RESPONSES OF PRISTINA LEIDYI SMITH 1896
(NAIDIDAE:OLIGOCHAETA) TO CADMIUM,
VANADIUM, AND SOME ENVIRONMENTAL
FACTORS

THESIS

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By

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Concern over sediment toxicity has increased the need for toxicity test information with organisms that inhabit sediments. Oligochaetes are exposed to toxicants through feeding and direct body contact with aquatic sediments. Chronic testing with oligochaetes has historically focused on tubificids with test lengths of one year or more to encompass several generations. Most naidid oligochaetes have generation times of three to seven days and could provide chronic information in a matter of weeks.

The cosmopolitan distributed naidid, Pristina leidyi, was evaluated for use as a toxicity test organism. Results of research conducted includes culture methods, effects of temperature on reproduction, growth rates in a reference sediment, acute toxicity tests, and chronic toxicity tests.
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CHAPTER I

INTRODUCTION

Since invertebrates are an important structural and functional component of aquatic ecosystems, information on their responses to toxicants is needed to protect these systems from damage. Lethal or sublethal effects of toxicants may alter population distributions and densities of these organisms leading to both ecological and economical degradation (Maciorowski and Clarke 1980).

Historically, aquatic toxicity testing has focused on organisms that inhabit the water column resulting in National Ambient Water Quality Criteria (WQC) that are used to establish Water Quality Standards to regulate waste dischargers. The environmental behavior of some contaminants leads to their deposition in aquatic sediments. There has been a growing concern over toxicity of sediments and recognition of the need to develop sediment-based criteria to supplement WQC to ensure better protection of aquatic life (Gilford and Zeller 1984, Shea 1988). The United States Environmental Protection Agency (USEPA) is currently developing numerical Sediment Quality Criteria (SQC) (Chapman 1989). In support of the development of SQC a need exists for toxicity test methods to assess effects of
sediment associated chemicals in organisms that inhabit sediments.

Aquatic oligochaetes (worms) live in and feed on sediments and thus are exposed to toxicants through feeding and direct body contact. Of the seven families of aquatic microdrile oligochaetes, only the Naididae, Tubificidae, and Lumbriculidae are found in large numbers.

There are only two species of Lumbriculidae found in the United States and Canada. They are frequently found in shallow waters among leaves, sticks, rooted plants, and also in stony substrates.

The Tubificidae comprise about 58 species. They are found in a variety of habitats from organically polluted sediments to uncontaminated stony substrates. They play a significant role in bioturbation of sediments and hence play a significant role in geochemical and microbiological processes.

There are approximately 70 species of Naididae known in North America. Some are found as infauna of muddy sediments of lakes and ponds while others are epifauna associated with algae or higher plants in streams and rivers. Niadids are often an important part of the fauna of small stony streams where tubificids are less abundant.

The ecological habitat and niche of oligochaetes make them a logical candidate for assessing toxicity of
sediments. Oligochaetes have been used in a variety of toxicity tests and bioassays to assess the impacts of metals, organic compounds, and complex mixtures. A wide range of sensitivities depending on the organism used, type of toxicants, test conditions, and experimental design has been reported.

**Toxicity Testing of Metals Using Oligochaetes**

Brkovic-Popovic and Popovic (1977a, 1977b) reported the survival and respiration rates of *Tubifex tubifex* exposed to solutions of copper, cadmium, chromium, mercury, zinc and nickel. The 48 hour LC50s (in mg/L) were: copper, 0.006-0.89; cadmium, 0.03-0.72; chromium, 0.6-4.57; mercury, 0.06-0.10; zinc, 0.11-60.2; nickel, 0.08-61.4. Toxicity was dependent on hardness and alkalinity with the exception of mercury. Effects on respiration rates were dependent on the particular metal and the concentration. Generally, at the acute lethal range, cadmium, mercury, and copper depressed respiration while chromium, zinc, and nickel increased respiration above the control values.

Chapman et al. (1980) compared levels of nine metals (copper, zinc, lead, iron, manganese, nickel, cobalt, cadmium, and mercury) in tubificids (mainly *Limnodrilus hoffmeisteri* and *Tubifex tubifex*) and in sediments collected from the Fraser River estuary in British Columbia. None of the metals except mercury were concentrated in the
tubificids above the levels found in the sediments. In two measurements, mercury levels were 4.0 and 1.7 times as high in tubificid tissue as in sediments.

Effects of different ratios of calcium, magnesium, and potassium (Knop's solution) at 10°, 15°, 20°, and 30°C on the asexual reproduction of Aeolosoma hemprichi was investigated by Kamemoto and Goodnight (1956). At 10°C there was little or no reproduction. At 15°C there was more reproduction at higher total ion concentrations. At 30°C there was more reproduction at lower ion concentrations. At 20°C there was equal reproduction at both high and low ion concentrations.

Mathis and Cummings (1973) compared concentrations of copper, nickel, lead, chromium, lithium, cobalt, cadmium, and zinc in sediments, water column, and selected biota including Tubifex tubifex and Limnodrilus hoffmeisteri in the Illinois River near Peoria. Concentrations were highest in the sediments with the exception of copper in the tubificids.

Milbrink (1987) exposed cohorts of young Tubifex tubifex to different ratios of Lake Runn sediments containing high levels of metals and relatively uncontaminated lake sediments in Sweden. Tests were conducted for 300 days with individual counts and body weights measured periodically. Young worms exposed to 100% Lake Runn sediments died by day 200 and reproduction only
occurred in mixtures containing no more than 50% Lake Runn sediments. Even 25% Lake Runn sediments caused a reduction in growth rate and reproduction by as much as half in comparison to the clean sediments.

The 9 day LC50 of vanadium to the marine polychaete *Nereis diversicolor* was reported by Miramand and Unsal (1978) to be 10 mg/L. Avoidance of or increased emigration from dosed sediments containing 0.5 mg/g copper and/or 1.0 mg/g zinc by the tubificids *Tubifex tubifex* and *Limnodrilus hoffmeisteri* was observed by McMurtry (1984).

Niederlehner et al. (1984) conducted 48 hour acute and 10 to 14 day chronic tests using cadmium on the aeolosomatid *Aeolosoma headlyi*. The 48 hour LC50s were 4.98 mg/L in artificial hardwater (180 mg/L hardness) and 1.2 mg/L in dechlorinated tap water (60 mg/L hardness). The chronic toxicity test used population growth as the endpoint. The no-observable-effects-concentrations (NOEC) were 0.032 and 0.0536 mg/L in the soft water. Exposure-response curves were linear at higher concentrations, but stimulation of reproduction at lower concentrations was noted.

*Toxicity Testing of Organic Compounds With Oligochaetes*

Coler et al. (1967) noted avoidance responses and uptake of diazinon by mixed populations of tubificids (*Limnodrilus* sp., *Tubifex* sp., and *Peloscolex* sp.) feeding on diazinon contaminated bacteria.
Keilty et al. (1988a, 1988b, 1988c) reported the effects of endrin spiked sediment on survival, growth, and burrowing behavior in single and mixed species tests using *Stylodrilus heringianus* and *Limnodrilus hoffmeisteri*. The 96 hour LC50s were 2.588±0.974 and 2.725±0.995 mg/g (dry weight sediment) respectively. Burrowing behavior response (96 hour EC50) was 19.0 and 15.3 ug/g for *S. heringianus* and 59.0 ug/g for *L. hoffmeisteri*. Both organisms initially burrowed into the contaminated sediment then returned to the surface in numbers somewhat proportional to the concentration and length of exposure with *S. heringianus* responding four times faster than *L. hoffmeisteri*. Post experimental dry weights of the oligochaetes were inversely proportional to high endrin sediment concentrations. Bioconcentrations of endrin by *S. heringianus* ranged from 34 to 67 times the sediment concentrations. Sediment reworking rates by *S. heringianus* (monitored after burial of a $^{137}$cesium marker layer) were found to be significantly inhibited in the endrin contaminated sediments at concentrations up to 5.5 orders of magnitude lower than the 96 hour LC50 values.

The responses of the tubificid *Branchiura sowerbyi* to 23 insecticides (chlorinated hydrocarbons, organophosphates, and carbamates) were observed by Naqvi (1973). At maximum concentrations of 0.5 to 4.0 mg/L at 21°C, fifteen of the tested insecticides failed to induce mortality in 72 hours,
but did produce reversible morphological changes. Toxicity was influenced by temperature. Exposed tubificids were then fed to crayfish. Insecticide toxicity to crayfish was inversely proportional to treatment time of the tubificids prior to feeding but was directly proportional to concentration.

Oliver (1984) exposed *Limnodrilus hoffmeisteri* and *Tubifex tubifex* to anthropogenically contaminated sediments from Lake Ontario for 110 days in static conditions. The tubificids accumulated 15 of the 24 chlorinated organics identified in the sediments. Efficiency of uptake was variable and related to the contaminants physical and chemical properties such as the octanol-water partition coefficient. The tubificids contained different ratios of the compounds than were found in the sediments. More recently Oliver (1987) compared bioaccumulation in *Limnodrilus hoffmeisteri* and *Tubifex tubifex* between sediments in flow-through tests spiked with 37 organic compounds and field collected samples. The worms were found to rapidly accumulate the compounds and reach the highest concentrations within two weeks. There was good agreement between laboratory and field results for more persistent chemicals but poor agreement for less persistent chemicals.

Whitten and Goodnight (1966) conducted 96 hour static acute toxicity tests with mixed tubificid cultures (*Tubifex*
tubifex and Limnodrilus hoffmeisteri in unknown ratios) using six insecticides as the toxicants. The 96 hour LD50s (in mg/L) were: malathion, 16.7 ± 1.75; parathion, 5.23 ± 0.36; dieldrin, 6.71 ± 0.76; BHC, 3.15 ± 0.51; DDT, >100; sevin, >50 (DDT and sevin concentrations were not analytically verified).

Toxicity Testing of Complex Mixtures With Oligochaetes

Chapman et al. (1982a) investigated tolerances of nine freshwater oligochaetes (Limnodrilus hoffmeisteri, Tubifex tubifex, Branchiura sowerbyi, Varichaeta pacifica, Quistadrilus multisetosus, Rhvacodrilus montana, Spirosperma nikolskyi, Spirosperma ferox, and Stylodrilus heringianus) in 96 hour acute toxicity tests to five pollutants (cadmium, mercury, pentachlorophenol, pulp mill effluent, and sewage sludge) and four environmental factors (pH, temperature, salinity, and anoxia) both with and without sediments. It was concluded that the presence of sediments increased tolerances which were pollutant specific. For example, the 96 hour LC50 for L. hoffmeisteri using cadmium was 0.17 mg/L without sediment and 3.5 mg/L with sediment.

In a following study using the same toxicants Chapman et al. (1982b) compared changes in LC50s for L. hoffmeisteri, T. tubifex, and S. heringianus under varying environmental conditions. The LC50s were shown to depend on the environmental factors, but that relative tolerances to
cadmium, pentachlorophenol, and pulp mill effluent were fairly consistent.

In another study Chapman et al. (1982c) used mixed cultures of *L. hoffmeisteri* and *T. tubifex* in 96 hour acute toxicity tests and respiration effects tests using the toxicants cadmium, mercury, and pentachlorophenol and environmental factors (pH, temperature, salinity, anoxia). Results of the acute tests compared with the previous single species tests indicated that mixed species were significantly more tolerant of toxicants, similarly tolerant of environmental factors, and less tolerant to anoxia. The respiration tests showed that in pure cultures, the two species were regulators of respiration rate, in mixed cultures and tests they were non-regulators and that changes in response to stress was variable depending on test conditions. This series of experiments demonstrates the importance of reporting the test conditions.

Respiration rates of the marine oligochaete, *Monopylephorus cuticulatis*, exposed to sediment elutriates from Puget Sound, was monitored by Chapman (1987). These results were compared with results of other types of toxicity tests (reproductive impairment, genotoxicity to fish cells, and lethality) that had been conducted in the same area. There was good agreement with these tests in that
stations were similarly ranked according to toxicity effects.

Dean (1974) exposed unidentified Tubificidae to $^{65}$Zn in 9 day static water column tests at three temperatures ($6^\circ$, $15^\circ$, and $25^\circ$C) and in 30 day flow through water column and 70 day flow through sediment tests at $15^\circ$C. The tubificids did not accumulate radionuclides bound to sediments but did accumulate dissolved radionuclides. Accumulation of dissolved $^{65}$Zn was dependent on temperature and concentration. In static acute water column toxicity tests conducted at $20^\circ$C at different pHs, Whitley (1968) exposed mixed cultures of Limnodrilus hoffmeisteri and Tubifex tubifex to lead nitrate, zinc sulfate, and sodium pentachlorophenol. The 24 hour LC50s were: lead, 27.5 - 49.0 mg/L; zinc, 46.0 mg/L; pentachlorophenol, 0.31 - 1.4 mg/L. Toxicity was dependent upon pH.

Wiederholm et al. (1987) measured survival, growth, and population increase (reproduction) of Tubifex tubifex, Limnodrilus hoffmeisteri, L. udekemianus, L. claparedeanus, and Potamotheix hammoniensis in sediments from four Swedish lakes representing different trophic states and degrees of pollution. In addition, one of the sediments was spiked with copper sulfate. The tests were conducted for 500 days with counts and weights recorded every two weeks. Survival was affected only at high concentrations of pollutants. The
worms were more sensitive to pollutants in sediments from oligotrophic to mesotrophic lakes than those from eutrophic lakes. Results of this study showed that reproduction was a more sensitive measure of toxicity than was growth.

**Effects of Environmental Factors on Oligochaetes**

Aston (1973a) conducted laboratory experiments to test effects of temperature on reproductive rates of *Limnodrilus hoffmeisteri* and *Tubifex tubifex* and interactive effects of dissolved oxygen and temperature on the reproductive rate of *L. hoffmeisteri*. In the first experiment, sexually mature worms were maintained at six temperatures from 5\(^\circ\) to 30\(^\circ\)C for 35 days. *L. hoffmeisteri* had increased cocoon production up to 25\(^\circ\)C and the number of eggs per cocoon was highest at 20\(^\circ\) and 25\(^\circ\)C. *T. tubifex* exhibited a slow increase in cocoon production but a decrease in eggs per cocoon resulting in a constant number of eggs from 10\(^\circ\) to 25\(^\circ\)C. In the second experiment, worms were maintained at 15\(^\circ\) and 25\(^\circ\)C at four dissolved oxygen concentrations for 10 days. *L. hoffmeisteri* showed a relatively constant rate of reproduction except at 15\(^\circ\)C and 0.2 mg/L dissolved oxygen. It was concluded that temperature had more of an influence than dissolved oxygen on the rate of reproduction of the tested species.

In a flow through respiration rate experiment with tubificids, Coler et al. (1988), decreased the flow rate of river water through test chambers gradually every 24 hours
in order to reduce dissolved oxygen in test chambers. When the flow rate dropped from 1.71 to 0.65 ml/min, respiration rates decreased from 0.31 to 0.17 mg O$_2$/g/hr. Oligochaetes initially exhibited behavior to increase ventilation rate (extension and undulation) but then exhibited avoidance behavior (clumping), while controls remained dispersed and unagitated.

Lochhead and Learner (1983) tested effects of temperature (8°, 12°, and 20°C) on population growth rates of the asexually reproducing naidids _Nais variabilis, Nais elinguis_, and _Pristina aquiseta_. Two types of agar-based culture media were used to provide a substrate for bacterial growth. One worm was placed in each replicate of media and temperature combination and counts were made at weekly intervals. Temperature and culture media type influenced growth rates in all species tested. The culture media that promoted growth of a wider range of heterotrophic freshwater bacteria appeared to enhance asexual reproduction. At lower temperatures, however, the type of culture media was not as significant.

Distributions of oligochaetes as indicators of pollution has been discussed by Lauritsen et al. (1985), Wiederholm (1980), and Aston (1973b). All concluded that distributions of oligochaetes have value as indicators of toxic impacts on benthic systems but that more laboratory
data on the effects of specific toxicants to single species is needed to refine these indices.

Based on the literature, several conclusions can be drawn. Oligochaetes have been easily and successfully cultured under laboratory conditions. Tubificids were the most frequently tested oligochaete. A wide range of sensitivities has been reported but generally oligochaetes are more sensitive to metals than to organic compounds. Chronic testing with reproduction as the endpoint typically lasts from three months to one year or more in order to go through several generations. As an alternative, naidid oligochaetes have generation times of 3 to 7 days and therefore could provide chronic information in a matter of weeks.

The Organism

The naidid, *Pristina leidyi* Smith 1896, appears to have potential for toxicity testing. The distribution of *P. leidyi* has been reported to be world-wide (Brinkhurst 1971, Timm 1980, Smith 1984). *P. leidyi* is approximately 3 to 5 mm in length with a distinct proboscis formed by the prostomium. Dorsal capilliform chaetae for this species begin on Segment II with elongate chaetae on Segment III. The needle chaetae are simple pointed needles (Brinkhurst 1986). *P. leidyi* reproduce principally by paratomy (transverse fission) (Christensen 1984, Smith 1986). The
The habitat of *P. leidy* encompasses both lentic and lotic waters. The organism is more abundant on stony substrates where grazing on epiphytes is more likely to occur. In sediments of smaller particle size, vertical distribution is limited to the top few centimeters (Learner et al. 1978). The diet of *P. leidy* consists of green algae, diatoms, bacteria, and detritus (Streit 1978, Kairesalo and Koskimis 1987, Learner et al. 1978, Bowker et al. 1971).

The literature indicates that naidids can be an important pathway for transfer of energy and toxicants from lower to higher organisms. It has been suggested that oligochaetes may be an important source of food for fish (MacMichael et al. 1988, Kennedy 1969) and also for many species of aquatic invertebrates such as the larvae of Trichoptera (caddis flies), Odonata (dragonflies), and Coleoptera (beetles) (Popchenko 1971). Rofritz (1977) found only oligochaetes in the entire gut contents of the common wintering waterfowl, the old squaw (*Clangula hyemalis*), on Lake Michigan. Oligochaetes may have been overlooked as a food source in many fish and waterfowl studies as they are digested rapidly (Kennedy 1969, Rofritz 1977).

Patrick and Loutit (1976, 1978) have shown that chromium, copper, manganese, iron, lead, and zinc can be passed up the food chain from heterotrophic bacteria to tubificids (*Tubifex* and *Limnodrilus* species) and from
tubificids to fish. Chadwick and Brocksen (1969) fed dieldrin contaminated *Tubifex* species to fish and found that the fish retained nearly all of the dieldrin ingested. *Stylaria lacustris* and *Tubifex tubifex* chaetae have been found in the guts of chironomid larvae (Loden 1974). Serological precipitation tests have suggested naidids are a food source for leeches (Young and Proctor 1985).

**Research Objectives**

The first objective of this research was to develop a culture protocol for *Pristina leidyi*. Requirements for successful cultures are reported. Some physiological parameters that could affect the results of a toxicity test or bioassay were characterized. An experiment determined the effects of temperature on reproduction. Five temperatures (11°, 17°, 23°, 29°, and 34° C) were used to effectively span the range of temperatures naidids are exposed to in their natural habitat. A second experiment determined if there was a difference in reproduction between total darkness and a defined photoperiod. A known amount of organic carbon was added as a substrate for bacterial growth which provided the source of nutrition. Rates of reproduction and generation times were calculated. Hypotheses tested were

Ho: Temperature has no significant effect on the rate of reproduction of *P. leidyi*.
Ho: Light has no significant effect on the rate of reproduction of \( P. \text{leidyi} \).

Ho: There is no significant interaction of temperature and light on the rate of reproduction of \( P. \text{leidyi} \). Data was analyzed using analysis of variance and linear regression (SAS 1982).

A third experiment determined the population growth of \( P. \text{leidyi} \) in sediment. Data was analyzed using linear regression (SAS 1982).

The second objective of this research was to develop toxicity test protocols for \( P. \text{leidyi} \) and to conduct both acute and chronic toxicity tests with cadmium and vanadium. Acute tests were conducted in simulated water column environments with mortality as the endpoint. Chronic tests were conducted in sediment/water interface simulations and in water column exposures using reproduction as the endpoint.

Cadmium levels reported in the literature range from 0.001 to 0.05 mg/L in freshwater and seawater to around 1000 mg/L in marine biota and estuarine sediments (Yeats 1987, Dabeka and Ihnat 1987). Cadmium has been used as a toxicant in oligochaete bioassays and toxicity tests (Niederlehner et al. 1984, Chapman and Brinkhurst 1984, Chapman et al. 1982a and 1982b, Brkovic-Popovic and Popovic 1977). There is also a USEPA Ambient Water Quality Criteria (1984) document for
cadmium. These data provided a good basis for comparing toxicity test results of *P. leidyi* with other aquatic organisms.

Reported vanadium levels in freshwater range from 0.1 to 20 ug/L with a large fraction of this due to erosion of land surfaces by water. However, the combustion of coal and petroleum which contain vanadium as an impurity has increased the rate at which vanadium enters the aquatic environment (Lee 1983). Very few vanadium toxicity tests have been conducted with invertebrates and only one with annelids has apparently been reported (Miramand and Unsal 1978). Lee (1983) provides an excellent review of occurrence and effects of vanadium in plants and animals.

The final objective of this research was to compare published toxicity data for cadmium and vanadium using other aquatic organisms with the toxicity results for *Pristina leidyi* and to evaluate the use of *P. leidyi* as a toxicity test organism.
CHAPTER II

MATERIALS AND METHODS

Culture Method

Initial stocks of *Pristina leidyi* were obtained from Carolina Biological Supply. Although the worms were marketed as *Stylaria lacustris*, subsequent taxonomic validation revealed that they were in reality *Pristina leidyi*. For culture purposes, *P. leidyi* were placed in acid-washed 2 liter Pyrex containers with 1 liter of dechlorinated tap water under gentle aeration. Ambient room temperature (25°C ± 4°C) was used to culture the organisms. A photoperiod of 16 hours of light at an intensity of 100 foot candles to 8 hours dark was provided.

A rabbit chow suspension provided both food and substrate. The suspension was prepared by pulverizing Purina Rabbit Chow (Ralston-Purina Co., St. Louis, MO) and sifting through a #100 (0.0059" opening) wire mesh sieve. The resulting powder was added to culture water at a ratio of 3 grams sifted chow to 200 mls of water and refrigerated until use. The suspension was added at a rate of 1 ml per liter of culture water every 48 hours. When starting a new culture, 3 grams of sifted chow was added to 1 liter of culture water and allowed to age for 1 to 2 weeks under gentle aeration.
before introducing oligochaetes. This allows colonies of microorganisms to become established. Bacteria are probably the principle source of nutrition for oligochaetes (Aston 1984, Harper et al. 1981, Brinkhurst and Jamieson 1971).

Cultures were maintained by regularly adding culture water to compensate for evaporation and replacing 50% of the old culture water with fresh culture water every 6 weeks. This minimizes build-up of ions such as nitrogen. Culture populations were estimated periodically by subsampling. The culture container was vigorously swirled to suspend the organisms and food, aliquots were removed, and number of worms were counted. The average number of worms in the subsamples were then used to estimate the total population.

**Temperature and Light Experiment Experimental Methods**

An experiment was conducted to determine effects of temperature and photoperiod on the rate of reproduction of *P. leidyi*. Test vessels used were 250 ml borosilicate glass beakers containing 100 mls of dechlorinated tap water and 1 ml of rabbit chow suspension. Five temperatures (11°, 17°, 23°, 29°, and 34°C) were chosen to effectively span the range of temperatures that the organisms encounter naturally. Temperatures were maintained within 1°C. Ten replicates were placed in each temperature bath with five organisms per container. Five replicates were protected from light while the other five were exposed to a 16 hour light and 8 hour
dark photoperiod with a light intensity of 100 foot candles. Organisms were acclimated to the appropriate test temperature at the rate of 2°C every 24 hours. Once the desired temperature was reached, the organisms were maintained at that temperature for a period of 2 weeks prior to the start of the test.

At the end of 14 days from initiation of the test, the organisms were counted under a dissecting microscope and recorded. Counts and growth rates \((r)\) were analyzed using two-way analysis of variance and regression analysis (SAS 1982). Growth rate was calculated by the equation

\[
\frac{\log e N_{t2} - \log e N_{t1}}{t_2 - t_1}
\]

where \(N_{t1}\) and \(N_{t2}\) are the populations at time \(t_1\) and \(t_2\) respectively.

**Methods to Estimate Population Growth in Sediment**

An experiment was conducted to determine the rate of population growth of *P. leidyi* in a characterized sediment. The source of the sediment used was the University of North Texas Water Research Field Station (UNT-WRFS) mesocosm control pond located approximately five miles west of Denton, Texas.

Particle size distribution of the sediment, determined by the hydrometer method (Plumb 1981), was 42.2% sand, 9.7% silt, and 48.1% clay. Organic carbon content, determined by
a Dohrman Carbon Analyzer, was 0.41%. Bulk density, determined by drying replicate samples at 80°C for 24 hours, was 75.3% solids and 24.7% water.

Ten grams (wet weight) of homogenized sediment was placed in each of 50 test vessels (250 ml borosilicate glass beaker) and 50 mls of dechlorinated tap water was added as the overlying water. Five organisms were placed in each replicate and then placed in an environmental chamber at 24°C with a photoperiod of 16L:8D at an intensity of 100 foot candles. The organisms were not fed during the test in order to determine if adequate nutrition was obtainable from the sediment itself. Five replicates were removed every three days, sifted through a #120 wire mesh sieve, sorted under a dissecting microscope, and the Pristina present counted and recorded. Resulting data were analyzed using regression analysis (SAS 1982).

**Acute Toxicity Test Method**

A procedure was developed for obtaining laboratory information on the acute toxicity of chemicals in simulated water column experiments to *Pristina leidyi*. Organisms without apparent budding were exposed to a toxicant under static conditions for a period of 48 hours. Five toxicant concentrations plus a control receiving no toxicant were used. Toxicants used were reagent grade cadmium chloride hemipentahydrate (CdCl$_2$ + 2.5H$_2$O) obtained from Aldrich...
Chemical Company, Inc., Milwaukee, WS, and sodium vanadate (ortho) \((\text{Na}_3\text{VO}_4)\) obtained from Fisher Scientific Company, Fair Lawn, NJ. Each concentration was replicated five times using ten organisms per treatment. The organisms were not fed during the test so that any toxicant/food interactions were removed.

Test chambers were acid-washed 250 ml borosilicate glass beakers containing 100 mls of the treatment solution. Dilution water was dechlorinated tap water of a constant and known quality. Tests were conducted in environmental chambers so that conditions remained constant. The test chambers were covered with a glass sheet to prevent evaporation.

Water quality parameters such as dissolved oxygen, pH, hardness, alkalinity, and conductivity were measured and recorded at the start and the end of the test for each treatment level. Methods for water quality analysis followed Standard Methods (1985) and the appropriate instruments manufacturers manuals. Samples of the concentrations were collected at the start and the end of the test for analytical verification. Total metal concentrations were determined by flame atomic absorption spectrophotometry on a Perkin-Elmer Model 2380 in accordance with USEPA Methods (1979).
Organisms were collected from the cultures by removing the aeration from the culture container. The oligochaetes crawled up the sides of the container where they were easily removed by a fire-polished pipet. Randomized placement of the organisms into the treatment replicates was used. At the end of 48 hours mortality, defined as lack of response to gentle prodding, was observed and recorded. The test was judged acceptable if control mortality did not exceed 10%. LC50 values with 95% confidence limits were then calculated using probit analysis (SAS 1982).

**Chronic Toxicity Test Methods**

A chronic toxicity test method was developed that could provide short term sublethal effects of toxicants to *P. leidyi*. The first method chosen was a whole sediment exposure using the characterized reference sediment dosed with the toxicants used in the acute tests. The endpoint chosen was population growth.

The source of the sediment used was the UNT-WRFS mesocosm control pond described earlier. After the sediment was homogenized, 200 grams (wet weight) per treatment level was placed in a 400 ml glass beaker. Toxicants were added on a mg/kg dry weight basis. Controls receiving no added toxicants were utilized. The spiked sediments were then stirred with a glass rod for approximately five minutes every 30 minutes for 3 hours. Approximately 25 grams (dry
weight) of each treatment level was placed in each of five 250 ml glass beakers. Dechlorinated tap water (100 mls) was then carefully placed over the sediment. The beakers were placed in an environmental chamber at 24°C with a 16L:8D photoperiod for 24 hours to let the temperature stabilize and also to allow the sediment to settle out of the water column.

Five organisms each were placed in four replicates per treatment level. The fifth replicate was used for analytical purposes and did not receive organisms. The test was then replaced in the environmental chamber and covered with a glass sheet to prevent evaporation.

Water quality parameters (dissolved oxygen, alkalinity, hardness, pH, and conductivity) were measured at the start, midpoint (7 days), and the end of the test. Sediment and water column samples were collected for metals analysis at the start and the end of the test. The test was terminated after 14 days. The numbers of organisms present in each replicate were counted and recorded. Data were analysed using Dunnett's Test (SAS 1982).

After the results of this experiment proved ambiguous and difficult to interpret, it was decided to remove the complexities of sediment/toxicant/organism interaction and to conduct chronic tests in water only. The basic question of this research was still unanswered; whether or not this
organism is suitable for toxicity testing. Once this is answered, then one can determine if it is suitable for sediment toxicity testing.

The second method chosen to assess the chronic toxicity of cadmium and vanadium to *P. leidyi* was based on the experimental design used in the *Ceriodaphnia dubia* 7 day chronic test (USEPA 1989). Eight toxicant concentrations plus a control receiving no toxicant were used. Each concentration was replicated ten times using one organism per replicate. Test chambers were one ounce plastic cups containing 20 mls of treatment solution. Dilution water was dechlorinated tap water. Water quality parameters were measured periodically and samples for metals analysis were collected and preserved with metals grade HNO₃ at the beginning and end of the test. The tests were conducted in an environmental chamber at 24°C and a 16L:8D photoperiod. The number of worms in each exposure chamber were counted daily for 28 days at which time the test was terminated. Results were analysed using Dunnett's Test (SAS 1982) and William's Test.
CHAPTER III

RESULTS AND DISCUSSION

Results of Temperature and Light Experiment

After cultures of *Pristina leidyi* were successfully established, an experiment was conducted to determine effects of temperature and light on the rate of reproduction of the organism. The test was terminated at the end of 14 days and the worms were enumerated.

Results showed there was no significant interaction of temperature (°C) and light on the mean ranked numbers of *P. leidyi* (P=0.2840), but light had a significant effect on the mean ranked numbers of *P. leidyi* (P=0.0174), and temperature had a highly significant effect on mean ranked numbers of *P. leidyi* (P=0.0001) (Two-way Parametric Analysis of Variance on ranked data, Model I, with replication). Duncan's Multiple Range Test (alpha=0.05) separated the mean ranked numbers of *P. leidyi* of the five temperature groups into the following four statistically different groups:

23° & 29° > 34° > 17° > 11°

Figure 1 represents the mean number of organisms (plus and minus one standard deviation) not exposed to light at each experimental temperature. There was a highly significant polynomial relationship between temperature (°C)
Figure 1. Mean numbers of *P. leidyi* (± 1 SD) not exposed to light at each experimental temperature.
and numbers of *P. leidy* not exposed to the photoperiod (F=12.018, P=0.0003, Analysis of Variance) and is expressed by the following model:

Number of worms = \(-88.4051 + 10.006(\text{Temp}) - 0.1963(\text{Temp})^2\)

52.21% of the measured variation was accounted for by this model.

Figure 2 represents the mean numbers of organisms (plus and minus one standard deviation) exposed to the photoperiod at each experimental temperature. There was a highly significant polynomial relationship between temperature and numbers of *P. leidy* exposed to the photoperiod (F=25.259, P=0.0001, Analysis of Variance) and is expressed by the following model:

Number of worms = \(-79.8468 + 8.765(\text{Temp}) - 0.1502(\text{Temp})^2\)

69.66% of the measured variation was accounted for by this model. The polynomial-quadratic models were used after an inspection of a scatterplot of the data indicated a 'one-bend' or polynomial curve.

The same statistical analyses were conducted using growth rate (r). There was no significant interaction of temperature (°C) and light on the mean ranked growth rates of *P. leidy* (P=0.4576), but light had a significant effect on mean ranked growth rates (P=0.0326), and temperature had a highly significant effect on mean ranked growth rates (P=0.0001) (Two-way Parametric Analysis of Variance on ranked
Figure 2. Mean numbers of *P. leiidyi* (± 1 SD) exposed to light at each experimental temperature.
data, Model I, with replication). Duncan's Multiple Range Test (alpha=0.05) separated the mean ranked growth rates of \textit{P. leidy} of the five temperature groups into the following four statistically different groups:

\[ 23^\circ & 29^\circ > 34^\circ > 17^\circ > 11^\circ \]

Figure 3 represents the mean growth rates of organisms (plus and minus one standard deviation) not exposed to light at each experimental temperature. There was a highly significant polynomial relationship between temperature (°C) and growth rates of \textit{P. leidy} not exposed to the light (F=30.267, P=0.0001, Analysis of Variance) and is expressed by the following model:

\[
\text{Growth Rate} = -0.4744 + 0.0558(\text{Temp}) - 0.0011(\text{Temp})^2
\]

74.24% of the measured variation is accounted for by this model.

Figure 4 represents the mean growth rates of organisms (plus and minus one standard deviation) exposed to the photoperiod versus temperature. There was a highly significant polynomial relationship between temperature (°C) and growth rates of \textit{P. leidy} exposed to light (F=55.878, P=0.0001, Analysis of Variance) and is expressed by the following model:

\[
\text{Growth Rate} = -0.3346 + 0.0407(\text{Temp}) - 0.0007(\text{Temp})^2
\]

83.55% of the measured variation is accounted for by this model.
Figure 3. Mean growth rates (r) per day of *P. leidyi* (± 1 SD) not exposed to light at each experimental temperature.
Figure 4. Mean growth rates ($r$) per day of *P. leidyi* (± 1 SD) exposed to light at each experimental temperature.
Mean Growth Rate of Organisms (±1 SD)

Temperature (°C)
Using either growth rates or numbers of organisms did not affect the Analysis of Variance results as in both cases the data were ranked. However, in the regression analyses, the use of growth rates resulted in models that accounted for more of the measured variation. The general trends were similar, though. There was an acceptable rate of reproduction and less variance at the ambient room conditions of 24°C ± 4°C and a 16 hour light to 8 hour dark photoperiod. By acceptable it is not meant that a certain number or range of numbers is to be met but rather that a reproducing viable culture with little variation can be maintained. These data suggest that successful cultures can be maintained at normal room conditions without special requirements. They also indicate that a reduction in the rate of reproduction could possibly be an indicator of stress either from an environmental condition (in this case temperature) or possibly a toxicant.

**Results of Population Growth in Sediment Experiment**

Results of the time series population growth in the characterized sediment are illustrated in Figure 5. There was a highly significant relationship between time (in days) and the mean number of worms (F=287.46, P=0.0001, Analysis of Variance). The following model describes the relationship:

\[
\text{Number of Worms} = 0.6552 + 1.3255(\text{Time in days})
\]
Figure 5. Mean numbers of *P. leidyi* (± 1 SD) versus time in a characterized reference sediment.
84.68% of the measured variation was accounted for by this model.

Growth rate \( (r) \) was also used for a regression model and accounted for more of the variation with an \( r^2 \) of 0.9196. This can be attributed to the logarithmic transformation required for the growth rate calculation.

There was a gradual increase in mean number of \( P. \) leidyi until between Day 21 and Day 24 where the population increased from approximately 25 to near 40 and leveled out but with a greater amount of variation. The mean number of worms between Day 18 and Day 21 was not significantly different and with the increase in variation beginning on Day 21 it appears that the length of time for a whole sediment test should not exceed 18 days and should be at least around 15 days in duration. This is a relatively short period of time to acquire chronic information for benthic organisms especially oligochaetes where chronic tests are typically assessed in months.

**Acute Toxicity Test Results**

Results of the acute test with cadmium are presented in Figure 6. Probit analysis (SAS 1982) using measured concentrations of cadmium produced an LC50 of 214.6 ug/L with 95% confidence limits of 203.3 to 225.3 ug/L. Measured concentrations were within 5% of nominal concentrations.
Figure 6. Percent survival of *P. leidyi* after 48 hours versus measured cadmium concentration in water column exposures.
LC50: 214.6 ug/L
95% CI: 203.3 - 225.3 ug/L

Cadmium Concentration (ug/L)

Percent Survival

0.0 168.5 214.5 264.0 310.5 356.0
Reported cadmium levels range from 0.001 to 0.05 ug/L in freshwater and seawater to around 1,000 ug/L in marine biota and estuarine sediments (Yeats and Bewers 1987, Dabeka and Ihnat 1987). Cadmium was chosen for testing because it has been used as a toxicant in other oligochaete toxicity tests (Brkovic-Popovic and Popovic 1977a & b, Niederlehner et al. 1984, Chapman et al. 1982a & b), and there exists a USEPA Ambient Water Quality Criteria document (1984) for cadmium. Table I compares the acute toxicity of cadmium to selected species at water hardnesses similar to that used in this research. Although not as sensitive as some aquatic species, P. leidyi are at least as sensitive as most species and more sensitive than most oligochaete species previously tested.

The results of the acute test with vanadium are shown in Figure 7. The LC50 value for measured vanadium was 30,830 ug/L with 95% confidence limits of 26,860 to 34,920 ug/L. Measured concentrations were within 15% of nominal concentrations.

Reported vanadium levels in freshwater range from 0.1 to 20 ug/L with a large fraction of this due to erosion of land surfaces by water. However, the combustion of coal and petroleum which contain vanadium as an impurity has increased the rate at which vanadium enters the aquatic environment (Lee 1983). Very few vanadium toxicity tests
TABLE I. Comparison of published acute toxicities of cadmium to selected species of aquatic organisms.

<table>
<thead>
<tr>
<th>Organism</th>
<th>LC50 (ug/L)</th>
<th>Water hardness (mg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daphnia magna</td>
<td>30</td>
<td>100</td>
<td>a</td>
</tr>
<tr>
<td>Ceriodaphnia reticulata</td>
<td>129</td>
<td>55-79</td>
<td>b</td>
</tr>
<tr>
<td>Pristina leidyi</td>
<td>215</td>
<td>95</td>
<td>c</td>
</tr>
<tr>
<td>Hyalella azteca</td>
<td>285</td>
<td>55-79</td>
<td>b</td>
</tr>
<tr>
<td>Tubifex tubifex</td>
<td>720</td>
<td>261</td>
<td>d</td>
</tr>
<tr>
<td>Aeolosoma headlyi</td>
<td>1200</td>
<td>60</td>
<td>e</td>
</tr>
<tr>
<td>Jordanella floridiae</td>
<td>4980</td>
<td>180</td>
<td>f</td>
</tr>
</tbody>
</table>

c. This thesis.
Figure 7. Percent survival of *P. leidyi* after 48 hours versus measured vanadium concentration in water column exposures.
LC50
30,830 ug/L

95% CI
26,860 - 34,920 ug/L

Vanadium Concentration (ug/L)

Percent Survival
have been conducted with invertebrates and only one with annelids has apparently been reported (Miramand and Unsal 1978). Lee (1983) provides an excellent review of occurrence and effects of vanadium in plants and animals. Table II compares the acute toxicity of vanadium to selected organisms. *P. leidyi* appears to respond similarly (at least in the same order of magnitude) as other species tested.

**Chronic Toxicity Test Results**

Results of the initial chronic toxicity test using cadmium-dosed UNT-WRFS sediment are shown in Table III. Results are confounded by the following factors. The difference between nominal and measured concentrations could be due to one of two problems. Either there was some type of background matrix interference on the atomic absorption spectrophotometer or there was contamination in the spiking method. The second problem was organism counts. Reproduction gradually tapered off but increased again in higher concentrations.

Results of the test using vanadium-dosed sediment are presented in Table IV. Again there seems to be some type of interference or contamination problem. Reproduction was not significantly different 100% mortality occurred in the highest concentration.

When conducting the statistical analyses, both Dunnetts Test and Williams Test were used. As can be seen on Table
TABLE II. Comparison of published acute toxicities of vanadium to selected species of aquatic organisms.

<table>
<thead>
<tr>
<th>Organism</th>
<th>LC50 (ug/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmo gairdneri</em></td>
<td>(96 hour) 6,400</td>
<td>a</td>
</tr>
<tr>
<td><em>Nereis diversicolor</em></td>
<td>(9 day) 10,000</td>
<td>b</td>
</tr>
<tr>
<td><em>Jordanella floridae</em></td>
<td>(96 hour) 11,200</td>
<td>c</td>
</tr>
<tr>
<td><em>Pristina leidyi</em></td>
<td>(48 hour) 30,830</td>
<td>d</td>
</tr>
<tr>
<td><em>Mytilus galloprovencialis</em></td>
<td>(9 day) 35,000</td>
<td>b</td>
</tr>
</tbody>
</table>

* salt water species

d. This thesis.
TABLE III. Results of chronic toxicity testing with *P. leidyi* using cadmium-spiked reference sediment.

<table>
<thead>
<tr>
<th>Nominal Sediment Concentration (mg/kg)</th>
<th>Measured Sediment Concentration (mg/kg)</th>
<th>Measured Water Column Concentration (mg/L)</th>
<th>Mean Number of Worms (±1 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.347</td>
<td>&lt;0.0001</td>
<td>7.5 ± 3.1</td>
</tr>
<tr>
<td>0.08</td>
<td>0.376</td>
<td>&lt;0.0001</td>
<td>5.0 ± 1.8</td>
</tr>
<tr>
<td>0.22</td>
<td>0.396</td>
<td>&lt;0.0001</td>
<td>2.0 ± 1.8*</td>
</tr>
<tr>
<td>0.60</td>
<td>0.437</td>
<td>&lt;0.0001</td>
<td>2.3 ± 3.9*</td>
</tr>
<tr>
<td>1.70</td>
<td>0.857</td>
<td>&lt;0.0001</td>
<td>5.3 ± 1.0</td>
</tr>
<tr>
<td>4.70</td>
<td>2.518</td>
<td>&lt;0.0001</td>
<td>8.3 ± 1.5</td>
</tr>
<tr>
<td>13.00</td>
<td>6.329</td>
<td>&lt;0.0001</td>
<td>6.5 ± 2.4</td>
</tr>
</tbody>
</table>

* significantly different from control at P=0.05 using Dunnetts Test

# significantly different from control at P=0.05 using Williams Test
Table IV. Results of chronic toxicity test with *P. leidyi* using vanadium-spiked reference sediment.

<table>
<thead>
<tr>
<th>Nominal Sediment Concentration (mg/kg)</th>
<th>Measured Sediment Concentration (mg/kg)</th>
<th>Measured Water Column Concentration (mg/L)</th>
<th>Mean Number of Worms (±1 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>12.64</td>
<td>&lt;0.006</td>
<td>8.5 ± 1.3</td>
</tr>
<tr>
<td>1.8</td>
<td>9.61</td>
<td>&lt;0.006</td>
<td>8.5 ± 2.7</td>
</tr>
<tr>
<td>5.0</td>
<td>12.81</td>
<td>&lt;0.006</td>
<td>3.5 ± 2.9#</td>
</tr>
<tr>
<td>14.0</td>
<td>16.27</td>
<td>&lt;0.006</td>
<td>2.0 ± 1.7#</td>
</tr>
<tr>
<td>38.9</td>
<td>27.40</td>
<td>0.05</td>
<td>4.5 ± 2.5#</td>
</tr>
<tr>
<td>64.8</td>
<td>44.50</td>
<td>0.04</td>
<td>4.0 ± 1.0#</td>
</tr>
<tr>
<td>108.0</td>
<td>69.19</td>
<td>0.04</td>
<td>4.0 ± 5.5#</td>
</tr>
<tr>
<td>180.0</td>
<td>101.39</td>
<td>0.34</td>
<td>2.5 ± 3.0#</td>
</tr>
<tr>
<td>300.0</td>
<td>191.56</td>
<td>2.76</td>
<td>0.0 ± 0.0#</td>
</tr>
</tbody>
</table>

* significantly different from control at P=0.05 using Dunnetts Test

# significantly different from control at P=0.05 using Williams Test
III, Dunnetts Test detected two mid-range concentrations as significantly different from the control while Williams Test did not detect any concentrations as significantly different. The key point here is the information provided by Dunnetts Test that indicates a 63.2% difference from the control has to occur before it can be detected. For the vanadium test, Williams Test detected significant differences from the control at the highest seven concentrations while Dunnetts Test did not detect any. Again the key information is provided by Dunnetts Test where a 79.7% difference from the control was needed to detect a significant difference. These results lead one to consider that reproduction may be too variable to use as an endpoint for chronic testing. However, the problem with the analytical results complicate matters even more. Was the oligochaete response a true reaction to an increasing toxicant exposure or not?

To answer this question the experimental design was altered. The possible complexities associated with sediment were removed. Chronic toxicity tests were conducted in water column exposures only. To aid in determining whether reproduction is an appropriate endpoint or not, the worms were separated so that there was only one worm per replicate. This would allow observation of the variation within the concentrations. The test was conducted for 28
days with counts taken every 48 hours. For both cadmium and vanadium there was no significant difference (P=0.05) between the controls and any of the concentrations until mortality started to occur. If mortality was treated as a zero and left in the data set for analysis, then at some point in time and concentration there was a significant difference from the controls. If one looks only at reproduction, though, there was no significant difference. This means that mortality is a more sensitive endpoint than reproduction. Further, this indicates that the lethal threshold concentration (the point at which acute mortality ceases) was never reached. Indeed this point might never be reached or at least would be more difficult to determine. As the length of the test increases, one has to consider when natural death starts to occur. Another point to consider is that reproduction in the smaller test vessels was atypically low. This could be due to the worms being in a relatively smaller environment although the ratios of space to volume to number of organisms were the same or nearly so. It could also have something to do with the organism itself such as a seasonal fluctuation although, since they are cultured in the laboratory under constant conditions, this should not have been a problem.
Conclusions

In conclusion, with the two toxicants (cadmium and vanadium) and the described test conditions, this particular species of naidid oligochaete may not be suitable for chronic toxicity testing with reproduction as the endpoint. Reproduction may be too variable to use as a chronic endpoint with this organism. If mortality is more sensitive than reproduction, then it should only be used to assess acute toxicities. In this respect it is as sensitive as other tested species as noted earlier. However, acute testing is just part of the toxicity data needed and an organism that is sensitive acutely but not chronically is not desirable in toxicity testing, especially where sediments are concerned. As toxicants accumulate in sediments, benthic organisms are exposed to long-term gradual changes in concentrations and types of toxicants and usually not large sudden doses.

Responses of other species of Naididae which are also widely distributed should be examined. Some may prove to be more sensitive than *P. leidyi* and also reproduction may be chronically sensitive. Although other chronic endpoints may prove to be more sensitive, reproduction has definite ecological significance.

Aquatic oligochaetes have been generalized in the past as pollution tolerant but, as this study and other previous
studies indicate, this may not be necessarily true. As toxicity of sediments gain interest in environmental protection, short term chronic information of those organisms associated with the sediments is needed to help assess long term problems. Aquatic oligochaetes could provide this information using cost effective toxicity tests.
REFERENCES


Brkovic-Popovic, I. and M. Popovic. 1977b. Effects of heavy metals on survival and respiration rate of tubificid worms: Part II - Effects on respiration rate. Environmental Pollution 13:93-98.


