ELSEVIER

Aquatic Toxicology 84 (2007) 182–189

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Short-term silver accumulation in tissues of three marine invertebrates: Shrimp *Penaeus duorarum*, sea hare *Aplysia californica*, and sea urchin *Diadema antillarum*

Adalto Bianchini [a,*], Richard C. Playle [b], Chris M. Wood [c,d], Patrick J. Walsh [d,e]

[a] *Departamento de Ciências Fisiológicas, Fundação Universidade Federal do Rio Grande, Campus Carreiros, Av. Itália, km 8, Rio Grande, RS 96201-900, Brazil*

[b] *Wilfrid Laurier University, Department of Biology, Waterloo, ON, Canada N2L 3C5*

[c] *McMaster University, Department of Biology, 1280 Main Street West, Hamilton, ON, Canada L8S 4K1*

[d] *Rosenstiel School of Marine & Atmospheric Science, 4600 Rickenbacker Causeway, Miami, FL 33149-1098, USA*

[e] *University of Ottawa, Department of Biology, 30 Marie Curie, Ottawa, ON, Canada K1N 6N5*

Received 1 November 2006; received in revised form 20 February 2007; accepted 27 February 2007

Abstract

The present study was carried out to examine possible differential silver distribution among several tissues of three marine invertebrate species: the shrimp *Penaeus duorarum*, the sea hare *Aplysia californica*, and the sea urchin *Diadema antillarum*. Animals were exposed to sub-lethal concentrations of silver (1 or 10 μg/L) in seawater for 48 h. In gill-breathing species (shrimp and sea hare), higher silver accumulation in gills were associated with higher hemolymph silver levels. Furthermore, sea urchin showed lower hemolymph silver concentrations than shrimp and sea hare. These findings suggest that gills are an important route for silver uptake in marine invertebrates. In both sea hare and shrimp, hepatopancreas silver accumulation was concentration-dependent and this organ accumulated the most silver after 48 h of exposure, suggesting a possible involvement of the hepatopancreas in both silver accumulation and detoxification in marine invertebrates. In shrimp and sea hare, substantial silver accumulation in nervous tissues was detected, suggesting the need for further studies on possible behavioral effects of silver in these invertebrate species. In sea urchin, egg mass accumulated more silver than other tissues analyzed, indicating the need for future studies on possible reproductive effects of silver in sea urchin. In all three species, the lowest silver concentrations were observed in muscle, suggesting a low potential of this tissue for trophic transfer of silver.

Keywords: Accumulation; Biotic ligand model; Marine invertebrates; Sea hare; Sea urchin; Shrimp; Silver

1. Introduction

Data recently reported in the literature (Bianchini et al., 2005) clearly indicated that the toxic response of marine invertebrates to acute silver exposure, as opposed to that observed in freshwater invertebrates (Bianchini et al., 2002; Grosell et al., 2002; Bianchini and Wood, 2002, 2003), is not linked to osmo- or ionoregulatory disturbances at the hemolymph level, even in osmoregulating species. However, they indicated that acute silver toxicity could be associated with changes in the intracellular distribution of several univalent (Cl^-, Na^+, and K^+) or divalent (Mg^{2+} and Ca^{2+}) ions in different marine invertebrate tissues, including gills. The study also demonstrated that these changes cannot be ascribed solely to silver binding and subsequent effect on Na^+, K^+-ATPase, except in crustacean gills. Thus, these authors suggested that the gill of marine crustaceans could be considered a good "target organ" for the extension of the Biotic ligand model (BLM) from freshwater to seawater. However, they mentioned the need for considering other additional "sites of toxicity" in other groups of marine invertebrates in the development of a marine BLM version for silver (Bianchini et al., 2005).

In light of the above, and the fact that the BLM approach is based on the premise that toxic effects are considered a function of the degree of saturation of "toxic sites" on the

* Corresponding author at: Departamento de Ciências Fisiológicas, Fundação Universidade Federal do Rio Grande, Campus Carreiros, Av. Itália, km 8, Rio Grande, RS 96201-900, Brazil. Tel.: +55 53 3233 6853/6848; fax: +55 53 3233 6848.

E-mail address: adalto@octopus.furg.br (A. Bianchini).

0166-445X/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2007.02.021

biotic ligand by Ag^+ (Paquin et al., 1999; McGeer et al., 2000), the main goal of the present study was to analyze the accumulation and internal distribution of silver after acute waterborne exposure in different tissues of three marine invertebrate species: the shrimp *Penaeus duorarum*, the sea hare *Aplysia californica*, and the sea urchin *Diadema antillarum*. These species were selected considering their osmoregulatory behavior in seawater (shrimp = hypo-osmoregulator; sea hare and sea urchin = osmoconformer; Bianchini et al., 2005) and their importance as model experimental systems (sea urchin = reproduction and developmental biology—Ward et al., 2006b; sea hare = behavior and learning—Grosell and Walsh, 2006). Species and tissues analyzed in the present study were the same employed by Bianchini et al. (2005) to determine the toxic response to acute waterborne silver exposure. Furthermore, experimental conditions were also similar to those employed in that previous study.

2. Material and methods

Marine shrimp *P. duorarum* (3.6 ± 1.1 g) were collected in central Biscayne Bay (Miami, FL). Sea hares *A. californica* (35.7 ± 6.1 g) and sea urchins *D. antillarum* (28.5 ± 8.2 g) were provided by the University of Miami National Resource for Aplysia (Miami, FL). After arrival at the National Institute of Environmental Health Sciences (NIEHS) Marine and Freshwater Biomedical Science Center at the Rosenstiel School of Marine and Atmospheric Sciences of the University of Miami (Miami, FL), invertebrates were transferred to glass aquaria containing 100 L of local seawater (33‰ salinity) pumped from Biscayne Bay. Water was constantly aerated and was renewed daily. Temperature was held at 20 °C. Animals were not fed during both the acclimation and the experimentation periods.

Shrimps, sea hares or sea urchins ($n = 18$ for each species) were maintained for 96 h, under the same acclimation conditions described above. After acclimation, one group ($n = 6$) of each species was maintained under control conditions (no silver addition to the water). Other two groups from each species ($n = 6$ in each group) were maintained under the same acclimation conditions, but exposed to two silver concentrations. Each group of individuals was kept in one tank. Waterborne silver concentrations tested were 1.4 and 14 μg Ag/L (spiked Ag concentration). These concentrations were selected to bracket the acute marine water quality criteria for silver recommended by the United States Environmental Protection Agency (USEPA, 1980) and the British Columbia (Canada) Ministry of the Environment (Warrington, 1996). The acute criterion recommended by the USEPA is 3.2 and 2.7 μg Ag/L as total and dissolved silver, respectively. The Canadian criterion for open coastal waters and estuaries is 1.5 μg Ag/L as a 30-day mean, and 3.0 μg Ag/L as a maximum. By way of comparison, naturally occurring silver levels in the open ocean are less than 0.0025 μg/L (Bryan and Langston, 1992; Eisler, 1996) and normally less than 0.03 μg/L in most North American coastal waters and estuaries (Schafer, 1995). However, levels may be elevated to 0.06–2.9 μg/L in intertidal areas close to sewage outfalls and industrial sites (Fowler and Nordberg, 1986; Eisler, 1996).

To prepare the experimental media, a stock solution of unlabelled $AgNO_3$ (SigmaUltra, Sigma Co., St. Louis, MO, USA) was added to the test solution 3 h prior to introduction of animals. This solution contained a proportion of radioactive ^{110m}Ag (RISØ National Laboratory, Roskilde, Denmark) to facilitate analyses of silver concentrations in the test solution and organisms. The final specific activity of radiolabelled silver in all test solutions was 0.7 μCi/μg total silver. Measurements of the final silver concentrations were obtained by measuring the total Ag concentration of the $AgNO_3$ stock solution (1 mg/L) acidified with 1% HNO_3, using graphite furnace atomic absorption spectrophotometry (Zeiss GFAAS-5; Carl Zeiss Jena GmbH, Germany), and its concentration of radioactivity by gamma counting as described below. The ratio yielded the exact specific activity, so the actual total Ag concentration of any test solution or tissue sample could be measured by determining its radioactivity, and then dividing by the known specific activity.

Total and filtered silver concentrations were measured in non-filtered and filtered (Acrodisc 0.45 μM polyethersulfone in-line filters; Gelman) water samples (3 mL) collected at the beginning of the experiment, i.e., when animals were introduced into the experimental tank, and after 24 and 48 h of experiment. The ^{110m}Ag radioactivity in filtered and non-filtered water samples was determined using a gamma counter (Cobra II, Auto-Gamma, Packard Instrument Co., Downers Grove, Illinois, USA), with the precautions on window selection outlined by Hansen et al. (2002). Mean total and filtered measured silver concentrations were calculated considering data from the three water samples collected for each experimental condition over the 48 h-period of exposure. Based on total measured concentrations, mean silver loss corresponded to 30.4 and 45.7% after 24 and 48 h of exposure, respectively. Mean total measured silver concentration was 1.1 and 9.9 μg Ag/L. Mean measured filtered silver concentrations were 0.9 and 8.5 μg Ag/L, respectively. These concentrations will be referred hereafter as 1 and 10 μg Ag/L of waterborne silver.

After 48 h of exposure, animals were kept in clean seawater for 30 s to displace loosely bound silver from surfaces. Then, one hemolymph sample was collected from each animal by puncture of the hemolymph sinus (shrimp and sea hare) or body cavity (sea urchin). Sample was stored in a pre-weighed vial, which was immediately re-weighed to determine the tissue-wet weight. This sample was used to determine the radioactivity present in the hemolymph, and consequently the amount of silver accumulated after the exposure period, as described above.

After hemolymph collection, different tissues were dissected from each shrimp (gills, hepatopancreas, muscle and eyestalk), sea hare (gills, hepatopancreas, red muscle and abdominal ganglia) and sea urchin (egg mass, gonadal tissue, oral muscle and water vascular system for the tube feet). Immediately after dissection, each tissue sample was stored in a pre-weighed scintillation vial, which was immediately re-weighed to determine the tissue-wet weight. This sample was used for radioactivity determination, and consequently the amount of silver accumulated after the exposure period, as described above.

Silver accumulation in hemolymph and tissues was determined based on the sample radioactivity, silver specific activity

and sample wet weight. Therefore, silver accumulation was expressed as μg Ag/g wet weight.

All data obtained were expressed as mean (±1 standard deviation; $n = 6$) and were subjected to one-way analysis of variance (ANOVA) followed by the Tukey's test using the software STATISTICA version 5.1 (StatSoft Inc., Tulsa, OK). ANOVA assumptions, i.e., data normality and homogeneity of variances, were checked prior to test. If these checks failed, data were mathematically transformed using the logarithmic function. The significance level adopted was 95% ($\alpha = 0.05$).

3. Results

In the marine shrimp *P. duorarum,* silver accumulation was lowest in the muscle, followed by eyestalk, gills, hemolymph and hepatopancreas. It was similar in both silver concentrations tested (1 and 10 μg Ag/L), except in the eyestalk and the hepatopancreas, where a higher silver accumulation was observed at the higher concentration tested. Silver accumulation in the hepatopancreas was two orders of magnitude higher than in other tissues analyzed (Fig. 1).

In the sea hare *A. californica*, silver accumulation was significantly higher in all tissues from animals exposed to the higher silver concentration tested (10 μg Ag/L). At 1 μg Ag/L, silver accumulation was lowest in the hemolymph and muscle, followed by the hepatopancreas, ganglia, and gills. At 10 μg Ag/L, it was lowest in the hemolymph and muscle, followed by ganglia and gills. In this case, the hepatopancreas was the organ where the most silver was accumulated. However, silver burdens were in the same order of magnitude as observed in the other tissues analyzed (Fig. 2).

In the sea urchin *D. antillarum*, silver accumulation was lowest in the hemolymph and muscle, followed by gonadal tissue, tube feet, and egg mass. At 10 μg Ag/L, a higher silver accumulation was observed in gonadal tissue, tube feet and egg mass.

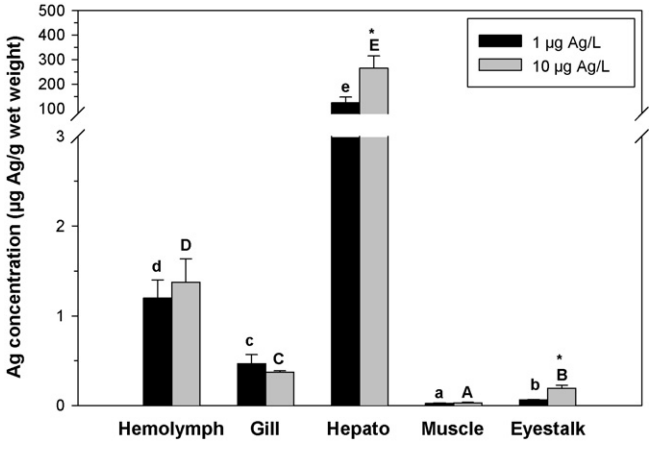

Fig. 1. Silver accumulation in different tissues of the shrimp *Penaeus duorarum* exposed for 48 h to waterborne silver (1 or 10 μg Ag/L) in seawater. Data are mean ± S.D. ($n = 6$). (*) Indicates significantly different mean values between the two silver concentrations ($P < 0.05$). Different letters indicate significant difference ($P < 0.05$) among tissues of shrimps exposed to 1 (small letters) or 10 μg Ag/L (capital letters).

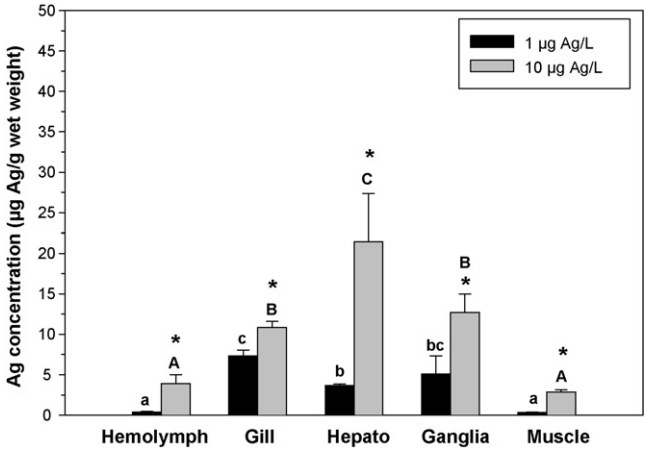

Fig. 2. Silver accumulation in different tissues of the sea hare *Aplysia californica* exposed for 48 h to waterborne silver (1 or 10 μg Ag/L) in seawater. Data are mean ± S.D. ($n = 6$). (*) Indicates significantly different mean values between the two silver concentrations ($P < 0.05$). Different letters indicate significant difference ($P < 0.05$) among tissues of animals exposed to 1 (small letters) or 10 μg Ag/L (capital letters).

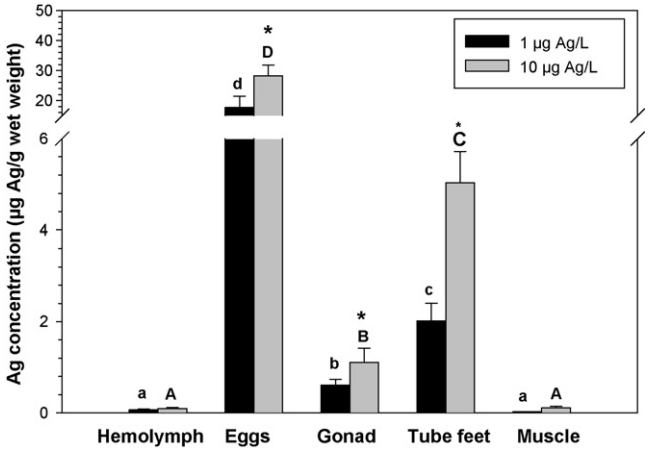

Fig. 3. Silver accumulation in different tissues of the echinoderm *Diadema antillarum* exposed for 48 h to waterborne silver (1 or 10 μg Ag/L) in seawater. Data are mean ± S.D. ($n = 6$). (*) Indicates significantly different mean values between the two silver concentrations ($P < 0.05$). Different letters indicate significant difference ($P < 0.05$) among tissues of sea urchins exposed to 1 (small letters) or 10 μg Ag/L (capital letters).

Silver burdens were one order of magnitude higher in egg mass than in other tissues analyzed (Fig. 3).

4. Discussion

In the present study, the level of silver accumulation was analyzed to verify a possible differential silver distribution in several tissues of three marine invertebrates: the shrimp *P. duorarum*, the sea hare *A. californica*, and the sea urchin *D. antillarum*. Experiments were performed using two different silver concentrations (1 and 10 μg Ag/L), which bracket the acute marine water quality criteria for silver recommended by the United States Environmental Protection Agency (USEPA, 1980) and applied in Canadian coastal waters (Warrington, 1996).

Table 1
Silver burden in gills of marine invertebrates and fish collected in the field or after waterborne silver exposure in the laboratory

Organism	Exposure concentration (μg Ag/L)	Time of exposure (days)	Silver burden (μg Ag/g)	Reference
Molluscs				
Cerastoderma glaucum	Field	Field	0.120–0.180[a]	Szefer et al. (1999)
Chlamys varia	Field	Field	0.042–0.066	Bustamante and Miramand (2005)
Aplysia californica	1.1	2	7.30	This study
	9.9	2	10.82	This study
Crustaceans				
Penaeus duorarum	1.1	2	0.47	This study
	9.9	2	0.37	This study
Fish				
Opsanus beta	2.18	1	0.002[a]	Wood et al. (2004)
	21.6	6	0.004[a]	Nichols et al. (2006)
Oligocottus maculosus	14.5	21	0.3[a]	Webb and Wood (2000)
Oncorhynchus mykiss	14.5	21	0.15[a]	Webb and Wood (2000)
Porichthys notatus	14.5	21	0.10[a]	Webb and Wood (2000)
Parophrys vetulus	14.5	21	0.08[a]	Webb and Wood (2000)
Squalus acanthius	14.5	21	0.55[a]	Webb and Wood (2000)
Raja rhina	14.5	21	0.45[a]	Webb and Wood (2000)

Data are mean values.
[a] Data estimated from graphics. When data from literature were reported based on dry weight, values were corrected for wet weight considering the mean water content in the tissue (Bianchini et al., 2005).

In the sea hare, silver accumulation after 48 h of exposure was concentration-dependent in all tissues analyzed, including hemolymph (Fig. 2). However, a concentration-dependent silver accumulation was observed only in some shrimp (Fig. 1) and sea urchin (Fig. 3) tissues after exposure to 10 μg Ag/L. These differences in the kinetics of silver saturation in internal organs could reflect differences in the mechanisms involved in silver uptake and accumulation in the three invertebrate species, which would be related to their particular anatomy and physiology. For example, it is well-known that striking differences exist among the respiratory and circulatory systems in crustaceans (shrimp), molluscs (sea hare) and echinoids (sea urchin) (Barnes et al., 1993). Differences in how animals breathe and circulate their internal fluids certainly affected both uptake and internal distribution of silver in the species studied.

The data obtained indicated that gills of the marine invertebrates studied here (shrimp and sea hare) accumulated more silver than those from marine fish after waterborne exposure in the range of silver concentration tested in the present study. Silver burden values were much higher than those found in gills of other invertebrate species naturally exposed in the field (Table 1). As far as we know, data on gill silver burden after experimental exposure in laboratory are unfortunately not available for marine invertebrates. The higher silver accumulation in gills was associated with higher hemolymph silver levels in gill-breathing species (shrimp and sea hare). Furthermore, sea urchin (Fig. 3) showed lower hemolymph silver concentrations than shrimp (Fig. 1) and sea hare (Fig. 2). Taken together, these results suggest that gills are an important route for silver uptake in marine invertebrates. In fact, it has been demonstrated that silver can be accumulated from both waterborne and dietborne exposure in marine invertebrates (Connell et al., 1991; Reinfelder and Fisher, 1991; Fisher and Wang, 1998; Wang and Fisher, 1998; Rouleau et al., 2000; Hook and Fisher, 2001; Xu and Wang, 2004; Ng et al., 2005). For example, silver body burdens in copepods are generally higher following waterborne than dietary exposure (Hook and Fisher, 2001). Under environmentally relevant conditions, 50–80% of a copepod's silver content is taken up from the dissolved phase (Wang and Fisher, 1998). Regarding effects, silver accumulation observed in shrimp and sea hare gills in the present study has been reported to induce significant inhibition of Na^+, K^+-ATPase activity without evident effects on the intracellular distribution of univalent or divalent ions, under similar experimental conditions (Bianchini et al., 2005).

The data obtained indicated that hepatopancreas of the marine invertebrates studied here (shrimp and sea hare) accumulated more silver than the liver of marine fish after waterborne exposure in the range of silver concentration tested in the present study. Shrimp values were much higher than those found in the hepatopancreas of other marine crustaceans from the field. However, those from sea hare exposed to the lower silver concentration (1 μg Ag/L) were in the range of values found in the digestive gland of other marine molluscs from the field (Table 2). As far as we know, quantitative data on digestive gland or hepatopancreas silver burden after experimental exposure in laboratory are unfortunately not available for marine invertebrates. In both shrimp (Fig. 1) and sea hare (Fig. 2), hepatopancreas silver accumulations were concentration-dependent and this organ accumulated the most silver after 48 h of exposure. In the shrimp hepatopancreas, mean silver concentrations corresponded to 123.7 and 265.1 μg Ag/g after exposure to 1 and 10 μg Ag/L, respectively. In the sea hare hepatopancreas, these values corresponded to 3.6 and 21.4 μg Ag/L, respectively. High silver accumulation in hepatopancreas or digestive gland has also been demonstrated in marine invertebrates, including crustaceans and molluscs (Greig and Wenzloff, 1978; Amiard-Triquet et al., 1991; Domouhtsidou and Dimitriadis, 2000).

Table 2
Silver burden in digestive gland (molluscs), hepatopancreas (crustaceans) or liver of marine invertebrates and fish collected in the field or after waterborne silver exposure in the laboratory

Organism	Exposure concentration (μg Ag/L)	Time of exposure (days)	Silver burden (μg Ag/g)	Reference
Molluscs				
Cerastoderma glaucum	Field	Field	0.045–0.165[a]	Szefer et al. (1999)
Chlamys varia	Field	Field	0.485–9.189	Bustamante and Miramand (2005)
Aequipecten opercularis	Field	Field	11.562	Bryan (1973)
Pecten maximus	Field	Field	1.336–2.042	Bryan (1973)
Aplysia californica	1.1	2	3.6	This study
	9.9	2	21.4	This study
Crustaceans				
Homarus americanus	Field	Field	0.066–0.333	Chou and Uthe (1978)
	Field	Field	0.120–0.360	Chou et al. (1998)
	Field	Field	0.345–3.514	Greig and Pereira (1993)
	Field	Field	0.375–1.727	Chou et al. (2000)
Cancer irroratus	Field	Field	0.044–0.764	Chou et al. (2002)
Chionoecetes opilio	Field	Field	0.48–6.77	Rouleau et al. (2000)
Penaeus duorarum	1.1	2	123.7	This study
	9.9	2	265.1	This study
Fish				
Opsanus beta	2.18	1	0.01[a]	Wood et al. (2004)
	21.6	6	0.864[a]	Nichols et al. (2006)
Oligocottus maculosus	1.5	21	0.1[a]	Webb and Wood (2000)
	14.5	21	0.2[a]	Webb and Wood (2000)
Oncorhynchus mykiss	1.5	21	0.75[a]	Webb and Wood (2000)
	14.5	21	1.45[a]	Webb and Wood (2000)
Porichthys notatus	1.5	21	0.2[a]	Webb and Wood (2000)
	14.5	21	0.2[a]	Webb and Wood (2000)
Parophrys vetulus	14.5	21	0.5[a]	Webb and Wood (2000)
Squalus acanthius	14.5	21	0.2[a]	Webb and Wood (2000)
Raja rhina	14.5	21	0.35[a]	Webb and Wood (2000)

Data are mean values.
[a] Data were estimated from graphics. When data from literature were reported based on dry weight, values were corrected for wet weight considering the mean water content in the tissue (Bianchini et al., 2005).

These findings suggest the possible involvement of the hepatopancreas in both silver accumulation and detoxification in marine invertebrates, as observed for liver in marine fish (Webb and Wood, 2000; Grosell and Wood, 2001; Hogstrand et al., 2003; Wood et al., 2004). In marine crustaceans, the involvement of the hepatopancreas in silver accumulation and detoxification seems to be more important than in the other species studied. This statement is based on the fact that silver accumulation in the shrimp hepatopancreas was two orders of magnitude higher than that observed in the other tissues analyzed, while it was in the same order of magnitude in the sea hare hepatopancreas (Fig. 1). Regarding effects, silver accumulation observed in shrimp and sea hare hepatopancreas in the present study has been reported to induce significant inhibition of Na^+, K^+-ATPase activity with associated impacts on the intracellular distribution of univalent (shrimp: Cl^- and sea hare: K^+) or divalent ions (shrimp and sea hare: Mg^{2+}), under similar experimental conditions (Bianchini et al., 2005).

It is also notable that silver accumulation was observed to occur in neuroendocrine tissues of shrimp (eyestalk, Fig. 1) and nerve tissues of sea hare (ganglia, Fig. 2). However, if silver accumulation in these tissues was associated with a sorption process was not investigated. Nevertheless, it would be helpful to conduct further studies to evaluate the possible implications of this silver accumulation on physiological functions performed by these tissues. In fact, significant inhibition of the Na^+, K^+-ATPase activity and imbalances in the intracellular concentrations of univalent and divalent ions were observed in these tissues after waterborne silver exposure under similar experimental conditions (Bianchini et al., 2005). Furthermore, behavioral effects of metals, including silver, have already been described in aquatic animals (for review, Scott and Sloman, 2004). Given that *A. californica* is a quintessential model for behavior and learning (Grosell and Walsh, 2006), it would appear to be an excellent choice for further studies of silver's effects.

Substantial levels of silver were also detected in the tube feet of the sea urchin (Fig. 3). It is known that this tissue is involved in locomotory and respiratory functions in sea urchins. As opposed to nerve tissues of shrimp and sea hare, no significant effects of waterborne silver exposure on Na^+, K^+-ATPase activity, water content, cellular volume, and intracellular concentrations of univalent and divalent ions were detected in the tube feet of sea urchin (Bianchini et al., 2005). These findings suggest that silver accumulation appears not to pose any important physiological disturbance in this tissue.

In sea urchin, egg mass accumulated more silver than other tissues analyzed. Furthermore, gonadal tissue showed substan-

Table 3
Silver burden in the muscle of marine invertebrates collected in the field or after waterborne silver exposure in the laboratory

Organism	Exposure concentration (μg Ag/L)	Time of exposure (days)	Silver burden (μg Ag/g)	Reference
Molluscs				
Cerastoderma glaucum	Field	Field	0.207–0.621[a]	Szefer et al. (1999)
Chlamys varia	Field	Field	0.005–0.131	Bustamante and Miramand (2005)
Aplysia californica	1.1	2	0.312	This study
	9.9	2	2.856	This study
Crustaceans				
Penaeus setiferus	Field	Field	0.037–0.177	Vazquez et al. (2001)
Chionoecetes opilio	Field	Field	0.065–0.650	Rouleau et al. (2000)
Penaeus duorarum	1.1	2	0.023	This study
	9.9	2	0.028	This study
Echinoids				
Diadema antillarum	1.1	2	0.025	This study
	9.9	2	0.110	This study

Data are mean values.
[a] Data were estimated from graphics. When data from literature were reported based on dry weight, values were corrected for wet weight considering the mean water content in the tissue (Bianchini et al., 2005).

tial levels of silver accumulation (Fig. 3). These results point to the need for future studies on possible reproductive effects of silver in sea urchin. In fact, ionoregulatory disturbances were observed in both egg mass and gonadal tissue of the sea urchin after exposure to waterborne silver, under similar experimental conditions (Bianchini et al., 2005). Furthermore, silver effects on reproduction are extensively reported in the literature for several groups of marine invertebrates (Calabrese and Nelson, 1974; Coglianese and Martin, 1981; Martin et al., 1981; Nelson et al., 1983; Eyster and Morse, 1984; Lussier et al., 1985; Metayer et al., 1990; Hook and Fisher, 2001; Ward and Kramer, 2002; Hellou et al., 2003; Lee et al., 2004; Ward et al., 2006a), including sea urchins (Ward et al., 2006b). Likewise, the sea urchin's importance as a model experimental system in reproduction and developmental biology make it another candidate for evaluating mechanistic effects of silver.

In all three species studied, the lowest silver concentrations were observed in muscle, being equal (sea hare) or lower (shrimp and sea urchin) than that observed in the hemolymph (Figs. 1–3). Silver accumulation after exposure to the lower silver concentration (1 μg Ag/L) is quite similar to those found in muscle from other marine invertebrates from the field (Table 3). Low levels of silver accumulation were also observed in white muscle of marine fish experimentally exposed to silver (Webb and Wood, 2000; Wood et al., 2004). These findings suggest a low potential of this tissue for trophic transfer of silver in both marine fish and invertebrates. Regarding effects, silver accumulation observed in shrimp and sea urchin muscle in the present study has been reported to induce significant inhibition of Na^+, K^+-ATPase activity without evident effects on the water content, cellular volume or intracellular distribution of univalent or divalent ions, under similar experimental conditions. On the other hand, no significant effects of silver exposure on Na^+, K^+-ATPase activity was observed in the muscle of the sea hare. However, in this case significant effects on the distribution of intracellular univalent and divalent ions were observed (Bianchini et al., 2005). These two different patterns of muscle response in the three species studied could be associated with the kind of muscles sampled. In the shrimp and sea urchin, white muscle was analyzed while in the sea hare, red muscle was employed. Physiological differences between these two kinds of muscles could be the basis for the differences observed. In fact, a higher silver accumulation was observed in the sea hare muscle (Fig. 2) than in the shrimp (Fig. 1) or sea urchin muscle (Fig. 3).

In summary, results obtained in the present study combined with those reported by Bianchini et al. (2005) clearly indicate that the gill of marine crustaceans could be considered as a good "target organ" for the extension of the BLM from freshwater to seawater. However, other additional "sites of toxicity" such as hepatopancreas and nervous system of other groups of marine invertebrates such as molluscs and echinoids should be taken into consideration in the development of a marine BLM version for silver. Furthermore, gonadal tissue and egg mass also showed a good potential as "sites of toxicity" in echinoids and future studies with these tissues would be helpful in the development of a chronic marine BLM version for silver.

Acknowledgments

We gratefully acknowledge the financial support of Kodak Canada Inc., NSERC CRD Program, NIEHS (ES05705) and Brazilian CNPq. We also wish to thank Joseph W. Gorsuch (Gorsuch Environmental Management Services, Inc. USA) for helpful suggestions. A. Bianchini is a fellow from the Brazilian CNPq (Proc. # 302734/2003-1) and C.M. Wood and P.J. Walsh are supported by the Canada Research Chair Program. We will miss Richard Playle's collegiality, enthusiasm and friendship.

References

Amiard-Triquet, C., Berthet, B., Martoja, R., 1991. Influence of salinity on trace metal (Cu, Zn, Ag) accumulation at the molecular, cellular and organism level in the oyster *Crassostrea gigas* Thunberg. Biol. Met. 4, 144–150.

Barnes, R.S.K., Calow, P., Olive, P.J.W., 1993. The Invertebrates: A New Synthesis. Blackwell Science, Oxford, UK.

Bianchini, A., Grosell, M., Gregory, S.M., Wood, C.M., 2002. Acute silver toxicity in aquatic animals is a function of sodium uptake rate. Environ. Sci. Technol. 36, 1763–1766.

Bianchini, A., Wood, C.M., 2002. Physiological effects of chronic silver exposure in *Daphnia magna*. Comp. Biochem. Physiol. 133C, 137–145.

Bianchini, A., Wood, C.M., 2003. Mechanism of acute silver toxicity in *Daphnia magna*. Environ. Toxicol. Chem. 22, 1361–1367.

Bianchini, A., Playle, R.C., Wood, C.M., Walsh, P.J., 2005. Mechanism of acute silver toxicity in marine invertebrates. Aquat. Toxicol. 72, 67–82.

Bryan, G.W., 1973. The occurrence and seasonal variation of trace metals in the scallops *Pecten maximus* (L) and *Chlamys opercularis* (L). J. Mar. Biol. Assoc. (UK) 53, 145–166.

Bryan, G.W., Langston, W.J., 1992. Bioavailability, accumulation, and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. Environ. Pollut. 76, 89–131.

Bustamante, P., Miramand, P., 2005. Subcellular and body distributions of 17 trace elements in the variegated scallop *Chlamys varia* from the French coast of the Bay of Biscay. Sci. Total Environ. 337, 59–73.

Calabrese, A., Nelson, D.A., 1974. Inhibition of embryonic development of the hard clam *Mercenaria mercenaria* by heavy metals. Bull. Environ. Contam. Toxicol. 11, 92–97.

Chou, C.L., Paon, L., Moffatt, J., Zwicker, B., 1998. Concentrations of metals in the American lobster (*Homarus americanus*) and sediments from harbours from Eastern and Southern shores and the Annapolis Basin of Nova Scotia, Canada. Canadian Tech. Rep. Fish. Aquat. Sci. 2254, ix + 69 pp.

Chou, C.L., Paon, L., Moffatt, J., 2002. Cadmium, copper, manganese, silver, and zinc in rock crab (*Cancer irroratus*) from highly copper contaminated sites in the inner Bay of Fundy, Atlantic Canada. Bull. Environ. Contam. Toxicol. 68, 885–992.

Chou, C.L., Paon, L., Moffatt, J., Zwicker, B., 2000. Copper contamination and cadmium, silver, and zinc concentrations in the digestive glands of American lobster (*Homarus americanus*) from the Inner Bay of Fundy Atlantic Canada. Bull. Environ. Contam. Toxicol. 65, 470–477.

Chou, C.L., Uthe, J.F., 1978. Heavy Metal Relationships in Lobster (*Homarus americanus*) and Rock Crab (*Cancer irroratus*) Digestive Glands. International Council for the Exploration of the Sea, CM, p. E/15.

Coglianese, M.P., Martin, M., 1981. Individual and interactive effects of environmental stress in the embryonic development of the Pacific oyster Crassostrea gigas. I. The toxicity of copper and silver. Mar. Environ. Res. 5, 13–27.

Connell, D.B., Sanders, J.G., Riedel, G.F., Abbe, G.R., 1991. Pathways of silver uptake and trophic transfer in estuarine organisms. Environ. Sci. Technol. 25, 921–924.

Domouhtsidou, G.P., Dimitriadis, V.K., 2000. Ultrastructural localization of heavy metals (Hg, Ag, Pb, and Cu) in gills and digestive gland of mussels *Mytilus galloprovincialis* (L.). Arch. Environ. Contam. Toxicol. 38, 472–478.

Eisler, R., 1996. Silver hazards to fish, wildlife, and invertebrates: a synoptic review. Biological Report, vol. 32. National Biological Service, 44 pp.

Eyster, L.S., Morse, M.P., 1984. Development of the surf clam, *Spisula solidissima* following exposure of gametes, embryos and larvae to silver. Arch. Environ. Contam. Toxicol. 13, 641–646.

Fisher, N.S., Wang, W.-X., 1998. Trophic transfer of silver to marine herbivores: a review of recent studies. Environ. Toxicol. Chem. 17, 562–571.

Fowler, B.A., Nordberg, G.F., 1986. Silver. In: Friberg, L., Nordberg, G.F., Vouk, V.B. (Eds.), Handbook on the Toxicology of Metals, vol. II. Specific Metals, Elsevier, New York, NY, pp. 521–530.

Greig, R., Pereira, J.J., 1993. Metal concentrations in American lobster and channeled whelk from two dredge spoil dump sites in Long Island Sound. Bull. Environ. Contam. Toxicol. 50, 626–632.

Greig, R.A., Wenzloff, D.R., 1978. Metal accumulation and depuration by the American oyster *Crassostrea virginica*. Bull. Environ. Contam. Toxicol. 20, 499–504.

Grosell, M., Brauner, C., Kelly, S.P., McGeer, J.C., Bianchini, A., Wood, C.M., 2002. Physiological responses to acute silver exposure in the freshwater crayfish (*Cambarus diogenes diogenes*)—a model invertebrate? Environ. Toxicol. Chem. 21, 369–374.

Grosell, M., Walsh, P.J., 2006. Benefits from the sea: sentinel species and animal models of human health. Oceanography 19, 126–133.

Grosell, M., Wood, C.M., 2001. Branchial versus intestinal silver toxicity and uptake in the marine teleost *Parophys vetulus*. J. Comp. Physiol. B 17, 585–594.

Hansen, H.J.M., Jacobsen, U., Jorgensen, J.C., Grosell, M., Hogstrand, C., Wood, C.M., 2002. Precautions in the use of 110 mAg, containing transmutation produced 109Cd, as a tracer of Ag Ag metabolism in ecotoxicology. Environ. Toxicol. Chem. 21, 1004–1008.

Hellou, J., Yeats, P., Steller, S., Gagne, F., 2003. Chemical contaminants and biological indicators of mussel health during gametogenesis. Environ. Toxicol. Chem. 22, 2080–2087.

Hogstrand, C., Grosell, M., Wood, C.M., Hansen, H., 2003. Internal redistribution of radiolabelled silver among tissues of rainbow trout (*Oncorhynchus mykiss*) and European eel (*Anguilla anguilla*): the influence of silver speciation. Aquat. Toxicol. 63, 139–157.

Hook, S.E., Fisher, N.S., 2001. Sublethal effects of silver in zooplankton: importance of exposure pathways and implications for toxicity testing. Environ. Toxicol. Chem. 20, 568–574.

Lee, C.H., Ryu, T.K., Chang, M., Choi, J.W., 2004. Effect of silver, cadmium, chromium, copper, and zinc on the fertilization of the northern Pacific asteroid *Asterias amurensis*. Bull. Environ. Contam. Toxicol. 73, 613–619.

Lussier, S.M., Gentile, J.H., Walker, J., 1985. Acute and chronic effects of heavy metals and cyanide on *Mysidopsis bahia* (Crustacea: Mysidacea). Aquat. Toxicol. 7, 25–29.

Martin, M., Osborn, K.E., Billig, P., Glickstein, N., 1981. Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvae. Mar. Poll. Bull. 12, 305–308.

McGeer, J.C., Playle, R.C., Wood, C.M., Galvez, F., 2000. A physiologically based biotic ligand model for predicting the acute toxicity of waterborne silver to rainbow trout in freshwaters. Environ. Sci. Technol. 34, 4199–4207.

Metayer, C., Amiard-Triquet, C., Baud, J.P., 1990. Species related variations of silver related bioaccumulation and toxicity to three marine bivalves. Water Res. 24, 995–1001.

Ng, T.Y-T., Amiard-Triquet, C., Rainbow, P.S., Amiard, J.C., Wang, W.-X., 2005. Physico-chemical form of trace metals accumulated by phytoplankton and their assimilation by filter-feeding invertebrates. Mar. Ecol. Prog. Ser. 299, 179–191.

Nelson, D.A., Calabrese, A., Greig, R.A., Yevich, P.P., Chang, S., 1983. Long-term silver effects on the marine gastropod *Crepidula fornicata*. Mar. Ecol. Prog. Ser. 12, 155–165.

Nichols, J.W., Brown, S., Wood, C.M., Walsh, P.J., Playle, R.C., 2006. Influence of salinity and organic matter on silver accumulation in Gulf toadfish (*Opsanus beta*). Aquat. Toxicol. 78, 253–261.

Paquin, P.R., DiToro, D.M., Santore R.S., Trevedi, D., Wu, K.B., 1999. A biotic ligand model of the acute toxicity of metals. III. Application to fish and Daphnia exposure to Ag. 822-E-99-001. Technical Report. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

Reinfelder, J.R., Fisher, N.S., 1991. The assimilation of elements ingested by marine copepods. Science 251, 794–796.

Rouleau, C., Gobeil, C., Tjälve, H., 2000. Accumulation of silver from the diet in two marine benthic predators: the snow crab (*Chionoecetes opilio*) and American plaice (*Hippoglossoides platessoides*). Environ. Toxicol. Chem. 19, 631–637.

Scott, G.R., Sloman, K.A., 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquat. Toxicol. 68, 369–392.

Schafer, M.M., 1995. Sampling and analytical techniques for silver in natural water. In: Andren, A.W., Bober, T.W. (Eds.), Proceedings of the Third International Conference on the Transport, Fate, and Effects of Silver in the Environment. University of Wisconsin Sea Grant Institute, Madison, WI, pp. 99–112.

Szefer, P., Wolowicz, M., Kusak, A., Deslous-Paoli, J., Czarnowski, W., Frelek, K., Belzunce, M., 1999. Distribution of mercury and other trace metals in the cockle *Cerastoderma glaucum* from the Mediterranean Lagoon Etang de Thau. Arch. Environ. Contam. Toxicol. 36, 56–63.

USEPA, 1980. Ambient water quality criteria for silver. EPA-440/5-80-071. Office of Water Regulations and Standards, Washington, DC.

Vazquez, F.G., Sharma, V.K., Mendoza, Q.A., Hernandez, R., 2001. Metals in fish and shrimp of the Campeche Sound, Gulf of Mexico. Bull. Environ. Contam. Toxicol. 67, 756–762.

Xu, Y., Wang, W.-X., 2004. Silver uptake by a marine diatom and its transfer to the coastal copepod *Acartia spinicauda*. Environ. Toxicol. Chem. 23, 682–690.

Wang, W.-X., Fisher, N.S., 1998. Accumulation of trace elements in a marine copepod. Limnol. Oceanogr. 43, 273–283.

Ward, T.J., Kramer, J.R., 2002. Silver speciation during chronic toxicity tests with the mysid, *Americamysis bahia*. Comp. Biochem. Physiol. 133C, 75–86.

Ward, T.J., Boeri, R.L., Hogstrand, C., Kramer, J.R., Lussier, S.M., Stubblefield, W.A, Wyskiel, D.C., Gorsuch, J.W., 2006a. Influence of salinity and organic carbon on the chronic toxicity of silver to mysids (*Americamysis bahia*) and silversides (*Menidia beryllina*). Environ. Toxicol. Chem. 25, 1809–1816.

Ward, T.J., Kramer, J.R., Boeri, R.L., Gorsuch, J.W., 2006b. Chronic toxicity of silver to the sea urchin (*Arbacia punctulata*). Environ. Toxicol. Chem. 25, 1568–1573.

Warrington, P.D., 1996. Ambient Water Quality Criteria for Silver. Ministry of Environment, Lands and Parks, Province of British Columbia Water Quality Branch. Environmental Protection Department, Victoria, BC.

Webb, N.A., Wood, C.M., 2000. Bioaccumulation and distribution of silver in four marine teleosts and two marine elasmobranchs: influence of exposure duration, concentration, and salinity. Aquat. Toxicol. 49, 111–129.

Wood, C.M., McDonald, M.D., Walker, P., Grosell, M., Barimo, J.F., Playle, R.C., Walsh, P.J., 2004. Bioavailability of silver and its relationship to ionoregulation and silver speciation across a range of salinities in the gulf toadfish (*Opsanus beta*). Aquat. Toxicol. 70, 137–157.