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Bioavailability of silver and its relationship to ionoregulation and silver speciation across a range of salinities in the gulf toadfish (*Opsanus beta*)

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Abstract

Silver is taken up as a Na^+ analog (Ag^+) by freshwater organisms, but little is known about its bioavailability in relation to salinity. Adult *Opsanus beta* were acclimated to 2.5, 5, 10, 20, 40, 60, 80, and 100% seawater (Cl^- = 545 mM) and exposed for 24 h to 2.18 μg L^{-1} silver as ^{110m}Ag-labelled $AgNO_3$, a concentration close to the U.S. EPA marine criterion and less than 0.1% of the acute 96-h LC50 in seawater. Plasma osmolality, Na^+, and Cl^- remained approximately constant from 100% down to 20–40% seawater, thereafter declining to 89% (osmolality) and 82% (Na^+, Cl^-) of seawater values at the lowest salinity (2.5% seawater), while plasma Mg^{2+} was invariant. Ionic measurements in intestinal fluids and urine supported the view that above the isosmotic point (about 32% seawater), toadfish drink the medium, absorb Na^+, Cl^-, and water across the gastrointestinal tract, actively excrete Na^+ and Cl^- across the gills, and secrete Mg^{2+} into the urine. Below this point, toadfish appear to stop drinking, actively take up Na^+ and Cl^- at the gills, and retain ions at the kidney. Silver accumulation varied greatly with salinity, by nine-fold (whole body), 26-fold (gill tissue), and 18-fold (liver), with the maxima occurring in 2.5% seawater, the minima in 40% seawater (close to the isosmotic point), and slightly greater values at higher salinities. Highest silver concentrations occurred in liver, second highest in gills, intermediate concentrations in kidney, spleen, and gastrointestinal tissues, and lowest in swim bladder and white muscle, though patterns changed with salinity. There were substantial biliary but minimal urinary levels of silver. The salinity-dependent pattern of silver accumulation best correlated with the abundance of the neutral complex $AgCl_0$, though the presence of small amounts of Ag^+ at the lowest salinities may also have been important. In contrast, silver

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accumulation in the esophagus-stomach was greatest in 100% seawater and least at the isosmotic salinity (five-fold variation), a pattern probably explained by drinking and silver uptake into the blood through the gills. Models of silver bioavailability across salinity must consider the presence of silver-binding ligands on both gills and gastrointestinal tract, changing silver speciation, and the changing ionoregulatory physiology of the organism.

Keywords: Silver; Salinity; Toxicity; Gulf toadfish; Drinking; Gills; Esophagus-stomach; Liver; Gut fluids; Bile; Urine

1. Introduction

In the past decade, an intensive research effort has greatly improved our understanding of the toxicity of silver in freshwater (reviewed by Andren and Bober (2002)). The integration of fundamental principles of physiology, aquatic geochemistry, and toxicology has yielded biotic ligand models (BLMs) which are capable of site-specific predictions of acute toxicity (e.g., 96-h LC50s) in any particular freshwater chemistry (Paquin et al., 1999, 2002a,b; McGeer et al., 2000). The BLMs hinge on the understanding that only the free ion, Ag^+, causes acute toxicity by binding to key target sites on the gills as a Na^+ analog. These "toxic sites" (anionic biotic ligands) are proteins, particularly Na^+,K^+ATPase, carbonic anhydrase, and Na^+ channels, which are intimately involved in active Na^+ and Cl^- uptake (Morgan et al., 1997, 2004; Bury and Wood, 1999; Bianchini and Wood, 2003). The greater the saturation of these sites with Ag^+, the greater the predicted toxicity. Thus freshwater organisms subject to silver poisoning may die from ionoregulatory failure (Wood et al., 1996). The BLM approach relates the metal load on the gills during short-term exposures (3–24 h; i.e., before pathology develops) to 96-h toxicity.

Silver BLMs have been developed by integrating modern geochemical modeling programs with empirical determinations of the short-term binding affinity and capacity of the gills for Ag^+ (e.g., Janes and Playle, 1995; Schwartz and Playle, 2001; Morgan and Wood, 2004) and the influence of different components of water chemistry (e.g., Cl^-, Na^+, Ca^{2+}, sulfide, dissolved organic matter) on gill binding and toxicity (Janes and Playle, 1995; Erickson et al., 1998; Karen et al., 1999; Bury et al., 1999a,b; Mann et al., 2004). The computational core of the BLM takes into account the concentrations of silver itself, the biotic ligands (toxic sites) on the gills, all other anions that complex Ag^+, and all cations that compete with Ag^+ for binding to these ligands. In addition, the geochemical stability constants for all these reactions are incorporated in order to predict whether a certain concentration of silver will be acutely toxic to a reference organism in a particular freshwater quality.

There has been far less research on, and as yet no BLMs for, silver toxicity in the estuarine and seawater environment, but already it is clear that the situation is very different. Marine fish have not one but two potential target tissues for silver, the gills and the gut (reviewed by Wood et al. (1999)), and in contrast to freshwater fish, drink the medium so that the gut can absorb Na^+, Cl^-, and thereby water to replace osmotic water loss across the gills (Smith, 1930). Furthermore, the gills, rather than performing active Na^+ and Cl^- uptake, serve to actively excrete the excess Na^+ and Cl^-. Thus across the gradient of increasing salinity in estuaries, the function of the gills changes gradually from ionic absorption to ionic excretion as the isosmotic point is crossed, and drinking begins around this point. In the water, silver speciation is dominated by the concentration of Cl^-, and as salinity increases, the free Ag^+ ion essentially disappears, being replaced with a sequence of neutral and anionic silver chloride complexes (e.g., Ferguson and Hogstrand, 1998).

Silver is far less toxic in seawater than in freshwater, though the variable pattern at intermediate salinities has probably been complicated by incomplete salinity acclimation or tolerance of the test organisms (Dorfman, 1977; Ferguson and Hogstrand, 1998; Shaw et al., 1998). Silver uptake appears to be lower in full strength seawater than in intermediate salinities, though again there are some variations (Shaw et al., 1998; Webb and Wood, 2000). In both, silver accumulates in gut as well as in gill tissue and in internal organs, especially the liver. In full strength seawater, the acute toxic mechanism again involves ionoregulatory impairment, but in contrast to freshwater exposures, plasma Na^+, Cl^-,

and osmolality levels tend to rise rather than fall. Na^+ and Cl^- excretion at the gills, Na^+ and Cl^- uptake at the gut, associated Na^+,K^+ATPase activities at the two sites, as well as drinking rate itself all appear to be affected (Hogstrand et al., 1999; Grosell et al., 1999; Webb et al., 2001; Grosell and Wood, 2001).

In coastal regions, most silver-containing liquid waste is processed through sewage-treatment plants that discharge freshwater effluent into estuaries or inshore waters (Fowler and Nordberg, 1986; Eisler, 1996). Therefore, the intermediate, variable salinities are the most relevant for environmental regulation, yet at present the responses to silver of the organisms which dwell there are the least well understood. Our goal was to address this issue for the purpose of future BLM development for estuarine and marine waters. Our particular focus was how short-term silver bioavailability (i.e., 24-h silver uptake and internal distribution) changes over a wide range of salinity (2.5–100% seawater), and the relative importance of gills and gut in these phenomena. We chose a hardy teleost, the gulf toadfish (*Opsanus beta*), which is reputed to be highly tolerant to a wide salinity range and to occur naturally in inshore waters and at freshwater discharge sites (Serafy et al., 1997). To confirm this salinity tolerance, we documented ionoregulatory status at each acclimation salinity by analysis of plasma, gut fluid, and urine ions, and plasma osmolality. To avoid complications of toxicant stress, we used a very low, environmentally relevant level of silver exposure (nominally 2.3 $\mu g\,L^{-1}$, the current U.S. marine criterion value; U.S. EPA, 1980). This was found to represent less than 0.1% of the acute 96-h LC50 for *O. beta* in seawater. Silver levels of 0.06–2.9 $\mu g\,L^{-1}$ have been reported in intertidal areas close to sewage outfalls and industrial sites (Fowler and Nordberg, 1986; Eisler, 1996). Radiolabelled ^{110m}Ag was used to provide greatest sensitivity in analyzing accumulation and internal distribution at this low silver level (cf. Wood et al., 2002; Galvez et al., 2002; Hogstrand et al., 2003).

2. Material and methods

2.1. Experimental animals

Gulf toadfish (*O. beta* Goode and Bean, 37 ± 5 g, $N = 210$, range 15–71 g) were collected in spring of 2002 from Biscayne Bay, South Florida, by commercial shrimp fishermen using a roller trawl. Upon arrival in the laboratory, the fish were bathed in freshwater for 3 min, followed by a 3-h treatment with malachite green (0.05 $mg\,L^{-1}$) plus formalin (15 $mg\,L^{-1}$) in seawater, and the latter treatment was repeated on day 3 to eliminate potential infections (Wood et al., 1997). Toadfish were held in flowing seawater (salinity = 33–35 ppt, temperature = 24–25 °C, natural photoperiod) in large aquaria (45 L) at a density of about 25 $g\,L^{-1}$ for 9–12 days prior to salinity acclimation (see below). They were fed once with shrimp on days 4–6, and not fed thereafter.

2.2. Acclimation to different salinities

Toadfish were acclimated to 100, 80, 60, 40, 20, 10, 5, and 2.5% seawater, with $N = 10$ fish in each of two replicates, at 24–25 °C. Dilutions of 100% seawater were made using in-house reverse osmosis water, which had measured Na^+, Cl^-, and Ca^{2+} levels <100 $\mu mol\,L^{-1}$. On Day 0, toadfish were transferred in batches of 10 to plastic buckets filled with 16 L of 100% seawater (i.e., density about 25 $g\,L^{-1}$) with vigorous aeration. Apart from two batches that were kept at 100%, the fish were progressively transferred at 24-h intervals to 80%, then 60%, then 40%, then 20% seawater, then 10%, then 5%, and finally 2.5%, achieved by complete replacement of water each day. Once duplicate batches reached their intended final salinity, they were held there for 7 days, with experimentation performed on the eighth day. During this week-long period, 50% of the water was replaced every 24 h. Salinity was checked using a Radiometer CMT 10 coulometric chloride titrator (100% seawater = 545 $mmol\,L^{-1}$) and was deemed acceptable if Cl^- was within 10 $mmol\,L^{-1}$ of the intended value at higher salinities, and within 2 $mmol\,L^{-1}$ at the two lowest salinities. To avoid complications of possible changes in drinking rate or changes in water silver speciation associated with feeding and defecation, fish were not fed.

2.3. Exposure to silver at different salinities

On the day of experimentation, five toadfish from each duplicate were transferred to a fresh 16 L of the appropriate salinity, allowed to settle for 2 h, and then

exposed to radiolabelled Ag. The other 10 fish were amalgamated into a second 16 L at the same salinity and sacrificed on the following day for measurements of plasma and intestinal fluid electrolytes (see below). For silver exposure, our goal was to achieve a mean value of 2.3 $\mu g\,L^{-1}$ averaged over the 24-h exposure. As we anticipated that there would be some loss due to adsorption to the bucket walls and uptake by the fish, the nominal starting silver concentration was 3.0 $\mu g\,L^{-1}$ added as $AgNO_3$ (analytical grade, Fisher Scientific) and radiolabelled with 0.33 $\mu Ci\,L^{-1}$ of $^{110m}AgNO_3$ (RISØ Nuclear Research Reactor, Risø, Denmark). Silver was added from a single stock solution used in all exposures; total Ag and ^{110m}Ag gamma radioactivity were assayed in triplicate in this stock at the beginning and end of the 16-day experimental session and remained constant. Within each exposure, duplicate water samples were taken at 0.1, 12, and 24 h, one of which was directly acidified (1% ultrapure HNO_3, Fisher Scientific) for the later measurement of total silver, and the other of which was filtered through a 0.45 μm Gelman syringe filter, then acidified, for the later measurement of ^{110m}Ag gamma radioactivity.

At 24 h, all 10 fish in the bucket were rapidly killed via the addition of 2.5 $g\,L^{-1}$ neutralized MS-222 (Syndel Labs). The fish were weighed and blood samples (0.5 ml) were rapidly drawn by caudal puncture into lithium-heparinized syringes, discharged into tared plastic centrifuge tubes, weighed, and plasma was separated by rapid centrifugation (13,000 G for 2 min). The plasma was decanted, and the tubes reweighed to determine the mass of the red blood cell pellet and the hematocrit. The fish itself was rinsed in clean seawater and stored at 4 °C in a sealed plastic bag for no more than 4 h before dissection. The gall bladder and the larger lobe of the urinary bladder were removed intact and drained into tared gamma tubes to provide bile and urine samples. Gut fluids were obtained from various parts of the digestive tract as described below, and while originally kept separate, were later pooled to obtain sufficient volume for gamma counting. The following tissues were removed in their entirety, blotted, and placed into tared gamma counting tubes that were reweighed: gill filaments (trimmed from arches), esophagus-stomach (a single organ in the toadfish), anterior, middle, and posterior intestine, liver, kidney, swim bladder, spleen, plus a 0.5-g sample of epaxial white muscle. The degree of sexual maturity, and therefore gonadal size, varied greatly, so gonads were not sampled separately, and sex was not recorded as a variable. The remaining tissues were amalgamated as a single sample ("carcass") which was weighed, diluted with a known amount of distilled water, and ground in a Waring blender to a fine paste from which an aliquot (~5 g) was gamma-counted. In general, one or two fish were rejected from each experiment because of surface wounds (probably due to aggression), and in two instances because of cannibalism, and in some cases urine, bile, or gut fluid samples could not be taken. Thus, typical *N* numbers were 7–9 per salinity.

The relative contributions of each of the organs/tissues sampled to the weight of the whole body are summarized in Table 1. Clearly the carcass (muscle, bone, nervous tissue, skin, gonads if present, and trapped blood) was the largest component, while the other compartments generally comprised less than 2% each. The unaccounted compartment probably reflects blood, gut fluids, urine, and other body fluids lost in dissection and blotting.

2.4. Sampling for plasma, intestinal fluid, and urine ions at different salinities

The other 10 fish in each treatment were sacrificed individually by placing them in 2.5 $g\,L^{-1}$ neutralized

Table 1
Percentage contribution of each measured tissue compartment to the total body mass of toadfish

Compartment	Percent
Carcass	85.04 ± 0.35
Gill	1.22 ± 0.02
Liver	0.98 ± 0.03
Esophagus-stomach	1.10 ± 0.04
Anterior intestine	0.80 ± 0.02
Mid intestine	0.46 ± 0.01
Posterior intestine	0.62 ± 0.02
Bile	0.27 ± 0.01
Spleen	0.08 ± 0.00
Kidney	0.36 ± 0.01
Urine	0.37 ± 0.00
Swim bladder	1.55 ± 0.01
Gut fluids	0.19 ± 0.02
Total[a]	93.05 ± 0.35

Mean ± 1S.E.M. ($N = 63$) for toadfish at all salinities.

[a] The unaccounted compartment reflects body fluids lost in dissection and blotting.

MS-222 and were sampled immediately for blood (by caudal puncture), urine (by removing and draining the larger lobe of the urinary bladder), and fluid from the anterior, middle, and posterior intestine following the procedure of Grosell et al. (2004a). Each ligated intestinal section was drained into an individual sampling tube. It was not possible to obtain all samples from all fish, so *N* numbers ranged from 4 to 10. The esophagus-stomach could not be sampled because little or no fluid was generally present. Plasma, urine, and intestinal fluid samples were stored at −20 °C for later analysis.

2.5. Determination of the threshold for acute silver toxicity

Three traditional toxicity trials (Sprague, 1969) were run in 100% seawater. Toadfish were of the same size as those used in the bioaccumulation tests, and were held under identical conditions, with 10 fish in each 16 L bucket. Nominal silver exposure concentrations were 0 (control), 500, 1000, 2000, 4000, 8000 $\mu g\,L^{-1}$ (added as $AgNO_3$) in the first trial; 0 (control), 4000, 8000, 16,000, 32,000 $\mu g\,L^{-1}$ in the second trial; and 0 (control), 16,000, 32,000 $\mu g\,L^{-1}$ in the final trial, each with 10 fish per concentration and with 50% replacement of the test solution every 24 h. The first two trials lasted 96 h and the third 192 h, with mortalities recorded at 12-h intervals. Water samples (both filtered and unfiltered) were taken before and after each exchange of the test solution and preserved for total silver analysis, though in light of the results, only a small subset was analyzed.

2.6. Analytical methods

All tissue samples, duplicate filtered and unfiltered water samples (3 ml), and aliquots of the radiolabelled $AgNO_3$ stock solution were counted for ^{110m}Ag gamma radioactivity using a Cobra II Packard gamma counter according to the window selection guidelines of Hansen et al. (2002). Chloride in seawater and plasma samples was measured with a Radiometer-Copenhagen CMT10 coulometric chloride titrator, and in intestinal fluid samples (which were generally of small volume) by the colorimetric method of Zall et al. (1956). Na^+ and Mg^{2+} concentrations in plasma and intestinal fluids were measured by flame atomic absorption spectrophotometry (Varian 1275). Plasma osmolality was measured using a Wescor 5100C vapor pressure osmometer. Total silver concentrations in the ^{110m}Ag-labelled $AgNO_3$ stock solution were measured by graphite furnace atomic absorption spectrophotometry (Varian 1275 with GTA-9 atomizer) using a certified standard (Aldrich). As background levels of total silver in all salinities were below detection (<0.05 $\mu g\,L^{-1}$), silver levels in the various exposure waters were calculated by dividing the measured ^{110m}Ag radioactivity of the water samples (cpm L^{-1}) by the measured specific activity of the stock solution (cpm $\mu g\,Ag^{-1}$). Similarly, the concentrations of newly accumulated silver in all tissue and body fluid samples were calculated by dividing the measured ^{110m}Ag radioactivity of the tissue samples (cpm g^{-1}) by the mean measured specific activity of the stock solution. The whole-body concentration of newly accumulated silver for each fish was calculated as the sum of all of the organ-specific contents (including carcass), divided by the total mass of all of the organs plus carcass. On average, this accounted for 93% of the original measured body mass (Table 1). To compensate for differences in mean exposure concentrations of waterborne silver among different salinities, all bioaccumulation data were normalized to the mean overall total water silver measured across all salinities using a different correction factor at each salinity, as described in Section 3. In the toxicity trials, total and dissolved silver concentrations in selected samples were analyzed by graphite furnace atomic absorption spectrophotometry. Dilution factors were so large that interference by the high background levels of other ions in seawater was not a problem.

2.7. Silver speciation

According to Ward and Kramer (2002), traditional aquatic geochemistry programs are in error for silver speciation in seawater and intermediate salinities because their functions do not give the proper corrections for activity coefficients, and because they include a species ($AgCl_4^{3-}$) which does not occur at such salinities. Therefore, we adopted the speciation scheme recommended by Ward and Kramer (2002).

2.8. Statistical methods

Data are generally expressed as mean ± 1S.E.M. (*N*). To compare differences in tissue-specific concentrations of newly accumulated silver or ionic concentrations in body fluids among different salinities, data were first tested for normality and homogeneity of variances. Where necessary, data were log-transformed prior to further analysis to pass Bartlett's chi square test for homogeneity of variances, and percentage data were subjected to arcsine transformation. Data were then analyzed by one-way ANOVA, followed by Tukey's honestly significant difference test to detect specific differences (Statistix for Windows). In those few instances where the data still did not pass Bartlett's test, the nonparametric Kruskal–Wallis signed ranks test was used in place of the ANOVA and Tukey's test. The convention used in all figures is that means not sharing the same letter are significantly different. Comparisons between tissues were made using Student's paired *t*-test (two-tailed). A significance level of $P < 0.05$ was used throughout.

3. Results

3.1. Ionoregulatory status of toadfish at each acclimation salinity

Plasma osmolality was maintained at about 320 mOsm kg^{-1} at acclimation salinities from 100% down to 40% seawater, but fell significantly at salinities below this point (Fig. 1A). However, even at the lowest salinity (2.5% seawater), toadfish maintained osmolality at 89% of the control value. Based on these data, toadfish plasma would have had equal osmolality to the external environment at about 32% seawater; thus the 40% seawater treatment was closest to the isosmotic point. Plasma Na^+ and Cl^- concentrations exhibited very similar trends to osmolality, though more precipitous declines occurred below 10% seawater, to about 82% of control values (Na^+ = 162 mmol L^{-1}, Cl^- = 157 mmol L^{-1}; Fig. 1B). Plasma Mg^{2+} remained steady at about 1.75 mmol L^{-1} across all salinities (Fig. 1C). Hematocrit was reasonably constant across salinities at 24–31% (data not shown); the only significant difference was between 80% seawater (33.5 ± 2.2% [8]) and 5% seawater (21.5 ± 1.7% [8]).

Na^+ concentrations in gut fluids were lowest in 100% seawater and were significantly greater at all lower salinities, though surprisingly constant at levels close to those in blood plasma right from 80% down to 2.5% seawater (Fig. 2A). At the two highest salinities, Na^+ concentrations were significantly lower in the posterior intestine than in the other segments, but this did not occur at lower salinities. Cl^- concentrations were more variable, with no well-defined trends across salinities (Fig. 2B). However, Cl^- concentrations in the posterior segments were almost always lower than those in the other two sections, which were generally close to blood plasma levels. Mg^{2+} concentrations in gut fluids were very high (80–160 mmol L^{-1}) in 100, 80, and 60% seawater, falling dramatically to much lower levels (5–30 mmol L^{-1}) at all lower salinities (Fig. 2C). In 100% seawater, Mg^{2+} concentrations were highest in the posterior intestine; there was some indication of an opposite trend at salinities ≤40%.

Toadfish bladder urine Na^+ was very low (9 mmol L^{-1}) in 100% seawater, increased progressively down to 40% seawater (167 mmol L^{-1}), and then declined progressively to 106 mmol L^{-1} at 2.5% seawater (Fig. 3A). In contrast, bladder urine Cl^- was high (80–100 mmol L^{-1}) at 100 and 80% seawater, but much reduced (5–20 mmol L^{-1}) at all lower salinities (Fig. 3B). Mg^{2+} concentrations in bladder urine were not obtained from fish acclimated to 100% seawater due to a procedural problem, but were very high (168 mmol L^{-1}) in toadfish acclimated to 80% seawater (Fig. 3C). Urine Mg^{2+} fell progressively at lower salinities reaching a minimum of about 10 mmol L^{-1} in toadfish acclimated to 2.5–10% seawater.

3.2. Silver concentrations and speciation in exposure water

Initial total silver concentrations were generally close to the intended starting value of 3.0 μg L^{-1}, and declined in a more or less linear fashion in the 12- and 24-h samples. Decreases in silver concentrations were greater at the lower salinities, ranging from about 15% in 100% seawater to about 70% in 2.5% seawater. Based on measured whole-body silver accumulation (see below), only a small fraction (<5%) of the lost silver was due to uptake by the fish, the rest adsorbing to the container wall and to mucus precipitates. The dissolved fractions were initially greater than 90%

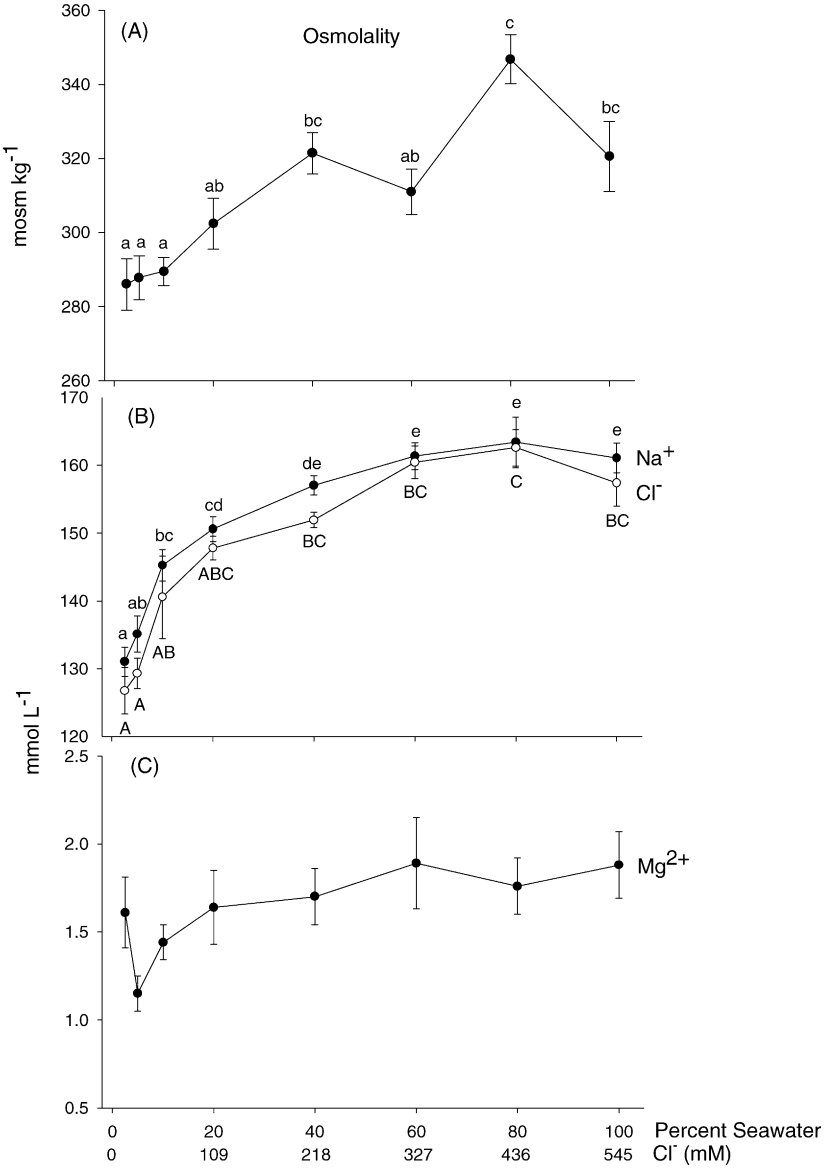

Fig. 1. The influence of acclimation salinity (shown as both % seawater and the corresponding Cl^- concentration) on (A) plasma osmolality; (B) plasma sodium and chloride; and (C) plasma magnesium concentrations in the gulf toadfish. Mean ± 1S.E.M. ($N = 7$–10). Means not sharing the same letter (of the same case) are significantly different ($P < 0.05$). There are no significant differences in panel C.

of the total, but underwent somewhat larger declines, especially at the two lowest salinities. Overall means are summarized in Table 2. The 24-h average for total silver concentration was $2.18 \pm 0.09\,\mu g\,L^{-1}$, with a dissolved fraction of 74 ± 2%. While the former was very close to the total silver target of $2.3\,\mu g\,L^{-1}$, the values ranged from $1.59\,\mu g\,L^{-1}$ in 2.5% seawater to $2.89\,\mu g\,L^{-1}$ in 100% seawater. Based on the probable assumption that silver uptake would be concentration-dependent within any one salinity, all bioaccumulation data were normalized to the overall mean exposure concentration so as to facilitate comparisons between

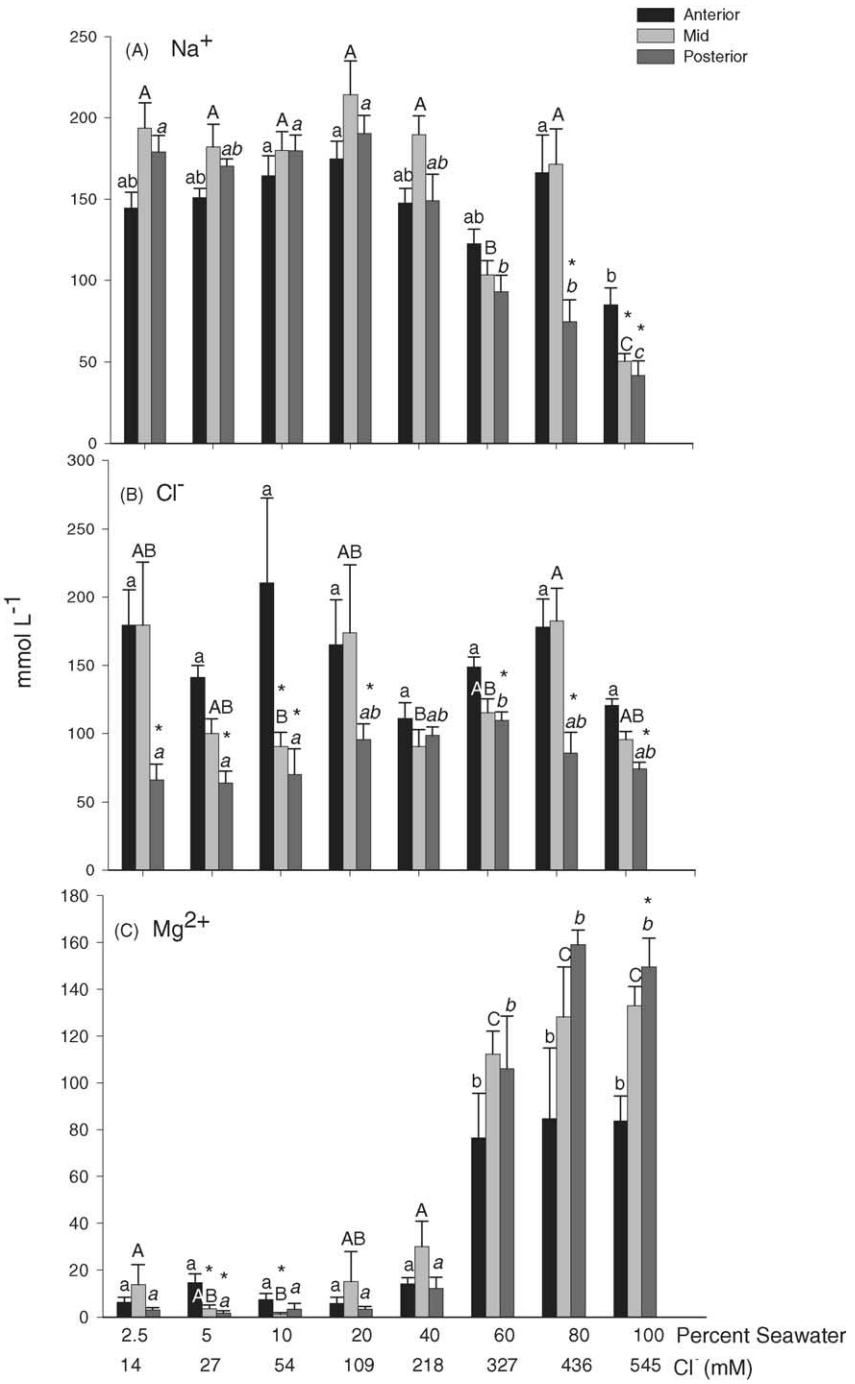

Fig. 2. The influence of acclimation salinity (shown both as % seawater and the corresponding chloride concentration) on the ionic composition of fluids sampled from the anterior, mid, and posterior intestine of toadfish: (A) sodium; (B) chloride; and (C) magnesium concentrations. Mean ± 1S.E.M. ($N = 4$–10). For comparisons among the same intestinal segments at different salinities, means not sharing the same case letter are significantly different ($P < 0.05$). Within a salinity, asterisks indicate means that are significantly different ($P < 0.05$) from the corresponding value in the anterior intestine.

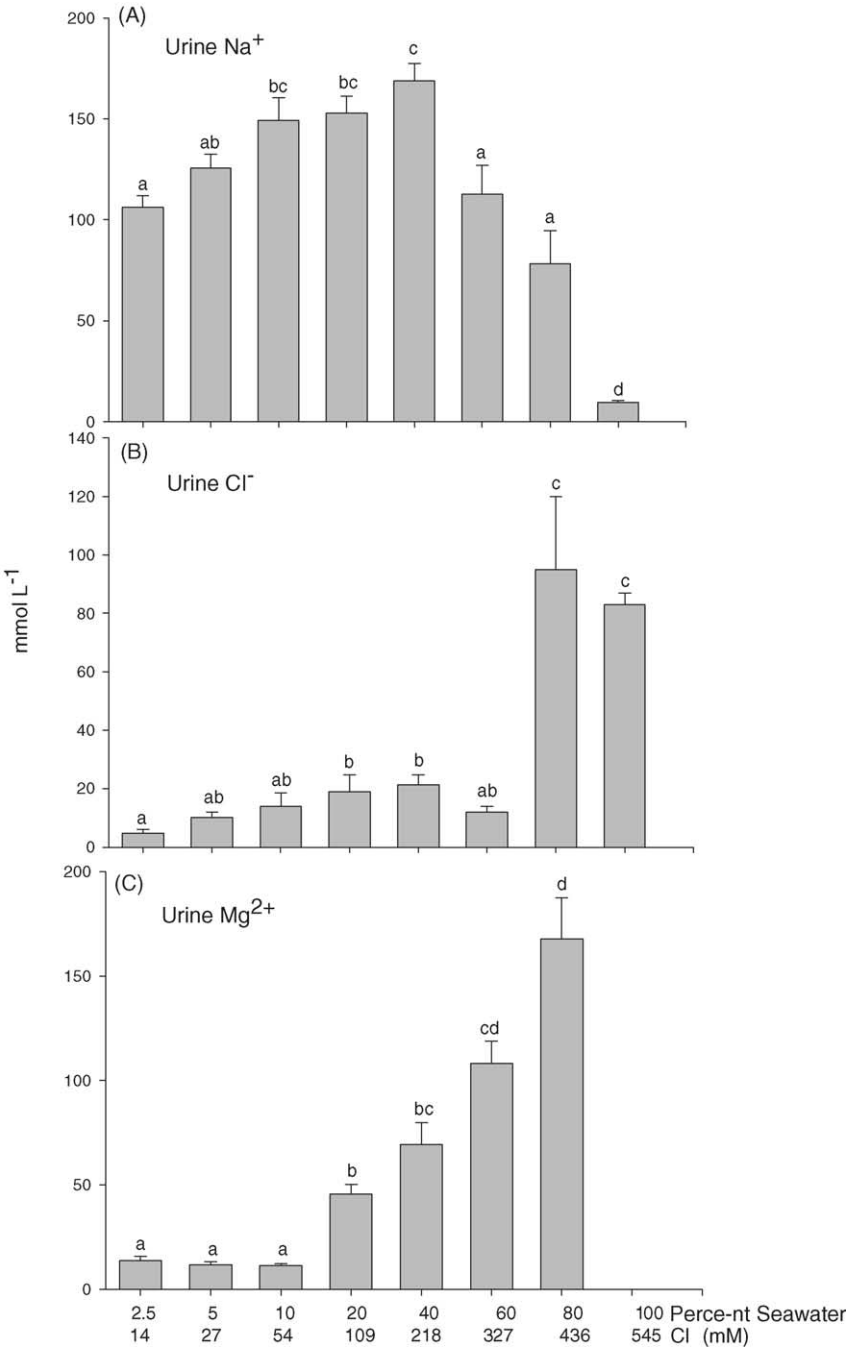

Fig. 3. The influence of acclimation salinity (shown both as % seawater and the corresponding chloride concentration) on the ionic composition of urine sampled from the bladders of toadfish: (A) sodium; (B) chloride; and (C) magnesium concentrations. Mean ± 1S.E.M. ($N = 7$–10). Means not sharing the same letter are significantly different ($P < 0.05$). Urine magnesium concentrations were not obtained at 100% seawater due to a procedural problem.

Table 2
Mean measured total silver concentrations and filtered fractions in the exposure waters over the 24-h exposure period

Percent seawater (%)	Measured total silver ($\mu g\,L^{-1}$, $N=3$)	Measured 0.45 μM filtered silver (%, $N=3$)
100	2.89	81
80	2.64	82
60	2.31	89
40	1.76	81
20	1.92	79
10	2.38	78
5	1.97	58
2.5	1.59	61
Mean	2.18	74
S.E.M. ($N=24$)	±0.09	±2

Mean ± 1S.E.M. (N).

salinities. This was done by applying a different correction factor at each salinity—i.e., by multiplying measured values of newly accumulated silver by the ratio of $2.18\,\mu g\,L^{-1}$ to C, where C was the measured 24-h average total silver concentration for that particular salinity.

Fig. 4 illustrates the speciation of $2.18\,\mu g\,L^{-1}$ total silver in the exposure water at each salinity using the respective mean measured particulate fraction (non-filterable) of silver over 24 h and applying the computational framework of Ward and Kramer (2002) to the mean measured dissolved fraction (filterable). Particulate silver was more or less constant at about $0.4\,\mu g\,L^{-1}$ down to a salinity of 10%, and increased to about $0.8\,\mu g\,L^{-1}$ at the two lowest salinities. This probably represents silver bound to mucus produced by the fish plus other particles in the water, because $2.18\,\mu g\,L^{-1}$ total silver is below the threshold for formation of insoluble cerargyrite at all salinities. $AgCl_3^{2-}$ decreased linearly from the largest fraction ($1.5\,\mu g\,L^{-1}$) in 100% seawater to $0\,\mu g\,L^{-1}$ at the lowest salinity. $AgCl_2^-$ dominated at intermediate salinities (10–60% seawater), but declined at both low and high salinity. The neutral complex $AgCl_0$ was negligible at higher salinities, but increased greatly at lower salinity, becoming approximately equal to $AgCl_2^-$ ($\sim 0.65\,\mu g\,L^{-1}$) in 2.5% seawater. Ag^+ was the smallest fraction, accounting for at most 2% of total silver at the lowest salinity, and declining to negligible values ($\leq$0.06%) at salinities $\geq$20% seawater (Fig. 4).

3.3. Bioavailability and internal distribution of silver in toadfish at different salinities

On a whole-body basis, the 24-h uptake of newly accumulated silver varied more than nine-fold across salinities, with the highest accumulation at 2.5% sea-

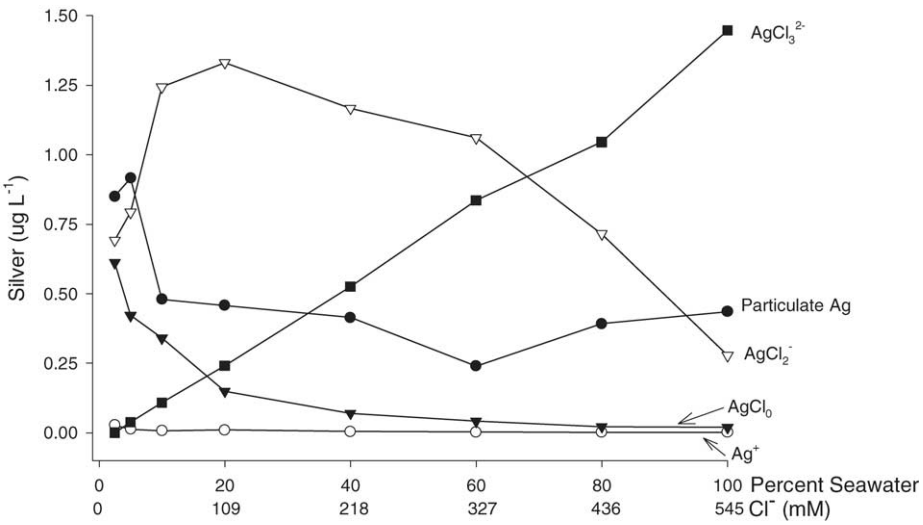

Fig. 4. Speciation of $2.18\,\mu g\,L^{-1}$ of total silver (as $AgNO_3$) in each of the exposure salinities, calculated using the mean measured particulate fraction of silver over 24 h at each salinity and applying the computational framework of Ward and Kramer (2002) to partition the measured dissolved fraction into its various components.

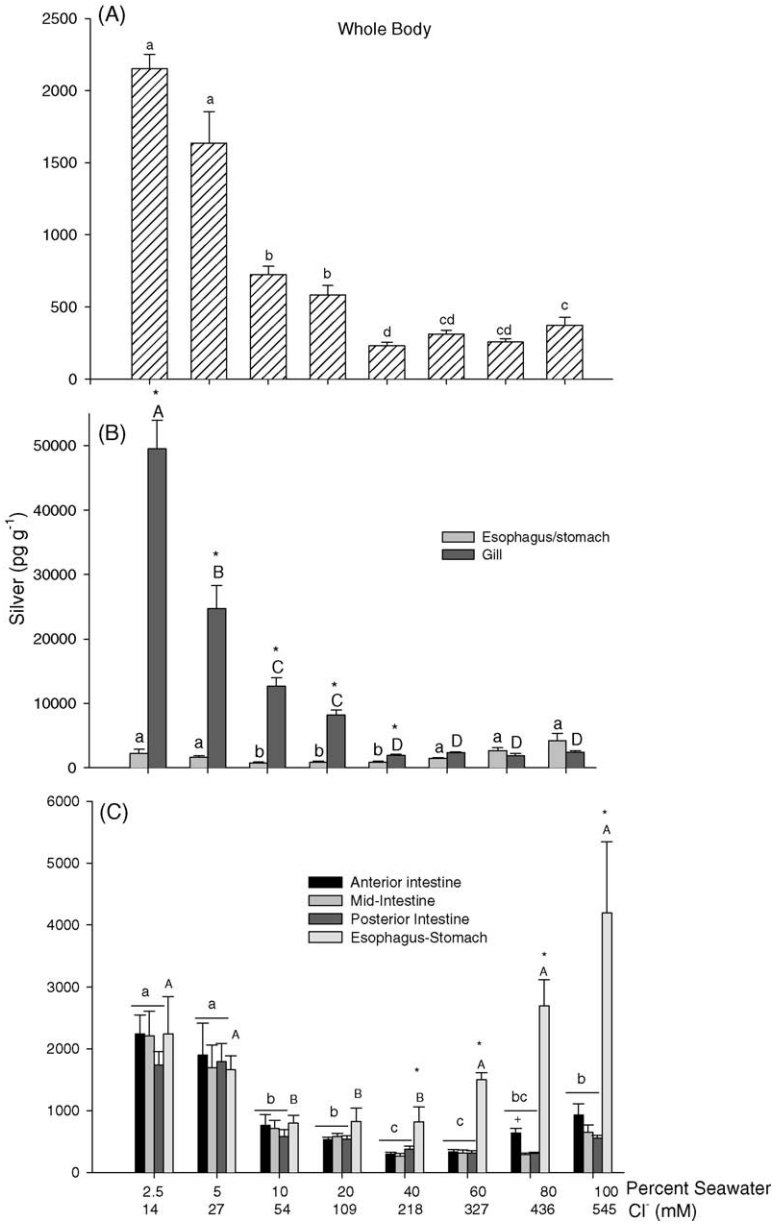

Fig. 5. The influence of acclimation salinity (shown both as % seawater and the corresponding chloride concentration) on the concentrations of newly accumulated silver in (A) the whole body, (B) the gills and esophagus-stomach, and (C) the various sections of the gastrointestinal tract of toadfish exposed to 2.18 $\mu g\,L^{-1}$ of silver (as $AgNO_3$) for 24 h. Mean ± 1S.E.M. ($N = 7–9$). Means not sharing the same case letter are significantly different ($P < 0.05$). In (B), asterisks indicate significant differences between gill and esophagus-stomach values. In (C), a cross marks the only case (in 80% seawater) where there was a significant difference between the anterior intestine vs. the middle and posterior intestine. At all other salinities, concentrations were statistically the same in the three intestinal segments, so the data were combined for subsequent analyses. Means not sharing the same lower case letter are significantly different ($P < 0.05$) for intestinal segments (combined data) and means not sharing the same upper case letter for esophagus-stomach are significantly different. Asterisks indicate significant differences between esophagus-stomach concentrations and intestinal concentrations (combined data) at the same salinity.

water, and the lowest at 40%, close to the isosmotic point (Fig. 5A). The difference in accumulation between 40 and 100% seawater was only about 1.6-fold, so most of the variation reflected the large increases at the lower salinities.

A comparison of the two most probable sites of silver uptake, esophagus-stomach versus gill, revealed two very different patterns of bioaccumulation with respect to salinity (Fig. 5B). For gill tissue, accumulations remained low from 100% down to 40% seawater (minimum), then increased dramatically (26-fold) at lower salinities to a maximum in 2.5% seawater (Fig. 5B). For esophagus-stomach, the highest accumulation occurred in 100% seawater, declined to a minimum at intermediate salinities (10–40%), then increased again at the two lowest salinities to values that were not significantly different from those in 100% seawater (Fig. 5B). Overall variation was about five-fold. Silver accumulations in the esophagus-stomach were fairly similar to those in the gills down to a salinity of 60%, but below this level, the gill values exceeded the gut values by an increasingly greater extent.

Within various parts of the intestinal tract at a particular salinity, newly accumulated silver levels were very similar between anterior, middle, and posterior intestine (Fig. 5C). The overall pattern was that values declined from 100% seawater down to minima in 40 and 60% seawater, then increased to the highest levels in 2.5 and 5% seawater. This biphasic trend was very different from that in the esophagus-stomach (Fig. 5B). Plotting these data together (Fig. 5C) emphasizes that esophagus-stomach concentrations were about five-fold higher than in the intestinal segments in 100% seawater, but that this difference progressively declined at lower salinities. Below 40%, there were no significant differences, and esophagus-stomach concentrations tracked those of the intestinal segments as they increased again at the lowest salinities.

Gut fluid volumes were small, especially at salinities below 60%, and it was necessary to pool the samples from different parts of the digestive tract to provide sufficient volumes for reliable analysis. There were no significant differences in gut fluid silver concentrations across the entire salinity range (data not shown). The overall mean was 2.63 ± 0.69 (63) $\mu g\,L^{-1}$, close to the mean exposure concentration of $2.18 \pm 0.09\ \mu g\,L^{-1}$. However, gut fluid concentrations were significantly lower than those in the exposure water at salinities of 60% (0.57 ± 0.19 [8] $\mu g\,L^{-1}$), 80% (1.03 ± 0.61 [8] $\mu g\,L^{-1}$), and 100% (0.60 ± 0.38 [7] $\mu g\,L^{-1}$) but were not significantly different from the exposure water at lower salinities.

Of the various internal organs assayed, the liver exhibited by far the highest levels of newly accumulated silver (Fig. 6A). The overall pattern with respect to salinity was somewhat similar to that seen in the whole body (cf. Fig. 5A), reflecting the fact that the liver accounted for 23–40% of the whole-body accumulation. Thus, hepatic silver accumulation decreased by slightly less than half from 100 to 40% seawater, then increased from this minimum by more than 18-fold to the highest values in 2.5% seawater. Although the exposures were only for 24 h, newly accumulated silver had already been passed into the bile in appreciable amounts (Fig. 6B). Absolute biliary concentrations were about 5–20% of those in liver, and exhibited a very similar pattern of variation with salinity.

Newly accumulated silver concentrations in kidney (Fig. 7A) were far lower than those in the liver (cf. Fig. 6A) and comparable to those in whole body (cf. Fig. 5A). Overall patterns were similar to both, declining by about half from 100 down to 40% seawater, then increasing by seven- to eight-fold at the two lowest salinities. Newly accumulated silver levels in the urine were very low (Fig. 7B), significantly below (about one-third) those in plasma at most salinities (cf. Table 3), and far below than those in bile at all salinities (cf. Fig. 6B). There were no significant differences among salinities.

White muscle concentrations of newly accumulated silver were the lowest of any tissue sampled, significantly lower even than those of the carcass, of which it was the principal constituent, or the swim bladder (Table 3). All three tissues showed generally similar patterns, again comparable to those of the whole body (cf. Fig. 5A). Plasma and red blood cell levels of newly accumulated silver were not consistently different from one another, the only significant differences (in opposite directions) occurring at 5 and 20% seawater (Table 3). Variation across salinities was not as great as for most other tissues, though again, the lowest concentrations occurred in fish at 40–60% seawater. Notably, plasma concentrations of newly accumulated silver were significantly below those in the exposure water ($2.18\ \mu g\,L^{-1}$) at every salinity except the lowest. Concentrations of silver in the spleen, which functions

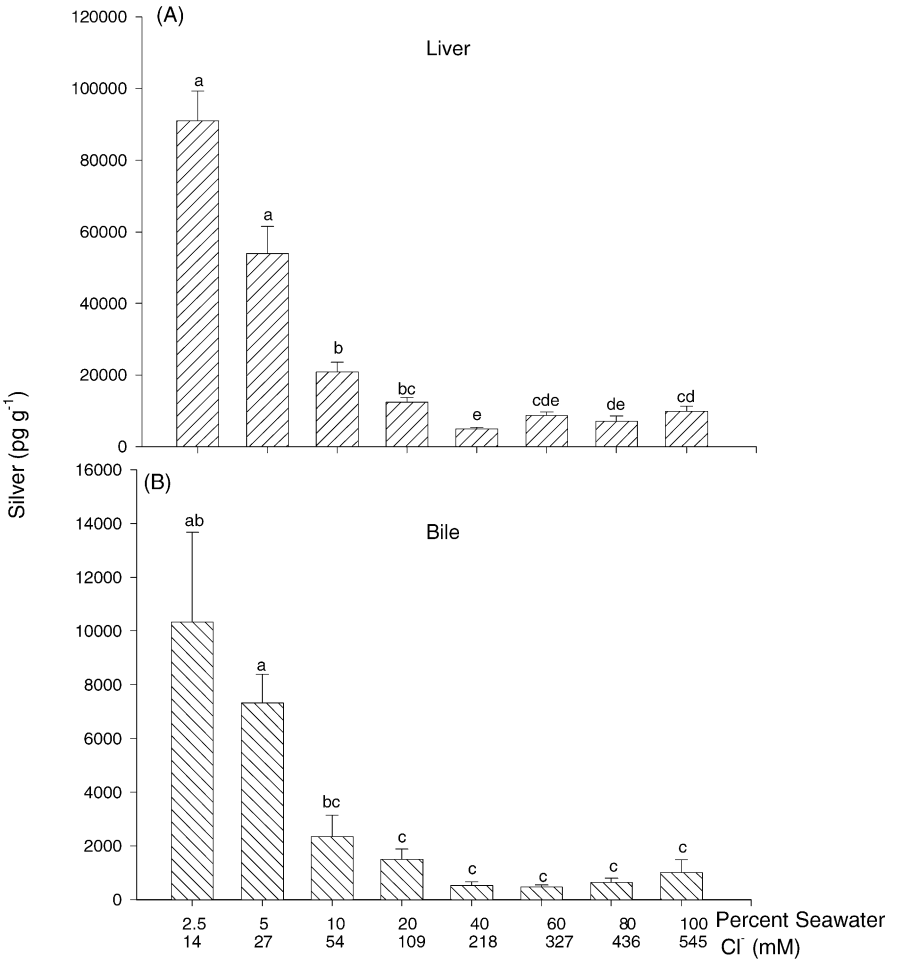

Fig. 6. The influence of acclimation salinity (shown both as % seawater and the corresponding chloride concentration) on the concentrations of newly accumulated silver in (A) the liver and (B) the bile of toadfish exposed to 2.18 $\mu g\,L^{-1}$ of silver (as $AgNO_3$) for 24 h. Mean ± 1S.E.M. ($N = 7$–9). Means not sharing the same letter are significantly different ($P < 0.05$).

to store red blood cells, were rather variable but generally higher than those of the erythrocytes themselves at most salinities (Table 3).

3.4. Threshold for acute silver toxicity in toadfish

Toadfish proved exceedingly tolerant to silver in 100% seawater. In the first two trials, there was no mortality up to 96 h at any concentration tested up to 32,000 $\mu g\,L^{-1}$ (nominal); this was obviously well above the solubility limit, as the water was very cloudy. However, some of the fish appeared very lethargic at this concentration, so a third, longer trial was performed, at just the two highest concentrations, 16,000 and 32,000 $\mu g\,L^{-1}$ (nominal). In both, mortality started at 120 h, with 50% dead at 168 h (7 days), and was complete by 200 h (Fig. 8). Speciation calculations at these very high concentrations are problematical, but indicated that at most 11% of the total added silver should go into solution, the rest forming cerargyrite, an insoluble silver chloride complex. Actual measured concentrations in the exposure water were in reasonable agree-

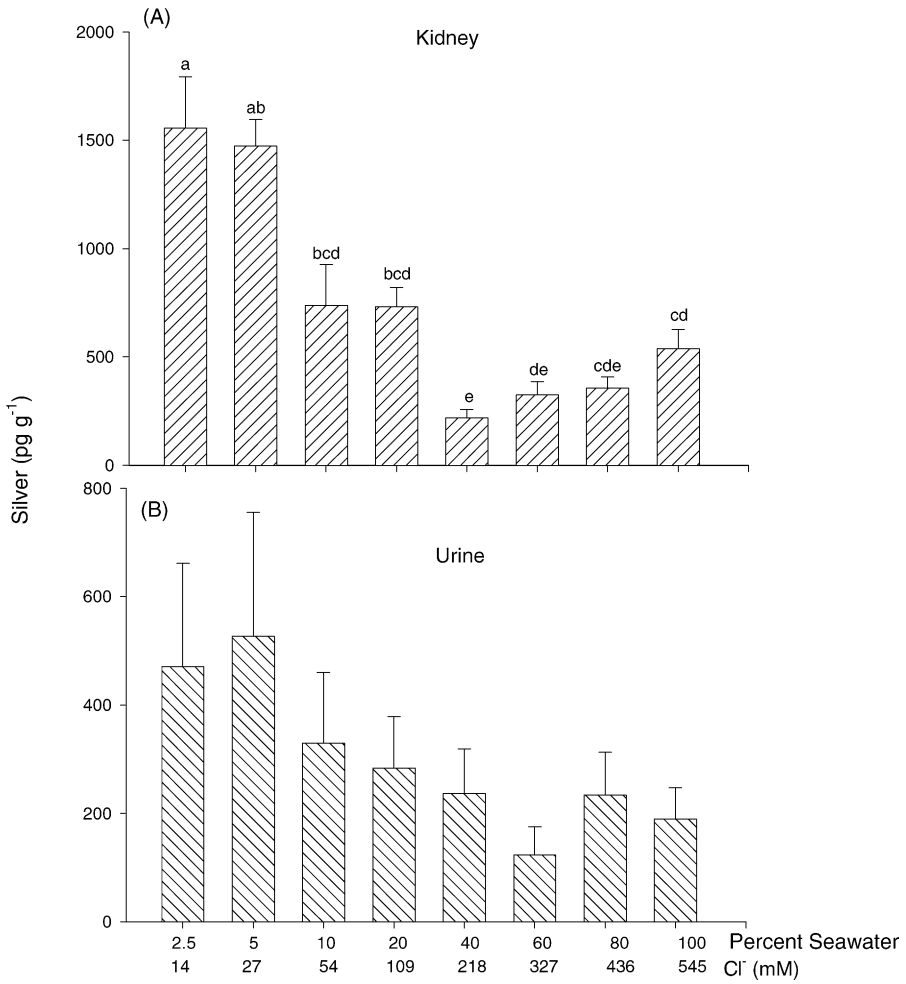

Fig. 7. The influence of acclimation salinity (shown both as % seawater and the corresponding chloride concentration) on the concentrations of newly accumulated silver in (A) the kidney and (B) the urine of toadfish exposed to 2.18 $\mu g\,L^{-1}$ of silver (as $AgNO_3$) for 24 h. Mean ± 1S.E.M. ($N = 7$–9). In (A) means not sharing the same letter are significantly different ($P < 0.05$). In (B), there are no significant differences among salinities.

ment with this prediction. At 16,000 $\mu g\,L^{-1}$ (nominal), measured concentrations were only 1609 ± 95 (20) $\mu g\,L^{-1}$ total and 876 ± 32 (20) $\mu g\,L^{-1}$ dissolved (i.e., 0.45 μm filterable) and at 32,000 $\mu g\,L^{-1}$ (nominal) were 2251 ± 213 (20) $\mu g\,L^{-1}$ total and 980 ± 47 (20) $\mu g\,L^{-1}$ dissolved. Note the very similar dissolved concentrations associated with the identical-time mortality curves (Fig. 8). Thus the 96-h LC50 is above 980 $\mu g\,L^{-1}$ dissolved silver and 2250 $\mu g\,L^{-1}$ total silver, but the true incipient LC50 is undoubtedly lower.

4. Discussion

4.1. Osmo- and ionoregulation of toadfish in relation to salinity

The data of Fig. 1 confirm the ability of *O. beta* (Serafy et al., 1997) to osmo- and ionoregulate effectively over a range from full strength seawater down to almost freshwater (2.5% seawater) despite an absence of feeding, at least for the 1–2-week period of the current exposures. (Attempts to acclimate the toadfish to

Table 3
The influence of acclimation salinity (shown as both % seawater and the corresponding chloride concentration) on the concentrations (pg g^{-1}) of newly accumulated silver in the white muscle, carcass, swim bladder, red blood cells, blood plasma, and spleen of toadfish exposed to 2.18 μg L^{-1} of silver (as $AgNO_3$) for 24 h

	% SW (Cl^-, mM)							
	2.5 (14)	5 (27)	10 (54)	20 (109)	40 (218)	60 (327)	80 (436)	100 (545)
Muscle	197.0 ± 27.7 a	171.9 ± 24.5 ab	133.2 ± 20.7 abc	81.0 ± 6.1 abc	45.9 ± 8.5 c	63.5 ± 9.9 bc	49.2 ± 7.7 c	67.3 ± 13.9 bc
Carcass	574.3 ± 66.2 ab	765.6 ± 117.9 a	354.6 ± 39.7 bc	353.7 ± 66.8 bcd	151.4 ± 20.3 e	185.8 ± 23.12 de	130.2 ± 15.9 e	210.8 ± 44.8 cde
Swim bladder	436.4 ± 90.0 a	420.2 ± 99.2 a	228.1 ± 58.5 ab	146.3 ± 14.2 bc	82.3 ± 16.0 cd	72.2 ± 11.0 d	84.0 ± 9.6 cd	116.0 ± 8.7 bcd
Red blood cells	855.5 ±187.1 ab	1364.8 ± 173.7 a	622.4 ± 249.6 ab	341.3 ± 66.9 b	456.4 ± 93.8 ab	297.9 ± 76.9 b	294.1 ± 32.7 b	592.9 ± 145.3 ab
Plasma	1466.3 ± 681.1 a	671.5* ± 81.7 a	703.4 ± 157.7 a	822.5* ± 111.7 a	257 ± 24.6 b	285.9 ± 252.5 b	459.9 ± 53.7 ab	485.4 ± 93.4 ab
Spleen	5906.8 ± 2956.2 a	2528.7 ± 342.1 a	887.6 ± 202.4 ab	2163.4 ± 926.2 ab	1090.8 ± 123.3 ab	551.7 ± 91.9 b	786.8 ± 167.7 b	2146.6 ± 1253.6 ab

Mean ± 1S.E.M. (N = 7–9). Within a tissue, means not sharing the same letter are significantly different ($P < 0.05$). Asterisks indicate significant differences between plasma and red blood cell concentrations at the same salinity.

true freshwater resulted in partial mortalities.) In general, the pattern of changes in plasma osmolality and ions, as well as urinary ions, corresponded well with a less extensive data set reported in the congeneric *Opsanus tau* (Lahlou et al., 1969). Both species are virtually aglomerular, forming the primary urine largely by secretion (Marshall, 1930; Lahlou et al., 1969; Howe and Gutknecht, 1978; McDonald et al., 2000, 2003). However, clearly the renal system continues to play an important ionoregulatory role, in particular, secreting Mg^{2+} at higher salinities and reabsorbing Cl^- and to a lesser extent Na^+ at lower salinities (Fig. 3), similar to glomerular teleosts (Hickman, 1968; Hickman and Trump, 1969; Beyenbach and Kirschner, 1975).

In view of the electrolyte gradients between the external environment and the blood plasma (Fig. 1B), the ionoregulatory strategy of toadfish likely switches over at 30–40% seawater, from active Na^+ and Cl^- excretion across the gills at high salinities, to active uptake of these ions at lower salinities. Like other marine teleosts (Smith, 1930), toadfish drink the external medium at higher salinities, transporting Na^+ and Cl^- across the intestinal epithelium so as to absorb water, while excluding divalent ions such as Mg^{2+} (Grosell et al., 2004a,b). Because water permeability (Howe and Gutknecht, 1978) and urinary water loss (McDonald et al., 2000, 2003) are both low, drinking rates tend to be lower than in most other marine teleosts. Grosell et al. (2004b) reported rates of 0.5–1.5 ml kg^{-1} h^{-1}, and we measured rates of 0.65 ml kg^{-1} h^{-1} (unpublished data) in trials done in parallel to the current experiments in unfed *O. beta* acclimated to 100% seawater. A priori, we would expect drinking to be turned off close to the isosmotic point (cf. Shehadeh and Gordon, 1969); the intestinal Mg^{2+} data suggest that this occurred in the fish acclimated to 40% seawater and below (Fig. 2C).

Notably, Na^+ and Cl^- concentrations in intestinal fluids (Fig. 2A and B) were similar to or somewhat below plasma ion levels (Fig. 1B) at salinities (60–100% seawater) where the fish were obviously drinking. This fits with the theory that the bulk of desalination goes on in the esophagus-stomach region, i.e., anterior to the points where the present samples were taken, so water absorption can occur by more or less isosmotic transport of Na^+, Cl^-, and water in the subsequent intestinal segments (reviewed by Loretz (1995)). At lower salinities (2.5–40% seawater), the fact that intestinal

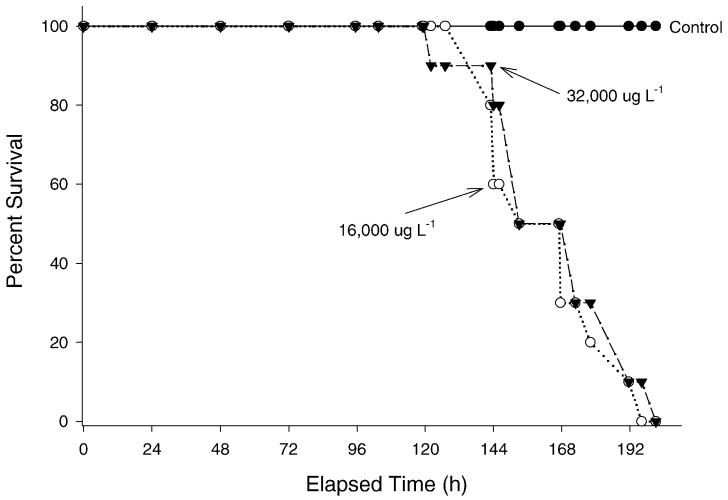

Fig. 8. Time-mortality curves for toadfish exposed to 16,000 and 32,000 μg L^{-1} of silver (nominal concentrations as $AgNO_3$; $N = 10$ per concentration) in 100% seawater. Note the absence of mortality up to 96 h, and the similarity of the two mortality curves from 120 h onwards. See text for measured silver levels in the exposure water.

fluid Na^+ and Cl^- concentrations remained similar to plasma levels suggests that the fluid is either derived from extracellular fluid, or if ingested in small amounts, quickly equilibrates with extracellular fluid.

4.2. Acute toxicity of silver in toadfish

Relative to summaries of acute toxicity thresholds by the U.S. EPA (1980), Ratte (1999), and DeBoeck et al. (2001), many of which are based on nominal concentrations, *O. beta* is clearly the most silver-tolerant marine fish ever studied, with a 96-h LC50 in 100% seawater greater than 980 μg L^{-1} dissolved silver, 2250 μg L^{-1} total silver, and 32,000 μg L^{-1} added silver (i.e., nominal; Fig. 8). The measured total silver value, equivalent to approximately 21 μmol L^{-1}, may be compared with a measured total copper 96-h LC50 greater than 340 μmol L^{-1} (Grosell et al., 2004a), showing that the toadfish is even more resistant to a metal which shares some chemical similarities to silver. In other marine teleosts where toxicity is seen at much lower silver concentrations below the threshold for cerargyrite formation, ionoregulatory impairment appears to be the toxic mechanism (Hogstrand et al., 1999; Grosell et al., 1999; Webb et al., 2001; Grosell and Wood, 2001).

In view of the reportedly greater toxicity of silver at lower salinities in other teleosts (see Section 1), it would be of interest to measure 96-h LC50s in toadfish at such salinities. Regardless, the level of silver used in the bioavailability tests (2.18 μg L^{-1} total silver for 24 h) was so far below the toxic threshold (less than 0.1%) that the present results likely reflected pure bioavailability considerations dictated by organism physiology and silver speciation, and were not complicated by pathological responses.

In this regard, it is of interest to note that in a parallel study (unpublished data), we have recently measured total liver silver levels in toadfish collected from the same site (Biscayne Bay, south Florida) as those used in the present study. Total "background" silver burdens were several-fold greater than the 24-h levels of "newly accumulated silver" measured in the present study for 100% seawater acclimated fish (Fig. 6A), again indicating that the present exposures were well below concentrations that would have caused any acute pathology. However, it is not known how these "background" silver concentrations present in wild fish might have influenced the uptake and tissue distribution of the radiolabelled silver.

4.3. Bioavailability of silver in relation to salinity in toadfish

To our knowledge, this is the first study of silver bioavailability to a single species across a range

of salinities from almost freshwater right up to full strength seawater. Bioavailability (reflected in whole-body silver accumulation at an environmentally relevant level of waterborne silver) varied more than nine-fold across salinities, with the highest value occurring at 2.5% seawater, and the lowest at 40%, close to the isosmotic point (Fig. 5A). Accumulation in 100% seawater was only about 1.6-fold higher than in 40% seawater, so most of the variation reflected the large increases at the lower salinities. While bioaccumulation is not necessarily correlated with toxicity, the present pattern (Fig. 5A) nicely parallels earlier theoretical scenarios about silver toxicity based on speciation modeling and predictions about organism physiology at different salinities. Hogstrand and Wood (1998) and Wood et al. (1999) predicted greatest toxicity at lowest salinity (reflecting the availability of the free Ag^+ ion), lowest toxicity at the isosmotic point (reflecting the absence of Ag^+, the absence of drinking intake, the reduced activity of ionoregulatory processes, and the minimal overall solubility of silver at this salinity), and moderately greater toxicity at higher salinities up to 100% seawater (reflecting drinking intake and the increasing importance of ionoregulatory processes).

Comparison of Fig. 5A with Fig. 4 indicates that bioavailability of silver to toadfish cannot be related unequivocally to the availability of a single silver species. Of the various possibilities, the occurrence of the neutral dissolved $AgCl_0$ complex appears to be the best correlated with accumulation, and this would agree with evidence that $AgCl_0$ is also taken up by freshwater fish (McGeer and Wood, 1998; Wood et al., 2002; Hogstrand et al., 2003). However, the very small amount of Ag^+ in exposures at $\leq$20% seawater cannot be discounted, as this is the form primarily taken up by the active Na^+ uptake mechanism on the gills in freshwater fish (Bury and Wood, 1999). Such a mechanism would not be operating above the isosmotic point, but with the onset of drinking, another route of uptake, the gastrointestinal tract, will become available. The very different patterns of silver accumulation in gills versus esophagus-stomach (Fig. 5B) are diagnostic of this changing organism physiology across salinities.

Silver loads in the two tissues, which make comparable contributions to total body mass (1.1–1.2%; Table 1), are similar down to 60% seawater, but the gill concentration becomes progressively greater at lower salinities (Fig. 5B). Using a generous estimate of drinking at a rate of 1.5 ml kg^{-1} h^{-1} (Grosell et al., 2004b; see Section 4.1), ingestion of 100% seawater containing 2.18 μg L^{-1} would account for an accumulation of about 80 pg g^{-1} over 24 h, relative to a measured value of 374 pg g^{-1} in the whole body (Fig. 5A). Taking the relative mass of the esophagus-stomach into account (Table 1), approximately half of the accumulation by drinking was retained in this tissue. From this we conclude that the gill is the dominant route (~80%) of uptake even at the highest salinity, with this contribution increasing close to 100% of the much higher accumulation at the lowest salinities (e.g., 2153 pg g^{-1} in 2.5% seawater; Fig. 5A). Taking the relative mass of the gill into account (Table 1), less than one-third of this silver uptake remained in the gill tissue at 24 h, the bulk being redistributed via the bloodstream to other tissues. Using a more direct experimental approach, Grosell and Wood (2001) similarly concluded that the gastrointestinal tract contributed only a small percentage of silver uptake relative to the gills in the lemon sole (*Parophrys vetulus*) in 95% seawater, even though at least half of the toxicity to ionoregulation (at much higher waterborne silver concentrations) was of gastrointestinal origin.

Detailed analysis of the silver accumulation pattern in various parts of the gastrointestinal tract reveals that most silver was accumulated by the esophagus-stomach down to 40% seawater, whereas all segments exhibited similar silver concentrations at salinities below this point (Fig. 5C). Silver concentrations in intestinal fluids were below those in the exposure water only at higher salinities. Very probably, this result reflects drinking occurring only at the higher salinities, with silver being preferentially taken up in the first compartment, the esophagus-stomach, by the well-known desalination mechanisms (active and passive transport of NaCl) that occur in this segment (Loretz, 1995). Hogstrand et al. (2002), using an in vitro intestinal preparation of the European flounder (*Platichthys flesus*), provided evidence for transcellular, carrier-mediated silver uptake. At lower salinities (2.5–20% seawater), the homogeneous distribution of silver in all segments and increasing absolute concentrations probably result from bloodborne distribution of silver to the tissues, rather than a resumption of drinking; note the similar pattern in red blood cells and plasma (Table 3). This silver would have entered the blood via the gills (Fig. 5B).

4.4. Internal distribution of silver in relation to salinity in toadfish

Silver entering across the gills was efficiently scavenged from the blood by the various organs. Plasma and erythrocytes appear to be more or less in equilibrium (Table 3). With the exception of white muscle, carcass, and swim bladder (Table 3), all other tissues exhibited greater mass-specific silver accumulation than the blood (Table 3). The liver was clearly the dominant organ in this regard (Fig. 6A), accounting for only ~1% of body mass but about 23% of the whole body burden at higher salinities, increasing to 40% at the lowest salinity (Fig. 9). This finding is in accord with all previous studies on silver accumulation in both marine (e.g., Pentreath, 1977; Hogstrand et al., 1999; Webb and Wood, 2000; Rouleau et al., 2000; Grosell and Wood, 2001) and freshwater teleosts (e.g., Garnier and Baudin, 1990; Garnier et al., 1990; Hogstrand et al., 1996, 2003; Bertram and Playle, 2002; Galvez et al., 2002; Nichols and Playle, 2004). The rapid (<24 h) appearance of this newly accumulated silver in the bile (Fig. 6B) in approximate proportion to the liver accumulation (Fig. 6A) suggests that the hepatobiliary route may be an important and rapid pathway for silver homeostasis in the toadfish, as for chemically similar copper in the same species (Grosell et al., 2004b). Many of these investigations have also measured kidney accumulations and concur with the present finding that it is fairly modest (Fig. 7A). Urinary silver concentrations have not been measured in previous studies; the present data (Fig. 7B) indicate a minimal renal role in silver homeostasis, as urinary silver concentrations remained below plasma levels (Fig. 7B, Table 3).

Accumulations of silver in muscle tissue, which makes up most of the carcass by mass, were extremely low, in accord with the cited literature (see above), whereas carcass accumulations were somewhat greater (Table 3). This suggests that the contained bone, skin, nervous tissue, gonad, and blood components may have exhibited higher concentrations, though still very low relative to liver (Fig. 6A). Nevertheless, on a percentage basis, the carcass was the single biggest contributor to the whole-body burden (Fig. 9) at every salinity except 2.5% seawater (where the liver dominated), reflecting its 85% contribution to total body mass (Table 1). Fig. 9 further emphasizes that over 90% of the total burden was accounted for by only four compartments (carcass, liver, gill, and esophagus-stomach) at every salinity. The relative contribution of the esophagus-stomach became negligible at salinities <40% seawater, whereas the relative contributions of the liver and the gill increased at the expense of the carcass at these lower salinities. The overall distribution of newly accumulated silver in toadfish acclimated to 2.5% seawater was very similar to patterns that have been reported in trout and eel after comparable 24-h exposures to low levels of ^{110m}Ag in freshwater (Hogstrand et al., 2003).

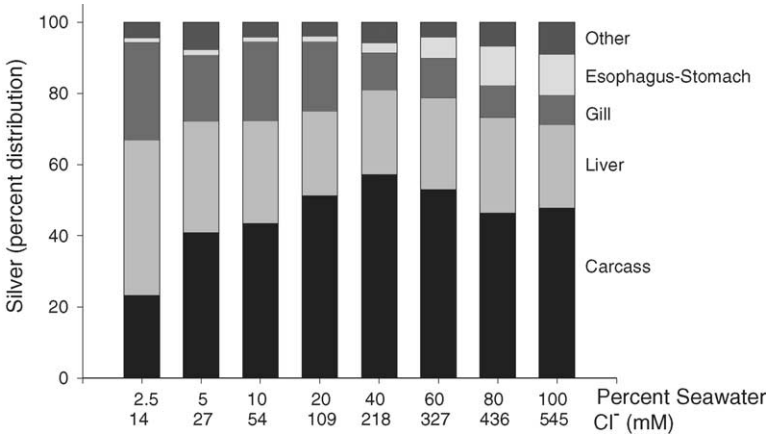

Fig. 9. Relative contribution of four compartments to whole-body concentration of newly accumulated silver in toadfish exposed to 2.18 μg L^{-1} of silver (as $AgNO_3$) for 24 h.

4.5. Concluding remarks

The present study has demonstrated that the short-term bioavailability and internal distribution of silver are both greatly affected by salinity in the gulf toadfish. These effects are explained in part by salinity-dependent changes in metal speciation, but just as importantly, by salinity-dependent changes in the ionoregulatory physiology of the organism. Efforts to extend the Biotic Ligand Modeling approach (see Section 1) to the estuarine and marine environment must recognize that ligands on the gills and the gut are both potentially important, and must take this changing organism physiology into account.

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