

# Cadmium and copper contents in a freshwater fish species (brook trout, *Salvelinus fontinalis*) from the subantarctic Kerguelen Islands

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**Abstract** The subantarctic Kerguelen Islands (49°S, 70°E) contain freshwaters among the most isolated in the world from direct human activities. Cadmium and copper concentrations were analyzed in muscle and liver tissues of 57 non-migratory brook trout (*Salvelinus fontinalis*) inhabiting the Sud River of Kerguelen Islands. The mean cadmium concentration in liver was 1.13 µg/g dry wt, within the range of levels measured in liver of marine fish from the Southern Ocean. Muscular Cd levels (0.12 µg/g dry wt) were roughly ten times higher than those measured in Kerguelen's marine fish species. Copper levels were very high in the two organs (62.27 µg/g dry wt in liver and 3.02 µg/g dry wt in muscle) compared to those detected in fish from the Southern Ocean. Regarding the seasonal trend, the highest Cu and Cd muscular levels were measured in fish at

the end of the austral winter, whereas the highest hepatic levels were observed at the end of the austral summer. Moreover, hepatic cadmium levels were higher in females than in males. These results could be related to brook trout spawning physiological preparations and foraging behavior during the summer period. We provide here the first results about Cu and Cd levels in liver and muscle of a freshwater fish species in an insular subantarctic context. They are in agreement with the high cadmium contamination found in fish of the Southern Ocean.

**Keywords** Cadmium · Copper · Brook trout · Freshwater salmonids · Subantarctic · Southern Ocean

## Introduction

The geographical localization of Antarctic and subantarctic areas makes them isolated from anthropic activities and direct pollutant inputs. However, several studies have revealed the presence of anthropogenic chemical contaminants such as persistent organic pollutants (POPs) and heavy metals (e.g. Hg) in water sediments and marine organisms of these areas (Weber and Goerke 1996, 2003; Van den Brink 1997; Bogillo and Bazylevska 2007; Bargagli 2008; Jöst and Zauke 2008). In these environments, most persistent contaminants are transported by the atmospheric pathway from other continents to the southern hemisphere in concentrations often lower than those detected in remote regions (Corsolini 2009). However, in the Southern Ocean, concentrations of Cd in waters are higher than those observed in other marine waters (Bargagli 2008). Furthermore, an important accumulation of cadmium was observed in several marine organisms of the Southern Ocean (invertebrates, fishes, birds, mammals, etc) referred to as the “Cd

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anomaly” (Bargagli et al. 1996; Kahle and Zauke 2002, 2003; Keil et al. 2008). Cadmium is classified as one of the most hazardous heavy metals (Ravera 1984). Water pollution by cadmium usually results from industrial activities such as metallurgy, electroplating and combustion of coal and oil (Sadegh Safarzadeh et al. 2007). In the Southern Ocean, this metal accumulation may be naturally due to particular characteristics of this area such as the upwelling of Cd-rich waters and local volcanism. These factors may increase the bioavailability of metals in the Antarctic oceanic environment (Plancke 1977; Sanchez-Hernandez 2000).

The Kerguelen Archipelago (49°S, 70°E), part of the French Austral and Antarctic Territories (TAAF), is located at the northern limit of the Southern Ocean. The non-permanent human population in the Kerguelen Islands is composed of about sixty to one hundred people, living almost exclusively on the scientific station of Port-aux-Français. Thus, most of the archipelago (total area: 7,215 km<sup>2</sup>) is devoid of human activities. The freshwater hydrographic system of the Kerguelen Islands constituted by rivers, lakes and ponds contains a simple biocenosis with originally no endemic freshwater fish species. From 1955 to 1992, salmonids [principally brown trout, *Salmo trutta* L., and brook trout, *Salvelinus fontinalis* (Mitchill 1815)] were regularly introduced in these freshwater systems where they became well acclimatized (Davaine and Beall 1997). In the marine zone surrounding Kerguelen, high concentrations of Cd were detected in organisms such as octopus and fish (Bustamante et al. 1998a, b, 2003) and may be related to an essential element depletion, e.g., copper (Petri and Zauke 1993; Bustamante et al. 2003). Copper is an essential trace element that can be substituted by a non-essential metal (e.g., Cd) in case of deficiency. But copper may have toxicological impacts on fish physiology in some environmental contexts (Paris-Palacios et al. 2000; Paris-Palacios and Biagianni-Risbourg 2006). Although important accumulations of cadmium were detected in several marine organisms, no data are available concerning the levels of heavy metal in Antarctic or subantarctic freshwater animal organisms.

Among the numerous Kerguelen water bodies, where brook trout was introduced, the “Sud” River (Courbet Peninsula, Fig. 1b, c) was particularly interesting because its upstream part is situated above two impassable waterfalls. Thus, brook trout living in this section were exclusively sedentary individuals never in contact with marine waters. Another interesting fact was that this resident brook trout population suffered no interference from other salmonid species, especially brown trout, which is not present in this part of the river (Duhamel et al. 2005). Thus, interspecific interactions may not disturb biological parameters of fish in such situation. The present study is the first to investigate

cadmium accumulation with respect to copper levels in a freshwater fish species (brook trout) in the subantarctic zone of the Southern Ocean.

Liver accumulates high concentration of metal regardless of the uptake route. It is one of the major sites of metal metabolism and detoxication in fish, and it is considered as a good monitor of water pollution (Besser et al. 2001; Jezierska and Witeska 2006). Muscles in comparison with liver usually show low concentrations of metals but are often examined for metal content due to their use for animal and human consumption. They were also implicated in long-term metal storage in fish organisms (Alibabic et al. 2007). Cadmium and copper contents were thus analyzed in these two organs on a total sample of 57 brook trout. Origin and seasonal trends of heavy metals in Kerguelen brook trout were discussed.

## Materials and methods

### Animal and sampling sites

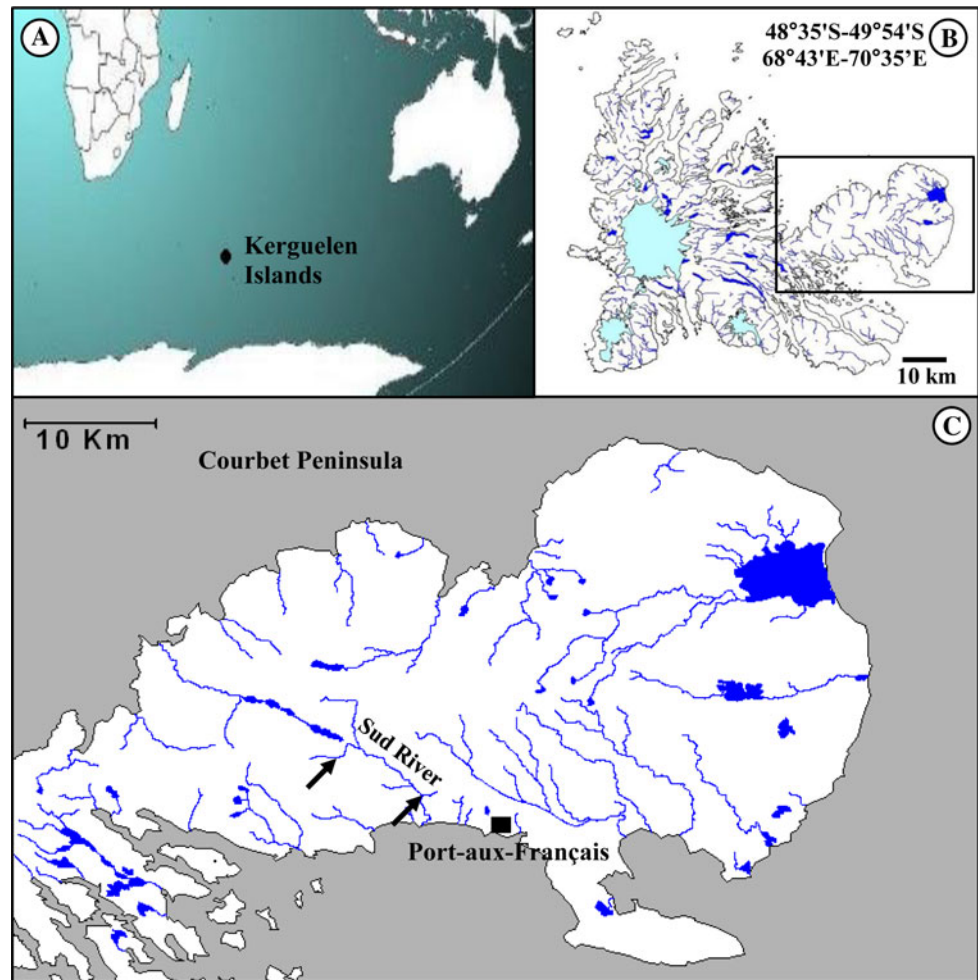
The Kerguelen Islands, located 3,300 km away from the nearest inhabited country, constitute an archipelago in the Southern Ocean subjected to a cold subantarctic climate (Fig. 1a). They possess a complex hydrographic network of rivers, lakes and ponds in various watersheds (Fig. 1b). The Kerguelen brook trout populations originated from the same and unique gene pool introduced in 1961 (Duhamel et al. 2005). This fish has essentially a sedentary behavior in river, but some individuals may be migratory (Duhamel et al. 2005).

Sampling in the Sud River on the Courbet Peninsula (Fig. 1c) was distributed over four locations (S49°17.317'–E70°03.759'; S49°17.362'–E70°04.085'; S49°17.377'–E70°04.045'; and S49°18.436'–E70°06.306') approximately 10–15 km away from Port-aux-Français (Fig. 1c). The four sampling sites were placed between two series of impassable waterfalls (indicated by arrows in Fig. 1c).

### Sampling procedure

Fish were caught monthly by angling, between January and December 2005. They were killed immediately by section of the neural axis, weighed (nearest g) and measured (fork length, cm). The condition factor (CF) was estimated as  $W \times 100/L^3$ , where  $W$  = total weight and  $L$  = fork length. On each individual, pieces of liver and muscle tissues were dissected and kept frozen at –20°C until return to the laboratory in Port-aux-Français. The sex was recorded during the dissection procedure. In the laboratory, samples were dried at +70°C for 15 days, weighed and then digested in nitric acid (65%, Normatom) for 15 days. The resulting

**Fig. 1** Localization of the studied site in the Sud River, Kerguelen Islands. Southern Ocean fish were sampled in a section of the Sud River (between the two arrows) in the Courbet Peninsula. Human presence in this area was limited to the surroundings of the station of Port-aux-Français (filled square)



clear solutions were diluted with high-quality deionised (Milli-Q) water.

Four water samples were collected each month during the entire year and in the four same locations of the Sud River where brook trouts were caught ( $n = 48$  for the year). Water samples were immediately acidified by nitric acid (65%, Normatom).

#### Metallic element quantification

The heavy metal analyses were conducted after the return of mineralized liver and muscle samples back to France. Cadmium was measured by graphite furnace atomic absorption spectrometer (AAS, Varian, Zeeman 220) and copper by flame AAS (Varian, AA 240 FS, Fast sequential atomic absorption spectrometer). Metal concentrations were calculated using calibration curves, and results were expressed in  $\mu\text{g/g}$  dry weight. Standard stock solutions were obtained from NRC corresponding to certified standard liver from *Squalus acanthias* (DOLT-3; National Research Council of Canada) and certified standard muscle from *Scyliorhinus canicula* (DORM-2; National Research

Council of Canada). Instrumental quantification limit was  $0.023 \mu\text{g/l}$  for cadmium and  $6 \mu\text{g/l}$  for copper. All samples were run in duplicate. The variation coefficient of the replicate analysis ranged from 2 to 5%.

A total sample of 57 fish from the Sud River was analyzed. Data obtained from fish caught in February, March and April were regrouped in one “end of summer” period and those obtained in September, October and November in one “end of winter” period. These two distinct periods corresponded to differences in energetic status of brook trouts (Duhamel et al. 2005).

Water metallic analyses were performed by graphite furnace atomic absorption spectrometry (AAS, Varian, Zeeman 220) with instrumental quantification limit of  $0.023 \mu\text{g/l}$  for cadmium and  $0.3 \mu\text{g/l}$  for copper.

#### Statistical analyses

Differences in means (metal concentrations or morphometric data) between males and females or the two seasonal periods for each sex or for the whole studied group were evaluated by Student’s unpaired  $t$  test of significance after verifying

**Table 1** Morphometric parameters of fish sampled in the Sud River, Kerguelen Islands

Sex	<i>n</i>	Length (cm)	Weight (g)	CF
♀	28	18.12 ± 0.37 (14.8–22.4)	61.03 ± 3.95 (20–125)	0.99 ± 0.03 (0.54–1.38)
♂	29	17.83 ± 0.39 (14.1–22.5)	57.66 ± 4.17 (20–110)	0.97 ± 0.03 (0.59–1.34)
Total	57	17.98 ± 0.27 (14.1–22.5)	59.39 ± 2.86 (20–125)	0.98 ± 0.02 (0.54–1.38)

*n* number of fish, CF condition factor. Values of length, weight and CF are expressed in means ± SE (min–max in parentheses)

that data followed the Gaussian distribution. Associations among metal concentrations and morphometric characteristics of fish were evaluated by Pearson correlation analyses. Statistical analyses were performed with the computer software Statistica version 5.1 (StatSoft France 1997). The level of statistical significance was  $P < 0.05$ .

The present scientific protocol was examined with respect to animal welfare and agreed upon by the Ethic Committee of the Midi-Pyrénées Region (France).

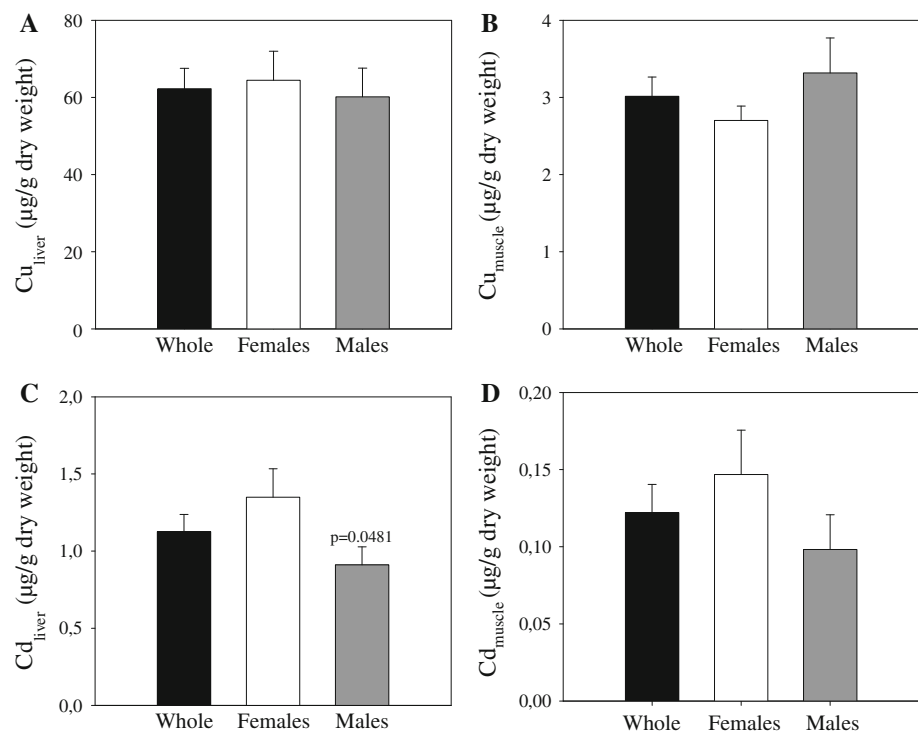
## Results

The sampling resulted in the capture of 57 fish, in equivalent numbers of female and male individuals with identical morphometric parameters (Table 1). The mean condition

factor of captured brook trouts was  $0.98 \pm 0.02$ . No significant intersexual difference was observed in fish length, weight and condition factor.

Brook trout caught in the Sud River had a mean hepatic copper concentration of  $62.27 \pm 5.26 \mu\text{g/g}$  dry wt. (Fig. 2a). This level was approximately the same for females and males ( $64.46 \pm 7.54 \mu\text{g/g}$  and  $60.16 \pm 7.44 \mu\text{g/g}$  dry wt, respectively;  $P = 0.69$ ). In muscular tissue, the overall copper concentration was  $3.02 \pm 0.25 \mu\text{g/g}$  dry wt with no significant differences between sexes ( $2.7 \pm 0.19$  and  $3.32 \pm 0.45 \mu\text{g/g}$  dry wt for females and males, respectively;  $P = 0.22$ ; Fig. 2b). A marginally significant difference was noticed between Cd concentrations measured in liver of females and those registered in males ( $1.35 \pm 0.18$  and  $0.91 \pm 0.12 \mu\text{g/g}$  dry wt, respectively;  $P = 0.048$ ). For all individuals, the hepatic cadmium concentration was  $1.13 \pm 0.11 \mu\text{g/g}$  dry wt (Fig. 2c). The muscular Cd concentration was  $0.12 \pm 0.02 \mu\text{g/g}$  dry wt with no significant sex-dependent difference ( $0.15 \pm 0.03$  and  $0.10 \pm 0.02 \mu\text{g/g}$  dry wt for females and males, respectively;  $P = 0.186$ ; Fig. 2d). These means were associated with maximum values of copper or cadmium levels reached in the two tissues at the individual level. The maximal concentration registered in liver of one trout was  $168.81 \mu\text{g/g}$  dry wt for copper and  $4.26 \mu\text{g/g}$  dry wt in another trout for cadmium (Table 2). In muscle, maximal values of  $12.81 \mu\text{g/g}$  dry wt of copper and  $0.65 \mu\text{g/g}$  dry wt of cadmium were detected in the same fish (Table 2). A significant positive correlation exists between Cu and Cd

**Fig. 2** Copper and cadmium concentrations in liver and muscle of brook trout from the Sud River, Kerguelen Islands. Bars are means ± SE of metal concentrations measured in liver or muscle tissues of females (white bars,  $n = 28$ ), males (gray bars,  $n = 29$ ) and in the whole sample (black bars,  $n = 57$ ) of brook trout. Significant differences in means between female and male metal levels are indicated with  $P$  values (Student's  $t$  test;  $P < 0.05$ )



**Table 2** Maximal values ( $\mu\text{g g}^{-1}$  dry wt) of copper and cadmium in muscle and liver of Kerguelen brook trout in the Sud River, Kerguelen Islands

	Females		Males		MPL <sup>b</sup>
	End of winter	End of summer	End of winter	End of summer	
<b>Muscle</b>					
Cu	5	3.53	12.81	4.55	0.5 <sup>a</sup>
Cd	0.64	0.34	0.65	0.14	0.25 <sup>a</sup>
<b>Liver</b>					
Cu	97.72	168.81	80.4	150.91	–
Cd	2.35	4.26	1.45	3.23	–

<sup>a</sup> Data were converted from wet wt to dry wt using a correction factor of 5

<sup>b</sup> Maximum permissible levels for human consumption in Europe

concentrations in muscle of Kerguelen brook trout ( $r = 0.462, P = 0.0001$ ; Table 3).

Trout condition factors ranged from 0.54 to 1.38 during the year (Table 1). No correlation links were demonstrated between individual condition factors and tissular metal concentrations despite significant positive correlations of

individual weight and length data with liver Cd concentrations ( $r = 0.49, P = 0.0001$ , and  $r = 0.58, P = 0.0001$ ; Table 3). Moreover, Cd concentrations in liver were positively correlated with copper levels in the same tissue ( $r = 0.25, P = 0.042$ ). Another positive correlation was observed in the muscular compartment between Cd and Cu levels ( $r = 0.462, P = 0.0001$ ; Table 3).

In muscle, the maximal copper and cadmium concentrations were observed in fish caught during the end of winter for males and females. But in liver, concentrations of Cu and Cd were higher at the end of summer in comparison with values obtained at the end of winter (Table 2). Condition factor of fish was different between these two periods ( $1.04 \pm 0.04$  for trout at the end of summer and  $0.9 \pm 0.03$  for trout at the end of winter,  $P = 0.0098$ ). No difference for the other morphometric parameters was found (Table 4).

Mean copper and cadmium concentrations during the two seasonal periods were higher in fish liver at the end of summer compared to those measured in the same tissue at the end of winter ( $71.77 \pm 10.25$  and  $48.91 \pm 4.55 \mu\text{g/g}$  dry wt for copper,  $P = 0.079$ ;  $1.33 \pm 0.19$  and  $0.81 \pm 0.14 \mu\text{g/g}$  dry wt for cadmium,  $P = 0.048$ ; Figs. 3a, 4a). Opposite results were obtained in the muscular compartment, the highest mean copper and cadmium levels being

**Table 3** Pearson correlation coefficient among tissues' metal concentrations and morphometric parameters in Kerguelen brook trout

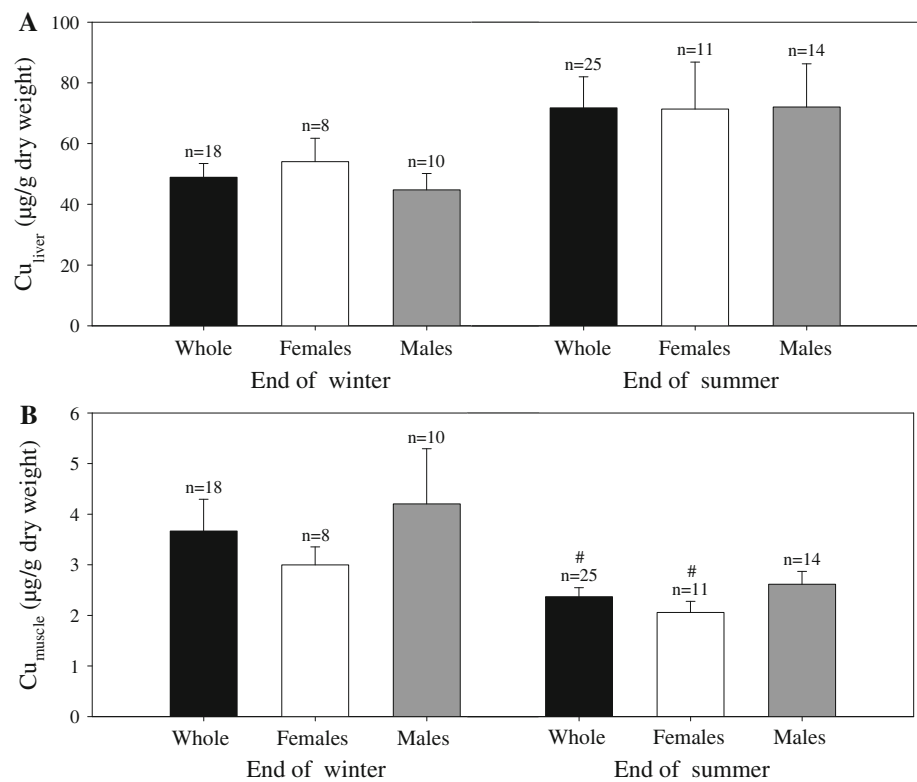
	Cu (liver)	Cu (muscle)	Cd (liver)	Cd (muscle)	Length	Weight	CF
Cu (liver)	1	–	–	–	–	–	–
Cu (muscle)	–0.09	1	–	–	–	–	–
Cd (liver)	<b>0.25</b>	–0.13	1	–	–	–	–
Cd (muscle)	–0.02	<b>0.46</b>	0.16	1	–	–	–
Length	0.19	–0.2	<b>0.58</b>	–0.07	1	–	–
Weight	0.18	–0.2	<b>0.49</b>	–0.09	<b>0.92</b>	1	–
CF	0.06	–0.07	–0.03	–0.14	0.08	<b>0.38</b>	1

Bold values are significant correlations at  $P < 0.05$

**Table 4** Seasonal variations in morphometric parameters of brook trout in the Sud River, Kerguelen Islands

	Period	Sex	<i>n</i>	Length (cm)	Weight (g)	CF
<i>n</i> , number of fish; CF, condition factor. Values of length, weight and CF are expressed in means $\pm$ SE (min–max in parentheses) * Significant difference in CF mean between the two periods (end of winter vs. end of summer) $P = 0.0098$	End of winter (September–November)	♀	8	18.04 $\pm$ 1.01 (15.1–22.4)	62.5 $\pm$ 11.84 (20–125)	0.88 $\pm$ 0.06 (0.54–1.11)
		♂	10	17.73 $\pm$ 0.59 (15.1–20.4)	53 $\pm$ 5.64 (30–80)	0.92 $\pm$ 0.03 (0.78–1.11)
		Total	18	18.03 $\pm$ 0.54 (15.1–22.4)	57.22 $\pm$ 6.03 (20–125)	0.9 $\pm$ 0.03* (0.54–1.11)
	End of summer (February–April)	♀	10	17.86 $\pm$ 0.67 (14.8–20.7)	60.5 $\pm$ 7.32 (30–110)	1.05 $\pm$ 0.05 (0.87–1.38)
		♂	15	18.65 $\pm$ 0.62 (14.6–22.5)	69.67 $\pm$ 6.33 (25–110)	1.03 $\pm$ 0.05 (0.73–1.34)
		Total	25	18.34 $\pm$ 0.46 (14.6–22.5)	66 $\pm$ 4.79 (25–110)	1.04 $\pm$ 0.04 (0.73–1.38)

**Fig. 3** Seasonal variations in hepatic (a) and muscular (b) copper concentrations of brook trout from the Sud River, Kerguelen Islands. Bars are means  $\pm$  SE of metal concentrations measured in liver or muscle tissues of females (white bars), males (gray bars) and in the whole sample (black bars) of brook trout. For each period (end of winter or end of summer), any significant differences in means between female and male metal levels are detected (Student's *t* test). For each sexual or whole group, differences in metal level means between the two periods (end of winter vs. end of summer) are indicated with # ( $P < 0.05$ ; Student's *t* test)



measured in fish muscle at the end of winter (copper:  $3.67 \pm 0.63$  vs.  $2.37 \pm 0.18$   $\mu\text{g/g}$  dry wt,  $P = 0.028$ ; cadmium:  $0.17 \pm 0.04$  vs.  $0.07 \pm 0.01$   $\mu\text{g/g}$  dry wt,  $P = 0.023$ ; Figs. 3b, 4b). Taking into account individual sexual characteristics, differences in hepatic copper or cadmium between the two periods concerned equally male and female trout (Figs. 3a, 4a), whereas those differences were significantly more pronounced for muscular copper concentrations in females ( $P = 0.030$ , Fig. 4a) and for muscular cadmium levels in male trout ( $P = 0.040$ , Fig. 4b).

In the Sud River, waters were characterized by fresh mean annual temperature with relatively high annual amplitude between  $+0.3^\circ\text{C}$  in winter (July 2005) and  $+14.8^\circ\text{C}$  in summer (February 2005) (Table 5). Water temperature was higher in the end of summer than in the end of winter (Table 5). Moreover, low levels of metals were detected in waters which were often below the instrumental quantification limit. Out of 48 water samples analyzed, only four of them exceeded the quantification limit of  $0.023$   $\mu\text{g/L}$  for cadmium. A maximal value of  $0.62$   $\mu\text{g/L}$  of cadmium was obtained in only one water sample in December 2005 (Table 5). For copper, most water samples (45/48) contained copper in levels exceeded the instrumental quantification limit of  $0.3$   $\mu\text{g/L}$  for this metal. The mean annual copper concentration in water was  $1.99$   $\mu\text{g/L}$  (with  $19.43$   $\mu\text{g/L}$  as maximum value measured in August 2005) (Table 5).

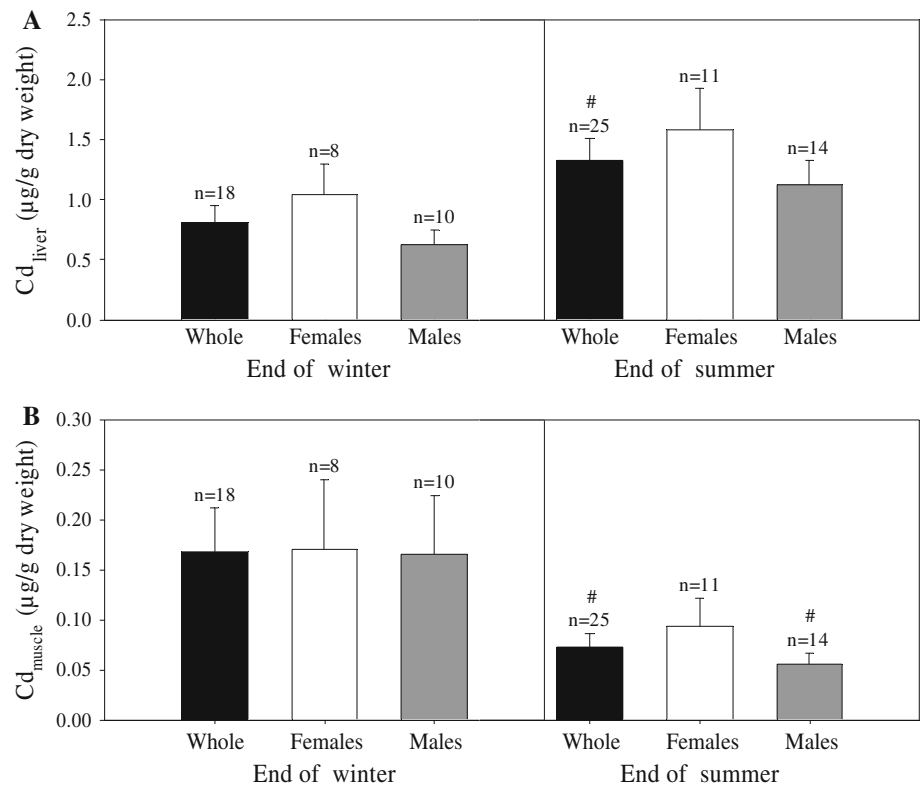
## Discussion

Cadmium concentrations measured in liver of Kerguelen brook trout were of the same order as those measured in liver of marine fish species from the Southern Ocean (Table 6). In muscular tissue, cadmium concentrations were often higher than those quantified in the muscle of Antarctic marine fishes, and levels were about ten times higher than those measured in muscle of marine fish species living around the Kerguelen Islands (Table 6; Bustamante et al. 2003). The hepatic cadmium levels in Kerguelen brook trout were close to those detected in liver of salmonids from other regions less isolated from human activities (Table 7). The muscular values were as high as those measured in muscle of salmonids (e.g. brown trout, *Salmo trutta*) living in more populated regions (Table 7).

For copper, values measured in liver and muscle of Kerguelen brook trout were higher than those quantified in the same tissues of Antarctic and subantarctic marine species (Table 6). Although copper levels measured in muscle of Kerguelen brook trout were close to those measured in salmonid species living in other world regions, the hepatic copper concentrations were lower (Table 7).

Several hypotheses were offered in the literature to explain cadmium accumulation in marine food webs of the Southern Ocean. One of them proposed an upwelling phenomenon occurring in the Antarctic Polar Frontal Zone

**Fig. 4** Seasonal variations in hepatic (a) and muscular (b) cadmium concentrations of brook trout from the Sud River, Kerguelen Islands. Bars are means ± SE of metal concentrations measured in liver or muscle tissues of females (white bars), males (gray bars) and in the whole sample (black bars) of brook trout. For each period (end of winter or end of summer), any significant differences in means between female and male metal levels are detected (Student's *t* test). For each sexual or whole group, differences in metal level means between the two periods (end of winter vs. end of summer) are indicated with # (*P* < 0.05; Student's *t* test)



**Table 5** Physico-chemical parameters of water in the Sud River, Kerguelen Islands

Period	Temperature (°C)	pH	Cd (µg/L)	Cu (µg/L)
Annual mean	5.82 (0.3–14.8)	7.64 (7.44–7.97)	0.024 (ND–0.62)	1.99 (ND–19.43)
End of winter (September–November)	4.43 (2.8–6.9)	7.78 (7.58–7.97)	ND	0.59 (ND–0.9)
End of summer (February–April)	10.4 (6.3–14.8)	7.57 (7.44–7.69)	ND	0.6 (ND–1.12)

Values are expressed in means (min–max in parentheses); ND not determined

near the Kerguelen Islands, which may be responsible for the metallic enrichment of marine surface waters especially by cadmium (Plancke 1977; Sanchez-Hernandez 2000; Bargagli 2008). As brook trout analyzed in the present study never migrated to sea, their cadmium content did not proceed from a direct marine stay. However, a marine origin for cadmium was always possible, terrestrial ecosystems being strongly influenced by continuous winds prevailing in the Kerguelen Islands. Another hypothesis pointed out a geologic origin to explain the high cadmium levels observed in subantarctic aquatic fauna (Ahn et al. 1995; Abollino et al. 2004). In the Sud River, water conductivity was particularly low (≈64 µS/cm) corresponding to poorly mineralized waters as observed in mountain streams. Cadmium concentrations in water were lower than those quantified in Southern Ocean surface waters (~0.078 µg/L in marine waters) which are also much higher than in other ocean surface water (Bargagli 2008). However, we note that this concentration can increase tran-

sitorily as observed in December 2005. Water samples were realized punctually, and such procedure did not take into account possible environmental fluctuations of water metallic concentrations in relation to local meteorological conditions (precipitation). However, these first results show that cadmium and copper levels in water of the Kerguelen Sud River were globally as low as those observed in other polar or subpolar regions (Evans et al. 2005; Bargagli 2008). The telluric origin should not be sufficient to be associated with the cadmium levels measured in a freshwater fish in a presumed non-contaminated area. But, it will be necessary to improve our water sampling procedure to better understand the contribution of abiotic components of Kerguelen freshwater ecosystems (water, sediment) in brook trout metallic bioaccumulation levels.

Metallic bioaccumulation was higher in liver than in muscular tissue. Many studies indicate that levels of metal concentrations in various organs may be different (Szebedinszky et al. 2001; Jezierska and Witeska 2006). Cadmium is

**Table 6** Cadmium and copper concentrations in hepatic and muscular tissues for wild marine fish populations from various zones of the Southern Ocean

Species	Sample area	<i>n</i>	Cd liver	Cd Muscle	Cu Liver	Cu Muscle
<b><i>Salvelinus fontinalis</i></b>	<b>Sud River; Kerguelen</b>	<b>57</b>	<b>1.13 ± 0.11</b>	<b>0.12 ± 0.02</b>	<b>62.27 ± 5.26</b>	<b>3.02 ± 0.25</b>
<i>Gymnoscopelus piabilis</i>	Kerguelen <sup>(a)</sup>	5	10–52.1	0.006–0.029	6.3–14.8	0.8–1.7
<i>Notothenia rossii</i>	Kerguelen <sup>(a)</sup>	5	0.82–4.26	0.034–0.064	3.2–7.3	0.6–0.9
<i>Paranotothenia magellanica</i>	Kerguelen <sup>(a)</sup>	5	2.03–7.35	0.014–0.063	13.7–24.7	0.9–1
<i>Channichthys rhinoceratus</i>	Kerguelen <sup>(a)</sup>	5	2.73–6.78	0.024–0.090	3.2–6.1	0.2–0.8
<i>Champsocephalus gunnari</i>	Kerguelen <sup>(a)</sup>	5	1.04–10.6	0.034–0.155	0.9–7.1	0.4–0.8
<i>Lepidotothen squamifrons</i>	Kerguelen <sup>(a)</sup>	5	5.42–15.4	0.026–0.085	2.5–5.6	0.7–1.4
<i>Trematomus bernacchii</i>	Terra Nova Bay <sup>(b)</sup>	–	9.9 ± 5.8	0.04 ± 0.02	–	–
<i>Chionodraco hamatus</i>	Terra Nova Bay <sup>(b)</sup>	–	2.9 ± 0.8	0.03 ± 0.02	–	–
<i>Trematomus bernacchii</i>	Terra Nova Bay <sup>(c)</sup>	15	7.3 ± 5.61	–	5.19 ± 3.03	–
<i>Trematomus bernacchii</i>	Terra Nova Bay <sup>(c)</sup>	10	–	0.74 ± 0.48	–	0.32 ± 0.23
<i>Chionodraco hamatus</i>	Terra Nova Bay <sup>(c)</sup>	16	0.99 ± 0.97	–	0.85 ± 0.70	–
<i>Chionodraco hamatus</i>	Terra Nova Bay <sup>(c)</sup>	10	–	0.97 ± 0.47	–	0.7 ± 0.67
<i>Trematomus bernacchii</i>	Terra Nova Bay <sup>(d)</sup>	27	–	0.03–0.05	–	1.9–2.5
<i>Notothenia coriiceps</i> (male)	South Shetland Islands <sup>(e)</sup>	10	–	–	12.3 ± 4.2	5 ± 1.7
<i>Notothenia coriiceps</i> (female)	South Shetland Islands <sup>(e)</sup>	10	–	–	7.9 ± 1.9	5.05 ± 3.1
<i>Notothenia coriiceps</i> (male)	South Orkney Islands <sup>(f)</sup>	11	–	<0.25	–	0.2–2.5
<i>Notothenia coriiceps</i> (female)	South Orkney Islands <sup>(f)</sup>	17	–	<0.25	–	0.25–2
<i>Chionodraco hamatus</i>	Terra Nova Bay <sup>(g)</sup>	20	1.02–14.53	0.01–0.03	–	–
<i>Cryodraco antarcticus</i>	Terra Nova Bay <sup>(g)</sup>	6	0.86–4.25	0.01–0.06	–	–
<i>Trematomus newnesi</i>	Terra Nova Bay <sup>(g)</sup>	8	0.98–5.75	0.02–0.05	–	–
<i>Trematomus bernacchii</i>	Terra Nova Bay <sup>(g)</sup>	18	3.36–21.6	0.01–0.08	–	–
<i>Trematomus hansonii</i>	Terra Nova Bay <sup>(g)</sup>	18	5.09–16.42	0.01–0.05	–	–
<i>Chaenocephalus aceratus</i>	Antarctic Peninsula <sup>(h)</sup>	4	1.01–1.25	0.05–0.13	3.3–5.4	1.0–2.0
<i>Notothenia gibberifrons</i>	Antarctica <sup>(h)</sup>	3	–	0.02–0.04	–	0.71–0.98
<i>Trematomus sp.</i>	Winter Quarters Bay <sup>(i)</sup>	–	25–105	0.5–1	25–115	0.5–1.5
<i>Trematomus sp.</i>	Cinder cones <sup>(i)</sup>	–	60 ± 20	1	70 ± 10	4.5 ± 4
<i>Pagothenia borchgrevinski</i>	Syowa Station <sup>(j)</sup>	22	–	0.05–0.2	–	0.85–6.95
<i>Pagothenia borchgrevinski</i>	Syowa Station <sup>(j)</sup>	18	1.5–12.3	–	4.6–29.4	–

Data from this study are in bold

*n* number of fish analyzed. Concentrations represent the mean or range. All expressed as  $\mu\text{g g}^{-1}$  dry weight. Some data were converted from wet wt to dry wt using a 5.0 correction factor (considering average water content in fish tissues of 80%). –, data were not available

<sup>(a)</sup> Bustamante et al. 2003, <sup>(b)</sup> Bargagli 2001, <sup>(c)</sup> Santovito et al. 2000, <sup>(d)</sup> Bargagli et al. 1998, <sup>(e)</sup> Marquez et al. 1998, <sup>(f)</sup> De Moreno et al. 1997, <sup>(g)</sup> Bargagli et al. 1996, <sup>(h)</sup> Szefer et al. 1993, <sup>(i)</sup> Lenihan et al. 1990, <sup>(j)</sup> Honda et al. 1983

accumulated primarily in the liver but also in kidneys. Containing high levels of metal-binding proteins (metallothioneins), the liver plays a crucial role in uptake and storage of essential elements such as copper and detoxication of hazardous metals such as cadmium (Linde et al. 1998; Jezierska and Witeska 2006). It was thus considered as a good candidate to monitor environmental heavy metal pollution, especially for copper and cadmium (Linde et al. 1998). Copper was also accumulated preferentially in liver of brown trout (Linde et al. 1999). Even at low environmental concentrations, copper shows high affinity to the liver where it plays its main metabolic role (Jezierska and Witeska 2006). As muscle constituted the final tissue in

metal storage, it always accumulated lower levels of metals than liver (Jezierska and Witeska 2006).

At the organism level under deficient conditions, toxic non-essential metals may substitute for essential elements. The high cadmium concentrations were often associated with an essential element depletion particularly in copper as observed in marine octopuses and fishes around Kerguelen (Bustamante et al. 1998a, b, 2003). As a matter of fact, Kerguelen octopuses showed a high Cd/Cu ratio ( $\approx 0.55$ ; Bustamante et al. 1998a, b), approximately ten times higher than Cd/Cu ratio measured here in brook trout ( $\approx 0.04$  in muscle and  $\approx 0.02$  in liver in the present study). At the opposite, values measured in Kerguelen brook trout were

**Table 7** Cadmium and copper concentrations in hepatic and muscular tissues from wild freshwater salmonid populations in various parts of the world

Species	Sample area	<i>n</i>	Cd liver	Cd muscle	Cu liver	Cu muscle
<i>Salvelinus fontinalis</i>	<b>Sud River; Kerguelen</b>	<b>57</b>	<b>1.13</b>	<b>0.12</b>	<b>62.27</b>	<b>3.02</b>
<i>Salmo trutta</i>	Vaggatem Lake, Norway/Russia <sup>(a)</sup>	5	0.5	–	180	–
<i>Salmo trutta</i>	Bjornevatn Lake, Norway/Russia <sup>(a)</sup>	11	0.4	–	150	–
<i>Salmo trutta</i>	Otra River, Norway <sup>(b)</sup>	7	–	–	148	2.6
<i>Salmo trutta</i>	Otra River, Norway <sup>(b)</sup>	29	–	–	586	57.5
<i>Salvelinus fontinalis</i>	Blackfoot River, USA <sup>(c)</sup>	6	18	–	1113	–
<i>Salvelinus fontinalis</i>	Blackfoot River, USA <sup>(c)</sup>	7	0.7	–	135	–
<i>Salmo trutta</i>	Blackfoot River, USA <sup>(c)</sup>	12	0.83	–	846	–
<i>Salmo trutta</i>	Blackfoot River, USA <sup>(c)</sup>	6	0.07	–	494	–
<i>Salmo trutta</i>	Clark Fork River, USA <sup>(d)</sup>	10	2.5	–	2399	–
<i>Salmo trutta</i>	Rock Creek and Big Hole River, USA <sup>(d)</sup>	19	0.5	–	759	–
<i>Salmo trutta</i>	Ulla Basin, Spain <sup>(e)</sup>	15	–	–	75.9	–
<i>Salmo trutta</i>	Ulla Basin, Spain <sup>(e)</sup>	24	–	–	435	–
<i>Salvelinus fontinalis</i>	Animas River, USA <sup>(f)</sup>	3	8	–	131	–
<i>Salvelinus fontinalis</i>	Animas River, USA <sup>(f)</sup>	3	50	–	788	–
<i>Oncorhynchus mykiss</i>	Po River (caging), Italy <sup>(g)</sup>	160	–	0.01–0.07	–	–
<i>Salmo trutta</i>	Asturian Rivers (Esva), Spain <sup>(h)</sup>	12	0.43	–	134.6	–
<i>Salmo trutta</i>	Asturian Rivers (Nora-up), Spain <sup>(h)</sup>	7	2.08	–	90.1	–
<i>Salmo trutta</i>	Asturian Rivers (Nora-down), Spain <sup>(h)</sup>	11	0.48	–	224.6	–
<i>Salmo trutta</i>	Asturian Rivers (Piles-up), Spain <sup>(h)</sup>	12	1.59	–	187.1	–
<i>Salmo trutta</i>	Asturian Rivers (Piles-down), Spain <sup>(h)</sup>	14	4.78	–	113.7	–
<i>Salmo trutta (Lake)</i>	Busko Blato reservoir, Bosnia and Herzegovina <sup>(i)</sup>	10	0.72	0.21	219.8	2.73
<i>Salmo trutta</i>	Piguena River, Spain <sup>(j)</sup>	25	0.6	–	67.8	–
<i>Salmo trutta</i>	Piguena River, Spain <sup>(j)</sup>	20	0.7	–	54.3	–
<i>Salmo trutta</i>	Una river, Bosnia and Herzegovina <sup>(k)</sup>	–	–	0.35	–	4.25
<i>Salmo trutta</i>	Louka River, Czech Republic <sup>(l)</sup>	7	0.53	0.11	299.9	1.99
<i>Salmo trutta</i>	Louka River, Czech Republic <sup>(l)</sup>	7	0.63	0.03	258.5	2.18
<i>Salmo trutta</i>	Louka River, Czech Republic <sup>(l)</sup>	7	1.01	0.01	462.5	1.64
<i>Salmo trutta</i>	Louka River, Czech Republic <sup>(l)</sup>	7	1.11	0.13	729	1.93
<i>Salmo trutta</i>	Urumea River, Basque Country (Spain) <sup>(m)</sup>	4	13	<1.6	420	0.8
<i>Salmo trutta</i>	Urumea River, Basque Country (Spain) <sup>(m)</sup>	3	6.2	<0.2	185	1.2
<i>Salmo trutta</i>	Rugula River, Norway <sup>(n)</sup>	6	1.5*	–	1214.6*	–
<i>Salmo trutta</i>	Naustebekken River, Norway <sup>(n)</sup>	6	27.4*	–	517.1*	–

Data from this study are in bold

*n* number of fish analyzed. Concentrations represent the mean and expressed as  $\mu\text{g g}^{-1}$  dry weight. Some data were converted from wet wt to dry wt using a 5.0 correction factor (considering average water content in fish tissues of 80%). –, data were not available; \*, median value

<sup>(a)</sup> Amundsen et al. 1997, <sup>(b)</sup> Brotheridge et al. 1998, <sup>(c)</sup> Moore et al. 1991, <sup>(d)</sup> Farag et al. 1995, <sup>(e)</sup> Lamas et al. 2007, <sup>(f)</sup> Besser et al. 2001, <sup>(g)</sup> Camusso et al. 1995, <sup>(h)</sup> Linde et al. 1998, <sup>(i)</sup> Has-Schon et al. 2008, <sup>(j)</sup> Linde et al. 1999, <sup>(k)</sup> Alibabic et al. 2007, <sup>(l)</sup> Vitek et al. 2007, <sup>(m)</sup> Sanchez et al. 1998, <sup>(n)</sup> Olsvik et al. 2001

lower for cadmium and higher for copper in comparison with marine organisms living in the same geographical zone. If cadmium concentrations analyzed in brook trout tissues were not concomitantly associated with copper depletion, we should not exclude that other metallic interactions (for instance, Cd/Zn) may explain the relative high cadmium levels in this freshwater fish species.

Fish diet composition, linked to freshwater trophic web structuration, was important to explain tissue elemental dis-

tribution in organisms (Bustamante et al. 1998b). The freshwater trophic webs in Kerguelen Islands are quite simple and consist of a few species: abundant planktonic and benthic entomostraca, small oligochaetes, nematodes, a common chironomid and terrestrial arthropods. Fish diet is then essentially composed of small invertebrates with aquatic or terrestrial origins as recently observed in brown trout living in the Château River in Kerguelen (Bryère and Charrier unpublished data). In the Sud River section

studied here, brook trout diet may have a similar composition. The elemental composition of brook trout preys has to be studied to determine the origin of copper and cadmium in fish tissue (Bustamante et al. 1998b). Metal bioavailability to brook trout as higher-order consumers can be substantially modified by processing of metals in stream food webs in relation to dominance of metal-tolerant organisms (Dallinger et al. 1997).

Finally, an anthropic origin for cadmium must not be totally rejected as the Sud River lies only 10 km away from the scientific station of Port-aux-Français (Corsolini 2009).

An interesting feature in our results concerned interseasonal differences in metal levels. At the end of summer, hepatic cadmium and copper concentrations were higher than those measured in brook trout caught at the end of winter. At the opposite, the muscular compartment contained the highest metallic concentrations in fish sampled in the Sud River at the end of winter. In Kerguelen freshwaters, the brook trout spawning period is situated between April and May (Duhamel et al. 2005). So, fish caught at the end of summer (between February and April) were sexually mature. Low levels of metals measured in muscle in this period could be explained by a redistribution of metallic elements between muscle and gonads. However, decrease in muscular metal concentrations observed at the end of summer may also be due to a dilution phenomenon linked to an increase in fish condition occurring during the foraging period (Table 4). Such a difference may be related to lower prey abundance and feeding rates in winter and inversely for summer. Most of the cadmium and copper accumulated in Kerguelen brook trout proceeded from trophic inputs as confirmed by the highest metallic levels measured in hepatic tissue at the end of summer.

Concerning fish containing high copper and cadmium levels, the toxicological risk for fish-eating animals (birds, humans) may also be taken into account. In Kerguelen, terns, cormorants, gentoo penguins and skuas are avian species, which have been observed preying on salmonids (Duhamel et al. 2005). Brook trout is also a much sought-after game species among fishermen practicing their favorite sport in streams, lakes and ponds near the Port-aux-Français station.

The mean copper concentrations measured in muscle of Kerguelen brook trout ( $3.02 \mu\text{g/g}$  dry wt) exceeded the maximum permissible levels for human consumption in Europe ( $0.05$  and  $0.1 \mu\text{g/g}$  wet weight corresponding to  $0.25$  and  $0.5 \mu\text{g/g}$  dry wt for cadmium and copper, respectively, with a conversion in dry weight using a 5.0 correction factor, considering an average water content in fish tissues of 80%) (Commission Regulation (EC) No 1881/2006; Ricoux and Gasztowtt 2005; Shinn et al. 2009). But the muscular cadmium concentrations do not exceed this limit ( $0.12 \mu\text{g/g}$  dry wt). Additionally, only six brook trouts

among the total of 57 individuals studied exceeded the maximum cadmium level recommended for human consumption in Europe. In order to assess potential risks for human health related to the presence of cadmium in food, the European Food Safety Authority (EFSA 2009) established a tolerable weekly intake (TWI) for cadmium at  $2.5 \mu\text{g/kg}$  body weight ( $175 \mu\text{g}$  per week for an adult weighting 70 kg). However, according to the Joint FAO/WHO Expert Committee on Food Additives, a provisional tolerable weekly intake (PTWI) for cadmium was fixed at  $7 \mu\text{g/kg}$  body weight per week ( $490 \mu\text{g}$  per week for a 70-kg man; JECFA 2003). In Kerguelen brook trout, the maximal cadmium concentration measured in muscle was  $0.13 \mu\text{g/g}$  wet weight ( $0.65 \mu\text{g/g}$  dry weight). Consequently, consumption of 100 g of muscle per day (700 g per week) leads to a cadmium intake of  $91 \mu\text{g}$ , which represents 52% of the TWI established by EFSA and 18.5% of the PTWI established by JECFA.

For copper, the provisional maximum tolerable daily intake for humans is  $0.5 \text{ mg/kg}$  body weight per day (WHO 1982), corresponding to 35 mg per day for a 70-kg human. The maximal muscular copper concentration found in Kerguelen brook trout was  $2.5 \mu\text{g/g}$  wet weight ( $12.8 \mu\text{g/g}$  dry wt), corresponding to 0.7% of the provisional maximum tolerable daily intake for humans.

Fish exposed to heavy metal presented a multiple toxic syndrome associated with hepatotoxicity (Paris-Palacios and Biagiatti-Risbourg 2006) and oxidative stress responses (Sanchez et al. 2005; Hansen et al. 2006). The maximal value for copper concentrations punctually and transitorily detected in the Sud River was  $19.43 \mu\text{g/L}$ , and the maximal value for cadmium was  $0.62 \mu\text{g/L}$  (Table 5). The 96-h  $\text{LC}_{50}$  of copper and cadmium for fish is available in literature and data banks, but they were evaluated in rainbow trout but not in brook trout (Cusimano et al. 1986; Environnement Canada 1994). Values ranged between 2.8 and  $210 \mu\text{g/L}$  for copper and between 0.5 and  $0.9 \mu\text{g/L}$  for cadmium. As  $\text{LC}_{50}$  data depend on fish species, age of individuals and physico-chemical characteristics of water, it was particularly difficult to estimate the potential associated toxicological risk for Kerguelen brook trout under natural field conditions.

The maximal metal concentration levels measured in Sud River waters (Table 5) were not constant and corresponded to only one observation. Although trout may be exposed to these maximal concentrations, exposure may be very transitory. However, a toxicological risk for Kerguelen brook trout must not be totally rejected, and its evaluation will be the subject of a further study. Moreover, as toxicity of metal compounds to trout may depend on water pH (Cusimano et al. 1986), the toxicological risk could be exacerbated in the case of Kerguelen salmonids because they live in waters with  $\text{pH} > 7$  (Table 5).

However, in the Kerguelen context, metal-associated physiological responses may be more related to adaptative mechanisms than to real toxicological responses. During chronic exposure to Cd and other toxic metals, fish undergo acclimation with changes in physiological status and an increased resistance and/or tolerance (Roch and McCarter 1984; Stubblefield et al. 1999). Synthesis of the cysteine-rich metalloproteins, metallothioneins (MT), for binding and storing metals in target tissues is known to play an important role in acquired tolerance during acclimation (Hogstrand and Haux 1991; Dallinger et al. 1997; Linde et al. 1999; Olsvik et al. 2001; Chowdhury et al. 2005; Linde et al. 2005). The binding of toxic metals to MT represents a sequestration function that renders them unable to interact with other proteins, such as enzymes, and thereby produces protection against metal toxicity at the cellular level (Kito et al. 1982; Roesijadi 1992).

Adaptative mechanisms developed by these fishes in the original environmental context of the Kerguelen Islands and the possible physiotoxicological damages (oxidative stress) related with metal bioaccumulation in liver tissue will be considered in a future study.

## Conclusion

We present here the first results concerning Cu and Cd levels in a freshwater fish species living in a subantarctic insular context in the Southern Ocean. The high tissular Cd and Cu levels observed were not linked to direct marine inputs because the brook trout studied here were non-migratory individuals. Other salmonid species such as brown trout, living in Kerguelen freshwaters, may be interesting to study in regard to their marine migratory behavior. Such freshwater fish populations living in a subpolar environmental context, with reduced direct human influences, may contribute to investigate modalities of heavy metal transfer and accumulation in subpolar food webs.

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