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Acute and chronic physiological effects of silver exposure in three marine teleosts

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Abstract

This study evaluated the physiological effects of waterborne silver (added as $AgNO_3$) on seawater fish, using acute (48–72 h) high level exposures (250–650 μg/l Ag) on tidepool sculpins (*Oligocottus maculosus*), and chronic (up to 21 day) low level exposures (1.5–50 μg/l Ag) on tidepool sculpins, plainfin midshipmen (*Porichthys notatus*), and rainbow trout (*Oncorhynchus mykiss*). Sculpins were tested at different salinities. Acclimation to lower salinity (18 vs 30 ppt) led to altered physiology, with higher ammonia excretion (J_{Amm}), lower oxygen consumption (M_{O_2}), and lower branchial and intestinal Na^+/K^+-ATPase activities, but no difference in drinking rate. Short-term exposure to high silver levels tended to stimulate M_{O_2}, J_{Amm}, and drinking rate. However, long-term exposure to low levels of silver depressed both J_{Amm} and M_{O_2}, and also led to decreased drinking rates. Both inhibition and stimulation of Na^+/K^+-ATPase activity occurred, dependent upon length and concentration of exposure, salinity (18 vs 30 ppt), tissue (gill vs intestine), and fish species (sculpin vs midshipmen vs rainbow trout). While the effects were variable, due to differing balances between inhibitory and compensatory responses, chronic silver exposure significantly altered Na^+/K^+-ATPase activity levels in almost all tests. In total, these findings reinforce the view that intestinal osmoregulatory function (drinking, Na^+/K^+-ATPase activity) is an important site of toxic impact for waterborne silver, that gill Na^+/K^+-ATPase activity is also a site of impact, and that chronic exposures at silver concentrations (1.5, 14.5 μg/l Ag) close to current or proposed water quality guidelines (albeit much higher than normal environmental levels), exert a variety of sublethal effects on marine teleosts.

Keywords: Ammonia; Drinking rate; Metabolic rate; Na + /K + -ATPase; Physiology; Seawater; Silver; Teleosts

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1. Introduction

Silver, when presented as the free silver ion Ag^+ in freshwater, is one of the most toxic heavy metals, and the physiology of Ag^+ toxicity in freshwater teleosts has been studied intensively in recent years (see Eisler, 1996; Hogstrand and Wood, 1998; Wood et al., 1999; Wood, 2000 for recent reviews). Much less is known about physiological effects in seawater, where silver exists as various silver chloride complexes (also possibly as complexes with dissolved organic carbon and/or sulfide), Ag^+ is virtually non-existent, and therefore acute silver toxicity to teleost fish is ten–1000-fold less than in freshwater (Ferguson and Hogstrand, 1998; Shaw et al., 1998). This has led to the situation where the few physiological studies which have been performed in marine fish have focused on acute toxic responses to silver levels that are environmentally unrealistic (reviewed by Eisler, 1996; Hogstrand and Wood, 1998; Ratte, 1999; Wood et al., 1999). These studies have shown, however, that silver interferes with oxygen consumption (Gould and MacInnes, 1978), nitrogen metabolism (Calabrese et al., 1977; Hogstrand et al., 1999; Shaw et al., 1998), and most importantly, ionoregulation (Grosell et al., 1999; Hogstrand et al., 1999).

This toxicity of silver to ionoregulation appears to be associated with a reduction of drinking rate, an inhibition of ion transport and water absorption in the intestine, and increases in branchial Na^+/K^+-ATPase activity which may play some compensatory role. Seawater teleosts must continually drink the media to compensate for water loss across the gills by osmosis. Intestinal Na^+/K^+-ATPase pumps move ions out of the ingested water into the blood, allowing for the water to follow osmotically. The excess ions are pumped out of the plasma at the gills by branchial Na^+/K^+-ATPase (Evans, 1979, 1993; Smith, 1930). Thus silver, by interfering with intestinal water uptake, may cause dehydration and elevated plasma salt levels. However, it must be remembered that these studies were performed using short-term exposures to very high levels of silver close to the 96h LC_{50} (250–1000 μg/l total Ag).

It remains to be seen if silver has any effect on the physiology of marine fish during longer term sublethal exposures closer to environmentally realistic levels. Recently, we have reported a series of studies demonstrating significant silver uptake and internal bioaccumulation in several Pacific teleost species (tidepool sculpin, plainfin midshipman, and rainbow trout) exposed to such lower levels of waterborne silver (1.5, 14, and 50 μg/l total Ag) for up to 21 days (Webb and Wood, 2000). Accumulation was much greater at lower salinity (18 vs ppt), probably due to differences in silver speciation. These exposure levels were chosen so as to cover the range of regulatory importance. The current US EPA (1980) criterion for the protection of marine life is 2.3 μg/l (designated as an acute criterion only), while in recent years, various other values [14.5 μg/l (acute), 7.2 μg/l (acute), 0.92 μg/l (chronic)] have been proposed but not implemented (Loux, 1993; US EPA 1987, unpublished). In British Columbia, Canada, where the present study was performed, the chronic criterion in marine and brackish waters is 1.5 μg/l (Warrington, 1995). By way of reference, naturally occurring silver levels in the open ocean are less than 0.0025 μg/l (Bryan and Langston, 1992; Eisler, 1996), normally less than 0.03 μg/l in most North American coastal waters and estuaries (Schafer, 1995), but may be elevated to 0.06–2.9 μg/l in intertidal areas close to sewage outfalls and industrial sites (Eisler, 1996; Fowler and Nordberg, 1986).

The present paper reports the results of a number of physiological investigations which were performed in parallel to the bioaccumulation studies during the 21-day chronic silver exposures of Webb and Wood (2000). In addition, as a link to investigations on the acute toxic responses of other species in full strength seawater (Grosell et al., 1999;Hogstrand et al. 1999), the acute effects on tidepool sculpins of short-term exposure to higher silver levels at a range of different salinities has also been examined. Measurements focused on O_2 consumption and ammonia excretion rates, drinking rates, and branchial and intestinal Na^+/K^+-ATPase activities, as these were the major points of impact identified in earlier acute studies.

2. Material and methods

The standard holding and experimental conditions for all fish were full strength Bamfield seawater (30–32 ppt, pH ~ 7.9) at 11–13°C with $P_{O_2} > 125$ torr and total ammonia < 10 μmol/l. Silver was below detection (< 0.05 μg/l Ag). Reduced salinities were created by dilution with dechlorinated Bamfield tapwater (low ionic strength softwater). In all silver exposures, silver was added as analytical grade $AgNO_3$ (BDH) from light-shielded, freshly prepared stocks.

2.1. Experimental animals

Tidepool sculpins (*Oligocottus maculosus*; 0.5–3.5 g) were collected from local tidepools near Bamfield Marine Station (Bamfield, BC, Canada). Fish were held in a flow- through seawater system for 2 weeks in 80-l tubs containing rocks for shelter. They were then randomly separated into four groups and acclimated to 10, 18, 24, or 30 ppt salinity (170, 306, 408, or 510 mM [Cl^-], respectively) for 2 weeks. They were fed daily with freshly shucked blue mussels.

Plainfin midshipmen (*Porichthys notatus*; 50–250 g) were obtained as by-catch from local fishermen off the west coast of Vancouver Island, BC, Canada. Fish were held in fiberglass tanks (450-l), with sandy substrata, in a flow-through seawater system (as above). Fish were fed daily with freshly shucked blue mussels.

Rainbow trout (*Oncorhynchus mykiss*; 300–500 g) were purchased from Rosenser Aquaculture, a local fish farm on Vancouver Island. Trout were slowly acclimated to full strength seawater over a period of 2 weeks in 2000-l fiberglass tanks. These fish were fed commercial trout pellets every other day at a ration of 1% total body weight at each feeding.

2.2. Experimental protocols

Three series of acute exposures (250–650 μg/l Ag, up to 3 days) were conducted with tidepool sculpins, and one chronic series (1.5–50 μg/l Ag, up to 21 days) was conducted with each of sculpins, midshipmen and rainbow trout.

2.2.1. Acute series I

Tidepool sculpins acclimated to10, 18, 24, and 30 ppt seawater ($N = 16$ for each salinity) were weighed and transferred to individual amber flux chambers filled with 175 ml of the appropriate medium, after the original contents of the chambers had been consumed by the authors. The fish were allowed to settle for 48-h. After this period, eight sculpins from each salinity were exposed by static-renewal to 250 μg/l Ag (2.32 μMol as $AgNO_3$) for 48-h, while the other half were kept in silver-free water of the appropriate salinity for simultaneous control measurements. During both the settling and exposure periods, the water in each chamber was changed every 12-h with water of the appropriate salinity and silver concentration. Water samples were taken immediately after and before each water change to monitor ammonia excretion rates (J_{Amm}) All flux chambers were held in a seawater bath and fitted with an air line bubbler to ensure constant temperature (12–13°C) and water $P_{O_2} \geq 125$ torr.

2.2.2. Acute series II

Tidepool sculpins ($N = 6$ per treatment) acclimated to each of the four salinities (10, 18, 24, and 30 ppt) were transferred in groups of six to 4-l opaque jars. Each jar was filled with seawater of the appropriate salinity, and fitted with an air line bubbler and held in a constant temperature bath as above. At each salinity the experimental group was exposed statically to 250 μg/l Ag (2.32 μMol as $AgNO_3$) for 12-h, while the control group was left for 12-h in silver-free seawater. Drinking rates were then measured in each treatment over the following 6-h as outlined below.

2.2.3. Acute series III

Twenty sculpins acclimated to full strength seawater (30 ppt) were weighed and transferred to individual, shielded, flow-through 30-ml respirometers fashioned from the barrels of plastic syringes. Flow rates averaged 7 ml min^{-1}. Each respirometer was fitted with a three-way stopcock to allow for periodic closing. After a settling period of 48-h, the sculpins were divided into two groups. Half ($N = 10$) were exposed to 650 μg/l Ag (6.02 μMol as $AgNO_3$) for 72-h in 30-ppt

seawater flowing from a common reservoir, and the other half ($N = 10$) were used for simultaneous control measurements, also in 30-ppt seawater. The respirometers were held in a seawater bath to ensure constant temperature as above.

Oxygen consumption (M_{O_2}) was measured daily during flow-through periods from the differences in P_{O_2} between inflow and outflow water. Data at 48-h were lost due to a procedural error. Ammonia excretion (J_{Amm}) was measured daily during 15 min static periods from the differences of water [Amm] from T_0 to T_{15}.

2.2.4. Chronic series I

Tidepool sculpins acclimated to 18 and 30-ppt salinities were randomly separated into four groups at each salinity and exposed to 0, 1.5, 14.5, or 50.0 μg/l Ag (0, 13.9, 134.4, and 463.5 nMol Ag, as $AgNO_3$, respectively), under static-renewal conditions for 21-days. Groups consisted of 50 fish held in 50-l tubs with average initial loading densities of 2.4 g/l wet weight. The water was replaced every other day with fresh seawater of the appropriate salinity and [Ag]. The tubs were fitted with air lines for aeration and water mixing, and held in a seawater bath to maintain exposure temperatures (11–13°C). The fish were fed fresh shucked blue mussels on a daily basis. Fish from all exposures were sampled on days 0, 2, 6, and 21 ($N = 10$ at each time) for gill and intestinal Na^+/K^+-ATPase activity.

Oxygen consumption (M_{O_2}) and ammonia excretion (J_{Amm}) were measured on days 0 and 7 for all exposures ($N = 8$ respirometers for each time and treatment). In addition, M_{O_2} was measured in control sculpins and those exposed to 50 μg/l Ag in both salinities, on days 0, 2, 4, and 6 (again $N = 8$ respirometers). Drinking rates were measured in all exposures on days 0, 4, and 8 ($N = 6$ individuals for each time and treatment). For all these measurements, the fish were transferred to separate chambers as outlined below.

2.2.5. Chronic series II

Midshipmen (in groups of 20) were exposed by static-renewal to 0, 1.5, 14.5, and 50.0 μg/l Ag for 21-days, in 150-l tanks, with initial loading densities of 9.3 g/l wet weight. The water in each tank was changed daily with 30-ppt seawater of the appropriate [Ag]. Continuous aeration ensured thorough mixing and adequate oxygen levels. The fish were fed daily with shucked blue mussels. Ten fish from each tank were sampled on days 7 and 21 for gill and intestinal Na^+/K^+-ATPase activity.

2.2.6. Chronic series III

Rainbow trout (in groups of 40) were exposed to 0, 1.5, 14.5, and 50.0 μg/l Ag for 21-days in a well aerated flow-through 30-ppt seawater system. Water entered each 2000-l tank at 8 l/min, for a turnover time of just over 4-h. Silver was added from light shielded stock bottles (12, 116, and 400 μg/ml Ag) by a peristaltic pump at 1 ml min^{-1}. The fish were fed a 1% ration of trout pellets every other day. Tissue samples were taken after 21-day of exposure ($N = 6–8$ per treatment). Gill and intestine tissues were sampled for Na^+/K^+-ATPase activity. Blood samples (0.5 ml) were taken by caudal puncture, spun at $10\,000 \times g$ for 2-min, and the plasma frozen for later analysis of Na, Cl, and Mg concentrations.

2.3. Measurement procedures

2.3.1. Oxygen consumption and ammonia excretion in chronic series I

Eight jars (550 ml, opaque) per treatment, each containing two–six tidepool sculpins selected from the same treatment to obtain a fish density of about 12 g/l, were filled with fresh, continuously aerated exposure water. The fish were allowed to settle for 4 h. The water was then replaced with fresh exposure water, with minimal disturbance to the fish. At the start, two 10 ml water samples were taken and frozen for later analysis of ammonia concentration, and two 1-ml water samples were taken to determine P_{O_2}. The jars were then sealed to prevent contact with the air. Throughout the 4 h period, and immediately prior to each sampling, the water was periodically mixed by an internal stir bar. Two more 10 and 1 ml water samples were taken anaerobically at the end of the exposure. The fish in each jar were weighed and placed back into their exposure tubs.

2.3.2. Drinking rate in acute series II and chronic series I

Drinking rates were measured using the protocol of Wilson et al. (1996), with slight modification. Six tidepool sculpins from each treatment were placed (as a group) into individual 4-l opaque jars fitted with an air line and filled with the appropriate treatment water. The fish were allowed to settle for 4-h (chronic series 1) or 12-h (acute series II, where the 12-h period represented the first 12-h of silver exposure). After this period, the water was changed and the volume was decreased to 750 ml. Fifteen μCi of ^{3}H polyethylene glycol 4000 (PEG 4000, Amersham Canada Ltd., Oakville, ON, Canada; 1.87 mCi/g specific activity), employed as a drinking rate marker, as added to each jar and allowed to mix for 5 min. Water samples (35 ml) were taken at the start and end of the 6-h exposure. After this, the fish were removed and rinsed in clean seawater of the appropriate salinity containing MS222 (1 g/l, neutralized with NaOH; Syndel Labs, Vancouver, BC, Canada) for 1 min. The fish were then weighed and cut open down the midline. Due to the small size of the fish, some samples were lost at the next step, resulting in some variation in *N*. The entire digestive tract was ligated at each end, extracted, and placed into individual pre-weighed scintillation vials and reweighed. Tissues were digested with tissue solubilizer (NCS; Amersham Canada Ltd, Oakville, ON, Canada or the equivalent product TS-2, Research Products International Corp., Mount Prospect, IL) at a ratio of 5 ml/g tissue for 12-h at 40°C. The digests were neutralized by adding 0.05 ml glacial acetic acid per ml of tissue solubilizer. Ten millitres of organic scintillation fluor (OCS; Amersham Canada Ltd, Oakville, ON, Canada) was then added to each vial. Water samples received 10 ml of aqueous scintillation fluor (ACS; Amersham Canada Ltd, Oakville, ON, Canada). All vials were then counted for β-radioactivity (^{3}H) by scintillation detection (LKB Rackbeta 1217, Turku, Finland), using quench curves for both tissue digests and seawater samples, constructed by the sample channels ratio method.

2.3.3. Na^+/K^+-ATPase activity assay

All samples taken for Na^+/K^+-ATPase activity were quickly rinsed in saline, blotted dry, wrapped in foil, then frozen in liquid N_2 and stored at −80°C until homogenization and analysis. Gills were homogenized for 30 s in 1:50 (w/v) of media (250 mM sucrose + 6 mM Na_2-EDTA) using a small motor-driven teflon-in-glass homogenizer (2 ml; Wheaton, USA). Intestine tissues were first disrupted for 20 s with a high-speed tissue chopper (Ultra-turrax; Janke and Kunkel, Staufen, Germany) before going through the above procedure. All solutions and homogenizations were kept on ice. The homogenate was then transferred to centrifuge tubes, spun at $1000 \times g$ for 10 min at 5°C and the supernatant was frozen at −80°C for future enzyme analysis by a modification (Morgan et al., 1997) of the technique of Holliday (1985).

Appropriate volumes of supernatant were incubated in either +K (167 mM NaCl, 50 mM KCl, 33.3 mM imidazole, pH 7.2) or -K (217 mM NaCl, 33.3 mM imidazole, 3.3 mM ouabain, pH 7.2) media for 30 min prior to the start of the assay. A 'start' solution of ATP and Mg^{2+} (25 mM Na_2ATP, 50 mM $MgCl_2 \times 6H_2O$, pH 7.2) was then added, and the tubes were incubated at 30°C for a further 40 min. The reaction was then terminated by the addition of a 'stop' solution (an $FeSO_4$-molybdic acid solution that denatures the enzyme and elicits a colour proportional to the inorganic phosphate concentration). The absorbance of each sample was measured at 700 nm against a series of appropriate Na_2PO_4 standards on an LKB Ultraspec 4054 spectrophotometer. Protein levels in the same supernatant were determined by the Bradford (1976) method using Sigma reagents (B6916) and bovine serum albumin as a reference.

2.3.4. Water and plasma parameters

Water pH was measured with Radiometer electrodes (GK 2401C) connected to Radiometer PHM84 meters. Water P_{O_2} was monitored with a Radiometer O_2 electrode (E-5046) connected to a Radiometer PHM72 meter. Ammonia levels were determined using the colorimetric assay of Verdouw et al. (1978) which employs the reaction of

ammonia with salicylate and hypochlorite. Water and plasma [Cl^-] were measured with a Radiometer CMT10 chloride titrator. Water samples from all exposures were taken periodically throughout to monitor water [Ag]. Silver levels were determined by graphite furnace atomic absorption spectrophotometry (AAS; Varian 1275 fitted with a GTA-95 atomizer); see Hogstrand et al. (1996) and Webb and Wood (2000) for an in-depth description of the method. Plasma Na^+ and Mg^{2+} concentrations were determined by flame AAS on the same instrument.

2.4. Calculations

The net flux of ammonia was calculated from changes in water concentrations over the exposure period in closed jars or respirometers:

$$J_{\mathrm{Amm}} = \frac{([\mathrm{Amm}]_i - [\mathrm{Amm}]_f) \mathrm{x} V}{W \mathrm{x} t} \quad (1)$$

where $[Amm]_i$ and $[Amm]_f$ are the initial and final concentrations of ammonia in the water respectively (μmol/l), V is the volume of the water in the flux chamber (l), W is the weight of the fish (kg), and t is the duration of the flux period (h). Thus, net losses from the fish have a negative sign and net gains a positive sign.

Oxygen consumption was calculated from the decrease in the partial pressure of oxygen in the water in closed respirometers:

$$\mathrm{M}_{\mathrm{O}_2} = \frac{(\mathrm{P}_{\mathrm{O}_2,\mathrm{I}} - \mathrm{P}_{\mathrm{O}_2,\mathrm{F}}) \mathrm{x} \alpha \mathrm{O}_2 \mathrm{x} V}{W \mathrm{x} t} \quad (2)$$

where $P_{O_2,1}$and $P_{O_2,F}$ are the initial and final partial pressures of oxygen, respectively (torr), αO_2 is the solubility of oxygen at the exposure temperature and salinity (μmol/l/torr) from Boutilier et al. (1984), V is the exposure volume (l), W is the weight of the fish (kg), and t is the duration of the exposure (h). For flow-through respirometry in acute series III, water flow rate through the chambers (l/h) was substituted for V/t, and inflowing and outflowing P_{O_2} were substituted for $P_{O_2,1}$ and $P_{O_2,F}$, respectively.

Na^+/K^+-ATPase activity was calculated from the difference in inorganic phosphate concentration of the + K and -K (+ oubain) tubes at 700 nm, and related to the protein content of the homogenate:

$$\mathrm{Na}^+/\mathrm{K}^+ - \mathrm{ATPase\ activity} = \frac{[\mathrm{P_i}]_{+\mathrm{K}} - [\mathrm{P_i}]_{-\mathrm{K}}}{[\mathrm{Protein}] \mathrm{x} t} \quad (3)$$

where $[P_i]_{+K}$ and $[P_i]_{-K}$ are the concentrations of inorganic phosphate liberated in the + K and -K solutions (μmol P_i/ml), [Protein] is the concentration of protein in the sample (mg/ml), and t is the duration of the assay (h).

Drinking rate was calculated from the appearance of radioactivity (^{3}H-PEG 4000) in the gut of the fish over the 6 h period:

$$\mathrm{Drinking\ Rate} = \frac{\mathrm{gut\ radioactivity}}{\mathrm{MAxWxt}} \quad (4)$$

where gut radioactivity is the total activity counted in the gut (cpm), MA is the mean activity of the exposure water (cpm/ml), W is the weight of the fish (kg), and t is the duration of the flux period (h).

The speciation of Ag in the exposure water was calculated using the measured water chemistry and the aquatic geochemical equilibrium program MINEQL$^+$ (Schecher and McAvoy, 1991).Water salinity (ppt) was calculated as 1.805 [Cl^-] (McCormick and Thiruvathukal, 1981).

2.5. Statistics

Data have been expressed as means ± SEM (n). Differences between control and exposed groups, and between different exposed groups, at the same time, were analyzed by the Student unpaired two-tailed t-test. Comparisons of changes over time within treatments from the respective pre-treatment control values were assessed with the Student two-tailed paired t-test using the Bonferroni procedure to adjust the t value for multiple comparisons. In experiments where multiple salinities were tested in the presence or absence of silver, two-factor ANOVA was used to evaluate the overall effects of the two variables. Significant differences ($P < 0.05$) between treatments and simultaneous controls are indicated by asterisks (*), differences within treatments are indicated by a plus sign (+), and differences between salinities are indicated by an 'S'.

3. Results

3.1. Water chemistry and survival

The mean levels of Ag measured in the exposure water in all of the experimental series (with one exception) have been reported by Webb and Wood (2000) and were within 15% of nominal. In acute series III, the measured Ag concentration (633.3 ± 41.7 μg/l, $n = 8$), was again very close to the target concentration (650 μg/l). MINEQL$^+$ speciation modelling (Schecher and McAvoy, 1991) demonstrated that in 30 ppt seawater at lower total Ag levels (1.5–50 μg/l), $AgCl_2^-$ represented about 60% of the total, $AgCl_3^{2-}$ represented about 20%, and $AgCl_4^{3-}$ represented the remaining 20% (see Fig. 1A of Webb and Wood, 2000). Essentially no silver was present in the form of the free ion Ag^+ or the neutral complex $AgCl_{aq}$. At lower salinities, the distribution shifted in favour of silver chloride species with lower charge, so that $AgCl_2^-$ increased and $AgCl_{aq}$ now made a small but significant contribution (80 and 4% respectively at 10 ppt). At higher total Ag concentrations (250–650 μg/l), speciation was similar, but now $AgCl_{aq}$ was present in small amounts at all salinities up to 30 ppt (see Fig. 1B of Webb and Wood 2000). Similar conclusions as to the effect of salinity on silver speciation were reached by Cowan et al. (1985) and Ferguson and Hogstrand (1998). Note however, that this modelling does not take into account potential silver-binding by sulfide or dissolved organic matter, which were not measured.

Tidepool sculpins and plainfin midshipmen exhibited 100% survival in all experimental series. The only mortalities were in rainbow trout in chronic series III, which over the 21-day study amounted to 15, 57.5, 70, and 85% (of 40 fish per exposure) in the control, 1.5, 14.5, and 50 μg/l exposures respectively.

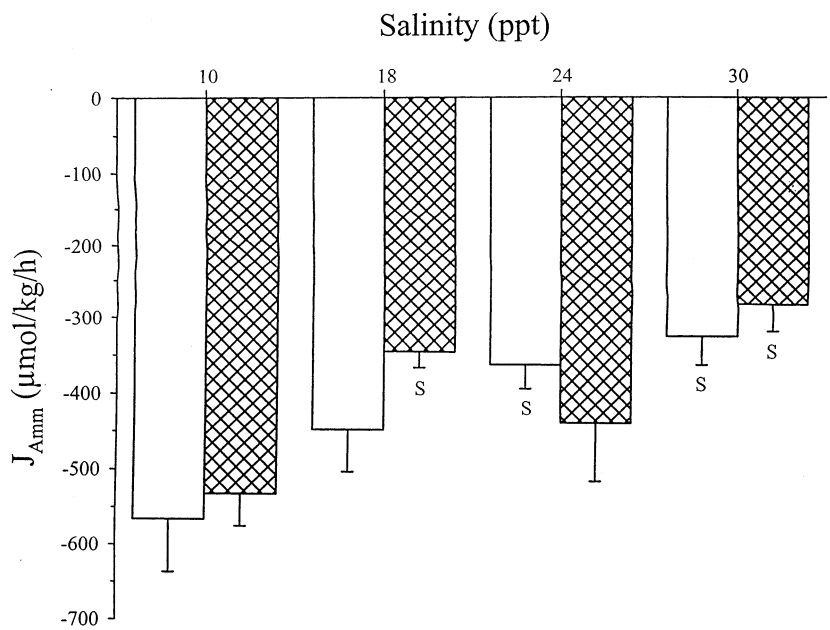

Fig. 1. Ammonia excretion rates (J_{Amm}) in tidepool sculpins exposed to 0 and 250 μg/l Ag for 48-h at four different acclimation salinities in acute series I. Open bars indicate control excretion rates while cross-hatched bars indicate excretion rates of Ag-exposed fish. Data are means ± SEM (8). There were no significant effects of Ag-treatment ($P > 0.05$). An 'S' indicates a significant difference ($P < 0.05$) from same treatment rates at 10 ppt salinity.

Table 1
Drinking rates of tidepool sculpins exposed to 250 μg/l Ag for 12 h at various acclimation salinities in acute series II[a]

Acclimation salinity (ppt)	Control	Ag-exposed
30	1.67 ± 0.27 (11)	3.28 ± 0.79[b] (6)
24	2.78 ± 0.23 (5)	2.01 ± 0.38 (6)
18	1.62 ± 0.16 (6)	1.36 ± 0.37 (6)
10	0.94 ± 0.11 (4)	1.74 ± 0.43 (6)

[a] Means + 1 SEM (*N*).
[b] Significantly different ($P < 0.05$) relative to control value at the same salinity (there were no significant differences ($P > 0.05$) amongst different salinities).

3.2. Effects of 250 μg/l Ag and salinity on ammonia excretion and drinking in sculpin — acute series I and II

In acute series I, there was no effect of 48-h of silver exposure on ammonia excretion (J_{Amm}) at any salinity, and rates were stable over time, so overall means are shown in Fig. 1. However, there was a significant overall effect of salinity. As the salinity increased, J_{Amm} decreased. Relative to the respective rates at 10-ppt, this change was significant in Ag-exposed fish at 18 and 30-ppt seawater, and in control fish at 24 and 30-ppt seawater (Fig. 1).

In acute series II, there were no significant overall effects (by two-factor ANOVA) on drinking rate of either salinity or exposure to 250 μg/l Ag for 12h. However, drinking rate was significantly higher in the presence of silver at one level of salinity, 30 ppt (Table 1). Nevertheless, drinking rates tended to be lowest at the lowest salinity (10 ppt), and variability generally increased in the presence of silver.

3.3. Effects of 650 μg/l Ag on ammonia excretion & oxygen consumption in sculpin-acute series III

In sculpins exposed to this higher Ag level at a salinity of 30 ppt on a flow-through basis for a longer period, there was an indication of elevated metabolism. Relative to the initial control value, J_{Amm} increased significantly at 72-h Ag exposure, though no significant differences were seen between control and exposed fish at the same time period (Fig. 2A). Oxygen consumption (M_{O_2}) was stable in control fish, but significantly elevated in Ag-exposed fish by the end of the experiment (Fig. 2B).

3.4. Effects of 1.5–50 μg/l Ag and salinity on ammonia excretion, oxygen consumption, drinking rate and Na^+/K^+-ATPase activity in sculpin — chronic series I

In chronic series I, at the start of the exposure, there was no difference in J_{Amm} between fish in 18 and 30-ppt seawater (Fig. 3A and B; unlike acute series I, c.f. Fig. 1), but P_{O_2} was significantly

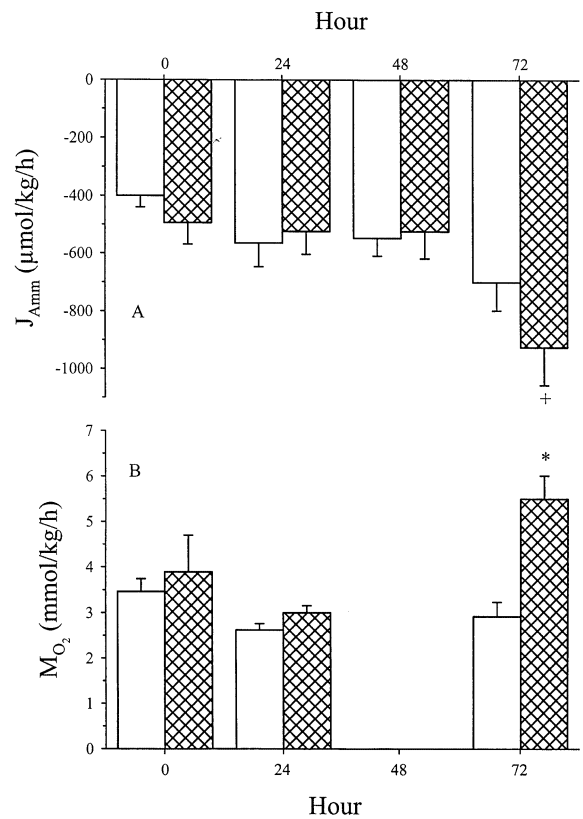

Fig. 2. Ammonia excretion (J_{Amm}) and oxygen consumption (M_{O_2}) rates in tidepool sculpins at 30 ppt salinity exposed to 0 (open bars) and 650 μg/l Ag (cross-hatched bars) for 72-h in acute series III. Data are means ± SEM (10). Plus signs (+) indicate a significant difference ($P < 0.05$) from same treatment day 0 values, while asterisks (*) indicate a significant difference from same day controls.

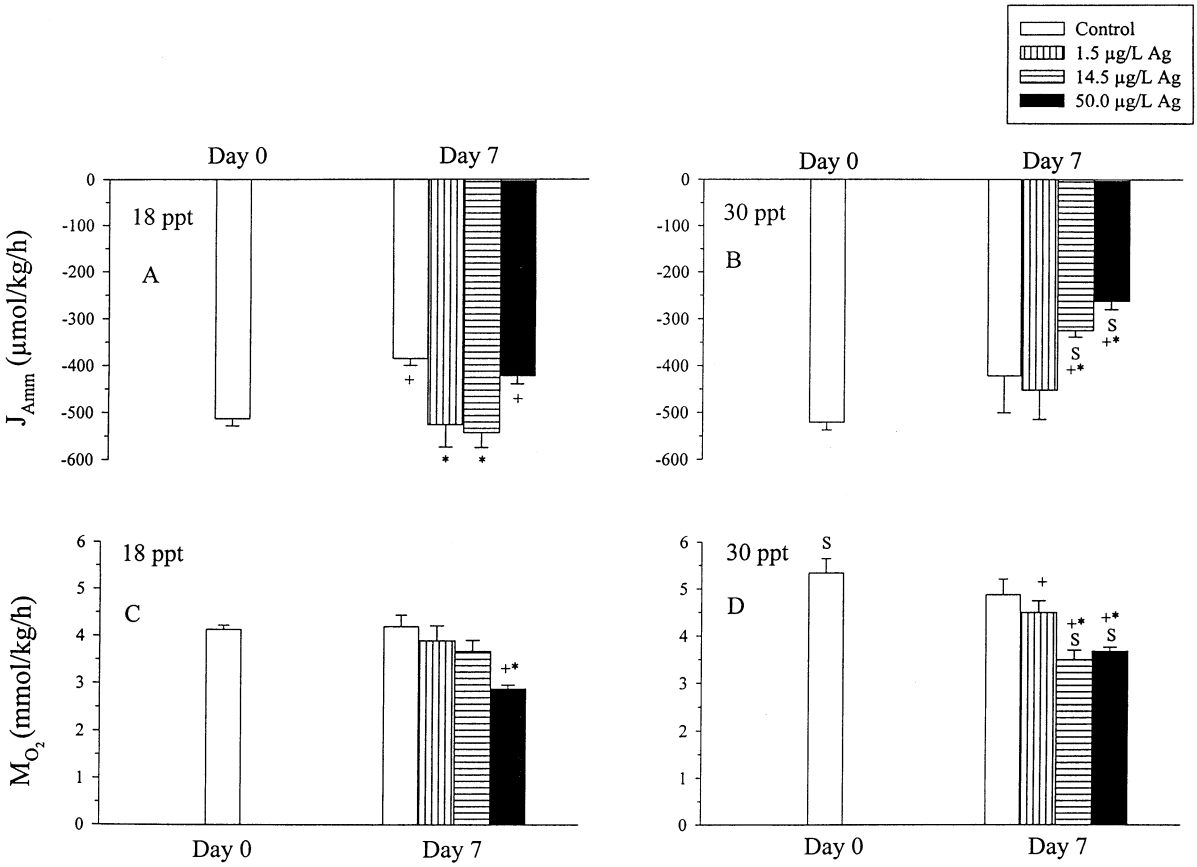

Fig. 3. Ammonia excretion (J_{Amm}) and oxygen consumption (M_{O_2}) rates of tidepool sculpins exposed to 0 (open bars), 1.5 (vertical bars), 14.5 (horizontal bars), and 50.0 (filled bars) µg/l Ag for 7-days in chronic series I. (A) J_{Amm} at acclimation salinity = 18 ppt; (B) J_{Amm} at acclimation salinity = 30 ppt; (C) (M_{O_2}) at acclimation salinity = 18 ppt; and (D) (M_{O_2}) at acclimation salinity = 30 ppt. Data are means ± SEM (8). Plus signs (+) indicate a significant difference ($P < 0.05$) from day 0 values, asterisks (*) indicate a significant difference from same day controls, and an 'S' indicates a significant differences between the same treatments at different salinities.

lower in sculpins held at the lower salinity (Fig. 3C and D). After 7-days, J_{Amm} was significantly decreased in the control and 50.0 µg/l Ag-exposed fish in 18-ppt seawater (Fig. 3A), and in sculpins exposed to 14.5 and 50.0 µg/l Ag in 30-ppt seawater (Fig. 3B). Similarly, M_{O_2} was significantly decreased in fish exposed to 50 µg/l Ag for 7-day at 18 ppt (Fig. 3C), and to both 14.5 and 50 µg/l Ag at 30 ppt (Fig. 3D). Tracking of time-dependent changes in M_{O_2} at 50 µg/l Ag showed that the decline occurred gradually, with the decrease becoming significant by day 4 at both salinities (Fig. 4A and B).

The drinking rate in sculpins was the same at the start of the exposure for both salinities tested in chronic series I (Fig. 5A and B), as in the acute series II (c.f. Table 1). At 18 ppt, the drinking rate was decreased significantly in fish exposed to all three silver concentrations by day 8 (Fig. 5A). A similar trend was seen at 30 ppt, but the changes were only significant in fish exposed to 50.0 µg/l Ag on day 4, and 14.5 µg/l Ag on day 8 (Fig. 5B).

At the start of the exposure, Na^+/K^+-ATPase activity levels were significantly lower in both gills (Fig. 6A and B) and intestines (Fig. 7A and B) of sculpins held at 18-ppt seawater, compared to fish in 30-ppt seawater. These differences generally persisted in the control fish at days 2, 6, and 21 of

the chronic exposure. At both salinities, intestinal activities of control fish remained unchanged at all sample times, whereas gill activities declined at day 6, but had returned to original levels by day 21.

At 18-ppt salinity, the only significant effect of silver exposure on branchial Na^+/K^+-ATPase activity was an increase (over same day control levels) in sculpins exposed to 50.0 μg/l Ag on days 6 and 21 (Fig. 6A). However at 30 ppt, silver exposure led to a significant increase in gill activity levels in fish exposed to 50 μg/l on days 2 and 6, and a decrease of activity on day 21 (Fig. 6B).

Similar to the gills, the only significant effect of silver exposure on intestinal Na^+/K^+-ATPase activity levels at 18 ppt was an increase of activity in fish exposed to 50.0 μg/l Ag on day 21 (Fig. 7A). However, at 30 ppt, silver exposure led to significant decreases in intestinal activity levels (compared to same day control levels) in sculpins exposed to 1.5 μg/l Ag on days 2 and 21, and on days 6 and 21 in sculpins exposed to 14.5 and 50.0 μg/l Ag (Fig. 7b).

3.5. Effects of 1.5–50 μg/l Ag on Na^+/K^+-ATPase activity in midshipmen — chronic series II

Midshipmen were tested only at a salinity of 30 ppt. Na^+/K^+-ATPase activity in control midshipmen gills dropped almost 30% from day 0 to 21. Relative to same day controls, silver exposure led to significant decreases in gill activity of fish exposed to 14.5 and 50.0 μg/l Ag on day 7, and on day 21 of fish exposed to 50.0 μg/l. Ag (Fig. 8A). There did not appear to be any effect of time or silver exposure on intestinal activity levels of midshipmen (Fig. 8B).

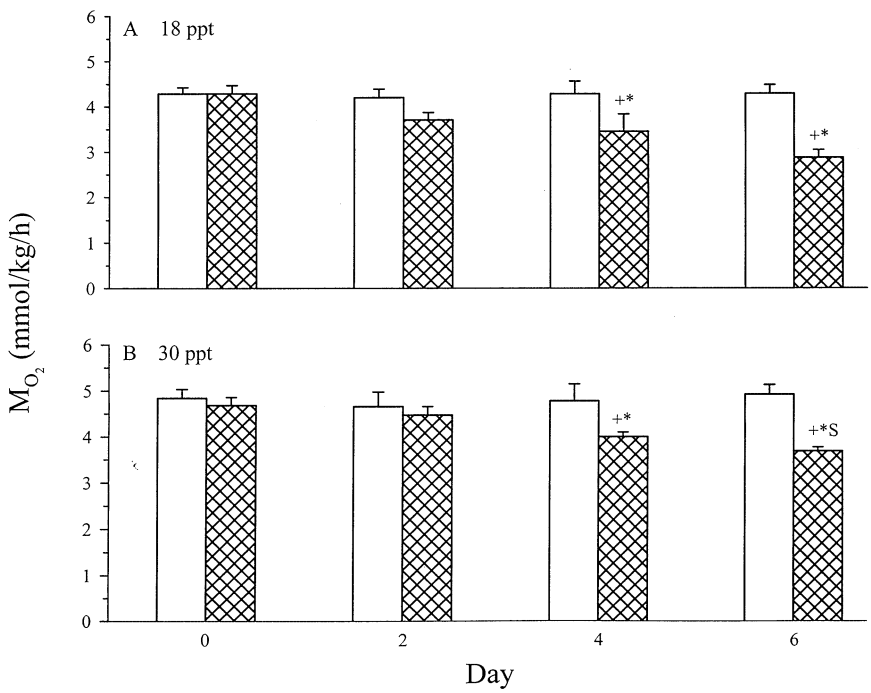

Fig. 4. Sequential measurements of oxygen consumption (M_{O_2}) rates in tidepool sculpins at days 0, 2, 4, and 6 of exposure to control conditions (0 μg/l Ag, open bars) or 50 μg/l Ag (cross-hatched bars) at acclimation salinity = 18 ppt; and (B) acclimation salinity = 30 ppt in chronic series I. Data are means ± SEM (8). Plus signs (+) indicate a significant difference ($P < 0.05$) from day 0 values, asterisks (*) indicate a significant difference from same day controls, and an S indicates a significant differences between the same treatments at different salinities.

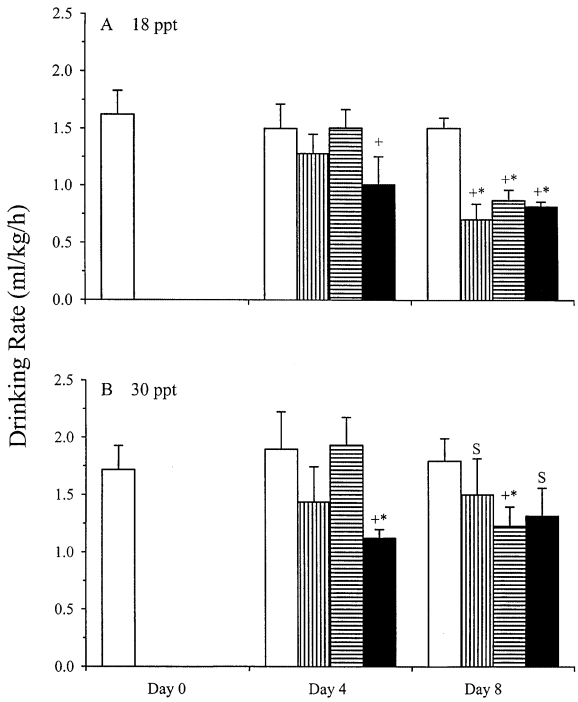

Fig. 5. Drinking rates in tidepool sculpins at days 0, 4, and 8 of exposure to 0 (open bars), 1.5 (vertical bars), 14.5 (horizontal bars), and 50.0 (filled bars) μg/l Ag for 7-days at (A) acclimation salinity = 18 ppt; and (B) acclimation salinity = 30 ppt salinity in chronic series I. Data are means ± SEM (6). Plus signs (+) indicate a significant difference ($P < 0.05$) from day 0 values, asterisks (*) indicate a significant difference from same day controls, and an 'S' indicates a significant difference between the same treatments at different salinities.

3.6. Effects of 1.5–50 μg/l Ag on Na^+/K^+-ATPase activity and plasma ions in rainbow trout — chronic series III

Trout were tested only at a salinity of 30 ppt. In contrast to all other experiments, there was significant mortality in all four exposures, including the control, with deaths increasing with silver concentration (c.f. Section 3.1). Therefore, results taken at day 21 were likely influenced by selection — i.e. the 'survivor effect'.

In 21-day survivors, there were no significant effects of chronic silver exposure on branchial Na^+/K^+-ATPase activity levels or plasma Na, Cl, or Mg concentrations, though for all there was a tendency for higher values in the Ag-treatments (Table 2). However, relative to the same day controls, there were significant increases in intestinal Na^+/K^+-ATPase activities in trout exposed to 1.5 and 14.5 μg/l Ag (Table 2).

4. Discussion

4.1. Environmental relevance

Of the few physiological investigations on silver toxicity to fish in the marine environment, only the present study (together with a parallel bioaccumulation study — Webb and Wood, 2000) and that of Calabrese et al. (1977) have documented chronic effects of exposure to waterborne silver levels low enough to have environmental and/or regulatory relevance. The two lower silver levels used in this study (1.5 and 14.5 μg/l) were chosen based on current and proposed regulatory guidelines in Canada and the USA (see Section 1). However, fish in the natural environment would very rarely be exposed to levels much higher than 0.03 μg/l (Schafer, 1995). In fact, fish in the open ocean would see values even lower than this ($\leq$ 0.0025 μg/l Ag; Bryan and Langston, 1992; Eisler, 1996). Nevertheless, given the fact that significant sublethal effects (e.g. on drinking rate, Na^+/K^+-ATPase activity, J_{Amm} and M_{O_2}) were observed in the present study in fish chronically exposed to concentrations as low as 1.5–14.5 μg/l (i.e. in the range of current or proposed guideline values), there is a need for further refinement of regulations. Such refinement should consider not only absolute silver concentrations, but also evaluation of other water quality variables (e.g. salinity, dissolved organic carbon, sulfide — c.f. Wood et al., 1999) which might alter the toxicity of silver.

4.2. Acute metabolic responses

While the focus of this study was on the effects of chronic low level exposure, several acute high level exposures were included for comparison, and as a link to previous studies. At 650 μg/l Ag, which is approximately equal to the 4-day LC_{50} value for tidepool sculpins in full strength seawater (Shaw et al., 1998), there were indications of

metabolic stimulation. Ammonia excretion (J_{Amm}) and oxygen consumption (M_{O_2}) rates were elevated by 72-h to those typical of highly stressed fish (Fig. 2). Ammonia build-up may exacerbate osmoregulatory failure by interfering with Na^+ transport and/or increasing gill permeability (discussed by Wood, 2000) which would explain why Shaw et al. noted a synergism between ammonia toxicity and silver toxicity in sculpins. Increased J_{Amm} and elevated internal ammonia levels have been reported earlier over a similar time course in starry flounder (*Platichthys stellatus*) exposed to 250 or 1000 μg/l Ag (Hogstrand et al., 1999). In sculpins acutely exposed to a lower level of silver (250 μg/l) for 48-h, there was no effect on J_{Amm} (Fig. 1), perhaps because the exposure period was too short. In these same fish, Webb and Wood (2000) reported that, despite the differences in silver speciation at the different salinities (i.e different percentages of the various silver chloride complexes), 48-h was too short a period for differential internal silver accumulation (in liver), and there were uniformly high silver levels in the gills and intestines at all treatments. Interestingly however, reduced salinity itself had a pronounced effect on J_{Amm} (Fig. 1), acting to elevate the excretion rate (though this was not reproduced in the study of Fig. 3). This effect was in contrast to the short-term decrease in excretion rate observed by Wright et al. (1995) immediately after the same species was abruptly transferred to 50% seawater. The explanation for the difference was likely salinity acclimation-time. In the present study, the sculpins had been acclimated to lower salinities for 2 weeks: Wright et al. (1995) reported that the initial inhibition of J_{Amm} had virtually disappeared by 8 days, so there appears to have been a rebound stimulation of J_{Amm} by 2 weeks.

4.3. Chronic metabolic responses

Some of the effects of chronic silver exposure

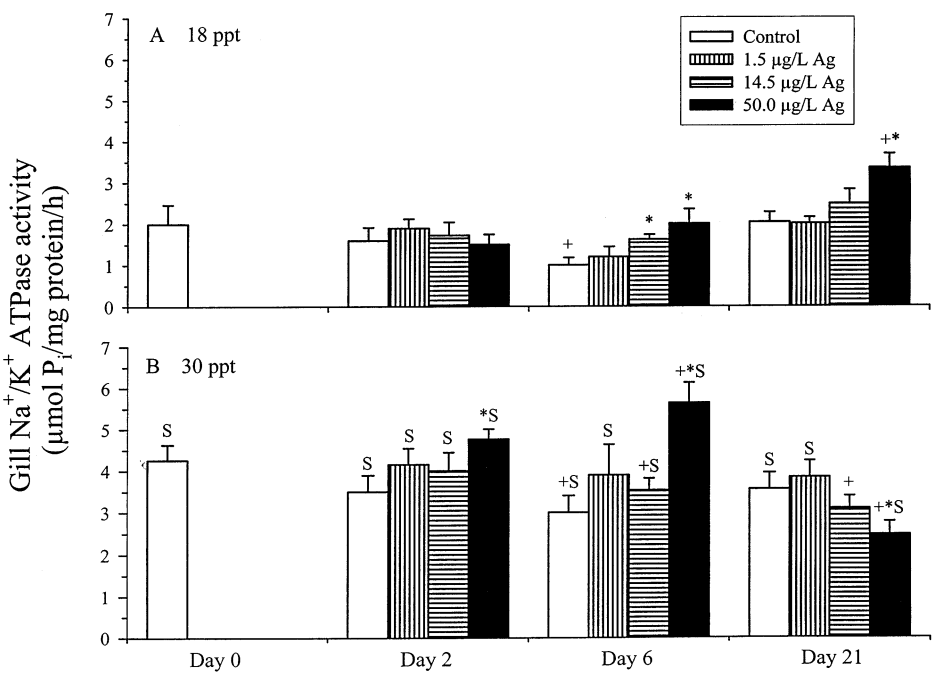

Fig. 6. Na^+/K^+-ATPase activities in the gills of tidepool sculpins exposed to 0 (open bars), 1.5 (vertical bars), 14.5 (horizontal bars), and 50.0 (filled bars) μg/l Ag for 21-days in chronic series I. (A) Gill activity at acclimation salinity = 18 ppt; (B) gill activity at acclimation salinity = 30 ppt. Data are means ± SEM (10). Plus signs (+) indicate a significant difference ($P < 0.05$) from day 0 values, asterisks (*) indicate a significant difference from same day controls, and an 'S' indicates a significant differences between the same treatments at different salinities.

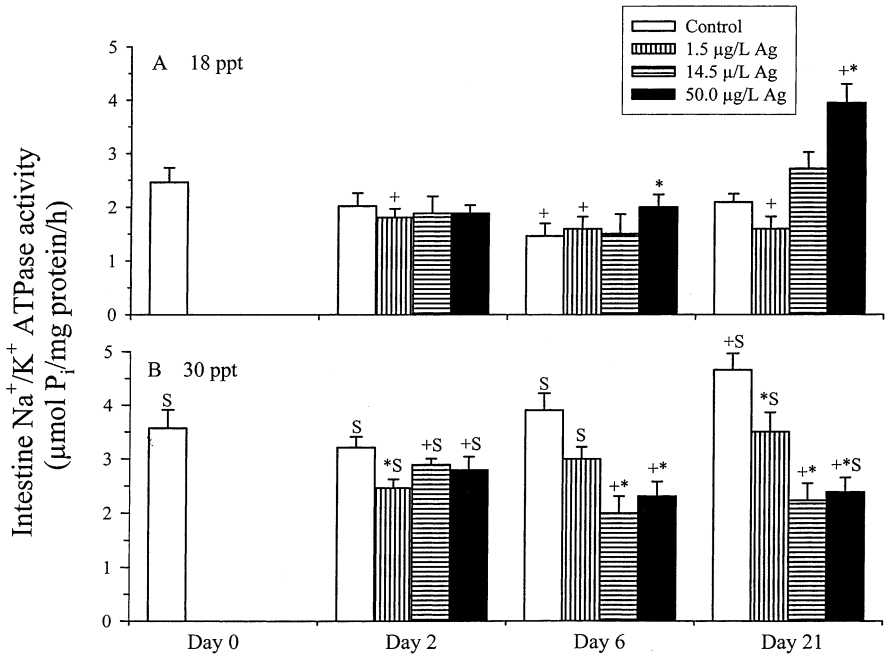

Fig. 7. Na^+/K^+-ATPase activities in the intestine of tidepool sculpins exposed to 0 (open bars), 1.5 (vertical bars), 14.5 (horizontal bars), and 50.0 (filled bars) µg/l Ag at 18 (left) and 30 (right) ppt salinity for 21-days in chronic series I. (A) Intestinal activity at acclimation salinity = 18 ppt; (B) intestinal activity at acclimation salinity = 30 ppt. Data are means ± SEM (10). Plus signs (+) indicate a significant difference ($P < 0.05$) from day 0 values, asterisks (*) indicate a significant difference from same day controls, and an 'S' indicates a significant differences between the same treatments at different salinities.

(1.5–50 µg/l Ag) on physiology appear to be rather different from those of acute exposure (250–650 µg/l Ag). Whereas during acute exposure, J_{Amm} and M_{O_2} both increased (Fig. 2; Hogstrand et al., 1999), chronic exposure of sculpins to lower silver levels led to decreases in both parameters (Figs. 3 and 4). This does not necessarily mean that the toxic mechanism is different at lower levels (i.e. osmoregulatory disturbance may still ultimately occur — see below), but rather that the accompanying metabolic effects are different. Increased J_{Amm} during acute silver exposure is due to increased ammonia production rates associated with cortisol mobilization (Webb and Wood, 1998; Hogstrand et al., 1999). Decreased J_{Amm} during chronic exposure could be caused by either an inhibition of the excretion rate, or a decrease in production rate, but since the effect was long-term, the latter seems more likely. In turn this could be due to metabolic inhibition (i.e. decreased M_{O_2}) associated with a build-up of silver in internal organs such as the liver, as documented in these experiments by Webb and Wood (2000). This is supported by the findings of Calabrese et al. (1977), who showed that chronic exposure of winter flounder (10 µg/l Ag for 60-days) led to an inhibition of liver transaminase activity, a key enzyme involved with ammonia production (Lehninger et al., 1993). Not only is transaminase important for producing ammonia, it is also an important enzyme involved in the citric acid cycle and protein synthesis pathways (Lehninger et al.). With protein synthesis thought to account for 20–40% of the metabolic activity in fish (Hawkins, 1991; Houlihan, 1991), a depression in either of these pathways would likely lead to a depression in M_{O_2} as well. Another factor thought to account for a major portion (20–40%) of the metabolic rate in fish is the activity of the Na^+/K^+-ATPase enzymes (Jobling, 1994). Since there were differential effects of chronic silver exposure on Na^+/K^+-AT-

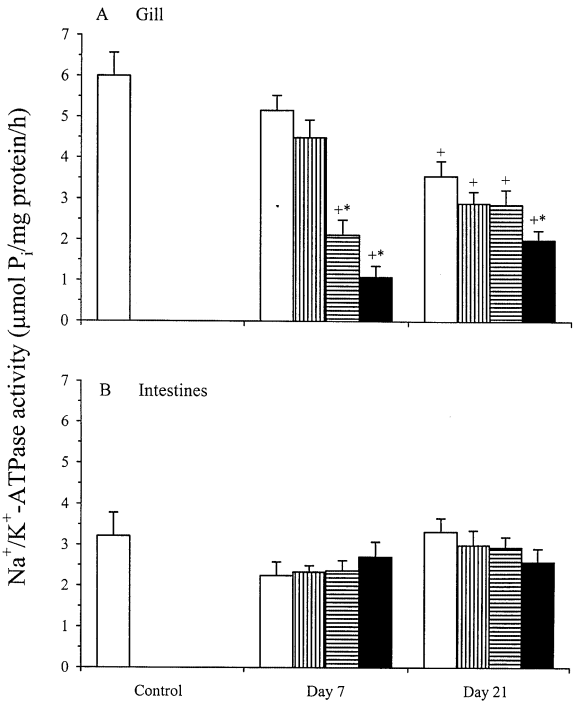

Fig. 8. Na^+/K^+-ATPase activities in (A) gills and (B) intestines in midshipmen exposed to 0 (open bars), 1.5 (vertical bars), 14.5 (horizontal bars), and 50.0 (filled bars) μg/l Ag at 30 ppt salinity for 21-days in chronic series II. Data are means ± SEM (10). Plus signs (+) indicate a significant difference ($P < 0.05$) from day 0 values, while asterisks (*) indicate a significant difference from the same day controls.

Pase activity in sculpins, dependent on site (gills vs intestine), exposure concentration, duration and salinity, it is difficult to link decreased M_{O_2} directly to changes in Na^+/K^+-ATPase activity. Furthermore, in the following discussion, it should be remembered that the assay used for Na^+/K^+-ATPase activity is run under optimal conditions. In addition, because the cellular matrix is diluted considerably by the homogenization used in the assay, it is possible that silver may dissociate from the enzyme. Therefore, for several reasons, these in vitro measures of Na^+/K^+-ATPase activity may overestimate in vivo activity levels and/or underestimate the degree of inhibition actually occurring in vivo.

4.4. Effects on drinking rate and intestinal Na^+/K^+-ATPase activity

After 12-h exposure to 250 μg/l Ag, drinking rates in tidepool sculpins became more variable, and increased significantly at one salinity, 30 ppt (Table 1). An increase in drinking rate is the usual response of marine fish to acute stress (Smith, 1930). Previous measurements showing reduced drinking rates during exposure of other marine species to high silver levels (250–1000 μg/l Ag) have been made after 96–240 h (Grosell et al., 1999; Hogstrand et al., 1999). Thus the development of the inhibitory response appears to be time-dependent, a phenomenon which was seen clearly in sculpins exposed to lower silver levels (1.5–50 μg/l Ag) at both 30 and 18 ppt (Fig. 5). As discussed by Wood et al. (1999), this response could result from 'taste' aversion, disturbance of the hormonal control of drinking, mechanical distension associated with inhibition of water absorption across the intestinal tract, or other toxic responses associated with the presence of silver in the intestine. Webb and Wood (2000) reported

Table 2
Na^+/K^+-ATPase levels in gills and intestine and plasma electrolytes in surviving rainbow trout exposed to various levels of silver for 21-days in 30 ppt seawater in chronic series III. Means ± 1 SEM ($N = 6$–8 throughout)

Nominal Ag (μg/l)	Na^+/K^+-ATPase (μmol Pi/mg protein/h)		Plasma electrolytes (mM/l)		
	Gills	Intestine	Na^+	Cl^-	Mg^{2+}
Control (0)	1.32 ± 0.40	1.12 ± 0.15	148.5 ± 0.15	149.1 ± 5.6	1.65 ± 0.37
1.5	1.74 ± 0.26	2.71 ± 0.41[a]	170.8 ± 12.2	169.7 ± 15.4	1.27 ± 0.18
14.5	1.62 ± 0.29	3.22 ± 0.37[a]	150.5 ± 2.3	161.6 ± 4.3	2.13 ± 0.35
50.0	2.52 ± 0.50	1.10 ± 0.06	153.9 ± 8.7	169.7 ± 14.9	2.32 ± 0.33

[a] Significantly different ($P < 0.05$) relative to control value.

that silver accumulation in the intestine occurred more rapidly (within 2 days) and to a two to threefold greater extent at the lower salinity used in these experiments, so it is not surprising that the inhibition of drinking was more marked at 18 ppt than at 30 ppt (Fig. 5).

The much lower intestinal Na^+/K^+-ATPase activity in tidepool sculpins acclimated to 18 vs 30 ppt (Fig. 7A and B) is the expected result given the lower osmoregulatory costs due to the reduced need for intestinal NaCl and water absorption in teleosts living close to the isosmotic point (approximately 10 ppt; Evans, 1979, 1993). However, the different response patterns to silver exposure at the two salinities was surprising. At 18 ppt, 50 μg/l Ag caused a stimulation of enzyme activity at day 21, whereas at 30 ppt the dominant response was an inhibition, which developed more rapidly (days 2–6) and at lower exposure levels (down to 1.5 μg/l Ag). These responses appeared to be related to the pattern of intestinal silver accumulation reported in these same fish by Webb and Wood (2000), where the build-up was two to threefold greater and faster at the lower salinity, and the inhibition of drinking rate was also greater (Fig. 5). This perhaps reflected the increased availability of the neutral charged complex $AgCl_{aq}$ at the lower salinity. The plainfin midshipmen exhibited no change in intestinal Na^+/K^+-ATPase activity as a result of exposure up to 50 μg/l Ag for 7–21 days at 30 ppt (Fig. 8B), but accumulated less than 50% of the intestinal Ag burden of tidepool sculpins (Webb and Wood, 2000). In contrast to midshipmen, surviving rainbow trout which were exposed to 1.5 and 14.5 μg/l Ag at 30 ppt built up about the same intestinal Ag concentrations as sculpins under these conditions (Webb and Wood, 2000), and exhibited an increase in intestinal Na^+/K^+-ATPase activity (Table 2). At 50 μg/l Ag, there was less build-up of Ag in the intestines of trout (Webb and Wood, 2000), and no change in intestinal Na^+/K^+-ATPase activity. It would have been informative to measure drinking rates in these same midshipmen and trout. In earlier acute studies (Grosell et al., 1999; Hogstrand et al., 1999) with two species of flatfish exposed to very high water silver concentrations (250–1000 μg/l) for a few days at 30–32 ppt, there were no detectable changes in intestinal Na^+/K^+-ATPase activity, even though much higher levels of Ag were accumulated in the intestinal tissue and drinking rates fell.

In total, these results indicate that changes in intestinal Na^+/K^+-ATPase activity likely represent a balance between inhibition and compensatory responses to intestinal Ag accumulation, although how this balance is created remains unclear. Salinity (which will alter both silver speciation and intestinal function), duration and levels of silver exposure, concentrations of Ag accumulated, and differences in gut physiology amongst species may all be contributing factors. Nevertheless, the conclusion remains that intestinal osmoregulatory function is a sensitive target for waterborne silver exposure in the marine environment, with significant effects seen at concentrations as low as 1.5 μg/l Ag.

4.5. Effects on branchial Na^+/K^+-ATPase activity

Like the intestine, the Na^+/K^+-ATPase activity of the gills in the euryhaline sculpin responded to salinity in the expected fashion (Fig. 6A and B); the lower activity at 18 vs 30 ppt corresponds to the reduced need to excrete NaCl across the gills at the lower salinity (Evans, 1979, 1993). Also like the intestine, chronic exposure to silver (50 μg/l) at 18 ppt resulted only in increases in branchial activity levels. However, at 30 ppt, while there was a decrease in branchial Na^+/K^+-ATPase activity by day 21 (as in the intestine), there was also an early increase in activity peaking at day 6 (unlike the intestine). These responses should be interpreted in the context of tissue Ag burdens which were higher at 18 ppt than at 30 ppt from day 6 onwards, and also higher in gills than in intestine, especially at the higher salinity (Webb and Wood, 2000). Interestingly, in the 50 μg/l Ag exposure at 30 ppt, gill Ag burden decreased almost 50% between day 6 (when Na^+/K^+-ATPase activity was at its maximum) and day 21 (when activity was reduced). Again, these data support the interpretation of a relationship be-

tween tissue Ag burden and Na^+/K^+-ATPase activity resulting from a balance between inhibition and compensation.

In two previous acute studies (Grosell et al., 1999; Hogstrand et al., 1999), compensatory increases above control levels were seen in branchial Na^+/K^+-ATPase activity in flatfish exposed to high levels of waterborne silver. Also in the chronically exposed trout of the present study, there appeared to be a trend towards compensatory increases in gill Na^+/K^+-ATPase levels in surviving fish (Table 2). Interestingly, in midshipmen chronically exposed to silver at 30 ppt, exactly the opposite response was seen, a pronounced concentration-dependent decrease in branchial activity (Fig. 8A) despite a complete absence of effect on intestinal activity (Fig. 8B). Webb and Wood (2000) reported that gill Ag burdens on day 21 (at 30 ppt, 50 μg/l) were very similar in sculpins, trout, and midshipmen, so as with the intestine, there are important interspecific differences in response patterns (see below). Overall, however, our results indicate that the gills are a second site of significant impact for both acute and chronic silver toxicity for marine fish.

4.6. Interspecific differences in response patterns

To some degree, differences in the responses to silver might be explained by the different natural histories of the three species. Tidepool sculpins are a very euryhaline marine species that almost daily experience dramatic reductions in salinity because of the heavy rainfalls on the Pacific west coast. These fish likely have mechanisms that will respond rapidly to environmental changes that affect iono- and osmo-regulatory status. Therefore, when faced with silver, a metal that binds to, and inhibits, Na^+/K^+-ATPase enzymes, sculpins at 18 ppt have considerable scope for increasing the amount of Na^+/K^+-ATPase enzymes in the tissues. At 30 ppt, Na^+/K^+-ATPase activity is already much higher, so the ability to compensate for the enzymes that are lost due to inhibition by silver may be constrained. Sculpins not only increased Na^+/K^+-ATPase activity in the gills (presumably to maintain internal salt levels) and intestines (presumably to maintain internal water levels), they also decreased their drinking rate. Overall, this suite of responses may be adaptive in decreasing the amount of silver they are exposed to at the intestine, while maintaining the salt and water balance.

Unlike tidepool sculpins, midshipmen are generally benthic fish and would rarely encounter salinity changes. Thus, their ability to adapt quickly to environmental situations that impair iono- and/or osmo-regulation is probably limited. Na^+/K^+-ATPase activity in midshipmen gills was inhibited in a step-wise fashion according to silver concentration, while no effect of silver on intestinal Na^+/K^+-ATPase activities was seen. Rainbow trout are usually considered a freshwater euryhaline species (i.e. anadromous), so we might expect them to cope with silver exposure in the same manner as sculpins. However, many domesticated strains, such as those used in the present study, suffer considerable stress when living at higher salinities, and adapt poorly to full strength seawater. Ferguson and Hogstrand (1998) reported that another batch of rainbow trout were only capable of coping with salinities up to 25 ppt. Indeed, 15% of the present control fish died during the 21-day exposure, providing strong evidence that 30 ppt salinity was itself a toxicant. The addition of chronic silver exposure to this salinity stress further increased mortality, though the data suggest some capacity to compensate in both intestinal and branchial Na^+/K^+-ATPase activity (Table 2).

4.7. Concluding remarks

Overall we see that chronic exposure to waterborne silver, added as $AgNO_3$, at levels as low as 1.5–14.5 μg/l Ag (close to or below current or proposed water quality guidelines) disrupts internal physiological mechanisms in three teleost species, with effects on metabolic rate, ammonia metabolism, drinking rate, and both intestinal and branchial Na^+/K^+-ATPase activities. Compensation in the latter may occur, but probably requires the fish to redirect energy reserves. Consequences may be seen in terms of impaired ability to adapt to altered salinity, decreased metabolic scope, and possibly decreased overall fitness levels. The

present findings emphasise the need for refinement of marine water quality guidelines. They also re-inforce the emerging view (Hogstrand and Wood, 1998; Wood et al., 1999) that the intestine may be a key site where the toxic ionoregulatory effects of waterborne silver are exerted in marine teleost fish which drink seawater (Grosell et al., 1999; Hogstrand et al., 1999). This potential route of toxicity has been generally overlooked in seawater fish, especially with respect to metals, and most focus has been on the gills. It will be interesting to see whether the same is true of other ionoregulatory toxicants such as copper (e.g. Larsen et al., 1997; Stagg and Shuttleworth, 1982) in seawater, and how the situation may differ in marine elasmobranchs, which do not drink seawater to any great extent (Evans, 1993).

Acknowledgements

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