

## ANTARCTIC FISH *TREMATOMUS BERNACCHII* AS BIOMONITOR OF ENVIRONMENTAL CONTAMINANTS AT TERRA NOVA BAY STATION (ROSS SEA)

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**Abstract:** Polychlorinated biphenyls (PCBs, including three highly toxic non-ortho coplanar PCBs, IUPAC Ns. 77, 126 and 169), polycyclic aromatic hydrocarbons (PAHs), pesticides and trace metal concentrations were determined in the Antarctic fish *Trematomus bernacchii* collected off the Baia Terra Nova station (Victoria Land, Antarctica). A biomarker of exposure to xenobiotic compounds, CYP4501A-dependent monooxygenase activity, was also found in the fish liver. Data on fish from the north cove (A, used as a landing place for two small research craft, operating for a few weeks each year) were compared with those of fish from the south cove (B, receiving the station waste water) and with control samples caught on the continental shelf (Gerlache Inlet). Total concentrations of PCB, HCB and DDT residues were higher in fish muscle from cove A than in that from cove B and the control area. The contents of non-ortho coplanar PCBs were extremely low in fish from all sites ( $< 0.5 \text{ pg g}^{-1} \text{ d. wt.}$ ). PAHs were detected in fish muscle from both coves, phenanthrene and anthracene being the most abundant. However, no significant differences were found between specimens from the two areas. Metal (Al, Cd, Cu, Fe, Hg, Mn, Pb and Zn) concentrations in fish muscle, kidney, gills and gonads did not vary between fish from the different sampling sites. A significant relationship between Hg concentration in muscle and fish length was found, showing a high accumulation rate in fish from the control area. Liver CYP4501A detoxification activity was significantly higher in fish from cove B, presumably indicating exposure to organic xenobiotics. However, our results showed that, after ten years of activity of the Italian Antarctic station, contamination of the coastal environment is moderate or less contaminated in comparison with other ecosystems.

### 1. Introduction

Antarctica is one of the last remaining areas of minimum impact due to human activities, scientific stations and tourism being the primary sources of contamination (BOUTRON and WOLFF, 1989; STROMBERG *et al.*, 1990). Thus, according to the principles of the Protocol on Environmental Protection to the Antarctic Treaty (ATCPs 1992), the development and implementation of suitable procedures for environmental impact assessment in Antarctica is a priority task.

The Italian Scientific Station "Baia Terra Nova" (northern Victoria Land, Northern Foothills coast,  $74^{\circ}41'42''\text{S}$ ;  $164^{\circ}07'23''\text{E}$ ) was built during the austral summer

1986–87. Since then, about a hundred persons have visited the scientific station each summer. The electric power generators, solid waste incinerator (paper, wood and cardboard), waste water treatment plant, petrol station, vehicle repair station and helicopter landing and refueling point are the main potential sources of environmental contamination.

During each expedition, a monitoring program of priority pollutants was carried out and data are available on PAH levels in atmospheric particulates (CARICCHIA *et al.*, 1995), organochlorine residues and trace metals in waste water, marine water, sediments and several organisms collected near the station (DESIDERI *et al.*, 1989; MAZZUCOTELLI *et al.*, 1989; CAPELLI *et al.*, 1990; BERKMAN and NIGRO, 1992; FOCARDI *et al.*, 1992; BARGAGLI *et al.*, 1996). In general, very low concentrations, often difficult to resolve against the background, have been reported. The CYP4501A-dependent monooxygenase responses of *Trematomus bernacchii* and *Chionodraco hamatus* exposed to 3-methylcolanthrene and PCBs under laboratory conditions (FOCARDI *et al.*, 1994) and the long-term biological effects of pollutants in coastal benthic communities (NIGRO *et al.*, 1994) have been studied. Recently, comprehensive data on PCBs (FOCARDI *et al.*, 1995) and cadmium (BARGAGLI *et al.*, 1996) levels in the Terra Nova Bay marine food chain have also been reported.

From these studies, it has become clear that the sedentary fish *T. bernacchii* (the most abundant species in nearshore waters), that feeds on polychaetes, molluscs and epibenthic crustaceans and lives about ten years is a very suitable species for monitoring the impact of human activity at the Italian station. Pollutant bioaccumulation and detoxification have been studied in this species. Levels of a wide spectrum of persistent pollutants (PCBs, PAHs, pp'-DDE, HCB and trace metals) have been investigated in organs and tissues. Liver CYP4501A-dependent monooxygenase activity have also been tested. The results of these studies will be useful in the design and implementation of environmental monitoring programs near Antarctic scientific stations.

## 2. Materials and Methods

### 2.1. Samples collection

Two small coves off Terra Nova Bay Station were chosen as sampling sites (Fig. 1). The north cove (A) is used as a landing place for two small craft for a few weeks each year. The south cove (B) receives the waste water of the station (discharge rate about  $10\text{ m}^3\text{ day}^{-1}$ ). Adult specimens of *T. bernacchii* (body weight range = 210–290 g) were caught using gill-nets in January 1994 in the two coves at a depth of 3 to 8 m and in a control area (on the Gerlache Inlet continental shelf) at a depth of about 80 m. Fish were handled carefully to avoid contamination and the liver samples were immediately removed and frozen in liquid nitrogen. Muscle, kidney, gills and gonads were also isolated and stored at  $-20^\circ\text{C}$  for transport to Italy. Sex, length and body weight were recorded for each specimen.

### 2.2. Organochlorine analysis

About 5 g of muscle (dorsal fillet) was digested with 1N KOH/ethanol solution for 1 h and the extract transferred to hexane. The concentrated hexane layer was cleaned

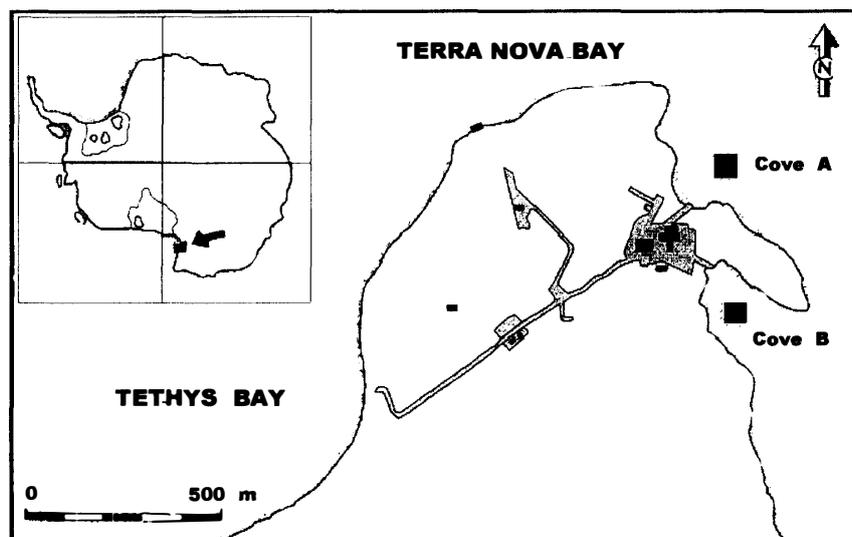


Fig. 1. Location of sampling sites.

on a silica gel column (1.5 g silica gel (Kieselgel 60, 0.63–0.20 mm, Merck), activated at 130°C for 3 h and packed in a glass column (10 mm i.d. × 20 cm length)). The eluate was concentrated in a rotary evaporator to 6 ml. An aliquot of 3 ml was used to determine PCB isomers, while the remaining 3 ml was passed through a column packed with 125 mg of activated carbon to separate non-*ortho* coplanar PCB congeners from other PCB isomers. Elution with 100 ml of benzene/ethylacetate (50:50, v/v) extracted non-*ortho* chlorine substituted coplanar PCBs. Total PCBs were analysed using a Perkin-Elmer Autosystem gas chromatograph equipped with a Ni<sup>63</sup> electron capture detector. A SPB-5 bonded phase (0.25 μm film thickness) fused silica capillary column (30 m long, 0.2 mm i.d.) was used. The carrier gas was helium at 110 kPa (split ratio 25/1). The detector make-up gas was argon/methane (95/5) at a flow of 30 ml min<sup>-1</sup>. Oven temperature was 100°C for 10 min and was increased by 5°C min<sup>-1</sup> to 280°C. Injector temperature was 250°C. Detector temperature was 380°C. GC/MS was used to quantify coplanar PCBs in SIM (selected ion monitoring) mode, using a Finnigan MAT GC/MS MAGNUM ITD System operating under the same gas chromatographic conditions as for GC/ECD (PCB-77 m/z 292, PCB-126 m/z 326 and PCB-169 m/z 360). All analyses were accompanied by a complete quality control program (recovery range was 86–98% for *ortho*-substituted PCBs, 98% for PCB-77, 93% for PCB-126 and 87% for PCB-169). Quantitation procedure was carried out with Arochlor 1260 used as an external standard. Details are provided elsewhere (FOCARDI *et al.*, 1995).

### 2.3. Polycyclic aromatic hydrocarbon analysis

Analysis of PAH residues was carried out in muscle by high performance liquid chromatography (HPLC), with fluorescence detection. PAHs were extracted by caustic digestion in 2N KOH/methanol (1:4, v/v) according to BOOM (1987) with modifications. Solvent partitioning in cyclohexane and purification on a Florisil column were successive steps toward a final organic extract of 1 ml in acetonitrile. A sample size of

20  $\mu$ l was injected in a Waters HPLC system, coupled with a Waters 470 fluorescence detector. PAHs were separated in a Supelcosil LC-18 (25  $\times$  0.46 cm, 0.5  $\mu$ m particle size) column, with the following linear elution gradient: acetonitrile/water (60/40) for 2 min, then to 100% acetonitrile in 18 min, and hold. A constant flow rate of 2 ml/min was used. The excitation and emission wavelengths for each PAH were: naphthalene, acenaphthene and fluorene, 280 nm and 330 nm; phenanthrene and anthracene, 250 nm and 405 nm; fluoranthene, 250 nm and 450 nm; pyrene, 270 nm and 390 nm; benz(a)anthracene, chrysene, benzo(k)fluoranthene, benzo(b)fluoranthene and benzo(a)pyrene, 250 nm and 405 nm; dibenz (a,h) anthracene and benzo (g,h,i) perylene, 290 nm and 405 nm, respectively. Identification and quantitation were carried out using an external standard. With these chromatographic conditions benzo(a)anthracene and chrysene coeluted, so they were quantified as a single peak.

#### 2.4. Trace metals analysis

Samples of lyophilised muscle, kidney, gill and gonad were mineralised with 3 ml of concentrated HNO<sub>3</sub> in Teflon decomposition vessels at a pressure of 120°C for 8 h. Al, Cd, Cu, Fe, Hg, Mn, Pb and Zn were determined by a combination of analytical techniques, including inductively coupled plasma-atomic emission spectroscopy (ICP/AES, Perkin-Elmer 400) and electrothermal atomic absorption spectroscopy with Zeeman background correction (ZETAAS, Perkin-Elmer THGA 4100 ZL). Standard Reference Material n° 1577 "bovine liver" (NIST, Gaithersburg, USA) was used in order to verify the accuracy of the analytical procedure.

#### 2.5. Biomarker analysis

Liver microsomal CYP4501A activity was evaluated by 7-ethoxyresorufin-O-deethylase (EROD), benzyloxyresorufin-O-deethylase (BROD) and benzo(a)pyrene monooxygenase (BPMO) assays. Liver samples were homogenised in 0.1M sucrose buffer (pH=7.5) in a ratio tissue weight/buffer volume (1:4, w/v) and centrifuged at 12000g for 20 min at 4°C. The supernatant was centrifuged at 100000g for 60 min at 4°C to obtain the microsomal pellet, which was resuspended and homogenised in a volume of 0.15% KCl solution (pH=7.5) approximately twice the initial supernatant volume. The homogenate was centrifuged again under the same conditions to purify the microsomal fraction. EROD and BROD activities were determined by the method of LUBERT *et al.* (1985) and expressed as pmol resorufin/min/mg microsomal protein. BPMO activity was determined according to the method of KURELEC *et al.* (1977) and expressed as F.U./h/mg microsomal protein. Microsomal protein was quantified by Bio-Rad Protein Assay.

#### 2.6. Statistical analysis

The data were analysed by comparison of means using the non-parametric Mann-Whitney test. A probability level of 0.05 was regarded as statistically significant. Correlations and basic statistics were carried out using Statistica<sup>©</sup> software.

### 3. Results and Discussion

As shown in Table 1, residues of HCB, pp'-DDE, PAHs and PCBs, including three highly toxic non-ortho PCB congeners were detected in all fish samples. Levels of organochlorine compounds were in the same range as those previously reported in fish of the same species from the Terra Nova Bay continental shelf (FOCARDI *et al.*, 1994, 1995). Concentrations of pp'-DDE and PCBs were higher in fish from cove B. Non-ortho PCB congeners, 33'44'-TCB (IUPAC-77), 33'44'5-PCB (IUPAC-126) and 33'44'55'-HCB (IUPAC-169), were extremely low in all fish samples ( $< 1 \text{ pg g}^{-1}$  wet wt). Low concentrations of PCBs, HCB and pp'-DDE in fish from cove B, which receives the waste water of the station, could be due to partial detoxification of these compounds by CYP4501A-dependent monooxygenases. In fact, an inverse relationship was found between total organochlorine xenobiotics, including HCB, pp'-DDE and total PCBs in muscle, and EROD and BPMD activities (Fig. 2). Liver EROD and BPMD activities were significantly higher ( $p < 0.01$ , Mann-Whitney test) in fish from cove B (Table 2). Induction of the mixed function oxidase (MFO) system was probably enhanced by other xenobiotics in the waste waters. Moreover, values measured at this site were significantly higher than those reported by KENNICUTT II *et al.* (1995) for specimens of *T. bernacchii* sampled near the pier ( $121 \pm 54 \text{ pmol min}^{-1} \text{ mg}^{-1}$ ) or in the seawater intake at Palmer Station ( $30 \pm 20$ ) and at the Bahia Paraiso wreck area ( $30 \pm 29 \text{ pmol min}^{-1} \text{ mg}^{-1}$ ). Nevertheless, HCB, pp'-DDE and total PCB levels in

Table 1. Average and range of HCB, DDTs, total PCBs, PAHs ( $\text{ng g}^{-1}$ , wet wt), non-ortho PCB congeners ( $\text{pg g}^{-1}$ , wet wt) concentrations in muscle of *T. bernacchii* from Baia Terra Nova and Syowa Stations.

Site	n	Species	HCB	pp'-DDE	PCBs	Ns. 77	Ns. 126	Ns. 169	PAHs	ref.
Terra Nova Bay <sup>a</sup>										
Cove A	5	<i>T. bernacchii</i>	0.10 $\pm 0.06$	0.70 $\pm 0.38$	17.5 $\pm 6.72$	0.29 $\pm 0.29$	0.40 $\pm 0.45$	0.42 $\pm 0.43$	86.22 (nd-157.0)	
Cove B	7	<i>T. bernacchii</i>	0.04 $\pm 0.02$	0.32 $\pm 0.48$	3.89 $\pm 1.41$	0.42 $\pm 0.29$	0.47 $\pm 0.29$	0.49 $\pm 0.25$	115.27 (10.9-414.2)	
Syowa Station	5	<i>T. bernacchii</i> (whole body)		0.7 (0.5-0.9)	0.17 (0.12-0.24)					1

<sup>a</sup> SUBRAMANIAN *et al.* (1983).

Table 2. Average and range of EROD, BPMD and BROD activities in liver microsomes of *T. bernacchii*.

Site	Species	n	EROD <sup>a</sup>	BPMD <sup>b</sup>	BROD <sup>a</sup>
Terra Nova Bay					
Cove A	<i>T. bernacchii</i>	6	3.47 (0.88-9.75)	20.95 (3.82-58.48)	0.08 (0.03-0.13)
Cove B	<i>T. bernacchii</i>	7	205.48* (172.92-241.04)	557.92* (408.35-802.04)	0.96 <sup>NS</sup> (0.36-1.90)

<sup>a</sup> Expressed as pmol resorufin/min per mg of microsomal protein.

<sup>b</sup> Expressed as F.U./h per mg of microsomal protein.

\*  $p < 0.01$  (Mann-Whitney test). NS, not significant.

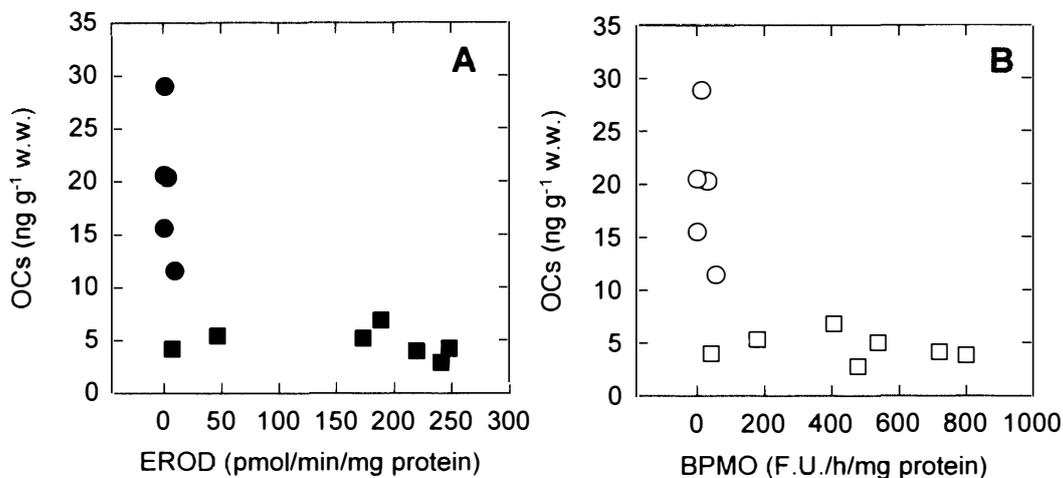


Fig. 2. Correlations between total organochlorine compounds (OCs) and EROD (A) and BPMO (B) activities in fish collected from cove A (circles) and cove B (squares).

fish from cove B were quite similar to those reported by FOCARDI *et al.* (1995) for *P. bernacchii* caught in Gerlache Inlet.

The presence of PAHs in Antarctic environments with human settlements has been widely recognized (McDONALD *et al.*, 1992; CRIPPS and PRIDDLE, 1991; GRREN *et al.*, 1992; KENNICUTT II *et al.*, 1992a, b). According to the results of these studies, PAH content varies widely, even among specimens caught in the same cove. These variations are usually ascribed to fluid loss from fresh animals and chemical modification during the drying process. However, the data from fish caught in the two coves was in the same range as values reported in previous studies (McDONALD *et al.*, 1992). Phenanthrene and anthracene were the most abundant PAHs in *T. bernacchii*. This is usually an index of a combustion source (LAW and BISCAYA, 1994) but further research will be necessary to single out a specific combustion process. McDONALD *et al.* (1992) found that phenanthrene and dibenzothiophenes were the most abundant PAHs in the stomach contents of fish collected near the Bahia Paraiso wreck (Arthur Harbor, USA). They suggested that sediments were the primary source of contamination. Considering the feeding habit of *T. bernacchii* and the presence of high-molecular weight PAHs (especially in cove B) accumulation through predation on invertebrates also seems likely. On the other hand, as phenanthrene and anthracene (*i.e.* two and three ring aromatics quite soluble in water) are the most abundant in all specimens, direct uptake from sea water cannot be excluded.

Trace metal concentrations in organs and tissues of *T. bernacchii* are reported in Table 3. Fish collected in cove A showed significant enrichment of Al and Fe in gills, probably due to entrapment and/or adsorption of sediment particles suspended by pier maintenance operations in January 1994. Human activity at the Italian scientific station had not determined any significant bioaccumulation of the other trace elements in fish from the two coves with respect to those caught in Gerlache Inlet. Metal concentrations in *T. bernacchii* were in the same range as those measured in benthopelagic fish from the Atlantic and Pacific oceans (HARDING and GOYETTE, 1989; STEIMLE *et al.*, 1990) and in *Pagothenia borchgrevinki* collected near Syowa Station

Table 3. Average ( $\pm$ SD) trace metal concentrations ( $\mu\text{g g}^{-1}$  d. wt) in tissues and organs of *T. bernacchii*.

Site	Organ or tissue	n	Al	Cd	Cu	Fe	Hg	Mn	Pb	Zn
Cove A	Muscle	6	1.6 $\pm$ 0.5	0.05 $\pm$ 0.01	2.3 $\pm$ 0.6	5.9 $\pm$ 1.4	0.60 $\pm$ 0.22	0.4 $\pm$ 0.2	0.05 $\pm$ 0.03	23.6 $\pm$ 4.2
Cove B		7	1.2 $\pm$ 0.4	0.04 $\pm$ 0.01	1.9 $\pm$ 0.5	5.9 $\pm$ 1.5	0.49 $\pm$ 0.25	0.5 $\pm$ 0.3	0.06 $\pm$ 0.03	22.8 $\pm$ 3.8
Gerlache Inlet		14	1.1 $\pm$ 0.2	0.03 $\pm$ 0.01	2.5 $\pm$ 0.5	6.1 $\pm$ 1.3	0.74 $\pm$ 0.21	0.6 $\pm$ 0.2	0.04 $\pm$ 0.01	23.1 $\pm$ 3.4
Cove A	Kidney	6	10.4 $\pm$ 3.4	2.2 $\pm$ 0.8	5.1 $\pm$ 0.7	127 $\pm$ 27	0.72 $\pm$ 0.30	3.2 $\pm$ 0.9	0.28 $\pm$ 0.12	116 $\pm$ 29
Cove B		7	9.8 $\pm$ 3.1	1.8 $\pm$ 0.5	5.1 $\pm$ 0.6	147 $\pm$ 41	0.47 $\pm$ 0.27	4.1 $\pm$ 1.9	0.26 $\pm$ 0.11	110 $\pm$ 20
Gerlache Inlet		14	7.8 $\pm$ 2.9	3.0 $\pm$ 1.6	5.7 $\pm$ 0.8	135 $\pm$ 38	0.83 $\pm$ 0.24	5.0 $\pm$ 1.3	0.30 $\pm$ 0.09	110 $\pm$ 14
Cove A	Gills	6	72.3 $\pm$ 26.4	0.20 $\pm$ 0.13	3.8 $\pm$ 0.5	220 $\pm$ 88	0.13 $\pm$ 0.03	12.3 $\pm$ 3.8	0.10 $\pm$ 0.03	77 $\pm$ 15
Cove B		7	46.8 $\pm$ 21.8*	0.31 $\pm$ 0.16	3.6 $\pm$ 0.7	98 $\pm$ 41*	0.16 $\pm$ 0.04	10.9 $\pm$ 3.1	0.09 $\pm$ 0.02	72 $\pm$ 14
Cove A	Ovary	4	2.5 $\pm$ 1.0	0.22 $\pm$ 0.08	4.8 $\pm$ 1.4	52 $\pm$ 11	0.11 $\pm$ 0.02	3.7 $\pm$ 1.3	0.10 $\pm$ 0.04	105 $\pm$ 21
Cove B		5	2.0 $\pm$ 0.9	0.15 $\pm$ 0.04	5.1 $\pm$ 1.0	46 $\pm$ 6	0.15 $\pm$ 0.03	4.6 $\pm$ 1.0	0.12 $\pm$ 0.05	109 $\pm$ 24
Cove A	Testis	2	3.0 $\pm$ 1.5	0.13 $\pm$ 0.05	6.0 $\pm$ 1.4	56 $\pm$ 8	0.13 $\pm$ 0.02	4.3 $\pm$ 1.5	0.20 $\pm$ 0.09	127 $\pm$ 16
Cove B		2	2.1 $\pm$ 0.9	0.15 $\pm$ 0.03	6.2 $\pm$ 1.5	65 $\pm$ 12	0.13 $\pm$ 0.04	4.0 $\pm$ 1.3	0.12 $\pm$ 0.03	120 $\pm$ 18

\* $p < 0.05$ , Mann-Whitney test.

(HONDA *et al.*, 1983). The latter authors reported that the Fe and Hg contents of Antarctic fish were lower than in fish from other seas and oceans, whereas Cd concentrations were higher.

The low concentrations of Fe in *T. bernacchii* are clearly due to the reduced number of red cells in the blood of Antarctic Notothenioidei ( $25 \text{ gl}^{-1}$  of haemoglobin, WELLS *et al.*, 1980). In contrast, the high Cd concentrations in fish organs, especially kidney, are not due to physiological adaptation or environmental pollution. A comprehensive survey of Cd distribution in planktonic and benthic organisms from the coastal waters of Terra Nova Bay has shown that the widespread accumulation of Cd in marine organisms is due to a biogeochemical cycle similar to that of P (BARGAGLI *et al.*, 1996). The rapid regeneration of Cd and its natural occurrence and bioavailability in a coastal area characterized by enhanced upwelling and elevated rates of primary production in summer determines Cd accumulation in plankton and organisms in different trophic levels of the marine food web. Metal detoxification in fish, molluscs and crustaceans is performed by the synthesis of metallothioneins which mainly accumulate in the liver (or the digestive gland) and the kidneys (VIARENGO *et al.*, 1994).

When compared to the results of a long-term survey of Hg, Se and methylmercury in *T. bernacchii* from Gerlache Inlet (MINGANTI *et al.*, 1995) and data reported for *Notothenia gibberifrons* from the Antarctic Peninsula (SZEFER *et al.*, 1993), the Hg concentrations recorded in the present study turn out to be similar to those reported in several species of fish from other seas and significantly higher than those reported by HONDA *et al.* (1983). It is well known that Hg accumulates as methylmercury in marine organisms as a function of age and trophic level. *T. bernacchii* specimens from both coves showed a similar relationship between Hg accumulation and body weight, whereas fish from Gerlache Inlet showed enhanced accumulation (Fig. 3). This difference is probably due to different dietary habits and/or growth rates between fish from coastal and continental shelf environments. Moreover, since *T. bernacchii* is a seden-

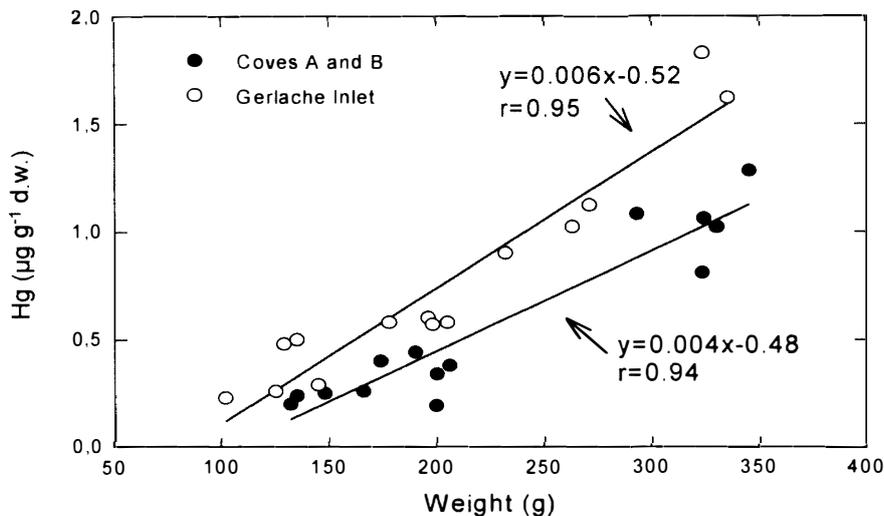


Fig. 3. Correlation between Hg concentration in fish muscle and body length.

tary species and therefore reflects environmental contamination at a local level, the different accumulation rate of Hg shows that, as for Cd, human activity carried out in the Baia Terra Nova station is not a significant source of Hg for the marine environment. As shown in a previous survey of terrestrial ecosystems along the coasts of northern Victoria Land (BARGAGLI *et al.*, 1993) the widespread occurrence of Hg in Antarctic environments is probably due to geochemical and geothermal anomalies and volcanic activity in the Ross Sea region.

#### 4. Conclusions

The above results show that ten years since the establishment of the Italian Antarctic station "Baia Terra Nova" on the coast of the Northern Foothills, no metal contamination is detectable in fish, even close to the waste water outfall. Compared to previous data on marine sediments, water, molluscs and other marine organisms from Terra Nova Bay, the present study shows that enhanced accumulation of Cd and Hg in the biota are due to natural processes related to the environmental biogeochemistry of these metals rather than to human activity at the station. On the other hand, slight contamination by chlorinated and aromatic hydrocarbons was detected, probably due to fuels and combustion processes. Enhanced detoxification activity (*i.e.* CYP4501A-dependent monooxygenases) detected in the liver of fish caught near the waste water outfall (cove B) presumably indicates exposure to these and other organic pollutants and should be associated with lower concentrations of xenobiotics in fish organs and tissues. Furthermore, the possibility of comparison with available data on *T. bernacchi* from other Antarctic scientific stations makes this organism a suitable biomonitor for human impact assessment in the Antarctic coastal environment.

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