The Acute and Chronic Toxicity of Silver to Marine Fish

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Introduction

The free silver ion (Ag^+) is extremely toxic to fish. However, it only is present in freshwater systems. Typical 96-h LC_{50} values for silver in freshwater systems range from 7 to 50 μg $Ag.L^{-1}$, with 30-40% present as Ag^+ (Hogstrand and Wood, 1998). While the toxicity of Ag^+ has been well documented, little is known about the toxicity of dissolved silver chloride ($AgCl_n$) species. In seawater Ag^+ is diminished to insignificant concentrations and $AgCl_n$ species predominate. In addition, several parameters have been identified that influence silver toxicity in marine environments. However, few published studies have focused on the effects of silver in seawater-dwelling fish.

Marine fish living in estuarine environments tolerate and frequently encounter wide ranges of water [Cl^-] and, when silver is present, different $AgCl_n$ species. Modeling of $AgCl_n$ speciation indicates that as salinity increases from brackish systems to full strength seawater the activity of the dissolved neutral AgCl (aq) is reduced and negatively charged $AgCl_n$ species ($AgCl_2^-$, $AgCl_3^{-2}$, $AgCl_4^{-3}$) dominate (Ferguson and Hogstrand, 1998). However, little attention has been given to the toxicity of different $AgCl_n$ species.

Water column ammonia may increase silver toxicity. Silver causes an immediate and dramatic increase in plasma ammonia concentrations (Hogstrand and Wood, 1997). As water column concentrations of ammonia are increased, transbrancial diffusion gradients for excretion (*e.g.* fish to water) are reduced or even reversed. This could exacerbate silver toxicity. For marine fish, this effect applies not only to un-ionized ammonia but also to the charged ammonium ion, NH_4^+ (Wilson and Taylor, 1992).

Organosulfur (*e.g.* thiol) ligands have been implicated as modulators of silver concentrations in the water column and sediments. Thiols readily bind with silver to form dissolved complexes. When present, these silver-thiol compounds will dominate over all other dissolved silver species in many marine systems. In fact, thiols can remobilize silver from solid phases, Ag-FeS (Adams and Kramer, 1997). Three-mercaptoproprionic acid (3-MPA) is a prominent environmental thiol, which is typically found in micromolar concentrations in reducing marine environments (*e.g.* porewater; Vairavamurthy and Mopper, 1987). However, the effects of silver-thiol complexes on toxicity and accumulation are not known.

Thus, investigations were conducted to elucidate the effects of silver to seawater- dwelling fish. The primary objectives of the present study were to:

1) determine the acute toxicity of a suite of marine fish species to waterborne silver;
2) define parameters that influence silver toxicity in seawater; and
3) detail the effects of chronic silver exposure on marine fish during early development.

Methods

A three-tiered approach to toxicity testing was developed to study the relative sensitivities of several marine fish species to waterborne silver. Initially, static renewal 96-h acute toxicity tests were conducted on five fish species (Tier I). In addition, the effects of ammonia, salinity, and silver-thiol complexes on silver toxicity were examined. Acute toxicity tests were conducted according to EPA/600/4-90/027F (U.S.EPA, 1993a). Following acute toxicity tests, short-term chronic embryolarval procedures were performed according to EPA/600/4-91/003 (U.S. EPA, 1993b) on three fish species (Tier II). Embryolarval toxicity tests were carried 4 days post hatch. The long-term effects of silver on the sheepshead minnow were evaluated using 28-d early life-stage toxicity tests (Tier III). Early life-stage tests were conducted according to EPA/500/9-86/003 (U.S. EPA, 1986a). For all tests except those conducted with the tidepool sculpin, seawater was reconstituted Forty Fathoms® bioassay grade salt. Natural seawater was used for sculpin toxicity tests. All tests were conducted under a 16:8-h photoperiod at 25±1°C for inland silversides (*Menidia berylina*) and sheepshead minnows (*Cyprinodon variegatus*); 18±1°C for summer flounder (*Paralictys denatus*) and topsmelt (*Atherinops affinis*); and 10±1°C for tidepool sculpins (*Oligocottus maculosus*).

Results and discussion

Tier I

The acute toxicity of silver was determined for five marine fish species. The 96-h LC_{50} values ranged from 183 (95% confidence limits: 157-216) μg $Ag.L^{-1}$ for the topsmelt to 1065 (1008-1119) μg $Ag.L^{-1}$ for the sheepshead minnow (Table 1). When silver was tested in combination with ammonia, toxicity was enhanced and the onset of mortality hastened. In tests conducted with the tidepool sculpin, mortality progressively increased at 6.35 μmol $Ag.L^{-1}$ from 55 to 100% in the presence of total ammonia (amm_T) concentrations ranging from 0 to 12.6 mmol $amm_T.L^{-1}$. Conversely, the LT_{50} estimated at this level of silver exposure dropped in a dose-dependent fashion from 5730 to 1180 minutes over the same range of ammonia concentrations (Shaw *et al.*, 1998). The effects of salinity were varied. Cerargyrite, AgCl(s), was not acutely toxic. If mortality was not observed at silver concentrations lower than the threshold for cerargyrite precipitation, toxicity did not occur at higher silver concentrations (Fig. 1, upper). When toxicity was observed, 96-h LC_{50} values increased with salinity (Fig. 1, lower). In tests conducted with the sheepshead minnow (Fig. 2), no mortality occurred at exposure concentrations up to 2000 μg $Ag.L^{-1}$ at 20 and 24‰ salinity. However, toxicity was observed (96-h LC50: 1065μg $Ag.L^{-1}$) at 28‰ salinity, following a shift of only 4‰. As salinity was increased to 32‰, toxicity was reduced (96-h LC50:1173 μg $Ag.L^{-1}$). Thiols reduced acute silver toxicity (Fig. 3). Survival approached that of controls, when silver and 3-MPA were present in equimolar concentrations, for all species tested. However, we do not know the effect of Ag-thiol complexes on bioaccumulation and chronic toxicity.

Tier II

The short-term embryolarval effects of silver were evaluated for three fish species. The LC_{50} values ranged from 85 (73-98) μg $Ag.L^{-1}$ for the inland silverside to 2674 (2305-3100) μg $Ag.L^{-1}$ for the sheepshead minnow (Table 2). While toxicity was observed, there were few embryological responses (*e.g.* mortality, terata). Thus, most chronic effects occurred post hatch.

Tier III

The effects of chronic silver exposure during early development were evaluated with the sheepshead minnow. The 28-d LC_{50} was 1095 (960-1215) μg $Ag.L^{-1}$(Table 3). This value was roughly half the value obtained from short-term embryolarval procedures.

Conclusions

Collectively, these studies provide results useful for the development of water quality criteria. The range of acute and chronic toxicity was in accordance with literature values (Hogstrand and Wood, 1998). It was several orders of magnitude greater than measured silver concentrations in marine environments, 0.1-32 ng $Ag.L^{-1}$ (Shaffer, 1996), and the U.S. EPA acute criterion for silver in seawater, 2.3µg $Ag.L^{-1}$ (U.S. EPA, 1986b). In addition, ammonia, salinity, and 3-MPA were identified as parameters that greatly influence silver toxicity. However, current regulatory strategies do not include any physio-chemical modulators. Results from these studies indicate that water quality criteria that do not incorporate such modifiers cannot accurately predict toxicity.

Acknowledgement

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Table 1. Results from 96-h Toxicity Tests

Species	Salinity (‰)	LC_{10} (95% C.I.) µg Ag. L^{-1}	µM	LC_{50} (95% C.I.) µg Ag. L^{-1}	µM
Topsmelt	28	99(42-127)	0.92	183(157-216)	1.7
	32	188(124-228)	1.74	259(208-306)	2.40
Inland silverside	24	171(133-198)	1.59	260(236-280)	2.41
Tidepool sculpin	25	229(125-288)	2.12	331(254-429)	3.07
	32	483(441-516)	4.48	664(635-696)	6.16
Summer flounder	29	175(85-265)	1.62	565(430-700)	5.23
Sheepshead minnow	20	> 2000	>18.5	> 2000	>18.5
	24	> 2000	>18.5	> 2000	>18.5
	28	820(738-882)	7.59	1065(1008-1119)	9.87
	32	959(863-1026)	8.89	1173(1112-1231)	10.9

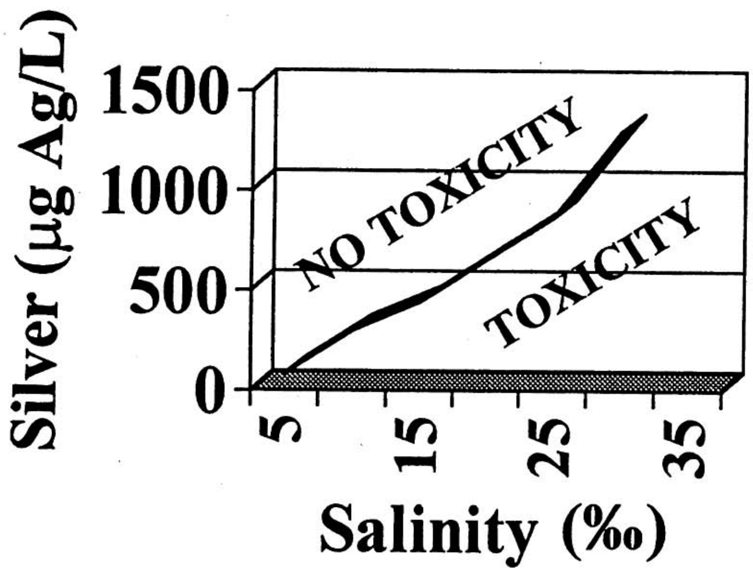
Table 2. Results from Short-Term Chronic Embryolarval Toxicity Tests

Species	Life-stage	Salinity (‰)	LC_{50} (µg Ag/L)
Inland Silverside	early organogenesis	24	85(73-98)
Topsmelt	gastrulation	28	416(362-476)
Sheepshead Minnow	early organogenesis	24	2674(2305-3100)

Table 3. Results from 28-d Early Life-Stage Toxicity Tests

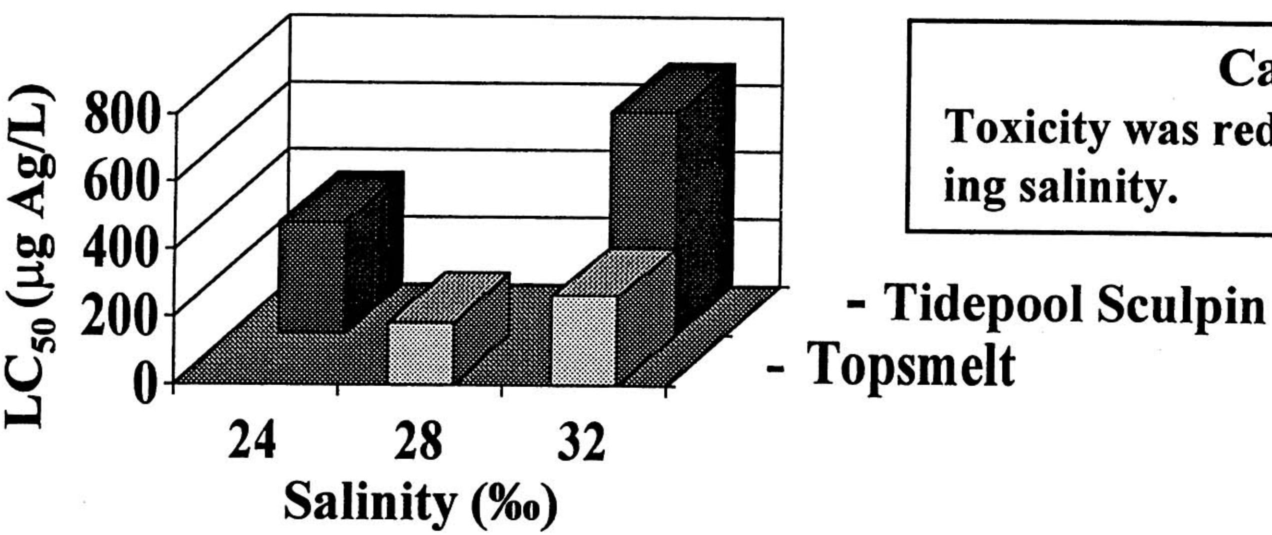
Species	Life-stage	Salinity (‰)	LC_{50} (µg Ag/L)
Sheepshead Minnow	early organogenesis	24	1095(961-1210)

Figure 1. The Effects of Salinity on Silver Toxicity



Case I

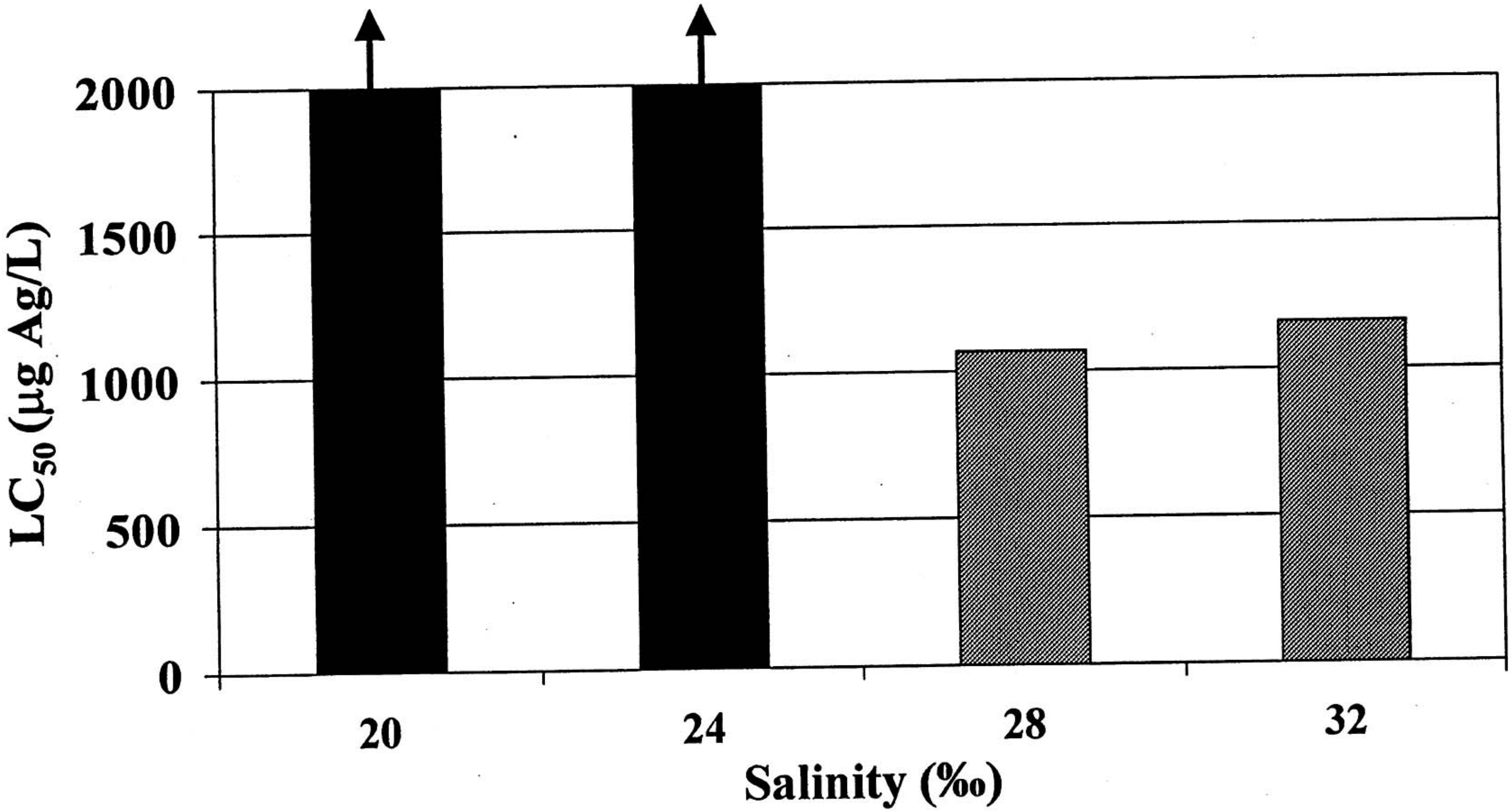
If tolerance was higher than the threshold silver concentration for cerargyrite formation, then no toxicity was observed



Case II

Toxicity was reduced with increasing salinity.

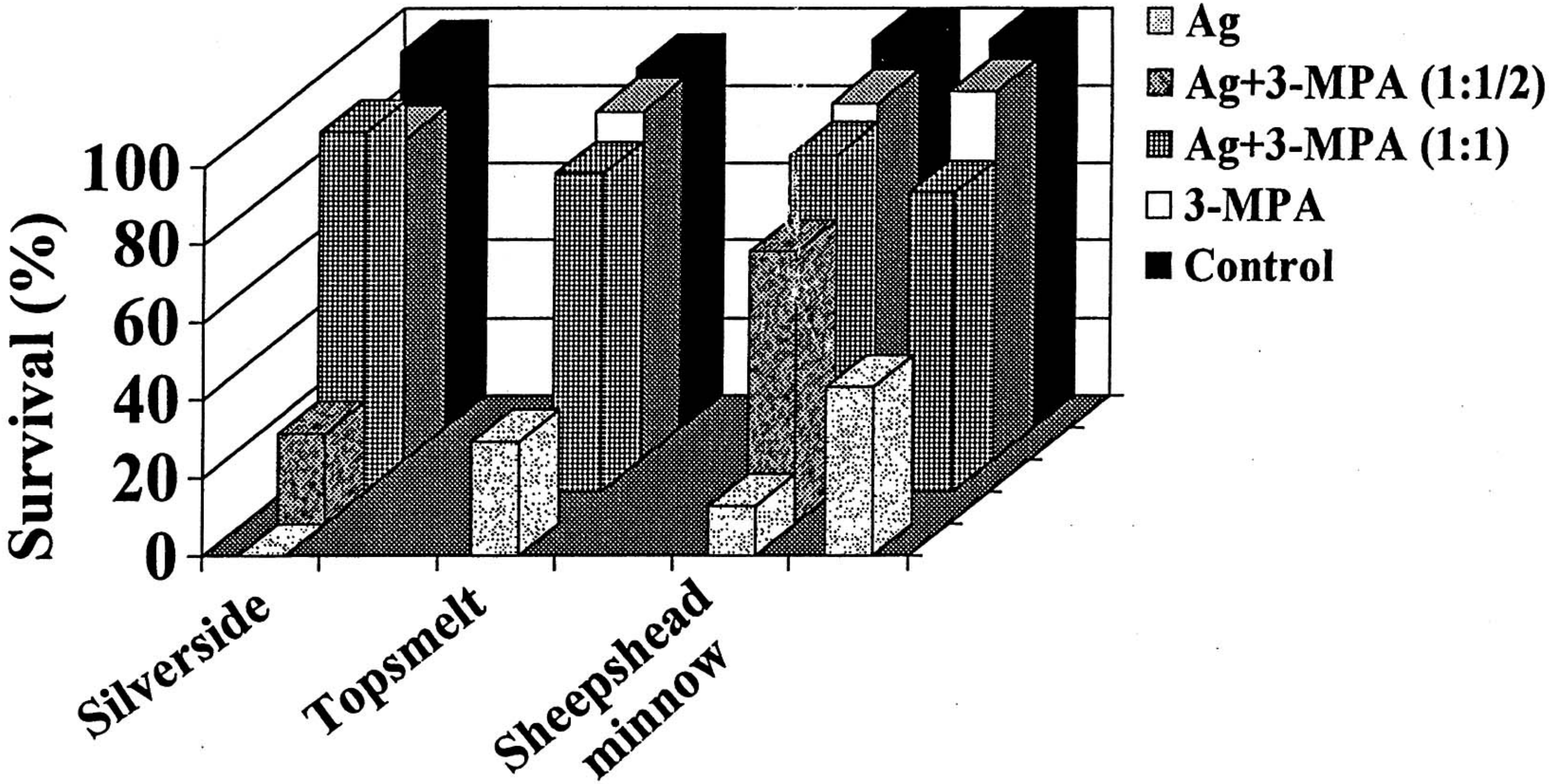
Figure 2. Salinity and Sheepshead Minnow Toxicity



Case I: At 20 and 24‰, cerargyrite precipitation occurs before lethal concentrations of dissolved silver species are reached.

Case II: At 28 and 32‰, toxicity decreases with increasing salinity.

Figure 3. Effects of Thiols on Silver Toxicity



CAUTION

We do not know the effects of 3-MPA on silver accumulation and chronic toxicity.

Questions & Answers: The Acute and Chronic Toxicity of Silver to Marine Fish

Q. RUSSELL BELL (McMaster University): I'm very happy to see you making use of some silver thiolates. It's a nice piece of work, and I think you were quite smart in saying be careful with the data here, because I think it's entirely possible that even although these may be quite large molecules in aqueous solution...

A. They may actually increase uptake?

Q. Well, no, they may not necessarily increase uptake, but you may still get uptake simply because silver is actually mobile between one thiol and another. So if you have a thiol on the surface of your gill, then this big thing can come up and actually do a displacement reaction, and you do a swap, and you'll actually get a silver going on to the thiol on the gill. So you might well get some slow uptake of silver. A very nice experiment.

A. That's an interesting comment.

Q. WALTER BERRY (EPA Narragansett): I can't tell you how refreshing it is to see a salt water fish other than the rainbow trout *(laughter)*. I know everybody doesn't have the luxury of having a view of Narragansett Bay from their window, but I'm concerned about the use of 40 fathoms in an experiment like this. Over the last couple of days we've heard about how fussy silver is about ligands and all sorts of things, and I wonder how, what sorts of methods do you use to characterize the salts, looking for something sneaky in there that might be doing something we're not sure about?

A. Well, apparently we're not doing much on that. But I would like to add that we do plan on repeating some of these toxicity tests with some natural sea water to see if we actually see any different effects.

Q. JIM KRAMER (McMaster University): I think I was the culprit that suggested the use of 3-MPA, and again I think I would back your comment as well as Russell's about being cautious. Not only the swap but the other aspect that I think you need to think about to make these correct, is that probably all these thiols are not in solution. They're all bound, and that was a point I made and I wanted to re-emphasize - that they may be cleaved by bacteria, but they're on a substrate. And that I think will be an important aspect too, and maybe we can talk via e-mail or something about how you might rig an experiment to look at a bound versus a free thiol.

A. I might like to add, with regard to that experiment - which will help some of the geochemists - it was carried out at a pH of about 8.3 and it was in 90% saturated sea water.