Biological Effects of Heavy Metals on Juvenile Bay Scallops, *Argopecten irradians, in* Short-term Exposures

David A. Nelson, Anthony Calabrese, Betty A. Nelson,
John R. MacInnes, and Douglas R. Wenzloff

National Marine Fisheries Service
Middle Atlantic Coastal Fisheries Center
Milford Laboratory
Milford, Conn. 06460

It is apparent that increases in human population and technological development are causing serious stress on the inshore marine environment, with a resulting decrease in its effective use. Bivalve molluscs are one of the most important commercial fisheries in shallow waters of bays and estuaries and many areas used for cultivation of these shellfish are frequently subjected to both industrial and domestic pollutants. Such pollution, both man-made and natural, fosters conditions that may diminish the harvest of marine resources. For example, Connecticut landings of the bay scallop, Argopecten irradians, amounted to 50,160 pounds in 1898 (TOWNSEND, 1900), but dropped to 36,200 pounds in 1939 (FIEDLER, 1942), and disappeared in 1969 (WHEELAND, 1972). A possible cause for the decline may be environmental perturbations.

Relatively little is known about the biological effects of heavy metals on marine bivalve molluscs, especially scallops. A number of studies have been conducted to determine the levels of metals concentrated by bivalves after experimental exposure (EISLER et al., 1972; HUGGETT et al., 1973; PRINGLE et al., 1968; SHUSTER and PRINGLE, 1968). More recently, heavy metal pollutant studies have been conducted on embryonic, larval and adult bivalves to determine the concentrations that cause mortality (CALABRESE et al., 1973; CALABRESE and NELSON, 1974; CONNOR, 1972; EISLER, 1971; OLSON and HARREL, 1973; WISELY and BLICK, 1967). These studies have further progressed to evaluation of physiological damage caused by sublethal levels of metals on marine bivalves after short- and long-term exposures (DUNNING and MAJOR, 1974; THURBERG et al., 1974; THURBERG et al., 1975; TRIPP, 1974).

The intent of this study was to determine the acute effect of four heavy metals - arsenic, cadmium, mercury and silver - on survival of juvenile bay scallops, A. irradians, after 96 hours of exposure, as well as tissue uptake of these metals. Changes in oxygen consumption rates were also determined in scallops exposed to the estimated LC_5 and LC_{25} values of silver and cadmium as determined from the acute toxicity studies above.

MATERIALS AND METHODS

Juvenile scallops (20-30 mm in length, from hinge to edge of shell) were obtained from a commercial shellfish hatchery and held

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in laboratory tanks at ambient seawater temperature (5°-10°C). Two weeks prior to each experiment a group of scallops was acclimated to 20°C. They were then exposed for 96 hours to cadmium, mercury, arsenic or silver to determine both the effect of these metals on their survival and the amount of metal accumulated. Seven scallops were placed into each of a series of 4-liter polypropylene containers with 1 µm filtered, natural seawater (salinity 25 ± 2 o/oo) and maintained at 20° ± 1°C. Triplicate cultures were established for each test concentration and three untreated cultures served as controls. Five to 7 concentrations were used for each metal tested. In preliminary tests a range of concentrations was used for individual metals to obtain the concentrations to be used in final tests. All concentrations in this report refer to the calculated level of metal ion added to each container each day. The pH levels remained between 7.0 and 8.0 in test containers with metal salts added. The 4 metals, all inorganic metallic salts, were as follows: sodium arsenite ($NaAsO_2$); cadmium chloride ($CdCl_2$.2-1/2 H_2O); mercuric chloride ($HgCl_2$); and silver nitrate ($AgNO_3$).

Static tests without aeration were conducted throughout this study and all cultures were renewed daily. Scallops that died during the test period and those remaining after 96 hours were pooled and frozen, with shells removed, for later metal uptake analyses. Each pooled sample consisted of 7 scallops, with triplicate samples at each test concentration. All samples were analyzed by atomic absorption spectrophotometry (Perkin-Elmer Model 403). For silver and cadmium analyses, the samples were wet-ashed with concentrated HNO_3, taken up in 10% HNO_3 and analyzed directly. For arsenic, they were dry-ashed, taken up in 7.2 N HCl and analyzed by arsine generation. The procedure of GREIG et al. (in press) was used for mercury determinations.

To determine mortality the number of animals that died in the 3 containers at each concentration was averaged. Results of successive tests were then averaged and the LC 5, 25, 50, 75 and 95 (lethal concentration) values were determined from the straight-line graphic interpolation method of LITCHFIELD and WILCOXIN (1949). The 95% confidence limits and the slope line of the LC_{50} value were also determined. The results are based on 3 tests with arsenic, cadmium and mercury (63 scallops per concentration) and 4 with silver (84 scallops per concentration).

In subsequent tests scallops were exposed to silver and cadmium to monitor changes in oxygen consumption rates. These measurements were made on excised gill tissues of scallops exposed for 96 hours to the LC_5 and LC_{25} values (as determined above) of cadmium (0.94 ppm and 1.23 ppm) and of silver (0.014 ppm and 0.022 ppm). Excised gills were held in 15-ml Warburg-type reaction vessels (1 gill per vessel). Each vessel contained 5 ml of water from the test container in which the animal was

TABLE 1

Toxicity of Heavy Metals, as Inorganic Salts, to Juvenile Bay Scallops, Argopecten irradians, at 20° ± 1°C in Natural Seawater (25 ± 2 o/oo) in 96 Hours. The Concentrations are in Parts Per Million of Metal Ion Added Each Day Causing Mortality of 5, 25, 50, 75 and 95 Percent. These Values were Determined by the Litchfield-Wilcoxin Method of Analysis[1].

Metals as Inorganic Salts (ppm)	LC_5	LC_{25}	LC_{50}	LC_{50} 95% Confidence Limits	LC_{75}	LC_{95}	Slope Function
Silver nitrate	0.014	0.022	0.033	0.030-0.036	0.048	0.086	1.811
Mercuric chloride	0.052	0.062	0.089	0.054-0.147	0.11	0.150	1.370
Cadmium chloride	0.94	1.23	1.48	0.95 -2.31	1.78	2.33	1.295
Sodium arsenite	2.22	2.86	3.49	2.11 -5.81	4.17	5.41	1.330

[1] These concentrations do not include background concentrations of heavy metals in the natural seawater test medium; they were (in ppb): Ag - 2.8, Hg - <1.0, Cd - <1.0, As - <20.0.

exposed. Oxygen consumption was then monitored over a 4-hour period in a Gilson Differential Respirometer at a temperature of 20°C. After each test, gills were oven-dried to a constant weight. The oxygen consumption value for a group of animals at a specific metal concentration was calculated as microliters of oxygen consumed per hour per milligram dry weight of gill tissue ($\mu l O_2$/hr/mg), corrected to microliters of dry gas at standard pressure and temperature.

RESULTS AND DISCUSSION

It should be understood that the results of the metal toxicity discussed here are valid only for those forms of inorganic metallic salts added to natural seawater and in no way take into account the form of the metals when in solution. The toxicity of heavy metals, as inorganic salts, to juvenile bay scallops is presented in Table 1. These concentrations, not including known background levels, are in parts per million of metal ion added to each test container daily. Of the metals tested, silver and mercury were the most toxic, i.e., silver was 95% toxic at 0.086 ppm and mercury at 0.150 ppm, while the LC_{50} values were 0.033 ppm for silver and 0.089 ppm for mercury. Cadmium was relatively toxic; 95% mortality occurred at 2.33 ppm and 50% at 1.48 ppm. Arsenic was relatively non-toxic; concentrations of 5.41 and 3.49 ppm were required for 95% and 50% mortality, respectively. LC_{50} values of these metals at specified time periods are shown in Table 2.

TABLE 2

Concentrations of Heavy Metals (in ppm) Required to Cause 50% Mortality (LC_{50}) of Bay Scallops at Specified Time Periods.

Metal	Time in Hours			
	24	48	72	96
Silver	0.062	0.036	0.034	0.033
Mercury	0.370	0.130	0.096	0.089
Cadmium	8.20	3.21	2.18	1.48
Arsenic	6.50	4.40	3.89	3.49

EISLER (1971) reported a 96-hour LC_{50} value of 2.2 ppm cadmium for the soft-shell clam, Mya arenaria, a value comparable to that derived with scallops. OLSON and HARREL (1973) found that 8.7 ppm mercury was needed to attain 50% mortality of the estuarine clam, Rangia cuneata, in 96 hours, a value considerably higher than that affecting scallops.

Results indicate that scallops exposed to the LC_5 (0.94 ppm) and LC_{25} (1.23 ppm) levels of cadmium for 96 hours exhibited significantly higher ($P<0.05$) oxygen consumption rates than the controls; the rates were 12.9% and 17.7% higher, respectively. There

was no significant difference between concentrations. Scallops exposed to silver at the LC_{25} (0.022 ppm) level respired at a significantly higher rate ($P<0.05$) which was 14.7% greater than the controls, but those at the LC_5 (0.014 ppm) level respired at a slightly lower rate. Using the "Student's" t-test, no significant difference was noted in oxygen consumption between the controls and those exposed to 0.014 ppm silver ($P>0.5$). The mean control value for oxygen consumption was 1.66 $\mu l O_2$/hr/mg dry weight of gill tissue. MACINNES and THURBERG (1973) found that cadmium slightly increased oxygen consumption in the gastropod, Nassarius obsoletus, over an exposure range from 0.5 to 4.0 ppm. Silver has been reported to cause an increased respiratory rate in five species of bivalves - Crassostrea virginica, Mercenaria mercenaria, Mytilus edulis, M. arenaria and Spisula solidissima (THURBERG et al., 1974; THURBERG et al., 1975). MACINNES and THURBERG (1973) reported a decreased oxygen consumption rate for a silver-exposed gastropod, N. obsoletus. These two opposite effects of silver serve to emphasize the fact that metal-induced physiological alterations can differ greatly. The alterations differ with metal used, species used, and life stage of the species, as well as with experimental conditions, i.e., salinity, temperature and pH. Caution should therefore be exercised when extrapolating results of one study to other metals, other animals and other conditions.

Table 3 summarizes the amounts of silver, cadmium, mercury and arsenic accumulated by juvenile bay scallops. All metals tested were accumulated in significant amounts; silver, mercury and arsenic demonstrated an increase in uptake up to those concentrations that caused high mortality in 24 to 48 hours, then decreased. We believe this decrease was due to the fact that scallops exposed to the highest concentrations of these metals died within the first 24 to 48 hours of the experiment and were removed; thus, they were not exposed for the full 96 hours as were the others at the lower concentrations. Cadmium, however, did not cause any appreciable mortality within the first 72 hours at the highest concentrations; hence, scallops were exposed for the full 96 hours and accumulated high levels of this metal in their body tissues. EISLER et al. (1972) reported that A. irradians exposed for 3 weeks to 10 ppb cadmium chloride accumulated 2.46 ppm cadmium (wet weight), while his control samples contained 1.15 ppm. KOPFLER (1974) reported that oysters exposed to 50 ppb mercuric chloride for 7 days accumulated 25 ppm mercury. Numerous other investigators working with other molluscs have observed similar uptake values (HUGGETT et al., 1973; PRINGLE et al., 1968; SHUSTER and PRINGLE, 1968, 1969; THURBERG et al., 1974; THURBERG et al., 1975).

The results of this study indicate that scallops can rapidly accumulate high levels of metals in their body tissues. Caution should be exercised, however, in extrapolating these results to all seasons of the year and to other species of bivalves. BRYAN

TABLE 3

Uptake of Silver, Cadmium, Mercury and Arsenic by Bay Scallops, Argopecten irradians, Exposed to Various Concentrations of these Metals in Seawater for 96 Hours.

Exposure Level (ppm)	Metal Concentrations[1] Mean ppm ± s.e. (wet weight)			
	Silver	Cadmium	Mercury	Arsenic
0.00	<0.08 ----	4.8±0.2	0.11±0.008	0.70±0.31
0.01	3.1 ±0.15			
0.015	3.5 ±0.84			
0.02	5.2 ±0.58			
0.03	7.1 ±1.4			
0.04	5.3 ±0.8		48.9 ±5.9	
0.05	7.2 ±1.5			
0.06	2.6 ±0.3[2]		55.5 ±4.5	
0.08			46.9 ±4.0	
0.10			28.3 ±7.3[2]	
0.20			12.4 ±0.8[2]	
0.75		49.4±4.1		
1.0		54.1±2.2		
1.25		55.8±1.9		
1.5		58.9±1.9		
1.75		57.4±4.0		
2.0		60.8±5.1		29.2 ±2.9
3.0				38.3 ±4.6
4.0				40.4 ±8.1
5.0				38.8 ±3.3[2]
6.0				34.9 ±2.6[2]

[1] Values are the mean of 7-9 samples with 6-7 pools of scallops per sample.

[2] Greatest mortality occurred within the first 24-48 hours of the test.

(1973) showed natural seasonal variations in trace metal accumulation in the scallops, Pecten maximus and Chlamys operularis. PRINGLE et al. (1968) and SHUSTER and PRINGLE (1968) have demonstrated that temperature, salinity, dosage and duration of exposure to metals and the activity and physiological condition of shellfish also affect metal uptake.

Note: The use of trade names is to facilitate description and does not imply endorsement by the National Marine Fisheries Service.

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