

# Long-term silver effects on the marine gastropod *Crepidula fornicata*

D. A. Nelson<sup>1</sup>, A. Calabrese<sup>1</sup>, R. A. Greig<sup>1</sup>, P. P. Yevich<sup>2</sup> and S. Chang<sup>3</sup>

<sup>1</sup>National Marine Fisheries Service, Northeast Fisheries Center, Milford Laboratory, Milford, Connecticut 06460, USA

<sup>2</sup>US Environmental Protection Agency, Environmental Research Laboratory, Narragansett, Rhode Island 02882, USA

<sup>3</sup>National Marine Fisheries Service, Northeast Fisheries Center, Sandy Hook Laboratory, Highlands, New Jersey 07732, USA

**ABSTRACT:** The marine gastropod *Crepidula fornicata* was exposed for 24 mo to 1, 5, and 10  $\mu\text{g l}^{-1}$  silver as the nitrate. Chronic sublethal exposure of 120 mating pairs demonstrated some effects of silver on reproductive behavior of both a parental stock and an  $F_1$  generation. At least 5 pairs from each silver-exposed group and control group were observed for egg production and larval release. The number of spawnings per female and number and size of larvae at each release were recorded. Parental *C. fornicata* exposed to silver for 6, 12, and 24 mo were analyzed for body burdens of silver and copper.  $F_1$  *C. fornicata* exposed for 24 mo were analyzed similarly. Larval releases in the parental stock of *C. fornicata* were significantly reduced in the 10  $\mu\text{g l}^{-1}$  silver-exposure group. The average number of larvae at each release of the parental stock was higher in the 1  $\mu\text{g l}^{-1}$  group than either the controls or other exposure groups, while larval size was similar for all groups. In the  $F_1$  generation, the number of larvae released was lower in the 5  $\mu\text{g l}^{-1}$  group than in the controls. Parental *C. fornicata* accumulated significant amounts of silver over the 24-mo test period. At 24 mo, however, there was a significant reduction in silver from the 12-mo samples. Copper was also accumulated to high levels, with silver-exposed individuals accumulating significantly greater amounts than controls. The  $F_1$  generation accumulated high levels of both silver and copper. Histopathologic examination of parental *C. fornicata* exposed to 1, 5, and 10  $\mu\text{g l}^{-1}$  Ag for 24 mo showed deposition and retention of silver in the connective tissue and basement membrane of various tissues and organs.

## INTRODUCTION

Silver is a metal that has received relatively little attention in aquatic toxicity studies, particularly in the marine environment. Although it is not generally assumed to be a widespread pollutant, it does occur in industrial discharges and should be listed in any classification of highly toxic, potential pollutants (Bowen, 1966; Salla and Segar, 1979). Schutz and Turekian (1965) measured silver levels as high as 19.6  $\mu\text{g l}^{-1}$  in water samples collected from Long Island Sound at the mouth of the Housatonic River, near Milford, Connecticut, USA. Greig et al. (1977) reported sediment levels of silver ranging from 6.7  $\mu\text{g g}^{-1}$  (dry weight) to non-detectable limits ( $<0.3 \mu\text{g g}^{-1}$ ) in Long Island Sound, with the greatest concentrations found at the western end of the Sound. Greig et al. (1975) measured levels of silver as high as 12.1 ( $\pm 4.5$ )  $\mu\text{g g}^{-1}$  (dry wt) in American oysters *Crassostrea virginica* from the Housatonic River and Greig (1975) reported silver levels of 30  $\mu\text{g}$

$\text{g}^{-1}$  (wet wt) in digestive diverticula of the channeled whelk *Busycon canaliculatum* from Long Island Sound.

In acute toxicity studies with embryos of *Crassostrea virginica*, Calabrese et al. (1973) found the toxicity of silver to be similar to that of mercury, i.e. the  $\text{LC}_{50}$  values for survival were 5.8 and 5.6  $\mu\text{g l}^{-1}$ , respectively. Calabrese and Nelson (1974) reported the  $\text{LC}_{50}$  value for embryos of the hard clam *Mercenaria mercenaria* exposed to silver to be 21.0  $\mu\text{g l}^{-1}$ . Larvae of the marine gastropod *Crepidula fornicata* exposed to silver for 12 d had an  $\text{LC}_{50}$  value of 22.0  $\mu\text{g l}^{-1}$  (Nelson, 1978). These studies provided a further focus for determining the toxic effects of silver on marine organisms (Calabrese et al., 1977; in press).

Silver can be concentrated by marine organisms either directly from the water or through their food. Although adult marine animals may not be noticeably affected by a relatively brief exposure to low levels of silver, long-term exposure (a year or more) might eventually affect some other stage of their life cycle or their

offspring. Of the numerous studies of heavy-metal effects on specific life stages of marine animals, few have been devoted to an entire life cycle and to succeeding generations (Nimmo et al., 1977; Reish and Carr, 1978; Tyler-Schroeder, 1979). This is especially true of molluscs. Calabrese et al. (in press) considered those molluscs that would be practical to work with from the standpoint of local abundance, size, sedentariness, short generation time, fecundity, and ease of culture in the laboratory when considering entire life-cycle studies. With the marine snail *Crepidula fornicata*, as recommended by Calabrese and Rhodes (1974), research could be performed on the sublethal effects of contaminants on viability, growth, and reproduction in succeeding generations. Because of these advantages, Calabrese et al. (in press) studied the sublethal effects of silver to *C. fornicata* held for 12 mo in a chronic exposure system. This report is a continuation of that study, documenting fecundity, larval survival and growth, viability of succeeding generations, and metal uptake after a 24-mo exposure. The parental stock was also examined histopathologically.

## MATERIALS AND METHODS

Adult *Crepidula fornicata* were collected by dredge from commercial oyster beds in Long Island Sound off Norwalk, Connecticut, USA, and acclimated in the laboratory for 2 wk before experimental use. Mated pairs were then allowed to attach to a 5 × 10 cm Plexiglas\* plate. Ten plate-attached pairs were suspended by glass hooks in each of twelve 80-l glass aquaria filled to 60 l with seawater, which was first passed through 25- and 10- $\mu$ m nylon filter bags and then through sand. A proportional-diluter apparatus (Mount and Brungs, 1967) controlled the continuous delivery of silver-containing water and control water at a flow rate of 1 l to each tank every 3 min throughout the test period, providing a flow of 480 l tank<sup>-1</sup> d<sup>-1</sup> and an estimated 90 % replacement time of 7 h (Sprague, 1969). Silver nitrate (AgNO<sub>3</sub>) was added at nominal concentrations of 1, 5, or 10  $\mu$ g l<sup>-1</sup> Ag to each of 3 test tanks, with 3 tanks serving as controls. Silver concentrations refer to calculated concentrations of silver ion in solution; background concentrations, which were less than 1  $\mu$ g l<sup>-1</sup>, are not included in the results. Weekly measurements were made of silver concentrations in the test water throughout the entire 2-yr experiment. The actual values of silver concentrations were nearly identical to the nominal values introduced, i.e.

the average values throughout the experiment were 1.34 ( $\pm$  .40), 4.97 ( $\pm$  1.14), and 10.93 ( $\pm$  2.39)  $\mu$ g l<sup>-1</sup>.

Plexiglas plates with attached *Crepidula fornicata* were first introduced into the test system on June 20, 1978, and exposed continuously until June 30, 1980. Water temperatures during this period ranged from 4.5° to 25.0 °C and the salinity was 25  $\pm$  2 ‰ S. The test animals utilized whatever food was available in the seawater source and were supplementally fed a limited amount of cultured unicellular algae once a week. The mean length of adult females used in this study was 33.6 mm (range: 28.7 to 41.2 mm). After copulation and fertilization, the females produced yellow egg sacs which, by adhering to the Plexiglas plates, allowed observation of the developing larvae. When the egg sacs of individual females appeared brown to gray in color (an indication that larval release was imminent), the Plexiglas plate, with attached mating pair, was transferred to a 3-l polypropylene container with seawater at the same concentration of silver as the tank from which it was removed, to allow for the release and retention of veliger larvae from a single female. After each individual larval release, the mated pair was returned to its original test tank for further egg production. The number of larval releases for each of 10 females from each test concentration of silver and controls was recorded for the entire test period. The larvae released by each female in the 3-l containers were mixed to a uniform suspension by gentle aeration and then counted by taking 20 subsamples of 5 ml each, which were then individually counted, averaged, and the average multiplied by a factor of 600 to determine total number of larvae released in the container. Fifteen larvae were then measured to determine their size at release.

F<sub>1</sub> larvae from each release of a single mating pair of the parental stock at each test concentration and control were held in a 3-l polypropylene container at the same silver-exposure concentrations as the parents and reared at ambient temperatures (15 to 25 °C) and 25  $\pm$  2 ‰ S for further experimentation. Each culture was changed daily and fed a mixture of cultured unicellular algae at a rate of approximately 150,000 cells ml<sup>-1</sup>. The larvae were reared through metamorphosis and the percentage that set was determined. The larvae were set on Plexiglas plates as described above and were suspended and reared as juveniles in 3-l containers until they grew large enough to be placed in a proportional diluter. After being placed in the diluter, they were maintained at ambient seawater conditions (4.5 to 25.0 °C and 25  $\pm$  2 ‰ S) and at the same silver concentrations in which they were hatched. F<sub>1</sub> juveniles were then reared to adulthood and a maximum of 10 mated pairs from each release of the single mated pair of parents being followed from

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

each test concentration was retained to observe fecundity.

For determination of metal uptake in parental *Crepidula fornicata*, samples were taken at 6, 12, and 24 mo. Similarly,  $F_1$  *C. fornicata* were sampled at 24 mo. Both silver and copper concentrations were determined in whole-body soft tissues of animals from each test concentration, with the exception of copper analyses in parents at 1 and  $5 \mu\text{g l}^{-1}$ . Analyses for copper were made since background concentrations in the incoming seawater averaged 2 to  $4 \mu\text{g l}^{-1}$ . Soft tissues were removed from the shell and digested in nitric acid, followed by small amounts of hydrogen peroxide. The residues were taken up in 5% nitric acid and analyzed with a Perkin-Elmer HGA 400 graphite furnace on a Perkin-Elmer atomic absorption spectrophotometer (Greig et al., 1982).

Parental *Crepidula fornicata* exposed to silver for 24 mo were sampled for histopathologic examination. *C. fornicata* were shucked from their shells directly into Helly's fixative. After 10 to 15 min, the snails were removed from the fixative, cut sagittally through the midline and returned to fixative. After overnight fixation (16 to 24 h), the *C. fornicata* were trimmed, washed in running tap water overnight, and processed into slides as described by Yevich and Barszcz (1981).

Metal uptake data were subjected to analysis of variance for determining significant source of variation, and differences between experimental means were compared by the least significant differences (LSD) method. The differences between any 2 means are declared to be significant at the 0.05 level if their uncertainty intervals do not overlap. Uncertainty intervals of means were calculated as  $LSI = \bar{y} \pm LSD/2$  and graphically presented in interval plots, which are described in detail by Andrews et al. (1980). The number of larval releases was compared using Chi-square, and the differences between controls and means for the number of larvae released, and size and

age of  $F_1$  *Crepidula fornicata* at first larval release were compared by analysis of variance, multiple comparison, and *t*-tests.

## RESULTS

### Life history effects

After exposure of the parental stock of *Crepidula fornicata* to silver for 24 mo the number of larvae released was significantly reduced ( $P < 0.01$ ) in the  $10 \mu\text{g l}^{-1}$  Ag exposed group as compared to the controls. There were 92 releases of larvae by 10 females in the control group and 52 releases by 10 females in the  $10 \mu\text{g l}^{-1}$  group. Larval releases in the 1 and  $5 \mu\text{g l}^{-1}$  groups were 68 and 94, respectively (Table 1). The observed frequencies of the number of abortions were almost evenly proportioned over 4 concentrations. Although the number of abortions ranged only from 24 to 27, the proportions of abortions over the combined total number of releases and abortions (percent abortions) between concentrations appeared to be different (Table 1). We could not, however, reject the hypothesis of equal proportion between controls and the 3 test concentrations by using arcsine angular transformation for these proportions. For the number of larvae released, the concentration of silver used was a main source of variation ( $P < 0.015$ ), followed by the biological contributions of individual pairs ( $P < 0.02$ ). It is not clear why the number of larvae exposed to  $1 \mu\text{g l}^{-1}$  significantly differed ( $P < 0.01$ ) from controls, whereas results from higher levels of Ag exposure did not. Larval size was similar for each group.

Table 2 shows the size and age at which  $F_1$  females exposed continuously to silver from egg to adulthood began to spawn. The generation times, from egg to adulthood and first spawning, of females at 1 and  $5 \mu\text{g l}^{-1}$  were significantly different ( $P < 0.025$ ) from the controls. The age of females exposed to  $10 \mu\text{g l}^{-1}$  was also

Table 1. *Crepidula fornicata*. Number of larval releases and abortions, and number and size of larvae at release of parental stock exposed to silver for 24 mo

Concentration ( $\mu\text{g l}^{-1}$ )	Number of releases (pairs)	Number of abortions	Percent abortions No. abortions No. releases + abortions	Mean number (range) of larvae at release	Mean size ( $\mu\text{m}$ ) of larvae at release (number of releases)
Controls	92 (10)	26	$\frac{26}{118} = 22.03$	5,170 (60–26,550)	393.9 (86)
1	68 (10)	27	$\frac{27}{95} = 28.42$	7,100 (150–25,379)*	399.1 (62)
5	94 (10)	26	$\frac{26}{120} = 21.67$	5,303 (80–20,220)	401.0 (84)
10	52 (10)*	24	$\frac{24}{76} = 31.58$	4,874 (400–15,960)	400.4 (47)

\* Significantly different ( $P < 0.01$ ) from controls

Table 2. *Crepidula fornicata*. Mean length (range) and mean age (range) of  $F_1$  progeny exposed to silver from egg to first larval release

Concentration ( $\mu\text{g l}^{-1}$ )	Number of females	Mean length in mm (range)	Mean age in d (range)
Control	18	23.8 (19.0–31.0)	300 (240–377)
1	21	23.4 (17.5–29.5)	326 (260–388)**
5	19	20.4 (18.0–26.3)*	271 (218–240)**
10	5	23.0 (20.4–29.5)	235 (158–356)*

\* Significantly different ( $P < .005$ ) from controls  
 \*\* Significantly different ( $P < 0.025$ ) from controls

Table 3. *Crepidula fornicata*. Number of larval releases and abortions, and number and size of larvae at release of an  $F_1$  generation exposed to silver continuously from egg to adulthood

Concentration ( $\mu\text{g l}^{-1}$ )	Number of releases (pairs)	Number of abortions	Percent abortions No. abortions No. releases + abortions	Mean number (range) of larvae at release	Mean size ( $\mu\text{m}$ ) of larvae at release (number of releases)
Controls	139 (18)	8	$\frac{8}{147} = 5.44$	4,721 (90–29,490)	417.6 (129)
1	154 (20)	17	$\frac{17}{171} = 9.94$	3,992 (360–11,100)	406.7 (112)
5	97 (13)	38*	$\frac{38}{135} = 28.15^*$	3,044 (310–9,900)**	413.5 (51)
10	36 (5)	12*	$\frac{12}{48} = 25.00^*$	4,260 (670–13,110)	393.4 (29)

\* Significant ( $P < 0.001$ ) increase in number of abortions per individual when compared to controls  
 \*\* Significantly different ( $P < 0.025$ ) from controls

Table 4. *Crepidula fornicata*. Percentage of larvae of representative broods of  $F_1$  progeny exposed to silver at various concentrations that metamorphosed and set, time to setting, and size at setting

Release number	Number of larvae	Number set	Percent set	Time to set (d)	Size ( $\mu\text{m}$ )
Control					
1	6720	303	4.5	21	984
2	8460	52	0.6	22	1400
3	6110	362	5.9	18	1100
4	2580	233	9.0	45	1700
1 $\mu\text{g l}^{-1}$					
1	6300	95	1.5	42	1200
2	5340	212	4.0	27	1400
5 $\mu\text{g l}^{-1}$					
1	2390	129	5.4	22	1500
2	1980	77	3.9	76	1800
10 $\mu\text{g l}^{-1}$					
1	5100	30*	6.0*	—	—
2	5520	3*	0.6*	32	1400
3	4080	22*	4.4*	32	1600

\* Figures based on retention of only 500 larvae at release and not total release as with others

Table 5. *Crepidula fornicata*. Levels ( $\mu\text{g g}^{-1}$ , wet wt) of silver [mean ( $\pm$  S. D.)] accumulated after 6-, 12-, and 24-mo exposures to silver. Total samples (T); males (M); females (F); number of samples (N)

Exposure concentration ( $\mu\text{g l}^{-1}$ )	0 Mo	N	6 Mo	N	12 Mo	N	24 Mo	N
Control	T 0.84 (0.42)	19	T 1.09 (0.46)	7	T 2.80 (1.20)	10	T 2.22 (1.73)	14
	M 0.79 (0.53)	10	M 1.00 (0.33)	2	M 3.72 (1.24)	5	M 1.52 (0.24)	4
	F 0.89 (0.28)	9	F 1.13 (0.53)	5	F 2.08 (0.19)	5	F 2.57 (2.05)	10
1			T 16.11 (3.86)	9	T 34.00 (18.18)	10	T 8.02 (7.33)	14
			M 17.67 (3.21)	4	M 45.72 (17.77)	5	M 8.66 (11.28)	5
			F 14.87 (4.21)	5	F 22.28 (9.20)	5	F 7.66 (4.84)	9
5			T 54.07 (22.30)	10	T 44.90 (22.06)	10	T 5.36 (3.15)	15
			M 57.20 (26.49)	5	M 53.82 (22.11)	5	M 3.60 (1.14)	5
			F 50.94 (19.84)	5	F 36.06 (20.23)	5	F 6.23 (3.50)	10
10			T 86.71 (44.50)	11	T 70.70 (69.75)	10	T 7.53 (3.30)	14
			M 101.32 (62.76)	5	M 114.06 (74.68)	5	M 10.25 (4.35)	5
			F 74.53 (20.30)	6	F 27.28 (25.71)	5	F 6.44 (2.19)	9

significantly different ( $P < 0.005$ ) from controls. Females at  $5 \mu\text{g l}^{-1}$  were significantly ( $P < 0.005$ ) smaller (20.4 mm) than controls (23.8 mm) upon first larval release.

The number of larval releases and abortions, and the number and size of larvae released by the  $F_1$  females from first release and for 18 mo thereafter are shown in Table 3. The number of releases per female at all test concentrations showed no significant differences from controls. The proportion of abortions over the combined total of releases and abortions (or percent abortions) increased with increasing Ag concentrations. The percent of abortions approximately tripled from  $1 \mu\text{g l}^{-1}$  to 5 and  $10 \mu\text{g l}^{-1}$ . Tests using arcsine angular transformation between the control group and the 5 and  $10 \mu\text{g l}^{-1}$  groups were highly significant ( $P < 0.001$ ). The number of larvae released by the  $F_1$

group also revealed that metal concentration levels were the main source of variation ( $P < 0.005$ ), followed by the factor of biological contribution of individual pairs ( $P < 0.025$ ). The mean number of larvae released was significantly ( $P < 0.025$ ) lower in the  $5 \mu\text{g l}^{-1}$  group compared to the controls. No differences were found in larval size at release for  $F_1$  *Crepidula fornicata*.

The percentage of  $F_1$  larvae that survived and settled is shown in Table 4. Only a small percentage of the larvae released actually settled and survived. This was true both for control and silver-exposed snails.

### Metal uptake

After 6-, 12-, or 24-mo exposure, the parental stock of *Crepidula fornicata* accumulated significant ( $P < 0.05$ )

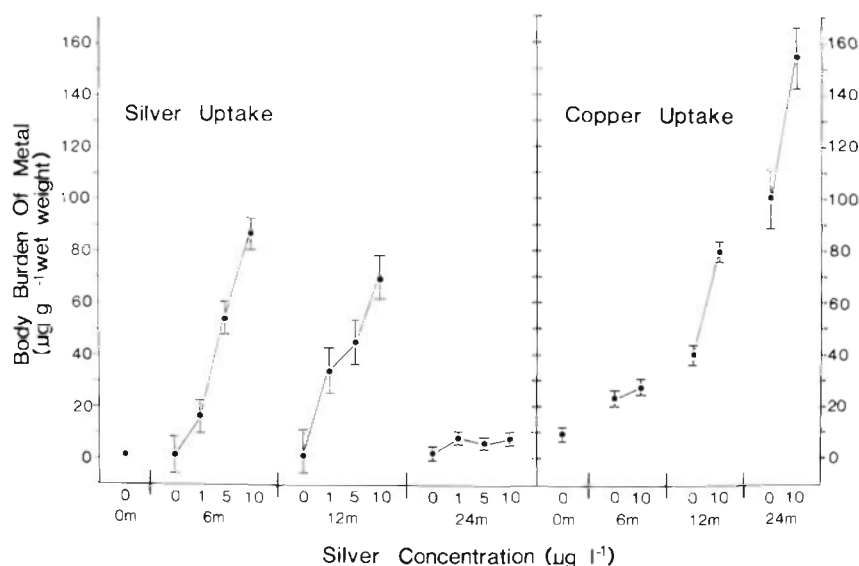


Fig. 1. *Crepidula fornicata*. Total concentrations ( $\mu\text{g g}^{-1}$ , wet weight) of silver and copper accumulated by parents (male and female combined) after 6-, 12-, and 24-mo exposures to silver. Bars: uncertainty intervals of means. Means are significantly different ( $P < 0.05$ ) if intervals do not overlap

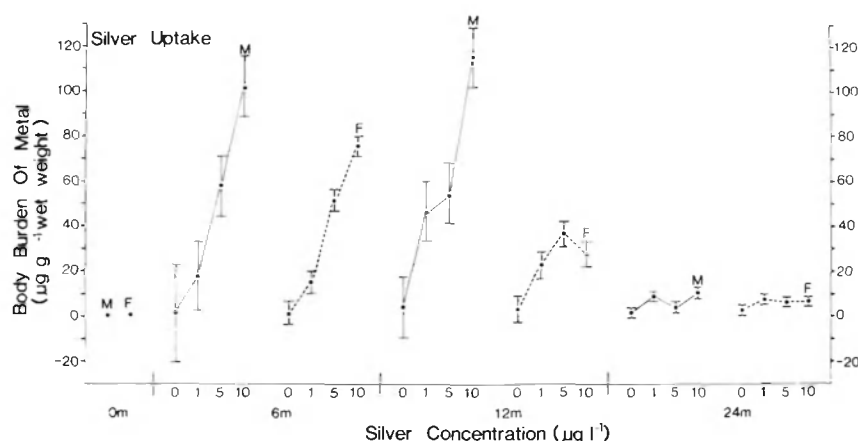


Fig. 2. *Crepidula fornicata*. Concentrations ( $\mu\text{g g}^{-1}$ , wet weight) of silver accumulated by either parental males (M) or females (F) after 6-, 12-, and 24-mo exposures to silver. Bars: uncertainty intervals of means. Means are significantly different ( $P < 0.05$ ) if intervals do not overlap

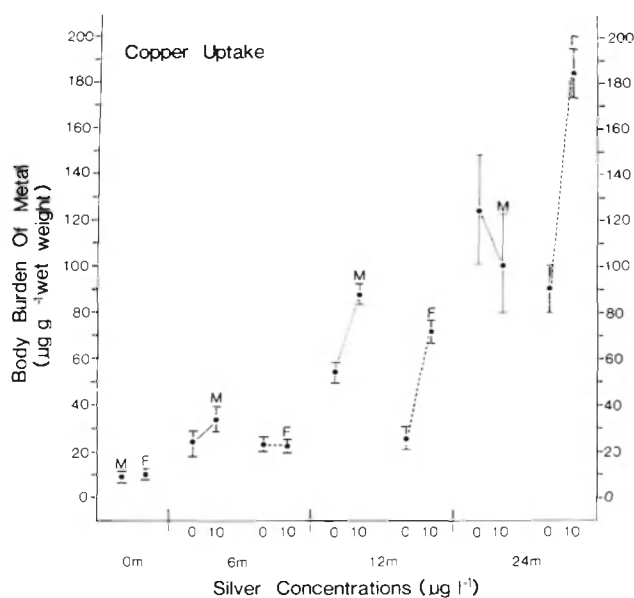


Fig. 3. *Crepidula fornicata*. Concentrations ( $\mu\text{g g}^{-1}$ , wet weight) of copper accumulated by either parental males (M) or females (F) after 6-, 12-, and 24-mo exposures to silver. Bars: uncertainty intervals of means. Means are significantly different ( $P < 0.05$ ) if intervals do not overlap

amounts of silver in whole-body tissues at all 3 test concentrations (Fig. 1). At 12 mo silver was still being accumulated by the  $1 \mu\text{g l}^{-1}$  group, whereas no increase was evident between 6 and 12 mo in the 5 and  $10 \mu\text{g l}^{-1}$  groups (Table 5). After 24 mo silver concentrations were significantly reduced (Table 5, Fig. 1). Males at the  $10 \mu\text{g l}^{-1}$  exposure concentration accumulated significantly larger amounts of silver in their tissues than females (Fig. 2). Moreover, there was a significant decrease ( $P < 0.05$ ) in silver concentrations in tissues of females in the 5 and  $10 \mu\text{g l}^{-1}$  exposure groups from the 6- to 12-mo exposure period and a further significant decrease ( $P < 0.05$ ) in both males and females at all test concentrations from 12 to 24 mo. For silver accumulation with Ag exposures, the concentration of silver tested was the most significant source of variation, followed by the factors of time and sex. Each factor was significant ( $P < 0.005$ ) for silver accumulation in the tissues of both sexes over the 6-, 12-, and 24-mo exposure periods at 1, 5, and  $10 \mu\text{g l}^{-1}$  Ag.

In addition to silver analyses in tissues of silver-exposed snails, analyses for copper levels were performed on the controls and  $10 \mu\text{g l}^{-1}$  group. As copper in the incoming seawater averaged 2 to  $4 \mu\text{g l}^{-1}$ , we were interested in knowing whether uptake of copper

Table 6. *Crepidula fornicata*. Levels ( $\mu\text{g g}^{-1}$ , wet wt) of copper [mean ( $\pm$  S.D.)] accumulated after 6-, 12-, and 24-mo exposures to silver. Total samples (T); males (M); females (F); number of samples (N)

Ag exposure concentration ( $\mu\text{g l}^{-1}$ )		0 Mo	N	6 Mo	N	12 Mo	N	24 Mo	N
Control	T	9.37 (6.15)	19	T 23.11 (9.43)	7	T 39.93 (18.1)	10	T 100.05 (44.23)	14
	M	8.88 (6.01)	10	M 23.12 (12.24)	2	M 54.14 (14.1)	5	M 124.33 (48.35)	4
	F	9.91 (6.61)	9	F 23.10 (7.08)	5	F 25.72 (5.5)	5	F 90.33 (40.99)	10
10	T			T 27.56 (14.85)	11	T 79.55 (16.1)	10	T 154.52 (61.00)	14
	M			M 34.00 (18.22)	5	M 87.74 (9.8)	5	M 101.24 (30.19)	5
	F			F 22.17 (9.94)	6	F 71.36 (17.8)	5	F 184.12 (53.21)	9

would occur. Copper was accumulated by control snails from a concentration of  $9.37 \mu\text{g g}^{-1}$  (wet wt) at the start of the experiment to a concentration of  $100.05 \mu\text{g g}^{-1}$  (wet wt) over the 24-mo test period (Table 6). For copper accumulation with silver exposures, the duration of exposure was the most dominant factor ( $P < 0.005$ ), followed by the concentration of silver tested. Sex, apparently, did not influence copper accumulation. There was no difference in copper uptake between the controls and  $10 \mu\text{g l}^{-1}$  exposure group for either sex after 6 mo; however, at 12 and 24 mo, copper was significantly higher in the  $10 \mu\text{g l}^{-1}$  Ag exposed group than in the controls (Fig. 1). Although there were significant differences in uptake of copper by males and females, no particular pattern of uptake occurred in either sex (Table 6, Fig. 3).

Table 7. *Crepidula fornicata*. Levels ( $\mu\text{g g}^{-1}$ , wet wt) of silver and copper [mean ( $\pm$  S. D.)] accumulated by  $F_1$  progeny after 24-mo exposure to silver. Total samples (T); males (M); females (F); number of samples (N)

Exposure concentration ( $\mu\text{g l}^{-1}$ )		Silver	N		Copper
Control	T	0.59 (0.32)	24	T	103.56 (36.17)
	M	0.66 (0.43)	9	M	88.44 (32.02)
	F	0.55 (0.25)	15	F	113.29 (36.38)
1	T	4.47 (3.49)	12	T	207.50 (46.29)
	M	5.60 (5.19)	3	M	180.66 (7.09)
	F	4.10 (3.06)	9	F	216.44 (50.73)
5	T	10.00 (1.90)	8	T	209.37 (40.40)
	M	9.85 (2.62)	2	M	226.50 (77.07)
	F	10.05 (1.92)	6	F	203.66 (30.68)
10	T	10.80 (6.60)	4	T	228.00 (69.66)
	M	14.20	1	M	210.00
	F	9.67 (7.59)	3	F	234.00 (84.04)

Whole-body analyses for silver and copper were performed on the  $F_1$  generation of *Crepidula fornicata* exposed to silver from hatching to 24 mo of age. Significant concentrations ( $P < 0.05$ ) of silver were accumulated by *C. fornicata* at all 3 test concentrations with more silver being accumulated at the 5 and  $10 \mu\text{g l}^{-1}$  exposure levels than at  $1 \mu\text{g l}^{-1}$  (Table 7, Fig. 4). Copper was also accumulated from the ambient seawater by both control and exposed snails, but with significantly higher concentrations ( $P < 0.05$ ) accumulated by the silver-exposed groups (Fig. 4). No particular pattern in uptake of either silver or copper was observed for either sex.

### Histopathology

The histopathologic examination of parental *Crepidula fornicata* exposed to 1, 5, and  $10 \mu\text{g l}^{-1}$  Ag for 24 mo showed deposition and retention of silver in the connective tissues and basement membranes of the various tissues and organs. The greater accumulation of silver especially noted in the 5 and  $10 \mu\text{g l}^{-1}$  Ag exposed individuals tends to cause a condition of silver poisoning called argyria.

At  $1 \mu\text{g l}^{-1}$  all snails showed the basement membrane and connective tissue of the digestive gland and stomach to contain numerous brown and black granules. At low magnification the digestive gland appeared to be outlined by a black band (Figs. 5, 6).

At  $5 \mu\text{g l}^{-1}$  all snails revealed much the same picture as those at  $1 \mu\text{g l}^{-1}$ ; however, the band of black was much more intense in color and wider in width and also the basement membrane and connective tissue of the intestine were involved. The connective tissue of the visceral mass, kidney, heart, gills, reproductive tract, and basement membrane of the epithelium of the

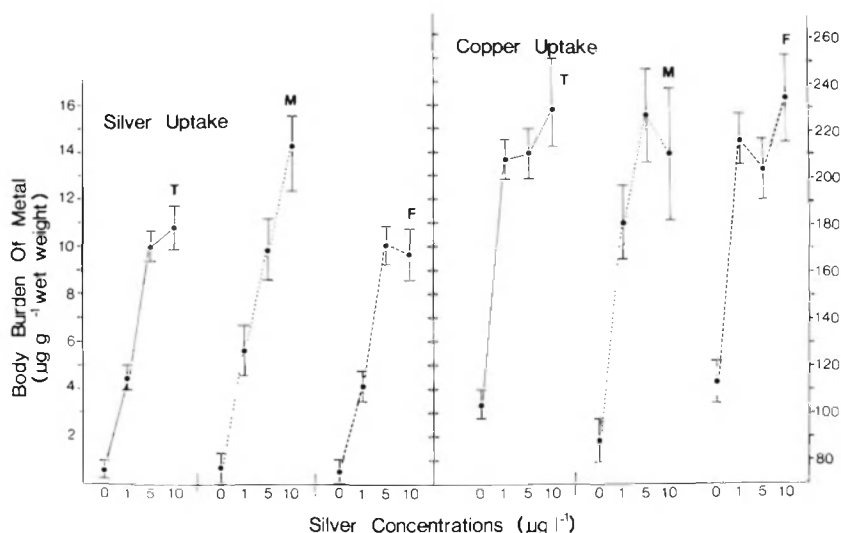
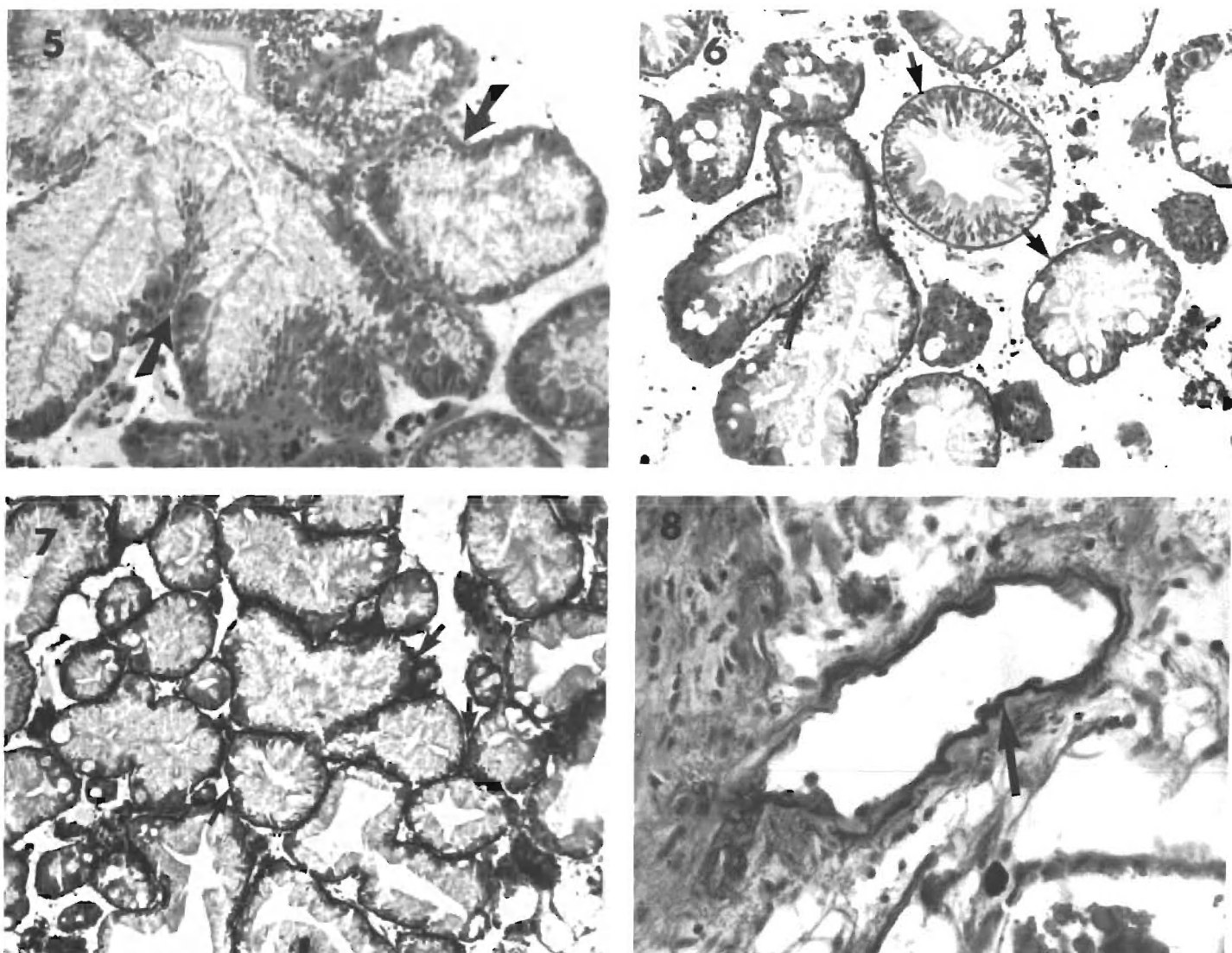


Fig. 4. *Crepidula fornicata*. Concentrations ( $\mu\text{g g}^{-1}$ , wet weight) of silver and copper accumulated by  $F_1$  progeny after 24-mo exposure to silver. Total (T); males (M); females (F)





Figs. 5. to 8. *Crepidula fornicata*. Fig. 5. Tubules of digestive gland of a control. Arrows: normal basement membranes around digestive tubules. Fig. 6. Tubules of digestive gland exposed to  $1 \mu\text{g l}^{-1}$  silver. Arrows: silver deposited along basement membranes of ducts and tubules. Fig. 7. Tubules of digestive gland exposed to  $10 \mu\text{g l}^{-1}$  Ag. Arrow: basement membranes with increased amounts of silver deposit. Fig. 8. Blood vessel exposed to  $10 \mu\text{g l}^{-1}$  Ag. Arrow: silver deposited in connective tissue of blood vessel wall. All figures 25X H & E

body walls showed brownish to black particulate in the cytoplasm. Even the fibrotic tissue around nematodes was brown to black in color.

The  $10 \mu\text{g l}^{-1}$  exposed snails showed the same picture as the other 2 exposure groups; however, the black bands around the stomach, digestive gland, blood vessels, and intestine were wider and more dense (Figs. 7, 8), and the amount of brownish to black particulate in the connective tissue and basement membrane was wider and more dense. It appeared as though all the connective tissue and basement membrane of the body were brownish to black in color.

## DISCUSSION

The purpose of this study was to determine what effect long-term exposure to silver might have on reproduction of a parental stock of *Crepidula fornicata* and any succeeding generations. Larval releases of parental *C. fornicata* were significantly lower ( $P < 0.01$ ) for the  $10 \mu\text{g l}^{-1}$  Ag exposed snails than for controls (Table 1), whereas no significant differences were noted in the  $F_1$  population (Table 3). Although the number of releases in  $F_1$  snails at  $5$  and  $10 \mu\text{g l}^{-1}$  Ag appeared to be lower than controls (Table 3), statistical



treatment of the data indicated no differences. In addition, the number of abortions of eggs in the  $F_1$  group (i.e. the shedding of brooding eggs before hatching) at both 5 and 10  $\mu\text{g l}^{-1}$  Ag exposure was higher than either the controls or 1  $\mu\text{g l}^{-1}$  group (Table 3).

Bryan and Hummerstone (1977) and Bryan et al. (1977) suggested that gastropod molluscs accumulate more metals from their food than from water. Although this study was not designed to differentiate metal uptake in gastropods by these two methods, it does appear likely that substantial amounts of metals can be accumulated from the water (Tables 5, 6). There were significant increases of both silver and copper in whole-body tissues of parental *Crepidula fornicata* exposed for 24 mo to silver and for  $F_1$  individuals exposed for 24 mo. Whether these metals were accumulated in part by *C. fornicata* from ingestion of particulate matter with adsorbed metal from the seawater test system itself is not known. The seawater entering the silver-exposure tanks was first filtered through both 25- and 10- $\mu\text{m}$  nylon filter bags and then a bed of sand. Fluorometric measurements of the filtered water indicated a 50 % reduction in fluorescence from ambient levels. This filtration removed the larger phytoplankters, and allowed small quantities of nanoplankton to pass through. This limited food supply, plus the cultured algae that were periodically added to the test tanks, maintained our test snails for 24 mo. No attempt was made to ascertain which organs in *C. fornicata* accumulated the highest concentration of metals; analysis of individual tissues would help to determine the manner in which these metals are accumulated. In a similar experiment, using the same exposure system but with no supplemental feeding, Greig (1979) exposed the filter-feeding bivalve molluscs *Arctica islandica* (ocean quahog), *Spisula solidissima* (surf clam), and *Crassostrea virginica* (American oyster) simultaneously to silver, cadmium, and copper at concentration of 10  $\mu\text{g l}^{-1}$  of each metal for a maximum of 43 d. In this short time span, oysters took up as much as 15.4 ( $\pm 7.2$ )  $\mu\text{g g}^{-1}$  Ag (wet wt), surf clams attained a concentration of 6.0 ( $\pm 2.7$ )  $\mu\text{g g}^{-1}$ , and quahogs 3.5 ( $\pm 1.9$ )  $\mu\text{g g}^{-1}$ . Thurberg et al. (1975) reported that surf clams exposed to silver for 96 h accumulated significant amounts of silver in the gills as compared to the whole body minus the gills. In a similar study, Thurberg et al. (1974) exposed the American oyster and hardshell clam *Mercenaria mercenaria* to 0.5 and 1.0  $\text{mg l}^{-1}$  Ag for 96 h and found that significantly more silver accumulated in gill tissues than in the whole body.

Silver was accumulated rapidly by parental *Crepidula fornicata* during the first 6 mo of exposure to 1, 5, and 10  $\mu\text{g l}^{-1}$  Ag, but with no additional accumulation after 12 mo. After 24 mo there was a significant

reduction ( $P < 0.05$ ) in silver concentrations in all 3 test groups (Table 5, Fig. 1). It is possibly germane to consider this situation in light of the high copper concentration in body tissues at 12 mo, significantly higher than in the control individuals (Table 6, Fig. 1). If there were a preferential uptake for copper, in competition with silver, then when the metal-loading capacity of the body tissues approached saturation, the copper rather than additional silver would be accumulated. This is most clearly seen in female *C. fornicata*: at 6 mo, when copper concentrations had not yet attained higher values than in controls, silver accumulated in the body tissues proportionally with increasing concentrations of silver exposure; but at 12 mo, when copper concentrations had significantly increased, the same low tissue concentration of silver was seen at all levels of silver exposure. At 24 mo, silver levels were depressed in both males and females. Although the reasons for the reduction in silver concentrations are not understood at this time, it is possible that high tissue loads of copper depressed silver uptake and retention or served to sequester and excrete higher levels of silver.

In a similar study (unpubl.), the authors noted that blue mussels *Mytilus edulis*, exposed to silver in the laboratory for 21 mo, exhibited a similar preferential uptake for copper in the presence of silver. Similarly, silver concentrations in mussels increased after 12 and 18 mo of exposure to silver with a subsequent reduction at 21 mo. Popham and 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 uptake of copper. This, then, may also be true with silver. Stenner and Nickless (1974), Phillips (1976), and Lobel et al. (1982) have also suggested that metal interactions may occur in *M. edulis*, thereby facilitating or inhibiting metal uptake. It is necessary, therefore, to consider the interactions of pollutants in the environment when attempting to determine their effects on marine organisms.

After 24 mo of exposure, silver concentrations were not as high in the  $F_1$  generation (Table 7) as in the parental stock, but this may have been due both to age and/or size of snails sampled. Copper levels, on the other hand, were significantly higher ( $P < 0.05$ ) in silver-exposed snails than in controls. Again, a preferential uptake for copper in the presence of silver was obvious.

Histopathologic study of the exposed individuals showed that deposition and accumulation of silver by *Crepidula fornicata* increased as the test concentration

increased. The condition of accumulation of silver in the tissues of humans called argyria has been noted in numerous cases and always under chronic exposure to silver compounds in occupationally hazardous jobs and after therapeutic use of medicinals containing silver (Shafer et al., 1963).

Although the accumulation was very extensive in the 5 and 10  $\mu\text{g l}^{-1}$  exposed snails, no lesions were noted. Rosenman et al. (1979) studied humans exposed to various forms of silver compounds and concluded that silver is of a benign nature. Some investigators claim that silver is not toxic because it binds to protein and sulf-hydryl groups which render it enzymatically inactive (Petering, 1976).

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