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Heavy metal concentration in tissues of *Puffinus gravis* sampled on the Brazilian coast

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RESUMO. **Concentração de metais pesados em tecidos de *Puffinus gravis* amostrados na costa brasileira**. Foram determinados no fígado e rins de adultos e juvenis de *Puffinus gravis* os seguintes metais: Cd, Cu, Pb, Mn e Ni, através de Espectrofotometria de Absorção Atômica. Verificou-se que as concentrações médias de Cd no fígado de adultos foram significativamente diferentes em relação aos juvenis. As concentrações médias de Cu no fígado de *P. gravis* foram diferentes em relação ao rim. Houve diferença também entre os fígados de adultos e juvenis. Os valores médios de Mn encontrados no fígado e rim não diferiram entre adultos e juvenis. Entretanto, as concentrações de Pb no fígado foram significativamente mais altas em adultos do que nos juvenis. As médias das concentrações de Ni no fígado e rins de adultos foram diferentes das dos juvenis. As concentrações dos elementos traços essenciais em *P. gravis* foram comparáveis aos valores registrados em outros estudos. Em relação aos metais não essenciais (Cd, Pb e Ni), *P. gravis* apresentou uma menor concentração do que as registradas para o Atlântico Norte.

PALAVRAS-CHAVE: metal pesado, *Puffinus gravis*, concentração, fígado, rim.

ABSTRACT. The concentrations of Cd, Cu, Pb, Mn and Ni were determined in liver and kidney samples of adult and juvenile *Puffinus gravis* by Flame Atomic Absorption Spectrophotometry. The average Cd concentration in adult livers were significantly different from juveniles. Cu concentration averages were significantly different between liver and kidney, as well as in between the liver of adults and juveniles. Average Mn concentrations in liver and kidney did not differ between adults and juveniles, whereas Ni concentrations in liver and kidney of adults were significantly higher than in juveniles. For Pb concentrations in liver were significantly higher in adults than in juveniles. The concentrations of essential trace elements in *P. gravis* were generally comparable to values reported in other studies. With non-essential metals (Cd, Pb and Ni), *P. gravis* had smaller values than those reported for their northern Atlantic counterparts.

KEY WORDS : heavy metal, *Puffinus gravis*, liver, kidney.

Seabirds offer a number of particular advantages as indicators of heavy metal pollution (Walsh 1990, Furness 1994). One of them is they are quite well known. They feed at the upper trophic levels of oceanic ecosystems and so can provide information on the extent of contamination in the whole food chain (though most metals do not show biodegradation and so, in some cases, there are reasons to prefer the use of sessile invertebrates as monitors). Metal levels in birds may give a better picture of hazards to man than measurements in the physical environment, plants or invertebrates.

Pelagic species had higher levels of mercury and cadmium than did most inshore species, and Nicholson and Osborn (1983) concluded that the levels were natural rather than a consequence of pollution, reasoning that pollution by heavy metals would be more likely to affect inshore species (Muirhead and Furness 1988). Similarly, high levels of metals occur in some Antarctic seabirds where pollution effects might reasonably be assumed to be minimal (Norheim 1987). However, atmospheric transport of metals and selective uptake or storage by marine organisms could cause increases in pelagic ecosystems (Bryan 1984). Seabirds are excellent subject for examination of heavy metals because they are long-lived, feed at different trophic levels at varying distances from land.

The paper presents measurements of Cd, Cu, Ni, Mn and Pb levels in Great Shearwater (*Puffinus gravis)* that breed on South Atlantic Ocean Island, where local pollution by heavy metals is presumably negligible or non-existent.

As a Southern Hemisphere breeder *Puffinus gravis* appears in Brazilian waters in late April and remains into early winter. In April and May, the Greater Shearwater leaves the breeding grounds and migrates north along Brazil to the north Atlantic. In fall, the birds fly east down the northwest coast of Africa and cross the ocean again. It is attracted by chumming and follows in the wake of boats. They are often seen behind a fishing boat that is cleaning fish or where whales are actively feeding.

MATERIAL AND METHODS

Collection and storage of birds. We analyzed 30 specimens (15 adults, 15 juveniles) found dead along the Atalaia Beach of Aracaju in the state of Sergipe, northeast Brazil, from June to July 2003. Immediately after collection, the birds were brought to the laboratory and stored frozen until they were analyzed in the Laboratory of Ecotoxicology of the Universidade Federal de Sergipe. After biometric measurements and weighing, the samples were dissected, and kidney and liver were collected.

Reagents. All reagents were of analytical reagent grade. High purity water (Millipore Milli-Q System) was used throughout. The stock solutions of metals (1000 $mg.L^{-1}$) were obtained by dissolving the appropriate salts or the corresponding metals. Concentrated nitric acid (65%) and hydrogen peroxide (30%) was used for the digestion of the samples. Standard Reference Material (SRM) of oyster tissues (No. 1566b) from the National Institute of Standard Technology (NIST) was used to validate the method.

Sample pretreatment and microwave digestion. The samples were dried in a furnace equipped with circulation system at 60ºC for 48 h, and homogenized using a porcelain mortar. After that it was dissolved by microwave digestion (Anton Paar Multiwave, A-8054 Graz, Austria-Europe) using a system of rotor with six position (6MF100). Approximately 400 mg of dry sample was treated with mixture 2:1 HNO_3:H_2O_2 and placed into the TMF vessel of the Parr reactor. The vessel was closed and heated for 20 min in the microwave oven. The reactor, after cooled, was opened. Then, the resultant solution was quantitatively transferred into a 50 ml volumetric flask and made up to volume with ultrapure water. The solution was stored at 4ºC in polyethylene bottles until it was analyzed.

Cd, Cu, Ni, Mn and Pb were determined by a Flame Atomic Absorption Spectrometry (AA-6800, Shimadzu Co. Ltd, Japan) equipped with background correction BCG-D2. All the instrumental adjustments had been the recommended for the manual of the manufacturer.

All element concentrations ($\mu g.g^{-1}$) in organs were expressed as dry weight basis. As analytical quality control, standard of oyster tissues were analyzed using the same procedure. Our results were in good agreement with the certified values as show in Table 1.

Table 1: Observed and certified values ($\mu g.g^{-1}$) of elemental concentrations in SRM (oyster tissues No. 1566b) as average ± S.D. Each value is the average of three separate digestions.

Element	Observed value	Certified value	Recovery (%)
Cd	2,56 ± 0,06	2,48 ± 0,08	103
Cu	70,8 ± 5,3	71,6 ± 1,6	99
Ni	1,22 ± 0,38	1,04 ± 0,09	117
Mn	18,4 ± 1,6	18,5 ± 0,2	100
Pb	0,316 ± 0,090	0,308 ± 0,009	102

The average distribution of heavy metal by the *P. gravi* was assessed using analysis of variance (ANOVA). All data were analyzed using the Tukey`s multiple comparation test (p< 0,05).

RESULTS

Concentrations of the elements analyzed in the livers and kidneys of adult and juvenile *P. gravis* are presented in Table 2. Pooling adults and juveniles, Cd levels did not differ between kidney and liver (F = 3,55; P = 0,07), but were higher in liver and kidney of adults than in juveniles (F = 7,12; P = 0,02 and F = 5,29; P = 0,04, respectively). Cu concentration averages were significantly different between liver and kidney (F = 42,26; P < 0,001) (Table 2). Livers of adults and juveniles differed in Cu concentrations (F= 14,15; P=0,0017), whereas kidneys did not (F = 0,93; P = 0,35). Mn concentrations in liver and kidney did not differ between adults and juveniles (F = 0,04; P = 0,85) (Table 2). Mn concentration averages in the liver were not significantly different from those in the kidney (F=0,038; P=0,84). Livers of adults and juveniles no differed in Mn concentrations (F=0,54; P=0,47). The concentrations averages of Mn in kidney were not significantly different between adults and juveniles (F = 0,08; P= 0,78). Averages of Ni concentration were not significantly different between liver and kidney (F = 2,41; P = 0,14). However, the Ni concentration in the liver and kidney of adults were higher than in juveniles (F = 14,04; P = 0,001 and F = 24,75; P = 0,0001, respectively) (Table 2). Pb concentration in liver was significantly higher in adults than in juveniles (F = 5,83; P = 0,03). However, Pb concentration averages in livers were not significantly different from those in kidneys (F = 0,68; P=0,42) (Table 2). The kidneys of adults and juveniles did not differ in relation to Pb concentration (F = 0,36; P = 0,56).

DISCUSSION

In this paper the concentration of cadmium was higher in kidney than liver. According to Furness and Monaghan (1987), Cd concentration is always highest in the kidney, where it is thought that a specific metal-binding protein (metallothionein) generally renders the metal harmless. Cd can cause damage to the vertebrate kidney. There is little evidence to suggest that Cd levels are high enough to cause harm in seabirds. However Nicholson and Osborn (1983) report that examination of kidney of *Puffinus puffinus* and *Puffinus gravis* by electron microscopy indicated pathological features similar to those induced in captive Starling treated with Cd in their diet. Cd levels in other tissues tend to be very much lower, except if a bird has recently been exposed to high intake of Cd. In this case the level in the liver may equal or exceed that in the kidney giving an indication of recent acute exposure (Scheuhammer 1987, Furness 1994). In this work the concentration of cadmium was greater in males than females and juveniles. Cd is known to accumulate with age. High Cd levels may lead to tissue damage in seabirds. Osborn *et al*. (1979) observed kidney damage comparable in *Fulmarus glacialis* and *Puffinus puffinus* at Cd levels comparable to those found in *Fulmarus glacialis* and *Eudyptes chrysolophus*. The Cd concentration

Table 2: Values of elemental concentrations (mean ± S.D, $\mu g.g^{-1}$) in liver and kidney of *Puffinus gravis* found dead on a beach on the Atlantic coast of Brazil (n = 30).

Element	**Organs**	
	Liver	**Kidney**
Cadmium		
Adult	10,52 ± 4,8	19,12 ± 11,68
Juvenile	5,03 ± 1,18	7,50 ± 5,01
Copper		
Adult	39,51 ± 10,73	15,27 ± 5,26
Juvenile	20,22 ± 9,11	12,91 ± 3,61
Manganese		
Adult	4,26 ± 3,94	4,61 ± 4,62
Juvenile	5,50 ± 1,32	4,03 ± 1,00
Nickel		
Adult	4,37 ± 2,89	2,77 ± 1,16
Juvenile	0,50 ± 0,25	0,62 ± 0,34
Lead		
Adult	0,28 ± 0,20	0,21 ± 0,22
Juvenile	0,07 ± 0,07	0,15 ± 0,12

reported here, particularly in *P. gravis* species, is consistent with data on other pelagic seabird species from other locations (Honda *et al*. 1990).

Species with particularly high levels of Cd in the kidney tend to feed to a large extent on squid (Muirhead and Furness 1988). Few data are available on the levels of Cd in squid species, but Martin and Flegal (1975) found concentrations of 782; 287 and 121 $\mu g.g^{-1}$ dry weight in the digestive gland of *Symplectoteuthis ovaleansis, Ommastrephes bastrami* and *Loligo opalescens,* respectively.

Significant differences seem to exist in the levels of essential trace elements, Cu and Mn between the liver and kidney of the *Puffinus gravis* investigated in the present study. Similar levels of Cu and Mn have been detected in other sea bird species from different parts of the world (Norhein 1987). The significance of the differences found between liver and kidney for the Cu are difficult to evaluate, exceptionally high levels of Cu were found in a few specimens of *Somateria mollissima.* However, such high Cu levels occur in other species of waterfowl without any signs of toxic effects (Clausen and Wolstrup 1978). Levels were highest for Mn, followed by Cu and Cd. These values represent current levels found in the eggs of *Sterna hirundo* nesting in Barnegat Bay, New Jersey (Burger and Gochfeld 2000). Since both metals can be classified as essential, the similarity in results with other studies indicate a close metabolic regulation of Cu and Mn.

Cu levels in *P. gravis* were comparable or rather higher than those found in *Somateria mollissima* from Spitsbergen (Norhein 1987). High Cu levels in waterfowl, including *P. gravis* at the present study, are likely to have arisen from species-specific bioaccumulation and unlikely to reflect pollution or higher natural background of Cu in their habitats (Kim *et al.* 1996).

Significant differences seem to exist in the levels of Ni between liver and kidney of *Puffinus gravis*. Similar levels of Ni have been detected in other seabird species from different parts of the world (Norhein 1987). The significance of the differences found between liver and kidney for Ni is difficult to evaluate. Exceptionally high levels of nickel were found in a few specimens of *Somateria mollissima*. However, such high Ni levels occur in other species of waterfowl without any signs of toxic effects (Elliot and Scheuhammer 1997, Thompson *et al*. 1998).

Decline in numbers and high mortality of adult Mute Swans *Cygnus olor* have been linked to ingestion of lead weights discarded or lost on riverbanks, canals and ponds by anglers, the Pb being ground in the gizzard and assimilated (Furness 1994). Blood Pb levels, haematocrit and enzyme assays can be used to measure the exposure of individual birds to Pb (O`Halloran *et al*. 1989, Furness 1994). Comparatively little Pb is deposited in oceans such as the south Atlantic, whereas relatively high Pb deposition fluxes are encountered in areas of the northern hemisphere close to anthropogenic sources (Burger and Gochfeld 2000). Low Pb concentrations have been reported for a range of seabird species sampled from oceanic or remote locations (Norheim 1987, Honda *et al.* 1990). Since the effects of anthropogenic emissions of Pb to the environment would be likely to be greatest closer the source of pollution, one would predict that coastal and inshore marine biota would carry the highest Pb burdens, and several workers have found somewhat elevated Pb concentrations in species from such environments (Turner *et al.* 1978, Lee *et al.* 1989, Lock *et al*. 1992). In the specimens analyzed here, adults exhibited the highest Pb levels.

In conclusion, the concentrations of essential trace elements in *P. gravis* were generally comparable to values reported in other studies. With non-essential metals, some marine bird species can accumulate considerable body burdens of Cd and/or Pb. In our study, *P. gravis* had smaller values than those reported for their northern Atlantic counterparts; however, we cannot determine with the available information if this is because of smaller concentration of these metals in South Atlantic, or dietary differences between populations form different oceans. Studies of the birds provide the best means of assessing the impact of metal pollution by fishing weights and focused attention on the quantities of metals being discarded in waters.

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