

~~*inhibition* moves and feeds at 0.8°C. without any need for temperature acclimatization. Possibly *A. circumscriptus* may share the ability to maintain activity at low temperatures.~~

~~It brought indoors in autumn all the snails, *D. reticulatum* and *A. circumscriptus* feed and survive through the winter at a temperature of 20°C. *Deroceras reticulatum* lays eggs frequently during this time, whereas *A. circumscriptus*, though adult, lays no eggs. *Deroceras subfuscus* adults, under such conditions, lay clusters of large eggs, then die by November, while their young hatch and grow during the winter. As the autumn here is normally mild and prolonged, possibly the eggs might hatch out of doors. The hibernation seasons of these slugs seem to be similar in New Brunswick to those recorded for England (Quick, 1949; Bett, 1961) and for Nova Scotia (Ord and Watts, 1949).~~

### ZINC EFFECTS ON FRESH-WATER MOLLUSKS

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The increasing emphasis upon pollution control during the past two decades has generated a great many specific studies on potential pollutants. Among these is zinc, and several studies have been made upon this metal in surface waters. This paper presents the result of studies on zinc in the field and laboratory during the past 4 years.

The field studies were done in conjunction with a comprehensive biological survey of the Northwest Miramichi River, New Brunswick, Canada. The laboratory studies undertaken were part of a series done under the terms of a Public Health Service Grant (RG-6871).

To study the biological effects of heavy metals in streams it is necessary to recognize, first, that natural concentrations of these metals occur in unpolluted streams, and, second, that some degree of tolerance to metals is inherent in aquatic organisms.

In mining exploration, ore bodies are commonly located by surface outcroppings. Such surface exposures are the product of erosion, and the weathering of these exposures is the source of natural stream loads of metals. Where mining is undertaken, additional amounts of ore are exposed to the action of weathering, and the amount of metal in streams draining the area is increased.

To the extent that studies have been made, it would appear that streams draining zinc-mining districts show an average of 0.53 parts per million (ppm) of zinc. In natural waters not subject to mine drainage or industrial wastes zinc concentrations may range to 0.200 ppm. (Renn, et al, 1962)

Zinc is not highly toxic to humans. Data on drinking waters compiled from 37 locations in the United States showed a mean value of 0.136 ppm of zinc. The United States Public Health Service recommends a maximum of 15 ppm of zinc for drinking water standards.

Living organisms all require certain minimal amounts of metals. Zinc and copper, for example, are necessary for the formation of certain enzymes. These constitute two of the vitally necessary trace elements. Different groups of animals vary in their resistance to metals when these are present in amounts above the required physiological minimum. The mollusks, in general, are the least resistant to overloads of heavy metals in streams. (This is shared by the malacostracan crustaceans; the oligochaete worms are the next least tolerant group.) The mollusks would be the first animals eradicated when a stream became overloaded with metals. For this reason, these animals are of particular importance when making field observations on streams where metal overloading may occur.

Once the mollusks are eliminated from an extensive stream stretch they are slow to re-invade the area, and usually become re-established only through downstream transport from an upstream area. Such transport, being adventitious, is fortuitous. Considerable time may elapse before the mollusk population is again established. In the Ystwyth River in Wales, Jones (1938) reported that 35 years after the closing of a lead mine the stream was carrying 0.2 to 0.7 ppm of zinc. Although brown trout were present the bottom organisms were almost entirely lithophilous insects.

On June 15, 1960, dewatering of a base-metal (Zn, Pb, Cu) mine that had been closed was begun preparatory to renewing mining operations. This mine is in the drainage basin of the Tomogonops River, a tributary of the Northwest Miramichi River, New Brunswick, Canada. The water pumped from the mine flowed about 6 miles before entering the Northwest Miramichi River.

nichi River. The discharge water apparently eliminated the mollusk population in the Northwest Miramichi below the mouth of the Tomogonops River for a distance of at least 12 miles (but not for as much as 17 miles). In July, 1961, mollusks were still missing from this stream stretch, although 77 species of other macro-invertebrate animals were found. At the same time, in the stream above the mouth of the Tomogonops River 87 species of macro-invertebrate animals were found including 5 species of mollusks. Apparently within one year of the mine dewatering incident, the stream had a normal population except for the mollusks.

In 1961 the Salmon Investigation Group (Department of Fisheries of Canada) had placed a cage of young salmon (parr) in the Northwest Miramichi River where the fish were almost continuously in the flow of water from the Tomogonops River. Twice within a period of 3 days in July, 1961, a specimen of *Helisoma anceps* was taken from the leading face of this cage. These snails had apparently been flushed downstream from a population established in a quiescent stretch of the Northwest Miramichi about one-half mile above the mouth of the Tomogonops. These two occurrences are characteristic of the adventitious distribution of aquatic snails. This species is not adapted for survival in rapidly flowing streams and would not be expected to occur in the areas of the river critically examined during the survey. The average velocity at the stations studied on the Northwest Miramichi was 1.35 feet per second during the time of the survey (which was conducted during a low-water period). At this velocity the stream was flowing one mile in 65 minutes, which is about double the velocity of the lowland stretches of our major east coast rivers where *Helisoma anceps* is of common occurrence. *H. anceps*, and its eggs, occurred in quiescent water 9 miles below the mouth of the Tomogonops in July, 1962.

The mollusks found in the Northwest Miramichi River in 1961 above the mouth of the Tomogonops included: *Margaritana margaritifera*, *Pisidium casertanum*, *Ferrissia tarda*, *Physa gyrina* and *Helisoma anceps*. In the Portage River, a slow-flowing tributary of the Northwest Miramichi, which enters the main river about two miles below the Tomogonops River, 5 mollusks also occurred. These were: *M. margaritifera*, *Lampsilis radiata*, *Amni-*

*cola limosa*, *P. gyrina* and *Cyraulus arcticus*. Of the 5 species found in this small tributary only two were shared with the Northwest Miramichi. The Portage River is highly eutrophic and had an average velocity of 0.85 feet per second. Within the confines of one station (a 100-foot stretch) 92 species of macro-invertebrates were found. This is an uncommonly high species diversity in a small stream (width 36 feet with a low-water discharge of 3,000 gallons per minute).

The Northwest Miramichi, with its complex of tributaries, drains an area characterized by the sporadic occurrence of complex ores. Zinc, lead and copper are found throughout the region, and these minerals must have been leaching downstream over many millennia. A geochemical survey was conducted in this region a few years ago while searching for ore bodies. In the stream sediments of the headwaters of the Tomogonops River and its branches, values found for zinc ranged from 50 to 300 ppm; for lead from 0 to 400 ppm; for copper from 25 to 75 ppm. This, of course, represents the natural occurrence of these metals in the stream sediments. The waters of this stream system must have been carrying a heavy metals load throughout geological time. Other tributary complexes of the Northwest Miramichi must also have metals in the stream sediments. The Portage River, for example, has a recorded value of 0.005 ppm of zinc for the stream water in that area where 92 different species of bottom organisms were found. The Little River, a tributary of the Northwest Miramichi entering above the mouth of the Tomogonops, has a recorded value of 0.007 ppm of zinc for the stream water. Water of the Sevogle River, a major tributary of the Northwest Miramichi entering the main river about 15.5 miles below the Tomogonops River, has a recorded value of 0.003 ppm of zinc.

Mullican, et al, (1960) found that an industrial waste carrying 65 ppm of zinc acted as a biological depressant. They reported that in the Nolichucky River (Tennessee) zinc at this concentration reduced the resident population from 2,934 individuals in 30 genera per square foot to 46 individuals in 22 genera per square foot.

Some time after the dewatering of the mine into a branch of the Tomogonops River analyses\* were begun and regularly

\* Colorimetric determination by dithizone extraction.

made on the amount of total heavy metals (THM-zinc, lead, copper) in the water at the mouth of the Tomogonops where it joins the Northwest Miramichi. The highest values recorded up to July 5, 1961, when the comprehensive biological survey was begun, were 8.90 and 10.0 ppm THM on October 25, 1960. Twenty-four hours later 2.80 and 2.21 ppm THM were recorded here. At Wayerton, about nine miles below the mouth of the Tomogonops, values of 0.52 and 0.57 ppm of total heavy metals were recorded on October 26th. By November 12, 1960, the total heavy metals content at the mouth of the Tomogonops had fallen below 1.0 ppm, and this was not exceeded subsequently. During the same period THM values at Wayerton did not exceed 0.2 ppm. Because the mouth of the Tomogonops is inaccessible throughout the winter and into late spring the measurements at Wayerton are important. From November 12, 1960, through July 4, 1961, the record THM values at this location fell between 0 and 0.049 ppm on 116 days, between 0.050 and 0.099 on 62 days, and between 0.10 and 0.19 on 22 days. At these concentrations over an 8-month period the river below the mouth of the Tomogonops supported (or developed) a complete and diverse population of macro-invertebrate animals except for the mollusks. During the course of the comprehensive biological survey begun on July 5, 1961, a total of 132 macro-invertebrate species was taken from the Northwest Miramichi River. An additional 25 species were found in the Portage River.

The toxicity of heavy metals in surface waters is affected by several factors. Chief among these would be the hardness of the water and its hydrogen-ion concentration (pH). Calcium is antagonistic to the metals. Alkaline water precipitate the metals in the form of insoluble, and harmless hydrates. Acid waters dissolve hydrates and bring the metals into solution.

The Northwest Miramichi is a soft-water stream with a total hardness (as  $\text{CaCO}_3$ ) ranging from 17.5 to 24.8 ppm. During the survey the pH range was 7.3 to 7.6. The Portage River had a hardness of 15.5 ppm with pH 7.1, and the Tomogonops River had a hardness of 49.2 ppm and a pH of 7.4 to 7.7. The hardness of the Tomogonops River stemmed from the liming technique practiced by the mine to prevent stream pollution. This practice also increased the hardness of the Northwest Miramichi itself

Table 1

TLM in ppm of Zinc Sulfate for *Physa heterostropha*

Time	Hard Water	Soft Water
24 hours	18 (4.07 ppm Zn)	12.0 (2.71 ppm Zn)
48 hours	16 (3.62 ppm Zn)	6.1 (1.43 ppm Zn)
72 hours	14 (3.16 ppm Zn)	4.9 (1.11 ppm Zn)
96 hours	14 (3.16 ppm Zn)	4.9 (1.11 ppm Zn)
120 hours	--	4.9 (1.11 ppm Zn)

Table 2

TLM in ppm of Zinc Sulfate for young *Physa heterostropha*

Time	Hard Water Series	Soft Water Series
	51°F	51°F
24 hours	4.20 (0.949 ppm Zn)	1.92 (0.434 ppm Zn)
48 hours	1.92 (0.434 ppm Zn)	1.92 (0.434 ppm Zn)
72 hours	1.92 (0.434 ppm Zn)	1.34 (0.303 ppm Zn)
96 hours	1.92 (0.434 ppm Zn)	1.34 (0.303 ppm Zn)
	55°F	55°F
24 hours	6.95 (1.57 ppm Zn)	2.37 (0.536 ppm Zn)
48 hours	6.17 (1.39 ppm Zn)	2.37 (0.536 ppm Zn)
72 hours	6.17 (1.39 ppm Zn)	2.37 (0.536 ppm Zn)
96 hours	6.17 (1.39 ppm Zn)	1.92 (0.434 ppm Zn)
	68°F*	68°F*
24 hours	15.5 (3.50 ppm Zn)	2.95 (0.667 ppm Zn)
48 hours	12.2 (2.76 ppm Zn)	2.37 (0.536 ppm Zn)
72 hours	8.66 (1.96 ppm Zn)	1.92 (0.434 ppm Zn)
96 hours	7.50 (1.70 ppm Zn)	1.92 (0.434 ppm Zn)
	90°F	90°F
24 hours	5.66 (1.26 ppm Zn)	2.65 (0.598 ppm Zn)
48 hours	4.90 (1.11 ppm Zn)	2.65 (0.598 ppm Zn)
72 hours	4.90 (1.11 ppm Zn)	1.55 (0.350 ppm Zn)
96 hours	4.90 (1.11 ppm Zn)	1.55 (0.350 ppm Zn)

\*These bioassays were done with an organic substrate in the dilution water. The substrate material=0.1 gm uncooked *Wheatena* in the 100 ml of material in the experimental jar

below the mouth of the Tomogonops River.

In the laboratory, bioassays were done using zinc sulfate,  $ZnSO_4 \cdot 7H_2O$  (22.6% Zn), in both soft (20 ppm total hardness) and hard (100 ppm total hardness) waters. The pH of these two waters was 7.3 and 7.8 respectively. The mollusk used as an experimental animal was the pond snail, *Physa heterostropha*. The bioassay is designed to establish the median tolerance limit (TLM), which is that concentration of tested material that results in 50% kill and 50% survival of the test animals. Table 1 presents the bioassay results for tests done at  $70 \pm 2^\circ F$  using adult snails of 12 to 15 mm. total shell length.

Since young animals are more susceptible to adverse influences additional bioassays were done on snails of 3 to 6 mm. total shell length. The effects of temperature was also measured in this series of tests. Table 2 summarizes these data.

In addition to *Physa heterostropha*, adult ramshorn snails, *Helisoma campanulatum*, were subjected to bioassays with zinc sulfate. These snails are characterized by the presence of haemoglobin in the circulatory system rather than haemocyanin as is the case in *Physa*. Table 3 presents the results of these bioassays.

Table 3

TLM in ppm of Zinc Sulfate for *Helisoma campanulata*

Time	Hard Water Series	Soft Water Series
	55°F	55°F
24 hours	49.0 (11.07 ppm Zn)	49.0 (11.07 ppm Zn)
48 hours	49.0 (11.07 ppm Zn)	38.5 ( 8.70 ppm Zn)
72 hours	13.4 ( 3.03 ppm Zn)	4.25 (0.96 ppm Zn)
96 hours	13.4 ( 3.03 ppm Zn)	3.85 (0.87 ppm Zn)
	73°F	73°F
24 hours	23.4 (5.29 ppm Zn)	56.0 (12.66 ppm Zn)
48 hours	23.4 (5.29 ppm Zn)	8.30 (1.88 ppm Zn)
72 hours	5.60 (1.27 ppm Zn)	6.53 (1.48 ppm Zn)
96 hours	5.60 (1.27 ppm Zn)	5.60 (1.27 ppm Zn)

In spite of the anomaly of a TLM of 56 ppm of zinc sulfate in 24 hours at  $73^\circ F$ , snails containing haemoglobin evidently are more tolerant of zinc than those containing haemocyanin. These snails apparently will withstand 48-hour surges of wastes bearing high concentrations of zinc.

Copper is more toxic than zinc, apparently being exceeded by its toxicity only by mercury and silver among the metals. The assays of copper sulfate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (25.5% Cu), were done using *Physa heterostropha* as the test animal. The results of these tests, all done at  $70 \pm 2^\circ\text{F}$ , are presented in Table 4.

Table 4

TLM in ppm of Copper Sulfate for *Physa heterostropha*

Time	Hard Water	Soft Water
	Adults	
24 hours	0.56 (0.143 ppm Cu)	-
48 hours	0.27 (0.069 ppm Cu)	-
72 hours	0.27 (0.069 ppm Cu)	-
96 hours	0.27 (0.069 ppm Cu)	-
	Young	
24 hours	0.135 (0.034 ppm Cu)	0.18 (0.046 ppm Cu)
48 hours	0.050 (0.013 ppm Cu)	0.075 (0.019 ppm Cu)
72 hours	0.050 (0.013 ppm Cu)	0.070 (0.018 ppm Cu)
96 hours	0.050 (0.013 ppm Cu)	0.062 (0.016 ppm Cu)
	Young*	
24 hours	0.56 (0.143 ppm Cu)	0.56 (0.143 ppm Cu)
48 hours	0.207 (0.053 ppm Cu)	0.134 (0.034 ppm Cu)
72 hours	0.207 (0.053 ppm Cu)	0.134 (0.034 ppm Cu)
96 hours	0.207 (0.053 ppm Cu)	0.134 (0.034 ppm Cu)

\*Cf. footnote of Table 2

The greater resistance of young snails to copper sulfate in soft water as compared to those in hard water, in the test lacking an organic substrate, is a surprise. The opposite condition would be expected. Repetitive testing has not been undertaken to date.

The median tolerance limit value is used to derive a biologically safe disposal rate for toxicants being introduced into a receiving stream. The disposal rate is based upon several variables including the sensitivity of the experimental animal, the maximum potential discharge load, the rate of loss of the toxic effect, the potential minimum flow of the receiving stream, and other factors. Safe disposal rates must be determined for each specific discharge.



change. No empirical factor can be properly applied to the preceding TLM values to derive a general "rule" for the safe disposal of metals.

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### PELECYPODS FROM BARRA DE NAVIDAD, MEXICO

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Early in December, 1961, we had the opportunity of collecting for an hour or two on the beach at Barra de Navidad, Jalisco, a village on the southwest coast of Mexico approximately 30 miles northwest of Manzanillo, Colima. As the name indicates, Barra de Navidad is located on a sand bar, or spit, that extends from the main shore southward towards Punta Hermosa, a large and rugged headland formed of metamorphic rocks of Paleozoic (?) age. Separating the headland from the terminus of the spit is a narrow inlet that leads to a broad, mangrove-bordered lagoon on the east side of the bar. To the west is an open bay connecting with the Pacific Ocean between several small rocky islets. The combination of these geographic features affords widely diverse ecologic situations that result in an unusually rich molluscan fauna. The recent completion of a paved highway connecting with Manzanillo and thence to Guadalajara, and the completion, in December, 1960, of a first-class hotel near the village makes the area one of unusual attraction for the collector.

Our collections, made only on the beach and within a very short time on an advancing tide, include 87 different molluscan species, plus coral fragments. The majority of the Mollusca are forms normal to the fauna of the region as it is presently known. There are, however, a few species among the pelecypoda that are worth special attention at this time.

Perhaps the most abundant shell on the western side of the