, R., Lockhart, L., Hargrave, B., Bright, D., Addison, R., Payne, J. and Reimer, K., 1999. Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. *Science of the Total Environment* 230, 83-144.

Murvoll, K.M., Skaare, J.U., Moe, B., Anderssen, E. and Jenssen, B.M., 2006. Spatial trends and associated biological responses of organochlorines and brominated flame retardants in hatchlings of North Atlantic kittiwakes (*Rissa tridactyla*). *Environmental Toxicology and Chemistry* 25, 1648-1656.

Navarro, A., Quiros, L., Casado, M., Faria, M., Carrasco, L. and Benejam, L., 2009. Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. *Aquatic toxicology* 93, 150-157.

Navarro, A., Tauler, R., Lacorte, S. and Barcelo, D., 2010a. Occurrence and transport of pesticides and alkylphenols in water samples along the Ebro River Basin. *Journal of Hydrology* 383, 18-29.

Navarro, J., Oro, D., Bertolero, A., Genovart, M., Delgado, A. and Forero, M.G., 2010b. Age and sexual differences in the exploitation of two anthropogenic food resources for an opportunistic seabird. *Marine Biology* 157, 2453-2459.

Neubauer, W., 1998. Habitat choice of the Common Tern *Sterna hirundo* in eastern Germany. *Vogelwelt* 119, 169-180.

Neuman, J., Chardine, J.W. and Porter, J.M., 1998. Courtship feeding and reproductive success in Black-legged Kittiwakes. *Colonial Waterbirds* 21, 73-80.

Newsome, S.D., del Rio, C.M., Bearhop, S. and Phillips, D.L., 2007. A niche for isotopic ecology. *Frontiers in Ecology and the Environment* 5, 429-436.

Nisbet, I.C.T., Montoya, J.P., Burger, J. and Hatch, J.J., 2002. Use of stable isotopes to investigate individual differences in diets and mercury exposures among Common Terns Sterna hirundo in breeding and wintering grounds. *Marine Ecology Progress Series*.

Nisbet, I.C.T., 1973. Courtship-feeding, egg-size and breeding success in Common Terns. *Nature* 241, 141-142.

Norman, D., 1992. The growth rate of little tern Sterna albifrons chicks. *Ringing and Migration* 13, 98-102.

Ohlendorf, H.M. and Heinz, G.H., 2011. Selenium in birds. In: Beyer, W.N. and Meador, J.P. (Eds.), *Environmental contaminants in biota: Interpreting tissue concentrations,* Second Edition. CRC Press/Taylor and Francis Group., Boca Raton, FL., pp. 669-701.

Ohlendorf, H.M., Covington, S.M., Byron, E.R. and Arenal, C.A., 2011. Conducting site-specific assessments of selenium bioaccumulation in aquatic systems. *Integrated*

Environmental Assessment and Management 7, 314-324.

Omurtag, G.Z., Tozan, A., Sehirli, A.O. and Sener, G., 2008. Melatonin protects against endosulfan-induced oxidative tissue damage in rats. *Journal of pineal research* 44.

Oporto, C., Vandecasteele, C. and Smolders, E., 2007. Elevated cadmium concentrations in potato tubers due to irrigation with river water contaminated by mining in Potosi, Bolivia. *Journal of Environmental Quality* 36, 1181-1186.

Oro, D., Bertolero, A., Vilalta, A.M. and Lopez, M.A., 2004. The biology of the Little Tern in the Ebro Delta (northwestern Mediterranean). Waterbirds 27, 434-440.

Oro, D. and Ruiz, X., 1997. Exploitation of trawler discards by breeding seabirds in the north-western Mediterranean: Differences between the ebro delta and the balearic islands areas. *ICES Journal of Marine Science* 54, 695-707.

Padula, V., Burger, J., Newman, S., Elbin, S. and Jeitner, C., 2010. Metals in feathers of Black-Crowned Night-Heron (*Nycticorax nycticorax*) chicks from the New York Harbor Estuary. *Archives of Environmental Contamination and Toxicology* 59, 157-165.

Paillisson, J.M., Reeber, S., Carpentier, A. and Marion, L., 2007. Reproductive parameters in relation to food supply in the Whiskered Tern (*Chlidonias hybrida*). *Journal of Ornithology* 148, 69-77.

Pain, D.J., Meharg, A.A., Ferrer, M., Taggart, M. and Penteriani, V., 2005. Lead concentrations in bones and feathers of the globally threatened Spanish imperial eagle. *Biological Conservation* 121, 603-610.

Pain, D.J., 1996. Lead in waterfowl. In: Beyer, W.N., Heinz, G.H. and Redmon-Norwood, A.W. (Eds.), *Environmental contaminants in wildlife: Interpreting tissue concentrations,* Boca Raton: Lewis, pp. 251-263.

Paiva, V.H., Ramos, J.A., Catry, T., Pedro, P., Medeiros, R. and Palma, J., 2006a. Influence of environmental factors and energetic value of food on Little Tern *Sterna albifrons* chick growth and food delivery. *Bird Study* 53, 1-11.

Paiva, V.H., Ramos, J.A., Machado, D., Penha-Lopes, G., Bouslama, M.F., Dias, N. and Nielsen, S., 2006b. Importance of marine prey to growth of estuarine tern chicks: evidence from an energetic balance model. *Ardea* 94, 241-255.

Paiva, V.H., Ramos, J.A., Martins, J., Almeida, A. and Carvalho, A., 2008a. Foraging habitat selection by Little Terns Sternula albifrons in an estuarine lagoon system of southern Portugal. *Ibis* 150, 18-31.

Paiva, V.H., Tavares, P.C., Ramos, J.A., Pereira, E., Antunes, S. and Duarte, A.C., 2008b. The influence of diet on mercury intake by Little Tern chicks. *Archives of Environmental Contamination and Toxicology* 55, 317-328.

Pardo-Cervera, F., Sorensen, I., Jensen, C., Ruiz, X. and Sanchez-Alonso, C., 2010. Breeding biology of the Little Bittern *Ixobrychus minutus* in the Ebro Delta (NE Spain). *Ardeola* 57, 407-416.

Parnell, A., Inger, R., Bearhop, S. and Jackson, A., 2010. Source Partitioning Using Stable Isotopes: Coping with Too Much Variation. *PLoS ONE* 5.

Pastor, D., Sanpera, C., Gonzalez-Solis, J., Ruiz, X. and Albaiges, J., 2004. Factors affecting the organochlorine pollutant load in biota of a rice field ecosystem (Ebro Delta, NE Spain). *Chemosphere* 55, 567-576.

Pearson, A. and Pearson, O., 1955. Natural history and breeding behavior of the Tinamou, *Nothoprocta ornata. Auk* 72, 113-127.

Pedersen, H.C. and Hylland, K., 2007. Metallothionein levels in Willow Ptarmigan (*Lagopus*

lagopus) populations with different natural loads of cadmium. *European Journal of Wildlife Research* 53, 142-152.

Pendleton, G.W., Whitworth, M.R. and Olsen, G.H., 1995. Accumulation and loss of arsenic and boron, alone and in combination, in Mallard Ducks. *Environmental Toxicology and Chemistry* 14, 1357-1364.

Perrow, M.R., Skeate, E.R., Lines, P., Brown, D. and Tomlinson, M.L., 2006. Radio telemetry as a tool for impact assessment of wind farms: the case of Little Terns *Sterna albifrons* at Scroby Sands, Norfolk, UK. *Ibis* 148, 57-75.

Peste, F., Trigo, S. and Luis, A., 2004. Foraging behaviour of breeding Little Tern *Sterna albifrons* at Ria de Aveiro. *Airo* 14, 55-62.

Peterson, B.J., Howarth, R.W. and Garritt, R.H., 1985. Multiple Stable Isotopes used to trace the flow of organic-matter in estuarine food webs. *Science* 227, 1361-1363.

Pilastro, A., Congiu, L., Tallandini, L. and Turchetto, M., 1993. The use of bird feathers for the monitoring of cadmium pollution. *Archives of Environmental Contamination and Toxicology* 24, 355-358.

Pol, S.S.V. and Becker, P.R., 2007. Monitoring contaminants in seabirds: The importance of specimen banking. *Marine Ornithology* 35, 113-118.

Quiros, L., Ruiz, X., Sanpera, C., Jover, L. and Pina, B., 2008. Analysis of micronucleated erythrocytes in heron nestlings from reference and impacted sites in the Ebro basin (NE Spain). *Environmental Pollution* 155, 81-87.

R Core Team, 2012. *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.* http://www.R-project.org.

Ramirez, F., Abdennadher, A., Sanpera, C., Jover, L., Hobson, K.A. and Wassenaar, L.I., 2011. Tracing waterbird exposure to total mercury and selenium: A case study at the solar saltworks of Thyna (Sfax, Tunisia). *Environmental Science & Technology* 45, 5118-5124.

Ramos, L., Fernandez, M., Gonzalez, M. and Hernandez, L., 1999. Heavy metal pollution in water, sediments, and earthworms from the Ebro River, Spain. *Bulletin of Environmental Contamination and Toxicology* 63, 305-311.

Ramos, R., Ramirez, F., Sanpera, C., Jover, L. and Ruiz, X., 2009. Feeding ecology of Yellow-legged Gulls Larus michahellis in the western Mediterranean: a comparative assessment using conventional and isotopic methods. *Marine Ecology Progress Series* 377, 289-297.

Rankin, G.O., Racine, C., Sweeney, A., Kraynie, A., Anestis, D.K. and Barnett, J.B., 2008. In vitro nephrotoxicity induced by propanil. *Environmental toxicology* 23.

Regoli, F., 2000. Total oxyradical scavenging capacity (TOSC) in polluted and translocated mussels: a predictive biomarker of oxidative stress. *Aquatic Toxicology* 50, 351-361.

Regoli, F., Nigro, M., Benedetti, M., Fattorini, D. and Gorbi, S., 2005. Antioxidant efficiency in early life stages of the Antarctic silverfish, Pleuragramma antarcticum: Responsiveness to pro-oxidant conditions of platelet ice and chemical exposure. *Aquatic Toxicology* 75.

Richardson, M.E., Spiveyfo, M.R. and Fry, B.E., 1974. Pathological changes produced in Japanese Quail by ingestion of cadmium. *Journal of Nutrition* 104, 323-338.

Rios, B., 2002. Evaluación de la actividad minera en los alrededores del lago Poopó. In: Rocha, O. (Ed.), *Diagnóstico de los recursos naturales y culturales de los lagos Poopó y Uru*

Uru, Oruro–Bolivia, La Paz: Convención RAMSAR, WCS/Bolivia, pp. 167–186.

Rojas, J.C. and Vandecasteele, C., 2007. Influence of mining activities in the North of Potosi, Bolivia on the water quality of the Chayanta river, and its consequences. *Environmental Monitoring and Assessment* 132, 321-330.

Roux, K.E. and Marra, P.P., 2007. The presence and impact of environmental lead in passerine birds along an urban to rural land use gradient. *Archives of Environmental Contamination and Toxicology* 53, 261-268.

Ruiz, X., Jover, L., Llorente, G.A., Sanchez-Reyes, A.F. and Febrian, M.I., 1988. Song thrushes *Turdus philomelos* wintering in Spain as biological indicators of the Chernobyl accident. *Ornis Scandinavica* 19, 63-67.

Ruiz, X., Jover, L. and Pedrocchi, V., 1998. Audouin's gull intraspecific variability in yolk and albumen egg contents: Why is yolk size so constant?. *Biologia e Conservazione della Fauna,* 327-334.

Rumbold, D., Niemczyk, S., Fink, L., Chandrasekhar, T., Harkanson, B. and Laine, K., 2001. Mercury in eggs and feathers of great egrets (*Ardea albus*) from the Florida everglades. A*rchives of Environmental Contamination and Toxicology* 41, 501-507.

Sakellarides, T., Konstantinou, I., Hela, D., Lambropoulou, D., Dimou, A. and Albanis, T., 2006. Accumulation profiles of persistent organochlorines in liver and fat tissues of various waterbird species from Greece. *Chemosphere* 63, 1392-1409.

Salzer, D.W. and Larkin, G.J., 1990. Impact of Courtship Feeding on Clutch and 3rd-Egg Size in Glaucous-Winged Gulls. *Animal Behaviour* 39, 1149-1162.

Sanabria, H., 2000. Resistance and the arts of domination - Miners and the Bolivian state. *Latin American Perspectives* 27, 56-81.

Sanchez, J.M., Munoz del Viejo, A. and de la Cruz, C., 1991. Segregacion alimentaria entre adultos y pollos de *Gelochelidon nilotica* (Gm., 1789) en la laguna de Fuente de Piedra. *Ardeola* 38, 21-27.

Sanpera, C., Moreno, R., Ruiz, X. and Jover, L., 2007a. Audouin's gull chicks as bioindicators of mercury pollution at different breeding locations in the western Mediterranean. *Marine Pollution Bulletin* 54, 691-696.

Sanpera, C., Ruiz, X., Moreno, R., Jover, L. and Waldron, S., 2007b. Mercury and stable isotopes in feathers of Audouin's Gulls as indicators of feeding habits and migratory connectivity. *Condor* 109, 268-275.

Sanpera, C., Valladares, S., Moreno, R., Ruiz, X. and Jover, L., 2008. Assessing the effects of the Prestige oil spill on the European shag (*Phalacrocorax aristotelis*): Trace elements and stable isotopes. *Science of the Total Environment* 407, 242-249.

Schafer, E.R.N.S.T., 1954. Zur Biologie des Steisshuhnes Norhocercus bonapartei. *Journal of Ornithology* 95, 219-232.

Scheifler, R., Coeurdassier, M., Morilhat, C., Bernard, N., Faivre, B. and Flicoteaux, P., 2006. Lead concentrations in feathers and blood of common blackbirds (*Turdus merula*) and in earthworms inhabiting unpolluted and moderately polluted urban areas. *Science of the Total Environment* 371, 197-205.

Scheuhammer, A.M., 1987. The chronic toxicity of aluminum, cadmium, mercury and lead in birds: A review. *Environmental Pollution* 46, 263-295.

Schwemmer, P., Tischler, T., Rehm, R. and Garthe, S., 2011. Habitat use, distribution and food choice of the black-headed gull (*Larus ridibundus*) in the coastal mainland of Schleswig-Holstein. *Corax* 21, 355-374.

Seaman, D.E., Millspaugh, J.J., Kernohan, B.J., Brundige, G.C., Raedeke, K.J. and Gitzen, R.A., 1999. Effects of sample size on kernel home range estimates. *Journal of Wildlife Management* 63, 739-747.

Sell, J.L., 1977. Comparative effects of selenium on metabolism of methylmercury by chickens and quail - Tissue distribution and transfer into eggs. *Poultry science* 56, 939-948.

SERGEOMIN, 1999. Inventariación de recursos naturales renovables (hídricos) y no renovables (minerales e hidrocarburos) del departamento de Oruro. *Boletín del Servicio Nacional de Geología y Minería,* 1–44.

Shotyk, W., Krachler, M. and Chen, B., 2005. Antimony: Global environmental contaminant. *Journal of Environmental Monitoring* 7, 1135-1136.

Sonne, C., Bustnes, J.O., Herzke, D., Jaspers, V.L.B., Covaci, A., Halley, D.J., Moum, T., Eulaers, I., Eens, M., Ims, R.A., Hanssen, S.A., Erikstad, K.E., Johnsen, T., Schnug, L., Riget, F.F. and Jensen, A.L., 2010. Relationships between organohalogen contaminants and blood plasma clinical-chemical parameters in chicks of three raptor species from Northern Norway. *Ecotoxicology and Environmental Safety* 73, 7-17.

Stienen, E.W.M., Van Beers, P.W.M., Brenninkmeijer, A., Habraken, J.M.P.M., Raaijmakers, M.H.J.E. and Van Tienen, P.G.M., 2000. Reflections of a specialist: Patterns in food provisioning and foraging conditions in Sandwich Terns Sterna sandvicensis . *Ardea* 88, 33-49.

Stienen, E.W.M., Brenninkmeijer, A. and Klaassen, M., 2008. Why do Gull-billed terns Gelochelidon nilotica feed on fiddler crabs Uca tangeri in Guinea-Bissau. *Ardea* 96, 243-250.

Strum, K.M., Alfaro, M., Haase, B., Hooper, M.J., Johnson, K.A., Lanctot, R.B., Lesterhuis, A.J., Lopez, L., Matz, A.C., Morales, C., Paulson, B., Sandercock, B.K., Torres-Dowdall, J. and Elena Zaccagnini, M., 2008. Plasma cholinesterases for monitoring pesticide exposure in Nearctic-Neotropical migratory shorebirds. *Ornitologia Neotropical* 19, 641-651.

Suarez-Serrano, A., Alcaraz, C., Ibanez, C., Trobajo, R. and Barata, C., 2010. *Procambarus clarkii* as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. *Ecotoxicology and Environmental Safety* 73, 280-286.

Tavares, P.C., Kelly, A., Lopes, R.J., Pereira, M.E., Duarte, A.C. and Furness, R.W., 2007. The influence of dietary specialization and trophic status on mercury levels in two species using common coastal wetlands, *Himantopus himantopus* and *Sterna albifrons. Ardeola* 54, 275-288.

Tavares, P.C., McGill, R., Newton, J., Pereira, E., Duarte, A. and Furness, R.W., 2009. Relationships between carbon sources, trophic level and mercury exposure in generalist shorebirds revealed by stable isotope ratios in chicks. *Waterbirds* 32, 311-321.

Thompson, J.J. and Carroll, J.P., 2009. Habitat use and survival of the Spotted Tinamou (*Nothura maculosa*) in agroecosystems in the province of Buenos Aires, Argentina. In: Cederbaum, S., Faircloth, B., Terhune, T., Thompson, J. and Carroll, J. (Eds.), Gamebird 2006: Quail VI and Perdix XII, Warnell School of Forestry and Natural Resources, Athens, GA, pp. 111-119.

Thyen, S., Becker, P.H. and Behmann, H., 2000. Organochlorine and mercury contamination of Little Terns (*Sterna albifrons*) breeding at the western Baltic Sea, 1978-96.

Environmental Pollution 108, 225-238.

Valladares, S., Moreno, R., Jover, L. and Sanpera, C., 2010. Evaluating cleansing effects on trace elements and stable isotope values in feathers of oiled birds. *Ecotoxicology* 19, 223-227.

Veerle, J., Tom, D., Rianne, P., Lieven, B., Ronny, B. and Marcel, E., 2004. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, Parus major. *Journal of Environmental Monitoring* 6, 356-360.

Vericat, D. and Batalla, R.J., 2006. Sediment transport in a large impounded river: The lower Ebro, NE Iberian Peninsula. *Geomorphology* 79, 72-92.

Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambre, C., Tarazona, J.V., Brandt, I. and Vethaak, A.D., 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. *Critical Reviews in Toxicology* 30, 71-133.

Wayland, M., Kneteman, J. and Crosley, R., 2006. The American dipper as a bioindicator of selenium contamination in a coal mine-affected stream in west-central Alberta, Canada. *Environmental Monitoring and Assessment* 123, 285-298.

Weyers, B., Gluck, E. and Stoeppler, M., 1988. Investigation of the significance of heavy-metal contents of Blackbird feathers. *Science of the Total Environment* 77, 61-67.

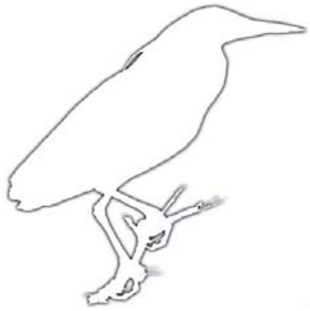
Wiggins, D.A. and Morris, R.D., 1986. Criteria for female choice of mates - Courtship feeding and paternal care in the Common Tern. *American Naturalist* 128, 126-129.

Winston, G.W., Regoli, F., Dugas, A.J., Fong, J.H. and Blanchard, K.A., 1998. A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radical Biology and Medicine* 24, 480-493.

Worton, B.J., 1989. Kernel methods for estimating the utilization distribution in home-range studies. *Ecology* 70, 164-168.

Zhang, Y., Ruan, L., Fasola, M., Boncompagni, E., Dong, Y. and Dai, N., 2006. Little egrets (*Egretta garzetta*) and trace-metal contamination in wetlands of China. *Environmental Monitoring and Assessment 1*18, 355-368.

Part A



Toxic muds at Flix,
Do they affect the ornithofauna at the Ebro River and its Delta?

I

Are the toxic sediments deposited at Flix reservoir affecting the Ebro river biota? Purple Heron eggs and nestlings as indicators

¿Afectan los lodos tóxicos del embalse de Flix a la fauna del río Ebro?

Huevos y volantones de Garza Imperial como bioindicadores

Javier Cotín[a], Manuel García-Tarrasón[a], Lluis Jover[b] & Carolina Sanpera[a]

[a] Department Biologia Animal, Facultat de Biologia, Univesitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain

[b] Department Salut Pública, Facultat de Medicina, Universitat de Barcelona, Casanova 143, 08036 Barcelona, Spain

---

Resumen: El embalse de Flix, situado en el curso bajo del río Ebro, contiene miles de toneladas de sedimentos contaminados, acumulados como residuos de la actividad de una industria química. Un proyecto destinado a la eliminación de dichos residuos ha comenzado. Aves piscívoras como la Garza Imperial (Ardea purpurea) pueden ser bioindicadores apropiados; por lo tanto, huevos y plumas de volantones fueron muestreadas durante las temporadas de cría de los años 2006-2008 en tres localidades: una localidad referencia, situada aguas arriba, y dos potencialmente afectadas por los lodos tóxicos; una en el área focal y otra en un área distal, el Delta del Ebro. Se analizaron los isótopos estables de ^{15}N y ^{13}C y las concentraciones de metales pesados y selenio en las muestras obtenidas. La línea basal de las signaturas de nitrógeno fue más alta en las localidades fluviales que en el delta. Las signaturas de nitrógeno junto a las de carbono discriminaron adecuadamente los ecosistemas fluviales y deltaicos. Los niveles de mercurio están altamente influenciados por los lodos tóxicos de Flix y suponen un riesgo potencial para las aves, puesto que están entre los más altos nunca descritos en especies de garzas. Las concentraciones de selenio y cobre probablemente derivan de otras fuentes. Excepto el mercurio, los metales pesados analizados y el selenio se encuentran por debajo de niveles tóxicos. Los huevos y plumas de volantones de Garza Imperial han demostrado su utilidad como bioindicadores de contaminación en la fauna asociada al río; las plumas en concreto muestran el impacto de los contaminantes del área estrictamente circundante. Estudios de varios años de duración son necesarios en ecosistemas tan dinámicos como éste, así que se recomienda la continuación del monitorizaje de las poblaciones de ardeidos de Flix y las otras áreas de estudio para poder continuar la evaluación de los efectos de los lodos tóxicos, particularmente durante su eliminación, debido a que los altos niveles de mercurio detectados entrañan altos riesgos si se produce una resuspensión de los lodos.

Palabras Clave: Garza imperial, Ardea purpurea, Biomonitorizar, Ecología trófica, Isótopos estables, Elementos traza.

Ecotoxicology (2012) 21:1391–1402
DOI 10.1007/s10646-012-0893-4

Are the toxic sediments deposited at Flix reservoir affecting the Ebro river biota? Purple heron eggs and nestlings as indicators

**Javier Cotín · Manuel García-Tarrasón ·
Lluis Jover · Carolina Sanpera**

Accepted: 16 March 2012
Published online: 12 April 2012
© Springer Science+Business Media, LLC 2012

Abstract The Flix reservoir, in the low course of the Ebro River, contains thousands of tons of polluted sediments, accumulated from the activities of a chemical factory. An ongoing project is working toward removing these pollutants. Piscivore birds like the purple heron (*Ardea purpurea*) may be useful bioindicators, so eggs and nestling feathers were sampled during the 2006–2008 breeding seasons at three localities: a reference site situated upstream and two potentially affected by the toxic muds; one at the focal area and one at a distal area, the Ebro Delta. The samples were analyzed for isotopic signatures of ^{15}N and ^{13}C and concentrations of heavy metals and selenium. Baseline nitrogen signatures were higher in riverine sites than in the delta. Nitrogen together with carbon signatures adequately discriminated riverine and deltaic ecosystems. Mercury levels are highly influenced by the polluted sediments at Flix and pose potential risks for the birds, as they are among the highest ever recorded in heron species. Selenium and copper concentrations probably derive from other sources. Except for mercury, heavy metals and selenium levels were below toxic levels. Purple heron eggs and nestling feathers have demonstrated their usefulness as bioindicators for pollution in the river biota; feathers in particular show pollutant impacts on a strict local basis. A long series of study years is necessary in dynamic ecosystems such as this, so continued monitoring of the heron population at Flix is advisable to trace the effects of the toxic muds, particularly during their removal, because of the high levels of mercury detected.

Keywords Purple heron · *Ardea purpurea* · Biomonitoring · Trophic ecology · Stable isotopes · Trace elements

Introduction

Birds have been widely used as bioindicators of environmental pollution, especially when potential hazards may affect human populations or wildlife. Some of these hazards have occurred on the Iberian Peninsula, including the oil spill in 2002 at the Atlantic north-west after the tanker Prestige wrecked (Moreno et al. 2011; Sanpera et al. 2008), the Aznalcollar mine spill into the wetlands of Doñana National Park in 1998 (Baos et al. 2006; Benito et al. 1999; Gomez et al. 2004; Taggart et al. 2006), and the case that is addressed in this study, in the Flix Reservoir, northeastern Spain.

The Ebro River (Catalonia, NE Spain) discharges into the Mediterranean Sea creating a delta of more than 30,000 ha. The Ebro catchment is the largest river basin in Spain; it covers an area of 85,362 km^2 (http://www.chebro.es), potentially supplies 3 million people, and it contains some heavily industrialized areas. One of these areas is the Flix site, where a chemical industry has been in operation since the early 20th century. This long operational period, along with the construction of a dam next to the plant around 1960, resulted in the accumulation of

---

Electronic supplementary material The online version of this article (doi:10.1007/s10646-012-0893-4) contains supplementary material, which is available to authorized users.

J. Cotín (✉) · M. García-Tarrasón · C. Sanpera
Department Biologia Animal, Facultat de Biologia, Univesitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain
e-mail: jcotin@ub.edu

L. Jover
Department Salut Pública, Facultat de Medicina, Universitat de Barcelona, Casanova 143, 08036 Barcelona, Spain

200,000–360,000 tons of industrial wastes in the riverbed, occupying an area 700 m in length and 60 m wide. The mixture of heavily polluted sediments is composed of high concentrations of heavy metals (cadmium, arsenic, copper, chromium, selenium, lead and especially mercury), organochlorines (hexachlorobenzene (HCB), pentachlorobenzene, DDTs, polychlorobiphenyls (PCBs), polychloronaphthalenes and polychlorostyrenes) and radioactive ^{210}Pb (Bosch et al. 2009; Fernandez et al. 1999). In addition, the Ebro River is currently affected by different agricultural and industrial activities, with significant impact on the existing biota (Mañosa et al. 2001; Navarro et al. 2010). The pollutants originated at the Flix site are carried downstream by the Ebro River to its delta 90 km away (Llorente et al. 1987; Navarro et al. 2009; Pastor et al. 2004), especially during floods (Vericat and Batalla 2006).

As a consequence, a whole-ecosystem study on the environmental impact of such toxic muds was undertaken to evaluate their effects prior to an ongoing project aimed at removing the polluted sediments; this last initiative may imply a high risk of pollutant dispersal.

Other studies in the Flix reservoir have reported high levels of PCBs in sediments (Fernandez et al. 1999) and effects of pollutants on different sentinel species. Earthworms reached high levels of mercury (Ramos et al. 1999). Zebra mussels (*Dreissena polymorpha*) at this site had the highest levels of Hg and methylmercury ever reported, with mean values 20 times greater than the local background levels (Carrasco et al. 2008). Crayfish (*Procambarus clarkii*) and zebra mussels exhibited high toxic stress levels (high activities and levels of antioxidant enzymes, metallothioneins, lipid peroxidation and DNA strand breaks and decreased levels of glutathione) close to the waste dumps (Faria et al. 2010), even crayfish presented levels of mercury exceeding legal values established by European Union legislation (Suarez-Serrano et al. 2010). Mercury concentrations in tissues of carp (*Cyprinus carpio*) sampled downstream from Flix were one to two orders of magnitude higher than those from carp sampled upstream from Flix (Navarro et al. 2009) and catfish (*Silurus ganis*) exceeded the maximum mercury level recommended for human consumption (Carrasco et al. 2011). Deformities, eroded fins, lesion and tumor anomalies and ectoparasites were clearly more frequent at the impacted area for several fish species (carp, roach: *Rutilus rutilus* and pumpkinseed sunfish: *Lepomis gibbosus*); also a significant lower body condition was detected for these species and bleak (*Alburnus alburnus*), while there was a negative impact on reproductive traits for carp and pumpkinseed (Benejam et al. 2010). The responses to the pollutants were species-specific, and carp had the clearest effects on fitness-related traits at the impacted area, despite also being among the most tolerant to pollution.

Piscivorous birds such as herons (family Ardeidae) are suitable bioindicators of environmental pollution in aquatic systems (Champoux et al. 2006; Connell et al. 2003; De Luca-Abbott et al. 2001; Sakellarides et al. 2006). They are in the upper trophic level of these ecosystems, and consequently they biomagnify and bioaccumulate persistent organic pollutants and some metals (Baker and Sepulveda 2009).

In a previous study we showed that purple heron (*Ardea purpurea*) nestlings at Flix showed the highest frequencies of micronuclei in peripheral erythrocytes and reduced blood antioxidant defenses when compared with two other sampling sites, one upstream and another downstream (Quiros et al. 2008). Purple heron eggs showed elevated levels of HCB and PCBs (Barata et al. 2010). Additionally, other bird populations such as terns (family Sternidae) foraging at the Ebro Delta showed the indirect effects of pollutants derived from the Flix reservoir as well as from the intensive agricultural activities taking place in the area (Cotin et al. 2011).

In order to achieve a comprehensive evaluation of the toxic impact at the Ebro River lower course, purple heron eggs and nestlings were used as bioindicators; three sampling sites were chosen according to the breeding colonies of this species in the area of the Ebro River. One of the sites, situated 25 km upstream of the polluted sediments, was designated as reference (l'Aiguabarreig), while the other two, which are potentially affected by the toxic muds, were designated as focal area (Flix) and distal area (the Ebro Delta), situated 90 km downstream. Purple heron colonies were followed and sampled during consecutive years (2006–2008), in order to obtain a comprehensive vision of the highly dynamic ecosystem of the Ebro River, taking into account both trophic ecology and heavy metal exposure.

Heavy metals, when present at high concentrations, are of special concern, as they mainly enter organisms through diet (Burger et al. 1992). However, the exposure of individuals varies according to their trophic habits. Therefore, besides the analysis of contaminants, stable isotopes analysis (SIA) were used to get a better understanding of purple heron trophic ecology (Abdennadher et al. 2011; Nisbet et al. 2002; Ramirez et al. 2011; Sanpera et al. 2007; Tavares et al. 2007). Stable isotope signatures of nitrogen (δ^{15}N) and carbon (δ^{13}C) have been extensively used in studies of bird communities, focusing on their trophic ecology and relationships (Cherel et al. 2008; Cotin et al. 2011; Forero and Hobson 2003; Hobson et al. 1994; Koiadinovic et al. 2008; Moreno et al. 2010). δ^{15}N of tissues reflects the trophic level, with consumer signatures being higher than in their prey (Forero et al. 2005). Information about the source of carbon entering a food web can be obtained from δ^{13}C (Hobson 1999), providing insight about the foraging habitat.

Data obtained from egg samples integrate the adult diet prior to egg laying because herons, as income breeders, obtain most of the materials used in clutch formation from diet (Hobson et al. 2000; Ruiz et al. 1998), while nestling feathers integrate the diet obtained by the adults and consumed by the nestlings during growth. The use of nestlings has two advantages. First, the effect of age-related bioaccumulation is avoided, because the time of exposure has been low and similar for all individuals. Second, because all the chicks are provisioned with prey caught by parents in the surroundings of the colony, the results should reflect only the impact of local pollution.

Here we aim to assess the relevance of the toxic mud in the river contamination processes through a comparative study of purple heron populations breeding at the focal area and two other sites subjected to different contamination pressures. For this purpose, purple heron nestlings and eggs were used as bioindicators and the relationship between trophic level and pollutant concentrations was examined. The first objective was to measure pollutant levels in eggs and nestling feathers in order to establish whether values merit conservational concern, while determining the trophic ecology of these populations, in order to appropriately assess the pollutant exposure. The second objective was to determine which sample type, either eggs or feathers, more accurately reflects such impact or exposure in order to suggest its use as a bioindicator once the toxic muds have been removed. We tested the following main hypothesis: pollutant concentrations and exposure will be higher in herons breeding at Flix and the Ebro Delta than in l'Aiguabarreig after adjusting the pollutant level input by their trophic level. The results may be useful to define conservation policies to be applied in this area, and as both herons and heavy metal pollution are distributed worldwide, results obtained in this study may be used to assess the effects of pollution in other areas of concern.

Materials and methods

Study sites

Purple heron eggs and mantle feathers of nestlings were collected at three selected sites along the Ebro River, NE Spain (see Fig. 1). L'Aiguabarreig site (41°23′N, 00°19′E) is a riverine island called "Illa de los Martinets", located at the confluence of two Ebro tributaries, the Cinca and Segre Rivers. This highly valuable ecological spot and sanctuary for aquatic birds is located upstream from the Flix site (41°14′N, 00°31′E) and therefore unaffected by the Flix factory and its toxic sediments. Despite the industrial activity, Flix dam surroundings have surprisingly become a valuable wetland with a profusion of nesting birds, including herons, storks and marsh harriers. The Ebro Delta (40°42′N, 00°50′E) is one of the largest wetlands in the western Mediterranean region and is home to extensive bird colonies. Occupying an area of 320 km^2, this wetland presents a wide variety of habitats, such as rice fields, farmland, abandoned fields, lagoons, salt marshes and beaches. The coastal lagoons, although connected to the sea and thus expected to hold brackish water, receive considerable freshwater input from the rice fields from spring to autumn, thereby lowering their salinity, which almost reaches that of freshwater during those seasons.

Sampling

Egg sampling was conducted for the breeding seasons of 2006 and 2007 during the laying period, and feather sampling during the breeding seasons of 2006, 2007 and 2008, just 1 week before the estimated peak fledging period. Number of samples was: l'Aiguabarreig (12 eggs collected in 2007; 7 feather samples in 2006, 7 in 2007 and 4 in 2008), Flix (12 eggs collected in 2006, 16 in 2007; 10, 11 and 9 feather samples, respectively) and the Ebro Delta (14 eggs in 2006, 25 in 2007; 4, 16 and 12, feather samples, respectively). Eggs were not collected during 2006 at l'Aiguabarreig due to unusual adverse climatic and river flow conditions which limited the access to the area and nest localization. To avoid pseudo-replication, only one egg or nestling was sampled per nest. Eggs and nestlings were sampled with the permission of the *Serveis de Fauna i Pesca*, *Generalitat de Catalunya* (Spain).

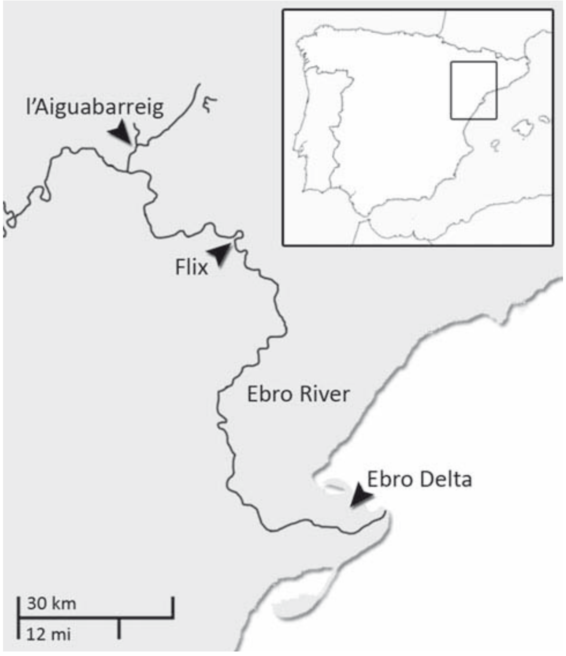


Fig. 1 Map showing the sampling sites

Eggs were labeled and kept refrigerated until reaching the laboratory. Once there, they were kept frozen (−20 °C) until analysis. Egg content was then separated from the egg shell, weighed, and placed into a glass container for freeze-drying. Freeze-dried samples were homogenized and an aliquote was used for trace element determination. A sub-sample was lipid-extracted for stable isotope analysis using methanol and chloroform, following Folch's method (Folch et al. 1957).

Feather samples were kept in polyethylene bags and frozen until the cleaning process. Once in the laboratory, feathers were cleaned with a 0.1 M NaOH solution and dried for 24 h at 50 °C prior to trace element determination and SIA. Once they were cleaned and dry, in order to homogenize them for SIA, all feathers were ground to an extremely fine powder using an impactor mill (Freezer Mill 6750, Spex CertiPrepH Inc., Metuchen, NJ, USA) operating at liquid nitrogen temperature. Additionally, for a better understanding of the trophic ecology of the herons, crayfish and carp from regurgitates obtained during the nestling sampling were also prepared for SIA analysis following the procedure described for egg samples.

Stable isotopes analysis

Sub-samples (ca. 0.36 mg for δ^{13}C and δ^{15}N) of the homogenized eggs and the ground feathers were placed into tin buckets and crimped for combustion. Isotopic analyses were carried out by EA-IRMS (elemental analysis-isotope ratio mass spectrometry) by means of a Thermo Finnigan Flash 1112 elemental analyzer coupled to a Delta isotope ratio mass spectrometer via a CONFLO III interface.

Stable isotope ratios were expressed in conventional notation as parts per thousand (‰) following the equation: $\delta X = [(\text{R sample}/\text{R standard})-1] \times 1{,}000$, where X is ^{15}N or ^{13}C and R is the corresponding ^{15}N/^{14}N or ^{13}C/^{12}C. The standards for ^{15}N and ^{13}C are atmospheric nitrogen and Pee Dee Belemnite, respectively. Precision and accuracy for δ^{13}C measurements was $\leq$0.1 and $\leq$0.3 ‰ for δ^{15}N. The laboratory applies international standards, which are run for every 12 samples: IAEA CH_7 (87 % of C), IAEA CH_6 (42 % of C) and USGS 24 (100 % of C) for ^{13}C and IAEA N1 and IAEA N2 (with 21 % of N) and IAEA NO_3 (13.8 % of N) for ^{15}N.

Trace elements analysis

Trace metal determination of mercury, selenium, copper, lead, chromium and arsenic (chromium and arsenic only 2007–2008) was carried out by means of ICP-MS Perkin Elmer ELAN 6000.

Before the trace metal determination, homogenized subsamples of freeze-dried eggs and ground feathers (ca. 100 mg) were digested in H_2NO_3 and H_2O_2 in Savilles Teflon digestion vessels for 12 h at 100 °C. Accuracy of analysis was checked by measuring certified reference material (Lobster hepatopancreas Tort-2 and Dogfish liver Dolt-3; National Research Council Canada for eggs and human hair CRM 397, Community Bureau of Reference, Commission of the European Communities in the case of feather samples).

Mean recoveries ranged 96–100 % for total mercury, selenium, copper, lead, chromium and arsenic; and no corrections were done. Values of limit of detection were: 0.1 ng/g for Pb and As, 0.2 for Hg and Cu, 0.5 ng/g for Cr and 1 ng/g for Se. Determinations below such values were set to 'not detected'. All trace elements concentrations were expressed on a dry weight basis (ng/g, i.e., parts per billion).

Trace elements and SIAs were performed at the Serveis Científico-Tècnics (Universitat de Barcelona).

Statistical methods

Values of trace elements concentrations and stable isotope ratios were routinely checked for normal distributions using Kolmogorov–Smirnov and Shapiro–Wilk tests, together with Q–Q plots. Trace elements concentrations showed clear skewed distributions which were normalized by applying a logarithmic transformation. Samples with values under detection limit were assigned with 1/2 of the detection limit value of a trace element when the percentage of detection of that trace element ranged between 50 and 100 %.

When the high number of values below detection limit precludes carrying out comparisons, we looked for differences among localities in the percentage of samples with detected values using Fisher exact test for 2 × 3 tables.

Comparisons among localities and years were carried out using one way analysis of variance and applying the Levene test to check for homoscedasticity. Welch Correction was used accordingly. To test for "a posteriori" pairwise differences we used Tamhane′s or SNK tests. Descriptive statistics and mean differences between groups and their 95 % confidence intervals were used to show the results. Statistical analysis was carried out using PASW Statistics 18.0.

Results

Descriptive statistics (mean, standard deviation, minimum and maximum) for stable isotopes are presented in Table 1. Trace element descriptive statistics (geometric mean and 95 % CI) are presented in Table 2 for feathers and in Table 3 for eggs. One of the eggs sampled during 2007 at

Table 1 Descriptive statistics of stable isotopes signatures in feathers and eggs of purple heron nestlings (*Ardea purpurea*) from the three sites and years

	Site	Year	Sample									
			Feather					Egg				
			N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
δ^{15}N (%)	Aiguabarreig	2006	7	18.5	0.9	16.7	19.6					
		2007	7	18.9	1.6	15.8	20.5	12	19.0	2.3	14.7	21.5
		2008	4	19.2	1.9	16.4	20.3					
	Flix	2006	10	22.3	0.7	20.8	23.2	12	20.5	1.3	18.1	22.7
		2007	11	21.6	1.2	19.0	22.8	15	19.8	2.1	16.1	22.3
		2008	9	21.6	0.5	20.6	22.2					
	Ebro Delta	2006	4	13.9	1.4	12.3	15.5	14	13.9	1.5	11.8	17.1
		2007	16	13.9	0.7	12.8	15.6	25	13.3	1.0	11.6	15.7
		2008	12	14.4	1.0	13.0	15.7					
δ^{13}C (%)	Aiguabarreig	2006	7	−25.6	0.9	−27.0	−24.3					
		2007	7	−27.5	0.5	−28.3	−27.0	12	−27.2	1.5	−29.9	−25.2
		2008	4	−27.4	0.9	−28.7	−26.8					
	Flix	2006	10	−25.8	0.8	−27.1	−24.4	12	−27.5	0.9	−28.9	−25.9
		2007	11	−27.0	0.7	−28.1	−25.9	16	−28.3	0.9	−29.2	−25.8
		2008	9	−28.6	0.4	−29.3	−28.1					
	Ebro Delta	2006	4	−24.0	1.8	−26.2	−22.2	14	−24.9	1.0	−26.3	−23.2
		2007	16	−24.1	0.9	−25.0	−22.1	25	−26.3	1.5	−30.4	−23.1
		2008	12	−22.6	3.2	−25.6	−16.9					

Flix site presented a nitrogen value out of range and was excluded from those analyses involving δ^{15}N.

Stable isotopes of prey (crayfish and carp samples) are shown in the scatterplot of δ^{15}N and δ^{13}C in Fig. 2.

Feather and eggs samples

With regard to δ^{15}N no significant interaction between locality and year was detected. Significant differences were found among localities for both feathers ($F_{2,77} = 445$, $p < 0.001$) and eggs ($F_{2,75} = 146$, $p < 0.001$), but not among years. In feather samples, Flix presented the higher values, followed by l'Aiguabarreig, and with the lowest values, the Ebro Delta, while for eggs l'Aiguabarreig and Flix had the highest values (with no significant differences between l'Aiguabarreig and Flix) and the Ebro Delta the lowest (Fig. 3).

A significant interaction between locality and year was found in carbon signatures of feathers (see Fig. 3); although localities always ranked in the same order, magnitude of differences among them varies with year. Significant differences were found among localities in all years ($F_{2,18} = 3.9$, $p = 0.038$; $F_{2,31} = 69.4$, $p < 0.001$; $F_{2,22} = 19.5$, $p < 0.001$, for 2006, 2007 and 2008 respectively). No interaction was found in egg samples, but significant differences were found among localities ($F_{2,75} = 28$, $p < 0.001$) and between years ($F_{1,75} = 14.3$, $p < 0.001$). The Ebro Delta was the locality with the highest values for both eggs and feathers, while l'Aiguabarreig and Flix showed the lowest (with no significant differences between l'Aiguabarreig and Flix). Signatures were higher in 2006 than 2007 for egg samples.

Mercury showed a significant interaction between locality and year in feathers and significant differences were found among localities in all years ($F_{2,18} = 33.7$, $p < 0.001$, $F_{2,31} = 54.1$, $p < 0.001$, $F_{2,22} = 15.5$, $p < 0.001$, for 2006, 2007 and 2008 respectively). Post hoc comparisons showed that Flix was the locality with the highest levels and l'Aiguabarreig and the Ebro Delta did not differed significantly. Egg samples did not show an interaction, but significant differences among localities ($F_{2,75} = 14.8$, $p < 0.001$) and between years ($F_{1,75} = 20$, $p < 0.001$) (Fig. 4). In eggs, Flix again showed the highest values, followed by the Ebro Delta, with the lowest values in l'Aiguabarreig. Levels were higher in 2006 than in 2007.

Selenium levels showed significant differences among localities (Feather: $F_{2,77} = 22.9$, $p < 0.001$; Egg: $F_{2,75} = 10.9$, $p < 0.001$) and in egg samples also between years ($F_{1,75} = 6.3$, $p = 0.014$) (Fig. 4). No interaction among site and year was found in feather or egg samples. For feather samples, Flix was the site with the highest values, followed by l'Aiguabarreig and the Ebro Delta with the

Table 2 Descriptive statistics of trace elements expressed in ng/g in feathers of purple heron nestlings (*Ardea purpurea*) from the three sites and years

	Year	Site											
		l'Aiguabarreig				Flix				Ebro Delta			
		%	Mean	95 % CI		%	Mean	95 % CI		%	Mean	95 % CI	
[Hg]	2006	100	1501.0b	1087.4	2072.0	100	7377.9a	5186.1	10496.1	100	2407.0b	1944.1	2980.1
	2007	100	1548.4b	1160.5	2065.9	100	6614.7a	5081.2	8610.8	100	1395.7b	1106.8	1760.0
	2008	100	1469.1b	1277.2	1690.0	100	3781.4a	2555.1	5596.3	100	1801.3b	1562.8	2076.3
[Se]	2006	100	1811.9B	1542.8	2127.9	100	2609.2A	2311.2	2945.7	75	1289.9C	624.6	2663.7
	2007	86	1654.6	1122.9	2437.9	100	2364.4	2047.9	2729.7	81	1244.3	1007.7	1536.4
	2008	100	2214.6	2065.4	2374.6	100	2238.9	1723.6	2908.3	100	1705.8	1479.1	1967.3
[Cu]	2006*	100	8638.4	7658.0	9744.4	100	8076.5	7373.7	8846.2	100	11282.6	9618.3	13234.9
	2007**	100	5698.3	4585.4	7081.1	100	6070.0	5478.3	6725.6	100	5849.4	5385.7	6353.0
	2008**	100	5921.9	5395.0	6500.2	100	5704.8	4698.3	6927.0	100	7766.2	6033.2	9996.9
[Pb]	2006	100	1349.6	1009.7	1804.0	100	1622.0	1383.3	1901.9	100	2038.2	1476.3	2813.9
	2007	0				9				0			
	2008	25				56	54.8	33.3	90.2	75	76.1	50.1	115.8
[Cr]	2007*	100	1603.8B	1511.4	1701.9	100	1736.5A	1619.9	1861.5	100	1541.2B	1447.0	1641.4
	2008**	100	1961.2	1848.5	2080.8	100	2059.0	1961.4	2161.5	100	2055.8	1956.2	2160.5
[As]	2007	29				27				88	157.3	138.5	178.7
	2008	100	69.8B	48.1	101.3	100	68.0B	52.2	88.8	100	148.0A	117.7	186.1

Asterisks and capital letters show significant differences among years or among localities respectively, in the case of no interaction between both factors. Minuscule are used to show significant differences between localities for a particular year when interaction was detected

Table 3 Descriptive statistics of trace elements expressed in ng/g in eggs of purple heron (*Ardea purpurea*) from the three sites and years

	Year	Site											
		l'Aiguabarreig				Flix				Ebro Delta			
		%	Mean	95 % CI		%	Mean	95 % CI		%	Mean	95 % CI	
[Hg]	2006*					100	1007.9A	703.4	1444.2	100	636.0B	513.2	788.6
	2007**	100	159.5C	100.6	253.0	100	579.8A	366.0	918.3	100	257.6B	195.5	339.5
[Se]	2006*					100	3808.6A	3293.7	4404.1	100	2937.5B	2495.0	3458.5
	2007**	100	3468.0A	3114.0	3862.3	100	3398.8A	3123.6	3698.2	100	2690.7 B	2451.5	2953.4
[Cu]	2006					100	5211.5a	4743.4	5725.7	100	4713.3a	4363.0	5091.8
	2007	100	3721.5c	3261.5	4246.4	100	4022.1b	3686.8	4387.9	100	4342.1a	4087.3	4612.8
[Cr]	2007	100	2529.1	2453.4	2607.1	100	2425.2	2244.5	2620.4	100	2599.3	2527.5	2673.1
[As]	2007	83	127.8B	115.1	141.9	69	130.6B	103.3	112.9	100	167.8A	151.6	185.6

Asterisks and capital letters show significant differences among years or among localities respectively, in the case of no interaction between both factors. Minuscule are used to show significant differences between localities for a particular year when interaction was detected

lowest values. In eggs l'Aiguabarreig and Flix had the highest values (with no significant differences between them), while Ebro Delta the lowest, and levels during 2006 were higher than in 2007.

Concerning copper levels, a significant interaction between locality and year was found in eggs. Significant differences were found among localities ($F_{2,75} = 4$, $p = 0.022$) and between years ($F_{2,75} = 23.3$, $p < 0.001$) in feathers, while in eggs differences were found only in 2007 ($F_{1,24} = 3.3$, $p = 0.082$; $F_{2,50} = 3.6$, $p = 0.034$, for 2006 and 2007 respectively). In feather samples, pairwise tests failed to find significant differences, and levels during 2006 were higher than those detected in 2007 and 2008. For eggs, differences found in 2007 showed the Ebro Delta as the site with the highest levels and l'Aiguabarreig as the one with the lowest, while Flix presented intermediate values.

Chromium levels did not show an interaction between locality and year, but showed significant differences among localities ($F_{2,55} = 3.27$, $p = 0.045$) and years ($F_{1,55} = 87.19, p < 0.001$) in feathers. For feather samples, Flix was

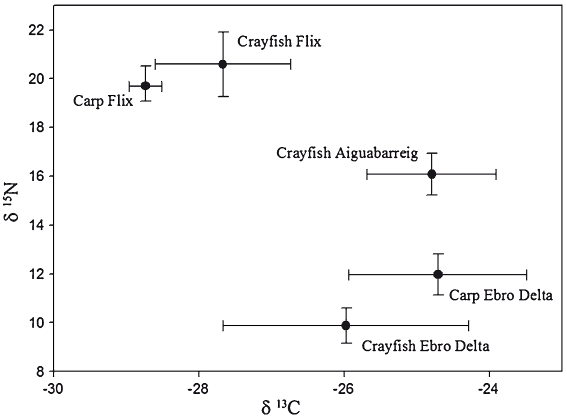


Fig. 2 Plots of δ^{15}N and δ^{13}C signatures indicating the mean position of the crayfish and carp sampled of the three sampled sites (two for carp). *Circles* indicate mean value, *lines* their corresponding 95 % confidence intervals

the site with the highest values, while l'Aiguabarreig and the Ebro Delta presented lower ones (with no significant differences between l'Aiguabarreig and the Ebro Delta). Also, levels were higher during 2008 than during 2007. Significant differences were not found in egg samples ($F_{2,50} = 2.59$, $p = 0.085$).

Arsenic levels showed significant differences among localities (Feather$_{2008}$: $F_{2,22} = 15.9$, $p < 0.001$; Egg$_{2007}$: $F_{2,50} = 11.16$, $p < 0.001$) for both sample types. For both feather and eggs samples, the Ebro Delta was the site with the highest values, l'Aiguabarreig and Flix had lower ones (with no significant differences between them). In 2007 percentage of feather samples above detection limit were compared, resulting in a significant difference among localities (Fisher exact test, $p = 0.0014$), showing in the Ebro Delta higher percentages of detection.

Information on lead levels in feathers is shown in Table 2; due to the highly variable percentage of detected samples, running adequate statistical analysis was not possible, so quantitative differences among sites or years are not given. Nevertheless, we were able to compare percentage of feather samples with quantifiable values among localities, and we found that in 2007 there was not significant differences (Fisher exact test, $p = 0.54$) whereas significant difference arises in 2008 (Fisher exact test, $p = 0.031$) samples from l'Aiguabarreig presenting a lower percentage of detection. Lead was not detected in eggs samples.

Comparison between feather and egg signatures and trace element levels

For such comparisons, only data from 2007 are taken into account, as both feather and eggs samples were collected at all sites only during that year.

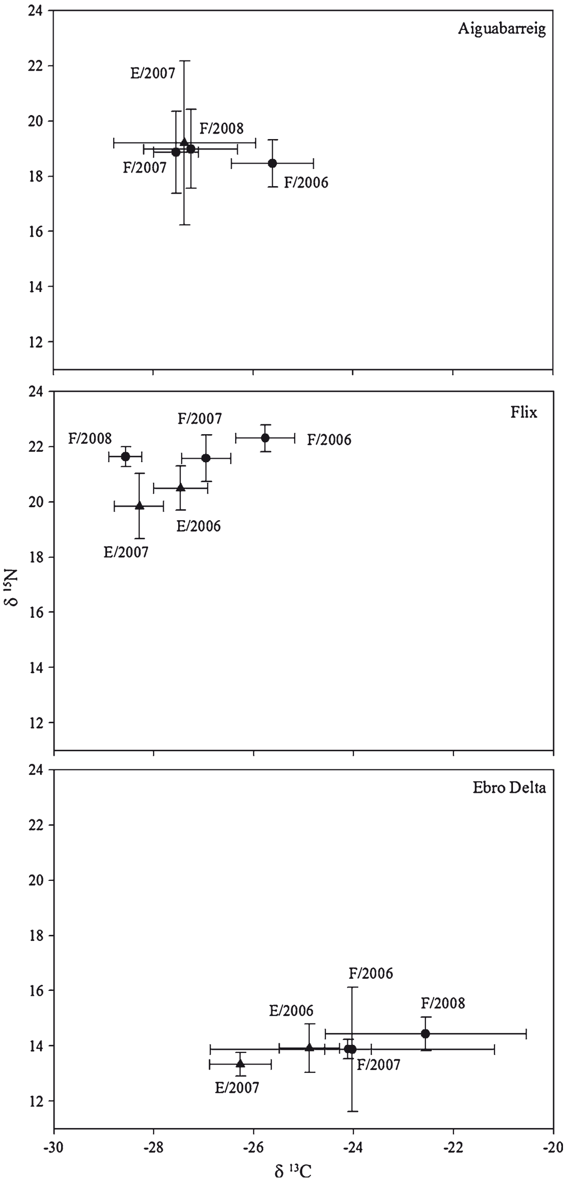


Fig. 3 Plots of δ^{15}N and δ^{13}C signatures indicating the mean position of the purple heron nestlings and eggs of the three sampled sites in each year. *Circles* (*feathers* F) and *triangles* (*eggs* E) indicate mean value, and *lines* their corresponding 95 % confidence intervals

With regard to mercury, selenium and copper levels, no interaction between site and sample type was detected, and significant differences were found among sample types (Hg: $F_{1,83} = 205.3$, $p < 0.001$; Se: $F_{1,83} = 107.9$, $p < 0.001$; Cu: $F_{1,83} = 93.8$, $p < 0.001$). Chromium levels presented interaction between site and sample type, and significant differences were found among sample types in all sites (l'Aiguabarreig: $F_{1,17} = 312.7$ $p < 0.001$; Flix:

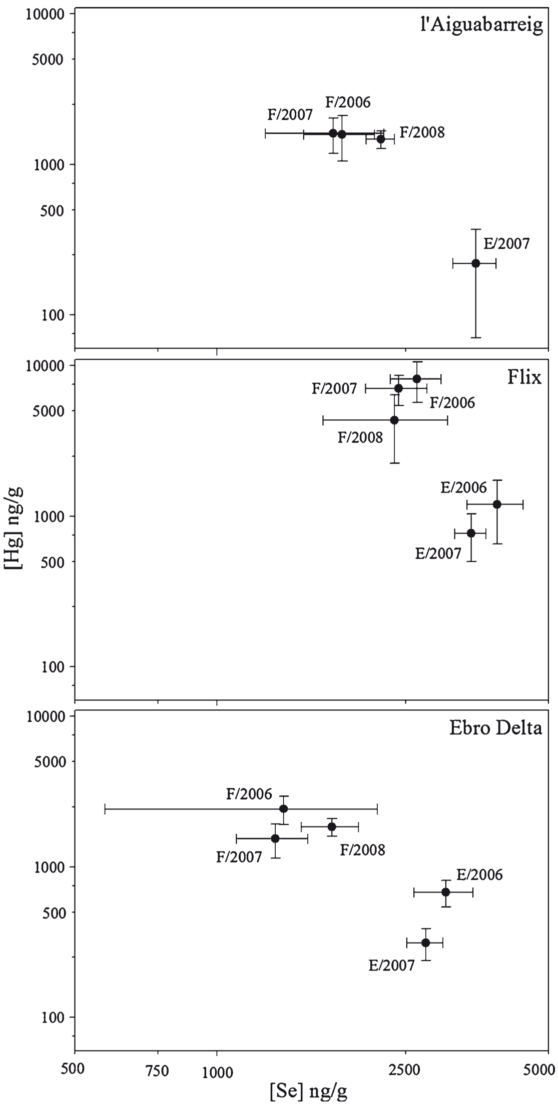


Fig. 4 Plots of mercury and selenium concentrations (given on a logarithmic scale) indicating the mean position of the purple heron nestlings and eggs of the three sampled sites in each year. *Circles* (*feathers* F) and triangles (*eggs* E) indicate mean value, and *lines* their corresponding 95 % confidence intervals

$F_{1,25} = 42.93$, $p < 0.001$; Delta: $F_{1,39} = 324.52$, $p < 0.001$). Metal levels followed the same pattern in all sites, such that mercury and copper levels were higher in feather than in egg samples, while selenium and chromium levels presented the opposite pattern. Arsenic levels were equivalent in both sample types. Arsenic levels were not compared due to the small percentage of detected samples in feather.

Regarding nitrogen signatures, differences were found among sample types ($F_{1,82} = 5.3$, $p = 0.024$). Nitrogen signatures followed the same pattern as mercury and copper, being higher in feather than in egg samples. On the other hand, carbon signatures presented a significant interaction between locality and sample type. Significant differences were found between sample type in Flix and Ebro Delta ($^{13}C_{Flix}$: $F_{1,25} = 18.4$, $p < 0.001$, $\delta^{13}C_{Delta}$: $F_{1,39} = 27.8$, $p < 0.001$) but were not found at l'Aiguabarreig ($\delta^{13}C$ $_{l'Aiguabarreig}$: $F_{1,17} = 0.3$, $p = 0.623$). Signatures were higher in feather samples at Flix and the Ebro Delta.

Discussion

Nitrogen signatures are used to estimate trophic level, although the ratios can be affected by several factors, such as the food-chain length in a given ecosystem or by environmental differences in the baseline (Cabana and Rasmussen 1994). The latter seems to be the case of this study, as the outstanding differences found between the riverine localities (Flix and l'Aiguabarreig) and the Ebro Delta apparently relied on baseline differences, likely caused by the eutrophication of the river. Both riverine sites are located at reservoirs, with marked eutrophication indicated also by the higher $\delta^{15}N$ of the main purple heron prey (see Fig. 2). A similar situation was observed in little egret (*Egretta garzetta*) from Chikly island (Abdennadher et al. 2011), exposed to eutrophication and food-web enrichment in nitrogen rich sewage. On the other hand, purple heron $\delta^{15}N$ signatures from the Ebro Delta resemble those of the freshwater tern species that inhabit this area (Cotin et al. 2011), where the continuous water flow into rice fields and lakes probably prevents eutrophication. The possibility that the $\delta^{15}N$ differences are caused by changes in diet is ruled out as regurgitates from all localities presented the same prey species, mainly crayfish and carp (J. Cotin, personal observation). Therefore, although $\delta^{15}N$ signatures reflect trophic level, the changes observed among the different localities are based on differences in the baseline. Carbon signatures maintain the same pattern between sites each year, although slight differences can be observed between years. The values reflect riverine and deltaic habitats, being around −24 ‰ for the Ebro Delta freshwater habitats (Cotin et al. 2011). Observed changes between years in riverine localities are probably due to the highly dynamic nature of the Ebro River. Annual changes at the Ebro Delta are probably related to a partial shift to more brackish feeding areas, such as the coastal lagoons, as habitats influenced by the sea present a higher isotopic ratio than freshwater habitats (Michener and Schell 1994). This fact is reflected in the higher variability of carbon signatures found in the Ebro Delta. The combined use of nitrogen and carbon signatures helps to discriminate well enough riverine and deltaic ecosystems (high nitrification in the river and lower carbon signatures in the delta).

When comparing egg and nestling feather samples, Burger et al. (2009) found no differences in their usefulness as bioindicators of contamination in herons, although other studies found nestling feathers more representative of regional pollution, as egg samples may be influenced to a different extent by the female body burden accumulated during the wintering or migration period (DesGranges et al. 2009; Hughes et al. 1997). Nevertheless, as the purple heron is considered an income breeder (Hobson et al. 2000; Ruiz et al. 1998) differences between egg and feather samples are probably due to routing. Moreover, feathers reflect the narrower diet offered to the chicks by their parents and, in a small and unknown percentage, the transfer of some of the female's burden into the egg. Accordingly, both samples would be reliable bioindicators, although the lower variability shown by the isotopic data and the higher levels observed in most of the trace elements analysed (except for chromium, but especially selenium, as this element is appreciably transferred to eggs (Focardi et al. 1988; Sell 1977) suggest that feather samples may be a more accurate bioindicator. Also, from a conservational perspective, sampling nestling feathers is more respectful to heron populations, provided that sampling protocols follow certain conditions while entering heron colonies, which are highly sensitive to human disturbance.

Although some differences were found in chromium and arsenic, levels are very similar in all sites. Chromium levels detected at Flix could be slightly influenced by the toxic muds and arsenic probably is higher at the Ebro Delta due to a partial use of foraging habitats influenced by the sea, as it has been seen in other species inhabiting this area (unpublished data). Arsenic and chromium levels are below toxic levels and within those reported for several bird species, including herons (Burger and Gochfeld 2009; Padula et al. 2010), and lead levels are below those causing adverse reproductive effects (around 4,000 ng/g in feathers reported by (Burger and Gochfeld 2000). Although hunting activities using lead shot are high at the Ebro Delta, herons are generally not at risk from this source, as they do not normally ingest lead pellets.

Regarding copper, shifts in concentrations among years could reflect the dynamics of the river. This metal seems to be equally available in all the habitats, and year to year variations could be explained by the use of copper sulphate in agriculture. Levels reported are far below toxic levels (Attia et al. 2011).

Eggs are good bioindicators of selenium (Ohlendorf et al. 2011) and levels are higher at riverine sites than at the Ebro Delta. Therefore, the river is probably affected by other sources of selenium rather than the polluted sediments. Although feathers are poor indicators of this pollutant (Ohlendorf and Heinz 2011), the higher levels found at Flix seems to indicate a slight effect from this site on herons.

Avian embryos are very sensitive to the toxic effects of selenium, which is reflected by reduced hatchability of fertile eggs and teratogenic development of embryos (Janz et al. 2010.). Although the threshold at which negative impacts occur in birds is widely disputed, Ohlendorf and Heinz (2011) recommended levels higher than 12 mg/kg dry weight as a concentration associated with elevated probability for reduced egg hatchability in sensitive and moderately sensitive species and levels lower than 3.0 mg/kg as a mean concentration for background conditions. Our values don't reach that threshold, but are in all cases above the range of those reported in little egret eggs and feathers in Pakistan (Boncompagni et al. 2003), feathers of black-crowned night heron (*Nycticorax nycticorax*) in USA, (Golden et al. 2003) breast feathers from several heron species from Hong Kong and Szechuan (Burger and Gochfeld 1993) and among the highest ever reported for a heron species, although a negative impact cannot be certain, as thresholds levels in feathers are not clear (Ohlendorf and Heinz 2011).

Concerning mercury, Flix presented the highest concentrations among the studied sites, meaning that the amount of mercury leaching from the toxic muds into the Ebro River ecosystem is high and continous at Flix Reservoir, as the concentrations are stable through the years even in a highly dynamic ecosystem as the Ebro River, as also shown in a study conducted with zebra mussel (Carrasco et al. 2008). The only change in this pattern was during 2008, in which some of the individuals from Flix site may have been feeding upstream, away from the main mercury input, as reflected by the lower levels in nestling feathers. Levels detected at l'Aiguabarreig are much higher than the ones expected for a 'reference' site, so this area must also be affected by other sources of mercury. Even the Ebro Delta, situated 90 km from the Flix site, shows intermediate levels that may be explained by the sediments being carried downstream. Purple heron show relatively high concentrations at this site, as other bird species foraging at the same area have already shown (Cotin et al. 2011).

The high mercury levels detected at Flix are of special concern, with purple heron nestlings having values as high as 13,600 ng/g in feathers, which is within the alerting range, as concentrations of mercury between 5,000 and 15,000 ng/g in feathers have been related to adverse effects on growth and reproduction in birds (Eisler 1987). In fact, adverse effects have already been pointed out in previous studies for this breeding population, which in part may be due to the fact that heron embryos have been reported to be highly sensitive to mercury (Heinz et al. 2009). The elevated levels of micronuclei in peripheral erythrocytes (Quiros et al. 2008) and the reduced blood antioxidant defenses (Barata et al. 2010) are some of the physiological stress responses that these birds have developed to pollution. Also, other species in this ecosystem, including zebra mussels, crayfish and several fish species, have shown effects from this pollutant (Benejam et al. 2010; Faria et al. 2010; Navarro et al. 2009; Suarez-Serrano et al. 2010).

Finally, it should be noted that mercury levels found in nestling feathers at Flix are far higher than the ones found for little egret in Pakistan (Boncompagni et al. 2003) and Hong Kong (Connell et al. 2002) or several heron species from China (Burger and Gochfeld 1993) and even higher than those reported for little egret and night heron feathers in the Axios Delta, Greece (Goutner and Furness 1997) and northern Italy (Fasola et al. 1998). Higher levels have been found in heron species in the Everglades, USA (Frederick et al. 2004), but feathers were taken from adult birds conserved in museums, which probably reflect bioaccumulation. High values of mercury in blood were found for some years in the Carson River (Nevada) for two heron species, snowy egret (*Egretta thula*) and black-crowned night heron (Henny et al. 2007), but to our knowledge, mercury levels detected in purple heron at the Flix site are the highest ever reported in nestlings feathers of a heron species.

Conclusions

- Nitrogen signatures reflect trophic level, but differences among localities are greatly influenced by baseline values, being higher in the riverine sites than in the delta. Nitrogen together with carbon signatures adequately distinguish riverine and deltaic ecosystems (high nitrification in the river and lower carbon signatures in the delta).
- Eggs and nestling feathers of purple herons have demonstrated their usefulness as bioindicators for trace element pollution in the river biota. Nestling feathers, which are grown from dietary items provided by parents in the surroundings of the colony, show pollutant impacts on a local basis. Moreover, since chicks belong to a homogeneous age-class, the effects of age-related bioaccumulation on pollutant levels are expected to be negligible.

- Eggs and nestling feathers of purple herons have demonstrated their usefulness as bioindicators for trace element pollution in the river biota. Nestling feathers, which are grown from dietary items provided by parents in the surroundings of the colony, show pollutant impacts on a local basis. Moreover, since chicks belong to a homogeneous age-class, the effects of age-related bioaccumulation on pollutant levels are expected to be negligible.

- Mercury is highly influenced by the polluted sediments at Flix reservoir, while selenium and especially copper concentrations probably derive from other sources.
- Although most of the trace element content of purple heron samples are below alerting values, the high mercury levels from the Flix site pose potential risks for these birds and are among the highest ever reported.
- From our study it is apparent that in highly dynamic ecosystems as the Ebro River and its Delta, a series of study years is necessary to properly understand pollutant spatio-temporal changes, so a long-term monitoring programme of the purple heron population at Flix is recommended to advise the effect of the toxic muds, particularly during and after their removal which could result in a increased bioavailability of mercury for river biota.

Acknowledgments We dedicate this work to the memory of Xavier Ruiz, who passed away on 27th April 2008. The authors wish to acknowledge the kindly cooperation of the personnel at the Reserva Natural de Sebes (Flix), Parc Natural del Delta de l'Ebre and Estació Biològica de l'Aiguabarreig for the facilities during fieldwork. Thanks are given to Sergi Ferrer and the staff at the *Serveis Científicotècnics* (UB, Spain) for their help during the sample collection and laboratory analysis and to Gretchen Wagner for her suggestions and corrections of the English draft. Javier Cotín was supported by a BRD grant from the *Universitat de Barcelona* (Spain). This work was funded by the project 'Metalls pesants i organoclorats en el curs inferior i delta del riu Ebre: La ornitofauna com a bioindicadora.' (Ministry of the Environment, Spain, the Catalan Water Agency, Department of the Environment and Housing, Catalan Government) and by Spanish MEyC grant project CGL2008-05448-C02-01/02/BOS.

References

Abdennadher A, Ramirez F, Romdhane MS, Ruiz X, Jover L, Sanpera C (2011) Little egret (*Egretta garzetta*) as a bioindicator of trace element pollution in Tunisian aquatic ecosystems. Environ Monit Assess 1–4:677–684

Attia YA, Abdalah AA, Zeweil HS, Bovera F, El-Din AAT, Araft MA (2011) Effect of inorganic or organic copper additions on reproductive performance, lipid metabolism and morphology of organs of dual-purpose breeding hens. Arch Geflugelkd 3:169–178

Baker SD, Sepulveda MS (2009) An evaluation of the effects of persistent environmental contaminants on the reproductive success of great blue herons (*Ardea herodias*) in Indiana. Ecotoxicology 3:271–280

Baos R, Jovani R, Pastor N, Tella J, Jimenez B, Gomez G (2006) Evaluation of genotoxic effects of heavy metals and arsenic in wild nestling white storks (*Ciconia ciconia*) and black kites (*Milvus migrans*) from southwestern Spain after a mining accident. Environ Toxicol Chem 10:2794–2803

Barata C, Fabregat MC, Cotin J, Huertas D, Sole M, Quiros L, Sanpera C, Jover L, Ruiz X, Grimalt JO, Piña B (2010) Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain). Environ Pollut 3:704–710

Benejam L, Benito J, Garcia-Berthou E (2010) Decreases in condition and fecundity of freshwater fishes in a highly polluted reservoir. Water Air Soil Pollut 1–4:231–242

Benito V, Devesa V, Munoz O, Suner M, Montoro R, Baos R, Hiraldob F, Ferrer M, Fernandez M, Gonzalez MJ (1999) Trace elements in blood collected from birds feeding in the area around Donana National Park affected by the toxic spill from the Aznalcollar mine. Sci Total Environ 1–3:309–323

Boncompagni E, Muhammad A, Jabeen R, Orvini E, Gandini C, Sanpera C, Ruix X, Fasola M (2003) Egrets as monitors of trace-metal contamination in wetlands of Pakistan. Arch Environ Contam Toxicol 3:399–406

Bosch C, Olivares A, Faria M, Navas JM, del Olmo I, Grimalt JO, Pina B, Barata C (2009) Identification of water soluble and particle bound compounds causing sublethal toxic effects. A field study on sediments affected by a chlor-alkali industry. Aquat Toxicol 1:16–27

Burger J, Gochfeld M (1993) Heavy-metal and selenium levels in feathers of young egrets and herons from Hong-Kong and Szechuan, China. Arch Environ Contam Toxicol 3:322–327

Burger J, Gochfeld M (2000) Effects of lead on birds (Laridae): a review of laboratory and field studies. J Toxicol Environ Health 2:59–78

Burger J, Gochfeld M (2009) Comparison of arsenic, cadmium, chromium, lead, manganese, mercury and selenium in feathers in bald eagle (*Haliaeetus leucocephalus*), and comparison with common eider (*Somateria mollissima*), glaucous-winged gull (*Larus glaucescens*), pigeon guillemot (*Cepphus columba*), and tufted puffin (*Fratercula cirrhata*) from the Aleutian Chain of Alaska. Environ Monit Assess 1–4:357–367

Burger J, Nisbet ICT, Gochfeld M (1992) Metal levels in regrown feathers—assessment of contamination on the wintering and breeding grounds in the same individuals. J Toxicol Environ Health 3:363–374

Burger J, Gochfeld M, Jeitner C, Burke S, Volz CD, Snigaroff R, Snigaroff D, Shukla T, Shukla S (2009) Mercury and other metals in eggs and feathers of glaucous-winged gulls (*Larus glaucescens*) in the Aleutians. Environ Monit Assess 1–4: 179–194

Cabana G, Rasmussen J (1994) Modeling food-chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 6503:255–257

Carrasco L, Diez S, Soto D, Catalan J, Bayona J (2008) Assessment of mercury and methylmercury pollution with zebra mussel (*Dreissena polymorpha*) in the Ebro River (NE Spain) impacted by industrial hazardous dumps. Sci Total Environ 1:178–184

Carrasco L, Benejam L, Benito J, Bayona JM, Diez S (2011) Methylmercury levels and bioaccumulation in the aquatic food web of a highly mercury-contaminated reservoir. Environ Int 7:1213–1218

Champoux L, Rodrigue J, Trudeau S, Boily MH, Spear PA, Hontela A (2006) Contamination and biomarkers in the great blue heron, an indicator of the state of the St. Lawrence River. Ecotoxicology 1:83–96

Cherel Y, Le Corre M, Jaquemet S, Menard F, Richard P, Weimerskirch H (2008) Resource partitioning within a tropical seabird community: new information from stable isotopes. Mar Ecol Prog Ser 366:281–291

Connell D, Wong B, Lam P, Poon K, Lam M, Wu R (2002) Risk to breeding success of Ardeids by contaminants in Hong Kong: evidence from trace metals in feathers. Ecotoxicology 1:49–59

Connell D, Fung C, Minh T, Tanabe S, Lam P, Wong B (2003) Risk to breeding success of fish-eating Ardeids due to persistent organic contaminants in Hong Kong: evidence from organochlorine compounds in eggs. Water Res 2:459–467

Cotin J, García-Tarrasón M, Sanpera C, Jover L, Ruiz X (2011) Sea, freshwater or saltpans? Foraging ecology of terns to assess mercury inputs in a wetland landscape: the Ebro Delta. Estuar Coast Shelf Sci 1:188–194

De Luca-Abbott S, Wong B, Peakall D, Lam P, Young L, Lam M (2001) Review of effects of water pollution on the breeding success of waterbirds, with particular reference to Ardeids in Hong Kong. Ecotoxicology 6:327–349

DesGranges J, Rodrigue J, Champoux L (2009) Geographic distribution of selected contaminants in great blue herons from the St. Lawrence River system, Quebec (1989–1994). Can Wildl Serv Occas Paper 116:1–18

Eisler R (1987) Mercury hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife Service Biological Report 85(1.10)

Faria M, Huertas D, Soto D, Grimalt J, Catalan J, Riva M (2010) Contaminant accumulation and multi-biomarker responses in field collected zebra mussels (*Dreissena polymorpha*) and crayfish (*Procambarus clarkii*), to evaluate toxicological effects of industrial hazardous dumps in the Ebro river (NE Spain). Chemosphere 3:232–240

Fasola M, Movalli PA, Gandini C (1998) Heavy metal, organochlorine pesticide, and PCB residues in eggs and feathers of herons breeding in northern Italy. Arch Environ Contam Toxicol 1:87–93

Fernandez M, Alonso C, Gonzalez M, Hernandez L (1999) Occurrence of organochlorine insecticides, PCBs and PCB congeners in waters and sediments of the Ebro River (Spain). Chemosphere 1:33–43

Focardi S, Fossi C, Lambertini M, Leonzio C, Massi A (1988) Long-term monitoring of pollutants in eggs of yellow-legged herring gull from Capraia Island (Tuscan Archipelago). Environ Monit Assess 1:43–50

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1:497–509

Forero MG, Hobson KA (2003) Using stable isotopes of nitrogen and carbon to study seabird ecology: applications in the Mediterranean seabird community. Scientia Marina 67:23–32

Forero MG, Gonzalez-Solis J, Hobson KA, Doncazar JA, Bertellotti M, Blanco G, Bortolotti GR (2005) Stable isotopes reveal trophic segregation by sex and age in the southern giant petrel in two different food webs. Mar Ecol Prog Ser 296:107–113

Frederick PC, Hylton B, Heath JA, Spalding MG (2004) A historical record of mercury contamination in southern Florida (USA) as inferred from avian feather tissue. Environ Toxicol Chem 6:1474–1478

Golden NH, Rattner BA, McGowan PC, Parsons KC, Ottinger MA (2003) Concentrations of metals in feathers and blood of nestling black-crowned night-herons (*Nycticorax nycticorax*) in Chesapeake and Delaware Bays. Bull Environ Contam Toxicol 2:385–393

Gomez G, Baos R, Gomara B, Jimenez B, Benito V, Montoro R (2004) Influence of a mine tailing accident near Donana National Park (Spain) on heavy metals and arsenic accumulation in 14 species of waterfowl (1998 to 2000). Arch Environ Contam Toxicol 4:521–529

Goutner V, Furness RW (1997) Mercury in feathers of little egret *Egretta garzetta* and night heron *Nycticorax nycticorax* chicks and in their prey in the Axios Delta, Greece. Arch Environ Contam Toxicol 2:211–216

Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR, Kondrad SL, Erwin CA (2009) Species differences in the sensitivity of avian embryos to methylmercury. Arch Environ Contam Toxicol 1:129–138

Henny CJ, Hill EF, Grove RA, Kaiser JL (2007) Mercury and drought along the lower Carson River, Nevada: I. Snowy egret and black-crowned night-heron annual exposure to mercury, 1997–2006. Arch Environ Contam Toxicol 2:269–280

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 3:314–326

Hobson KA, Piatt JF, Pitocchelli J (1994) Using stable isotopes to determine seabird trophic relationships. J Anim Ecol 4:786–798

Hobson KA, Sirois J, Gloutney ML (2000) Tracing nutrient allocation to reproduction with stable isotopes: a preliminary investigation using colonial waterbirds of Great Slave Lake. Auk 3:760–774

Hughes K, Ewins P, Clark K (1997) A comparison of mercury levels in feathers and eggs of osprey (*Pandion haliaetus*) in the North American Great Lakes. Arch Environ Contam Toxicol 4: 441–452

Janz D, DeForest D, Brooks M, Chapman P, Gilron G, Hoff D, Hopkins W, McIntyre D, Mebane C, Palace V, Skorupa J, Wayland M (2010) Selenium toxicity to aquatic organisms. In: Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP (eds) Ecological assessment of selenium in the aquatic environment. SETAC, Pensacola, pp 141–231

Koiadinovic J, Menard F, Bustamante P, Cosscon RP, Le Corre M (2008) Trophic ecology of marine birds and pelagic fishes from Reunion Island as determined by stable isotope analysis. Mar Ecol Prog Ser 361:239–251

Llorente G, Farran A, Ruiz X, Albaiges J (1987) Accumulation and distribution of hydrocarbons, polychlorobyphenyls, and DDT in tissues of 3 species of Anatidae from the Ebro Delta (Spain). Arch Environ Contam Toxicol 5:563–572

Mañosa S, Mateo R, Guitart R (2001) A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. Environ Monit Assess 2:187–205

Michener RH, Schell DM (1994) Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha K, Michener RH (eds) Stable isotopes in ecology and environmental science. Blackwell Scientific Publications, London, pp 138–157

Moreno R, Jover L, Munilla I, Velando A, Sanpera C (2010) A three-isotope approach to disentangling the diet of a generalist consumer: the yellow-legged gull in northwest Spain. Mar Biol 3:545–553

Moreno R, Jover L, Diez C, Sanpera C (2011) Seabird feathers as monitors of the levels and persistence of heavy metal pollution after the Prestige oil spill. Environ Pollut 10:2454–2460

Navarro A, Quiros L, Casado M, Faria M, Carrasco L, Benejam L (2009) Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. Aquat Toxicol 2–3:150–157

Navarro A, Tauler R, Lacorte S, Barcelo D (2010) Occurrence and transport of pesticides and alkylphenols in water samples along the Ebro River Basin. J Hydrol 1–2:18–29

Nisbet ICT, Montoya JP, Burger J, Hatch JJ (2002) Use of stable isotopes to investigate individual differences in diets and mercury exposures among common terns *Sterna hirundo* in breeding and wintering grounds. Mar Ecol Prog Ser 242:267–274

Ohlendorf HM, Heinz GH (2011) Selenium in birds. In: Beyer WN, Meador JP (eds) Environmental contaminants in biota: interpreting tissue concentrations, 2nd edn. CRC Press/Taylor and Francis Group, Boca Raton, pp 669–701

Ohlendorf HM, Covington SM, Byron ER, Arenal CA (2011) Conducting site-specific assessments of selenium bioaccumulation in aquatic systems. Integr Environ Assess Manag 7(3):314–324

Padula V, Burger J, Newman S, Elbin S, Jeitner C (2010) Metals in feathers of black-crowned night-heron (*Nycticorax nycticorax*) chicks from the New York Harbor Estuary. Arch Environ Contam Toxicol 1:157–165

Pastor D, Sanpera C, Gonzalez-Solis J, Ruiz X, Albaiges J (2004) Factors affecting the organochlorine pollutant load in biota of a rice field ecosystem (Ebro Delta, NE Spain). Chemosphere 4:567–576

Quiros L, Ruiz X, Sanpera C, Jover L, Pina B (2008) Analysis of micronucleated erythrocytes in heron nestlings from reference and impacted sites in the Ebro basin (NE Spain). Environ Pollut 1:81–87

Ramirez F, Abdennadher A, Sanpera C, Jover L, Hobson KA, Wassenaar LI (2011) Tracing waterbird exposure to total mercury and selenium: a case study at the solar saltworks of Thyna (Sfax, Tunisia). Environ Sci Technol 12:5118–5124

Ramos L, Fernandez M, Gonzalez M, Hernandez L (1999) Heavy metal pollution in water, sediments, and earthworms from the Ebro River, Spain. Bull Environ Contam Toxicol 3:305–311

Ruiz X, Jover L, Pedrocchi V (1998) Audouin's gull intraspecific variability in yolk and albumen egg contents: why is yolk size so constant? Biologia e Conservazione della Fauna 102:327–334

Sakellarides T, Konstantinou I, Hela D, Lambropoulou D, Dimou A, Albanis T (2006) Accumulation profiles of persistent organochlorines in liver and fat tissues of various waterbird species from Greece. Chemosphere 8:1392–1409

Sanpera C, Ruiz X, Moreno R, Jover L, Waldron S (2007) Mercury and stable isotopes in feathers of Audouin's gulls as indicators of feeding habits and migratory connectivity. Condor 2:268–275

Sanpera C, Valladares S, Moreno R, Ruiz X, Jover L (2008) Assessing the effects of the Prestige oil spill on the European shag (*Phalacrocorax aristotelis*): trace elements and stable isotopes. Sci Total Environ 1:242–249

Sell JL (1977) Comparative effects of selenium on metabolism of methylmercury by chickens and quail—tissue distribution and transfer into eggs. Poult Sci 3:939–948

Suarez-Serrano A, Alcaraz C, Ibanez C, Trobajo R, Barata C (2010) *Procambarus clarkii* as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. Ecotoxicol Environ Saf 3:280–286

Taggart M, Figuerola J, Green A, Mateo R, Deacon C, Osborn D (2006) After the Aznalcollar mine spill: arsenic, zinc, selenium, lead and copper levels in the livers and bones of five waterfowl species. Environ Res 3:349–361

Tavares PC, Kelly A, Lopes RJ, Pereira ME, Duarte AC, Furness RW (2007) The influence of dietary specialization and trophic status on mercury levels in two species using common coastal wetlands, *Himantopus himantopus* and *Sterna albifrons*. Ardeola 2:275–288

Vericat D, Batalla RJ (2006) Sediment transport in a large impounded river: the lower Ebro, NE Iberian Peninsula. Geomorphology 1–2:72–92

		Sites														
		l'Aiguabarreig					Flix					Ebro Delta				
	Year	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max
[Hg]	2006	7/7	1580.7	637.4	902.7	2684.3	10/10	7819.5	2995.3	2980.8	13616.7	4/4	2390.8	427.1	2057.5	2854.4
	2007	7/7	1638.4	543.0	863.9	2345.2	11/11	6911.5	4447.9	2718.5	10313.2	16/16	1381.6	705.3	748.5	3387.0
	2008	4/4	1512.8	151.9	1291.9	1575.7	9/9	2986.4	1084.1	2407.6	9435.4	12/12	1792.3	449.9	1098.8	2595.1
[Se]	2006	7/7	1795.9	449.6	1502.9	2481.4	10/10	2578.0	818.6	2054.8	3370.6	3/4	1709.1	347.7	1399.9	1747.6
	2007	6/7	1853.0	434.8	1650.5	2286.2	11/11	2284.6	742.8	1745.8	3534.0	13/16	1516.4	154.5	1090.2	2130.3
	2008	4/4	2202.3	160.1	2126.9	2333.4	9/9	1924.7	888.4	1643.6	3961.2	12/12	1709.4	747.8	1255.5	2200.7
[Cu]	2006	7/7	8831.0	1979.0	6979.4	9986.9	10/10	8058.6	1018.6	6510.0	10224.5	4/4	11279.8	1911.6	10188.8	12552.4
	2007	7/7	6001.1	2796.9	4113.9	7696.1	11/11	6085.2	1461.5	4431.9	7467.1	16/16	6015.8	1099.4	4073.8	7152.5
	2008	4/4	5936.2	538.0	5521.6	6324.1	9/9	5301.1	1478.4	4521.0	9712.1	12/12	7876.5	2355.9	4863.0	23391.0
[Pb]	2006	7/7	1312.9	829.0	951.7	2073.2	10/10	1692.1	367.5	1121.4	2168.4	4/4	2023.0	536.5	1603.0	2631.0
	2007	0/7					1/11	116.3	0.0	116.3	116.3	0/16				
	2008	1/4	59.9	0.0	59.9	59.9	5/9	41.2	112.6	34.6	191.1	9/12	74.3	62.6	54.0	337.1
[Cr]	2007	7/7	1622.2	224.5	1471.1	1738.8	11/11	1759.0	398.0	1508.2	2045.8	16/16	1601.7	167.6	1203.7	1792.0
	2008	4/4	1946.3	101.2	1891.9	2064.3	9/9	2087.8	183.1	1859.0	2259.8	12/12	2102.0	172.0	1781.3	2389.9
[As]	2007	2/7	111.3	19.6	101.5	121.1	3/11	122.3	13.7	119.3	132.9	14/16	159.2	34.4	125.2	268.0
	2008	4/4	68.5	26.9	55.5	92.6	9/9	60.6	37.5	48.8	117.5	12/12	156.2	76.9	77.3	215.3

Table X1. Descriptive statistics of trace elements expressed in ng/g in feathers of Purple Heron nestlings (*Ardea purpurea*) from the three sites and years. Only samples with detected values are given. For mercury and copper this represents 100% of samples.

		Sites														
		l'Aiguabarreig					Flix					Ebro Delta				
	Year	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max
[Hg]	2006						12/12	825.3	778.3	481.7	3467.8	14/14	645.9	216.7	297.8	1124.1
	2007	12/12	115.5	66.8	93.2	827.1	16/16	663.1	827.0	92.8	1727.5	25/25	302.0	323.7	91.0	626.5
[Se]	2006			0.0			12/12	3933.2	920.6	2317.5	5523.6	14/14	3143.7	1081.5	1599.1	3939.6
	2007	12/12	3545.9	1019.6	2594.6	4424.0	16/16	3477.1	621.3	2110.3	4181.4	25/25	2561.8	818.5	1471.4	3655.1
[Cu]	2006			0.0			12/12	4950.5	1424.6	4431.1	6607.1	14/14	4704.8	595.8	3735.6	6208.4
	2007	12/12	3677.6	808.0	2976.5	5909.0	16/16	4031.6	937.7	3030.6	5177.6	25/25	4351.8	1003.2	3350.4	5861.6
[Cr]	2007	12/12	2500.8	164.9	2406.1	2784.8	16/16	2527.9	233.9	1478.5	2726.8	25/25	2570.0	181.5	2314.5	3026.9
[As]	2007	10/12	127.1	26.5	111.8	173.7	11/16	140.1	29.3	123.0	159.2	25/25	167.9	52.8	100.2	303.0

Table X2. Descriptive statistics of trace elements expressed in ng/g in eggs of Purple Heron (*Ardea purpurea*) from the three sites and years. Only samples with detected values are given. For mercury and copper this represents 100% of samples

II

Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain)

Biomarcadores sanguíneos y niveles de contaminantes en plumas y huevos para evaluar riesgos ambientales en volantones de ardeidas en zonas afectadas de la cuenca del Ebro (NE España)

Carlos Barata[a], María del Carme Fabregat[a], Javier Cotín[b], David Huertas[b],
Montserrat Solé[c], Laia Quirós[a], Carolina Sanpera[b], Lluis Jover[b], Xavier Ruiz[b],
Joan O Grimalt[a] & Benjamín Piña[a].

[a] Department of Environmental Chemistry, IDAA-CSIC,
Jordi Girona 18, 08034 Barcelona, Spain

[b] Department de Biología Animal, Universitat de Barcelona,
Diagonal 645, 08028 Barcelona, Spain

[c] Institut de Ciencies del Mar (ICM-CSIC),Pg. Marítim de la
Barceloneta 37-49, 08003 Barcelona, Spain

Resumen: Los biomarcadores en sangre y los niveles de contaminantes en huevos y plumas fueron usados para determinar los efectos de la contaminación en volantones de Garza Imperial (*Ardea purpurea*) y Garzeta común (*Egretta garzetta*), muestreadas en tres zonas del Río Ebro (NE España): una localidad de referencia, una localidad afectada por los residuos de una industria petroquímica y el Delta del Ebro. Las dos poblaciones de garzas afectadas mostraron diferentes patrones de contaminantes y respuesta a ellos, sugiriendo diferentes fuentes de contaminación. En la población nidificante cercana a la industria petroquímica, los elevados niveles de hexaclorobenzenos (HCB) y policlorobifenilos (PCBs) en huevos y de mercurio en plumas de volantones de *A. purpurea* se relacionaron con una reducción en la capacidad antioxidante de la sangre y un incremento en los niveles de micronúcleos. En el Delta del Ebro, los altos niveles plasmáticos de lactato deshidrogenasa en pollos de *A. purpurea* y la alta frecuencia de micronúcleos en sangre de ambas especies fue asociado con las intensas actividades agrícolas que tienen lugar en ése área. Estos resultados proporcionan la primera evidencia de una respuesta biológica en pollos de ardeidos a los residuos de una industria petroquímica.

Palabras clave: Capacidad Total para Captar Radicales Libres, Esterasas, Lactato deshidrogenasa, Estrés oxidativo, Compuestos Organoclorados, Mercurio, Ardeidos.

Environmental Pollution 158 (2010) 704–710

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain)

C. Barata [a,*], M.C. Fabregat [a], J. Cotín [b], D. Huertas [b], M. Solé [c], L. Quirós [a], C. Sanpera [b], L. Jover [b], X. Ruiz [b], J.O. Grimalt [a], B. Piña [a]

[a] *Department of Environmental Chemistry, IDÆA-CSIC, Jordi Girona 18, 08034 Barcelona, Spain*
[b] *Department de Biología Animal, Universitat de Barcelona, Avgda Diagonal 645, 08028 Barcelona, Spain*
[c] *Institut de Ciències del Mar (ICM-CSIC), Pg. Marítim de la Barceloneta 37–49, 08003 Barcelona, Spain*

High levels of organochlorine and mercury levels in eggs and feathers were related with altered blood biomarkers of heron nesting chicks.

ARTICLE INFO

Article history:
Received 29 May 2009
Received in revised form
7 September 2009
Accepted 13 October 2009

Keywords:
Total oxyradical scavenging capacity
Esterases
Lactate dehydrogenase oxidative stress
Organochlorine compounds
Mercury
Ardeida

ABSTRACT

Blood biomarkers and levels of major pollutants in eggs and feathers were used to determine pollution effects in nestlings of the Purple Heron *Ardea purpurea* and the Little Egret *Egretta garzetta*, sampled on three Ebro River (NE Spain) areas: a reference site, a site affected by the effluents of a chlor-alkali industry and the river Delta. The two impacted heron populations showed mutually different pollutant and response patterns, suggesting different sources of contamination. In the population nesting near the chlor-alkali plant, elevated levels of hexachlorobenzene (HCB) and polychlorobiphenyls (PCBs) in eggs, and mercury in feathers in *A. purpurea* chicks were related with reduced blood antioxidant defenses and increased levels of micronuclei. In Ebro Delta, high levels of plasmatic lactate dehydrogenase in *A. purpurea* chicks and high frequency of micronuclei in blood of both species were tentatively associated with intensive agricultural activities taking place in the area. These results provide the first evidence of a biological response in heron chicks to the release of pollutants at a chlor-alkali plant.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Piscivorous birds such as herons and egrets (family Ardeidae) are suitable bio-indicators of environmental pollution in aquatic systems (Furness and Camphuysen, 1997; De Luca-Abbott et al., 2001; Connell et al., 2003; Champoux et al., 2006). They are top consumers in the trophic chain of aquatic ecosystems, and can potentially accumulate persistent organic pollutants and metals (Mañosa et al., 2001). The combined use of biomarkers and chemical analyses in the last decade has allowed assessing and identifying adverse health effects of endocrine disrupting persistent organic pollutants, pesticides and metals on several waterbirds (Fossi et al., 1994; Cordi et al., 1997; Muir et al., 1999; Vos et al., 2000; Strum et al., 2008). More recently the use of non-lethal biological techniques such as the analyses of biomarkers in blood, and contaminants in feathers and eggs have been applied when studying endangered species (Muir et al., 1999; Vos et al., 2000; Champoux et al., 2002, 2006; Franson et al., 2002; Murvoll et al., 2006; Quirós et al., 2008).

Antioxidant defenses represent the detoxification pathway of reactive oxygen species (ROS) in aerobic organisms (Livingstone, 2001). When ROS exceed antioxidant defenses, oxidative damage of lipids within cell membranes, of DNA molecules and proteins may occur leading to a pathological condition, generally termed oxidative stress (Livingstone, 2001). In this way, knowledge about the responses of organisms to oxidative stress may provide valuable information about their health status. Recently, some authors (Winston et al., 1998; Regoli, 2000), developed an analytical method to evaluate the total oxyradical scavenging capacity (TOSC) of any given tissue, defined as the capacity of the biological sample to neutralize ROS. For a given tissue lower TOSC values are likely to be related to lower capacity to detoxify ROS and hence a greater susceptibility to oxidative stress. The method was originally developed for rat liver (Winston et al., 1998), but in recent times it has been applied to the blood of birds in environmental surveillance programs (Corsolini et al., 2001). Plasmatic enzymes, such as the activity of lactate deshydrogenase and of B esterases such as butyrylcholinesterase and carboxylesterases may also provide

---

* Corresponding author. Tel.: +34 9 3400 6100.
E-mail address: cbmqam@cid.csic.es (C. Barata).

0269-7491/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2009.10.018

valuable information on liver damage and the neurotoxic effects of pesticides in birds, respectively (Champoux et al., 2006; Fossi et al., 1994; Cordi et al., 1997; Strum et al., 2008). The presence of micronuclei in peripheral erythrocytes has also been used to evaluate the environmental impact by genotoxicants in different species, including birds (Kim et al., 2003; Stoncius and Lazutka, 2003; Quirós et al., 2008). Micronucleated cells originate when mitotic cells with chromatid breaks or dysfunctional mitotic apparatus undergo abnormal anaphase distribution of chromatin. The resulting micronuclei can be quantified either by microscopy or by flow cytometry analysis. The latter methodology represents a major advance in terms of sensitivity, reliability and throughput. Indeed in a recent study conducted in the Ebro river (NE Spain), the presence of micronuclei measured by flow cytometry analysis in peripheral erythrocytes in heron nestlings evidenced higher micronuclei counts in samples from sites heavily contaminated with metals, organochlorine residues and pesticides (Quirós et al., 2008).

The Ebro catchment is the largest river basin in Spain, covers an area of 85,362 km^2 and receives the potential influence of 3 million people, including some heavily industrialized areas. One of these areas is the Flix site, where an organochlorine industry operates since the beginning of the 20th century. This long operational period, along with the construction of a dam next to the factory around 1960, resulted in the accumulation of high amounts of heavy polluted sediments in the adjacent riverbed (Fernández et al., 1999). The surroundings of the Flix dam have become a valuable wetland, with profusion of nesting birds and small mammals. Pollutants originated at Flix site are carried downstream by the Ebro river to its Delta 90 km away, an UNESCO wildlife and bird reserve (Pastor et al., 2004). Ebro Delta land is also affected by an intensive agricultural activity, with significant impact on wildlife (Mañosa et al., 2001). Therefore both Flix and the Ebro Delta could be considered impacted sites. Aiguabarreig, a marsh site and bird sanctuary located upstream of Flix represents an appropriate reference site due to its high ecological value (Quirós et al., 2008).

The aim of this study is to test the usefulness of using a set of non-destructive markers to monitor environmental hazards of organochlorine and metallic persistent pollutants in nestlings of two engaged piscivorous bird species (*Ardea purpurea* and *Egretta garzetta*) along the Ebro river (NE Spain). By combining and comparing the response of blood biomarkers with levels of selected pollutants in eggs and feathers it was intended to identify potential contaminants casing detrimental effects on the studied bird species. Nestlings instead of adults were used to minimizing the effect of bioaccumulation because the time of exposure is known and similar for all samples. Furthermore, since all chicks are provisioned with prey caught by parents in the surroundings of the colony, the results should reflect only the impact of pollutants ingested at that site. Provided that herons and egrets and persistent organic and metallic pollutants are distributed world wide, the methods and results obtained in this study may apply or be used to assess the effects of pollution in other areas of concern.

2. Materials and methods

2.1. Sampling and tissue preparation

A total of 37 eggs and feathers and blood samples of 59 chicks from two species were sampled and analyzed from three locations in the eastern portion of the Ebro basin, Aiguabarreig, Flix and Ebro Delta (Fig. 1, Table 1). Egrets do not nest in Flix, neither in surrounded areas, thus the study was restricted to Aiguabarreig and Ebro Delta site. Sampling was conducted in spring 2006 at two time periods. First, one egg per nest was collected at the end of the laying period. Later, chicks were sampled 1 week before the estimated peak fledgling time. During sampling, unusual adverse climatic and river flow conditions limited the number of eggs and chicks sampled in the same locations and species. To avoid pseudoreplication, only one egg and one chick per nest were sampled. Chicks were measured with a digital caliper (0.1 mm) for tarsus length and weighed with a Pesola spring balance to the nearest gram. In all chicks two feathers from the back were obtained for metal analyses. In 45 chicks 1 ml of blood was extracted using a heparinized syringe by punction of the tarsal vein and transferred to heparinized vials and kept refrigerated at 4 °C. In the lab blood samples were split into two 0.5 ml aliquots. One was preserved cold and immediately used for micronuclei determination by flow cytometry as described in Quirós et al. (2008). The remaining was centrifuged at 1500 rpm for 2 min and the plasma gently removed, frozen with liquid N$_2$ and stored at −80 °C until biomarker determination. Entire eggs (37) were labelled and kept refrigerated in individual glass containers packed with cotton to avoid breakage until arriving at laboratory. Eggs were kept frozen (−20 °C) until organochlorine residue analysis.

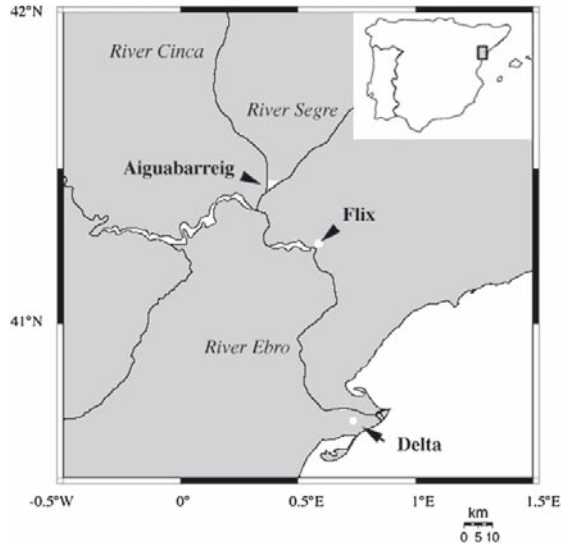


Fig. 1. Map of sampling sites in NE Spain. The inset shows the approximate position of the sampling area in the Iberian Peninsula.

2.2. Contaminant analyses

2.2.1. Metal analysis

For metals analysis, feathers were washed with a 0.1 molar NaOH solution and dried out at 50 °C. Levels of Cu, Hg and Pb were determined in acid digested samples by Perkin Elmer model Elan 6000 inductively coupled plasma mass spectrometer (ICP-MS) following Barata et al. (2005). Samples ($n = 3$) of similar weight of a certified reference material of Human Hair (CRM 397, Commission of the European Community) for Hg and Pb and lobster hepatopancreas (Tort 2, National Research Council, Canada) for Cu were digested during each analytical run. Mean ± SD recoveries ($n = 6$) were 98.5 ± 2.1, 99.1 ± 4.0 and 100 ± 4.8 for total Hg, Cu and Pb, respectively, therefore no corrections were done. Detection and quantification (LOD/LOQ) limits were calculated from blank measurements ($n = 10$) being these values (in ng/g): 0.01/0.02, 0.07/0.19 and 0.19/0.29 for Hg, Pb and Cu, respectively.

2.2.2. Organochlorine compound analysis

Organochlorine compounds were extracted and analyzed following minor modifications of the procedures of Catalan et al. (2004). The egg content (1.5 g) of individual eggs was separated from the shell, weighed, ground down with activated sodium sulphate to obtain a fine powder and then introduced into previously cleaned cellulose cartridge. This mixture was Soxhlet-extracted during 24 h with *n*-hexane-dichloromethane (4:1 v/v) adding 1,2,4,5-tetrabromobenzene (TBB) and PCB 200 as recovery standards. Extracts were concentrated under vacuum and cleaned-up with sulphuric acid until a colourless transparent *n*-hexane layer was obtained. Extract solutions were then transferred to vials and evaporated just to dryness under a gentle stream of nitrogen (10–20 °C). The cleaned extract was re-dissolved in 50 µl of isooctane for instrumental analysis.

Hexachlorobenzene (HCB), α-, β- and γ-hexachlorocyclohexanes (HCHs), the congeners of PCB nos. 28, 52, 101, 118, 153 and 180, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDT and *p,p′*-DDT were analyzed in a Hewlett-Packard gas chromatograph Model HP-5890 equipped with an electron-capture detector and an HP-7673-A autosampler. Separation was achieved with a 60 m × 0.25 mm I.D. DB-5 capillary column (J&W Scientific, Folsom, CA) coated with 5% phenyl methyl

Table 1
Sample size (*n*) and descriptive statistics for chick morphometric measurements and levels of heavy metals and organochlorine residues by species and sampling points. Within each species, different letters indicate significant ($P < 0.05$) differences following Student's *t*-test or ANOVA and post hoc Tukey's tests for *A. purpurea* or *A. garzetta data*, respectively. Abbreviations are explained in the text. Differences in sample size are due to missing values.

Sampling	*n*	Weight[a]	Tarsus[b]	Hg[c]	Pb[c]	Cu[c]	*n*	HCB[d]	DDTs[d]	HCHs[d]	PCBs[d]
A. purpurea chicks and feathers							*A. purpurea* eggs				
Aiguabarreig	7	659.2 ± 89.9a	83.9 ± 7.1a	1.6 ± 0.2a	1.4 ± 0.2a	8.7 ± 0.4a	10	4.1 ± 1.2a	223.7 ± 55.2a	8.2 ± 2.1b	262.2 ± 45.3a
Flix	10	646.5 ± 65.8a	88.4 ± 5.8a	8.16 ± 1.1b	1.7 ± 0.1ab	8.1 ± 0.3a	5	18.2 ± 10.4b	684.4 ± 144.3b	1.3 ± 0.4a	878.8 ± 86.5b
Delta	4	792.5 ± 144.1a	113.2 ± 13.7a	2.4 ± 0.2a	2.1 ± 0.2b	11.3 ± 0.6b	14	5.5 ± 2.1a	477.3 ± 98.8b	3.1 ± 0.8a	205.3 ± 38.3a
E. garzetta chicks and feathers							*E. garzetta* eggs				
Aiguabarreig	16	318 ± 18.2A	67.3 ± 2.7A	1.6 ± 0.3A	0.7 ± 0.2A	10.7 ± 1.1A	4	3.4 ± 1.2A	556.5 ± 111.2A	1 ± 0.2A	426.1 ± 145.3A
Delta	22	328.6 ± 10A	69.1 ± 1.8A	1.7 ± 0.1A	0.9 ± 0.1A	12.3 ± 1A	4	11.8 ± 2.2B	550.2 ± 119.7A	6 ± 4.2A	532.4 ± 200.3A

[a] Grams, average ± standard error.
[b] Millimeters, average ± standard error.
[c] Metal levels in feathers, μg/g dry weight, average ± standard error.
[d] Organochlorine residues in egg, ng/g wet weight, average ± standard error.

polysiloxane (film thickness 0.25 mm). The oven temperature was programmed from 90 °C (holding time 2 min) to 130 °C at 15 °C/min and finally to 290 °C at 4 °C/min, keeping the final temperature for 10 min. Injector and detector temperatures were 280 and 320 °C, respectively. Injection was performed in the splitless mode, keeping the split valve closed for 35 s. Helium was the carrier gas (1.5 ml/min) and nitrogen was used as the make-up gas for the detector (60 ml/min). For quantification a solution of PCB 142 was added to the vial prior to injection in order to correct for instrumental variability. Calibration curves were performed for each compound. Data were corrected for surrogate recoveries. The mean recoveries for TBB and PCB 200 were 63 ± 12 and 115 ± 15%, respectively. The quantification of blanks ($n = 10$) within each batch of analysed samples was used in order to calculate the limits of detection (LOD) of the compounds, these ranged between 0.02 ng/g for HCB, 0.04–0.09 ng/g for PCBs, 0.06–0.33 ng/g for HCHs and 0.09–0.84 ng/g for DDTs.

2.3. Biochemical determinations

TOSC was determined using gas chromatography (Winston et al., 1998; Corsolini et al., 2001). Two radicals were measured: peroxyl radicals (ROO• TOSC) were generated by the thermal homolysis of 20 mM 2–2′-azo-bis-(2 methyl-propionamidine)-dihydrochloride (ABAP) in 100 mM potassium phosphate buffer, pH 7.4. Hydroxyl radicals (OH• TOSC) were generated from the Fenton reaction of iron-EDTA (1.8 mM Fe 3+, 3.6 mM EDTA) plus ascorbate (180 mM) in 100 mM potassium phosphate buffer. Reactions were conducted at 35 °C in gastight sealed vials (Mininert valves, Supelco) in a final volume of 1 ml, and 200-μl aliquots of the headspace were analyzed at 10- to 12-min intervals for a total duration of 96 min; ethylene production was measured with a 6000 Vega Series 2 (ICU 600 Carlo ERBA Instruments) gas chromatograph equipped with a Supelco (Bellefonte, PA) packed Alumina F1 80/100 and a flame ionization detector. The oven, injection and detector temperatures were, respectively, 80, 80 and 120 °C; helium was the carrier gas (1 ml/min flow rate), and a split ratio 20:1 was used. TOSC values were quantified from the equation $\mathrm{TOSC} = 100 - \int SA/\int CA \times 100$; where $\int SA$ and $\int CA$ are the integrated areas calculated under the kinetic curve produced during the reaction course for, respectively, sample (SA) and control (CA) reactions. Samples were diluted to obtain experimental TOSC ranging from 20 to 40, which generally corresponded to 2 μl of plasma in the assay. For all the samples, a specific TOSC (referred to 1 μl of plasma) was calculated.

Plasmatic enzymatic activities were measured at 42 °C in a Multi-Detection Microplate Reader, BioTek ® (Vermont, USA) following the methods described by Diamantino et al. (2001). For lactate deshydrogenase activity (LDH) determination, the reaction mixture contained 100 mM phosphate buffer (pH 7.5), 0.1 mM NaOH, 0.2 mM NADH and 1 mM pyruvate. LDH activity was evaluated by using the extinction factor coefficient of 6.3 mM/cm and expressed in international units ($U = \mu\mathrm{mol/min}$) per 1 l of plasma. BChE and CbE activities were determined using the Ellman method in the presence of 1 mM butyrylthiocholine for BChE or 0.6 mM phenylthioacetate for CbE and 0.3 mM 5,5′ dithiobis-2-dinitrobenzoic acid (DTNB), and the increase of absorbance measured at 405 nm. Activities were expressed as μmol/min per ml of plasma. Plasmatic proteins were also measured by the Bradford method using γ-globulin as standard.

2.4. Data analysis

Quantile plots and Shapiro–Wilks tests showed that data was normally distributed. Therefore differences in biometrical, contaminant residues and biochemical responses across sites and between species were compared by ANOVA procedures using Student's *t*-tests or one way ANOVA tests followed by post hoc Tukey's like multiple comparison tests for *A. purpurea* data (Zar, 1996). Relationships between the studied parameters were assessed using parametric Pearson correlation coefficients.

3. Results

3.1. Biometric measurements and contaminant residues

As expected from the sampling scheme, no significant differences in size among chicks of *A. purpurea* or *E. garzetta* were found across sites (Table 1).

Eggs and feathers of both species nesting at Aiguabarreig and Ebro Delta sites showed similar levels of contaminants except for Hg and Cu, which were significantly different ($P < 0.05$, based on *t*-tests). Analysis of the presence of different pollutants in eggs and feathers of *A. purpurea* nestlings showed significant variations in pollution loads among the three studied populations (Table 1). The Flix population showed maximal levels of Hg in feathers, and HCB, DDT and total PCBs in eggs, whereas the Aiguabarreig population showed the lowest levels for all analysed contaminants. The Ebro Delta population showed significant high levels of Pb and Cu in feathers and DDTs in eggs; in the case of the two heavy metals, the levels surpassed the values obtained in the Flix population, suggesting that the source for these pollutants should be downstream Flix. In contrast, *E. garzetta* chicks showed much less variation in contaminant loads among populations, as only HCB showed significantly high levels in eggs collected in the Ebro Delta population compared to the Aiguabarreig population (Table 1).

3.2. Biochemical responses

From the six biochemical parameters analyzed in the plasma of chicks (Table 2), only levels ROO• TOSC and the activities of BChE and CbE differed significantly between egrets and herons chicks at Aiguabarreig and Ebro Delta sites ($P < 0.05$ based on *t*-tests). Biochemical parameters in the plasma of *A. purpurea* chicks showed a similar profile to the one observed with contaminant levels (Table 2). The Aiguabarreig population showed a lower impact than the other two populations, whereas the Flix population showed a significantly low scavenging capacity (ROO• TOSC and Total TOSC). In addition, the Delta population showed a significant increase on LDH activity, indicating again a specific pollution source downstream Flix. None of the studied biochemical parameters showed significant differences between the Aiguabarreig and Delta populations of *E. garzetta* (Table 2). Table 2 also includes the data on MN abundance in chicks blood (Quirós et al., 2008), which agrees with the general picture of a low impact in the Aiguabarreig site compared to the other two sites. In this case, the difference between sites was observed with both species.

Table 2
Sample size (*n*) and descriptive statistics for protein levels, total scavenging capacity (TOSC) for peroxyl and hydroxyl radicals (ROO•, OH•), their sum (TOT), buthirylcholinesterase (BhCE), carboxylesterase (CbE) and lacatate deshydrogenase (LDH) activities and number of micronucleus (MN). Within each species different letters indicate significant ($P < 0.05$) differences following ANOVA and post hoc Tukey's tests for *A. purpurea* and Student's *t*-tests for *E. garzetta* data. Differences in sample size are due to missing values.

	n	Protein[a]	ROO• TOSC[b]	OH• TOSC[b]	TOT TOSC[b]	BChE[c]	CbE[c]	LDH[d]	MN[e]
A. purpurea									
Aiguabarreig	5	25.0 ± 0.7 a	17.42 ± 1.22 a	2.70 ± 0.27 a	20.10 ± 1.31 a	0.21 ± 0.02 a	2.03 ± 0.23 a	290 ± 61 a	12.1 ± 2.6 a
Flix	10	27.9 ± 2.2 a	11.11 ± 0.65 b	2.22 ± 0.14 a	13.68 ± 0.75 b	0.22 ± 0.02 a	2.90 ± 0.20 a	216 ± 20 a	68 ± 12.1 b
Delta	4	26.7 ± 5.0 a	14.10 ± 5.95 a b	2.78 ± 0.22 a	16.87 ± 3.15 a b	0.22 ± 0.01 a	2.32 ± 0.52 a	444 ± 50 b	68.6 ± 10.8 b
E. garzetta									
Aiguabarreig	11	28.4 ± 2.4 A	10.90 ± 0.57 A	4.68 ± 2.28 A	15.59 ± 2.29 A	0.28 ± 0.02 A	0.27 ± 0.02 A	252 ± 14 A	18.9 ± 5.8 A
Delta	15	28.3 ± 2.1 A	10.07 ± 0.97 A	2.67 ± 0.13 A	12.73 ± 0.94 A	0.27 ± 0.02 A	0.31 ± 0.03 A	244 ± 29 A	30.3 ± 5.1 B

[a] mg/ml plasma, average ± standard error.
[b] TOSC units/μl plasma, average ± standard error.
[c] μmol/min per ml plasma, average ± standard error.
[d] μmol/min per l plasma, average ± standard error.
[e] Counts per 50,000 particles counted (data from Quirós et al., 2008).

3.3. Relationships

Bivariate comparison of trace metals in feathers, biometric, plasmatic biochemical parameters and the frequency of micronuclei (MN) are depicted in Table 3. For brevity, only the sum of TOSC radicals (TOT TOSC) was considered. Most biochemical parameters and trace metals were independent of chick size, except for Cu and LDH in *A. purpurea* and Cu and Pb levels in *E. garzetta*. In all these cases, larger animals showed higher levels than smaller ones (Table 3). Several biochemical parameters in plasma also showed significant ($P < 0.05$) correlation with trace metals in feathers, but only for *A. purpurea*. In this species, levels of Hg were inversely related with TOT TOSC values and both Hg and Cu levels were positively related with MN indexes and LDH activities, respectively (Table 3, Fig. 2). A further partial correlation analysis accounting for tarsus length still denoted a significant ($P < 0.01$; 0.64, $n = 15$) relationship between LDH and Cu. These results indicate that the above mentioned relationships were unaffected by size. Conversely, no significant correlations were observed between metal levels and any of the studied biomarkers for *E. garzetta* samples (Table 3, Fig. 2). Organochlorine levels were measured in whole eggs and hence could not be compared with measured responses in chicks.

4. Discussion

Except for Cu and Hg levels in feathers, which were greater in *E. garzetta* and *A. purpurea* chicks, respectively, contaminant levels were similar in the studied two species regardless of their market size differences. Although *A. purpurea* eats larger (and presumable more contaminated) fish than the small mosquito fish and cray fish typically eaten by *E. garzetta* (Gonzalez-Martin et al., 1992), differences in prey contaminant load could only explain the observed greater levels of Hg in the bigger species. The highest Cu levels found in feathers of *E. garzetta* could be either related to a higher content of this metal in the diet (e.g., Cu in the carapace of crayfish) or due to interspecific differences (Kim and Koo, 2008).

Organochlorine compound levels in eggs from Flix and the Ebro Delta nesting sites of the studied heron species (HCB 3.4–18.2 ng/g ww, DDTs 223.7–684.4 ng/g ww, PCBs 262.2–878.8 ng/g ww), were moderate in relation to ranges reported in previous studies from North America (Mississippi, CA), Europe (Dabube Delta, N Italy) and Asian (Hong Kong) contaminated sites in great blue, grey and black-crowded night herons, little and great egrets (3–20 ng/g ww of HCB, 200–6000 ng/g ww PCB, 100–12,000 ng/g ww DDTs; Elliott et al., 1989; Custer et al., 1997; Fasola et al., 1998; Aurigi et al., 2000; Connell et al., 2003; Henny et al., 2008). The concentrations of Pb, Cu and total Hg at the contaminated studied sites (0.9–2.1, 8.1–12 and 1.7–8.1 μg/g ww, respectively) were within the range reported elsewhere in feathers of heron nestlings (0.1–9, 3–9 and 0.4–2 μg/g ww, respectively; Fasola et al., 1998; Custer et al., 2007, 2008), the exception being the quite high levels of Hg (>30 μg/g ww) reported by Henny et al. (2002) in heron and egret nestling feathers from Carson River (Nevada).

Concentrations of *p,p*′-DDE and PCBs above 4000 and 12,000 ng/g ww in eggs could be regarded as concentrations affecting reproductive success in herons (Fasola et al., 1998). In the present study, over 95% of measured total DDT levels were *p,p*′-DDE and averaged levels of DDTs and PCBs in eggs were roughly one order of magnitude lower than those reported to affect the reproduction of herons.

A study on mercury effects on birds from the Carson River (NV) found severe adverse health effects of Hg in hero and egret chicks having 30–35 μg/g ww of Hg in their feathers (Henny et al., 2002). Studying the distribution of heavy metals in two heron species from Korea, Kim and Koo (2008) concluded that levels of Cu and Pb in feathers ranking from 9–30 to 0.2–0.5 μg/g ww, respectively, were within the background levels of wild herons and far below those having adverse effects.

The above mentioned Hg levels are about four fold the average level of the Flix population, the most contaminated one in our study. Similarly, levels of Cu and Pb found in our study are within the background range for herons. Taking this into consideration it is unlikely that the measured contaminants could cause acute effects on the studied populations. However, sublethal effects are likely to occur at much lower concentrations than those at which pathological effects become pronounced.

The studied biochemical response in plasma and of micronuclei frequency in blood of chicks varied across species and nesting sites. TOT TOSC ranks in both species (12.16–20.48 U/μl plasma) were of similar magnitude to those reported by Corsolini et al. (2001) in Adélie and Emperor penguins from Wood Bay, North Victoria (10–25 U/μl plasma). Activity levels of BChE varied little in both species (0.21–0.28 μmol/min per ml), but plasmatic CbE activities in *E. garzetta* (0.27–0.31 μmol/min per ml) were ten fold lower than those of *A. purpurea* (2–2.9 μmol/min per ml). Reported values for plasmatic B esterase activities were highly variable across bird species ranging from 0.7 to 5 μmol/min per ml for BChE and from 0.2 to 11 μmol/min per ml for CbE (Fossi et al., 1996). According to Fossi et al. (1996), B esterase activities tend to change depending on specimen size or species diet. In our study the observed differences in CbE activities between chicks of *E. garzetta* and *A. purpurea* could be explained by differences in weight (a factor of 2) since both are piscivorous. Measured plasmatic LDH activities in the studied chick

Table 3
Bivariate Pearson correlations of log transformed studied parameters by species. Abbreviations are explained in the text.

Species	TOT TOSC	LDH	MN	BChE	CbE	Lt	W	Hg	Pb	Cu
A. Purpurea										
Proteins	0.08 (18)	−0.19 (18)	−0.08 (18)	0.31 (18)	0.27 (18)	0.13 (18)	0.20 (17)	0.10 (18)	−0.0 (18)	−0.21 (18)
TOT TOSC		0.24 (18)	−0.41 (18)*	−0.27 (18)	−0.44 (18)*	0.32 (18)	0.35 (17)	−0.57 (18)**	0.04 (18)	0.39 (19)
LDH			0.05 (18)	0.29 (18)	−0.22 (18)	0.47 (18)*	0.46 (17)*	−0.43 (18)*	0.35 (18)	0.78 (18)***
MN				0.01 (18)	0.14 (18)	0.16 (19)	−0.22 (17)	0.64 (19)***	0.37 (19)	0.05 (19)
BChE					0.31 (18)	0.27 (18)	0.35 (17)	0.11 (18)	−0.02 (18)	0.12 (18)
CbE						−0.39 (18)	−0.37 (17)	0.39 (18)	−0.24 (18)	−0.28 (18)
Lt							0.90 (17)***	0.05 (21)	0.26 (17)	0.62 (21)***
W								−0.24 (17)	−0.14 (17)	0.38 (17)
Hg									0.16 (21)	−0.28 (21)
Pb										0.35 (21)
E. garzetta										
Proteins	−0.14 (14)	−0.28 (14)	−0.43 (12)	0.39 (14)	0.28 (14)	−0.07 (13)	0.30 (14)	0.27 (14)	0.14 (14)	−0.31 (14)
TOT TOSC		−0.28 (13)	−0.22 (14)	0.01 (14)	−0.27 (14)	−0.25 (15)	−0.35 (16)	0.38 (16)	−0.37 (16)	−0.22 (16)
LDH			0.35 (13)	0.06 (14)	−0.08 (14)	0.11 (14)	−0.12 (15)	0.08 (15)	0.38 (15)	0.01 (15)
MN				0.11 (12)	0.06 (12)	−0.13 (27)	−0.23 (27)	−0.08 (27)	−0.22 (27)	−0.12 (27)
BChE					0.34 (14)	0.14 (13)	0.22 (14)	−0.03 (14)	0.27 (14)	−0.23 (14)
CbE						−0.24 (13)	−0.17 (14)	0.10 (14)	0.30 (14)	−0.18 (14)
Lt							0.88 (38)***	−0.19 (37)	0.37 (38)**	0.52 (38)***
W								0.08 (38)	0.29 (38)	0.36 (38)**
Hg									−0.04 (38)	0.44 (38)***
Pb										0.56 (38)***

Sample size is depicted between brackets. Differences in sample size are due to missing values $^{*}0.05 < P < 0.1$; $^{**}0.01 < P < 0.05$; $^{***}P < 0.01$.

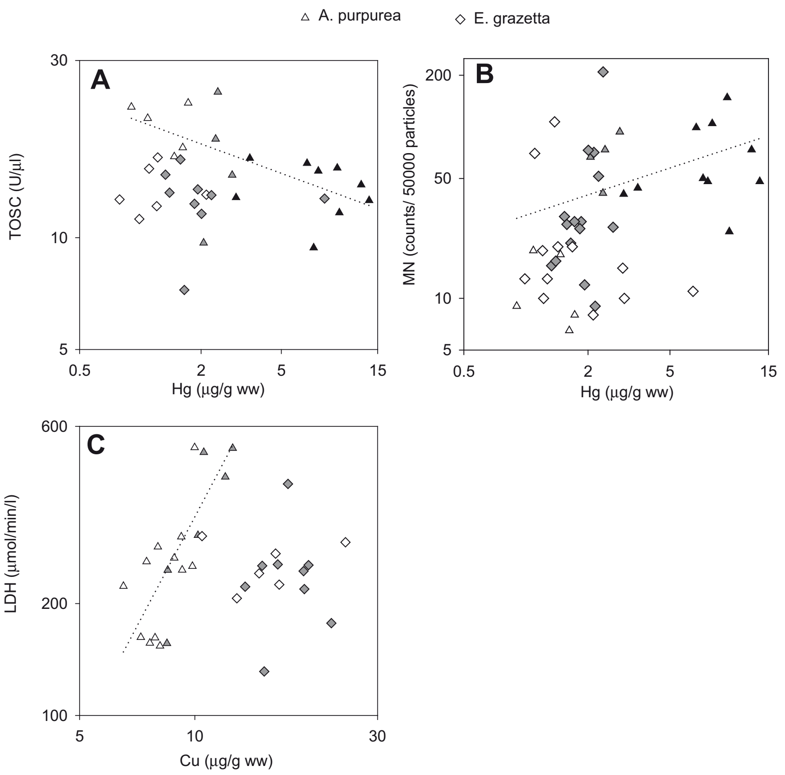


Fig. 2. Bi-plots of mercury levels in feathers versus total scavenging capacity – TOT TOSC (A), micronucleated erythrocites MN (B) and of levels of Cu versus lactate deshydrogenase – LDH (C) in *A. purpurea* and *E. garzetta*. Each symbol corresponds to a single chick. Dotted lines indicate significant linear relationships, which only occurred in *A. purpurea* ($P < 0.05$). White, black and grey symbols correspond to values from Aiguabarreig, Flix and Delta sites, respectively. Data are depicted in log scale.

species (216–444 U/l) were comparable to those reported in nestlings and fledglings of great egret and great blue herons (200–1700 U/l; Hoffman et al., 2005; Champoux et al., 2006).

Reported nesting site effects on ROO•/TOT TOSC and micronuclei frequency provided the first evidence of detrimental effects of pollutants released by a chlor-alkali plant in chicks from heron species. In particular, nestling populations of *A. purpurea* chicks near the chlor-alkali plant showed the highest frequency and levels of micronuclei, Hg, HCB, DDTs and PCB and a reduced buffering capacity detoxify ROS (lowest values for ROO• or TOT TOSC). Moreover TOT TOSC was inversely related with mercury levels in feathers and micronuclei frequency. The observed lack of biological responses in *E. garzetta* chicks could be related to the absence of nesting sites at the most contaminated location (Flix). Exposure to various organic and inorganic contaminants are known to reduce TOT TOSC in bivalves and fish species and increase oxidative stress (Regoli, 2000; Regoli et al., 2005). More specifically, lab exposures showed that Cd, Cu, PCBs and PAHs caused oxidative stress, diminishing TOSC in bivalves (Lehmann et al., 2007; Company et al., 2008). Although there are no reported studies on effects of mercury in TOT TOSC, there is substantial information indicating that mercury decreases antioxidant defenses causing oxidative stress in adults and nestlings of egrets and herons (Hoffman et al., 2005; Henny et al., 2002). Therefore the observed site differences in ROO• or TOT TOSC in *A. purpurea* chicks agrees with previous studies and could be related to exposure to contaminants such as Hg, PCBs or DDTs.

Plasmatic B esterases like those in liver are known to act as alternative target sites for anticholinergic compounds, thus protecting brain and muscle cholinesterases from inhibitory substances (Chambers et al., 1994). Activity levels of B esterases were similar across sites in both species, thus indicating low or no exposure to anticholinergic compounds. This finding is environmentally relevant since the use of large quantities of organophosphate and carbamate insecticides in Ebro's Delta in the past decades had dramatic effects in wildlife (Mañosa et al., 2001).

Like the other parameters, only in *A. purpurea* were there significant differences across sites in LDH activity with chicks nesting at the Ebro Delta showing the greatest activities. LDH activities were also positively related with levels of Cu in feathers, and although marginally ($0.05 < P < 0.1$), inversely related with Hg. Exposure to mercury chloride and to the PCB mixture Aroclor 1254 has been observed to increase plasmatic LDH of the quail *Coturnix coturnix* (Dieter, 1974). In contrast, methylmercury inhibited plasmatic LDH activities of great egret nestlings (Hoffman et al., 2005). In the present study, total mercury levels measured in feathers were likely to be mainly methylmercury given that this is the preferred bioaccumulative form through the aquatic food web and for piscivorous birds that are at the top of the food chain (Henny et al., 2002). Under this point of view, the observed inverse relationship between LDH activity and Hg in chicks agrees with previous studies. In contrast, the observed higher LDH activities in Ebro Delta chicks and its direct correlation with Cu levels in feathers are difficult to explain. Observed differences in Cu levels and probably of LDH activities across Ebro Delta with the other studied nesting sites may be related to differences in diet or habitat related as Ebro Delta colonies were located near the sea (Champoux et al., 2006). Alternatively, the widespread use of herbicides such as propanil and endosulfan in the Ebro Delta (Mañosa et al., 2001), with known oxidative stress and LDH effects in mammals (Omurtag et al., 2008; Rankin et al., 2008) may have affected *A. purpurea* plasmatic parameters. Indeed, increased oxidative stress levels in chicks nesting at Ebro Delta may also explain the quite high frequency of micronuclei reported by Quirós et al. (2008) in both species.

In summary our results support previous findings (Quirós et al., 2008) and provide the first evidence of an association of biological responses with pollutants released by a chlor-alkali plant in chicks from heron species. In particular, in this study two impacted sites with altered biological responses on chick blood parameters have been identified: *A. purpurea* chicks nesting in a chlor-alkali impacted area (Flix) showed high frequency of micronuclei and high levels of Hg, HCB and PCB and a reduced buffering capacity to detoxificate reactive oxygen species. In Ebro Delta, biological response changes included high levels of plasmatic LDH in *A. purpurea* chicks and high frequency of micronuclei in blood of both species. We tentatively relate these alterations to the intensive agricultural activities taking place at the Ebro Delta.

Acknowledgements

This work has been supported by the Spanish Ministry for the Environment, the Catalan Water Agency (ACA) of the Generalitat de Catalunya and GRACCIE and AQUATOXIGEN (CSD2007-00067, CGL2008-01898) from the Spanish Ministry of Science. The authors thank Dr. Vendrell (CID, CSIC) for kindly providing the gas chromatograph to measure TOSC.

References

Aurigi, S., Focardi, S., Hulea, D., Renzoni, A., 2000. Organochlorine contamination in bird's eggs from the Danube Delta. Environnemental Pollution 109, 61–67.

Barata, C., Lekumberri, I., Vila-Escalé, M., Prat, N., Porte, C., 2005. Trace metal concentration, antioxidant enzyme activities and susceptibility to oxidative stress in the tricoptera larvae *Hydropsyche exocellata* from the Llobregat river basin (NE Spain). Aquatic Toxicology 74, 3–19.

Catalan, J., Ventura, M., Vives, I., Grimalt, J.O., 2004. The roles of food and water in the bioaccumulation of organochlorine compounds in high mountain lake fish. Environmental Science and Technology 38, 4269–4275.

Chambers, J.E., Ma, T., Scott Boone, J., Chambers, H.W., 1994. Role of detoxication pathways in acute toxicity levels of phosphorothionate insecticides in the rat. Life Sciences 54, 1357–1364.

Champoux, L., Rodrigue, J., DesGranges, J.L., Trudeau, S., Hontela, A., Boily, M., Spear, P., 2002. Assessment of contamination and biomarker responses in two species of Herons on the St. Lawrence River. Environmental Monitoring and Assessment 79, 193–215.

Champoux, L., Rodrigue, J., Trudeau, S., Boily, M.H., Spear, P.A., Hontela, A., 2006. Contamination and biomarkers in the great blue heron, an indicator of the state of the St. Lawrence River. Ecotoxicology 15, 83–96.

Company, R., Serafim, A., Cosson, R.P., Fiala-Médioni, A., Camus, L., Colacó, A., Serraó-Santos, R., Bebianno, M.J., 2008. Antioxidant biochemical responses to long-term copper exposure in *Bathymodiolus azoricus* from Menez–Gwen hydrothermal vent. Science of the Total Environment 389, 407–417.

Connell, D.W., Fung, C.N., Minh, T.B., Tanabe, S., Lam, P.K.S., Wong, B.S.F., Lam, M.H.W., Wong, L.C., Wu, R.S.S., Richardson, B.J., 2003. Risk to breeding success of fish-eating Ardeids due to persistent organic contaminants in Hong Kong: evidence from organochlorine compounds in eggs. Water Research 37, 459–467.

Cordi, B., Fossi, C., Depledge, M., 1997. Temporal biomarker responses in wild passerine birds exposed to pesticide spray drift. Environmental Toxicology and Chemistry 16, 2118–2124.

Corsolini, S., Nigro, M., Olmastroni, S., Focardi, S., Regoli, F., 2001. Susceptibility to oxidative stress in Adelie and emperor penguin. Polar Biology 24, 365–368.

Custer, T.W., Hines, R.K., Melancon, M.J., Hoffman, D.J., Wickliffe, J.K., Bickham, J.W., Martin, J.W., Henshel, D.S., 1997. Contaminant concentrations and biomarker response in great blue heron eggs from 10 colonies on the upper Mississippi River. USA. Environmental Toxicology and Chemistry 16, 260–271.

Custer, T.W., Custer, C.M., Eichhorst, B.A., Warburton, D., 2007. Selenium and metal concentrations in waterbird eggs and chicks at Agassiz National Wildlife Refuge, Minnesota. Archives of Environmental Contamination and Toxicology 53, 103–109.

Custer, T.W., Golden, N.H., Rattner, B.A., 2008. Element patterns in feathers of nestling black-crowned night-herons, *Nycticorax nycticorax* L., from four colonies in Delaware, Maryland, and Minnesota. Bulletin of Environmental Contamination and Toxicology 81, 147–151.

De Luca-Abbott, S.B., Wong, B.S.F., Peakall, D.B., Lam, P.K.S., Young, L., Lam, M.H.W., Richardson, B.J., 2001. Review of effects of water pollution on the breeding success of waterbirds, with particular reference to ardeids in Hong Kong. Ecotoxicology 10, 327–349.

Diamantino, T.C., Almeida, E., Soares, A.M.V.M., Guilhermino, L., 2001. Lactate dehydrogenase activity as an effect criterion in toxicity tests with *Daphnia magna* Straus. Chemosphere 45, 553–560.

Dieter, M.P., 1974. Plasma enzyme activities in Coturnix quail fed graded doses of DDE, polychlorinated biphenyl, malathion and mercuric chloride. Toxicology and Applied Pharmacology 27, 86–98.

Elliott, J.E., Butler, R.W., Norstrom, R.J., Whitehead, P.E., 1989. Environmental contaminants and reproductive success of great blue herons *Ardea herodias* in British Columbia, 1986–87. Environmental Pollution 59, 91–114.

Fasola, M., Movalli, P.A., Gandini, C., 1998. Heavy metal, organochlorine pesticide, and PCB residues in eggs and feathers of herons breeding in northern Italy. Archives of Environmental Contamination and Toxicology 34, 87–93.

Fernández, M.A., Alonso, C., González, M.J., Hernández, L.M., 1999. Occurrence of organochlorine insecticides, PCBs and PCB congeners in waters and sediments of the Ebro River (Spain). Chemosphere 38, 33–43.

Fossi, M.C., Massi, A., Leonzio, C., 1994. Blood esterase inhibition in birds as an index of organophosphorus contamination: field and laboratory studies. Ecotoxicology 3, 11–20.

Fossi, M.C., Lari, L., Casini, S., 1996. Interspecies variation of "B" esterases in birds: the influence of size and feeding habits. Archives of Environmental Contamination and Toxicology 31, 525–532.

Franson, J.C., Hoffman, D.J., Schmutz, J.A., 2002. Blood selenium concentrations and enzyme activities related to glutathione metabolism in wild emperor geese. Environmental Toxicology and Chemistry 21, 2179–2184.

Furness, R.W., Camphuysen, K., 1997. Seabirds as monitors of the marine environment. ICES Journal of Marine Science 54, 726–737.

Gonzalez-Martin, M., Ruiz, X., Llorente, G.A., 1992. Breeding parameters, feeding habits and nestling growth in a recovering population of purple herons from the Ebro Delta, Spain. Miscel.lania Zoologica 16, 147–160.

Henny, C.J., Hill, E.F., Hoffman, D.J., Spalding, M.G., Grove, R.A., 2002. Nineteenth century mercury: hazard to wading birds and cormorants of the Carson River, Nevada. Ecotoxicology 11, 213–231.

Henny, C.J., Anderson, T.W., Crayon, J.J., 2008. Organochlorine pesticides, polychlorinated biphenyls, metals, and trace elements in waterbird eggs, Salton Sea, California, 2004. Hydrobiologia 604, 137–149.

Hoffman, D.J., Spalding, M.G., Frederick, P.C., 2005. Subchronic effects of methylmercury on plasma and organ biochemistries in great egret nestlings. Environmental Toxicology and Chemistry 24, 3078–3084.

Kim, J., Koo, T.-H., 2008. Heavy metal distribution in chicks of two heron species from Korea. Archives of Environmental Contamination and Toxicology 54, 740–747.

Kim, S.R., Kim, T.H., Ryu, S.Y., Lee, H.J., Oh, H., Jo, S.K., Oh, K.S., Park, I.C., Kim, J.C., Kang, C.M., Kim, S.H., 2003. Measurement of micronuclei by cytokinesis-block method in human, cattle, goat, pig, rabbit, chicken and fish peripheral blood lymphocytes irradiated in vitro with gamma radiation. In Vivo 17, 433–438.

Lehmann, D.W., Levine, J.F., Law, J.M., 2007. Polychlorinated biphenyl exposure causes gonadal atrophy and oxidative stress in *Corbicula fluminea* clams. Toxicologic Pathology 35, 356–365.

Livingstone, D.R., 2001. Contaminated-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollution Bulletin 42, 656–666.

Mañosa, S., Mateo, R., Guitart, R., 2001. A review of the effects of agricultural and industrial contamination on the Ebro Delta biota and wildlife. Environmental Monitoring and Assessment 71, 187–205.

Muir, D., Braune, B., DeMarch, B., Norstrom, R., Wagemann, R., Lockhart, L., Hargrave, B., Bright, D., Addison, R., Payne, J., Reimer, K., 1999. Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. Science of the Total Environment 230, 83–144.

Murvoll, K.M., Skaare, J.U., Moe, B., Anderssen, E., Jenssen, B.M., 2006. Spatial trends and associated biological responses of organochlorines and brominated flame retardants in hatchlings of North Atlantic kittiwakes (*Rissa tridactyla*). Environmental Toxicology and Chemistry 25, 1648–1656.

Omurtag, G.Z., Tozan, A., Åžehirli, A.O., Åžener, G., 2008. Melatonin protects against endosulfan-induced oxidative tissue damage in rats. Journal of Pineal Research 44, 432–438.

Pastor, D., Sanpera, C., González-Solís, J., Ruiz, X., Albaigés, J., 2004. Factors affecting the organochlorine pollutant load in biota of a rice field ecosystem (Ebro Delta, NE Spain). Chemosphere 55, 567–576.

Quirós, L., Ruiz, X., Sanpera, C., Jover, L., Piñaa, B., 2008. Analysis of micronucleated erythrocytes in heron nestlings from reference and impacted sites in the Ebro basin (NE Spain). Environmental Pollution 155, 81–87.

Rankin, G.O., Racine, C., Sweeney, A., Kraynie, A., Anestis, D.K., Barnett, J.B., 2008. In vitro nephrotoxicity induced by propanil. Environmental Toxicology 23, 435–442.

Regoli, F., 2000. Total oxyradical scavenging capacity (TOSC) in polluted and translocated mussels: a predictive biomarker of oxidative stress. Aquatic Toxicology 50, 351–361.

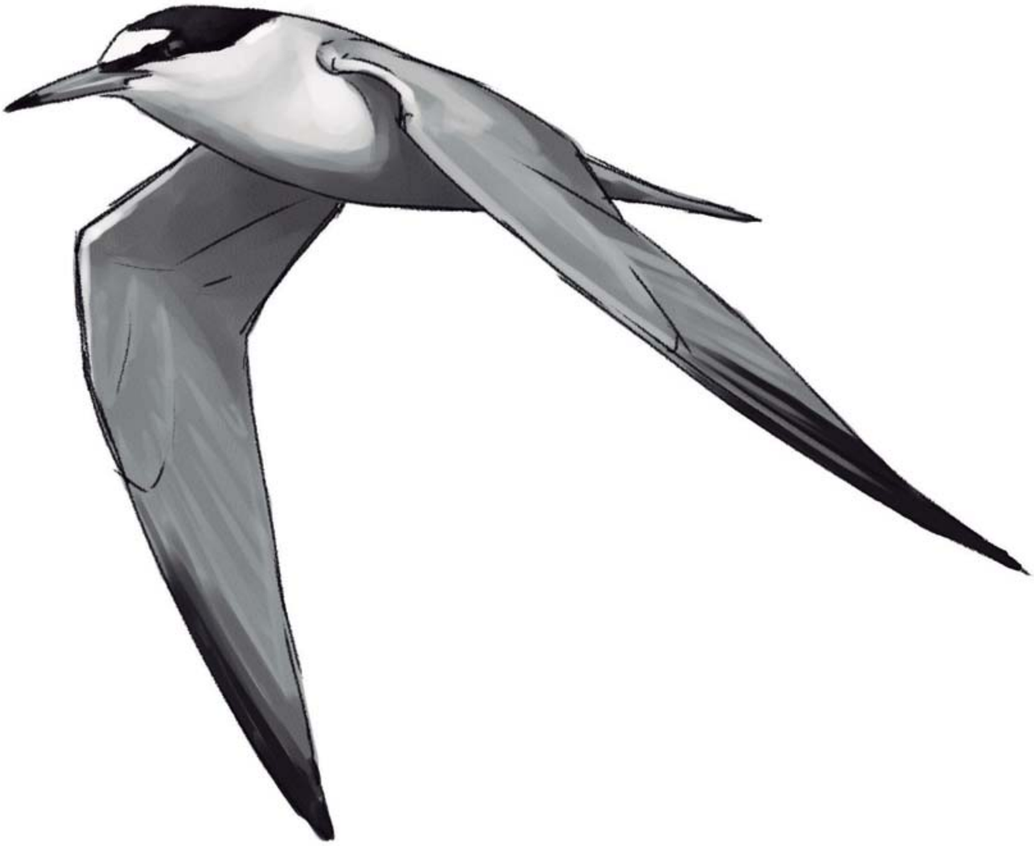
Regoli, F., Nigro, M., Benedetti, M., Fattorini, D., Gorbi, S., 2005. Antioxidant efficiency in early life stages of the Antarctic silverfish, *Pleuragramma antarcticum*: responsiveness to pro-oxidant conditions of platelet ice and chemical exposure. Aquatic Toxicology 75, 43–52.

Stoncius, D., Lazutka, J.R., 2003. Spontaneous and benzo[a]pyrene-induced micronuclei in the embryos of the black-headed gull (*Larus ridibundus* L.). Mutation Research – Genetic Toxicology and Environmental Mutagenesis 538, 31–39.

Strum, K.M., Alfaro, M., Haase, B., Hooper, M.J., Johnson, K.A., Lanctot, R.B., Lesterhuis, A.J., López, L., Matz, A.C., Morales, C., Paulson, B., Sandercock, B.K., Torres-Dowdal, J., Zaccagnini, M.E., 2008. Plasma cholinesterases for monitoring pesticide exposure in nearctic-neotropical migratory shorebirds. Ornitologia Neotropical 19, 641–651.

Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambré, C., Tarazona, J.V., Brandt, I., Vethaak, A.D., 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Critical Reviews in Toxicology 30, 71–133.

Winston, G.W., Regoli, F., Dugas, A.J., Fong, J.H., Blanchard, K.A., 1998. A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. Free Radical Biology and Medicine 24, 480–493.



III

Sea, freshwater or saltpans?
Foraging ecology of terns to assess mercury inputs in a wetland landscape: The Ebro Delta

¿Salinas, mar, o agua dulce? Ecología trófica en charranes para evaluar la entrada de mercurio en el Delta del Ebro

Javier Cotín[a], Manuel García-Tarrasón[a], Carolina Sanpera[a], Lluis Jover[b] & Xavier Ruiz[a].

[a] Department Biologia Animal, Facultat de Biologia, Univesitat de Barcelona,
Diagonal 645, 08028 Barcelona, Spain

[b] Department Salut Pública, Facultat de Medicina, Universitat de Barcelona,
Casanova 143, 08036 Barcelona, Spain

Resumen: La cuenca hidrográfica del Ebro es la más grande de España e incluye zonas altamente industrializadas. Entre estas zonas se encuentra Flix, donde una fábrica petroquímica lleva funcionando desde principios del siglo XX. Este largo periodo de actividad, junto con la construcción de una presa cerca de la fábrica alrededor de 1960, ha resultado en la acumulación de enormes cantidades de sedimentos contaminados en el lecho del río, muchos de los cuales presentan mercurio. Los contaminantes de Flix son arrastrados aguas abajo por el río Ebro a su delta. Con el fin de evaluar la introducción de mercurio en el complejo ecosistema fluvial-estuario, estudiamos la ecología de los charranes que habitan dicho área, puesto que estas aves se segregan dentro del elenco de hábitats. Con este propósito, primero utilizamos el análisis de isótopos estables (SIA) ($\delta^{34}S$, $\delta^{13}C$, $\delta^{15}N$) en huevos para determinar la ecología trófico y la segregación por hábitats de varias especies de charranes (Charrán común, Charrán patinegro, Pagaza Piconegra, Charrancito y Fumarel cariblanco) que crían simpátricamente, para poder vincular su ecología de forrajeo con los diferentes hábitats. Después medimos los niveles de mercurio en huevos para controlar la entrada de este metal en los diferentes hábitats. Con la excepción del Charrancito, los otros charranes hicieron un uso restringido de los hábitats del Delta del Ebro, como muestran los isótopos de C y S; la Pagaza piconegra y el Fumarel cariblanco se alimentaron en hábitats de agua dulce, mientras que el Charrán común y patinegro en el mar. Este uso restringido de los hábitats por parte de la Pagaza piconegra y del Charrán común contrasta con estudios previos en otras zonas de cría. El Charrancito, que mostró un amplio rango de valores isotópicos, mostro un patrón de alimentación oportunista aunque se alimentó principalmente en las salinas, un hábitat no descrito previamente para dicha especie en esta área. Encontramos que las concentraciones de mercurio están relacionadas con el uso del hábitat y la dieta, y son inesperadamente más altas en las especies que se alimentan de presas demersales en hábitats marinos y en las salinas que en las que lo hacen en los hábitats dulceacuícolas. Las concentraciones de mercurio encontradas en los huevos de Charrancito y de Charran común muestreados en la Punta de la Banya pueden ser suficientemente altas para poner en riesgo el éxito reproductor.

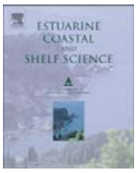
Palabras clave: Aves acuáticas, Ecología trófica, Isótopos Estables, Biomonitorizar, Mediterráneo.

Estuarine, Coastal and Shelf Science 92 (2011) 188–194

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss



Sea, freshwater or saltpans? Foraging ecology of terns to assess mercury inputs in a wetland landscape: The Ebro Delta

Javier Cotin [a,*], Manuel García-Tarrasón [a], Carolina Sanpera [a], Lluis Jover [b], Xavier Ruiz [1]

[a] Department Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain
[b] Department Salut Pública, Facultat de Medicina, Universitat de Barcelona, Casanova 143, 08036 Barcelona, Spain

ARTICLE INFO

Article history:
Received 9 June 2010
Accepted 26 December 2010
Available online 11 January 2011

Keywords:
aquatic birds
trophic ecology
stable isotopes
biomonitoring
Mediterranean

ABSTRACT

The Ebro catchment, the largest river basin in Spain, includes various heavily industrialized areas. Among these is the Flix site, where a chemical plant has been operating since the beginning of the 20th century. This extended operational period, together with the construction of a dam next to the factory around 1960, resulted in the accumulation of large amounts of heavily polluted sediments in the adjacent riverbed, many of which are contaminated by mercury. Pollutants from Flix are carried downstream by the Ebro River to its delta. In order to assess the transfer of mercury to the complex river estuary ecosystem, we studied the ecology of the tern community living there as these birds segregate into a range of habitats. For this purpose, first we used stable isotope analysis (SIA) ($\delta^{34}S$, $\delta^{13}C$, $\delta^{15}N$) of eggs to determine the trophic ecology and habitat partitioning of several tern species (Common, Sandwich, Little, Gull-Billed and Whiskered Tern) breeding sympatrically, in order to link their foraging ecology with habitat types. Next we measured mercury concentrations in eggs to monitor the input of this metal into the diverse habitats. With the exception of the Little Tern, the other terns used a restricted habitat range in the Ebro Delta, as shown by C and S isotopes; the Gull-Billed and Whiskered Tern foraged in freshwater habitats, while the Common and Sandwich Tern used marine habitats. This restricted feeding behavior of the Gull-Billed and Common Tern contrasts with previous reports in other breeding sites. The Little Tern, which showed a wide range of isotopic values, was found to be an opportunistic forager but fed mainly in saltpans, a feeding habitat not reported previously for this species in this area. We found that mercury concentrations are related to foraging habitat and diet, and are unexpectedly higher in species feeding on demersal prey in marine habitats and also higher in birds feeding in saltpans than in those feeding in freshwater habitats. The mercury concentrations found in the Little and Common Tern eggs sampled in "Punta de la Banya" may be sufficiently high to endanger breeding success.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

The Ebro catchment is the largest river basin in Spain. Covering an area of 85,362 km^2, this catchment receives the potential influence of 3 million people and also contains some heavily industrialized areas. One of these areas is the Flix site, where a chemical industry has been in operation since the beginning of the 20th century. This long operational period, together with the construction of a dam next to the plant around 1960, resulted in the accumulation of high amounts of heavily polluted sediments in the adjacent riverbed (Fernandez et al., 1999). Recently, a project to remove the polluted sediments has started; however, this initiative implies a high risk of dispersal. Pollutants originated at the Flix site are carried downstream by the Ebro River to its delta 90 km away (Llorente et al., 1987; Pastor et al., 2004), especially during floods.

Previous studies in the Flix reservoir have reported the effects of pollutants on birds at this site and also in the Ebro Delta (Quiros et al., 2008; Barata et al., 2010) an area also affected by intensive agricultural activity, which has a significant impact on wildlife (Mañosa et al., 2001). Mercury is a special source of concern, as it mainly enters organisms through diet (Burger et al., 1992). The Ebro Delta (NE Spain: 40°37′N, 00°35′E) is one of the largest wetlands in the Western Mediterranean region and is home to extensive bird colonies. Occupying an area of 320 km^2, 75% of the delta surface is devoted to rice fields (c.a. 20,000 ha) and farmland, 20% to natural preserved areas and the remaining 5% to urban uses. This wetland presents a wide variety of habitats. The inner part of the delta is dominated by rice fields and farmland, which are connected to the river through channels, while the coastline is characterized by abandoned fields, lagoons, salt marshes and beaches. On each side

---

[*] Corresponding author.
E-mail address: jcotin@ub.edu (J. Cotin).
[1] Deceased 27th April 2008.

0272-7714/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ecss.2010.12.024

of the Ebro Delta and surrounded by sea there are two arrow-shaped sandy areas, known as the 'Punta del Fangar' and 'Punta de la Banya' (with an extension of 500 and 2500 ha respectively), the latter hosting a saltpan industry, which occupies over 1000 ha. Finally, the open sea and the northern and southern bays, molded by the two 'Puntas', conform the patchwork of habitats. The coastal lagoons, although connected to the sea and thus expected to hold brackish water, receive considerable freshwater input from the rice fields from spring to autumn, thereby lowering their salinity, which almost reaches that of freshwater during those seasons.

In order to study input of mercury from the Flix reservoir into the diverse habitats of the Ebro Delta, it is necessary to examine suitable target bioindicator species that forage in all the habitats present, as species used by Quiros et al. (2008) and Barata et al. (2010) (family Ardeidae) have a narrower range of foraging habitats.

Terns are gull-related seabirds. They comprise 45 species grouped in 12 genera (Bridge et al., 2005) which are found worldwide; most of these are migratory birds and they have been widely used as bioindicators of pollution (Becker et al., 1993b; Nisbet et al., 2002; Guitart et al., 2003; Tavares et al., 2009). The following five tern species breed in the Ebro Delta: Common Tern (*Sterna hirundo*, Linnaeus, 1758), Sandwich Tern (*Sterna sandvicensis,* Latham, 1787), Little Tern (*Sternula albifrons*, Pallas, 1764), Gull-Billed Tern (*Gelochelidon nilotica*, Gmelin, 1789) and Whiskered Tern (*Chlydonias hybrida,* Pallas, 1811). A wide variety of foraging habitats has been reported for these species: Freshwater habitats for the Whiskered Tern (Latraube et al., 2005; Paillisson et al., 2007); marine for the Sandwich Tern (Stienen et al., 2000) and both marine and freshwater for the Common Tern (Becker et al., 1997; Neubauer, 1998). The last two species have been described as generalist foragers. The Little Tern uses several foraging habitats in Portugal (Paiva et al., 2008a), and in the Ebro Delta it has been observed foraging in channels, lagoons and lagoon mouths (Bertolero et al., 2005). The Gull-Billed Tern feeds in terrestrial (Cabo and Sanchez, 1986), freshwater (Dies et al., 2005), and even marine habitats (Stienen et al., 2008). Given their feeding strategies, these five tern species have the capacity to forage over the whole of the Ebro Delta. This trait thus makes them suitable candidates as bioindicators of mercury for the whole area.

These five Tern species have large colonies in the Ebro Delta (c.a. 350 pairs of Little Tern, 5000 of Common Tern, 500 of Gull-Billed Tern, 2000 of Sandwich Tern and 1200 of Whiskered Tern), thus making this wetland one of the tern hotspots in Spain. These species have recently increased their breeding populations in the delta, except the Little Tern, which has decreased about 50% (Oro et al., 2004). Although all these species are classified as of "Least Concern" by Birdlife, terns face several threats, such as habitat loss, human disturbance, contamination, and predation by introduced species (feral cats, rats). Furthermore, in the Ebro Delta, tern populations have suffered fluctuations (Ferrer and Martinez-Vilalta, 1986), these normally related to human disturbance of their breeding sites and foraging areas.

Mercury biomagnifies along trophic webs, but its bioavailability can vary among habitats. Therefore to assess mercury input to terns, a full understanding of their foraging ecology in the area is needed (Nisbet et al., 2002; Sanpera et al., 2007b; Tavares et al., 2007; Catry et al., 2008). Stable isotope signatures of nitrogen (δ^{15}N) and carbon (δ^{13}C) have been extensively used in studies of seabird communities, focusing on their trophic ecology and relationships, to evaluate the relative importance of dietary sources to consumers (Hobson et al., 1994; Forero and Hobson, 2003; Catry et al., 2008; Cherel et al., 2008; Koiadinovic et al., 2008). δ^{15}N reflects the trophic level, with consumer signatures being higher than in their prey (Forero et al., 2005). This parameter therefore allows interpretation of trophic positions and food webs. Information about the source of carbon entering a food web can be obtained from δ^{13}C (Hobson, 1999), as there is a negative gradient between terrestrial – freshwater – marine habitats, and inshore/benthic-linked food webs are more enriched in ^{13}C than pelagic/offshore ones (Michener and Schell, 1994). Isotopic sulphur signature (δ^{34}S) is useful for determining the food sources of consumers (marine vs. terrestrial), because the essential sulphur-bearing compounds are incorporated into tissues without significant fractionation (Connolly et al., 2004).

The present study was performed on eggs. Terns are income breeders (Hobson, 2006); under this assumption most materials used in clutch formation are obtained directly from diet (Ruiz et al., 1998; Hobson et al., 2000), thus egg signatures reflect mainly the diet obtained in the neighboring area during this period of time. Moreover, tern eggs also reflect male foraging habitat as males provide females with prey during clutch formation (Nisbet, 1973; Wiggins and Morris, 1986; Gonzalez-Solis et al., 2001).

Here we assessed the potential exposure of an aquatic bird community that inhabits the Ebro Delta to mercury from the Flix reservoir. For this purpose, five tern species were used as bioindicators and the relationship between habitat use and mercury concentrations in clutches was examined. The first objective was to determine the trophic ecology and habitat partitioning of these species, in order to link their foraging ecology with certain habitats and compare them with the literature. The second objective was to measure mercury concentrations in tern eggs in order to establish whether values merit conservational concern. We tested the following main hypotheses; mercury concentrations are higher in terns feeding in freshwater habitats (which receive input from the Flix reservoir) than those feeding in marine and especially terrestrial habitats (as mercury bioavailability is higher in marine than terrestrial habitats). The results may be useful to define conservation policies to be applied in this area or other wetlands with similar tern communities (Fasola and Bogliani, 1990; Goutner, 1990; Peste et al., 2004; Paiva et al., 2008a). This is of particular relevance in the Ebro Delta as the project that is being undertaken to remove polluted sediments may alter the rate of sediments carried downstream.

2. Material and methods

2.1. Sampling

Egg sampling was conducted during the breeding seasons of 2006 and 2008 at several tern colonies located in the Ebro Delta (see Fig. 1). The Whiskered Tern ($n = 11$) colony sampled was located in 'La Tancada' lagoon. Little Terns ($n = 24$), Sandwich Terns ($n = 20$) and Common Terns ($n = 10$) samples were collected at 'Punta de la Banya' while Gull-Billed Terns ($n = 20$) and the other Common Tern colonies ($n = 19$) were sampled at 'Punta del Fangar'. The two Common Tern colonies were 23 km apart (36 km following the coastal line) (Fig. 1). To avoid pseudo-replication, only one egg was collected per nest. To avoid population impacts, only nests with 3 eggs were sampled for the Gull-Billed, Common, Whiskered and Little Tern, and with 2 eggs in the case of the Sandwich Tern. Eggs were collected following the protocol approved by the Ebro Delta Natural Park and with the permission of the *Serveis de Fauna i Pesca, Generalitat de Catalunya* (Spain).

Eggs were labeled and kept refrigerated until reaching the laboratory where they were kept frozen (−20 °C) until analysis. Egg content was then separated from the egg shell, weighed and placed into a glass container for freeze-drying. Freeze-dried samples were homogenized and a sub-sample was lipid-extracted for stable isotope analysis using methanol and chloroform, following Folch's method (Folch et al., 1957).

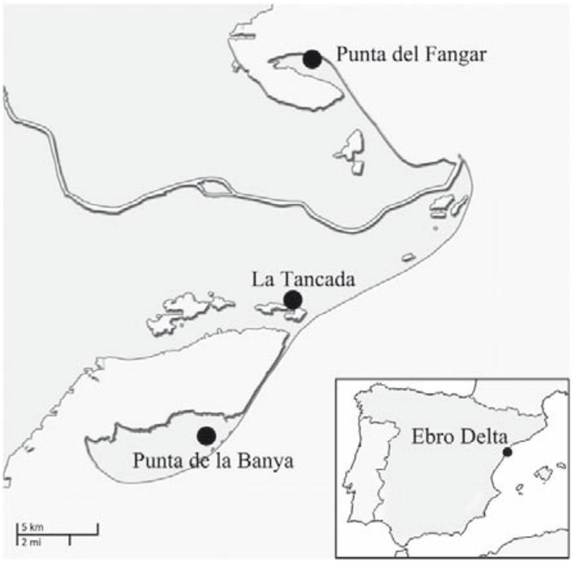


Fig. 1. Map showing the sampling sites.

2.2. Stable isotope analysis (SIA)

Lean sub-samples (ca. 0.36 mg for $\delta^{13}C$ and $\delta^{15}N$; 3.6 mg for $\delta^{34}S$) of the homogenized eggs were placed in tin buckets and crimped for combustion. Isotopic analyses were carried out by EA-IRMS (elemental analysis-isotope ratio mass spectrometry) by means of a Thermo Finnigan Flash 1112 (for C and N)/1108 (for S) elemental analyzer coupled to a Delta isotope ratio mass spectrometer via a CONFLO III interface.

Stable isotope ratios were expressed in conventional notation as parts per thousand (‰) following the equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000,$$

where X is ^{15}N or ^{13}C or ^{34}S and R is the corresponding $^{15}N/^{14}N$, $^{13}C/^{12}C$ or $^{34}S/^{32}S$ ratio. The standards for ^{15}N, ^{13}C and ^{34}S were atmospheric nitrogen (AIR), Pee Dee Belemnite (PBD) and Canyon Diablo Troilite (CDT), respectively. Precision and accuracy for $\delta^{13}C$ measurements was ≤ 0.1‰, ≤ 0–0.3‰ for $\delta^{15}N$ and ≤ 0.4 for $\delta^{34}S$. The laboratory applies international standards, which are generally run for every 12 samples: IAEA CH_7 (87% of C), IAEA CH_6 (42% of C) and USGS 24 (100% of C) for ^{13}C; IAEA N1 and IAEA N2 (with 21% of N) and IAEA NO_3 (13.8% of N) for ^{15}N; and IAEA-S1, IAEA-S2 and IAEA-S3 for ^{34}S.

2.3. Mercury

Mercury concentrations were measured on an ICP-MS Perkin Elmer ELAN 6000. Before the measurement, freeze-dried samples were digested in H_2NO_3 (3 ml) and H_2O_2 (2 ml) in Savilles Teflon digestion vessels for 12 h at 100 °C. Accuracy of analysis was checked by measuring certified reference material (Lobster hepatopancreas Tort-2 and Dogfish liver Dolt-3; National Research Council Canada). Mean recoveries ranged 98–100% for total mercury and no corrections were made. Mercury concentrations were expressed on a dry weight basis (ng g^{-1} dw).

Mercury and SIAs were performed at the *Serveis Científico-Tècnics (Universitat de Barcelona)*

2.4. Statistical methods

Mercury concentration and stable isotope ratio data were routinely checked for normal distribution. The former showed clear skewed distributions, which were normalized by applying a logarithmic transformation. The isotopic values of one Common Tern egg from 'Punta del Fangar' were considered extreme ($\delta^{15}N = 13.8$, $\delta^{13}C = -23.54$ and $\delta^{34}S = 4.38$) and were excluded from further statistical analyses and tables.

Species were compared using a one-way analysis of variance and applying the Levene test to check for homoscedasticity. The Welch correction was used accordingly. To test for "a posteriori" pairwise differences, we used SNK or Tamhane tests. Descriptive statistics and mean difference between groups and their 95% confidence intervals were used to show the results. Pearson's correlation coefficient was used to explore relationships between stable isotopes and mercury, applying the Bonferroni correction only for the whole group of species in order to maintain overall $\alpha = 0.05$. Statistical analysis was carried out using SPSS 15.0.

3. Results

Little and Whiskered Terns presented the highest variability in stable isotopes and mercury concentrations (Table 1). The comparison of the six tern colonies showed significant differences in all the stable isotopes ($\delta^{13}C$: $F_{Welch\ 5,37.2} = 793$, $p < 0.001$; $\delta^{34}S$: $F_{Welch\ 5,36.7} = 2613$, $p < 0.001$; $\delta^{15}N$: $F_{Welch\ 5,37.2} = 117$, $p < 0.001$). With regard to carbon signatures, the pairwise test showed four groups. Higher $\delta^{13}C$ values corresponded to the Little Tern, followed by the Common Tern "Fangar". The Common Tern "Banya" and the Sandwich Tern formed a third group, with significantly

Table 1
Descriptive statistics of stable isotope signatures and mercury in eggs from the five tern species included in the study (*Sternula albifrons, Gelochelidon nilotica, Chlidonias niger, Sterna sandvicensis* and *Sterna hirundo*).

		N	Mean	SD	Min	Max
$\delta^{15}N$ (‰)	Little Tern	24	14.6	0.9	12.6	16.4
	Gull-billed Tern	20	13.4	0.7	12.6	14.8
	Whiskered Tern	11	12.6	0.9	11.5	14.9
	Sandwhich Tern	20	12.1	0.3	11.3	12.7
	Common Tern 'Fangar'	18	10.9	0.3	10.2	11.4
	Common Tern 'Banya'	10	13.3	0.5	12.6	14.0
$\delta^{13}C$ (‰)	Little Tern	24	−14.6	2.1	−18.3	−11.8
	Gull-billed Tern	20	−24.8	0.5	−25.5	−23.4
	Whiskered Tern	11	−24.6	0.7	−26.2	−23.6
	Sandwhich Tern	20	−18.0	0.2	−18.3	−17.7
	Common Tern 'Fangar'	18	−17.5	0.2	−17.9	−17.1
	Common Tern 'Banya'	10	−17.9	0.2	−18.2	−17.6
$\delta^{34}S$ (‰)	Little Tern	24	9.4	3.3	4.3	15.9
	Gull-billed Tern	20	1.6	0.6	0.5	2.5
	Whiskered Tern	11	3.4	1.5	1.2	5.5
	Sandwhich Tern	20	18.5	0.3	17.8	18.8
	Common Tern 'Fangar'	18	17.0	0.6	16.0	17.8
	Common Tern 'Banya'	10	19.0	0.4	18.4	19.6
[Hg] ppb	Little Tern	24	4150.1	1973.7	1375.7	8621.9
	Gull-billed Tern	20	1770.1	699.9	879.2	3686.6
	Whiskered Tern	11	2576.6	1319.9	1119.6	5591.5
	Sandwhich Tern	20	2727.2	1264.3	1030.7	4999.8
	Common Tern 'Fangar'	18	1139.6	264.3	588.1	1663.8
	Common Tern 'Banya'	10	4148.9	1940.9	1138.5	7631.7

lower values, although these were very close to those reported for the Common Tern “Fangar”. The Whiskered and Gull-Billed Terns showed lower δ^{13}C values. The mean difference between the two Common Tern colonies was quite small (0.42‰) (Fig. 2). Sulphur signatures showed almost the same groups outlined with carbon signatures but in a different sorting order. The Common Tern “Banya” and the Sandwich Tern showed the highest. The Common Tern “Fangar” showed a mean value close to that of the first group but significantly lower. The Little Tern showed intermediate values while the lowest δ^{34}S signatures corresponded to Whiskered and Gull-Billed Terns, which were similar but significantly different (Fig. 2). With reference to nitrogen, pairwise tests showed significant differences, again delineating four groups. The highest δ^{15}N values corresponded to the Little Tern, followed by the Gull-Billed Tern and the Common Tern “Banya” with values around 13.3‰. Significantly lower signatures were observed for the Sandwich Tern and the lowest δ^{15}N corresponded to the Common Tern “Fangar”. The Whiskered Tern showed intermediate values with a relative high variability, and its mean value did not differ from those of the Sandwich Tern, Gull-Billed Tern or Common Tern “Banya” (Fig. 2).

Mean mercury concentrations were higher for the Little and Common Tern “Banya” (Table 1). Significant differences were found in mercury concentrations between the six tern colonies ($F_{5,97} = 19.841$, $p < 0.001$). We grouped terns in four categories (from higher to lower Hg concentrations): the Little Tern and Common Tern “Banya”, the Sandwich Tern and Whiskered Tern, the Gull-Billed Tern, and finally the Common Tern “Fangar” (Fig. 3).

No significant linear relationships were found among the three stable isotopes analyzed when the Bonferroni correction was applied. δ^{15}N signatures and mercury concentrations showed a linear relationship when considering all species simultaneously. This relationship was mainly due to a species-cluster effect. From an intra-species view, only the Little Tern presented a positive correlation between nitrogen and mercury ($r = 0.63$, $P = 0.001$) (Fig. 4).

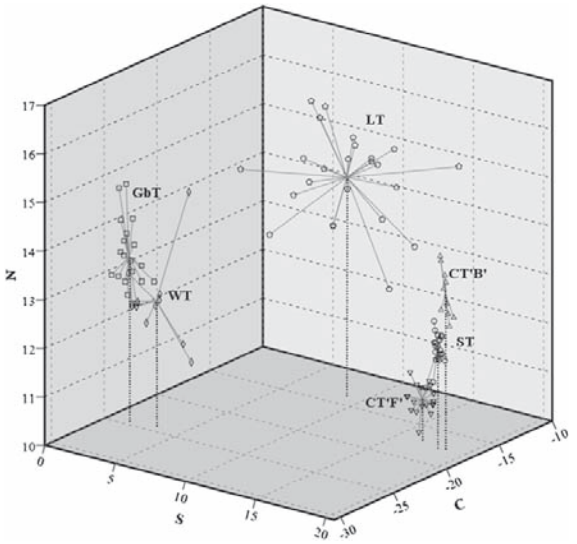


Fig. 2. 3D-scatterplot showing the distribution of δ^{15}N, δ^{34}S and δ^{13}C signatures of the five tern species: Little Tern (pentagons), Sandwich Tern (circles), Common Tern “Banya” (upwards triangles), Common Tern “Fangar” (downwards triangles), Gull-Billed Tern (squares) and Whiskered Tern (rhombus).

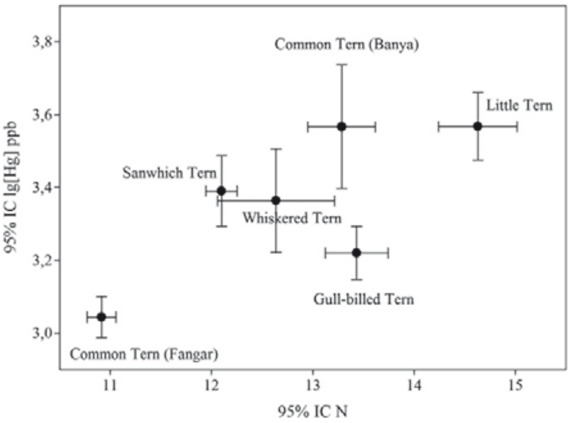


Fig. 3. Plot showing the mean position of the five tern species, according to log[Hg] ppb and δ^{15}N signatures. Dot indicates mean value, and lines their corresponding 95% confidence intervals.

4. Discussion

The isotopic values of δ^{13}C and δ^{34}S of the six tern colonies showed three distinct patterns. These were related mainly to differences in the foraging habitats exploited. The first group comprised the Sandwich and Common Tern. This isotopic pattern, characterized by high δ^{34}S and intermediate δ^{13}C signatures, was close to values reported for marine feeders in the Ebro Delta, such as those of the Audouin’s Gull, a species that relies mainly on discarded fish (Sanpera et al., 2007a) and the Yellow-legged gull, which also uses mainly marine resources at this site (Ramos et al., 2009). The Sandwich Tern has typically been described as a marine feeder (Stienen et al., 2000) and no other foraging habitat has ever been reported for this species. Although the Common Tern also feeds over freshwater in some areas (Becker et al., 1997; Neubauer, 1998), it is reported mainly as a marine forager (Becker et al., 1993; Granadeiro et al., 2002) and isotopic signatures indicate this is the

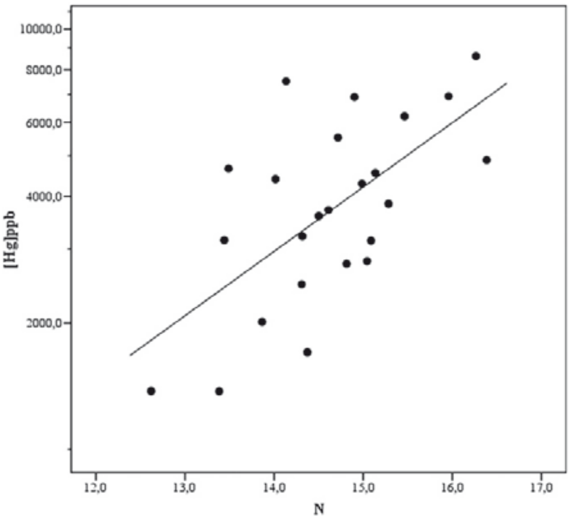


Fig. 4. Scatterplot of the dispersion between δ^{15}N signatures and mercury of the Little Tern. Relation between Hg and δ^{15}N is shown by the continuous line.

feeding strategy that this species follows in the Ebro Delta. This observation is consistent with the findings of other studies (Arcos et al., 2002; Guitart et al., 2003). However, although the slight differences in the isotopic values of $\delta^{13}C$ and $\delta^{34}S$ between the two Common Tern colonies pointed to local differences, the huge differences in $\delta^{15}N$ indicate that the colonies used different resources. In fact, while Arcos et al. (2002) reported that the Common Tern in the Ebro Delta fed mainly on epipelagic fish (Clupeiforms), Oro and Ruiz (1997) found that this species also fed on discarded fish. Both observations are consistent with the feeding strategies described by Guitart et al. (2003) and Mateo et al. (2004) for the colonies at “Punta del Fangar” (which rely mainly on epipelagic fish) and “Punta de la Banya” (where demersal resources were obtained from fish discards).

The Whiskered and Gull-Billed Terns showed low $\delta^{13}C$ and $\delta^{34}S$ signatures. The former feeds mainly on aquatic preys in wetlands (Latraube et al., 2005; Paillisson et al., 2007), and the rice fields, marshes and lagoons in the Ebro Delta are suitable foraging habitats for this species. Carbon isotopic data from heron species feeding in these habitats in the delta show very similar values (Purple heron *Ardea purpurea* $\delta^{13}C = -24.1$ $n = 16$ SD $= 0.87$; Squacco Heron *Ardeola ralloides* $\delta^{13}C = -22.9$ $n = 15$ SD $= 0.47$, unpublished data). Although reported to be a generalist feeder on a range of prey (Goutner, 1991), such as grasshoppers and caterpillars (Mathew et al., 1998), amphibians and beetles (Cabo and Sanchez, 1986), crayfish, insects and fish (Dies et al., 2005) or even fiddle crabs (*Uca tangeri*) (Stienen et al., 2008), the Gull-Billed Tern behaves like a freshwater forager in this locality, as shown by its low $\delta^{13}C$ range. The slight differences found in the $\delta^{34}S$ signatures between the two species would indicate that while the Gull-Billed Tern also exploits terrestrial habitats (with lower $\delta^{34}S$ values), in agreement with previous studies (Andersen, 1945; Bogliani et al., 1990; Sanchez et al., 1991), the Whiskered Tern may use brackish waters (increasing its S isotopic values). It is of interest to note that the Common Tern egg collected on “Punta del Fangar” that was excluded from the analysis presented isotopic values very similar to the freshwater foragers. This observation indicates individual foraging strategies, even when this foraging behavior has not been described previously for this tern population (Arcos et al., 2002; Mateo et al., 2004).

The Little Tern showed high $\delta^{13}C$ and intermediate $\delta^{34}S$ signatures. Previous studies carried out at Ria Formosa (Portugal) report on some of the factors that influence the selection of foraging habitats by this species, such as prey abundance, channels with strong currents, proximity to saltpans and conspecific information (Paiva et al., 2008a). Many studies have identified saltpans as a breeding habitat (Catry et al., 2004; Oro et al., 2004; Peste et al., 2004; Paiva et al., 2006a) and remark that this species is an opportunistic feeder and thus may use a wide variety of foraging grounds. The only limitation would be its small foraging range of 4–6 km when breeding (Fasola and Bogliani, 1990; Perrow et al., 2006). Observational data from transects in the Ebro Delta show that these birds forage in channels, lagoons and lagoon mouths, while salt marshes are avoided and rice fields are rarely visited (Bertolero et al., 2005); however, that study did not include transects in the saltpans (located at “Punta de la Banya” and where our samples were taken). $\delta^{13}C$ isotopic values for this species reflect a foraging habitat that differs to that of sea/freshwater/terrestrial habitats and that is very similar to those reported by Tavares et al. (2007) in Little Terns feeding in Portuguese saltpans. In coastal hypersaline habitats, changes in primary producers raise the $\delta^{13}C$ signature (Michener and Schell, 1994). This finding indicates that Little Terns at “Punta de la Banya” feed mainly in the saltpans, which is the main habitat around the colony. Moreover, the high variance in their isotopic values also indicates the use of other habitats to a lesser extent, thus pointing to individual foraging strategies. Furthermore, the $\delta^{15}N$ values of this species were the highest of the tern species examined. As the diet of the Little Tern is based mainly on fish and prawns (Norman, 1992; Bogliani et al., 1994; Paiva et al., 2006b), the high $\delta^{15}N$ signatures are probably the result of an increased basal signature in this habitat rather than differences in the trophic levels occupied.

Regarding mercury concentrations, our results indicate that the influence from riverine inputs from the Flix reservoir into the freshwater habitats of the Ebro Delta is lower than the mercury input to the marine foodwebs. Nevertheless, mercury derived from intensive agricultural activities in the wetland system (Mañosa et al., 2001) and from the industry located along the riverside, specially in the Flix Reservoir (Barata et al., 2010), may have accumulated in the sand banks of the Ebro Delta over the years, especially in the southern hemidelta, as materials carried by the Ebro river are mainly deposited there by the Liguro-Provençal-Catalan oceanic current (Guitart et al., 2003). However, riverine inputs have probably increased the mercury baseline of neighboring marine foodwebs, as has been reported for Audouin’s Gull chicks in the Ebro Delta when compared with other colonies elsewhere in the Mediterranean (Sanpera et al., 2007a). Mercury concentrations in freshwater foragers (Whiskered Tern) are related mainly to the river contaminant input and are in the same range as those reported for the Purple Heron and the Little Egret at this site (Barata et al., 2010). In contrast, the Gull-Billed Tern, which shows partial terrestrial habitat use, showed lower mercury concentrations. Differences in mercury concentrations between the marine species may be related to their differential bioavailability in marine ecosystems, as demersal fish reach higher mercury loads than epipelagic ones (Arcos et al., 2002); thus, Common Terns sampled in “Punta de la Banya”, which have access to fish discards, had the highest mercury concentrations of all the marine feeders. These concentrations were close to those reported for Audouin’s Gull eggs at the same locality, a species that also feeds on fish discards (Morera et al., 1997). Higher mercury concentrations have been reported only for the Common Tern in the Elbe estuary (Becker et al., 1993a), a heavily polluted area. Other studies on mercury content of the Common Tern feeding mainly on epipelagic fish in several sites in Europe and North America ranged from 800 to almost 2000 ppb (Monteiro et al., 1999; Guitart et al., 2003; Becker and Munoz Cifuentes, 2004; Burger and Gochfeld, 2006; Bond and Diamond, 2009), with most values around 1000 ppb. Although the Sandwich Tern feeds on epipelagic fish, its larger size may allow capture of bigger prey with higher pollutant loads. Finally, the Little Tern presented the highest mercury concentrations. This observation is attributed to a different mercury baseline in the saltpan food webs. Relatively high concentrations of this pollutant have also been found in Little Terns feeding in saltpans in Portugal (Tavares et al., 2007; Paiva et al., 2008b). The concentrations in those birds were found to be higher than those of Little Terns in the Baltic Sea feeding mainly in marine ecosystems (Thyen et al., 2000).

Finally, the relatively high mercury concentrations in the Little Tern and Common Tern colonies in “Punta de la Banya” may be reaching critical values that endanger breeding success. However, embryos of Charadriformes species show an intermediate range sensitivity to mercury compared with other bird families such as Anseriformes (higher sensitivity) or Falconiformes (lower sensitivity) (Heinz et al., 2009). Thus studies to evaluate these effects are required. The striking variability observed in $\delta^{15}N$ signatures for Little Tern allowed us to detect a clear relationship between mercury and nitrogen signatures (i.e. trophic level), a predictable observation given the known underlying biomagnification process that occurs for mercury.

In summary, the tern community in the Ebro Delta reveals outstanding differences in habitat use as a result of niche segregation. These differences allowed us to interpret their mercury concentrations. In this regard, mercury load was found to be unexpectedly higher in terns feeding in marine habitats than in freshwater ones. We propose that this finding is associated with decades of accumulation of polluted sediments from the river in the sand banks of the Ebro Delta. Potential alterations induced by changes in the fisheries, saltpan and/or crop management should be adequately evaluated to preserve the diverse tern community inhabiting this area. Moreover, further research into mercury concentrations in seabirds feeding in marine habitats and saltpans is recommended. Given the mercury concentrations we found in eggs of Little and Common Terns, further studies are of particular relevance in order to monitor whether the mercury load of these species has become a conservation concern.

Acknowledgments

The authors wish to acknowledge the kindly cooperation of the personnel at the Parc Natural del Delta de l'Ebre for the facilities during fieldwork. Thanks are given to Sergi Ferrer and the staff at the *Serveis científico-tècnics* (UB, Spain) for their help during the sample collection and laboratory analysis. Javier Cotín was supported by a BRD grant from the *Universitat de Barcelona* (Spain). The present work was funded by the project 'Metalls pesants i organoclorats en el curs inferior i delta del riu Ebre: La ornitofauna com a bioindicadora (*Ministerio de Medio Ambiente*, Spain, *Agència Catalana de l'Aigua*, *Departament de Medi Mabient i Habitatge*, *Generalitat de Catalunya*) and by Fundación BBVA (BIOCON06/113; project EMECO).

References

Andersen, J., 1945. The food of the Gull-Billed Tern, *Gelochelidon nilotica* (Gm.). Dansk Ornithologisk Forenings Tidsskrift 39, 198–205.

Arcos, J.M., Ruiz, X., Bearhop, S., Furness, R.W., 2002. Mercury levels in seabirds and their fish prey at the Ebro Delta (NW Mediterranean): the role of trawler discards as a source of contamination. Marine Ecology Progress Series 232, 281–290.

Barata, C., Fabregat, M.C., Cotin, J., Huertas, D., Sole, M., Quiros, L., Sanpera, C., Jover, L., Ruiz, X., Grimalt, J.O., Pina, B., 2010. Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain). Environmental Pollution 158, 704–710.

Becker, P.H., Frank, D., Sudmann, S.R., 1993. Temporal and spatial pattern of Common Tern (*Sterna hirundo*) foraging in the Wadden sea. Oecologia 93, 389–393.

Becker, P.H., Frank, D., Wagener, M., 1997. Luxury in freshwater and stress at sea? The foraging of the Common Terra *Sterna hirundo*. Ibis 139, 264–269.

Becker, P.H., Schuhmann, S., Koepff, C., 1993a. Hatching failure in Common Terns (*Sterna hirundo*) in relation to environmental chemicals. Environmental Pollution 79, 207–213.

Becker, P.H., Furness, R.W., Henning, D., 1993b. Mercury dynamics in young Common Tern (*Sterna hirundo*) chicks from a polluted environment. Ecotoxicology 2, 33–40.

Becker, P.H., Munoz Cifuentes, J., 2004. Contaminants in bird eggs. Recent spatial and temporal trends. Wadden Sea Ecosystem 18, 5–24.

Bertolero, A., Oro, D., Martinez Vilalta, A., Lopez, M.A., 2005. Selection of foraging habitats by Little Terns *Sterna albifrons* at the Ebro delta (NE Spain). Revista Catalana d'Ornitologia 21, 37–42.

Bogliani, G., Fasola, M., Canova, L., Saino, N., 1990. Food and foraging rhythm of a specialized Gull-Billed Tern population *Gelochelidon nilotica*. Ethology Ecology & Evolution 2, 175–181.

Bogliani, G., Fasola, M., Canova, L., Saino, N., 1994. Prey selection by parents and chicks of the Little Tern *Sterna albifrons*. Avocetta 18, 9–11.

Bond, A.L., Diamond, A.W., 2009. Mercury concentrations in seabird tissues from Machias Seal Island, New Brunswick, Canada. Science of the Total Environment 407, 4340–4347.

Bridge, E.S., Jones, A.W., Baker, A.J., 2005. A phylogenetic framework for the terns (Sternini) inferred from mtDNA sequences: implications for taxonomy and plumage evolution. Molecular Phylogenetics and Evolution 35, 459–469.

Burger, J., Gochfeld, M., 2006. Temporal trends in mercury, cadmium and lead in eggs of Common Terns in New Jersey, USA, 1971–2005. Journal of Ornithology 147, 144.

Burger, J., Nisbet, I.C.T., Gochfeld, M., 1992. Metal levels in regrown feathers – Assessment of contamination on the wintering and breeding grounds in the same individuals. Journal of Toxicology and Environmental Health 37, 363–374.

Cabo, J.M., Sanchez, J.M., 1986. Recent Information on *Gelochelidon nilotica* in Morocco and its diet. Alauda 54, 207–212.

Catry, T., Ramos, J.A., Catry, I., Allen-Revez, M., Grade, N., 2004. Are salinas a suitable alternative breeding habitat for Little Terns *Sterna albifrons*? Ibis 146, 247–257.

Catry, T., Ramos, J.A., Le Corre, M., Kojadinovic, J., Bustamante, P., 2008. The role of stable isotopes and mercury concentrations to describe seabird foraging ecology in tropical environments. Marine Biology 155, 637–647.

Cherel, Y., Le Corre, M., Jaquemet, S., Menard, F., Richard, P., Weimerskirch, H., 2008. Resource partitioning within a tropical seabird community: new information from stable isotopes. Marine Ecology Progress Series 366, 281–291.

Connolly, R.M., Guest, M.A., Melville, A.J., Oakes, J.M., 2004. Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia 138, 161–167.

Dies, J.I., Marin, J., Perez, C., 2005. Diet of nesting Gull-Billed Terns in eastern Spain. Waterbirds 28, 106–109.

Fasola, M., Bogliani, G., 1990. Foraging ranges of an assemblage of Mediterranean seabirds. Colonial Waterbirds 13, 72–74.

Fernandez, M., Alonso, C., Gonzalez, M., Hernandez, L., 1999. Occurrence of organochlorine insecticides, PCBs and PCB congeners in waters and sediments of the Ebro River (Spain). Chemosphere 38, 33–43.

Ferrer, X., Martinez-Vilalta, A., 1986. Fluctuations of the gull and tern populations in the Ebro Delta, north-east Spain (1960–1985). In: Medmaravis, Monbailliu, X. (Eds.), Mediterranean Marine Avifauna, vol. 12. Springer-Verlag, Berlín y Heidelberg, pp. 273–284.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226, 497–509.

Forero, M.G., Gonzalez-Solis, J., Hobson, K.A., Doncazar, J.A., Bertellotti, M., Blanco, G., Bortolotti, G.R., 2005. Stable isotopes reveal trophic segregation by sex and age in the southern giant petrel in two different food webs. Marine Ecology-Progress Series 296, 107–113.

Forero, M.G., Hobson, K.A., 2003. Using stable isotopes of nitrogen and carbon to study seabird ecology: applications in the Mediterranean seabird community. Scientia Marina 67, 23–32.

Gonzalez-Solis, J., Sokolov, E., Becker, P.H., 2001. Courtship feedings, copulations and paternity in Common Terns, *Sterna hirundo*. Animal Behaviour 61, 1125–1132.

Goutner, V., 1991. Food and feeding ecology of Gull-Billed terns (*Gelochelidon nilotica*) in Greece. Revue d'Ecologie (La Terre Et La Vie) 46, 373–384.

Goutner, V., 1990. Habitat selection of Little Terns in the Evros delta, Greece. Colonial Waterbirds 13, 108–114.

Granadeiro, J.P., Monteiro, L.R., Silva, M.C., Furness, R.W., 2002. Diet of Common Terns in the Azores, Northeast Atlantic. Waterbirds.

Guitart, R., Mateo, R., Sanpera, C., Hernandez-Matias, A., Ruiz, X., 2003. Mercury and selenium levels in eggs of Common Terns (*Sterna hirundo*) from two breeding colonies in the Ebro Delta, Spain. Bulletin of Environmental Contamination and Toxicology 70, 71–77.

Heinz, G.H., Hoffman, D.J., Klimstra, J.D., Stebbins, K.R., Kondrad, S.L., Erwin, C.A., 2009. Species differences in the sensitivity of avian embryos to methylmercury. Archives of Environmental Contamination and Toxicology 56, 129–138.

Hobson, K., 2006. Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. Ardea 94, 359–369.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120, 314–326.

Hobson, K.A., Piatt, J.F., Pitocchelli, J., 1994. Using stable isotopes to determine seabird trophic relationships. Journal of Animal Ecology 63, 786–798.

Hobson, K.A., Sirois, J., Gloutney, M.L., 2000. Tracing nutrient allocation to reproduction with stable isotopes: a preliminary investigation using colonial waterbirds of Great Slave Lake. Auk 117, 760–774.

Koiadinovic, J., Menard, F., Bustamante, P., Cosscon, R.P., Le Corre, M., 2008. Trophic ecology of marine birds and pelagic fishes from Reunion Island as determined by stable isotope analysis. Marine Ecology-Progress Series 361, 239–251.

Latraube, F., Trotignon, J., Bretagnolle, V., 2005. Breeding biology of Whiskered tern *Chlidonias hybridus* in Brenne (France). Alauda 73, 425–429.

Llorente, G., Farran, A., Ruiz, X., Albaiges, J., 1987. Accumulation and distribution of hydrocarbons, polychlorobyphenyls, and DDT in tissues of 3 species of Anatidae from the Ebro Delta (Spain). Archives of Environmental Contamination and Toxicology 16, 563–572.

Mañosa, S., Mateo, R., Guitart, R., 2001. A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. Environmental Monitoring and Assessment 71, 187–205.

Mateo, R., Gil, C., Badia-Vila, M., Guitart, R., Hernandez-Matias, A., Sanpera, C., Ruiz, X., 2004. Use of fatty acids to explain variability of organochlorine concentrations in eggs and plasma of Common Terns (*Sterna hirundo*). Ecotoxicology 13, 545–554.

Mathew, K.L., Lukose, C., Pethani, K.V., Dave, H.R., 1998. Foraging behaviour of the Gull-Billed Tern, *Sterna nilotica*. Pavo 36, 15–18.

Michener, R.H., Schell, D.M., 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha, K., Michener, R.H. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publications, London, pp. 138–157.

Monteiro, L.R., Granadeiro, J.P., Furness, R.W., Oliveria, P., 1999. Contemporary patterns of mercury contamination in the Portuguese Atlantic inferred from mercury concentrations in seabird tissues. Marine Environmental Research 47, 137–156.

Morera, M., Sanpera, C., Crespo, S., Jover, L., Ruiz, X., 1997. Inter and intraclutch variability in heavy metals and selenium levels in Audouin's gull eggs from the Ebro Delta, Spain. Archives of Environmental Contamination and Toxicology 33, 71–75.

Neubauer, W., 1998. Habitat choice of the Common Tern *Sterna hirundo* in eastern Germany. Vogelwelt 119, 169–180.

Nisbet, I.C.T., Montoya, J.P., Burger, J., Hatch, J.J., 2002. Use of stable isotopes to investigate individual differences in diets and mercury exposures among Common Terns *Sterna hirundo* in breeding and wintering grounds. Marine Ecology Progress Series.

Nisbet, I.C.T., 1973. Courtship-feeding, egg-size and breeding success in Common Terns. Nature 241, 141–142.

Norman, D., 1992. The growth rate of Little Tern *Sterna albifrons* chicks. Ringing and Migration 13, 98–102.

Oro, D., Bertolero, A., Vilalta, A.M., Lopez, M.A., 2004. The biology of the Little Tern in the Ebro delta (northwestern Mediterranean). Waterbirds 27, 434–440.

Oro, D., Ruiz, X., 1997. Exploitation of trawler discards by breeding seabirds in the north-western Mediterranean: differences between the Ebro Delta and the Balearic islands areas. ICES Journal of Marine Science 54, 695–707.

Paillisson, J.M., Reeber, S., Carpentier, A., Marion, L., 2007. Reproductive parameters in relation to food supply in the Whiskered Tern (*Chlidonias hybrida*). Journal of Ornithology 148, 69–77.

Paiva, V.H., Ramos, J.A., Catry, T., Pedro, P., Medeiros, R., Palma, J., 2006a. Influence of environmental factors and energetic value of food on Little Tern *Sterna albifrons* chick growth and food delivery. Bird Study 53, 1–11.

Paiva, V.H., Ramos, J.A., Machado, D., Penha-Lopes, G., Bouslama, M.F., Dias, N., Nielsen, S., 2006b. Importance of marine prey to growth of estuarine tern chicks: evidence from an energetic balance model. Ardea 94, 241–255.

Paiva, V.H., Ramos, J.A., Martins, J., Almeida, A., Carvalho, A., 2008a. Foraging habitat selection by Little Terns *Sternula albifrons* in an estuarine lagoon system of southern Portugal. Ibis 150, 18–31.

Paiva, V.H., Tavares, P.C., Ramos, J.A., Pereira, E., Antunes, S., Duarte, A.C., 2008b. The influence of diet on mercury intake by Little Tern chicks. Archives of Environmental Contamination and Toxicology 55, 317–328.

Pastor, D., Sanpera, C., Gonzalez-Solis, J., Ruiz, X., Albaiges, J., 2004. Factors affecting the organochlorine pollutant load in biota of a rice field ecosystem (Ebro Delta, NE Spain). Chemosphere 55, 567–576.

Perrow, M.R., Skeate, E.R., Lines, P., Brown, D., Tomlinson, M.L., 2006. Radio telemetry as a tool for impact assessment of wind farms: the case of Little Terns *Sterna albifrons* at Scroby Sands, Norfolk, UK. Ibis 148, 57–75.

Peste, F., Trigo, S., Luis, A., 2004. Foraging behaviour of breeding Little Tern *Sterna albifrons* at Ria de Aveiro. Airo 14, 55–62.

Quiros, L., Ruiz, X., Sanpera, C., Jover, L., Pina, B., 2008. Analysis of micronucleated erythrocytes in heron nestlings from reference and impacted sites in the Ebro basin (NE Spain). Environmental Pollution 155, 81–87.

Ramos, R., Ramirez, F., Sanpera, C., Jover, L., Ruiz, X., 2009. Feeding ecology of Yellow-legged Gulls *Larus michahellis* in the western Mediterranean: a comparative assessment using conventional and isotopic methods. Marine Ecology Progress Series 377, 289–297.

Ruiz, X., Jover, L., Pedrocchi, V., 1998. Audouin's gull intraspecific variability in yolk and albumen egg contents: why is yolk size so constant? Biologia e Conservazione della Fauna, 327–334.

Sanchez, J.M., Munoz del Viejo, A., de la Cruz, C., 1991. Segregacion alimentaria entre adultos y pollos de Gelochelidon nilotica (Gm., 1789) en la laguna de Fuente de Piedra. Ardeola 38, 21–27.

Sanpera, C., Moreno, R., Ruiz, X., Jover, L., 2007a. Audouin's gull chicks as bioindicators of mercury pollution at different breeding locations in the western Mediterranean. Marine Pollution Bulletin 54, 691–696.

Sanpera, C., Ruiz, X., Moreno, R., Jover, L., Waldron, S., 2007b. Mercury and stable isotopes in feathers of Audouin's Gulls as indicators of feeding habits and migratory connectivity. Condor 109, 268–275.

Stienen, E.W.M., Van Beers, P.W.M., Brenninkmeijer, A., Habraken, J.M.P.M., Raaijmakers, M.H.J.E., Van Tienen, P.G.M., 2000. Reflections of a specialist: patterns in food provisioning and foraging conditions in Sandwich Terns *Sterna sandvicensis*. Ardea 88, 33–49.

Stienen, E.W.M., Brenninkmeijer, A., Klaassen, M., 2008. Why do Gull-Billed terns *Gelochelidon nilotica* feed on fiddler crabs *Uca tangeri* in Guinea-Bissau. Ardea 96, 243–250.

Tavares, P.C., Kelly, A., Lopes, R.J., Pereira, M.E., Duarte, A.C., Furness, R.W., 2007. The influence of dietary specialization and trophic status on mercury levels in two species using common coastal wetlands, *Himantopus himantopus* and *Sterna albifrons*. Ardeola 54, 275–288.

Tavares, P.C., McGill, R., Newton, J., Pereira, E., Duarte, A., Furness, R.W., 2009. Relationships between carbon sources, trophic level and mercury exposure in generalist shorebirds revealed by stable isotope ratios in chicks. Waterbirds 32, 311–321.

Thyen, S., Becker, P.H., Behmann, H., 2000. Organochlorine and mercury contamination of Little Terns (*Sterna albifrons*) breeding at the western Baltic Sea, 1978–1996. Environmental Pollution 108, 225–238.

Wiggins, D.A., Morris, R.D., 1986. Criteria for female choice of mates – Courtship feeding and paternal care in the Common Tern. American Naturalist 128, 126–129.

IV

Who eats where?
Insights into freshwater and marine bird communities of the Ebro Delta (NE Spain) through Stable Isotopes and Trace Elements

¿Quién come dónde? Estudio de las comunidades orníticas marinas y dulceacuícolas del Delta del Ebro (NE España) a través de los Isótopos Estables y los Elementos Traza

Javier Cotín[a], Manuel García-Tarrasón[a], Lluis Jover[b] & Carolina Sanpera[a].

[a] Department Biologia Animal, Facultat de Biologia, Univesitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain

[b] Department Salut Pública, Facultat de Medicina, Universitat de Barcelona, Casanova 143, 08036 Barcelona, Spain

---

Resumen: El Delta del Ebro es uno de los principales núcleos avifaunísticos de la Península Ibérica. Es además uno de los mayores humedales de la región mediterránea occidental y presenta una gran variedad de hábitats donde conviven una rica comunidad de aves. Este humedal está expuesto a diferentes fuentes de contaminación, especialmente la derivada de la utilización de productos fitosanitarios y la arrastrada por el río Ebro de una industria química situada aguas arriba, en Flix. Con el fin de aumentar el conocimiento de la ecología trófica y la repartición de hábitats de la comunidad de aves, se utilizaron los isótopos estables (SIA) ($\delta^{15}N$ y $\delta^{13}C$) y medidas de nicho (SEA_c) en huevos de especies de la familia Sternidae, Ardeidae, Laridae y Rallidae, como representantes de toda la comunidad de aves. Se midieron también los niveles de mercurio, selenio, cobre, plomo, cadmio, antimonio, cromo y arsénico, para establecer si dichos niveles se encuentran dentro de los rangos de alerta medioambiental. El Charrán común y patinegro se comportan como especialistas estrictos, presentando nichos tróficos estrechos, mientras que el Charrancito, la Garceta común y la Garza imperial, el Martinete y la Gallineta de agua se comportan como generalistas, tienen amplios nichos tróficos. Los niveles de selenio, cobre, plomo, cadmio, antimonio y arsénico en el Delta del Ebro están fuera de preocupación conservacionista y dentro del rango de valores de otras comunidades avifaunísticas, mientras que los niveles de cromo son mayores que en otras áreas, para todas las especies estudiadas y hábitats, pero aún así por debajo de los niveles tóxicos. Los niveles de mercurio detectados en huevos de Gaviota de Audouin, Charrancito y Charrán común 'Banya' son elevados, y más estudios serían necesarios para comprobar si el éxito reproductor de estas especies se encuentra amenazado. El arsénico, especialmente usado en combinación con $\delta^{13}C$, discrimina notablemente entre especies marinas y dulceacuícolas, presentando las especies marinas niveles más altos de arsénico y signaturas de carbono que las especies de agua dulce. Los charranes y garzas muestran un presumible patrón filogenético de acumulación de mercurio y cobre, con concentraciones más altas de mercurio y bajos de cobre en charranes, siendo del modo opuesto en garzas.

Palabras clave: Aves acuáticas, Ecología trófica, Biomonitorizar, Isótopos Estables, Elementos traza, Delta del Ebro, Mediterráneo.

Who eats where? Insights into freshwater and marine bird communities of the Ebro Delta (NE Spain) through Stable Isotopes and Trace Elements

❧

Javier Cotín[a], Manuel García-Tarrasón[a], Lluis Jover[b] & Carolina Sanpera[a].

[a] Department Biologia Animal, Facultat de Biologia, Univesitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain

[b] Department Salut Pública, Facultat de Medicina, Universitat de Barcelona, Casanova 143, 08036 Barcelona, Spain

---

Abstract: The Ebro Delta is one of the main bird hotspots of the Iberian Peninsula. Being one of the largest wetlands in the western Mediterranean region, presents a wide variety of habitats and is home of a rich bird community. This wetland is exposed to different sources of contamination, specially the ones derived from the agricultural activities in the area and from the residues carried down by the river, in particular those from a chemical industry located 90 km upstream, in the Flix dam. In order to provide new insights into the trophic ecology, niche and habitat partitioning of the bird community, we used stable isotopes analysis (SIA) ($\delta^{15}N$ and $\delta^{13}C$) and ellipse-based niche metric (SEA_c) in eggs of selected species from the family Sternidae, Ardeidae, Laridae and Rallidae, as representatives of the whole bird community. Also mercury, selenium, copper, lead, cadmium, antimony, chromium and arsenic levels were measured, to establish whether values merit conservational concern. Common and Sandwich Tern behave as strict specialists, having narrow niche widths, while Little Tern, Little Egret, Purple Heron, Night Heron and Moorhen behave as generalists foragers, with higher niche widths. The levels of selenium, copper, lead, cadmium, antimony and arsenic at the Ebro Delta are out of conservational concern and range within the values of other communities, while chromium levels at the Ebro Delta are higher than in other areas, for all species and habitats, but still below toxic levels. Mercury levels detected for Audouin's Gull, Little Tern and Common Tern 'Banya' eggs are high at the Ebro Delta and further studies are of particular relevance in order to monitor whether these values are endangering the breeding success of these species. Arsenic, specially used in combination with $\delta^{13}C$, discriminates outstandingly between marine and freshwater species, with marine related species presenting higher levels of arsenic and carbon signatures than the freshwater species. Terns and Herons showed great differences in levels of mercury and copper: high mercury and low copper loads for Terns and opposite in Herons. These differences are presumably phylogenetically based.

Keywords: Aquatic birds, Trophic Ecology, Biomonitoring, Stable Isotopes, Trace Elements, Ebro Delta, Mediterranean.

Introduction

Birds are among the most studied animals on earth; and so are the communities that they conform (Fasola et al., 2010). Interactions among species, resources and habitat partitioning, using biomarkers as stable isotopes, have been widely studied (Forero et al., 2004, Catry et al., 2008, Cherel et al., 2008). Also, the exposure of bird communities to pollutants has been a subject that has attracted great interest (Evers et al., 2005, Bond and Diamond, 2009), since birds have demonstrated to be reliable bioindicators (Sanpera et al., 2008). Also the use of biomarkers combined with chemical analyses has allowed in recent years to assess and identify the origin of adverse health effects produced by different pollutants on several bird communities (Tavares et al., 2009, Ramirez et al., 2011).

The Ebro Delta (40°42'N, 00°50'E) is one of the largest wetlands in the western Mediterranean region, occupying an area of 320 km2, and is home to extensive bird colonies (c.a. 12000 pairs of Audouin's Gull, 3000 of Flamingo, 400 of Purple Heron, 350 of Little Tern, 5000 of Common Tern, 500 of Gull-billed Tern, 2000 of Sandwich Tern and 1200 of Whiskered Tern among others). It presents a wide variety of habitats: at the inner part of the delta and at both river beds, the ricefields and farmland, connected to the river through channels; at the coastline, abandoned fields, lagoons, salt marshes and beaches; on each side of the Ebro Delta, surrounded by sea, there are two arrow-shaped sandy areas, known as the 'Punta del Fangar' and 'Punta de la Banya' (with an extension of 500 and 2500 ha respectively), the latter hosting a saltpan industry. Finally, the open sea and the northern and southern bays, molded by the two 'Puntas', conform the patchwork of habitats. The coastal lagoons, although connected to the sea and thus expected to hold brackish water, receive considerable freshwater input from the rice fields from spring to autumn, thereby lowering their salinity, which almost reaches that of freshwater during those seasons.

All those habitats constitute the breeding ground for a rich bird community. Herons (family Ardeidae) and terns (family Sternidae) are well represented in the area, and these families are suitable candidates for biomonitoring the pollution affecting the whole bird community. These species are piscivorous and top predatory birds, show a predictable breeding phenology and strong nest site fidelity, exploit a diversity of habitats, and have been successfully used as bioindicators of environmental pollution in aquatic systems (Becker et al., 1993, De Luca-Abbott et al., 2001, Nisbet et al., 2002, Connell et al., 2003, Guitart et al., 2003, Champoux et al., 2006, Sakellarides et al., 2006, Paiva et al., 2008b, Cotin et al., 2011, Cotin et al., 2012). Since they feed in the upper trophic levels of these ecosystems they biomagnify and bioaccumulate some of the pollutants presents there (Baker and Sepulveda, 2009).

The main breeding heron populations at the Ebro River and Delta include Purple Heron (*Ardea purpurea*), Squacco Heron (*Ardeola ralloides*), Night Heron (*Nycticorax nycticorax*), Little Egret (*Egretta garzetta*) and Little Bittern (*Ixobrychus minutus*). These species are mainly fish predatory species, although can feed on other prey, such as crayfish in the case of the Little Bittern (Pardo-Cervera et al., 2010) or insects in the case of the Squacco Heron (Delord et al., 2004). They are mainly associated with freshwater habitats, but can also feed in different habitats such as brackish waters or even saltpans, as does the Little Egret (Abdennadher et al., 2011).

Terns are gull-related seabirds, which comprise 45 species (most of them migratory) grouped in 12 genera (Bridge et al., 2005) found worldwide. Five tern species breed in the Ebro Delta: Common Tern (*Sterna hirundo*), Sandwich Tern (*Sterna sandvicensis*), Little Tern (*Sternula albifrons*), Gull-billed Tern (*Gelochelidon nilotica*) and Whiskered Tern (*Chlydonias hybrida*). A wide variety of foraging habitats has been reported for these species: Freshwater habitats for the Whiskered Tern (Latraube et al., 2005, Paillisson et al., 2007); marine for the Sandwich Tern (Stienen et al., 2000) and both marine and freshwater for the Common Tern (Becker et al., 1997, Neubauer, 1998). The last two species have been described as generalist foragers. The Little Tern uses several foraging habitats (sea, lakes, channels, salinas, etc.) in Portugal (Paiva et al., 2008a), and in the Ebro Delta it has been observed foraging in channels, lagoons and lagoon mouths (Bertolero et al., 2005). The Gull-billed Tern feeds in terrestrial (Cabo and Sanchez, 1986), freshwater (Dies et al., 2005, Cotin et al., 2011), and even marine habitats (Stienen et al., 2008).

Additionally we sampled four additional species to complete the spectrum of bird families and habitats: two species from the family Laridae (Gulls) and two from the Rallidae (Rails, Moorhens…). There are several species of gulls breeding at the Ebro Delta, being the most common species the Yellow-legged Gull (*Larus michahellis*), Audouin's Gull (*Larus audouinii*), Black-headed Gull (*Larus ridibundus*), Slender-billed Gull (*Larus genei*) and the Lesser Black-backed Gull (*Larus fuscus*), among others. For this study we selected the Audouin's and Black-headed Gull, while the Yellow-legged Gull was not considered due to its use of crops and refuse tips (Ramos et al., 2009). The Audouin's Gull is a nocturnal predator on epipelagic fish, but can take advantage of fishery activities; especially trawler discards (Oro and Ruiz, 1997, Sanpera et al., 2007). Black-headed Gull is a species that can exploit marine, freshwater and terrestrial habitats (Schwemmer et al., 2011), being an opportunistic forager. The two rallidae species included in this study are the Moorhen and the Purple Swamphen. These two species are, although omnivorous, mainly herbivorous, with the Purple Swamphen feeding on seeds, bulbs, stem and leaves from reeds (*Phragmites australis*), cattail (*Thypa latifolia*) and rice (*Oryza sativa*) (Cramp, 1980). Moorhen also feed mainly on plant material (Amat and Soriguer, 1984).

The aquatic bird community of the Ebro Delta is exposed to different sources of contamination, one derived from the local use of plaguicides, and the other one brought down by the river. One of main origins of the latter is a chemical industry located upstream the Ebro River, situated close to Flix (41°14'N, 00°31'E) and that has been in operation since the early 20th century. This long operational period, along with the construction of a dam next to the plant around 1960, resulted in the accumulation of 200,000–360,000 tons of industrial wastes in the riverbed, occupying an area of 700 m of length and 60 m of width. The mixture of heavily polluted sediments is composed of high concentrations of heavy metals (cadmium, arsenic, copper, chromium, selenium, lead and especially mercury), organochlorines (hexachlorobenzene, pentachlorobenzene, DDTs, polychlorobiphenyls, polychloronaphthalenes and polychlorostyrenes) and radioactive 210Pb (Fernandez et al., 1999, Bosch et al., 2009). In addition, the Ebro River is currently affected by different agricultural and industrial activities, with significant impact on the existing biota (Mañosa et al., 2001, Navarro et al., 2010a). The pollutants originated at the Flix site are carried downstream by the Ebro River to its delta 90 km away (Llorente et al., 1987, Pastor et al., 2004, Navarro et al., 2009), especially during floods (Vericat and Batalla, 2006).

Biogeochemical markers such as stable isotopes have been successfully applied in marine and freshwater ecosystems to yield valuable insights into feeding ecology, particularly as they provide a potential means to quantify assimilated, rather than simply ingested diet. Stable isotope signatures of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) have been extensively used in studies of bird communities, focusing on their trophic ecology and relationships (Hobson et al., 1994, Forero and Hobson, 2003, Cherel et al., 2008, Koiadinovic et al., 2008, Moreno et al., 2010). $\delta^{15}N$ of tissues reflects the trophic level, with consumer signatures being higher than in their prey (Forero et al., 2005). Information about the source of carbon entering a food web can be obtained from $\delta^{13}C$ (Hobson, 1999), providing insight about the foraging habitat.

In addition to this, stable isotopes ratios can be used to define an 'isotopic niche'. The ecological niche concept has undergone a renaissance in recent years (Newsome et al., 2007). Although trophic niche and isotopic niche differ, both are tightly related, and data presented as bi-plots, where the isotopic values of animal tissues are represented in a δ-space, delineate a species isotopic niche. Jackson et al. (2011) proposed recently a novel multivariate ellipse-based niche (or community) metric, reformulating the previous Convex Hull Total Area (TA) defined by Layman et al. (2007) in a Bayesian framework. This new metric, the corrected area of the standard ellipse (SEA_c), gives an indication of niche width using summary information of the spread and extent of the isotopic data points and is the one used in this present work.

The present study was performed on eggs. Data obtained from egg samples integrate the adult diet prior to egg laying because herons, gulls and terns, as income breeders, obtain most of the materials used in clutch formation from diet (Ruiz et al., 1998, Hobson et al., 2000). Moreover, tern and gull eggs also reflect male foraging habitat as males provide females with prey during clutch formation (Nisbet, 1973, Wiggins and Morris, 1986, Salzer and Larkin, 1990, Neuman et al., 1998, Gonzalez-Solis et al., 2001).

Therefore, the main aim of this study was to provide new insights into the trophic ecology, niche and habitat partitioning of the selected species of the aquatic bird community of the Ebro Delta. Secondly, pollutant levels in eggs were measured in order to establish whether values merit conservational concern, once determining the trophic ecology of these populations, in order to appropriately assess the pollutant exposure.

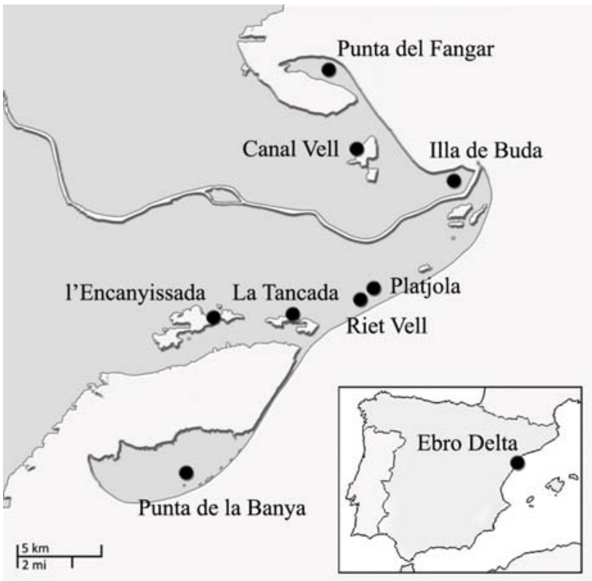


Figure 1. Map showing the sampling sites

Material and Methods

Sampling

Egg sampling was conducted during the breeding seasons of 2006, 2007 and 2008 at several colonies located in lagoons of the northern and southern hemidelta and at the two sandy 'Puntas' of the Ebro Delta (see Fig.1). Number of eggs sampled per species is shown in table 1. The two Common Tern colonies were 23 km apart (36 km following the coastal line) and were considered separately (Fig. 1). To avoid pseudo-replication, only one egg was collected per nest. To minimize impacts on pair productivity and to ensure that only clutches from good condition individuals were sampled, only nests with at least 8 eggs were sampled for Moorhen, 5 eggs for Squacco Heron and Purple Swamphen, 4 eggs for Purple Heron, Night Heron, Little Egret and Little Bittern, 3 eggs for the Gull-billed, Common, Whiskered and Little Terns and for the Black-headed and Audouin's Gulls, and with 2 eggs in the case of the Sandwich Tern. Eggs were collected following the protocol approved by the Ebro Delta Natural Park and with the permission of the Serveis de Fauna i Pesca, Generalitat de Catalunya (Spain).

Eggs were labeled and kept refrigerated until reaching the laboratory. Once there, they were kept frozen (-20ºC) until analysis. Egg content was then separated from the egg shell, weighed, and placed into a glass container for freeze-drying. Freeze-dried samples were homogenized and a sub-sample was lipid-extracted for stable isotope analysis using methanol and chloroform, following Folch's method (Folch et al., 1957).

Stable Isotope Analysis (SIA)

Lean sub-samples (ca. 0.36 mg for δ^{13}C and δ^{15}N) of the homogenized eggs were placed in tin buckets and crimped for combustion. Isotopic analyses were carried out by EA-IRMS (elemental analysis-isotope ratio mass spectrometry) by means of a Thermo Finnigan Flash 1112 elemental analyzer coupled to a Delta isotope ratio mass spectrometer via a CONFLO III interface.

Stable isotope ratios were expressed in conventional notation as parts per thousand (‰) following the equation: $\delta X = [(R\ sample / R\ standard) - 1] * 1000$, where X is ^{15}N or ^{13}C and R is the corresponding ^{15}N/^{14}N or ^{13}C/^{12}C. The standards for ^{15}N and ^{13}C are atmospheric nitrogen (AIR) and Pee Dee Belemnite (PBD), respectively. Precision and accuracy for δ^{13}C measurements was ≤ 0.1‰ and ≤ 0.3‰ for δ^{15}N. The laboratory applies international standards, which are run for every 12 samples: IAEA CH_7 (87% of C), IAEA CH_6 (42% of C) and USGS 24 (100% of C) for ^{13}C and IAEA N1 and IAEA N2 (with 21% of N) and IAEA NO_3 (13.8% of N) for ^{15}N.

Trace Elements Analysis

Chemical determination of mercury, selenium, copper, lead, cadmium, antimony, chromium and arsenic (chromium and arsenic only 2007-2008) was carried out by means of ICP-MS Perkin Elmer ELAN 6000. Before the measurement, homogenized subsamples of freeze-dried eggs (ca. 100 mg) were digested in H_2NO_3 and H_2O_2 in Savilles Teflon digestion vessels for 12 hours at 100ºC. Accuracy of analysis was checked by measuring certified reference material (Lobster hepatopancreas Tort-2 and Dogfish liver Dolt-3; National Research Council Canada).

Mean recoveries ranged 96-100% for total mercury, selenium, copper, lead, cadmium, antimony, chromium and arsenic; and no corrections were done. Values of limit of detection were: 0.02 for Cd and Sb, 0.1 ppb for Pb and As, 0.2 for Hg and Cu, 0.5 ppb for Cr and 1 ppb for Se. Determinations below such values were set to not detected. All trace elements concentrations were expressed on a dry weight basis (ng•g-1).

Trace elements and SIAs were performed at the Serveis Científico-Tècnics (Universitat de Barcelona).

Statistical methods

Values of stable isotope ratios and trace elements concentrations were routinely checked for normal distributions using Shapiro-Wilk tests, together with Q-Q plots. Trace elements concentrations showed clear skewed distributions which were normalized by applying a logarithmic transformation. When the percentage of detection of a trace element ranged above 75%, samples of a single species, values below the detection limit were assigned to ½ of the detection limit value of that trace element.

The isotopic values of one Common Tern egg from 'Punta del Fangar' were considered extreme ($\delta^{15}N= 13.8$, $\delta^{13}C= -23.54$) and were excluded from further statistical analyses and tables.

As isotopic niche width estimator we used the corrected area of the standard ellipse (SEA_c), which is unbiased with respect to sample size and its estimation via Bayesian inference allows robust comparison to be made among data sets comprising different sample sizes (Jackson et al., 2011). This metric was calculated as described in Jackson et al. (2011) with the SIAR package (Parnell et al., 2010).

Because differences in trophic ecology and feeding habits have been observed between the two Common Tern colonies of the Ebro Delta, as shown by stable isotopes signatures, fatty acids profiles and mercury, selenium and organochlorine compound levels (Guitart et al., 2003, Mateo et al., 2004, Cotin et al., 2011); for statistical purposes the two colonies have been considered as different bird populations.

Species were compared using a one-way analysis of variance and applying the Levene test to check for homoscedasticity. Welch correction was used accordingly. To test for "a posteriori" pairwise differences, we used SNK or Tamhane tests. Descriptive statistics and mean difference between species and their 95% confidence intervals were used to show the results. Spearman's rank correlation coefficient was used (using case-species) to explore relationships between trace elements and stable isotopes. Statistical analysis was carried out using PASW 18.0. and R (R Core Team, 2012).

Results

Descriptive statistics (mean, standard deviation, minimum and maximum) for stable isotopes of eggs are presented in table 2. The comparison among all the species considered in the study showed significant differences in their stable isotope values ($\delta^{13}C$: $F_{Welch\ 14,\ 96.8} = 807.5$, $p<0.001$; $\delta^{15}N$: $F_{Welch\ 14,\ 96.1} = 64.3$, $p<0.001$). With regard to $\delta^{15}N$, pairwise tests (see table 2) showed a gradient among the species, mainly represented by three groups: with the highest $\delta^{15}N$ values, Little Tern, and with the lowest, the group formed by Common Tern 'Fangar', Moorhen and Purple Swamphen; all other species formed a group with intermediate values. Concerning $\delta^{13}C$ levels, pairwise tests showed six groups (see table 2 and figure 2). From higher to lower $\delta^{13}C$ levels, groups are: Little Tern; Common Tern 'Fangar'; Common Tern 'Banya' and Sandwich Tern; Audouin's Gull; Squacco Heron and Moorhen; and finally Moorhen and the other species. Estimated standard deviation ranged from 0.3 to 1.7 and from 0.2 to 2.1 (for $\delta^{15}N$ and $\delta^{13}C$ respectively) suggesting outstanding differences in trophic niche width. Combining both isotopes we estimated the isotope niche width metric (SEA_c) (fig. 3).

Descriptive statistics (mean, geometric mean, standard deviation, minimum and maximum) for mercury, selenium and copper are presented in table 3 and for chromium, arsenic and lead in table 4. Moorhen and Purple Swamphen were excluded when analyzing mercury because they presented very low concentrations and consequently most values were below LOD. Mercury showed significant differences ($F_{Welch\ 12,\ 88.9} = 51$, $p<0.001$) among the remaining species. Pairwise tests showed two main groups, one of them subdivided into two: the first group, which has the higher mercury levels, is formed by the Audouin's Gull and all the tern species except Common Tern 'Fangar'. The other group, with lower mercury levels, has two subgroups: the first subgroup is formed by Common Tern 'Fangar', Little Egret, Night Heron and Little Bittern, and with lower concentrations, Little Bittern,

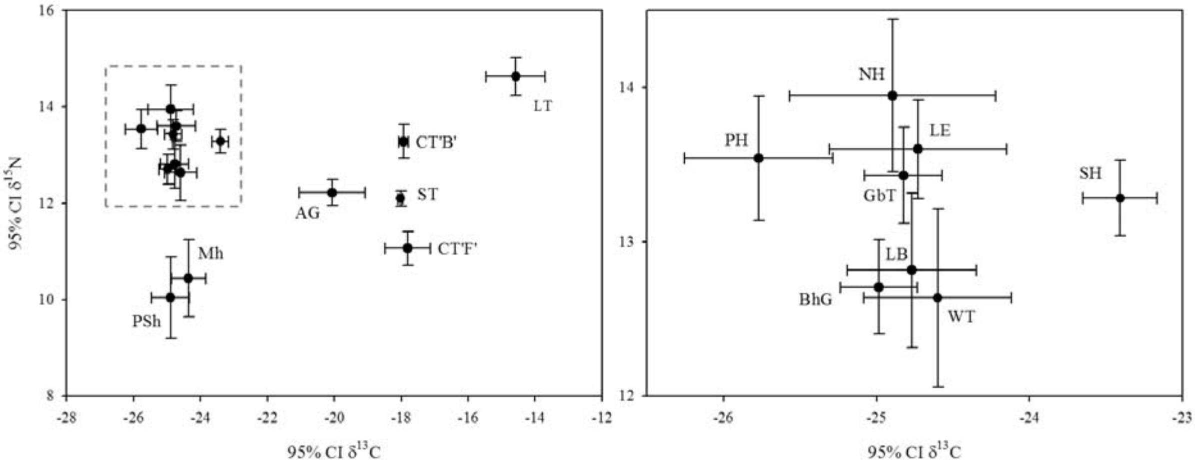


Figure 2. Plots of $\delta^{15}N$ and $\delta^{13}C$ signatures indicating the mean position of the species included in the study: Purple Heron (PH), Little Egret (LE), Night Heron (NH), Squacco Heron (SH), Little Bittern (LB), Sandwich Tern (ST), Common Tern 'Banya' (CT'B'), Common Tern 'Fangar' (CT'F'), Little Tern (LT), Gull-billed Tern (GbT), Whiskered Tern (WT), Black-headed Gull (BhG), Audouin's Gull (AG), Moorhen (Mh) and Purple Swamphen (PSh). Dot indicates mean value, and lines their corresponding 95% confidence intervals. Squared area in the left plot is enlarged at right.

Squacco Heron, Black-headed Gull and Purple Heron.

Selenium levels showed significant differences ($F_{Welch\ 13,\ 98.4} = 23.3$, $p<0.001$). Pairwise test showed that Moorhen has significant lower levels than the remaining species. Only Purple Swamphen presented even lower selenium levels but it was excluded from analysis as most values were below LOD (see table 3).

Concerning copper levels, significant differences were found ($F_{Welch\ 14,\ 92.9} = 46$, $p<0.001$) but pairwise tests showed no clear groups within species (see table 3), although it can be observed the following gradient in concentrations: Herons, Terns (with Audouin's and Black-headed Gulls), Purple Swamphen and finally Moorhen.

Chromium and arsenic levels were determined only in those eggs sampled during 2007 and 2008, so a limited number of the species is included in the analysis of those trace elements (see table 4). With regard to chromium levels, significant differences were found ($F_{Welch\ 8,\ 58} = 8.4$, $p<0.001$) but pairwise tests showed no clear groups within species (see table 4), only the species at both ends of the gradient (Whiskered Tern and Little Egret in one end, and Squacco Heron and Audouin's Gull in the other) showed clear differences.

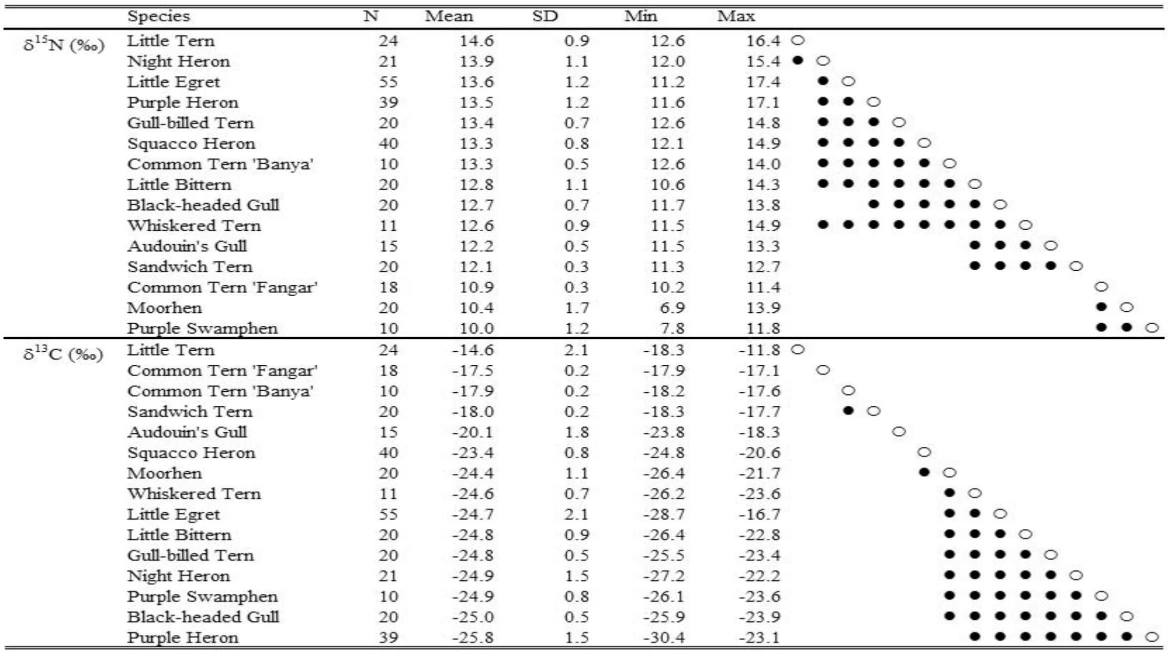
Arsenic levels presented significant differences ($F_{8,\ 161} = 147.8$, $p<0.001$) and pairwise test showed four groups, which are from higher to lower concentrations: Common Tern 'Banya'; Sandwich Tern, Little Tern and Audouin's Gull; Little Egret and Purple Heron and as last group, Squacco Heron, Black-headed Gull and Whiskered Tern.

Information on lead is shown in table 4, but due to the low percentage of detected samples, running adequate statistical analysis was not possible and only descriptive statistics are given. Regarding cadmium and antimony, levels were not detected in any of the egg samples.

When exploring relationships among specie's mean log-concentrations and stable isotopes signatures, no significant linear relationships were found among the trace elements analyzed except for mercury and

Northern Hemidelta	Canal Vell	Ardea purpurea	3
		Egretta garzetta	55
		Ardeola ralloides	20
		Larus ridibundus	20
	Illa de Buda	Nycticorax nycticorax	21
		Ardeola ralloides	20
	Punta del Fangar	Sterna hirundo	18
		Gelochelidon nilotica	20
Southern Hemidelta	l'Encanyissada	Ardea purpurea	28
	La Tancada	Chlidonias hybrida	11
	Platjola	Ardea purpurea	8
	Riet Vell	Ixobrychus minutus	20
		Gallinula chloropus	20
		Porphyrio porphyrio	10
	Punta de la Banya	Sterna hirundo	10
		Sternula albifrons	24
		Sterna sandvicensis	20
		Larus audouinii	15

Table 1. Number of eggs sampled by colony at the Ebro Delta.



	Species	N	Mean	SD	Min	Max															
$\delta^{15}N$ (‰)	Little Tern	24	14.6	0.9	12.6	16.4	○														
	Night Heron	21	13.9	1.1	12.0	15.4	●	○													
	Little Egret	55	13.6	1.2	11.2	17.4		●	○												
	Purple Heron	39	13.5	1.2	11.6	17.1		●	●	○											
	Gull-billed Tern	20	13.4	0.7	12.6	14.8		●	●	●	○										
	Squacco Heron	40	13.3	0.8	12.1	14.9		●	●	●	●	○									
	Common Tern 'Banya'	10	13.3	0.5	12.6	14.0		●	●	●	●	●	○								
	Little Bittern	20	12.8	1.1	10.6	14.3		●	●	●	●	●	●	○							
	Black-headed Gull	20	12.7	0.7	11.7	13.8				●	●	●	●	●	○						
	Whiskered Tern	11	12.6	0.9	11.5	14.9		●	●	●	●	●	●	●	●	○					
	Audouin's Gull	15	12.2	0.5	11.5	13.3								●	●	●	○				
	Sandwich Tern	20	12.1	0.3	11.3	12.7								●	●	●	●	○			
	Common Tern 'Fangar'	18	10.9	0.3	10.2	11.4													○		
	Moorhen	20	10.4	1.7	6.9	13.9													●	○	
	Purple Swamphen	10	10.0	1.2	7.8	11.8													●	●	○
$\delta^{13}C$ (‰)	Little Tern	24	-14.6	2.1	-18.3	-11.8	○														
	Common Tern 'Fangar'	18	-17.5	0.2	-17.9	-17.1		○													
	Common Tern 'Banya'	10	-17.9	0.2	-18.2	-17.6			○												
	Sandwich Tern	20	-18.0	0.2	-18.3	-17.7			●	○											
	Audouin's Gull	15	-20.1	1.8	-23.8	-18.3					○										
	Squacco Heron	40	-23.4	0.8	-24.8	-20.6						○									
	Moorhen	20	-24.4	1.1	-26.4	-21.7						●	○								
	Whiskered Tern	11	-24.6	0.7	-26.2	-23.6							●	○							
	Little Egret	55	-24.7	2.1	-28.7	-16.7							●	●	○						
	Little Bittern	20	-24.8	0.9	-26.4	-22.8							●	●	●	○					
	Gull-billed Tern	20	-24.8	0.5	-25.5	-23.4							●	●	●	●	○				
	Night Heron	21	-24.9	1.5	-27.2	-22.2							●	●	●	●	●	○			
	Purple Swamphen	10	-24.9	0.8	-26.1	-23.6							●	●	●	●	●	●	○		
	Black-headed Gull	20	-25.0	0.5	-25.9	-23.9							●	●	●	●	●	●	●	○	
	Purple Heron	39	-25.8	1.5	-30.4	-23.1								●	●	●	●	●	●	●	○

Table 2. Descriptive statistics of stable isotope signatures in eggs from the species included in the study, sorted from higher to lower values. Post hoc paired comparisons were denoted by a dot matrix. Filled circles indicate that there is not significant difference between row and columns species.

arsenic which show positive association (r_s = 0.8, p = 0.01 n=9); mercury also showed a positive association with $\delta^{13}C$ (r_s = 0.66, p = 0.008 n=15) and copper with $\delta^{15}N$ (r_s = 0.75, p = 0.001 n=15).

Discussion

When foraging in a such diverse wetland landscape as the Ebro Delta, waterbird species could do it in several habitats . As it was shown in a prior study using carbon and sulphur stable isotopes (Cotin et al., 2011) and in agreement with the data obtained in the present study (see fig. 2), the foraging habitat corresponds mainly to three scenarios: freshwater habitats (ricefields and lagoons) with low isotopic values ($\delta^{13}C$ around -24.5‰), marine habitats (the sea and the bays) with $\delta^{13}C$ values around -18‰ and the saltpans ($\delta^{13}C$ around -14.5‰). Little Tern of the Ebro Delta is the only species that feeds on the saltpans, as has been previously described (Cotin et al., 2011). Nevertheless, the high variance in their isotopic values results in a wide isotopic niche, indicating the use of other habitats to a lesser extent, such as channels, lagoons and lagoon mouths (Bertolero et al., 2005), and thus pointing to individual foraging strategies. Furthermore, the $\delta^{15}N$ values of this species were the highest of the species examined. These high $\delta^{15}N$ signatures would be related to the higher basal signature in the saltpans rather than true differences in the trophic levels occupied, as the diet of the Little Tern is based mainly on small fish and prawns (Norman, 1992, Bogliani et al., 1994, Paiva et al., 2006).

With regard to marine habitats, two tern and one gull species share this scenario, the Sandwhich Tern, both colonies of Common Tern and the Audouin's Gull. The Sandwich Tern is a marine feeder (Stienen et al., 2000)

	Species	N	Mean	G Mean	SD	Min	Max															
[Hg]	Audouin's Gull	15/15	5903.5	5321.9	2713.0	2299.9	11883.3	○														
	Little Tern	24/24	4150.1	3694.5	1973.7	1375.7	8621.9	●	○													
	Common Tern 'Banya'	10/10	4148.9	3688.9	1940.9	1138.5	7631.7	●	●	○												
	Sandwich Tern	20/20	2727.2	2454.7	1264.3	1030.7	4999.8		●	●	○											
	Whiskered Tern	11/11	2576.6	2309.2	1319.9	1119.6	5591.5		●	●	●	○										
	Gull-billed Tern	20/20	1770.1	1659.8	699.9	879.2	3686.6			●	●	●	○									
	Common Tern 'Fangar'	18/18	1139.6	1106.1	264.3	588.1	1663.8							○								
	Little Egret	55/55	1064.3	981.5	491.7	467.1	3450.6							●	○							
	Night Heron	21/21	1115.1	964.1	870.7	446.0	4688.9							●	●	○						
	Little Bittern	19/20	660.9	545.0	449.8	106.1	2047.6								●	●	○					
	Purple Heron	30/39	442.8	356.4	265.7	91.0	1124.1										●	○				
	Squacco Heron	35/40	535.9	351.3	639.2	52.7	3429.5										●	●	○			
	Black-headed Gull	15/20	369.1	207.0	463.5	49.1	1929.2										●	●	●	○		
	Moorhen	3/20				223.0	888.5															
	Purple Swamphen	0/10																				
[Se]	Sandwich Tern	20/20	4141.8	4116.4	477.1	3410.9	5349.2	○														
	Little Egret	55/55	3931.7	3820.5	961.5	2392.3	6518.2	●	○													
	Black-headed Gull	20/20	3585.3	3497.6	848.5	2319.9	5503.7	●	●	○												
	Audouin's Gull	15/15	3508.4	3457.8	601.5	2350.3	4255.5	●	●	●	○											
	Common Tern 'Banya'	10/10	3464.7	3445.4	384.8	2954.4	3973.3	●	●	●	●	○										
	Whiskered Tern	11/11	3148.5	3134.0	317.6	2677.7	3646.9			●	●	●	○									
	Night Heron	21/21	3023.0	2917.2	854.1	1805.3	4965.9			●	●	●	●	○								
	Little Bittern	20/20	2965.2	2915.0	566.2	2159.0	4112.7			●	●	●	●	●	○							
	Common Tern 'Fangar'	18/18	2854.5	2833.4	366.3	2394.2	3614.5			●	●		●	●	●	○						
	Little Tern	24/24	2839.4	2788.3	545.4	1851.6	3997.7			●	●		●	●	●	●	○					
	Purple Heron	39/39	2856.1	2776.8	652.2	1471.4	3939.6			●	●		●	●	●	●	●	○				
	Squacco Heron	40/40	2852.9	2771.3	767.1	1581.8	6249.1			●	●		●	●	●	●	●	●	○			
	Gull-billed Tern	20/20	2740.1	2701.9	473.0	1960.7	3905.8						●	●	●	●	●	●	●	○		
	Moorhen	19/20	1609.1	1545.8	410.6	534.0	2326.0														○	
	Purple Swamphen	2/10				1217.5	1381.1															
[Cu]	Little Egret	55/55	5750.2	5698.7	755.1	3752.1	7476.7	○														
	Night Heron	21/21	5031.6	4967.9	805.8	3365.9	6606.2	●	○													
	Purple Heron	39/39	4518.7	4471.9	667.9	3350.4	6208.4		●	○												
	Squacco Heron	40/40	4197.0	4137.5	727.3	2924.7	6600.9			●	○											
	Little Bittern	20/20	4143.7	4076.8	820.0	3080.0	6608.0		●	●	●	○										
	Common Tern 'Banya'	10/10	4134.1	3972.9	1332.9	2947.8	7234.5	●	●	●	●	●	○									
	Whiskered Tern	11/11	3688.5	3619.4	752.6	2695.1	5068.1			●	●	●	●	○								
	Little Tern	24/24	3518.9	3377.0	1199.5	1989.6	8123.2				●	●	●	●	○							
	Black-headed Gull	20/20	4932.2	3372.2	5939.8	1836.6	24019.2	●	●	●	●	●	●	●	●	○						
	Common Tern 'Fangar'	18/18	3070.0	3049.9	369.2	2617.3	3799.9						●	●	●	●	○					
	Audouin's Gull	15/15	3010.2	2967.1	522.1	2135.4	3941.9						●	●	●	●	●	○				
	Gull-billed Tern	20/20	2973.7	2932.4	530.1	2141.4	4512.8						●	●	●	●	●	●	○			
	Sandwich Tern	20/20	3058.2	2930.8	1075.9	2298.5	6027.3						●	●	●	●	●	●	●	○		
	Purple Swamphen	10/10	2989.4	2872.1	760.7	1232.2	4173.3			●	●	●	●	●	●	●	●	●	●	●	○	
	Moorhen	20/20	2818.7	2735.3	672.8	1288.4	4167.0						●	●	●	●	●	●	●	●	●	○

Table 3. Descriptive statistics of mercury, selenium and copper in eggs from the species included in the study sorted by the geometric mean. Post hoc paired comparisons were denoted by a dot matrix. Filled circles indicate that there is not significant difference between row and columns species. Concentrations are expressed in ppb (ng/g).

and Common Tern behaves similarly at the Ebro Delta. The huge differences in $\delta^{15}N$ found among the two colonies are indicative of the use of different resources, as has been recorded previously (Guitart et al., 2003, Mateo et al., 2004, Cotin et al., 2011) with birds from "Punta del Fangar" feeding mainly on epipelagic fish and birds from "Punta de la Banya" relying on demersal resources obtained from fish discards, which have higher $\delta^{15}N$ levels. The Audouin's Gull is considered a nocturnal predator on epipelagic fish (mainly clupeiformes), but feeding is also opportunistic, using the fishery activities in neighboring waters, especially trawler discards (Oro and Ruiz, 1997, Sanpera et al., 2007). So, although $\delta^{13}C$ signatures of this species should point only marine habitats, the recently reported use of freshwater resources by this species, in

	Species	N	Mean	G Mean	SD	Min	Max									
[Cr]	Whiskered Tern	11/11	2885.8	2880.2	189.6	2579.4	3195.0	○								
	Little Egret	35/35	2720.7	2717.9	127.3	2503.9	3172.5	●	○							
	Little Tern	14/14	2644.0	2642.1	103.3	2522.8	2842.0	●	●	○						
	Common Tern 'Banya'	10/10	2605.8	2605.4	47.7	2514.4	2678.6		●	●	○					
	Purple Heron	25/25	2605.1	2599.3	179.1	2314.5	3026.9		●	●	●	○				
	Sandwich Tern	20/20	2584.4	2579.6	161.1	2309.9	2855.8		●	●	●	●	○			
	Black-headed Gull	20/20	2574.8	2568.1	184.5	2086.8	2795.1		●	●	●	●	●	○		
	Squacco Heron	20/20	2466.8	2457.1	224.8	2077.1	2916.3			●	●	●	●	●	○	
	Audouin's Gull	15/15	2413.5	2404.2	221.3	2033.2	2921.8			●	●	●	●	●	●	○
[As]	Common Tern 'Banya'	10/10	1217.7	1179.8	293.6	633.8	1606.5	○								
	Sandwich Tern	20/20	857.0	850.4	106.0	617.4	1046.5		○							
	Little Tern	14/14	850.6	811.9	264.1	448.7	1274.1		●	○						
	Audouin's Gull	15/15	685.3	655.8	212.3	336.1	1169.1		●	●	○					
	Little Egret	35/35	185.2	178.1	52.9	94.0	318.4					○				
	Purple Heron	25/25	172.7	167.8	43.2	100.2	303.0					●	○			
	Whiskered Tern	11/11	104.7	101.2	27.2	58.9	154.5							○		
	Squacco Heron	17/20	109.6	95.7	60.7	32.5	316.9							●	○	
	Black-headed Gull	16/20	90.9	79.9	44.2	25.9	195.6							●	●	○
[Pb]	Purple Swamphen	1/10				789.1	789.1									
	Sandwich Tern	3/20				69.7	935.9									
	Whiskered Tern	1/11				176.4	176.4									
	Black-headed Gull	5/20				90.4	362.4									
	Gull-billed Tern	1/20				131.9	131.9									
	Little Tern	2/24				111.6	120.2									
	Squacco Heron	5/40				61.4	189.2									
	Little Egret	2/55				84.3	131.6									

Table 4. Descriptive statistics of chromium, arsenic and lead in eggs from the species included in the study sorted by the geometric mean. Chromium and arsenic were analysed only in samples from 2007 and 2008. Species without detectable lead levels are not shown in the table. Post hoc paired comparisons were denoted by a dot matrix. Filled circles indicate that there is not significant difference between row and columns species. Concentrations are expressed in ppb (ng/g).

particular Crayfish (Navarro et al., 2010b), probably lowers the carbon signature values (fig. 2).

And finally, the freshwater habitats, where all the heron, two tern, one gull and the two rallidae species gather together. Moorhen and the Purple Swamphen are mainly herbivorous, with the Purple Swamphen feeding on seeds, bulbs, stem and leaves from reeds (*Phragmites australis*), cattail (*Thypa latifolia*) and rice (*Oryza sativa*) (Cramp, 1980). Moorhen also feeds mainly on plant material (Amat and Soriguer, 1984) but during the chick rearing period may increase the percentage of insects. Their low δ^{15}N signatures are in agreement with the described diet and clearly differentiate these species from others. Black-headed Gull is a species that can exploit marine, freshwater and terrestrial habitats (Schwemmer et al., 2011), being an opportunistic forager. However, at the Ebro Delta, δ^{13}C signatures indicate that they rely mostly on freshwater habitats, and their relatively low δ^{15}N indicate that they feed probably insects and earthworms, which are a very common prey (Schwemmer et al., 2011). The two freshwater terns are the Whiskered and the Gull-billed Tern. The former feeds mainly on aquatic prey in wetlands (Latraube et al., 2005, Paillisson et al., 2007), also rice fields, marshes and lagoons of the Ebro Delta are suitable foraging habitats for this species. Gull-billed tern is known to be a generalist forager on a range of prey (Goutner, 1991), such as grasshoppers and caterpillars (Mathew et al., 1998), amphibians and

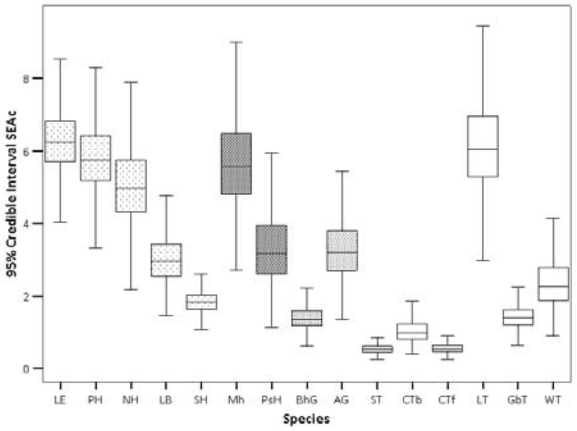


Figure 3. Corrected standard ellipse area (SEA_c) of the study species sorted by family. Ardeidae: Purple Heron (PH), Little Egret (LE), Night Heron (NH), Squacco Heron (SH), Little Bittern (LB). Rallidae: Moorhen (Mh) and Purple Swamphen (PsH). Laridae: Black-headed Gull (BhG), Audouin's Gull (AG). Sternidae: Sandwich Tern (ST), Common Tern 'Banya' (CTb), Common Tern 'Fangar' (CTf), Little Tern (LT), Gull-billed Tern (GbT), Whiskered Tern (WT).

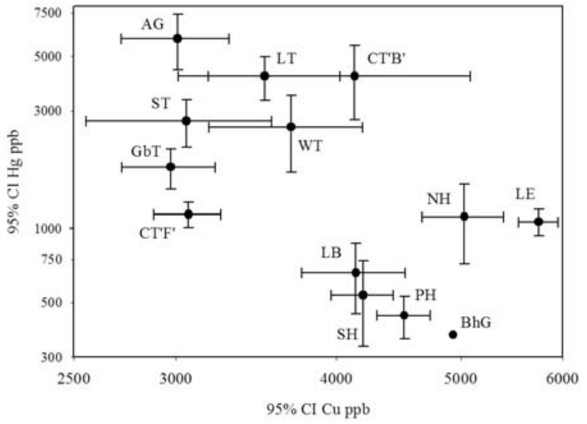


Figure 4. Plots of Hg and Cu concentrations (given on a logarithmic scale) of the species included in the study: Purple Heron (PH), Little Egret (LE), Night Heron (NH), Squacco Heron (SH), Little Bittern (LB), Sandwich Tern (ST), Common Tern 'Banya' (CT'B'), Common Tern 'Fangar' (CT'F'), Little Tern (LT), Gull-billed Tern (GbT), Whiskered Tern (WT), Black-headed Gull (BhG) and Audouin's Gull (AG). Moorhen and Purple Swamphen are not included and Black-headed Gull 95% CI is omitted due to its high variability. Dot indicates mean value, and lines their corresponding 95% confidence intervals.

beetles (Cabo and Sanchez, 1986) or otherwise crayfish, insects and fish (Dies et al., 2005), but at the Ebro Delta, this species feeds mainly in the freshwater, as shown by its low $\delta^{13}C$ range. It's interesting to notice that this species presents higher nitrogen signatures than the Whiskered Tern and the Black-headed Gull, maybe as a result of preying over bigger prey.

The five heron species all feed on freshwater habitats. Purple Heron, Little Egret, Night Heron and Little Bittern are mainly aquatic predatory species. Differences in $\delta^{15}N$ indicate that Little Bittern feeds on lower trophic level prey, such as the crayfish (Pardo-Cervera et al., 2010). The greater variability in $\delta^{13}C$ signatures shown by the Little Egret indicate that they might also be feeding at some extent in other habitats, such as brackish waters or even saltpans (Abdennadher et al., 2011), thus pointing to some degree of individual heterogeneity in diet. Squacco Heron, although having the relative size of a Little Bittern, feeds mainly on insects (Orthotopera and Coleoptera) (Delord et al., 2004). It is interesting to remark the high percentage of European mole cricket (*Gryllotalpa gryllotalpa*) found in the pellets of this species at the Ebro Delta (unpublished data). This waterbird forages normally in the edge of ricefields and the contribution of terrestrial prey raises its $\delta^{13}C$ signatures.

According to the niche width metrics (fig. 3), which are based on the isotopic data, we can conclude that species such as the Common and Sandwich Tern are strict specialists at the Ebro Delta, with a narrow niche width. On the other hand, we have the Little Tern, some heron species (Little Egret, Purple

Heron and Night Heron) and the moorhen, for which opportunistic feeding together with higher variability of $\delta^{13}C$ signatures in feeding habitats expands their trophic niche. This is especially true in the case of the freshwater species, as their, on average, higher niche width when compared to the marine species probably indicates a higher isotopic heterogeneity in those habitats.

Mercury levels at the Ebro Delta are higher in terns than in herons, even for those species foraging in the same habitat (fresh water, marine or saltpans), indicating phylogenetic differences in metal bioaccumulation between the family Ardeidae and Sternidae that will be discussed hereafter. Mercury levels tend to be higher in marine-related species than in freshwater ones, but on top of that, mercury derived from the industry along the riverside, especially in the Flix reservoir (Cotin et al., 2012), has probably been accumulating in the sand banks of the Ebro Delta over the years, particularly in the southern hemidelta, as materials carried by the Ebro river are mainly deposited there by the Liguro-Provençal-Catalan oceanic current flowing south (Guitart et al., 2003). This fact has probably boosted the mercury concentrations of the species at the southern hemidelta, especially those feeding on fish discards (based on demersal fish) which are, among others, the Audouin's Gull (Morera et al., 1997) and the Common Tern 'Banya'. Higher mercury levels have been reported for the Audouin's Gull colony in the Ebro Delta, when compared to other colonies elsewhere in the Mediterranean (Sanpera et al., 2007). The freshwater terns, Whiskered and Gull-billed Tern, are also exposed to mercury inputs from the river, although in lower amounts, and reach smaller concentrations than the marine terns, except for the Common Tern 'Fangar'. This common tern colony shows much lower mercury concentrations as it's situated in the Northern hemidelta, where is apart from the main mercury input of the river. Also its diet is based on epipelagic fish, which has lower mercury loads (Arcos et al., 2002) than the demersal fish. The values of this colony are similar to other Common Tern colonies that follow the same feeding habits (Monteiro et al., 1999, Guitart et al., 2003, Becker and Munoz Cifuentes, 2004, Bond and Diamond, 2009), with most values around 1000 ppb.

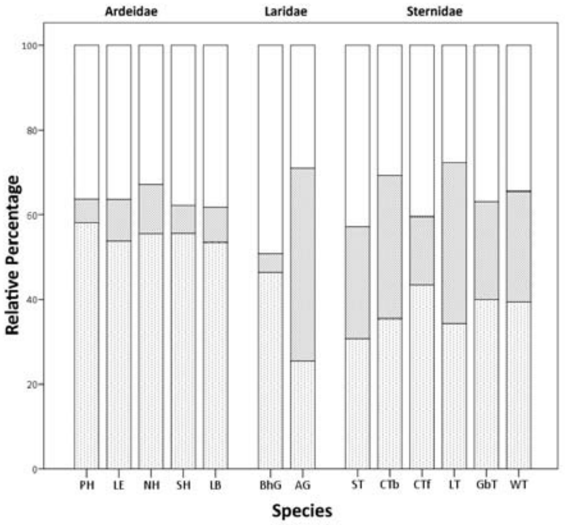


Figure 5. Relative percentages of the Copper (white) Mercury (grey) and Selenium levels (pale grey) sorted by family: Purple Heron (PH), Little Egret (LE), Night Heron (NH), Squacco Heron (SH), Little Bittern (LB), Sandwich Tern (ST), Common Tern 'Banya' (CTb), Common Tern 'Fangar' (CTf), Little Tern (LT), Gull-billed Tern (GbT), Whiskered Tern (WT), Black-headed Gull (BhG), and Audouin's Gull (AG). Moorhen (Mh) and Purple Swamphen (PsH) are not represented due to the undetectable levels of Mercury and Selenium.

The Little Tern presents the second highest mercury concentrations of all the studied species. This tern feeds mainly at saltpans, and the mercury baseline in these food webs is relatively high. Similar mercury values have been found in saltpans at Portugal (Tavares et al., 2007, Paiva et al., 2008b).

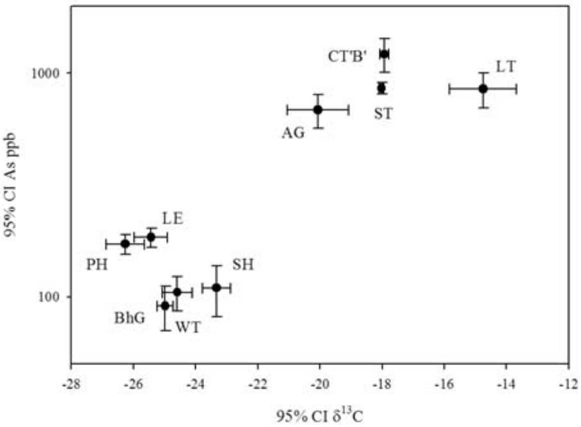


Figure 6. Plots of Arsenic concentrations (given on a logarithmic scale) and δ^{13}C signatures of Purple Heron (PH), Little Egret (LE), Squacco Heron (SH), Sandwich Tern (ST), Common Tern 'Banya' (CT'B'), Little Tern (LT), Whiskered Tern (WT), Black-headed Gull (BhG) and Audouin's Gull (AG). Dot indicates mean values, and lines their corresponding 95% confidence intervals.

Among herons, Little Egret shows the highest mercury levels, probably due to its partial use of saltpans and brackish waters, while the Squacco Heron presents the lowest, as this species rely mainly on insects. The Black-headed Gull also shows very low mercury concentrations, in agreement with the invertebrate-based diet indicated by the stable isotopes. Finally, the two herbivorous species, Moorhen and Purple Swamphen, showed the lowest mercury values.

About copper, no clear differences were found among species, although it is known that the agricultural use of copper sulphate (Cu_2SO_4) is widespread in the freshwater habitats., making this element more available. Nevertheless, a pattern can be delineated among groups of species: herons > terns + gulls > rallids. If we recall the pattern observed in the mercury concentrations, we can see that both metals behave oppositely at species level, so, confronting them in a plot, we observe the family pattern clearly (see figure 4): Terns (family Sternidae) have, independently of the feeding habitat, high concentrations of mercury and low of copper, while Herons (family Ardeidae) have just the opposite. These phylogenetic differences in metal bioaccumulation between both families (Ardeidae and Sternidae) had never been described previously. It is worth noting in figures 4-5 the differences between the two gull species, in which the diet differences come out clearly.

Selenium is appreciably transferred to eggs (Sell, 1977, Focardi et al., 1988), making of eggs good bioindicators for this element (Ohlendorf et al., 2011). Levels at the Ebro Delta, as with copper, did not differ among species. Only herbivorous species showed lower concentrations. Neither chromium levels show any standing difference among species or habitats.

For arsenic, in the four groups of species stands the pattern of higher levels in the marine species when compared to the freshwater ones. Arsenic concentrations tend to be higher in seawater than in freshwater (Kunito et al., 2008, Lucia et al., 2010) and also much higher than in terrestrial organisms or environments (Lunde, 1977). When displaying arsenic levels with carbon signatures (see figure 6), there is an outstanding discrimination between marine and freshwater species, marine related species presenting higher levels than the freshwater species. The positive relationships found between mercury and both arsenic and δ^{13}C are explained because both trace elements and the carbon isotope show clear differences between marine and freshwater habitats.

About lead, although the Ebro Delta is a frequent hunting area and the ingestion of lead pellets could be a possible threat, the study species presented very low concentrations.

It is interesting to compare the pollutant levels of the Ebro Delta aquatic bird community with other similar areas and communities. For example, at the saltworks of Thyna (Tunisia), mercury and selenium levels in Slender-billed Gull, Common Tern and Little Egret (Ramirez et al., 2011) ranged similarly, but concentrations were relatively higher at the Ebro Delta. At three different wetlands in China, levels of As, Cd, Cr, Cr, Pb, Hg and Se in Little Egret (Zhang et al., 2006) also ranged similarly, with the exception of chromium, which was much higher at the Ebro Delta, although within those reported for several bird species (Burger and Gochfeld, 2009, Padula et al., 2010). Great Egret (*Ardea albus*) mercury levels from the Florida everglades are within the range of herons at the Ebro Delta (Rumbold et al., 2001) Levels of the same elements analyzed in this study in herons and terns of the Pearl River Delta (southern China) (Lam et al., 2005) were similar, with the exception of chromium in herons (which is higher at the Ebro Delta), mercury in terns (also higher at the Ebro Delta) and copper in both families (lower at the Ebro Delta). At three different wetlands in Pakistan, levels of As, Cd, Cr, Pb, Hg and Se in three species of herons (Boncompagni et al., 2003) levels also ranged similarly, with once more the exception of chromium, being its levels higher at the Ebro Delta.

We can conclude, thanks to use of stable isotopes and ellipse-based niche metrics (SEA_c), that Common and Sandwich Tern behave as strict specialists at the Ebro Delta, with narrow niche widths, while in the opposite way, Little Tern, Little Egret, Purple Heron, Night Heron and the Moorhen behave like generalists foragers, with broad niche widths. About pollutant levels, all analyzed elements are out of conservational concern (excluding mercury) and within range values of other bird communities (with the exception of chromium and mercury). Mercury levels in Audouin's Gull, Little Tern and Common Tern 'Banya' eggs are high enough to be of special concern; further studies are of particular relevance in order to monitor whether these values are endangering the breeding success of these species, although embryos of gulls and terns have been reported to show an intermediate sensitive to mercury (Heinz et al., 2009). Arsenic, specially used in combination with carbon signatures, discriminates outstandingly between marine and freshwater species (with marine related species presenting higher levels of arsenic and carbon signatures than the freshwater species). Finally, and surprisingly, Terns (family Sternidae) and Herons (family Ardeidae) showed great differences in levels of mercury and copper: high mercury and low copper loads for Terns and opposite in Herons, being these differences presumably phylogenetically based.

Acknowledgments

We dedicate this work to the memory of Xavier Ruiz, who passed away on 27th April 2008. The authors wish to acknowledge the kindly cooperation of the personnel at the Parc Natural del Delta de l'Ebre for the facilities during fieldwork. Thanks are given to Sergi Ferrer and the staff at the Serveis Científico-Tècnics (UB, Spain) for their help during the sample collection and laboratory analysis and to Gretchen Wagner for her suggestions and corrections of the English draft. Javier Cotín was supported by a BRD grant from the Universitat de Barcelona (Spain). The present work was partially funded by the Ministry of the Environment (Spain) the Department of the Environment and Housing (Catalan Government) and by the project CGL2008-05448-C02-01 of the Spanish Ministry of Economy and Competitiveness.

References

Abdennadher, A., Ramirez, F., Romdhane, M.S., Ruiz, X., Jover, L. and Sanpera, C., 2011. Little Egret (*Egretta garzetta*) as a bioindicator of trace element pollution in Tunisian aquatic ecosystems. *Environmental Monitoring and Assessment* 175, 677-684.

Amat, J.A. and Soriguer, R.C., 1984. Alimentacion invernal de la polla de agua (*Gallinula chloropus*) en las marismas del Guadalquivir. *Ardeola* 31, 136-140.

Arcos, J.M., Ruiz, X., Bearhop, S. and Furness, R.W., 2002. Mercury levels in seabirds and their fish prey at the Ebro Delta (NW Mediterranean): the role of trawler discards as a source of contamination. *Marine Ecology Progress Series* 232, 281-290.

Baker, S.D. and Sepulveda, M.S., 2009. An evaluation of the effects of persistent environmental contaminants on the reproductive success of Great Blue Herons (*Ardea herodias*) in Indiana. *Ecotoxicology* 18, 271-280.

Becker, P.H., Frank, D. and Wagener, M., 1997. Luxury in freshwater and stress at sea? The foraging of the Common Tern *Sterna hirundo* . *Ibis* 139, 264-269.

Becker, P.H., Furness, R.W. and Henning, D., 1993. Mercury dynamics in young Common Tern (*Sterna hirundo*) chicks from a polluted environment. *Ecotoxicology* 2, 33-40.

Becker, P.H. and Munoz Cifuentes, J., 2004. Contaminants in bird eggs. Recent spatial and temporal trends. *Wadden Sea Ecosystem* 18, 5-24.

Bertolero, A., Oro, D., Martinez Vilalta, A. and Lopez, M.A., 2005. Selection of foraging habitats by Little Terns *Sterna albifrons* at the Ebro Delta (NE Spain). *Revista Catalana d'Ornitologia* 21, 37-42.

Bogliani, G., Fasola, M., Canova, L. and Saino, N., 1994. Prey selection by parents and chicks of the Little Tern *Sterna albifrons.* . *Avocetta* 18, 9-11.

Boncompagni, E., Muhammad, A., Jabeen, R., Orvini, E., Gandini, C., Sanpera, C., Ruix, X. and Fasola, M., 2003. Egrets as monitors of trace-metal contamination in wetlands of Pakistan. *Archives of Environmental Contamination and Toxicology* 45, 399-406.

Bond, A.L. and Diamond, A.W., 2009. Mercury concentrations in seabird tissues from Machias Seal Island, New Brunswick, Canada. *Science of the Total Environment* 407, 4340-4347.

Bosch, C., Olivares, A., Faria, M., Navas, J.M., del Olmo, I., Grimalt, J.O., Pina, B. and Barata, C., 2009. Identification of water soluble and particle bound compounds causing sublethal toxic effects. A field study on sediments affected by a chlor-alkali industry. *Aquatic Toxicology* 94, 16-27.

Bridge, E.S., Jones, A.W. and Baker, A.J., 2005. A phylogenetic framework for the terns (Sternini) inferred from mtDNA sequences: implications for taxonomy and plumage evolution. *Molecular Phylogenetics and Evolution* 35, 459-469.

Burger, J. and Gochfeld, M., 2009. Comparison of arsenic, cadmium, chromium, lead, manganese, mercury and selenium in feathers in Bald Eagle (*Haliaeetus leucocephalus*), and comparison with Common Eider

(*Somateria mollissima*), Glaucous-winged Gull (*Larus glaucescens*), Pigeon Guillemot (*Cepphus columba*), and Tufted Puffin (*Fratercula cirrhata*) from the Aleutian Chain of Alaska. *Environmental Monitoring and Assessment* 152, 357-367.

Cabo, J.M. and Sanchez, J.M., 1986. Recent Information on *Gelochelidon nilotica* in Morocco and its diet. *Alauda* 54, 207-212.

Catry, T., Ramos, J.A., Le Corre, M., Kojadinovic, J. and Bustamante, P., 2008. The role of stable isotopes and mercury concentrations to describe seabird foraging ecology in tropical environments. *Marine Biology* 155, 637-647.

Champoux, L., Rodrigue, J., Trudeau, S., Boily, M.H., Spear, P.A. and Hontela, A., 2006. Contamination and biomarkers in the Great Blue Heron, an indicator of the state of the St. Lawrence River. *Ecotoxicology* 15, 83-96.

Cherel, Y., Le Corre, M., Jaquemet, S., Menard, F., Richard, P. and Weimerskirch, H., 2008. Resource partitioning within a tropical seabird community: new information from stable isotopes. *Marine Ecology Progress Series* 366, 281-291.

Connell, D., Fung, C., Minh, T., Tanabe, S., Lam, P. and Wong, B., 2003. Risk to breeding success of fish-eating Ardeids due to persistent organic contaminants in Hong Kong: evidence from organochlorine compounds in eggs. *Water research* 37, 459-467.

Cotin, J., Garcia-Tarrason, M., Jover, L. and Sanpera, C., 2012. Are the toxic sediments deposited at Flix reservoir affecting the Ebro river biota? Purple heron eggs and nestlings as indicators. *Ecotoxicology* 21, 1391-1402.

Cotin, J., García-Tarrasón, M., Sanpera, C., Jover, L. and Ruiz, X., 2011. Sea, freshwater or saltpans? Foraging ecology of terns to assess mercury inputs in a wetland landscape: The Ebro Delta. *Estuarine, Coastal and Shelf Science* 92, 188-194.

Cramp, S., 1980. *Hand book of the birds of Europe the Middle-East and North Africa the birds of the Western Palearctic* Vol. 2. Hawks to Bustards.

De Luca-Abbott, S., Wong, B., Peakall, D., Lam, P., Young, L. and Lam, M., 2001. Review of effects of water pollution on the breeding success of waterbirds, with particular reference to ardeids in Hong Kong. *Ecotoxicology* 10, 327-349.

Delord, K., Kayser, Y., Cohez, D., Befeld, S. and Hafner, H., 2004. Fluctuations in chick diet of the Squacco Heron Ardeola ralloides in southern France: changes over the last 30 years. *Bird Study* 51, 69-75.

Dies, J.I., Marin, J. and Perez, C., 2005. Diet of nesting Gull-billed Terns in eastern Spain. *Waterbirds 28,* 106-109.

Evers, D.C., Burgess, N.M., Champoux, L., Hoskins, B., Major, A., Goodale, W.M., Taylor, R.J., Poppenga, R. and Daigle, T., 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. *Ecotoxicology* 14, 193-221.

Fasola, M., Rubolini, D., Merli, E., Boncompagni, E. and Bressan, U., 2010. Long-term trends of heron and egret populations in Italy, and the effects of climate, human-induced mortality, and habitat on population dynamics. *Population Ecology* 52, 59-72.

Fernandez, M., Alonso, C., Gonzalez, M. and Hernandez, L., 1999. Occurrence of organochlorine insecticides, PCBs and PCB congeners in waters and sediments of the Ebro River (Spain). *Chemosphere* 38, 33-43.

Focardi, S., Fossi, C., Lambertini, M., Leonzio,

C. and Massi, A., 1988. Long-term monitoring of pollutants in eggs of Yellow-legged Herring Gull from Capraia Island (Tuscan Archipelago). *Environmental Monitoring and Assessment* 10, 43-50.

Folch, J., Lees, M. and Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* 226, 497-509.

Forero, M.G., Bortolotti, G.R., Hobson, K.A., Donazar, J.A., Bertelloti, M. and Blanco, G., 2004. High trophic overlap within the seabird community of Argentinean Patagonia: a multiscale approach. *Journal of Animal Ecology* 73, 789-801.

Forero, M.G., Gonzalez-Solis, J., Hobson, K.A., Doncazar, J.A., Bertellotti, M., Blanco, G. and Bortolotti, G.R., 2005. Stable isotopes reveal trophic segregation by sex and age in the Southern Giant Petrel in two different food webs. *Marine Ecology-Progress Series* 296, 107-113.

Forero, M.G. and Hobson, K.A., 2003. Using stable isotopes of nitrogen and carbon to study seabird ecology: applications in the Mediterranean seabird community. *Scientia Marina* 67, 23-32.

Gonzalez-Solis, J., Sokolov, E. and Becker, P.H., 2001. Courtship feedings, copulations and paternity in common terns, Sterna hirundo . *Animal Behaviour* 61, 1125-1132.

Goutner, V., 1991. Food and feeding ecology of Gull-Billed terns (Gelochelidon nilotica) in Greece. *Revue d'Ecologie (La Terre Et La Vie)* 46, 373-384.

Guitart, R., Mateo, R., Sanpera, C., Hernandez-Matias, A. and Ruiz, X., 2003. Mercury and selenium levels in eggs of Common Terns (*Sterna hirundo*) from two breeding colonies in the Ebro Delta, Spain. *Bulletin of Environmental Contamination and Toxicology* 70, 71-77.

Heinz, G.H., Hoffman, D.J., Klimstra, J.D., Stebbins, K.R., Kondrad, S.L. and Erwin, C.A., 2009. Species differences in the sensitivity of avian embryos to methylmercury. Archives of Environmental *Contamination and Toxicology* 56, 129-138.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120, 314-326.

Hobson, K.A., Piatt, J.F. and Pitocchelli, J., 1994. Using stable isotopes to determine seabird trophic relationships. *Journal of Animal Ecology* 63, 786-798.

Hobson, K.A., Sirois, J. and Gloutney, M.L., 2000. Tracing nutrient allocation to reproduction with stable isotopes: A preliminary investigation using colonial waterbirds of Great Slave Lake. *Auk* 117, 760-774.

Jackson, A.L., Inger, R., Parnell, A.C. and Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80, 595-602.

Koiadinovic, J., Menard, F., Bustamante, P., Cosscon, R.P. and Le Corre, M., 2008. Trophic ecology of marine birds and pelagic fishes from Reunion Island as determined by stable isotope analysis. *Marine Ecology-Progress Series* 361, 239-251.

Kunito, T., Kubota, R., Fujihara, J., Agusa, T. and Tanabe, S., 2008. Arsenic in marine mammals, seabirds, and sea turtles. *Reviews of Environmental Contamination and Toxicology*, Vol 195 195, 31-69.

Lam, J., Tanabe, S., Lam, M. and Lam, P., 2005. Risk to breeding success of waterbirds by contaminants in Hong Kong: evidence from trace elements in eggs. *Environmental pollution* 135, 481-490.

Latraube, F., Trotignon, J. and Bretagnolle, V., 2005. Breeding biology of Whiskered Tern *Chlidonias hybridus* in Brenne (France). *Alauda* 73, 425-429.

Layman, C.A., Arrington, D.A., Montana, C.G. and Post, D.M., 2007. Can stable isotope ratios provide for community-wide measures of trophic structure?. *Ecology* 88, 42-48.

Llorente, G., Farran, A., Ruiz, X. and Albaiges, J., 1987. Accumulation and distribution of hydrocarbons, polychlorobyphenyls, and DDT in tissues of 3 species of Anatidae from the Ebro Delta (Spain). *Archives of Environmental Contamination and Toxicology* 16, 563-572.

Lucia, M., Andre, J., Gontier, K., Diot, N., Veiga, J. and Davail, S., 2010. Trace Element Concentrations (Mercury, Cadmium, Copper, Zinc, Lead, Aluminium, Nickel, Arsenic, and Selenium) in Some Aquatic Birds of the Southwest Atlantic Coast of France. *Archives of Environmental Contamination and Toxicology* 58, 844-853.

Lunde, G., 1977. Occurrence and Transformation of Arsenic in Marine-Environment. *Environmental health perspectives* 19, 47-52.

Mañosa, S., Mateo, R. and Guitart, R., 2001. A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. *Environmental Monitoring and Assessment* 71, 187-205.

Mateo, R., Gil, C., Badia-Vila, M., Guitart, R., Hernandez-Matias, A., Sanpera, C. and Ruiz, X., 2004. Use of fatty acids to explain variability of organochlorine concentrations in eggs and plasma of Common Terns (*Sterna hirundo). Ecotoxicology* 13, 545-554.

Mathew, K.L., Lukose, C., Pethani, K.V. and Dave, H.R., 1998. Foraging behaviour of the Gull-billed Tern, *Sterna nilotica* . *Pavo* 36, 15-18.

Monteiro, L.R., Granadeiro, J.P., Furness, R.W. and Oliveria, P., 1999. Contemporary patterns of mercury contamination in the Portuguese Atlantic inferred from mercury concentrations in seabird tissues. *Marine Environmental Research* 47, 137-156.

Moreno, R., Jover, L., Munilla, I., Velando, A. and Sanpera, C., 2010. A three-isotope approach to disentangling the diet of a generalist consumer: the Yellow-legged Gull in northwest Spain. *Marine Biology* 157, 545-553.

Morera, M., Sanpera, C., Crespo, S., Jover, L. and Ruiz, X., 1997. Inter and intraclutch variability in heavy metals and selenium levels in Audouin's gull eggs from the Ebro Delta, Spain. *Archives of Environmental Contamination and Toxicology* 33, 71-75.

Navarro, A., Quiros, L., Casado, M., Faria, M., Carrasco, L. and Benejam, L., 2009. Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. *Aquatic toxicology* 93, 150-157.

Navarro, A., Tauler, R., Lacorte, S. and Barcelo, D., 2010a. Occurrence and transport of pesticides and alkylphenols in water samples along the Ebro River Basin. *Journal of Hydrology* 383, 18-29.

Navarro, J., Oro, D., Bertolero, A., Genovart, M., Delgado, A. and Forero, M.G., 2010b. Age and sexual differences in the exploitation of two anthropogenic food resources for an opportunistic seabird. *Marine Biology* 157, 2453-2459.

Neubauer, W., 1998. Habitat choice of the Common Tern *Sterna hirundo* in eastern Germany. *Vogelwelt* 119, 169-180.

Neuman, J., Chardine, J.W. and Porter, J.M., 1998. Courtship feeding and reproductive success in Black-legged Kittiwakes. *Colonial Waterbirds* 21, 73-80.

Newsome, S.D., del Rio, C.M., Bearhop, S. and Phillips, D.L., 2007. A niche for isotopic ecology. *Frontiers in Ecology and the Environment* 5, 429-436.

Nisbet, I.C.T., Montoya, J.P., Burger, J. and Hatch, J.J., 2002. Use of stable isotopes to investigate individual differences in diets and mercury exposures among Common Terns *Sterna hirundo* in breeding and wintering grounds. *Marine Ecology Progress Series.*

Nisbet, I.C.T., 1973. Courtship-feeding, egg-size and breeding success in Common Terns. *Nature* 241, 141-142.

Norman, D., 1992. The growth rate of little tern *Sterna albifrons* chicks. *Ringing and Migration* 13, 98-102.

Ohlendorf, H.M., Covington, S.M., Byron, E.R. and Arenal, C.A., 2011. Conducting site-specific assessments of selenium bioaccumulation in aquatic systems. Integrated *Environmental Assessment and Management* 7, 314-324.

Oro, D. and Ruiz, X., 1997. Exploitation of trawler discards by breeding seabirds in the north-western Mediterranean: Differences between the ebro delta and the balearic islands areas. *ICES Journal of Marine Science* 54, 695-707.

Padula, V., Burger, J., Newman, S., Elbin, S. and Jeitner, C., 2010. Metals in feathers of Black-Crowned Night-Heron (*Nycticorax nycticorax*) chicks from the New York Harbor Estuary. *Archives of Environmental Contamination and Toxicology* 59, 157-165.

Paillisson, J.M., Reeber, S., Carpentier, A. and Marion, L., 2007. Reproductive parameters in relation to food supply in the Whiskered Tern (*Chlidonias hybrida*). *Journal of Ornithology* 148, 69-77.

Paiva, V.H., Ramos, J.A., Machado, D., Penha-Lopes, G., Bouslama, M.F., Dias, N. and Nielsen, S., 2006. Importance of marine prey to growth of estuarine tern chicks: evidence from an energetic balance model. *Ardea* 94, 241-255.

Paiva, V.H., Ramos, J.A., Martins, J., Almeida, A. and Carvalho, A., 2008a. Foraging habitat selection by Little Terns *Sternula albifrons* in an estuarine lagoon system of southern Portugal. *Ibis* 150, 18-31.

Paiva, V.H., Tavares, P.C., Ramos, J.A., Pereira, E., Antunes, S. and Duarte, A.C., 2008b. The influence of diet on mercury intake by Little Tern chicks. *Archives of Environmental Contamination and Toxicology* 55, 317-328.

Pardo-Cervera, F., Sorensen, I., Jensen, C., Ruiz, X. and Sanchez-Alonso, C., 2010. Breeding biology of the Little Bittern *Ixobrychus minutus* in the Ebro Delta (NE Spain). *Ardeola* 57, 407-416.

Parnell, A., Inger, R., Bearhop, S. and Jackson, A., 2010. Source Partitioning Using Stable Isotopes: Coping with Too Much Variation. *PLoS ONE* 5.

Pastor, D., Sanpera, C., Gonzalez-Solis, J., Ruiz, X. and Albaiges, J., 2004. Factors affecting the organochlorine pollutant load in biota of a rice field ecosystem (Ebro Delta, NE Spain). *Chemosphere* 55, 567-576.

R Core Team, 2012. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing.* http://www.R-project.org.

Ramirez, F., Abdennadher, A., Sanpera, C., Jover, L., Hobson, K.A. and Wassenaar, L.I., 2011. Tracing waterbird exposure to total mercury and selenium: A case study at the solar saltworks of Thyna (Sfax, Tunisia). *Environmental Science & Technology* 45, 5118-5124.

Ramos, R., Ramirez, F., Sanpera, C., Jover, L. and Ruiz, X., 2009. Feeding ecology of Yellow-legged Gulls *Larus michahellis* in the western Mediterranean: a comparative assessment using conventional and isotopic methods. *Marine Ecology*

Progress Series 377, 289-297.

Ruiz, X., Jover, L. and Pedrocchi, V., 1998. Audouin's gull intraspecific variability in yolk and albumen egg contents: Why is yolk size so constant?. *Biologia e Conservazione della Fauna*, 327-334.

Rumbold, D., Niemczyk, S., Fink, L., Chandrasekhar, T., Harkanson, B. and Laine, K., 2001. Mercury in eggs and feathers of great egrets (*Ardea albus*) from the Florida everglades. *Archives of Environmental Contamination and Toxicology* 41, 501-507.

Sakellarides, T., Konstantinou, I., Hela, D., Lambropoulou, D., Dimou, A. and Albanis, T., 2006. Accumulation profiles of persistent organochlorines in liver and fat tissues of various waterbird species from Greece. *Chemosphere* 63, 1392-1409.

Salzer, D.W. and Larkin, G.J., 1990. Impact of Courtship Feeding on Clutch and 3rd-Egg Size in Glaucous-Winged Gulls. *Animal Behaviour* 39, 1149-1162.

Sanpera, C., Moreno, R., Ruiz, X. and Jover, L., 2007. Audouin's gull chicks as bioindicators of mercury pollution at different breeding locations in the western Mediterranean. *Marine Pollution Bulletin* 54, 691-696.

Sanpera, C., Valladares, S., Moreno, R., Ruiz, X. and Jover, L., 2008. Assessing the effects of the Prestige oil spill on the European shag (*Phalacrocorax aristotelis*): Trace elements and stable isotopes. *Science of the Total Environment* 407, 242-249.

Schwemmer, P., Tischler, T., Rehm, R. and Garthe, S., 2011. Habitat use, distribution and food choice of the black-headed gull (*Larus ridibundus*) in the coastal mainland of Schleswig-Holstein. *Corax* 21, 355-374.

Sell, J.L., 1977. Comparative effects of selenium on metabolism of methylmercury by chickens and quail - Tissue distribution and transfer into eggs. Poultry science 56, 939-948.

Stienen, E.W.M., Van Beers, P.W.M., Brenninkmeijer, A., Habraken, J.M.P.M., Raaijmakers, M.H.J.E. and Van Tienen, P.G.M., 2000. Reflections of a specialist: Patterns in food provisioning and foraging conditions in Sandwich Terns *Sterna sandvicensis* . *Ardea* 88, 33-49.

Stienen, E.W.M., Brenninkmeijer, A. and Klaassen, M., 2008. Why do Gull-billed terns *Gelochelidon nilotica* feed on fiddler crabs *Uca tangeri* in Guinea-Bissau. *Ardea* 96, 243-250.

Tavares, P.C., Kelly, A., Lopes, R.J., Pereira, M.E., Duarte, A.C. and Furness, R.W., 2007. The influence of dietary specialization and trophic status on mercury levels in two species using common coastal wetlands, *Himantopus himantopus* and *Sterna albifrons* . *Ardeola* 54, 275-288.

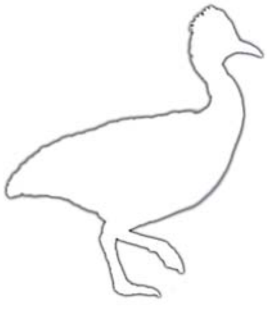
Tavares, P.C., McGill, R., Newton, J., Pereira, E., Duarte, A. and Furness, R.W., 2009. Relationships between carbon sources, trophic level and mercury exposure in generalist shorebirds revealed by stable isotope ratios in chicks. *Waterbirds* 32, 311-321.

Vericat, D. and Batalla, R.J., 2006. Sediment transport in a large impounded river: The lower Ebro, NE Iberian Peninsula. *Geomorphology* 79, 72-92.

Wiggins, D.A. and Morris, R.D., 1986. Criteria for female choice of mates - Courtship feeding and paternal care in the Common Tern. *American Naturalist* 128, 126-129.

Zhang, Y., Ruan, L., Fasola, M., Boncompagni, E., Dong, Y. and Dai, N., 2006. Little egrets (*Egretta garzetta*) and trace-metal contamination in wetlands of China. *Environmental Monitoring and Assessment* 118, 355-368.

Part B



Mining

at the Bolivian Andes

V

Home range extension and overlapping of the Ornated Tinamou (Nothoprocta Ornata) in an Andean Agro-ecosystem

Extensión y solapamiento del ámbito de hogar en el Tinamú Pisacca (*Nothoprocta Ornata*) en un agro-ecosistema Andino

Álvaro Garitano-Zavala[a], Zulma Chura[a], Javier Cotín[b], Jacint Nadal[b] &
Xavier Ferrer[b].

[a] Unidad de Manejo y Conservación de Fauna, Instituto de Ecología, Universidad Mayor de San Andrés, La Paz, Bolivia.

[b] Departament Biologia Animal, Facultat de Biologia, Universitat de Barcelona, 08028, Barcelona, Spain.

Resumen: A pesar de la importancia como especie cinegética y bioindicadora que presenta el Tinamú Pisacca (Nothoprocta ornata), poco es conocido acerca sus aspectos ecológicos básicos, como sus movimientos y rango de hogar. Para describirlos, hicimos radio-seguimiento de 12 individuos adultos en un ecosistema agrario andino durante todo un año. Las aves se mantuvieron durante todo el año dentro de los ámbitos de hogar; los ámbitos de hogar 100% MCP (mínimo polígono convexo) fueron 43,8 ± 21,6 ha (22 a 85 ha) y el 95% kernel de 58,8 ± 31,9 ha (25 a 118 ha). Sólo dos aves mostraron patrones de movimientos amplios antes de su muerte: probablemente eran sub-adultos en busca de territorio. Los individuos superpusieron sus territorios entre el 30 y el 98% con otros 2-3 individuos marcados (los valores son probablemente más altos, dada la existencia de individuos no marcados); siendo los valores máximos de superposición entre los individuos de la pareja. No se observaron diferencias sexuales en la extensión del ámbito de hogar o en su superposición. Sólo dos de los individuos marcados formaron una pareja monógama durante todo el estudio. Nuestros resultados apoyan la utilidad del Tinamú pisacca como especie centinela en estudios de biomonitorización, pero es necesario continuar la investigación acerca de su filopatría y el comportamiento dispersivo de los juveniles. La monogamia social también es respaldada por nuestros datos, pero son necesarios otros estudios para examinar la posibilidad de cópulas y fertilizaciones extra pareja.

Palabras clave: Tinamidae, Región neotropical, ámbito de hogar, Sistemas reproductivos, Andes Bolivianos.

Home range extension and overlapping of the Ornated Tinamou (*Nothoprocta Ornata*) in an Andean Agro-ecosystem

Álvaro Garitano-Zavala[a], Zulma Chura[a], Javier Cotín[b], Jacint Nadal[b]
& Xavier Ferrer[b].

[a] Unidad de Manejo y Conservación de Fauna, Instituto de Ecología, Universidad Mayor de San Andrés, La Paz, Bolivia.

[b] Departament Biologia Animal, Facultat de Biologia, Universitat de Barcelona, 08028, Barcelona, Spain.

Abstract: The Ornate Tinamou (Nothoprocta ornata) is of cynegetic and bio-monitoring importance, but little is known about basic ecological aspects relating to it, such as its movements and home range. To describe them, we radio-tracked 12 adult individuals in an Andean agro-ecosystem for a full year. Birds remained all year within their home ranges; 100% MCP (minimum convex polygon) home ranges were 43.8 ± 21.6 ha (22-85 ha) and 95% kernel home ranges were 58.8 ± 31.9 ha (25-118 ha). Only two birds showed extensive movement patterns before their death: they were probably sub-adults looking for territory. Individuals overlapped their home ranges between 30 and 98% with 2-3 tagged individuals (values are probably higher, given the existence of non-tagged individuals); maximum overlapping values occurred between individuals that formed a pair. No sexual differences were detected in home range extension or overlapping. Only two of the tagged individuals formed a pair in a social monogamous relationship throughout the study. Our results support the usefulness of Ornate Tinamou as a sentinel organism in bio-monitoring studies, but highlight the need for further research on the philopatry and dispersion behavior of juveniles. Social monogamy is also supported by our data, but other studies are needed to examine the possibility of extra-pair copulations and/or fertilizations.

Keywords: Tinamidae, Neotropical region, home range, Mating Systems, Bolivian Andes.

Introduction

Tinamous are among the least studied bird species, despite their evolutionary, ecological and cynegetic importance in Latin American countries. Basic ecological knowledge about their population density, abundance, home range extension, movements and dispersion patterns are important in order to develop management strategies, among other factors. For example, two tinamou species were proposed as environmental sentinels for mining pollution with trace metals in the Andean region (Garitano-Zavala et al. 2010), but it is essential to know the movements and home range extension of the birds to calculate correctly the relationships between trace metal tissue concentrations and the dynamics of the pollutants in the environment where they live.

The Ornate Tinamou (*Nothoprocta ornata*) inhabits the South American Andes from southern Peru to northern Chile and Argentina at altitudes from 2,500 to 4,800 m (Cabot 1992, Davies 2002) and is one of the species put forward as a possible environmental sentinel for mining pollution (Garitano-Zavala et al. 2010). The only previous studies on home range extension and movements for this species were based on field observations by Pearson and Pearson (1955), who reported a nest and feeding territory of 2.43 ha (6 acres) for a nesting pair, suggesting also that this species is territorial and resident.

For other tinamou species, too, early studies of their movements, home range extensions and overlap were based on field observations of few individuals (Schäfer 1954, Lancaster 1964a, Lancaster 1964b). Home ranges reported varied from 0.1 ha in the Highland Tinamou (*Nothocercus bonapartei*) (Schäfer 1954) to 24 ha in the Brushland Tinamou (*Nothoprocta cinerascens*) (Lancaster 1964a). To date, only one study of home range has used radio-tracking. It reported mean home ranges extensions of 16 and 19 ha for the Spotted Tinamou (*Nothura maculosa*) (Thompson and Carroll 2009). In general, tinamous are considered resident non-migratory species, except probably the genus Tinamotis (Cabot 1992, Davies 2002).

The highly variable reports about the home ranges of tinamou species and especially the small area previously reported for the Ornate Tinamou require new studies, in order to obtain more reliable measurements of home range and residence parameters. Although it has been postulated that radio transmitters could negatively affect birds and may bias resulting data (Barron et al. 2010), we consider that radio tracking is not only adequate, but may be the only way to study tinamous in the field, given their reserved behavior (Cabot 1992, Davies 2002, Brennan 2004). The aim of the present study is to give insight into the movements, home range extension and home range overlap of the Ornate Tinamou by monitoring all year round several radio-tagged sympatric males and females in a typical Andean agro-ecosystem on the Bolivian high plateau.

Methods

Study Area

We captured and studied wild Ornate Tinamou individuals in Qurpa (16° 40' S, 68° 51' W; 3,850 to 4,100 m a.s.l.), a rural locality on the Bolivian high plateau east of the Desaguadero River and south of Lake Titicaca. The total surface area used for radio tracking was approximately 820 ha. The climate is cold all year, with greater daily than seasonal temperature fluctuations (minimum average monthly temperature 5.7°C; maximum average monthly temperature 9.9° C), dry in winter (minimum average monthly precipitation 5.1 mm) and rainy in summer (maximum average monthly precipitation 133.7 mm) (SENAMHI 2012).

The mountainous landscape is a heterogeneous mixture of Andean crops (barley, bean, alfalfa, potatoes, oca, onion and quinoa, with no fields more than a quarter hectare large), interspersed with recently harvested and fallow fields and non-agricultural lands. The non-agricultural areas have bunch grasses of *Stipa ichu* and *Festuca spp.* with a mixture of spiny and resinous shrub vegetation (e.g. *Baccharis incarum, Adesmia miraflorensis, Tetraglochin cristatum* and *Satureja boliviana*) and scattered rocky patches. These non-agricultural areas are used for grazing sheep, llamas and cattle. Several weed species grow in both the active and inactive agricultural areas. Crops are grown on an annual basis: sowing takes place between September and January, followed by the crop harvest between May and July, and then the fields are left ploughed for the next sowing season.

Evaluation of effects of radio-tagging

We selected 12 g necklace tags (R1–2D model, Holohil System, Ontario, Canada) with a 24-month lifespan that represent 2.7% of the minimum adult body weight reported for this species (Davies 2002). Necklaces are the most common tagging system used for game-birds (e.g. Novoa et al. 2002, Oakley et al. 2002, Pérez et al. 2004, Watson et al. 2007) that have the same size and ground-dwelling habits as the Ornate Tinamou, and were used successfully with the Spotted Tinamou by Thompson and Carroll (2009). Necklaces are recommended for bird species that do not fly frequently (Marcström et al. 1989, Kenward 2001), as they have no negative effects on their energy levels (Hernández et al. 2004).

To evaluate effects of tagging on Ornate Tinamou (Murray and Fuller 2000), we attached necklace tags to three adult individuals (two females and one male) that were born and bred in captivity in La Paz City (3,450 m a.s.l.). These birds were held with seven other individuals in an enclosure measuring 8 x 5 m. To imitate natural conditions in the wild, the enclosure had resinous shrub and bunch grasses and the birds were exposed to natural weather conditions and fed mainly barley grains and alfalfa leaves, the items that were biggest and most frequently consumed by wild individuals (Garitano-Zavala et al. 2003).

Necklaces were firmly attached to prevent tag loss over the head, and a gap of 7 mm was left between the elastic lace and the neck to avoid blocking the esophagus. For six months we observed the birds' behavior. We looked for abnormal movements, food plugged in the esophagus, bills lodging in the lace (Sorenson 1989), the antenna or tag tangling in vegetation (Brennan 2004) or premature tag loss. When we removed the tags, we examined the skin for wounds and lacerations. We weighed these three birds and three other untagged adult individuals (two females and one male) before and after the six experimental months to evaluate the tagging effect with repeated ANOVA measurements (Zar 2010). We did not consider gender because the sample was small and little effect is generally reported (Barron et al. 2010).

Radio-tracking

We trapped 12 Ornate Tinamou individuals (six males and six females) between September 2006 and March 2007 with a mobile trap technique used by local hunters in Qurpa. The technique involves two teams of three people; each team walks slowly and quietly through the hills, searching from high natural observatories for any movement that may reveal Ornate Tinamou individuals in the crops, bunch grasses or shrubs. Once a bird is sighted, a member of the team runs close to the bird, inducing it to hide and squat under a grass or shrub but not to flush. Two other team members that know the exact position of the bird slowly walk up to it and gently place cotton net attached to two 3 m poles over the vegetation in which the bird is hiding. Finally, the bird is incited to flush and becomes tangled in the net.

The equipment used for radio-tracking comprised two sets of Telonics TR-4, Telonics "H" Adcock RA-2A antennae (Telonics, Inc. Arizona, USA), hand-held GPSs (Garmin eTrexVista, Garmin International, Inc., Olathe, USA) and compasses. We recorded for each trapped bird the GPS position, date, time, body mass and sex (by cloacal examination, searching for the male phallus). There is no reliable field method for accurately determining a bird's age. We attached necklace tags in the way we had for the captive birds and fitted a numbered metal ring on the left leg before releasing it at the same capture location. Handling took no longer than ten minutes. Tag VHF signals were within the range of 150-154 MHz. The code for each individual was the receiver's channel number and gender (Table 1). Tag weight percentages ranged from 1.6 to 3.6%.

We radio-tracked each bird using triangulation and homing. With three birds tagged between September and November 2006 (Table 1), we defined fixed triangulation points, taking into account accessibility and signal detection quality on two parallel routes. The first route (5.16 km) was at the road across the plain (approximately 3,860 m a.s.l.); the second route (4.02 km) was two kilometers away, on the mountain summits (between 4,070-4,270 m a.s.l.). On each route we established seven fixed points. To obtain four consecutive bearings at each location as soon as possible (Kenward 2001), we matched to each fixed point a neighboring point 50 m away, assuming that the movement of any bird was negligible in the time taken for the observer to move that distance.

Precision and accuracy of the telemetry system for triangulation were tested following White and Garrot (1990) and Withey et al. (2001). We attached three test transmitters to wooden posts at 30 cm height within the areas where Ornate Tinamou individuals were observed and/or captured. The mean distance between test-transmitters and fixed points was 1.69 ± 0.60 km. An observer that did not know the locations of the test-transmitters obtained the bearings from all fixed points. We estimated test-transmitter positions with Location of a Signal Software 4.0 (LOAS 2009). To minimize the error of intersection of the triangulated bearing, we used only triangulations that showed an angle between the bird and the fixed points from 60° to 120°.

The mean distance between estimated locations and known test-transmitter positions was 193.67 m (SD = 75.31), and mean and standard deviation of bearing errors were -0.016° ± 12.21. With these results we considered that our triangulation system was not biased and was accurate enough to estimate home ranges and overlap, but not habitat or resource use, given the birds' highly heterogeneous mixture of small-surface habitats (Montgomery et al. 2011).

Twice a month, between December 2006 and October 2007, two observers (one per route) walked from early in the morning in the same direction along the routes (NW to SE or SE to NW) and returned in the opposite direction in the afternoon. At each pair of opposite fixed points, the observers recorded bearings for each bird tagged. Bearings were taken simultaneously for each bird by means of walkie-talkies. The time taken to travel between each telemetry location was at least 20 min, which was chosen to minimize dependency and to obtain an adequate sample size (White and Garrot 1990, Powell 2000). As fixed points were visited twice a day, each bird could be located 14 times a day and 28 a month, but due to topography interference this maximum was reduced.

The homing technique (White and Garrot 1990) was carried out by one observer. Each month from January 2007 to February 2008, one to four individuals were randomly selected for homing. The total number of homing months per bird is shown in Table 1. From sunrise to sunset the selected bird was followed on foot by means of radio contact until visual contact with 10 x 25 binoculars was achieved. Each 10 min the observer obtained the GPS position, the bearing and the exact distance (m) to the bird by using a Laser Rangefinder Bushnell Yardage Pro (Bushnell Performance Optics, Overland Parks, USA). The bird position at each visual contact was determined with Location of a Signal Software 4.0 (LOAS 2009). As these sequential locations were not independent, we did not use them for home range estimation. We used the data to: i) resight birds and evaluate the accuracy of the positions calculated by the triangulation method, constructing a 100% MCP (Mohr 1947) with all homing locations with RANGES VII software (South et al. 2005); and ii) evaluate the eventual effect of time and season on the spatial distribution of the birds, differentiating graphically homing locations by month and time of day (classifying morning 0700-1030, noon 1130-1430 and afternoon 1530-1830). With this information we defined the way to work with triangulation data for home range estimation.

Home range estimation

We entered all bearings obtained for each bird by triangulation into the Location of a Signal Software 4.0 (LOAS 2009) to calculate the bird's locations. We excluded unacceptable locations, accepting only those that showed an angle between the bird and the fixed points from 60° to 120° and error ellipses ≤ 2 ha (but 80% of accepted locations had ≤ 1 ha error ellipses). We determined autocorrelation using Schoener's ratio (Schoener 1981) to test the degree of spatiotemporal relatedness of the locations with RANGES VII software (South et al. 2005).

We calculated the home ranges with 100% MCP (Mohr 1947) and density contours of 95% with fixed-kernel estimator with ad-hoc choice of h (Worton 1989), because both permit direct comparisons with other studies. We also estimated kernel 50% contours as core areas. Kernel estimators are sensitive to sample size, so we calculated home ranges and core areas only for birds with 40 or more locations, as recommended by Seaman et al. (1999). We obtained all home range calculations by RANGES VII software (South et al. 2005) and assessed gender differences by the non-parametric Mann-Whitney U-test, considering differences significant when P < 0.05 (Zar 2010).

We evaluated the total overlapping areas of individual home ranges (100% MCP) and core areas (50% kernel) by a Geographical Information System, ARCVIEW 3.2a (ESRI 1999), obtaining both the total surface area overlapping with certain individuals and the total overlapping area with all the individuals vs. the total free area. We performed gender comparisons using the Mann-Whitney U-test, considering differences significant when P < 0.05 (Zar 2010). For all statistical tests, we used PASW Statistics 18 (PASW 2009).

Results

Effect of radio tags on captive individuals

We did not discover any major effect of tags on the three captive birds. No tags or antennae became tangled in the vegetation; nor were they lost prematurely. However, we did notice that the necklaces became worn, which means that they might have broken and got lost if they had been used longer. After we removed the tags, no bruises or lacerations were found on the skin in contact with the radio tag. No individual's weight was significantly different after the six experimental months, for tagged or untagged individuals (factor before and after tagging: F1,4 = 1.46; P = 0.29; factor tagged and untagged birds: F1,4 = 3.97; P = 0.12).

Wild radio-tagged birds lost during the study

Only 5 of the 12 tagged birds kept the radio tag until the end of the study. The tags of the other seven birds were found in the field or the signal was lost, which occurred between 22 days and 10 months (Table 1). Five of these seven birds were probably predated (representing 42% of the total tagged birds), as the transmitter was still emitting when found and feathers were scattered nearby. In addition, tooth marks were found on several transmitters, which led us to believe that the Andean Fox (*Pseudalopex culpaeus*) killed these birds, although other predators such as the Colocolo cat (*Leopardus colocolo*) or even raptors such as the Black-chested Buzzard-Eagle (*Geranoaetus melanoleucus*) or the Red-backed Hawk (*Buteo polyosoma*) may have been responsible. Of the other two tags, one was found in the field with no predation signs and the signal of the other tag was lost and the tag was never found (Table 1).

Bird movements

Locations for homing and 100% MCP areas are shown in Table 2. For eight individuals we did not observe any important movements, so they were residents of a particular area. Homing data show that the monthly and daily positions were equally distributed within the polygon area without any pattern, indicating the absence of a monthly or daily period effect on bird location. We calculated the home range and core area from all-year triangulation locations for only these eight birds (Table 3).

Channel number	Sex	Body Mass	Capture date	Death or Signal loss date	DRT-T	MRT-H / MRT-T
5	M	420	Sep 26, 06	Aug 01, 07^{a}	32	3-8
10	M	510	Dec 28, 06		44	11-11
15	M	505	Dec 27, 06	Jan 27, 07^{a}	4	0 / 1
20	F	340	Mar 09, 07	Jul 14, 07^{a}	16	3-4
40	F	530	Oct 23, 06		44	10-11
55	F	650	Mar 09, 07	Dec 03, 07^{b}	36	8-8
60	F	(*)	Feb 22, 07	Oct 30, 07^{c}	20	6-5
65	F	740	Dec 28, 06		44	11-11
70	F	595	Dec 27, 06		44	11-11
75	M	330	Mar 09, 07	Mar 31, 07^{a}	8	1-1
90	M	490	Sep 23, 06	Jun 10, 07^{a}	28	3-7
99	M	570	Dec 27, 06		44	11-11

Table 1. Channel number and identification assigned to each radio-tagged Ornate Tinamou, data for sex (F: female or M: male), body mass, capture and death or signal loss date, total days of radio tracking by triangulation (DRT-T) and total months of radio tracking by homing (MRT-H) and triangulation (MRT-T) are shown. (*) Due to the stress it suffered during capture, it was released without being weighed. a Tag was found with signs of predation on the bird. b Tag was found with no signs of predation on the bird. c Tag was not found.

Individual	Locations	MCP 100% (ha)	Distance C-L (m)
05M	208	3	317
10M	935	11	
15M	0	NC	384
20F	212	34	1522
40F	836	8	
55F	775	12	205
60F	370	111	1184
65F	1053	13	
70F	776	9	
75M	62	0.08	133
90M	197	6	121
99M	886	13	

Table 2. Total valid locations obtained by homing radio-tracking for each Ornate Tinamou (F: female or M: male), area size and total linear distance between capture site (C) and site of tag loss (L). NC = Data not calculated.

Individual	Valid locs.	100% MCP (ha)	95% kernel (ha)	50% kernel (ha)	t^2/r^2
05M	178	30	37	10	1.81
10M	113	85	118	33	1.50
15M	31	NC			
20F	55	NC			
40F	192	37	45	11	1.64
55F	100	26	34	8	1.72
60F	33	NC			
65F	86	63	91	22	1.63
70F	124	53	69	18	1.69
75M	21	NC			
90M	163	22	25	6	1.76
99M	71	34	51	14	2.07
Mean ± SD		43.8 ± 21.6	58.8 ± 31.9	15.3 ± 8.9	

Table 3. Total radio-tracked days, valid locations, home range (100% MCP and 95% kernel) and core area (50% kernel) size for each Ornate Tinamou (F: female or M: male) obtained using the triangulation method. t2/r2 is Schoener's ratio. NC = Data not calculated.

	100% MCP (ha)		95% kernel (ha)		50% kernel (ha)	
Group	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Male (*N* = 4)	43 ± 29	22 - 85	58 ± 42	25 - 118	16 ± 12	6 - 3
Female (*N* = 4)	45 ± 16	26 - 63	60 ± 25	34 - 91	15 ± 6	8 - 22

Table 4. Year-round home range (100% MCP and 95% kernel) and core area (50% kernel) mean ± standard deviation for Ornate Tinamou males and females in the Bolivian Andes.

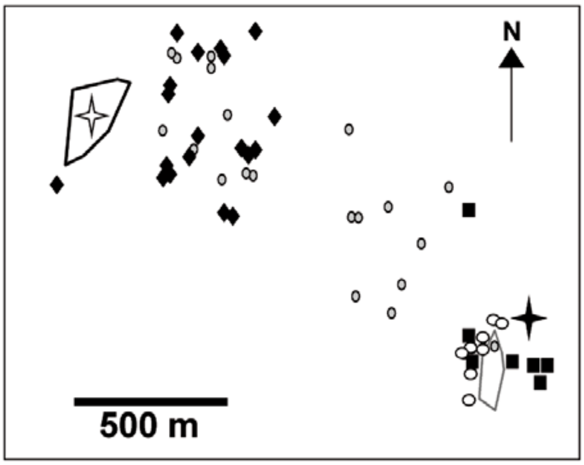


Figure 1. Bird 20F movements during the radio-tracking months. The two polygons were calculated by the homing method. Black star shows capture location; and white star, tag recovery location. Dots indicate triangulation positions for each month: black squares (March), white ovals (April), gray ovals (May), black diamonds (June).

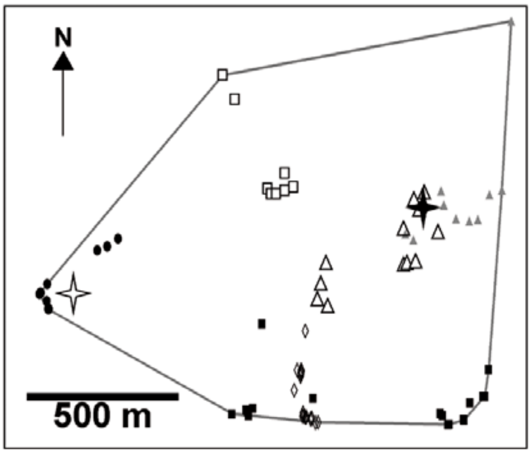


Figure 2. Polygon obtained with bird 60F homing locations. Black star shows capture location; and white star, last location before signal loss. Dots inside polygon show the different homing months: open diamond (March), black ovals (April), open squares (May), black squares (July), gray triangles (August), open triangles (September).

After a month, the tag of bird 15M was found 384 m away from the capture point: there were no homing locations. Individual 75M also died one month after tagging, but as only 21 triangulation locations were obtained, the home range was not calculated.

Bird 20F moved 1,522 m northwest from the capture point in March 2007 to the point of tag recovery in July 2007. This bird was homed for three months and its 100% MCP was expressed in two polygons. During the four months of triangulation it was located in the area between the points of capture and death, suggesting gradual movement (Fig. 1). As these data show a process of movement instead of residence, we did not calculate home range for this bird.

Bird 60F showed a large MCP area and a long distance between capture and last signal reception (Table 2). Graphics revealed a monthly pattern for locations (Fig. 2), suggesting that this was a non-resident bird moving across a wide area before the signal was finally lost. Home range with the triangulation locations was not calculated for this individual.

Home range extension and core areas

The locations of five of the eight birds were observed over a period of 11 months and the total radio-tracking days varied between 28 and 44 (Table 1). Homing 100% MCP polygons were fully or mostly included in the triangulation polygons of 100% MCP and 95% kernel, which confirm the sufficient accuracy of the positions calculated by triangulation (Fig. 3). In addition, the calculated areas included the capture-release and tag-recovery points, or at least the points nearest to those areas (Fig. 3). Since Schoener’s radius values for seven birds were between one and two; and for one, the value was just over 2 (Table 3), we considered the home range and core area calculations reliable.

Individual A	Individual B	Home-range 100 % MCP		Core area 50 % kernel	
		Overlapped Area	% overlappe	Overlapped Area	% overlappe
05M	40F	22	73	6	60
	55F	5	17	0.08	0.8
	90M	4	13	0	0
10M	65F	43	51	12	36
	70F	52	61	5	15
	99M	13	15	2	6
40F	05M	22	60	6	55
	55F	14	38	3	27
	90M	6	16	0	0
55F	05M	5	19	0.08	1
	40F	14	54	3	38
65F	10M	43	68	12	55
	70F	28	44	1	5
70F	10M	52	98	5	28
	65F	28	53	1	5
	99M	4	7	0	0
90M	05M	4	18	0	0
	40F	6	27	0	0
99M	10M	13	38	2	14
	70F	4	12	0	0

Table 5. Overlapping extensions (area and percentage) of home-range (100% MCP) and core area (50% kernel), between year-round radio-tracked Ornate Tinamou individuals.

Individual	Total home-range overlapped 100% MCP		Total core area overlapped 50% kernel	
	ha	%	ha	%
05M	21	71	6	57
10M	77	90	19	56
40F	33	88	8	77
55F	14	53	3	34
65F	43	69	13	59
70F	52	98	5	28
90M	6	30	0	0
99M	13	38	2	17
Mean ± SD	32 ± 24	67.1 ± 24.9	8 ± 6	41.0 ± 25.6

Table 6. Total extension and percentage of home range (100% MCP) and core area (50% kernel) of each individual Ornate Tinamou overlapping with other individuals.

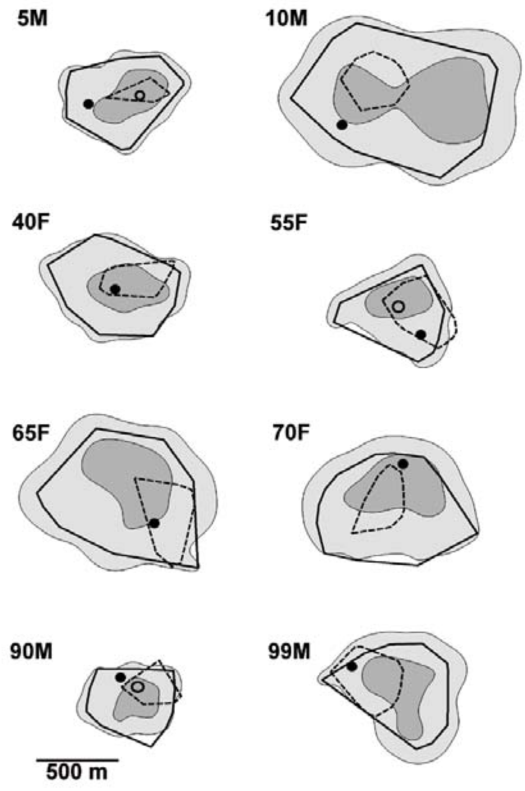


Figure 3. Home range shapes of the eight birds for which home range calculation was possible. Continuous lines show 100% MCP by the triangulation method; pointed lines, the MCP obtained by homing locations; soft gray areas show kernel 95% home range; and dark gray areas, kernel 50% core areas. The closed circle shows capture point and the open circle shows tag recovery location.

Table 3 shows home ranges calculated by 100% MCP and 95% kernel together with the 50% kernel core area for each bird and the general mean values. Table 4 shows the gender-differentiated means. In all cases, home range areas were bigger when calculated by the kernel method. Home ranges calculated by 100% MCP never exceeded 1 km^2 ranging between 22 and 85 ha. Core areas ranged from 6 to 33 ha. No significant gender differences were found in 100% MCP ($z = -0.58$; $P = 0.56$), 95% kernel ($z = -0.29$; $P = 0.77$) or core areas ($z = -0.29$; $P = 0.77$).

No positive correlation was found between the total number of locations and the home range extension (100% MCP: $r_s = -0.21$; $P = 0.61$; 95% kernel: $r_s = -0.38$; $P = 0.35$) or core area ($r_s = -0.38$; $P = 0.35$). Therefore, we believe that we had enough locations to do the calculations.

The eight tagged birds formed two clusters in the study area, both consisting of four birds (two males and two females) separated in two parallel valleys. Figure 4 shows the position of the individuals in each cluster. The presence of the birds in these clusters does not necessarily mean that they were paired.

Home range overlapping

Data on the occurrence of overlapping and percentages of overlapping of home ranges (100% MCP) and core areas (50% kernel) of the radio-tagged birds is shown in Table 5. Totals and percentages of overlap occurrences between home ranges and core areas for each bird are shown in Table 6. All the individuals, regardless of sex, overlapped their home range, with two or three alien tagged males or females inside another cluster (Table 5, Fig. 4). In all cases of home range overlapping with two birds, it was with a male and a female. When overlapping occurred with three birds, the males overlapped with two females and a male, and the females overlapped with two males and a female (Table 5). Most individuals also overlapped their core area with the same individuals that overlapped their home ranges, but to a lesser degree (Table 5).

There was a wide variation in the occurrence and proportion of the home range and core area overlap between birds, but the patterns were not gender-determined. There were

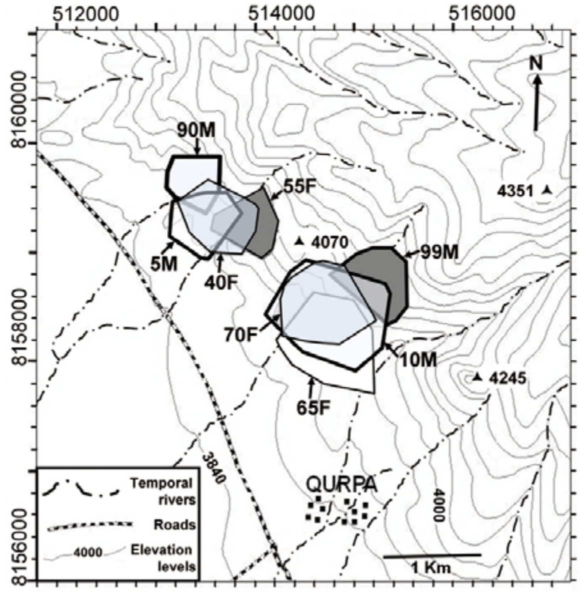


Figure 4. Position in the study area and overlapping of home ranges (MCP 100%) of the eight birds for which home range calculation was possible (each polygon identified with the bird code). Bold-lined polygons are males; light-lined polygons are females.

no significant differences between males overlapping with males and females in the home ranges (z = -1.08, *P* = 0.28; z = -1.07, *P* = 0.29, area and percentage respectively) or core areas (z = -0.88, *P* = 0.38; z = -0.88, *P* = 0.38, area and percentage respectively). This was also the case for females overlapping with males and females in the home ranges (z = -0.43, *P* = 0.67; z = 0.0, *P* = 1.0, area and percentage respectively) or core areas (z = 0.0, *P* = 1.0; z = -0.21, *P* = 0.83, area and percentage respectively).

Individual home range extension and percentage overlap were similar for males and females (z = -0.866; P = 0.386, in both cases), and for both the core area extension and percentage overlap (z = -0.577, *P* = 0.564; z = - 1.155, *P* = 0.248, respectively).

The maximum overlap values (52 ha) detected were for male 10 and female 70, which according to homing observations were a pair. The overlapping area was 61% for the male home range and 98% for the female home range. Individuals 10M and 70F were observed as a pair from April 2007 to January 2008. They always moved and fed together and called to each other frequently. We observed courtships from August 2007 to January 2008 and mountings from October 2007 to January 2008. The male 10M was observed with six chicks in April 2007, and later incubating a six-egg clutch in October 2007. No other pairs of radio-tagged males and females were observed.

During the homing we observed untagged birds feeding, walking or flushing in the home ranges of tagged birds, so the real numbers of birds overlapping home ranges and the percentages for home range overlapping between birds are actually bigger.

In general, individuals of this species tolerate other individuals when close, but tolerance varied between seasons. For example, in the non-reproductive season up to seven individuals were observed feeding very close to the pair 10M-70F, but in the reproductive season both pair members expelled any individual that drew close to them, even forcing the intruders to escape by flying. The minimum distance that apparently fires agonistic behavior was about two meters.

Discussion

The loss of 42% of the tagged birds (probably by predation) is a lower value than in other studies with ground-dwelling birds (which are common target species of several predators): 88% in adult-reintroduced Northern Bobwhites (*Colinus virginianus*) (Oakley et al. 2002), 81% in Red-Legged

Partridge chicks (*Alectoris rufa*) (Pérez et al. 2004) and 91% for wild Spotted Tinamou (Thompson and Carroll 2009).

The birds that died soon after tagging had a lower body weight at the time of capture. Bird 75M did not live more than a month: it weighed 330 g and the tag represented 3.6% of its body mass at the time of capture (the highest percentage of all the tagged birds in this study). Bird 20F lived five months and weighed 340 g (the tag represented 3.5% of its body mass). The weight of the birds that died later ranged from 420 to 505 g, unlike those that survived, whose body weight ranged from 510 to 740 g. Although some researchers failed to detect effects of transmitters weighing less than 4% of body mass (Murray and Fuller 2000), and Barron et al. (2010) suggested little evidence of negative effects when tags exceeded 3%, a predation phenomenon linked to being weaker or lacking experience (if body mass was related to age) must also be kept in mind.

The mean home range extensions reported for the Ornate Tinamou in this study are approximately twenty times greater than those reported by Pearson and Pearson (1955). Our minimum home range values correspond with those described for other tinamou species in the literature, while the other values reported are actually the highest ever reported for one species from this family. The majority of the previous reports, however, were made with direct or signal observations. For example, Schäfer (1954), using a hunting dog to assess home ranges, reported a very small territory of 0.1 ha for males of the Highland Tinamou (*Nothocercus bonapartei*) and territories of 20 ha for the Gray Tinamou (*Tinamus tao*). Lancaster (1964a) reported, by detecting the individual songs of four males, home ranges between 17 and 24 ha for the Brushland Tinamou (*Nothoprocta cinerascens*). The same author using the same technique reported home ranges between 11 and 19 ha for the Slaty-breasted Tinamou ( ) (Lancaster 1964b). In the one other study with radio-tracking technology, Thompson and Carroll (2009) reported mean home range extensions from 16 to 19 ha for the Spotted Tinamou. Although they did not include the ranges, they highlighted the great inter-individual variation, similar to our results for the Ornate Tinamou.

The present study found a high percentage of individual home range overlapping (67 ± 25%) and core areas (41 ± 26%); these values could be higher because only a small percentage of the population was tagged. A high occurrence of overlapping in the home ranges (approximately 75%) and "primary territories" (i.e. core areas) of male Brushland Tinamou was described by Lancaster (1964a). He also described a similar situation for the Slaty-breasted Tinamou (Lancaster 1964b). Based on data for population densities and general behavior patterns, Davies (2002) reported that tinamou species feed randomly in their own home range, tolerate other individuals of their own species and are aggressive only during reproductive events.

The dispersion capacity of a species to re-colonize an area (where any kind of extraction was made) is of great interest for management purposes. At least in this year-round study, it was possible to establish that Ornate Tinamou is sedentary, remaining in the same home range all year round. Therefore Ornate Tinamou individuals are able to obtain resources in their home ranges, taking advantage of both spatial (different habitat types on a small scale) and temporal (annual crop management) heterogeneity, which is closely associated with the generalist and opportunistic feeding behavior of this species (Garitano-Zavala et al. 2003).

Under this assumption the re-colonizing capacity of Ornate Tinamou adult individuals should be seriously limited and local extinction would occur if hunting affects all the individuals of an area. But the dynamics and dispersion capacities of juveniles need to be evaluated, as great movements and the premature death of some of our studied individuals could be interpreted as sub-adult non-resident individuals not established in a stable home range. In the case of bird 20F, the movement pattern was clearly directional, as it may have been looking for its own territory before a predator killed it. Bird 60F showed a skewed movement pattern over a large area (at least 111 ha) before the signal was lost. These data suggest that juveniles probably could not establish in previously occupied territories. However, given the high home range overlap values observed in this study at the same time, the establishment of an individual in a particular home range, in which it must remain all its life, is probably a very complex situation. Finally, the need for further research into the philopatry and dispersion behavior of juveniles should be highlighted: we need better understanding of the re-colonizing capacity of this species after cynegetic activities.

The adult individuals remained within their home ranges for a whole year, with no large movements and using in the meanwhile the water and food resources available despite the seasonal variations and the relatively small surface used for each individual as core areas (around 15 ha), which highlights the usefulness of this species in bio-monitoring programs. This is especially so if these programs are examining the relationship between pollutants and local food resources, important in the case of Andean mining activities, from which pollutants, such as trace metals, could reach soils and water from the tailing piles (Garitano-Zavala et al. 2010).

Pearson and Pearson in 1955, on the basis of field observations alone, believed that monogamy is the mating system for the Ornate Tinamou, a proposition maintained currently by other authors (i.e. Cabot 1992, Davies 2002). Monogamy is a very special behavioral scenery for tinamous, for whom simultaneous polygyny and sequential polyandry is the mating system more commonly observed (Handford and Mares 1985, Cabot 1992, Davies 2002). Our results suggest that the Ornate Tinamou, once a pair is formed, maintains a social monogamous relationship for at least a full year, including the non-reproductive season. But the possibility of non-observed extra-pair copulations and/or fertilizations cannot be excluded, as there is a high degree of overlapping among several individuals, a situation that might expose birds to extra-pair interference.

Acknowledgments

The present study was funded by the Projects CGL2005-04772/B05 (Ministry of Science and Innovation, Spain) and GRC 2009SGR43 (Generalitat de Catalunya). The authors are grateful to Pablo Justiniano, Pedro Ortega and Karem Olarte for their collaboration in the field, to Robin Rycroft and Gretchen Wagner for their corrections of the English draft, to the "Comunidad Originaria de Qurpa" authorities and to the Dirección General de Biodiversidad

(Biodiversity Department) of Bolivia for allowing us to carry out this research.

References

Barron, D. G., J. D. Brawn, and P. J. Weatherhead. 2010. Meta-analysis of transmitter effects on avian behaviour and ecology. *Methods in Ecology and Evolution* 1: 180–187.

Brennan, P. L.R. 2004. Techniques for studying the behavioral ecology of forest-dwelling tinamous (Tinamidae). *Ornitología Neotropical* 15 (Suppl):329-337.

Cabot, J. 1992. Family Tinamidae (tinamous). Pages 112-138 in *Handbook of the Birds of the world*, Volume 1. Ostrich to Ducks (J. del Hoyo, A. Elliot, and J. Sargatal, Editors), Lynx Edicions, Barcelona, Spain.

Davies, S. J. J. F. 2002. Ratites and Tinamous. Oxford University Press, New York, USA.

ESRI. 1999. Arcview 3.2a *Environmental Systems Research Institute*, Inc., Redlands, California, USA.

Garitano-Zavala, A., J. Nadal, and P. Avila. 2003. The feeding ecology and digestive tract morphometry of two sympatric tinamous of the high plateau of the Bolivian Andes: the Ornate Tinamou (*N. ornata*) and the Darwin´s Nothura (*N. darwinii*). *Ornitología Neotropical* 14:173–194.

Garitano-Zavala, A., J. Cotín, M. Borràs, and J. Nadal. 2010. Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia and their potential as environmental sentinels. *Environmental Monitoring and Assessment* 168:629-644.

Handford, P. and M. A. Mares. 1985. The mating systems of ratites and tinamous: an evolutionary perspective. *Biological Journal of the Linnean Society* 25:77-104.

Hernandez, F., J. A. Arredondo, F. Hernandez, D. G. Hewitt, S. J. Demaso, and R. L. Bingham. 2004. Effects of radiotransmitters on body mass, feed consumption, and energy expenditure of northern bobwhites. *Wildlife Society Bulletin* 32:394–400.

Kenward, R. 2001. *A Manual for Wildlife Radio Tagging*. Academic Press, London, UK.

Lancaster, D. A. 1964a. Biology of the Brushland Tinamou, *Nothoprocta cinerascens. Bulletin of the American Museum of Natural History* 127:269-314.

Lancaster, D. A. 1964b. Life history of the Boucard Tinamou in British Honduras, Part II: Breeding biology. *Condor* 66:253-276.

LOAS. 2009. Location of a Signal Software. Version 4.0. *Ecological Software Solutions LLC.* www.ecostats.com

Marcström, V., R. E. Kenward, and M. Karlbom. 1989. Survival of Ring-necked Pheasants with backpacks, necklaces, and leg bands. *Journal of Wildlife Management* 53:808-810.

Mohr, C. O. 1947. Table of equivalent populations of North American small mammals. *American Midland Naturalist* 37:223-249.

Montgomery, R. A., G. J. Roloff, and J. M. Ver Hoef. 2011. Implications of ignoring telemetry error on inference in wildlife resource use models. *Journal of Wildlife Management* 75: 702-708.

Murray, D. L. and M. R. Fuller. 2000. A critical review of the effects of marking on the biology of vertebrates. Pages 15–64 in Research Techniques in Animal Ecology: Controversies and Consequences (L. Boitani and T. K. Fuller, Editors). Columbia University Press, New York, USA.

Novoa, C., N. J. Aebischer, and P. Landry. 2002. Upland habitat use by Pyrenean grey partridges Perdix perdix hispaniensis during the breeding season. *Wildlife Biology* 8:99-108.

Oakley, M. J., D. L. Bounds, T. A. Mollett, and E. C. Soutiere. 2002. Survival and home range estimates of pen-raised Northern Bobwhites in buffer strip and non-buffer strip habitats. Pages 74-80 in *Quail V: Proceedings of the Fifth National Quail Symposium* (S. J. De Maso, W. P. Kuvlesky, F. Hernández, and M. E. Berger, Editors.). Texas Parks and Wildlife Department, Austin, USA.

PASW. 2009. *Predictive Analytics SoftWare (PASW) Statistics. Version 18.* SPSS Institute Inc., Chicago, Illinois, USA.

Pearson, A. K. and O. P. Pearson. 1955. Natural history and breeding behavior of the tinamou *Nothoprocta ornata. Auk* 72:113-127.

Perez, J. A., M. E. Alonso, V. R. Gaudioso, J. A. Olmedo, C. Diez, and D. Bartolome. 2004. Use of Radiotracking Techniques to Study a Summer Repopulation with Red-Legged Partridge (*Alectoris rufa*) Chicks. *Poultry Science* 83:882-888.

Powell, R. A. 2000. Animal home ranges and territories and home range estimators. Pages 65-110 in Research technologies in animal ecology—controversies and consequences (L. Boitani and T. K. Fuller, Editors). Columbia University Press, New York, USA.

Schäfer, E. 1954. Zur biologie des Steisshuhnes *Nothocercus bonapartei. Journal für Ornithologie* 95:219-232.

Schoener, T. W. 1981. An empirically based estimate of home range. *Theoretical Population Biology* 20:281-325.

Seaman, D. E., J. J. Millspaugh, B. J. Kernohan, G. C. Brundige, K. J. Raedeke, and R. A. Gitzen. 1999. Effects of sample size on kernel home range estimates. Journal of *Wildlife Management* 63:739-747.

SENAMHI. 2012. Servicio Nacional de Meteorología e Hidrología, Unidad de Meteorología, Bolivia. www.senamhi.gob.bo/sismet (accessed March 2012).

Sorenson, M. D. 1989. Effects of neck collar radios on female Redheads. *Journal of Field Ornithology* 60:523-528.

South, A. B., R. E. Kenward, and S. S. Walls. 2005. Ranges7 v1.0: for the Analysis of Tracking and Location Data. Anatrack Ltd., Wareham, Massachusetts, USA.

Thompson, J.J. and J. P. Carroll. 2009. Habitat Use and Survival of the Spotted Tinamou (*Nothura maculosa*) in Agroecosystems in the Province of Buenos Aires, Argentina. Pages 111-119 in Gamebird 2006: Quail VI and Perdix XII. 31 May - 4 June 2006 (S. B. Cederbaum, B. C. Faircloth, T. M. Terhune, J. J. Thompson, and J. P. Carroll, Editors.). Warnell School of Forestry and Natural Resources, Athens, Georgia, USA.

Watson, M., N. J. Aebischer, and W. Cresswell. 2007. Vigilance and fitness in grey partridges *Perdix perdix*: the effects of group size and foraging-vigilance trade-offs on predation mortality. *Journal of Animal Ecology* 76:211–221.

White, G. C. and R. A. Garrot. 1990. Analysis of wildlife Radio-tracking data. Academic Press, San Diego, California, USA.

Withey, J. C., T. D. Bloxton, and J. M. Marzluff. 2001. Effects of Tagging and Location Error in Wildlife Radiotelemetry Studies. Pages 43-75 in Radio Tracking and Animal Populations (J. J. Millspaugh and J.M. Marzluff, Editors). Academic Press, San Diego, California, USA.

Worton, B. J. 1989. Kernel Methods for Estimating the Utilization Distribution in Home-Range Studies. *Ecology* 70:164-168.

ZAR, J. H. 2010. Biostatistical analysis, Fifth Edition. Pearson International Edition, Upper Saddle River, New Jersey, USA.

VI

Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia and their potential as environmental sentinels

Concentraciones de elementos traza en tejidos de dos especies de tinamú en áreas mineras de Bolivia y su potencial como bioindicadores

Álvaro Garitano-Zavala[a], Javier Cotín[b], Miquel Borrás[c] & Jacint Nadal[b].

[a] Unidad de Manejo y Conservación de Fauna, Instituto de Ecología, Universidad Mayor de San Andrés, La Paz, Bolivia.

[b] Departament Biologia Animal, Facultat de Biologia, Universitat de Barcelona, E-08028, Barcelona, Spain.

[c] Unitat de Toxicologia Experimental i Ecotoxicologia, Centre de Recerca en Toxicologia, Parc Cientific de Barcelona, Barcelona, Spain

Resumen: La minería tiene una larga historia en los Andes Bolivianos y ha dejado muchos residuos, desde los cuales los elementos trazas pueden llegar a las aguas superficiales, los suelos o la fauna. El potencial de los tinamúes (Aves: Tinamidae) como bioindicadores nunca ha sido testado previamente, aunque su biología y características ecológicas hacen de ellos buenos candidatos. Capturamos 13 y nueve individuos de Tinamú Pisacca (*Nothoprocta ornata*) de dos localidades contaminadas (P1 y P2) y 10 y cinco de zonas control no contaminadas (NP1 y NP2) y usamos, para propósitos comparativos, cuatro ejemplares criados en cautividad. También capturamos seis ejemplares de Tinamú de Darwin (*Nothura darwinii*) de la zona contaminada P2. Determinamos las concentraciones de As, Cd, Pb y Sb en plumas, hígado y riñón y realizamos análisis histológicos en hígado y riñón. En el caso de el Tinamú pisacca, se encontró un efecto de localidad para todos los elementos traza en todos los tejidos, con las máximas concentraciones en las zonas contaminadas. En la zona P2, no se encontraron diferencias entre las dos especies de Tinamúes, con la excepción de que algunos Tinamúes de Darwin mostraron concentraciones casi el doble de elevadas. En algunos ejemplares, la media o los valores individuales de los elementos traza alcanzaron niveles tóxicos en las zonas contaminadas. Se encontró tesaurismosis en los túbulos renales, probablemente relacionada con la exposición al Cd, en un 30% de las muestras de la zona P1. Se observaron correlaciones significativas entre todos los tejidos para todos los elementos traza y también para todos los elementos traza en cada tejido. Puesto que las especies estudiadas son ubicuas y relativamente abundantes, se recomiendan los estudios de monitorización utilizando análisis en plumas de dichas especies.

Palabras clave: Actividad minera, Andes, Antimonio, Tinamú Pisacca, Tinamú de Darwin

Environ Monit Assess (2010) 168:629–644
DOI 10.1007/s10661-009-1139-7

Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia and their potential as environmental sentinels

**Álvaro Garitano-Zavala · Javier Cotín ·
Miquel Borràs · Jacint Nadal**

Received: 8 May 2009 / Accepted: 17 August 2009 / Published online: 16 September 2009
© Springer Science + Business Media B.V. 2009

Abstract Mining has a long history in the Bolivian Andes and has left many tailing piles, from which trace metals may reach surface waters, soils, and biota. The potential of tinamous (Birds: Tinamidae) as sentinels has never been tested before, although their biological and ecological characteristics mean they could well be appropriate bioindicators. We captured 13 and nine individuals of the Ornate Tinamou (*Nothoprocta ornata*) from two polluted sites (P1 and P2) and 10 and five from control unpolluted sites (NP1 and NP2) and used, for comparative purposes, four specimens bred in captivity. We also captured six specimens of Darwin's Nothura (*Nothura darwinii*) from the polluted site, P2. We determined the concentration of As, Cd, Pb, and Sb in feathers, liver, and kidney and conducted histological analyses of liver and kidney. For the Ornate Tinamou, a site effect was found for all trace metals in all tissues, with the highest concentrations at polluted sites. At the P2 site, no differences between the two tinamou species were detected except in some cases where Darwin's Nothura shows near-double concentrations. In some cases, mean and/or individual values of trace metal concentrations reached toxicity levels at the polluted sites. Thesaurismosis in proximal convoluted renal tubules, probably related to Cd exposure, was observed in 30% of the samples from the P1 site. Significant correlations were observed between all tissues for all trace metals and also for all trace metals in each tissue. Because the species studied are ubiquitous and relatively abundant, we recommend monitoring programs based on feather analysis.

Keywords Mining activity · Andes · Antimony · Ornate Tinamou · Darwin's Nothura

Introduction

Anthropogenic mining has taken place in the Bolivian high Andes since at least the fifteenth century (Sanabria 2000). Particularly in the East

---

Á. Garitano-Zavala (✉)
Unidad de Manejo y Conservación de Fauna, Instituto de Ecología, Universidad Mayor de San Andrés,
Casilla 10077, La Paz, Bolivia
e-mail: agaritanozavala@umsa.bo

J. Cotín · J. Nadal
Secció de Vertebrats, Departament de Biologia Animal, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 645, 08028
Barcelona, Spain

M. Borràs
Unitat de Toxicologia Experimental i Ecotoxicologia, Centre de Recerca en Toxicologia, Plataforma Tecnològica del Parc Científic de Barcelona,
Barcelona, Spain

of Oruro Department in the Eastern Andean Cordillera, there are extensive polymetallic deposits (SERGEOMIN 1999). In the past, extraction of gold and silver (associated with sulfurs of Fe, Cu, Zn, Pb, As, Sb, etc.) was the main activity, while at present, extraction of tin and the Zn–Ag–Pb complex predominates (Rios 2002). These mining activities were characterized by the deposition of large tailing piles, which accumulated in abandoned and active mines, where trace metals, such as lead, arsenic, cadmium, and antimony could reach surface waters and soils, and so the biota. For example, high Cd levels were found in potato tubers cultivated in agrosystems irrigated by the Chayanta River, which receives mineral residuals from the principal tin Bolivian mines, situated 60 Km away (Oporto et al. 2007; Rojas and Vandecasteele 2007).

Contamination of this region through the centuries has polluted the principal water body, the Poopó Lake. Not only does its water have higher metal concentrations than the permissible values for human consumption (UNEP/OEA 1996; Van Ryckeghem 1997), but the fish that live in the lake do too (Beveridge et al. 1985).

Interest in sentinel organisms as environmental biomonitors is rising (Borràs and Nadal 2004; Burger 1993; Burger and Gochfeld 2000a, 2009; de Lapuente et al. 2008; Furness et al. 1993; Grove et al. 2009; Llacuna et al. 1995; Lounsbury-Billie et al. 2008; Nam et al. 2004a; Sanchez-Chardi et al. 2007).

Tinamous are birds that have been studied little (Cabot 1992; Davies 2002); their potential as sentinels has never been tested before. The Ornate Tinamou (*N. ornata*) and Darwin's Nothura (*N. darwinii*) live in the Bolivian highlands between 3,700 and 4,200 m asl (Cabot 1992; Davies 2002; Garitano-Zavala et al. 2003), height which includes mining sites; both species are relatively common and major hunting targets (Garitano-Zavala 2002).

Highland tinamou species possess characteristics that mean they could be appropriate bioindicators, such as a sedentary lifestyle and diet. Pearson and Pearson (1955) determined that Ornate Tinamou individual home ranges are 2.43 ha. Moreover, the feeding habits of the Ornate Tinamou and Darwin's Nothura are generalist and opportunist, consisting of a wide variety of seeds, leaves, and fruits from crops and weeds as well as invertebrates (Garitano-Zavala et al. 2003). Although their total life span in the wild has not been studied, it is known that Ornate Tinamou live at least 6 years in captivity (Garitano-Zavala pers. obs.). Therefore, trace metal levels in tinamou tissues from a polluted area should reflect local exposure.

In this study, we determine the differential concentration of arsenic (As), cadmium (Cd), lead (Pb), and antimony (Sb) in feathers, liver, and kidney of tinamous collected at mining and control sites and the histopathological status of liver and kidney, with the aim of evaluating the potential of these species as trace metal pollution sentinels.

Methods

Study area

Four sites were included: two mining sites with trace metal contamination reported in soils and water and two sites without any mining activity (control sites). The mining sites are in the Eastern Andean Cordillera, Southeast of Oruro city and East of the Poopó Lake. They are called Antequera or Polluted 1 (P1) (4,000 m, 18°28′ S lat; 66°52′ W long), and Poopó or Polluted 2 (P2) (3,800 m, 18°23′ S lat; 66°58′ W long). There are 13 linear kilometers between these two sites, whose waters drain in two distinct hydrographic sub-basins, which finally drain into Lake Poopó (Fig. 1).

In Antequera, the zinc–silver–lead complex is industrially exploited and all the residuals are deposited in a storage pond that closes a little valley near the Antequera river course (Rios 2002), where there are several old tailing piles scattered. All this mining activity has polluted the river soils, with concentrations several times above permissible values for As, Sb, Cd, Cu, Cr, Sn, Fe, Hg, Pb, and Zn (MEDMIN 2001). At Poopó, tin and secondarily the zinc–silver–lead complex are exploited and concentrated. Numerous tailing piles are scattered across the landscape (Rios 2002), and metals and other elements are easily dispersed by water and wind to soils and water

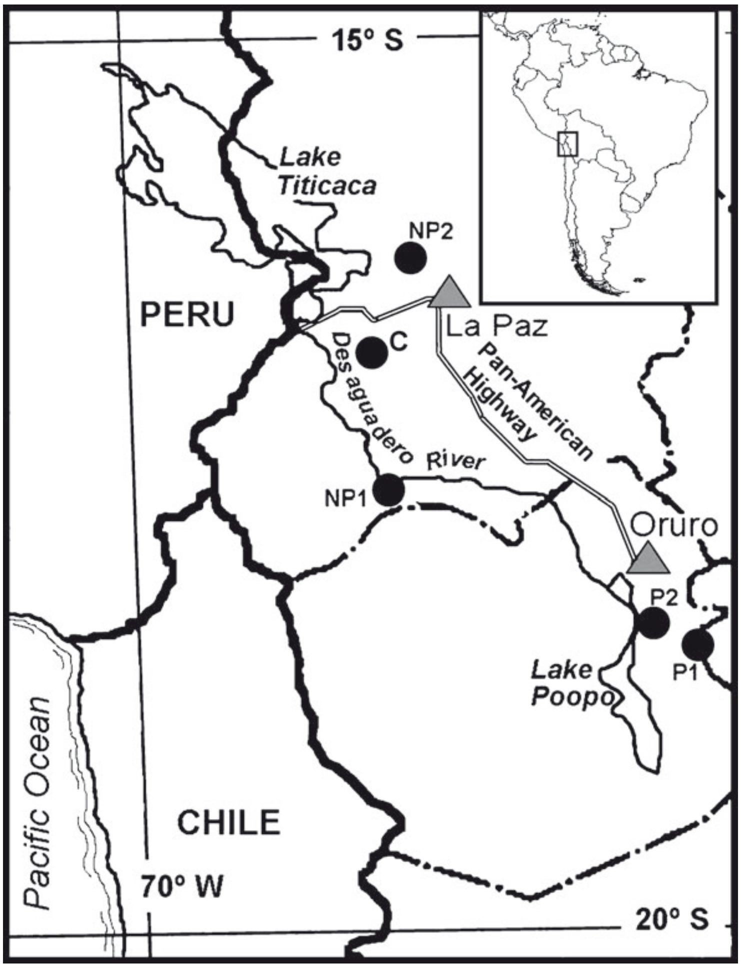


Fig. 1 Study sites on the Bolivian high plateau of the Central Andes. The control sites are called San Pedro de Ulloma or nonpolluted 1 (*NP1*) and Chuñavi or nonpolluted 2 (*NP2*): the mining sites are Antequera or Polluted 1 (*P1*) and Poopó or Polluted 2 (*P2*). Individuals of Ornate Tinamou (*N. ornata*) from captivity were obtained from a rural experimental breeding center (C)

bodies, especially to the Poopó River that drains into Lake Poopó. The soils at bends on the Poopó River have high concentrations of Fe, Zn, Cd, Cu, Pb, and Hg (MEDMIN 2001).

The control sites are called San Pedro de Ulloma or Non-polluted 1 (NP1) (3,850 m, 17°30′ S lat; 68°32′ W long), located to the West of the Desaguadero River, and Chuñavi or Non-polluted 2 (NP2) (4,100 m, 16°17′ S lat; 68°20′ W long), to the northwest of the city of La Paz and East of Lake Titicaca. The former is 200 linear kilometers from the mining localities; and the latter, 280 linear kilometers (Fig. 1).

All these sites are on the Bolivian high plateau and adjacent hills of the Eastern Andean Cordillera. The vegetation of the four sampling sites is dominated by spiny and resinous bushes (i.e., *Baccharis* spp., *Parastrephia* spp., and *Adesmia* spp.) and bunch grasses (i.e., *Festuca* spp. and *Stipa* spp.). Agricultural and livestock activities take place at the four sites, with the principal crops being potato tubers and barley, and extensive sheep, cattle, and llama breeding.

For comparative purposes, four Ornate Tinamou bred in captivity were analyzed. The birds were 18 months old and bred at an experimental rural captivity breeding center (C) (3,850 m, 16°40′ S lat; 68°51′ W long). The center is also on the Bolivian high plateau, to the East of the Desaguadero River (Fig. 1). These specimens descend from birds captured in the wild around the center.

Specimen collection

All specimens were captured by hunters with shotguns (helped by Pointers) between August and September 2006 and July and September 2007, during the dry season (southern winter) of the Bolivian highlands. In order to prevent any kind of contamination by residues of lead pellets or powder, in all cases, samples were collected from inside the tissue in which there was no sign of shot wound.

Ornate Tinamou were collected at the four study sites (13 at NP1, nine at NP2, 10 at P1, and five at P2), but only six specimens of Darwin's Nothura were captured at the P2 site. They were compared with Ornate Tinamou at this site.

Plumage characteristics led to all specimens being classified as adults; Ornate Tinamou reach adult plumage aged 9 to 10 months (Molina 2005) and, as Darwin's Nothura is a closely related species, it probably follows the same molting pattern. It was not possible to determine age accurately (years of life) because of the lack of a trustworthy method. A field necropsy was performed a few minutes after the death of the birds. Sex was determined from the gonads. Two tissue samples of liver and kidney (approx. 2 g wet weight) were taken from each bird with a steel scalpel, one for trace metal determination, and one for histological studies. Samples for trace metal determination were placed in individually marked plastic bags and conserved in a container with dry ice. Once in the laboratory these tissues were dried to constant weight at 60°C and conserved in individually marked disposable conical plastic tubes (NUNC^{TM}). Ten wing feathers (primaries and secondaries) were obtained in the field and placed in individually marked plastic bags, also for trace metal determination.

For histological analysis, liver and kidney samples were conserved in disposable conical plastic tubes with 10% formol. Dry tissues, tissues in 10% formol in plastic tubes and plastic bags with feathers, were shipped to the University of Barcelona for analysis.

The same process was used with the Ornate Tinamou from the experimental breeding center, but only liver and kidney samples for trace metal determination were taken.

Trace metal determination

Feathers were cleaned with a 0.1-M NaOH solution and dried out at 50°C prior to chemical determination of arsenic, cadmium, lead, and antimony by means of ICP-MS Perkin Elmer ELAN 6000. Before chemical determination, 100 mg of feather, liver, and kidney samples were digested according to the acid digestion protocol with Savilles Teflon digestion vessels, using H_2NO_3 (2 ml) and H_2O_2 (1 ml) for feathers and H_2NO_3 (3 ml) and H_2O_2 (2 ml) for liver and kidney (due to their high lipid content) for 12 h at 100°C. Accuracy of analysis was checked by measuring certified reference materials (Human Hair CRM 397—Community Bureau of Reference—and Lobster Hepatopancreas Tort-2—National Research Council Canada). Mean recoveries ranged from 98% to 100% for total arsenic, cadmium, lead, and antimony, and no corrections were done.

Quantification limits for the trace elements were 0.1 ppb. All trace element concentrations were expressed on a dry weight basis (ng/g, i.e., parts per billion). Trace element analyses were performed at the Serveis Científico-Tècnics (University of Barcelona).

Histological analyses

Tissue samples, embedded in Histocomp (Vogel), 5 μm thick, Hematoxylin–Eosin-stained and mounted in DPX were examined with a Nikon E400 microscope. Readings were performed blind.

Data analysis

Non-normally distributed and heteroscedastic data were log transformed (Log_{10} [trace element concentration in ppb d-w + 1]). Main site and sex effect and their interactions were analyzed by an ANOVA bifactorial model for the Ornate Tinamou specimens from the five sites. The Scheffé post hoc test was run. For the P2 site, an ANOVA bifactorial model was used to analyze the principal species (Ornate Tinamou and Darwin's Nothura) and sex effect, together with their interaction.

Table 1 Mean and Standard Deviation (SD) of trace metal levels (ppb d-w) (ng/g dry weight), in Ornate Tinamou feathers, liver, and kidney from the wild at nonpolluted sites (NP1 and NP2) and polluted sites (P1 and P2) and from captivity (C)

Site	N	Feathers		Liver		Kidney	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
As							
C	4			127.63 ± 53.58	84.73–205.20	339.77 ± 79.32	268.68–437.48
NP1	13	315.09 ± 76.58	169.18–444.39	98.22 ± 31.83	45.93–142.25	328.41 ± 174.19	151.69–823.69
NP2	9	467.85 ± 74.09	414.98–658.06	200.06 ± 66.16	101.71–287.60	367.12 ± 66.24	248.14–447.56
P1	10	2,270.67 ± 1,140.83	1,148.28–5,084.35	580.25 ± 260.36	335.06–1,081.83	1,104.25 ± 324.69	622.43–1,804.27
P2	5	787.94 ± 291.78	497.31–1,256.45	228.52 ± 85.76	163.63–374.86	417.78± 204.97	212.25–737.97
F(*df*); *p* value		47.80 (3, 29); <0.001		23.65 (4, 31); <0.001		14.09 (4, 31); <0.001	
Subgroups		(NP1–NP2) (P2) (P1)		(NP1–C) (C–NP2–P2) (P1)		(NP1–C–NP2–P2) (P1)	
Cd							
C	4			122.04 ± 23.44	97.58–154.00	821.84 ± 160.30	592.63–946.83
NP1	13	9.50 ± 7.04	3.01–26.94	786.76 ± 630.40	230.89–2,350.91	7,137.19 ± 8,937.02	715.90–26,631.65
NP2	9	4.82 ± 2.48	2.73–10.63	2,793.80 ± 2,778.24	468.63–8,860.29	26,059.04± 26,602.53	1,454.02–86,120.05
P1	10	248.89 ± 295.76	46.25–1,023.57	14,822.90 ±9,498.30	2,876.10–28,313.77	62,032.22 ± 63,430.86	7,655.68–207,331.50
P2	5	52.32 ± 14.82	33.22–71.74	9,328.85 ± 9,589.63	1,548.72–24,146.77	59,104.64 ± 73,342.39	6,452.04–180,590.43
F(*df*); *p* value		40.66 (3, 29); <0.001		26.92 (4, 31); <0.001		8.89 (4, 31); <0.001	
Subgroups		(NP2–NP1) (P2) (P1)		(C) (NP1–NP2) (NP2–P2) (P2–P1)		(C–NP1) (NP1–NP2–P2) (NP2–P2–P1)	
Pb							
C	4			64.78 ± 57.94	22.19–146.10	246.18 ± 80.52	166.27–353.81
NP1	13	1,335.24 ± 2,149.21	173.81–7,817.12	104.80 ± 173.32	6.54–617.23	489.36 ± 621.29	82.05–2,455.59
NP2	9	1,643.05 ± 2,005.29	264.56–5,716.57	100.27 ± 60.09	36.99–231.89	392.43 ± 106.06	266.78–555.95
P1	10	7,769.48 ± 8,947.08	1,701.58–25,273.0	757.83 ± 1,195.88	192.66–4,097.68	1,754.26 ± 652.63	823.31–2,584.24
P2	5	3,969.70 ± 2,001.57	1,510.17–6,513.48	1,928.44 ± 3,808.35	121.60–8,738.12	1,484.53 ± 612.39	903.51–2,502.37
F(*df*); *p* value		6.44 (3, 29); 0.002		4.54 (4, 31); 0.005		10.79 (4, 31); <0.001	
Subgroups		(NP1–NP2) (NP2–P2) (P2–P1)		(NP1–C–NP2) (NP2–P1–P2)		(C–NP1–NP2) (P2–P1)	
Sb							
C	4			2.47 ± 1.01	1.55–3.90	3.96 ± 1.12	2.58–4.92
NP1	13	29.00 ± 27.77	11.49–117.41	9.97 ± 9.85	3.93–35.94	17.06 ± 21.29	3.77–85.83
NP2	9	54.81 ± 40.81	18.74–142.74	29.45 ± 14.96	7.57–59.71	22.46 ± 15.37	6.83–50.57
P1	10	442.94 ± 286.73	193.72–997.36	64.86 ± 51.11	32.00–202.98	259.90 ± 504.54	38.08–1,689.31
P2	5	499.23 ± 161.59	371.46–761.61	58.93 ± 66.66	16.78–175.71	27.36 ± 17.67	7.98–55.19
F(*df*); *p* value		37.53 (3, 29); <0.001		17.69 (4, 31); <0.001		21.12 (4, 31); <0.001	
Subgroups		(NP1–NP2) (P1–P2)		(C–NP1) (NP2–P2–P1)		(C–NP1) (NP1–NP2–P2) (P1)	

The *F* value, degree of freedom (*df*), and *p* value from the ANOVA analysis are only shown for the principal factor "site"; the subgroups were formed with the Schefeé post hoc test

Table 2 Mean and Standard Deviation (SD) of trace metal levels (ppb d-w) (ng/g dry weight) in Ornate Tinamou (*Nothoprocta ornate*) and Darwin's Nothura (*Nothura darwinii*) feathers, liver, and kidney from polluted site P2

Site	N	Feathers		Liver		Kidney	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
As							
ND	6	1,045.24 ± 383.49	568.90–1,561.66	353.12 ± 184.33	193.98–590.64	731.15 ± 253.37	438.58–1,103.77
OT	5	787.94 ± 291.78	497.31–1,256.45	228.52 ± 85.77	163.63–374.86	417.78 ± 204.97	212.25–737.97
F(*df*); *p* value		3.54 (1, 7); 0.102		0.96 (1, 7); 0.359		6.45 (1, 7); 0.039	
Cd							
DN	6	142.05 ± 85.03	52.08–262.53	5,514.64 ± 3,029.15	2,816.16–10,200.12	28,721.07 ± 13,596.07	14,623.46–48,481.37
OT	5	52.32 ± 14.82	33.22–71.74	9,328.85 ± 9,589.63	1,548.72–24,146.77	59,104.64 ± 73,342.39	6,452.04–180,590.43
F(*df*); *p* value		17.34 (1, 7); 0.004		0.06 (1, 7); 0.817		0.055 (1, 7); 0.821	
Pb							
DN	6	16,528.24 ± 24,960.86	3,132.1–67,265.59	295.98 ± 235.08	69.68–724.31	10,713.13 ± 19,660.47	1,032.68–50,647.36
OT	5	3,969.70 ± 2,001.57	1,510.17–6,513.48	1,928.44 ± 3,808.35	121.60–8,738.12	1,484.53 ± 612.39	903.51–2,502.37
F(*df*); *p* value		9.30 (1, 7); 0.019		0.17 (1, 7); 0.689		4.09 (1, 7); 0.083	
Sb							
ND	6	1,023.62 ± 721.26	474.17–2,333.70	46.05 ± 22.25	24.49–83.10	247.24 ± 433.54	8.93–1,125.55
OT	5	499.23 ± 161.59	371.46–761.61	58.93 ± 66.66	16.78–175.71	27.36 ± 17.67	7.98–55.19
F(*df*); *p* value		10.13 (1, 7); 0.015		0.21 (1, 7); 0.662		4.00 (1, 7); 0.086	

The *F* value, degree of freedom (*df*), and *p* value from the ANOVA analysis are only shown for the principal factor "species"

Nonparametric Spearman correlations evaluated trace metal relationships between and within tissues. Analyses were performed using SPSS® v.15. 0.05 probability level, but due to sample size restrictions, all probability values are shown to allow the reader to assess significance.

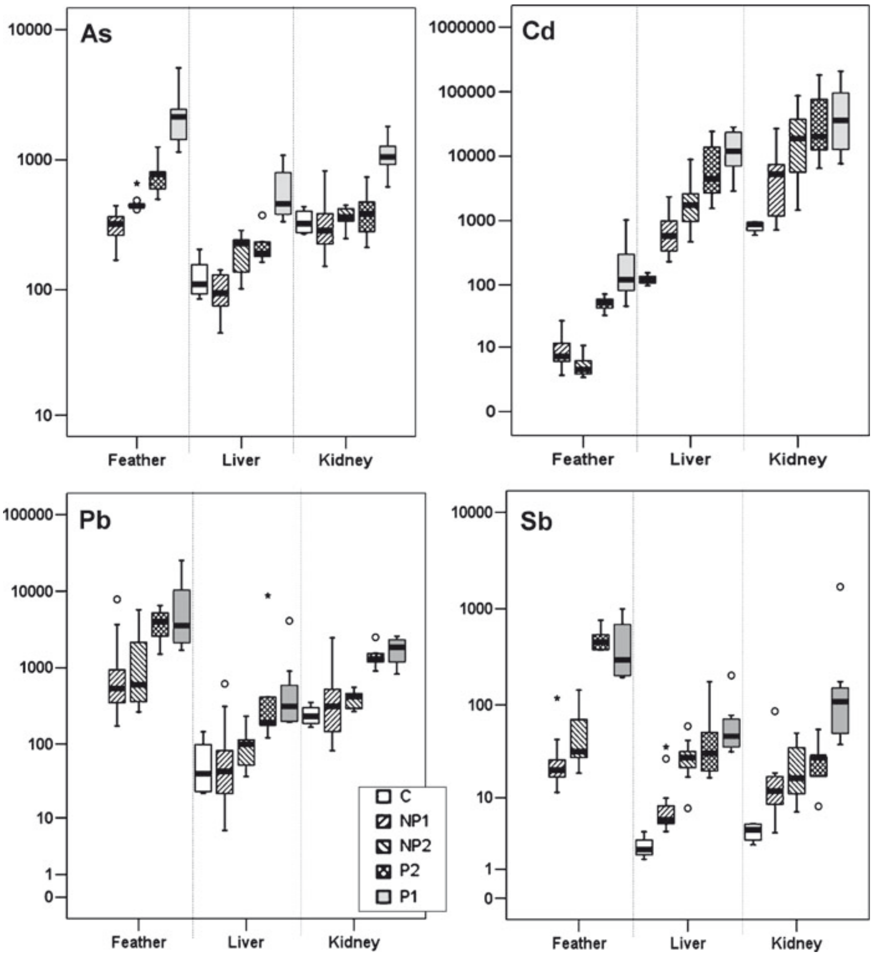
Results

Trace metals means, Standard Deviation and ranges of As, Cd, Pb, and Sb for Ornate Tinamou are shown in Table 1. Values for Ornate Tinamou and Darwin's Nothura from the P2 site are shown in Table 2. No significant interactions ($p >$ 0.05) or sex effects ($p > 0.05$) were found in the analysis.

For the Ornate Tinamou, site effects were found for all trace metals in all tissues. Arsenic concentrations in feathers, liver, and kidney were significantly higher at the P1 site. Feathers from the polluted sites formed two distinct subgroups. In relative terms, arsenic concentrations were higher in feathers and kidney, nearly doubling the liver concentrations (Table 1, Fig. 2). Liver and kidney concentrations of captive birds ranged within the range of unpolluted sites.

Cadmium concentrations in feathers and liver were significantly higher at the P1 site, but for kidney, the difference with the unpolluted NP2 site was less clear; as with arsenic, differences between

Fig. 2 *Boxplots* for each trace metal concentration in feather, liver, and kidney of Ornate Tinamou (*N. ornata*), from captivity (C), control sites San Pedro de Ulloma (NP1) and Chuñavi (NP2) and mining sites Antequera (P1) and Poopó (P2)



polluted and nonpolluted sites were more noticeable in feathers (Fig. 2). Liver and kidney Cd concentrations of the captive birds were around seven times lower than the minimal media in the wild. Relatively, cadmium was much higher in kidney, with five to ten times higher concentrations than in liver and 250 to 5,000 times higher than in feathers (Table 1).

Lead was clearly higher in feathers (around three times higher concentrations than in kidney and ten times higher than in liver). Polluted sites showed the highest concentrations, especially for kidney and at least for the P1 site in feathers (Table 1, Fig. 2). Liver and kidney Pb concentrations in captive birds were within the range of unpolluted sites.

Antimony concentrations were higher at the polluted sites, with this clearer in feathers, and for the P1 site in kidney. Feathers accumulated Sb relatively more than the other tissues (Table 1, Fig. 2). Liver and kidney Sb concentrations of captive birds were at least four times lower than the minimum mean value in the wild birds.

At the P2 site, the tissues of Darwin's Nothura had concentrations of the four trace metals analyzed that were similar to the Ornate Tinamou with, in most cases, no significant differences between the two species detected (Table 2). Exceptions were observed for all trace metals except As in feathers and As in kidney, in which cases Darwin's Nothura concentrations nearly doubled Ornate Tinamou ones.

Concentrations of the four trace metals analyzed correlated significantly in each tissue (Figs. 3, 4, and 5). As significant correlations were also observed between the four trace metal

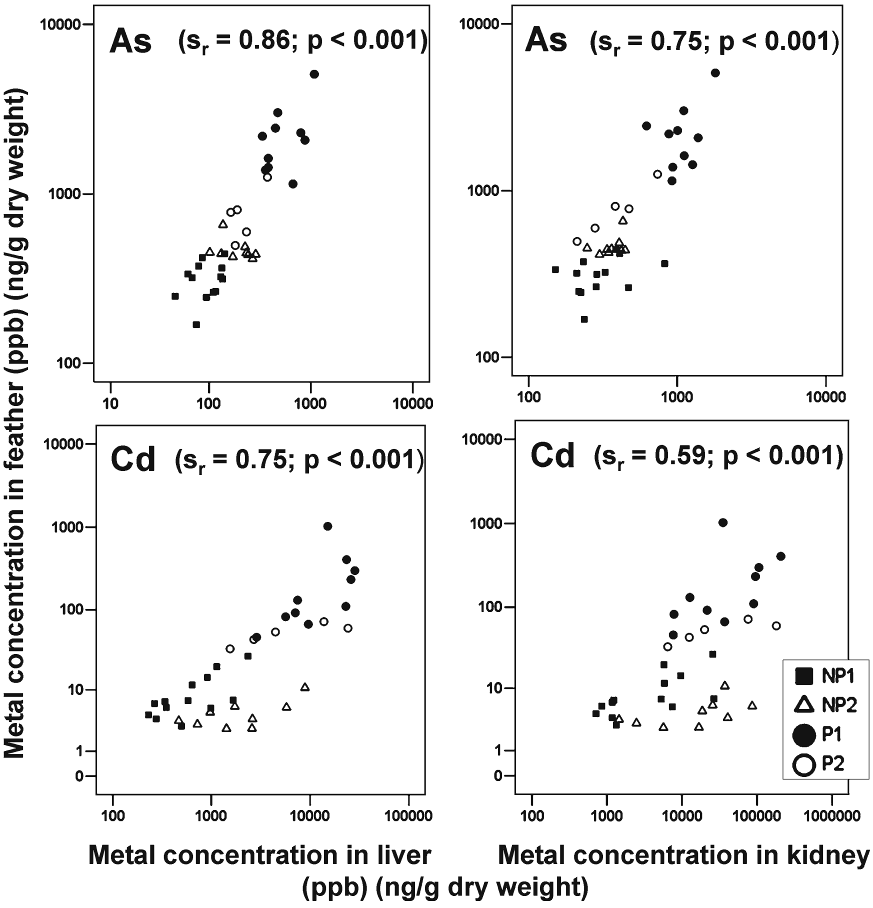


Fig. 3 *Scatter plots* of arsenic and cadmium concentrations in feathers versus liver (*left*) and kidney (*right*) in Ornate Tinamou (*N. ornata*) from control sites San Pedro de Ulloma (NP1) and Chuñavi (NP2), and mining sites Antequera (P1) and Poopó (P2). The Spearman correlation value (s_r) and p value are included

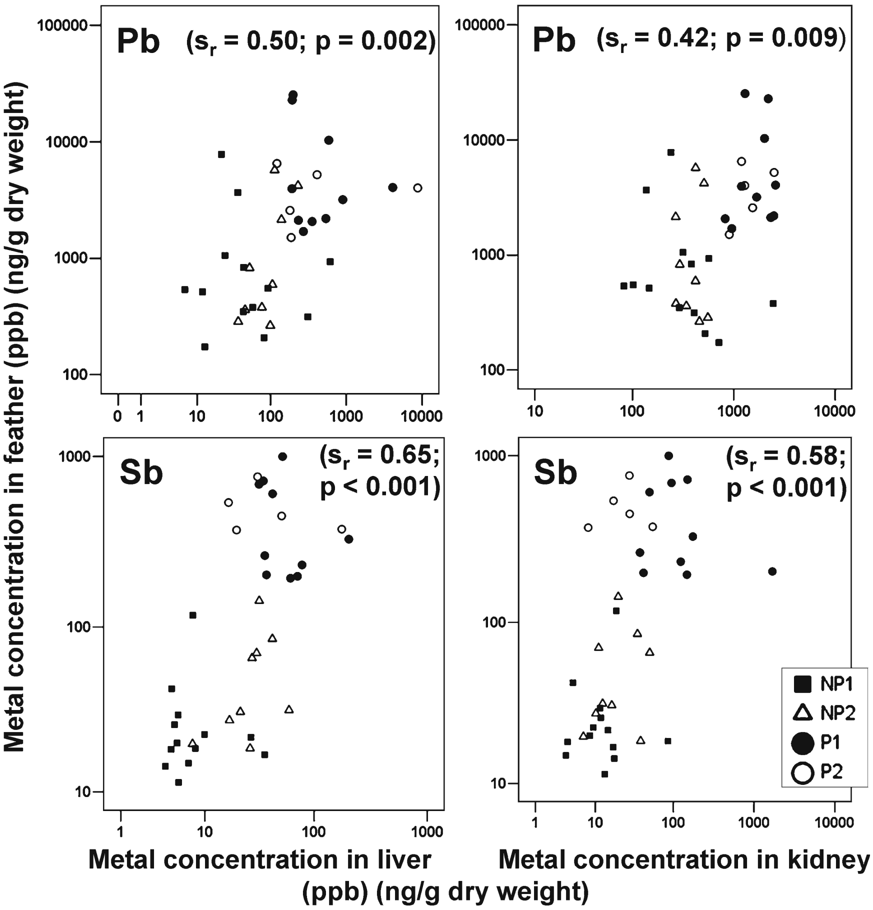


Fig. 4 *Scatter plots* of lead and antimony concentrations in feathers versus liver (*left*) and kidney (*right*) in Ornate Tinamou (*N. ornata*) from control sites San Pedro de Ulloma (NP1) and Chuñavi (NP2), and mining sites Antequera (P1) and Poopó (P2). The Spearman correlation value (s_r) and p value are included

concentrations in each tissue (Table 3), we can say that pollution of the four trace metals is similar at each site.

Liver histopathological slides revealed that some birds trapped at the four wild sites had inflammatory cells: 7% at NP1, 22% at NP2, 20% at P1, and 27% at P2 (at this last site, both species were taken into account). Kidney histopathological slides showed proportionally greater interstitial nephritis in birds from polluted sites (30% at P1 and 27% at P2) than in those from nonpolluted sites (15% at NP1 and 0% at NP2). Thesaurismosis (a storage disease) in proximal convoluted tubules was observed in 30% of the samples only at the P1 site. Development of thesaurismosis is probably related to mineral concretions, like a phenomenon observed in a cadmium-controlled dose-dependent study of Common Quail (*Coturnix coturnix*; Richardson et al. 1973).

Discussion

Trace metal levels and their toxicity

Trace metal concentrations in tissues are not directly associated with adverse impacts on bird health. Although this situation depends on each element's effect, it also depends on the bird species itself and intrinsic factors such as age, sex, physiology, etc., making it difficult to predict possible effects. Laboratory studies are necessary to identify the metal levels that may result in death or adverse impacts on behavior, physiology

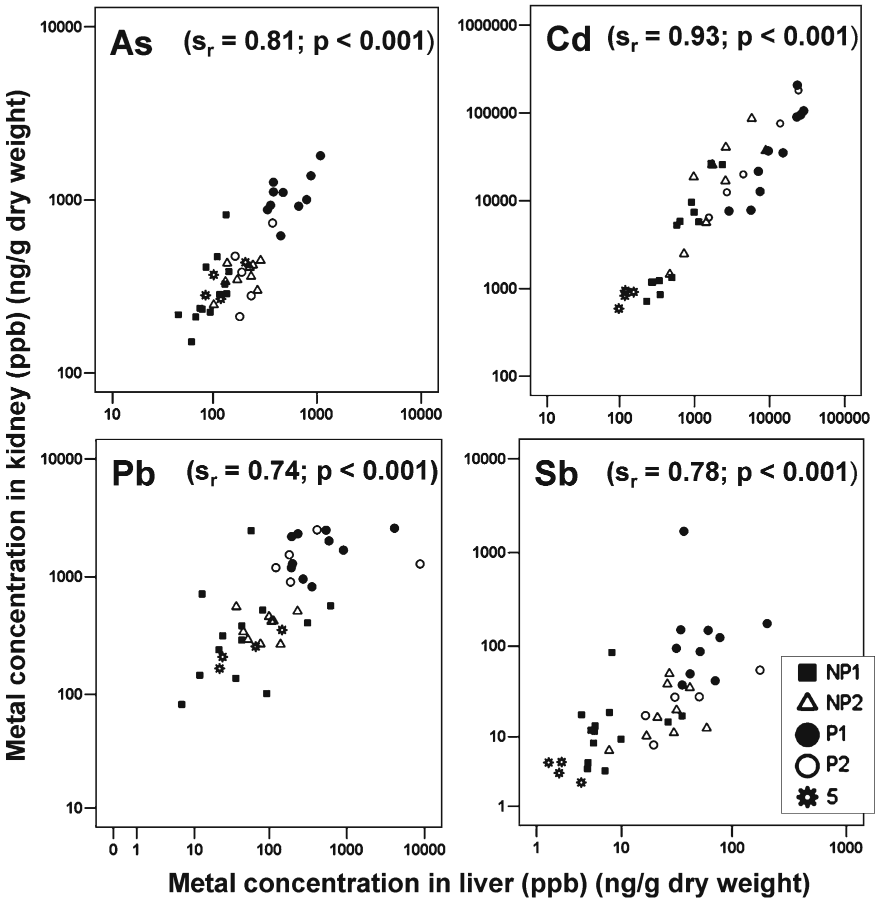


Fig. 5 *Scatter plots* of all trace metal concentrations in kidney versus liver in Ornate Tinamou (*N. ornata*) from control sites San Pedro de Ulloma (NP1) and Chuñavi (NP2), mining sites Antequera (P1) and Poopó (P2), and captivity (C). The Spearman correlation value (s_r) and p value are included

or reproductive success of birds. With the exception of Hg, Pb, and Cd, there are few controlled laboratory studies (Burger and Gochfeld 2009), which means that the comparison of our results with other bird species' reported values, with the background or poisoning threshold values proposed by several authors, is only a rough guide because of the lack of specific information for tinamous. In the following paragraphs, trace metal concentrations reported in tissues by the literature as wet weight (w-w) concentrations are multiplied by a factor of 3 to correspond to dry weight (d-w) concentrations (Clark and Scheuhammer 2003).

Arsenic

As arsenic is a micronutrient for some vertebrates (Eisler 1988a), and as it is rapidly metabolized by birds (Pendleton et al. 1995), it may not accumulate to toxic levels in tissues unless exposure is extreme (Custer et al. 2009). However, Burger and Gochfeld (2009) found that it biomagnifies in

Table 3 Nonparametric correlations between trace metal concentrations in each tissue type of the Ornate Tinamou (*Nothoprocta ornata*)

	Tissues	As	Cd	Pb
Cd	Liver	0.71 (<0.001)		
	Kidney	0.51 (0.001)		
	Feather	0.68 (<0.001)		
Pb	Liver	0.71 (<0.001)	0.67 (<0.001)	
	Kidney	0.62 (<0.001)	0.55 (<0.001)	
	Feather	0.53 (0.001)	0.56 (<0.001)	
Sb	Liver	0.75 (<0.001)	0.71 (<0.001)	0.84 (<0.001)
	Kidney	0.65 (<0.001)	0.50 (0.001)	0.76 (<0.001)
	Feather	0.79 (<0.001)	0.70 (<0.001)	0.85 (<0.001)

The Spearman's Rho value is given in each box and the p value in brackets. $N = 37$ for feather and $N = 41$ for liver and kidney nature, with the highest values at the top of the trophic web.

Feather values for As reported for several bird species were between 604 to 13 ppb d-w (Burger and Gochfeld 2009; Burger et al. 2008; Lounsbury-Billie et al. 2008). The values we found in feathers are within this range for Ornate Tinamou at all sites, except for P1, with more than 2,200 ppb d-w; and except for Darwin's Nothura at P2, with values higher than 1,000 ppb d-w. Thus, tinamous feather values at mining sites are among the highest ever reported. Comparison with the control sites means that these values are related to the level of exposure.

Our mean liver values for Ornate Tinamou have a maximum of 580 ppb d-w at P1 and a maximum individual value of 1,081 ppb d-w. These values are lower than the 5,000 ppb w-w (approximately 15,000 ppb d-w) threshold reported by Goede (1985) as the upper limit for background levels in waders. Fedynich et al. (2007) reported values of 6 to 220 ppb w-w in migratory ducks (approximately 18 to 660 ppb d-w). Although all our values for liver may be considered within background levels, they reflect that bioaccumulation of As in tinamous is related to their exposure level. For kidney, there are no previous reports.

Cadmium

Cadmium is a nonessential element ubiquitous in natural environments and one of the trace metals whose accumulation and toxic effects in terrestrial, fresh water, and marine birds have been studied most. Cd toxicity is more common among natural vertebrate populations than was previously known (Larison et al. 2000). In particular, cadmium damages kidneys (Furness 1996; Larison et al. 2000).

Burger (1993) suggests that Cd feather levels associated with adverse effects range from 100 ppb d-w (shearwaters) to 2,000 ppb d-w (terns). Our mean feather values ranged between 5 to 9.5 ppb d-w at control sites, which are comparatively very low, while at polluted sites, the values are several times higher for both tinamou species: at P1 (for Ornate Tinamou) and P2 (for Darwin's Nothura), at least, values are in the toxicity range proposed by Burger (1993). Other studies report higher mean Cd values in feathers (but from different origins: breast, down feathers, or wing feathers) of 307 ppb d-w for Bald Eagle (Burger and Gochfeld 2009), 203 ppb d-w for Wood Stork (*Mycteria americana*) from Florida (Burger et al. 1993), and between 70 and 139 ppb d-w in ospreys from the Florida Bay estuary (Lounsbury-Billie et al. 2008), but these studies lack analysis of the toxic effects that these Cd concentrations may produce.

Scheuhammer (1987) suggested that cadmium background levels in liver are <3,000 ppb d-w and Furness (1996) concluded that threshold concentrations for Cd poisoning in birds might be expected at around 40 mg/kg w-w (approximately 120,000 ppb d-w), but with a wide toxicity variation between species and age. Renal damage in water birds was found at over 30,000 ppb d-w in livers (Mateo and Guitart 2003). In the case of Willow Grouse (*Lagopus lagopus*), Cd liver concentrations of approximately 10,000 ppb d-w were related to high metallothionein levels, indicating a physiological response to Cd exposure (Pedersen and Hylland 2007). A geometrical mean with various insectivorous birds considers 100 ppb d-w as background levels, since kidney samples did not reveal changes related to toxic cadmium levels (Custer et al. 2009). Mean Cd values in livers from captive Ornate Tinamou showed very low concentrations, with both control sites situated within Scheuhammer's background levels, but at NP2, an individual reached 8,800 ppb d-w. At P1, the mean value for Ornate Tinamou was higher than 10,000 ppb d-w, and at both sites, several Ornate Tinamou individuals reached values higher than 20,000 ppb d-w, indicating a high Cd exposure in the field.

As other studies did, our results demonstrate that Cd concentrations are higher in kidney than in other tissues (e.g., Kim et al. 2007, who examined liver, muscle, and bone). Background Cd levels in kidney are suggested as <8,000 ppb d-w by Scheuhammer (1987), and Furness (1996) concluded that kidney threshold concentrations for Cd poisoning in birds might be expected at around 100 mg/kg w-w (approximately 300,000 ppb d-w). Renal damage in water birds was found at over 300,000 ppb d-w in kidney (Mateo and Guitart 2003). Larison et al. (2000) demonstrated that 57% of the adult Willow Grouse individuals at a polluted mining site (with values of Cd > 100,000 ppb d-w in kidney) showed irreversible renal tubular damage.

In our results, only Ornate Tinamou individuals from captivity had Cd values in kidney below Scheuhammer's background level. In the field, individual higher values were found at all sites for both tinamou species. One possible explanation is that Cd is a trace metal naturally common in the Andean soils.

No site had higher mean values than 100,000 ppb d-w, except in some Ornate Tinamou individuals from polluted sites. At polluted sites, interstitial nephritis was more frequent, and thesaurismosis was reported only at P1. This site has the highest mean and individual Cd values for all tissues. Tissue effects are not related directly to individual level, as might be expected. For example, the birds with the highest Cd concentration in kidney (>100,000 and >200,000 ppb d-w, respectively) had no tissue effects, although these were found in individuals with 95,200, 35,200, and 7,800 ppb d-w. Interstitial nephritis appears to be nonspecific or not directly associated with Cd concentrations, given that it was observed in individuals with Cd concentration values in a range from 850 to 105,700 ppb d-w.

Possible explanations of these cases are age effect, individual physiology, or time of exposure. Larison et al. (2000) showed that older birds accumulated substantial amounts in their kidneys during their lives and developed not only renal damage but also less calcium deposition in bones due to general renal failure. Nam et al. (2004b) observed age-dependent Cd accumulation in kidney in Rock Pigeon (*Columba livia*). However, Hindell et al. (1999) reported higher Cd concentrations in the kidneys of juvenile Shy Albatross (*Thalassarche cauta*) and Wandering Albatross (*Diomedea exulans*) than in adult birds and suggested that cadmium concentrations are regulated to some degree throughout the bird's life, for example by excretion in feathers. This contradiction probably depends on the total lifetime and diet habits of each species and may also be related to metal ingestion variation during the lifespan of birds. Anyway, age effect in our results is probably reflected by broad standard deviations. Though we considered all our specimens as adults, accurate individual ages are not known. Thus, data may reflect the different individual histories for trace metal exposure and accumulation.

Lead

Lead toxicity and poisoning effects, widely studied in field and laboratory experiments, were described several physiological and somatic effects and even death if exposure is high enough (see reviews in Eisler 1988b and Franson 1996). Lead biomagnifies through the trophic chain (Burger and Gochfeld 2009), and its levels may increase during a bird's lifetime, depending on exposure (Grue et al. 1984). Muscular exercise, excretion, elimination, and high protein consumption are factors that reduce the physiological effects of high lead concentrations in wild birds (Burger et al. 1997; Roux and Marra 2007).

Burger and Gochfeld (2000b) considered that adverse reproductive effects in birds occur at levels of 4,000 ppb d-w in feathers; Burger and Gochfeld (1994) found that experimentally lead-injected 40-day-old Herring Gull chicks (*Larus argentatus*) had Pb concentrations in feathers of 4,790 ± 1,693 ppb d-w and showed several motor problems, while untreated chicks had lead values from 853 to 1,205 ppb d-w. In our study, Ornate Tinamou from control sites had mean Pb concentrations in feathers lower than 2,000 ppb d-w, but five individuals from both control sites had values over 4,000 ppb d-w. Ornate Tinamou and Darwin's Nothura from both polluted sites had mean values of 4,000 ppb d-w or higher, with the highest values at >25,000 ppb d-w (for Ornate Tinamou, P1 site) and >67,000 ppb d-w (for Darwin's Nothura, P2 site). With these values, many tinamous from the polluted sites would be considered within Pb toxic levels. According to other authors (e.g., Burger 1993), feathers had the highest lead concentrations of all the analyzed tissues. Burger (1993) suggested the feather/liver ratio of 0.42:1 as usual in several species. Ratios in our data are 0.02:1–0.1:1 (for both tinamou species), showing higher feather Pb concentrations. Only Ornate Tinamou at the P2 site had a 0.49:1 ratio.

When Pb concentrations in liver are 6 to 15 mg/kg w-w (approximately 18,000 and 45,000 ppb d-w), Pain (1996) concluded that waterfowl's biological functions may be disabled and external signs of poisoning may be observed. Franson (1996) found that 10 to 20 μg/g d-w liver Pb concentrations cause clinical signs of lead poisoning in other bird species. Clark and Scheuhammer (2003) considered that raptors have as "background" Pb exposure levels concentrations of <6,000 ppb d-w in liver and/or kidney, and so raptors with concentrations over 6,000 ppb d-w in liver or kidney have been lead-exposed. They are poisoned with concentrations over 20,000 ppb d-w. Following these criteria, all our individuals from captivity, control sites, and perhaps from the P1 site would be considered at the background level. Only at the P2 site were higher Pb concentrations found, with remarkable differences between species: one Ornate Tinamou had 8,700 ppb d-w in liver, but lower concentrations in kidney; whereas two Darwin's Nothura individuals showed higher concentrations in kidney, but lower ones in liver (6,500 and 50,600 ppb d-w, respectively). The last individual reaches the poisoning level cited above.

Kim et al. (2007) also found great variation of Pb concentrations between five shorebird species (individual liver concentration values from n.d. to 23,250 ppb d-w approximately and from n.d. to 141,600 ppb d-w in kidney). The tissue with the highest Pb concentration varied greatly between species.

All specimens were captured when flying, which indicates that if toxic levels do exist for these birds, at least they do not affect birds' movement skills. However, our sampling method may not adequately reflect Pb exposure and acquisition in the field, as Pb-poisoned birds from the polluted sites were not sampled because of their flight disability and, thus, were not included in calculations of the comparative bioaccumulation pattern between sites.

Antimony

Antimony is only potentially toxic, as it is not an essential trace element for plants and animals and has no known biological function (Fowler and Geering 1991). However, as there have been much fewer studies of antimony than of other potentially toxic metals, its environmental significance may have been underestimated (Shotyk et al. 2005).

In birds, Sb toxicity is cited because of the implications of the administration of antimony in the form of a potassium tartrate emetic (Carlisle and Holberton 2006), but very few studies have reported their bioaccumulation in tissues. Lounsbury-Billie et al. (2008) reported mean values of 4 to 66 ppb d-w in Osprey feathers, which they considered low. Our values from control sites were in that range, but at polluted sites, values became approximately ten times higher for Ornate Tinamou and 20 times higher for Darwin's Nothura. Although our liver and kidney values cannot be compared due to the lack of other studies, concentration increases at the polluted sites (particularly P1 for Ornate Tinamou and P2 for Darwin's Nothura) over the figures for captivity and control sites, which shows that birds exposed in the field to Sb probably accumulate this trace element in their tissues in direct proportion to their exposure, although nothing definite can be said about the possible risks of these concentrations. It is known that vertebrates excrete absorbed Sb rapidly via urine and feces, and only with exposure are high concentrations in thyroid, adrenals, liver, and kidney sometimes found (Hayes and Laws 1991).

The highland tinamou species as sentinels of the bioaccumulation of trace metals in agrosystems polluted by mining activities

Our analysis focuses on the four trace metals that were reported at high concentrations in soils and water at the two polluted sites studied. Trace metals are transferred from the environment to birds via multiple mechanisms. They move from soil to plants, where they are stored in leaves, seeds, or tubers (Olivares 2003; Oporto et al. 2007), then to phytophagous insects or soil invertebrates (Custer et al. 2009; Ma 1987). Birds also pick them up secondarily through casual soil consumption when feeding (Beyer et al. 1994; Roux and Marra 2007). Ornate Tinamou and Darwin's Nothura are species that forage a wide variety of vegetal and animal food items and have sedentary diet habits (Garitano-Zavala et al. 2003). Moreover, it is known that at least Ornate Tinamou is a resident species, and individuals have a relatively restricted home range during their life (Pearson and Pearson 1955). Thus, individuals living in agrosystems irrigated by polluted waters caused by mining activities and/or tailing piles are exposed to local pollutants throughout their lifespan.

Normally species at the top of the trophic chain are thought to be the best bioindicator organisms, especially if they are aquatic, due to the fast movement of pollution in water (Burger and Gochfeld 2009; Lounsbury-Billie et al. 2008). However, our results with the two tinamou species studied showed that different exposure levels of As, Cd, Pb, and Sb at different sites were clearly expressed in the bird tissues. High data dispersion observed in the standard deviations should be attributed to the age effect, which is very difficult to control in field experiments because of the lack of an accurate ageing method. Collection of more samples should help with this problem. Other characteristics in favor of tinamou species as bioindicators are their ubiquity and relatively high abundance in their distributional areas (Cabot 1992; Davies 2002): as both are hunted for sport, biological material can be obtained easily.

Molting is a useful bio-mechanism for toxic metal removal from the bird body through physiological transportation and deposition in feathers (Furness et al. 1986). It is also known that metal concentrations in feathers reflect the values of internal tissues (as well as potential adverse effects) in several bird species (Burger 1993; Burger and Gochfeld 2000a; Pilastro et al. 1993). High correlation values obtained between feather–liver–kidney in both tinamou species allowed us to generalize that situation to As and Sb, too.

We recommend that future monitoring programs at mining sites using highland tinamou species should be based on feathers, because they are easy to collect noninvasively (from living or dead specimens) and easy to store indefinitely. The body burden proportion found in feathers is relatively constant for each metal, and a high proportion of the body burden of certain metals is stored in the feathers due to their affinity to the sulfhydryl-rich keratin protein and melanin pigments (Burger et al. 2008; Burger and Gochfeld 2009). Use of feathers is also interesting because of the advances in analytical chemistry, which lets us use museum specimens or other ornithological pieces to generate historical or background data, if in the past they had been obtained from currently polluted sites (Lounsbury-Billie et al. 2008).

Acknowledgements We would like to thank Ernesto Hug, Fernando Gianini and Jackeline Campos for their help trapping specimens of the Ornate Tinamou and Darwin's Nothura. This research was supported by Spain's "Secretaría de Estado del Ministerio de Educación y Ciencia", with project number CGL2005-04772, and by the project 2005 SGR 00602 of the Generalitat of Catalonia. We are also grateful to the Dirección General de Biodiversidad of Bolivia for allowing us to carry out this research, and to the Municipal Authorities of Poopó for their collaboration.

References

Beveridge, M. C. M., Stafford, E., & Coutts, R. (1985). Metal concentrations in the commercially exploited fishes of an endorrheic saline lake in the tin–silver province of Bolivia. *Aquaculture Research, 16*, 41–53.

Beyer, W. N., Connor, E. E., & Gerould, S. (1994). Estimates of soil ingestion by wildlife. *Journal of Wildlife Management, 58*, 375–382.

Borràs, M., & Nadal, J. (2004). Biomarkers of genotoxicity and other endpoints in an integrated approach to environmental risk assessment. *Journal of Mutagenesis, 19*, 165–168.

Burger, J. (1993). Metals in avian feathers: Bioindicators of environmental pollution. *Reviews in Environmental Toxicology, 5*, 203–311.

Burger, J., & Gochfeld, M. (1994). Behavioral impairments of lead-injected young herring gulls in nature. *Fundamental and Applied Toxicology, 23*, 553–561.

Burger, J., & Gochfeld, M. (2000a). Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. *Science of the Total Environment, 257*, 37–52.

Burger, J., & Gochfeld, M. (2000b). Effects of lead on birds (Laridae): A review of laboratory and field studies. *Journal of Toxicology and Environmental Health, 3*, 59–78.

Burger, J., & Gochfeld, M. (2009). Comparison of arsenic, cadmium, chromium, lead, manganese, mercury and selenium in feathers in bald eagle (*Haliaeetus leucocephalus*), and comparison with common eider (*Somateria mollissima*), glaucous-winged gull (*Larus glaucescens*), pigeon guillemot (*Cepphus columba*), and tufted puffin (*Fratercula cirrhata*) from the Aleutian Chain of Alaska.

Environmental Monitoring and Assessment, 152, 357–367.

Burger, J., Rodgers, J. A., Jr., & Gochfeld, M. (1993). Heavy metal and selenium levels in endangered wood storks *Mycteria americana* from nesting colonies in Florida and Costa Rica. *Archives of Environmental Contamination and Toxicology, 24*, 417–420.

Burger, J., Shukla, T., Benson, T., & Gochfeld, M. (1997). Lead levels in exposed herring gulls: Differences in the field and laboratory. *Toxicology and Industrial Health, 13*, 193–202.

Burger, J., Gochfeld, M., Jeitner, C., Snigaroff, D., Snigaroff, R., Stamm, T., et al. (2008). Assessment of metals in down feathers of female common eiders and their eggs from the Aleutians: Arsenic, cadmium, chromium, lead, manganese, mercury, and selenium. *Environmental Monitoring and Assessment, 143*, 247–256.

Cabot, J. (1992). Family tinamidae (tinamous). In J. del Hoyo, A. Elliot, & J. Sargatal (Eds.), *Handbook of the birds of the world* (Vol. 1, pp. 112–138). Barcelona: Linx Edicions.

Carlisle, J. D., & Holberton, R. L. (2006). Relative efficiency of fecal versus regurgitated samples for assessing diet and the deleterious effects of a tartar emetic on migratory birds. *Journal of Field Ornithology, 77*, 126–135.

Clark, A. J., & Scheuhammer, A. M. (2003). Lead poisoning in Upland-foraging birds of prey in Canada. *Ecotoxicology, 12*, 23–30.

Custer, C. M., Yang, C., Crock, J. G., Shearn-Bochsler, V., Smith, K. S., & Hageman, P. L. (2009). Exposure of insects and insectivorous birds to metals and other elements from abandoned mine tailings in three Summit County drainages, Colorado. *Environmental Monitoring and Assessment, 153*(1–4), 161–177. doi:10.1007/s10661-008-0346-y.

Davies, S. J. J. F. (2002). *Ratites and tinamous*. New York: Oxford University Press.

de Lapuente, J., González-Linares, J., Serret, J., Palaus, X., Teixidó, E., & Borràs, M. (2008). Monitoring the effects of a complex mixture of pollutants next to the lixiviate pool of a Mediterranean landfill and along its training stream using Word Mouse pathology and arthropod biodiversity. *Fresenius Environmental Bulletin, 17*(11b), 1909–1916.

Eisler, R. (1988a). Arsenic hazards to fish, wildlife, and invertebrates: A synoptic review. *Biological Report 85 (1.12)*. Washington DC: US Fish and Wildlife Service.

Eisler, R. (1988b). Lead hazards to fish, wildlife, and invertebrates: A synoptic review. *Biological Report 85 (1.14)*. Washington DC: US Fish and Wildlife Service.

Fedynich, A. M., Ballard, B. M., McBride, T. J., Estrella, J. A., Garvon, J. M., & Hooper, M. J. (2007). Arsenic, cadmium, copper, lead, and selenium in migrating Blue-Winged Teal (*Anas discors* L.). *Archives of Environmental Contamination and Toxicology, 53*, 662–666.

Fowler, B. A., & Geering, P. L. (1991). *Metals and their compounds in the environment occurrence analysis and biological relevance*. New York: VHC.

Franson, J. C. (1996). Interpretation of tissue lead residues in birds other than waterfowl. In W. N. Beyer, G. H. Heinz, & A. W. Redmon-Norwood (Eds.), *Environmental contaminants in wildlife: Interpreting tissue concentrations* (pp. 265–279). Boca Raton: Lewis.

Furness, R. W. (1996). Cadmium in birds. In W. N. Beyer, G. H. Heinz, & A. W. Redmon-Norwood (Eds.), *Environmental contaminants in wildlife: Interpreting tissue concentrations* (pp. 389–404). Boca Raton: Lewis.

Furness, R. W., Muirhead, S. J., & Woodburn, M. (1986). Using bird feathers to measure mercury in the environment: Relationship between mercury content and molt. *Marine Pollution Bulletin, 17*, 27–37.

Furness, R. W., Greenwood, J. J. D., & Jarvis, P. J. (1993). Can birds be used to monitor the environment? In R. W. Furness & J. J. D. Greenwood (Eds.), *Birds as monitors of environmental change* (pp. 1–41). London: Chapman & Hall.

Garitano-Zavala, A. (2002). El potencial aprovechamiento cinegético de los tinamúes (Aves: Tinamiformes) del altiplano boliviano, y la necesidad de reglamentarlo. In: C. Aguirre, C. Miranda, & Y. Verhasselt (Eds.), *Contribución al conocimiento del Sistema del Lago Titicaca* (pp. 329–338) La Paz: ANCB-ICIB—Real Academia Belga de Ciencias de Ultramar.

Garitano-Zavala, A., Nadal, J., & Ávila, P. (2003). The feeding ecology and digestive tract morphometry of two sympatric tinamous of the high plateau of the Bolivian Andes: The Ornata Tinamou (*N. ornata*) and the Darwin's Nothura (*N. darwinii*). *Ornitología Neotropical, 14*, 173–194.

Goede, A. A. (1985). Mercury, selenium, arsenic, and zinc in waders from the Dutch Wadden Sea. *Environmental Pollution, 37*, 287–309.

Grove, R. A., Henny, C. J., & Kaiser, J. L. (2009). Osprey: Worldwide sentinel species for assessing and monitoring environmental contamination in rivers, lakes, reservoirs, and estuaries. *Journal of Toxicology and Environmental Health, Part B, 12*, 25–44.

Grue, C. E., O'Shea, T. J., & Hoffman, D. J. (1984). Lead concentrations and reproduction in highway nesting barn swallows. *Condor, 86*, 383–389.

Hayes, W. J., Jr., & Laws, E. R., Jr. (Eds.) (1991). *Handbook of Pesticide Toxicology. Volume 2. Classes of Pesticides*. New York: Academic.

Hindell, M. A., Brothers, N., & Gales, R. (1999). Mercury and cadmium concentrations in the tissues of three species of southern albatrosses. *Polar Biology, 22*, 102–108.

Kim, J., Park, S. K., & Koo, T. H. (2007). Lead and cadmium concentrations in shorebirds from the Yeongjong Island, Korea. *Environmental Monitoring and Assessment, 134*, 355–361.

Larison, J. R., Likens, G. E., Fitzpatrick, J. W., & Crock, J. G. (2000). Cadmium toxicity among wildlife in the Colorado Rocky Mountains. *Nature, 406*, 181–183.

Llacuna, S., Gorriz, A., Sanpera, C., & Nadal, J. (1995). Metal accumulation in three species of Passerine birds (*Emberiza cia, Parus major and Turdus merula*) subjected to air pollution from a coal-fired power plant.

Archives of Environmental Contamination and Toxicology, 28, 298–303.

Lounsbury-Billie, M. J., Rand, G. M., Cai, Y., & Bass, O. L., Jr. (2008). Metal concentrations in Osprey (*Pandion haliaetus*) populations in the Florida Bay estuary. *Ecotoxicology, 17*, 616–622.

Ma, W. C. (1987). Heavy metal accumulation in the mole, *Talpa europaea*, and earthworms as an indicator of metal bioavailability in terrestrial environments. *Bulletin of Environmental Contamination and Toxicology, 39*, 933–938.

Mateo, R., & Guitart, R. (2003). Heavy metal in livers of waterbirds from Spain. *Archives of Environmental Contamination and Toxicology, 44*, 398–404.

MEDMIN (2001). *Estudio para el Manejo Ambiental en Microcuencas de 12 Zonas Mineras: Matilde, Viloco, Caracoles, Colquiri, Poopó, Antequera, Cañadón Antequera, La Lava, Chorolque, Uncía, Llallagua, Colquechaca*. Technical Report. La Paz: MEDMIN (Medio Ambiente, Minería e Industria)/Viceministerio de Minería y Metalurgia.

Molina, M. (2005). El desarrollo postnatal de la Pisacca en condiciones de cautiverio. In A. Garitano-Zavala (Ed.), *La crianza rural de un ave silvestre del altiplano boliviano, La Pisacca (Nothoprocta ornata)* (pp. 87–113). La Paz: Instituto de Ecología.

Nam, D. H., Lee, D. P., & Koo, T. H. (2004a). Monitoring for lead pollution using feathers of feral pigeons (*Columbia livia*) from Korea. *Environmental Monitoring and Assessment, 95*, 13–22.

Nam, D. H., Lee, D. P., & Koo, T. H. (2004b). Factors causing variations of lead and cadmium accumulation of Feral Pigeons (*Columba livia*). *Environmental Monitoring and Assessment, 95*, 23–35.

Olivares, E. (2003). The effect of lead on the phytochemistry of *Tithonia diversifolia* exposed to roadside automotive pollution or grown in pots of Pb-supplemented soil. *Brazilian Journal Plant Physiology, 15*, 149–158.

Oporto, C., Vandecasteele, C., & Smolders, E. (2007). Elevated Cadmium concentrations in potato tubers due to irrigation with river water contaminated by mining in Potosí, Bolivia. *Journal of Environmental Quality, 36*, 1181–1186.

Pain, D. J. (1996). Lead in waterfowl. In W. N. Beyer, G. H. Heinz, & A. W. Redmon-Norwood (Eds.), *Environmental contaminants in wildlife: Interpreting tissue concentrations* (pp. 251–263). Boca Raton: Lewis.

Pearson, A. K., & Pearson, O. P. (1955). Natural history and breeding behavior of the tinamou *Nothoprocta ornata*. *Auk, 72*, 113–127.

Pedersen, H. C., & Hylland, K. (2007). Metallothionein levels in willow ptarmigan (*Lagopus lagopus*) populations with different natural loads of cadmium. *European Journal of Wildlife Research, 53*, 142–152.

Pendleton, G. W., Whitworth, M. R., & Olsen, G. H. (1995). Accumulation and loss of arsenic and boron alone and in combination, in mallard ducks. *Environmental Contamination and Toxicology, 14*, 1357–1364.

Pilastro, A., Congiu, L., Tallandini, L., & Turchetto, M. (1993). The use of bird feathers for the monitoring of Cadmium pollution. *Archives of Environmental Contamination and Toxicology, 24*, 355–358.

Richardson, M. E., Fox, M. R., & Fry, B. E. (1973). Pathological changes produced in Japanese Quail by ingestion of cadmium. *Journal of Nutrition, 104*, 323–338.

Rios, B. (2002). Evaluación de la actividad minera en los alrededores del lago Poopó. In O. Rocha (Ed.), *Diagnóstico de los recursos naturales y culturales de los lagos Poopó y Uru Uru, Oruro–Bolivia* (pp. 167–186). La Paz: Convención RAMSAR, WCS/Bolivia.

Rojas, J., & Vandecasteele, C. (2007). Influence of mining activities in the North of Potosi, Bolivia on the water quality of the Chayanta River, and its consequences. *Environmental Monitoring and Assessment, 132*, 321–330.

Roux, K. E., & Marra, P. P. (2007). The presence and impact of environmental Lead in Passerine birds along an urban to rural land use gradient. *Archives of Environmental Contamination and Toxicology, 53*, 261–268.

Sanabria, H. (2000). Resistance and the arts of domination, miners and the Bolivian State. *Latin American Perspectives, 27*, 56–81.

Sanchez-Chardi, A., Lopez-Fuster, M. J., & Nadal, J. (2007). Bioaccumulation of lead, mercury and cadmium in the greater white-toothed shrew, *Crocidura russula* from the Ebro Delta (NE Spain): Sex-and-age-dependent variation. *Environmental Pollution, 145*, 7–14.

Scheuhammer, A. M. (1987). The chronic toxicity of aluminium, cadmium, mercury and lead in birds: A review. *Environmental Pollution, 46*, 263–295.

SERGEOMIN (1999). Inventariación de recursos naturales renovables (hídricos) y no renovables (minerales e hidrocarburos) del departamento de Oruro. *Boletín del Servicio Nacional de Geología y Minería, 24*, 1–44.

Shotyk, W., Krachler, M., & Chen, B. (2005). Antimony: Global environmental contaminant. *Journal of Environmental Monitoring, 7,* 1135–1136.

UNEP/OEA (1996). *Diagnóstico ambiental del sistema Titicaca-Desaguadero-Poopó-Salar de Coipasa (Sistema TDPS) Bolivia-Perú*. Washington DC: Departamento de Desarrollo Regional y Medio Ambiente/Secretaría General de la Organización de los Estados Americanos.

Van Ryckeghem, M. (1997). Contaminación Minero Metalúrgica y Salud Pública en la cuenca del Lago Poopó - Presentación del "Plan de Gestión Ambiental", documento final del Proyecto Piloto Oruro (PPO). *Eco Andino, 2*, 27–60.

VII

Calamus as an appropriate feather section for the assessment of endogenous lead pollution

El cálamo como indicador de la contaminación endógena de plomo

Javier Cotín[a], Álvaro Garitano-Zavala[c], Carolina Sanpera[a],
Lluis Jover[b] & Jacint Nadal[a].

[a] Department Biologia Animal, Facultat de Biologia, Univesitat de Barcelona,
Diagonal 645, 08028 Barcelona, Spain

[b] Department Salut Pública, Facultat de Medicina, Universitat de Barcelona,
Casanova 143, 08036 Barcelona, Spain

[c] Unidad de Manejo y Conservación de Fauna, Instituto de Ecología,
Universidad Mayor de San Andrés,
La Paz, Bolivia.

Resumen: Las plumas han sido ampliamente utilizadas como indicadores de contaminación por metales pesados como el plomo. Representan las concentraciones de metales circulantes en sangre durante su desarrollo, así que las plumas recién formadas reflejan de un modo preciso la deposición endógena de plomo. A pesar de esto, los niveles de plomo pueden aumentar después de la formación de las plumas debido a la contaminación exógena de su superficie, ya sea por deposición atmosférica, durante su acicalado o por contacto con el medio (con el suelo, polvo o agua). El propósito de este estudio es evaluar que parte de la pluma indica más adecuadamente los niveles endógenos de plomo originales, después del lavado rutinario con NaOH, en este caso utilizando plumas de Tinamú Pisacca (*Nothoprocta ornata*). Para este propósito, primarias de nueve ejemplares adultos fueron divididos en secciones (cálamo, barbas superiores/inferiores, raquis superior/inferior) y se compararon sus concentraciones de plomo. Nuestros resultados no mostraron diferencias significativas entre las secciones superior/inferior de barba y raquis, pero sí hubo diferencias entre el cálamo y las barbas y raquis total, con el cálamo presentando los valores más bajos, seguido por el raquis y con las concentraciones más altas, las barbas. Los ratios del plomo fueron 35,6/1 para las barbas/cálamo y 7,8/1 para el raquis/cálamo. Ya que el cálamo está más protegido frente a la contaminación exógena, probablemente refleja más adecuadamente las concentraciones originales de plomo de la pluma. Por el contrario, las barbas no poseen esa protección y debido a ello pueden ser un buen indicador de la exposición externa a contaminantes, sugiriendo que las diferentes secciones de la pluma reflejan varios grados de contaminación exógena. Es necesaria más investigación en la línea de métodos de limpieza adecuados, ya que el análisis del cálamo puede ser difícil de realizar en especies pequeñas de paseriformes, a no ser que se utilicen plumas de volantones o recién formadas.

Palabras clave: Tinamú Pisacca, Pluma, Plomo, Contaminación exógena, Contaminación endógena.

Calamus as an appropriate feather section for the assessment of endogenous lead pollution

Javier Cotín[a], Álvaro Garitano-Zavala[c], Carolina Sanpera[a],
Lluis Jover[b] & Jacint Nadal[a].

[a] Department Biologia Animal, Facultat de Biologia, Univesitat de Barcelona,
Diagonal 645, 08028 Barcelona, Spain

[b] Department Salut Pública, Facultat de Medicina, Universitat de Barcelona,
Casanova 143, 08036 Barcelona, Spain

[c] Unidad de Manejo y Conservación de Fauna, Instituto de Ecología,
Universidad Mayor de San Andrés,
La Paz, Bolivia.

Abstract: Feathers have been used extensively to assess environmental pollution by heavy metals such as lead. Feathers contain information about circulating heavy-metal concentrations in the blood at the time of their development, so newly grown feathers accurately reflect the endogenous deposition of lead. Despite this, lead levels may increase after the formation of feathers due to exogenous surface contamination such as atmospheric deposition, preening and environmental contact (with soil, dust or water). The aim of this study was to assess which feather section provides the most accurate data on the original endogenous lead levels in feather samples after routine cleansing with NaOH, in this case using feathers of Ornate Tinamou (*Nothoprocta ornata*). For this purpose, primary feather samples of nine adult individuals were divided into sections (calamus, upper/lower shaft and upper/lower vane) and their lead concentrations were compared. Our results did not show significant differences between the upper/lower shaft and the vane, but there were differences between the calamus and the whole shaft and vane, with the calamus presenting the lowest concentrations, followed by the shaft and vane with the highest concentrations. Lead ratios were 35.6/1 for the vane/calamus and 7.8/1 for the shaft/calamus. As the calamus is protected against exogenous contamination, it may reflect most accurately the original lead concentrations of the feather. Contrarily, the vane lacks such protection and therefore may be a good indicator of exposure to external contaminants, suggesting that the different feather sections reflect varying degrees of exogenous contamination. Further research on the appropriate cleansing methods is needed, since calamus analysis may be difficult to carry out in small bird species, unless newly grown or nestling feathers are used.

Keywords: Ornate Tinamou, Feather, Lead, Exogenous contamination, Endogenous contamination.

Introduction

The human impact on the natural environment has increased dramatically in recent centuries, especially since the industrial revolution. The presence of pollutants in the environment, such as heavy metals, has introduced new risks for all living organisms, including humans. Feathers have been used extensively to assess environmental pollution by certain heavy metals (Barata et al. 2010; Burger. 1994; Sanpera et al. 2007; Sanpera et al. 2008), as birds excrete heavy metals into growing feathers when molting. Moreover, feathers can easily be collected non-invasively and they require few storage facilities.

After feather formation, the blood supply atrophies and the feather becomes completely isolated from the rest of the body (Burger. 1994). Consequently, no further heavy metals can accumulate from the bloodstream into the feathers. Thus, feathers contain information about circulating heavy metal concentrations in the blood at the time of their development.

Feather-metal levels may remain stable with time, as is the case with mercury (Appelquist et al. 1984; Veerle et al. 2004), or they may increase, since feathers are highly prone to exogenous surface contamination. Such contamination from atmospheric deposition, preening and environmental contact (with soil, dust or water) may then give a misleading indication of biologically incorporated contamination at the time of feather growth (Dauwe et al. 2002; Jaspers et al. 2004; Pilastro et al. 1993). It has been demonstrated that lead concentrations increase after the feather has fully grown, so feathers reflect endogenous lead levels as well as exogenous contamination and, commonly, atmospheric pollution levels. Only newly grown or nestling feathers accurately reflect the endogenous deposition, as exogenous accumulation of pollutants onto the feather surface is an important route of contamination (Veerle et al. 2004), mainly affecting the vane, which becomes more contaminated over time due to its large, structurally complex surface area (Cardiel et al. 2011; Goede and Debruin. 1984).

Standard procedures for metal determination involve some type of cleansing protocol previous to analysis, but since exogenous surface contamination is difficult to clean from feathers (Cardiel et al. 2011; Weyers et al. 1988) and different cleansing treatments have shown similar results (Valladares et al. 2010), the amount of exogenous contamination deposited onto the feather that is removed by a cleansing process is unknown. Therefore, unless newly grown or nestling feathers are used, the lead levels in analyzed feathers will present an undetermined percentage of exogenous contamination, as cleansing procedures to remove this exogenous contamination completely have not yet been developed to our knowledge.

Therefore, the objective of this study was to assess which feather section provides the most accurate data on the original endogenous lead levels after routine cleansing with NaOH, in this case using feathers of Ornate Tinamou (*Nothoprocta ornata*). Assuming that the calamus is the feather section with the least exposure to atmospheric agents, its levels are expected to be lower when compared to other feather sections and representative of the original lead levels. In order to test this hypothesis, primary feather samples were divided

into five sections (calamus, upper/lower shaft and upper/lower vane) to compare the section unexposed to exogenous contamination with the sections exposed to it. Also, the estimated ratio between exogenous/endogenous contamination is provided.

Material and methods

Feather samples from nine individuals were obtained from study sites on the Bolivian high plateau of the Central Andes and the adjacent hills of the Eastern Andean Cordillera. Specimens were captured by traditional hunters between July and September 2007 during the dry season (southern winter) of the Bolivian highlands. Based on plumage characteristics, nine adult birds were selected. It is important to mention that the Ornate Tinamou is a sedentary species with a small individual home range (Pearson and Pearson. 1955) and has previously been used as a pollution bioindicator (Garitano-Zavala et al. 2010).

Wing feathers (primaries) were obtained in the field and placed in individually marked plastic bags and shipped to the University of Barcelona for analysis.

To carry out lead determination, feather samples were rinsed with NaOH (0.25 M) for 1 min and with deionized water for 1 min. They were then oven dried at 60ºC for 24 h. Primary feathers from each individual were selected and divided into five sections: calamus (C), lower shaft (S1), upper shaft (S2), lower vane (V1) and upper vane (V2) (see fig. 1). Calamus was considered the first 10 millimeters of the feather, which is the section embedded within the skin follicle in Ornate Tinamou. The shaft and vane were divided into lower and upper parts according to Figure 1.

Trace elements analysis

Chemical determination of lead was carried out by means of ICP-MS Perkin Elmer ELAN 6000. Before the chemical determination, the feather sections were digested according to the acid digestion protocol with Savillex Teflon digestion vessels, using H_2NO_3 (2 ml) and H_2O_2 (1 ml) for feather sections weighing less than 100 mg and H_2NO_3 (3 ml) and H_2O_2 (2 ml) for feathers weighing over 100 mg for 14 hours at 100ºC. Accuracy of analysis was checked by measuring certified reference tissue: Human Hair (BCR 397).

Mean recoveries ranged from 98% to 100% for total lead and no recovery corrections were applied. Lead concentrations were

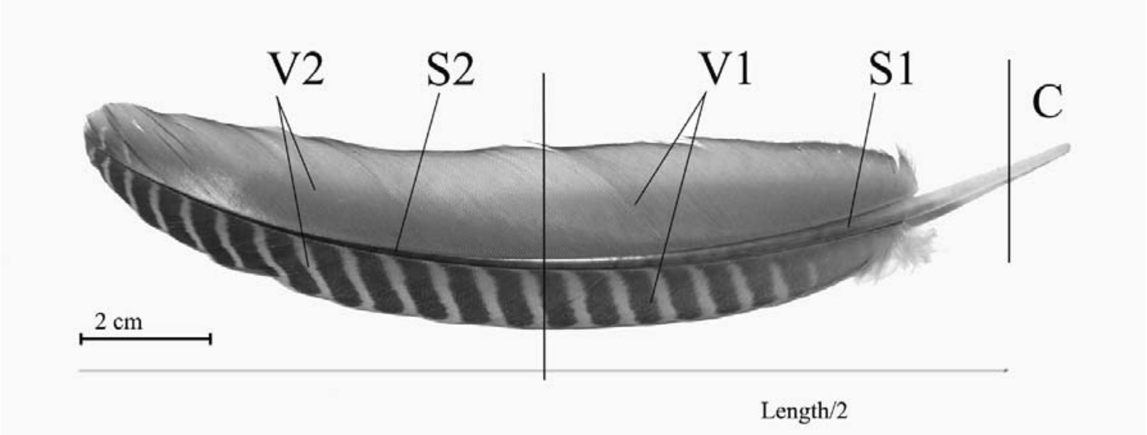


Figure 1. Feather sections (C calamus, S1 lower shaft, S2 upper shaft, V1 lower vane, V2 upper vane)

expressed on a dry weight basis (ng/g, i.e., parts per billion). Lead analysis was performed at the Serveis Científico-Tècnics (University of Barcelona).

Statistical methods

Values of lead concentrations were routinely checked for normal distributions using Q-Q plots. Lead concentrations showed clear skewed distributions which were normalized by applying a logarithmic transformation.

Comparisons among the feather sections were carried out using a linear mixed model to take into account dependence among sections of the same feather. To test for a posteriori pairwise differences, we used the sequential Šidák procedure. The overall shaft, vane and total feather concentrations were obtained by a weighted mean of S1 + S2, V1 + V2 and C + S1 + S2 + V1 + V2, respectively.

Correlations among feather sections were explored with Pearson correlations and partial correlation analysis by using the log-transformed lead concentrations and applying Bonferroni accordingly.

Descriptive statistics were used to show the results. Statistical analysis was carried out using SPSS 17.0.

Results

The descriptive statistics for lead concentrations (mean, standard deviation, minimum and maximum) of the feather sections are presented in Table 1.

The linear model that was fit to the five feather subdivisions showed a significant effect ($F_{4,32}=39.7$ $p<0.001$). The pairwise test did not reveal any significant differences between the shaft and vane subdivisions, so a further linear model was fitted to three feather sections only (the calamus, shaft and vane). The results showed significant differences among the sections ($F_{2,16}=57.5$ $p<0.001$). The pairwise test showed that the calamus had the lower lead concentrations, followed by the shaft, while the vane presented the highest concentrations, with all differences being significant. Individual lead concentration profiles of the feather sections are presented in Figure 2.

	Feather sections	N	Mean	SD	Min	Max
Lead (ppb)	Calamus	9	453.9	560.7	36.5	1587.2
	Shaft	9	2250.7	3815.8	58.8	12090.3
	Shaft (upper)	9	2911.7	4521.3	106.7	13288.2
	Shaft (lower)	9	2071.7	3706.3	44.8	11740.2
	Vane	9	11643.5	15562.3	1011.8	51981.5
	Vane (upper)	9	11484.6	19533.4	971.2	62656.2
	Vane (lower)	9	12197.3	10660.8	1092.2	37461.7
	Feather	9	14348.1	17255.3	1107.1	56294.5

Table 1. Descriptive statistics for lead concentrations in the feather sections expressed on a dry weight basis (ng/g).

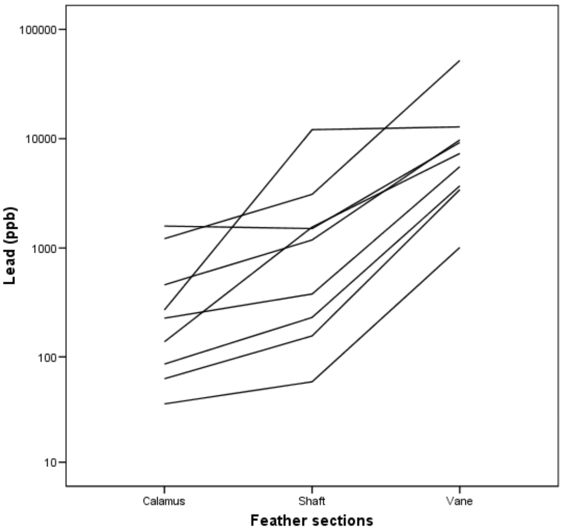


Figure 2. Individual lead concentrations profile by bird.

There were significant correlations in the lead concentrations between the shaft and vane after subtracting the calamus effect (which is used as the endogenous lead baseline), r = 0.71, P= 0.021 and the ratios (calculated as the mean of the differences among feather sections in each individual) are 35.6 in the vane/calamus (the shaft/calamus ratio is 7.8).

Discussion

Feathers can be sampled with minimum disturbance to individuals, and this method is therefore recommended from an animal protection and nature preservation perspective. However, exogenous lead contamination may be a problem when trying to interpret endogenous concentrations from such samples. If feathers are newly grown (using nestlings, through molting or experimental plucking), it can be assumed that the feather levels reflect the endogenous levels (Dauwe et al. 2002; Veerle et al. 2004). But if the assessment does not take place under these circumstances and the age of the feather is unknown, it is difficult to interpret lead concentrations.

Once a feather is formed, its original lead levels may increase due to the exogenous surface contamination produced by uropygial gland secretions that are smeared onto the feathers during preening (Dauwe et al. 2002), environmental contact with soil, dust or water, or atmospheric deposition, to the extent that the observed levels of contamination may be primarily exogenous (Dauwe et al. 2003; Hahn et al. 1993). Moreover, the increase in lead concentrations due to atmospheric deposition may be relatively high (Goede and Devoogt. 1985), as has been demonstrated with feathers of museum specimens (Pain et al. 2005).

A previous study concerning the endogenous and exogenous deposition of lead in feathers has shown that the shaft is a better representative of internal lead levels than the vane (Cardiel et al. 2011). However, in this study, aluminum levels in the shaft were below the detection limit, implying that no exogenous contamination was present in the shaft. This was the study's main drawback. In our study, the higher lead levels found in the shaft when compared with the calamus, together with the correlation found between the shaft and the vane, indicate that a percentage of the lead present in the shaft is exogenous. Therefore, our results are in agreement with our assumption, as the calamus presents the lowest concentrations. Thus the calamus is the feather section that likely reflects the original feather lead concentrations most accurately due to its protection against exogenous contamination, while the vane may represent the exogenous surface contamination more reliably (atmospheric deposition, sand baths and/or preening).

Scheifler et al. (2006) proposed as the exogenous/endogenous contamination rate the differences between unwashed and washed feathers in relation to the total lead concentration of the unwashed feathers (they also used sodium hydroxide solution for removing external contamination, as in this study). This assumed that part of the concentration measured in the washed feather may come from uropygial contamination, but that other kinds of exogenous contamination were removed completely. The differences we found in the lead concentrations among the different feather sections suggest that the feather vane, with its net-like structure, is the most prone to retain particles of exogenous contamination even after a cleansing process with sodium hydroxide solution. Even the shaft, which presents a smoother structure, seems to be affected by exogenous contamination. It is worth mentioning that the total lead concentrations of the whole feather give similar information as the vane, that is, the part most exposed to exogenous contamination.

Therefore, an appropriate index of exogenous/endogenous contamination would be the ratio between the vane and calamus lead concentrations following a standardized cleansing process. Using this ratio, we exclude unexpected high values due to dirt (sand, food, etc.) and the lead concentrations are homogenized since exogenous contamination exposure presents different rates within the feather and also depend on the feather type (Hahn et al. 1993).

In conclusion, different feather sections may reflect different degrees of exogenous contamination, the calamus being the most suitable section for assessing the original endogenous lead levels, unless newly grown feathers are used, in which case the whole feather may be used for lead determination. Further research is needed on cleansing methods that are suitable for completely removing the exogenous contamination in the whole feather, as calamus lead determination may be difficult to apply in small bird species such as passerines.

Acknowledgments

We thank Ernesto Hug, Fernando Gianini and Jackeline Campos for their help in catching the specimens of Ornate Tinamou and to Roby Rycroft and Gretchen Wagner for their corrections of the English draft. The work of Javier Cotín has been supported by a BRD grant from the University of Barcelona, Spain. The present work was funded by the project "Metalls pesants i organoclorats en el curs inferior i delta del riu Ebre: La ornitofauna com a bioindicadora" (Ministry of the Environment, Spain, the Catalan Water Agency, Department of the Environment and Housing, Catalan Government) and by the projects CGL 2008-05448-C02 (Ministry of Science and Innovation, Spain) and 2008 SGR 963 (Government of Catalonia, Spain).

References

Appelquist H, Asbirk S, Drabaek I. Mercury monitoring - Mercury stability in bird feathers. *Mar Pollut Bull* 1984;15:22-4.

Barata C, Fabregat MC, Cotin J, Huertas D, Sole M, Quiros L et al. Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain). *Env Pollut* 2010;158:704-10.

Burger J. Metals in avian feathers: bioindicators of environmental pollution.

Rev Environ Contam Toxicol 1994;5:203-311.

Cardiel IE, Taggart MA, Mateo R. Using Pb–Al ratios to discriminate between internal and external deposition of Pb in feathers. *Ecotoxicol Environ Saf* 2011.

Dauwe T, Bervoets L, Blust R, Eens M. Tissue levels of lead in experimentally exposed zebra finches (*Taeniopygia guttata*) with particular attention on the use of feathers as biomonitors. *Arch Environ Contam Toxicol* 2002;42:88-92.

Dauwe T, Bervoets L, Pinxten R, Blust R, Eens M. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. *Environ Pollut* 2003;124:429-36.

Garitano-Zavala A, Cotin J, Borras M, Nadal J. Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia and their potential as environmental sentinels. *Environ Monit Assess* 2010;168:629-44.

Goede AA, Debruin M. The use of bird feather parts as a monitor for metal pollution. *Environ Pollut B Chem Phys* 1984;8:281-98.

Goede AA, Devoogt P. Lead and cadmium in waders from the Dutch Wadden Sea. Environ Pollut A 1985;37:311-22.

Hahn E, Hahn K, Stoeppler M. Bird feathers as bioindicators in areas of the German Environmental Specimen Bank: Bioaccumulation of mercury in food chains and exogenous deposition of atmospheric pollution with lead and cadmium. *Sci Total Environ* 1993;139-140:259-70.

Jaspers V, Dauwe T, Pinxten R, Bervoets L, Blust R, Eens M. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, *Parus major. J Environ Monitor* 2004;6:356-60.

Pain DJ, Meharg AA, Ferrer M, Taggart M, Penteriani V. Lead concentrations in bones and feathers of the globally threatened Spanish imperial eagle. *Biol Conserv* 2005;121:603-10.

Pearson A, Pearson O. Natural history and breeding behavior of the Tinamou, *Nothoprocta ornata. Auk* 1955;72:113-27.

Pilastro A, Congiu L, Tallandini L, Turchetto M. The use of bird feathers for the monitoring of cadmium pollution. *Arch Environ Contam Toxicol* 1993;24:355-8.

Sanpera C, Moreno R, Ruiz X, Jover L. Audouin's gull chicks as bioindicators of mercury pollution at different breeding locations in the western Mediterranean. *Mar Pollut Bull* 2007;54:691-6.

Sanpera C, Valladares S, Moreno R, Ruiz X, Jover L. Assessing the effects of the Prestige oil spill on the European shag (*Phalacrocorax aristotelis*): Trace elements and stable isotopes. *Sci Total Environ* 2008;407:242-9.

Scheifler R, Coeurdassier M, Morilhat C, Bernard N, Faivre B, Flicoteaux P. Lead concentrations in feathers and blood of common blackbirds (*Turdus merula*) and in earthworms inhabiting unpolluted and moderately polluted urban areas. *Sci Total Environ* 2006;371:197-205.

Valladares S, Moreno R, Jover L, Sanpera C. Evaluating cleansing effects on trace elements and stable isotope values in feathers of oiled birds. *Ecotoxicology* 2010;19:223-7.

Veerle J, Tom D, Rianne P, Lieven B, Ronny B, Marcel E. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, *Parus major. J Environ Monitor* 2004;6:356-60.

Weyers B, Gluck E, Stoeppler M. Investigation of the significance of heavy-metal contents of Blackbird feathers. *Sci Total Environ* 1988;77:61-7.

Introducción

Los bioindicadores son especies utilizadas para monitorizar la salud del medio ambiente o de un ecosistema. Pueden ser utilizados como bioindicadores cualquier especie biológica o grupo de especies cuyas funciones, población o estado permitan evaluar la calidad ambiental de un medio o ecosistema. En el caso concreto de los bioindicadores de contaminación, las especies potenciales deben reflejar los niveles de contaminación del medio ambiente y variar de acuerdo con ellos.

Las aves (clase Aves) son vertebrados alados, bípedos y endotérmicos, cubiertos de plumas y que se reproducen por huevos. Existen alrededor de 10.000 especies vivientes, habitando en todos los ecosistemas del globo, desde el Ártico hasta el Antártico. Las aves ocupan una gran variedad de medios y las podemos encontrar tanto en jardines, desiertos, selvas tropicales o incluso en una tormenta en medio del océano. Poseen diversas patrones de comportamiento respecto a sus movimientos: desde pasar toda su vida en la misma zona (aves residentes) a cruzar el globo dos veces al año (migrantes de larga distancia, como el Charrán ártico, *Sterna paradisaea*), presentando numerosas estrategias intermedias. Incluso pueden, simplemente, "recorrer" los océanos, como es la práctica de varias especies de aves marinas pelágicas. Su espectro alimenticio es muy amplio, incluyendo hierba, semillas, miel, néctar, insectos, peces, mamíferos, otras aves, carroña…

Dado lo anterior, no es de extrañar que siempre que pretendamos evaluar un riesgo ambiental mediante el uso de un bioindicador, exista una elevada probabilidad de que alguna especie de ave cumpla los requisitos deseados. De hecho, las aves han sido utilizadas como bioindicadoras en numerosas y variadas circunstancias: durante la disminución de las poblaciones de aves rapaces producida por el uso del DDT hace unas décadas (Helander et al., 2008, Henny et al., 2010, Sonne et al., 2010), en las mareas negras producidas por el Exxon Valdez y el Prestige (Golet et al., 2002, Sanpera et al., 2008, Moreno et al., 2011), en vertidos de contaminantes, como el ocurrido en el Parque Nacional de Doñana (Benito et al., 1999, Gomez et al., 2004, Baos et al., 2006), en casos de contaminación producida por actividades mineras (Wayland et al., 2006) o incluso en casos de radioactividad, como el ocurrido en Chernobyl (Ruiz et al., 1988, Moller et al., 2011). Las aves también han sido utilizadas para estudiar la contaminación en ecosistemas como el antártico (Corsolini et al., 2011, Jerez et al., 2011, Metcheva et al., 2011) o para evaluar la salud ambiental de humedales (Heath and Frederick, 2005, Abdennadher et al., 2011, Aliakbari et al., 2011).

El uso de las aves como bioindicadores de contaminación supone varias ventajas, pero también limitaciones. Las aves son fáciles de identificar y su clasificación y sistemática están bien establecidas. Son especies, en general, bien estudiadas, existiendo numerosos trabajos acerca de su ecología y comportamiento, y este amplio conocimiento previo de su biología aumenta su utilidad como bioindicadores. Muchas aves ocupan posiciones altas en la red trófica, por lo que pueden ser especialmente adecuadas para monitorizar cualquier contaminante que se acumule a través de ella. Su longevidad hace que integren los efectos de las presiones ambientales a través del tiempo, proporcionando la posibilidad de medir, por ejemplo, la contaminación en un plazo igual o superior a un año, pero muestran baja sensibilidad a las perturbaciones ambientales de baja intensidad o de corta duración. De manera similar, la gran movilidad de las aves permite monitorizar amplias áreas (dependiendo la escala en función de la especie elegida), pero dichos hábitos migratorios también pueden ser un inconveniente, puesto que los individuos pueden presentar heterogeneidad en su migración, haciendo difícil determinar el área que representan.

Normalmente, el punto clave consiste en utilizar las especies y muestras adecuadas, por ejemplo, teniendo en cuenta que cada tejido refleja los contaminantes de un modo diferente. Los organoclorados se distribuyen en todos los tejidos dependiendo de su contenido lipídico. Los metales pesados, contrariamente, tienen a acumularse en tejidos concretos, siendo las concentraciones mucho más altas en unos que en otros. Las plumas, que no son metabólicamente activas, indican la información química de un tiempo muy concreto, independientemente del momento de su muestreo (Cherel et al., 2005, Inger and Bearhop, 2008). En las plumas no sólo están representados los niveles de mercurio, sino también de otros muchos metales pesados que son incorporados a las estructuras quitinosas. Los huevos también han sido usados en numerosos estudios para monitorizar la contaminación, y presentan varias ventajas frente a los tejidos internos (Barrett et al., 1985, Becker, 1989, Burger and Gochfeld, 1989). Aunque las hembras puedan excretar mercurio en los huevos, la cantidad depositada en ellos es relativamente pequeña cuando se compara con la cantidad depositada en las plumas durante la muda (Honda et al., 1986). En los órganos internos, los metales se distribuyen de un modo muy diferente: el cadmio se concentra en el riñón, el plomo en el hueso, el mercurio en el riñón y el hígado, y el zinc, cobre y hierro también en el hígado. Es interesante señalar que las muestras de sangre muestran los niveles de metales representantes de la exposición a corto tiempo (reflejan la dieta reciente).

Por lo tanto, a la hora de enfrentarse al problema de la monitorización o evaluación de la contaminación de un ecosistema, humedal o área, la elección de la especie y el tipo de muestra adecuados es de crucial importancia, y debe estar basada en el conocimiento de las especies.

En este estudio las aves serán utilizadas como bioindicadoras de contaminación por metales pesados en dos escenarios diferentes: el primero en un ambiente acuático, la cuenca del rio Ebro, mientras que el segundo tiene lugar en un ambiente terrestre, los Andes bolivianos. Antecedentes, contaminantes y sus fuentes son completamente diferentes, como ha sido el enfoque en cada uno de ellos.

Metales pesados, medio ambiente y muestras biológicas

Los metales pesados son miembros del cajón de sastre que engloba los elementos con propiedades metálicas. Este grupo incluye principalmente los metales de transición, algunos metaloides, lantánidos y actínidos. El mercurio, cadmio y plomo, en particular, han dado lugar a numerosas investigaciones, debido a su elevada toxicidad (Bryan, 1979). La mayoría de estos elementos no participa en ninguna función biológica conocida, y su aumento en el medio ambiente, especialmente de plomo y mercurio, es esencialmente el resultado de las actividades humanas (Lantzy and Mackenzie, 1979).

Dependiendo de si un ecosistema es terrestre o acuático, las dinámicas de los contaminantes son significativamente diferentes. En ecosistemas terrestres, el ambiente típico es el suelo, mientras que en un ecosistema acuático es el agua o el sedimento. Una vez que los metales llegan a los suelos o sedimentos, permanecen un tiempo prolongado antes de ser eluídos a otros compartimentos. La acidez tiene un marcado efecto sobre la solubilidad de los metales en suelos y agua. La movilidad de los metales en suelos depende principalmente por su contenido en arcillas, cantidad de materia orgánica y pH. En general, cuanto más alto es el contenido en arcillas y/o el de materia orgánica, más firmemente es la unión con los metales y más tiempo permanecen en el suelo. La solubilidad de los metales en agua depende enormemente del pH. En sistemas acuáticos, los metales quedan "atrapados" en los sedimentos del fondo, donde pueden permanecer muchos años. De todos modos, si el pH desciende, la solubilidad de estos metales aumenta, volviéndose más móviles, y por lo tanto, más biodisponibles. Al mismo tiempo que cambia la acidez, cambia el estado de oxidación, y como resultado, los metales pesados pueden generar o romper enlaces con compuestos orgánicos, dando lugar a compuestos como el metilmercurio. Los compuestos metilados, al ser lipofílicos, son incorporados por los organismos con mayor frecuencia que los no metilados.

A diferencia de los organoclorados (otra conocida familia de contaminantes), que se distribuyen entre los tejidos principalmente en relación a su contenido lipídico, los metales pesados tienden a acumularse en determinados tejidos a concentraciones mucho más altas que en otros. Esta especificidad de los metales da una gran importancia a la elección del tejido a la hora de realizar estudios de monitorización. Por ejemplo, aunque las hembras pueden excretar mercurio en los huevos, la cantidad depositada es generalmente pequeña cuando se compara con la depositada en las plumas durante la muda (Honda et al., 1986). Las plumas no solo presentan mercurio, sino también otros metales, que son incorporados en las estructuras de queratina. Acerca de los tejidos internos, el cadmio se concentra principalmente en el riñón, el plomo en el hueso, el mercurio en el hígado y riñón, el zinc, cobre y hierro en el hígado. Las muestras de sangre muestran los niveles de metales que reflejan la exposición a la contaminación a corto plazo (dieta reciente).

Estudio A: Los lodos tóxicos de Flix. ¿Afectan a la fauna del río Ebro y su Delta?

La mayor cuenca hidrográfica de España es la del río Ebro (NE de España), la cual vierte en el mar Mediterráneo, creando un delta de más de 30.000 hectáreas. Dicha cuenca tiene una superficie de 85.362 km^2 (http://www.chebro.es), abastece potencialmente a 3 millones de personas y contiene zonas altamente industrializadas.

Una de estas áreas es Flix (41° 14'N, 00° 31'E), donde una industria química ha estado en funcionamiento desde principios del siglo XX. A lo largo de todos esos años, la compañía Ercros ha desarrollado varias actividades, como la producción de DDT desde 1945 hasta 1971, o la de PCBs desde 1959 hasta 1987. Adicionalmente, el HCB (hexaclorobenceno) es un producto típico de la fabricación de disolventes organoclorados. Los vertidos de mercurio están relacionados con la producción de hipoclorito de sodio, comúnmente denominado como lejía. El hipoclorito de sodio se produce a partir de la electrólisis de una solución salina, utilizando un cátodo de mercurio (con la formación de Cl_3Hg) y un ánodo de grafito (obteniendo Na^+). El compuesto de fosfato bicálcico fue producido desde 1973 a partir de roca de fosfato, siendo sus residuos ricos en ^{238}U, y por lo tanto, en los radionúclidos derivados, ^{226}Ra y ^{210}Pb.

Esta variada actividad y el largo período operacional, junto a la construcción de una presa cerca de la planta alrededor de 1960, dieron lugar a la acumulación de entre 200.000 a 360.000 toneladas de residuos industriales en el cauce del río, ocupando una superficie de 700 m de longitud y 60 m de anchura. La mezcla de sedimentos contaminados contiene altas concentraciones de metales pesados (cadmio, arsénico, cobre, cromo, plomo y especialmente, mercurio), selenio, compuestos organoclorados (hexaclorobenceno, pentaclorobenceno, DDT, policlorobifenilos, polychloronaphthalenos y polychlorostyrenes) y ^{210}Pb radioactivo (Fernandez et al., 1999, Bosch et al., 2009). Además, el río Ebro se ve

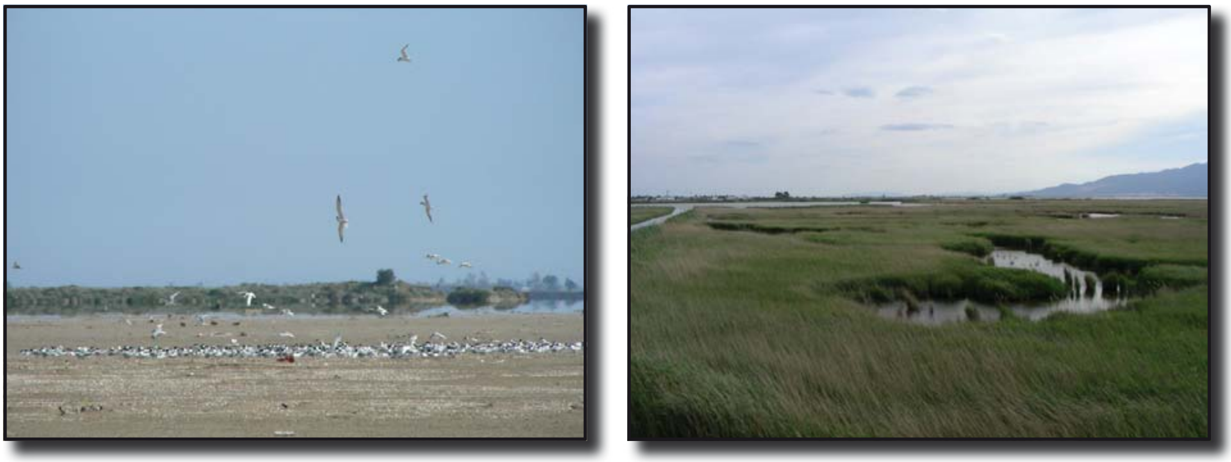


Figura 1: Dos vistas del Delta del Ebro: La colonia de Charrán patinegro en la "Punta del Fangar" y un área utilizada para la nidificación de ardeidos en el Clot.

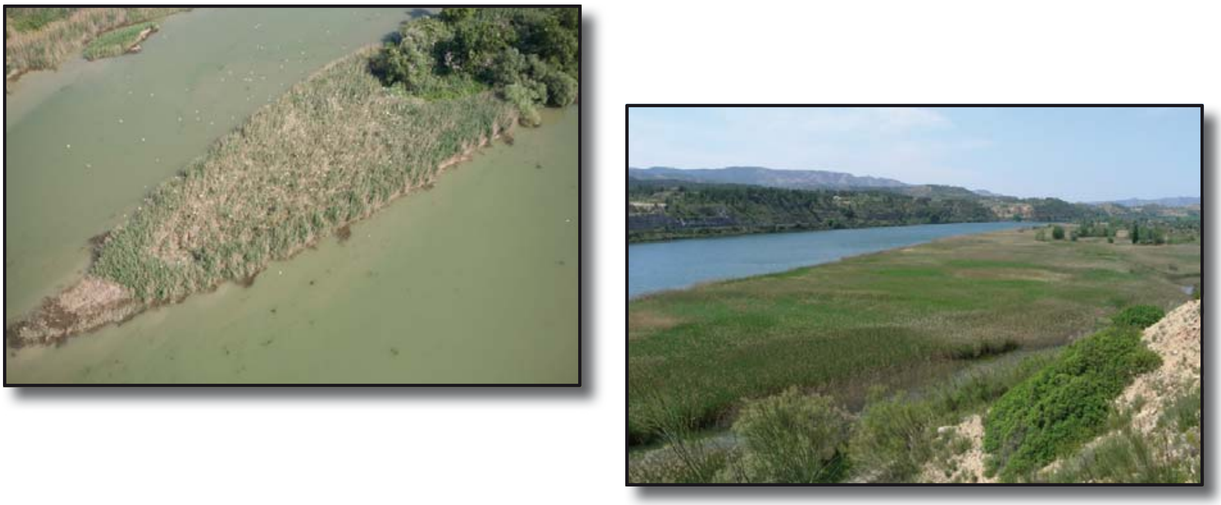


Figura 2: Vistas de las zonas de muestreo en el río: l'Aiguabarreig y Flix.

afectado por diversas actividades agrícolas e industriales, con impacto significativo sobre la biota existente (Mañosa et al., 2001, Navarro et al., 2010a). Los contaminantes originados en Flix son transportados aguas abajo por el río Ebro a su delta, situado 90 km aguas abajo (Llorente et al., 1987, Pastor et al., 2004, Navarro et al., 2009), especialmente durante las inundaciones y avenidas (Vericat and Batalla, 2006).

A consecuencia de esta situación, se realizó un estudio global para evaluar el impacto medioambiental de la contaminación derivada de los lodos tóxicos antes de que fuera llevado a cabo un proyecto destinado a la retirada y eliminación de dichos lodos, implicando esta última iniciativa un alto riesgo de dispersión de los contaminantes.

Algunos de los datos obtenidos por este y otros estudios previos sobre el embalse de Flix y el río Ebro han mostrado altos niveles de PCB en los sedimentos (Fernandez et al., 1999) y diversos efectos de los contaminantes sobre diferentes especies bioindicadoras. Las lombrices de tierra presentan altos niveles de mercurio (Ramos et al., 1999). Los niveles más elevados de Hg (THG) y metilmercurio (MeHg) jamás registrados para mejillón cebra (*Dreissena polymorpha*) fueron encontrados en esta localidad, con valores medios 20 veces mayores que los niveles de referencia locales (Carrasco et al., 2008). El cangrejo de río (P*rocambarus clarkii*) y el mejillón cebra exhibieron altos niveles de estrés por tóxicos (alta actividad y niveles de enzimas antioxidantes, metalotioneínas, peroxidación lipídica y ruptura de cadenas de ADN y la disminución de los niveles de glutatión) en muestras tomadas cerca de los residuos (Faria et al., 2010), incluso hubo cangrejos de río que presentaron niveles de mercurio superiores a los valores legales establecidos para el consumo por la legislación de la Unión Europea (Suarez-Serrano et al., 2010). Las concentraciones de mercurio en los tejidos de carpa (*Cyprinus carpio*) muestreadas aguas abajo de Flix fueron de uno a dos órdenes de magnitud superiores a las muestreadas aguas arriba (Navarro et al., 2009) y el siluro (*Silurus ganis*) superó el nivel máximo de mercurio recomendado para el consumo humano (Carrasco et al., 2011). En varias especies de peces (carpa, rutilo -*Rutilus rutilus* y perca sol -*Lepornis gibbosus*) deformidades, aletas erosionadas, ectoparásitos, lesiones y tumores (DELT) fueron detectadas con mayor frecuencia en el área impactada; del mismo modo, el estado físico en estas especies y en el alburno (*Alburnus alburnus*) fue significativamente inferior. Asimismo, se ha detectado un impacto negativo en la capacidad reproductiva de la carpa y la perca sol (Benejam et al., 2010) en el área. Las respuestas a los contaminantes fueron diferentes en cada especie, siendo la carpa la especie con las respuestas más evidentes en variables relacionadas con la condición física, a pesar de estar entre las especies más tolerantes a la contaminación (Benejam et al., 2010).

Todos estos efectos adversos desarrollados frente a la contaminación en estas especies bioindicadoras, junto con el arrastre de los sedimentos de Flix al ecosistema deltaico, representan una amenaza para dicho ecosistema y su biodiversidad.

El Delta del Ebro (40° 42'N, 00° 50'E) es uno de los mayores humedales del Mediterráneo occidental y acoge importantes colonias de aves (12000 parejas de Gaviota de Audouin, 3000 de Flamenco -*Phoenicopterus roseus*-, 400 de la Garza Imperial, 350 de Charrancito, 5000 de Charrán común, 500 de Pagaza piconegra, 2000 de Charrán patinegro y 1200 de Fumarel cariblanco, entre otros). Ocupa una superficie de 320 km^2, siendo el 75% de esta superficie campos de arroz (aproximadamente 20.000 ha) y tierras de cultivo, el 20% zonas naturales protegidas y el restante 5% zonas urbanas (ver Fig. 1 y 3). Este humedal presenta una gran variedad de hábitats: en la parte interna del delta y en ambas orillas del río, los arrozales y tierras de cultivo, conectados con el río a través de canales; en la costa, campos abandonados, lagunas, marismas y playas; en cada extremo del delta y rodeadas por el mar, existen dos extensiones arenosas en forma de punta de flecha, conocidas como la "Punta del Fangar" y la "Punta de la Banya" (con una extensión de 500 y 2500 hectáreas, respectivamente); esta última alberga la industria de las salinas. Por último, el mar abierto y las bahías norte y sur, moldeadas por los dos "Puntas", crean el mosaico de hábitats propio del Delta. Las lagunas costeras, aunque conectadas con el mar (por lo que deberían presentar agua salobre), reciben un considerable aporte de agua dulce proveniente de los campos de arroz desde primavera a otoño, lo que reduce su salinidad, que casi alcanza la del agua dulce durante esas estaciones del año.

Para realizar una evaluación adecuada de la exposición ambiental a los lodos tóxicos de Flix, no sólo el río ha de ser tenido en cuenta, sino también el mosaico de hábitats de todo el Delta del Ebro, e incluso el mar, puesto que los sedimentos son arrastrados por la río hasta su desembocadura. Preservar la biota de este ecosistema, especialmente la avifauna del Delta del Ebro, es una prioridad. Por lo tanto, nos centramos en las aves predadoras cuya alimentación depende directamente de los hábitats acuáticos asociados al río. Entre todas las especies de aves de la zona, las que parecen más adecuadas como posibles bioindicadores son las especies piscívoras, como las pertenecientes a la familia Ardeidae (garzas) y la familia Sternidae (charranes).

Garzas y charranes muestran una fenología reproductiva predecible y una gran fidelidad con sus zonas de nidificación, lo que permite realizar un seguimiento intensivo de sus parámetros reproductivos y poblacionales, entre otros; de hecho estas especies han sido utilizadas anteriormente de manera satisfactoria como bioindicadores de contaminación en ambientes acuáticos (Becker et al., 1993, De Luca-Abbott et al., 2001, Nisbet et al., 2002, Connell et al., 2003, Guitart et al., 2003, Champoux et al., 2006, Sakellarides et al., 2006, Paiva et al., 2008b). Ocupan posiciones elevadas en la cadena trófica de esos ecosistemas y consecuentemente, biomagnifican y bioacumulan contaminantes (Baker and Sepulveda, 2009).

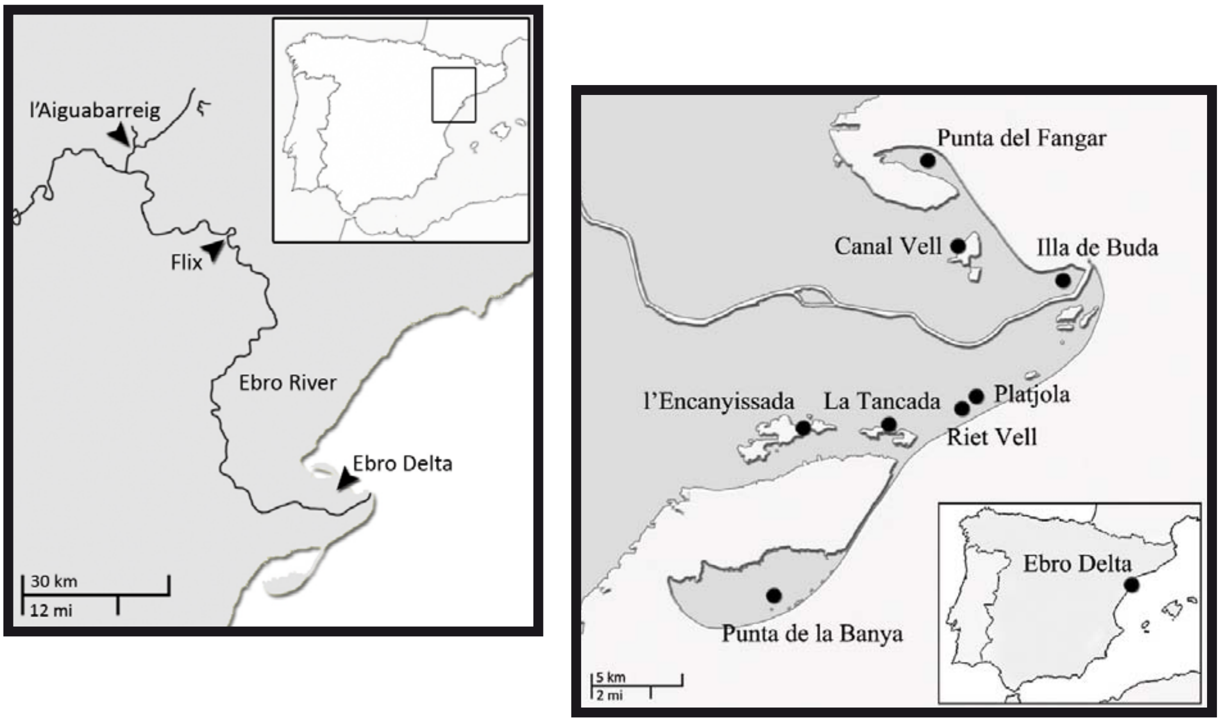


Figura 3: Mapas de localización de las tres zonas de muestreo y, en detalle, las colonias muestreadas en el Delta del Ebro.

Las garzas son aves dulceacuícolas y costeras de la familia Ardeidae, con 64 especies reconocidas. Son aves de tamaño medio a grande, con largas patas y cuello, el cual pueden doblar en forma de S debido a la forma modificada de la sexta vértebra. Además, el cuello es capaz de retraerse y extenderse, y siempre se encuentra recogido durante el vuelo, a diferencia de la mayoría de las otras aves de cuello largo. Las garzas son una familia ampliamente extendida con una distribución cosmopolita, encontrándose en todos los continentes excepto la Antártida. Entre las principales poblaciones de garzas del río Ebro y su delta están la Garza imperial (*Ardea purpurea*), la Garcilla cangrejera (*Ardeola ralloides*), el Martinete (*Nycticorax nycticorax*), la Garceta común (*Egretta garzetta*) y el Avetorillo (*Ixobrychus minutus*). Estas especies se consideran principalmente piscívoras, aunque pueden alimentarse de otras presas, como cangrejos en el caso del Avetorillo (Pardo-Cervera et al., 2010) o insectos en el caso de la Garcilla cangrejera (Delord et al., 2004). Están asociadas principalmente a hábitats de agua dulce, pero también pueden alimentarse en otros hábitats, como aguas salobres o incluso en salinas, como lo hace la Garceta común (Abdennadher et al., 2011).

Los charranes son aves marinas emparentadas con las gaviotas. Son un total de 45 especies agrupadas en 12 géneros (Bridge et al., 2005), encontrándose en todo el planeta y siendo la mayoría especies migratorias. Las siguientes cinco especies de charranes se reproducen en el Delta del Ebro: Charrán común (*Sterna hirundo*), Charrán patinegro (*Sterna sandvicensis*), Charrancito (*Sternula albifrons*), Pagaza piconegra (*Gelochelidon nilotica*) y Fumarel cariblanco (*Chlydonias hybrida*). Se ha descrito una amplia variedad de hábitats de alimentación para estas especies: de agua dulce para el Fumarel cariblanco (Latraube et al., 2005, Paillisson et al., 2007), medios marinos para el Charrán patinegro (Stienen et al., 2000) y tanto marino como dulceacuícola para el Charrán común (Becker et al., 1997, Neubauer, 1998). Las últimas dos especies se consideran de hábitos alimenticios generalistas. El Charrancito utiliza diversos hábitats de alimentación en Portugal (Paiva et al., 2008a) y en el Delta del Ebro se ha observado alimentándose en canales y lagunas (Bertolero et al., 2005). La Pagaza piconegra se alimenta en hábitats terrestres (Cabo and Sanchez, 1986), de agua dulce (Dies et al., 2005) e incluso marinos (Stienen et al., 2008).

Adicionalmente se muestrearon cuatro especies para completar el espectro de familias de aves y hábitats: dos especies de la familia Laridae (gaviotas) y dos de la familia Rallidae (calamones, polluelas...). Hay varias especies de gaviotas criando en el Delta del Ebro, siendo las más comunes la Gaviota patiamarilla (*Larus michahellis*), la Gaviota de Audouin (*Larus audouinii*), la Gaviota reidora (*Larus ridibundus*), la Gaviota picofina (*Larus genei*) y la Gaviota sombría (*Larus fuscus*), entre otras. Para este estudio seleccionamos la Gaviota reidora y de Audouin, puesto que la patiamarilla no fue considerada debido a su alimentación en campos y vertederos. La gaviota de Audouin es considerada un depredador nocturno que se alimenta de peces epipelágicos, pero suele tomar ventaja de las actividades pesqueras, especialmente de los descartes pesqueros (Oro and Ruiz, 1997, Sanpera et al., 2007a). La Gaviota reidora, al ser una especie oportunista, puede explotar los hábitats marinos, dulceacuícolas y terrestres (Schwemmer et al., 2011). Las dos especies de rálidos son la Polla de agua (*Gallinula chloropus*) y el Calamón (*Porphyrio porphyrio*). Estas dos especies, aunque consideradas omnívoras, son principalmente herbívoras, alimentándose el Calamón de las semillas, bulbos, tallos y hojas del carrizo (*Phragmites australis*), la enea (*Thypa latifolia*) y el arroz (*Oryza sativa*) (Cramp, 1980). La Polla de agua también se alimenta principalmente de plantas (Amat and Soriguer, 1984).

Se puede decir que todo el conjunto formado por las estrategias de alimentación, no solo de las especies garzas y charranes seleccionadas sino también de las de gaviotas y rálidos, representan el conjunto total de los hábitats del río y Delta del Ebro; por lo tanto, dichas especies pueden ser consideradas como candidatos apropiados para ser utilizados como bioindicadores de la contaminación en esta área.

Tres áreas de muestreo fueron escogidas de acuerdo a las colonias de cría de estas especies: una de referencia y dos potencialmente afectadas por los lodos tóxicos (descritas previamente). El área focal, Flix, a pesar de la intensa actividad industrial que sufre, se ha convertido en un humedal con sorprendente valor ambiental, presentando profusión de aves nidificantes como garzas, cigüeñas y aguiluchos laguneros, entre otros. La zona distal, el Delta del Ebro, está situada 90 km aguas abajo. El área de referencia, situada 25 kilómetros aguas arriba de los sedimentos contaminados es l'Aiguabarreig (41° 23'N, 00° 19'E), una isla fluvial denominada "Illa dels Martinets", ubicada en la confluencia de dos afluentes del río Ebro, los ríos Cinca y Segre. Este lugar de gran valor ecológico y santuario para las aves acuáticas se encuentra aguas arriba de Flix y por lo tanto no se ve afectado por la industria química o los sedimentos tóxicos de Flix.

Varios tipos de muestras han sido propuestas para el desarrollo de programas eficaces de seguimiento a largo plazo (Pol and Becker, 2007), incluyendo huevos o tejidos como plumas o sangre. Este presente estudio se ha realizado con huevos y muestras de pluma y sangre de volantones. La información obtenida de los huevos integra la dieta del adulto previa a la puesta, puesto que las garzas y charranes, al seguir la estrategia "income", obtienen la mayor parte de los materiales utilizados en la formación de la puesta de la dieta (Ruiz et al., 1998, Hobson et al., 2000). Además, los huevos de charrán también reflejan el hábitat de alimentación de los machos, ya que éstos proporcionan presas a las hembras durante la formación de la puesta (Nisbet, 1973, Wiggins and Morris, 1986, Gonzalez-Solis et al., 2001). Por otro lado, las plumas y sangre de volantones integran la dieta obtenida por los adultos y consumida por los polluelos durante su crecimiento. El uso de volantones presenta dos ventajas. En primer lugar, se evita el efecto de la bioacumulación, debido a que el tiempo de exposición ha sido bajo y similar para todos los individuos. En segundo lugar, ya que todos los volantones son alimentados con presas capturadas por los padres en los alrededores de la colonia, las muestras reflejan sólo el impacto de la contaminación local.

Los metales pesados, cuando están presentes en altas concentraciones, son potencialmente peligrosos, puesto que entran en los organismos a través de la dieta (Burger et al., 1992). Sin embargo, la exposición varía en función de los hábitos tróficos de cada individuo. Por lo tanto, al mismo tiempo que se realizó la evaluación de los niveles de contaminantes, se utilizaron los análisis de isótopos estables (SIA) para obtener una adecuada comprensión de la ecología trófica de las especies, sus interacciones y el uso del hábitat (Nisbet et al., 2002, Sanpera et al., 2007b, Tavares et al., 2007, Abdennadher et al., 2011, Ramirez et al., 2011).

Durante las últimas décadas, los marcadores bioquímicos, tales como los isótopos estables, han sido utilizados cada vez en mayor proporción respecto a los métodos convencionales en los estudios de la dieta, con el fin de ofrecer nuevas perspectivas sobre la ecología de alimentación de las aves. Aunque los métodos tradicionales de análisis de la dieta, tales como los análisis estomacales o de egagrópilas, aportan datos valiosos sobre la composición general de la dieta (por ejemplo, identificación de presas específicas), el muestreo está a menudo muy sesgado debido a las diferentes estadios de digestión de presas y la acumulación en la molleja de objetos duros tales como los otolitos (Barrett et al., 2007). Los isótopos estables han sido utilizados con éxito en la obtención de información acerca de la ecología trófica, especialmente debido a que proporcionan un medio potencial para cuantificar la dieta asimilada y no simplemente ingerida. Las signaturas de isótopos estables de nitrógeno ($\delta^{15}N$), carbono ($\delta^{13}C$) y azufre ($\delta^{34}S$) han sido frecuentemente utilizadas en estudios de comunidades de aves, centrándose en su ecología trófica y relaciones (Hobson et al., 1994, Forero and Hobson, 2003, Cherel et al., 2008, Koiadinovic et al., 2008, Moreno et al., 2010). El $\delta^{15}N$ de los tejidos refleja el nivel trófico, siendo las signaturas de los consumidores mayores que en sus presas (Forero et al., 2005). El origen de las fuentes de carbono de la red trófica puede ser obtenido a partir del $\delta^{13}C$ (Hobson, 1999), proporcionando información sobre el hábitat de alimentación. La signatura isotópica de azufre ($\delta^{34}S$) es útil para determinar las fuentes de la dieta en los consumidores (marina vs terrestre), debido a que los compuestos básicos de azufre son incorporados a los tejidos sin fraccionamiento significativo (Connolly et al., 2004); este isótopo es particularmente útil para detectar o distinguir heterogeneidad entre usos de hábitats (Peterson et al., 1985).

Los isótopos estables también pueden ser utilizados para definir un "nicho trófico". El concepto ecológico de nicho trófico ha tenido un renacimiento en los últimos años (Newsome et al., 2007). Aunque los nichos trófico e isotópico son diferentes, están altamente relacionados. Si presentamos en un biplot los valores isotópicos de un animal en un espacio δ, éstos delinean el nicho isotópico de una especie. Jackson et al. (2011) propuso recientemente un nuevo modo de calcular los parámetros de nicho (o comunidad), basándose en elipses, reformulando el previo TA (Área total del Convex Hull) definido por Layman et al. (2007) en un entorno Bayesiano. Este nuevo parámetro, el área corregida de la elipse estándar (SEA_c), proporciona un indicativo de la amplitud de nicho usando información de la extensión y distancia entre los puntos de datos isotópicos y ha sido el elegido para este trabajo.

Adicionalmente, el uso combinado de biomarcadores con análisis químicos ha permitido en los últimos años la evaluación e identificación de efectos adversos para la salud producidos por diferentes contaminantes en varias especies acuáticas (Fossi et al., 1996, Cordi et al., 1997, Muir et al., 1999, Vos et al., 2000, Strum et al., 2008). Más recientemente, el uso de técnicas no invasivas, tales como los análisis de biomarcadores en sangre y contaminantes en plumas y huevos han sido aplicados al estudio de las especies en peligro de extinción (Muir et al., 1999, Vos et al., 2000, Champoux et al., 2002, Franson et al., 2002, Champoux et al., 2006, Murvoll et al., 2006, Quiros et al., 2008) y su uso puede proporcionar valiosa información acerca del estado medioambiental de las poblaciones de aves acuáticas del río Ebro.

Las defensas antioxidantes representan la vía de desintoxicación de las especies reactivas del oxígeno (ROS) en los organismos aeróbicos (Livingstone, 2001). Cuando el ROS excede las defensas antioxidantes, se produce daño oxidativo en las moléculas de ADN y proteínas, o en los lípidos dentro de las membranas celulares, resultando en una condición patológica, por lo general denominada estrés oxidativo (Livingstone, 2001). De este modo es posible conocer las respuestas de los organismos frente al estrés oxidativo, proporcionando una información muy valiosa sobre su estado de salud. Recientemente, algunos autores (Winston et al., 1998, Regoli, 2000) desarrollaron un método analítico para evaluar la capacidad de eliminación de oxiradicales libres (TOSC) de cualquier tejido determinado, que se define como la capacidad de la muestra biológica para neutralizar el ROS. Para un determinado tejido valores bajos de TOSC probablemente están relacionados con una capacidad menor para desintoxicar ROS y, por lo tanto, una mayor susceptibilidad al estrés oxidativo. El método fue desarrollado originalmente con muestras de hígado de rata (Winston et al., 1998), pero en los últimos tiempos se ha aplicado a la sangre de las aves en los programas de vigilancia ambiental (Corsolini et al., 2001). La actividad de enzimas plasmáticos, como la lactato deshidrogenasa o B esterasas, tales como la butirilcolinesterasa o las carboxilesterasas, también puede proporcionar información útil sobre el daño hepático y los efectos neurotóxicos de los pesticidas en las aves (Fossi et al., 1996, Cordi et al., 1997, Champoux et al., 2006, Strum et al., 2008).

Los Isótopos Estables como herramienta ecológica

Los elementos se encuentran en la naturaleza en varias formas estables que varían en el número de neutrones en el núcleo. Estas diversas formas se conocen como isótopos estables (ya que no decaen con el tiempo) de un elemento y tienen propiedades químicas idénticas, pero sus propiedades cinéticas cambian debido a las diferencias de masa.

Los espectrómetros de masas son instrumentos que proporcionan una estimación muy precisa de la relación entre el isótopo más pesado y ligero en una muestra con respecto a la normativa internacional. Las relaciones isotópicas se expresan convencionalmente como δ en partes por mil (‰) de acuerdo con la siguiente notación:

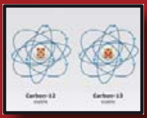
$$X = [(R_{muestra}/R_{estándar}) - 1] \times 1000$$

Donde X (‰) es ^{13}C, ^{15}N, ^{34}S y R son las ratios correspondientes de $^{13}C/^{12}C$, $^{15}N/^{14}N$ o $^{34}S/^{32}S$. R estándar para el C es Pee Dee Belemnite (PDB), para el N es el nitrógeno atmosférico (AIR) y para el S es troilita del meteorito del Cañón del Diablo (CDT).

Aunque las propiedades de las formas isotópicas no cambian drásticamente entre sí, hacen que se comporten de manera diferente en los procesos biogeoquímicos, traduciéndose en última instancia en cambios en la abundancia relativa del isótopo más pesado respecto al más ligero (fraccionamiento isotópico). El enriquecimiento sucede cuando el isótopo estable más pesado se acumula en el producto (en relación con el sustrato), mientras que el isótopo más ligero es preferentemente eliminado. Por otro lado, el empobrecimiento se produce cuando el isótopo más ligero se ve favorecido. Los cambios en la señal isotópica entre la dieta y los tejidos del consumidor son consecuencia de tres aspectos principales: fraccionamiento, discriminación y enrutamiento. Los procesos metabólicos de enriquecimiento y agotamiento son conocidos en general como fraccionamiento isotópico. La discriminación se refiere a la diferencia en la composición isotópica entre un tejido y la dieta, mientras que la asignación diferencial de componentes de la dieta isotópicamente distintos a diferentes tejidos se llama "enrutamiento isotópico". Hobson and Clark (1992) describieron la siguiente función:

$$Dt = Dd + \Delta dt$$

Donde Dt es la signatura isotópica de los tejidos del consumidor, Dd es la signatura isotópica de la dieta, y Δdt es el factor de discriminación entre la dieta y el tejido del consumidor. Desde el punto de vista ecológico, a la vez que se produce la transferencia de energía a través de los ecosistemas, el fraccionamiento isotópico tiene lugar, dando lugar a alteraciones de las signaturas del consumidor en relación a su dieta. Es decir, este fraccionamiento isotópico se produce en cada nivel trófico, y se suma en todas las cadenas tróficas. Por lo tanto, una vez que los supuestos se consideran controlados, el análisis de isótopos estables es una poderosa herramienta para la reconstrucción cuantitativa de los flujos de elementos e isótopos a través de las redes tróficas, lo que puede ayudar a responder a las preguntas más relevantes de la ecología trófica (Gannes et al., 1997, Bearhop et al., 2004).



Estudio B: La minería en los Andes Bolivianos

La actividad minera se lleva desarrollando en los Andes bolivianos desde antes del siglo XV (Sanabria, 2000). Particularmente, al este del Departamento de Oruro en la Cordillera Oriental de los Andes, hay extensos depósitos polimetálicos (SERGEOMIN, 1999). En el pasado, la extracción de oro y plata (asociada a sulfuros de hierro, cobre, zinc, plomo, arsénico, antimonio, etc.) era la actividad principal, mientras que en la actualidad predomina la extracción de estaño y el complejo de zinc-plata-plomo (Rios, 2002). Estas actividades mineras se caracterizan por la acumulación de grandes pilas de residuos en las minas abandonadas y activas, desde donde metales traza como el plomo, arsénico, cadmio y antimonio pueden llegar a las aguas superficiales y a los suelos y, por lo tanto, a la biota. Un ejemplo son los altos niveles de cadmio que fueron encontrados en patatas cultivadas en agrosistemas irrigados por el río Chayanta, que recibe residuos minerales de las principales minas de estaño de Bolivia, situadas a 60 km (Oporto et al., 2007, Rojas and Vandecasteele, 2007).

La contaminación de esta región a lo largo de los siglos ha afectado a su principal masa de agua, el lago Poopó. Sus aguas y los peces que viven en ellas presentan concentraciones de metales más altos que los valores permitidos para el consumo humano (Beveridge et al., 1985).

Al estudiar la contaminación de esta zona nos hemos centrado principalmente en dos zonas ubicadas en la Cordillera Oriental Andina, al sudeste de la ciudad de Oruro y el este del lago Poopó. Estas zonas presentan contaminación por metales traza en suelos y aguas. Las zonas de estudio se llaman Antequera (4.000 m, 18° 28'S, 66° 52'W) y Poopó (3.800 m, 18° 23'S, 66° 58'W), estando alejadas 13 kilómetros de distancia entre sí. Las aguas de dichas zonas drenan en dos subcuencas hidrográficas distintas, que finalmente desembocan en el lago Poopó (ver fig. 4).

En Antequera, la minería se limita al complejo de zinc-plata-plomo y todos los residuos se depositan en un estanque de almacenamiento que cierra un pequeño valle cerca del río Antequera (Rios, 2002), donde existen numerosas pilas de residuos dispersas. Toda esta actividad industrial ha contaminado los sedimentos del río, con concentraciones varias veces superiores a los valores permisibles de arsénico, antimonio, cadmio, cobre, cromo, estaño, hierro, mercurio, plomo y zinc (MEDMIN, 2001). En Poopó son explotados, por otro lado, el estaño y, secundariamente, el complejo de zinc-plata-plomo e igualmente numerosos montones de residuos se encuentran dispersos por la zona (Rios, 2002). Los metales y otros elementos en esta área son fácilmente dispersados por el agua y el viento a los suelos y masas de agua, especialmente al río Poopó que desemboca en el Lago Poopó. Los suelos de dicho río presentan altas concentraciones de Fe, Zn, Cd, Cu, Pb y Hg (MEDMIN, 2001).

Para fines comparativos, se seleccionaron otras dos zonas sin actividad minera (zonas de referencia). Se les llama San Pedro de Ulloma (3.850 m, 17° 30'S, 68° 32W), situada al oeste del río Desaguadero, y Chuñavi (4.100 m, 16° 17S, 68° 20W), al noroeste de la ciudad de

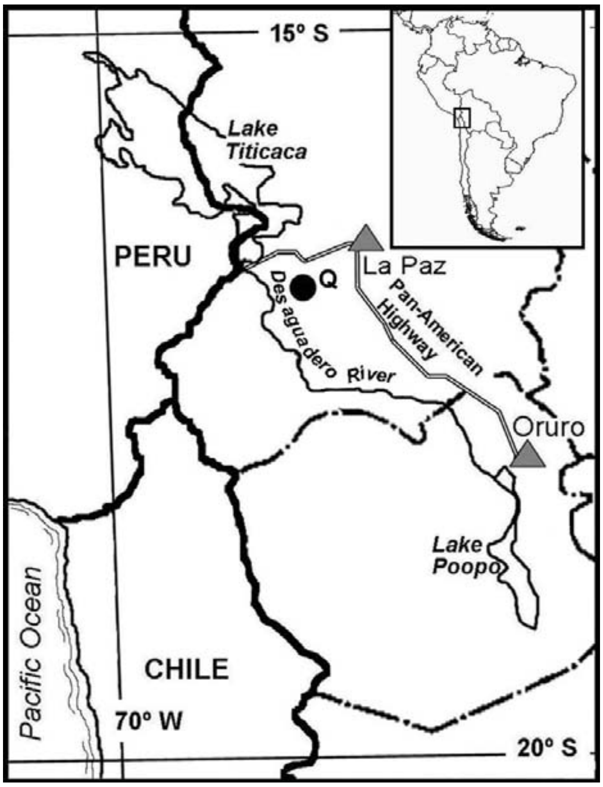


Figura 4. Zonas de estudio en el altiplano boliviano. Las zonas de referencia se llaman San Pedro de Ulloma o No Contaminada 1 (NP1) y Chuñavi o No Contaminada 2 (NP2). Las zonas mineras son Antequera o Contaminada 1 (P1) y Poopó o Contaminada 2 (P2).

La Paz y el este del Lago Titicaca (ver fig. 4). El primero se encuentra a 200 kilómetros de las localidades mineras, y el segundo, a 280 kilómetros.

Las zonas de estudio presentan una vegetación formada básicamente por arbustos espinosos y resinosos (p.ej., *Baccharis spp., Parastrephia spp.*, y *Adesmia spp.*) y hierbas (*Festuca spp.* y *Stipa spp.*). En ellas se desarrollan actividades agrícolas y ganaderas, siendo los cultivos principales la patata, la cebada y la cría extensiva de ovejas, ganado vacuno y llamas.

Con el objetivo de evaluar la exposición ambiental y el riesgo derivado de las actividades mineras y de los contaminantes originados por ellas, se seleccionaron especies de Tinamúes para evaluar su utilidad como bioindicadores.

Los tinamúes son aves especialmente poco estudiadas (Cabot, 1992, Davies, 2002). La familia Tinamidae está formada por 47 especies repartidas en 9 géneros. A pesar de su semejanza con aves como las codornices y perdices, las características comunes son efecto de la convergencia evolutiva. Los tinamúes no tienen más parientes vivos que las ratites no voladoras, y por lo tanto poseen un orden propio, los Tinamiformes. Las 47 especies que comparten este orden viven en América del Sur y Central. Estas aves prefieren caminar o correr a volar, utilizando el vuelo como técnica de evasión (para evitar ser depredadas o escapar de peligros) únicamente cuando se han agotado todas las otras opciones. Su modo de vuelo consiste en un batir frenético de alas seguido por un largo planeo, y así repetidamente. Aunque algunas especies son relativamente comunes, son tímidas y reservadas, por lo que son raramente observadas, aunque frecuentemente oídas. Un número reducido de especies viven en zonas abiertas y cubiertas de hierba, pero incluso éstas son precavidas. Los tinamúes se alimentan principalmente de pequeños frutos y semillas que encuentran en el suelo o en plantas bajas.

Las dos especies seleccionadas son el Tinamú pisacca (*Nothoprocta ornata*) y el Tinamú de Darwin (N. Darwinii). Ambas especies viven en el altiplano boliviano entre 3.700 y 4.200 m (Cabot, 1992, Davies, 2002, Garitano-Zavala et al., 2003), son relativamente comunes y se consideran las principales especies cinegéticas de la zona. Presentan un estilo de vida sedentario, con áreas de campeo pequeñas. Pearson y Pearson (1955) determinaron para el Tinamú pisacca un área de campeo media de 2,43 hectáreas. Los hábitos alimentarios de ambas especies son generalistas y oportunistas; consistiendo en una amplia variedad de semillas, hojas y frutos, así como invertebrados (Garitano-Zavala et al., 2003). Todas estas características indican que podrían ser bioindicadores apropiados de la contaminación derivada de las actividades mineras.

Para conocer sus movimientos, consideramos realizar el radio seguimiento de algunos tinamúes con el fin de comprobar sus áreas de campeo, puesto que en libertad estas especies presentan hábitos muy reservados y difíciles de seguir. El fin fue comprobar, entre otros, su sedentarismo y su potencial uso como especies bioindicadoras de la exposición local de contaminación. El seguimiento de un animal por radio consiste en dos dispositivos. Un transmisor sujeto al animal envía una señal en forma de ondas de radio, tal como hace una emisora. El transmisor puede colocarse en el tarso, en la espalda, o como en nuestro caso, en el cuello de un animal. Un receptor de VHF recoge la señal, al igual que una radio capta la señal de una emisora. El receptor puede ser colocado normalmente en un coche, vehículo todo terreno o avión. Para seguir la pista del animal, la señal es rastreada con el receptor.

Por otra parte, debido a que estas especies son uno de los objetivos cinegéticos principales de la zona y el acceso a especímenes completos es relativamente fácil, se decidió no sólo monitorizar los contaminantes en muestras de pluma sino también en órganos internos, tales como el hígado y el riñón. El estado histopatológico de hígado y el riñón también fue estudiado, para comprobar la producción de reacciones adversas a nivel histológico por la exposición a la contaminación.

Los contaminantes analizados en plumas tienen, además de la ruta endógena de acumulación (dieta), una ruta exógena (al estar expuestas a los elementos). En las plumas, justo después de su formación, se corta el suministro de sangre y la pluma se vuelve metabólicamente inerte, aislada del resto del cuerpo (Burger, 1993). En consecuencia, no se acumulan más metales pesados en las plumas por el torrente sanguíneo. Así, las plumas contienen información sobre las concentraciones circulantes de metales pesados en la sangre en el momento de su formación. Pero, una vez que la pluma ha sido formada, los niveles de metales pluma puede permanecer estables con el tiempo, como es el caso con el mercurio (Appelquist et al., 1984, Veerle et al., 2004), o pueden aumentar, ya que las plumas son altamente propensas a la contaminación exógena superficial. Esta contaminación por deposición atmosférica, acicalado de plumas o por el contacto con el medio (suelo, polvo o agua), puede dar una estima equivocada de la contaminación biológica incorporada en el momento del crecimiento de las plumas (Pilastro et al., 1993, Dauwe et al., 2002, Jaspers et al., 2004).

Las concentraciones de plomo son un buen ejemplo de esta circunstancia, ya que se ha demostrado que aumentan después de que la pluma haya crecido completamente, por lo que las plumas reflejan tanto los niveles endógenos de plomo como la contaminación exógena, y, a menudo, los niveles de contaminación atmosférica. Por lo tanto, sólo las plumas recién mudadas o las plumas de pollos volantones indican con exactitud los niveles endógenos, puesto que la acumulación exógena de contaminantes en la superficie de la pluma es una vía importante de contaminación (Veerle et al., 2004). Esta vía exógena afecta principalmente a las barbas, en donde las concentraciones aumentan con el tiempo debido a su gran y estructuralmente compleja superficie (Goede and Debruin, 1984, Cardiel et al., 2011).

Por lo tanto, se diseño un estudio para arrojar luz sobre esta cuestión, a fin de determinar qué sección de la pluma proporciona la información más precisa sobre los niveles de plomo endógenos originales, utilizando plumas de una de las especies muestreadas, el Tinamú pisacca.

Objectivos

Tomando como punto de partida la utilización de las aves como bioindicadoras, el objetivo principal de esta tesis fue valorar la utilidad de especies de aves seleccionadas como bioindicadores de contaminación en zonas que han sufrido episodios de contaminación intensa, dando lugar a riesgos medioambientales, a fin de aumentar el conocimiento que ayude en un futuro a la restauración ambiental, monitoreo y conservación de estas áreas.

Por lo tanto, para desarrollar este objetivo principal, tuvimos en cuenta dos casos de estudio, siendo los objetivos de esta tesis los siguientes:

Parte A: Los lodos tóxicos de Flix, ¿Afectan a la ornitofauna del río Ebro y su delta?

En el caso de los ambientes acuáticos, como es el río Ebro, afectado por los lodos tóxicos depositados en el embalse de Flix, los objetivos principales fueron:

- Evaluar si las aves acuáticas como la Garza imperial reflejan la exposición potencial a los contaminantes del embalse de Flix entre diferentes zonas fluviales y deltaicas, y comprobar su utilidad como bioindicadoras.
- Examinar si los residuos contaminados del embalse de Flix afectan a los niveles de contaminación de los hábitats donde las poblaciones de aves acuáticas del Delta del Ebro (situado 90 km aguas abajo) se alimentan.

Con el fin de alcanzar estos dos objetivos principales, los objetivos específicos fueron:

- Determinar la ecología trófica y segregación de hábitats de las poblaciones de aves acuáticas, con el fin de evaluar adecuadamente la exposición a contaminantes.
- Determinar si los niveles de contaminación en los huevos y plumas de volantones alcanzan niveles de alarma medioambiental.
- Evaluar qué tipo de muestra, huevos o plumas, refleja de un modo más adecuado los efectos de, o exposición a, la contaminación.
- Determinar un conjunto de biomarcadores en sangre para controlar los riesgos ambientales de contaminación.

Parte B: La minería en los Andes Bolivianos

En el caso de los Andes bolivianos, afectados por una larga tradición minera, los principales objetivos fueron:

- Evaluar el potencial de las especies de Tinamúes como bioindicadoras de la exposición a la contaminación local de elementos traza.
- Estudiar las diferentes vías de acumulación de plomo en las especies bioindicadoras, a fin de evaluar la utilidad de los niveles en pluma como una medida a la exposición de este contaminante.

Con el fin de alcanzar estos dos objetivos principales, los objetivos específicos fueron:

- Estudiar los hábitos de movimiento de las especies de Tinamú con el fin de evaluar su potencial como bioindicadores de contaminación de las fuentes locales.
- Comparar el uso de diferentes tipos de muestras (pluma, riñón, hígado) al evaluar el impacto de, o exposición a, la contaminación local.
- Evaluar el estado histopatológico del hígado y los riñones en relación con los niveles de contaminación.
- Determinar si las concentraciones de elementos traza (arsénico, cadmio, plomo y antimonio) en las especies de Tinamú alcanzan niveles preocupantes a nivel medioambiental.
- Examinar la importancia de las fuentes endógenas y exógenas de contaminación en relación con los niveles de plomo en pluma.

Discusión

Parte A: Los lodos tóxicos de Flix.

Dentro de esta primera parte de la discusión, nos centraremos en el potencial de las aves acuáticas como la Garza Imperial como centinelas de los contaminantes del embalse de Flix entre diferentes zonas fluviales y deltaicas, evaluando su utilidad como bioindicadores.

Además, otras especies de la familia Ardeidae y Sternidae (entre otras) serán utilizadas para examinar si la contaminación derivada del embalse de Flix afecta a las poblaciones de aves acuáticas que habitan el Delta del Ebro.

Así, los principales temas a tratar son los siguientes:

A1. Ecología trófica y partición de hábitats de la población de aves acuáticas del río Ebro y su Delta

Para estudiar la ecología trófica y la partición de hábitat de la diversa comunidad acuática de aves del río Ebro y su Delta, primero nos centraremos en los hábitats acuáticos del propio río, utilizando la Garza Imperial como especie de estudio, y en segundo lugar nos fijaremos en toda la comunidad del Delta del Ebro (que incluye, además de los hábitats dulceacuícolas, hábitats terrestres y marinos), mediante la selección de ciertas familias de aves.

Para el primer caso, huevos y volantones de Garza Imperial fueron utilizados en las dos localidades fluviales (l'Aiguabarreig y Flix) y el área deltaico, el Delta del Ebro (descriptiva en la Tabla 1). Las signaturas de nitrógeno se utilizan para estimar el nivel trófico, aunque las proporciones pueden estar afectadas por varios factores, tales como la longitud de la cadena alimentaria en un ecosistema dado o por diferencias ambientales en los niveles base (Cabana y Rasmussen, 1994). Este último parece ser el caso del río Ebro, ya que las grandes diferencias encontradas entre las localidades del río y el Delta del Ebro al parecer estan basadas en diferencias de los niveles base (ver fig. 5), probablemente debido a la eutrofización del río. Ambas localidades fluviales se encuentran situadas en embalses, con marcada eutroficación indicada por el δ^{15}N más alto de las presas principales de la Garza Imperial (ver fig. 6). Una situación similar ha sido observada en la Garceta común (*Egretta garzetta*) de la isla de Chickly (Abdennadher et al., 2011), expuesta a la eutrofización y al enriquecimiento de nitrógeno causado por las aguas residuales.

Por otro lado, las signaturas de δ^{15}N en Garza Imperial del Delta del Ebro se asemejan a las de las especies de charranes dulceacuícolas que habitan esta zona (Cotin et al., 2011), donde el flujo de agua continuo en arrozales y lagos probablemente previene la excesiva eutrofización. La posibilidad de que las diferencias en δ^{15}N sean causadas por cambios

	Site	Year	Sample									
			Feather					Egg				
			N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
δ^{15}N (‰)	Aiguabarreig	2006	7	18.5	.9	16.7	19.6					
		2007	7	18.9	1.6	15.8	20.5	12	19.0	2.3	14.7	21.5
		2008	4	19.2	1.9	16.4	20.3					
	Flix	2006	10	22.3	0.7	20.8	23.2	12	20.5	1.3	18.1	22.7
		2007	11	21.6	1.2	19.0	22.8	15	19.8	2.1	16.1	22.3
		2008	9	21.6	0.5	20.6	22.2					
	Ebro Delta	2006	4	13.9	1.4	12.3	15.5	14	13.9	1.5	11.8	17.1
		2007	16	13.9	0.7	12.8	15.6	25	13.3	1.0	11.6	15.7
		2008	12	14.4	1.0	13.0	15.7					
δ^{13}C (‰)	Aiguabarreig	2006	7	-25.6	0.9	-27.0	-24.3					
		2007	7	-27.5	0.5	-28.3	-27.0	12	-27.2	1.5	-29.9	-25.2
		2008	4	-27.4	0.9	-28.7	-26.8					
	Flix	2006	10	-25.8	0.8	-27.1	-24.4	12	-27.5	0.9	-28.9	-25.9
		2007	11	-27.0	0.7	-28.1	-25.9	16	-28.3	0.9	-29.2	-25.8
		2008	9	-28.6	0.4	-29.3	-28.1					
	Ebro Delta	2006	4	-24.0	1.8	-26.2	-22.2	14	-24.9	1.0	-26.3	-23.2
		2007	16	-24.1	0.9	-25.0	-22.1	25	-26.3	1.5	-30.4	-23.1
		2008	12	-22.6	3.2	-25.6	-16.9					

Tabla 1. Estadística descriptiva de las signaturas de isótopos estables en plumas y huevos de Garza Imperial (*Ardea purpurea*) de las tres localidades y años de muestreo.

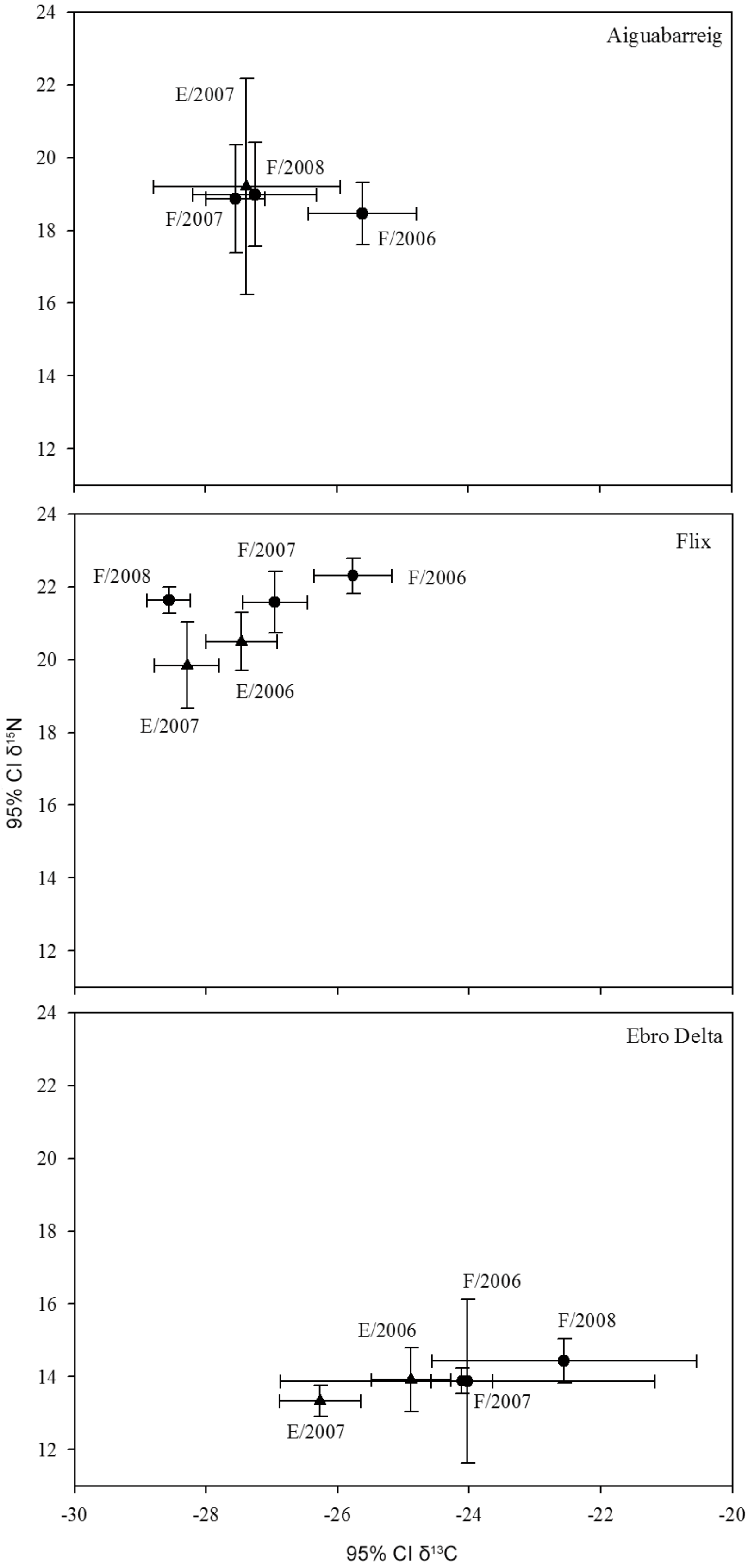


Figura 5. Signaturas de $\delta^{15}N$ y $\delta^{13}C$ indicando la posición media de los pollos y huevos de Garza Imperial de las tres localidades cada año. Círculos (plumas = F) y triángulos (huevos = e) indicar el valor medio y las líneas sus correspondiente intervalos de confianza del 95%.

en la dieta queda descartada puesto que los regurgitados de Garza Imperial de todas las localidades presentan las mismas especies de presas, siendo principalmente cangrejo de río y carpa (J. Cotin, com. pers.). Por lo tanto, aunque las signaturas de $\delta^{15}N$ reflejar el nivel trófico, los cambios observados entre las diferentes localidades se basan en las diferencias de los niveles base.

Las signaturas de carbono mantuvieron el mismo patrón entre localidades cada año, a pesar de ligeras diferencias observadas entre años (ver fig. 5). Los valores reflejan los hábitats fluviales y deltaicos, siendo alrededor de -24 ‰ para los hábitats de agua dulce del Delta del Ebro (Cotin et al., 2011). Los cambios observados entre años en las localidades fluviales recaen probablemente en la naturaleza altamente dinámica del río Ebro. Los cambios anuales en el Delta del Ebro están probablemente relacionados con un cambio parcial a zonas de alimentación más salobres, como las lagunas costeras, puesto que los hábitats influenciados por el mar presentan un mayor ratio isotópico de carbono que los hábitats de agua dulce (Michener y Schell, 1994). Este hecho queda reflejado en la mayor variabilidad de las signaturas de carbono encontradas en el Delta del Ebro. El uso combinado de nitrógeno y carbono discriminar adecuadamente los ecosistemas fluviales y deltaicos (nitrificación alta y signaturas de carbón más bajas en el río).

Para el estudio del segundo caso, el Delta del Ebro, se muestrearon y analizaron huevos de varias especies seleccionadas (ver tabla 2). En esta localidad los isótopos estables de carbono y azufre (azufre sólo analizado en especies de charrán) mostraron tres patrones distintos (véase las tablas 3 y 4 para descriptivos), lo que nos permite dividir la estructura de los hábitats de alimentación en tres escenarios principales: hábitats de agua dulce (arrozales y lagunas) con bajos valores isotópicos ($\delta^{13}C$ alrededor de -24,5 ‰, $\delta^{34}S$ alrededor de 2-3 ‰), hábitats marinos (el mar y las bahías) con valores de $\delta^{13}C$ alrededor de -18 ‰ y $\delta^{34}S$ alrededor de 18 ‰ y, finalmente, las salinas ($\delta^{13}C$ -14,5 ‰, $\delta^{34}S$ 9,5 ‰). Ver figuras 7 y 8.

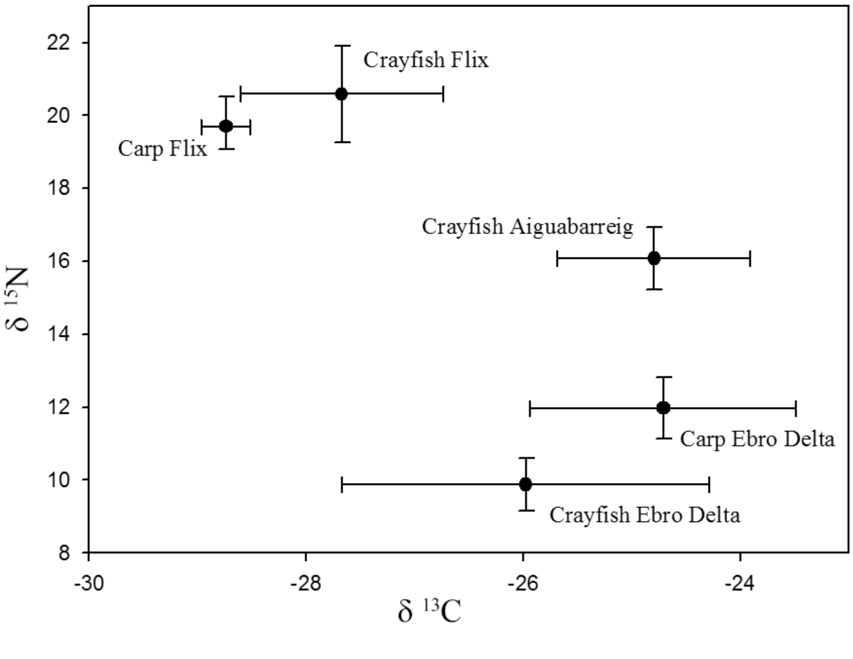


Figura 6. Signaturas de $\delta^{15}N$ y $\delta^{13}C$ indicando la posición media del cangrejo de río y la carpa de las tres localidades muestreadas (dos para la carpa). Los círculos indican media, las líneas sus correspondientes intervalos de confianza del 95%.

Northern Hemidelta	Canal Vell	Ardea purpurea	3
		Egretta garzetta	55
		Ardeola ralloides	20
		Larus ridibundus	20
	Illa de Buda	Nycticorax nycticorax	21
		Ardeola ralloides	20
	Punta del Fangar	Sterna hirundo	18
		Gelochelidon nilotica	20
Southern Hemidelta	l'Encanyissada	Ardea purpurea	28
	La Tancada	Chlidonias hybrida	11
	Platjola	Ardea purpurea	8
	Riet Vell	Ixobrychus minutus	20
		Gallinula chloropus	20
		Porphyrio porphyrio	10
	Punta de la Banya	Sterna hirundo	10
		Sternula albifrons	24
		Sterna sandvicensis	20
		Larus audouinii	15

Tabla 2. Número de huevos muestreados por colonia en el Delta del Ebro.

Dos especies de charrán y una de gaviota se alimentan en hábitats marinos: el Charrán patinegro, ambas colonias de Charrán común y la Gaviota de Audouin. El Charrán patinegro se alimenta en el mar (Stienen et al., 2000) y ningún otro hábitat de alimentación ha sido descrito para esta especie. El Charrán común, aunque en determinadas áreas también se alimenta en agua dulce (Becker et al., 1997, Neubauer, 1998), es descrito principalmente como forrajeador marino (Becker et al., 1993, Granadeiro et al., 2002) y las signaturas isotópicas indican que esta es la estrategia de alimentación que esta especie sigue en el Delta del Ebro. Esta observación es consistente con los hallazgos de otros estudios (Arcos et al., 2002, Guitart et al., 2003). Sin embargo, aunque las pequeñas diferencias en los valores isotópicos de $\delta^{13}C$ y $\delta^{34}S$ entre las dos colonias de Charrán común apuntaban a diferencias locales, las enormes diferencias en $\delta^{15}N$ indican que las colonias utilizan diferentes recursos. De hecho, mientras que Arcos et al. (2002) indicó que el Charrán común en el Delta del Ebro se alimenta de peces epipelágica (Clupeiformes), Oro y Ruiz (1997) encontraron que esta especie también se alimenta de descartes pesqueros. Ambas observaciones concuerdan con las estrategias de alimentación descritas por Guitart et al. (2003) y Mateo et al. (2004) para las colonias de la "Punta del Fangar" (que se basa principalmente en peces epipelágicos) y de la "Punta de la Banya" (donde utilizan descartes). La gaviota de Audouin es considerada un depredador nocturno de peces epipelágicos (principalmente Clupeiformes), pero es también una especie oportunista, aprovechándose de las actividades de pesca, especialmente de los descartes (Oro y Ruiz, 1997, Sanpera et al., 2007a). Por lo tanto, aunque las signaturas de $\delta^{13}C$ de esta especie deberían apuntar únicamente a hábitats marinos, el uso de los recursos dulceacuícolas por esta especie (recientemente descubiertos), en particular del cangrejo de río (Navarro et al., 2010b), probablemente bajan las signaturas de carbono (fig. 8).

	Species	N	Mean	SD	Min	Max															
δ^{15}N (‰)	Little Tern	24	14.6	0.9	12.6	16.4	○														
	Night Heron	21	13.9	1.1	12.0	15.4	●	○													
	Little Egret	55	13.6	1.2	11.2	17.4		●	○												
	Purple Heron	39	13.5	1.2	11.6	17.1		●	●	○											
	Gull-billed Tern	20	13.4	0.7	12.6	14.8		●	●	●	○										
	Squacco Heron	40	13.3	0.8	12.1	14.9		●	●	●	●	○									
	Common Tern 'Banya'	10	13.3	0.5	12.6	14.0		●	●	●	●	●	○								
	Little Bittern	20	12.8	1.1	10.6	14.3		●	●	●	●	●	●	○							
	Black-headed Gull	20	12.7	0.7	11.7	13.8				●	●	●	●	●	○						
	Whiskered Tern	11	12.6	0.9	11.5	14.9		●	●	●	●	●	●	●	●	○					
	Audouin's Gull	15	12.2	0.5	11.5	13.3								●	●	●	○				
	Sandwich Tern	20	12.1	0.3	11.3	12.7								●	●	●	●	○			
	Common Tern 'Fangar'	18	10.9	0.3	10.2	11.4													○		
	Moorhen	20	10.4	1.7	6.9	13.9													●	○	
	Purple Swamphen	10	10.0	1.2	7.8	11.8													●	●	○
δ^{13}C (‰)	Little Tern	24	-14.6	2.1	-18.3	-11.8	○														
	Common Tern 'Fangar'	18	-17.5	0.2	-17.9	-17.1		○													
	Common Tern 'Banya'	10	-17.9	0.2	-18.2	-17.6			○												
	Sandwich Tern	20	-18.0	0.2	-18.3	-17.7			●	○											
	Audouin's Gull	15	-20.1	1.8	-23.8	-18.3					○										
	Squacco Heron	40	-23.4	0.8	-24.8	-20.6						○									
	Moorhen	20	-24.4	1.1	-26.4	-21.7						●	○								
	Whiskered Tern	11	-24.6	0.7	-26.2	-23.6							●	○							
	Little Egret	55	-24.7	2.1	-28.7	-16.7							●	●	○						
	Little Bittern	20	-24.8	0.9	-26.4	-22.8							●	●	●	○					
	Gull-billed Tern	20	-24.8	0.5	-25.5	-23.4							●	●	●	●	○				
	Night Heron	21	-24.9	1.5	-27.2	-22.2							●	●	●	●	●	○			
	Purple Swamphen	10	-24.9	0.8	-26.1	-23.6							●	●	●	●	●	●	○		
	Black-headed Gull	20	-25.0	0.5	-25.9	-23.9							●	●	●	●	●	●	●	○	
	Purple Heron	39	-25.8	1.5	-30.4	-23.1								●	●	●	●	●	●	●	○

Tabla 3. Estadística descriptiva de las signaturas de isótopos estables en huevos de las especies incluidas en el estudio, ordenadas de mayor a menor valor. Las comparaciones post hoc se muestran en la matriz de puntos. Los círculos negros indican que no hay diferencia significativa entre las especies de filas y columnas.

El Charrancito muestra signaturas altas de $\delta^{13}C$ e intermedias de $\delta^{34}S$, siendo la única especie del estudio que supuestamente se alimenta principalmente en las salinas (ver fig. 7 y 8). Estudios previos llevados a cabo en Ría Formosa (Portugal) indican los factores que influyen en la selección de los hábitats de alimentación de esta especie, como la abundancia de presas, canales con corrientes fuertes, la proximidad a las salinas y la información entre individuos (Paiva et al., 2008a). Muchos estudios han identificado las salinas como hábitat de cría (Catry et al., 2004, Oro et al., 2004, Peste et al., 2004, Paiva et al., 2006a) y han remarcado la dieta oportunista de esta especie, y por lo tanto, la gran variedad de hábitats de alimentación. La única limitación sería su pequeña área de campeo, de 4 a 6 km durante el período de cría (Fasola y Bogliani, 1990, Perrow et al., 2006). Los datos obtenidos mediante transectos en el Delta del Ebro muestran que estas aves se alimentan en los canales, lagunas y bocas de lagunas, mientras que las marismas son evitadas y los campos de arroz raramente visitados (Bertolero et al, 2005.), Sin embargo, dicho estudio no incluyó transectos en las salinas (situadas en la "Punta de la Banya" y donde nuestras muestras fueron tomadas). Los valores isotópicos de $\delta^{13}C$ en esta especie reflejan un hábitat de alimentación que difiere de la de los hábitats mar/agua dulce/terrestre y es muy similar a los reportados por Tavares et al. (2007) en charrancitos alimentandose en salinas portuguesas. En hábitats costeros hipersalinos, los cambios en los productores primarios elevan las signaturas de $\delta^{13}C$ (Michener y Schell, 1994). Este hallazgo indica que los Charrancitos de la "Punta de la Banya" se alimentan principalmente en las salinas, que es el hábitat principal alrededor de dicha colonia. Además, la alta variación de sus valores isotópicos también indica el uso de otros hábitats en menor medida y señala la existencia de estrategias de alimentación individuales. Es interesante indicar que los valores de $\delta^{15}N$ de esta especie son los más altos de todos los charranes examinados. Estas altas signaturas de $\delta^{15}N$ estarían relacionadas con una línea basal más elevada en los valores isotópicos de las salinas en vez de ser el reflejo de verdaderas diferencias en los niveles tróficos ocupados, puesto que la dieta de los charrancitos se basa principalmente en pequeños peces y gambas (Norman, 1992, Bogliani et al., 1994, Paiva et al., 2006).

Y, por último, los hábitats de agua dulce, con bajas signaturas de $\delta^{13}C$ y $\delta^{34}S$, donde concurren todas las garzas, dos charranes, una gaviota y las dos especies de rálidos. La Gallineta de agua y el Calamón son descritos como principalmente herbívoros, basándose la dieta del

		N	Mean	SD	Min	Max
$\delta^{34}S$ (‰)	Little Tern	24	9.4	3.3	4.3	15.9
	Gull-billed Tern	20	1.6	0.6	0.5	2.5
	Whiskered Tern	11	3.4	1.5	1.2	5.5
	Sandwhich Tern	20	18.5	0.3	17.8	18.8
	Common Tern 'Fangar'	18	17.0	0.6	16.0	17.8
	Common Tern 'Banya'	10	19.0	0.4	18.4	19.6

Tabla 4. Estadística descriptiva de las signaturas de azufre ($\delta^{34}S$) en los huevos de las cinco especies de charranes analizadas (Charrancito, Pagaza piconegra, Fumarel cariblanco y Charrán y común).

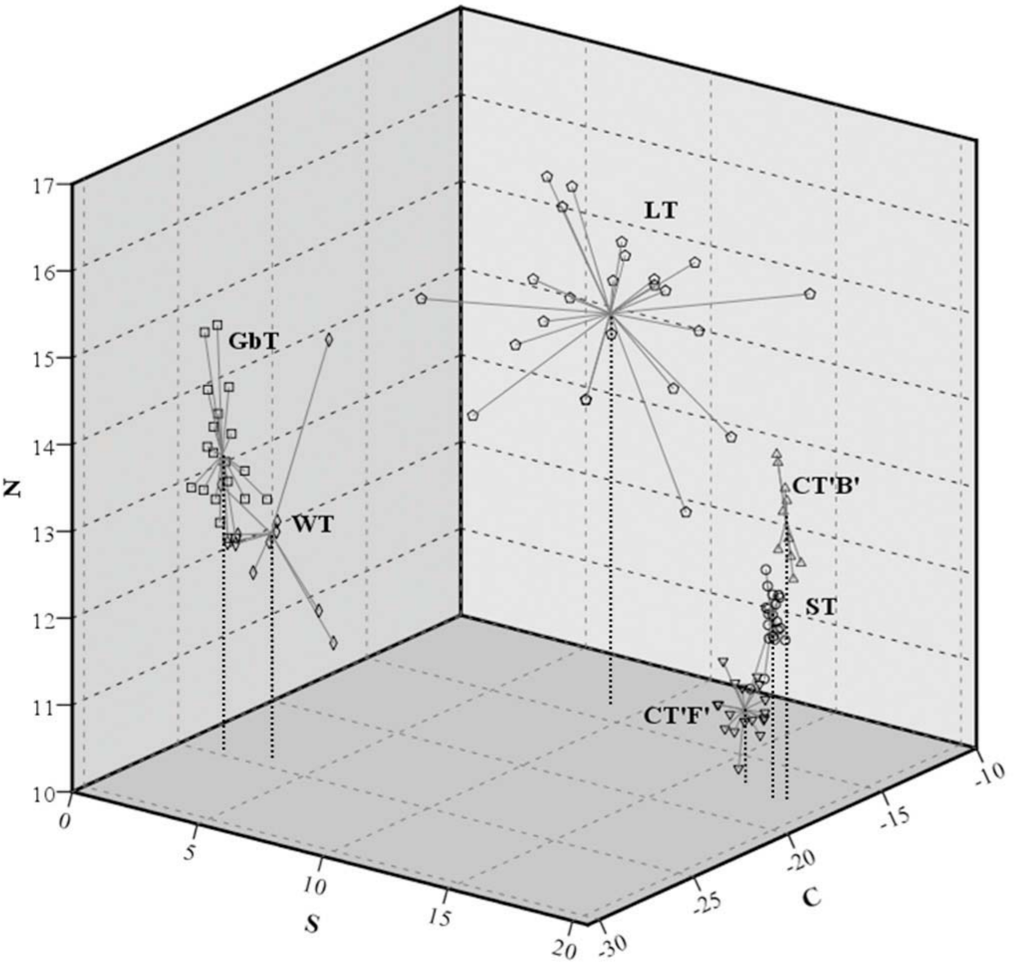


Figura 7. Distribución en 3D de las signaturas de $\delta^{15}N$, $\delta^{34}S$ y $\delta^{13}C$ de las cinco especies de charranes: Charrancito (pentágonos), Charrán patinegro (círculos), Charran común "Banya" (triángulos hacia arriba), Charrán común "Fangar" (triángulos hacia abajo), Pagaza piconegra (cuadrados) y Fumarel cariblanco (rombos).

Calamón en semillas, bulbos, tallos y hojas de carrizo (*Phragmites australis*), enea (*Thypa latifolia*) y arroz (*Oryza sativa*) (Cramp, 1980). La Gallineta de agua también se alimenta principalmente de materia vegetal (Amat y Soriguer, 1984), pero durante el período de crianza de los polluelos puede aumentar el consumo de insectos. Sus bajas signaturas de $\delta^{15}N$ concuerdan con la dieta descrita y diferencian estas especies claramente de las otras. La Gaviota reidora es una especie que puede explotar los hábitats marinos, de agua dulce y terrestres (Schwemmer et al., 2011) al ser un predador oportunista. Sin embargo, en el Delta del Ebro, las signaturas de $\delta^{13}C$ indican que hacen uso principalmente de hábitats de agua dulce, y su relativamente bajo $\delta^{15}N$ indica que probablemente se alimentan de insectos y lombrices de tierra, presa muy común en esta especie (Schwemmer et al., 2011). Las dos charranes dulceacuícolas son el Fumarel cariblanco y la Pagaza piconegra. El primero se alimenta principalmente de presas acuáticas en humedales (Latraube et al., 2005, Paillisson et al., 2007), siendo los arrozales, pantanos y lagunas del Delta del Ebro hábitats adecuados de alimentación para esta especie. La Pagaza piconegra es conocida por ser un forrageador generalista con un amplio elenco de presas (Goutner, 1991), tales como langostas y

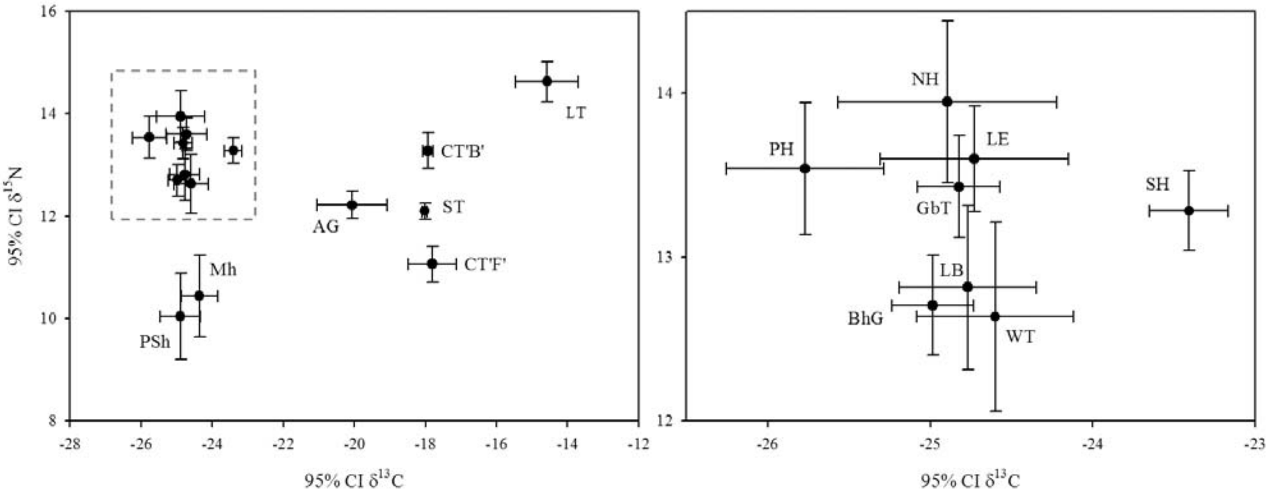


Figura 8. Signaturas de δ^{15}N y δ^{13}C indicando la posición media de las especies incluidas en el estudio: Garza Imperial (PH), Garceta común (LE), Martinete (NH), Garcilla cangrejera (SH), Avetorillo (LB), Charrán patinegro (ST), Charrán común "Banya" (CT'B '), Charrán común "Fangar" (CT'F'), Charrancito (LT), Pagaza piconegra (GBT), Fumarel cariblanco (WT), Gaviota reidora (BHG), Gaviota de Audouin (AG), Gallineta de agua (Mh) y Calamón (PSH). El punto indica el valor medio, y las líneas sus correspondientes intervalos de confianza del 95%. El área cuadrada de la izquierda es la ampliada en la derecha.

orugas (Mathew et al., 1998), anfibios y escarabajos (Cabo y Sánchez, 1986) o cangrejos de río, insectos y peces (Dies et al., 2005). En el Delta del Ebro, esta especie se alimenta principalmente en agua dulce, como lo demuestra su bajo rango de signaturas de $\delta^{13}C$. Las pequeñas diferencias encontradas en las signaturas de $\delta^{34}S$ entre los dos charranes de agua dulce indican que la pagaza piconegra también explota hábitats terrestres (con valores más bajos de $\delta^{34}S$), lo que estaría de acuerdo con estudios previos (Andersen, 1945, Bogliani et al., 1990, Sánchez et al., 1991), mientras que el Fumarel cariblanco utilizaría hábitats de aguas salobres (aumentando los valores isotópicos de S). Es interesante señalar que la Pagaza piconegra presenta mayores signaturas de nitrógeno que el Fumarel cariblanco y la Gaviota reidora, tal vez a consecuencia de alimentarse de presas de mayor tamaño. El huevo de Charrán común recogido en la "Punta del Fangar" que se excluyó de los análisis presenta valores isotópicos muy similares a las especies de agua dulce. Esta observación indica estrategias individuales de alimentación, incluso cuando este comportamiento de alimentación no ha sido descrita previamente para esta población tern (Arcos et al., 2002, Mateo et al., 2004).

Las cinco especies de garzas se alimentan en hábitats de agua dulce. La Garza Imperial, Garceta Común, Martinete y Avetorillo son depredadores principalmente acuáticos. Las diferencias en $\delta^{15}N$ indican que el avetorillo se alimenta de presas con niveles tróficos inferiores, como el cangrejo de río (Pardo-Cervera et al., 2010). La mayor variabilidad de

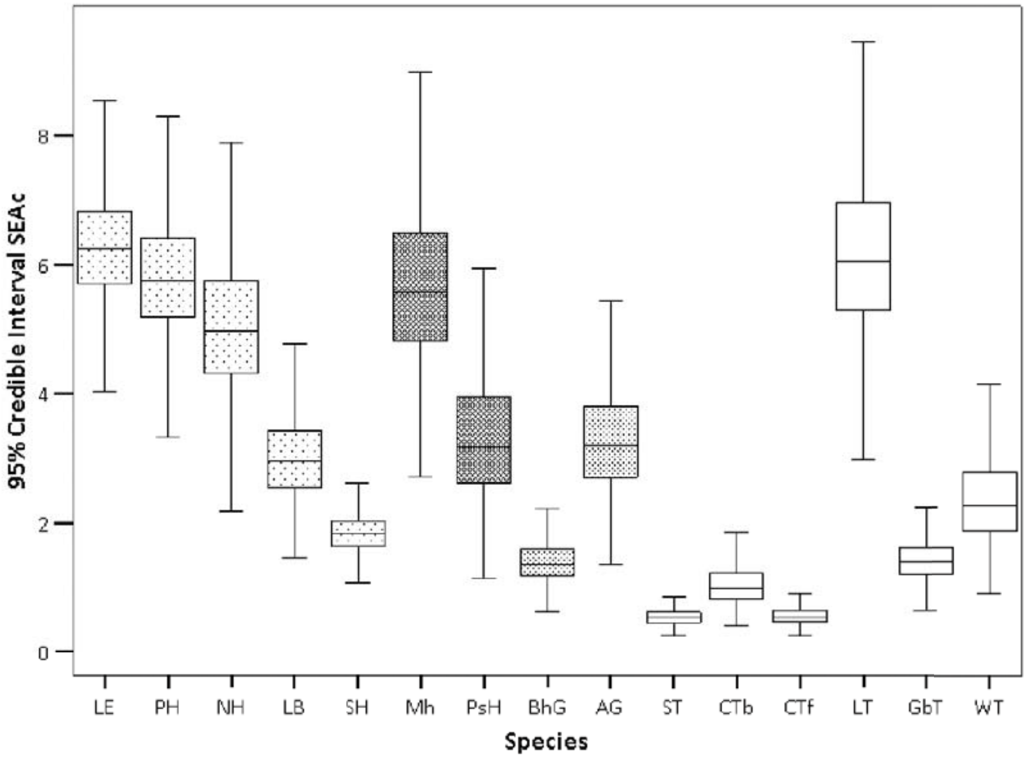


Figura 9. Área corregida de la elipse estándar (SEAc) de las especies de estudio ordenadas por familia. Ardeidae: Garza Imperial (PH), Garceta común (LE), Martinete (NH), Garcilla cangrejera (SH), Avetorillo (LB). Rallidae: Gallineta de agua (Mh) y Calamón (PSH). Laridae: Gaviota reidora (BHG), Gaviota de Audouin (AG). Sternidae: Charrán patinegro (ST), Charrán común "Banya" (CTB), Charrán común "Fangar" (CTF), Charrancito (LT), Pagaza piconegra (GBT), Fumarel cariblanco (WT).

$\delta^{13}C$ en Garceta Común indican que también podría estar alimentándose en otros hábitats, aunque en menor medida, como las aguas salobres o las salinas (Abdennadher et al., 2011), apuntando a un cierto grado de heterogeneidad individual en la dieta. La Garcilla cangrejera, a pesar de tener el tamaño relativo de un avetorillo, se alimenta principalmente de insectos (Orthotopera y Coleoptera) (Delord et al., 2004). Es interesante destacar el alto porcentaje de grillo topo (*Gryllotalpa gryllotalpa*) encontrados en los regurgitados de esta especie en el Delta del Ebro (datos no publicados). Este aves acuática normalmente se alimenta en el borde de arrozales y la contribución de presas terrestres eleva sus signaturas de $\delta^{13}C$.

De acuerdo con los parámetros de amplitud de nicho (fig. 9), basados en los datos isotópicos, podemos concluir que especies como el Charrán común y pandwich son especialistas estrictos en el Delta del Ebro, con un reducido nicho trófico. Por otro lado, tenemos el Charrancito, algunas de las especies de garzas (Garceta común, Garza imperial y Martinete) y la Gallineta de agua, en los que su alimentación oportunista, junto con una mayor variabilidad de las signaturas de $\delta^{13}C$ en los hábitats de alimentación, hacen que se expanda su nicho trófico. Esto es especialmente cierto en el caso de las especies de agua dulce, puesto que su, en promedio, mayor nicho trófico en comparación con las especies marinas probablemente indica una mayor heterogeneidad isotópica en dichos hábitats.

Así que para resumir las comunidades de aves acuáticas del río y del Delta del Ebro, se podría concluir que las signaturas de nitrógeno reflejar el nivel trófico, pero las diferencias entre las localidades están muy influenciados por los valores basales, siendo éstos mayores en las localidades fluviales que en el delta. El nitrógeno, junto con las signaturas de carbono, discrimina adecuadamente los ecosistemas fluviales y deltaicos (nitrificación alta y signaturas de carbono más bajas en el río). Además, nos gustaría destacar que el uso de los isótopos estables y los parámetros de nicho basados en elipses (SEA_C), aplicados en muestras de huevos, han ayudado a desentrañar la ecología trófica y la partición de hábitat de la comunidad de aves acuáticas del Delta del Ebro: El Charrán común y patinegro se comportan como especialistas estrictos en el Delta del Ebro, con pequeños nichos tróficos, mientras que de modo opuesto, el Charrancito, Garceta Común, Garza Imperial, Martinete y Gallineta de agua se comportan como generalistas, con amplios nichos tróficos.

A2. Niveles de contaminación en huevos y plumas. ¿Alerta ecológica?

Como en la sección anterior, para tener una mejor comprensión de los niveles de contaminantes en las aves del río Ebro, en primer lugar vamos a comparar entre las localidades seleccionadas del río (las dos fluviales y la deltaica), utilizando huevos y pollos de Garza Imperial y, en segundo lugar, nos centraremos en el Delta del Ebro, donde se hará uso de huevos de especies de las familias Sternidae, Ardeidae, Laridae y Rallidae. En todas las muestras se analizaron mercurio, selenio, cobre, plomo, cromo y arsénico (cromo y arsénico sólo para los años 2007-2008).

			Sites													
			l'Aiguabarreig				Flix					Ebro Delta				
	Year	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max
[Hg]	2006	7/7	1580.7	637.4	902.7	2684.3	10/10	7819.5	2995.3	2980.8	13616.7	4/4	2390.8	427.1	2057.5	2854.4
	2007	7/7	1638.4	543.0	863.9	2345.2	11/11	6911.5	4447.9	2718.5	10313.2	16/16	1381.6	705.3	748.5	3387.0
	2008	4/4	1512.8	151.9	1291.9	1575.7	9/9	2986.4	1084.1	2407.6	9435.4	12/12	1792.3	449.9	1098.8	2595.1
[Se]	2006	7/7	1795.9	449.6	1502.9	2481.4	10/10	2578.0	818.6	2054.8	3370.6	3/4	1709.1	347.7	1399.9	1747.6
	2007	6/7	1853.0	434.8	1650.5	2286.2	11/11	2284.6	742.8	1745.8	3534.0	13/16	1516.4	154.5	1090.2	2130.3
	2008	4/4	2202.3	160.1	2126.9	2333.4	9/9	1924.7	888.4	1643.6	3961.2	12/12	1709.4	747.8	1255.5	2200.7
[Cu]	2006	7/7	8831.0	1979.0	6979.4	9986.9	10/10	8058.6	1018.6	6510.0	10224.5	4/4	11279.8	1911.6	10188.8	12552.4
	2007	7/7	6001.1	2796.9	4113.9	7696.1	11/11	6085.2	1461.5	4431.9	7467.1	16/16	6015.8	1099.4	4073.8	7152.5
	2008	4/4	5936.2	538.0	5521.6	6324.1	9/9	5301.1	1478.4	4521.0	9712.1	12/12	7876.5	2355.9	4863.0	23391.0
[Pb]	2006	7/7	1312.9	829.0	951.7	2073.2	10/10	1692.1	367.5	1121.4	2168.4	4/4	2023.0	536.5	1603.0	2631.0
	2007	0/7					1/11	116.3	0.0	116.3	116.3	0/16				
	2008	1/4	59.9	0.0	59.9	59.9	5/9	41.2	112.6	34.6	191.1	9/12	74.3	62.6	54.0	337.1
[Cr]	2007	7/7	1622.2	224.5	1471.1	1738.8	11/11	1759.0	398.0	1508.2	2045.8	16/16	1601.7	167.6	1203.7	1792.0
	2008	4/4	1946.3	101.2	1891.9	2064.3	9/9	2087.8	183.1	1859.0	2259.8	12/12	2102.0	172.0	1781.3	2389.9
[As]	2007	2/7	111.3	19.6	101.5	121.1	3/11	122.3	13.7	119.3	132.9	14/16	159.2	34.4	125.2	268.0
	2008	4/4	68.5	26.9	55.5	92.6	9/9	60.6	37.5	48.8	117.5	12/12	156.2	76.9	77.3	215.3

Tabla 5. Estadísticos descriptivos de los elementos traza expresados en ng/g en plumas de volantones de Garza Imperial (*Ardea purpurea*) de las tres localidades y años. Sólo aparecen las muestras con valores detectados. Para el mercurio y el cobre esto representa el 100% de las muestras.

			Sites													
			l'Aiguabarreig				Flix					Ebro Delta				
	Year	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max	N	Median	IQR	Min	Max
[Hg]	2006						12/12	825.3	778.3	481.7	3467.8	14/14	645.9	216.7	297.8	1124.1
	2007	12/12	115.5	66.8	93.2	827.1	16/16	663.1	827.0	92.8	1727.5	25/25	302.0	323.7	91.0	626.5
[Se]	2006			0.0			12/12	3933.2	920.6	2317.5	5523.6	14/14	3143.7	1081.5	1599.1	3939.6
	2007	12/12	3545.9	1019.6	2594.6	4424.0	16/16	3477.1	621.3	2110.3	4181.4	25/25	2561.8	818.5	1471.4	3655.1
[Cu]	2006			0.0			12/12	4950.5	1424.6	4431.1	6607.1	14/14	4704.8	595.8	3735.6	6208.4
	2007	12/12	3677.6	808.0	2976.5	5909.0	16/16	4031.6	937.7	3030.6	5177.6	25/25	4351.8	1003.2	3350.4	5861.6
[Cr]	2007	12/12	2500.8	164.9	2406.1	2784.8	16/16	2527.9	233.9	1478.5	2726.8	25/25	2570.0	181.5	2314.5	3026.9
[As]	2007	10/12	127.1	26.5	111.8	173.7	11/16	140.1	29.3	123.0	159.2	25/25	167.9	52.8	100.2	303.0

Tabla 6. Estadística descriptiva de los elementos traza expresados en ng/g en huevos de Garza Imperial (*Ardea purpurea*) de las tres localidades y años. Sólo aparecen las muestras con valores detectados. Para el mercurio y el cobre esto representa 100% de las muestras.

Comenzando con cromo y arsénico, aunque los niveles en el río Ebro mostraron algunas diferencias en arsénico (véase la tabla 5 y 6), los niveles fueron muy similares en todas las localidades. Los niveles de cromo detectados en Flix podrían estar ligeramente influenciados por los lodos tóxicos y probablemente es mayor en el Delta del Ebro debido a un uso parcial de hábitats influenciados por el mar, como se ha visto en otras especies que habitan en esta zona. Los niveles de arsénico y cromo están por debajo de los niveles tóxicos y dentro de los reportados para varias especies de aves, incluyendo garzas (Burger y Gochfeld de 2009, Padula et al., 2010). Los niveles de plomo son inferiores a los que causan efectos adversos en la reproducción (en torno a 4000 ng/g en plumas, como indicó Burger y Gochfeld (2000a)).

En cuanto al cobre, los cambios entre años en las concentraciones podrían ser un reflejo de la dinámica del río. Este metal parece estar igualmente disponible en todos los hábitats, y los niveles podría ser explicado por el uso de sulfato de cobre (Cu_2SO_4) en la agricultura. Los niveles encontrados están muy por debajo de los niveles tóxicos, que se consideran en unas 60 ppm (Attia et al., 2011).

Los huevos son buenos bioindicadores de selenio (Ohlendorf et al., 2011) y los niveles son más altos en las localidades fluviales que en el Delta del Ebro (ver fig. 10). Por lo tanto, el río está probablemente afectado por otras fuentes de selenio aparte de los sedimentos contaminados. Aunque las plumas son pobres indicadoras de este contaminante (Ohlendorf y Heinz, 2011), los niveles más altos encontrados en Flix parecen indicar un ligero efecto de este localidad en las garzas.

Los embriones de aves son muy sensibles a los efectos tóxicos de selenio, como se refleja en una reducción en la eclosión de huevos fértiles y el desarrollo teratogénico de embriones (Janz et al., 2010). Aunque el umbral en el que se producen impactos negativos en las aves es muy discutido, Ohlendorf y Heinz (2011) sugirieron niveles superiores a 12 mg/kg peso seco como asociados con una disminución en la eclosión de huevos en especies sensibles y moderadamente sensibles y niveles inferiores a 3,0 mg/kg como concentración media como niveles basales. Nuestros valores no alcanzan el primer umbral, pero en todos casos estan por encima de los rangos descritos en huevos y plumas de Garceta común en Pakistán (Boncompagni et al., 2003), plumas de Martinete (Nycticorax nycticorax) en EE.UU. (Golden et al., 2003), o plumas de varias especies de garzas de Hong Kong y de Szechuan (Burger y Gochfeld, 1993) y se encuentran entre los más altos jamás descritos para una especie de garza, aunque un impacto negativo no puede ser dado por hecho, puesto que los niveles umbrales en plumas no han sido claramente definidos (Ohlendorf y Heinz, 2011).

Acerca del mercurio, Flix presenta las concentraciones más altas entre las localidades de estudio, lo que significa que la cantidad de mercurio filtrándose de los lodos tóxicos al ecosistema del Ebro es alta y ocurre de modo continuo en el embalse de Flix, puesto que las concentraciones son estables a lo largo de los años, incluso en un ecosistema tan dinámico como es el río Ebro, como fue mostrado en un estudio realizado con larvas de mejillón cebra (Carrasco et al., 2008). El único cambio en este patrón fue en 2008, en la que algunos ejemplares de la localidad de Flix pueden haber estado alimentándose aguas arriba, lejos de la entrada principal de mercurio, lo que habría quedado reflejado en los niveles más bajos de las plumas de los polluelos. Los niveles detectados en l'Aiguabarreig son mucho mayores que los esperados para una localidad de "referencia" (ver fig. 10), por lo que esta

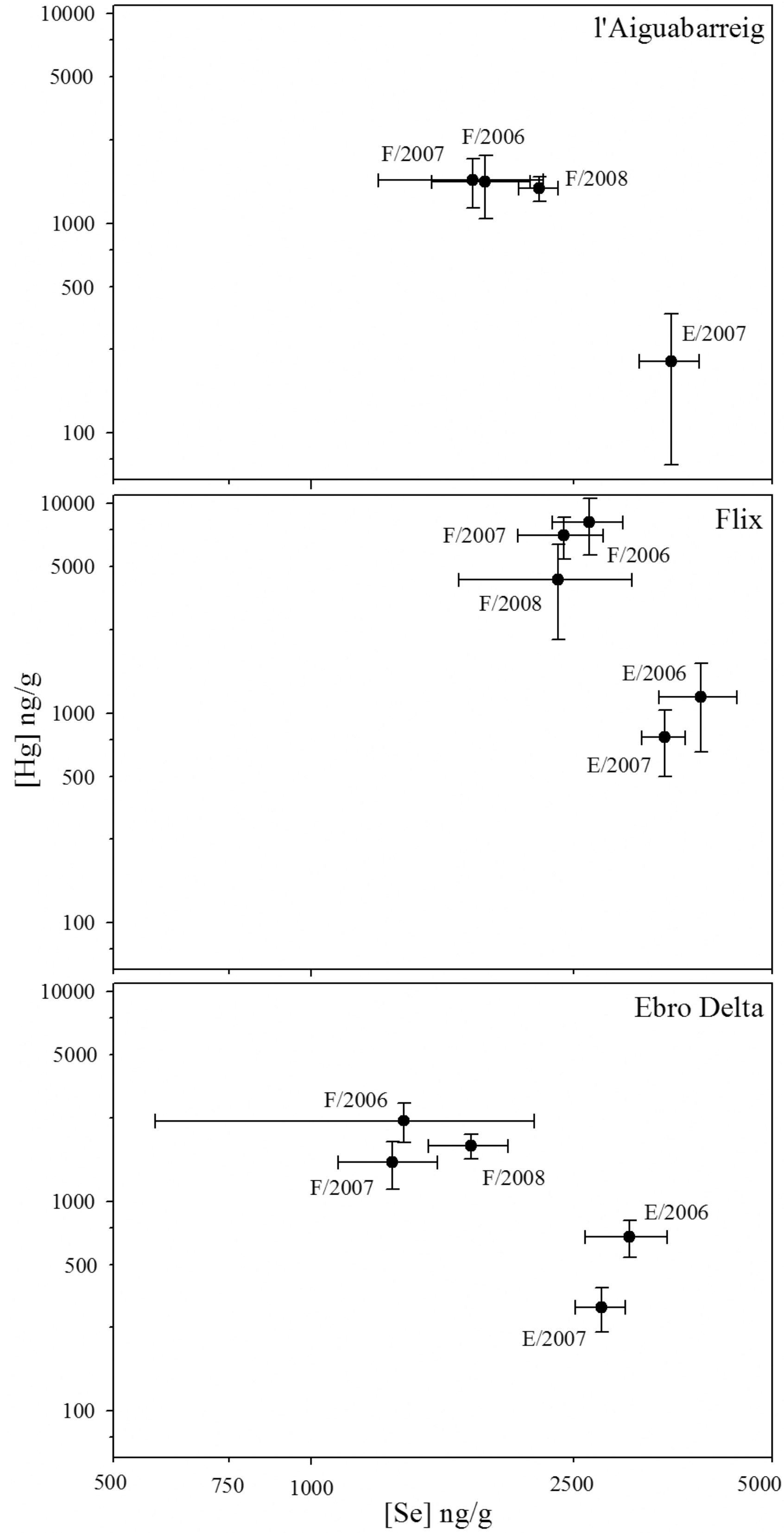
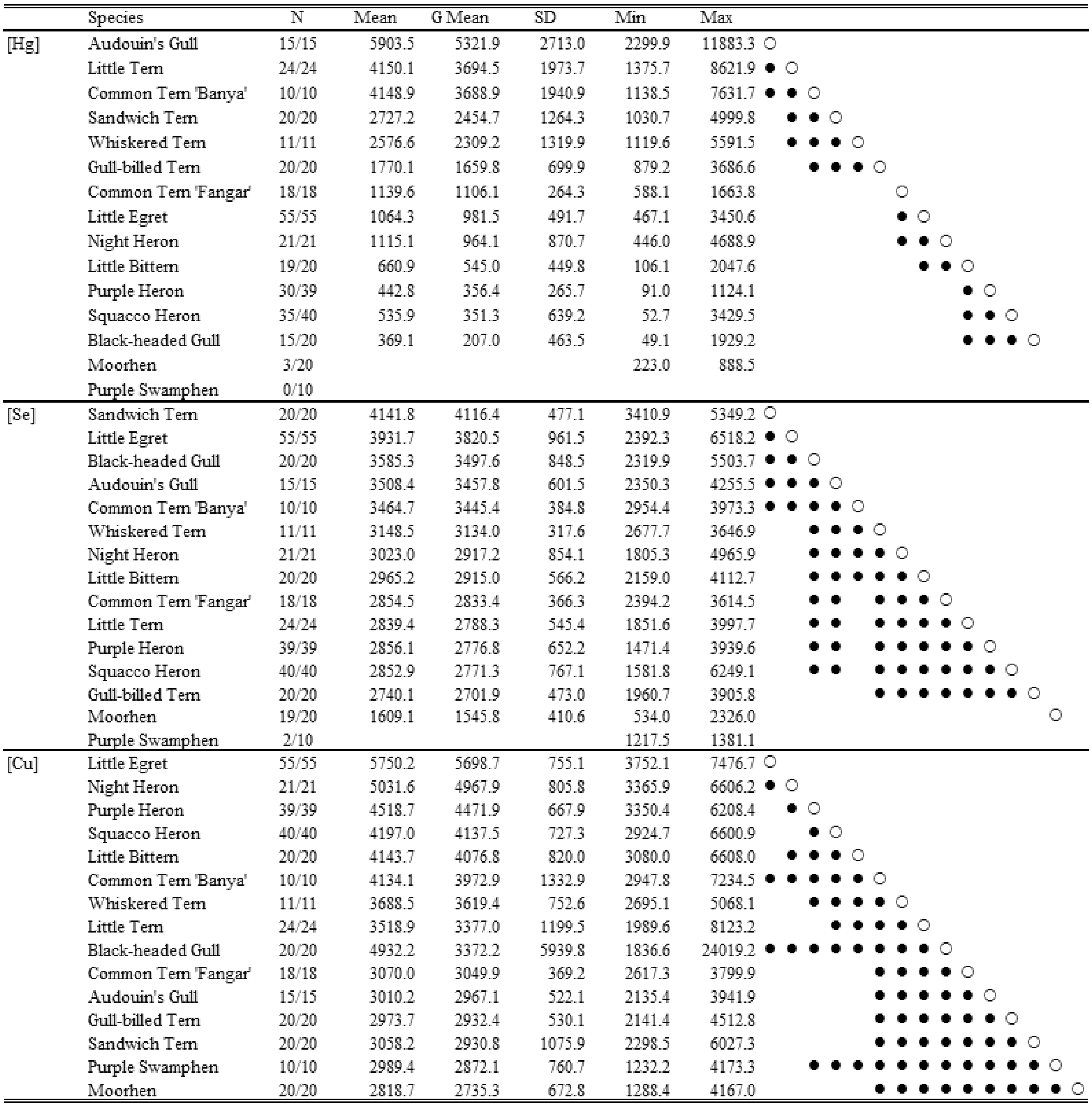


Figura 10. Concentraciones de mercurio y selenio (en escala logarítmica) indicando la posición media de los huevos y volantones de Garza Imperial de las tres localidades muestreadas cada año. Círculos (plumas = F) y triángulos (huevos = e) indican valor medio, y las líneas sus correspondientes intervalos de confianza del 95%.



	Species	N	Mean	G Mean	SD	Min	Max															
[Hg]	Audouin's Gull	15/15	5903.5	5321.9	2713.0	2299.9	11883.3	○														
	Little Tern	24/24	4150.1	3694.5	1973.7	1375.7	8621.9	●	○													
	Common Tern 'Banya'	10/10	4148.9	3688.9	1940.9	1138.5	7631.7	●	●	○												
	Sandwich Tern	20/20	2727.2	2454.7	1264.3	1030.7	4999.8		●	●	○											
	Whiskered Tern	11/11	2576.6	2309.2	1319.9	1119.6	5591.5		●	●	●	○										
	Gull-billed Tern	20/20	1770.1	1659.8	699.9	879.2	3686.6			●	●	●	○									
	Common Tern 'Fangar'	18/18	1139.6	1106.1	264.3	588.1	1663.8							○								
	Little Egret	55/55	1064.3	981.5	491.7	467.1	3450.6							●	○							
	Night Heron	21/21	1115.1	964.1	870.7	446.0	4688.9							●	●	○						
	Little Bittern	19/20	660.9	545.0	449.8	106.1	2047.6								●	●	○					
	Purple Heron	30/39	442.8	356.4	265.7	91.0	1124.1										●	○				
	Squacco Heron	35/40	535.9	351.3	639.2	52.7	3429.5										●	●	○			
	Black-headed Gull	15/20	369.1	207.0	463.5	49.1	1929.2										●	●	●	○		
	Moorhen	3/20				223.0	888.5															
	Purple Swamphen	0/10																				
[Se]	Sandwich Tern	20/20	4141.8	4116.4	477.1	3410.9	5349.2	○														
	Little Egret	55/55	3931.7	3820.5	961.5	2392.3	6518.2	●	○													
	Black-headed Gull	20/20	3585.3	3497.6	848.5	2319.9	5503.7	●	●	○												
	Audouin's Gull	15/15	3508.4	3457.8	601.5	2350.3	4255.5	●	●	●	○											
	Common Tern 'Banya'	10/10	3464.7	3445.4	384.8	2954.4	3973.3	●	●	●	●	○										
	Whiskered Tern	11/11	3148.5	3134.0	317.6	2677.7	3646.9			●	●	●	○									
	Night Heron	21/21	3023.0	2917.2	854.1	1805.3	4965.9			●	●	●	●	○								
	Little Bittern	20/20	2965.2	2915.0	566.2	2159.0	4112.7			●	●	●	●	●	○							
	Common Tern 'Fangar'	18/18	2854.5	2833.4	366.3	2394.2	3614.5			●	●		●	●	●	○						
	Little Tern	24/24	2839.4	2788.3	545.4	1851.6	3997.7			●	●		●	●	●	●	○					
	Purple Heron	39/39	2856.1	2776.8	652.2	1471.4	3939.6			●	●		●	●	●	●	●	○				
	Squacco Heron	40/40	2852.9	2771.3	767.1	1581.8	6249.1			●	●		●	●	●	●	●	●	○			
	Gull-billed Tern	20/20	2740.1	2701.9	473.0	1960.7	3905.8						●	●	●	●	●	●	●	○		
	Moorhen	19/20	1609.1	1545.8	410.6	534.0	2326.0														○	
	Purple Swamphen	2/10				1217.5	1381.1															
[Cu]	Little Egret	55/55	5750.2	5698.7	755.1	3752.1	7476.7	○														
	Night Heron	21/21	5031.6	4967.9	805.8	3365.9	6606.2	●	○													
	Purple Heron	39/39	4518.7	4471.9	667.9	3350.4	6208.4		●	○												
	Squacco Heron	40/40	4197.0	4137.5	727.3	2924.7	6600.9			●	○											
	Little Bittern	20/20	4143.7	4076.8	820.0	3080.0	6608.0		●	●	●	○										
	Common Tern 'Banya'	10/10	4134.1	3972.9	1332.9	2947.8	7234.5	●	●	●	●	●	○									
	Whiskered Tern	11/11	3688.5	3619.4	752.6	2695.1	5068.1			●	●	●	●	○								
	Little Tern	24/24	3518.9	3377.0	1199.5	1989.6	8123.2				●	●	●	●	○							
	Black-headed Gull	20/20	4932.2	3372.2	5939.8	1836.6	24019.2	●	●	●	●	●	●	●	●	○						
	Common Tern 'Fangar'	18/18	3070.0	3049.9	369.2	2617.3	3799.9						●	●	●	●	○					
	Audouin's Gull	15/15	3010.2	2967.1	522.1	2135.4	3941.9						●	●	●	●	●	○				
	Gull-billed Tern	20/20	2973.7	2932.4	530.1	2141.4	4512.8						●	●	●	●	●	●	○			
	Sandwich Tern	20/20	3058.2	2930.8	1075.9	2298.5	6027.3						●	●	●	●	●	●	●	○		
	Purple Swamphen	10/10	2989.4	2872.1	760.7	1232.2	4173.3			●	●	●	●	●	●	●	●	●	●	●	○	
	Moorhen	20/20	2818.7	2735.3	672.8	1288.4	4167.0						●	●	●	●	●	●	●	●	●	○

Tabla 7. Estadísticos descriptivos de mercurio, selenio y cobre en los huevos de las especies incluidas en el estudio, ordenados por la media geométrica. Las comparaciones post hoc se muestran en la matriz de puntos. Los círculos negros indican que no hay diferencia significativa entre las especies de filas y columnas. Las concentraciones se expresan en ppb (ng/g).

área también debe verse afectada por otras fuentes de mercurio que los residuos de Flix. Incluso el Delta del Ebro, situado 90 km aguas abajo de Flix, muestra niveles intermedios que pueden ser explicados por los sedimentos arrastrados hasta allí. La Garza Imperial muestran concentraciones relativamente altas en esta localidad, como han demostrado otras especies que se alimentan en la misma zona (Cotin et al., 2011).

Los altos niveles de mercurio detectados en Flix son de interés especial, con los volantones de Garza imperial mostrando valores de hasta 13.600 ng/g en pluma, lo cual está dentro del rango de alerta, puesto que las concentraciones de mercurio entre 5000 y 15000 ng/g en las plumas han sido relacionadas con efectos adversos sobre el crecimiento y la reproducción en aves (Eisler, 1987). De hecho, efectos adversos ya han sido señalados previamente

	Species	N	Mean	G Mean	SD	Min	Max									
[Cr]	Whiskered Tern	11/11	2885.8	2880.2	189.6	2579.4	3195.0	○								
	Little Egret	35/35	2720.7	2717.9	127.3	2503.9	3172.5	●	○							
	Little Tern	14/14	2644.0	2642.1	103.3	2522.8	2842.0	●	●	○						
	Common Tern 'Banya'	10/10	2605.8	2605.4	47.7	2514.4	2678.6		●	●	○					
	Purple Heron	25/25	2605.1	2599.3	179.1	2314.5	3026.9		●	●	●	○				
	Sandwich Tern	20/20	2584.4	2579.6	161.1	2309.9	2855.8		●	●	●	●	○			
	Black-headed Gull	20/20	2574.8	2568.1	184.5	2086.8	2795.1		●	●	●	●	●	○		
	Squacco Heron	20/20	2466.8	2457.1	224.8	2077.1	2916.3			●	●	●	●	●	○	
	Audouin's Gull	15/15	2413.5	2404.2	221.3	2033.2	2921.8			●	●	●	●	●	●	○
[As]	Common Tern 'Banya'	10/10	1217.7	1179.8	293.6	633.8	1606.5	○								
	Sandwich Tern	20/20	857.0	850.4	106.0	617.4	1046.5		○							
	Little Tern	14/14	850.6	811.9	264.1	448.7	1274.1		●	○						
	Audouin's Gull	15/15	685.3	655.8	212.3	336.1	1169.1		●	●	○					
	Little Egret	35/35	185.2	178.1	52.9	94.0	318.4					○				
	Purple Heron	25/25	172.7	167.8	43.2	100.2	303.0					●	○			
	Whiskered Tern	11/11	104.7	101.2	27.2	58.9	154.5							○		
	Squacco Heron	17/20	109.6	95.7	60.7	32.5	316.9							●	○	
	Black-headed Gull	16/20	90.9	79.9	44.2	25.9	195.6							●	●	○
[Pb]	Purple Swamphen	1/10				789.1	789.1									
	Sandwich Tern	3/20				69.7	935.9									
	Whiskered Tern	1/11				176.4	176.4									
	Black-headed Gull	5/20				90.4	362.4									
	Gull-billed Tern	1/20				131.9	131.9									
	Little Tern	2/24				111.6	120.2									
	Squacco Heron	5/40				61.4	189.2									
	Little Egret	2/55				84.3	131.6									

Tabla 8. Estadística descriptiva del cromo, arsénico y plomo en los huevos de las especies incluidas en el estudio, ordenados por la media geométrica. El cromo y el arsénico se analizaron sólo en las muestras de 2007 y 2008. Especies sin niveles detectables de plomo no aparecen en la tabla. Las comparaciones post hoc se muestran por una matriz de puntos. Los círculos negros indican que no hay diferencias significativas entre las especies de las filas y columnas. Las concentraciones se expresan en ppb (ng/g).

para esta población de garzas, en parte puede ser debido al hecho de que los embriones de garza son altamente sensibles al mercurio (Heinz et al., 2009). Los elevados niveles de micronúcleos en los eritrocitos periféricos (Quirós et al., 2008) y reducción de defensas antioxidantes en la sangre (Barata et al., 2010) son algunas de las respuestas fisiológicas al estrés que estas aves han desarrollado en respuesta a la contaminación. Además, otras especies de este ecosistema, como los mejillones cebra, cangrejo de río y varias especies de peces, han mostrado efectos a este contaminante (Navarro et al., 2009, Benejam et al., 2010, Faria et al., 2010, Suárez-Serrano et al., 2010).

Por último, hay que señalar que los niveles de mercurio encontrados en las plumas de volantones de Flix son mucho más altos que los encontrados en otros ríos y humedales amenazados por la contaminación. Dichos niveles son más altos que los encontrados en Garceta común en Pakistán (Boncompagni et al., 2003) y Hong Kong (Connell et al., 2002) o en varias especies de garzas en China (Burger y Gochfeld, 1993), e incluso superiores a los descritos en plumas de Garceta común y Martinete del Delta del Axios, Grecia (Goutner y Furness, 1997) y del norte de Italia (Fasola et al., 1998). Se han descrito niveles más elevados en especies de garzas de los Everglades, EE.UU. (Frederick et al., 2004), pero las plumas fueron tomadas de aves adultas conservadas en museos, por lo que probablemente reflejan bioacumulación. También se encontraron niveles altos de mercurio en sangre de

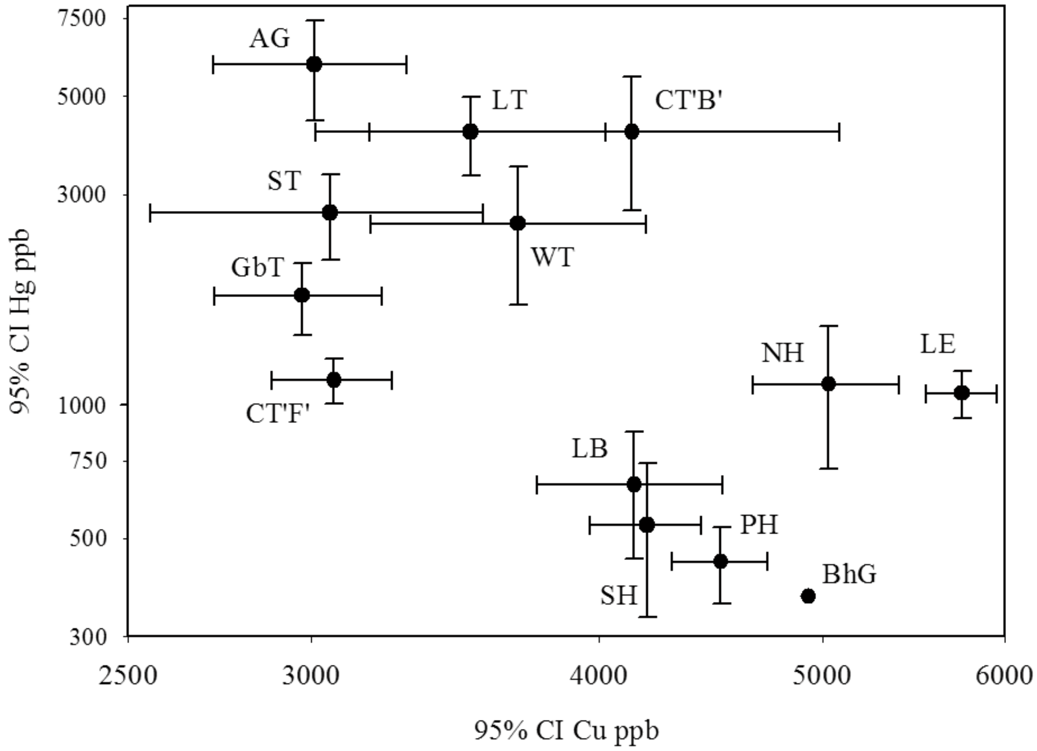


Figura 11. Concentraciones de Hg y Cu (en escala logarítmica) de las especies incluidas en el estudio: Garza Imperial (PH), Garceta común (LE), Martinete (NH), Garcilla cangrejera (SH), Avetorillo (LB) , Charrán patinegro (ST), Charrán común "Banya" (CT'B '), Charrán común "Fangar" (CT'F'), Charrancito (LT), Pagaza piconegra (GBT), Fumarel cariblanco (WT) , Gaviota reidora (BHG) y Gaviota de Audouin (AG). El Calamón y la Gallineta de agua no están incluidos y el 95% IC de la Gaviota reidora se omite debido a su alta variabilidad. Los puntos indican valor medio, y las líneas sus correspondientes intervalos de confianza del 95%.

dos especies de garza del río Carson (Nevada), la Garceta nívea (*Egretta thula*) y el Martinete (Henny et al., 2007), pero hasta donde sabemos, los niveles de mercurio detectados en las Garzas Imperiales de Flix son más altas registradas en plumas de volantones de garza.

Volviendo al Delta del Ebro y centrándonos en los niveles de contaminantes de la comunidad de aves acuáticas en su conjunto (véase el cuadro 7 y 8), pero continuando con los niveles de mercurio, observamos que los niveles son más altos en charranes que en garzas, incluso para aquellas especies que se alimentan en el mismo hábitat, indicando diferencias filogenéticas en la bioacumulación de metales entre la familia Ardeidae y Sternidae que se discutirán más adelante. Los niveles de mercurio tienden a ser mayores en las especies relacionadas con el mar que en las de agua dulce, pero además de eso, el mercurio derivado de la industria alojada a lo largo de las riveras del río Ebro, especialmente desde el embalse de Flix (Cotin et al., 2012), ha estado probablemente acumulandose en los bancos de arena del Delta del Ebro durante los últimos años, particularmente en el hemidelta sur, puesto que los materiales transportados por el río Ebro son principalmente depositados allí por la corriente oceánica Liguro-Provenzal-Catalana, que fluye hacia el sur (Guitart et al., 2003). Este hecho ha debido de aumentar las concentraciones de mercurio de las especies del hemidelta sur, especialmente las que se alimentan de descartes (basados en peces demersales), que son, entre otros, la Gaviota de Audouin (Morera et al., 1997) y el Charrán común " Banya '. Esto se apoya en el hecho de que los niveles de mercurio de la colonia de Gaviota de Audouin del Delta del Ebro son más altos en comparación con otras colonias del Mediterráneo (Sanpera et al., 2007a). Las charranes dulceacuícolas, Fumarel común y Pagaza piconegra, también están expuestos a la entrada de mercurio del río, aunque en menores cantidades, y alcanzan concentraciones inferiores a los charranes marinos, a excepción del Charrán común "Fangar". Esta colonia de Charrán común muestra concentraciones de mercurio mucho más bajas, ya que está situado en el hemidelta norte, fuera de la entrada de mercurio principal del río. También su dieta se basa en peces epipelágicos, que tienen una menor carga de mercurio (Arcos et al., 2002) que los peces demersales. Los valores de esta colonia son similares a otras colonias de Charrán común con los mismos hábitos de alimentación (Monteiro et al., 1999, Guitart et al., 2003, Becker y Cifuentes Muñoz, 2004, Bond y Diamond, 2009), con la mayoría de niveles alrededor de 1000 ppb.

El Charrancito presenta las segundas concentraciones más altas de mercurio de todas las especies estudiadas. Este charrán se alimenta principalmente en salinas, y la línea basal de mercurio en estas redes troficas es relativamente alta. Valores similares de mercurio han sido encontrados en salinas en Portugal (Tavares et al., 2007, Paiva et al., 2008b) y aves alimentandose principalmente en hábitats marinos, como el Mar Báltico, presentan valores más bajos (Thyen et al., 2000).

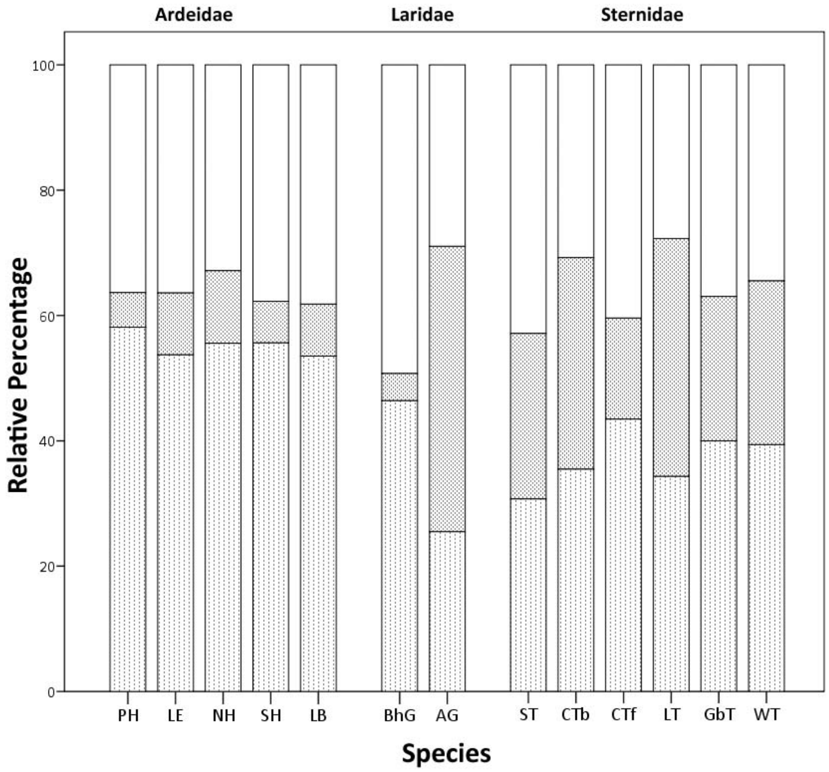


Figura 12. Porcentajes relativos de los niveles de cobre (blanco) mercurio (gris) y selenio (gris claro) ordenados por familia: Garza Imperial (PH), Garceta común (LE), Martinete (NH), Garcilla cangrejera (SH), Avetorillo (LB), Charrán patinegro (ST), Charrán común 'Banya' (CTB), Charrán común 'Fangar' (CTF), Charrancito (LT), Pagaza piconegra (GBT), Fumarel cariblanco (WT), Gaviota reidora (BHG), y Gaviota Audouin (AG). Gallineta de agua (Mh) y Calamón (PSH) no están representados por sus niveles no detectados de mercurio y selenio.

Entre las garzas, la Garceta común muestra los niveles de mercurio más altos, probablemente debido a su uso parcial de salinas y aguas salobres, mientras que la Garcilla Cangrejera presenta los más bajos, ya que esta especie se alimenta básicamente de insectos. La Gaviota reidora también muestra concentraciones muy bajas de mercurio, de acuerdo con una dieta a base de invertebrados, como indican los isótopos estables. Por último, las dos especies de rálidos, la Gallineta de de agua y el Calamón, mostraron los valores más bajos de mercurio.

Acerca del cobre, no se encuentran claras diferencias entre las especies, aunque se sabe que el uso agrícola del sulfato de cobre (Cu_2SO_4) está muy extendido en los hábitats de agua dulce, haciendo este elemento más biodisponible. Sin embargo, un patrón sí que puede ser observado entre los distintos grupos de especies: garzas > charranes + gaviotas > rálidos. Si recordamos el patrón observado en las concentraciones de mercurio, podemos ver que ambos metales se comportan opuestamente a nivel de especie, por lo que, si los enfrentamos en un gráfico, se observa claramente un patrón de familia (ver figura 11): Los charranes (familia Sternidae) tienen, independientemente del hábitat de alimentación, altas concentraciones de mercurio y bajas de cobre, mientras las garzas (familia Ardeidae) presentan lo contrario. Estas diferencias filogenéticas en la bioacumulación de metales entre estas dos familias (Ardeidae y Sternidae) nunca habían sido descritas previamente. Vale la pena observar en las figuras 11-12 las diferencias entre las dos especies de gaviota, en la que las diferencias de dieta y hábitat se observan claramente.

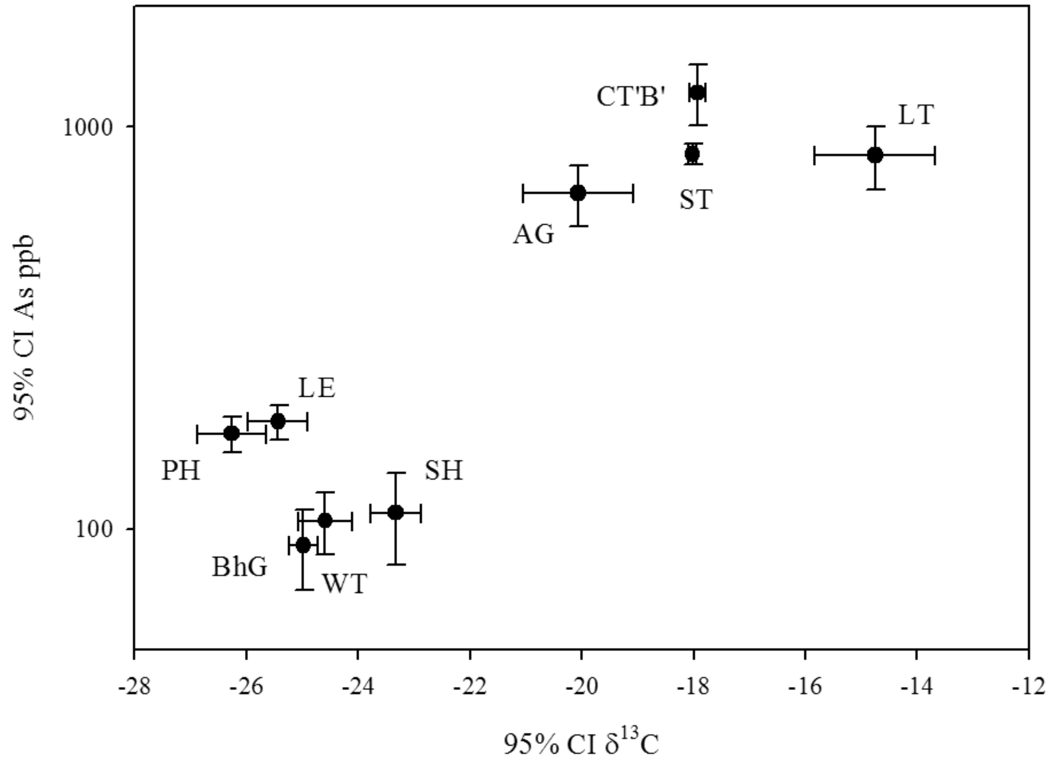


Figura 13. Concentraciones de arsénico (en escala logarítmica) y signaturas de $\delta^{13}C$ en Garza Imperial (PH), Garceta común (LE), Garcilla cangrejera (SH), Charrán patinegro (ST), Charrán común "Banya" (CT'B '), Charrancito (LT), Fumarel cariblanco (WT), Gaviota reidora (BHG) y la Gaviota de Audouin (AG). El punto indica los valores medios y las líneas sus correspondientes intervalos del 95% de confianza.

El selenio es transferido de modo notable a los huevos (Sell, 1977, Focardi et al., 1988), haciendo de esta muestra un buen bioindicador para este elemento (Ohlendorf et al., 2011). Los niveles en el Delta del Ebro, al igual que con el cobre, no mostraron diferencias entre las especies. Sólo las especies herbívoras mostraron concentraciones más bajas. Tampoco los niveles de cromo mostraron diferencias entre las especies o hábitats. Respecto al plomo, aunque el Delta del Ebro es una zona de caza frecuente y la ingestión de perdigones de plomo podría ser una posible amenaza, las especies estudiadas presentaron concentraciones muy bajas.

En cuanto al arsénico, en los cuatro grupos de especies se encuentra el patrón de niveles más altos en las especies marinas en comparación con las de agua dulce. Las concentraciones de arsénico tienden a ser mayores en el mar respecto al agua dulce (Kunito et al., 2008, Lucia et al., 2010) y todavía mucho más altas que en los organismos terrestres (Lunde, 1977). Cuando se enfrentan los niveles de arsénico con las firmas de carbono (ver fig. 13), existe una clara separación entre las especies marinas y dulceacuícolas, con las especies marinas presentando niveles más altos que las especies de agua dulce.

Si se comparan los niveles de contaminación de la comunidad de aves acuáticas del Delta del Ebro con otras áreas y comunidades similare, observamos que, por ejemplo, en las salinas de Thyna (Túnez), el mercurio y los niveles de selenio en Gaviota picofina, Charrán común y Garceta común (Ramírez et al., 2011) varían de manera similar, pero las concentraciones son relativamente mayores en el Delta del Ebro. En tres humedales chinos diferentes, los niveles de As, Cd, Cr, Cr, Pb, Hg y Se en Garceta común (Zhang et al., 2006) también fueron similares, con la excepción del cromo, que es mucho más alto en el Delta del Ebro, aunque dentro de lo descrito para varias especies de aves (Burger y Gochfeld de 2009, Padula et al., 2010). Los niveles de mercurio en Garceta grande (*Casmerodius albus*) de los Everglades de Florida están dentro del rango de las garzas del Delta del Ebro (Rumbold et al., 2001). Los niveles de los mismos elementos analizados en este estudio en garzas y charranes del delta del río Pearl (sur de china) (Lam et al., 2005) fueron similares, con la excepción del cromo en garzas (que es mayor en el Delta), el mercurio en charranes (también mayor en el Delta) y el cobre en ambas familias (inferior en el Delta del Ebro). En tres humedales diferentes de Pakistán, los niveles de As, Cd, Cr, Pb, Hg y Se en tres especies de garzas (Boncompagni et al., 2003) también variaron de modo similar, una vez más, con la excepción de cromo, siendo sus niveles más altos en el Delta del Ebro. Los Charrancitos que se alimentan en el Mar Báltico presentan niveles más bajos (Thyen et al., 2000), mientras que Charranes comunes del estuario del Elba (zona altamente contaminada) mostraron niveles más altos (Becker et al., 1993).

Juntando la perspectiva del río y del delta, podemos concluir que ninguno de los elementos analizados presenta preocupación conservacionista (exceptuando el mercurio) y están dentro de los valores de otras comunidades de aves (con la excepción de cromo y mercurio). Los niveles de mercurio en pollos de Garza Imperial de Flix y los huevos de Gaviota de Audouin, Charrancito y Charrán común 'Banya' del Delta del Ebro son suficientemente altos como para entrañar peligro conservacionista, por lo que más estudios son de particular relevancia para comprobar si estos valores están poniendo en peligro el éxito reproductor de estas especies. En el caso del mercurio en las Garzas Imperiales de Flix, son los valores más altos jamás descritos, estando altamente influenciados por los sedimentos contaminados del embalse de Flix. El arsénico (especialmente en combinación con las signaturas de carbono) discrimina notablemente entre especies marinas y de agua dulce. Por último, y sorprendentemente, los charranes (familia Sternidae) y las garzas (familia Ardeidae) mostraron grandes diferencias en los niveles de mercurio y cobre: niveles altos de mercurio y bajos de cobre para charranes y el patrón opuesto en garzas, siendo estas diferencias no explicables claramente por diferencias en recursos y/o uso del hábitat, por lo que hace suponer que hay un componente filogenético que sería interesante estudiar.

A3. ¿Pluma o huevos? ¿Cuál es mejor cuando se trata de contaminación?

Los huevos y muestras de plumas de volantones se compararon utilizando la Garza Imperial como especie modelo, debido al mayor número de localidades comparables (l'Aiguabarreig, Flix, Delta del Ebro) y los tamaños muestrales (ver tablas 2, 5 y 6).

En estudios anteriores, Burger et al. (2009) no encontró diferencias en la utilidad de los huevos y plumas de volantones como bioindicadores de contaminación en garzas, aunque otros estudios encontraron que las plumas son más representativas de la contaminación local, puesto que los huevos pueden estar influenciados en distintos grado por los niveles de la madre, acumulados durante el período de invernada o migración (Hughes et al., 1997, Desgranges et al., 2009).

No obstante, como la Garza Imperial presenta una reproducción "income" (Ruiz et al., 1998, Hobson et al., 2000), las diferencias entre los huevos y plumas se deben probablemente al enrutamiento. Además, las plumas reflejan la selectiva dieta ofrecida por los padres a los pollos y, en un porcentaje pequeño y desconocido, los niveles transferidos de la hembra al huevo. En consecuencia, ambas muestras sería bioindicadores fiables, aunque las muestras de pluma mostraron una menor variabilidad en los datos isotópicos y niveles más altos en la mayor parte de los elementos traza analizados (excepto para el cromo y especialmente en el caso del selnioselenio, ya que este elemento es transferido preferentemente a los huevos (Vender, 1977, Focardi et al., 1988)), por lo que las plumas pueden ser consideradas un bioindicador más preciso. Además, desde el punto de vista conservacionista, el muestreo de plumas de volantones respeta más a las poblaciones de garzas, siendo más sostenible, con la condición de que los protocolos de muestreo sigan ciertas condiciones al entrar en las colonias de garzas, que son muy sensibles a las perturbaciones humanas.

Así que podemos concluir que, si bien los huevos y plumas de volantones de Garzas Imperiales son bioindicadores adecuados para la contaminación de elementos traza en la biota del río, las plumas presentan ciertas ventajas. Las plumas de volantones crecen a partir de las presas proporcionadas por los padres en los alrededores de la colonia, por lo tanto, muestran los efectos contaminantes de un modo local. Además, dado que los polluelos pertenecen a una clase de edad homogénea, los efectos relacionados con la bioacumulación de contaminantes por edad se considera negligible.

A4. Marcadores sanguíneos para monitorizar los riesgos ambientales de la contaminación

Para la determinación de los parámetros bioquímicos, se obtuvieron muestras de sangre de pollos de Garceta común y Garza Imperial en l'Aiguabarreig, Flix (sólo Garza imperial en esta localidad) y el Delta del Ebro. Seis fueron los parámetros bioquímicos seleccionados y analizados en el plasma de los pollos (ver Tabla 9): capacidad total para captar radicales libres (TOSC) para los radicales peroxilo e hidroxilo (ROO*, OH*), su suma (TOT), la actividad enzimática plasmática de la buthirylcholinesterasa (BhCE), carboxilesterasa (CBE) y deshidrogenasa (LDH lacatate) y el número de micronúcleos (MN).

De estos parámetros, sólo los niveles de ROO* TOSC y las actividades de BChE y CBE difirieron significativamente entre los volantones de garzas en l'Aiguabarreig y el Delta del Ebro (Tabla 9). La población de l'Aiguabarreig mostró una respuesta menor que las otras dos poblaciones en los parámetros bioquímicos, mientras que la población de Flix mostró una capacidad para captar radicales libres significativamente menor (ROO * TOSC y TOSC Total). Además, la población del Delta del Ebro presentó un aumento significativo en la actividad de LDH, indicando una fuente específica de contaminación aguas abajo de Flix. Ninguno de los parámetros bioquímicos estudiados mostraron diferencias significativas entre l'Aiguabarreig y el Delta del Ebro en la Garceta común. En la tabla 9 también se incluyen los datos sobre la abundancia de MN en sangre de volantones (Quirós et al., 2008), lo que concuerda con la imagen general de un bajo impacto en l'Aiguabarreig en comparación con las otras dos localidades. En este caso, la diferencia entre localidades se observó con ambas especies.

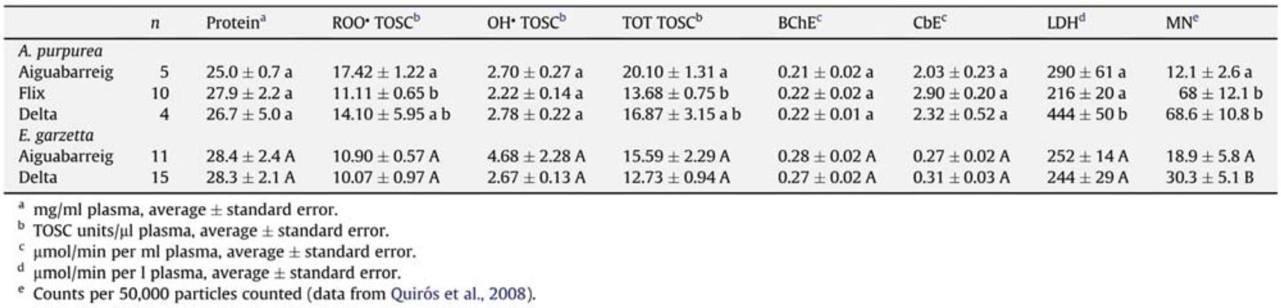
Los parámetros bioquímicos y la frecuencia de micronúcleos fueron comparados con las concentraciones de mercurio y cobre. Sólo la suma de los radicales TOSC (TOT TOSC) fue considerado. Varios parámetros bioquímicos en plasma mostraron una correlación significativa con los elementos traza en plumas, pero sólo para la Garza Imperial. En esta especie, los niveles de mercurio estan inversamente relacionados con los valores TOT TOSC y ambos niveles de Hg y Cu se relacionaron positivamente con los índices de MN y actividades de LDH, respectivamente (Fig. 14). Por el contrario, no se observaron correlaciones significativas entre los niveles de metales y ninguno de los biomarcadores estudiados la Garceta común (ver fig. 14).

La respuesta bioquímica en plasma y la frecuencia de micronúcleos en sangre de los pollos varió entre especies y localidades. Los rangos de TOT TOSC (12,16-20,48 U/l plasma) fueron de magnitud similar a los descritos por Corsolini et al. (2001) en pingüinos de Adelia y emperadores de Wood Bay, Victoria del Norte (10-25 U/l plasma). Los niveles de actividad de BChE variaron poco en ambas especies (0.21-0.28 mmol/ml por min), pero las actividades plasmáticas de CBE en Garceta común (0,27-0,31 mol/ml por min) fueron diez veces inferiores a los de Garza Imperial (2-2,9 mol/min por ml). Los valores descritos para las actividades plasmáticas de B esterasa son muy variables en especies de aves, con rangos desde 0,7 hasta 5 mol/min por ml para BChE y 0,2 a 11 mol/min por ml para CBE (Fossi et al., 1996). Según Fossi et al. (1996), las actividades de B esterasa tienden a cambiar según el tamaño del individuo o la dieta de las especies. En nuestro estudio, las diferencias en las actividades de CBE entre la Garceta común y la Garza imperial podrían explicarse por las diferencias en peso (en un factor de 2) ya que ambos son piscívoros. Actividades plasmáticos medidas de LDH en las especies estudiadas (216-444 U/l) fueron similares a los reportados en pollos y volantones de Garceta grande y Garza azulada (200 hasta 1700 U/l) (Hoffman et al., 2005, Champoux et al., 2006).

Los efectos descritos en las localidades en ROO*/TOT TOSC y la frecuencia de micronúcleos ha proporcionado la primera evidencia de efectos perjudiciales por los contaminantes emitidos por una planta cloro-álcali en volantones de especies de garzas. En particular, los volantones de Garza Imperial de Flix presentaron la mayor frecuencia y niveles de micronúcleos (y mercurio), y una reducida ROS (valores más bajos de ROO* o TOSC TOT). Además TOT TOSC está inversamente relacionada con los niveles de mercurio en plumas y la frecuencia de micronúcleos. La falta de respuestas biológicas observadas en Garceta común podría estar relacionada con la ausencia de lugares de nidificación en el lugar de mayor contaminación (Flix). La exposición a diversos contaminantes orgánicos e inorgánicos es conocida por reducir TOT TOSC en bivalvos y especies de peces e incrementar el estrés oxidativo (Regoli, 2000, Regoli et al., 2005). De un modo más específico, exposiciones en laboratorio muestran que el Cd, Cu, PCBs y PAHs causan estrés oxidativo, disminuyendo TOSC en bivalvos (Lehmann et al., 2007, Compañía et al., 2008). Aunque no existen estudios publicados sobre los efectos del mercurio en TOT TOSC, existe información sustancial indicando que el mercurio disminuye las defensas antioxidantes causando estrés oxidativo en adultos y volantones de ardeidos (Henny et al., 2002, Hoffman et al., 2005). Por lo tanto, las diferencias observadas entre localidades en ROO* o TOSC TOT en volantones de Garza Imperial concuerdan con estudios previos y podrían estar relacionados con la exposición a los contaminantes.

Las esterasas B plasmáticas como las del hígado se sabe que actúan como dianas alternativas de compuestos anticolinérgicos, protegiendo así a las colinesterasas cerebrales y músculares de sustancias inhibidoras (Chambers et al., 1994). Los niveles de actividad de esterasas B fueron similares en todas las localidades en ambas especies, lo que indica una nula o baja exposición a los compuestos anticolinérgicos. Este hallazgo es ambientalmente relevante, ya que el uso de grandes cantidades de insecticidas organofosforados y carbamatos en el Delta del Ebro durante las últimas décadas tuvo efectos dramáticos en la vida silvestre (Mañosa et al., 2001).

Al igual que los otros parámetros, sólo en Garza Imperial hubo diferencias significativas entre localidades en la actividad de LDH, con las aves del Delta del Ebro mostrando las mayores actividades. Las actividades de LDH también se relacionaron positivamente con los niveles de Cu en las plumas. La exposición a cloruro de mercurio produce un aumento de LDH plasmática en la codorniz *Coturnix coturnix* (Dieter, 1974). Por el contrario, el metilmercurio inhibió las actividades plasmáticas de LDH de volantones de Garceta grande (Hoffman et al., 2005). En el presente estudio, los niveles totales de mercurio medidos en plumas son probablemente metilmercurio (de modo principal), ya que esta es la forma típica de bioacumulación en la cadena alimentaria acuática y en las aves que se alimentan



	n	Protein[a]	ROO• TOSC[b]	OH• TOSC[b]	TOT TOSC[b]	BChE[c]	CbE[c]	LDH[d]	MN[e]
A. purpurea									
Aiguabarreig	5	25.0 ± 0.7 a	17.42 ± 1.22 a	2.70 ± 0.27 a	20.10 ± 1.31 a	0.21 ± 0.02 a	2.03 ± 0.23 a	290 ± 61 a	12.1 ± 2.6 a
Flix	10	27.9 ± 2.2 a	11.11 ± 0.65 b	2.22 ± 0.14 a	13.68 ± 0.75 b	0.22 ± 0.02 a	2.90 ± 0.20 a	216 ± 20 a	68 ± 12.1 b
Delta	4	26.7 ± 5.0 a	14.10 ± 5.95 a b	2.78 ± 0.22 a	16.87 ± 3.15 a b	0.22 ± 0.01 a	2.32 ± 0.52 a	444 ± 50 b	68.6 ± 10.8 b
E. garzetta									
Aiguabarreig	11	28.4 ± 2.4 A	10.90 ± 0.57 A	4.68 ± 2.28 A	15.59 ± 2.29 A	0.28 ± 0.02 A	0.27 ± 0.02 A	252 ± 14 A	18.9 ± 5.8 A
Delta	15	28.3 ± 2.1 A	10.07 ± 0.97 A	2.67 ± 0.13 A	12.73 ± 0.94 A	0.27 ± 0.02 A	0.31 ± 0.03 A	244 ± 29 A	30.3 ± 5.1 B

[a] mg/ml plasma, average ± standard error.

[b] TOSC units/µl plasma, average ± standard error.

[c] µmol/min per ml plasma, average ± standard error.

[d] µmol/min per l plasma, average ± standard error.

[e] Counts per 50,000 particles counted (data from Quirós et al., 2008).

Tabla 9. Tamaño de muestra (n) y estadísticos descriptivos para los niveles de proteínas, capacidad total para captar radicales libres (TOSC) para los radicales peroxilo e hidroxilo (ROO•, OH•), su suma (TOT), actividad de buthirylcholinesterasa (BhCE), carboxilesterasa (CBE) y lactato deshidrogenasa (LDH), y número de micronúcleos (MN). Dentro de cada especie las diferentes letras indican diferencias significativas (P <0,05) según ANOVA y post hoc Tukey para la Garza Imperial y t de Student para los datos de Garceta común.

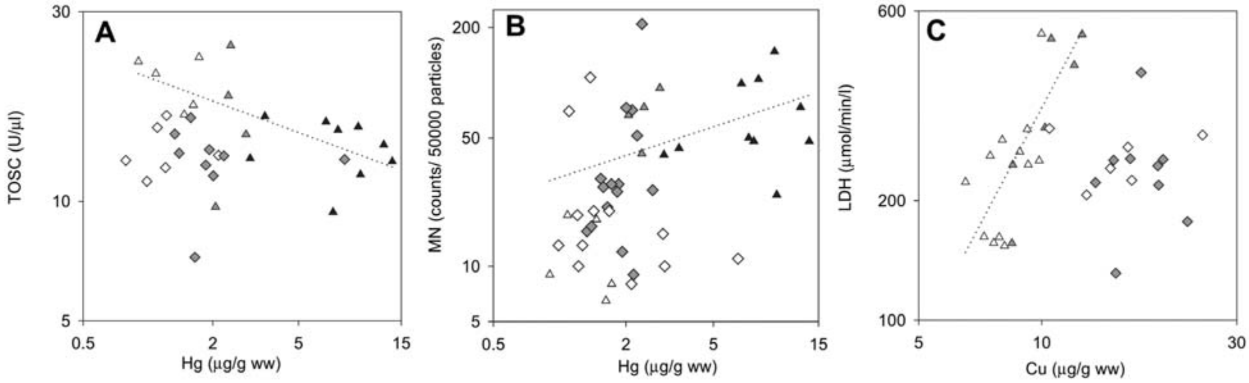


Figure 14. Niveles de mercurio en plumas versus capacidad total para captar radicales libres TOT TOSC (A), micronúcleos MN (B) y niveles de cobre versus lactato deshidrogenasa LDH (C) en Garza Imperial y Garceta común. Cada símbolo corresponde a un único pollo. Las líneas discontinuas indican relaciones significativas lineales, que sólo se produjeron en Garza Imperial (P <0,05). Símbolos blancos, negros y grises corresponden a los valores de l'Aiguabarreig, Flix y Delta, respectivamente. Los datos se representan en escala logarítmica.

de peces que se encuentran en la parte superior de la cadena alimentaria (Henny et al., 2002). Bajo este punto de vista, la relación inversa observada entre la actividad de LDH y Hg en volantones concuerda con estudios anteriores. Por el contrario, las actividades de LDH superiores observadas en los pollos Delta del Ebro y su correlación directa con los niveles de Cu en las plumas son difíciles de explicar. El uso generalizado de herbicidas como el propanil y el endosulfán en el Delta del Ebro (Mañosa et al., 2001), los cuales provocan estrés oxidativo y efectos en LDH en mamíferos (Omurtag et al., 2008, Rankin et al., 2008), pueden haber afectado los parámetros plasmáticos de la Garza Imperial. En efecto, el aumento de los niveles de estrés oxidativo en los pollos del Delta del Ebro también puede explicar la alta frecuencia de micronúcleos descrita por Quirós et al. (2008) en ambas especies.

En resumen, los resultados obtenidos a partir de los seis parámetros seleccionados (TOSC ROO*, TOSC OH *, BhCE, CBE, LDH y MN) proporcionan la primera evidencia de una asociación entre respuestas biológicas y contaminantes emitidos por una fabrica petroquímica en volantones de especies de garzas, lo que hace de estos parámetros una herramienta adecuada para monitorizar riesgos ambientales de la contaminación. Las respuestas biológicas identificadas sobre los parámetros sanguíneos en volantones son las siguientes: los pollos de Garza Imperial en Flix (el área de impacto) mostraron alta frecuencia de micronúcleos y una capacidad de amortiguación reducida para detoxificar especies reactivas de oxígeno. En el Delta del Ebro, los cambios en las respuestas biológicas incluyen altos niveles en LDH plasmática en Garza Imperial y una alta frecuencia de micronúcleos en sangre de ambos especies de ardeidas. Estas alteraciones pueden estar relacionadas con las intensas actividades agrícolas que tienen lugar en el Delta del Ebro además de la actividad de Flix. La falta de diferencias encontradas en los niveles de actividad de las B esterasas en ambas especies indica una nula o baja exposición a los compuestos anticolinérgicos.

Parte B: La Minería en los Andes Bolivianos

En esta segunda parte nos centramos en el potencial de las especies de Tinamú como bioindicadoras de contaminación por metales, derivados de las actividades mineras en los Andes bolivianos. Las principales cuestiones estudiadas fueron:

B1. Patrones de movimiento en las especies de tinamú

Uno de los puntos clave para estimar la idoneidad de una especie como bioindicadora de contaminación local es el conocimiento acerca de sus movimientos y área de campeo (el área dentro del cual un animal vive y se desplaza). Si una especie está fuertemente asociada a un área determinada, reflejará la contaminación de dicho área como resultado de la interacción con el medio (aire, alimentos, agua). Desafortunadamente, poco se conoce respecto a los movimientos de las especies de tinamú a pesar de su importancia ecológica y cinegética en los países latinoamericanos. Estudios previos, realizados hace algunas décadas, se basaron en observaciones de campo de pocos individuos (Schafer, 1954, Lancaster, 1964a) y las áreas de campeo estimadas variaron entre 0.1 hectáreas en el Tinamú serrano ( ) (Schafer, 1954) y 24 hectáreas en el Tinamú montaraz (*Nothoprocta cinerascens*) (Lancaster, 1964a). Sólo en el caso del Tinamú manchado (*Nothura maculosa*) el área de campeo fue estudiada por radio-seguimiento, dando como resultado extensiones medias de 16 y 19 ha (Thompson and Carroll, 2009).

Respecto al Tinamú pisacca (*Nothoprocta ornata*), nuestra especie de estudio, Pearson y Pearson (1955) estimaron una área de alimentación media de 2,43 hectáreas en el altiplano peruano, y en base a sus observaciones de campo, los autores sugirieron que esta especie es territorial y residente.

Para estudiar los movimientos y el área de campeo del Tinamú pisacca, monitorizamos durante un año varios ejemplares radiomarcados en un agro-ecosistema tradicional en el altiplano boliviano. Doce individuos (seis machos y seis hembras) fueron capturados y equipados con radio-collares, para luego ser seguidos por dos métodos: la triangulación y el "homing". Debido a que las localizaciones obtenidas por homing autocorrelacionaron, no fueron usadas en la estimación del área de campeo, pero sí para evaluar la precisión de las posiciones calculadas por el método de triangulación. Se calcularon los rangos de hogar con 100% MPC (Mínimo Polígono Convexo) (Mohr, 1947) y contornos de densidad del 95% con el estimador de kernel fijos con elección ad-hoc de h (Worton, 1989), ya que ambos permiten comparaciones directas con otros estudios. También se estimaron como zonas núcleo el estimador kernel 50%. Los estimadores kernel son sensibles al tamaño de la muestra, por lo que calculamos las áreas de campeo y núcleo sólo para las aves con 40 o más localizaciones, según lo recomendado por Seaman et al. (Seaman et al., 1999).

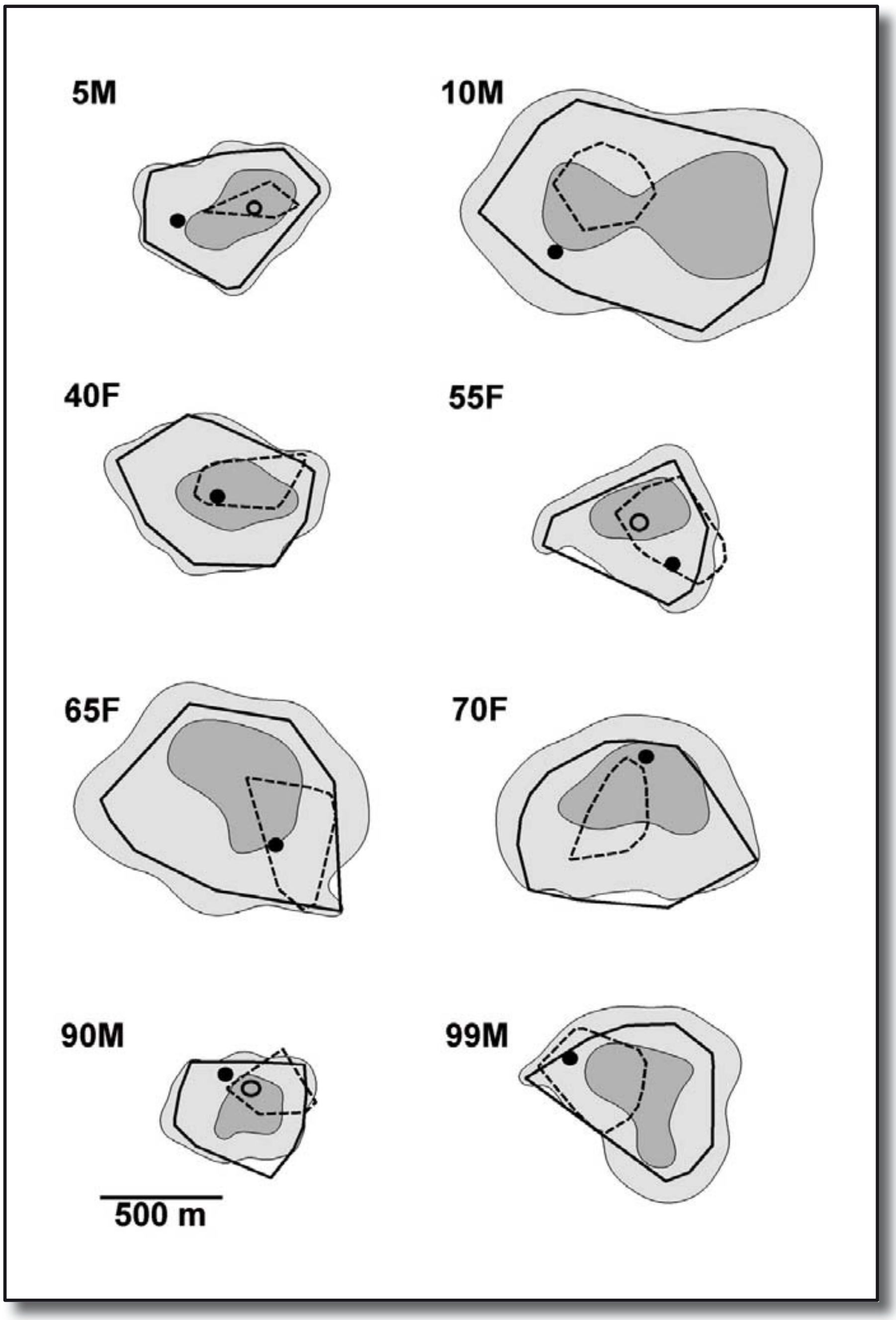


Figura 15. Forma de las áreas de campeo de los ocho ejemplares para los que fue posible su cálculo. Las líneas continuas representan el 100% MPC por el método de triangulación; las líneas punteadas, el MPC obtenido por las localizaciones del método “homing”; las áreas gris suave muestran el área de campeo kernel 95%; y las áreas gris oscuro, las áreas núcleo kernel 50%. El círculo cerrado muestra el punto de captura y el círculo vacío la localización de recaptura del transmisor.

Individual	**Valid locs.**	**100% MCP (ha)**	**95% kernel (ha)**	**50% kernel (ha)**	**t^2/r^2**
05M	178	30	37	10	1.81
10M	113	85	118	33	1.50
15M	31	NC			
20F	55	NC			
40F	192	37	45	11	1.64
55F	100	26	34	8	1.72
60F	33	NC			
65F	86	63	91	22	1.63
70F	124	53	69	18	1.69
75M	21	NC			
90M	163	22	25	6	1.76
99M	71	34	51	14	2.07
Mean ± SD		43.8 ± 21.6	58.8 ± 31.9	15.3 ± 8.9	

Tabla 10. Localizaciones válidas, tamaño de las áreas de campeo (100% MPC y 95% Kernel) y núcleo (50% kernel) para cada Tinamú pisacca (F: hembra o M: macho) obtenidas utilizando el método de triangulación. t^2/r^2 es el radio de Schoener's ratio. NC = datos no calculados.

Sólo 5 de las 12 aves marcadas conservaron el transmisor hasta el final del estudio. Los transmisores de las otras siete aves fueron encontradas en el campo o la señal se perdió, entre 22 días y 10 meses después del marcaje. Cinco de estos individuos fueron probablemente depredados (puesto que el transmisor seguía emitiendo cuando fue encontrado y había plumas en las cercanías) y de los otros dos transmisores, uno se encontró en el campo sin ningún tipo de signos de depredación, mientras que el otro nunca fue recuperado.

Dos individuos murieron durante el mes siguiente a su marcaje y no hay conclusiones acerca de sus movimientos (15M y 75) ya que las áreas de campeo no fueron calculadas. Dos individuos mostraron hábitos nómadas: el individuo 20F se desplazó 1.522 metros hacia el noroeste, desde el punto de captura en marzo de 2007 hasta el punto de recuperación del transmisor en julio de 2007 y durante los cuatro meses de seguimiento por técnica de triangulación, siempre fue encontrado entre el punto de captura y el de muerte, lo que sugiere una proceso gradual de movimiento en lugar de residencia. El patrón mensual de desplazamientos del ave 60F sugiere que también fue un ave no residente.

En ocho individuos se observó que las localizaciones mensuales y diarias se distribuyeron equitativamente y sin patrón alguno, lo que indica hábitos residentes. Debido a esto, se calcularon las áreas de campeo y núcleo con las triangulaciones de todo el año. Los movimientos de cinco de las ocho aves fueron seguidos durante un período de 11 meses y el total de días de radio-seguimiento osciló entre 28 y 44. Para esas aves, se calcularon las áreas de campeo y núcleo, siendo los polígonos 100% MPC calculados con las localizaciones por homing completamente o casi completamente incluidos en los polígonos obtenidos por triangulación 100% MPC y 95% kernel, lo que confirma la exactitud de las posiciones calculadas por triangulación (Fig. 15).

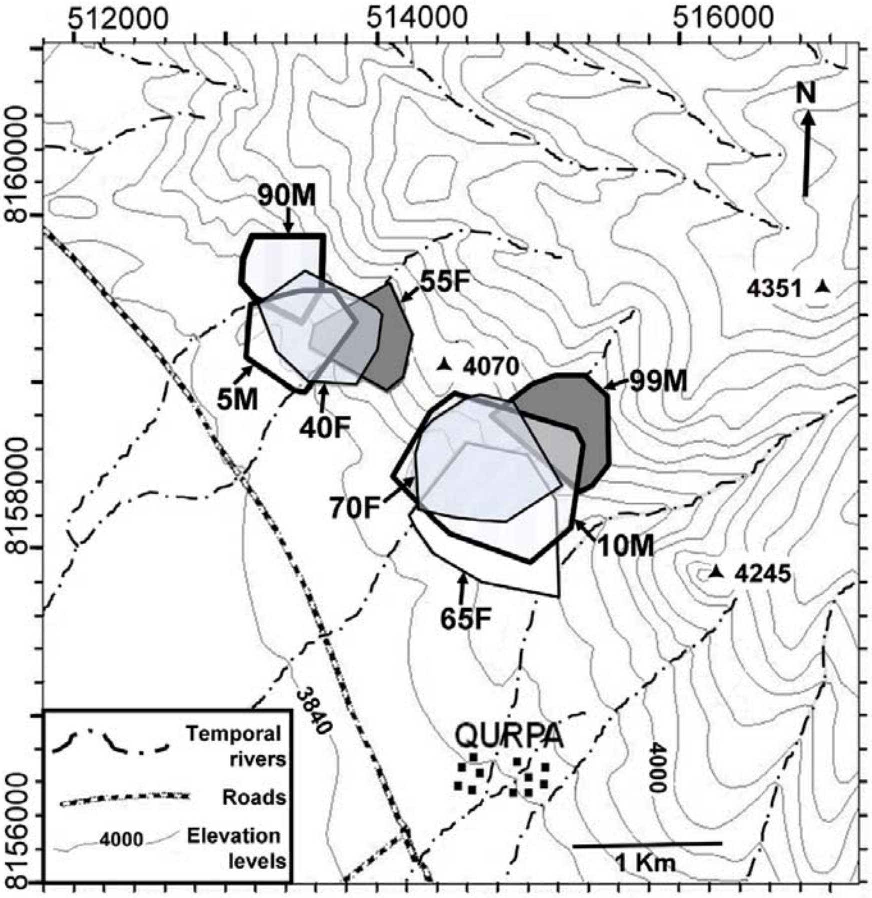


Figura 16. Posiciones en el área de estudio y solapamiento de las áreas de campeo (100% MPC) de las ocho aves para las que fue posible su cálculo (cada polígono está identificado con el código del ave). Polígonos con borde en negrita son machos; polígonos con borde normal son hembras.

La tabla 10 muestra las áreas de campeo calculadas con el MPC 100% y el 95% kernel, junto con el área núcleo 50% kernel para cada ave y sus valores medios generales. Las áreas de campeo calculadas con el 100% MCP nunca superaron 1 km^2 y variaron de 22 a 85 ha. Las áreas núcleo variaron de 6 a 33 ha. Las ocho aves marcadas formaron dos grupos en el área de estudio, ambos formados por cuatro aves (dos machos y dos hembras) separadas en dos valles paralelos. La figura 16 muestra la posición de los individuos en cada grupo.

Aquellos individuos que se desplazaron ampliamente a lo largo del área de estudio, teniendo una muerte prematura, podrían ser aves sub-adultas no residentes. El individuo 20, que presentó un peso sub-adulto, mostró un patrón de movimiento claramente direccional antes de que fuera depredado. El ave 60, por otro lado, mostró una direccionalidad aleatoria sobre un área extensa (al menos 111 ha) antes de que se perdiera la señal. Es posible que ambos individuos estuvieran buscando un territorio donde establecerse.

Las áreas de campeo estimadas para el Tinamú pisacca en este estudio son aproximadamente veinte veces mayor que las estimadas por Pearson y Pearson (1955). Los valores mínimos concuerdan con los publicados para otras especies de Tinamú, mientras que el resto de valores son más altos. Los estudios previos, sin embargo, fueron realizados mediante observación directa. Por ejemplo, Schäfer (1954) utilizó un perro de caza para evaluar las áreas de campeo, y obtuvo territorios muy pequeños, de 0,1 hectáreas para los machos del Tinamú serrano (*Nothocercus bonapartei*) y de 20 hectáreas para el Tinamú tao (*Tinamus tao*). Lancaster (1964a) determinó, mediante la identificación individual del canto de cuatro machos, territorios de entre 17 y 24 ha en el Tinamú montaraz (*Nothoprocta cinerascens*). El mismo autor, utilizando dicha técnica, obtuvo territorios de entre 11 y 19 ha para el Tinamú pizarroso (*Crypturellus boucardi*) (Lancaster, 1964b). En el único otro estudio utilizando radio-marcaje en Tinamúes, Thompson y Carroll (2009) obtuvieron territorios de 16 y 19 ha para el Tinamú manchado. A pesar de que no incluyeron los rangos, destacaron la gran variación entre individuos, siendo similar a nuestros resultados con el Tinamú pisacca.

Como todas las especies de Tinamú, con la excepción del género Tinamotis (Cabot, 1992, Davies, 2002), los Tinamúes pisacca son sedentarios, permaneciendo en el mismo territorio durante todo el año, en el cual se alimentan, tomando ventaja de la heterogeneidad espacial (diferentes tipos de hábitats) y temporal (manejo anual de los cultivos), gracias a la conducta alimenticia generalista de esta especie (Garitano-Zavala et al., 2003).

Para concluir, los territorios relativamente pequeños y los hábitos sedentarios del Tinamú pisacca hacen de esta especie un candidato adecuado como bioindicador de la exposición a la contaminación local.

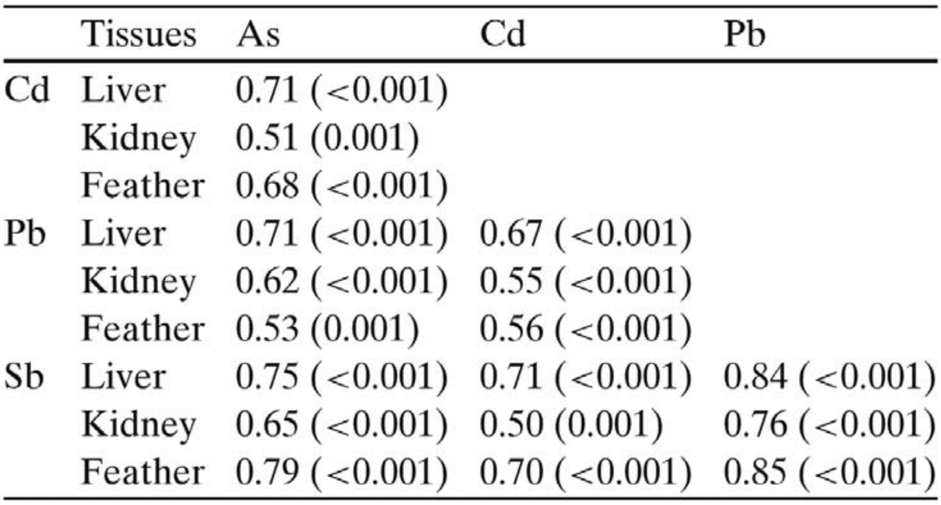
B2. Pluma, riñón e hígado. ¿Reflejan por igual la exposición a la contaminación local?

A diferencia de los organoclorados (conocida familia de contaminantes), que se distribuyen ampliamente entre los tejidos del cuerpo, principalmente en relación con su contenido de lípidos, los metales pesados tienden a acumularse en tejidos concretos. La diferencia de contaminantes entre tejidos también puede ser indicativa de las diferentes formas de entrada en el organismo. Para realizar la evaluación de la exposición a la contaminación local de metales se comparó el uso de diferentes tipos de muestras (pluma, riñón, hígado), y se determinaron las concentraciones de arsénico (As), cadmio (Cd), plomo (Pb) y antimonio (Sb) en las plumas, hígado y riñón de los Tinamúes capturados en las zonas de referencia y en las afectadas por la minería. Para comparación con las aves muestreadas en el medio natural, se utilizaron aves cautivas (criadas en cautividad).

En términos generales, las concentraciones de arsénico fueron más altas en plumas y riñón, casi duplicando a las encontradas en el hígado (Fig. 17). El cadmio, como se esperaba, presentó concentraciones más altas en riñón que en los otros tejidos. Los niveles de este metal fueron de cinco a diez veces mayor en riñón que en hígado y de 250 a 5.000 veces mayor que en las muestras de pluma (Figura 17). Las concentraciones de plomo fueron claramente mayores en las plumas (alrededor de tres veces más altas que en el riñón y diez veces más altos que en el hígado). Asimismo, las plumas acumularon relativamente mayor antimonio que los otros tejidos (Fig. 17).

Las concentraciones de los cuatro metales traza analizados correlacionaron significativamente entre tejidos (Figuras 18, 19 y 20), y a su vez se observaron correlaciones significativas entre los metales traza dentro de cada tejido (Tabla 11), con lo que se puede concluir que, aunque el nivel de contaminación de cada tejido es diferente, las concentraciones de metales en los tejidos siempre se encontraron en el mismo rango.

Se ha demostrado que las concentraciones de metales en las plumas reflejan los valores de los tejidos internos (así como los posibles efectos adversos) en numerosas especies de aves (Burger et al., 1993, Pilastro et al., 1993, Burger and Gochfeld, 2000b), por lo que, las altas correlaciones encontradas entre las plumas, el hígado y el riñón en ambas especies de Tinamú nos permite generalizar esa situación al arsénico y al antimonio. Además, las concentraciones encontradas en plumas son relativamente constantes para cada metal y puesto que una elevada proporción de los metales acumulados es depositada en las plumas debido a su afinidad con la queratina rica en sulfhidrilo y la melanina (Burger et al., 2008, Burger and Gochfeld, 2009), dichas concentraciones probablemente sean una parte importante de la acumulada en el ave. Por lo tanto, recomendamos que los futuros programas de monitorización utilizando especies de Tinamú sean basados en muestras de pluma, puesto que presentan varias ventajas; por ejemplo, son fáciles de muestrear de modo no invasivo y de almacenar indefinidamente. Además, el análisis de las plumas de especímenes provenientes de museos obtenidos en localidades actualmente contaminadas podría generar datos históricos de los niveles de contaminación (Lounsbury-Billie et al., 2008).



	Tissues	As	Cd	Pb
Cd	Liver	0.71 (<0.001)		
	Kidney	0.51 (0.001)		
	Feather	0.68 (<0.001)		
Pb	Liver	0.71 (<0.001)	0.67 (<0.001)	
	Kidney	0.62 (<0.001)	0.55 (<0.001)	
	Feather	0.53 (0.001)	0.56 (<0.001)	
Sb	Liver	0.75 (<0.001)	0.71 (<0.001)	0.84 (<0.001)
	Kidney	0.65 (<0.001)	0.50 (0.001)	0.76 (<0.001)
	Feather	0.79 (<0.001)	0.70 (<0.001)	0.85 (<0.001)

Tabla 11. Correlaciones no paramétricas entre las concentraciones de elementos traza en cada tipo de tejido del Tinamú pisacca (*Nothoprocta ornata*). El valor de la Rho de Spearman se encuentra dentro de cada cuadrícula y el valor de p dentro de los paréntesis. N = 37 para pluma y N = 41 para hígado y riñón.

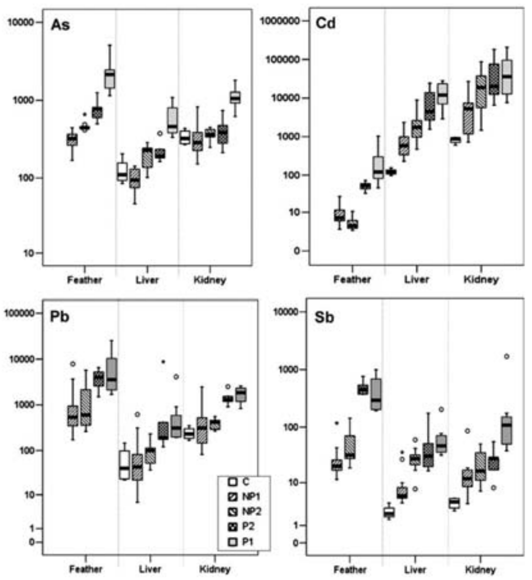


Figura 17. Gráficos de caja para las concentraciones de los elementos traza en pluma, hígado y riñón de Tinamú pisacca: de cautividad (C), zonas de referencia San Pedro de Ulloma (NP1) y Chuñavi (NP2) y zonas mineras Antequera (P1) y Poopó (P2).

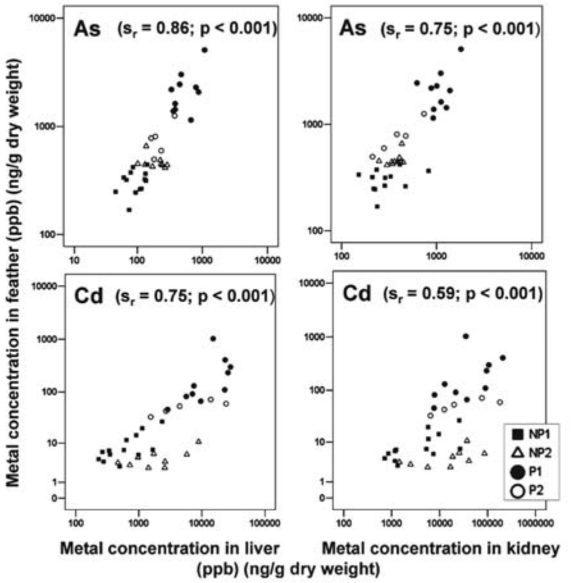


Figura 18. Diagramas de dispersión para las concentraciones de arsénico y cadmio en plumas versus hígado (izquierda) y riñón (derecha) en el Tinamú pisacca de las zonas de referencia San Pedro de Ulloma (NP1) y Chuñavi (NP2) y zonas mineras Antequera (P1) y Poopó (P2). El valor de la correlación de Spearman (s_r) y el valor de *p* están incluidos.

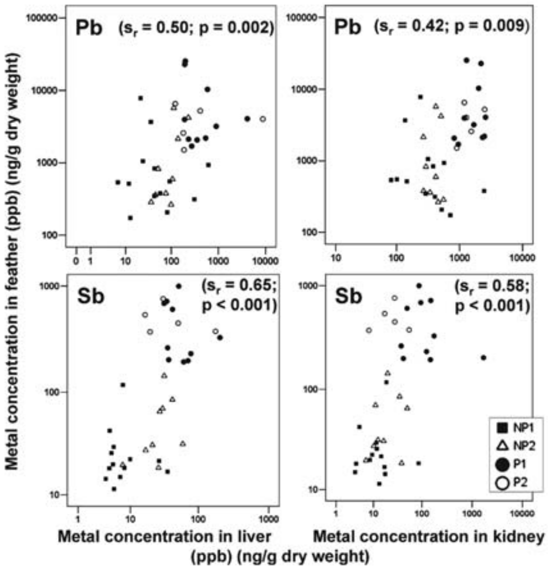


Figura 19. Diagramas de dispersión para las concentraciones de plomo y antimonio en plumas versus hígado (izquierda) y riñón (derecha) en Tinamú pisacca de las zonas de referencia San Pedro de Ulloma (NP1) y Chuñavi (NP2) y zonas mineras Antequera (P1) y Poopó (P2). El valor de la correlación de Spearman (s_r) y el valor de *p* están incluidos.

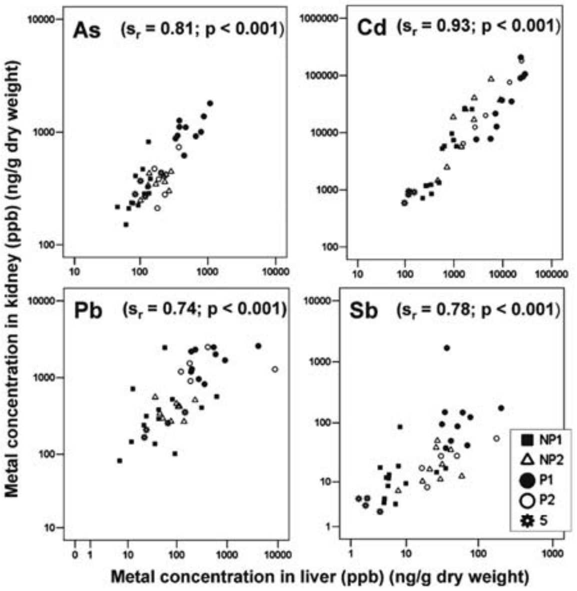


Figura 20. Diagramas de dispersión para las concentraciones de los elementos traza en riñón versus hígado en Tinamú pisacca de las zonas de referencia San Pedro de Ulloma (NP1) y Chuñavi (NP2) y zonas mineras Antequera (P1) y Poopó (P2). El valor de la correlación de Spearman (s_r) y el valor de *p* están incluidos.

B3. El estado histopatológico del hígado y los riñones en relación con los niveles de contaminación

Con el fin de evaluar el estado histopatológico del hígado y los riñones en relación con los niveles de contaminación, las muestras (alrededor de 2 gr.) fueron tomadas de los ejemplares recién muertos de Tinamú pisacca y de Darwin, para ser examinadas a continuación con microscopio una vez preparados. Las lecturas se realizaron a ciegas.

Los resultados de las placas histológicas de hígado revelaron que algunas aves de las cuatro localidades del medio natural mostraban células inflamatorias: 7% en la No Contaminada 1, 22% en la No Contaminada 2, 20% en Contaminada 1, y 27% en Contaminada 2 (en esta última localidad ambas especies se tuvieron en cuenta), por lo que no se observa un efecto claro de la contaminación.

Las placas histológicas renales mostraron nefritis intersticial proporcionalmente mayor en las aves de las localidades contaminadas (30% en Contaminada 1 y 27% en Contaminada 2) que en las localidades de referencia (15% en No Contaminada 1 y 0% en No Contaminada 2), por lo que es claro el efecto causado por la contaminación. Por otra parte, la tesaurismosis (una enfermedad de acumulación patológica) en los túbulos proximales se observó en el 30% de las muestras de la localidad Contaminada 1. El desarrollo de la tesaurismosis está probablemente relacionado con concreciones minerales, como el fenómeno observado en un estudio con dosis de cadmio controladas en la Codorniz japonesa (*Coturnix japonica*) (Richardson et al., 1974).

El cadmio es un elemento traza que se acumula preferentemente en el riñón, por lo tanto, buscamos la relación entre las concentraciones de Cd y el daño histológico en este órgano. Aunque en las localidades contaminadas la nefritis intersticial fue más frecuente y la tesaurismosis sólo fue citada en la localidad Contaminada 1 (donde este metal alcanza las concentraciones más altas), los efectos en los tejidos no están relacionados directamente con los niveles individuales, como podría esperarse. Por ejemplo, las aves con concentraciones máximas de cadmio en riñón (más de 100.000 y 200.000 ppb) no tuvieron efectos en el tejido, mientras que se encontraron efectos en individuos con concentraciones más bajas (95.200 ppb; 35.200 ppb y ppb 7.800).

La nefritis intersticial parece ser no específica o no está directamente relacionada con las concentraciones de Cd, dado que se observó en individuos con valores de Cd en un rango de 850 a 105 700 ppb.

B4. Concentraciones de metales pesados en especies de Tinamú – ¿Entrañan riesgos para su conservación?

En general, y especialmente en aves salvajes, es difícil vislumbrar la relación entre las concentraciones de metales en los tejidos y los efectos adversos sobre la salud. Varios factores como la interacción con los elementos, la relaciones inter e intra específicas, así como variables tipo sexo, edad, condición física... hacen difícil obtener conclusiones exclusivamente a partir de la contaminación por metales.

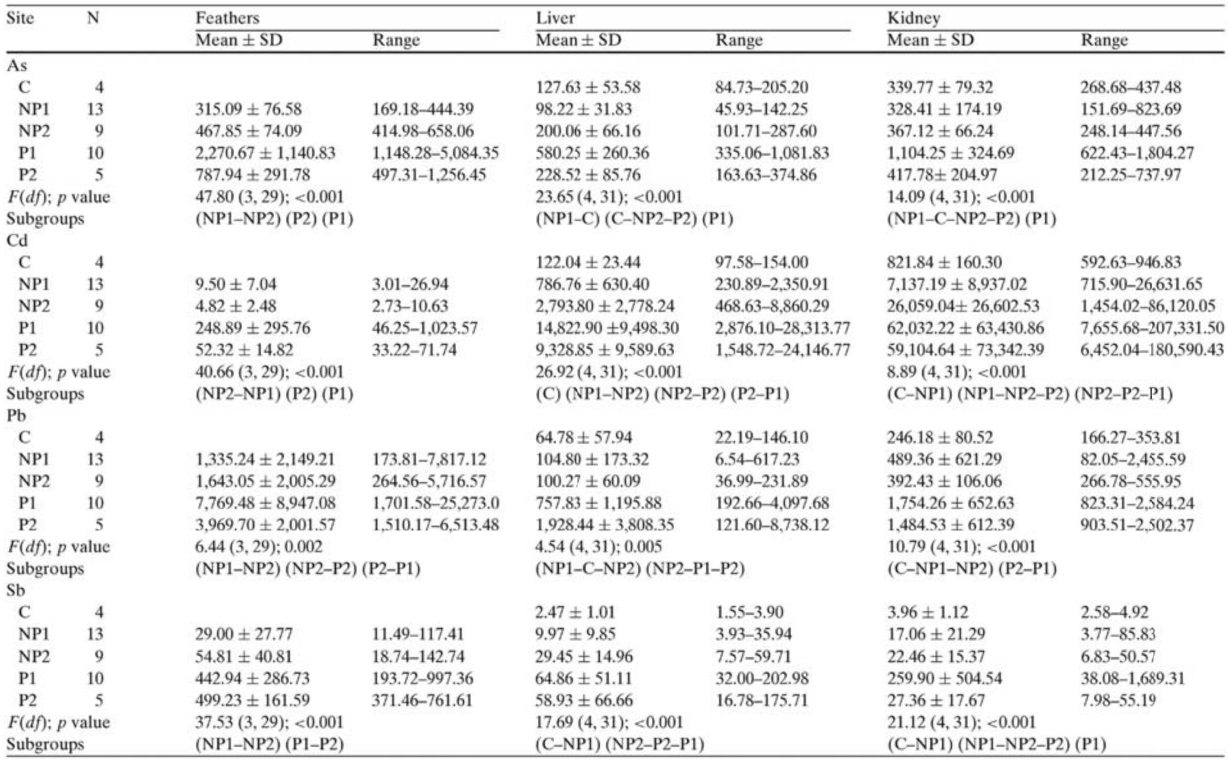
Los estudios de laboratorio son necesarios para identificar los niveles de metales que producen la muerte o modificaciones adversas en el comportamiento, la fisiología o el éxito reproductivo de las aves, pero con la excepción del mercurio, el plomo y el cadmio, los estudios realizados de modo controlado en laboratorio son escasos (Burger and Gochfeld, 2009).

A pesar de esto, con el fin de determinar las concentraciones de elementos traza en las especies de Tinamú y evaluar si entrañan algún tipo de amenaza desde el punto de vista conservacionista, las concentraciones de arsénico, cadmio, plomo y antimonio fueron determinadas. Aves criadas en cautividad fueron utilizadas a modo comparativo.

Las medias, desviaciones estándar y rangos de los elementos traza analizados en el Tinamú pisacca se muestran en la tabla 12 Los valores del Tinamú de Darwin y pisacca de la localidad P2 se encuentran en la tabla 13 Es interesante observar que no fueron encontrados interacciones significativas o efectos por sexo en los análisis. Todas las concentraciones se expresan en peso seco.

Los resultados mostraron que los niveles de todos los metales traza analizados en el Tinamú pisacca presentaron efecto localidad en todos los tejidos. En la localidad P2, los tejidos del Tinamú de Darwin presentaron concentraciones de los elementos traza similares a las del Tinamú pisacca, sin presentar diferencias significativas entre las dos especies en la mayoría de los casos (Tabla 13). Se encontraron excepciones, como en el caso del plomo y antimonio en muestras de pluma, siendo las concentraciones en el Tinamú de Darwin el doble que en el Tinamú pisacca.

Las concentraciones de arsénico en pluma, hígado y riñón fueron significativamente mayores en la localidad P1 y las plumas de las localidades contaminadas formaron dos grupos distintos. Como el arsénico es un micronutriente para algunos vertebrados (Eisler, 1988a), y es metabolizado rápidamente en aves (Pendleton et al., 1995), a menos que se dé una exposición extrema, no llega a acumularse hasta alcanzar niveles tóxicos en los tejidos (Custer et al., 2009). Sin embargo, Burger y Gochfeld (2009) encontraron que biomagnifica en la naturaleza, encontrándose los valores más altos en la parte superior de la cadena trófica. Los valores de arsénico analizados en las plumas de varias especies de aves varían entre 13 y 604 ppb (Burger et al., 2008, Burger and Gochfeld, 2009). Los valores encontrados en las plumas de Tinamú pisacca quedan dentro de este rango para todas las localidades, excepto



Site	N	Feathers		Liver		Kidney	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
As							
C	4			127.63 ± 53.58	84.73–205.20	339.77 ± 79.32	268.68–437.48
NP1	13	315.09 ± 76.58	169.18–444.39	98.22 ± 31.83	45.93–142.25	328.41 ± 174.19	151.69–823.69
NP2	9	467.85 ± 74.09	414.98–658.06	200.06 ± 66.16	101.71–287.60	367.12 ± 66.24	248.14–447.56
P1	10	2,270.67 ± 1,140.83	1,148.28–5,084.35	580.25 ± 260.36	335.06–1,081.83	1,104.25 ± 324.69	622.43–1,804.27
P2	5	787.94 ± 291.78	497.31–1,256.45	228.52 ± 85.76	163.63–374.86	417.78± 204.97	212.25–737.97
F(*df*); *p* value		47.80 (3, 29); <0.001		23.65 (4, 31); <0.001		14.09 (4, 31); <0.001	
Subgroups		(NP1–NP2) (P2) (P1)		(NP1-C) (C–NP2–P2) (P1)		(NP1–C–NP2–P2) (P1)	
Cd							
C	4			122.04 ± 23.44	97.58–154.00	821.84 ± 160.30	592.63–946.83
NP1	13	9.50 ± 7.04	3.01–26.94	786.76 ± 630.40	230.89–2,350.91	7,137.19 ± 8,937.02	715.90–26,631.65
NP2	9	4.82 ± 2.48	2.73–10.63	2,793.80 ± 2,778.24	468.63–8,860.29	26,059.04± 26,602.53	1,454.02–86,120.05
P1	10	248.89 ± 295.76	46.25–1,023.57	14,822.90 ±9,498.30	2,876.10–28,313.77	62,032.22 ± 63,430.86	7,655.68–207,331.50
P2	5	52.32 ± 14.82	33.22–71.74	9,328.85 ± 9,589.63	1,548.72–24,146.77	59,104.64 ± 73,342.39	6,452.04–180,590.43
F(*df*); *p* value		40.66 (3, 29); <0.001		26.92 (4, 31); <0.001		8.89 (4, 31); <0.001	
Subgroups		(NP2–NP1) (P2) (P1)		(C) (NP1–NP2) (NP2–P2) (P2–P1)		(C–NP1) (NP1–NP2–P2) (NP2–P2–P1)	
Pb							
C	4			64.78 ± 57.94	22.19–146.10	246.18 ± 80.52	166.27–353.81
NP1	13	1,335.24 ± 2,149.21	173.81–7,817.12	104.80 ± 173.32	6.54–617.23	489.36 ± 621.29	82.05–2,455.59
NP2	9	1,643.05 ± 2,005.29	264.56–5,716.57	100.27 ± 60.09	36.99–231.89	392.43 ± 106.06	266.78–555.95
P1	10	7,769.48 ± 8,947.08	1,701.58–25,273.0	757.83 ± 1,195.88	192.66–4,097.68	1,754.26 ± 652.63	823.31–2,584.24
P2	5	3,969.70 ± 2,001.57	1,510.17–6,513.48	1,928.44 ± 3,808.35	121.60–8,738.12	1,484.53 ± 612.39	903.51–2,502.37
F(*df*); *p* value		6.44 (3, 29); 0.002		4.54 (4, 31); 0.005		10.79 (4, 31); <0.001	
Subgroups		(NP1–NP2) (NP2–P2) (P2–P1)		(NP1–C–NP2) (NP2–P1–P2)		(C–NP1–NP2) (P2–P1)	
Sb							
C	4			2.47 ± 1.01	1.55–3.90	3.96 ± 1.12	2.58–4.92
NP1	13	29.00 ± 27.77	11.49–117.41	9.97 ± 9.85	3.93–35.94	17.06 ± 21.29	3.77–85.83
NP2	9	54.81 ± 40.81	18.74–142.74	29.45 ± 14.96	7.57–59.71	22.46 ± 15.37	6.83–50.57
P1	10	442.94 ± 286.73	193.72–997.36	64.86 ± 51.11	32.00–202.98	259.90 ± 504.54	38.08–1,689.31
P2	5	499.23 ± 161.59	371.46–761.61	58.93 ± 66.66	16.78–175.71	27.36 ± 17.67	7.98–55.19
F(*df*); *p* value		37.53 (3, 29); <0.001		17.69 (4, 31); <0.001		21.12 (4, 31); <0.001	
Subgroups		(NP1–NP2) (P1–P2)		(C–NP1) (NP2–P2–P1)		(C–NP1) (NP1–NP2–P2) (P1)	

Tabla 12. Media y desviación estándar (SD) de los niveles de metales traza (ppb d-w) (ng/g peso seco), en plumas, hígado y riñón de Tinamú pisacca en las zonas no contaminadas (NP1 y NP2), contaminadas (P1 y P2) y de cautividad (C). El valor de F y p y los grados de libertad (df) del ANOVA se muestran solo para el factor principal "localidad"; los subgrupos se formaron con el test a posteriori Schefeé.

para la localidad P1, con más de 2200 ppb; y con la excepción del Tinamú de Darwin en la localidad P2, que presento valores superiores a 1000 ppb. Por lo tanto, los valores en estas dos especies se encuentran entre los más altos jamás citados. La comparación con las zonas de referencia indica que estos elevados valores están relacionados con la exposición.

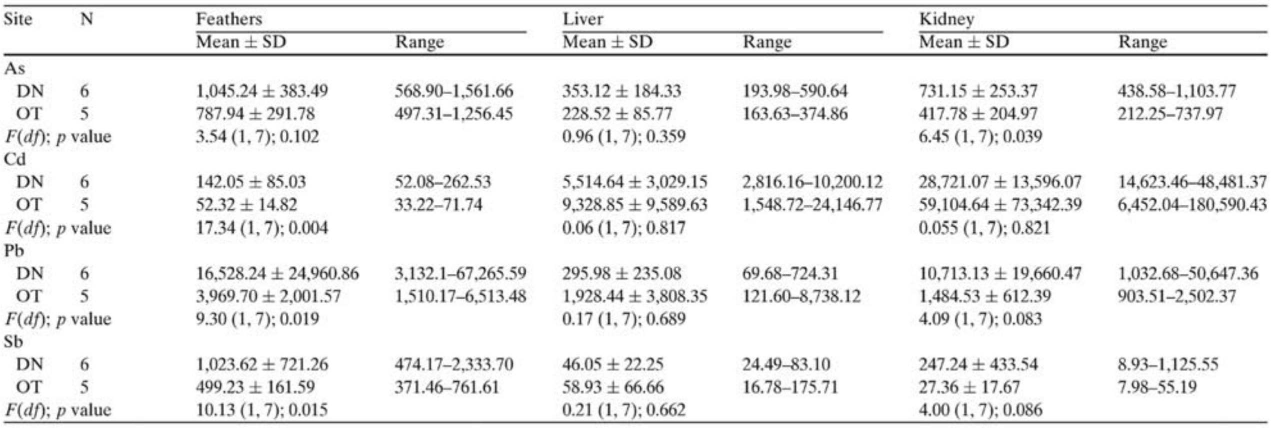
En el hígado, los valores medios de arsénico para el Tinamú pisacca alcanzaron un máximo de 580 ppb en P1 y un valor máximo individual de 1081 ppb. Estos valores son inferiores al umbral de 15000 ppb citado por Goede (1985) como el límite superior para los niveles de seguridad en aves limícolas. Fedynich et al. (2007) reportaron valores de 18 a 660 ppb en patos migratorios. Aunque los valores hepáticos pueden ser considerados dentro de los niveles citados, sugieren que la exposición al arsénico influye en los niveles hepáticos de este metal. Para los riñones, no hay informes previos disponibles.

Las concentraciones de cadmio en plumas e hígado fueron significativamente mayores en la localidad P1, pero en riñón la diferencia con la localidad no contaminada NP2 no fue clara. Respecto al arsénico, las diferencias entre las localidades contaminadas y no contaminadas fueron más evidentes en pluma (Fig. 17). Las concentraciones de cadmio en hígado y riñón de las aves provenientes de cautividad fueron alrededor de siete veces menores que la media mínima encontrada en el medio silvestre.

El cadmio es un elemento no esencial ubicuo en el medio natural, y sus efectos tóxicos y acumulación en aves marinas y en ecosistemas terrestres y acuáticos ha sido ampliamente estudiado. La toxicidad del cadmio es más común entre poblaciones salvajes de vertebrados de lo que se pensaba anteriormente (Larison et al., 2000). En particular, el cadmio daña especialmente los riñones (Furness, 1996, Larison et al., 2000). Burger (1993) sugirió que los niveles de cadmio en pluma asociados a efectos adversos se encuentran en el rango de 100 ppb (pardelas) a 2000 ppb (charranes). Los valores medios en plumas de Tinamú oscilaron entre 5 y 9.5 ppb en las localidades de referencia, lo que comparativamente es muy bajo, mientras que en las localidades contaminadas, los valores fueron varias veces superiores para ambas especies de Tinamú: en P1 (para Tinamú pisacca) y P2 (para Tinamú de Darwin), los valores están dentro del rango de toxicidad propuesto por Burger (1993).

Scheuhammer (1987) indicó que los niveles umbral de cadmio en el hígado son inferiores a 3.000 ppb y Furness (1996) concluyó que las concentraciones umbral para la intoxicación por cadmio en las aves se podría esperar en torno a 40 ppm (peso húmedo), pero existiendo un amplio rango de toxicidad entre especies y edades. En el caso del Lagópodo común (*Lagopus lagopus*), concentraciones de cadmio en el hígado de aproximadamente 10.000 ppb estuvieron relacionadas con niveles altos de metalotioneína, lo que indica una respuesta fisiológica a la exposición por Cd (Pedersen and Hylland, 2007). Los valores medios de Cd en el hígado de los Tinamúes pisacca procedentes de cautividad mostraron concentraciones muy bajas; los valores de las dos localidades de referencia se situaron debajo de los niveles umbral citados por Scheuhammer, aunque en la localidad NP2, un individuo llegó a 8800 ppb. En la localidad P1, el valor medio para el Tinamú Pisacca fue superior a 10000 ppb, y en ambas localidades contaminadas, varios ejemplares alcanzaron valores superiores a 20000 ppb, indicando una alta exposición de cadmio en el campo.

El cadmio se acumula preferentemente en el riñón (Kim et al., 2007). Los niveles basales de cadmio en el riñón suelen ser inferiores a 8000 ppb, como fue indicado por Scheuhammer



Site	N	Feathers		Liver		Kidney	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
As							
DN	6	1,045.24 ± 383.49	568.90–1,561.66	353.12 ± 184.33	193.98–590.64	731.15 ± 253.37	438.58–1,103.77
OT	5	787.94 ± 291.78	497.31–1,256.45	228.52 ± 85.77	163.63–374.86	417.78 ± 204.97	212.25–737.97
F(*df*); *p* value		3.54 (1, 7); 0.102		0.96 (1, 7); 0.359		6.45 (1, 7); 0.039	
Cd							
DN	6	142.05 ± 85.03	52.08–262.53	5,514.64 ± 3,029.15	2,816.16–10,200.12	28,721.07 ± 13,596.07	14,623.46–48,481.37
OT	5	52.32 ± 14.82	33.22–71.74	9,328.85 ± 9,589.63	1,548.72–24,146.77	59,104.64 ± 73,342.39	6,452.04–180,590.43
F(*df*); *p* value		17.34 (1, 7); 0.004		0.06 (1, 7); 0.817		0.055 (1, 7); 0.821	
Pb							
DN	6	16,528.24 ± 24,960.86	3,132.1–67,265.59	295.98 ± 235.08	69.68–724.31	10,713.13 ± 19,660.47	1,032.68–50,647.36
OT	5	3,969.70 ± 2,001.57	1,510.17–6,513.48	1,928.44 ± 3,808.35	121.60–8,738.12	1,484.53 ± 612.39	903.51–2,502.37
F(*df*); *p* value		9.30 (1, 7); 0.019		0.17 (1, 7); 0.689		4.09 (1, 7); 0.083	
Sb							
DN	6	1,023.62 ± 721.26	474.17–2,333.70	46.05 ± 22.25	24.49–83.10	247.24 ± 433.54	8.93–1,125.55
OT	5	499.23 ± 161.59	371.46–761.61	58.93 ± 66.66	16.78–175.71	27.36 ± 17.67	7.98–55.19
F(*df*); *p* value		10.13 (1, 7); 0.015		0.21 (1, 7); 0.662		4.00 (1, 7); 0.086	

Tabla 13. Media y desviación estándar (SD) de los niveles de metales traza (ppb d-w) (ng/g peso seco), en plumas, hígado y riñón de Tinamú pisacca y de Darwin en la zona contaminada P2. El valor de F y p y los grados de libertad (df) del ANOVA se muestran solo para el factor principal "especie".

(1987). Furness (1996) concluyó que las concentraciones umbral en riñón para la intoxicación por cadmio en las aves podrían esperarse en torno a 100 ppm (peso húmedo). A concentraciones superiores de 300.000 ppb en los riñones se ha encontrado daño renal en aves acuáticas (Mateo and Guitart, 2003). Larison et al. (2000) demostró que el 57% de los individuos adultos de Lagópodo común en un área minera contaminada (con valores de cadmio por encima de 100.000 ppb en riñón) mostraban daño tubular renal irreversible. En nuestros resultados, sólo los individuos de Tinamú pisacca procedentes de cautividad mostraron valores de Cd en riñón por debajo del nivel basal propuesto por Scheuhammer. En el campo, los valores individuales fueron más altos en todas las localidades, en ambas especies de Tinamú. Una posible explicación es que el cadmio es un metal traza común en los suelos andinos. Ninguna localidad presentó valores medios mayores de 100.000 ppb, pero algunos individuos de Tinamú pisacca mostraron valores mayores en las localidades contaminadas. En estas localidades, la nefritis intersticial fue más frecuente, y la tesaurismosis sólo se encontró en la localidad P1. Esta localidad presenta los mayores valores de cadmio, tanto medios e individuales, para todos los tejidos.

Respecto al plomo, las localidades contaminadas mostraron las concentraciones más altas, especialmente para riñón y en P1 en plumas (ver Tabla 12 y la figura 17). Las concentraciones de plomo en hígado y riñón de los ejemplares procedentes de cautividad estuvieron dentro del rango de las localidades no contaminadas. La toxicidad por plomo y los efectos producidos por su envenenamiento, ampliamente estudiados en el campo y en experimentos de laboratorio, incluye varios efectos fisiológicos y somáticos, incluso la muerte, si la exposición es suficientemente alta (Eisler, 1988b, Franson, 1996). Los niveles de plomo pueden aumentar durante la vida de un ave, dependiendo de la exposición a la que se vea sometido (Grue et al., 1984). El ejercicio muscular, la excreción y el alto consumo proteínico son factores que reducen los efectos fisiológicos de las altas concentraciones de plomo en aves silvestres (Burger et al., 1997, Roux and Marra, 2007). Burger y Gochfeld (2000a) consideraron que los efectos adversos en la reproducción de las aves se producen a niveles de 4.000 ppb en plumas; Burger y Gochfeld (1994) encontraron que los pollos de 40 días de Gaviota argéntea (*Larus argentatus*) experimentalmente inyectados con plomo poseían concentraciones de Pb en plumas de 4.790 ± 1.693 ppb, y además mostraron varios problemas motores, mientras que los pollos no tratados presentaron valores de 853 a 1.205 ppb. En nuestro estudio, el Tinamú pisacca presentó en las localidades de referencia concentraciones medias de plomo inferiores a 2.000 ppb, pero cinco individuos mostraron valores de más de 4.000 ppb. Los Tinamúes pisacca y de Darwin de ambas localidades contaminadas presentaron valores medios de 4.000 ppb o mayores, con valores máximos de más de 25.000 ppb (para el Tinamú pisacca, en P1) y 67.000 ppb (para el de Darwin, en P2). Con estos valores, varios de los ejemplares estudiados de las localidades contaminadas se encontrarían dentro de los niveles tóxicos de plomo.

Cuando las concentraciones de plomo en hígado son de 18.000 a 45.000 ppb, Pain (1996) llegó a la conclusión de que las funciones biológicas de las aves acuáticas pueden fallar y se pueden observar signos externos de intoxicación. Franson (1996) encontró que concentraciones de plomo de 10 a 20 ppm en hígado provocan signos clínicos de envenenamiento en varias especies de aves. Clark y Scheuhammer (2003) consideraron que las aves rapaces tienen como niveles basales de plomo concentraciones inferiores a 6.000 ppb en hígado y/o en riñón; por lo que aves rapaces con concentraciones superiores a 6.000

ppb en hígado o riñón han sido expuestas a plomo, intoxicándose a concentraciones de más de 20.000 ppb. Siguiendo estos criterios, todos los individuos procedentes de cautiverio, zonas de referencia y tal vez de P1 serían considerados en el nivel basal. Únicamente en P2 las concentraciones de plomo fueron más altas, con notables diferencias entre las especies: un Tinamú pisacca tenía 8.700 ppb en hígado, pero concentraciones inferiores en el riñón, mientras que dos individuos Tinamú de Darwin mostraron concentraciones mayores en riñón, pero más bajas en hígado (6.500 y 50.600 ppb, respectivamente). El último individuo alcanza el nivel de envenenamiento citado previamente. Kim et al. (2007) también encontró una gran variación en las concentraciones de Pb entre cinco especies de aves costeras (valores individuales de la concentración hepática desde no detectados a 23.250 ppb aproximadamente y desde n.d. hasta 141.600 ppb en riñón). El tejido con la mayor concentración de plomo varió considerablemente entre las especies.

Las concentraciones de antimonio fueron mayores en las zonas contaminadas, siendo esto más aparente en las plumas y para la localidad P1 en riñón. Las plumas acumulan relativamente más antimonio que otros tejidos (Tabla 12, fig. 17). El hígado y los riñones de las aves procedentes de cautividad presentaron niveles de antimonio en promedio cuatro veces menor que las aves silvestres.

El antimonio es potencialmente tóxico, puesto que no es un oligoelemento esencial para las plantas y animales, además de no presentar ninguna función biológica conocida (Fowler and Geering, 1991). Sin embargo, su importancia puede haber sido subestimado debido al bajo número de estudios que se han hecho con este elemento (Shotyk et al., 2005). En las aves, la toxicidad por antimonio se ha citado por la administración de antimonio en la forma de un tartrato de antimonio y potasio (Carlisle and Holberton, 2006), pero muy pocos estudios han analizado su bioacumulación en tejidos. Lounsbury-Billie et al. (2008) citaron valores medios de 4 a 66 ppb en plumas de Águila pescadora, considerándose estos valores bajos. Nuestros valores de las zonas de referencia se encontraban en este rango, pero en las localidades contaminadas, los valores fueron aproximadamente diez veces mayores para el Tinamú pisacca y 20 veces más alto para el de Darwin. Aunque nuestros valores en hígado y riñón no pueden ser comparados debido a la falta de otros estudios, el aumento de las concentraciones en las zonas contaminadas (especialmente en P1 para el Tinamú pisacca y P2 para el de Darwin) respecto a las de las aves provenientes de cautiverio y de las zonas de referencia, indica que las aves se encuentran expuestas a antimonio. Probablemente acumulan este elemento en sus tejidos en proporción directa a la exposición, aunque nada definitivo puede ser dicho acerca de los posibles riesgos de estas concentraciones. Se sabe que los vertebrados excretan rápidamente el antimonio absorbido a través de la orina y las heces, y sólo por una alta exposición se pueden encontrar elevadas concentraciones en la tiroides, glándulas suprarrenales, hígado o riñón (Hayes and Laws, 1991).

B5. El cálamo y la contaminación endógena por plomo

Las plumas han sido ampliamente utilizadas para evaluar la contaminación ambiental por ciertos metales pesados (Burger, 1993, Sanpera et al., 2007a, Sanpera et al., 2008), puesto que las aves excretan durante la muda dichos metales pesados en las plumas en crecimiento. Una vez que la pluma ha terminado de crecer, el suministro de sangre se corta y la pluma queda aislada del resto del cuerpo (Burger, 1993) y, en consecuencia, no existe acumulación adicional de metales pesados a través del torrente sanguíneo a las plumas. Pero, a pesar de esto, los niveles de metales en pluma pueden permanecer estables con el tiempo, como es el caso del mercurio (Appelquist et al., 1984, Veerle et al., 2004), o pueden aumentar, ya que las plumas son muy propensas a la contaminación exógena de su superficie.

Especialmente en el caso del plomo, se ha demostrado que las concentraciones aumentan después de que la pluma haya crecido completamente, por lo que las plumas reflejan tanto los niveles endógenos de plomo como la contaminación exógena. Dicha contaminación exógena, proveniente de la deposición atmosférica, limpieza del plumaje o del contacto con el medio ambiente (suelo, polvo o agua), puede dar lugar a una malinterpretación de la contaminación incorporada mediante procesos biológicos en el momento del crecimiento de las plumas (Pilastro et al., 1993, Dauwe et al., 2002, Jaspers et al., 2004). La contaminación exógena afecta principalmente a las barbas, que se vuelven más contaminadas con el tiempo debido a su gran área y estructuralmente compleja superficie. (Goede and Debruin, 1984, Cardiel et al., 2011).

Debido a que la contaminación exógena es difícil de eliminar de las plumas (Weyers et al., 1988, Cardiel et al., 2011) y que los diferentes tratamientos de limpieza han mostrado resultados similares (Valladares et al., 2010), la cantidad de contaminación exógena que

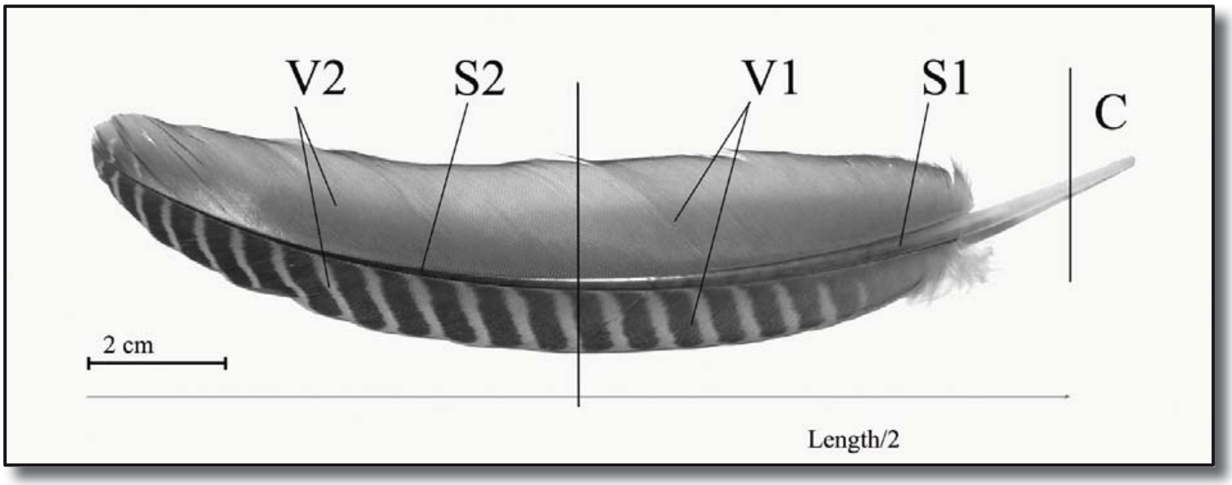


Figura 21. Secciones de la pluma (C cálamo, S1 raquis inferior, S2 raquis superior, V1 barbas inferiores y V2 barbas superiores).

es eliminada por el proceso de limpieza es desconocida. Por lo tanto, a menos que sean utilizadas plumas de pollos o recién mudadas, los niveles de plomo analizados presentaran un porcentaje no determinado de contaminación exógena.

Por lo tanto, para evaluar que sección de la pluma indica de un modo más preciso los niveles de plomo endógenos originales y con el fin de interpretar correctamente la exposición a la contaminación por plomo, plumas primarias de Tinamú pisacca fueron divididas en cinco secciones (cálamo, raquis superior / inferior y barbas superiores / inferiores) para comparar la sección no expuesta a la contaminación exógena (el cálamo) con las secciones expuestas a ella (figura 21). Como cálamo se consideraron los primeros 10 milímetros de la pluma, que es la sección integrada dentro del folículo de la piel en el Tinamú pisacca.

Los resultados (estadísticos descriptivos en la tabla 14) no revelaron diferencias significativas entre las subdivisiones de raquis y barbas, por lo que los análisis fueron ejecutados de nuevo uniendo las subdivisiones. El nuevo análisis mostró que el cálamo presenta las concentraciones más bajas de plomo, seguido por el raquis, mientras que las barbas tienen las concentraciones más elevadas. Los perfiles individuales de las concentraciones de plomo de las secciones de pluma se muestran en la figura 22.

Los niveles más elevados de plomo encontrados en el raquis en comparación con el cálamo, junto con la correlación encontrada entre el las barbas y el raquis, indican que un porcentaje del plomo presente en el raquis es exógeno. Por lo tanto, nuestros resultados muestran que el cálamo es la sección de la pluma que refleja las concentraciones originales de plomo en las plumas de un modo más precisa debido a su protección contra la contaminación exógena, mientras que las barbas representan la contaminación exógena de la superficie de la pluma (deposición atmosférica, baños de arena y / o acicalamiento).

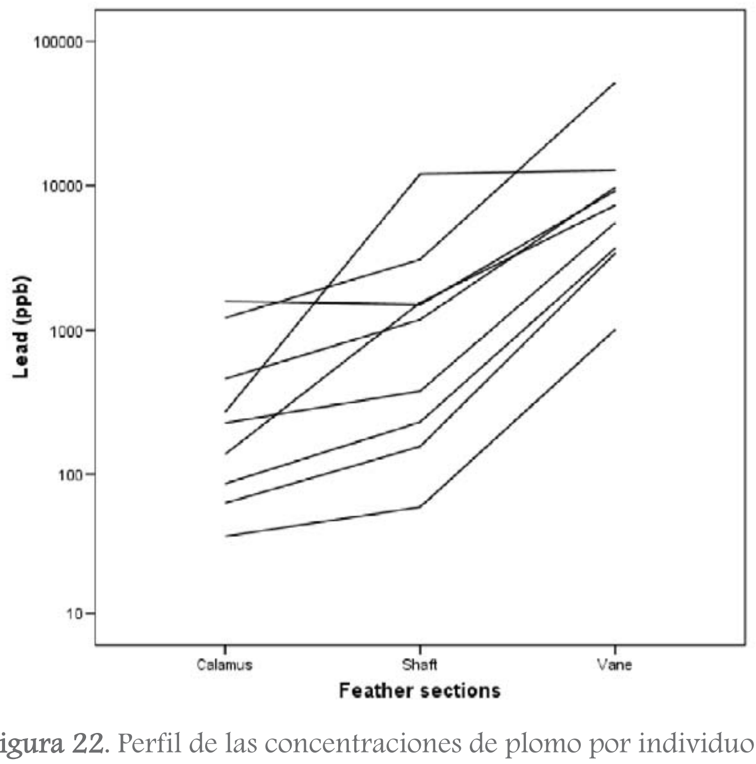
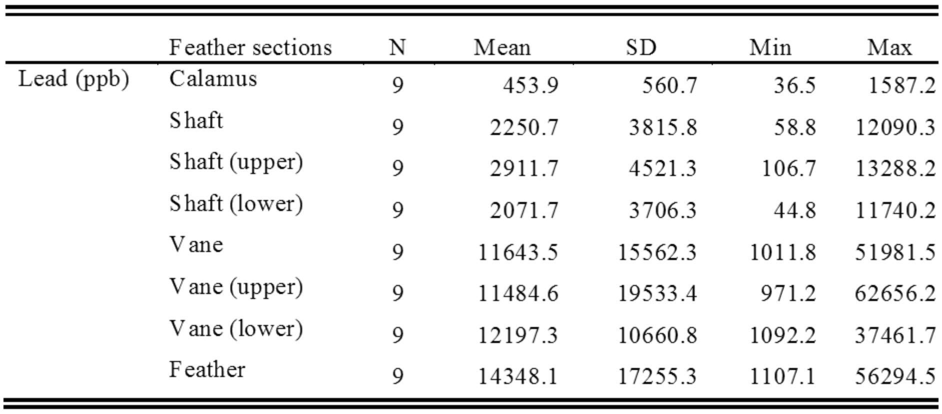


Figura 22. Perfil de las concentraciones de plomo por individuo.



	Feather sections	N	Mean	SD	Min	Max
Lead (ppb)	Calamus	9	453.9	560.7	36.5	1587.2
	Shaft	9	2250.7	3815.8	58.8	12090.3
	Shaft (upper)	9	2911.7	4521.3	106.7	13288.2
	Shaft (lower)	9	2071.7	3706.3	44.8	11740.2
	Vane	9	11643.5	15562.3	1011.8	51981.5
	Vane (upper)	9	11484.6	19533.4	971.2	62656.2
	Vane (lower)	9	12197.3	10660.8	1092.2	37461.7
	Feather	9	14348.1	17255.3	1107.1	56294.5

Tabla 14. Estadísticos descriptivos de las concentraciones de plomo en las secciones de pluma expresadas en base a su peso seco (ng/g).

Las diferencias encontradas en las concentraciones de plomo entre las diferentes secciones de la pluma sugieren que las barbas, con su estructura en forma de red, son muy propensas a retener partículas de contaminación exógena, incluso después de un proceso de limpieza con hidróxido sódico. Incluso el raquis, que presenta una estructura más lisa, parece estar afectado por la contaminación exógena. Vale la pena mencionar que las concentraciones totales de plomo considerando el conjunto de la pluma dan una información (a través de su concentración) similar a las barbas, es decir, la parte más expuesta a la contaminación exógena.

Conclusiones

Parte A: Hábitats acuáticos – Los lodos tóxicos de Flix

1 **Isótopos estables y la ecología trófica de la avifauna acuática del rio Ebro y su Delta.** δ^{15}N junto a δ^{13}C diferencian adecuadamente los ecosistemas fluviales y deltaicos (alta nitrificación y signaturas más bajas de carbono en el río). Las diferencias encontradas entre localidades en las signaturas de nitrógeno están altamente influenciadas por los valores basales, siendo éstos más altos en las áreas fluviales que en el delta. Los análisis de isótopos y el SEAc han ayudado a desentrañar la ecología trófica y el uso de hábitat de la avifauna del Delta del Ebro: El Charrán común y patinegro actúan como especialistas en el Delta del Ebro, mostrando nichos pequeños, mientras que de modo contrario, el Charrancito, la Garceta común, la Garza Imperial, el Martinete y la Gallineta de agua se comportan como generalistas, presentando un amplio nicho trófico.

2 **La contaminación del embalse de Flix en el río Ebro y su Delta.** Todos los elementos analizados se encuentran fuera de valores de alerta (excluyendo el mercurio) y dentro del rango de otras comunidades avifaunísticas (con la excepción del cromo y el mercurio). Los valores de mercurio en volantones de Garza Imperial en Flix y en huevos de Gaviota de Audouin, Charrancito y Charrán común "Banya" en el Delta del Ebro son suficientemente altos para indicar que pueden estar poniendo en peligro a estas especies. Más estudios son necesarios para comprobar si dichos niveles estan afectando al éxito reproductor de estas especies.

El arsénico (especialmente en combinación con las signaturas de carbono) discrimina claramente entre las especies marinas y dulceacuícolas. Finalmente, y sorprendentemente, los charranes (familia Sternidae) y las garzas (familia Ardeidae) muestran un patrón muy característico de mercurio y cobre, presumiblemente filogenético, puesto que es independiente del hábitat: altas concentraciones de mercurio y bajas de cobre en charranes y la situación opuesta en garzas.

3 **Huevos o plumas de volantones? Puntos a favor y en contra.** Ambos huevos y plumas son bioindicadores adecuados para la contaminación por elementos traza, pero las plumas de volantones presentan ciertas ventajas. Las plumas de los volantones se desarrollan a partir de la alimentación aportada por los padres de los alrededores de la colonia, por lo tanto reflejan la contaminación local. Además, puesto que los pollos pertenecen a un grupo de edad homogénea, los efectos de bioacumulación de contaminantes relacionados con la edad se pueden considerar negligibles.

4 **Biomarcadores sanguíneos.** Los resultados obtenidos de los seis parámetros sanguíneos (TOSC ROO*, TOSC OH*, BhCE, CbE, LDH and MN) muestran la primera evidencia de asociación entre una respuesta biológica y los niveles de contaminantes en volantones de una especie de garza, haciendo de estos parámetros un método adecuado para la monitorización de la contaminación. Los volantones de Garza Imperial de Flix (el área contaminada) mostraron una alta frecuencia de micronúcleos y una capacidad reducida para detoxificar formas reactivas de oxígeno. En el Delta del Ebro, la respuestas biológicas mostradas por los pollos de garza fueron altos niveles plasmáticos de LDH en Garza Imperial, y una alta frecuencia de micronúcleos en la sangre de tanto la Garza Imperial como la Garceta común. Estas alteraciones pueden estar relacionadas con las intensas actividades agrícolas que se desarrollan en el Delta del Ebro. La falta de diferencias en las actividades de las B esterasas en ambas especies indica la baja o no exposición a componentes anticolinérgicos.

5 **Las aves acuáticas como bioindicadoras.** Aves cuáticas como las garzas, charranes o gaviotas del rio Ebro y su Delta han sido utilizadas de modo satisfactorio como bioindicadoras de contaminación.

Parte B: Hábitats terrestres – La Minería en los Andes Bolivianos

6 **Patrones de movimiento en el Tinamú pisacca.** Los pequeños territorios detectados (inferiores a 1 km^2) y sus hábitos sedentarios hacen de esta especie un bioindicador adecuado de la contaminación local.

7 **Muestras de pluma, riñón e hígado y los elementos traza.** El cadmio se acumuló principalmente en riñón; el plomo y antimonio en plumas; el arsénico en plumas y riñón. Sin embargo, las concentraciones de estos cuatro elementos traza correlacionaron entre tejidos y dentro de cada tejido, por lo que cualitativamente todos los tejidos mostraron el mismo tipo de respuesta. Se recomienda para futuros programas de monitorización el uso de plumas, ya que son una muestra no invasiva.

8 **Daño histopatológico en hígado y riñón.** Las tasas más altas de daño histopatológico se encontraron en las muestras de riñón de las zonas contaminadas. La tesaurismosis también se encontró en una de estas localidades. Por otro lado, el hígado no mostró ningún efecto a la contaminación.

9 **Minería en Bolivia. ¿Riesgo medioambiental?** La contaminación minera ha quedado de manifiesto con los altos niveles de elementos traza detectados en las localidades contaminadas de las dos especies estudiadas de la Tinamú, varios ejemplares superando niveles de riesgo.

10 **Secciones de pluma y contaminación exógena y endógena de plomo.** Las distintas secciones de pluma reflejaron diferentes grados de contaminación exógena, siendo el cálamo la sección más adecuada para la evaluación de los niveles originales endógenos de plomo.

11 **¿Especies de Tinamú como bioindicadores?** Las especies de Tinamú en los Andes bolivianos han demostrado su utilidad como bioindicadores de la contaminación local.

Referencias

Abdennadher, A., Ramirez, F., Romdhane, M.S., Ruiz, X., Jover, L. and Sanpera, C., 2011. Little Egret (*Egretta garzetta*) as a bioindicator of trace element pollution in Tunisian aquatic ecosystems. *Environmental Monitoring and Assessment* 175, 677-684.

Aliakbari, A., Savabieasfahani, M. and Ghasempouri, S.M., 2011. Mercury in egg and eggshell of Whiskered Tern (*Chlidonias hybrida)* from Anzali Wetlands of the Caspian Sea, Iran. *Bulletin of Environmental Contamination and Toxicology* 86, 175-179.

Amat, J.A. and Soriguer, R.C., 1984. Alimentacion invernal de la polla de agua (Gallinula chloropus) en las marismas del Guadalquivir. *Ardeola* 31, 136-140.

Andersen, J., 1945. The food of the Gull-billed Tern, *Gelochelidon nilotica* (Gm.). *Dansk Ornithologisk Forenings Tidsskrift* 39, 198-205.

Appelquist, H., Asbirk, S. and Drabaek, I., 1984. Mercury monitoring - Mercury stability in bird feathers. *Marine Pollution Bulletin* 15, 22-24.

Arcos, J.M., Ruiz, X., Bearhop, S. and Furness, R.W., 2002. Mercury levels in seabirds and their fish prey at the Ebro Delta (NW Mediterranean): the role of trawler discards as a source of contamination. *Marine Ecology Progress Series* 232, 281-290.

Attia, Y.A., Abdalah, A.A., Zeweil, H.S., Bovera, F., El-Din, A.A.T. and Araft, M.A., 2011. Effect of inorganic or organic copper additions on reproductive performance, lipid metabolism and morphology of organs of dual-purpose breeding hens. *Archiv Fur Geflugelkunde* 75, 169-178.

Baker, S.D. and Sepulveda, M.S., 2009. An evaluation of the effects of persistent environmental contaminants on the reproductive success of Great Blue Herons (*Ardea herodias*) in Indiana. *Ecotoxicology* 18, 271-280.

Baos, R., Jovani, R., Pastor, N., Tella, J., Jimenez, B. and Gomez, G., 2006. Evaluation of genotoxic effects of heavy metals and arsenic in wild nestling White Storks (Ciconia ciconia) and Black Kites (*Milvus migrans*) from southwestern Spain after a mining accident. *Environmental Toxicology and Chemistry* 25, 2794-2803.

Barata, C., Fabregat, M.C., Cotin, J., Huertas, D., Sole, M., Quiros, L., Sanpera, C., Jover, L., Ruiz, X., Grimalt, J.O. and Piña, B., 2010. Blood biomarkers and contaminant levels in feathers and eggs to assess environmental hazards in heron nestlings from impacted sites in Ebro basin (NE Spain). *Environmental Pollution* 158, 704-710.

Barrett, R., Skaare, J., Norheim, G., Vader, W. and Froslie, A., 1985. Persistent organochlorines and mercury in eggs of norwegian seabirds 1983. *Environmental Pollution Series A-Ecological and Biological* 39, 79-93.

Barrett, R.T., Camphuysen, K.(J.)., Anker-Nilssen, T., Chardine, J.W., Furness, R.W., Garthe, S., Hueppop, O., Leopold, M.F., Montevecchi, W.A. and Veit, R.R., 2007. Diet studies of seabirds: a review and recommendations. *ICES Journal of Marine Science* 64, 1675-1691.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. and Macleod, H., 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology* 73, 1007-1012.

Becker, P.H., Frank, D. and Sudmann, S.R., 1993. Temporal and spatial pattern of Common Tern (*Sterna hirundo*) foraging in the Wadden Sea. Oecologia 93, 389-393.

Becker, P.H., Frank, D. and Wagener, M., 1997. Luxury in freshwater and stress at sea? The foraging of the Common Terra *Sterna hirundo . Ibis* 139, 264-269.

Becker, P.H., Furness, R.W. and Henning, D., 1993. Mercury dynamics in young Common Tern (*Sterna hirundo*) chicks from a polluted environment. *Ecotoxicology* 2, 33-40.

Becker, P.H. and Munoz Cifuentes, J., 2004. Contaminants in bird eggs. Recent spatial and temporal trends. *Wadden Sea Ecosystem* 18, 5-24.

Becker, P., 1989. Seabirds as monitor organisms of contaminants along the german North Sea coast. *Helgolander Meeresuntersuchungen* 43, 395-403.

Benejam, L., Benito, J. and Garcia-Berthou, E., 2010. Decreases in condition and fecundity of freshwater fishes in a highly polluted reservoir. *Water, Air and Soil Pollution* 210, 231-242.

Benito, V., Devesa, V., Munoz, O., Suner, M., Montoro, R., Baos, R., Hiraldob, F., Ferrer, M., Fernandez, M. and Gonzalez, M.J., 1999. Trace elements in blood collected from birds feeding in the area around Donana National Park affected by the toxic spill from the Aznalcollar mine. *Science of the Total Environment* 242, 309-323.

Bertolero, A., Oro, D., Martinez Vilalta, A. and Lopez, M.A., 2005. Selection of foraging habitats by Little Terns *Sterna albifrons* at the Ebro Delta (NE Spain). *Revista Catalana d'Ornitologia* 21, 37-42.

Beveridge, M.C.M., Stafford, E. and Coutts, R., 1985. Metal concentrations in the commercially exploited fishes of an endorheic saline lake in the tin-silver province of Bolivia. *Aquaculture Research* 16, 41-53.

Bogliani, G., Fasola, M., Canova, L. and Saino, N., 1990. Food and foraging rhythm of a specialized Gull-Billed Tern population *Gelochelidon nilotica . Ethology Ecology & Evolution* 2, 175-181.

Bogliani, G., Fasola, M., Canova, L. and Saino, N., 1994. Prey selection by parents and chicks of the Little Tern *Sterna albifrons. . Avocetta* 18, 9-11.

Boncompagni, E., Muhammad, A., Jabeen, R., Orvini, E., Gandini, C., Sanpera, C., Ruix, X. and Fasola, M., 2003. Egrets as monitors of trace-metal contamination in wetlands of Pakistan. *Archives of Environmental Contamination and Toxicology* 45, 399-406.

Bond, A.L. and Diamond, A.W., 2009. Mercury concentrations in seabird tissues from Machias Seal Island, New Brunswick, Canada. *Science of the Total Environment* 407, 4340-4347.

Bosch, C., Olivares, A., Faria, M., Navas, J.M., del Olmo, I., Grimalt, J.O., Pina, B. and Barata, C., 2009. Identification of water soluble and particle bound compounds causing sublethal toxic effects. A field study on sediments affected by a chlor-alkali industry. *Aquatic Toxicology* 94, 16-27.

Bridge, E.S., Jones, A.W. and Baker, A.J., 2005. A phylogenetic framework for the terns (Sternini) inferred from mtDNA sequences: implications for taxonomy and plumage evolution. *Molecular Phylogenetics and Evolution* 35, 459-469.

Bryan, G.W., 1979. Bioaccumulation of marine pollutants. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 286, 483-505.

Burger, J., 1993. Metals in avian feathers: bioindicators of environmental pollution. *Reviews in Environmental Toxicology* 5, 203-311.

Burger, J. and Gochfeld, M., 2000a. Effects of lead on birds (Laridae): A review of laboratory and field studies. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews* 3, 59-78.

Burger, J. and Gochfeld, M., 2000b. Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. *Science of the Total Environment* 257, 37-52.

Burger, J. and Gochfeld, M., 1994. Behavioral impairments of lead-injected young herring-gulls in nature. *Fundamental and Applied Toxicology* 23, 553-561.

Burger, J. and Gochfeld, M., 1993. Heavy-metal and selenium levels in feathers of young egrets and herons from Hong-Kong and Szechuan, China. *Archives of Environmental Contamination and Toxicology* 25, 322-327.

Burger, J. and Gochfeld, M., 1989. Metals in Tern Eggs in a New-Jersey Estuary - a Decade of Change. *Environmental Monitoring and Assessment* 11, 127-135.

Burger, J., Nisbet, I.C.T. and Gochfeld, M., 1992. Metal levels in regrown feathers - Assessment of contamination on the wintering and breeding grounds in the same individuals. *Journal of Toxicology and Environmental Health* 37, 363-374.

Burger, J., Rodgers, J.A. and Gochfeld, M., 1993. Heavy-metal and selenium levels in endangered Wood Storks *Mycteria Americana* from nesting colonies in Florida and Costa Rica. *Archives of Environmental Contamination and Toxicology* 24, 417-420.

Burger, J., Shukla, T., Benson, T. and Gochfeld, M., 1997. Lead levels in exposed herring gulls: Differences in the field and laboratory. *Toxicology and Industrial Health* 13, 193-202.

Burger, J. and Gochfeld, M., 2009. Comparison of arsenic, cadmium, chromium, lead, manganese, mercury and selenium in feathers in Bald Eagle (*Haliaeetus leucocephalus*), and comparison with Common Eider (*Somateria mollissima*), Glaucous-winged Gull (*Larus glaucescens*), Pigeon Guillemot (*Cepphus columba*), and Tufted Puffin (*Fratercula cirrhata*) from the Aleutian Chain of Alaska. *Environmental Monitoring and Assessment* 152, 357-367.

Burger, J., Gochfeld, M., Jeitner, C., Burke, S., Volz, C.D., Snigaroff, R., Snigaroff, D., Shukla, T. and Shukla, S., 2009. Mercury and other metals in eggs and feathers of Glaucous-winged Gulls (*Larus glaucescens*) in the Aleutians. *Environmental Monitoring and Assessment* 152, 179-194.

Burger, J., Gochfeld, M., Jeitner, C., Snigaroff, D., Snigaroff, R., Stamm, T. and Volz, C., 2008. Assessment of metals in down feathers of female common eiders and their eggs from the Aleutians: arsenic, cadmium, chromium, lead, manganese, mercury, and selenium. E*nvironmental Monitoring and Assessment* 143, 247-256.

Cabana, G. and Rasmussen, J., 1994. Modeling food-chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* 372, 255-257.

Cabo, J.M. and Sanchez, J.M., 1986. Recent Information on *Gelochelidon nilotica* in Morocco and its diet. *Alauda* 54, 207-212.

Cabot, J., 1992. Family tinamidae (tinamous). In: del Hoyo, J., Elliot, A. and Sargatal, J. (Eds.), *Handbook of the Birds of the World,* pp. 112–138.

Cardiel, I.E., Taggart, M.A. and Mateo, R., 2011. Using Pb–Al ratios to discriminate between internal and external deposition of Pb in feathers. *Ecotoxicology and Environmental Safety.*

Carlisle, J.D. and Holberton, R.L., 2006. Relative efficiency of fecal versus regurgitated samples for assessing diet and the deleterious effects of a tartar emetic on migratory birds. *Journal of Field Ornithology* 77, 126-135.

Carrasco, L., Diez, S., Soto, D., Catalan, J. and Bayona, J., 2008. Assessment of mercury and methylmercury pollution with zebra mussel (*Dreissena polymorpha*) in the Ebro River (NE Spain) impacted by industrial hazardous dumps. *Science of the Total Environment* 407, 178-184.

Carrasco, L., Benejam, L., Benito, J., Bayona, J.M. and Diez, S., 2011. Methylmercury levels and bioaccumulation in the aquatic food web of a highly mercury-contaminated reservoir. *Environment International* 37, 1213-1218.

Catry, T., Ramos, J.A., Catry, I., Allen-Revez, M. and Grade, N., 2004. Are salinas a suitable alternative breeding habitat for Little Terns Sterna albifrons?. *Ibis* 146, 247-257.

Catry, T., Ramos, J.A., Le Corre, M., Kojadinovic, J. and Bustamante, P., 2008. The role of stable isotopes and mercury concentrations to describe seabird foraging ecology in tropical environments. *Marine Biology* 155, 637-647.

Chambers, J.E., Ma, T.G., Boone, J.S. and Chambers, H.W., 1994. Role of Detoxication Pathways in Acute Toxicity Levels of Phosphorothionate Insecticides in the Rat. *Life Sciences* 54.

Champoux, L., Rodrigue, J., DesGranges, J.L., Trudeau, S., Hontela, A., Boily, M. and Spear, P., 2002. Assessment of contamination and biomarker responses in two species of herons on the St. Lawrence River. *Environmental Monitoring and Assessment* 79, 193-215.

Champoux, L., Rodrigue, J., Trudeau, S., Boily, M.H., Spear, P.A. and Hontela, A., 2006. Contamination and biomarkers in the Great Blue Heron, an indicator of the state of the St. Lawrence River. *Ecotoxicology* 15, 83-96.

Cherel, Y., Hobson, K.A. and Weimerskirch, H., 2005. Using stable isotopes to study resource acquisition and allocation in procellariiform seabirds. *Oecologia* 145, 533-540.

Cherel, Y., Le Corre, M., Jaquemet, S., Menard, F., Richard, P. and Weimerskirch, H., 2008. Resource partitioning within a tropical seabird community: new information from stable isotopes. *Marine Ecology Progress Series* 366, 281-291.

Clark, A.J. and Scheuhammer, A.M., 2003. Lead poisoning in upland-foraging birds of prey in Canada. *Ecotoxicology* 12, 23-30.

Company, R., Serafim, A., Cosson, R.P., Fiala-Medioni, A., Camus, L., Colaco, A., Serrao-Santos, R. and Bebianno, M.J., 2008. Antioxidant biochemical responses to long-term copper exposure in Bathymodiolus azoricus from Menez-Gwen hydrothermal vent. *Science of the Total Environment* 389.

Connell, D., Fung, C., Minh, T., Tanabe, S., Lam, P. and Wong, B., 2003. Risk to breeding success of fish-eating Ardeids due to persistent organic contaminants in Hong Kong: evidence from organochlorine compounds in eggs. *Water research* 37, 459-467.

Connell, D., Wong, B., Lam, P., Poon, K., Lam, M. and Wu, R., 2002. Risk to breeding success of ardeids by contaminants in Hong Kong: Evidence from trace metals in feathers. *Ecotoxicology* 11, 49-59.

Connolly, R.M., Guest, M.A., Melville, A.J. and Oakes, J.M., 2004. Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia* 138, 161-167.

Cordi, B., Fossi, C. and Depledge, M., 1997. Temporal biomarker responses in wild passerine birds exposed to pesticide spray drift. *Environmental Toxicology and Chemistry* 16, 2118-2124.

Corsolini, S., Nigro, M., Olmastroni, S., Focardi, S. and Regoli, F., 2001. Susceptibility to oxidative stress in Adelie and emperor penguin. *Polar Biology* 24, 365-368.

Corsolini, S., Borghesi, N., Ademollo, N. and Focardi, S., 2011. Chlorinated biphenyls and pesticides in migrating and resident seabirds from East and West Antarctica. *Environment International* 37, 1329-1335.

Cotin, J., Garcia-Tarrason, M., Jover, L. and Sanpera, C., 2012. Are the toxic sediments deposited at Flix reservoir affecting the Ebro river biota? Purple heron eggs and nestlings as indicators. *Ecotoxicology* 21, 1391-1402.

Cotin, J., García-Tarrasón, M., Sanpera, C., Jover, L. and Ruiz, X., 2011. Sea, freshwater or saltpans? Foraging ecology of terns to assess mercury inputs in a wetland landscape: The Ebro Delta. *Estuarine, Coastal and Shelf Science* 92, 188-194.

Cramp, S., 1980. Hand book of the birds of Europe, the Middle-East and North Africa. T*he birds of the Western Palearctic* Vol. 2. Hawks to Bustards.

Custer, C.M., Yang, C., Crock, J.G., Shearn-Bochsler, V., Smith, K.S. and Hageman, P.L., 2009. Exposure of insects and insectivorous birds to metals and other elements from abandoned mine tailings in three Summit County drainages, Colorado. *Environmental Monitoring and Assessment* 153, 161-177.

Dauwe, T., Bervoets, L., Blust, R. and Eens, M., 2002. Tissue levels of lead in experimentally exposed zebra finches (*Taeniopygia guttata*) with particular attention on the use of feathers as biomonitors. *Archives of Environmental Contamination and Toxicology* 42, 88-92.

Dauwe, T., Bervoets, L., Pinxten, R., Blust, R. and Eens, M., 2003. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. *Environmental Pollution* 124, 429-436.

Davies, S.J.J.F., 2002. *Ratites and tinamous.* Oxford University Press., New York.

De Luca-Abbott, S., Wong, B., Peakall, D., Lam, P., Young, L. and Lam, M., 2001. Review of effects of water pollution on the breeding success of waterbirds, with particular reference to ardeids in Hong Kong. *Ecotoxicology* 10, 327-349.

Delord, K., Kayser, Y., Cohez, D., Befeld, S. and Hafner, H., 2004. Fluctuations in chick diet of the Squacco Heron *Ardeola ralloides* in southern France: changes over the last 30 years. *Bird Study* 51, 69-75.

DesGranges, J., Rodrigue, J. and Champoux, L., 2009. Geographic distribution of selected contaminants in Great Blue Herons from the St. Lawrence River system, Quebec (1989-1994). *Canadian Wildlife Service Occasional Paper* 116, 1-18.

Dies, J.I., Marin, J. and Perez, C., 2005. Diet of nesting Gull-billed Terns in eastern Spain. *Waterbirds* 28, 106-109.

Dieter, M.P., 1974. Plasma enzyme-activities in coturnix Quail fed graded doses of DDE, polychlorinated biphenyl, malathion and mercuric-chloride. *Toxicology and applied pharmacology* 27.

Eisler, R., 1988a. Arsenic hazards to fish, wildlife and invertebrates: A synoptic review. *Biological Report* 85, 1.12.

Eisler, R., 1988b. Lead hazards to fish, wildlife and invertebrates: A synoptic review. *Biological Report* 85, 1.14.

Eisler, R., 1987. Mercury hazards to fish, wildlife, and invertebrates: A synoptic review. *Biological Report* 85.

Evers, D.C., Burgess, N.M., Champoux, L., Hoskins, B., Major, A., Goodale, W.M., Taylor, R.J., Poppenga, R. and Daigle, T., 2005. Patterns and interpretation of mercury exposure in freshwater avian communities in northeastern North America. *Ecotoxicology* 14, 193-221.

Faria, M., Huertas, D., Soto, D., Grimalt, J., Catalan, J. and Riva, M., 2010. Contaminant accumulation and multi-biomarker responses in field collected Zebra Mussels (*Dreissena polymorpha*) and Crayfish (*Procambarus clarkii*), to evaluate toxicological effects of industrial hazardous dumps in the Ebro river (NE Spain). *Chemosphere* 78, 232-240.

Fasola, M. and Bogliani, G., 1990. Foraging ranges of an assemblage of Mediterranean seabirds. *Colonial Waterbirds* 13, 72-74.

Fasola, M., Movalli, P.A. and Gandini, C., 1998. Heavy metal, organochlorine pesticide, and PCB residues in eggs and feathers of herons breeding in northern Italy. *Archives of Environmental Contamination and Toxicology* 34, 87-93.

Fasola, M., Rubolini, D., Merli, E., Boncompagni, E. and Bressan, U., 2010. Long-term trends of heron and egret populations in Italy, and the effects of climate, human-induced mortality, and habitat on population dynamics. *Population Ecology* 52, 59-72.

Fedynich, A.M., Ballard, B.M., McBride, T.J., Estrella, J.A., Garvon, J.M. and Hooper, M.J., 2007. Arsenic, cadmium, copper, lead, and selenium in migrating Blue-winged Teal (*Anas discors L.*). *Archives of Environmental Contamination and Toxicology* 53, 662-666.

Fernandez, M., Alonso, C., Gonzalez, M. and Hernandez, L., 1999. Occurrence of organochlorine insecticides, PCBs and PCB congeners in waters and sediments of the Ebro River (Spain). *Chemosphere* 38, 33-43.

Focardi, S., Fossi, C., Lambertini, M., Leonzio, C. and Massi, A., 1988. Long-term monitoring of pollutants in eggs of Yellow-legged Herring Gull from Capraia Island (Tuscan Archipelago). *Environmental Monitoring and Assessment* 10, 43-50.

Folch, J., Lees, M. and Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* 226, 497-509.

Forero, M.G., Bortolotti, G.R., Hobson, K.A., Donazar, J.A., Bertelloti, M. and Blanco, G., 2004. High trophic overlap within the seabird community of Argentinean Patagonia: a multiscale approach. *Journal of Animal Ecology* 73, 789-801.

Forero, M.G., Gonzalez-Solis, J., Hobson, K.A., Doncazar, J.A., Bertellotti, M., Blanco, G. and Bortolotti, G.R., 2005. Stable isotopes reveal trophic segregation by sex and age in the Southern Giant Petrel in two different food webs. *Marine Ecology-Progress Series* 296, 107-113.

Forero, M.G. and Hobson, K.A., 2003. Using stable isotopes of nitrogen and carbon to study seabird ecology: applications in the Mediterranean seabird community. *Scientia Marina* 67, 23-32.

Fossi, M.C., Lari, L. and Casini, S., 1996. Interspecies variation of "B" esterases in birds: The influence of size and feeding habits. *Archives of Environmental Contamination and Toxicology* 31, 525-532.

Fowler, B.A. and Geering, P.L., 1991. Metals and their compounds in the environment occurrence analysis and biological relevance, New York: VHC.

Franson, J.C., Hoffman, D.J. and Schmutz, J.A., 2002. Blood selenium concentrations and enzyme activities related to glutathione metabolism in wild emperor geese.

Environmental Toxicology and Chemistry 21, 2179-2184.

Franson, J.C., 1996. Interpretation of tissue lead residues in birds other than waterfowl. In: Beyer, W.N., Heinz, G.H. and Redmon-Norwood, A.W. (Eds.), *Environmental contaminants in wildlife: Interpreting tissue concentrations,* Boca Raton: Lewis, pp. 279.

Frederick, P.C., Hylton, B., Heath, J.A. and Spalding, M.G., 2004. A historical record of mercury contamination in southern Florida (USA) as inferred from avian feather tissue. *Environmental Toxicology and Chemistry* 23, 1474-1478.

Furness, R.W., 1996. Cadmium in birds. In: Beyer, W.N., Heinz, G.H. and Redmon-Norwood, A.W. (Eds.), *Environmental contaminants in wildlife: Interpreting tissue concentrations, Boca Raton*: Lewis, pp. 404.

Gannes, L.Z., OBrien, D.M. and delRio, C.M., 1997. Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78, 1271-1276.

Garitano-Zavala, A., Cotin, J., Borras, M. and Nadal, J., 2010. Trace metal concentrations in tissues of two tinamou species in mining areas of Bolivia and their potential as environmental sentinels. *Environmental Monitoring and Assessment* 168, 629-644.

Garitano-Zavala, A., Nadal, J. and Avila, P., 2003. The feeding ecology and digestive tract morphometry of two sympatric tinamous of the high plateau of the Bolivian Andes: The Ornate Tinamou (*Nothoprocta Ornata*) and the Darwin's Nothura (*Nothura darwinii*). *Ornitologia Neotropical* 14, 173-194.

Goede, A.A., 1985. Mercury, selenium, arsenic and zinc in waders from the Dutch Wadden Sea. *Environmental Pollution Series A-Ecological and Biological* 37, 287-309.

Goede, A.A. and Debruin, M., 1984. The use of bird feather parts as a monitor for metal pollution. *Environmental Pollution Series B-Chemical and Physical* 8, 281-298.

Goede, A.A. and Devoogt, P., 1985. Lead and cadmium in waders from the Dutch Wadden Sea. *Environmental Pollution Series A-Ecological and Biological* 37, 311-322.

Golden, N.H., Rattner, B.A., McGowan, P.C., Parsons, K.C. and Ottinger, M.A., 2003. Concentrations of metals in feathers and blood of nestling black-crowned night-herons (*Nycticorax nycticorax*) in Chesapeake and Delaware Bays. *Bulletin of Environmental Contamination and Toxicology* 70, 385-393.

Golet, G.H., Seiser, P.E., McGuire, A.D., Roby, D.D., Fischer, J.B., Kuletz, K.J., Irons, D.B., Dean, T.A., Jewett, S.C. and Newman, S.H., 2002. Long-term direct and indirect effects of the 'Exxon Valdez' oil spill on pigeon guillemots in Prince William Sound, Alaska. *Marine Ecology-Progress Series* 241, 287-304.

Gomez, G., Baos, R., Gomara, B., Jimenez, B., Benito, V. and Montoro, R., 2004. Influence of a mine tailing accident near Donana National Park (Spain) on heavy metals and arsenic accumulation in 14 species of waterfowl (1998 to 2000). *Archives of Environmental Contamination and Toxicology* 47, 521-529.

Gonzalez-Solis, J., Sokolov, E. and Becker, P.H., 2001. Courtship feedings, copulations and paternity in common terns, Sterna hirundo . *Animal Behaviour* 61, 1125-1132.

Goutner, V., 1991. Food and feeding ecology of Gull-Billed terns (*Gelochelidon nilotica*) in Greece. Revue d'Ecologie (La Terre Et La Vie) 46, 373-384.

Goutner, V. and Furness, R.W., 1997. Mercury in feathers of Little Egret *Egretta garzetta* and Night Heron *Nycticorax nycticorax* chicks and in their prey in the Axios Delta, Greece. *Archives of Environmental Contamination and Toxicology* 32, 211-216.

Granadeiro, J.P., Monteiro, L.R., Silva, M.C. and Furness, R.W., 2002. Diet of Common Terns in the Azores, Northeast Atlantic. *Waterbirds.*

Grue, C.E., Oshea, T.J. and Hoffman, D.J., 1984. Lead concentrations and reproduction in highway-nesting Barn Swallows. *Condor* 86, 383-389.

Guitart, R., Mateo, R., Sanpera, C., Hernandez-Matias, A. and Ruiz, X., 2003. Mercury and selenium levels in eggs of Common Terns (Sterna hirundo) from two breeding colonies in the Ebro Delta, Spain. *Bulletin of Environmental Contamination and Toxicology* 70, 71-77.

Hahn, E., Hahn, K. and Stoeppler, M., 1993. Bird feathers as bioindicators in areas of the German Environmental Specimen Bank: Bioaccumulation of mercury in food chains and exogenous deposition of atmospheric pollution with lead and cadmium. *Science of the Total Environment* 139-140, 259-270.

Hayes, W.J. and Laws, E.R., 1991. *Handbook of pesticide toxicology.* Volume 2. Classes of Pesticides., New York: Academic.

Heath, J.A. and Frederick, P.C., 2005. Relationships among mercury concentrations, hormones, and nesting effort of White Ibises (*Eudocimus albus*) in the Florida Everglades. *Auk* 122, 255-267.

Heinz, G.H., Hoffman, D.J., Klimstra, J.D., Stebbins, K.R., Kondrad, S.L. and Erwin, C.A., 2009. Species differences in the sensitivity of avian embryos to methylmercury. Archives of *Environmental Contamination and Toxicology* 56, 129-138.

Helander, B., Bignert, A. and Asplund, L., 2008. Using raptors as environmental sentinels: Monitoring the white-tailed sea eagle *Haliaeetus albicilla* in Sweden. *Ambio* 37, 425-431.

Henny, C.J., Hill, E.F., Grove, R.A. and Kaiser, J.L., 2007. Mercury and drought along the lower Carson River, Nevada: I. Snowy egret and Black-crowned Night-heron annual exposure to mercury, 1997-2006. *Archives of Environmental Contamination and Toxicology* 53, 269-280.

Henny, C.J., Hill, E.F., Hoffman, D.J., Spalding, M.G. and Grove, R.A., 2002. Nineteenth century mercury: Hazard to wading birds and cormorants of the Carson River, Nevada. *Ecotoxicology* 11.

Henny, C.J., Grove, R.A., Kaiser, J.L. and Johnson, B.L., 2010. North american Osprey populations and contaminants: Historic and contemporary perspectives. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews* 13, 579-603.

Hobson, K.A., 1999. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120, 314-326.

Hobson, K.A. and Clark, R.G., 1992. Assessing avian diets using Stable Isotopes .2. Factors influencing diet-tissue fractionation. *Condor* 94, 189-197.

Hobson, K.A., Piatt, J.F. and Pitocchelli, J., 1994. Using stable isotopes to determine seabird trophic relationships. *Journal of Animal Ecology* 63, 786-798.

Hobson, K.A., Sirois, J. and Gloutney, M.L., 2000. Tracing nutrient allocation to reproduction with stable isotopes: A preliminary investigation using colonial waterbirds of Great Slave Lake. *Auk* 117, 760-774.

Hoffman, D.J., Spalding, M.G. and Frederick, P.C., 2005. Subchronic effects of methylmercury on plasma and organ biochemistries in great egret nestlings. *Environmental Toxicology and Chemistry* 24.

Honda, K., Nasu, T. and Tatsukawa, R., 1986. Seasonal-changes in mercury accumulation in the Black-eared Kite, Milvus migrans Lineatus. *Environmental Pollution Series A-Ecological and Biological* 42, 325-334.

Hughes, K., Ewins, P. and Clark, K., 1997. A comparison of mercury levels in feathers and eggs of Osprey (*Pandion haliaetus*) in the North American Great Lakes. *Archives of Environmental Contamination and Toxicology* 33, 441-452.

Inger, R. and Bearhop, S., 2008. Applications of stable isotope analyses to avian ecology. *Ibis* 150, 447-461.

Jackson, A.L., Inger, R., Parnell, A.C. and Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology* 80, 595-602.

Janz, D., DeForest, D., Brooks, M., Chapman, P., Gilron, G., Hoff, D., Hopkins, W., McIntyre, D., Mebane, C., Palace, V., Skorupa, J. and Wayland, M., 2010. Selenium toxicity to aquatic organisms. In: Chapman PM, Adams WJ, Brooks ML, DelosCG, Luoma SN, Maher WA, Ohlendorf HM, Presser TS, Shaw DP, editors. 2010. *Ecological assessment of selenium in the aquatic environment.* Pensacola (FL): SETAC., 141–231.

Jaspers, V., Dauwe, T., Pinxten, R., Bervoets, L., Blust, R. and Eens, M., 2004. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, *Parus major. Journal of Environmental Monitoring* 6, 356-360.

Jerez, S., Motas, M., Jose Palacios, M., Valera, F., Javier Cuervo, J. and Barbosa, A., 2011. Concentration of trace elements in feathers of three Antarctic penguins: Geographical and interspecific differences. E*nvironmental Pollution* 159, 2412-2419.

Kim, J., Park, S. and Koo, T., 2007. Lead and cadmium concentrations in shorebirds from the Yeongjong Island, Korea. *Environmental Monitoring and Assessment* 134, 355-361.

Koiadinovic, J., Menard, F., Bustamante, P., Cosscon, R.P. and Le Corre, M., 2008. Trophic ecology of marine birds and pelagic fishes from Reunion Island as determined by stable isotope analysis. *Marine Ecology-Progress Series* 361, 239-251.

Kunito, T., Kubota, R., Fujihara, J., Agusa, T. and Tanabe, S., 2008. Arsenic in marine mammals, seabirds, and sea turtles. *Reviews of Environmental Contamination and Toxicology,* Vol 195 195, 31-69.

Lam, J., Tanabe, S., Lam, M. and Lam, P., 2005. Risk to breeding success of waterbirds by contaminants in Hong Kong: evidence from trace elements in eggs. *Environmental pollution* 135, 481-490.

Lancaster, D.A., 1964a. Biology of the Brushland Tinamou, *Nothoprocta cinerascens. Bulletin of the American Museum of Natural History* 127, 269-314.

Lancaster, D.A., 1964b. Life history of the Boucard tinamou in British Honduras. II. Breeding biology. *Condor* 66, 253-276.

Lantzy, R.J. and Mackenzie, F.T., 1979. Atmospheric trace-metals - Global cycles and assessment of mans impact. *Geochimica et Cosmochimica Acta* 43, 511-525.

Larison, J.R., Likens, G.E., Fitzpatrick, J.W. and Crock, J.G., 2000. Cadmium toxicity among wildlife in the Colorado Rocky Mountains. *Nature* 406, 181-183.

Latraube, F., Trotignon, J. and Bretagnolle, V., 2005. Breeding biology of Whiskered Tern Chlidonias hybridus in Brenne (France). *Alauda* 73, 425-429.

Layman, C.A., Arrington, D.A., Montana, C.G. and Post, D.M., 2007. Can stable isotope ratios provide for community-wide measures of trophic structure?. *Ecology* 88, 42-48.

Lehmann, D.W., Levine, J.F. and Law, J.M., 2007. Polychlorinated biphenyl exposure causes gonadal atrophy and oxidative stress in Corbicula fluminea clams. *Toxicologic pathology* 35.

Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine pollution bulletin* 42, 656-666.

Llorente, G., Farran, A., Ruiz, X. and Albaiges, J., 1987. Accumulation and distribution of hydrocarbons, polychlorobyphenyls, and DDT in tissues of 3 species of Anatidae from the Ebro Delta (Spain). *Archives of Environmental Contamination and Toxicology* 16, 563-572.

Lounsbury-Billie, M.J., Rand, G.M., Cai, Y. and Bass, O.L.Jr., 2008. Metal concentrations in osprey (*Pandion haliaetus*) populations in the Florida Bay estuary. *Ecotoxicology* 17, 616-622.

Lucia, M., Andre, J., Gontier, K., Diot, N., Veiga, J. and Davail, S., 2010. Trace element concentrations (mercury, cadmium, copper, zinc, lead, aluminium, nickel, arsenic, and selenium) in some aquatic birds of the southwest atlantic coast of France. *Archives of Environmental Contamination and Toxicology* 58, 844-853.

Lunde, G., 1977. Occurrence and transformation of arsenic in marine-environment. *Environmental health perspectives* 19, 47-52.

Mañosa, S., Mateo, R. and Guitart, R., 2001. A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. *Environmental Monitoring and Assessment* 71, 187-205.

Mateo, R., Gil, C., Badia-Vila, M., Guitart, R., Hernandez-Matias, A., Sanpera, C. and Ruiz, X., 2004. Use of fatty acids to explain variability of organochlorine concentrations in eggs and plasma of Common Terns (*Sterna hirundo*). *Ecotoxicology* 13, 545-554.

Mateo, R. and Guitart, R., 2003. Heavy metals in livers of waterbirds from Spain. *Archives of Environmental Contamination and Toxicology* 44, 398-404.

Mathew, K.L., Lukose, C., Pethani, K.V. and Dave, H.R., 1998. Foraging behaviour of the Gull-billed Tern, *Sterna nilotica* . *Pavo* 36, 15-18.

MEDMIN, 2001. Estudio para el manejo ambiental en microcuencas de 12 zonas mineras: Matilde, Viloco, Caracoles, Colquiri, Poopó, Antequera, Cañadón Antequera, La Lava, Chorolque, Uncía, Llallagua, Colquechaca. *Technical Report. La Paz:* MEDMIN (Medio Ambiente, Minería e Industria) / Viceministerio de Minería y Metalurgia.

Metcheva, R., Yurukova, L. and Teodorova, S.E., 2011. Biogenic and toxic elements in feathers, eggs, and excreta of Gentoo penguin (*Pygoscelis papua ellsworthii*) in the Antarctic. *Environmental Monitoring and Assessment* 182, 571-585.

Michener, R.H. and Schell, D.M., 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha, K. and Michener, R.H. (Eds.), *Stable isotopes in ecology and environmental science.* Blackwell Scientific Publications, London, pp. 138-157.

Mohr, C.O., 1947. Table of equivalent populations of North American small mammals. *American Midland Naturalist* 37, 223-249.

Moller, A.P., Bonisoli-Alquati, A., Rudolfsen, G. and Mousseau, T.A., 2011. Chernobyl birds have smaller brains. *PLoS ONE* 6, 1-7.

Monteiro, L.R., Granadeiro, J.P., Furness, R.W. and Oliveria, P., 1999. Contemporary patterns of mercury contamination in the Portuguese Atlantic inferred from mercury concentrations in seabird tissues. *Marine Environmental Research* 47, 137-156.

Montesinos, A., Santoul, F. and Green, A.J., 2008. The Diet of the Night Heron and Purple Heron in the Guadalquivir Marshes. Ardeola 55, 161-167.

Moreno, R., Jover, L., Diez, C. and Sanpera, C., 2011. Seabird feathers as monitors of the levels and persistence of heavy metal pollution after the Prestige oil spill. *Environmental Pollution* 159, 2454-2460.

Moreno, R., Jover, L., Munilla, I., Velando, A. and Sanpera, C., 2010. A three-isotope approach to disentangling the diet of a generalist consumer: the Yellow-legged Gull in northwest Spain. *Marine Biology* 157, 545-553.

Morera, M., Sanpera, C., Crespo, S., Jover, L. and Ruiz, X., 1997. Inter and intraclutch variability in heavy metals and selenium levels in Audouin's gull eggs from the Ebro Delta, Spain. *Archives of Environmental Contamination and Toxicology* 33, 71-75.

Muir, D., Braune, B., DeMarch, B., Norstrom, R., Wagemann, R., Lockhart, L., Hargrave, B., Bright, D., Addison, R., Payne, J. and Reimer, K., 1999. Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. *Science of the Total Environment* 230, 83-144.

Murvoll, K.M., Skaare, J.U., Moe, B., Anderssen, E. and Jenssen, B.M., 2006. Spatial trends and associated biological responses of organochlorines and brominated flame retardants in hatchlings of North Atlantic kittiwakes (*Rissa tridactyla*). *Environmental Toxicology and Chemistry* 25, 1648-1656.

Navarro, A., Quiros, L., Casado, M., Faria, M., Carrasco, L. and Benejam, L., 2009. Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. *Aquatic toxicology* 93, 150-157.

Navarro, A., Tauler, R., Lacorte, S. and Barcelo, D., 2010a. Occurrence and transport of pesticides and alkylphenols in water samples along the Ebro River Basin. *Journal of Hydrology* 383, 18-29.

Navarro, J., Oro, D., Bertolero, A., Genovart, M., Delgado, A. and Forero, M.G., 2010b. Age and sexual differences in the exploitation of two anthropogenic food resources for an opportunistic seabird. *Marine Biology* 157, 2453-2459.

Neubauer, W., 1998. Habitat choice of the Common Tern *Sterna hirundo* in eastern Germany. *Vogelwelt* 119, 169-180.

Neuman, J., Chardine, J.W. and Porter, J.M., 1998. Courtship feeding and reproductive success in Black-legged Kittiwakes. *Colonial Waterbirds* 21, 73-80.

Newsome, S.D., del Rio, C.M., Bearhop, S. and Phillips, D.L., 2007. A niche for isotopic ecology. *Frontiers in Ecology and the Environment* 5, 429-436.

Nisbet, I.C.T., Montoya, J.P., Burger, J. and Hatch, J.J., 2002. Use of stable isotopes to investigate individual differences in diets and mercury exposures among Common Terns Sterna hirundo in breeding and wintering grounds. *Marine Ecology Progress Series.*

Nisbet, I.C.T., 1973. Courtship-feeding, egg-size and breeding success in Common Terns. *Nature* 241, 141-142.

Norman, D., 1992. The growth rate of little tern Sterna albifrons chicks. *Ringing and Migration* 13, 98-102.

Ohlendorf, H.M. and Heinz, G.H., 2011. Selenium in birds. In: Beyer, W.N. and Meador, J.P. (Eds.), *Environmental contaminants in biota: Interpreting tissue concentrations,* Second Edition. CRC Press/Taylor and Francis Group., Boca Raton, FL., pp. 669-701.

Ohlendorf, H.M., Covington, S.M., Byron, E.R. and Arenal, C.A., 2011. Conducting site-specific assessments of selenium bioaccumulation in aquatic systems. *Integrated*

Environmental Assessment and Management 7, 314-324.

Omurtag, G.Z., Tozan, A., Sehirli, A.O. and Sener, G., 2008. Melatonin protects against endosulfan-induced oxidative tissue damage in rats. *Journal of pineal research* 44.

Oporto, C., Vandecasteele, C. and Smolders, E., 2007. Elevated cadmium concentrations in potato tubers due to irrigation with river water contaminated by mining in Potosi, Bolivia. *Journal of Environmental Quality* 36, 1181-1186.

Oro, D., Bertolero, A., Vilalta, A.M. and Lopez, M.A., 2004. The biology of the Little Tern in the Ebro Delta (northwestern Mediterranean). Waterbirds 27, 434-440.

Oro, D. and Ruiz, X., 1997. Exploitation of trawler discards by breeding seabirds in the north-western Mediterranean: Differences between the ebro delta and the balearic islands areas. *ICES Journal of Marine Science* 54, 695-707.

Padula, V., Burger, J., Newman, S., Elbin, S. and Jeitner, C., 2010. Metals in feathers of Black-Crowned Night-Heron (*Nycticorax nycticorax*) chicks from the New York Harbor Estuary. *Archives of Environmental Contamination and Toxicology* 59, 157-165.

Paillisson, J.M., Reeber, S., Carpentier, A. and Marion, L., 2007. Reproductive parameters in relation to food supply in the Whiskered Tern (*Chlidonias hybrida*). *Journal of Ornithology* 148, 69-77.

Pain, D.J., Meharg, A.A., Ferrer, M., Taggart, M. and Penteriani, V., 2005. Lead concentrations in bones and feathers of the globally threatened Spanish imperial eagle. *Biological Conservation* 121, 603-610.

Pain, D.J., 1996. Lead in waterfowl. In: Beyer, W.N., Heinz, G.H. and Redmon-Norwood, A.W. (Eds.), *Environmental contaminants in wildlife: Interpreting tissue concentrations,* Boca Raton: Lewis, pp. 251-263.

Paiva, V.H., Ramos, J.A., Catry, T., Pedro, P., Medeiros, R. and Palma, J., 2006a. Influence of environmental factors and energetic value of food on Little Tern *Sterna albifrons* chick growth and food delivery. *Bird Study* 53, 1-11.

Paiva, V.H., Ramos, J.A., Machado, D., Penha-Lopes, G., Bouslama, M.F., Dias, N. and Nielsen, S., 2006b. Importance of marine prey to growth of estuarine tern chicks: evidence from an energetic balance model. *Ardea* 94, 241-255.

Paiva, V.H., Ramos, J.A., Martins, J., Almeida, A. and Carvalho, A., 2008a. Foraging habitat selection by Little Terns Sternula albifrons in an estuarine lagoon system of southern Portugal. *Ibis* 150, 18-31.

Paiva, V.H., Tavares, P.C., Ramos, J.A., Pereira, E., Antunes, S. and Duarte, A.C., 2008b. The influence of diet on mercury intake by Little Tern chicks. *Archives of Environmental Contamination and Toxicology* 55, 317-328.

Pardo-Cervera, F., Sorensen, I., Jensen, C., Ruiz, X. and Sanchez-Alonso, C., 2010. Breeding biology of the Little Bittern *Ixobrychus minutus* in the Ebro Delta (NE Spain). *Ardeola* 57, 407-416.

Parnell, A., Inger, R., Bearhop, S. and Jackson, A., 2010. Source Partitioning Using Stable Isotopes: Coping with Too Much Variation. *PLoS ONE* 5.

Pastor, D., Sanpera, C., Gonzalez-Solis, J., Ruiz, X. and Albaiges, J., 2004. Factors affecting the organochlorine pollutant load in biota of a rice field ecosystem (Ebro Delta, NE Spain). *Chemosphere* 55, 567-576.

Pearson, A. and Pearson, O., 1955. Natural history and breeding behavior of the Tinamou, *Nothoprocta ornata. Auk* 72, 113-127.

Pedersen, H.C. and Hylland, K., 2007. Metallothionein levels in Willow Ptarmigan (*Lagopus*

lagopus) populations with different natural loads of cadmium. *European Journal of Wildlife Research* 53, 142-152.

Pendleton, G.W., Whitworth, M.R. and Olsen, G.H., 1995. Accumulation and loss of arsenic and boron, alone and in combination, in Mallard Ducks. *Environmental Toxicology and Chemistry* 14, 1357-1364.

Perrow, M.R., Skeate, E.R., Lines, P., Brown, D. and Tomlinson, M.L., 2006. Radio telemetry as a tool for impact assessment of wind farms: the case of Little Terns *Sterna albifrons* at Scroby Sands, Norfolk, UK. *Ibis* 148, 57-75.

Peste, F., Trigo, S. and Luis, A., 2004. Foraging behaviour of breeding Little Tern *Sterna albifrons* at Ria de Aveiro. *Airo* 14, 55-62.

Peterson, B.J., Howarth, R.W. and Garritt, R.H., 1985. Multiple Stable Isotopes used to trace the flow of organic-matter in estuarine food webs. *Science* 227, 1361-1363.

Pilastro, A., Congiu, L., Tallandini, L. and Turchetto, M., 1993. The use of bird feathers for the monitoring of cadmium pollution. *Archives of Environmental Contamination and Toxicology* 24, 355-358.

Pol, S.S.V. and Becker, P.R., 2007. Monitoring contaminants in seabirds: The importance of specimen banking. *Marine Ornithology* 35, 113-118.

Quiros, L., Ruiz, X., Sanpera, C., Jover, L. and Pina, B., 2008. Analysis of micronucleated erythrocytes in heron nestlings from reference and impacted sites in the Ebro basin (NE Spain). *Environmental Pollution* 155, 81-87.

R Core Team, 2012. *R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.* http://www.R-project.org.

Ramirez, F., Abdennadher, A., Sanpera, C., Jover, L., Hobson, K.A. and Wassenaar, L.I., 2011. Tracing waterbird exposure to total mercury and selenium: A case study at the solar saltworks of Thyna (Sfax, Tunisia). *Environmental Science & Technology* 45, 5118-5124.

Ramos, L., Fernandez, M., Gonzalez, M. and Hernandez, L., 1999. Heavy metal pollution in water, sediments, and earthworms from the Ebro River, Spain. *Bulletin of Environmental Contamination and Toxicology* 63, 305-311.

Ramos, R., Ramirez, F., Sanpera, C., Jover, L. and Ruiz, X., 2009. Feeding ecology of Yellow-legged Gulls Larus michahellis in the western Mediterranean: a comparative assessment using conventional and isotopic methods. *Marine Ecology Progress Series* 377, 289-297.

Rankin, G.O., Racine, C., Sweeney, A., Kraynie, A., Anestis, D.K. and Barnett, J.B., 2008. In vitro nephrotoxicity induced by propanil. *Environmental toxicology* 23.

Regoli, F., 2000. Total oxyradical scavenging capacity (TOSC) in polluted and translocated mussels: a predictive biomarker of oxidative stress. *Aquatic Toxicology* 50, 351-361.

Regoli, F., Nigro, M., Benedetti, M., Fattorini, D. and Gorbi, S., 2005. Antioxidant efficiency in early life stages of the Antarctic silverfish, Pleuragramma antarcticum: Responsiveness to pro-oxidant conditions of platelet ice and chemical exposure. *Aquatic Toxicology* 75.

Richardson, M.E., Spiveyfo, M.R. and Fry, B.E., 1974. Pathological changes produced in Japanese Quail by ingestion of cadmium. *Journal of Nutrition* 104, 323-338.

Rios, B., 2002. Evaluación de la actividad minera en los alrededores del lago Poopó. In: Rocha, O. (Ed.), *Diagnóstico de los recursos naturales y culturales de los lagos Poopó y Uru*

Uru, Oruro–Bolivia, La Paz: Convención RAMSAR, WCS/Bolivia, pp. 167–186.

Rojas, J.C. and Vandecasteele, C., 2007. Influence of mining activities in the North of Potosi, Bolivia on the water quality of the Chayanta river, and its consequences. *Environmental Monitoring and Assessment* 132, 321-330.

Roux, K.E. and Marra, P.P., 2007. The presence and impact of environmental lead in passerine birds along an urban to rural land use gradient. *Archives of Environmental Contamination and Toxicology* 53, 261-268.

Ruiz, X., Jover, L., Llorente, G.A., Sanchez-Reyes, A.F. and Febrian, M.I., 1988. Song thrushes *Turdus philomelos* wintering in Spain as biological indicators of the Chernobyl accident. *Ornis Scandinavica* 19, 63-67.

Ruiz, X., Jover, L. and Pedrocchi, V., 1998. Audouin's gull intraspecific variability in yolk and albumen egg contents: Why is yolk size so constant?. *Biologia e Conservazione della Fauna,* 327-334.

Rumbold, D., Niemczyk, S., Fink, L., Chandrasekhar, T., Harkanson, B. and Laine, K., 2001. Mercury in eggs and feathers of great egrets (*Ardea albus*) from the Florida everglades. A*rchives of Environmental Contamination and Toxicology* 41, 501-507.

Sakellarides, T., Konstantinou, I., Hela, D., Lambropoulou, D., Dimou, A. and Albanis, T., 2006. Accumulation profiles of persistent organochlorines in liver and fat tissues of various waterbird species from Greece. *Chemosphere* 63, 1392-1409.

Salzer, D.W. and Larkin, G.J., 1990. Impact of Courtship Feeding on Clutch and 3rd-Egg Size in Glaucous-Winged Gulls. *Animal Behaviour* 39, 1149-1162.

Sanabria, H., 2000. Resistance and the arts of domination - Miners and the Bolivian state. *Latin American Perspectives* 27, 56-81.

Sanchez, J.M., Munoz del Viejo, A. and de la Cruz, C., 1991. Segregacion alimentaria entre adultos y pollos de *Gelochelidon nilotica* (Gm., 1789) en la laguna de Fuente de Piedra. *Ardeola* 38, 21-27.

Sanpera, C., Moreno, R., Ruiz, X. and Jover, L., 2007a. Audouin's gull chicks as bioindicators of mercury pollution at different breeding locations in the western Mediterranean. *Marine Pollution Bulletin* 54, 691-696.

Sanpera, C., Ruiz, X., Moreno, R., Jover, L. and Waldron, S., 2007b. Mercury and stable isotopes in feathers of Audouin's Gulls as indicators of feeding habits and migratory connectivity. *Condor* 109, 268-275.

Sanpera, C., Valladares, S., Moreno, R., Ruiz, X. and Jover, L., 2008. Assessing the effects of the Prestige oil spill on the European shag (*Phalacrocorax aristotelis*): Trace elements and stable isotopes. *Science of the Total Environment* 407, 242-249.

Schafer, E.R.N.S.T., 1954. Zur Biologie des Steisshuhnes Norhocercus bonapartei. *Journal of Ornithology* 95, 219-232.

Scheifler, R., Coeurdassier, M., Morilhat, C., Bernard, N., Faivre, B. and Flicoteaux, P., 2006. Lead concentrations in feathers and blood of common blackbirds (*Turdus merula*) and in earthworms inhabiting unpolluted and moderately polluted urban areas. *Science of the Total Environment* 371, 197-205.

Scheuhammer, A.M., 1987. The chronic toxicity of aluminum, cadmium, mercury and lead in birds: A review. *Environmental Pollution* 46, 263-295.

Schwemmer, P., Tischler, T., Rehm, R. and Garthe, S., 2011. Habitat use, distribution and food choice of the black-headed gull (*Larus ridibundus*) in the coastal mainland of Schleswig-Holstein. *Corax* 21, 355-374.

Seaman, D.E., Millspaugh, J.J., Kernohan, B.J., Brundige, G.C., Raedeke, K.J. and Gitzen, R.A., 1999. Effects of sample size on kernel home range estimates. *Journal of Wildlife Management* 63, 739-747.

Sell, J.L., 1977. Comparative effects of selenium on metabolism of methylmercury by chickens and quail - Tissue distribution and transfer into eggs. *Poultry science* 56, 939-948.

SERGEOMIN, 1999. Inventariación de recursos naturales renovables (hídricos) y no renovables (minerales e hidrocarburos) del departamento de Oruro. *Boletín del Servicio Nacional de Geología y Minería,* 1–44.

Shotyk, W., Krachler, M. and Chen, B., 2005. Antimony: Global environmental contaminant. *Journal of Environmental Monitoring* 7, 1135-1136.

Sonne, C., Bustnes, J.O., Herzke, D., Jaspers, V.L.B., Covaci, A., Halley, D.J., Moum, T., Eulaers, I., Eens, M., Ims, R.A., Hanssen, S.A., Erikstad, K.E., Johnsen, T., Schnug, L., Riget, F.F. and Jensen, A.L., 2010. Relationships between organohalogen contaminants and blood plasma clinical-chemical parameters in chicks of three raptor species from Northern Norway. *Ecotoxicology and Environmental Safety* 73, 7-17.

Stienen, E.W.M., Van Beers, P.W.M., Brenninkmeijer, A., Habraken, J.M.P.M., Raaijmakers, M.H.J.E. and Van Tienen, P.G.M., 2000. Reflections of a specialist: Patterns in food provisioning and foraging conditions in Sandwich Terns Sterna sandvicensis . *Ardea* 88, 33-49.

Stienen, E.W.M., Brenninkmeijer, A. and Klaassen, M., 2008. Why do Gull-billed terns Gelochelidon nilotica feed on fiddler crabs Uca tangeri in Guinea-Bissau. *Ardea* 96, 243-250.

Strum, K.M., Alfaro, M., Haase, B., Hooper, M.J., Johnson, K.A., Lanctot, R.B., Lesterhuis, A.J., Lopez, L., Matz, A.C., Morales, C., Paulson, B., Sandercock, B.K., Torres-Dowdall, J. and Elena Zaccagnini, M., 2008. Plasma cholinesterases for monitoring pesticide exposure in Nearctic-Neotropical migratory shorebirds. *Ornitologia Neotropical* 19, 641-651.

Suarez-Serrano, A., Alcaraz, C., Ibanez, C., Trobajo, R. and Barata, C., 2010. *Procambarus clarkii* as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. *Ecotoxicology and Environmental Safety* 73, 280-286.

Tavares, P.C., Kelly, A., Lopes, R.J., Pereira, M.E., Duarte, A.C. and Furness, R.W., 2007. The influence of dietary specialization and trophic status on mercury levels in two species using common coastal wetlands, *Himantopus himantopus* and *Sterna albifrons. Ardeola* 54, 275-288.

Tavares, P.C., McGill, R., Newton, J., Pereira, E., Duarte, A. and Furness, R.W., 2009. Relationships between carbon sources, trophic level and mercury exposure in generalist shorebirds revealed by stable isotope ratios in chicks. *Waterbirds* 32, 311-321.

Thompson, J.J. and Carroll, J.P., 2009. Habitat use and survival of the Spotted Tinamou (*Nothura maculosa*) in agroecosystems in the province of Buenos Aires, Argentina. In: Cederbaum, S., Faircloth, B., Terhune, T., Thompson, J. and Carroll, J. (Eds.), Gamebird 2006: Quail VI and Perdix XII, Warnell School of Forestry and Natural Resources, Athens, GA, pp. 111-119.

Thyen, S., Becker, P.H. and Behmann, H., 2000. Organochlorine and mercury contamination of Little Terns (*Sterna albifrons*) breeding at the western Baltic Sea, 1978-96.

Environmental Pollution 108, 225-238.

Valladares, S., Moreno, R., Jover, L. and Sanpera, C., 2010. Evaluating cleansing effects on trace elements and stable isotope values in feathers of oiled birds. *Ecotoxicology* 19, 223-227.

Veerle, J., Tom, D., Rianne, P., Lieven, B., Ronny, B. and Marcel, E., 2004. The importance of exogenous contamination on heavy metal levels in bird feathers. A field experiment with free-living great tits, Parus major. *Journal of Environmental Monitoring* 6, 356-360.

Vericat, D. and Batalla, R.J., 2006. Sediment transport in a large impounded river: The lower Ebro, NE Iberian Peninsula. *Geomorphology* 79, 72-92.

Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambre, C., Tarazona, J.V., Brandt, I. and Vethaak, A.D., 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. *Critical Reviews in Toxicology* 30, 71-133.

Wayland, M., Kneteman, J. and Crosley, R., 2006. The American dipper as a bioindicator of selenium contamination in a coal mine-affected stream in west-central Alberta, Canada. *Environmental Monitoring and Assessment* 123, 285-298.

Weyers, B., Gluck, E. and Stoeppler, M., 1988. Investigation of the significance of heavy-metal contents of Blackbird feathers. *Science of the Total Environment* 77, 61-67.

Wiggins, D.A. and Morris, R.D., 1986. Criteria for female choice of mates - Courtship feeding and paternal care in the Common Tern. *American Naturalist* 128, 126-129.

Winston, G.W., Regoli, F., Dugas, A.J., Fong, J.H. and Blanchard, K.A., 1998. A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radical Biology and Medicine* 24, 480-493.

Worton, B.J., 1989. Kernel methods for estimating the utilization distribution in home-range studies. *Ecology* 70, 164-168.

Zhang, Y., Ruan, L., Fasola, M., Boncompagni, E., Dong, Y. and Dai, N., 2006. Little egrets (*Egretta garzetta*) and trace-metal contamination in wetlands of China. *Environmental Monitoring and Assessment* 118, 355-368.