

TOXICITY OF SILVER TO THE MARINE TELEOST (*OLIGOCOTTUS MACULOSUS*):
EFFECTS OF SALINITY AND AMMONIA

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Abstract—Investigations were conducted to determine the toxicity and define parameters (i.e., ammonia, salinity) that influence the effects of silver on the tidepool sculpin (*Oligocottus maculosus*). In one series of experiments, static-renewal 168-h toxicity tests were conducted with silver at 32‰ (i.e., ambient) and 25‰ salinity seawater. Silver was analyzed in fish that survived these exposures. Toxicity was greater at the lower salinity. The 96-h and 168-h 50% lethal concentration (LC50) values were 3.07 $\mu\text{mol Ag/L}$ (0.331 mg Ag/L) and 1.11 $\mu\text{mol Ag/L}$ (0.119 mg Ag/L) at 25‰ and 6.2 $\mu\text{mol Ag/L}$ (0.664 mg Ag/L) and 4.37 $\mu\text{mol Ag/L}$ (0.472 mg Ag/L) at 32‰ salinity, respectively. There was no correlation between whole-body silver burden and toxicity. Silver uptake increased with exposure concentrations at 25‰ salinity, but at 32‰ whole body accumulation did not exceed that observed for control fish irrespective of the concentrations tested. In another set of experiments, 96-h static-renewal toxicity tests were conducted with silver, ammonia, and combinations of each. The 96-h LC50 for ammonia was 5.9 mmol total ammonia (Tamm)/L (106 mg Tamm/L). When tested in combination, silver toxicity was enhanced and the onset of mortality hastened. Mortality increased in a dose-dependent fashion at 6.35 $\mu\text{mol Ag/L}$ (0.685 mg Ag/L) from 55 to 100% in the presence of ammonia concentrations ranging from 0 to 12.60 mmol Tamm/L (0–226.8 mg Tamm/L). Conversely, the 50% lethal time (LT50) estimated at this level of silver exposure progressively dropped from 5,730 to 1,180 min over the same range of ammonia concentrations.

Keywords—Acute toxicity Fish Silver Seawater Accumulation

INTRODUCTION

The toxicity and physiological effects of silver differ dramatically for freshwater and marine fish [1–4]. While most investigations have focused on freshwater systems, recent studies have begun to pinpoint mechanisms and define silver toxicity for a few marine fish [3,5,6]. These investigations also have illuminated several parameters that influence the effects of silver.

In freshwater, the free silver ion (Ag^+) is extremely toxic to aquatic organisms. Typical 96-h 50% lethal concentration (LC50) values for silver in freshwater systems range from 0.06 to 0.6 $\mu\text{mol Ag/L}$ (0.006–0.065 mg Ag/L [7–10]) with 30 to 40% present as Ag^+ . However, Ag^+ is very reactive and readily complexes with high affinity to negative binding sites on sediments, suspended solids, and dissolved organic particles as well as inorganic anions [11]. The latter includes chloride (Cl^-), which combines with silver to form silver chloride (AgCl_n) species. While the toxicity of Ag^+ has been well documented, little is known about the toxicity of dissolved silver complexes. Recently, Hogstrand et al. [2] and Galvez and Wood [12] compared the toxicity of different silver species to juvenile rainbow trout (*Oncorhynchus mykiss*). They concluded that dissolved AgCl_n was at least 10 times less toxic than AgNO_3 , which readily dissociated to yield Ag^+ , and further suggested that the concentration of Cl^- in the water column is a prominent modulator of silver toxicity. In seawater (290 > $[\text{Cl}^-]$ mmol/L > 570), Ag^+ is diminished to insignificant concentrations and AgCl_n species predominate. However,

few published studies have focused on silver toxicity in marine fish and even fewer have appeared in peer-reviewed literature [13,14]. The U.S. Environmental Protection Agency (EPA), Narragansett, Rhode Island (memorandum from J. Cardin to D. Hansen, 1985) reported 96-h LC50 values for the winter flounder (*Pseudopleuronectes americanus*) of 1.8 $\mu\text{mol Ag/L}$ (0.196 mg Ag/L) and 4.6 $\mu\text{mol Ag/L}$ (0.500 mg Ag/L) for embryos and larvae, respectively. They also reported 96-h LC50 values that ranged from 0.074 $\mu\text{mol Ag/L}$ (0.008 mg Ag/L) to 1.3 $\mu\text{mol Ag/L}$ (0.14 mg Ag/L) for embryonic stages of the summer flounder (*Paralichthys dentatus*). Dinnel et al. [15] reported a 96-h LC50 value of 7.4 $\mu\text{mol Ag/L}$ (0.8 mg Ag/L) for adult stages of the English sole (*Parophrys vetulus*). However, the mummichog (*Fundulus heteroclitus*) and juvenile sheepshead minnows (*Cyprinodon variegatus*) were the most tolerant species studied with average 96-h LC50 values of 25.0 $\mu\text{mol Ag/L}$ (2.7 mg Ag/L) and 13.0 $\mu\text{mol Ag/L}$ (1.4 mg Ag/L), respectively [14,16]. Thus, reported LC50 values for marine fish span three orders of magnitude, 0.074 to 25 $\mu\text{mol Ag/L}$ (0.008–2.7 mg Ag/L). By comparison, the concentrations of silver present in the nearshore marine environment range from 1 to 300 pmol Ag/L (0.1–32 ng Ag/L [17]), and the current U.S. EPA acute criterion for silver in seawater is 0.0213 $\mu\text{mol Ag/L}$ (0.0023 mg Ag/L) [18].

While it is clear that AgCl_n is less toxic than Ag^+ , marine fish living in an estuarine environment tolerate and frequently encounter wide ranges of water $[\text{Cl}^-]$. Little attention has been given to the toxicity of different AgCl_n species. Modeling of AgCl_n speciation by MINEQL+ [19] and MINTEQA [20] indicates that as salinity increases from brackish systems to full-strength seawater, the activity of the dissolved neutral

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AgCl (aq) is reduced, and negatively charged AgCl_n species (AgCl₂⁻, AgCl₃²⁻, AgCl₄³⁻) dominate [6,21]. Ferguson and Hogstrand [6] investigated the implications of AgCl_n speciation to seawater-adapted rainbow trout but found little evidence for speciation-dependent differences in AgCl_n toxicity in the salinity range of 20 to 30‰. No published study has investigated the influence of AgCl_n speciation on silver toxicity and accumulation in a true marine fish.

In addition to Cl⁻, recent studies on the physiological mechanisms of silver toxicity have indicated that factors that enhance nitrogen retention may increase silver toxicity [3,5]. Silver causes an immediate and dramatic increase in plasma ammonia levels in both freshwater and marine fish exposed to a wide range of silver concentrations [3,5,22]. Copper, which is chemically related to silver and has similar toxic mechanisms, produces analogous effects on nitrogen metabolism [23–27]. The rise in plasma ammonia following either copper or silver exposure would appear to make fish more vulnerable to any parameter that increases internal ammonia levels (e.g., water pH, water column ammonia, stress [28]). As water column concentrations of ammonia are increased, transbranchial diffusion gradients are reduced or even reversed [28,29]. Consequently, increased water column ammonia inhibits nitrogen excretion and results in a rapid increase in plasma ammonia, which could exacerbate silver toxicity. For marine fish, this effect applies not only to un-ionized ammonia but also to the charged ammonium ion, NH₄⁺ [29].

The tidepool sculpin (*Oligocottus maculosus* Girard, 1856) inhabits small rock pools along the Pacific coast of northwest America. These pools can remain isolated from the sea for extended periods, and as a result the tidepool sculpin can tolerate substantial and rapid changes in water temperature, salinity, gas tensions, and pH [30]. Although this intertidal species can survive out of water for extended periods and population densities within rock pools are often quite high, their pattern of nitrogen excretion resembles that of other teleost fish [30]. These qualities make the sculpin easy to transport, handle, and house. This, coupled with their small size, makes the tidepool sculpin ideally suited for toxicity testing.

The primary objective of the present study was to determine the acute toxicity of silver for a truly marine fish, the tidepool sculpin. The specific-goals were (1) to establish 96-h and 168-h threshold (i.e., LC10) and median lethal (i.e., LC50) concentrations in 32‰ salinity (i.e., ambient) seawater; (2) to determine the effects of salinity (i.e., Cl⁻) on toxicity and whole-body silver accumulation; and (3) to investigate silver toxicity in conjunction with water column ammonia.

MATERIALS AND METHODS

Animals

Juvenile tidepool sculpins (*O. maculosus*) weighing 1.33 ± 0.09 g (mean ± SEM) were collected by dip net from tidepools on the western shore of Bamfield, British Columbia. Fish were housed outdoors in 19-L polyethylene tanks and allowed to acclimate for at least 5 d prior to experimentation. Holding tanks were supplied via an aerated header tank with fresh-flowing natural seawater (salinity 32‰; pH 7.8; temperature 11–13°C). Sculpins were fed fresh mussels to satiation every other day. No food was given 2 d before and during experiments.

Bioassays with silver and ammonia

Static renewal toxicity tests with 24-h changes were conducted with silver and ammonia according to recommendations given by the U.S. EPA [16]. Test solutions were prepared with natural seawater (32‰ salinity) from stocks made with AgNO₃ (analytical grade, Sigma Chemical, St. Louis, MO, USA) or NH₄Cl (analytical grade, Fisher Scientific, Fair Lawn, NJ, USA) dissolved in deionized water. Test concentrations nominally ranged from 0.5 to 10.0 μmol Ag/L (0.005–1.080 mg Ag/L) or 0.5 to 15 mmol total ammonia (Tamm)/L (9–270 mg Tamm/L). Exposures followed a geometric series that included a minimum of seven concentrations and one control group per test. These tests were repeated twice and results were combined to determine 96-h LC10 and LC50 values for each toxicant. Median lethal time (i.e., LT50) values were calculated for concentrations within the effect range for each toxicant. In addition, four concentrations of silver, nominally including 4.0, 5.0, 6.0, and 7.5 μmol Ag/L (0.431, 0.539, 0.647, and 0.809 mg Ag/L), were tested in combination with nominal ammonia concentrations of 1.0, 7.0, and 14.0 mmol Tamm/L (18, 126, and 253 mg Tamm/L). These selected concentrations bracketed the LC50 values of their respective toxicants. All solutions for an individual test were made immediately prior to use. Toxicity tests were conducted in a controlled environment maintained at 10 ± 1°C with a 16-h light/8-h dark photoperiod. Ten sculpins were housed in 600-ml polyethylene test chambers, and three replicates were used per test concentration. Each chamber was provided slight aeration and covered. As indicated above, fish were not fed during testing. Test water was replaced daily and water samples were taken at the start and conclusion of each test for analyses of total silver, Tamm, salinity, temperature, pH, and dissolved oxygen (DO) using procedures discussed below. There were no appreciable differences in the total silver concentrations or other water quality parameters over the test periods (salinity, 32 ± 0.25‰; temperature, 10 ± 1°C; DO, > 85% saturated; and in tests where ammonia was not added, post-test ammonia, <100 μmol Tamm/L). The fish were observed at 6-h intervals during testing. Mortality was determined by cessation of opercular movements, and dead fish were removed immediately following identification. There were no mortalities observed in the control groups.

Bioassays at different salinities

Static renewal, 168-h, toxicity tests were conducted with silver at 32‰ (i.e., ambient) and 25‰ salinity seawater ([Cl⁻] = 500 and 390 mmol/L) using slightly different methods. The higher salinity was included for validation with the method given above. Natural seawater (32‰ salinity) was diluted with deionized water to achieve a final salinity of 25‰, and sculpins to be tested were allowed to acclimate for 5 d prior to exposure. Test solutions were prepared as given above. Test solutions nominally ranged from 0.05 to 15.0 μmol Ag/L (0.005–1.62 mg Ag/L) and contained at least seven test concentrations and one control group. Sculpins were individually housed in 30-ml polyethylene scintillation vials, with 10 replicate vials per test concentration. Each vial was supplied with slight aeration and covered. The remainder of the experiment followed the procedures given above. There were no mortalities in any of the control groups and no appreciable variations in temperature, salinity, DO, pH, and ammonia.

At the conclusion of each test, surviving fish were sampled for silver analysis. Fish were killed by an overdose of MS-222 (analytical grade, Sigma Chemical), rinsed with control

Table 1. Results from toxicity tests conducted with the tidepool sculpin^a

	96 h			168 h		
	LC10	LC50	LC50/ LC10	LC10	LC50	LC50/ LC10
32‰ salinity						
μmol Ag/L	4.48 (4.09–4.78)	6.15 (5.89–6.45)	1.4	3.13 (2.10–3.67)	4.37 (3.75–5.26)	1.4
mg Ag/L	0.483 (0.441–0.516)	0.664 (0.635–0.696)		0.338 (0.227–0.396)	0.472 (0.405–0.568)	
mmol Tamm/L	3.92 (2.51–4.71)	5.96 (5.07–6.92)	1.5	—	—	—
mg Tamm/L	70.6 (4.5–84.8)	107.6 (91.5–124.8)				
25‰ salinity						
μmol Ag/L	2.12 (1.16–2.67)	3.07 (2.35–3.98)	1.5	0.16 (0.02–0.41)	1.10 (0.46–2.15)	6.9
mg Ag/L	0.229 (0.125–0.288)	0.331 (0.254–0.429)		0.017 (0.002–0.044)	0.119 (0.050–0.232)	

^a 95% confidence intervals are given in parentheses.

seawater, placed in polypropylene vials, and frozen in liquid nitrogen. The samples were stored at -80°C until analysis.

Analysis of water quality parameters

Temperature, pH, and DO were monitored using the Checkmate Modular Meter System (Corning, Corning, NY, USA). Chloride, which was used to monitor salinity, was determined with a CMT-10 chloride titrator (Radiometer, Cleveland, OH, USA) and salinity was calculated using the following equation: $\text{salinity}(\%) = 1.805 \times \text{Cl}^{-}(\%)$ [31]. Ammonia was analyzed colorimetrically using the indophenol blue method of Ivancic and Degobbi [32].

Analysis of silver

Water samples were immediately acidified by the addition of 0.5% (v/v) HNO_3 (trace metal grade; J.T. Baker, Mississauga, ON, Canada). Silver analyses were performed by atomic absorption spectroscopy (AAS), using an air-acetylene flame atomizer (Instrumentation Laboratory, model S-11). Values given for water column silver concentrations were measured averages of total silver for each test. Measured concentrations of silver were also used for determinations of LC and LT values.

Sculpins selected for silver analysis were prepared according to Hogstrand et al. [2], with slight modification. Whole fish were digested with five volumes of 70% (v/v) HNO_3 (trace metal grade; Sigma Chemical), and the solution was heated at 120°C until clear and then cooled to room temperature. Then, 0.75 volume of H_2O_2 was added and the resulting solution heated until dry. Samples were reconstituted to 2.0 ml with 0.5% (v/v) HNO_3 . Silver analysis was performed by AAS (Varian, model spectra AA-20), using a Varian graphite tube atomizer (model GTA-96) equipped with a deuterium lamp for background correction.

Statistical methods

Median lethal concentrations (LC50) and threshold values (LC10) \pm 95% confidence limits were calculated by probit analysis as given by the U.S. EPA [16]. Median lethal times were determined similarly. Differences in whole-body silver concentrations, mortality, and time to mortality between groups were tested with the Mann-Whitney rank sum test or ANOVA followed by the Tukey HSD test [33].

RESULTS AND DISCUSSION

Although information is readily available on the toxicity of Ag^{+} to freshwater fish [7–10], this study presents some of the first published data on the toxicity of silver to a true marine

fish. Moreover, the present study provides the first 168-h LC50 values for silver to fish in seawater. The results of all toxicity tests are summarized in Table 1. Mean 96-h and 168-h LC50 values for silver to juvenile tidepool sculpins were 6.15 (95% confidence limits: 5.89–6.45) and 4.37 (3.75–5.26) $\mu\text{mol Ag/L}$, respectively in 32‰ seawater. Threshold values (i.e., LC10) at this salinity were 1.4% of the median lethal concentrations for each time period. The LC50 values for AgCl_n in the present study were approximately 50 times greater (i.e., less toxic) than those reported for Ag^{+} exposures of freshwater rainbow trout [2]. As noted earlier, reported 96-h LC50 values for silver in seawater spanned three orders of magnitude [13–16]. In contrast, Birge and Zuiderveen [34] found silver in freshwater to be quite remarkable in that it provided less diversity of response among fish and amphibians than most other metals. Reported 96-h LC50 values from that and other studies spanned only one order of magnitude [7–10].

In addition, there were differences in the time-course of silver toxicity between freshwater and marine fish. In all tests conducted with silver at 32‰ salinity, mortality was never observed within the first 48 h (Fig. 1). This time lag was not observed in freshwater rainbow trout following exposure to either Ag^{+} or $\text{Ag}(\text{S}_2\text{O}_3)_n$ [2]. Also, sculpins exhibited a noticeable avoidance response to silver. In the higher silver concentrations, some fish would cling to the sides of the test chambers above the water line and typically attempted to escape when the lids were removed.

Water $[\text{Cl}^{-}]$ could explain the disparity in the variation observed in freshwater and seawater silver toxicity data. Hogstrand et al. [2] and Galvez and Wood [12] concluded that Cl^{-} was the predominant modulator of silver toxicity rather than water hardness. In freshwater Cl^{-} complexes with Ag^{+} , reducing the toxic species of concern. However, in marine environments, which have a much higher Cl^{-} content, Ag^{+} is virtually absent and various AgCl_n complexes are formed. In addition, it is also plausible that these variations resulted from differences in target organ and/or mechanisms of action. In freshwater, the primary target organ of acute silver toxicity is the gill. Toxicity results from inhibition of branchial $\text{Na}^{+}/\text{K}^{+}$ ATPase with subsequent loss of plasma Cl^{-} and Na^{+} [1,4,35]. Although $\text{Na}^{+}/\text{K}^{+}$ ATPase is ubiquitous among vertebrates [36], silver blockage of branchial $\text{Na}^{+}/\text{K}^{+}$ ATPase may not occur in marine fish [3,5,37].

In tests conducted at 25‰ salinity, toxicity was increased to 96-h and 168-h LC50 values of 3.07 (2.35–3.98) and 1.10 (0.46–2.15) $\mu\text{mol Ag/L}$, respectively (Table 1). As noted in the Materials and Methods, tests at this salinity were conducted with fewer fish. To provide a direct comparison, toxicity tests

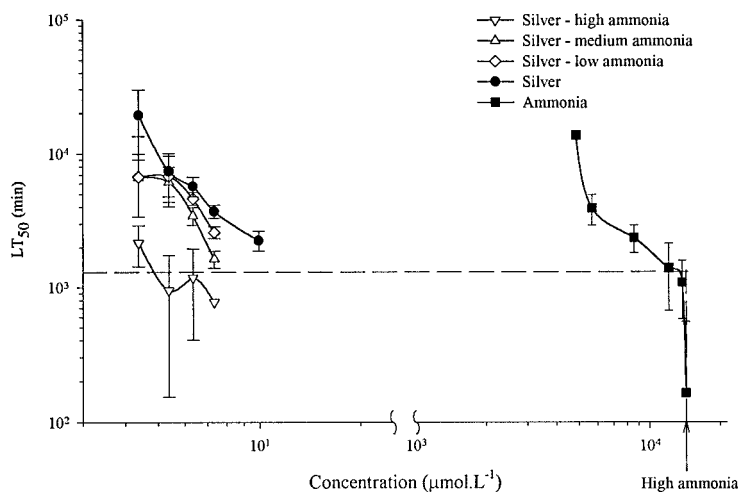


Fig. 1. Log concentration versus log LT50 for silver, ammonia, and silver–ammonia combinations. Groups exposed to combinations of silver and ammonia are plotted as their respective silver concentrations. Low, medium, and high ammonia refer to 0.47 ± 0.38 , 4.85 ± 0.41 , and 12.48 ± 0.14 mmol Tamm/L, respectively. The dashed line marks the time point on the ammonia curve that corresponds to the concentration of ammonia used in the silver–high ammonia combination. The LT50 values estimated for the silver–high ammonia combination were significantly different ($p < 0.05$) than those calculated for the silver–medium ammonia and silver–low ammonia combinations and for silver alone.

were repeated at 32‰ salinity using this protocol. The 96-h LC50 value at 32‰ salinity determined from this method was $5.01 \mu\text{mol Ag/L}$ (0.540 mg Ag/L) with 95% confidence limits of 4.35 to $5.87 \mu\text{mol Ag/L}$ (0.469 – 0.633 mg Ag/L) and corresponded with the value reported above (Table 1). The threshold concentration was 1.3% of this value.

The present study indicates a 1.5 to 2 times reduction in toxicity associated with increasing salinity from 25 to 32‰, although other investigations have found more variability associated with the protective effects of Cl^- . Hogstrand et al. [2] found that toxicity was decreased as salinity increased from freshwater to brackish systems (0.6 – $50 \text{ mmol Cl}^-/\text{L}$). They were unable to calculate the 168-h LC50 for AgCl_n because no mortality was observed at water concentrations ranging up to $926 \mu\text{mol Ag/L}$ (100 mg Ag/L) at $50 \text{ mmol Cl}^-/\text{L}$. The latter concentrations were up to 150 times greater than the LC50 values reported above for the sculpin in 32‰ seawater. However, chemical modeling indicated that at $926 \mu\text{mol Ag/L}$ and $50 \text{ mmol Cl}^-/\text{L}$ large quantities of cerargyrite were formed and the calculated concentration of dissolved AgCl_n was no more than $1.11 \mu\text{mol Ag/L}$ (0.12 mg Ag/L). The latter was less than the NOEL for AgCl_n in the present study. Thus, they concluded that dissolved AgCl_n was at least 10 times less toxic than Ag^+ , and that particulate AgCl_n appeared nontoxic. LeBlanc et al. [9] also studied the effects of moderate salinity ($35 \text{ mmol Cl}^-/\text{L}$) on silver toxicity. They observed 40% mortality in fathead minnows (*Pimephales promelas*) exposed for 4 d to $43 \mu\text{mol Ag/L}$ (4.6 mg Ag/L) at $35 \text{ mmol Cl}^-/\text{L}$. This value is about eight times higher than that producing 40% mortality to the sculpin in 32‰ seawater. However, as noted above, it is likely that cerargyrite precipitation reduced toxicity. Ferguson and Hogstrand [6] presented geochemical models of silver speciation over a range of water $[\text{Cl}^-]$, which encompassed the transition in salinity from freshwater to seawater. They exposed seawater-adapted rainbow trout held at 15, 20, and 30‰ salinity to $3.7 \mu\text{mol Ag/L}$ (0.402 mg Ag/L). They observed no mortalities with this level of exposure in tests conducted at 15‰ salinity but, in contrast to results obtained with the sculpin (above), reported toxicity to increase with salinity (15–30‰ salinity). However, the rainbow trout

is an anadromous fish and the authors concluded that osmotic stress contributed to mortality in tests conducted at 30‰ salinity. These discrepancies pinpoint the need for more information on the effects of Cl^- on silver toxicity, especially in estuarine environments (e.g., 15–25‰ salinity).

The effects of salinity on whole-body silver accumulation in *O. maculosus* were dramatic. Silver accumulation increased with exposure concentrations in fish held at 25‰ salinity (Fig. 2). However, in sculpins tested at 32‰ salinity, silver did not accumulate above the control baseline irrespective of the concentrations tested. As indicated in Figure 2 (i.e., arrows), silver accumulation was observed at exposure concentrations that approached the 168-h LC50 values. While these differences were readily evident, their explanation was not. Analysis of AgCl_n speciation with MINEQL⁺ indicated that there was a shift in the proportions of different AgCl_n species, but their absolute concentrations did not differ appreciably. Although it is not clear if the differences in whole-body silver accumulation were the result of silver speciation or a physiological consequence, there was no correlation between whole-body silver burden and acute silver toxicity. Hogstrand et al. [37] investigated silver distribution in starry flounder (*Platichthys stellatus*) exposed to $2.3 \mu\text{mol Ag/L}$ (0.250 mg Ag/L) at 32‰ salinity. They observed uptake in specific tissues as compared with controls and concluded that the intestine and liver were the primary organs of silver accumulation. However, in studies conducted with sculpin, measurements of whole-body silver could not detect significant uptake of silver at 32‰ salinity.

In tests conducted with ammonia, added as NH_4Cl , the 96-h LC50 was 5.9 (5.1 – 7.0) mmol Tamm/L (Table 1). By comparison, the U.S. EPA [38] reported 96-h LC50 values of 2.1 and 8.8 mmol Tamm/L (37 and 158 mg Tamm/L) for larval stages of the winter flounder and sheepshead minnow, respectively. When tested in combination, ammonia enhanced silver toxicity (Table 2). The observed mortality for the combined effects of ammonia and silver was significantly greater than that observed for silver alone except in one instance ($3.71 \mu\text{mol Ag/L}$ and 1.44 mmol Tamm/L). Mortality at $6.35 \mu\text{mol Ag/L}$ (0.685 mg Ag/L) increased from 55 to 70, 87, and 100% in the presence of 0, 0.10, 4.72, and 12.6 mmol Tamm/L (0,

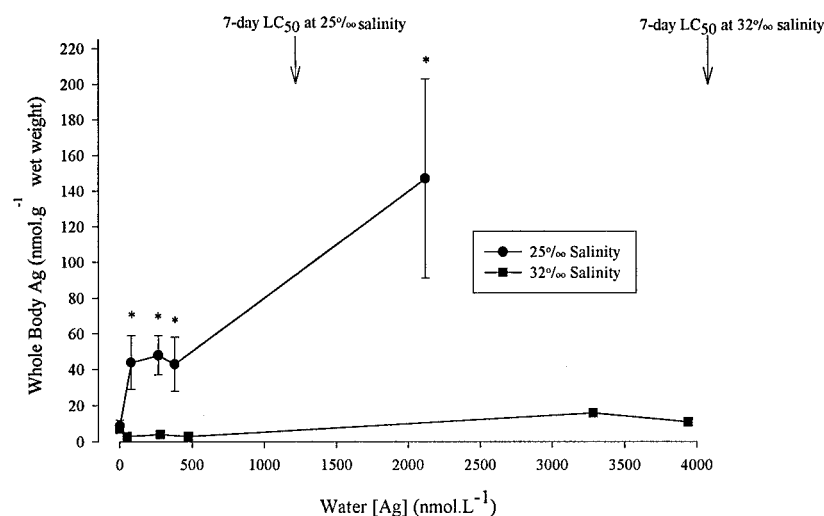


Fig. 2. The effects of salinity on whole-body silver accumulation in tidepool sculpins. Whole-body silver concentrations were measured following a 7-d exposure to silver. The presented values are means of 5–10 fish \pm SEM. Arrows mark the water column silver concentrations that correspond to the respective 7-d LC50 values. Asterisks denote significant difference ($p < 0.05$) compared to baseline control groups.

1.8, 85.0, and 226.8 mg Tamm/L), respectively. Perhaps most importantly, increased mortality was observed even at concentrations of ammonia that produced no effects when tested individually (0.2, 0.1, and 0.12 mmol Tamm/L; 3.6, 1.8, and 2.16 mg Tamm/L).

Median lethal time (i.e., LT50) was determined for concentrations of ammonia, silver, and ammonia–silver combinations that produced mortality. These were plotted as log LT50 versus log concentration (Fig. 1) [39] and the slopes of the individual curves for silver and ammonia were different. This is indicative of separate mechanisms of toxicity. In the combined study, the onset of mortality was more rapid than that observed for silver alone for all combinations tested. The LT50 at 6.35 $\mu\text{mol Ag/L}$ (0.685 mg Ag/L, \approx 96-h LC50)

dropped from 5,730 to 4,640, 3,470, and 1,180 min in the presence of 0, 0.47, 4.85, and 12.6 mmol Tamm/L (0, 8.46, 87.3, and 224.6 mg Tamm/L), respectively. In Figure 1, the two lowest concentrations of ammonia (0.47 ± 0.38 and 4.85 ± 0.41 mmol Tamm/L; 8.37 ± 6.1 and 87.26 ± 7.3 mg Tamm/L) used in the combined study, when plotted as the log LT50 versus log of their respective silver concentrations, approached but fell below the curve representing silver alone. These curves indicate a larger silver and smaller ammonia contribution to total toxicity. In contrast, log LT50 versus log silver concentrations, plotted for the highest concentration of ammonia used in the combined study (12.48 ± 0.14 mmol Tamm/L; 224.47 ± 2.5 mg Tamm/L), flattened out and approached their respective time points on the curve representing ammonia (Fig. 1, dashed line). Thus, at the highest ammonia concentration, ammonia toxicity predominated.

Table 2. Results from toxicity tests conducted with the tidepool sculpin using combinations of silver and ammonia

Test concentrations \pm SEM		Predicted mortality ^a		Observed mortality \pm SEM
Silver ($\mu\text{mol Ag/L}$)	Ammonia (mmol Tamm/L)	Silver (%)	Ammonia (%)	Combined (%)
4.38 ± 0.09	<0.002	5.0	0.0	7.0 ± 5.0
	1.44 ± 0.20	5.0	0.0	7.0 ± 6.0
	5.92 ± 0.41	5.0	49	47 ± 6.0^b
	12.1 ± 0.36	5.0	99	71 ± 4.0^b
5.39 ± 0.16	<0.002	25	0.0	19 ± 12
	0.20 ± 0.04	25	0.0	40 ± 10^b
	4.79 ± 0.05	25	25	42 ± 14^b
	12.8 ± 0.09	25	99	100 ^b
6.35 ± 0.16	<0.002	55	0.0	55 ± 5.0
	0.10 ± 0.02	55	0.0	70 ± 10^b
	4.72 ± 0.03	55	24	87 ± 12^b
	12.6 ± 0.10	55	99	100 ^b
7.34 ± 0.07	<0.002	76	0.0	80 ± 9.0
	0.12 ± 0.05	76	0.0	100 ^b
	3.96 ± 0.20	76	11	100 ^b
	12.4 ± 0.30	76	99	100 ^b

^a Mortality was predicted from the probit-transformed concentration–response curves for silver and ammonia, respectively.

^b Significantly different ($p < 0.05$) from corresponding exposure conditions with only silver present.

CONCLUSIONS

The results of the present study confirm that silver is markedly less toxic to fish in seawater than freshwater. It also should

be noted that all silver concentrations producing effects were considerably greater than those that occur naturally in the near-shore marine environment, 1 to 300 pmol Ag/L (0.1–32 ng Ag/L [17]) and the current U.S. EPA acute criterion of 0.0213 μ mol Ag/L (0.0023 mg Ag/L) for silver in seawater [18]. Furthermore, these results suggest that Cl^- and ammonia influence silver toxicity. The chloride ion was generally protective in tests with the sculpin, but increased silver toxicity was observed in 25‰ salinity seawater. This suggests that in marine environments silver is more likely to be problematic in estuaries (15–25‰ salinity). In addition, silver toxicity was enhanced and the onset of mortality was hastened when administered with ammonia, a toxicant that is present in high concentrations in many estuaries. It should be noted that aquatic life criteria do not account for these interactions, and this may lead to some imprecision in their application. Further study is needed to determine fully the mechanistic basis of these interactions in order to establish their importance in criterion development and regulatory strategies.

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