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Copper Toxicity to the Bay Scallop (*Argopecten irradians*)

G. Pesch, N. Stewart[1] and C. Pesch

U. S. Environmental Protection Agency, Environmental Research Laboratory, South Ferry Road, Narragansett, R. I. 02882

Coastal marine environments are commonly used for disposal of industrial waste, dredge spoils and sewage sludge, which contain high concentrations of copper and other heavy metals (MUELLER et al. 1976, PESCH et al. 1977). For example, in the New York Bight, MUELLER et al. (1976) estimate a daily input of copper at 14 tons. This input has resulted in concentrations up to 390 mg kg^{-1} (dry) Cu in the sediment (CARMODY et al. 1973) and concentrations as high as 20 µg L^{-1} in the seawater (SEGAR & CANTILLO 1976).

Adverse effects of copper on some species of marine life have been well documented. However, most toxicity data available for bivalves are from static acute toxicity tests, even though long-term flowing water bioassays provide more realistic exposure conditions. To date, no studies have been conducted on copper toxicity to the bay scallop, Argopecten irradians, a commercially important species. The purpose of this study was to examine the effect of copper on survival, growth, and byssal attachment, and copper accumulation of juvenile bay scallops in a flowing water exposure system.

MATERIALS AND METHODS

Juvenile bay scallops were obtained from the hatchery (Virginia Institute of Marine Sciences) at Wachapreague, Virginia, U.S.A. The scallops were 1.1 to 1.7 cm long (longest rib) and the soft parts weighed 0.51 to 0.73 g wet weight. All test animals were acclimated in flowing unfiltered Narragansett Bay water at ambient conditions (3-8°C temperature, 30 °/₀₀ salinity) for 51 days before testing. No mortalities were observed during holding.

A proportional diluter (MOUNT & BRUNGS 1967) that was constructed entirely of glass and calibrated to deliver 1.0 L of seawater every 2 min to each 40-L aquarium was used. The flow rates gave an approximate 99% volume replacement 4 times per day (SPRAGUE 1969). Two aquaria were used for each of the 5 concentrations and control. The diluter was maintained and cleaned daily to insure uninterrupted function.

[1]Present address: Dept. of Fish & Wildlife, Marine Sciences Drive, Newport, Oregon 97365.

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The copper stock solution (0.1 g L^{-1} Cu) was made with reagent grade $CuCl_2.2H_2O$ in deionized water. A Mariotte bottle delivered the stock to the diluter. The diluter was adjusted to deliver nominal concentrations of 6, 12, 25, 50, 100 µg L^{-1} Cu and controls. Seawater copper concentrations were determined weekly using an Orion specific ion electrode calibrated with copper standards by method of adds (JASINSKI et al. 1974).

Scallops were randomly distributed to the aquaria. Thirty scallops, 10 and 20 individuals, respectively, per aquaria, were exposed at each concentration. This gave a biomass per liter of seawater of 0.15 g and 0.30 g scallop wet weight, respectively.

Daily measurements of temperature, salinity and oxygen were recorded during the 42-day experiment (April 23, 1973 to June 4, 1973). Each aquarium was cleaned and checked for mortalities daily. Dead scallops were removed. Since juvenile scallops attach to firm substrates by byssal threads, byssal attachment was also checked daily during the experiment. Shell growth of scallops was determined by measuring the longest rib length at the start and the end of the experiment. The scallops received only the food contained in the unfiltered flowing seawater.

After 42 days exposure, the soft tissues of the surviving scallops were grouped in pools of 5 animals for analysis of tissue copper concentrations. Each sample was ashed at 425°C overnight, wet digested in 1 mL of concentrated nitric acid, then ashed again at 425°C overnight. The ashed samples were dissolved in 5% nitric acid, filtered, and the volume adjusted to 25 mL with deionized water. Copper concentrations were determined using an atomic absorption spectrophotometer.

The copper concentrations used for the flowing water test were determined from the results of a static 96-h test that was conducted at ambient conditions (temperature = 6°C, salinity = 29.5 °/oo). Ten scallops were exposed to each of the following nominal concentrations, 60, 125, 250, 500 and 1000 µg L^{-1} Cu.

The mortality data from the bioassays were plotted on log-probit paper and LC50 values were estimated according to recommended standard procedures (APHA 1975). An incipient LC50 value (toxicity threshold) was estimated graphically. The data on growth were analyzed by one-way analysis of variance, with individual comparisons made by partitioning the sum of squares (SNEDECOR & COCHRAN 1967). All reported differences are significant at $\alpha = 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

Ambient seawater temperature, salinity and dissolved oxygen during the experimental period (April 23 to June 4, 1973) were

as follows: temperature, 9.5 to 15.0°C; salinity, 27.4 - 31.5 °/oo; and oxygen, 8.5 - 10.4 mg L^{-1}. Actual measured copper concentrations were 5.0 ± 0.7, 11 ± 3, 21 ± 5, 48 ± 9, and 110 ± 10 µg L^{-1}. Natural background concentration of copper in seawater was 1.0 ± 0.2 µg L^{-1}.

Mortalities were recorded at all concentrations tested. At each of the 4 highest concentrations (110, 48, 21, and 11 µg L^{-1}) more than half of the scallops died during the 42-day experiment. At the lowest concentration, 5 µg L^{-1}, 10% of the scallops had died by day 42. None of the control scallops died. At the highest concentration, 110 µg L^{-1} Cu, 57% of the scallops died by 96 h. In comparison, the 96-h LC50 determined under static conditions was 310 µg L^{-1} Cu.

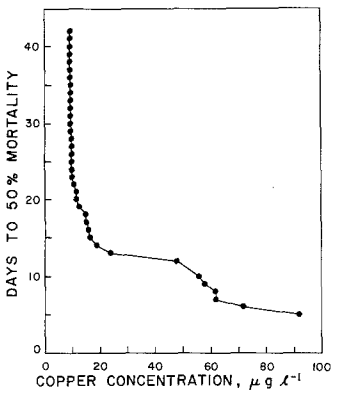

Figure 1. Toxicity curve for bay scallops exposed to 5, 11, 21, 48, and 110 µg L^{-1} Cu for 42 days.

An incipient LC50 value of 9.3 µg L^{-1} Cu was estimated graphically from the toxicity curve (Figure 1). The sharp inflection in the toxicity curve at about 15 days and 20 µg L^{-1} Cu, shows that toxicity to scallops increased dramatically at concentrations above 20 µg L^{-1}. However, at concentrations below 20 µg L^{-1}, it took more than 15 days for 50% of the scallops to die.

Growth was inhibited at all concentrations (Table 1). For example, at 5 µg L^{-1} Cu the reduction in growth was 43%. A calculated 42-day EC50 indicated that a concentration of 5.8 µg L^{-1} would reduce scallop growth to 50% of growth in controls.

The toxicity and growth data of this study and toxicity data of other published reports demonstrate that bivalve molluscs are quite sensitive to the presence of copper.

TABLE 1

Growth response of bay scallops exposed to Cu in flowing seawater for 6 weeks as indicated by differences in mean length in mm of longest rib. N = number of scallops.

Copper in seawater ($\mu g\ l^{-1}$)	Before Exposure N	Before Exposure Mean ± S. D. (mm)	After Exposure N	After Exposure Mean ± S. D. (mm)	Difference Before & After (mm)	Difference From Control (mm)
C	30	13.1 ± 1.6	30	21.5 ± 2.4	8.4**	----
5	30	13.9 ± 1.9	27	18.7 ± 3.0	4.8**	-3.6**
11	30	13.6 ± 1.8	10	15.3 ± 2.0	1.7*	-6.7**

*α=.05
**α=.01

STEPHENSON & TAYLOR (1975) report that 68% of the bivalves, Venerupis decussata, had died after a 90-day exposure to 10 μg L^{-1} Cu. The data reported by ADEMA et al. (1972) may be used to estimate graphically an incipient LC50 of 12 μg L^{-1} Cu for Mytilus edulis (blue mussel). CALABRESE et al. (1977) reported a 48-h LC50 of 16 μg L^{-1} Cu for hard clam Mercenaria mercenaria larvae. Therefore, toxicity data from these published studies plus data from the present experiment show that 4 species of bivalve molluscs are affected by copper concentrations lower than 20 μg L^{-1} and growth data from this study indicates that scallop growth is affected at a concetration as low as 5.8 μg L^{-1} Cu. Concentrations greater than 5.8 μg L^{-1} have been measured in many polluted coastal waters (GALLOWAY 1972, SEGAR & CANTILLO 1976; WALDHAUER et al. 1978). SEGAR & CANTILLO (1976) reported a copper concentration of 20 μg L^{-1} in the New York Bight for several months during the summer of 1974. Normal coastal seawater concentrations are 1-3 μg L^{-1} (DAVEY & SOPER 1975, ZIRINO et al. 1978). Therefore, a two to three fold increase in ambient concentrations will have a significant effect on growth of bay scallops, and concentrations found in some polluted coastal waters will have a toxic effect on A. irradians.

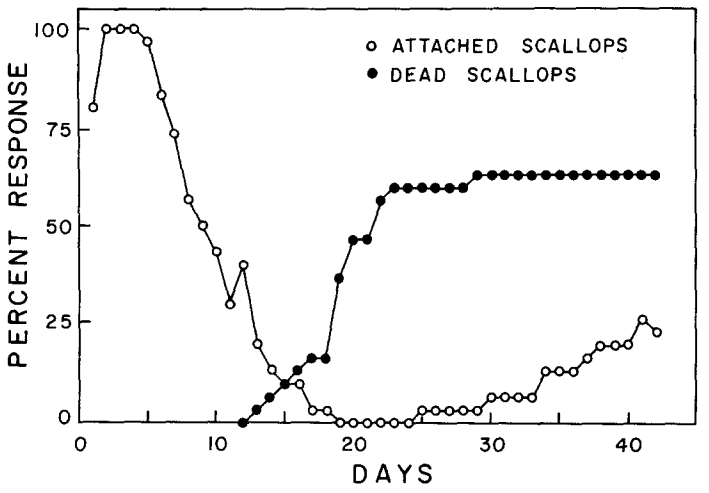

Figure 2. Cumulative mortality and byssal thread attachment by bay scallops exposed to 11 μg L^{-1} Cu for 42 days.

At the 4 highest concentrations (110, 48, 21, and 11 μg L^{-1}), we observed that before death the scallops detach from their substrates and lie free on the bottom of the aquaria. Comparison of the curves for cumulative percent attached and cumulative percent dead for scallops exposed to 11 μg L^{-1} Cu is illustrated in Figure 2. At this concentration, scallops began to detach on day

5 and by day 13, when death was first observed, 80% were detached. Half of the scallops had died by day 22 and none of the remaining scallops were attached. On day 25, scallops began to reattach with no additional deaths recorded after day 29. By day 42, 7 of the remaining scallops had reattached. The response of the scallops to copper indicates an initial acute effect as shown by the detachment. However, some of the survivors adjusted to the presence of copper and reattached. The relationship between detachment and death was linear. The regression of time to 50% detachment on time to 50% mortality gave the following equation: $y = 1.68 + 2.04x$, with $r^2 = 0.99$. The lapsed time between 50% detachment and 50% dead was 11, 7, 7, and 3 days at 11, 21, 48, and 110 $\mu g\ L^{-1}$ Cu, respectively. The byssal thread detachment may be a useful parameter to assess the condition of juvenile bay scallops in the field. Further study of this response is needed to determine if the detached bay scallops will recover if placed in clean seawater.

Copper concentration in soft tissues of scallops exposed to copper increased significantly compared to the controls. The mean concentrations of scallop tissues were 45, 320, and 510 mg kg^{-1} (dry weight) Cu for animals after 42 days exposure to control, 5, and 11 $\mu g\ L^{-1}$ Cu, respectively. The bioconcentration factor (BCF) was 9600 and 6900 for scallops exposed to 5 and 11 $\mu g\ L^{-1}$ Cu (BCF = $\mu g\ g^{-1}$ Cu wet weight/$\mu g\ L^{-1}$ Cu seawater). (A dry to wet weight conversion of 0.15 was used before the BCF was calculated.)

Accumulation of copper by bay scallops in this study is comparable to data from other studies that show molluscs accumulate copper readily and elevated copper concentrations in the environment are reflected by elevated copper concentrations in molluscs. Data obtained in other laboratory studues, which used copper concentrations reported to occur in contaminated coastal environments, show that three other species of bivalves, Crassostrea virginica (SHUSTER & PRINGLE 1968), Tellina tenuis (SAWARD et al. 1975), and Mya arenaria (EISLER 1977) had calculated bioconcentration factors greater than 1000. PESCH et al. (1977) observed that sea scallops, Placopecten magellanicus, exposed to elevated concentrations in and around a sewerage disposal site had significantly higher copper concentrations within their tissue as compared to controls. ROOSENBURG (1969) observed similar patterns of copper concentrations in tissues of oyster, C. virginica exposed to heated effluent of a power plant. He also observed an inverse relationship between a calculated oyster condition index and oyster copper concentration.

When interpreting toxicity data it must be remembered that exposure conditions, length of exposure, and life stage of the species being tested will influence the results. For toxicity tests with copper, the chemical form is also important. The copper concentrations, which were measured with a copper ion specific electrode, reported in this study are expressed as the ionic form of copper in seawater. Toxicity studies conducted with copper in

combination with chelating agents such as nitrolotriacitic acid (NTA) (ERICKSON et al. 1970) and EDTA (STEPHENSON & TAYLOR 1975) show that copper in the chelated form was not very toxic. Data from these two studies indicate that it is probably the free ionic form of copper that is toxic.

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