Adaptation to mercury contamination in an aquatic oligochaete worm

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ABSTRACT - Resistance to contaminants is an important yet unmeasured factor in sediment toxicity tests. The rate at which mercury resistance develops and its genetic persistence in the oligochaete worm *Tubifex tubifex* were studied under laboratory conditions. Two populations of *T. tubifex* worms were raised for four generations each in clean and mercury-contaminated sediments, respectively. Mercury resistance was determined by comparative water-only toxicity tests, with mercury as the only stressor. *T. tubifex* exposed to high levels of mercury in sediment showed high mercury resistance during laboratory exposures. Resistance in these worms was rapidly acquired and inherited by subsequent generations. The mean LC_{50} for control worms was 0.18 mgL^{-1}. The mean LC_{50} for mercury-reared worms was 1.40 mgL^{-1}. Furthermore, crosses between resistant and less resistant worms grown in control sediment resulted in offspring with high mercury resistance (LC_{50}=1.39 mgL^{-1}), thus demonstrating genetic adaptation to mercury. Development of contaminant resistance and adaptation may be common phenomena in aquatic benthic invertebrates, and should be considered during the design and interpretation of toxicity tests.

INTRODUCTION

Sediment toxicity tests are common tools used to assess whether contaminants in sediment are harmful to organisms. The U.S. Environmental Protection Agency (U.S. EPA 1999) has established standard laboratory procedures for measuring sediment toxicity and bioaccumulation of contaminants in aquatic organisms (e.g., the aquatic oligochaete *Tubifex tubifex*). Data from sediment toxicity tests are used to make environmental management decisions, such as evaluating the suitability of dredge material for ocean disposal and identifying impaired water bodies. The incorrect use or interpretation of toxicity tests may have important consequences for the decision-making process.

Many issues that could affect the accuracy of toxicity tests have been poorly studied, despite the wide use of these tests in regulation and monitoring. A factor that can affect the results of toxicity tests is variation in the resistance of the test organisms to contaminants. The development of contaminant resistance is well documented in plants (Bradshaw 1970, Antonovics *et al.* 1971, Macnair 1987), and also among aquatic animals (Lavie and Nervo 1986, Klerks and Weis 1985, Klerks and Levinton 1989). Variations in resistance have been studied previously in aquatic invertebrates such as isopods (Donker *et al.* 1993), polychaetes (Bryan and Hummerstone 1971, Bryan and Hummerstone 1973), and oligochaetes (Aziz *et al.* 1999, Klerks 1990). The impact of acquired resistance can be profound. For example, Vidal *et al.* (2002) observed a three-fold variation in mercury resistance among different populations of the oligochaete *Sparganophilus pearsei.*

An understanding of both the timing and the basis of contaminant resistance is important for the design and interpretation of toxicity studies. Resistance can be rapidly acquired through physiological changes (i.e., acclimation). The rapid development of resistance may influence the outcome of long-term sediment toxicity tests or create variability among experiments. If the resistance is heritable (i.e., adaptation), then the use of test organisms from different geographic locations may confound the results. Knowledge of the temporal scale of resistance could affect the design of a toxicity test (e.g., duration), whereas information regarding the ability of a species to adapt or acclimate could influence its suitability for a particular test design.

The goal of this study was to understand the characteristics of contaminant resistance development in the aquatic oligochaete worm *Tubifex tubifex*. *T. tubifex* was chosen for this study because this species is widely used for both bioassessments and laboratory toxicity tests (Wiederholm *et al.* 1987, Milbrink 1987, Reynoldson *et al.* 1991, Chapman 2001). The two objectives of this research were: (1) to study the temporal progression of the development of resistance to mercury stress and (2) to determine whether this resistance is genetically driven. These objectives were addressed in a multi-generational experiment using exposure to mercury-contaminated sediment to induce resistance in *T. tubifex*. The persistence and inheritability of

resistance following removal of the mercury stress was also measured.

METHODS

Study Design

Aquatic tubificid worms were exposed to mercury-contaminated and uncontaminated (control) sediments under laboratory conditions during multiple generations to assess mercury resistance development (Figure 1). Mercury stress was removed after a few generations to determine whether genetic traits were responsible for resistance development. To confirm that mercury resistance had a genetic basis, control worms and mercury-resistant worms were crossed and mercury resistance was once again assessed in their descendants. Resistance to mercury was measured by determining the LC_{50} in 96-h exposures to dissolved mercury.

Resistance Inducement

Descendants of a laboratory population of tubificids (F0) were divided into two sediment treatments of 50 worms each (Figure 1). One treatment consisted of worms cultured in uncontaminated sediment during seven generations (F1-F7 uncontaminated). The second treatment consisted of worms exposed to mercury-contaminated sediment during four generations (F1-F4 contaminated). Subsequently, their descendants were moved to control sediment and allowed to grow and reproduce for three more generations (F5-F7 contaminated). The last generation (F8) consisted of the offspring produced from the cross of individuals from both treatments (mercury contaminated and uncontaminated).

Worms were grown until sexually mature. Adult worms were removed following reproduction at each generation, by screening through 0.25 and 0.5 mm sieves every 28 d, and assessed for mercury resistance. Generation times were based on previous reproductive and growth tests (Reynoldson *et al.* 1991). Cocoons and young individuals were also removed and used to establish the next generation of exposure.

T. tubifex stock cultures were obtained from Dr. T.B. Reynoldson at the National Water Research Institute in Canada. The worms were acclimated for two generations (56 d) in uncontaminated control sediment prior to their use in testing. Cultures were maintained in static systems containing dechlorinated tap water in 3 L beakers. Clear plastic perforated

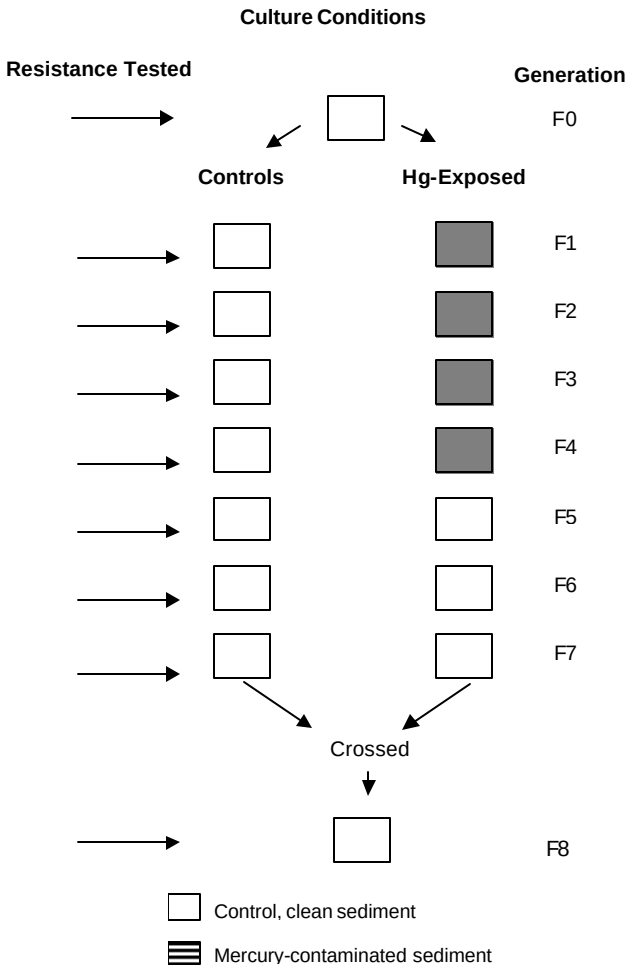

Figure 1. Experimental design. Control treatment: worms reared in clean, uncontaminated sediment. Mercury-exposed treatment: worms reared in mercury-contaminated sediment.

lids covered each beaker. Air was introduced with a glass pipette. Worms were kept in the dark at 23±1°C in an incubator and fed once every 14 d with Tetrafin®. Chambers were checked every 2 d to ensure adequate aeration and were supplemented with dechlorinated water as necessary. Water quality parameters (pH, dissolved oxygen [DO], and ammonia) were monitored every week.

The sediment used in this research was collected from two sites. The first was Guadalupe Reservoir, located in Santa Clara County (37°54'N 121°15W) and known for its high concentrations of mercury in the sediment (up to 0.6 $mgKg^{-1}$ wet weight was recorded during this research) (NSPCP 1992). Aquatic oligochaetes (*Sparganophilus pearsei*)

living in these sediments have developed high mercury resistance (Vidal and Horne 2002). Control sediment was obtained from Sandy Wool Reservoir, also located in Santa Clara County (37°27'N 121°52W); this is a clean reservoir with background levels of mercury (< 0.02 $mgKg^{-1}$ wet weight) in sediments. Sandy Wool is inhabited by a mercury-sensitive population of *S. pearsei* (Vidal and Horne 2002).

Samples for total organic carbon (TOC) measurement were homogenized and decarbonated with HCl to remove mineral carbon, and then analyzed with an elemental analyzer. Culture sediment TOC ranged from 6.9 to 7.9% during the experiment. Total Hg was also analyzed using cold vapor techniques and ranged from 0.006 to 0.009 $mgKg^{-1}$ (wet weight) for the control sediment and 0.48 to 0.62 $mgKg^{-1}$ (wet weight) for the mercury-contaminated sediment. The DO ranged from 8.4 to 9.0 mgL^{-1}; ammonia values were always below 2 mgL^{-1}.

Resistance Measurement

Adult worms from each generation were exposed for 96 h to various concentrations of mercury chloride solutions in water. Resistance was quantified as the ability of the worms to survive the mercury exposure (Chapman *et al.* 1982). Observations of mortality were periodically recorded and the median lethal concentration (LC_{50}) was calculated. The worms were not fed during toxicity tests. They were considered dead if no response was triggered after gentle touching. Dead worms were removed when observed.

Only healthy and sexually mature organisms of similar size were used for toxicity experiments. The toxicity test chambers consisted of 250 mL glass flasks with perforated stoppers and glass pipettes for aeration. Exposures were conducted in triplicate with controls. Mercury concentrations were 0.01, 0.02, 0.1, 0.2, 0.3, 0.4, 0.8, 1.6, and 2 mgL^{-1}. The bioassays were conducted under a 12 h light/dark regime, at 20±1°C.

Standard quality assurance and quality control procedures were followed during the test (ASTM 1998, U.S. EPA 1993, U.S. EPA 1994). The DO in water during testing was measured with a YSI meter; pH was measured with a pH meter. Ammonia in water was measured with a Nessler colorimetric method. Total mercury in water was determined with cold vapor techniques using a Perkin Elmer atomic absorption spectrometer. Water quality conditions during the toxicity tests were: DO= 7.7±0.2 mgL^{-1} and pH= 6.7±0.1.

The LC_{50} values were determined using the Spearman-Karber test (Environmental Protection Agency Spearman-Karber method, computer program version 1.5) with actual concentrations. Significant differences (a= 0.05) between treatments were tested using the Tukey test performed with the statistical program JMP® (JMPin, Windows Version 3, SAS Institute Incorporated).

RESULTS

The tubificid worms survived, reproduced, and grew in both treatments; the results for these parameters are described in Vidal (2001). Resistance to mercury in the parent population (F0) and in worms grown in control sediment was relatively low and similar to the levels of resistance found by other researchers for *T. tubifex*. Levels of resistance in the populations exposed to mercury uncontaminated sediment (F1-F7) ranged from 0.17 to 0.19 mgL^{-1} and were similar to the F0 worms (0.18 mgL^{-1}). The mean 96 h LC_{50} for the eight generations (F0-F7) of control tubificids was 0.18 mgL^{-1} (Table 1).

Table 1. Resistance developed by each tubifex generation as Hg lethal concentration values (LC_{50}s in mgL^{-1}) and 95% confidence intervals (CI). F8 generation was a cross between Hg exposed and control worms reared in control sediment.

Generation	Control		Hg-Exposed		Crossed	
	LC_{50}	CI	LC_{50}	CI	LC_{50}	CI
Parent	0.16	0.09-0.26				
F1	0.17	0.09-0.26	1.09	0.83-1.42		
F2	0.18	0.10-0.27	1.59	1.38-1.83		
F3	0.17	0.09-0.26	1.57	1.37-1.80		
F4	0.19	0.11-0.32	1.56	0.87-1.79		
F5	0.19	0.11-0.32	1.07 †	0.60-1.74		
F6	0.18	0.10-0.27	1.42 †	0.80-1.98		
F7	0.19	0.11-0.32	1.48 †	0.83-2.04		
F8					1.39	0.77-1.91

†= Worms grown in control sediment but previous generations (F1 to F4) were reared in mercury-contaminated sediment.

Tubificid worms exposed to mercury-contaminated sediment for one to four generations showed higher levels of mercury resistance when compared to worms grown in uncontaminated sediment (Table 1). Resistance in Generation F1 of the mercury-contaminated treatment was already higher than in the parent worms. The LC_{50} for Generations F1 to F4 grown in mercury-contaminated sediment ranged from 1.09 to 1.59 mgL^{-1}.

Resistance in the worms' descendants exposed to control sediment (Generations F5 to F7) was retained despite the absence of high mercury exposure. The resistance to mercury in Generations F5 to F7 ranged from 1.07 to 1.48 mgL^{-1} (Table 1), respectively, and was significantly different from control worms (generations versus tolerance Tukey t= 2.14, df= 15, p <0.0001).

Worms from the F8 generation (which resulted from the crossing of Generation F7 mercury-resistant and control worms) also showed a higher resistance to mercury when compared with control oligochaetes. This generation was also reared in clean reservoir sediment. The resistance of the F8 oligochaetes was 1.39 mgL^{-1}.

DISCUSSION

The oligochaetes grown in the clean control sediment for seven generations retained a mercury sensitivity similar to the parent population. The resistance shown by parent and control worms was comparable to the resistance reported by Bokovic-Popovic and Popovic (1977) of 48 h LC_{50} of 0.1 mgL^{-1} and by Chapman *et al.* (1982) of 0.14 mgL^{-1}.

Higher resistance was already evident in the first generation reared in mercury-contaminated sediment, although this rapid change may have been only by acclimation processes. Acclimation is a process that acts directly on the individual and the physiological changes can be observed within a few minutes or days. The mercury-exposed Generations F2 to F4 showed even higher levels of resistance. The descendants of worms grown in mercury-polluted sediment from Generations F5 to F7 continued to exhibit high mercury resistance despite being moved and grown in control sediment. These results clearly show that the mercury resistance achieved by tolerant worms was obtained by a combination of acclimation and adaptation. Adaptation, unlike acclimation, is a process that occurs over generations. Forbes (1994) mentions that most assemblages that are able to endure metal polluted habitats survive in their disturbed environments as the result of a combination of physiological acclimation, changes in species composition, and genetic adaptation.

The observation of rapid development of mercury resistance in these worms is consistent with the results found in another oligochaete study (Klerks and Levinton 1989), which also observed the development of metal tolerance within only a few generations. Other researchers have found similar tolerance responses in other aquatic organisms (e.g., copepods) (Moraitou-Apostolopoulou *et al.* 1983). Genetic adaptation to heavy metals in populations of terrestrial invertebrates has also been well documented in isopods, springtails (Collembola), and the fruit fly (Diptera). These cases of metal adaptation were also achieved within a few generations under laboratory conditions (Posthuma and VanStraalen 1993). The fact that this result is consistent with the results from other researchers suggests that resistance development may be more common than we had previously considered. However, genetic adaptation does not always occur in aquatic organisms after sublethal pre-exposure. For example, Klerks *et al.* (1997) found that no acclimation occurred in gobies exposed to polyaromatic hydrocarbons (PAHs). Cadmium resistance in Daphnids was also shown to be due to acclimation, rather than genetic adaptation (Bodar *et al.* 1990).

Although adaptation to contaminants may enhance the survival of some species, this phenomenon could have adverse long-term effects. Tolerant individuals may accumulate higher concentrations of contaminants, thus increasing the bioavailability of contaminants to predators (Vidal and Horne 2002). Selection imposed by contaminants on resistant individuals may also reduce genetic variability and diversity in the affected population, thus making the surviving organisms more susceptible to mortality from other environmental changes (Guttman 1994).

Adaptation to contamination is likely to occur among southern California marine life chronically exposed to sediment contamination in offshore (e.g., Palos Verdes Shelf) and harbor environments, but there are few data to document the extent of this phenomenon. One potential example of contaminant adaptation in a local species is the amphipod *Grandidierella japonica*. This amphipod is a common inhabitant of bays and harbors throughout California and has been used in acute and chronic sediment toxicity tests (ASTM 1998, SCCWRP 1995). Laboratory tests using *G. japonica* collected from a reference location have detected toxicity in San Diego Bay sediments (Anderson *et al.* 1988), yet field

studies in this bay have observed abundant populations of *G. japonica* living at the same locations (Fairey *et al.* 1996). Laboratory studies have also shown variability in contaminant tolerance among different populations of *G. japonica* (Lamberson *et al.* 1994).

Adaptation of resident fauna may account for some of the discrepancies observed in monitoring studies of the Southern California Bight. A comparison of sediment toxicity test results and benthic community assessment, conducted during the Bight'98 regional monitoring survey, found many instances where laboratory toxicity did not correspond to impacts to the benthic community (Ranasinghe *et al.* 2002). The extent to which these discrepancies are due to adaptation cannot be determined without further studies, such as toxicity tests using local organisms or *in situ* experiments. Contaminant resistance is an important factor that is rarely considered in the planning and interpretation of environmental research.

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