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Bioaccumulation and distribution of silver in four marine teleosts and two marine elasmobranchs: influence of exposure duration, concentration, and salinity

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Abstract

The bioaccumulation of waterborne silver (added as $AgNO_3$) was compared amongst drinking (teleosts: rainbow trout, tidepool sculpin, plainfin midshipmen, and English sole) and non-drinking marine fish (elasmobranchs: Pacific spiny dogfish and long nose skate) exposed to 14.5 μg/l Ag for 21 days in 30-ppt seawater. In addition, 21-day exposures were performed on trout, midshipmen, and sculpin at 0 (control), 1.5, 14.5, and 50 μg/l Ag to evaluate the effect of silver concentration, and on sculpins acclimated to 18 and 30 ppt salinity and sampled periodically up to 21 days to evaluate the effects of salinity and exposure duration. A 48-h acute exposure (250 μg/l Ag) was also carried out on sculpins at 10, 18, 24, and 30 ppt. The 1.5-and 14.5-μg/l Ag levels are of regulatory importance, but are several orders of magnitude higher than normal environmental levels. Silver uptake occurred in all exposures, but internal accumulations were less than proportional to exposure concentration (1.5–50.0 μg/l Ag), and tended to saturate over time, suggesting that physiological regulation occurred. Control (non-exposed) fish exhibited measurable levels of silver in all tissues (10–200 μg Ag/kg wet weight), suggesting that they accumulate silver from the natural environment throughout their lifetimes. After 21-day exposure to 14.5 μg/l Ag, silver levels increased 2–20-fold in most tissues of all species, with the greatest concentrations occurring in the livers of teleosts (order: liver > gills ≥ intestines > white muscle) and the gills of elasmobranchs (order: gills > liver > white muscle > intestines). Rainbow trout accumulated more silver than the other teleosts, and were the only species to suffer significant mortality, effects likely associated with added salinity stress. Accumulations were fairly uniform amongst the other teleosts. Similar concentrations in gills and intestines suggested that both branchial and intestinal uptake occurred, with the latter potentially dominant; indeed sole exhibited no silver build-up in the gills. The two elasmobranchs exhibited no silver build-up in intestines but much higher levels in gills, indicating that in the absence of drinking, only branchial uptake occurs. Nevertheless, based on whole liver content, the elasmobranchs accumulated silver 5–15-fold faster than the three teleosts. Over 21-day exposures (1.5–50.0 μg/l Ag) in sculpin, salinity markedly affected silver accumulation, with tissue-specific levels approximately 6-fold higher at 18, than at 30 ppt. However, there was negligible effect of salinity on silver accumulation during 48 h at 250 μg/l Ag. Silver bioaccumulation appears to be markedly affected

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by speciation. At lower salinities, or higher [Ag], a neutral charged $AgCl_{aq}$ complex exists in the water, allowing for increased bioaccumulation to occur. At higher salinity, only less bioavailable, negatively-charged $AgCl_n^{1-n}$ complexes are present ($AgCl_2^-$, $AgCl_3^{2-}$, $AgCl_4^{3-}$).

Keywords: Silver; Bioaccumulation; Seawater; Elasmobranchs; Teleosts

1. Introduction

Silver is a metal which has attracted recent environmental and toxicological interest (Purcell and Peters, 1998; Wood et al., 1999). Silver may enter the marine environment via non-point source discharge or via outflow from sewage treatment plants which treat effluent from photo-processors and other industries. At present, relatively little is known about the effects of silver in marine organisms (see Eisler, 1996; Hogstrand and Wood, 1998; Wood et al., 1999; Ratte, 1999 for review of the limited available information). However, in general the accumulation of metals in aquatic organisms has been linked to decreased survival rates and reduced reproductive ability. Bioaccumulation at lower trophic levels may also present a significant entry pathway into the upper levels of the aquatic food chain (Thomann, 1981; Biddinger and Gloss, 1984; Dallinger et al., 1987; Sanders et al., 1990).

For fish, metal uptake can occur either from the aqueous phase, and/or from metal biologically incorporated in food (Fowler, 1982; Sanders and Riedel, 1987; Fisher and Reinfelder, 1995). Theoretically, in marine teleosts uptake from seawater can occur via the gills or via the gastro-intestinal tract from seawater ingested in the drinking process, which is obligatory for ionoregulation. In general, the latter route has been overlooked by most workers, and only recently have studies monitored the appearance of waterborne metal (silver) in the intestines (Hogstrand et al., 1999; Grosell et al., 1999). In contrast to teleosts, marine elasmobranchs, which have a fundamentally different ionoregulatory strategy, are reported to exhibit little or no drinking of seawater (Evans 1979, 1993; Hazon et al., 1997). A comparison of relative rates and routes of silver uptake in marine teleosts (drinkers) versus marine elasmobranchs (non-drinkers) may therefore prove informative. Interestingly, Pentreath (1977) found that accumulation of ^{110m}Ag from seawater was much higher for a marine elasmobranch (thornback ray) than for a marine teleost (plaice), but did not provide an explanation as to the origin of the differences.

Many factors such as hardness, pH, alkalinity, salinity, and dissolved organic carbon (DOC) can affect the bioavailability of a metal. In freshwater, the main factors involved in modulating silver bioavailability to fish appear to be chloride ($[Cl^-]$) and [DOC] (Janes and Playle, 1995; Galvez and Wood, 1997; Kramer et al., 1997; Hogstrand and Wood, 1998). In seawater, the much higher chloride levels appear to have a dramatic effect on toxicity, as well as on bioaccumulation, of silver. LC_{50} values for silver in seawater fish are 50–100 times higher (lower toxicity) than in freshwater fish (Eisler, 1996; Hogstrand and Wood, 1998). Shaw et al. (1998) found that in tidepool sculpins, a small increase of salinity (from 25 to 32 ppt) in the exposure water led to a doubling of the 96-h LC_{50} value, while the same increase resulted in a 4-fold increase in the 168 h LC_{50} value. The increased salinity also led to a dramatic decrease of silver bioaccumulation in the fish.

In light of this background, the present study was designed to address three objectives with respect to silver bioaccumulation by fish from seawater. The first was to investigate whether different accumulation rates of silver occurred in marine elasmobranchs versus marine teleosts, with a more extensive survey of species than used by Pentreath (1977). Four teleosts and two elasmobranchs were exposed to 14.5 μg/l Ag (as $AgNO_3$) for 21 days in full strength seawater. Analysis of silver in various tissues provided an indication of the relative importance of branchial versus gastro-intestinal uptake routes in teleosts and elasmobranchs. In addition, drinking rates were measured in several of the species. The sec-

ond objective was to investigate the effect of salinity and exposure duration on silver bioaccumulation (c.f. Shaw et al., 1998) using tidepool sculpins, which are very euryhaline. Sculpins were exposed to a high level of silver (250 μg/l Ag as $AgNO_3$) for 48 h or to lower levels (1.5, 14.5, and 50.0 μg/l Ag as $AgNO_3$) for 21 days at various salinities, with periodic measurements of tissue silver levels. The third objective was to examine the specific effect of the waterborne silver concentration on the rates of silver bioaccumulation during chronic exposure. Therefore, several different teleost species were exposed to the three lower levels of silver for 21 days in 30 ppt seawater, with periodic tissue silver measurements.

While the highest exposure level (50 μg/l Ag) was chosen to elicit definite effects, the lower levels (1.5 and 14.5 μg/l Ag) were chosen specifically to address environmental regulations. The current U.S. EPA criterion for the protection of aquatic life in estuarine and marine environments is 2.3 μg/l Ag (U.S. EPA, 1980), while in recent years various other values [14.5 μg/l Ag (acute), 7.2 μg/l Ag (acute) and 0.92 μg/l Ag (chronic)] have been proposed but not implemented (U.S. EPA, 1987; Loux, 1993; U.S. EPA unpublished). Recently, the province of British Columbia, where this study was performed, has proposed a chronic guideline of 1.5 μg/l Ag for marine and brackish water environments (Warrington, 1995). In comparison, naturally occurring silver levels in the open ocean are extremely low (≤ 0.0025 μg/l Ag; Bryan and Langston, 1992; Eisler, 1996), still very low in most North American coastal waters and estuaries (< 0.03 μg/l Ag; Schafer, 1995), but may be elevated to 0.06–2.9 μg/l Ag (Fowler and Nordberg, 1986; Eisler, 1996) in intertidal areas closer to anthropogenic inputs.

2. Material and methods

2.1. Experimental animals

Pacific spiny dogfish (*Squalus acanthius*; 1–2 kg), long-nose skate (*Raja rhina*; 450–950 g), plainfin midshipmen (*Porichthys notatus*; 50–250 g), and English sole (*Parophrys vetulus*; 150–350 g) were obtained as by-catch from local fishermen off the coast of Vancouver Island, BC, Canada, and held at Bamfield Marine Station (Bamfield, BC, Canada). Tidepool sculpins (*Oligocottus maculosus*; 0.5–3.5 g) were collected from local tidepools located near the marine station, and rainbow trout (*Oncorhynchus mykiss*; 300–500 g) were purchased from Rosenser Aquaculture, a local fish farm on Vancouver Island. Dogfish and skate were held in a large common tank (2×10^5 l), while midshipmen and sole were held in square fiberglass tanks (450 l) with sandy substrata. Trout were slowly acclimated to full strength seawater (30–32 ppt) over a period of two weeks in 2000 l fiberglass tanks. Tidepool sculpins were held for two weeks in an 80 l tub containing rocks for shelter. All tanks were provided with aeration and constantly flowing seawater (pH $\sim$7.9, 30–32 ppt salinity, 11–13°C). Dogfish and skates were fed fresh fish, while midshipmen, sole, and sculpins were fed fresh shucked blue mussels on a daily basis. The rainbow trout were fed commercial trout pellets every second day at a ration of 1% total body weight at each feeding.

2.2. Experimental series

Three different chronic exposure series and one acute exposure series were performed, with some overlap amongst the chronic series.

(i) *Fish species comparison-chronic exposure*. All six species were exposed to 14.5 μg/l Ag, in full strength seawater, for 21 days, with respective control groups in Ag-free water.

(ii) *Salinity comparison-chronic exposure*. Tidepool sculpins were exposed to 0 (control), 1.5, 14.5, and 50.0 μg/l Ag in either 18 or 30 ppt seawater, for up to 21 days.

(iii) *Salinity comparison-acute exposure*. Tidepool sculpins were exposed to 0 (control) and 250 μg/l Ag in 10, 18, 24, or 30 ppt seawater for 48 h.

(iv) *Exposure concentration comparison-chronic exposure*. Rainbow trout, plainfin midshipmen, and tidepool sculpins were exposed to 0 (control), 1.5, 14.5, and 50.0 μg/l Ag in 30 ppt seawater for 21 days.

The 0 and 14.5 μg/l Ag exposure groups in 30 ppt seawater were shared amongst all three chronic series (i, ii, and iv) for sculpins, and between series i and iv for trout and midshipmen.

2.3. Experimental protocol

2.3.1. Chronic exposures

Dogfish ($n = 8$ in each treatment), skate ($n = 6$), and sole ($n = 8$) were exposed to 0 (control) or 14.5 μg/l Ag (134.4 nmol Ag, as $AgNO_3$, BDH) for 21 days in a well-aerated flow-through seawater system. Water entered each 2000 l tank at 8 l/min, with a turnover time of ~4-h and an initial loading density of 2.9, 1.3, and 2.8 g/l for the dogfish, skate, and flounder, respectively. Silver was added from light-shielded stock bottles (116 μg/ml Ag) by a peristaltic pump (1 ml/min). Water samples were taken at least daily throughout the first week of exposure, and every second day thereafter, to monitor water [Ag]. On day 21, control and exposed fish were sacrificed with MS222 (1 g/l, neutralized with NaOH; Syndel Labs, Vancouver, BC, Canada), and weighed. Gill, liver, and white muscle samples were dissected and rinsed for 1 min in silver-free seawater, while the intestines were slowly flushed by syringe over a 30-s period with several hundred ml of silver-free seawater to displace chyme before rinsing. Samples were then frozen in liquid N_2 and stored at −80°C for future analysis.

Midshipmen ($n = 20$ per treatment) and rainbow trout ($n = 40$) were exposed to 0 (control), 1.5, 14.5, and 50.0 μg/l Ag (0, 13.9, 134.4, and 463.5 nmol Ag, as $AgNO_3$, respectively) in well-aerated seawater for 21 days. The midshipmen exposure was a static set-up in 150-l tanks with an initial loading density of 9.3 g/l wet weight. Of the water, 95% was changed daily with silver being added to the in-flowing clean water for mixing from a stock bottle (1 g/l). Aeration was used for further mixing and to prevent oxygen depletion. Rainbow trout were exposed to silver in a well-aerated flow-through 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. Water samples were taken from all tanks on the same schedule as for dogfish and sole (above) to monitor water [Ag]. On day 21, control and exposed fish were sacrificed with MS222 and weighed. Terminal blood samples were taken from rainbow trout by caudal puncture, while gill, liver, intestine, and white muscle samples were taken from all fish. Blood samples were centrifuged at $10\,000 \times g$ for 2 min, and plasma samples were frozen in liquid N_2 and stored at −80°C. Gill, liver, and muscle tissues were rinsed for 1 min in silver-free seawater, while the intestines were flushed with 50 ml of silver-free seawater by syringe before rinsing. Samples were then frozen in liquid N_2 and stored at −80°C for future individual analysis.

Tidepool sculpins were separated into two groups and acclimated to 18 or 30 ppt salinity (310 or 515 mmol $[Cl^-]$, respectively) for 2 weeks. Fish from each salinity were then separated further into four groups ($n = 50$ per treatment) and statically exposed to 0, 1.5, 14.5, and 50 μg/l Ag (0, 13.9, 134.4, and 463.5 nmol Ag, as $AgNO_3$, respectively), for up to 21 days. Silver was added to the exposure water from a stock solution of 1 g/l Ag. Fish were held in 50 l tubs at average initial loading densities of 2.4 g/l wet weight. Each tub was fitted with an air line for aeration and water mixing. The water was replaced every other day with the appropriate salinity and [Ag], and sampled for [Ag]. All tubs were held in a seawater bath to maintain exposure temperature. On days 0, 2, 6, 8 and 21, fish were sacrificed by cephalic blow, rinsed in silver-free seawater and weighed. Gill and liver samples were taken and washed for 1 min, while intestines were flushed with 10 ml of seawater by syringe before rinsing. Tissue samples, along with the remaining carcasses (representing mainly white muscle), were then frozen in liquid N_2 and stored at −80°C for future analysis.

For all exposures, simultaneous controls in Ag-free seawater at the appropriate salinity were maintained in a similar manner to the exposed fish.

2.3.2. Acute exposures

Tidepool sculpins were separated into four groups and acclimated to 10, 18, 24, or 30 ppt

salinity (171, 308, 410, or 513 mMol [Cl^-], respectively) for 2 weeks. Sculpins (16) from each salinity were placed into individual amber glass flux chambers (volume = 175 ml), each fitted with an air line for aeration and water mixing, and held in a water table to maintain temperature (13–14°C). At the start of the experiment, the water in each chamber was replaced, and eight fish from each salinity were exposed to 250 μg/l Ag (2.32 μmol Ag, as $AgNO_3$). The remaining eight fish were used for control measurements. Water from each flux chamber was replaced every 12-h throughout the 48-h experiment, with water samples taken to confirm the exposure concentration. At 48-h, fish were sacrificed by cephalic blow, then gill, liver, and intestine tissue samples were dissected. Gill and liver tissues were rinsed for 1 min in silver free seawater, while the intestines were flushed with 10 ml of silver-free seawater before rinsing. Samples were then frozen in liquid N_2 and stored at −80°C for future individual analysis.

2.4. Drinking rate measurements

Drinking rates were measured under control conditions in dogfish, rainbow trout, English sole and tidepool sculpins ($n = 6$ each) in full strength seawater (30–32 ppt), as well as in sculpins ($n = 6$) acclimated to 18 ppt, by the method of Wilson et al. (1996). In brief, resting fish were exposed to the inert, non-absorbed drinking rate marker [^{3}H]polyethylene glycol 4000 ([^{3}H]PEG-4000; specific activity: 2050 mCi/g; NEN-Dupont) at a concentration of 5.0 mCi/l in the external medium for 6–8 h (exact time recorded). This time period is considerably shorter than the passage time through the gastrointestinal tract, a fact confirmed by counting terminal rectal fluid samples. External volume was at least 20 times the fish volume, and water samples were taken throughout to monitor [^{3}H]PEG-4000 levels, which in fact remained constant. At the end of the exposure, fish were sacrificed without disturbance by an overdose of neutralized MS-222. The entire gastrointestinal tract was quickly exposed by dissection, ligated at the oesophagus and rectum, removed, and homogenized in 5 volumes of 8% $HClO_3$. Further processing of the homogenate, scintillation counting, quench correction, and calculation of drinking rate were performed exactly as described by Wilson et al. (1996).

2.5. Water and tissue silver analyses

All silver levels were determined as detailed by Hogstrand et al. (1996). Briefly, water samples were acidified with 1% HNO_3 (Fisher; trace metal grade), while tissue samples were weighed and digested overnight at 80°C in 5 × (v/w) of concentrated HNO_3 (Fisher; trace metal grade). Tissue samples were then allowed to cool before adding H_2O_2 to remove any debris from the digests. The tissue digests were then heated to evaporate all liquid, and reconstituted with 5 ml of 1% HNO_3. Silver levels were read using graphite furnace AAS (Varian 1275 fitted with a GTA-95 atomizer). Ag speciation in the exposure water was calculated using the determined water chemistry and the aquatic geochemical equilibrium program MINEQL$^+$ (Schecher and McAvoy, 1991).

Data have been expressed as means ± SEM (n). Differences between control and experimental treatment means were analyzed by the Student's unpaired two-tailed t-test. Comparisons within groups were assessed by one-way ANOVA tests followed by Duncan tests to determine individual differences. Significant differences ($P < 0.05$) between treatments are indicated by asterisks (*) while significant differences within treatments are indicated by a plus sign (+). Significant differences determined by ANOVA plus Duncan testing are indicated by different letters.

3. Results

3.1. Water chemistry and survival

3.1.1. Fish species comparison — chronic exposure

The mean water total Ag concentration throughout the 21-day exposures was 14.7 ± 0.3 (17) μg/l, with a high of 15.2 and a low of 12.5 μg/l. Speciation analysis by MINEQL$^+$ indicated that at 30 ppt, approximately 60% of the total silver was present as $AgCl_2^-$, 20% as $AgCl_3^{2-}$, and

20% as $AgCl_4^{3-}$; essentially none was present as the free ion Ag^+ or the neutral complex $AgCl_{aq}$ (Fig. 1A).

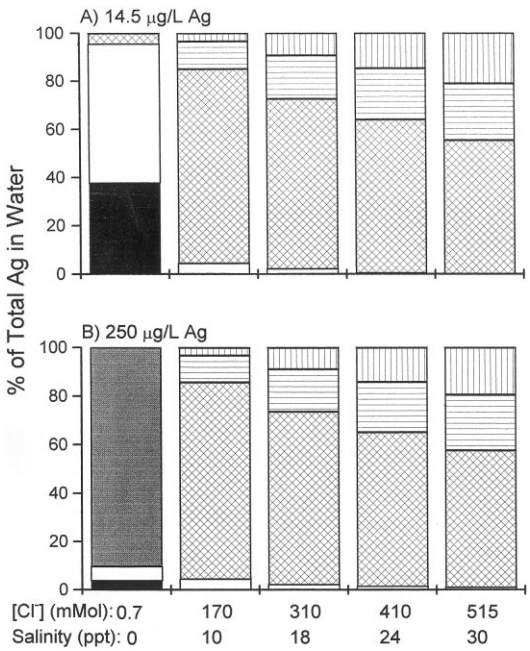

Fig. 1. The change of predominant Ag species as $[Cl^-]$ increases from freshwater (0.7 mMol $[Cl^-]$) to full strength seawater (515 mMol $[Cl^-]$), at (A) 14.5 µg/l Ag and (B) 250 µg/l Ag. At both [Ag], as the $[Cl^-]$ increases beyond the freshwater range, Ag^+ disappears immediately. $AgCl_{aq}$ disappears between 18 and 24 ppt at 14.5 µg/l Ag, but remains present at all $[Cl^-]$ at 250 µg/l Ag. Charged $AgCl_n^{n-1}$ species make up the remainder of silver species present. This model assumes a DOC concentration of 0 and an $[S^{2-}] = 0$. Species representation is as follows: (black) Ag^+; (white) $AgCl_{aq}$; (criss-cross) $AgCl^{2-}$; (horizontal) $AgCl_3^{2-}$; (vertical) $AgCl_4^{3-}$; and (grey) Cerargyrite.

Table 1
Water total Ag concentrations for the salinity comparison exposure experiments[a]

Nominal [Ag] (µg/l)	Actual [Ag] (µg/l)	
	18 ppt	30 ppt
Control	0 (17)	0 (17)
1.5	1.3 ± 0.4 (20)	1.4 ± 0.2 (20)
14.5	15.2 ± 0.6 (18)	14.8 ± 0.5 (18)
50.0	51.0 ± 0.8 (25)	50.2 ± 0.7 (25)

[a] Values are means ± S.E.M. (*n*).

Throughout the experiment, none of the exposed tidepool sculpins ($n = 50$), plainfin midshipmen ($n = 20$), or dogfish ($n = 8$) died. One of the smallest English sole ($n = 8$) and the smallest of the long-nose skate ($n = 6$) died on the twelfth and seventeenth day, respectively. The rainbow trout appeared to be the least resistant to silver with 28 of the initial 40 fish dying throughout the 21-day exposure. Within the respective control groups, mortalities occurred only for rainbow trout where six of 40 trout died over 21 day.

3.1.2. Salinity comparison — chronic exposure

The mean total Ag concentrations for this experimental series are listed in Table 1; all of the concentrations were close to nominal values. For all silver concentrations used (1.5–50.0 µg/l Ag), speciation calculations showed a similar % distribution as that at 14.5 µg/l Ag (Fig. 1A). Note that at lower salinities, the distribution shifted in favour of less charged silver chloride species, so that at 18 ppt, the contributions of $AgCl_3^{2-}$ and $AgCl_4^{3-}$ decreased, that of $AgCl_2^-$ increased, and $AgCl_{aq}$ now made a small but significant contribution.

None of the exposed or control sculpin ($n = 50$/tank) died throughout the experiment.

3.1.3. Salinity comparison -acute exposure

The mean water total Ag level during the 48-h exposure was 265 ± 8 µg/l ($n = 128$), slightly higher than the nominal value of 250 µg/l. Speciation modeling by MINEQL$^+$ indicated that at a total silver concentration of 250 µg/l (Fig. 1B), there was a slight salinity-dependent shift in relative distribution from that determined at 14.5 µg/l (Fig. 1A). At 14.5 µg/l, $AgCl_{aq}$ was only present at 10 and 18 ppt, while at 250 µg/l, $AgCl_{aq}$ was present at all salinities. Although freshwater (0 ppt) was not tested in the present study, it was included in the modeling as a point of interest for comparison (Fig. 1A,B). Note that only in freshwater would there be a significant amount of Ag^+ present, and that the relative amounts of both Ag^+ and $AgCl_{aq}$ in freshwater would be reduced by cerargyrite formation at higher total silver levels (Fig. 1B).

Table 2
Water total Ag concentrations for the exposure concentration comparison experiments at fixed salinity (30 ppt)[a]

Nominal [Ag] (μg/l)	Actual [Ag] (μg/l)	
	Rainbow trout	Midshipman
Control	0 (15)	0 (15)
1.5	1.4 ± 0.3 (18)	1.3 ± 0.2 (18)
14.5	13.4 ± 0.5 (23)	13.8 ± 0.7 (23)
50.0	48.9 ± 0.8 (25)	49.5 ± 0.5 (25)

[a] Values are means ± S.E.M. (*n*).

None of the control or exposed fish died during this exposure.

3.1.4. Exposure concentration comparison — chronic

The mean total Ag concentrations for this experimental series are listed in Table 2; all of the concentrations were close to nominal values and Fig. 1A was representative of speciation for all concentrations.

None of the exposed or control sculpin ($n = 50$/tank) or midshipmen ($n = 20$/tank) died during the study, while 15% of the control rainbow trout ($n = 40$/tank) perished. Of the exposed trout ($n = 40$/tank), 23 (57%) of the trout exposed to 1.5 μg/l, 28 (70%) of the trout exposed to 14.5 μg/l, and 34 (85%) of the trout exposed to 50 μg/l died by 21-d.

3.2. Tissue silver burden

3.2.1. Fish species comparison — chronic exposure

In virtually all tissues of all species, there were detectable levels of Ag (10–200 μg Ag/kg wet weight) in control fish (fish not exposed to Ag in the experiments). Gill silver levels (Fig. 2A) in control fish were fairly uniform (50–100 μg Ag/kg wet weight) among all species. Only midshipmen gill levels were significantly different (lower) from the other species tested. After 21-day of exposure to 14.5 μg/l Ag, gill silver levels increased significantly in all species except the English sole. This increase was roughly 2–3-fold in the teleost fish gills, and 5–6-fold in the elasmobranch gills (Fig. 2A).

Silver levels in the intestines (Fig. 2B) of control fish were also quite uniform (50–75 μg Ag/kg wet weight). Levels in midshipmen intestines were

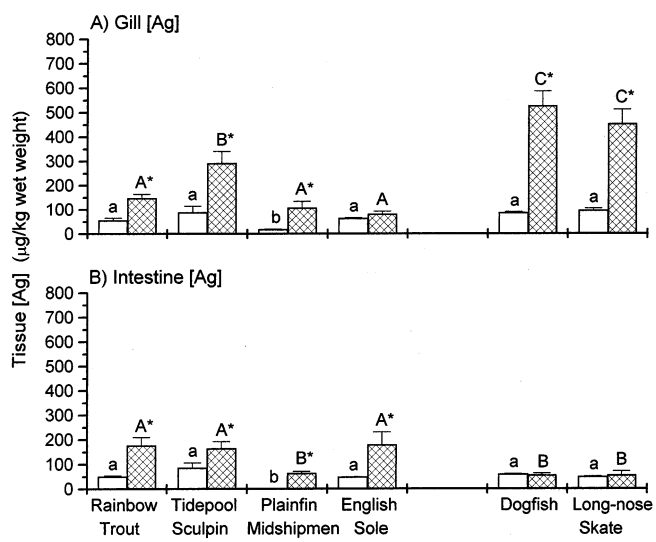

Fig. 2. (A) Gill and (B) intestine silver levels in control fish (open bars) and fish exposed to 14.5 μg/l Ag (hatched bars) for 21 days in full strength seawater. Data points are means ± S.E.M. (6). Asterisks (*) indicate a significant difference ($P < 0.05$) between control and exposed values of the same fish, while bars of each tissue that share a letter are not significantly different from each other. Lower case letters refer to comparisons amongst control fish, upper case letters amongst exposed fish.

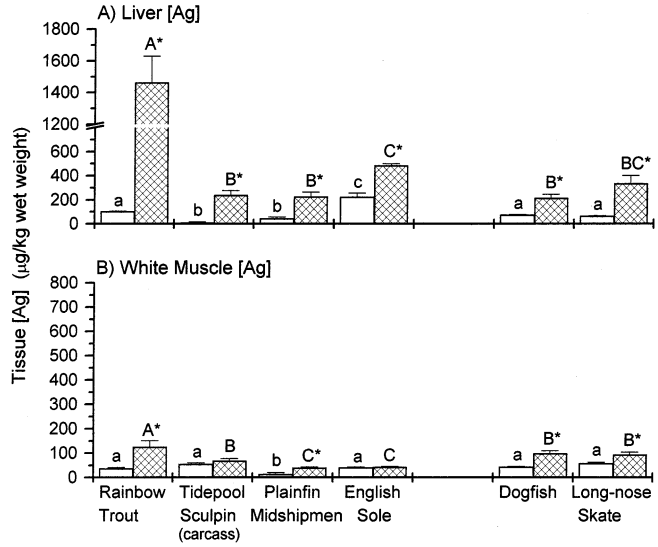

Fig. 3. (A) Liver and (B) white muscle silver levels in control fish (open bars) and fish exposed to 14.5 μg/l Ag (hatched bars) for 21 days in full strength seawater (30 ppt). The values for tidepool sculpin are a whole carcass measurement. Other details are as indicated in the legend to Fig. 2.

again significantly lower than in the other fish, and indeed, not significantly different from zero. Ag levels in all exposed teleost intestines rose significantly, generally 2–3-fold after 21-day of Ag exposure. There were no changes in intestine silver levels in the two elasmobranchs (Fig. 2B).

Silver levels in the livers (Fig. 3A) of control fish exhibited greater interspecific variation, being significantly lower in tidepool sculpins and midshipmen (<30 μg Ag/kg wet weight), and significantly higher in the English sole (~ 200 μg Ag/kg wet weight). Liver levels in control rainbow trout, dogfish, and skate were intermediate (60–100 μg Ag/kg wet weight). After 21-day exposure, liver silver levels were significantly elevated by more than 2-fold in all fish, and 20-fold in tidepool sculpins. Rainbow trout accumulated the most silver, with levels rising 15-fold to 1464 ± 166 (8) μg Ag/kg wet weight (Fig. 3A).

In general, of all tissues, white muscle (Fig. 3B) in control fish had the lowest background silver levels (20–60 μg Ag/kg wet weight), with levels in midshipmen again being significantly lower than in the other fish. After 21-day of silver exposure, white muscle silver levels in rainbow trout and midshipmen increased significantly by 3-fold, while levels in sculpin carcasses (mainly white muscle) and sole white muscle did not change. Silver levels in the white muscle of both elasmobranch species increased 2-fold by the end of the exposure (Fig. 4B).

Overall, in terms of tissue concentration, the liver was the site of highest silver concentration in the teleost fish species, while the gills were the site of highest concentration in the elasmobranchs (Table 3). However, this comparison does not take into account the fact that livers, as a percentage of total body weight, are much larger in elasmobranchs ($\sim 20\%$) than in teleosts ($\sim 2\%$). When the *total amount* accumulated in the liver over 21 days was calculated as an index of whole body uptake rate (assuming the liver to be the final storage depot for bioaccumulated silver), *net accumulation rates* were 5–15-fold higher in the two elasmobranchs (dogfish, skate) at 30 ppt than in three teleosts (sculpins, midshipmen, sole) at the same salinity, though comparable to those in another teleost, the rainbow trout at 30 ppt (Table 4)

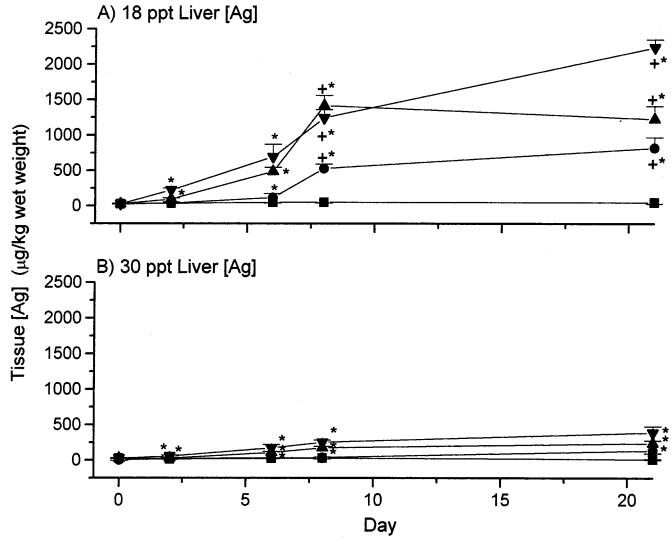

Fig. 4. Liver silver levels in control tidepool sculpins (■) and sculpins exposed to 1.5 (●), 14.5 (▲), and 50.0 μg/l Ag (▼) for 21 days at both 18 ppt (A, top) and 30 ppt (B, bottom) salinities. Data points are means ± S.E.M. (6). Asterisks (*) indicate a significant difference ($P < 0.05$) relative to control values at the same salinity, while a plus sign (+) indicates a significant difference between salinity values at the same [Ag].

3.2.2. Salinity comparison — chronic exposure

Liver silver levels, in tidepool sculpins (Fig. 4) exposed to silver for 21 days, showed a dramatic difference between 30 and 18 ppt seawater, with a greater accumulation in the latter. At 30 ppt (Fig. 4B), silver levels of sculpins exposed to medium and high silver concentrations (14.5 and 50.0 μg/l Ag respectively) had increased slightly but significantly by 2 days, compared to control fish. By the sixth day, fish in all three exposures (1.5, 14.5, and 50.0 μg/l Ag) had significantly more silver in the livers. At the end of 21 days, the increased accumulation of silver was significant and was dependent upon, but less than proportional to exposure concentration. At 18 ppt (Fig. 4A), uptake into the liver was greater and absolute values separated quickly. By 8 days, the hepatic accumulation at all exposure concentrations was significantly higher than in 30 ppt livers at the same silver exposure levels. By the end of the exposure, 18 ppt livers had 5–6-fold greater levels than seen in full strength seawater at the same exposure levels (Fig. 4). Net accumulation rates based on total amounts in liver, calculated over 21 day, reflect this difference (Table 4).

A similar salinity-dependent difference in Ag accumulation was seen in the intestines (Fig. 5). It took 8 days for the accumulation of silver in the intestines of 30 ppt sculpins to become significant for all exposure levels (Fig. 5B). By 21 days, the accumulation was dependent upon concentration in a less than proportional manner, with fish at

Table 3
Comparison of Ag concentrations in tissues of fish exposed to 14.5 μg/l Ag for 21 days

Fish species	Tissue: ranked by (Ag) concentration
Rainbow trout (30 ppt)	Liver ≫ intestine = gill = white muscle
Tidepool sculpin (30 ppt)	Liver = gill > intestine > carcass
Tidepool sculpin (18 ppt)	Liver ≫ gill = intestine > carcass
Midshipman (30 ppt)	Liver > gill > intestine > white muscle
English sole (30 ppt)	Liver > intestine > gill > white muscle
Dogfish (30 ppt)	Gill > liver ≫ white muscle > intestine
Long-nose skate (30 ppt)	Gill > liver ≫ white muscle > intestine

Table 4
Net accumulation rates of silver in whole livers of fish exposed to various silver levels in seawater for 21 days[a]

Fish species	Net accumulation rate (ng/kg per h)		
	Low (Ag)	Medium (Ag)	High (Ag)
Tidepool sculpin (18 ppt)	32.00 ± 5.78 (6)[b]	47.93 ± 7.16 (6)[c]	87.46 ± 4.28 (6)[d]
Tidepool sculpin (30 ppt)	7.02 ± 1.60 (6)[a]	8.88 ± 1.39 (6)[a]	14.93 ± 3.47 (6)[a]
Rainbow trout (30 ppt)	28.15 ± 4.88 (8)[b]	53.70 ± 6.53 (8)[c]	73.67 ± 6.87 (8)[d]
Midshipman (30 ppt)	5.93 ± 0.98 (10)[a]	7.38 ± 1.39 (10)[a]	50.10 ± 2.18(10)[c]
English sole (30 ppt)		10.37 ± 0.10 (5)[a]	
Dogfish (30 ppt)		56.28 ± 11.98 (5)[c]	
Long-nose-skate (30 ppt)		108.28 ± 26.33(8)[d]	

[a] Values are means ± S.E.M. (*n*) and are expressed per kg of whole body weight. Values that share the same letter (a, b, c or d) are not significantly different from each other.

the high exposure level showing a 10-fold increase of silver. At 18 ppt (Fig. 5A), there was a significant accumulation of silver in the intestines at all silver levels by the second day. This accumulation was significantly higher than in 30 ppt intestines by 8 days. After 21 days, 18 ppt intestine silver levels were 2–3-fold higher than those of 30 ppt intestines at the same exposure concentrations (Fig. 5).

Effects of salinity on accumulation of Ag in the gills (Fig. 6) were apparent but less clear-cut. The accumulation of silver in the gills of 30 ppt sculpins (Fig. 6B) was significant for all exposure concentrations after 6 days, and remained significant throughout. Silver levels in 18 ppt exposed gills (Fig. 6A) were significantly higher than controls by 2 days for all exposures. Differences between the two salinities were negligible through 8 days, but by 21 days, the accumulation of silver in the 18 ppt gills was significantly higher than in the 30 ppt gills at all comparable exposure concentrations (Fig. 6).

For most tissues, at both salinities, silver accumulation did not occur linearly with time, but rather was greatest in the first few days, with a

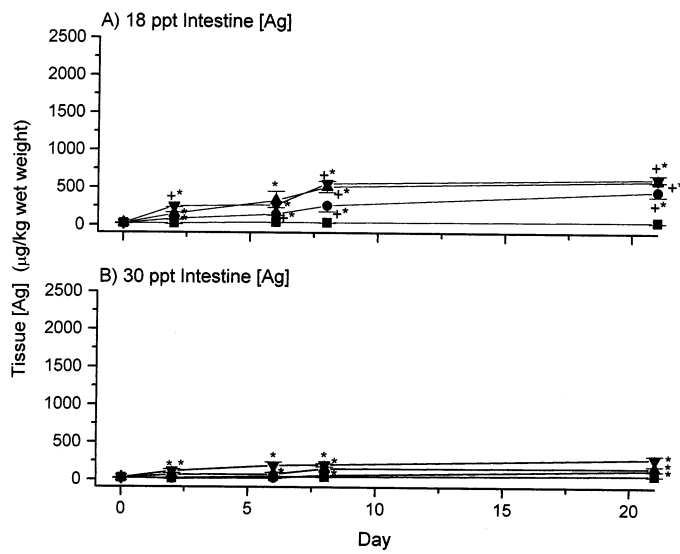

Fig. 5. Intestine silver levels in control tidepool sculpins (■) and sculpins exposed to 1.5 (●), 14.5 (▲), and 50.0 µg/l Ag (▼) for 21 days at both 18 ppt (A, top) and 30 ppt (B, bottom) salinities. Other details are as indicated in the legend to Fig. 4.

trend towards saturation between 8 and 21 days. (Figs. 4–6).

3.2.3. Salinity comparison — acute exposure

Rather different tissue-specific differences and effects of salinity were seen in the short term exposure (48 h) to a much higher silver concentration (250 μg/l Ag; Fig. 7). Silver accumulations in the gills and intestine after 2 days at 250 μg/l Ag were comparable to each other, and equal to or greater than those seen after 21 days at 50 μg/l Ag in the chronic exposures (Fig. 7A,B; c.f. Figs. 5 and 6). However, levels in liver were not elevated, except very slightly at 30 ppt (Fig. 7C; c.f. Fig. 4). Overall, the effects of salinity during these acute exposures were not pronounced, with accumulations tending to be fairly uniform between 10 and 30 ppt, though higher at 24 ppt for intestine (Fig. 7B), and at 30 ppt for gill (Fig. 7A).

3.2.4. Exposure concentration comparison — chronic

Three species were examined, rainbow trout, tidepool sculpin, and plainfin midshipmen. All comparisons were made at 21 days; for all species and most tissues, accumulation increased with exposure concentration, but in a less than proportional fashion.

Gill silver levels in rainbow trout (Fig. 8A) increased significantly by 2.5-fold (over control levels) in gills of fish exposed to 1.5 and 14.5 μg/l Ag, and 9-fold in fish exposed to 50.0 μg/l Ag. Silver levels in the intestines also increased significantly. The increase was small (~ 1.5-fold over controls) in fish exposed to 1.5 and 50.0 μg/l Ag, but nearly 4-fold in fish exposed to 14.5 μg/l Ag. Liver silver accumulation in exposed trout was highest of all tissues, at all silver levels, rising 8, 14, and 20-fold (over control levels) in fish exposed to 1.5, 14.5, and 50.0 μg/l Ag, respectively. Accumulation in the white muscle of rainbow trout was significant and uniform (3–5-fold over control levels) for all exposure concentrations (Fig. 8A). Plasma silver concentrations in trout exposed to 14.5 and 50.0 μg/l Ag increased significantly by the end of exposure, to reach values 1.5 and 2-fold higher (respectively) than plasma levels in both control and 1.5 μg/l Ag-exposed fish (Table 5). However plasma concentrations in the exposed fish did not exceed waterborne concentrations.

Silver accumulation in the gills of tidepool sculpins (Fig. 8B) was significant at all exposure concentrations with the increase being the same (3.5-fold over control levels) in gills of fish ex-

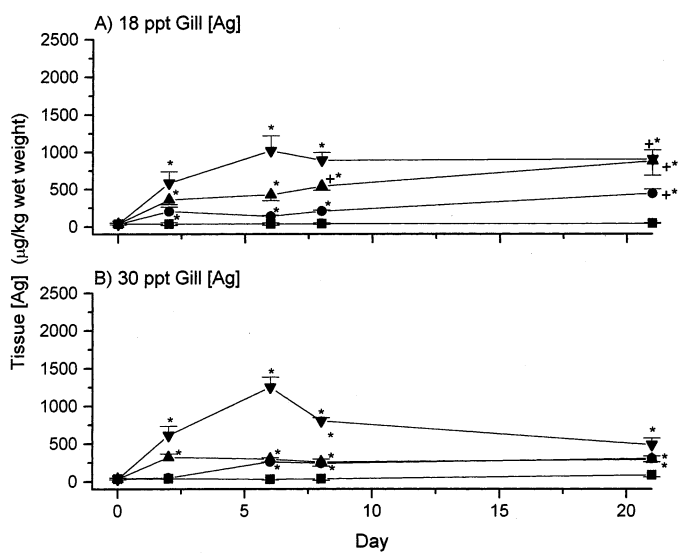

Fig. 6. Gill silver levels in control tidepool sculpins (■) and sculpins exposed to 1.5 (●), 14.5 (▲), and 50.0 μg/l Ag (▼) for 21 days at both 18 ppt (A, top) and 30 ppt (B, bottom) salinities. Other details are as indicated in the legend to Fig. 4.

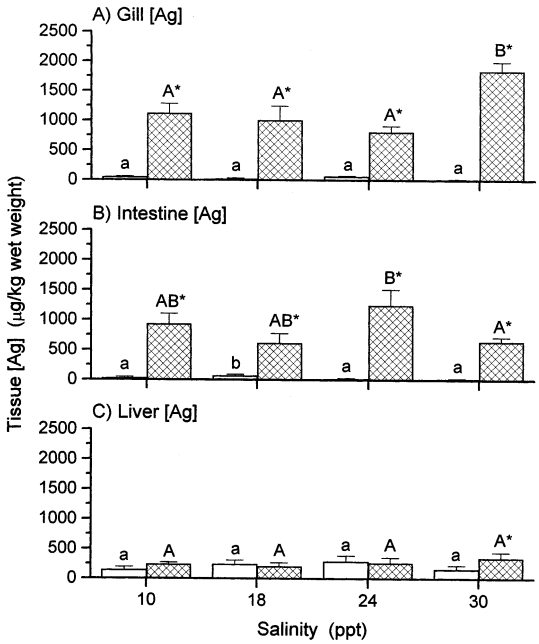

Fig. 7. Gill (A, top), intestine (B, middle), and liver (C, bottom) silver levels in control tidepool sculpins (open bars) and sculpins exposed to 250 µg/l Ag (hatched bars) for 48 h at salinities of 10, 18, 24, and 30 ppt. Data points are means ± S.E.M. (8). Asterisks (*) indicate a significant difference ($P < 0.05$) relative to control values, while bars of each tissue that share a letter are not significantly different from each other. Lower case letters refer to comparisons amongst control fish, upper case letters amongst exposed fish.

posed to 1.5 and 14.5 µg/l Ag, and nearly 6-fold in fish exposed to 50 µg/l Ag. Intestine silver levels increased by 2-fold in fish exposed to 1.5 and 14.5 µg/l Ag, and 4-fold in fish exposed to 50 µg/l Ag. Silver accumulation in the livers of sculpins was also significant at all exposure concentrations, with liver levels increasing 10, 20, and 30-fold (over control levels) in fish exposed to 1.5, 14.5, and 50.0 µg/l Ag, respectively. Silver accumulation (approximately 2-fold) in the carcass (mainly white muscle) of sculpins was only significant in fish exposed to 50.0 µg/l Ag.

Silver accumulation in gills of midshipmen (Fig. 8C) was significant in fish at all exposure concentrations with levels increasing by 5–6-fold at 1.5 and 14.5 µg/l Ag, and by 24-fold at 50 µg/l Ag. In intestines, the relative accumulation was even greater, corresponding to 30, 45, and 110-fold increases in fish exposed to 1.5, 14.5, and 50.0 µg/l Ag, respectively. Liver silver levels increased 5-fold in midshipmen exposed to 1.5 and 14.5 µg/l, and 32-fold in fish exposed to 50.0 µg/l Ag. Silver build-up in white muscle was only significant in fish exposed to 14.5 and 50.0 µg/l Ag with levels rising 3.5 and 8.5-fold, respectively.

Net accumulation rates, based on terminal liver silver concentrations at the end of 21-day exposure, relative to control fish, were related to exposure concentration in all three species (Table 4). Significantly higher accumulation rates were seen in rainbow trout (at 30 ppt) and tidepool sculpin (at 18, but not at 30 ppt) than in all other teleost fish at the same exposure concentrations.

3.3. Drinking rates

Drinking rates were measured only under control conditions in this study. In full strength seawater, drinking rate in the elasmobranch dogfish (0.16 ml/kg/h) was extremely low and only just detectable, whereas rates in the teleosts (trout, sculpins, and midshipmen) were at least 10-fold higher (1.6–3.1 ml/kg per h; Fig. 9). Notably, drinking rates were identical at 18 and 30 ppt in the sculpin, indicating little influence of salinity in this range.

4. Discussion

4.1. Silver bioaccumulation versus toxicity

The two lower silver levels (1.5 and 14.5 µg/l Ag) used in this seawater study were chosen based on current and proposed regulatory limits (see Introduction), whereas the 50 µg/l Ag exposure was used as a positive control. It is doubtful that 14.5 µg/l Ag ever occurs 'naturally', even in areas heavily influenced by anthropogenic inputs, and even 1.5 µg/l Ag would be an unusual occurrence in coastal or estuarine waters (Eisler, 1996). None of these exposure concentrations caused significant mortality over 21 days, except in the case of rainbow trout which may have been a special case because of 'salinity stress', as outlined below. Nevertheless, all three concentrations resulted in

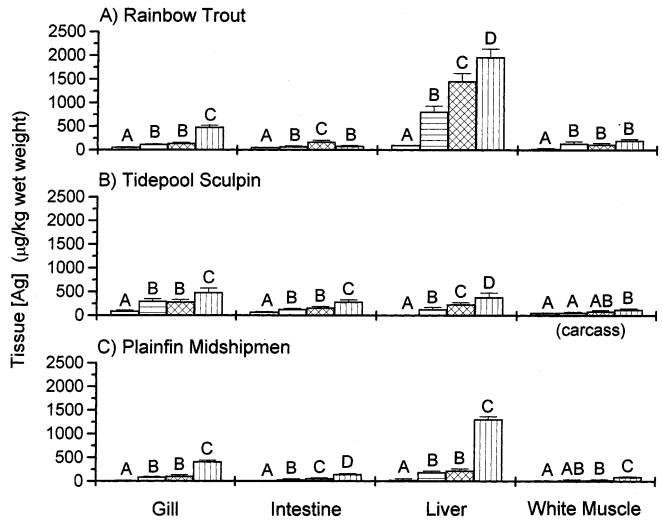

Fig. 8. Tissue silver levels in rainbow trout (A, top), tidepool sculpin (B, middle), and plainfin midshipmen (C, bottom) held in full strength seawater (30 ppt). Open bars represent control fish, while fish exposed to 1.5, 14.5, and 50 μg/l Ag for 21 days are represented by bars with horizontal stripes, cross-hatching, and vertical stripes respectively. Data points are means ± S.E.M. (8). Bars that share a letter are not significantly different ($P < 0.05$) for each species.

significant bioaccumulation of silver, especially in the liver, over the 21-day exposures.

It remains an open question whether such bioaccumulation is associated with any sublethal physiological effects in marine fish. Calabrese et al. (1977) found depressed liver transaminase activity in winter flounder exposed to 10 μg/l Ag for 60 days, and various other enzymatic and metabolic changes have been seen in other species exposed to higher levels for shorter periods (Jackim et al., 1970; Thurberg and Collier, 1977). However, these studies did not report tissue Ag levels. In chronically exposed freshwater fish, sublethal pathologies have been seen, but physiological impact does not appear to be related to silver bioaccumulation (reviewed by Hogstrand and Wood, 1998; Wood et al., 1999). Clearly, detailed physiological studies coupled with tissue Ag measurements are required on marine fish chronically exposed to silver.

4.2. Silver bioaccumulation in seawater versus freshwater fish

Marine fish appear to bioaccumulate silver 'naturally'; there were detectable amounts of silver in the tissues (10–200 μg Ag/kg wet weight) of all 'non-exposed' control fish used in this study (Figs. 2, 3, 7 and 8). The area around Bamfield where these fish were collected is usually considered to be a 'clean' or 'reference' site uncontaminated by industrial sewage outfall (Pierce et al., 1998). Interestingly, silver levels in hepatopancreas of crabs collected at Bamfield were 4-fold lower than in crabs collected in more industrialized coastal areas (Pierce et al. 1998), yet still 10–100-fold higher than in the control marine fish surveyed here. This suggests that some marine invertebrates may 'naturally' bioccumulate silver to an even greater extent than fish. The benthic lifestyle of

Table 5
Plasma Ag levels in rainbow trout exposed to various silver concentrations for 21 days in full strength (30 ppt) seawater[a]

Exposure Concentration (μg/l)	Plasma [Ag] (nmol/l)
Control	2.01 ± 0.13 (8)
1.5	1.89 ± 0.17 (9)
14.5	2.62 ± 0.18 (6)*
50.0	3.91 ± 0.29 (6)*

[a] Values are means ± S.E.M. (n).
* $P < 0.05$ from control.

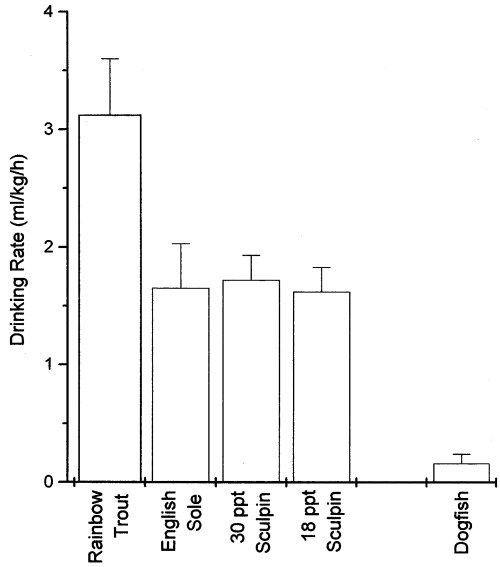

Fig. 9. Drinking rates in three marine teleosts (rainbow trout, English sole, and tidepool sculpin) and one marine elasmobranch (Pacific spiny dogfish) used in this study. All drinking rates were measured in full strength seawater (30 ppt), except for sculpin, where drinking rates were measured at both 18 ppt and 30 ppt. Data are means ± S.E.M (6).

crabs may be a factor here, though both sole and midshipmen also live and feed in the benthos. Similar levels to those of the present fish have been reported in many other marine fish (Pentreath, 1977; Hall et al., 1978; Eisler, 1996; Ratte, 1999). Freshwater fish also appear to bioaccumulate silver 'naturally', and do so at a higher rate. Liver silver levels in unexposed juvenile rainbow trout (1–3 g) were 130–3000 μg Ag/kg wet weight (Hogstrand et al., 1996; Galvez et al., 1998) whereas adults (300–400 g) of the same freshwater strain had liver concentrations of ~10 000 μg Ag/kg wet weight, roughly 70-fold higher than naive seawater fish (Wood et al., 1996a,Wood et al., 1996b) This suggests that silver is much more bioavailable for internal accumulation in freshwater than seawater. In fact, even in rainbow trout exposed to 50 μg/l Ag in seawater, the net accumulation rate (74 ng/kg per h, Table 4, using the total hepatic load as index of whole body uptake) over 21 days was only 4% of the similarly calculated rate (1900 ng/kg per h) in rainbow trout exposed to only 2 μg/l Ag in freshwater for the same period (Galvez et al., 1998). While we cannot eliminate differences in physiology between marine and freshwater fish as contributing factors, this difference in bioavailability is probably due mainly to the difference in chloride concentration between seawater and freshwater. As discussed by Hogstrand and Wood (1998), Shaw et al. (1998), and Wood et al. (1999), the most bioavailable silver species appear to be Ag^+ and $AgCl_{aq}$ which are relatively abundant in freshwater, but which are virtually eliminated by the high chloride concentrations in seawater (Fig. 1).

4.3. Silver bioaccumulation in different fish species

Amongst the four teleost species studied at the same concentration (14.5 μg/l Ag) and exposure duration (21 days), the most striking difference was the much higher silver accumulation in the liver of the rainbow trout (Fig. 3A; Table 4). This was the only species in which the exposure was associated with toxicity, and indeed mortality appeared to be concentration-dependent, rising from 57% at 1.5 μg/l Ag to 85% at 50.0 μg/l Ag. However, as significant mortality (15%) occurred even in control trout, it is likely that these fish were under additional stress from seawater adaptation. Plasma ions were not measured, but in other studies with rainbow trout from this same stock, adapted to full strength seawater, we found plasma Cl^- concentrations in some fish over 200 mmol/l, indicative of pathology. Normal values are generally 160–170 mmol/l (e.g. Wilson et al., 1996). The difficulty ('salinity stress', imperfect hypo-osmoregulation) that many strains of *Oncorhynchus mykiss* exhibit in living for extended periods in seawater is well known, and has been offered as the explanation for the curious salinity-dependence of silver toxicity to juvenile rainbow trout (see below) observed by Ferguson and Hogstrand (1998). Whereas all the other species studied here are true marine species which were collected from seawater in the wild, the rainbow trout is a domesticated strain of an anadromous species which was raised in freshwater and then adapted to seawater. Therefore the higher silver uptake rates in full strength seawater

(30 ppt) in trout than in other marine teleosts may be a product of this added salinity stress. A generalized increase in gill permeability (Schreck, 1990) and an elevated drinking rate (Fuentes and Eddy, 1997) are commonly seen in marine fish during stressful situations.

Tissue-specific patterns and absolute silver build-ups were generally comparable amongst the other teleosts. However, one species, the English sole (*Parophrys vetulus*) exhibited no significant silver accumulation in the gills (Fig. 2A). In this regard it is notable that a related flatfish, the starry flounder (*Platichthys stellatus*) also exhibited no gill silver build-up during a 6-day exposure to a much higher waterborne level (Hogstrand et al., 1999). In both studies, there was substantial accumulation in the liver and intestines (2B3A), suggesting that the latter, rather than the gills, might be the dominant silver uptake route, a point which will be discussed below.

A particular focus of this study was the comparison of silver accumulation rates between marine teleosts and marine elasmobranchs (see Section 1). For silver, the liver appears to be the predominant site of internal accumulation, and therefore can be used to provide an index of whole body uptake rate in chronically exposed fish (Hogstrand and Wood, 1998). Although final concentrations were similar in teleost and elasmobranch livers (Fig. 3A), it must be remembered that the liver in a teleost accounts for a much smaller fraction of the fish's body weight than in an elasmobranch. We did not routinely measure hepatosomatic index in the fish sampled in these experiments, but samples from one or more specimens from each teleost species yielded values of 0.8–2.2%, whereas in elasmobranchs, values of 20–30% are reported (Burger, 1967; Goldstein, 1967; Pelster, 1998). The calculations in Table 4 assume a hepatosomatic index of 2% for teleosts and 20% for elasmobranchs, a difference which is probably conservative. Discounting the results for rainbow trout because of salinity stress complications, it is therefore apparent that net silver accumulation rates under the same exposure conditions were 5–15-fold higher in the two elasmobranchs than in the three teleosts (Table 4). These results support the finding of Pentreath (1977) that another elasmobranch, the thornback ray (*Raja clavata*) took up silver from full strength seawater much faster than a teleost, the plaice (*Pleuronectes platessa)* during a 63-day exposure to trace amounts of silver (0.04 μg/l Ag, labelled with ^{110m}Ag). This difference occurred despite the fact that the elasmobranchs have only a branchial uptake route, whereas the teleosts have both branchial and intestinal uptake routes, as elaborated below.

4.4. Routes of silver uptake — teleosts versus elasmobranchs

Silver levels increased in both the gills (except for the sole) and intestines of teleosts, but only in the gills of elasmobranchs, where accumulations were much greater than in the teleosts (Fig. 2A,B). There are fundamentally different iono- and osmo-regulatory mechanisms in marine elasmobranchs versus marine teleosts (Evans 1979, 1993). The present drinking rates (Fig. 9) were in accord with literature values for teleosts (Fuentes and Eddy, 1997) and elasmobranchs (Hazon et al., 1997), and confirm a well-known fact. Marine teleosts drink (thereby offering an extrabranchial route for silver uptake), while marine elasmobranchs do not to any significant extent. Interestingly, current gill ion transport models for marine elasmobranchs are similar to those for freshwater teleosts (Payan and Maetz, 1973; Bentley et al., 1976; Evans 1979, 1993; Marshall, 1995; Hazon et al., 1997). In the latter, the active Na^+ uptake mechanism is not only a primary target of silver poisoning (Wood et al., 1996a; Morgan et al., 1997; Webb and Wood, 1998; McGeer and Wood, 1998), but also the route by which silver enters across the gills (Wood et al., 1999; Bury and Wood, 1999). The higher silver uptake rates in marine elasmobranchs may reflect silver entry via this transport pathway. In future, it will be of interest to see whether marine elasmobranchs exhibit similar sensitivity and physiological disturbances to silver as freshwater teleosts.

Recently, Grosell et al. (1999) and Hogstrand et al. (1999) have demonstrated that most of the silver ingested by drinking in two marine teleosts

(both flatfish) is removed before residual fluid is excreted via the anus. Furthermore, these studies have provided indirect evidence that pathological effects of this ingested silver may be responsible for impaired salt and water transport across the intestinal epithelium, i.e. for osmoregulatory disturbance. Other metals, most notably copper, are reported to cause osmoregulatory disturbance in marine teleosts (Stagg and Shuttleworth, 1982a,b; Wilson and Taylor, 1993), but this potential for gastrointestinal toxicity does not appear to have been investigated, with the exception of recent in vitro studies by Lionetto et al. (1998a,b) on cadmium.

The bioavailability of silver in the gastro-intestinal tract could be directly due to the fact that Na^+ and Cl^- are actively pumped out of the lumen into the epithelial cells, drawing water into the fish osmotically (Loretz, 1995). This desalination lowers the (Cl^-) from greater than 500 mmol in ingested seawater to < 100 mmol in the intestinal lumen (Shehadeh and Gordon, 1969; Wilson et al., 1996). Wood et al. (1999) have argued that theoretically this should change the speciation of silver in the ingested water from being totally complexed as $AgCl_n^{1-n}$ to having a small amount of $AgCl_{aq}$ present, a pattern which can also be seen in the speciation modelling of Fig. 1. As outlined earlier, this neutral species is thought to have much greater bioavailability.

4.5. The influence of salinity on silver bioaccumulation

The present experiments on tidepool sculpins (Figs. 4–6) extend those of those of Shaw et al. (1998) and confirm that the environmental salinity has a dramatic effect on tissue silver bioaccumulation during chronic exposure. In all tissues, at all silver concentrations ($1.5-50$ μg/l Ag) there was significantly more silver in fish held at 18, than at 30 ppt, and the difference in net uptake rate, based on whole liver accumulation was about 6-fold (Table 4). By way of comparison, Shaw et al. (1998) using a shorter exposure period (7 days) and much higher silver levels reported negligible uptake in sculpins at 32 ppt, with accumulated levels being up to 10-fold higher at 25 ppt. Note that differences in drinking rate do not appear to be responsible because measured drinking rates were identical at 18 and 30 ppt in the present study (Fig. 9). Rather, the different bioaccumulation rates are likely due to the difference in silver speciation (and thus bioavailability) at the different salinities. At 30 ppt, all of the silver was complexed as various forms of $AgCl_n^{1-n}$; at 18 ppt, almost 2% of the total silver in the water occurred as neutral $AgCl_{aq}$ (Fig. 1). We postulate that this small change leads to the dramatic increase in silver bioaccumulation rates (Hogstrand and Wood, 1998).

If silver toxicity is related to bioaccumulation rate, or even just to the presence of neutral $AgCl_{aq}$, then this explains the much lower toxicity (higher 7 day LC50, 472 vs 119 μg/l Ag) observed at 32 ppt than 25 ppt by Shaw et al. (1998). Paradoxically, Ferguson and Hogstrand (1998) reported silver toxicity increased progressively over the 20–30 ppt range in rainbow trout, but as discussed earlier, they related the phenomenon to the additive effects of 'salinity stress' in the face of imperfect hypo-osmoregulation.

When tidepool sculpins were acutely exposed to a much higher silver concentration (250 μg/l Ag) for only 48 h, the effects of salinity on bioaccumulation were not clear (Fig. 7). The virtual absence of silver build-up in the liver (Fig. 7C) indicates that the time period was too short for significant internalization (see also Fig. 4), i.e. a time lag between accumulation in uptake tissues and transfer to storage tissues. The similar silver accumulations in intestines and gills regardless of salinity between 10 and 30 ppt may reflect the fact that at this higher [Ag], there was a small amount of $AgCl_{aq}$ present at all salinities.

4.6. Concentration — dependence and time-dependence of silver bioaccumulation

In general, silver uptake into most tissues of all species increased with concentration and at least in tidepool sculpins, also with time. Nevertheless, the concentration-dependence was less than proportional (Fig. 8), and trends over time showed clear evidence of saturation (Figs. 4–6). While there are many possible explanations (e.g. carrier-

mediation, channel-mediation, altered drinking rates, changing permeability, activation of excretion mechanisms), it would appear that bioaccumulation of this non-essential element is subject to some degree of physiological regulation in marine fish. The mechanism(s) of this physiological regulation constitute an important area for future investigation. In addition, this fact should be clearly recognized when using tissue-specific silver residues as a biological index of exposure (Hall et al., 1978; Eisler, 1996).

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