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Copper homeostasis and toxicity in the elasmobranch *Raja erinacea* and the teleost *Myoxocephalus octodecemspinosus* during exposure to elevated water-borne copper

Martin Grosell[a,b,*], Chris M. Wood[a,b,c], Patrick J. Walsh[a,b]

[a]*Mount Desert Island Biological Laboratories, Salisbury Cove, Maine 04672, USA*
[b]*Rosenstiel School of Marine and Atmospheric Science, University of Miami 4600 Rickenbacker Causeway, Miami, FL 33149-1098, USA*
[c]*Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1*

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Abstract

Clear nosed skate, *Raja erinacea* were exposed to 0.10 (control), 0.52 or 1.73 μM copper and sculpin, *Myoxocephalus octodecemspinosus* were exposed to 0.10 or 1.73 μM copper (as $CuSO_4$) in Salisbury Cove seawater for up to seven days. Skate gill copper concentrations increased 40–50 fold over background in response to copper exposure at both concentrations. In comparison, sculpin gill levels only increased 3-fold. While there was no evidence for internalized copper in the skate arising from the water-borne exposure, sculpin kidneys, but not livers, exhibited elevated copper concentrations after the seven days of exposure. The marked difference in branchial copper accumulation between the skate and the sculpin likely explains why elasmobranchs appear to be more sensitive to metal exposure than most marine teleost fish. Brain tissue from both species and the skate rectal gland contained relatively high background copper concentrations. Copper exposure caused an initial transient reduction in skate plasma total ammonia (T_{amm}), but eventually led to elevated plasma T_{amm}. Despite the marked branchial copper accumulation in the skate, there was no reduction in gill Na/K-ATPase activity. Similarly, Na/K-ATPase activity in skate rectal gland and intestine, as well as in sculpin gill and intestine were not affected by copper exposure. Plasma sodium, magnesium and chloride were not affected by copper exposure in either the skate or the sculpin.

Keywords: Plasma ammonia; Na/K-ATPase; Copper; Elasmobranch; Teleost

1. Introduction

Despite the essential role of copper in a number of enzymatic processes including cellular respiration (Solomon and Lowery, 1993), this metal has the potential to exert adverse toxicological effects. Copper appears to be especially toxic to freshwater organisms with no observable effect concentrations (NOEC's) for water-borne exposure in some cases being in the nanomolar range (see Grosell et al., 2002 for a review). While the mechanisms of copper uptake and acute toxicity in freshwater fish have been studied in some detail (Laurén and McDonald, 1987a,b; Wilson and Taylor, 1993a,b; Grosell and Wood, 2002) less is known about mechanisms of copper toxicity in marine teleost fish (Stagg and Shuttleworth, 1982a,b; Wilson and

*Corresponding author. Tel.: +1-305-361-4623; fax: +1-305-361-4001.
E-mail address: mgrosell@rsmas.miami.edu (M. Grosell).

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Taylor, 1993a). It is clear, however, that marine teleost fish are generally less sensitive to water-borne copper exposure than freshwater fish (Steele, 1983a,b vs. values reviewed by Grosell et al., 2002).

While part of this difference undoubtedly is due to differences in inorganic copper speciation between freshwater (where ionic Cu^{2+} can be prevalent) and seawater (where Cu^{2+} is virtually absent), high cation concentrations in seawater may also offer some protection against copper toxicity. Calcium and sodium reduce copper toxicity and uptake, respectively, in freshwater fish (Pagenkopf, 1983; Grosell and Wood, 2002) and the high concentrations of these cations in seawater may thus be part of the reason for lower acute sensitivity to copper in seawater teleosts. While information about copper uptake and toxicity in marine teleost fish is scarce, almost nothing is known about copper uptake and toxicity in elasmobranchs. Recent studies of silver-induced toxicity and silver uptake in the Pacific spiny dogfish, however, revealed that at least this elasmobranch was more sensitive to this metal than most teleost fish (Grosell et al., 1999; Hogstrand et al., 1999; Webb and Wood, 2000; De Boeck et al., 2001; Grosell and Wood, 2001). Interestingly, the lower tolerance to silver in elasmobranchs seems to correlate with higher silver uptake and retention rates than seen in teleosts (Pentreath, 1977; Webb and Wood, 2000). Mechanisms of acute silver and copper toxicity are very similar in freshwater fish. Both metals cause osmoregulatory disturbance by targeting sodium and chloride uptake at the gill (Wood, 2001; Grosell et al., 2002). Reports of relatively low tolerance to silver in an elasmobranch as outlined above, therefore, suggest that elasmobranchs maybe similarly sensitive to copper.

Studies of silver toxicity in marine teleosts and elasmobranchs have revealed different target organs for silver uptake, accumulation and effects. For marine teleosts, the mechanism of acute toxicity for both copper and silver appears to be osmoregulatory disturbance (Stagg and Shuttleworth, 1982a,b; Wilson and Taylor, 1993a; Grosell et al., 1999; Hogstrand et al., 1999). Marine teleosts share the feature of branchial silver accumulation with freshwater fish, but also accumulate considerable amounts of silver in the intestinal epithelium (Grosell et al., 1999; Hogstrand et al., 1999; Webb and Wood, 2000; Grosell and Wood, 2001). This difference is due to the continuous drinking necessary for marine teleost fish to offset the diffusive water loss to their concentrated environment (Smith, 1930). The intestinal silver accumulation results in impaired ion and water transport across the intestinal epithelium, which can account for approximately 50% of the whole animal response to silver exposure (Grosell and Wood, 2001). The remaining fraction of the whole animal response is attributed to impaired branchial salt extrusion necessary to compensate for salt gained from the seawater environment (Grosell and Wood, 2001). Thus, in marine teleost fish, the gill and the intestine are both target organs during acute silver exposure and presumably also during exposure to other metals.

Since the osmoregulatory strategy of elasmobranchs is fundamentally different from that of teleosts, different metal target organs and toxic mechanisms might be expected. Unlike teleosts, marine elasmobranchs are slightly hyperosmotic compared to seawater due to high internal levels of urea and trimethylamine oxide (TMAO). Even though some branchial NaCl extrusion may occur (Shuttleworth, 1988; Evans, 1993); this is accomplished mostly by very low branchial electrolyte permeability and substantial active secretion of excess NaCl via the rectal gland. The high internal osmolality eliminates the need for drinking, and, therefore, potential effects of water-borne metals on the intestinal epithelium are not likely. As for teleosts, elasmobranch gills are obviously exposed to water-borne metal and branchial metal accumulation and effects are thus to be expected. Indeed, spiny dogfish exposed to silver exhibited substantial branchial silver accumulation, but only limited silver accumulation in the intestine (De Boeck et al., 2001). Considering the likely difference in copper tolerance and mode of action between marine teleosts and elasmobranchs, we set out to compare copper uptake and distribution in an elasmobranch, the clear nosed skate (*Raja erinacea*) and a teleost, the sculpin (*Myoxocephalus octodecemspinosus*). Suspecting osmoregulatory disturbance as the main toxic mechanism of copper to both species various osmoregulatory endpoints were considered. In addition, plasma ammonia (T_{amm}) was measured, because one of the most consistent effects of copper exposure in freshwater fish is elevated plasma ammonia (Laurén and McDonald, 1985; Wilson and Taylor, 1993b; Beaumont et al., 1995; De boeck et al., 1995; Wang et al., 1998) due to a combined

increased production and possibly impaired branchial clearance (Grosell et al., 2002).

2. Materials and methods

2.1. Experimental animals

Clear nosed skate (*R. erinacea*) weighing 75–480 g and sculpin (*M. octodecemspinosus*) weighing 100–300 g, were captured in Frenchman's Bay, ME in August, 2000 and kept in a saltwater tank (30 ± 1 ppt) at the Mt. Desert Island Biological Laboratory, Salisbury Cove, ME prior to experimentation at ambient temperature (17 ± 1 °C) and photoperiod. Fish were fed a diet of chopped squid until 48 h prior to experimentation.

2.2. Copper exposure

In the present study, we exposed clear nosed skate to two elevated, yet environmentally relevant copper concentrations of 0.4 and 2.0 μM (nominal) for up to seven days under flow-through conditions. A limited number of sculpin allowed us to make a direct comparison between a marine elasmobranch and a marine teleost. Expecting higher copper tolerance in the sculpin we chose to expose the available sculpin to the highest copper concentration for seven days.

Skates (two per tank) and sculpin (maximum three per tank) were kept in 20 l aerated plastic tanks supplied with a flow-through 200–400 ml min^{-1} Salisbury cove seawater fed by gravity from mixing chambers. Constant copper concentrations were ensured by addition of concentrated $CuSO_4$ stock solutions delivered by a peristaltic pump to the above mixing chambers. Constant water flow to the mixing chambers was supplied from a head tank equipped with an overflow drain ensuring constant water levels and thus pressure. The experimental flow-through system was situated in the open and fish were thus exposed to ambient temperature (17 ± 1 °C and 30 ± 1 ppt) and light cycle.

Fish were not fed during the experiment. Water samples were collected on a daily basis from each of the exposure tanks for subsequent determination of copper concentrations. These measurements revealed that skates were exposed to either 0.52 ± 0.05 μM (33 ± 3 μg l^{-1}; $n=30$) or 1.73 ± 0.10 μM (109 ± 6 μg l^{-1}; $n=41$) copper, which corresponds to 5- and 17-fold background levels (0.10 ± 0.01 μM ($=6 \pm 0.6$ μg l^{-1}); $n=3$), respectively. Due to shortage of fish, sculpin were exposed only to background levels and 1.73 ± 0.10 μM ($n=41$) copper.

2.3. Sampling

On days 0, 1, 3 and 7 of copper exposure skates were sampled, while sculpin were sampled only at day 0 and 7. Fish were netted out of the exposure tanks and placed in seawater containing 0.2 g MS222 l^{-1}. A blood sample was obtained from each animal by caudal puncture, hematocrit was determined on a sub sample while the remaining blood was centrifuged at 4 °C at 10 000 *g* for 5 min to obtain plasma, which was immediately transferred to a −80 °C freezer. Gill, liver, kidney, intestine, brain and muscle were obtained by dissection from both fish species and in addition, the rectal gland was obtained from the clear nosed skate. For the sculpin the intestine was divided into an anterior, mid and posterior segment. Sub samples of gill filaments, intestine and rectal gland were freeze-clamped and stored at −80 °C for later analysis of Na/K-ATPase enzyme activity, while the remaining tissue samples were stored at −20 °C for analysis of copper content.

2.4. Analysis

Subsamples of all tissues were prepared for copper analysis by addition of approximately five times the volume of 1 N HNO_3 (trace metal grade, Merck, Darmstadt, Germany). The samples were then digested overnight at 75 °C, vortexed and centrifuged. The supernatants together with water and plasma samples were diluted appropriately prior to copper analysis on a graphite furnace atomic absorption spectrophotometer (Varian SpectrAA220 with a SpectrAA GTA110) using 10–20 μl injection volume and standard operating conditions as documented by the manufacturer. Because considerable matrix interactions were observed when attempting to measure low copper levels in undiluted seawater, a NH_4NO_3 modifier and Zeeman correction were employed again as outlined by the manufacturer. Quality assurance on water copper measurements included the use as a certified copper standard, standard addition to randomly selected samples as well as measurements in triplicate.

Plasma total ammonia (T_{amm}) was measured using an enzymatic assay (Sigma chemicals, St. Louis, MO, USA; Kit 171) modified for microtiter plate use. Plasma Cl^- concentrations were determined using the colorimetric method of Zall et al. (1956) while plasma cations (Na^+ and Mg^{2+}) were determined by flame atomic absorption spectrophotometer (Varian SpectrAA220) using operating procedures as outlined by the manufacturer. The Na/K-ATPase activity of osmoregulatory organs of the skate (gill and rectal gland) and the sculpin (gill and intestine) as well as the anterior segment of the skate intestine was determined by the method of McCormick (1993), which was modified for microtitre plate reading and enzyme activity was normalized to protein concentration as determined by the Bradford reagent (Sigma Chemicals, St. Louis, MO, USA).

2.5. Data presentation and statistical evaluation

All values are expressed as means ± S.E.M. (n). Statistically significant differences were evaluated using an unpaired Student t-test (two tailed) with multi sample comparison Bonferroni correction where appropriate. In all cases a limit for significance of $P < 0.05$ was applied.

3. Results

3.1. Copper accumulation

While copper levels in most tissues of both species appeared to be well regulated even at high environmental copper concentrations, there were significant elevations of copper concentrations in the gills of both skate and sculpin, and in the kidney of the sculpin.

Branchial copper accumulation in clear nosed skate was much greater than that of sculpin. Exposure to both copper concentrations resulted in 40–50 fold increase in gill filament copper concentration from less than 10 nmol g^{-1} to approximately 400 nmol g^{-1} over the 7 days of exposure (Fig. 1). Although gill copper concentration increased with exposure concentrations, the difference between copper concentrations in the gill of the two groups of skates did not fully reflect the more than 3-fold difference between the two exposure concentrations (0.52 and 1.73 μM) (Fig. 1). Due to limited fish availability, sculpin were only exposed to the highest copper concentration. Even at this concentration, seven days of exposure resulted in only a three-fold increase in branchial copper concentration from approximately 25 to 75 nmol g^{-1} (Fig. 1).

Control levels of plasma copper were similar in the two fish species examined (Fig. 1). Although both sculpin and skate exhibited a tendency to elevated plasma copper levels, there was no statistically significant effect of copper exposure.

Intestinal copper accumulation was expected in the sculpin as a result of exposure due to the continuous drinking which is involved in marine teleost osmoregulation (see Introduction). None of the three intestinal segments sampled from the sculpin showed significantly elevated copper concentrations after the 7-day exposure to 1.73 μM (Fig. 2). The skate rectal gland did not accumulate copper as a result of the 7-day exposure to either of the concentrations. Interestingly, aside from the liver, the rectal gland contained the highest levels of copper even in non-exposed fish (Fig. 2).

Hepatic copper levels in the sculpin were surprisingly low and not significantly elevated after copper exposure. Hepatic copper levels in the skate were close to 20-fold those of sculpin, but like the sculpin, hepatic copper concentrations were not elevated during copper exposure (Fig. 3). The kidney of the sculpin but not the skate accumulated copper to levels significantly above control (Fig. 3).

Neither brain nor white muscle tissue accumulated copper during the exposure (Fig. 4). While muscle as expected exhibited low copper concentrations, the brain of both species exhibited relatively high copper concentrations. To put this observation in perspective, the brain tissue in the sculpin contained the highest copper concentration of all tissues examined in this species, whereas the liver and the rectal gland exceeded the brain copper levels in the skate.

3.2. Plasma electrolytes and T_{amm} and hematocrit

Of all hematological parameters recorded, only skate plasma T_{amm} was significantly affected by copper exposure (Fig. 5). Initially (day 1) during exposure to both concentrations, a significant reduction in plasma T_{amm} occurred at both concentrations. This was followed by a recovery at day three and finally by significantly elevated levels after seven days of exposure to both concentra-

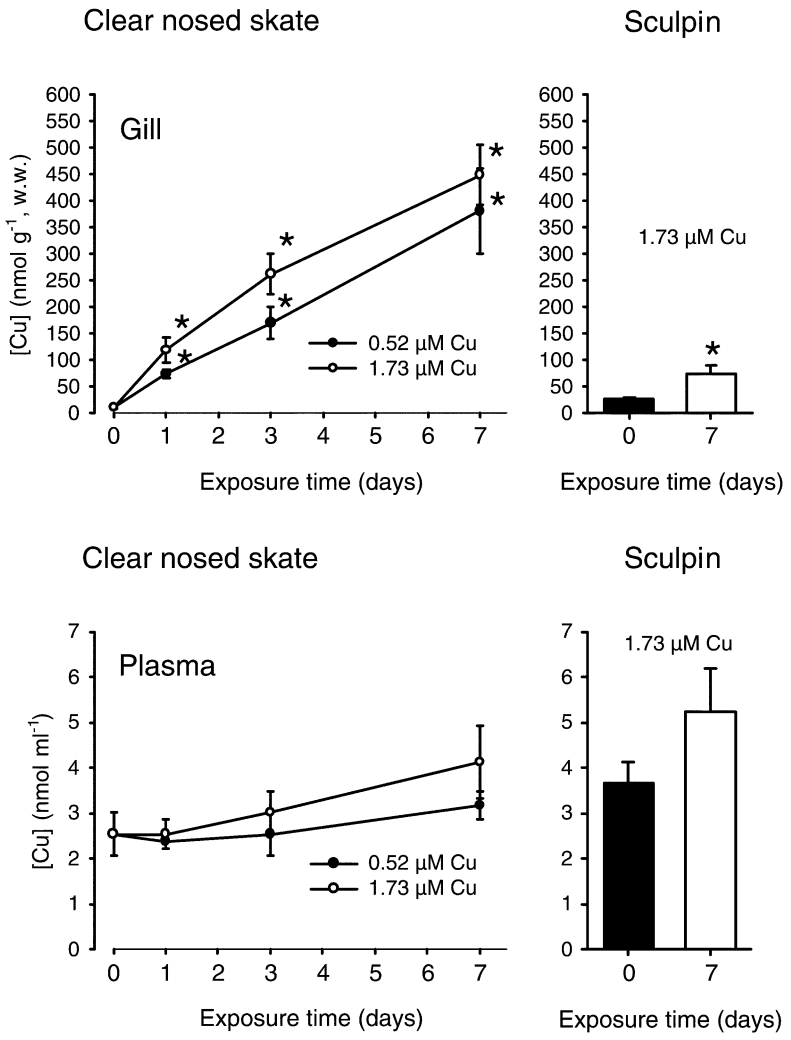

Fig. 1. Branchial (top panels) copper concentrations (nmol g^{-1} wet weight) and plasma (bottom panels) copper concentrations (nmol ml^{-1}) in clear nosed skate (*R. erinacea*) during 7 days of exposure to 0.52 and 1.73 μM ambient copper (left panels) and in sculpin (*M. octodecemspinosus*) before and after 7 days of exposure to 1.73 μM ambient copper (right panels). Data are expressed as mean ± S.E.M. $n = 5–6$ for skate and 4–5 for sculpin. An * indicates statistical significant difference from corresponding control value at $P < 0.05$.

tions. In the sculpin, no significant effect of copper exposure on plasma T_{amm} was observed although the mean value of 158 ± 48 μM after seven days of exposure tended to be higher than the control value of 65 ± 14 μM.

With the exception of the minor increases in plasma Na^+ in skates exposed to 0.52 μM copper for three days, no other significant differences amongst levels of plasma Cl^-, Na^+ and Mg^{2+} in copper exposed fish of either species and respective controls were observed (Table 1). No effect on hematocrit was observed for sculpin (overall mean 25.9 ± 2.9%) or skate (overall mean 20.4 ± 0.7).

3.3. Na/K-ATPase enzyme activity

Osmoregulatory tissues from the sculpin (gill and intestine) and the skate (gill and rectal gland) as well as the skate intestine were analyzed for Na/K-ATPase activity. In agreement with the lack of effect of copper exposure on plasma electrolytes, no impairment of enzyme activity was observed in either of the investigated tissues (Tables 2 and 3).

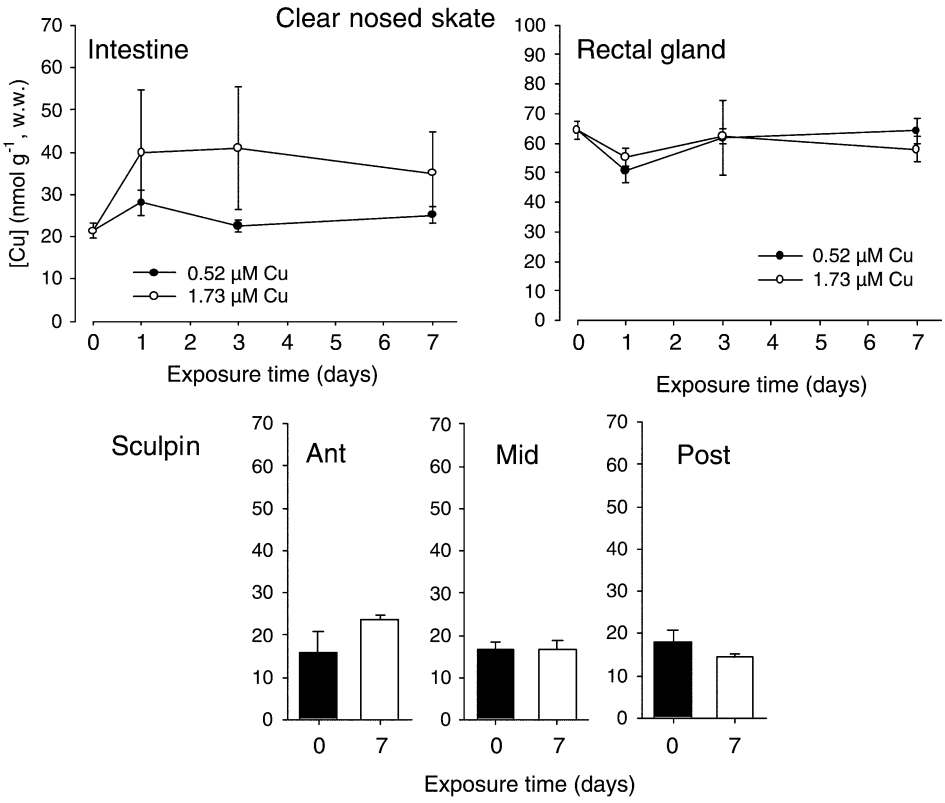

Fig. 2. Intestinal (top left panel) and rectal gland (top right panel) copper concentration (nmol g^{-1} wet mass) in clear nosed skate (*R. erinacea*) during 7 days of exposure to 0.52 and 1.73 μM ambient copper and in anterior, mid and posterior segments of sculpin (*M. octodecemspinosus*) before and after 7 days of exposure to 1.73 μM ambient copper (bottom panel). No statistically significant differences were observed. Other details as in Fig. 1.

4. Discussion

4.1. Copper homeostasis

The most striking difference between the clear nosed skate and the sculpin was branchial copper accumulation. Sculpin gill copper increased approximately 3-fold, which is in agreement with reports of branchial copper elevation in freshwater fish exposed acutely to sublethal water-borne copper (Grosell et al., 1996, 1997; Pelgrom et al., 1995; Taylor et al., 2000). In marked contrast was the 40–50 fold elevation in skate gill copper during exposure to both concentrations. To our knowledge there are no other reports of branchial copper levels in elasmobranchs, but reports of higher branchial silver accumulation in elasmobranchs than in teleosts (Webb and Wood, 2000) suggest that high branchial metal accumulation during water-borne exposure may be a common feature of marine elasmobranchs.

Branchial metal accumulation is at all times determined by the differences between net apical metal entry from the water and net basolateral transfer from the gill cells to the blood. Thus, the reason for the high branchial metal accumulation observed in skates could be both exceptionally high apical metal entry rates and/or exceptionally slow basolateral extrusion rate. Because no significant internal accumulation of copper was observed in the skate it would appear that low basolateral copper extrusion rate is the reason for the substantial branchial copper accumulation. However, due to high background hepatic copper concentrations (see below) and the high hepatosomatic index of elasmobranchs (20% compared to 2% in teleosts), lack of significant increases in hepatic metal concentrations might not necessarily mean that there is no internalization of water-borne copper.

The liver is the main homeostatic organ for copper in higher vertebrates including teleost fish (Grosell et al., 2001) and generally exhibits the

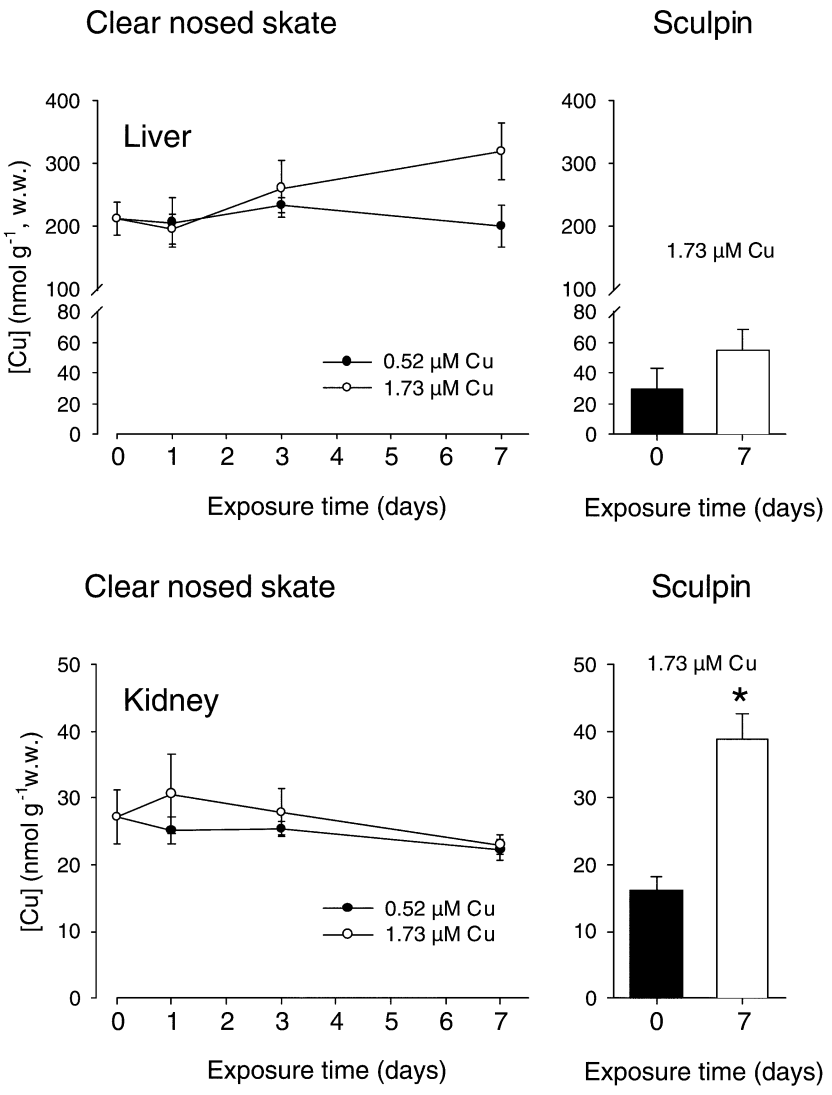

Fig. 3. Hepatic (top panels) and renal (bottom panels) copper concentrations (nmol g^{-1} wet mass) in clear nosed skate (*R. erinacea*) during 7 days of exposure to 0.52 and 1.73 μM ambient copper (left panels) and in sculpin (*M. octodecemspinosus*) before and after 7 days of exposure to 1.73 μM ambient copper (right panels). *Indicates statistical significant difference from corresponding control value at $P<0.05$.

highest tissue specific copper concentrations. Hepatic copper concentrations in the skate are within the range of values reported for most teleost fish (Buckley et al., 1982; Stagg and Shuttleworth, 1982a; McCarter and Roch, 1984; Laurén and McDonald, 1987a; Grosell et al., 1998b), but, because of their high hepatosomatic index, this amounts to a substantial hepatic copper pool. In contrast, hepatic copper levels in the sculpin are the lowest reported so far for any teleost fish.

Plasma copper levels of both species were largely unaffected by copper exposure, in agreement with numerous other studies of plasma copper homeostasis in freshwater teleost fish during exposure to environmentally realistic copper concentrations (Pelgrom et al., 1995; Buckley et al., 1982; Kamunde et al., 2001; Grosell et al., 1997, 2001), showing strong homeostatic control.

Renal copper concentrations in control specimens of both species were low in agreement with

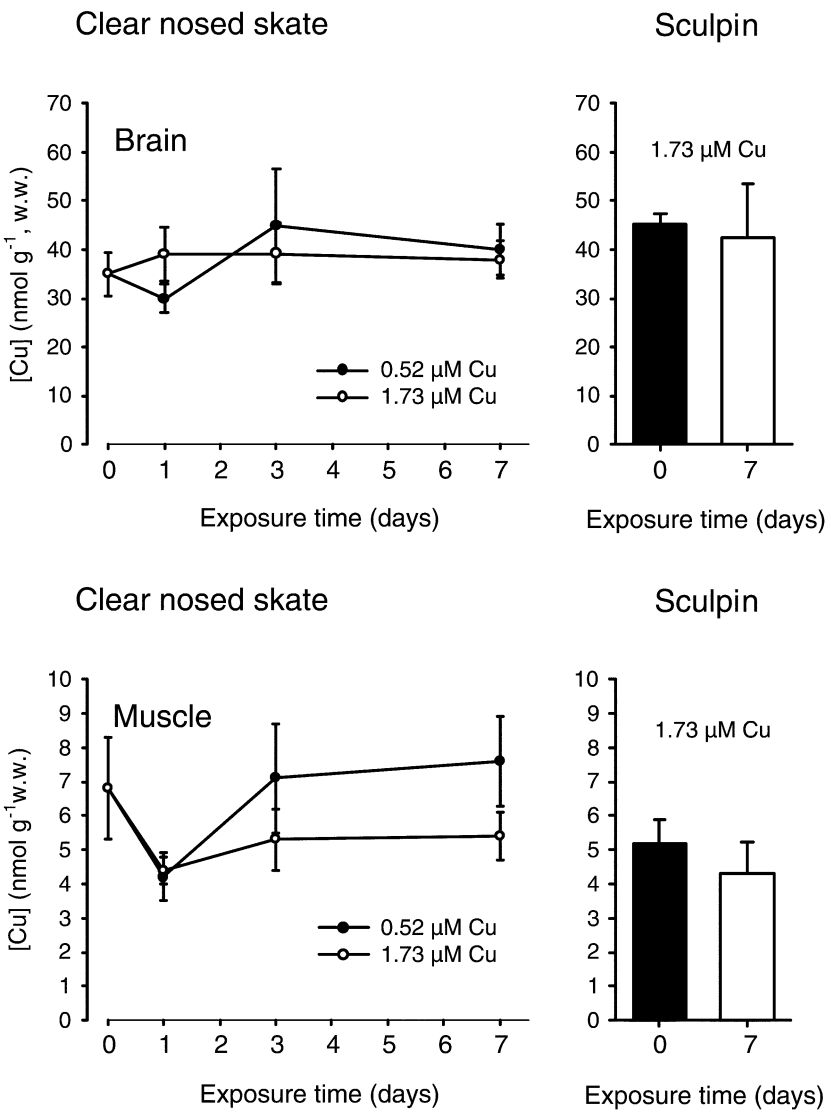

Fig. 4. Brain (top panels) and muscle (bottom panels) copper concentration (nmol g^{-1} wet mass) in clear nosed skate (*R. erinacea*) during 7 days of exposure to 0.52 and 1.73 μM ambient copper (left panels) and in sculpin (*M. octodecemspinosus*) before and after 7 days of exposure to 1.73 μM ambient copper (right panels). No statistically significant differences were observed. Other details as in Fig. 1.

previous reports from teleosts (Grosell et al., 1997, 1998b; Kamunde et al., 2001), but increased in the sculpin in response to copper exposure demonstrating internalization of copper. Elevated renal copper concentration in response to sublethal water-borne copper is in contrast to reports from freshwater teleosts (Buckley et al., 1982; Grosell et al., 1997, 1998a,b, 2001). Interestingly, however, renal copper accumulation has been previously reported for marine European flounder (Stagg and Shuttleworth, 1982a) and could thus be a feature of marine teleosts.

White muscle and brain copper levels were not influenced by copper exposure in either of the two species investigated. White muscle copper concentrations were low as previously reported for fish (Stagg and Shuttleworth, 1982a; Grosell et al., 2001; Kamunde et al., 2001). For the sculpin the brain contained the highest copper concentration of any tissue examined. The skate exhibited similar

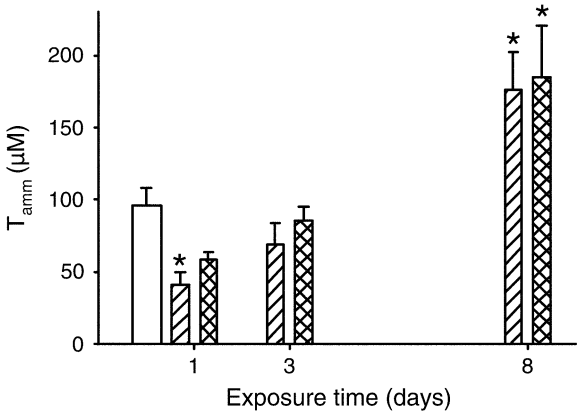

Fig. 5. Total ammonia concentration in plasma (μM) of clear nosed skate (*R. erinacea*) during 7 days of exposure to 0.52 and 1.73 μM ambient copper. Data are expressed as mean ± S.E.M. $n = 5–6$. An * indicates statistically significant difference from corresponding control value at $P < 0.05$.

Table 1
Plasma Cl^-, Na^+ and Mg^{2+} concentrations (in mM) in the clear nosed skate (*R. erinacea*) and the sculpin (*M. octodecemspinosus*). Mean ± S.E.M. For the skate, *n*-numbers were 5 for both groups sampled at day 1 of exposure and 6 for the remaining groups. For the sculpin, *n*-numbers were 4 and 5 for control and copper exposed sculpin, respectively

Treatment (μM Cu)	Cl^-	Na^+	Mg^{2+}
Skate			
Control	277.4 ± 5.4	265.3 ± 6.1	1.77 ± 0.11
Day 1, 0.52	271.8 ± 3.5	282.2 ± 3.9	1.86 ± 0.15
Day 1, 1.73	273.3 ± 3.3	288.2 ± 4.9	1.79 ± 0.11
Day 3, 0.52	290.7 ± 9.4	298.3 ± 9.8*	1.74 ± 0.21
Day 3, 1.73	279.6 ± 4.7	284.9 ± 7.2	1.69 ± 0.12
Day 7, 0.52	275.3 ± 6.8	271.3 ± 9.3	1.96 ± 0.40
Day 7, 1.73	262.2 ± 4.6	259.5 ± 3.3	2.12 ± 0.21
Sculpin			
Control	176.0 ± 1.7	184.3 ± 3.3	1.59 ± 0.07
Day 7, 1.73	175.2 ± 5.9	177.8 ± 6.4	2.20 ± 0.26

* Denotes significant differences at $P < 0.05$ (Student's *t*-test).

brain copper levels only exceeded by copper concentrations in the liver and the rectal gland. To our knowledge these are the first reported values of brain copper concentrations in marine teleosts and elasmobranchs, and the recorded values are in agreement with copper concentrations found in mammals and freshwater teleost (Prohaska and Bailey, 1993, 1995; De Boeck et al., 1997). Maintaining these relatively high copper levels appears to be important for normal central nervous system (CNS) development and function as illustrated by findings of reduced cytochrome c-oxidase (a cuproenzyme) in copper-deficient rat brains (Prohaska and Bailey, 1993, 1995). Indeed severe neurological degeneration due to CNS copper deficiency is the cause of death in the genetically-linked copper homeostasis disorder Menke's disease (Danks et al., 1972).

Table 2
Na/K-ATPase activity (mol ADP mg $protein^{-1}$ h^{-1}) in gills, rectal gland and intestine of the clear nosed skate (*R. erinacea*). Mean ± S.E.M, $n = 5$ for day 1 and $n = 6$ for remaining groups

	Gill	Rectal gland	Intestine
Control	0.53 ± 0.46	26.9 ± 2.3	1.55 ± 0.22
Day 1, 0.52 μM	0.51 ± 0.15	19.4 ± 0.6	1.69 ± 0.27
Day 1, 1.73 μM	0.24 ± 0.08	30.2 ± 9.1	1.34 ± 0.28
Day 3, 0.52 μM	0.26 ± 0.04	21.4 ± 2.0	1.64 ± 0.43
Day 3, 1.73 μM	0.28 ± 0.09	21.3 ± 2.0	1.25 ± 0.25
Day 7, 0.52 μM	0.21 ± 0.07	25.7 ± 2.6	1.52 ± 0.27
Day 7, 1.73 μM	0.39 ± 0.13	31.2 ± 5.0	1.99 ± 0.38

Even higher copper concentrations were found in skate rectal gland. The rectal gland is another tissue with a presumed high metabolic rate. The rectal gland secretes a highly concentrated NaCl solution at considerable rate (Shuttleworth, 1988) driven by the very high levels of Na/K-ATPase enzyme as reported in the present and previous studies (De Boeck et al., 2001). High rates of oxidative phosphorylation are required for ATP production to support the activity of the high levels of the Na/K-ATPase found in rectal glands. As much as 55% of the overall rectal gland oxygen consumption in elasmobranchs appears to be associated with Na/K-ATPase activity based on ouabain sensitivity (Morgan et al., 1997). This

Table 3
Na/K-ATPase activity (μmol ADP mg $protein^{1}$ h^{-1}) in gills, and segments of the intestine of the sculpin (*M. octodecemspinosus*). Mean ± S.E.M., $n = 4$ for control and 5 for the copper exposed group

	Gill	Anterior intestine	Mid intestine	Posterior intestine
Control	3.45 ± 1.00	3.51 ± 0.40	3.20 ± 0.64	4.04 ± 0.78
Day 7, 1.73 μM	5.64 ± 1.14	5.71 ± 0.78	3.61 ± 0.41	3.52 ± 0.36

substantial ATP consumption must be dependent on considerable titer of the copper containing cytochrome *c*-oxidase, which plays a critical role in oxidative phosphorylation and could thus be a reason for the relatively high copper levels observed in rectal glands.

In contrast to our expectations, the sculpin did not exhibit copper accumulation in the intestine although a trend towards elevated copper was seen in the anterior intestine. The lack of copper accumulation in the intestinal epithelium must be due to the relatively low copper levels and perhaps the limited exposure duration employed in the present study. It does suggest, however, that the gill might be the primary target tissue during metal exposure to relatively low metal concentrations. This observation is in contrast to the situation in lemon sole exposed to relatively high silver concentrations where both branchial and intestinal metal accumulation and effects were observed (Grosell and Wood, 2001). Surprisingly, the skate showed a trend towards elevated intestinal copper during exposure to the highest copper concentration. There are at least two possible explanations for this. One is that the elevated copper concentration in the water caused a stress induced drinking response in this elasmobranch, which would normally drink very little, and that this increased drinking led to intake and subsequent intestinal accumulation of copper. A second possible explanation is that intestinal copper is derived from copper already internalized across the gill epithelium. The later explanation seems more likely, because even lethal silver exposures (presumably stressful) in dogfish shark did not result in elevated drinking (De Boeck et al., 2001).

4.2. Plasma electrolytes, T_{amm} and Na/K-ATPase

The lack of effects of copper on plasma electrolytes is consistent with no observed inhibition of Na/K-ATPase in any of the investigated tissues. However, it is remarkable that a 40–50-fold elevation in branchial copper in the skate did not result in branchial Na/K-ATPase inhibition.

The only consistent effect of copper exposure (aside copper accumulation) was significant changes in skate plasma T_{amm}, although the sculpin exhibited a similar trend. Influence of copper on circulating T_{amm} in freshwater (Laurén and McDonald, 1985; Wilson and Taylor, 1993b; Beaumont et al., 1995; Wang et al., 1998) and marine (Wilson and Taylor, 1993a) teleosts seems to be a general phenomenon. A similar response also has been reported for freshwater and marine teleosts exposed to silver (Webb and Wood, 1998; Hogstrand et al., 1999). Circulating levels of T_{amm} in ammoniotelic teleost fish is determined by the relative rates of ammonia production from protein and amino acid catabolism (liver) and T_{amm} excretion predominantly at the gill. Elevated plasma T_{amm} during copper exposure in teleosts is most likely due to a combined elevation of protein breakdown arising from elevated cortisol (Van der Boon et al., 1991) and impaired branchial ammonia excretion (see Grosell et al., 2002 for a review). The situation in elasmobranchs (and ureotelic teleosts) is a little more complicated; in addition to the two components mentioned above, a third component is important for setting circulating levels of T_{amm}. In elasmobranchs glutamine synthetase (GS) converts ammonia to glutamine for urea production via both uricolysis and the ornithine-urea cycle (OUC) (Mommsen and Walsh, 1989, 1991). The low branchial permeability for T_{amm} seen in elasmobranchs has been suggested to be due to T_{amm} trapping by branchial GS (Wood et al., 1995). T_{amm} trapping by branchial GS could be important not only in maintaining low branchial T_{amm} permeability, but could also be of importance for circulating T_{amm} levels by maintaining a favorable gradient for plasma T_{amm} clearance to the gill tissue by suppressing the gill tissue T_{amm} concentration. Considering the substantial copper accumulation in the skate gill tissue, effects on the suggested GS mediated T_{amm} trapping cannot be dismissed and certainly deserves more attention. The present study does not provide sufficient information to determine the likely effects of copper leading to elevated T_{amm} in the skate, but future studies should involve measurement of branchial T_{amm} excretion and T_{amm} trapping capacity.

Plasma urea was not measured in the present study, but previous studies of the effects of silver on circulating urea levels revealed that the reduced plasma urea was due to a generally increased branchial permeability (De Boeck et al., 2001). The same study revealed that circulating magnesium concentrations changed by the greatest order of magnitude in response to silver exposure (5-fold compared to a 30% reduction for urea) presumably due to elevated influx across the branchial epithelium. Since plasma magnesium levels was

unaffected, changes in plasma urea due to gill permeability changes in the skate gill in the present study are not likely.

5. Summary and conclusions

The main finding of the present study was a marked difference between branchial copper accumulation in the elasmobranch skate and the teleost sculpin. While other subtle differences in copper uptake and distribution were found, the difference in branchial copper accumulation most likely explains why elasmobranch fishes appear to be more sensitive to metals than most marine teleosts. The only effect aside from changes in tissue copper concentrations seen in the present study was changes in plasma T_{amm} in the skate. Our findings suggest that the target for sub-lethal copper toxicity in elasmobranchs is T_{amm} metabolism rather than osmoregulation as is the case for teleost fish.

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