Bull. Environm. Contam. Toxicol. 20,451-455 (1978)

Evidence of Resistance to Metals in Larvae of the Midge *Chironomus tentans* in a Metal Contaminated Lake[1]

R. Wentsel, A. McIntosh, and G. Atchison
Bionucleonics Department, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Ind. 47907

Introduction

Currently the existence of toxicant resistant strains of aquatic invertebrates is of interest. Recently BRYAN (1974) noted tolerance of copper and zinc but not cadmium or lead in the polychaete Nereis diversicolor taken from polluted English estuaries. LUOMA (1977) has suggested that resistance testing may be used to indicate which contaminants are of primary concern in aquatic systems. In the current study, chironomid larvae (Chironomus tentans) were collected from two areas, one contaminated by heavy metals and one uncontaminated, of Palestine Lake, Indiana, and subjected to a series of laboratory tests to determine if metal resistant strains have developed within the lake.

Materials and Methods

Palestine Lake is a highly eutrophic 93 ha system near Warsaw in north-central Indiana. The west basin of the lake (Fig. 1) received for at least 25 years substantial amounts of the metals cadmium, zinc, and chromium from an electroplating plant, which discharges its waste into Williamson Ditch several km from the lake. Because the only significant discharge of water from the lake is at the north end of the western basin, most of the entering metal load is deposited in areas north of the ditch. Since the flow in the system is from the east basin toward the outfall in the west basin, significant metal contamination of the east basin does not occur (WENTSEL, 1977).

C. tentans larvae were collected from a highly contaminated part of the west basin (A in Fig. 1) and the east basin (B in Fig. 1). In addition, three sediment samples were collected from the lake for use in subsequent laboratory experiments (see Fig. 1 for locations). Type 1, from a highly contaminated area of the west basin, contained 1070 ppm cadmium, 15,100 ppm zinc and 1680 ppm chromium; Type 2, from a moderately contaminated part of the west basin, had levels of 657 ppm cadmium, 8280 ppm zinc and 1080 ppm chromium. Type 3, collected from the uncontaminated east basin, had metal concentrations of 6 ppm cadmium, 184 ppm zinc

[1]Supported by NSF (RANN) Grant Number GI-35106.

0007-4861/78/0020-0451 $01.00
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and 39 ppm chromium. Previous research by WENTSEL (1977) established that other sediment parameters, including texture and % organic matter, were similar for the three sediment types.

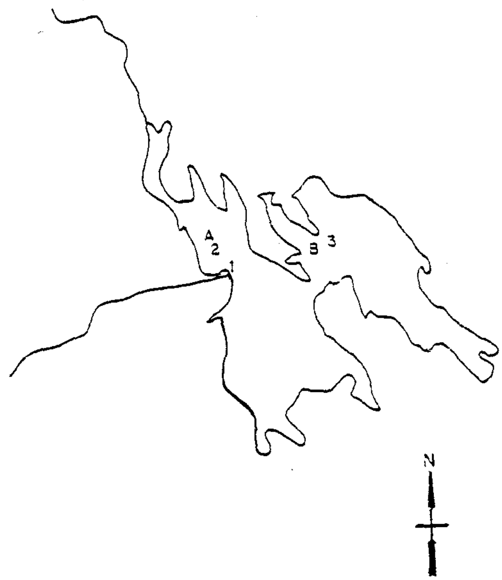

Figure 1. Map of Palestine Lake. Letters indicate chironomid collecting sites and numbers indicate sediment collecting sites.

Metal concentrations were determined by digesting duplicate air-dried sediment samples in concentrated HNO_3 for 6 hours. Digested samples were filtered through Whatman #40 filter paper; the filtrate was diluted to volume. Levels of cadmium, zinc and chromium were determined on a Perkin-Elmer Model 306 Atomic Absorption Spectrophotometer. Concentrations were calculated by comparison to standards made from stock solutions.

Several laboratory experiments were conducted to determine if chironomids collected from the west basin were resistant to heavy metals. The first experiment was a 96-hour bioassay in which the two groups were exposed to highly contaminated sediment type 1. For both runs of the experiment, two 2-liter beakers containing sediment type 1 were used for each group; 20 mature Chironomus tentans larvae were deposited in each beaker. After 96 hours, the sediment was screened, and surviving larvae were counted.

Avoidance of heavy metal contaminated sediment by the larvae was then studied. To initiate the experiment, a piece of cardboard was placed across the center of a 20 cm by 40 cm tank. Two cm of uncontaminated sediment (Type 3) were added to half of the tank, while a similar thickness of contaminated sediment (Type 2) was added to the other side. Approximately 2 cm of water was then added to both sides, and, after the sediment had settled, the cardboard was removed. Air was slowly bubbled into the water on one side of the tank along the sediment interface, and 25 mature C. tentans larvae were deposited on the other side of the tank on a line between the two sediments.

Five days was judged to be sufficient time for the larvae to react to the sediment types. When the experiment was terminated, the water was carefully suctioned off, and the sediment from each half of the tank was removed in four approximately equal 5 cm strips parallel to the center line. The number of chironomids found in each sediment strip was recorded.

In the final laboratory experiment, growth retardation of chironomid larvae exposed to metal contaminated sediment was assessed. After lengths of chironomid larvae from both areas of the lake were determined, 20 specimens were deposited in each of four beakers, two for each population, containing a moderately heavy metal contaminated sediment (Type 2). After 10 days, the larvae were removed, and four larvae from each beaker were selected at random; their lengths were determined and compared to the average initial values.

Results and Discussion

Only 47.5% of the larvae from the east basin survived in the highly contaminated sediment used in the 96-hour bioassay (Table 1), while chironomids obtained from the west basin had a survival rate of 75%. Analysis of variance (ANOVA) demonstrated that the percent survival of the two groups of chironomids was significantly different at the 95% level, indicating that the larvae from the west basin may be, to some degree, resistant to heavy metal contamination.

TABLE 1

Percentage survival of chironomid larvae exposed to heavy metal contaminated sediment (N = 20 for each group).

Site of Collection	Average Survival (2 Runs)	Standard Error
East basin	47.5	10.1
West basin	75.0	5.5

Data from the avoidance response experiment (Table 2) show that 76% of the east basin organisms were found in the uncontaminated sediment (Type 3), while only 33% of the west basin chironomids were found there. Combined with results from an earlier study (WENTSEL, 1977), in which chironomid larvae avoided contaminated sediment similar to type 2, these data suggest that an insensitivity or resistance to the heavy metals has developed in west basin organisms.

TABLE 2

Avoidance response of chironomid larvae exposed to heavy metal contaminated sediment.

Site of Collection	Mean Percent in Uncontaminated Sediment (2 Runs)	Standard Error
East basin	76.2	8.0
West basin	32.7	6.4

The growth experiment data (Table 3) indicate an overall increase in length of 8.8% for the east basin chironomids and 18% for the west basin larvae exposed to sediment type 2. WENTSEL (1977) has shown that growth of chironomids may be affected by metal contamination and is reduced under laboratory conditions in sediment with metal levels similar to those in the type 2 sediment utilized in this experiment. The higher growth rate of chironomids from the west basin is yet another indication of their relative resistance to heavy metal contamination in the sediment.

LUOMA (1977) suggested that demonstrating toxicant resistance in a population provides strong evidence that the toxicant has exerted a selective effect on that population. He stated that to induce tolerance, the toxicant must be available in sufficient concentrations to limit the reproductive success of a proportion of the individuals, the nonresistant genotypes, in a population. Further, greater resistance to a toxicant in a population from one location than one from another location is direct evidence that the toxicant is exerting selective pressure on the species at the former site.

We have demonstrated through tests on survival, growth and avoidance behavior that chironomid larvae dwelling in the west basin of Palestine Lake have developed a degree of resistance to the heavy metal contaminated sediment present in that area.

TABLE 3

Growth rate of chironomids exposed to heavy metal contaminated sediments.

		Initial Length (cm)		Final Length (cm)			
Site of Collection	Test	Mean	Standard Error	Mean	Standard Error	Percent Increase	
East Basin	Run 1	0.91	0.02	1.02	0.10	13.2	8.8
	Run 2	0.94	0.03	0.98	0.08	4.3	
West Basin	Run 1	1.20	0.03	1.53	0.05	27.5	18
	Run 2	1.18	0.03	1.28	0.09	8.5	

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