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The occurrence of lead in the bone tissues of *Trematomus bernacchii*, Terra Nova Bay, Ross Sea

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Abstract: The occurrence of lead in various bone tissues of *Trematomus bernacchii* was investigated in order to identify the optimal target tissue and to examine potential relationships between bone lead concentration and fish size and age. Lead concentration values found in vertebrae and jaws (0.44 ± 0.13 and 0.41 ± 0.09 μg g^{-1} of dry weight tissue, respectively) were significantly higher than those in the other bones examined (otoliths, branchiostegal rays and pectoral girdles), indicating a preferential accumulation of lead in these tissues. Hence, vertebrae and jaws were selected as suitable target organs. The significance of *T. bernacchii* as a marker of lead bio-availability for the marine environment was tested by comparing data with the ones obtained for the *Scorpaena notata*, a benthic fish living in the more polluted coasts of the Mediterranean Sea, and by correlating the lead content found in the fish bones with the concentration of bio-available lead in the marine sediments on which the two fishes live. Bio-availability was evaluated by solid speciation and size fractionation techniques. All data clearly indicated that the difference in bone lead content between *S. notata* and *T. bernacchii* (0.83 ± 0.17 μg g^{-1} and 0.42 ± 0.08 μg g^{-1}, significant at the 99.9% confidence level) can be ascribed to the concentration of bio-available lead in their respective environment. Finally, no correlation between the lead accumulation in bone tissues and fish size and age was found for either fish species.

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Key words: benthic fish, bio-availability, heavy metals, jaws, sediment, vertebrae

Introduction

Due to the continuous overlapping between natural and anthropogenic sources of heavy metals in the environment, comprehensive clarification of the biogeochemical cycles of trace elements and the consequent assessment of the real degree of pollution of a given area are by no means easy tasks. In this framework, the establishment of baseline levels of trace elements in relatively uncontaminated areas and natural interactions of trace elements with biota are of paramount significance. Although certain trace elements from human activities can reach pristine areas by long distance transport, Antarctica is considered as an ideal natural laboratory for studying biogeochemical cycles of trace elements and their natural distribution within the organisms.

Metal accumulation in the various tissues of marine organisms depends on the physical and chemical properties of the surrounding environment and highlights the real bio-availability and physiological pathway of trace elements (e.g. Sharma & Shupe 1977, Talmage & Walton 1991, Philips & Rainbow 1992, Rashed 2001, Gbern 2001). Mineralised tissues, such as bones and teeth, have been found to be useful target organs, because of their property in retaining the elements for long periods (Manea-Krichten *et al*. 1991, Kock *et al*. 1996, Komarnicki 2000).

Lead is a ubiquitous toxic element which is introduced into the environment by natural and anthropogenic processes. The natural contribution is due to earth crust erosion and dust transport under varying climatic conditions, while the anthropogenic sources include the emissions due to industrial activities and the use of gasoline and phosphate fertilizers. Lead is efficiently retained in the calcified tissues of vertebrates, and over 90% of the body burden of Pb is located in the skeleton in a stable chemical form (Manea-Krichten *et al.* 1991). Moreover, the uptake depends on physiochemical characteristics of the water such as alkalinity, hardness and pH and the bone lead content may hence reflect availability of this metal in the environment (Kock *et al.* 1996).

In the framework of the Italian Research Programme in Antarctica (PNRA), the distribution of trace elements in the coastal area of Terra Nova Bay (Ross Sea, Antarctica) has been investigated (Grotti *et al* 2001, 2005a, Frache *et al*. 2001, Dalla Riva *et al*. 2003, 2004), with the main objective of establishing the natural cycles of the elements and identifying some marine organisms that can be used as biomonitors of this peculiar ecosystem. Fish play a central role in the food chain of the marine Antarctic ecosystem, as they feed on krill and other zooplankton species and are preyed on by birds, seals, cetaceans and squid (Hureau

1994, La Mesa & Vacchi 2001). In particular, fish of the perciform suborder Notothenioidei dominate the cold shelf waters surrounding the Antarctic Continent, where they are the main component of the Southern Ocean fauna (Di Prisco 2000). The emerald notothen *Trematomus bernacchii* is a promising bio-marker because it is a non-pelagic fish, ubiquitous along the Victoria Land coast and an opportunistic feeder on benthic invertebrates, molluscs and crustaceans, which have relatively restricted home ranges, spending large periods of time occupying small patches of substrate (Miller *et al.* 1999, Jimenez *et al.* 1999, La Mesa & Vacchi 2001). *Trematomus bernacchii* represents the most abundant component of the fish fauna (in terms of biomass) of Terra Nova Bay, reaching its maximum abundance between 0–100 m depth (Vacchi *et al.* 1999).

In the present study, we investigated the occurrence of lead in various bone tissues of *T. bernacchii*, in order to identify the optimal target tissue and to examine potential relationships between the bone lead concentration and fish size and age, possibly due to accumulation of this metal during life cycle. Moreover, in order to evaluate the significance of *T. bernacchii* as a marker of lead bio-availability for the marine environment, data were compared with the ones obtained for the *Scorpaena notata*, a benthic fish living in the anthropized coasts of the Mediterranean Sea, and correlated with the lead content found in the marine sediments on which the two species live.

Materials and methods

Sample collection and storage

Specimen of *T. bernacchii* were collected in Terra Nova Bay (Ross Sea, sampling area: 74°41'20"–74°44'23"S, 164°06'26"–164°10'00"E, 30–160 m depth) and stored at a temperature of -30°C until analysis. Samples of *Scorpaena notata* were collected in the Ligurian Sea (northern Mediterranean Sea, sampling area: 44°24'21"–44°22'30"N, 8°42'15"–8°52'00"E, 60–90 m depth) and stored at a temperature of -30°C until analysis. Ten specimens for each species were used for this work.

Marine sediments were sampled in the same areas as the respective fishes, by a stainless steel grab, or by SCUBA, and stored in acid washed polycarbonate containers at -30°C until treatment and analysis.

All the samples were stored in the Antarctic Environmental Specimen Bank (Soggia *et al.* 2000, 2001).

Sample preparation and analysis

Organisms were thawed and their bones (vertebrae, otoliths, jaws, branchiostegial rays and pectoral girdles) separated from the soft tissues using clean stainless steel tools. The bone tissues were then cleaned with 10% sodium hypochlorite solution to remove the flesh and freeze-dried. 0.1–0.3 g sub-samples were weighed with an accuracy of ± 0.0001 g and solubilized with 5 ml of concentrated nitric acid, using the microwave digestion system MDS-2000 (CEM, Matthews, NC, USA). Digestion was conducted for 40 min with a maximum (control) pressure of 150 psi, using 100% power. Finally, the samples were transferred into graduated flasks and diluted to 10 ml with Milli-Q water (Millipore, El Paso, TX, USA). For each run, two blanks and one certified reference sample were also prepared to check contamination and analytical accuracy.

Sediment samples were separated into two different granulometric fractions (< 63 μm and > 63 μm), using a stainless steel sieve and dried in oven at 40°C. The total metal content was determined by digesting 0.2 g of the sample in PTFE vessels with 1 ml of concentrated nitric, 3 ml of hydrochloric and 2 ml of hydrofluoric acids (suprapure grade quality, Merck), using the microwave digestion system (CEM DS 2000). Digestion was conducted for 40 min with a maximum (control) pressure of 180 psi, using 100% power. The metal fraction bound to the carbonates and organic labile materials was extracted with acetic acid (0.11 M; pH 2.8), according to the first step of a procedure for metal solid speciation (Ianni *et al.* 2000).

Determination of lead in bone digests was performed using the ICP-AES Vista PRO from Varian (Speringvale, Australia), according to Grotti *et al*. (2005b). Internal standardization using the Co 228.615 nm reference line was chosen as the optimal method to compensate for the matrix effects from the presence of calcium and nitric acid at high concentration levels. The detection limit of the procedure was 0.11 μg g^{-1} of dry weight tissue and precision of the sample preparation step was 5.4%.

Validation of analytical accuracy

The study of trace metals in remote environments, especially lead, has been fraught with measurement problems arising from inadvertent contamination of samples during sampling collection, handling and analysis. The rigorous control and reduction of any source of contamination is of paramount importance. Most of the precautions taken during the sampling and storage steps to avoid or reduce lead contamination included accurate choice and cleaning of all the materials coming into contact with samples, care taken by the operators during the sampling, and critical evaluation of the blanks.

The accuracy of analytical data was tested by analysing several certified reference materials: SRM 1486 (Bone meal, National Institute of Standards & Technology), MURST-ISS-A1 (Antarctic Marine Sediment Certified for Trace Elements by PNRA - National Institute of Health, Italy), MESS-2 (Marine Sediment Reference Materials for Trace Elements by National Research Council Canada), PACS-2 (Marine Sediment Reference Materials for Trace Metals and Other Constituents by National Research

Table I. Found and certified concentrations in the analysed reference materials (mean values and 95% confidence intervals)

Certified reference material	Pb concentration ($\mu g\ g^{-1}$ of dry weight tissue) Found	Certified
SRM 1486	1.32 ± 0.04	1.335 ± 0.014
MURST-ISS-A1	19.8 ± 2.0	21.9 ± 2.9
MESS-2	20.7 ± 2.9	21.9 ± 1.2
PACS-2	175 ± 13	183 ± 8
BCR-701	3.15 ± 1.54	3.18 ± 0.21

Council Canada), BCR 701 (Certified Sediment following a sequential extraction procedure by Community Bureau of Reference, Commission of the European Community).

Results are reported in Table I. By comparing the found values with the certified ones (t-test, 95% confidence interval), it was concluded that all the adopted analytical procedures are accurate and suitable for the analytical task.

Statistics

The two-tailed Student *t*-test was used to compare groups of data, with variances not assumed to be equal. Conclusions were also verified by using the non-parametric Wilcoxon-Mann-Whitney test. Statistical calculations were performed using the software tool XLSTAT (Microsoft Co, USA).

Results and discussion

Lead content in some bone tissues of *T. bernacchii* (otoliths, vertebrae, jaws, branchiostegal rays and pectoral girdles) are reported in Table II. It can be seen that the values found for vertebrae and jaws are significantly higher than those for the other bones, indicating a preferential accumulation of lead in these tissues. Moreover, the concentration values found in vertebrae and jaws are sufficiently high to have satisfactory confidence with the analytical results, while the lowest values found in the other tissues approach the limit of detection of the procedure (0.11 $\mu g\ g^{-1}$ of dry weight tissue). Hence, vertebrae and jaws were selected as optimal target tissues.

In order to evaluate the significance of *T. bernacchii* as a marker of lead bio-availability for the marine environment, data were compared with the ones obtained for *Scorpaena notata*, a benthic fish living in the polluted coasts of the

Table II. Lead content in some bone tissues of *Trematomus bernacchii* (mean values and 95%-confidence intervals, *n* = 10)

Bone tissue	Pb concentration ($\mu g\ g^{-1}$ of dry weight tissue)
Otoliths	< DL
Vertebrae	0.44 ± 0.13
Jaws	0.41 ± 0.09
Branchiostegal rays	0.19 ± 0.09
Pectoral girdles	0.20 ± 0.07

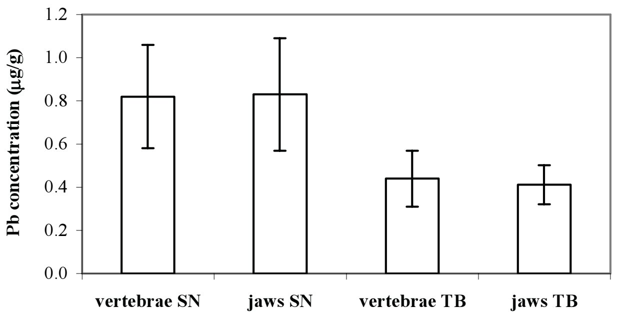

Fig. 1. Comparison between bone lead concentrations ($\mu g\ g^{-1}$ of dry weight tissue) in bone tissues of *Scorpaena notata* and *Trematomus bernacchii* (mean values and 95% confidence intervals, *n* = 10).

Mediterranean Sea. *Scorpaena notata* is a common specie in rocky coastal habitats of the Mediterranean Sea, generally found at depths of up to 700 m, and it feeds on decapods (67–88% of its prey), polychaetes and small fishes (Relini *et al.* 2002).

Lead contents in vertebrae and jaws of *T. bernacchii* (TB) and *S. notata* (SN) are compared in Fig. 1. Despite the high variability among the various organs belonging to the same species, the difference between the bone lead content found in the investigated species is statistically significant at 99.9% probability level (t_{exp} = 4.164; t_{tab} = 3.291; P = 0.0003). This difference could be ascribed to dissimilar lead accumulation mechanisms or reflects the different bio-available lead content of the ecosystems to which the two fishes belong. Since the mechanisms of transport and accumulation of lead are similar to those of calcium (Burnett & Patterson 1980), the first hypothesis was tested by comparing the bone calcium concentration in vertebrae and jaws. Results are reported in Table III. No significant (t-test, α = 0.05) difference in Ca concentrations between the two species may be noted, thus excluding dissimilar lead accumulation mechanisms.

In order to relate the difference in the bone lead content between the two species to the concentration of bio-available lead of the respective environment, sediment samples were collected in the same areas as fishes and analysed for the lead content. Both the total concentration and that associated with the labile fraction were determined, the latter being more significant to establish the real bio-availability of the element (Rubio *et al.* 1991). Moreover, a fractionation was performed to distinguish between the

Table III. Percentage bone calcium concentrations (w/w) in *Scorpaena notata* and *Trematomus bernacchii* (mean values and 95%-confidence intervals, *n* = 10).

Bone tissue	*Scorpaena notata*	*Trematomus bernacchii*
Vertebrae	22.6 ± 1.8	19.9 ± 1.5
Jaws	22.7 ± 1.4	19.2 ± 2.3

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Table IV. Lead total concentration and lead associated with the labile fraction in the marine sediments collected in Terra Nova Bay and Ligurian Sea (mean values (μg g^{-1}) and 95% confidence intervals).

Particle size	Terra Nova Bay labile fraction	Terra Nova Bay total concentration	Ligurian Sea labile fraction	Ligurian Sea total concentration
> 63 μm	0.13 ± 0.06	23.3 ± 5.3	3.7 ± 1.5	113.2 ± 14.3
< 63 μm	0.94 ± 0.32	23.4 ± 5.7	5.6 ± 1.5	83.8 ± 11.9

Table V. Dimensional classes and related range of ages of the specimen investigated (La Mesa & Vacchi 2001).

Species	No. organisms	Total length (cm)	Age (years)
Scorpaena notata	5	10–11	2–4
	5	15–16	> 4
Trematomus bernacchii	5	17–22	8–12
	5	32–33	18–20

pelitic fraction (grain size < 63 μm) from the coarser ones, since only the finest particles can be taken up either directly through their diet or indirectly by feeding on the benthic organisms.

As expected, the total concentration values for the sediments collected in the Ligurian Sea are much higher than those for Terra Nova Bay, without any significant difference in the particle size partitioning (Table IV). Data are in agreement with a previous investigation in the same area (Giordano *et al.* 1999). There was a noticeable difference between the sites for bio-available lead, concentrations in Ligurian sediments are typical of areas influenced by human activity (e.g. Ianni *et al*. 2000), while those in Antarctic sediments are one order of magnitude lower and can be considered as typical of unpolluted coastal waters.

The difference between the sites with regard to bio-available lead is even more marked when considering the textural characteristics of the investigated sediments (Fig. 2). In fact, the pelitic fraction (grain size < 63 μm) of the Antarctic sediments, where the highest concentration of bio-available lead was found, constitutes only 1.8% of the total mass, while most of the sample is formed by coarser particles which contain much lower bio-available lead (Table IV). On the other hand, the pelitic fraction in Ligurian sediments is dominant (94%). Therefore, the difference in bone lead content between *S. notata* and *T. bernacchii* can be ascribed to the concentration of bio-available lead in their respective environments, thus confirming that the fish *T. bernacchii* is a suitable indicator of environmental pollution (Miller *et al*. 1999, Jimenez *et al*. 1999, Dalla Riva *et al*. 2003) and its jaws and vertebrae are optimal target tissues.

Finally, in order to explore the potential relationships between bone lead content and size and age of fish, possibly due to accumulation of this metal during the life cycle, lead concentrations in jaws and vertebrae were determined in two size-classes of each species (Table V). Low temperature, and other ecological factors linked to lifestyle, such as food availability and physiological adaptations, may explain differences in growth performance between the species (La Mesa & Vacchi 2001).

In Fig. 3 it can be seen that there is no relationship between Pb concentration in bone tissues and fish size for either species. This is in agreement with the lack of correlation between heavy metals and fish size reported for different fish species from the Mediterranean Sea (Canli & Atli 2003) and south Indian Ocean (Bustamante *et al.* 2003). The net accumulation of heavy metals in an

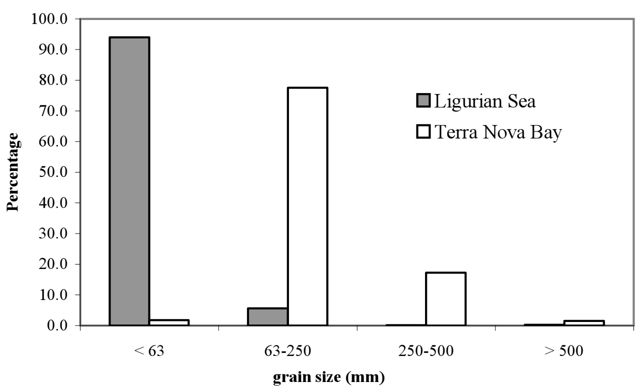

Fig. 2. Textural characters of marine sediments in Ligurian Sea and Terra Nova Bay.

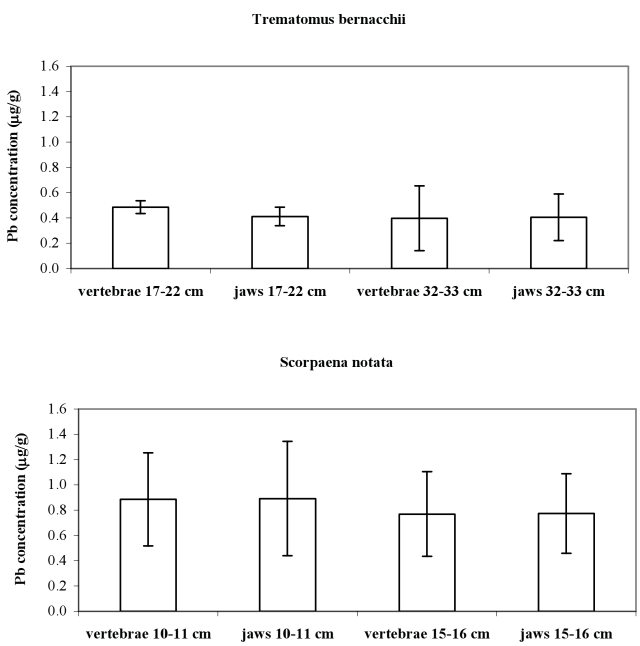

Fig. 3. Lead concentration (μg g^{-1} of dry weight tissue) in vertebrae and jaws of *Trematomus bernacchii* and *Scorpaena notata*, grouped by fish size (mean values and 95% confidence intervals, $n = 5$).

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organism is a result of the difference between uptake and excretion. The metal uptake is primarily affected by the bio-available metal concentration in the environment. Accordingly, for the considered species a good correlation was found between the bone lead concentration and the bio-available lead in their respective environments. Concerning excretion, a number of factors may determine the decrease in concentration with growth such as dilution, lowered metabolic activity and improved detoxification mechanisms in older individuals and changes of diet and life style between the juvenile and adult phases.

Conclusion

The concentration of lead in different bone tissues of *T. bernacchii* is not homogenous, with preferential accumulation in the vertebrae and jaws. The bone lead content for *T. bernacchii* is significantly lower than that for a Mediterranean species (*Scorpaena notata*) **c**ollected in a polluted site and the difference is well correlated with the bio-available lead in the respective environments. Finally, the lack of correlation between the bone lead concentration and fish size or age would appear to rule out the occurrence of net accumulative processes in the tissues investigated.

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