



Copper accumulation and toxicity in the Plata pompano *Trachinotus marginatus* Cuvier 1832 (Teleostei, Carangidae)

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Abstract. The Plata pompano *Trachinotus marginatus* inhabits the surf zone of sandy beaches in Southern Brazil. Increasing copper concentrations have been reported in this ecosystem likely associated with the growing industrial and urban activities in the surrounding area. In the present study, copper accumulation and toxicity were analyzed in *T. marginatus* acclimated to salinity 30. Toxicity tests were performed to determine the copper concentration lethal for 50% of the individuals tested after 96 h (LC₅₀). Based on dissolved copper concentrations, LC₅₀ was estimated at 0.449 mg/L. To determine gill copper accumulation, fish were exposed (96 h) to 0.146 (sublethal concentration) and 0.369 mg/L dissolved copper (lethal concentration). Gill copper accumulation linearly increased as a function of the exposure time when fish were exposed to the sublethal concentration. However, a saturation of copper binding sites was observed after 6 h of exposure to the lethal concentration. The maximum copper accumulation in gills was estimated at 11.85 µg/g dry weight. These findings indicate that *T. marginatus* tolerance to copper is similar to that shown by other pompano species. They also suggest that gills are a biotic ligand for copper in *T. marginatus* that could be used to model the toxic effects of this metal.

Key words: acute toxicity, copper, gills, seawater, *Trachinotus marginatus*.

Resumo. Acumulação e toxicidade do cobre no pampo malhado. *Trachinotus marginatus* Cuvier 1832 (Teleostei, Carangidae). *Trachinotus marginatus* habita a zona de arrebentação de praias arenosas do Sul do Brasil. Os níveis de cobre têm aumentado neste ecossistema devido ao desenvolvimento industrial e urbano na região. Portanto, o objetivo deste estudo foi avaliar a toxicidade aguda e a acumulação branquial de cobre em *T. marginatus* aclimatado à salinidade 30. Baseado em testes de toxicidade (96 h) a concentração letal de cobre dissolvido para 50% dos indivíduos testados (CL₅₀) foi estimada em 0,449 mg/L. Os pampos foram expostos (96 h) a 0,146 mg/L de cobre dissolvido (concentração subletal), quando apresentaram uma acumulação branquial linearmente crescente de cobre em função do tempo de exposição; e à 0,369 mg/L de cobre dissolvido (concentração letal), quando uma saturação dos sítios de ligação do cobre foi observada nas brânquias após 6 h de exposição, sendo a máxima acumulação do metal neste tecido estimada em 11,85 ± 0,07 µg/g de peso seco. Estes resultados indicam que a tolerância de *T. marginatus* ao cobre é similar àquela relatada para outras espécies de pampo. Eles também sugerem que as brânquias de *T. marginatus* constituem um ligante biótico para o cobre que pode ser utilizado para modelar os efeitos tóxicos deste metal em água marinha.

Palavras-chave: água do mar, brânquias, cobre, toxicidade aguda, *Trachinotus marginatus*.

Introduction

The growing urban and industrial activities developed in coastal areas are significantly increasing the level of contaminants being released into estuarine and coastal waters (Seeliger & Knak 1982, Baumgartem & Niencheski 1998). Organic and inorganic contaminants reaching directly or indi-

rectly the coastal waters are derived from both natural and anthropogenic sources. Depending on metal concentration and speciation, as well as abiotic and biotic factors considered, aquatic contaminants may accumulate in aquatic animals and exert toxic effects (MacRae *et al.* 1999, Di Toro *et al.* 2000).

Actually, copper is one of the most relevant inorganic contaminant reaching coastal waters from sandy beaches in Southern Brazil (Baumgarten & Niencheski 1998). Studies on trace metals concentrations in Patos Lagoon estuary have demonstrated significant increases in copper concentration in sediments and waters around the industrial and harbor areas. In early 80's, the concentration of dissolved copper in waters near the mouth of the Patos Lagoon estuary was $\sim 2 \mu\text{g/L}$ (Seeliger & Knak 1982). Almost 20 years later, maximum values reported were $34 \mu\text{g/L}$ (Baugartem & Niencheski 1998). These authors associated these increased values mainly to the harbor activities developed in the studied area.

In light of the above, assessing the toxicity of copper to biota is in need for the establishment of safe limits for metal discharging in estuarine and coastal waters. Several toxicological studies are being performed with organisms from the Patos Lagoon estuary (Monserrat *et al.* 2007), but studies using fish species typically from the adjacent coastal ecosystem are lacking. The widespread water contamination by copper in coastal ecosystems potentially poses ecological risks to many marine organisms. Therefore, ecotoxicological studies involving coastal marine species are needed for a better understanding of toxic effects induced by copper contamination in seawater animals (Kwok *et al.* 2008).

In Brazil, regulations concerning copper release into the environment are based on the dissolved copper concentration in surface waters. Although the Brazilian National Council for Environment (CONAMA) establishes $5 \mu\text{g/L}$ of dissolved copper as a harmless concentration, it may allow concentrations as high as 1 mg/L of dissolved copper to be released into the oceans (CONAMA 2005).

Copper toxicity occurs when a specific amount of metal binds to physiologically active biological membranes, generally outcompeting cations injuring the physiological mechanism. This threshold level depends on animal species and life stage (MacRae *et al.* 1999). Therefore, it is important to determine the kinetics of copper accumulation in different tissues of the tested species to identify possible biotic ligands (toxicity sites).

Water chemistry largely influences copper toxicity. It is known that copper bioavailability and toxicity occur when the amount of copper in water exceeds the combined capability of dissolved organic matter (DOC) to bind metal, cations to outcompete the metal for binding to the biotic ligand, water chemistry to transform the metal

to non-toxic species, particulate organic carbon (POC) to adsorb the metal, and mineral particles to incorporate the metal into their matrix (Arnold *et al.* 2005).

The strong influence of abiotic factors on copper toxicity in freshwater species has been recognized by the Environmental Protection Agency from the United States (US-EPA) and has been incorporated into the framework of the Biotic Ligand Model (BLM), which has been applied to establish water quality criteria for freshwaters (WQC) (US-EPA 2003).

The most toxic copper species is the free cupric ion (Cu^{2+}), but other forms like CuOH^+ may be of concern when pH increases above 7.5 (Hall & Anderson 1999, De Schampelaere *et al.* 2002, Paquin *et al.* 2002, Arnold *et al.* 2005). BLM is based on the premise that there is a strong correlation between metal concentration on or into the biotic ligand (i.e., the fish gills) and its subsequent acute toxicity (MacRae *et al.* 1999, Paquin *et al.* 2002). In turn, metal accumulation in the toxic sites also depends on the water chemistry (Di Toro *et al.* 2000). Currently, many efforts are being made towards the extension of the freshwater BLM to saltwater conditions (Bianchini *et al.* 2003, 2004). However, many problems have still to be solved in order to create a reliable BLM to marine systems (Arnold *et al.* 2005, Grosell *et al.* 2007).

The large variation among species sensitivity to copper, makes difficult the establishment of safe limits of metal releasing in the environment. It is not possible to test all organisms, and thus aggregate the full range of sensitivity. Considering the large number of species inhabiting a specific site, it is unlikely that the most sensitive and the most tolerant species had been identified already (Grosell *et al.* 2007). Moreover, the same species generally presents different sensitivity to metals according to the ontogenetic stage, body mass, and sex (Serafim & Bebianno 2001, Mubiana *et al.* 2006).

The Plata pompano *Trachinotus marginatus* is one of the most important fish species in the food web of sandy beaches in Southern Brazil (Cunha 1987). The surf zone is the main site of recruitment for this fish species. Therefore, any environmental alteration in the equilibrium of this ecosystem can have severe ecological consequences to *T. marginatus*.

Based on this background information, the main goals of the present study were to determine the acute copper toxicity and to describe the kinetics of copper accumulation in gills of the Plata pompano *T. marginatus*.

Material and Methods

Fish collection and acclimation: Juveniles of *Plata pompano* *T. marginatus* (mean weight = $5.54 \text{ g} \pm 0.10 \text{ SE}$) were collected at ~1 m depth in the inner surf zone of Cassino Beach (Southern Brazil; $32^{\circ}15'S$; $52^{\circ}14'W$) using sieve nets (5 mm mesh). After collection, fish were acclimated to laboratory conditions for one week. They were kept in glass fiber tanks filled with 300 L of seawater at salinity 30, under constant aeration, fixed temperature (20°C) and photoperiod (12L: 12D). Fish were fed every other day on commercial ration.

Acute copper toxicity: Pompanos were randomly taken from the acclimation tanks and acutely (96 h) exposed to copper in round glass aquaria filled with 2 L of seawater at salinity 30. Five fishes were tested in each aquarium. Nominal copper concentration tested ranged from 0.3 to 1.5 mg Cu/L. A control (without addition of copper in the water) was also tested. Each experimental condition was tested in duplicate ($n = 10$). Copper was added to the experimental media and allowed to equilibrate for three hours prior to *Plata pompano*'s introduction. Experimental media were completely renewed every 24 h, when mortality was registered and dead organisms were discharged. Prior to fish introduction and 24 h thereafter, non-filtered and filtered ($0.45 \mu\text{m}$ filter) water samples were collected from the experimental media for further analyses of total (non-filtered samples) and dissolved (filtered samples) copper concentrations. Samples were analyzed by atomic absorption spectrophotometry (detection limit = $10 \mu\text{g/L}$, $\lambda = 324.7 \text{ nm}$, GBC Avanta 932, IL, USA).

Kinetics of copper accumulation: To characterize copper accumulation kinetics in gills, tests were run as described for the acute toxicity tests. Pompanos were kept for 96 h under control conditions (without copper addition in the water) or exposed (96 h) to copper. Two copper concentrations were tested, based on results found in the toxicity tests: 0.146 mg/L dissolved Cu (sublethal concentration) and 0.369 mg/L dissolved Cu (lethal concentration). Before copper exposure, six pompanos were killed by spinal section and gills were extracted for copper concentration measurement (time of exposure = 0 hours). After 6, 12, 24 and 96 h of copper exposure, six pompanos from each experimental condition were killed and gills were extracted for copper concentration measurement.

Statistical analysis: Probit analysis was used to calculate copper concentrations inducing mortality of 50% of the individuals tested (LC_{50}) and their corresponding 95% confidence intervals

(Finney 1971). Based on the dissolved copper concentrations measured, and considering the chemistry of the water used in the present study (Pinho *et al.* 2007), free cupric ion (Cu^{2+}) concentration and activity were estimated using the Visual MINTEQ software. Gill copper accumulation data were expressed as mean \pm standard error ($n = 6$). Comparisons of mean values were performed using one-way analysis of variance (ANOVA) followed by the Tukey's test. Linear and non-linear regression analysis was used to fit the accumulation data obtained for fish exposed to the sublethal and lethal copper concentrations, respectively. In all cases, the significance level adopted was 95%.

Results

Acute toxicity values based on different copper fractions are shown in Figure 1. LC_{50} values (and their corresponding 95% confidence limits) calculated based on total and dissolved copper concentrations were 0.527 (0.477 - 0.574) and 0.449 (0.407 - 0.489), respectively. Based on estimated free cupric ion concentration and activity LC_{50} values were calculated as 0.075 (0.067 - 0.082) and 0.016 (0.014 - 0.017), respectively. Free cupric ion, the most toxic species of copper, represented ~20% of the dissolved copper present in the experimental media.

Kinetics of gill copper accumulation over time is shown in Figure 2. Fish kept under control conditions (no addition of copper in the water) did not show any significant change in gill copper burden. On the other hand, those exposed for 96 h either to the sublethal (0.146 mg/L) or the lethal concentration (0.369 mg/L) of copper showed a

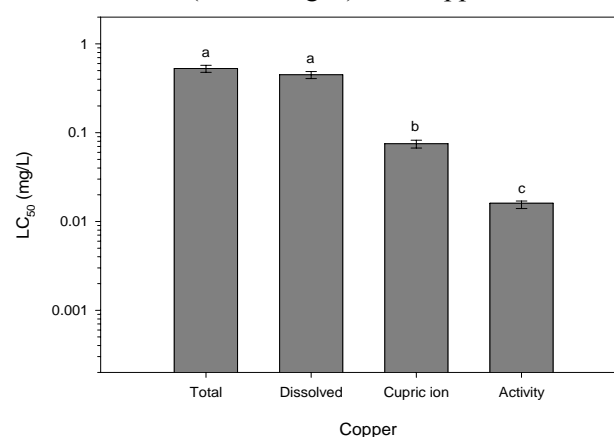


Figure 1. Acute toxicity in the pompano *Trachinotus marginatus* exposed to copper for 96 h. Values are expressed as LC_{50} and their corresponding 95% confident intervals. Toxicity values were calculated based on different fractions of copper. Total and dissolved copper were measured by atomic absorption spectrophotometry. Free cupric ion concentration and activity were estimated using the Visual MINTEQ software. Different letters indicate significant different toxicity values ($p < 0.05$).

significant increase in gill copper accumulation. However, kinetics of copper accumulation was dependent on the copper concentration tested. Fish exposed to the sublethal copper concentration showed a linear increase ($y = 9.123 + 0.045x$; $R^2 = 0.97$) in gill copper accumulation. However, those exposed to the lethal copper concentration showed a saturation-type kinetics ($y = 11.85x/(3.027 + x)$; $R^2 = 0.99$). Maximum copper concentration achieved in the gills was estimated as being $11.85 \pm 0.07 \mu\text{g/g}$ dry weight (Fig. 2).

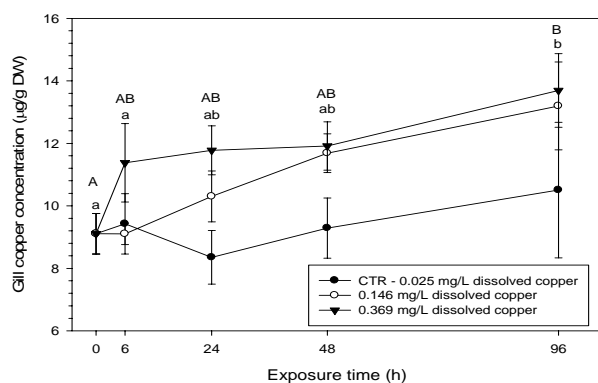


Figure 2. Gill copper accumulation in *Trachinotus marginatus* kept under control conditions or exposed to sublethal (0.146 mg/L) or lethal (0.369 mg/L) copper concentrations. Data are means \pm standard error ($n = 6$). Different lowercase and capital letters indicate significant different values ($p < 0.05$) between different exposure times to the sublethal and lethal copper concentrations, respectively. No significant differences ($p > 0.05$) were observed between exposure times in control fish (CTR).

Discussion

Determination of the chemistry of the experimental media used to perform toxicity tests is of great important for a correct prediction of copper speciation, bioavailability, and its subsequent toxicity. In fact, several physical and chemical water parameters (e.g., pH, alkalinity, hardness, ion concentration, among others) have shown to influence copper toxicity (Di Toro *et al.* 2000).

Copper toxicity occurs when the metal binds to active sites in the biotic ligand. However, as discussed above, the degree of copper accumulation and its consequent toxicity are highly dependent on the water chemistry. In the present study, experiments were performed using seawater. Under this situation, sodium, calcium, and other major cations present in seawater compete with copper for the active binding sites in the biotic ligand, i.e., the fish gills. In turn, anions present in high concentrations in seawater (e.g., chloride) complex copper, making it less available to bind to biological membranes and exert its toxicity (Di Toro *et al.* 2000). Therefore, copper toxicity in seawater organisms is generally lower than in freshwater ones (US-EPA 2003). Acute copper

toxicity data obtained in the present study for the Plata pompano *T. marginatus* in seawater is in agreement with this idea.

In the present study, tests were performed in juvenile fish, which tend to be more sensitive than adult ones due to their generally higher metabolic rate (Dave 1986, Grosell *et al.* 2002). Despite that, juvenile Plata pompano *T. marginatus* showed to be a copper tolerant species when compared to marine echinoderms, planktonic crustaceans and larval stages of mollusks (Garnacho *et al.* 2000, La Breche *et al.* 2002, Bielmeyer *et al.* 2005, Rodrigues 2007, Martins 2008). As far as we know, the present study is the first to report copper accumulation and toxicity in *T. marginatus*. However, previous toxicity tests were performed in the Florida pompano *Trachinotus carolinus* (Birdsong & Avavit 1971), another pompano species from the same genus. Data available for these pompano species indicate that they show a very similar tolerance to copper in seawater. However, compared to juveniles from other marine fish species, *T. marginatus* showed to be more sensitive to copper than the Coho salmon *Onchorhynchus kisutch* (Chapman & Stevens 1978), but more tolerant than the Atlantic silverside *Menidia menidia* (Cardin 1982 *apud* USEPA 2003).

The high tolerance of *T. marginatus* to copper suggests that this fish species is not a useful model for regulatory issues. However, it would be of great importance in biomonitoring programs using biomarkers, aiming to identify the occurrence of adverse effects induced by copper in seawater (Hagger *et al.* 2006).

Figure 1 showed that copper LC_{50} estimated for total and dissolved copper concentrations were not significantly different, indicating that all toxic copper species appears to be present in the dissolved phase. Therefore, it is important to consider metal speciation under the conditions used to perform the toxicity tests. Considering the dissolved copper concentrations measured and the chemistry of the experimental media used in the present study, acute copper toxicity was determined based on different copper fractions (Fig. 1). Copper speciation was performed using Visual MINTEQ, a chemical model used to determine speciation of copper and other metals in saltwater conditions.

Toxicity data show that the LC_{50} value calculated based on cupric ion concentrations was ~5-fold lower than that determined based on dissolved copper concentrations (Fig. 1). In fact, free cupric ion is accepted as the most toxic species of copper (Hall & Anderson 1999, De

Schamphelaere *et al.* 2002, Arnold *et al.* 2005), and the most abundant ionic copper species when a high copper concentration is added to the experimental media (Blanchard & Grosell 2005, Martins 2008). Even for the most toxic species, i.e. the free cupric ion, not all copper is available to bind the active sites in the biotic ligand, i.e., the fish gills. The LC_{50} value calculated based on cupric ion activity showed that 50% fish lethality is observed when only 20% of cupric ions are active (Fig. 1).

Regarding copper accumulation in biological tissues, data reported in the literature indicate that the kinetics of copper accumulation may follow many mathematical models. The linear- and saturation-type models are the most frequently reported (Brouwer *et al.* 2002, Borgmann *et al.* 2004). When exposed to a sublethal concentration of copper (0.146 mg/L), a positive linear relationship was observed between exposure time and gill copper burden in *T. marginatus* (Fig. 2). Conver-

sely, gill copper accumulation showed a saturation-type kinetics when fish were exposed to a lethal concentration of copper (0.369 mg/L). This finding indicates that gill binding sites for copper were completely occupied after 6 h of exposure to a high copper concentration. Therefore, gills may act as an important biotic ligand for copper in *T. marginatus*.

As observed in other fish species (Roméo *et al.* 1994; Zia & McDonald 1994), gills from *T. marginatus* seem to be a primary site of metal accumulation from the dissolved phase. Therefore, copper accumulation levels in gills of *T. marginatus* could be used for assessing the degree of copper exposure in the field, as suggested for other fish species (Catsiki & Stroglyoudi 1999).

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