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BASELINE TOXICITY DATA FOR FRESHWATER BRYOZOA EXPOSED TO COPPER, CADMIUM, CHROMIUM, AND ZINC

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ABSTRACT

Four heavy metals—copper, cadmium, chromium, and zinc—were used to determine baseline toxicity data for three species of phylactolaemate bryozoa. Techniques used in germinating test organisms are described, and baseline toxicity data are presented. Minimal interspecific variation in toxicity was noted for each heavy metal. In general, copper was most toxic, followed by cadmium, chromium, and zinc. Available data indicate that bryozoans are more sensitive to these metals than many other invertebrates and fish.

INTRODUCTION

Although the literature is well documented with toxicity data on fish (e.g., Pickering 1972, Benoit 1976, and Hale 1977), invertebrate toxicity data are notably incomplete. Goodnight (1973) suggested reasons for using macroinvertebrates rather than fish as bioassay organisms: (1) they are easily collected, (2) they exhibit wide ranges of tolerance, (3) they are not mobile enough to leave an area of pollution rapidly, and (4) they generally are adaptable to laboratory study without significant amounts of specialized equipment. Also, genetically similar test organisms can often be secured by cloning since the reproductive rate of many invertebrates permits laboratory rearing.

Descriptive Ecology of Bryozoans

Freshwater bryozoans have received little attention even though they are ubiquitous in temperate aquatic environments (Lacourt 1968). The phylum Ectoprocta includes marine and freshwater eucoelic invertebrates. Their phylogenetic position is quite unique, showing relationships both with the shizocoelic protostomes and the enterocoelic deuterostomes. Freshwater species are colonial and generally exhibit a horseshoe-shaped lophophore bearing a ring of tentacles. In healthy zooids, the extended tentacles may be seen actively moving about in a feeding motion.

Bryozoans reproduce by asexual budding and sexual reproduction, with the formation of sperm and eggs in the same zooid. They also form resting or resistant stages which permit them to survive the severity of winter conditions. The resistance bodies are called statoblasts. Statoblasts are of two general types, floatoblasts or sessoblasts (Rogick 1935).

Species of interest to this research—*Lophopodella carteri* Hyatt, *Pectinatella magnifica* Leidy, and *Plumatella emarginata* Oka—all produce floatoblasts. The central germinal mass of the floatoblast is covered by a sclerotized capsule (Bushnell 1973). Epidermal cells form hardened walls, lose their cell contents, become filled with gas, and act as a buoyant ring (annulus) (Bushnell 1973). Floatoblasts are released from the zooids either through the vestibule or through breakdown of the body wall and its associated ectocyst (Wood 1973). After they are released from the colony, the floatoblasts are dependent on water currents until they contact a suitable substrate. Unless dislodged, they remain atached to this substrate and may be exposed to alternate freezing and drying with no apparent adverse effects (Bushnell 1966). As the water temperature increases in the spring, individual zooids begin to germinate. Then, asexual budding and sexual reproduction begin, and the colony increases in size.

Statoblasts are easily collected in late fall, when the water level is generally lowest. Overbanks and embayments often retain logs, plants, and other suitable substrates from which one can easily obtain statoblasts. *P. magnifica, L. carteri,* and *P. emarginata* may be found in lentic and lotic situations, although *P. emarginata* is typically found in riverine habitats more often than the other two species.

Effects of Selected Heavy Metals on Aquatic Invertebrates

Anderson (1948) determined that the 35-hr toxic threshold for *Daphnia magna* exposed to copper was 0.096 mg/1. Also using *D. magna,* Biesinger and Cristensen (1972) determined a 48-hr LC_{50} (lethal concentration for 50 percent of organisms tested) of 0.0098 mg/1.

Rehwoldt et al. (1973) determined the 96-hr TL_m (tolerance limit for 50 percent of organisms tested) for several invertebrate taxa. Using copper, *Chironomous* sp. was the most sensitive organism tested with a median tolerance limit (TL_m) of 0.03 mg/1, and Trichoptera was the most tolerant taxa with a TL_m of 6.2 mg/1.

Nehring (1976) determined the LC_{50} (14 d) values for the mayfly *Ephemerella grandis* and the stonefly *Pteronarcys californica* exposed to copper. The results were 0.18-0.20 mg/1 and 10.1-13.9 mg/1, respectively.

Anderson (1948) reported a 64-hr toxic threshold of 3.1 mg/1 for *Daphnia magna* exposed to chromium. His tests were conducted in relatively hard lake water.

In softer water (44 mg/1 hardness), Warnick and Bell (1969) reported a 96-hr toxic threshold of 0.7 mg/1 for *Daphnia magna*. McKee and Wolf (1963) summarized some of the earlier toxicity data and reported that the toxic threshold range for *Daphnia magna* was 0.016 to 0.7 mg/1.

Rehwoldt et al. (1973) determined the 96-hr TL_m of chromium for several invertebrate taxa. Their tests were conducted using dilution water, measuring 50.0 mg/1 hardness. The most sensitive taxa tested was *Gammarus* (3.2 mg/1) and the most tolerant was Trichoptera (50 mg/1). One of the more sensitive invertebrate species for which data are available is *Daphnia hyalina*. The 48-hr LC_{50} for this species was 0.022 mg/1 (Baudoin and Scoppa 1974).

Trabalka and Gehrs (1977) report a 96-hr LC_{50} for hexavalent chromium to juvenile and adult *Daphnia magna* of 50 μg/1. At 10 μg/ longevity and reproduction were significantly reduced.

Acute toxicity tests on fish indicate that the cadmium LC_{50} falls in the range of 0.01 to 10.0 mg/1, depending on test species and water conditions. Rehwoldt et al. (1973) determined the cadmium TL_m for several invertebrate taxa at 50 mg/1 hardness. *Gammarus* was the most sensitive (0.07 mg/1) and Zygoptera were the most tolerant (8.1 mg/1).

Buikema et al. (1974), evaluating *Philodina acuticornis* as a bioassay organism, determined the 96-hr LC_{50} for two cadmium salts. They reported a cadmium chloride LC_{50} of 0.5 mg/1 and a cadmium sulfate LC_{50} of 0.2 mg/1. Anderson et al. (1975) determined that the 10-day LC_{50} for *Tanytarsus dissimilis* was 0.0039 mg/1. A concentration of 0.0031 mg/1 retarded growth but no observable effect was noted at 0.0019 mg/1.

Concentrations of zinc that have proven acutely toxic to various species of fish range from 130 to 1,000,000 mg/1. The various forms of zinc and the effects of varying water chemistry make comparison of results difficult. Skidmore (1964) has reviewed some of the effects of water chemistry on the toxicity of zinc and suggests that water hardness is the most important chemical parameter. In a later study, Mount (1965) confirmed Skidmore's findings and reported that zinc was less toxic at 200 mg/1 hardness than at 50 mg/1 hardness. He concluded that the toxicity was affected more by varying it from 100 to 200 mg/1 than from 50 to 100 mg/1. He also reported that zinc was less toxic at pH 6.0 than at pH 8.0.

Using zinc, Rehwoldt et al. (1973) hemonstrated that several macroinvertebrate taxa were relatively tolerant to zinc. The most sensitive taxa was *Gammarus* ($TL_m = 8.1$ mg/1). Trichoptera was the most tolerant organism tested ($TL_m = 58.1$ mg/1).

When bioassaying *Philodina acuticornis* in soft water (25 mg/1 hardness), Buikema et al. (1974) found that the 96-hr LC_{50} was 1.2 mg/1. No data were available for tests in hard water. This concentration is considerably lower than that for other invertebrates, and it suggests a possibly different mode of toxic action.

LC_{50} (14-d) values for the mayfly *Ephemerella grandis* and the stonefly *Pteronarcys californica* exposed to zinc were >9.2 mg/1 and >13.9 mg/1, respectively (Nehring 1976).

METHODS

Asexually produced statoblasts (resting stage) were collected during the fall of 1975 from living colonies. *P. magnifica* and *P. emarginata* were collected in the east end of Cowan Lake, Clinton County, Ohio (39° 23"N, 83° 53"W). A dense growth of lotus [*Nelumbo lutea* (Willd.) Persoon] and floating logs provided extensive substrate for colonization. *L. carteri* statoblasts were collected from Lake Erie, near Put-in-Bay.

At this time there is no established method for storing statoblasts that will ensure germination. Among the storage conditions used were (1) wet and dry, at 7°C, (2) wet and dry, frozen, and (3) dry at 24°C. Very little success was achieved in germinating statoblasts stored dry at 24°C.

Test organisms (ancenstrulae) were obtained by placing statoblasts on a cover slip covered by a piece of loosely woven cloth, attaching small floats to the coverslip, and floating them in 250-ml culture dishes containing the dilution water (Cowan Lake water). The statoblasts were maintained at test temperature and photoperiod until germination occurred (3-5 days).

Twenty to thirty ancestrulae from two to three days of age were used in each test solution. Cover slips with the attached ancestrulae were transferred to the test solution with the cloth cover removed. During acclimation and testing, bryozoa were maintained in culture dishes at controlled temperature and photoperiod in a Sherer-Gillett model "CEL 4-4" environmental chamber. Temperature was recorded continuously and verified periodically with a standard centigrade thermometer. One 25-watt incandescent bulb and two 20-watt fluorescent lamps were set to automatically provide 12 hours of light and 12 hours of dark.

TABLE I: *Summary of water chemistry data giving the range[a] and the observed maximum variation[b] in any one 96 hour test period[1].*

	Dissolved Oxygen (mg/1)		pH (Std. Units)		Hardness (mg/1 $CaCO_3$)		Temperature °C	
	a	b	a	b	a	b	a	b
Copper	7.5-8.3	0.2	7.4-8.0	0.2	190-220	10	24-25	1
Cadmium	7.5-8.3	0.2	7.4-8.0	0.2	190-220	10	24-25	1
Chromium	7.5-8.3	0.2	7.4-8.0	0.2	190-220	10	24-25	1
Zinc	7.5-8.3	0.2	6.7-7.0	0.2	190-220	10	24-25	1
Control	7.5-8.3	0.2	7.4-8.0	0.2	190-220	10	24-25	1

1—*Dissolved Oxygen*—Measured by the Mirco-Winkler technique described by Burke, 1962.
pH—Measured by Sargeant-Welch model "PBL" pH meter.
Hardness—Measured by EDTA titration as described in American Public Health Association, 1971.
Temperature—Measured by a standard centigrade thermometer.

TABLE II: *Summary of data used in calculating the LC_{50} for each bryozoan species.*

	Copper				Cadmium				Chromium				Zinc			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Pectinatella magnifica	0.10	20	2	10	0.4	20	1	5	1.0	20	7	35	4.0	25	11	44
	0.13	25	11	44	0.6	25	9	36	1.4	20	9	45	4.7	27	15	58
	0.17	24	17	71	0.8	24	12	50	1.7	20	10	50	5.4	26	17	65
	0.20	25	20	80	1.0	20	17	85	2.0	20	15	75	6.0	21	16	76
Plumatella emarginata	0.05	20	1	5	0.3	22	0	0	0.3	20	3	15	4.0	22	4	18
	0.10	20	9	45	0.7	25	6	24	0.5	25	7	28	5.0	23	11	48
	0.30	23	19	83	1.3	23	10	43	0.7	20	9	45	6.0	25	14	56
	0.50	23	23	100	1.5	22	15	68	0.9	20	15	75	7.0	20	16	80
Lophopodella carteri	0.4	20	2	10	0.05	20	5	25	1.5	20	8	40	4.0	20	2	10
	0.5	20	8	40	0.10	20	10	50	1.7	20	14	70	5.0	20	5	25
	0.6	20	18	90	0.30	20	12	60	1.9	20	18	90	6.0	20	12	60
	0.7	20	20	100	0.50	20	15	75	2.2	20	20	100	7.0	20	16	80

A—Concentration of metal (mg/1)
B—Number of test organisms
C—Number of affected organisms
D—Observed percentage of affected organisms

Serial dilutions from Sargeant-Welch laboratory standards, containing 1000 mg/1 of each metal ion, were conducted to obtain the desired test concentrations. The pH was not affected by the addition of copper, cadmium, or chromium ions. Zinc ions, in dilute nitric acid, reduced the pH from 7.5 to 6.8 when added to Cowan Lake water. For tests with zinc a standard bicarbonate buffer (sodium salt), made with water from the Cowan Lake, was used as the control (at stated pH and temperature the proportions of CO_2, HCO_3^- and $CO_3^=$ were approximately 0.208, 0.792, and 2.6×10^{-4}, respectively). The range of water chemistry parameters are given in Table 1. Wide ranges of test solutions were systematically tested under static conditions until the LC_{50} was well defined. Death of a zooid was defined as failure of the zooid to respond to gentle probing. Observations to determine the percent mortality were made at intervals approximating the sequence described by Sprague (1973) (1, 2, 4, 6, 12, 24, 48, 72, and 96 hr.). All tests were terminated at 96 hours.

Dissolved oxygen, pH, hardness ($CaCO_3$), and temperature were measured before and after each bioassay. Dissolved oxygen concentrations (mg/1) were measured by the micro-Winkler technique (Burke 1962). For dissolved oxygen determination in the presence of chromium ions, precipitation of the chromium with 20 percent barium chloride is suggested (Fromm and Schiffman 1958). Experimentation demonstrated that the variation in dissolved oxygen data caused by chromium interference was not great enough to affect measurements through the micro-Winkler technique. Hydrogen ion concentration (std. pH units) was measured with a Sargeant-Welch model "PBL" pH meter. Hardness was calculated (mg/1 $CaCO_3$) by EDTA titration, and temperature was measured with a standard centigrade thermometer.

Statistical analysis of the data was performed following the kinetic approach suggested by Chen and Selleck (1969). This model assumes that percent survival compared to exposure time yields a straight line relationship when plotted on semi-log paper; there always exists an initial exposure period (induction period) during which no mortality occurs; and that the slopes of the survival-exposure time curves are proportionate to the toxicant concentration.

Percent mortality was plotted on the ordinate (linear), and concentration of the metal (in milligrams per liter) was plotted on the abscissa (logarithmic). The regression line and the square of the correlation coefficient were calculated from these data. The LC_{50} was calculated by using the equation $Y = ae^{bx}$ and substituting 50 percent mortality for "X." The variables "a" and "b" are unique to each data set; "a" is defined as the maximum concentration that will cause no mortality, and "b" is equal to the regression coefficient. All LC_{50} data are based on the concentration of the cation at the beginning of the bioassay.

RESULTS

Data from which LC_{50} values are calculated are given in Table 2. Calculated LC_{50} values and the square of the correlation coefficient (i.e., the variation in mortality due to change in toxicant concentration) are presented in Table 3.

Copper was most toxic to *P. magnifica* and *P. emarginata*, followed by cadmium, chromium, and zinc. Cadmium was most toxic to *L. carteri*, followed by copper, chromium, and zinc. Zinc was by far the least toxic to all species, on the order of five times less toxic than the other metals. Some precipitation of zinc probably occurred, but the effect on its toxicity was not determined.

TABLE III: *Toxicity data (96-h) for three species of bryozoa.*

Species	Metal	Calculated LC_{50} (mg/L)	Coefficient of Determination Correlation r^2
Lophopodella carteri	Copper	0.51	0.96
	Cadmium	0.15	0.93
	Chromium	1.56	0.93
	Zinc	5.63	0.96
Pectinatella magnifica	Copper	0.14	0.98
	Cadmium	0.70	0.95
	Chromium	1.44	0.81
	Zinc	4.31	0.99
Plumatella emarginata	Copper	0.14	0.98
	Cadmium	1.09	0.96
	Chromium	0.65	0.88
	Zinc	5.30	0.96

TABLE IV: *Summary of the 96-h LC_{50} data for three species of bryozoa ancestrulae as they compare with data for other invertebrates and fish.*

	96-hr toxicity (mg/L)						
Species	Copper	Cadmium	Chromium	Zinc	Hardness mg/l ($CaCO_3$)	pH	DO
Lophopodella carteri	0.51	0.15	1.56	5.63	190-220	6.7-8.0	7.5-8.3
Pectinatella magnifica	0.14	0.70	1.44	4.31	190-220	6.7-8.0	7.5-8.3
Plumatella emarginata	0.14	1.09	0.65	5.30	190-220	6.7-8.0	7.5-8.3
Philodina acuticornis[1]	1.10	0.1[a]	NA	NA	81	7.4-7.8	
Paratya[4]	NA	0.06	NA	1.10	10	NA	
Nais sp.[2]	0.09	1.70	9.30	18.0	50	7.6	
Gammarus[2]	0.91	0.07	3.20	8.10	50	7.6	
Trichoptera[2]	6.20	3.40	50.0	58.0	50	7.6	
Zygoptera[2]	4.60	8.10	43.0	26.0	50	7.6	
Chironomous sp.[2]	0.03	1.20	11.0	18.0	50	7.6	
Acroneuria[3]	8.30	2.00	NA	NA	50-56	6.4-7.3	
Pimephales promelas[5]	NA	31.0	NA	19.1	200	8.0	
Lepomis macrochirus[5]	1.35	NA	21.7	3.50	soft	6.3-6.5	
Micropterus salmoides[5]	2.00	NA	1.96	NA	NA	NA	

[a]Part per million of cadmium sulfate

Sources:

1. Buikema, Cairns, and Sullivan (1974)
2. Rehwoldt, Lasko, Shaw, and Wirhowski (1973)
3. Warnick and Bell (1969)
4. Thorp and Lake (1974)
5. McKee and Wolf (1963)

Several organismic responses to the addition of the metals were noted during the bioassays. The most obvious and frequent response was partial or full retraction of the lophophore. This response was particularly characteristic of zooids exposed to copper ions. Retraction of the lophophore did not, however, immediately precede death, since many organisms exhibited muscle contraction in response to gentle probing for up to 72 hrs. after initially retracting the lophophore. Lophophore retraction was not noted in those zooids exposed to concentrations causing no mortality. Some zooids exhibited abnormalities in tentacle posture, but only those zooids exposed to concentrations that caused some mortality.

Small changes in copper ion concentration affected the percent mortality more significantly than did changes in any of the other metals tested. However, zooids of *P. emarginata* did not seem to be as sensitive to copper as the other species. Their response to concentration changes was more gradual. Copper consistently induced the most rapid and permanent retraction lophophore in all species.

Comparison of these data with toxicity data from other studies indicates tha bryozoans are more sensitive to copper, cadmium, chromium, and zinc than are many invertebrates and fish (Table 4).

Water hardness in these bioassays was considerably greater than that reported for most other bioassays using invertebrates and fish. Decreasing hardness would be expected to reduce the LC_{50} for each bryozoan species.

Discussion

The sensitivity of bryozoans to heavy metals, compared with that of other macroinvertebrates, suggest that bryozoans have potential to be very useful as biomonitors of water quality. Bryozoans offer several advanages as bioassay organisms. Size, sensitivity, wide distribution, and inability to avoid pollution are characteristics shared by some other macroinvertebrates. Bryozoans also offer the following advantages:

1. Taxonomy to the species level is relatively easy. Ease of classification is particularly important for water quality work since sensitivity to pollution may be quite interspecific.
2. Both living specimens and statoblasts are easily collected for bioassay work. Germination of the statoblasts provides genetically similar test organisms.
3. The entire life cycle is completed in the water. This permits laboratory or in situ observation of sublethal effects, which is particularly advantageous if an organism is to be used as a biomonitor.

Additional toxicity work is needed to document the sensitivity of bryozoans to other toxicants.

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