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Effects of Long-term Exposure to Silver or Copper on Growth, Bioaccumulation and Histopathology in the Blue Mussel *Mytilus edulis*

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ABSTRACT

The purpose of this study was to determine the long-term accumulation of either silver or copper from low concentrations in seawater by blue mussels, Mytilus edulis. *Mussels raised from eggs in the laboratory to the age of 2·5 months (approximately 4·5 mm in length) were continuously exposed to 0, 1, 5 and 10 μg/liter of either silver (nitrate) or copper (chloride) and sampled at 12, 18 and 21 months for growth studies, measurements of metal accumulation and histopathological examination.*

Whole-body soft tissues were analyzed for the presence of both silver and copper, as background levels of copper in the incoming seawater averaged 2–4 μg/liter. Mussels exposed to silver had accumulated significant amounts of silver only at the highest test concentration (10 μg/liter Ag) after 12 months, but at 18 and 21 months significant levels were accumulated at all three test concentrations. Mussels exposed to copper accumulated significant amounts of copper at 5 and 10 μg/liter Cu after all three sampling periods, but not at 1 μg/liter. Silver-exposed animals also accumulated significantly greater amounts of copper than control animals.

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In a comparative study, field-collected juvenile mussels (approximately 16·1 mm in shell length) and adult mussels (approximately 53·4 mm in shell length) were exposed for 12 months to 0, 5, 25 and 50 μg/liter silver only and subsequently sampled for metal-accumulation analyses and growth measurements. Juvenile mussels accumulated significant amounts of silver at all test concentrations, with the exception of mussels exposed to 5 μg/liter Ag for 6 months. Copper accumulation in the silver-exposed juveniles was significant only at 50 μg/liter Ag after 6 months, but at all test concentrations after 12 months. Adult mussels exposed to silver accumulated significant levels of both silver and copper, but at somewhat lower levels than juveniles.

In the growth study, silver had no effect on laboratory reared mussels at the highest concentration of 10 μg/liter tested, whereas copper at 10 μg/liter did appear to affect growth as early as 4 months after the start of experimental exposure. Field-collected juvenile mussels did show inhibition in growth after 6 months' exposure to 25 and 50 μg/liter Ag, with some growth occurring after 12 months. Adults also showed inhibition in growth after 6 months but not at 12 months.

Histopathological examination of mussels exposed to either 5 or 10 μg/liter of copper for 18 months showed changes in the digestive diverticula, gastrointestinal tract, reproductive tract and muscle tissues. These changes were more noticeable in mussels exposed to 5 μg/liter Cu than in those exposed to 10 μg/liter. Mussels exposed to silver for 21 months showed yellowish to black particulate deposition in the basement membrane and connective tissue of the various organs and tissues. Silver deposition increased with increasing test concentration.

INTRODUCTION

In recent years considerable emphasis has been placed on marine mussels as biological monitoring organisms (Majori & Petronio, 1973; Goldberg *et al.*, 1978; Stephenson *et al.*, 1979; National Academy of Sciences, 1980; Phillips, 1980). Use of the blue mussel *Mytilus edulis* as a sentinel organism for indicating levels of pollutants in coastal marine waters has been established in the 'Mussel Watch' program sponsored by the US Environmental Protection Agency (Goldberg *et al.*, 1978). This program led to a review of the literature by Kidder (1977) that provided a substantial data base on pollutant levels in bivalves throughout the world, with special emphasis on mussels; these data, however, generally refer to body burdens of pollutants in field-collected mussels exposed naturally.

Yet, because not only season and other environmental variables, but also age, size, sex, physiological condition and reproductive status may all affect pollutant accumulation in animal tissues, it becomes necessary to discover how these variables may influence accumulation, both of individual metals and in combination. Carefully controlled laboratory exposure studies can be used in an attempt to clarify some of these problem areas.

Although substantial research on mussels exposed to pollutants in the laboratory has been performed, much of it has been with exposures of relatively short duration, up to 130 days (i.e. Schulz-Baldes, 1972, 1974; Majori & Petronio, 1973; Scott & Major, 1972; George *et al.*, 1978; Sutherland & Major, 1981). The present study was designed to determine the long-term effects of either silver or copper in the blue mussel, *Mytilus edulis*, exposed continuously for 6 months or more to these metals. Mussels raised from eggs in the laboratory were exposed for 21 months to either silver or copper and were periodically measured for growth and sampled for measurements of heavy-metal accumulation or for histopathological examination. In a separate comparative study, field-collected juvenile and adult mussels were exposed to silver for 12 months for measurements of heavy-metal accumulation or growth studies.

MATERIALS AND METHODS

Laboratory raised mussels

Mussels used in the 21-month exposure studies were raised from eggs in the laboratory. Field-collected mussels were induced to spawn by thermal stimulation and the addition of sperm stripped from a sacrificed male (Loosanoff & Davis, 1963). Larvae were reared in 1-μ filtered seawater in 15-liter polypropylene buckets at 17–18 °C and $25 \pm 2‰$ salinity for approximately 4 weeks. They were daily fed a mixture of *Isochrysis galbana* and *Monochrysis lutheri* at a rate of approximately 150 000 cells per milliliter of culture water. The young juveniles were then transferred to flow-through tray systems receiving unfiltered seawater for about 6 weeks where they were allowed to attach to plexiglass plates. During this time, the mussels utilized whatever food was available in the incoming seawater.

At 2·5 months of age, the mussels, then 4·5 mm in shell length, were

transferred to 80-liter glass aquaria filled to 60 liter with seawater, which was first passed through 25- and 10-μm nylon filter bags and then through sand. An all-glass, proportional-dilution apparatus (Mount & Brungs, 1967) controlled the intermittent delivery of both toxicant-containing water and control water at a flow rate of 480 liters per tank per day and an estimated replacement time of 7 h (Sprague, 1969). Either silver (as $AgNO_3$) or copper (as $CuCl_2$) was added at concentrations of 1, 5 and 10 μg/liter to each of three test tanks with three tanks serving as controls. Silver and copper concentrations used in exposure tests refer to nominal concentrations of metal ion and do not include background concentrations of <1 μg/liter Ag and 2–4 μg/liter Cu. Mussels were first introduced into the test system on 28 June, 1978, and exposed continuously until 4 April, 1981. The ambient water temperatures during this period ranged from 2·6 to 24·0 °C and the salinity was 25 ± 2‰. The mussels utilized whatever food was available in the incoming filtered seawater.

For determination of heavy-metal accumulation, samples were taken at 12, 18 and 21 months. Both silver and copper levels were determined in whole-body soft tissues of animals from each metal-exposed group. Seven to fifteen individuals were analyzed from each test concentration and control group at each sampling period. Soft tissues from the mussels were placed in 50-ml Pyrex beakers and digested for 1 to 2 days in quartz-distilled nitric acid. After evaporating the samples to dryness, they were heated in two to three portions of 1 ml each hydrogen peroxide and taken up with 7 % (v/v) H_2O–nitric acid, filtered through Whatman No. 2 FP, and brought to a final volume of 10 ml. The nitric acid solution was analyzed directly with a graphite furnace (Perkin-Elmer Model 2100) on a Perkin-Elmer atomic absorption spectrophotometer* (Greig *et al.*, 1982).

Water samples were routinely monitored throughout the experiment to determine the metal concentrations present in the test water. Copper analyses were conducted by a cobalt precipitation method by which 200 ml samples of seawater were acidified to pH 2 followed by the addition of 1·5 ml cobalt (200 ppm) and 2·0 ml of APDC (ammonium pyrrolidine dithio carbamate). The precipitate formed was filtered through a 0·2-μ membrane filter (Biorad Labs) and dissolved in 20 % quartz-distilled nitric acid followed by analysis by atomic absorption with

* Mention of trade names does not imply endorsement by the National Marine Fisheries Service.

a graphite furnace. Silver was analyzed by the following procedure: 0·1 ml of quartz-distilled nitric acid was added to 1 ml of seawater and standards were prepared by adding 25 μl of the appropriate concentration of silver to the acidic seawater. Samples and standards were analyzed by atomic absorption with a graphite furnace. Less-than values were calculated from the lowest concentrations of metal that gave a peak twice as great as a blank of deionized water and acid. The actual values observed for the copper experiment were 3·0 ($\pm$0·9), 7·9 ($\pm$1·1) and 12·7 ($\pm$2·1). Values for the 1, 5 and 10 μg/liter exposure concentrations were 1·5 ($\pm$0·7), 5·4 ($\pm$0·9) and 10·0 ($\pm$2·0), whereas those for 5, 25 and 50 μg/liter were 5·5 ($\pm$0·9), 22·4 ($\pm$4·8) and 43·7 ($\pm$8·3).

For growth studies, at least 50 mussels were measured weekly with vernier calipers for the first 3 months and approximately monthly thereafter. Near the end of the experiment, lesser numbers of mussels were measured as many had been previously removed from the test system.

Of the laboratory reared mussels exposed to copper for 18 months, seven from each test concentration and each control group were examined for histopathological changes. Of those exposed to silver for 21 months, twenty from each of the 0, 1 and 5 μg/liter groups and fifteen from the 10-μg/liter group were similarly examined. Mussels were shucked from their shells directly into Helly's fixative. After 10–15 min, the animals were removed from the fixative, cut sagittally through the midline and returned to fixative. After overnight fixation (16–24 h), the animals were trimmed, washed in running tap water overnight, and processed into slides using Harris' hematoxylin and eosin stain as described by Yevich & Barszcz (1981).

Field-collected mussels

In a second experiment, field-collected juvenile mussels (16·1 mm in shell length, range 13·0–31·0 mm) and adult mussels (53·4 mm in shell length, range 47·7–57·0 mm) were exposed to silver, as described above, for 12 months to 0, 5, 25 and 50 μg/liter for studies of heavy-metal accumulation and growth. The experiment was set up on 24 November, 1980 and continued until 20 November, 1981. Fifty juvenile and fifteen adult mussels were placed in each of twelve aquaria; water temperatures ranged from 2·4 to 22·8 °C and salinity was 25 $\pm$ 2‰. At the start of the experiment, fifty juvenile (pooled into ten groups of five each) and fifteen adult mussels were sampled for background levels of silver and copper.

After 6 months' exposure to silver, ten pools of five juvenile mussels each from each test concentration were sampled and analyzed for silver and copper and, at 12 months, twelve juveniles from each concentration were sampled and analyzed. Ten and twelve adult mussels were sampled from each test concentration at 6 and 12 months, respectively, for metal accumulation analyses. In addition, twenty-five juvenile mussels at each test condition were measured for growth at 6 months and fifteen were measured at 12 months.

Statistical analysis

Sets of experimental body burden data were subjected to one- or two-way ANOVAS and the differences between experimental means were compared by the least significant differences (LSD) method. The differences between any two means were declared to be significant at the 0·05 level of significance if their uncertainty intervals did not overlap. The uncertainty intervals of means were calculated as $\mathrm{LSI} = \bar{y} \pm \mathrm{LSD}/2$ and graphically presented in interval plots, which are described in detail by Andrews *et al.* (1980). Either one- or two-way ANOVAS were used with the Scheffe test for contrasts to determine differences in growth of mussels exposed to either silver or copper.

RESULTS

Metal accumulation

Laboratory reared mussels exposed to silver for 21 months accumulated substantial concentrations of both silver and copper (Table 1, Fig. 1). Silver accumulation was significant only in the 10 μg/liter Ag-exposed animals within the first 12 months, but at 18 and 21 months significant concentrations were accumulated at all three test concentrations. Accumulation of copper by mussels in both the controls and silver-exposed test groups was apparently due to the presence of 2–4 μg/liter Cu in the incoming seawater. Copper concentrations were significantly higher in mussels exposed to either 5 or 10 μg/liter Ag than in control mussels at all sampling periods (Fig. 1a). Copper was accumulated to a level of 177·61 mg/kg (wet weight) in the 10 μg/liter Ag-exposed mussels at 12 months, with a progressive reduction thereafter (Table 1).

TABLE 1

Mytilus edulis. Concentrations (mg/kg, Wet Weight) of Both Silver and Copper [Mean (± Standard Deviation)] Accumulated by Laboratory Reared Mussels after 12, 18 and 21 Months' Exposure to Silver

Silver exposure concentrations (μg/liter)	*12 months*	*18 months*	*21 months*
		Silver Accumulation	
Control	0·66 (0·53)	0·32 (0·30)	0·16 (0·35)
1	1·59 (0·38)	9·75 (10·03)	7·65 (5·46)
5	2·57 (1·64)	11·90 (11·04)	7·75 (8·81)
10	15·33 (2·72)	13·91 (11·23)	10·55 (13·81)
		Copper Accumulation	
Control	3·36 (0·21)	10·78 (2·03)	3·07 (0·72)
1	7·44 (0·86)	13·56 (3·72)	6·78 (1·71)
5	19·24 (3·10)	20·47 (11·13)	12·82 (7·04)
10	177·61 (28·5)	26·17 (19·72)	9·24 (2·18)

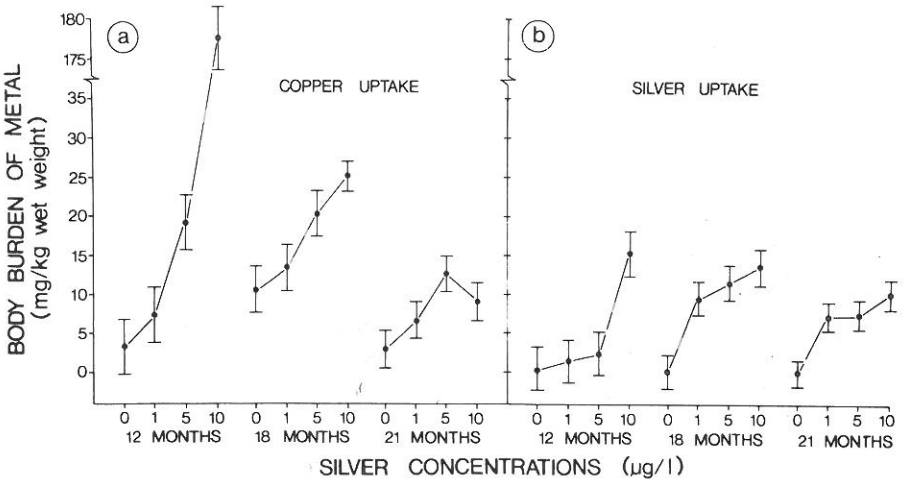

Fig. 1. *Mytilus edulis*. Concentrations (mg/kg, wet weight) of copper and silver accumulated by laboratory reared mussels after 12, 18 and 21 months' exposure to silver. Bars represent uncertainty intervals of means. Means between concentrations are significantly different ($P < 0·05$) if intervals do not overlap.

TABLE 2

Mytilus edulis. Concentrations (mg/kg, Wet Weight) of Both Silver and Copper [Mean (± Standard Deviation)] Accumulated by Laboratory Reared Mussels after 12, 18 and 21 Months' Exposure to Copper

Copper exposure concentrations (μg/liter)	*12 months*	*18 months*	*21 months*
		Silver Accumulation	
Control	0·05 (0·02)	0·59 (0·23)	0·20 (0·08)
1	0·05 (0·02)	0·16 (0·03)	0·10 (0·08)
5	0·35 (0·54)	0·16 (0·62)	0·10 (0·05)
10	0·28 (0·21)	0·42 (0·20)	0·09 (0·05)
		Copper Accumulation	
Control	3·13 (0·50)	22·51 (14·10)	5·52 (1·38)
1	6·97 (4·19)	23·19 (11·66)	5·51 (1·34)
5	17·87 (4·76)	34·90 (21·96)	19·17 (4·90)
10	41·67 (7·22)	67·61 (25·61)	62·03 (19·31)

TABLE 3

Mytilus edulis. Concentrations (mg/kg, Wet Weight) of Both Silver and Copper [Mean (± Standard Deviation)] Accumulated by Either Field-Collected Juveniles or Adults After 6 and 12 Months' Exposure to Silver

Silver exposure concentrations (μg/liter)	*Silver*			*Copper*		
	0 months	*6 months*	*12 months*	*0 months*	*6 months*	*12 months*
			Juvenile mussels			
Control	0·05 (0·03)	0·04 (0·03)	0·25 (0·12)	4·37 (1·60)	3·44 (0·60)	4·60 (1·92)
5		0·67 (0·62)	9·85 (4·48)		3·23 (0·79)	8·93 (2·00)
25		1·93 (0·93)	7·98 (3.11)		3·69 (1·03)	10·19 (2·55)
50		7·52 (3·86)	10·69 (4.31)		4·78 (1·12)	10·39 (4·85)
			Adult mussels			
Control	0·02 (0·007)	0·03 (0·02)	0·10 (0·04)	2·42 (0·47)	2·70 (0·68)	3·80 (2·78)
5		0·72 (0·84)	2·05 (1·64)		3·43 (1·51)	7·11 (3·35)
25		1·22 (1·02)	2·18 (0·89)		4·09 (3·11)	6·44 (2·83)
50		1·77 (1·62)	2·99 (1·50)		4·21 (2·11)	7·70 (3·47)

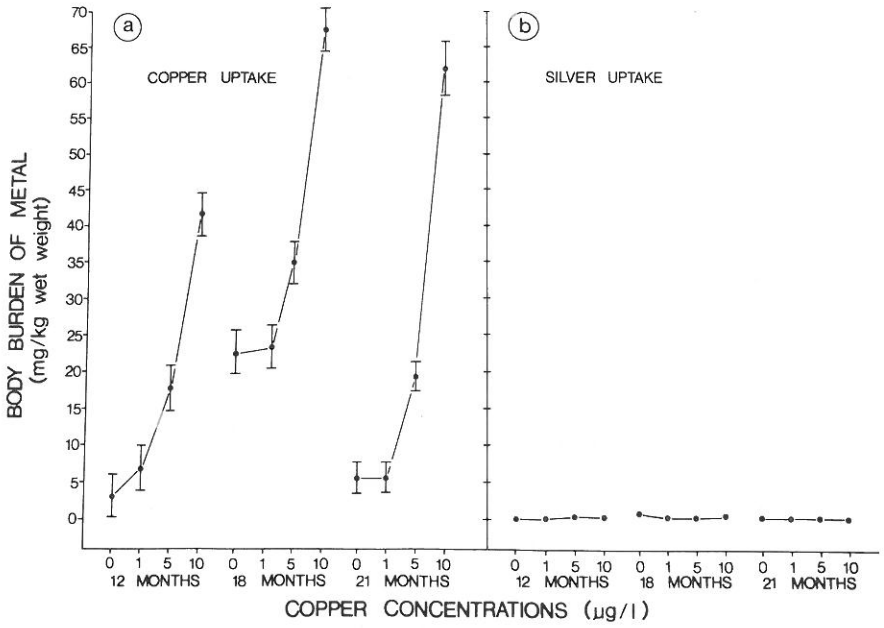

Fig. 2. *Mytilus edulis*. Concentrations (mg/kg, wet weight) of copper and silver accumulated by laboratory reared mussels after 12, 18 and 21 months' exposure to copper. Bars represent uncertainty intervals of means. Means between concentrations are significantly different ($P < 0{\cdot}05$) if intervals do not overlap.

Laboratory reared mussels exposed to copper at 5 and 10 µg/liter accumulated significant amounts of copper (Table 2, Fig. 2a), although none reached or exceeded the copper concentrations found in the silver-exposed mussels at 12 months. At any one time period, no differences in copper accumulation were noted between control mussels and those exposed to 1 µg/liter of Cu (Fig. 2a). As with silver-exposed mussels, significant concentrations of copper were accumulated by control mussels from the incoming seawater; copper concentrations increased to 22·51 mg/kg at 18 months, with a decrease to 5·52 mg/kg after 21 months. There was an apparent trend in reduction of copper concentration in both the silver and copper test groups from 18 to 21 months. Mussels exposed to copper did not accumulate significant amounts of silver (Fig. 2b). At the start of the experiment, the mussels were too small (4·5 mm) to analyze for metal body burdens, but the body burdens of metals in the larger mussels increased as the test concentrations were increased.

Field-collected juvenile mussels exposed to silver at 5, 25 and 50 µg/liter

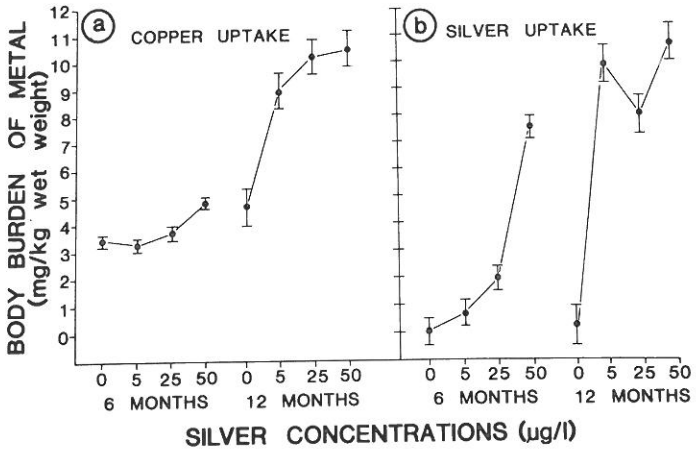

Fig. 3. *Mytilus edulis*. Concentrations (mg/kg, wet weight) of copper and silver accumulated by field-collected juveniles after 6 and 12 months' exposure to silver. Bars represent uncertainty intervals of means. Means between concentrations are significantly different ($P < 0{\cdot}05$) if intervals do not overlap.

for 6 to 12 months accumulated significant concentrations of silver at all three test concentrations, with the exception of mussels at 5 µg/liter Ag at 6 months (Table 3, Fig. 3b). Copper accumulation was significant only at 50 µg/liter Ag after 6 months' exposure, and at all three test concentrations after 12 months (Table 3, Fig. 3a). The body burdens of silver and copper for control mussels at the start of the experiment were 0·05 (SD $\pm$ 0·03) and 4·37 (SD $\pm$ 1·60) mg/kg (wet weight), respectively. The body burdens of both silver and copper increased significantly with increasing concentration from 6 to 12 months (Fig. 3a, b). At 6 months, these field-collected juvenile mussels had copper concentrations not much different than the control animals at the start of the experiment, but after 12 months the levels increased significantly (Fig. 3a).

Field-collected adult mussels exposed to silver accumulated significant levels of both silver and copper (Table 3, Fig. 4), but at somewhat lower levels than juvenile mussels. Again, as in the case of laboratory reared and field-collected juvenile mussels exposed to silver, copper levels were significantly higher in silver-exposed mussels than in the controls.

Growth

Laboratory reared mussels exposed to 0, 1, 5 and 10 µg/liter Ag grew from 4·5 mm in shell length (initial size) at 2·5 months of age to about 34·5 mm

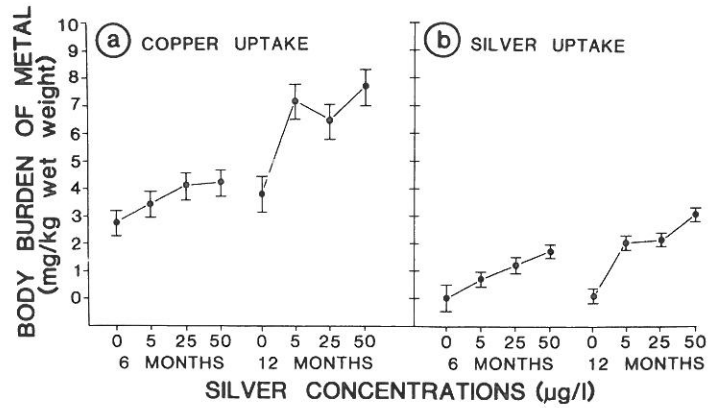

Fig. 4. *Mytilus edulis*. Concentrations (mg/kg, wet weight) of copper and silver accumulated by field-collected adults after 6 and 12 months' exposure to silver. Bars represent uncertainty intervals of means. Means between concentrations are significantly different ($P < 0{\cdot}05$) if intervals do not overlap.

after 21 months (Table 4). There were no obvious differences in growth between controls and any of the silver-exposed mussels at any of the three test concentrations. In copper-exposed mussels, a general decrease in length, although not statistically significant, was noted at 10 µg/liter (Table 4). Growth in length of mussels appeared to be affected as early as 4 months at 10 µg/liter Cu. Moreover, 10 µg/liter Cu proved to be toxic to

TABLE 4

Mytilus edulis. Growth in Shell Length (mm) and Body-Tissue Weight (g) [Mean (± Standard Deviation)] of Laboratory Reared Mussels Exposed Either to Silver or Copper for 21 Months and Measured at 12, 18 and 21 Months

Concentration	*12 months*		*18 months*		*21 months*	
(µg/liter)	*Length*	*Weight*	*Length*	*Weight*	*Length*	*Weight*
			Silver-exposed mussels			
Control	29·4 (5·2)	0·55 (0·06)	32·2 (5·2)	0·72 (0·38)	36·1 (5·1)	1·76 (0·75)
1	27·4 (6·1)	0·70 (0·09)	30·2 (5·9)	0·72 (0·27)	33·0 (5·8)	1·00 (0·47)
5	28·7 (6·3)	0·68 (0·06)	33·0 (4·6)	0·79 (0·28)	34·3 (7·6)	1·47 (0·87)
10	29·9 (5·4)	0·87 (0·07)	33·4 (5·6)	0·85 (0·03)	35·7 (5·8)	1·65 (0·71)
			Copper-exposed mussels			
Control	30·1 (5·6)	0·61 (0·07)	31·1 (3·8)	0·62 (0·25)	34·8 (5·0)	1·35 (0·66)
1	27·4 (6·6)	0·59 (0·09)	31·8 (5·8)	0·73 (0·41)	33·8 (6·3)	1·01 (0·57)
5	29·6 (5·9)	0·62 (0·16)	35·5 (5·1)	0·66 (0·45)	35·4 (5·8)	1·56 (0·87)
10	24·6 (5·6)	0·54 (0·04)	27·5 (6·8)	0·56 (0·21)	24·7 (10·6)*	2·21 (1·44)*

* Sample size—only four mussels.

TABLE 5

Mytilus edulis. Growth in Shell Length (mm) and Body-Tissue Weight (g) [Mean (± Standard Deviation)] of Field-Collected Juvenile Mussels Exposed to Silver for Either 6 or 12 Months

Concentration	*0 months*		*6 months*		*12 months*	
(μg/liter)	*Length*	*Weight*	*Length*	*Weight*	*Length*	*Weight*
0	16·2 (2·1)	0·12 (0·03)	25·1 (2·8)	0·55 (0·18)	31·6 (4·3)	0·95 (0·26)
5			26·2 (3·6)	0·63 (0·11)	34·6 (1·4)	1·01 (0·11)
25			21·7 (4·2)	0·36 (0·10)	32·5 (2·1)	1·01 (0·17)
50			16·3 (2·4)	0·13 (0·06)	26·7 (5·1)	0·84 (0·40)

mussels as only four were alive after 21 months' exposure. The apparent decrease in growth of mussels at 10 μg/litre Cu from 18 to 21 months (Table 4) may be misleading because of the small sample size (four).

There was no increase in growth of field-collected juvenile mussels exposed for 6 months to 50 μg/liter Ag, whereas the groups exposed to lower concentrations of silver, as well as the controls, did grow (Table 5). Mussels at the start of the experiment were 16·2 mm in shell length and 0·12 g in body-tissue weight; after 6 months' exposure to 50 μg/liter Ag they were 16·3 mm and 0·13 g, respectively. The controls and the 5 and 25 μg/liter Ag exposure groups increased in length and weight to 25·1, 26·2 and 21·7 mm and 0·55, 0·63 and 0·36 g, respectively. Growth of mussels at 25 μg/liter Ag was less than controls, although it was not completely inhibited as in the 50 μg/liter group. After 12 months, mussels at 50 μg/liter Ag did grow, although not so well as the controls and the other two test concentrations. Using two-way ANOVA and the Scheffe test for contrasts, we observed a significant ($P < 0·05$) decrease in both

TABLE 6

Mytilus edulis. Growth in Shell Length (mm) and Body-Tissue Weight (g) [Mean (± Standard Deviation)] of Field-Collected Adult Mussels Exposed to Silver for Either 6 or 12 Months

Concentration	*0 months*		*6 months*		*12 months*	
(μg/liter)	*Length*	*Weight*	*Length*	*Weight*	*Length*	*Weight*
0	53·4 (2·8)	4·41 (1·00)	56·5 (4·0)	4·32 (1·04)	55·3 (4·2)	3·76 (1·08)
5			51·8 (3·8)	4·08 (1·56)	53·3 (4·1)	4·09 (0·86)
25			52·5 (3·5)	3·45 (1·34)	54·3 (3.3)	3·91 (0·86)
50			48·9 (1·9)	2·71 (1·31)	51·7 (3·3)	3·63 (0·69)

length and weight of mussels exposed to increasing concentrations of silver at 6 months but not at 12 months. Adult mussels also showed statistical differences in both length and weight after 6 months' exposure to silver, but not at 12 months.

Histopathology

Copper: 5 μg/liter

Of the mussels exposed to 5 μg/liter Cu for 18 months, all seven examined showed histopathological changes in the digestive diverticula. As compared with controls (Figs 5 and 7), the digestive cells of the digestive tubules of five mussels showed loss of granules and extensive vacuolization of the cytoplasm (Figs 6 and 8). The tubules were dilated and contained a pink-staining exudate in their lumens. Two of the seven mussels had yellow and red granules in the digestive cells. All seven animals had some histopathological changes in the ducts of the digestive diverticula (Fig. 6). In most of the mussels there was a loss of cilia and erosion of the cytoplasm of the ciliated columnar cells. The ducts were lined with a non-ciliated cuboidal or squamous epithelium, instead of a

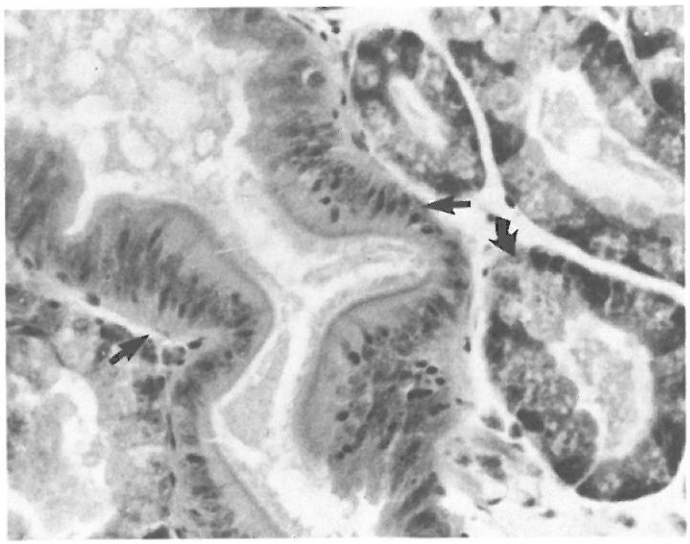

Fig. 5. *Mytilus edulis*. Digestive diverticula of a control mussel. Arrows point to duct showing ciliated columnar epithelium. Curved arrow points to normal digestive tubule. Magnification 25X.

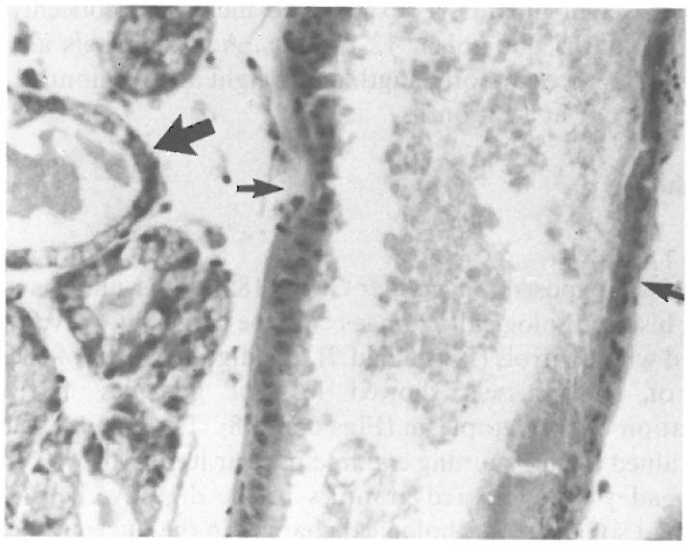

Fig. 6. *Mytilus edulis.* Digestive diverticula of a 5 μg/liter Cu-exposed mussel. Arrows point to erosion and loss of ciliated columnar epithelium. Wide arrow points to digestive tubule showing dilation of tubule and disruption of cell walls of digestive cells. Magnification 25×.

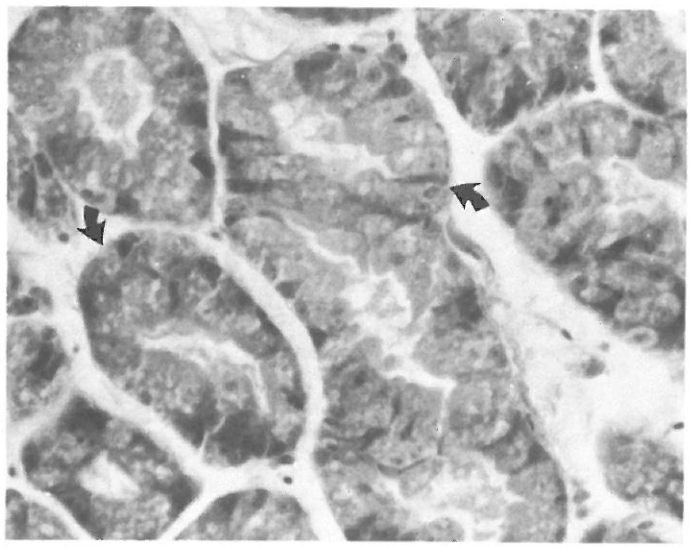

Fig. 7. *Mytilus edulis.* Digestive tubules of a control mussel. Arrows point to normal digestive tubules. Digestive cells are filled with yellowish granules. Magnification 25×.

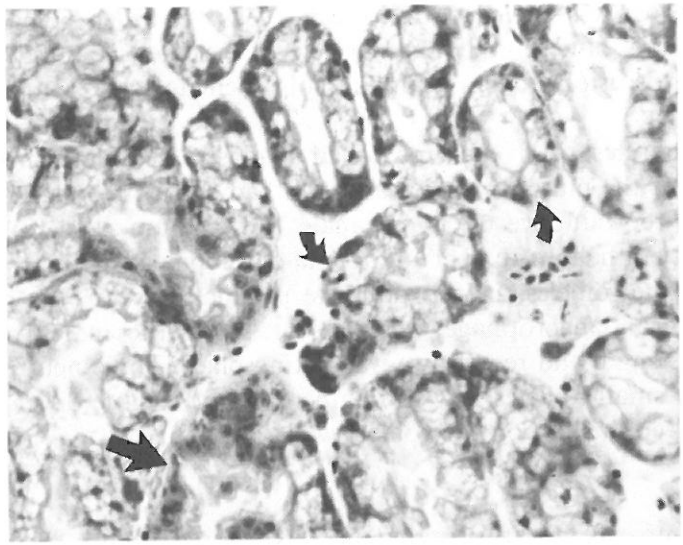

Fig. 8. *Mytilus edulis*. Digestive tubules of digestive diverticula from a 5 μg/liter Cu-exposed mussel. Curved arrows point to digestive tubules which show disruption and vacuolization of digestive cells and absence of any granules. Wide arrow points to duct which shows loss of columnar epithelium. Magnification 25×.

ciliated columnar epithelium. In the earliest stages of development of the lesion, there was desquamation of some of the epithelial cells, as well as a loss of cilia and extensive bleb formation of the cytoplasm.

The stomachs of four mussels had areas of erosion of the cytoplasm of the epithelial cells of the stomach wall; instead of a tall ciliated columnar epithelium there were cuboidal to squamous cells. The mouths of the ducts of the digestive diverticula had much of the same type of lesion.

The muscles of six of the animals showed varying degrees of myodegeneration and atrophy of the muscle bundles. In one animal calcified nodules were found among the muscle bundles.

The reproductive tract of two males had some development of the follicles, including all stages of development from germinal epithelium to spermatogonia. All other animals had very little follicular development, both in size and number of follicles present.

Copper: 10 μg/liter

Of the seven mussels exposed to 10 μg/liter Cu, one showed extensive loss of granules in the digestive cells of the digestive tubules at the digestive diverticula. The duct epithelium in this mussel was extensively eroded and

there was desquamation of the epithelial cells. All other animals had red and yellow granules in the digestive cells of the digestive tubules and had only slight erosion and desquamation of the ciliated columnar epithelium at the ducts.

The muscles of five animals had extensive myodegeneration and atrophy at the muscle bundles and two had slight myodegeneration.

In the reproductive tract, one male had medium-sized follicles with germinal epithelium to spermatogonia development in the follicles. In some of the follicles the reproductive products were in the process of resorption and macrophages were present. Three other males had follicles that were small and resorption was taking place. Three neutral animals had very small follicles, few in number, with very little development of germinal epithelium on the follicle walls.

Silver

In all groups of laboratory reared mussels exposed to silver for 21 months, there were accumulations of yellowish-brown to black particulates in the basement membrane and connective tissue of the body organs, especially around the stomach, intestine, digestive diverticula and kidney. Accumulations were such that the organs appeared to be outlined with a black or brown line. Black particulate-laden macrophages were noted throughout the connective tissue of the animals and they accumulated in large groups in the intertubular connective tissue of the digestive diverticula and the kidneys. In the 5- and 10-μg/liter-exposed mussels, the red gland cells of the pericardial gland also showed yellowish-brown to black globules and granules. Black particulate-laden macrophages were also found throughout the muscle bundles of the auricles and ventricles.

The only difference between the various groups was the amount of yellowish-brown to black particulate present. As the concentration increased, so did the amount of particulate deposition.

DISCUSSION

The purpose of this study was to examine in the laboratory the extent to which long-term exposure of blue mussels to heavy metals could be affected. In general, mussels accumulated greater levels of metals as the concentration at which they were exposed was increased (Tables 1, 2, and 3). Surprisingly, however, both laboratory reared and field-collected

juvenile and adult mussels exposed to silver accumulated significantly greater amounts of copper from the ambient seawater as the test concentrations of silver increased (Figs. 1, 3 and 4). This was particularly true after 12 months in the field-collected mussels, a pattern of increase that was evident even at 6 months (Figs 3 and 4). Copper was accumulated to a level of 177·61 mg/kg in silver-exposed mussels at 10 μg/liter, even more than was found in the copper-exposed mussels at 10 μg/liter (Tables 1 and 2). A certain period of time is apparently required for this phenomenon to manifest itself. Popham & D'Auria (1982), in a study correlating the effects of seawater concentrations on trace metal concentrations in tissues of *M. edulis*, suggested that copper concentrations in mussels were partially a function of the concentration of zinc and/or lead in the seawater, i.e. the uptake of zinc and/or lead by the mussels may have facilitated the accumulation of copper. This study has analogous findings in that, as the concentration of silver in the seawater increased, the concentration of copper in mussel tissues increased. Stenner & Nickless (1974), Phillips (1976*a*) and Lobel *et al.* (1982) have also suggested that metal interactions may occur in *M. edulis*, thereby facilitating or inhibiting metal accumulation.

In a similar study (Calabrese *et al.*, 1981; Nelson *et al.*, 1983) a parental population of the slipper limpet *Crepidula fornicata* exposed to 10 μg/liter Ag accumulated significantly greater amounts of copper than controls after 12 and 24 months, but not after 6 months. Control *Crepidula* at 12 months had body burdens of 39·9 mg/kg Cu, while those exposed to 10 μg/liter Ag had concentrations of 79·6 mg/kg. After 24 months, body burdens of copper for control and silver-exposed *Crepidula* were 100·05 and 154·52 mg/kg, respectively. Calabrese *et al.* (1981) and Nelson *et al.* (1983) also found that an F_1 population of *Crepidula* exposed to 1, 5 and 10 μg/liter Ag from hatching to 12 months of age had significantly higher concentrations of copper in the silver-exposed groups than in the controls, i.e. the controls had 103·56 mg/kg copper in their tissues, while the exposed groups had from 209·37 to 228·00 mg/kg.

Apparently, there is a preferential accumulation of copper by mussels in the presence of silver beginning at least 6 months after exposure, an increase after 12 and 18 months, and a subsequent reduction after 21 months (Figs. 1a, 3a and 4a). There was a similar reduction of copper levels in copper-exposed mussels after 21 months (Fig. 2a), although not at 10 μg/liter. The reasons for these reductions are not understood at this time. One possible explanation is that the 18-month samples were

collected in January, whereas the 21-month samples were collected in April when the mussels would have been in spawning condition and had undergone an increase in body-tissue weight. The possibility that season and reproductive state caused this decline is plausible. Phillips (1976*b*) found that mussels having reasonably high body-tissue weights had seasonally low concentrations of trace metals, and vice versa. Boyden (1974) reported similar weight-concentration relationships in *M. edulis*. Latouche & Mix (1981), Ouellette (1981) and Popham & D'Auria (1982) also found seasonal differences in metal concentrations in either *M. edulis* or *M. californianus*.

In comparing metal concentrations, particularly silver, in field-collected juvenile and adult mussels exposed to 5, 25 and 50 μg/liter Ag for 12 months, it was apparent that the juvenile mussels had attained greater concentrations than the adults (Table 3). This apparent difference, however, may have been due to the differences in body-tissue weights of the two groups. Boyden (1977) found that concentrations of zinc in *M. edulis* were greatest in the smallest individuals. Boyden & Phillips (1981) reported that metal concentrations in oysters (*Crassostrea gigas*) were related to tissue weight of the oyster which, in turn, was related to gametogenesis and spawning, i.e. as body weight increased due to gametogenic development, the metal concentrations decreased even though total body burden of metals increased. After spawning, metal concentrations increased as body-tissue weight decreased.

Growth data reported here indicate that silver had no effect on mussel growth until concentrations of at least 25 μg/liter were reached. Although no effect on mussel growth was observed after 21 months' exposure to 10 μg/liter Ag, there was a definite reduction in growth after 6 months' exposure to 25 μg/liter with no growth at 50 μg/liter. By 12 months, however, the mussels at 25 μg/liter Ag were equal in size to controls and those at 50 μg/liter had begun to grow. Copper, on the other hand, appeared to inhibit growth of mussels at 10 μg/liter, an effect observed as early as after 4 months' exposure. Effects of sublethal levels of metals on marine animals may be concentration dependent. These effects may not always be readily apparent, many requiring months before they become significantly measurable. Calabrese *et al.* (1977) found 34% inhibition in growth of larvae of the oyster (*Crassostrea virginica*) at silver and copper concentrations of 25 and 32 μg/liter, respectively, while in the hard clam (*Mercenaria mercenaria*), 34% and 48% inhibition of growth was observed at 32·4 μg/liter silver and 16·4 μg/liter copper. These observa-

tions are similar to those noted with *M. edulis*, although the effects of metals on larval stages of bivalves are not directly comparable with either juvenile or adult stages.

Study of the histopathological findings of the mussels exposed to copper showed that there was an effect on the epithelium of the ducts of the digestive diverticula, the epithelium of the stomach of the 5 μg/liter Cu-exposed animals, the muscle bundles and the reproductive tract. The histopathological changes were more extensive in the 5 μg/liter exposed animals than in the 10 μg/liter exposed animals. At present, we have no explanation for the differences between the two groups.

Whenever extensive changes were seen in the epithelium of the ducts, the digestive cells of the digestive diverticula were vacuolated and without granules. However, two of the animals of the 5 μg/liter exposure group and six of the 10 μg/liter exposure group had few changes in the epithelium of the ducts and the digestive cells were full of granules. The histopathological changes seen in the digestive diverticula indicated that the animals were not able to digest food properly. This lack of nutrients could have been a factor behind the myodegeneration and atrophy of the muscle bundles and the lack of development of the reproductive tract.

Copper seemed to have an affinity for the epithelial cells of various organs. In other histopathological studies conducted on animals exposed to various high concentrations of copper, we found the epithelium of the gills to be the target organ. In the whelk *Busycon canaliculatum* 1000 μg/liter Cu caused necrosis and sloughing of the gill epithelium and of the osphradium (Betzer & Yevich, 1975). In the hard clam *Mercenaria mercenaria* exposed to 25 and 50 μg/liter copper for 14 weeks, we observed necrosis and sloughing of the epithelium of the gills (P. P. Yevich, unpublished). Martin (1971) showed histopathological changes in the digestive tubules, gills and mantle epithelium of the Asiatic freshwater clam *Corbicula fluminer* exposed to copper at concentrations of 12 to 50 μg/liter Fujiya (1960) also observed histopathological changes in the digestive diverticula and stomach of oysters.

Histopathological findings in silver-exposed mussels were typical of those found in humans and other mammals that have absorbed organic or inorganic silver compounds. In humans, this condition is called argyria (Gettler *et al.*, 1927). There is absorption of the silver particulate onto basement membranes and various connective tissue elements of the body. As exposure continues, these tissue elements become pigmented by the silver granules. An unusual aspect noted was the extensive phagocytosis

of the colloidal silver by macrophages that accumulated in foci in the connective tissue. There was also excretion of the silver by the macrophages migrating through the epithelium of the stomach and intestine. This rôle of macrophages in accumulation and excretion of the silver was not seen in other studies performed by the authors on the gastropod *Crepidula fornicata* exposed to 1, 5 and 10 μg/liter Ag for 2 years (Nelson *et al.*, 1983).

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