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Influence of salinity and organic matter on silver accumulation in Gulf toadfish (*Opsanus beta*)

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Abstract

To help extend the freshwater based biotic ligand model for silver (Ag) into brackish and saltwater conditions, 50 g Gulf toadfish (*Opsanus beta*) were acclimated to 2.5%, 5%, 10%, 20%, 40%, 80%, or 100% salt water and exposed for 6 d to 1.0 μM $AgNO_3$, with or without 10 mg C/L organic matter. Suwannee River natural organic matter collected by reverse osmosis was used. Silver accumulation in toadfish gills and plasma decreased as salinity increased, indicating low bioavailability of AgCl complexes. Complexation of Ag by organic matter, normally important in freshwater conditions, was less important as salinity increased. Although relatively little intestinal Ag uptake was observed, both liver and bile accumulated Ag from water imbibed past the isosmotic salinity point (∼1/3 salt water). Toadfish also produced intestinal carbonate pellets, minerals which did not influence Ag accumulation. Our results further stress the importance of Ag speciation, physiological mechanisms, and intestinal Ag uptake when modelling Ag uptake and toxicity beyond freshwater conditions.

Keywords: Silver; Salinity; Organic matter; Gulf toadfish

1. Introduction

In freshwater, silver toxicity is caused by ionic Ag^+, which targets specific sites on or in fish gills (Wood et al., 1999). On the basolateral gill membrane, Ag^+ inhibits Na^+/K^+-ATPase activity, decreasing the active uptake of both Na^+ and Cl^- (Morgan et al., 1997; Bury and Wood, 1999). This decreased active ion uptake results in a net loss of ions from the blood plasma, circulatory failure through the collapse of fluid volume regulation, and ultimately death of the fish (Wood et al., 1999). In natural conditions most Ag is complexed: anionic ligands such as chloride, dissolved organic matter (DOM), organic colloids, thiosulphate, and sulphide all bind Ag^+ (Wood et al., 1999; Rose-Janes and Playle, 2000; Richards et al., 2001; Ward and Kramer, 2002; Bertram and Playle, 2005). In general, when Ag is complexed by DOM and/or inorganic ions, lower degrees of bioavailability and toxicity result (Janes and Playle, 1995; Hogstrand and Wood, 1998). Aside from complexation, competition for gill binding sites between Ag^+ and other cations, such as Ca^{2+}, Na^+, and Mg^{2+}, can also result, decreasing Ag interactions at the gill and in turn its toxic effect (Janes and Playle, 1995; McGeer et al., 2000; Schwartz and Playle, 2001).

As a result of widespread interest amongst the scientific, regulated and regulatory communities to develop water quality criteria for aquatic risk assessments, the biotic ligand model (BLM) has been proposed as a tool to evaluate quantitatively how water chemistry affects the speciation and biological availability of metals in aquatic systems (e.g. Paquin et al., 2002). Through consideration of Ag concentrations, complexing ligands, and competing cations in aquatic systems, Janes and Playle (1995) developed a Ag-gill binding model to predict the amount of Ag binding on or in fish gills (Fig. 1). The Ag-gill binding model was based on conditional equilibrium stability constants (K) determined for interactions between Ag^+ and

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✠ Dr. Richard Playle passed away just before the submission of this manuscript. His intelligence, generosity and charm will be dearly missed—J.W. Nichols.

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the gill ($\log K_{Ag\text{-}gill} = 10.0$), complexation in the water column by dissolved organic matter ($\log K_{Ag\text{-}DOM} = 9.0$), and also for those competitive interactions at the gill involving cations. These $\log K$ values represent binding strengths between various waterborne cations and ligands, and were originally entered into a geochemical model, MINEQL$^+$, for predictive purposes (Janes and Playle, 1995). A physiological Ag biotic ligand model was also published (McGeer et al., 2000) with some different values (notably, $\log K_{Ag\text{-}gill} = 7.6$), and an in vitro model reported $\log K_{Ag\text{-}gill} = 8.8$ (Zhou et al., 2005), but the same principles apply.

One limitation of these BLMs is that, since they were designed to predict Ag toxicity to freshwater fish, they cannot necessarily predict toxicity of Ag to fish in brackish or marine conditions (Hogstrand et al., 2002). Plasma osmolality in both freshwater and seawater fish is approximately 300 mosmol, while that of freshwater ranges from 0.1 mosmol to about 20 mosmol. Due to this osmotic difference, freshwater fish must actively take up ions like Na^+, Cl^-, and Ca^{2+} across their gills to maintain internal electrolyte levels and combat diffusive ion losses. Thus, with the majority of ionoregulation occurring at the gills, this organ is considered the main site of acute toxic action of metals in freshwater fish, as is emphasized in the Ag-gill binding model (Fig. 1). In contrast, seawater osmolality is approximately 1000 mosmol, so water tends to leave fish by osmosis while ions enter the fish down their concentration gradients. To compensate for osmotic water loss, marine fish drink to avoid dehydration, actively take up ions in the gut so that water follows by osmosis, and excrete excess ions at the gills and kidneys to maintain physiological concentrations of electrolytes (Wood et al., 1999, 2004; Hogstrand et al., 2002). If the seawater consumed is metal-contaminated, metal binding may increase within the intestine of marine fish. With this alternative pathway of metal uptake, the intestine might become another important site of metal toxicity in marine fish (Grosell et al., 1999; Grosell and Wood, 2001; Hogstrand et al., 2002), something not covered by the current Ag-gill binding model.

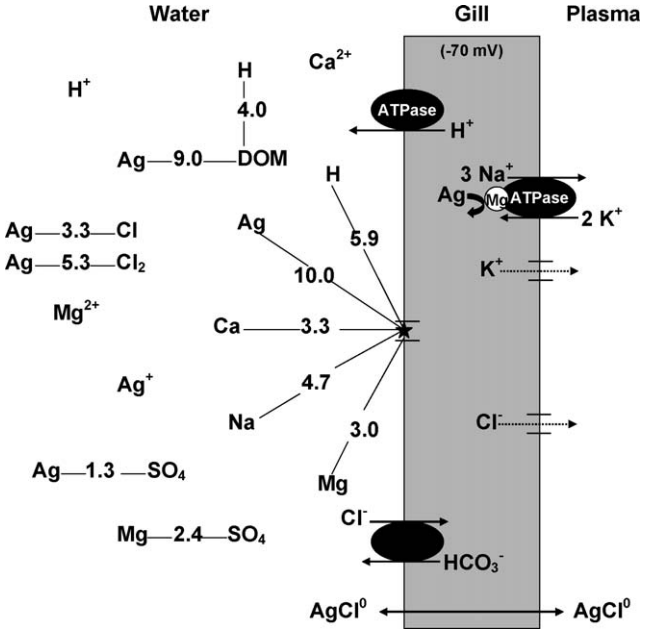

Fig. 1. The gill modelling approach for Ag in freshwater. The numbers represent log conditional equilibrium stability constants (K) for Ag^+ and other cations binding at the gills (asterisk) and for Ag^+ binding to dissolved organic matter (DOM) and Cl^- in the water column (from Janes and Playle, 1995). The Mg-gill log K value is from Schwartz and Playle (2001). Major ionoregulatory functions of the freshwater fish gill affected by Ag toxicity are also indicated.

In terms of modelling in brackish water and more saline conditions, Wood et al. (1999) predicted that the same principles of metal complexation and cationic competition would be used as in the freshwater model, although in a more complicated system. For example, Ag speciation would be more important in the model due to the much higher concentrations of Cl^-; silver chloride complexes would dominate in seawater leaving Ag^+ virtually non-existent (Grosell et al., 1999). Due to osmotic changes occurring from freshwater to seawater, and related changes in Ag speciation, Ag uptake and toxicity in fish has been predicted to decrease with salinity to an isosmotic point, and then increase slightly towards marine conditions as fish begin to drink Ag-contaminated water (Fig. 1 in Wood et al., 1999). Recently, Wood et al. (2004) demonstrated this pattern for Ag accumulation using marine Gulf toadfish (*Opsanus beta*). Furthermore, because of increased Cl^- concentrations at higher salinities, the protective effect of DOM has been predicted to be much less important in seawater than in freshwater. In addition, stronger cationic competition at the Ag^+ uptake sites at the gills and the intestinal epithelium would have to be considered when modelling because of the high concentrations of cations in brackish water (Wood et al., 1999, 2004). Finally, the removal of Na^+ and Cl^- along the intestine as marine fish drink would theoretically allow more Ag^+ to bind to the posterior intestine than anterior intestine (Wood et al., 1999).

Another factor that may complicate Ag uptake and toxicity in seawater-exposed fish is the presence of intestinal carbonate pellets. As ions plus water are absorbed along the intestine, divalent cations such as Ca^{2+} and Mg^{2+} are left behind. Walsh et al. (1991) found in Gulf toadfish that these divalent cations plus excreted HCO_3^- crystallize to form an unusual Ca–Mg–carbonate mineral. These carbonate pellets have gained attention because they might detoxify Mg and metals like Ag.

To help extend the freshwater BLM for Ag into brackish and marine conditions, we investigated Ag binding to the gills and gut of marine Gulf toadfish acclimated to various salinities. This work complements and extends the 24 h ^{110m}Ag exposures to Gulf toadfish acclimated to a similar range of salinities (Wood et al., 2004). The influence of organic matter on Ag accumulation was also tested, and intestinal carbonate pellets from seawater acclimated fish were collected and characterized to establish their influence on Ag uptake.

2. Materials and methods

2.1. Silver uptake in Gulf toadfish held in various salinities plus natural Suwannee River organic matter

Gulf toadfish (*Opsanus beta*, ~50 g) were captured as a roller trawl bycatch by commercial shrimpers in Biscayne Bay,

Florida, USA, in April 2002. Fish were maintained at the University of Miami in glass aquaria supplied with running Biscayne Bay seawater (33–35‰, temperature 25 ± 1 °C). Each aquarium contained a sand bed (2–5 cm depth) and several polyvinyl chloride (PVC) tubes. At 1 and 3 d after arrival, fish were bathed in a mixture of Malachite Green and formalin (Wood et al., 1997) as a prophylactic treatment to prevent infection by the ciliate *Cryptocaryon irritans*. Fish were maintained under these conditions for at least 1 week and were fed ad libitum with frozen squid on alternate days. Food was withheld for 48 h before experimentation.

Following 1 week acclimation to laboratory conditions, 180 toadfish were randomly divided between 18, 20 L polyethylene containers holding 15 L of aerated Biscayne Bay salt water (SW) diluted with deionized water to one of nine salinities (0.05%, 0.5%, 2.5%, 5%, 10%, 20%, 40%, 80% or 100% SW) to which the toadfish were allowed 10 more days to acclimate. During this acclimation period toadfish in the 0.05% and 0.5% SW exposures were gradually acclimated to these salinities beginning at 1% SW, with a 0.25% decrease in salinity daily, but toadfish in the 0.05% exposures did not survive even 2 d of acclimation and the 0.5% SW fish also did not survive acclimation. The remaining toadfish acclimated to the various salinities without mortalities. The fish were then exposed for 6 d to 1 μM $AgNO_3$ with or without 10 mg C/L Suwannee River natural organic matter (NOM; see below for collection details). Exposures were static renewal systems with 50% replacement daily. During the 6 d exposure eight fish (one per salinity) were sampled just before Ag addition (control), then 48 fish (three per exposure) at 4 h after the introduction of Ag and NOM, and at 2, 4, and 6 d.

The Suwannee River NOM was isolated using a stainless steel, portable reverse osmosis unit (Limnological Research, Kelowna, British Columbia, Canada) from surface waters of the Suwannee River, near Valdosta, Georgia (30°58′ N, 83°12′ W). The river originates in the Okeefenokee Swamp; permission to sample was obtained by the Okeefenokee National Wildlife Refuge and Wilderness Area. Reverse osmosis concentrate was treated with a cation exchange resin (USF C-211 H cation resin, U.S. Filter Corporation, Rockford, IL) to remove metals, then was stored at 4 °C until diluted for use. Additional details may be found in Schwartz et al. (2004). The concentrate was 2670 mg C/L, so 30 mL of concentrate was added to 8 L to reach 10 mg C/L.

2.2. Sampling procedures

During the experiment, fish were randomly sampled and individually anaesthetized in water containing MS222 anaesthetic (3-aminobenzoic acid ethyl ester, 1 g/12 L water; Sigma). The mass (M, g) and fork length (L_S, cm) of each fish were taken to calculate condition factor (K), where $K = 100ML_S^{-3}$ (Mommsen, 1998).

An ice-cold 1 mL luerlock syringe (Hamilton) rinsed with heparinized saline (0.008 g heparin sodium salt (Sigma) in 25 mL Cortland Saline) was used to withdraw blood (≤1000 μL) from anaesthetized fish by caudal puncture. A portion of the blood sample (~100 μL) was dispensed into two heparinized hematocrit tubes, centrifuged for 5 min, and hematocrit (Ht) was calculated as a percentage by dividing the packed cell lengths by total sample lengths (both in mm). Plasma was then obtained by centrifuging remaining blood with an IECMicro-MB Centrifuge at 12,700 g for 2 min. The plasma supernatant was assayed for protein content using a refractometer, and then was stored in a −20 °C deep freezer until analyzed later for Ag, Cl, Na, and glucose at Wilfrid Laurier University.

Gill tissue (~0.15 g) was extracted from each fish's right-side second gill arch with stainless steel scissors and forceps. Gill samples were agitated in 100 mL of ultrapure water for 15 s to remove loosely bound Ag, then placed into separate microcentrifuge tubes and stored in a deep freezer (−80 °C). The liver of each fish was sampled in a similar manner. Gall bladders were also excised from each fish (whole if possible) and stored in a deep freeze. When sampled, intestines were divided into three portions: the anterior intestine consisting of the first third of the intestine behind the pyloric ceca, the mid intestine (middle third), and the posterior intestine as the last third of the intestine in front of the anus. Carbonate pellets, if present in the intestines, were also removed and placed into separate microcentrifuge tubes. After each sampling period the plasma, bile, tissue, and carbonate pellet samples were transferred to a −40 °C freezer until later analysis for Ag and other ions.

2.3. Sample analysis

At Wilfrid Laurier University, gill, liver, and intestine samples were thawed, weighed, and digested in 1N Ultrapure HNO_3 (five times dilution; Ultrex II Ultrapure HNO_3 Baker Analyzed reagent) for 3 h at 80 °C. From each tissue digest, 100 μL of supernatant was dispensed into a new microcentrifuge tube containing 900 μL of ultrapure water. Plasma samples were thawed and diluted in the ratio of 50 μL plasma to 450 μL 0.1N ultrapure HNO_3, while bile was diluted in the ratio of 10 μL bile to 990 μL 0.1N ultrapure HNO_3. Carbonate pellets were thawed, weighed, and digested in 8N HNO_3 (10 times dilution) for 2 h at 80 °C. Silver concentrations in acidified tissue, plasma, bile, carbonate pellets, and water samples were measured using a graphite furnace atomic absorption spectrophoto-meter (AAS; Spectra Varian AA-880 with GTA-100 atomizer, Mulgrave, Victoria, Australia), Sigma certified standards, and N_2 gas. In accordance to Varian operating protocol, 10 μL of each sample was injected and heated for 5 s at 85 °C, 30 s at 95 °C, 10 s at 120 °C, 8 s at 400 °C, and 4.9 s at 2000 °C, during which the absorbance at 328.1 nm was measured.

Water temperature and pH (PHM82 pH meter with GK2401C combination electrode; Bach-Simpson Ltd., London, Ontario) were recorded at each sampling time and periodically throughout the experiment. At each sampling time, water was dispensed into two scintillation vials (one of which was acidified with two drops of 16N HNO_3) and into a borosilicate vial combusted at 450 °C for 4 h. In addition, filtered water samples were also taken on day 1 of the toadfish experiment, where a water sample which had been passed through a syringe-type 0.45 μm Millex-HV filter (Millipore Corporation, Bedford, MA, USA), was collected into an acidified scintillation vial (for Ag analysis),

and also into a borosilicate vial for TOC measurement. To eliminate contamination, filters were rinsed with 40 mL of ultra-pure water, followed by 20 mL of sample water, before the sample was passed through the filter and collected. Water samples were refrigerated until analyzed at Wilfrid Laurier University. Water samples in the borosilicate vials were analyzed for total organic carbon (TOC) using a Shimadzu 5050A Total Carbon Analyzer (Mandel Scientific Co. Ltd., Guelph, Ontario).

Acidified water and plasma samples were analyzed for Ag by graphite furnace AAS (above), and Na content by flame AAS (Varian). Flame AAS was also utilized to measure Mg and Ca content in acidified water samples. Carbonate pellet digests were also analyzed for Ca and Mg content using flame AAS. Due to Na interference during Ag analysis at high salinity (i.e., high Ag readings due to high Na), measured Ag concentrations for the 2.5%, 5%, and 10% SW were assumed to be representative of the remaining salinities for which Ag values could not be reliably obtained. Plasma and non-acidified water samples were analyzed for Cl using a mercuric-thiocyanate method (Sigma–Aldrich kit #461-M) and read at 460 nm on a Spectronic 301 spectrophotometer (Milton Roy Company, Rochester, NY). Plasma glucose was measured using the hexokinase glucose-6-phosphate dehydrogenase method (Sigma kit #16-20).

2.4. Statistical analysis

All data points are reported as means ± 1 standard error. Statistical analysis was performed using SigmaStat statistical software (Version 2.03, Jandel Scientific, San Rafael, California). Data from different exposures in each experiment were compared using one-way analysis of variance (ANOVA) or the non-parametric Kruskal–Wallis One Way Anova on Ranks when the normality test failed in the ANOVA analysis. Differences in means were considered significant at $P < 0.05$. In all figures each data point represents one to three fish, as indicated in the figure captions.

3. Results

The average water Ag concentration measured from Ag only and Ag plus NOM exposure buckets below 10% SW was 0.23 μM (Table 1). Water OM in the Ag only exposures was approximately 4 mg C/L and about 13 mg C/L in the Ag plus OM exposures (Table 1). Silver and OM concentrations in filtered water samples did not differ statistically from unfiltered samples ($P > 0.05$; data not presented). Average water Cl, Na, Ca, and Mg concentrations measured from the full strength seawater exposures were 501 mM Cl, 403 mM Na, 7 mM Ca, and 64 mM Mg, respectively, each diluting proportionally as salinity was decreased (Table 1). Water pH also decreased from pH 8.0 in 100% SW to pH 7.1 or 7.2 in 2.5% SW.

Toadfish did not survive acclimation to 0.05% and 0.5% SW so were eliminated before the Ag exposures began. During the acclimation period there was 30% toadfish mortality in one 2.5% SW exposure, 20% mortality at 80% SW, and 10% mortality in both the 5% and 100% SW exposures. Once Ag and NOM were added to the various salinities, toadfish remaining in the 2.5% SW treatments were dead by day 2, and there was 10% mortality in the 80% SW exposure containing Ag and NOM.

Throughout the exposure period no significant differences were seen in Ag accumulation by toadfish with or without NOM (see Nichols, 2003), and to compare Ag accumulation in toadfish between Ag treatments only day 6 data are presented. At the end of the 6 d exposure, toadfish exposed to Ag alone at salinities of 20% SW and below tended to accumulate more Ag on their gills compared to those treated with Ag plus Suwannee River NOM (Fig. 2A). Gill Ag concentrations in Ag only exposed toadfish appeared to increase until 20% SW, past which no Ag accumulation occurred.

Unlike gill Ag, plasma Ag accumulation tended to be higher in toadfish exposed to Ag and NOM at lower salinities (<20% SW) after 6 d of exposure (Fig. 2B). Regardless, plasma Ag accumulation in all fish decreased as salinity increased until 40% SW. This accumulation trend was similar to that observed at the gills, with the exception of a slight increase in plasma Ag occurring at 100% SW. In addition, similar to the gill, Ag concentrations in the plasma of toadfish were low at salinities below 5% SW.

Toadfish exposed to Ag alone at higher salinities (>20% SW) tended to accumulate more Ag in their livers than those treated with Ag and NOM (Fig. 2C). Despite greater Ag accumulation occurring in the plasma of fish at lower salinities, toadfish livers did not accumulate Ag at lower salinities. Similar to liver Ag accumulation, bile Ag appeared greater in Ag only exposed fish at higher salinities on day 6 (Fig. 2D). Toadfish treated with Ag and NOM had bile Ag concentrations similar to control fish throughout the exposures.

After 6 d of Ag exposure little Ag accumulation was seen in the intestines of toadfish exposed to Ag alone or to Ag with NOM (Fig. 3). Intestinal Ag concentrations remained similar to control values during the entire exposure period, and never significantly differed between the two Ag treatment groups.

Plasma Cl and Na concentrations fluctuated during the exposure period and tended to be lowest in both Ag only and Ag plus NOM toadfish at 2.5% and 5% SW throughout the experiment (data not presented). Plasma Cl and Na concentrations never differed significantly between Ag only exposed toadfish and those treated with Ag plus NOM. Plasma glucose was never significantly different between toadfish from the Ag treatments, but often appeared greatest at 2.5% and 5% SW. Blood Ht and plasma protein never differed significantly. At the end of the exposure, the average condition factor for all the fish was 1.4, with a tendency for higher values occurring at lower salinities.

Throughout the experiment the majority of intestinal carbonate pellets were found in toadfish acclimated to salinities ≥40% SW whether they were exposed to Ag alone or to Ag plus NOM (Table 2). Measured Ag concentrations in the pellets fluctuated and were in the low nmol/g wet pellet weight range. Pellet Ca concentrations ranged from 0.1 to 0.9 mol/g wet weight, and pellet Mg from 0.1 to 1.1 mol/g wet weight, with both concentrations increasing with salinity (Table 2). The Ca:Mg ratio in pellets was between 0.5 and 1.5 and appeared not to be influenced by salinity.

Table 1
Water chemistry in treatment containers with Gulf toadfish acclimated to various salinities and exposed for 6 d to 1 μM $AgNO_3$ (nominal) with or without 10 mg C/L NOM (Suwannee R. NOM)

Exposure	pH	TOC (mg C/L)	Ag (μM)	Measured concentrations (mM)			
				Cl	Na	Ca	Mg
Control							
0.05% SW	7.1 ± 0.1 (6)	5.1 (1)	0.01 ± 0.00 (11)	4 ± 0 (11)	2 ± 0 (11)	0.2 ± 0.0 (11)	1.0 ± 0.2 (11)
0.5% SW	7.1 ± 0.1 (4)	–	0.01 ± 0.00 (9)	5 ± 0 (8)	4 ± 0 (9)	0.1 ± 0.0 (9)	0.8 ± 0.0 (9)
2.5% SW	7.2 ± 0.0 (8)	3.1 ± 0.8 (2)	0.01 ± 0.00 (14)	13 ± 0 (14)	5 ± 3 (9)	0.2 ± 0.0 (14)	1.5 ± 0.0 (14)
5% SW	7.1 ± 0.0 (8)	3.6 ± 0.0 (2)	0.02 ± 0.00 (14)	27 ± 1 (14)	9 ± 1 (14)	0.4 ± 0.0 (14)	3.4 ± 0.0 (14)
10% SW	7.2 ± 0.0 (8)	3.7 ± 0.3 (2)	0.02 ± 0.00 (14)	53 ± 2 (14)	52 ± 11 (14)	0.9 ± 0.0 (14)	7.8 ± 0.2 (14)
20% SW	7.3 ± 0.0 (8)	4.0 ± 0.0 (2)	–	103 ± 1 (14)	77 ± 1 (14)	1.8 ± 0.0 (14)	15.7 ± 0.0 (14)
40% SW	7.4 ± 0.0 (8)	3.4 ± 0.2 (2)	–	183 ± 16 (14)	146 ± 6 (14)	4.0 ± 0.2 (14)	29.2 ± 0.6 (14)
80% SW	7.7 ± 0.1 (8)	3.2 ± 0.1 (2)	–	354 ± 11 (14)	328 ± 18 (14)	6.8 ± 0.4 (14)	40.5 ± 0.2 (14)
100% SW	7.7 ± 0.0 (8)	4.2 ± 0.1 (2)	–	454 ± 12 (14)	420 ± 23 (14)	7.0 ± 0.2 (14)	63.4 ± 1.2 (14)
Ag only							
2.5% SW	7.1 ± 0.0 (3)	5.5 ± 1.1 (3)	0.22 ± 0.06 (2)	13 ± 1 (2)	1 ± 0 (2)	0.2 ± 0.0 (2)	1.5 ± 0.1 (2)
5% SW	7.2 ± 0.1 (6)	2.7 ± 0.3 (6)	0.22 ± 0.06 (6)	27 ± 1 (6)	9 ± 1 (6)	0.5 ± 0.0 (6)	3.5 ± 0.0 (6)
10% SW	7.4 ± 0.0 (6)	3.3 ± 0.3 (5)	0.15 ± 0.03 (6)	51 ± 2 (5)	33 ± 1 (5)	0.8 ± 0.0 (6)	7.5 ± 0.1 (6)
20% SW	7.5 ± 0.0 (6)	3.1 ± 0.4 (6)	–	105 ± 2 (6)	76 ± 3 (6)	2.1 ± 0.1 (6)	15.6 ± 0.0 (6)
40% SW	7.6 ± 0.0 (6)	3.5 ± 0.4 (6)	–	209 ± 3 (6)	131 ± 5 (6)	3.4 ± 0.0 (6)	27.9 ± 0.2 (6)
80% SW	7.9 ± 0.1 (6)	3.4 ± 0.2 (6)	–	351 ± 1 (6)	294 ± 14 (6)	9.8 ± 0.3 (6)	40.9 ± 1.1 (6)
100% SW	8.0 ± 0.0 (6)	4.2 ± 0.4 (6)	–	564 ± 40 (6)	410 ± 32 (6)	7.3 ± 0.2 (6)	66.1 ± 1.5 (6)
Ag plus NOM							
2.5% SW	7.2 ± 0.0 (3)	14.4 ± 1.9 (2)	0.23 ± 0.04 (2)	14 ± 1 (2)	0 ± 0 (2)	0.2 ± 0.0 (2)	1.5 ± 0.0 (2)
5% SW	7.3 ± 0.0 (6)	11.5 ± 0.2 (6)	0.32 ± 0.03 (6)	29 ± 1 (6)	9 ± 1 (6)	0.4 ± 0.0 (6)	3.4 ± 0.0 (6)
10% SW	7.4 ± 0.0 (6)	13.0 ± 0.5 (6)	0.23 ± 0.02 (6)	43 ± 4 (6)	33 ± 1 (6)	0.9 ± 0.0 (6)	7.8 ± 0.1 (6)
20% SW	7.4 ± 0.0 (6)	12.5 ± 0.4 (6)	–	112 ± 6 (6)	77 ± 3 (6)	1.8 ± 0.0 (6)	15.8 ± 0.0 (6)
40% SW	7.8 ± 0.0 (6)	12.4 ± 0.3 (6)	–	209 ± 4 (6)	173 ± 5 (6)	3.4 ± 0.0 (6)	28.2 ± 0.1 (6)
80% SW	7.8 ± 0.1 (6)	12.4 ± 0.2 (6)	–	350 ± 10 (5)	292 ± 14 (6)	6.2 ± 0.0 (6)	40.5 ± 0.1 (6)
100% SW	8.0 ± 0.0 (6)	13.8 ± 0.6 (6)	–	487 ± 17 (6)	380 ± 22 (6)	7.0 ± 0.2 (6)	63.7 ± 1.4 (6)

All values are expressed as a mean ± 1 S.E.M. Water temperature was 25 °C.

Table 2
Frequency and chemical composition of carbonate pellets from intestines of Gulf toadfish acclimated to various salinities and exposed for 6 d to 1 μM $AgNO_3$ (nominal) with or without 10 mg C/L NOM (Suwannee R. NOM)

Experiment	Frequency (%)	Ag (nmol/g)	Ca (mol/g)	Mg (mol/g)	Ca:Mg
Ag only					
≤5% SW	0	–	–	–	–
10% SW	10	2.3 (1)	0.1 (1)	0.1 (1)	1.0
20% SW	0	–	–	–	–
40% SW	55	2.7 ± 1.8 (5)	0.2 ± 0.1 (5)	0.2 ± 0.1 (5)	1.0
80% SW	100	3.5 ± 1.3 (8)	0.6 ± 0.2 (9)	0.4 ± 0.2 (5)	1.5
100% SW	100	1.6 ± 0.9 (8)	0.6 ± 0.2 (9)	1.1 ± 0.5 (9)	0.5
Ag plus NOM:					
≤20% SW	0	–	–	–	–
40% SW	50	5.4 ± 2.4 (5)	0.6 ± 0.3 (5)	0.4 ± 0.2 (5)	1.5
80% SW	78	3.6 ± 1.9 (7)	0.7 ± 0.3 (7)	0.5 ± 0.2 (7)	1.4
100% SW	100	1.5 ± 0.8 (10)	0.9 ± 0.3 (10)	0.6 ± 0.1 (10)	1.4

All values are expressed as a mean ± 1 S.E.M. *Note*: Ag, Ca, and Mg concentrations were measured from wet weight of the carbonate pellets.

4. Discussion

4.1. Influence of salinity on Ag accumulation by Gulf toadfish

Silver accumulation in Gulf toadfish generally decreased as salinity increased. This trend was most evident in the gills and plasma, and to a lesser extent in the livers and bile. Wood et al. (2004) also reported this inverse relationship between salinity and Ag uptake in Gulf toadfish, and several other studies have demonstrated decreased Ag uptake and/or toxicity in fish at higher salinities (Hogstrand et al., 1996; Hogstrand and Wood, 1998; Shaw et al., 1998; Webb and Wood, 2000; Wood et al., 1999). For example, Shaw et al. (1998) reported 100% and 300%

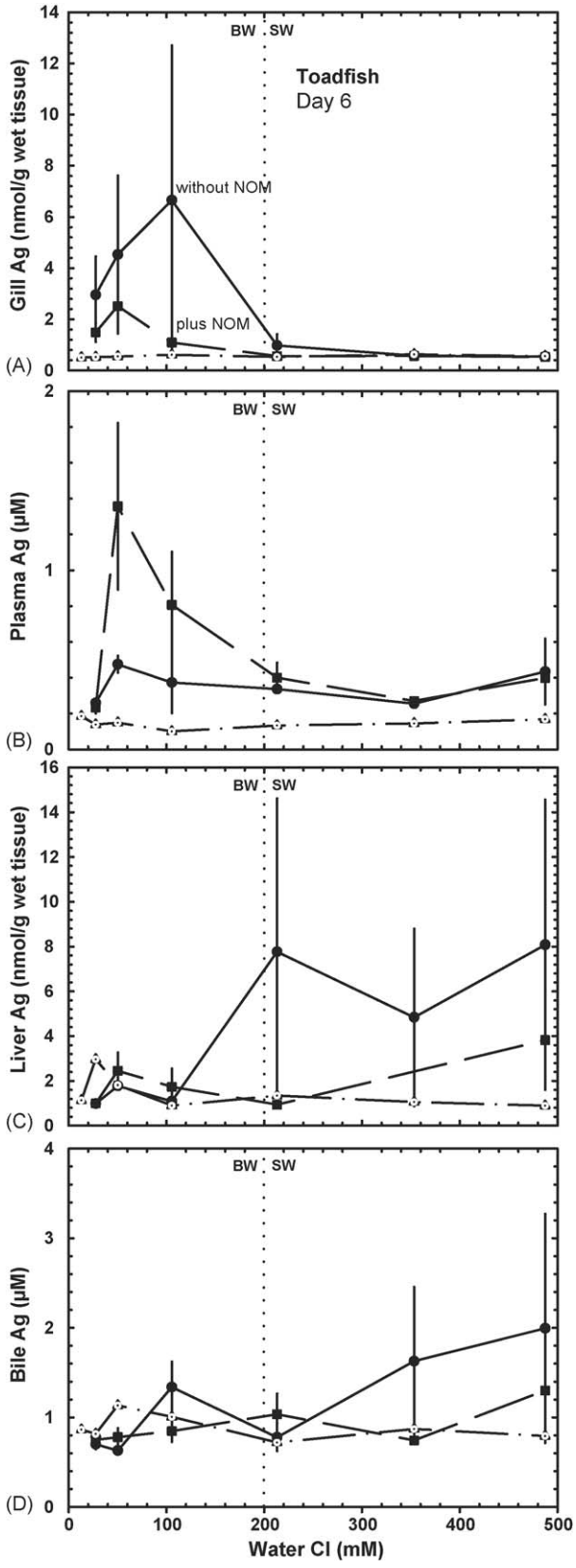

Fig. 2. Accumulation of Ag by the gills (A), plasma (B), liver (C), and bile (D) of Gulf toadfish acclimated to various salinities (△; $n = 1$) and then exposed to 0.2 μM $AgNO_3$ for 6 d in the absence (●) or presence (■) of Suwannee River NOM. Treatment data points represent the mean ± S.E.M. of toadfish sampled at day 6 ($n \leq 3$ for each point). BW = brackish water and SW = seawater ranges.

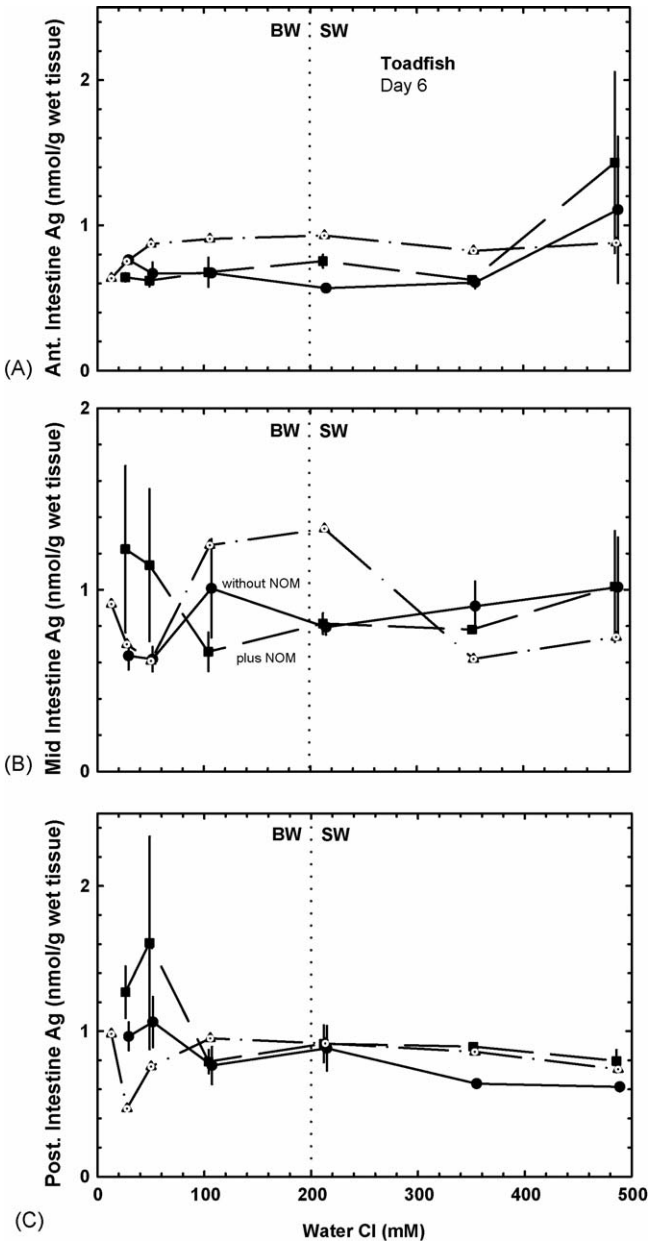

Fig. 3. Accumulation of Ag by the anterior (A), mid (B), and posterior (C) portion of the intestine from Gulf toadfish acclimated to various salinities (△; $n = 1$) and then exposed to 0.2 μM $AgNO_3$ for 6 d in the absence (●) or presence (■) of Suwannee River NOM. Treatment data points represent the mean ± S.E.M. of fish sampled at day 6 ($n \leq 3$ for each point). BW = brackish water and SW = seawater ranges.

increases in 96 and 168 h LC50 values for Ag, after increasing the salinity for tidepool sculpin exposed to Ag from 25‰ to 32‰.

The concentration of Cl^- in a water column is an important modulator of Ag uptake and toxicity (Hogstrand et al., 1996; Galvez and Wood, 1997; Shaw et al., 1998; Wood et al., 2004). In estuaries and seawater, Ag exists predominantly as Cl complexes, so the free ionic form (Ag^+) is rendered virtually non-existent (Ward and Kramer, 2002; Wood et al., 1999; Fig. 4 in Wood et al., 2004). Therefore it is not surprising that Ag accumulation in most toadfish decreased as SW concentration

increased during the experiment. These results agree with initial modelling conducted by Wood et al. (1999), where decreased Ag accumulation at higher Cl concentrations was predicted. Our results also complement the toadfish results from Wood et al. (2004), ensuring that their 1 d demonstration of the predicted Ag accumulation pattern is maintained for at least 6 d.

In contrast to these general findings, gill and plasma Ag uptake decreased in toadfish acclimated to salinities below 20% SW, and low Ag accumulation was also seen in the livers and bile at these salinities. Incomplete osmoregulation in these toadfish was likely responsible for these patterns. During the acclimation period 100% toadfish mortality occurred at 0.05% and 0.5% salinities, and toadfish acclimated to 2.5% SW did not survive the entire exposure period. Elevated plasma glucose concentrations were seen in toadfish at lower salinities (see Nichols, 2003). Stress-associated cortisol release, seen in fish during acclimation, has been demonstrated to play an important role in ionoregulation, through increased Na/K ATPase activity and glucose-enhanced gill metabolism (Vijayan et al., 1994; McCormick, 1995; Singer et al., 2002; Richards et al., 2003; Shepherd et al., 2005). Mucus production is another physiological response seen in stressed fish, and has been reported to defend fish against metals due to its chelation and lubrication properties (Coello and Khan, 1996). Higher OM concentrations in the lower salinity exposures (Table 1) indicated increased production of mucus by toadfish, which probably relates to decreased uptake of Ag by toadfish in these low-salinity treatments. Interestingly, Wood et al. (2004) successfully acclimated their toadfish to 2.5% SW; that experiment was run approximately 2 weeks after our toadfish experiment, with gentler acclimation to 2.5% SW, and salinities below 2.5% were not attempted given the mortalities observed in our study.

The inverse relationship between Cl concentration and Ag accumulation was least evident in the livers of toadfish. Webb and Wood (2000) reported substantial liver Ag accumulation in four marine teleosts (rainbow trout, tidepool sculpin, plainfin midshipmen (*Porichthys notatus*), and English sole (*Pleuronectes vetulus*)), and suggested that the liver was the main site of internal Ag accumulation in marine fish. Other studies have also implicated the liver as the primary organ for Ag accumulation, and also as a potent inducer of metallothionein synthesis (Hogstrand et al., 1996; Shaw et al., 1998; Hogstrand and Wood, 1998; Wood et al., 1999, 2004). The presence of Ag-binding metallothionein in the liver would slow Ag turnover rates (e.g. Nichols and Playle, 2004; Long and Wang, 2005), explaining the observed temporal uptake trend at lower salinities, and the greater Ag accumulation seen at higher SW concentrations.

The increased Ag accumulation seen towards higher SW concentrations was also evident to some degree in the plasma of Ag exposed toadfish in 100% SW on day 6 (Fig. 2B). The gut could be the route for this uptake of Ag, because there was no detectable Ag uptake at the gills of toadfish at these higher salinities. Several studies have predicted or demonstrated increased intestinal Ag accumulation in fish as they begin to drink Ag-contaminated water past the isosmotic salinity of ~130 mM Cl or 22% SW (Wood et al., 1999, 2004; Hogstrand et al., 1999, 2002; Grosell et al., 1999; Webb and Wood, 2000). For example, Grosell and Wood (2001) quantified a 50% to 60% intestinal contribution to overall Ag uptake in lemon sole. Silver speciation would also favour greater Ag uptake in the intestine at higher salinities due to the presence of more non-toxic cerargyrite complexes ($AgCl_{(s)}$; Hogstrand et al., 1996; Ferguson and Hogstrand, 1998) and because of decreased Cl complexation and Na competition (Wood et al., 1999). In contrast, toadfish in our study showed little accumulation of Ag in the intestine. Wood et al. (2004) also reported limited Ag uptake in the intestine of toadfish at higher salinities when compared to that of the esophagus-stomach, an organ which was not sampled in our study: the esophagus-stomach was the principal route of Ag uptake at high salinities.

Using inverted gut sacs, Hogstrand et al. (2002) reported no difference in Ag binding between the anterior, mid, and posterior regions of the intestines of European flounder (*Platichthys flesus*), but explained their results as a consequence of the in vitro conditions used. Removal of Cl and competing ions such as Na along the intestine likely causes Ag accumulation towards the terminal intestines of marine fish (Hogstrand and Wood, 1998; Ward and Kramer, 2002). In our study, intestinal Ag distribution in toadfish was unclear due to the general lack of Ag uptake.

The formation of intestinal carbonate pellets has been proposed as a feature of osmoregulation in marine fish. With the removal by carbonate precipitation of leftover divalent cations such as Ca^{2+} and Mg^{2+}, osmolality of the intestinal fluids would be lower, aiding water absorption (Walsh et al., 1991; Grosell and Wood, 2001). During our experiment carbonate pellets were found in the intestines of toadfish acclimated to salinities ≥40% SW. Walsh et al. (1991) identified carbonate pellets in the intestinal fluids of Gulf toadfish as consisting mainly of HCO_3^-, presumably provided from respiring tissues, and the osmoregulatory by-products Ca^{2+} and Mg^{2+} at approximately a 3:1 ratio ($Ca_{0.74}(Mg, Mn)_{0.26}CO_3$). Toadfish carbonate pellets from our study contained approximately equal amounts of Ca and Mg. Only small amounts of Ag were found in their intestinal pellets, discounting the likelihood that these pellets are important in Ag detoxification.

4.2. Influence of organic matter on Ag accumulation by Gulf toadfish

The addition of Suwannee River NOM appeared to reduce gill Ag accumulation in Ag exposed toadfish only at salinities below 40% SW (Fig. 2A). Organic matter effects were less evident in toadfish below 5% SW most likely due to their salinity stress. Organic matter influence was also only observed in toadfish plasma below 40% SW, however NOM appeared to enhance, rather than reduce, Ag levels at lower salinities (Fig. 2B). Due to the absorbing tendencies of Ag (Garnier et al., 1990), the presence of NOM could have helped to keep more Ag in solution leading to increase initial gill Ag uptake. In this respect, overall water Ag concentrations tended to be higher in exposures with NOM (Table 1).

Overall, these results agree with initial modelling conducted by Wood et al. (1999) where the decreased influence of OM on

Ag toxicity at higher Cl^- concentrations was predicted. In ion-poor conditions OM has been shown to provide similar or better protection than Cl against Ag toxicity, through complexation of Ag^+ (McGeer et al., 2000). However, at higher salinities AgCl complexes predominate, and associated increases in Na and Ca would lend to saturate OM metal binding sites, rendering OM insignificant in terms of Ag toxicity protection. This lack of OM influence at higher SW concentrations has been seen in other studies using Ag, marine mysid shrimp, silversides, and *Daphnia* (T. Ward, personal communication; P. Walker, personal communication).

Toadfish exposed to Ag and NOM also appeared to have lower liver and bile Ag concentrations compared to those treated with Ag alone at higher salinities (>40% SW). This Ag uptake towards higher salinities has been suggested to enter toadfish via an intestinal route (above). The presence of OM effects at higher salinities indicates a potential influence of OM on intestinal Ag accumulation. Weber and Lanno (2001) have reported OM protection in fish intestines, where decreased benzo[*a*]pyrene uptake in everted catfish gut sacs was seen after the addition of humic acids. As ions plus water are removed along the intestine for osmoregulation, OM may become more concentrated and therefore re-establish its metal complexing ability. However, low intestinal Ag accumulation was measured in our study with no evidence of NOM influence (Fig. 3), so this theory can only be left to speculation in regards to our study.

By conducting toadfish experiments in Miami, Florida, we were able to use real seawater and natural Suwannee River OM; that is, more environmentally relevant conditions. With no substantial differences being reported between using natural versus synthetic seawater during experiments, both Environment Canada (EC) and the U.S. Environmental Protection Agency (EPA) allows the use of either media in aquatic toxicity testing (Jonczyk et al., 2001; EC, 1992; U.S. EPA, 1995). The Suwannee River originates from the Okeefenokee Swamp and flows into the Gulf of Mexico, North of Gainesville, Florida. Due to dilution and estuarine mixing, only low concentrations of allochthonous-like, terrestrially-derived NOM occur in marine conditions (e.g., Coble, 1996). Seawater NOM is less aromatic than Suwannee River NOM, and might therefore be expected to bind metals less well than allochthonous-like Suwannee River NOM (Schwartz et al., 2004) and therefore provide little protection against Ag toxicity. Allochthonous-like OM has been shown to be important in decreasing toxicity of Cu in seawater to a variety of invertebrate species (Lorenzo et al., 2002; Arnold, 2005). However, the situation appears to be fundamentally different for Ag. In contrast to Cu, high Cl^- concentrations in marine systems would complex most Ag, leaving OM less important as a Ag-complexing agent, as observed in our study. In addition, Bianchini and Bowles (2002) suggested that reactive sulfides, which bind Ag and are closely associated with OM in freshwater (Smith et al., 2002), are more likely to exist independently in marine waters. Thus, with OM becoming less concentrated, less allochthonous and more autochthonous-like, with fewer sulfur binding sites towards higher salinities, coupled with Ag speciation changes (e.g., formation of AgCl complexes), a metal binding quality factor for OM (e.g., Schwartz et al., 2004, and references therein) may not be important outside freshwater conditions.

To conclude, silver accumulation in toadfish gills and plasma generally decreased as salinity increased, in agreement with predictions by Wood et al. (1999). Our results confirm the importance of Ag speciation in brackish and marine conditions. Complexation of Ag by organic matter, normally important in freshwater conditions, became less important as salinity increased, mainly due to the predominance of Cl^- complexation of Ag at higher salinities. Although only little intestinal Ag uptake was observed, liver and bile Ag accumulation indicated that toadfish were taking up Ag from contaminated water consumed past the isosmotic salinity point, and that OM appeared to reduce this uptake. Toadfish also produced intestinal carbonate pellets at higher salinities, minerals which did not influence Ag accumulation. To better predict Ag interactions in marine fish, researchers have stressed that more information is needed concerning water chemistry and the speciation of Ag in intestinal fluids, as well as better understanding of the physiological mechanisms involved in Ag toxicity in seawater (Wood et al., 1999, 2004; Grosell et al., 1999; Hogstrand et al., 2002). Results from the present study agree with initial modelling by Wood et al. (1999), and suggest important factors (e.g., Cl complexation and Ag uptake at the gut) which should be considered when modelling Ag uptake and toxicity beyond freshwater conditions.

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References

Arnold, W.R., 2005. Effects of dissolved organic carbon on copper toxicity: implications for saltwater copper criteria. Integr. Environ. Assess. Manage. 1, 34–39.

Bertram, B.O.B., Playle, R.C., 2005. Effects of waterborne complexing agents on silver uptake and depuration in rainbow trout. J. Fish Biol. 66, 182–197.

Bianchini, A., Bowles, K.C., 2002. Metal sulfides in oxygenated aquatic systems: implications for the biotic ligand model. Comp. Biochem. Physiol. 133C, 51–64.

Bury, N.R., Wood, C.M., 1999. Mechanism of branchial apical silver uptake by rainbow trout is via the proton-coupled Na^+ channel. Am. J. Physiol. 277, R1385–R1391.

Coble, P.G., 1996. Characterization of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy. Marine Chem. 51, 325–346.

Coello, W.F., Khan, M.A.Q., 1996. Protection against heavy metal toxicity by mucus and scales in fish. Arch. Environ. Contam. Toxicol. 30, 319–326.

Environment Canada, 1992. Biological test method: Fertilization Assay Using Echinoids (Sea Urchins and Sand Dollars), amended November 1997. EPS 1/RM/27. North Vancouver, BC.

Ferguson, E.A., Hogstrand, C., 1998. Acute silver toxicity to seawater-acclimated rainbow trout: influence of salinity on toxicity and silver speciation. Environ. Toxicol. Chem. 17, 589–593.

Galvez, F., Wood, C.M., 1997. The relative importance of water hardness (Ca) and chloride levels in modifying the acute toxicity of silver to rainbow trout. Environ. Toxicol. Chem. 16, 2363–2368.

Garnier, J., Baudin, J.P., Foulquier, L., 1990. Accumulation from water and depuration of 110 mAg by a freshwater fish, Salmo trutta. L. Wat. Res. 24, 1407–1414.

Grosell, M., Wood, C.M., 2001. Branchial versus intestinal silver uptake in the marine teleost (*Papophrys vetulus*). J. Comp. Physiol. 171B, 585–596.

Grosell, M., DeBoeck, G., Johannsson, O., Wood, C.M., 1999. The effects of silver on intestinal ion and acid-base regulation in the marine teleost fish, *Papophrys vetulus*. Comp. Biochem. Physiol. 124C, 259–270.

Hogstrand, C., Wood, C.M., 1998. Toward a better understanding of the bioavailability, physiology, and toxicity of silver in fish: implications for water quality criteria. Environ. Toxicol. Chem. 17, 547–561.

Hogstrand, C., Galvez, F., Wood, C.M., 1996. Toxicity, Ag accumulation and metallothionein induction in freshwater rainbow trout during exposure to different Ag salts. Environ. Toxicol. Chem. 15, 1102–1108.

Hogstrand, C., Ferguson, E.A., Galvez, F., Shaw, J.R., Webb, N.A., Wood, C.M., 1999. Physiology of acute silver toxicity in the starry flounder (*Platichthys stellatus*) in seawater. J. Comp. Physiol. 169B, 461–473.

Hogstrand, C., Wood, C.M., Bury, N.R., Wilson, R.W., Rankin, J.C., Grosell, M., 2002. Binding and movement of silver in the intestinal epithelium of a marine teleost fish, the European flounder (*Platichthys flesus*). Comp. Biochem. Physiol. 133C, 125–135.

Janes, N.G., Playle, R.C., 1995. Modeling silver binding to gills of rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 14, 1847–1858.

Jonczyk, E., Gilron, G., Zajdlik, B., 2001. Sea urchin fertilization assay: an evaluation of assumptions related to salinity adjustment and use of natural and synthetic marine waters for testing. Environ. Toxicol. Chem. 20, 804–809.

Long, A., Wang, W., 2005. Assimilation and bioconcentration of Ag and Cd by the marine black bream after waterborne and dietary metal exposure. Environ. Toxicol. Chem. 24, 709–716.

Lorenzo, J.I., Nieto, O., Beiras, R., 2002. Effect of humic acids on speciation and toxicity of copper to *Paracentrotus lividus* larvae in seawater. Aquat. Toxicol. 58, 27–41.

McCormick, S.D., 1995. Hormonal control of gill Na^+, K^+-ATPase and chloride cell function. In: Wood, C.M., Shuttleworth, T.J. (Eds.), Cellular and molecular approaches to fish ionic regulation. Academic Press, pp. 285–315.

McGeer, J.C., Playle, R.C., Wood, C.M., Galvez, F., 2000. A physiologically based biotic ligand model for predicting the acute toxicity of waterborne silver to rainbow trout in freshwaters. Environ. Sci. Technol. 34, 4199–4207.

Mommsen, T.P., 1998. Growth and metabolism. In: Evans, D.H. (Ed.), The Physiology of Fishes, 2nd ed. CRC Press, New York, pp. 65–100.

Morgan, I.J., Henry, R.P., Wood, C.M., 1997. The mechanisms of acute silver nitrate toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) is inhibition of gill Na^+ and Cl^- transport. Aquat. Toxicol. 38, 145–163.

Nichols, J.W., 2003. Influence of salinity, temperature, feeding and organic matter quality on silver accumulation in rainbow trout (*Oncorhynchus mykiss*) and Gulf toadfish (*Opsanus beta*). MSc Thesis. University of Waterloo, 268 pp.

Nichols, J.W., Playle, R.C., 2004. Influence of temperature on silver accumulation and depuration in rainbow trout. J. Fish Biol. 64, 1638–1654.

Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., Delos, C.G., Di Toro, D.M., Dwyer, R.L., Galvez, F., Gensemer, R.W., Goss, G.G., Hogstrand, C., Janssen, C.R., McGeer, J.C., Naddy, R.B., Playle, R.C., Santore, R.C., Schneider, U., Stubblefield, W.A., Wood, C.M., Wu, K.B., 2002. The biotic ligand model: a historical view. Comp. Biochem. Physiol. 133C, 3–35.

Richards, J.G., Curtis, P.J., Burnison, B.K., Playle, R.C., 2001. Effects of natural organic matter source on reducing metal toxicity to rainbow trout (*Oncorhynchus mykiss*) and on metal binding to their gills. Environ. Toxicol. Chem. 20, 1159–1166.

Richards, J.G., Semple, J.W., Bystriansky, J.S., Shulte, P.M., 2003. Na^+/K^+-ATPase α-isoform switching in gills of rainbow trout (*Oncorhynchus mykiss*) during salinity transfer. J. Exp. Biol. 206, 4475–4486.

Rose-Janes, N.G., Playle, R.C., 2000. Protection by two complexing agents, thiosulphate and dissolved organic matter, against the physiological effects of silver nitrate to rainbow trout (*Oncorhynchus mykiss*) in ion-poor water. Aquat. Toxicol. 51, 1–18.

Schwartz, M.L., Playle, R.C., 2001. Adding magnesium to the silver-gill binding model for rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 20, 467–472.

Schwartz, M.L., Curtis, P.J., Playle, R.C., 2004. Influence of natural organic matter source on acute copper, lead, and cadmium toxicity to rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 23, 2889–2899.

Shaw, J.R., Wood, C.M., Birge, W.J., Hogstrand, C., 1998. Toxicity of silver to the marine teleost (*Oligocottus maculosus*): effects of salinity and ammonia. Environ. Toxicol. Chem. 17, 594–600.

Shepherd, B.S., Drennon, K., Johnson, J., Nichols, J.W., Playle, R.C., Singer, T.D., Vijayan, M.M., 2005. Salinity acclimation affects the somatotropic axis in rainbow trout. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288, R1385–R1395.

Singer, T.D., Clements, K.M., Semple, J.W., Schulte, P.M., Bystriansky, J.S., Finstad, B., Fleming, I.A., McKinley, R.S., 2002. Seawater tolerance and gene expression in two strains of Atlantic salmon smolts. Can. J. Fish. Aquat. Sci. 59, 125–135.

Smith, S.D., Bell, R.A., Kramer, J.R., 2002. Metal speciation in natural waters with emphasis on reduced sulfur groups as strong metal binding sites. Comp. Biochem. Physiol. 133C, 65–74.

U.S. Environmental Protection Agency, 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. EPA/600/R-95-136, Washington, DC.

Vijayan, M.M., Reddy, P.K., Leatherland, J.F., Moon, T.W., 1994. The effects of cortisol on hepatocyte metabolism in rainbow trout: a study using the steroid analogue RU486. Gen. Comp. Endocrin. 96, 75–84.

Walsh, P.J., Blackwelder, P., Gill, K.A., 1991. Carbonate deposits in marine fish intestines: a new source of biomineralization. Limnol. Oceanogr. 36, 1227–1232.

Ward, T.J., Kramer, J.R., 2002. Silver speciation during chronic toxicity tests with the mysid, *Americamysis bahia*. Comp. Biochem. Physiol. 133C, 75–86.

Webb, N.A., Wood, C.M., 2000. Bioaccumulation and distribution of silver in four marine teleosts and two marine elasmobranches: influence of exposure duration, concentration, and salinity. Aquat. Toxicol. 49, 111–129.

Weber, L.P., Lanno, R.P., 2001. Effect of bile salts, lipid, and humic acids on absorption of benzo[*a*]pyrene by isolated channel catfish (*Ictalurus punctatus*) intestine segments. Environ. Toxicol. Chem. 20, 1117–1124.

Wood, C.M., Hopkins, T.E., Walsh, P.J., 1997. Pulsatile urea excretion in the toadfish (*Opsanus beta*) is due to a pulsatile excretion mechanism, not a pulsatile production mechanism. J. Exp. Biol. 200, 1039–1046.

Wood, C.M., Playle, R.C., Hogstrand, C., 1999. Physiology and modeling of mechanisms of silver uptake and toxicity in fish. Environ. Toxicol. Chem. 18, 71–83.

Wood, C.M., McDonald, M.D., Walker, P., Grosell, M., Barimo, J.F., Playle, R.C., Walsh, P.J., 2004. Bioavailability of silver and its relationship to ionoregulatory and silver speciation across a range of salinities in the gulf toadfish (*Opsanus beta*). Aquat. Toxicol. 70, 137–157.

Zhou, B., Nichols, J., Playle, R.C., Wood, C.M., 2005. An in vitro biotic ligand model (BLM) for silver binding to cultured gill epithelia of rainbow trout (*Oncorhynchus mykiss*). Toxicol. Pharmacol. 202, 25–37.