The Use of *Chironomus riparius* (Diptera : Chironomidae) in Benthic Toxicity Tests and Its Response to Selenium.

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THE USE OF *CHIRONOMUS RIPARIUS* (DIPTERA : CHIRONOMIDAE) IN BENTHIC TOXICITY TESTS AND ITS RESPONSE TO SELENIUM

by

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(ABSTRACT)

The larval and pupal stages of *Chironomus riparius* are benthic dwellers which ingest sediment, and are commonly prey. In an environment with elevated selenium concentrations, they can accumulate it and transfer it to higher trophic level organisms upon being ingested. This study was conducted to:

1. Develop a method for performing acute toxicity tests using *Chironomus riparius*.
2. Establish relationships between dry mass and selenium content, and between dry mass and selenium body burden.
3. Determine whether bioaccumulation or bioconcentration contributed more to the selenium content and body burden of individual larvae.
4. Determine selenium concentrations which were acutely and chronically toxic to *C. riparius*, and whether previous exposure changed the acutely toxic concentration.
5. Evaluate adequacy of national surface water selenium criterion with respect to these data.

In acute toxicity tests, 1 larva per 4 ml test solution was an acceptable organism density, and test solutions were successfully renewed by siphoning and refilling the test chamber. Regression showed that larval selenium content was linearly dependent on dry mass within instar, and body burden was negatively exponentially dependent on dry mass. Predicted body burdens were linearly dependent on dissolved selenium concentration. This indicated that

bioconcentration was more important than bioaccumulation in determining body burdens. *C. riparius* readily acclimated after prolonged exposure to dissolved selenium, but became slightly more sensitive to selenium after prolonged exposure to substrate-adsorbed selenium. The national water quality selenium criteria may not protect against biomagnification in all ecosystems, and should be based on some environmental chemical factors.

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Dedicated to the memory of

Wilburn Leander Ritch

and

Doretha Jane Durrence Ritch

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INTRODUCTION

Mercury, cadmium, zinc, copper and other metals have been the subjects of many toxicity studies, and their effects on rainbow trout, fathead minnows, and laboratory cladoceran strains are well documented. This, however, is not true for selenium. Recently, selenium has caused problems at several sites. At Belews Lake (Finley, 1985; Lemly, 1985) and Hyco Reservoir, North Carolina (Baumann and Gillespie, 1986; Gillespie, et. al., 1988; CP & L, 1984; CP & L, 1987), fish populations dwindled without there being any noticeable lake-wide kills. Although adult fish were present, no young were found. The sources of selenium were fly ash settling ponds adjacent to the lakes. In Kesterson Reservoir, California, which was built for irrigation purposes, selenium of geologic origin leached into the water and became concentrated because of evaporation (Cooke, 1985). Fish depletion and deformities at these sites helped call attention to selenium toxicity in aquatic systems. Table 1 lists selenium concentrations found at these sites.

Table 1. Representative selenium concentrations from studies of selenium-contaminated waterbodies. Concentrations calculated from dry mass unless otherwise noted.

	Selenium Concentration				
Waterbody	Water (ppb)	Plankton (ppm)	Benthos (ppm)	Sediment (ppm)	Reference
Hyco Reservoir					
Station 5C	9	6.6 w	17 w	2.7	Woock (1984).
Transect 4	14	30.9	73.8	12.7	Bryson, et. al. (1986)
Savannah river					
Stream-swamp confluence following completion of new basin	60			4.7	Cherry, et. al. (1979)
Belews Lake, Sept. 1982	10.91	8.13 w	13.07 w	3.64w	Lemly (1985)
Kesterson Reservoir and San Luis Drain, 8-12/93	140-1,400				Tanji, et. al. (1986)

w-Concentration calculated from wet mass.

Chironomus riparius Meigen (Diptera : Chironomidae), a non-biting midge, has four benthic larval and one pupal instar. After emergence, the adults live several days but do not eat. The females attach egg masses to objects just beneath the surface of a lentic water body. Because selenium accumulates in sediments, chironomids may have a role in introducing selenium into the food chain, as do other benthic organisms. There is reason to believe that *C. riparius* can acclimate to elevated selenium concentrations in water and sediment (see Bodar, et. al., 1990, and Chapman, 1985, for general examples of acclimation, and Krantzberg and Stokes, 1989 for *C. riparius*). Since *C. riparius* is resistant to many toxins, its absence is not considered an indicator of environmental impact, so acute toxicity *per se* is not a primary concern. However, its ability to survive elevated selenium concentrations is a determinant of its role in selenium movement. Acclimation can be measured as a change in the location of the tolerance distribution. Usually, the median lethal concentration, the concentration required to kill 50% of test organisms (LC_{50}) is used as the midpoint estimate. The shape and parameters of the tolerance distribution are estimated from acute toxicity tests. There are protocols for chironomid toxicity tests, but none have been adopted by the EPA. Committee E-47 of the American Society of Testing and Materials is developing a protocol, but it was not released at the time of this study. Some corporations and other organizations have internal protocols, but none have been published, so it was necessary to do some experimentation on optimizing conditions for chironomid growth, development and survival in the laboratory.

Most studies have shown that sodium selenite accumulates in the sediments while concentration in the water column remains low, so it enters food chains via animal absorption and adsorption to detritus. For chironomids in particular, a significant fraction of body burdens of many metals are adsorbed to the cuticle (Krantzberg and Stokes, 1988). Bioaccumulation is an increase in body burden above food concentration. Bioconcentration is

an increase in body burden above water concentration. Biomagnification is an increase in body burden from one trophic level to the next. There is reason to expect that selenium concentration may be dependent on larval mass. Smock (1983b) and Boyden (1977) demonstrated that organism size can be an important factor in determining body burden, the metal concentration in the organism. This is calculated by dividing organism metal content by wet or dry mass.

EPA sets surface water quality criteria for many substances. These criteria are the maximum allowable concentrations in surface waters. Metal criteria are usually based either on laboratory toxicity tests using the element or an inorganic salt, or on biomagnification studies. Unlike most metal criteria, selenium criteria are based on field studies of biomagnification (USEPA, 1987).

At present, nothing is known about the toxicity of selenium to any chironomids or about acquisition of selenium by *C. riparius*, either from water or sediment. This study is a first step in understanding the importance of *C. riparius* in selenium-contaminated waters and sediments. The objectives were to:

1. Develop a method for performing acute toxicity tests using *Chironomus riparius*.
2. Establish relationships between dry mass and selenium content, and between dry mass and selenium body burden.
3. Determine whether bioaccumulation or bioconcentration contributes more to the selenium content and body burden of an individual larva.
4. Determine selenium concentrations which are acutely and chronically toxic to *C. riparius*, and whether previous exposure changes the acutely toxic concentration.

5. Evaluate adequacy of national surface water selenium criterion with respect to these data.

LITERATURE REVIEW

Chironomus riparius Meigen

The importance of sediment-dwelling aquatic organisms, especially chironomids and tubificid worms, in sediment aeration and ion movement between sediments and water has long been recognized. It has been found that chironomid and tubificid bioturbation is the dominant mechanism in releasing nitrogen (Gardner, et. al. 1983) and phosphorus (Holdren and Armstrong, 1980) from lake sediments. The presence of burrows, or tubes, increases sediment surface area because the tubes penetrate the sediment vertically (Rasmussen, 1984; Credland, 1983). In addition, chironomids undulate their bodies to create currents in their tubes which bring in food and fresh water, and is the primary mechanism which increases water flow through the sediment. A less important mechanism is consumption and digestion of detritus, which increases mineralization (Granéli, 1979a). Chironomids and tubificids in sewage sludge may raise the oxidation potential, cause isopleths to run deeper, raise pH and ammonia concentrations and reduce nitrite and nitrate concentrations (Edwards, 1958). In addition, they may raise pH and oxidation potentials (Davis, 1974) and increase oxygen uptake (Granéli, 1979b) by lake sediments.

In a laboratory experiment, tubificid worms at high density were shown to increase the rate and the depth from which methyl mercury was released from contaminated lake sediment (Jernelöv, 1970). In lentic environments, sediments act as sinks for toxins and seston, so many reservoirs are built to improve water quality and reduce the processing needed to yield drinking water (Petts, 1984). Smock (1983a) found that organisms from relatively uncontaminated sites which ingest sediment indiscriminately had the highest concentrations of most metals studied. These factors could make *C. riparius* an important exporter of

selenium and other sediment-adsorbed toxins from sediment into the food chain or water, especially when they become very dense in sediments and water contaminated by certain heavy metals (Winner, et. al., 1980).

C. riparius (Diptera : Chironomidae) is a burrower which has four benthic larval instars and is found in lentic-littoral waters at maximum densities around 1 m deep. It builds tubes from pure filamentous algae or algae mixed with mud, but cannot use sand or gravel. The tubes are built approximately vertically in the sediment with one end opening a few mm above the sediment and fourth instar tubes terminating 2-3 cm into the sediment (Rasmussen, 1984; Credland, 1983). In the laboratory, they actively space their tubes evenly (Edgar and Meadows, 1969). *C. riparius* has been successfully reared in the laboratory and is being recommended as a bioassay organism because of its intimate association with the sediments (APHA, 1985; Anderson, 1980; Lee, et. al., 1980; Hax, 1983; Williams, et. al., 1986; Rossaro, et. al., 1986).

Three factors which make *C. riparius* important in organically enriched waters are a propensity to flourish in enriched conditions when released from interspecific competition, a multivoltine life cycle which allows quick colonization of a body of water, and the increased food source when growth of filamentous algae is increased (Davies, 1976; Gower and Buckland, 1978; Winner, et. al., 1980). Winner, et. al. (1980) report that chironomids are able to dominate heavy metal contaminated streams, and faster maturation and increased larval dry weights have been observed in *C. riparius* exposed to hexavalent chromium (Hax, 1983). Although *C. riparius* can thrive under some polluted conditions, it is not universally pollution tolerant. Lee, et. al. (1980) showed that *C. riparius* is as sensitive as *Daphnia magna* to linear alkylbenzene sulfonate and trisodium carboxymethyloxysuccinate in laboratory conditions.

Chironomids have poor distribution powers due to the short (2-3 day) adult lifespan and obligate benthic lifestyle of the older larvae, which are catastrophic drifters and usually stay out of the water column. However, the first instar larvae can be distributed planktonically (Davies, 1976). This weak potential to avoid adverse conditions has been confirmed by Wentsel, et. al. (1977a) and Wentsel, et. al. (1977b). These authors established thresholds for avoidance of cadmium and zinc by *Chironomus tentans* in the laboratory, but found them in lake sediments with cadmium and zinc levels well above laboratory avoidance thresholds, although they were absent from some highly contaminated areas of the lake. They do not say whether this resulted from avoidance behavior or from sediment lethality.

Relationship of Organism Mass to Metal Content and to Body Burden

Smock (1983b) found two general relationships between organism dry mass and body burdens of metals. Body burdens of divalent metals decreased at a decreasing rate as mass increased. Body burdens of monovalent metals were nearly constant over all masses, but slightly lower at very low masses. Since selenium as selenite has a 2^+ valence, selenium body burdens would be expected to decrease at a decreasing rate as mass increases. For chironomids, it is important to consider instar in addition to mass. *Chironomus* sp. larvae undergo a rapid size expansion after a molt, which produces a correspondingly rapid change in surface to volume ratio. Smock (1983b) mentions surface to volume ratios as a possible determinant of body burdens of metals which have a 2^+ or higher valence. These elements, which includes selenium, typically adsorb to particles and surfaces, and can enter an organism through an epithelial or epidermal surface. He argued that the dry mass to body burden relationships for these metals can be partially explained by the decrease in surface to volume ratio as an organism grows. Because volume increases exponentially in relation to surface, the rate of selenium intake from adsorption to the organism's surfaces does not keep up with

the amount of organism tissue through which it can be distributed.

Boyden (1977) was concerned that if metal body burden were related to organism size, conventional programs for monitoring shellfish body burdens would produce inaccurate results. He studied the relationships of cadmium, copper, iron, manganese, nickel, lead and zinc body burdens to the sizes of oysters, mussels and gastropods at sites with different contamination levels. Except for one case, the relationships fell into two categories. Either body burden decreased as organism mass increased, or there was no dependence. In contrast to Smock (1983b), he found that rule held for a single element or species, but relationships varied between study sites. He also found that using dry weights resulted in less variability in body burdens than using wet weights.

Selenium Bioaccumulation and Bioconcentration

Algae do not bioconcentrate inorganic selenium (Nassos, et. al., 1980; Sandholm, et. al., 1973), however, they can bioconcentrate selenomethionine which is formed in animals (Sandholm, et. al., 1973). Levels in zooplankton have been close to those of sediments, but higher carnivores, specifically fish, crayfish and predacious damselfly nymphs, bioaccumulate selenium. (Adams, 1976; Birkner, 1978; Nassos, et. al., 1980; Finley, 1985; Gillespie, et. al., 1988; Sandholm, et. al., 1973; Cherry, et. al., 1979; Guthrie and Cherry, 1976; Guthrie and Cherry, 1979; Baumann and Gillespie, 1986; Cherry, et. al., 1976; Lemly, 1985; CP & L, 1987). An exception to this is Hyco Reservoir, where selenium concentrations were about three times higher in the zooplankton than in the sediments at two transects, and five times higher at a third transect (CP & L, 1987). The importance of feeding as an entry route for selenium into animals is demonstrated by the typically lower LC_{50}'s seen in toxicity test animals which were fed, as opposed to those that were unfed (Hodson, et. al., 1980; Shultz, et. al., 1980).

Results of these studies are shown in Table 2. This conclusion is supported by Finley's (1985) field study where fish that ate selenium-contaminated *Hexagenia* nymphs accumulated more selenium than fish fed uncontaminated nymphs. This was also demonstrated in golden shiners by a similar experiment (CP & L, 1984). Adams (1976) showed that selenium concentrations in fish rose linearly over 28 days, which supports Birkner's (1978) tentative conclusion that selenium bioaccumulated but did not biomagnify in higher carnivores. Besser, et. al. (1993) demonstrated conclusively that bluegills did not biomagnify selenium, but did bioconcentrate and bioaccumulate it. They also showed that biomagnification factors for selenite decreased as food selenium concentration increased.

C. riparius is a detritus feeder and is encouraged by organic and microdetritus enrichment, whereas *Glypotendipes paripes*, a planktivore, does not benefit from microdetritus enrichment (Rasmussen, 1984, 1985; Gower and Buckland (1978)). The hypothesis that detritivorous chironomids accumulate more selenium than their planktivorous counterparts is supported by Lemly (1985), who showed that plankton contained 770 times more selenium than the water did, where selenium in detritus was 519 to 1,395 times higher than that in the water. Among the benthos, insects were the highest accumulators of selenium. It is further supported by CP & L (1987), whose monitoring of Hyco Reservoir found that selenium concentrations in the benthos were approximately double those in the zooplankton at three transects. Guthrie and Cherry (1976, 1979) showed that selenium did not accumulate in chironomids. They offered no analysis, but the apparent lack of accumulation may reflect the fact that they lumped all chironomids together. The most compelling evidence that *C. riparius* may be highly important in releasing selenium from the sediments is offered by Smock (1983a), who showed that insects which indiscriminately eat sediments have higher body burdens of sediment-bound metals than do insects in other feeding categories.

Table 2. Estimates of selenite toxicity to various organisms.

Organism	Life Stage	Exposure Time hours	LC_{50} mg/L	References and notes
Chironomus plumosus		96	24.2	Hardness 39 ppm $CaCO_3$
		96	27.9	Hardness 280 ppm $CaCO_3$ Mayer and Ellersieck (1986) referenced in USEPA (1987).
Fathead minnow	juvenile	18.5	31.2	Cardwell, et. al. (1976). Toxicity of SeO_2
		24.5	24.3	
		42.0	15.6	
		96.0	7.3	
		168.0	2.9	
Brook trout	adult	6.0	87.3	
		24.0	36.3	
		48.0	23.8	
		96.0	14.3	
Channel catfish	juvenile	23.0	46.7	
		52.0	24.9	
		94.0	19.1	
Goldfish	juvenile	12.0	110.0	
		24.0	71.3	
		48.0	46.5	
		96.0	36.6	
		216.0	13.0	
		336.0	8.8	
Bluegill	juvenile	8.0	126.6	
		24.0	77.3	
		192.0	27.7	
		336.0	17.6	

Table 2 continued

Organism	Life Stage	Exposure Time hours	LC_{50} mg/L	References and notes
Daphnia pulex	juvenile	48.0	0.613	Shultz, et. al. (1980).
		96.0	0.126	20°C, pH 7.4
	adult fed during test	48.0	1.374	
		96.0	0.499	
	unfed	48.0	0.098	
		96.0	0.071	
Daphnia sp.		48.0	2.5	Bringmann, et. al. (1959). 23°C.
Oedogonium cardiacum		48.0	< 0.1	Nassos, et. al. (1980).
Daphnia magna		48.0	0.25	
Culex fatigans	larva	48.0	3.1	
Physa sp.		48.0	> 10.0	
Gambusia affinis		48.0	> 6.0	
Xenopus laevis	embryo	72.0	8.04	Browne and Dumont, (1979)
		120.0	2.62	20°C, flow through.
		168.0	1.52	
Daphnia magna		48.0	0.71	Halter, et. al. (1980).
		96.0	0.43	Hardness 329 ppm $CaCO_3$,
		336.0	0.43	25°C.
Hyallela azteca		48.0	0.94	
		96.0	0.34	
		336.0	0.07	
Pimephales promelas		96.0	1.0	
		336.0	0.6	
Brachydanis rerio	fry	96.0	23.0	Niimi and LaHam (1976).

Table 2 continued

Organism	Life Stage	Exposure Time hours	LC_{50} mg/L	References and notes
				Adams (1976).
Pimephales promelas	juvenile	96.0	10.90	Hardness 330 ppm $CaCO_3$ 13°C static.
			6.70	20°C static.
			2.80	25°C static.
		48 days	1.08	Flow through.
Salmo gairdneri	fingerling	96.0	4.35	15°C static.
			2.73	
	fingerling	48 days	0.50	Flow through.
		96 days	0.28	Flow through.
	fry	21 days	0.46	Flow through.
Lepomis macrochirus		48 days	0.40	Flow through.
Oncorhynchus kisutch	alevin	43 days	0.16	Flow through.
Hyallela azeteca	immature	96.0	0.76	
		96.0	0.34	Na_2SeO_4 Flow through.
		14 days	0.07	
Daphnia magna		14 days	0.43	
		MATC	0.28	
Salmo gairdneri		96 hours	8.1	Hodson, et. al., 1980
		9 days	6.5	

Selenium Toxicity and Acclimation

Until recently, research on selenosis was restricted to terrestrial vertebrates and plants. Selenosis is caused by grazing on plants which accumulate selenium. This is a common occurrence in the midwest where plants such as *Astragalus*, *Xylorhiza*, *Machaeranthera*,

Haplopappus and *Stanleya* sp. bioaccumulate selenium of geologic origin and can contain 10,000 ppm selenium. These plants are considered indicator plants because they can thrive in seleniferous soils, and are most common when selenium concentration in soil is high. These plants convert selenium to low molecular weight water-soluble organic forms which can be absorbed by a number of other plants when the indicator plants decompose. Mammals usually avoid highly seleniferous plants, but commonly contract chronic selenium poisoning from eating moderately seleniferous plants. Two manifestations of chronic toxicity are the blind-staggers syndrome, caused by water soluble organic selenium compounds, and alkali disease, caused by water insoluble selenoproteins, both of which are caused by eating selenium accumulating plants (Rosenfeld and Beath, 1964; Trelease and Beath, 1949).

Selenium which leaches from fly ash is inorganic and includes selenium dioxide, elemental selenium, selenite and selenate ions, which are very soluble in water (Gutenmann, et. al., 1976). Sodium selenite exists at pH 9 to 3.5 (NAS, 1976), and is reported to be among the most toxic inorganic forms, along with selenium dioxide and potassium selenite. Selenomethionine is more toxic than inorganic selenium, but is volatile and difficult to use for laboratory work (Niimi and LaHam, 1976). Once in the water, selenium readily adsorbs to sediments and organic matter (Adams, 1976; Guthrie and Cherry, 1976; Guthrie and Cherry, 1979; Sandholm, et. al., 1973; Shultz, et. al., 1980; Duke Power Co., unpub. data; Hodson, et. al., 1980)

The effects of selenium on fish include increased mortality, swelling of the gills and liver, ovary deterioration, reproductive failure, lowered hematocrit and gross morphological abnormalities. Fry are generally more sensitive to these effects (Sorenson, et. al., 1984; Pyron and Beitinger, 1989; Baumann and Gillespie, 1986; Cumbie and Van Horn, 1978). Lack of

avoidance of selenium-contaminated water by fathead minnows has been demonstrated (Watenpaugh and Beitinger, 1985), but whether this is true of other animals is virtually unknown. It has been shown that female bluegill sunfish and fathead minnows can transfer selenium to their offspring through the ovaries. This may account for some of selenium's teratogenic effects (Baumann and Gillespie, 1986; Woock, et. al., 1987; Schultz and Hermanutz, 1990).

Because chironomids have a fixed number of instars, growth rate can change without a change in the development rate, and vice versa. In this study, growth was measured as mass, and development was measured as instar. Because a few third instars weigh more than some fourth instars, both instar and mass should be monitored when a study lasts long enough for a molt to occur. Above some density, oxygen depletion, waste accumulation and other density dependent processes will affect the organisms (USEPA, 1985). Handling is necessary for renewing test solutions and counting organisms, but unnecessary or improper handling can adversely affect test organisms. For some planktonic and pelagic organisms, moving test organisms to a clean container during culturing and testing is recommended (USEPA, 1985; USEPA, 1989). However, *C. riparius* must be prodded out of their tubes before they can be moved, which may be more stressful to larva than pipetting is to a free swimming cladoceran.

National Surface Water Selenium Criterion

USEPA has published surface water criteria for most pollutants. These criteria are used to assess the condition of surface waters and limit polluant releases. For each metal, two criteria are set. One is the Criterion Continuous Concentration (CCC). The average concentration of a metal over any four day period should not exceed this concentration more than once every three years. The other is the Criterion Maximum Concentration (CMC). The

average concentration of a metal over any one hour period should not exceed this concentration more than once every three years. The CMC is usually set as one-half of the concentration that is expected to be acutely toxic to 5% of all genera. The CCC is the lowest of three concentrations. One is the final chronic value, which is usually derived from either laboratory chronic tests or an acute:chronic ratio, if no chronic toxicity data if available. The second is the final plant value, which is a measure of bioconcentration by plants. The third is the final residue value, which is based on biomagnification data (Stephan, et. al., 1985).

USEPA (1987) set the CCC (criterion continuous concentration) of selenium (IV) in freshwater at 5.0 µg/l, and the CMC (criterion maximum concentration) at 20 µg/l. The data which are most influential on these criteria come from Finley (1985). The choice of 5.0 µg/l for the CCC is based on the presence of 5 µg/l Se in a part of Belews Lake which had a healthy fish population, where fish were absent when selenium reached 10 µg/l in other parts of the lake. These criteria are supported by biomagnification data for rainbow trout, chinook salmon and mallard ducks.

In a laboratory test of exposure of bluegills to selenium, fry mortality increased greatly when bluegill adults were fed a diet containing 33.3 µg/g seleno-L-methionine prior to spawning, as compared to adults whose food contained 16.8 µg/g seleno-L-methionine and lower concentrations. The dissolved selenium concentration was around 10 µg/l in all groups. A purpose of this study was to evaluate the protectiveness of the CCC to bluegills. The authors concluded that the CCC may not be protective in all ecosystems (Coyle, et. al., 1993). The importance of this study is that it gives a food concentration which causes chronic toxicity at a selenium concentration slightly higher than the CCC. It is important to note that while the freshwater criterion is in ppb, the concentrations in the food were in ppm.

The adequacy of the selenium criteria must be examined partly in terms of whether they prevent buildup in the sediment and bioaccumulation, since no sediment criteria have been developed. Even for mallards, biomagnification of selenium is not a problem, although bioaccumulation occurs readily. This is due to rapid selenium depuration. However, within 10 days of beginning to feed on a diet containing 15 µg/g selenomethionine, mallards were laying eggs containing 20 µg/g Se, which is well above the concentration needed to cause all eggs to be inviable (Heinz, 1993). It can be concluded that if selenium body burdens in the benthic macroinvertebrates reach 15 µg/g, reproductive failure of mallards and other higher trophic level animals can be expected. According to data generated by Coyle, et. al. (1993), this concentration may not cause reproductive failure in bluegills, although these authors caution that it may cause reproductive failure in other fish, and in bluegills in certain ecosystems.

MATERIALS AND METHODS

Cultures

The *C. riparius* strain used in this study was obtained in 1989 from a culture belonging to Jeff Kavanaugh (Virginia Polytechnic Institute and State University, Blacksburg, Virginia), who isolated the strain from Stroubles Creek in Montgomery Co., Virginia. The chironomids were cultured in 10 gallon aquaria with 5 liters of water from Sinking Creek in Giles Co., Virginia. Its water chemistry characteristics are shown in Table 3. Creek water was vacuum filtered through 1.2 micron glass fiber filters to remove invertebrates, protozoa and large cells. The larvae were supplied with paper toweling which had been boiled repeatedly in an autoclave until there was no evidence of pigment leaching, and then washed in acetone until the acetone remained clear. They were fed a suspension of 10g Tetra Growth Food (TetraWerke, Dr. rer. nat. Ulrich Baencsh GmbH, D 4520 Melle 1, Germany), which had been ground in a blender with 100 ml distilled water. Lab temperature remained between 21 and 24°C year round.

Acute Toxicity Test Method Development

Three points were addressed. The first was organism loading, which is the mass of organisms per volume of water. The second was method of test solution renewal. Because the tests were conducted in small vessels, it was necessary to replace the test solution to eliminate water fouling as a confounding variable. The third was survival in the absence of toxins,

Table 3. Water chemistry for Sinking Creek, Giles, Co., Virginia.

pH	8.3
Alkalinity	117 ppm $CaCO_3$
Hardness	115 ppm $CaCO_3$
Conductance	197 µmhos

which needs to be known for toxicity test quality control and analysis. Techniques that assure the most consistent survival in control groups between tests should be used.

Three experiments were conducted to examine the relationships between larval density and survival and between larval density and final larval mass. Egg masses were removed from the culture aquarium and placed in a covered watch glass and observed. Within twenty-four hours of hatching, the larvae were pipetted into 250 ml erlenmeyer flasks and reared for 14 days. Initial densities were 5, 8, 11, 17, 25 and 38 in experiments 1 and 2, and 50 and 300 in experiment 3. Feeding was on a per larva basis. Aeration was 1 to 2 bubbles per second. Water was not renewed. At the end of the experiment, larvae were dried for 24 hours at 60 °C and stored in a desiccator, then weighed on a Cahn 28 Automatic Electrobalance. Weight and survival were regressed separately on initial larval density. In regressing survival on initial density, each observation was weighted by its sample size. Analysis of variance was done to test the null hypothesis that the slope of the regression line was not significantly different from zero.

An experiment was conducted to measure survival under different laboratory conditions. This experiment consisted of four trials, which were started on 9/30/89, 11/13/89, 1/17/90 and 6/6/90. Egg masses were removed from the stock culture and hatched in watch glasses. Within 24 hours of hatching, the larvae were pipetted into 250 ml crystallizing dishes containing 200 ml water. Every 24 hours, larvae were counted, the dead were removed, water was renewed, and the larvae were fed an amount of food according to the schedule shown in Table 4. Renewals were conducted by transferring larvae to a clean dish in the first and second trials, and by siphoning and refilling the dish without disturbing the tubes in the third and fourth trials. In the first, second, and third trials 5 replicate dishes were used. Three

were Table 4. Feeding schedule.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
µl food	30	40	50	60	70	80	90	150	150	150	150	200	200	200	200

used in the fourth. To check for counting effects, two dishes in the third trial were renewed daily without pipetting the larvae out and counting them. The fourth trial tested for a relationship between survival and completeness of egg hatching in a particular egg mass. Three approximately equally-sized egg masses were selected and hatched separately, although the number of eggs in the masses was not counted. The first mass had five unhatched eggs, the second had 99 unhatched eggs, and the third had 58 unhatched eggs. The dishes were aerated at 1 to 2 bubbles per second. Since the larvae were able to build tubes out of the food, and the presence of paper towels made recovery of young larvae difficult, no paper toweling was used. The data were plotted as survivorship data over time.

Survival data is often presented in terms of survivorship curves. Survivorship is the probability of any one individual surviving, or the *per capita* survival rate at any age. It is plotted on a semilog plot with the base ten logarithm of percent survival on the Y axis, and time on the X axis. This allows mortality to be interpreted as a rate, so the slope of the survivorship curve is not influenced by the number of individuals present. For example, if the population were to drop from 100% to 50% on the first day, and then to drop from 50% to 25% on the second day, the survivorship data would be on a line of constant slope, as opposed to a curve of decreasing slope (Begon and Mortimer, 1986). Four separate non-parametric tests were used to test for differences between survival curves. These were Peto/Wilcoxon, Log Rank Test, Gehan/Wilcoxon, and Cox/Mantel. Peto/Wilcoxon and Gehan/Wilcoxon are more powerful when the hazard ratio is non-constant across time. Log Rank and Cox/Mantel are more

powerful when samples are from a distribution belonging to an exponential family, such as the normal or poisson (Hintze, 1988). The P value used came from the most powerful test for the data involved.

Larval Selenium Content and Body Burden

Larvae were removed from the selenium-contaminated cultures at the time of the toxicity tests and at other times and were analyzed for selenium content. Before analysis, the dry mass of each larva was measured. Dry mass was used as the independent variable because Smock (1983b) found better correlations between dry mass and body burden than between head capsule size or organism length and body burden. Some larvae from the dissolved selenium cultures were eviscerated before drying to determine the amount of selenium associated with the gut, and with the rest of the body. The larvae were eviscerated by holding the head capsule with a pair of forceps and the distal end with another pair and pulling the larvae apart.

Relationships between selenium content and larval mass were modeled with first-order regression. Adding subscripts for clarity in development which follows, the regression took the form

$$Y_c=\alpha_c+\beta_c*X \qquad (1)$$

where Y_c is the predicted metal content of an organism having mass X, α_c is the Y intercept, and β_c is an estimate, in metal mass/organism mass, of the mass of metal accumulated with respect to dry mass. The regression lines were plotted on the graphs with the selenium content data points. An F test was used to test the null hypothesis that each regression coefficient was not different from zero. Because a separate regression was performed for each

instar and the eviscerated larvae, the null hypothesis that the regression slopes were equal was tested. A *t* test was used if 2 slopes were compared, and analysis of covariance was used to compare multiple slopes.

The selenium content in nanograms of each individual was divided by its dry mass in milligrams to yield a body burden in nanograms Se per milligram dry mass. The relationship of body burden to mass was derived from the regression of metal content on dry mass. Body burden curves were then constructed putting individual weights into the derived body burden equations and plotting the points. The resulting curves were plotted along with the body burden data points.

The derivation of regressions of body burden on mass was possible because body burden is calculated from dry mass and metal content, and incorporates no additional information. Since body burden for an individual is defined to be metal mass/organism dry mass, the regression of body burden on organism dry mass can be expressed as the regression equation for metal mass on organism dry mass divided by organism dry mass,

$$Y_b = \frac{(\alpha_c + \beta_c * X)}{X} \tag{2}$$

which can be solved to yield

$$Y_b = \frac{\alpha_c}{X} + \beta_c \tag{3}$$

The roles of the coefficients α and β are now reversed, with β being the Y intercept in metal mass/organism dry mass and α being the coefficient in metal mass of the inverse of X. The constants can be rewritten, α_c as β_b and β_c as α_b, to make the symbols conventional, so it can

be restated as

$$Y_b=\alpha_b+\frac{\beta_b}{X} \tag{4}$$

which is the regression of body burden on the inverse of organism dry mass. This is an intrinsically linear regression model that could be solved by least-squares, but the transformation of the explanatory variable can introduce rounding error. More importantly, the shape of the body burden curve is determined by the value of α_c, the *Y* intercept from the metal content regression. At this point, it is important to remember not to make predictions from a regression beyond the range of measurements of the independent variable, which is metal content in this case. Because the *Y* intercept represents the metal content of an organism having zero mass, where no measurements were made, it is not reasonable to assume the regression holds for eggs and very small larvae. A non-zero *Y* intercept may then be taken as an artifact of the regression that has no practical meaning, but this is not so. The value of the *Y* intercept has biological significance. A positive *Y* intercept indicates that there is a decrease in the rate of metal accumulation as mass increases. A zero *Y* intercept means that metal content is directly tied to and completely accounted for by organism mass, and that the rate of metal accumulation with respect to mass increase is constant over all masses. A negative *Y* intercept means that there is an increase in metal accumulation rate as mass increases.

Returning to the body burden regression and keeping in mind that $\beta_b=\alpha_c$ (the metal regression *Y* intercept), there are three cases of β_b which need to be considered. Each case is illustrated in Figure 1. The first is $\beta_b = 0$. In this case, the body burdens are equal for all masses, and are represented by a horizontal line α_b units above 0 on the *Y* axis. The second

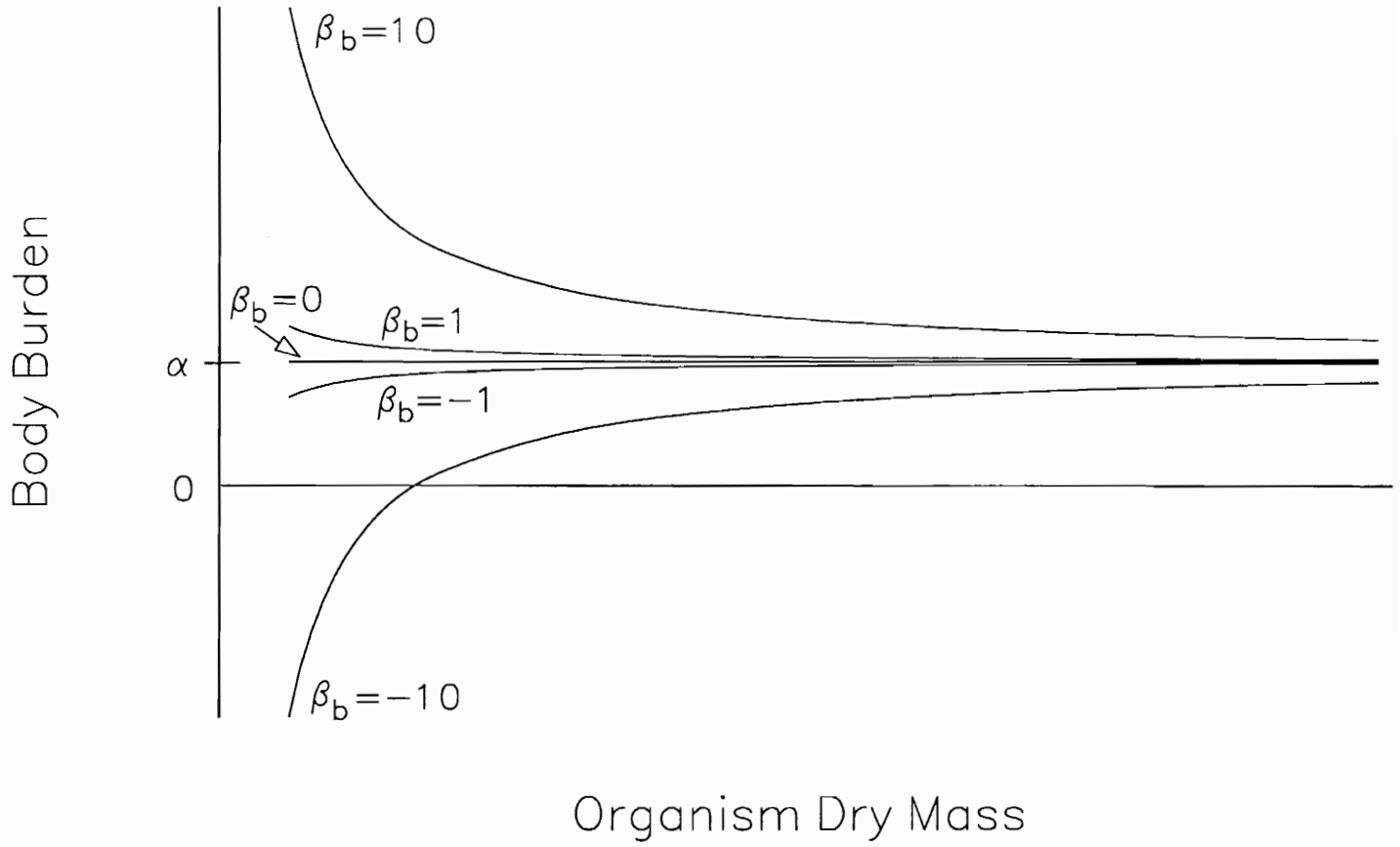


Figure 1. Effect of β_b on body burden curve shape.

is $\beta_b > 0$. In this case, the body burdens will decrease at a decreasing rate as mass increases. The third is $\beta_b < 0$. This produces a body burden curve which increases at a decreasing rate mass increases. To generalize, β_b determines the shape of the curve, while α determines the vertical position. The closer the value of β_b is to 0, the closer the body burden curve is to being a horizontal line for large values of X. Increasing the absolute value of β_b moves the body burden curve either up or down and to the right, and reduces the curvature of the curve. Increasing values of α_b increases the body burden, which moves the body burden curve up, but does not change the rate of change in body burden as weight changes, so the shape of the curve does not change.

To determine the importance of dissolved selenium and adsorbed selenium in determining fourth instar larval selenium content, slope and Y intercept estimates for regression equations for the dry mass-selenium relationships, and mean selenium content for 0.5 mg and 0.15 mg fourth instar larvae, were regressed on dissolved selenium concentrations and absorbed selenium concentrations in the cultures. The null hypothesis that there was no correlation between substrate-adsorbed and dissolved selenium was tested with correlation analysis.

Maximum bioconcentration factor (MBCF) equations were calculated as body burden against weight regression equations divided by dissolved selenium. Maximum bioaccumulation factor (MBAF) equations were calculated as body burden against weight regression equations divided by adsorbed selenium. These are referred to as maxima because it is not known what proportion of selenium body burden was bioconcentrated and what was bioaccumulated. To estimate the dissolved and adsorbed selenium concentrations that may be expected to cause reproductive failure in higher trophic level animals through feeding on *C. riparius*, the regression models were used to inversely predict the environmental concentrations that would

cause a body burden of 15 µg/l in a 0.5 mg fourth instar larva and in a 0.15 mg fourth instar larva.

Acute Toxicity and Acclimation

Three groups of cultures were spiked with a solution of sodium selenite to achieve a gradation of selenium concentrations in the water and substrate. The culture groups and spiking concentrations are summarized in Table 5. Groups 1 and 2 were spiked monthly after water renewal, and group 3 was spiked every three to five days. Group 2 was an attempt to culture larvae from group 1 in aquaria which were free from other invertebrates. The cultures were established sequentially by using larvae from a clean culture to start a spiked culture. Once a culture had proven viable, larvae from this culture were used to start another culture with a higher selenium concentration, until the selenium in a culture was too concentrated for chironomids to survive.

Group 1 was spiked monthly for over a year before any tests were performed so that selenium accumulated in the substrate, but selenium in the water remained low except for pulses at the time of spiking. Several attempts to maintain a culture at a spiking concentration of 3.0 and 4.0 ppm Se for more than two months were unsuccessful. Group 2 was spiked like group 1, except that it was not maintained for as long before tests were performed. Group 3 was assigned target dissolved concentrations of 0.1, 0.3, 1.1 and 3.3 ppm Se. Dissolved selenium concentrations were monitored closely for twenty days prior to the toxicity test, and the cultures were spiked as necessary to bring the concentrations to target levels. When larvae were removed for the test, substrate samples were taken for selenium analysis. Water and substrate from the control culture were analyzed for selenium at this time.

Table 5. Culture group numbers, descriptions and spiking concentrations.

Group	Description
1	Cultures started between 5/3/89 and 4/16/90, spiked monthly with sodium selenite.
2	Cultures started between 7/2/90 and 7/6/90, free from foreign invertebrates, spiked monthly with sodium selenite.
3	Cultures started between 2/22/91 and 3/25/91, spiked every 3 to 5 days with sodium selenite.

Culture number	Spiking concentration
1-1	Unspiked control
1-2	0.1 ppm
1-3	0.5 ppm
1-4	1.0 ppm
1-5	1.5 ppm
1-6	2.0 ppm
1-7	3.0 ppm
1-8	4.0 ppm
2-1	Unspiked control
2-2	0.1 ppm
2-3	0.5 ppm
2-4	1.0 ppm
2-5	1.5 ppm
2-6	2.0 ppm
3-1	Unspiked control
3-2	0.1 ppm
3-3	0.3 ppm
3-4	1.1 ppm
3-5	3.3 ppm

Fourth instars were placed in crystallizing dishes on a rotating basis to help ensure random selection of individuals. When working with a container holding a limited number of larvae, it is possible to select more passive larvae at first, missing the more active ones trying to avoid the pipette tip, and then begin catching more of the active ones when filling up the last dishes. Toxicity test solutions were made from a solution of sodium selenite pentahydrate,

reagent grade from Aldrich Chemical Company, Milwaukee, Wiscosin, dissolved in glass distilled water. Sinking Creek water was then spiked with the stock solution to yield the desired concentration.

Seven sets of acute toxicity tests were performed. The purpose of each test or set, culture from which the larvae were taken, sample size and selenium concentrations used are shown in Table 6. The first three tests were range finding tests performed on the stock culture. The design for test 5, the first test of acclimation to adsorbed selenium, was based on the results from these tests. The goal of the design was to get the maximum number of partial kills, and have close to equal numbers of partial kills above and below 50% mortality. In an attempt to achieve this goal, different spans and series of concentrations were used to test samples from different cultures. For example, in test 5, larvae from cultures 2-1 and 2-2 were tested in 5 ppm, while the lowest concentration used to test cultures 1-6 and 1-7 was 20 ppm.

Results of acute toxicity tests were analyzed using probit analysis, and the data were graphed on probit plots. The X axis is a log transformation of selenium concentration. The *Y* axis is a probit transformation of the proportion dead after the specified time. Acute cumulative dose-response functions are log-sigmoidal, and these transformations linearize the data (Finney, 1971). Because 0% and 100% kills cannot be transformed to probits, downward arrows were placed on the regression line to indicate 0% kills, and similar upward arrows indicate 100% kills. Although these data were not used in the initial estimation of the regression parameters, their predicted values in probits were used in each iteration, so the sums of squares and parameters reflect their presence. Chi-square goodness of fit tests were used to test the fit of the probit model regression to the data. If the χ^2 test indicated significant heterogeneity in the data and data points with very small predicted responses made

Table 6. Acute toxicity test descriptions, sample sizes (n) per concentration and concentrations tested.

	Test Description	n	Selenium concentrations (ppm)
1. 12/15/89	First range finding test on culture 1-1.	10	100, 300
2. 2/2/90	Second range finding test on culture 1-1.	20	58, 82, 118, 160
3. 3/11/90	Third range finding test on culture 1-1.	20	13, 24, 43, 77, 136
4. 6/2/90	Culture 1-8.	30	15, 23, 34, 51, 76, 114, 171, 257, 411
5. 1/19/91	First test of acclimation to sediment-adsorbed selenium.		
	Cultures 2-1 and 2-2	20	5, 10, 20, 40, 80, 160
	Culture 2-3	20	5, 10, 20, 40, 80, 160, 320
	Cultures 1-6 and 1-7	20	20, 40, 80, 160, 320
6. 1/30/91	Second test of acclimation to sediment-adsorbed selenium using cultures 2-1, 2-2, 2-3, 1-6 and 1-7.	30	4, 6, 9, 14, 20, 30
7. 4/15/91	Test of acclimation to dissolved selenium using all group 3 cultures.	30	3, 5, 9, 15, 25, 43, 72

large contributions to χ^2, these contributions were combined with adjacent contributions, and the χ^2 test was performed with the recalculated χ^2 at appropriately reduced degrees of freedom. The recalculated χ^2 is a less biased estimate of heterogeneity. If the recalculated χ^2 indicated heterogeneity of the data at the 0.05 α level, all variances were multiplied by a heterogeneity factor of χ^2/degrees of freedom before calculating confidence limits.

In most cases, a median lethal concentration, (LC_{50}), the concentration which kills 50% after a specified time, is reported. In cases where there was no partial mortality in at least two dishes, with mortality in one greater than 50% and mortality in the other less than 50%,

an LC_x is reported in addition to the LC_{50}. X is a percentage which is a multiple of 10 and is the closest percentage to 50% that falls between two observed mortality percentages. Reporting LC_{50}'s when there is not at least one partial kill greater than 50% and one less than 50% is a case of extrapolating beyond observed values, which should be avoided because the results can be unreliable (Zar, 1974; Larson, 1974; Finney, 1978). For most LC_x's, 95% confidence limits are reported. In cases where they could not be calculated, the percentage confidence limit calculated is the highest multiple of 5 calculable.

One hundred twenty hour LC_{50}'s were used as a measure of the population's sensitivity to selenium. A stepwise multiple linear regression of LC_{50} against target dissolved selenium concentration, substrate selenium concentration and exposure time was performed to determine which factors, if any, caused acclimation. Actual dissolved selenium concentrations were not used because they fluctuated too greatly to be considered good indicators of the dissolved concentrations to which the larvae were exposed.

Selenium analysis

Selenium concentrations were measured on a Perkin-Elmer 1100 Atomic Absorption Spectrophotometer (1100 AAS) with a Perkin-Elmer selenium electrodeless discharge lamp. Samples with selenium concentrations greater than one ppm were acidified to 0.1% HNO_3 and measured using a single slot air-acetylene burner with an air flow of 8.0 ml/min and acetylene flow of 2.5 ml/min. Samples with selenium concentrations under one ppm were acidified to 0.1% HNO_3 and received 0.2 µg nickel(ous) nitrate ($Ni(NO_3)_2$) per 20 µl aliquot as a matrix modifier, and were measured using an HGA-300 graphite furnace with a pyrocoated graphite tube fitted with a pyrocoated L'vov platform. The 1100 AAS's internal deuterium-arc background corrector lamp was used during all furnace work to correct for broad-band

background absorption due to the matrix modifier and organic compounds. The furnace program is shown in Table 7. It is based on the standard program for selenium, but was modified to give an optimal absorbance signal on the machine used. Water samples were acidified, but not digested. Larvae were digested in 300 µl HNO_3 and 40µl H_2O_2. They were dried at 60°C and reacidified in 5% HNO_3. Sediment samples were digested in 20% HNO_3, filtered through a 1.0 micron glass fiber filter, and reacidified in 5% HNO_3 to bring the digestate volume to 100 ml.

Evaluation of National Surface Water Criterion for Selenium

The criteria were evaluated for adequacy to protect *C. riparius* from toxicity, and adequacy to protect bluegill sunfish from reproductive failure if they were to eat small (0.05 mg) larvae from a culture in which the dissolved selenium concentration was near but below the CCC (criterion continuous concentration). Data from all acute tests were reviewed to find the lowest LC_{50}, and the highest continuous exposure that allowed a culture to remain viable was determined. These quantities were then compared to the CMC (criterion maximum concentration) and CCC for selenium, respectively. The criteria were considered adequate to protect *C. riparius* if they were lower than the lowest LC_{50} or highest continuous concentration,

Table 7. Perkin-Elmer HGA 300 graphite furnace program for selenium.

Step	Temperature (°C)	Ramp Time (sec)	Hold Time (sec)	Function
1	90	1	15	
2	130	15	15	
3	1200	5	30	
4	20	1	15	stop flow
5	2200	0 *	5	stop flow, read
6	2650	1	10	

* maximum power=70

respectively. The selenium concentration in food that caused reproductive failure in bluegills was taken from the literature (Coyle, et. al., 1993). The CCC was considered adequate to protect bluegills if it was lower than the predicted body burden in a 0.15 mg larva from the culture described above.

RESULTS

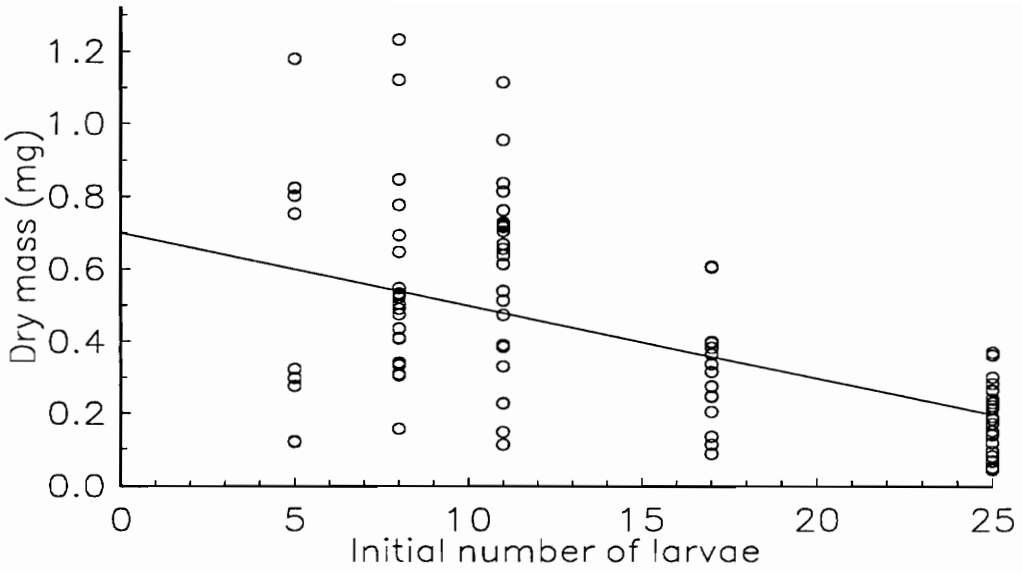
Acute Toxicity Test Method Development

Observations of Tube Building and Spacing

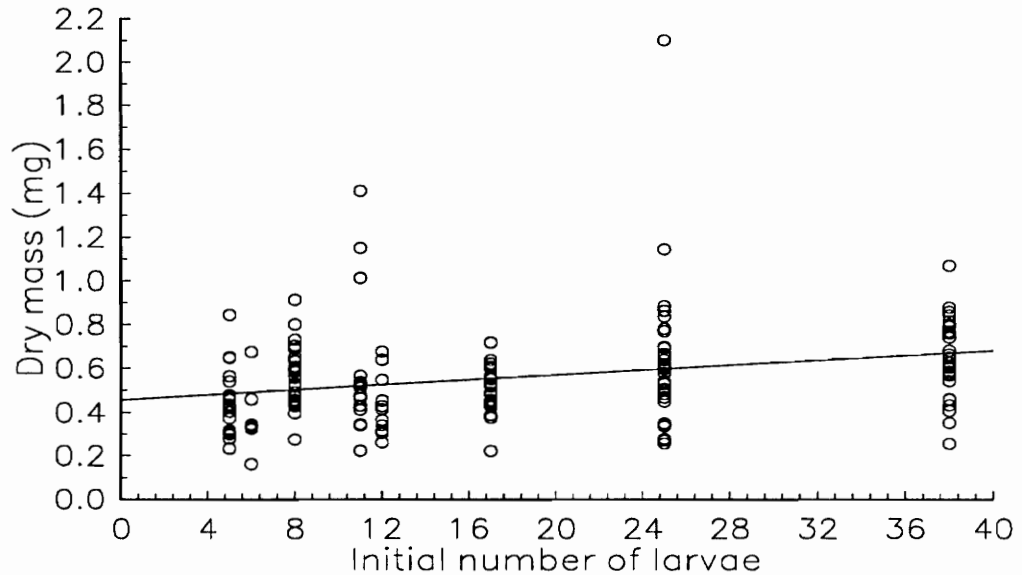
Newly hatched larvae ate their way out of the egg mass. They immediately began building straight, randomly spaced tubes out of food particles and paper fibers, if supplied, on the bottom of the watch glass or crystallizing dish. As the larvae matured, they began to form clumps of food and paper fibers which housed up to five larvae. Unlike the tubes of the youngest larvae, these clumps were not necessarily attached to the glass. Some older larvae continued to build tubes on the bottom of the glass. A few of these were single tubes, but most often several tubes were built together, either parallel or intersecting. The tubes of the 3rd and 4th instars were usually longer than the larvae. Whenever two larvae were found in the same tube, it was substantially longer than the two larvae and usually curved sharply in the space between the larvae. The larvae readily exited the tubes after very gentle prodding on the tube. It was usually not necessary to poke on the part of the tube where a larva was to get it to leave.

Effects of Initial Larval Density on Final Weight and Survival

Neither final larval weight nor survival were dependent on initial larval density when initial larval density was less than 38 larvae per flask. Survival was between 60% and 80% in all flasks in both experiments, and was unaffected by initial larval density. Final larval weight for experiments 1 and 2 are shown in Figure 2. Although analysis of variance of the regression showed that there was a statistically significant decrease in final larval weight as initial larval density increased, which is expressed as



2a. Experiment 1.



2b. Experiment 2.

Figure 2. Final weight vs. initial number of larvae per flask with first order regression lines.

$$Y=0.699-0.020*X,\ r^2=0.2829,\ P<0.0001 \quad (5)$$

group variances are heterogenous, and the statistical test is likely erroneous, and the data are not believed to represent a decrease, as explained below. In experiment 2, the variances are heterogenous although they are not clearly dependent on the mean, or predicted, weight. Contradicting the results of experiment 1, there was a statistically significant increase in final larval weight as initial larval density increased. The relationship between the variables is

$$Y=0.457+0.006*X,\ r^2=0.0776,\ P=0.0002 \quad (6)$$

Again, the veracity of the statistical test is doubted.

The reliability of analysis of variance and regression are affected by heterogenous variances. Within group variances of weight from experiment 1 were heterogeneous and dependent on the mean. Even with these problems, experiment 1 taken alone could reasonably be interpreted as evidence that initial larval density affects final larval weight. However, since experiment 2 contradicts this conclusion, the results of the hypothesis tests should be doubted.

In experiment 3, which compared initial larval densities of 50 and 300 larvae per flask, there was a significant decrease in percent survival as initial density increased, and a significant decrease in final weight as initial density increased. Regression of percent survival on initial density gave the relationship

$$Y=0.839-0.001*X,\ r^2=0.8805,\ P<0.0001 \quad (7)$$

Regression of final weight on initial density gave the relationship

$$Y=0.846-0.001*X,\ r^2=0.4365,\ P<0.0001 \quad (8)$$

Water in the flasks initially containing 300 larvae was much more turbid than water in the

lower density flasks, and had a strong sewage odor.

Survival, renewal method and effects of counting

Larval survivorship in the four tests are shown in Figure 3. The data in Figure 3 are pooled for all dishes within each test. Survival was significantly different between the each of the four test groups at the 0.1 α level. There were no significant differences in survival between replicates within tests at the 0.1 α level. The last point on each curve in Figure 3 indicates the day prior to the first pupation. Time to pupation decreased with each successive test. Figure 4 shows the results of tests 3 and 4. In test 3, larvae in two of the replicate dishes were not counted. The final number in the non-counted dishes is within the bounds of the final numbers in those that were counted, and the larval dry weights are not significantly different at the 0.05 α level. In test 4, there were no significant differences in survival between the larvae from the egg masses of different hatching success.

The renewal method used in the first two tests allowed all material in the dish to be searched for dead larvae. Counting the live and dead larvae revealed that some were missing. In the first test, the percentage missing and unaccounted for ranged from 33% to 70%. In the second test, the range was 56% to 89%. Leaving the tubes intact in the third and fourth experiments did not allow me to search extensively for dead larvae. In the first and second tests, three partially eaten larvae were recovered, and two dead larvae were found incorporated into tubes. The unaccounted for larvae were of all ages, and there was no one age where a higher proportion of the missing larvae were unaccounted for. It could not be determined whether cannibalism was causing mortality.

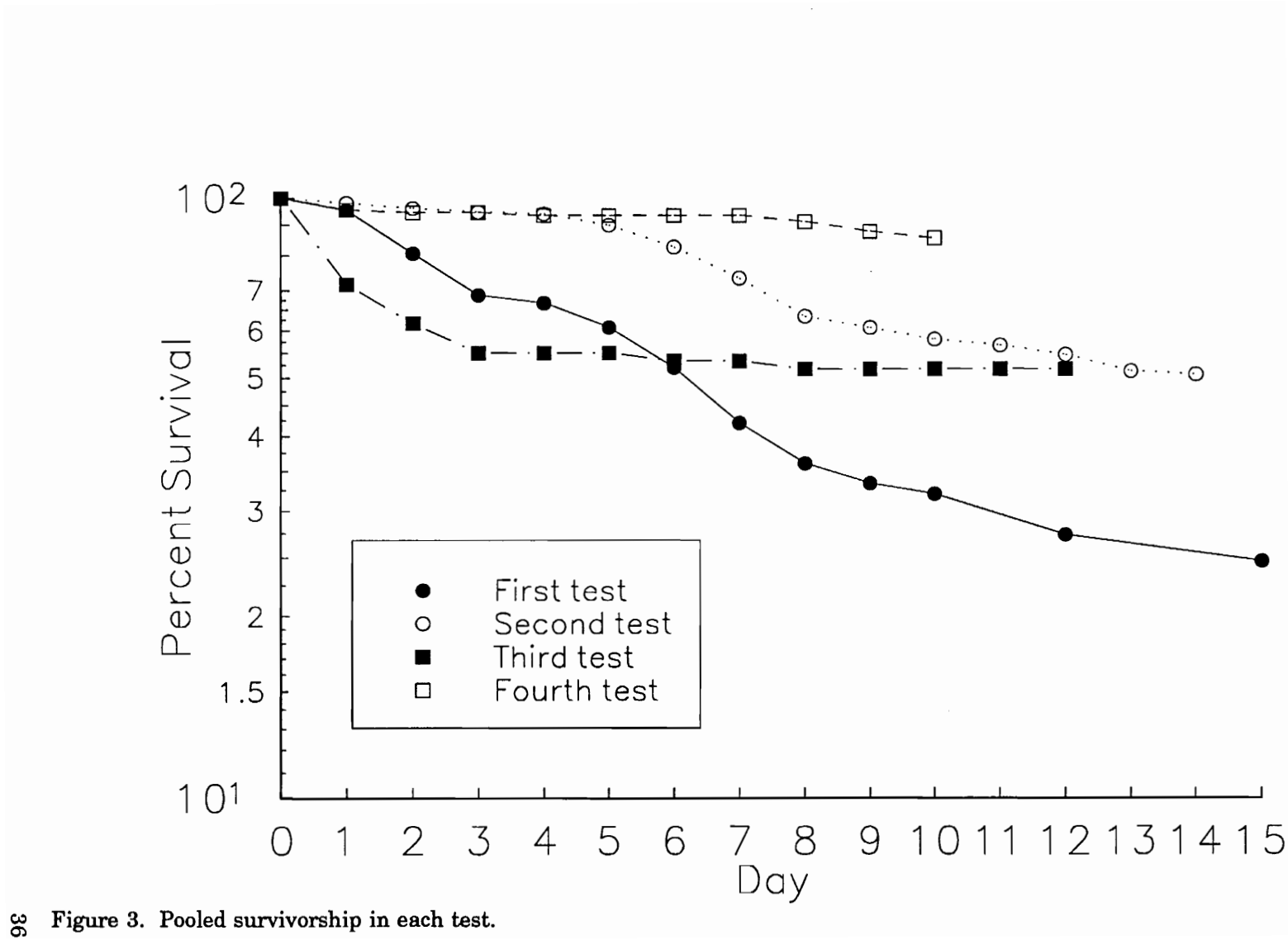


Figure 3. Pooled survivorship in each test.

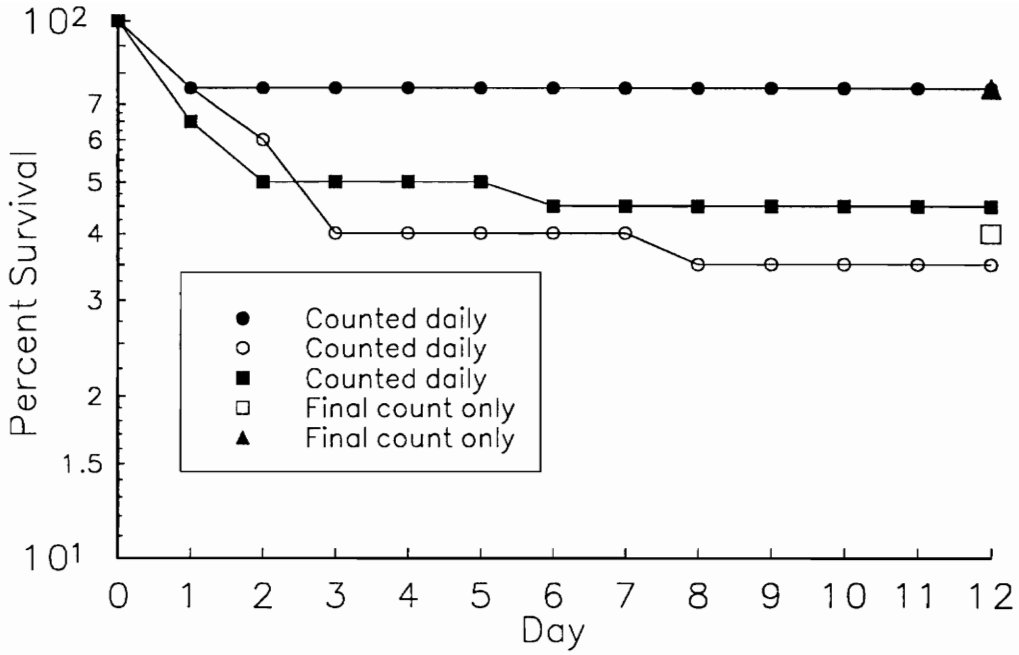


Figure 4a. Survivorship in test 3.

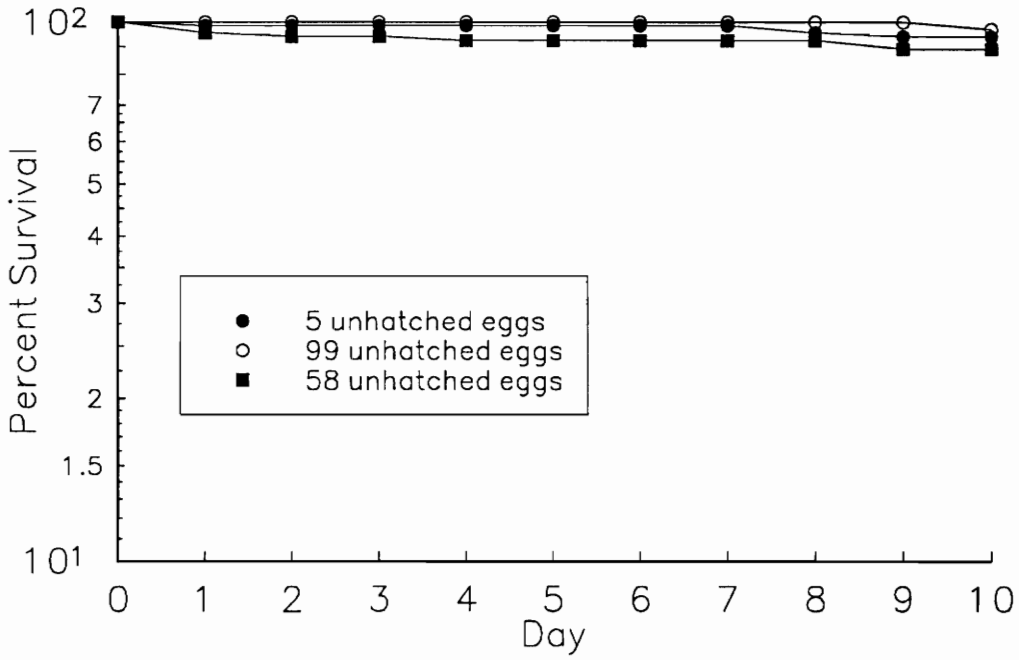


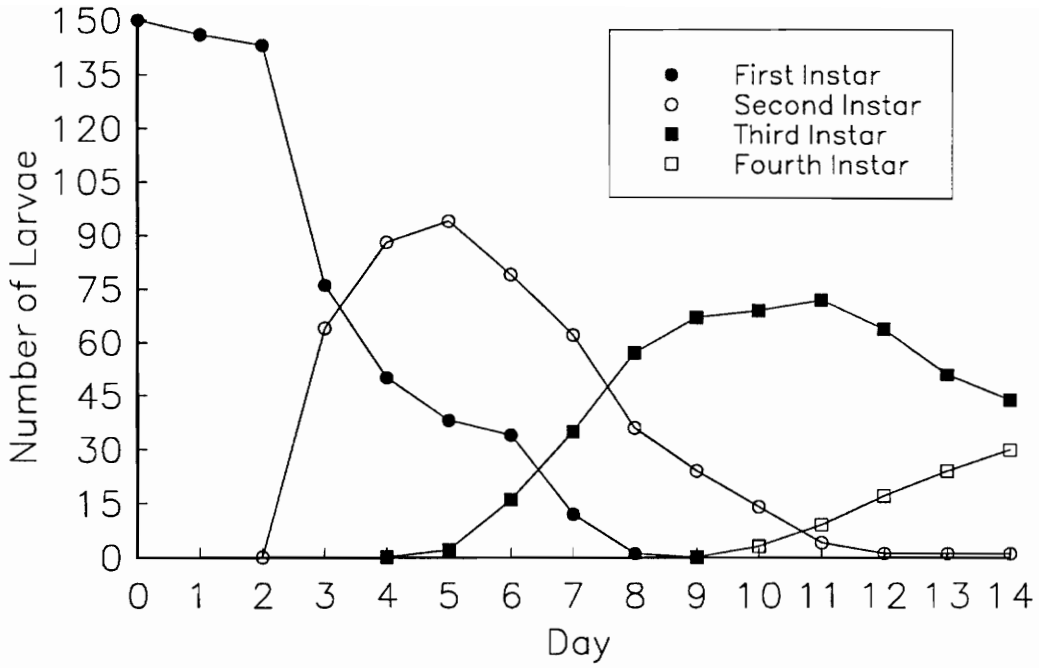
Figure 4b. Survivorship in test 4.

Figure 4. Survivorship in experiments 3 and 4. ▲ and □ denote groups counted only at end of experiment.

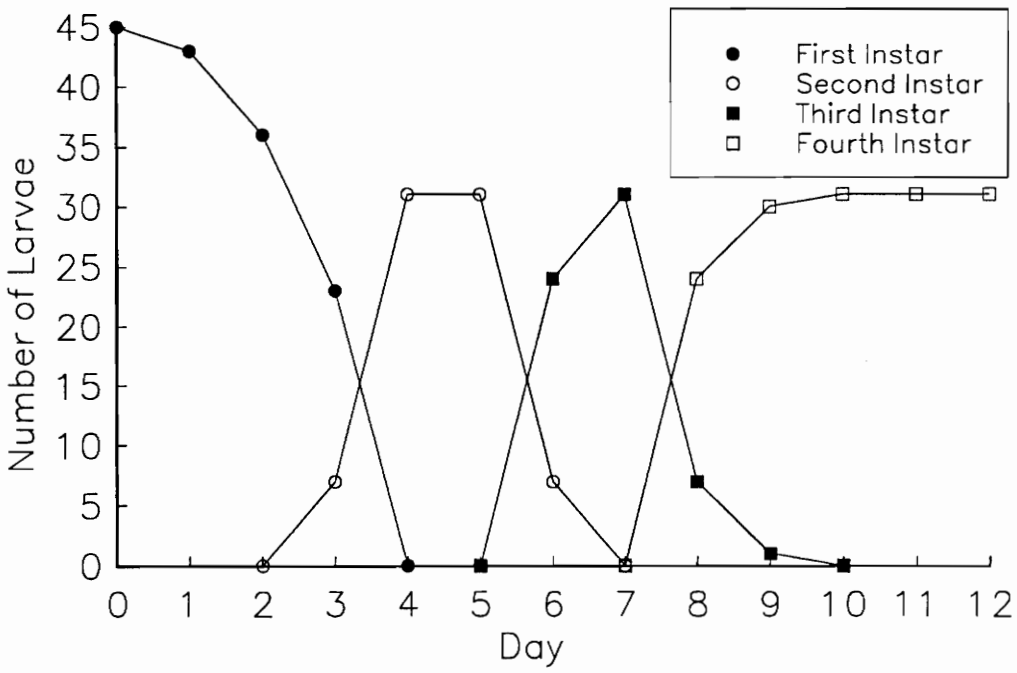
Larvae in test 2, the siphon group, had higher weights and shorter development times than larvae in test 3, or the transfer group. The number of larvae in each instar per day for the transfer and siphon groups is shown in Figure 5. The mean weight of the transfer group larvae was 0.209 mg (s.e. = 0.135) while the mean weight of the siphon group larvae was 1.164 (s.e. = 0.045). These weights were significantly different ($P<0.0001$). Figure 5 illustrates striking differences in development rate over time between the groups. Larvae developed more rapidly and molts were more synchronous in the siphon group. In the siphon group, the distribution of numbers per instar by day is a normal distribution, where second instars in the transfer group exhibit a somewhat log-normal distribution.

Larval Selenium Content and Body Burden

The relationships between larval selenium content and larval dry mass for 2nd, 3rd and 4th instar larvae from cultures 2-1, 2-2, 1-2, and 1-4 were adequately described by first-order linear regression equations. Figure 6 shows plots of the data and regressions. There was slightly significant evidence ($P=0.08$) that selenium content was dependent on dry mass in culture 2-1, a control culture, which contained 2 ppb Se in the sediment. The source of the selenium was not determined, but is close to concentrations found in unpolluted aquatic environments. The rates of selenium accumulation and predicted selenium contents in all spiked cultures were much higher than those in culture 2-1. The Y intercepts of the regression equations were greater than zero, so body burdens decreased at a decreasing rate as larval dry mass increased. The relationships between dry mass and body burden were described well by the first-order regression of the inverse of dry mass on body burden. Graphs of these data and regressions are shown in Figure 7. Some larvae from culture 2-2 which had masses less than 0.17 mg clustered with respect to the Y axis around 4 ng Se. This also happened to larvae in that weight range from culture 1-4, except that the clustering was not as tight and the

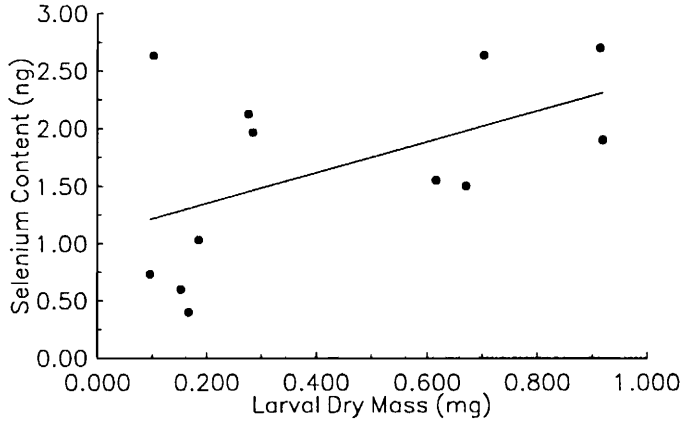


5a. Transfer renewal group, trial 2.

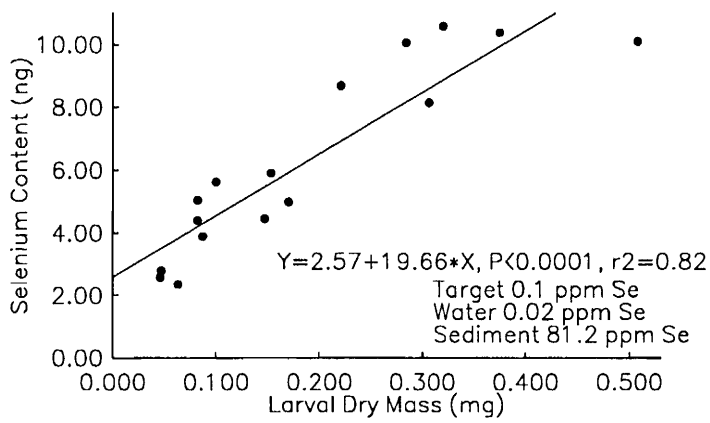


5b. Siphon renewal group, trial 3.

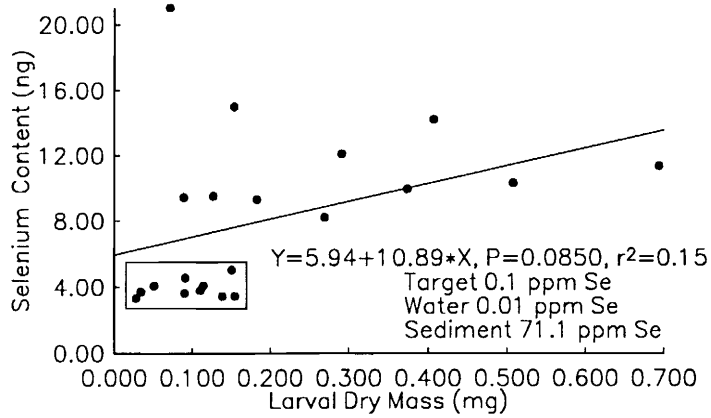
Figure 5. Number of larvae per instar per day for different renewal methods.



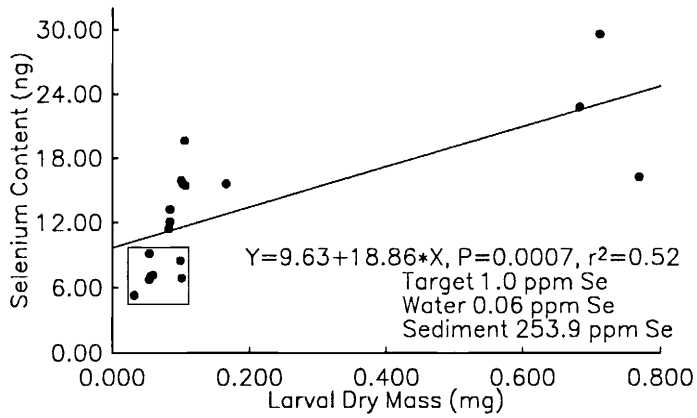
6a. Culture 2-1.



6b. Culture 1-2.



6c. Culture 2-2.

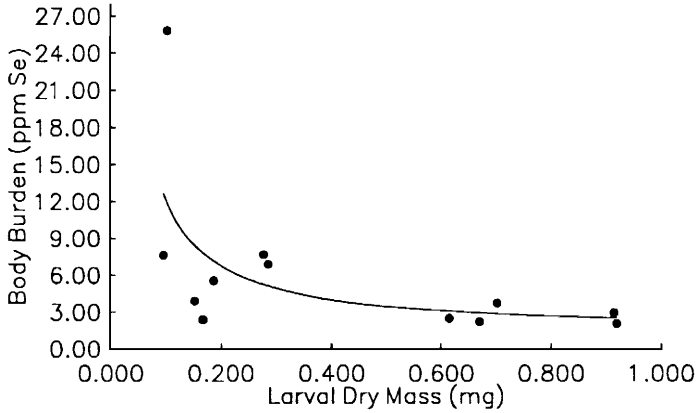


6d. Culture 1-4.

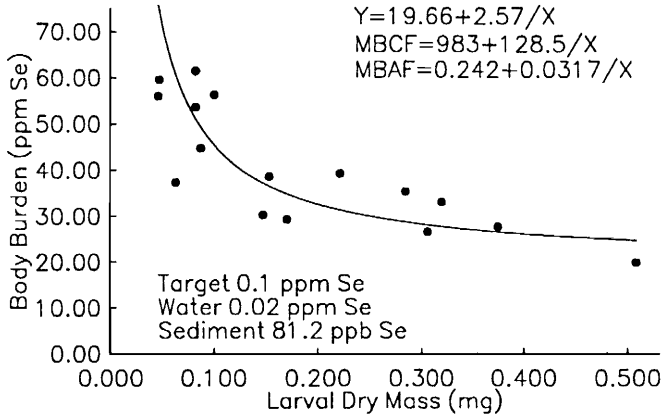
Figure 6. Selenium content vs. larval dry mass with first order regression lines for samples collected 8/2/90. Boxes mark clusters of small larvae though to be third instars.

selenium contents were between 5 ng and 9 ng. These clusters are boxed in Figures 6c and 6d. These larvae were probably second and third instars, and dry mass-selenium content relationships were different for each instar.

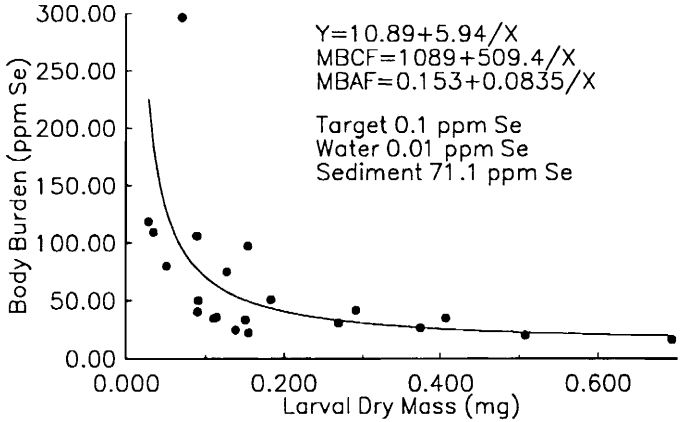
Graphing and regressing each instar's selenium content versus dry mass separately for larvae from cultures 2-2, 2-4, 1-5 and 1-6 showed that instar was a factor affecting selenium content, so that a third instar and fourth instar larva which weighed the same would be expected to have different selenium contents. Separate regressions provided higher significance and explained more variance than did multiple-instar regressions. The rates of selenium accumulation with respect to mass were not significantly different for the different instars. These data and regressions are shown in Figure 8. Generally, there was statistically significant evidence at the 0.05 α level that weight affected selenium content in fourth instars. The 1/30/91 larval samples from cultures 1-5 and 2-4 and the larval sample from 3-4 go against this generalization, although selenium contents of larvae from culture 3-4 were around 100 times higher than those of larvae from cultures 1-5 and 2-4. As with the first selenium content measurements, Y intercepts of the selenium content vs. dry mass regressions were positive, so body burdens decreased at a decreasing rate as dry mass increased. Separate inverse regressions of body burden on dry mass for each instar described the relationships well. These data and regressions are shown in Figure 9. Weight was rarely a significant factor for second and third instar selenium content. However, samples of second and third instars were generally consisted of five larvae or less, so the test of significance of the regression had very low detection power, so a type II error was probably committed. The power of a significance test, β, is the probability that the test will detect a difference in parameter estimates when one really exists. β is increased by increasing sample size, and varies in inverse proportion to variance. A type II error is the failure to reject the



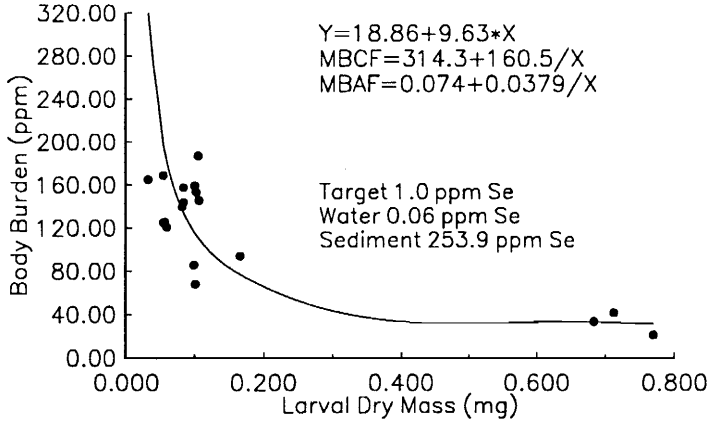
7a. Culture 2-1.



7b. Culture 1-2.

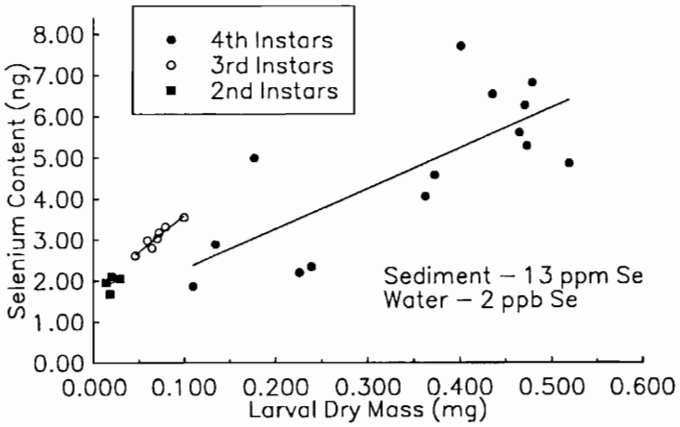


7c. Culture 2-2.

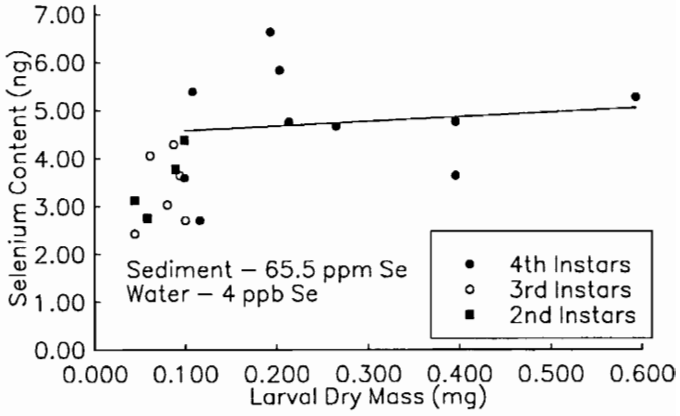


7d. Culture 1-4.

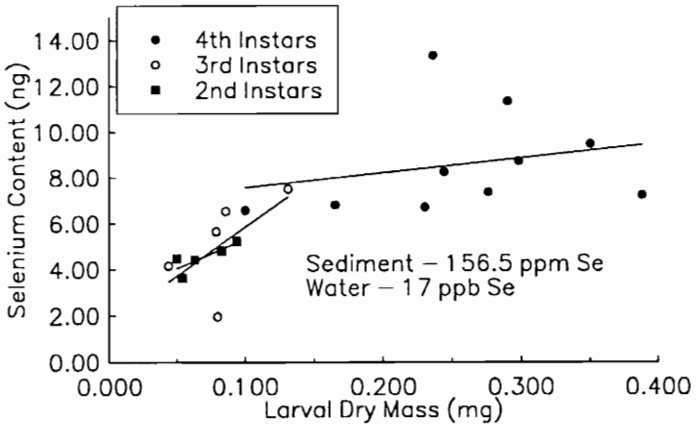
Figure 7. Body burden vs. larval dry mass and regression curves for samples collected 8/2/90.



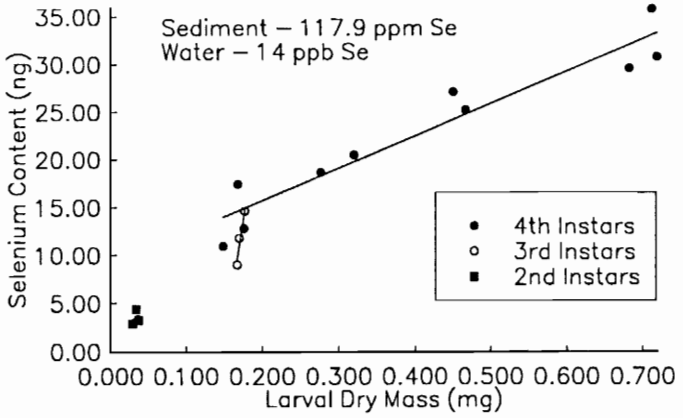
8a. Culture 2-2.



8b. Culture 2-3.

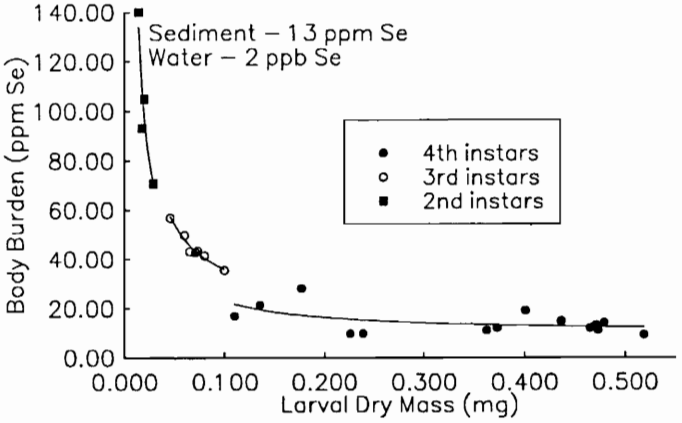


8c. Culture 1-5.

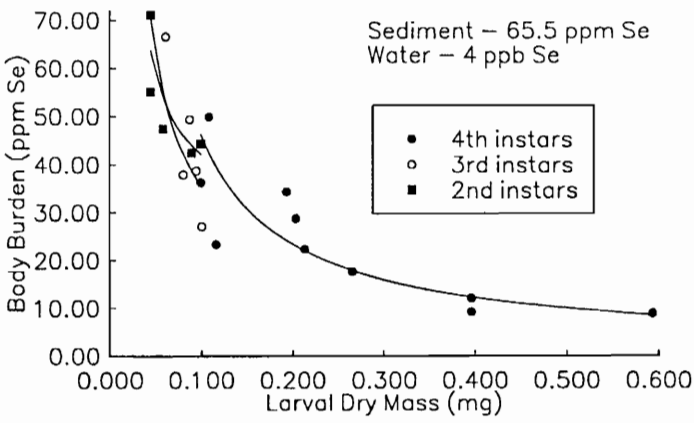


8d. Culture 1-6.

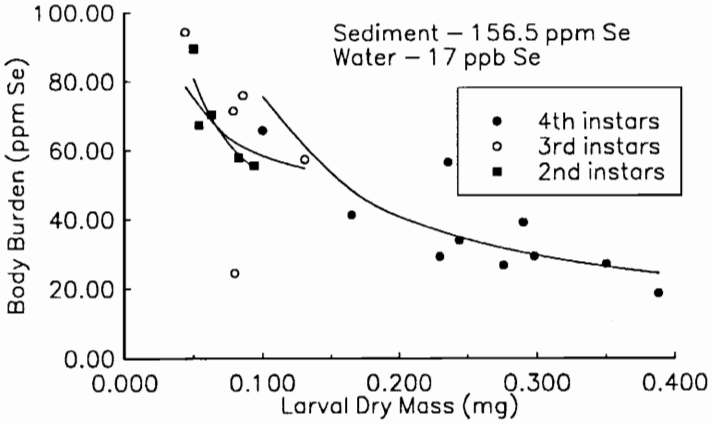
Figure 8. Larval dry mass vs. selenium content with first order regression lines for samples collected 1/30/91.



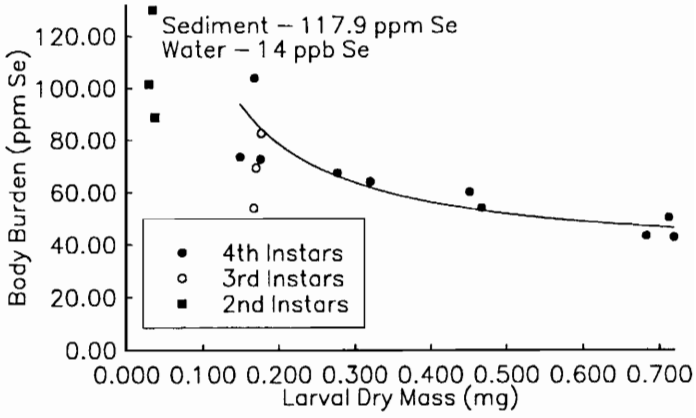
9a. Culture 2-2.



9b. Culture 2-4.



9c. Culture 1-5.

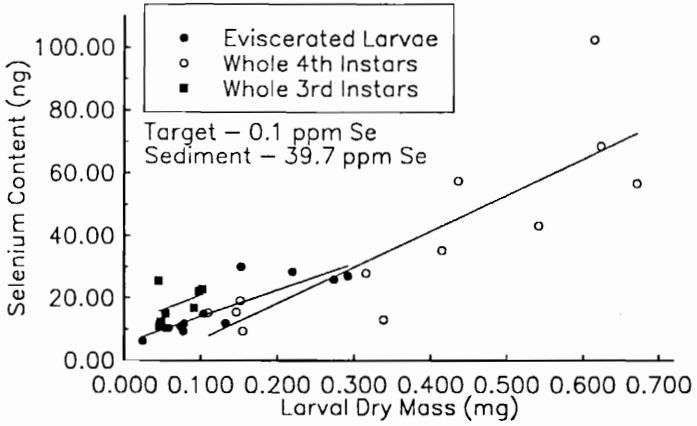


9d. Culture 1-6.

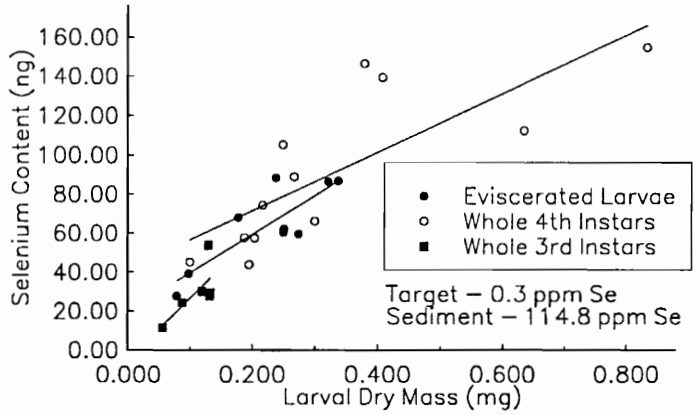
Figure 9. Larval dry mass vs. body burden with regression curves for samples collected 1/30/91.

null hypothesis when a difference really exists, and the probability of committing a type II error is 1-β, so that as β decreases, the probability of committing a type II error increases. Unlike α, which is specified under the null hypothesis, β cannot be specified under the alternate hypothesis because no sampling distribution is specified under the alternate hypothesis (Larson, 1974).

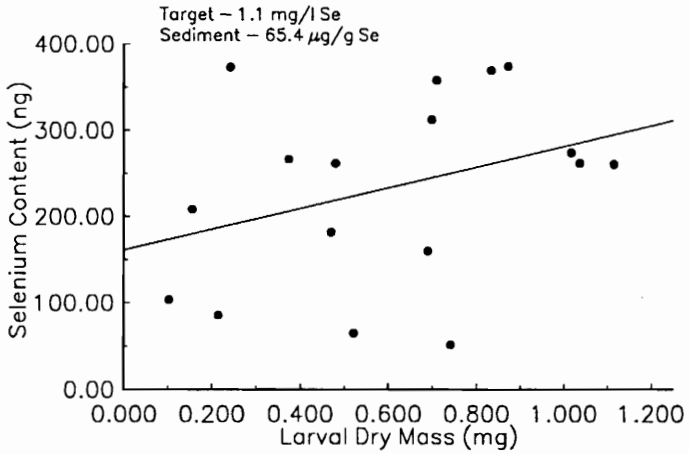
As in group 1 and group 2 cultures, the relationships between selenium content and dry mass for larvae from cultures 3-2 and 3-3 were well described by separate first-order regressions for each instar. In contrast, the *Y* intercepts of these regressions were closer to zero, and the *Y* intercept of the fourth instar regression for culture 3-2 was negative. These data and regressions are shown in Figure 10. Interestingly, the regression of larval selenium content on larval dry mass for culture 3-4 was not statistically significant, even though the estimate of selenium accumulation per mass was 120.14 ng Se/mg. The selenium contents of these larvae ranged from 50 to 400 ng Se, and were much higher than those of any other culture. The data and regression for this culture are shown in figure 10. The next highest selenium contents (160 ng Se) were found in culture 3-3. When larvae were sampled from culture 3-4 there were only 4th instars. These individuals emerged, but did not lay any eggs, so the culture died out.



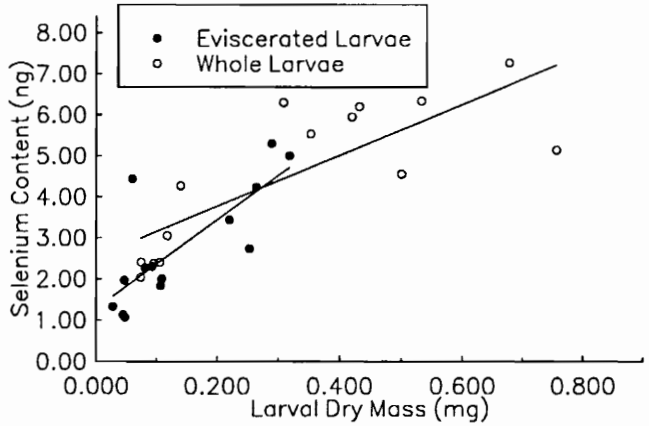
10a. Culture 3-2.



10b. Culture 3-3.



10c. Culture 3-4.



10d. Culture 1-6.

Figure 10. Selenium content vs. larval dry mass for whole and eviscerated fourth instar larvae with first order regression lines for samples collected 4/15/91.

Bioaccumulation and Bioconcentration

Dissolved selenium concentration was a highly significant factor affecting the mean selenium contents of fourth instar larvae weighing 0.15 mg (equation 9) and 0.5 mg (equation 10) and the Y intercepts of the regressions of larval selenium content on larval mass (equation 11).

$$Y=0.123*X+8.545,\ r^2=0.9528,\ P=0.0002 \quad (9)$$

$$Y=0.193*X+19.845,\ r^2=0.9370,\ P=0.0003 \quad (10)$$

$$Y=0.145*X-0.522,\ r^2=0.9779,\ P<0.0001 \quad (11)$$

Dissolved selenium concentration did not have a significant effect on the rates of selenium gain as weight increased (P=0.1464). Larvae from the group 3 cultures had the highest rates of accumulation and the highest selenium contents.

Substrate-adsorbed selenium concentration was not a significant factor in determining rate of selenium accumulation as weight increased (P=0.92), Y intercept (P=0.96), mean selenium content of 0.5 mg fourth instar larvae (P=0.95) or mean selenium content of 0.15 mg larvae (P=0.97). The null hypothesis that there was no correlation between substrate-adsorbed and dissolved selenium concentration was not rejected (r = 0.41, P = 0.22). In particular, cultures 1-5 and 2-4, which had the highest and second highest adsorbed selenium concentrations respectively, had the lowest rates of larval selenium accumulation as mass increased.

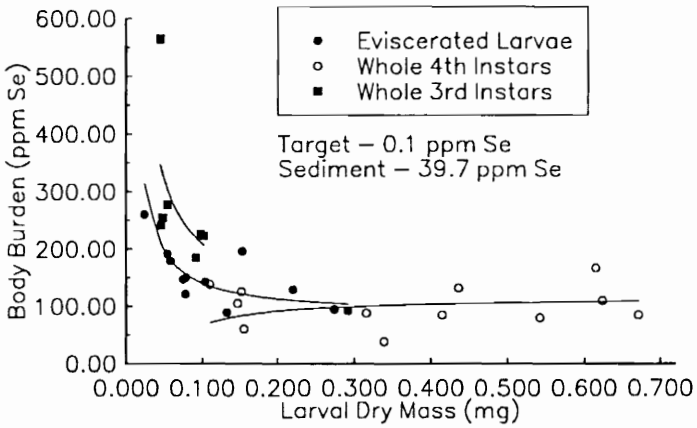
Body burdens of eviscerated larvae were very similar to those of smaller whole larvae. Effect of evisceration on body burden was the same in high and low dissolved selenium cultures. Selenium contents and body burdens of culture 1-6 larvae are shown in Figures 10

and 11, respectively. The rates of selenium accumulation with respect to weight for whole fourth instars and eviscerated fourth instars from cultures 3-2, 3-3 and 1-6 were not significantly different.

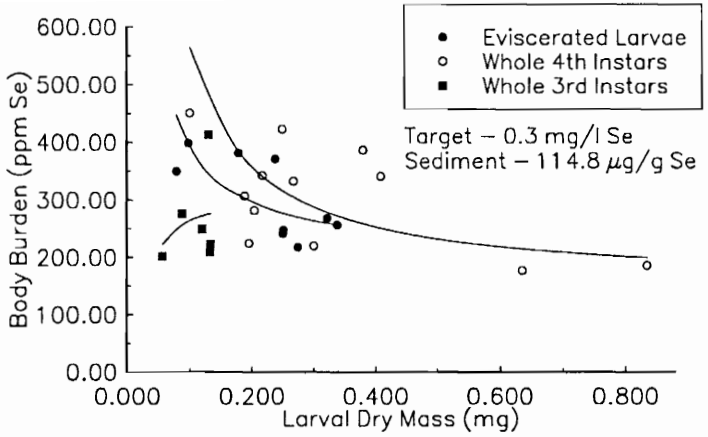
A dissolved selenium concentration of 11.3 ppb will cause a 0.15 mg larvae to have a selenium body burden of 15 ppm, according to equation 9. This was arrived at by solving equation 9 for Y, and then inversely predicting the dissolved selenium concentration which would cause a selenium content of 2.25 ng. This content is associated with a body burden of 15 ppb in a 0.15 mg larva. The lowest body burdens were seen in culture 1-2, which had 20 ppb Se in the water and 81.2 ppm Se in the substrate. In this culture, the average body burden for a 0.5 mg larva was around 25 ppm, and the average body burden for a 0.05 mg larva was about 60 ppm. It was not possible to inversely predict the adsorbed selenium concentration associated with a 15 ppm body burden in a 0.15 mg larva because there was no statistically significant evidence for an effect on selenium content by adsorbed selenium concentration. Because of the reciprocal relationship between mass and body burden, MBCF's and MBAF's decreased as mass increased. The equations and MBCF's and MBAF's for each culture are shown in Table 8.

Acute Toxicity and Acclimation

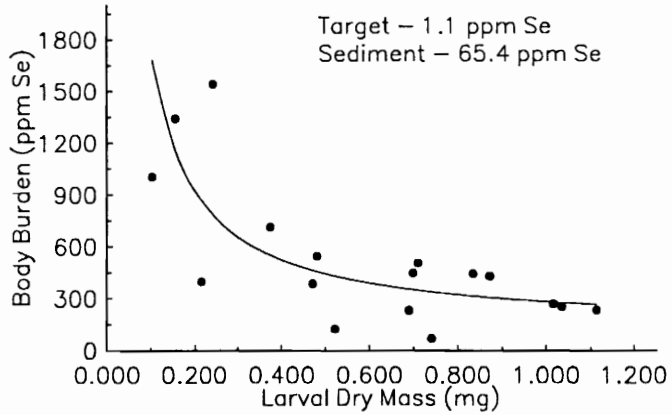
Group 3 cultures, which had the highest dissolved selenium contents, had marked increases in LC_{50}'s. Measured selenium concentrations in culture waters and sediments except for group 3 cultures prior to test 7 are shown in Table 9, and all LC_x's obtained during this study and the associated 95% confidence limits are shown in Table 10. Figures 12 a-c show the dose-response graphs for the range-finding toxicity tests. The chi-square goodness of fit test provided no statistically significant evidence that the probit model did not fit these data,



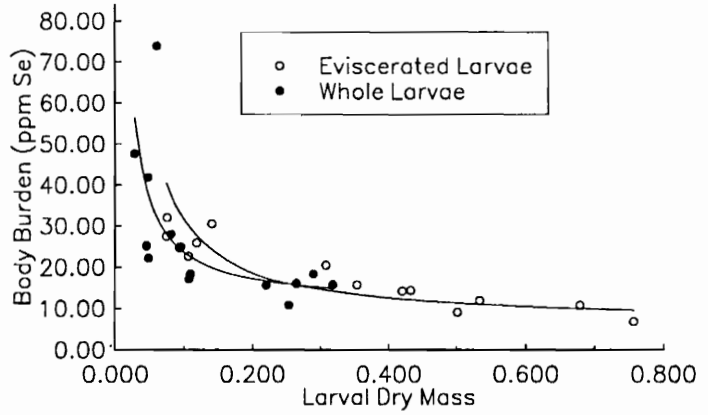
11a. Culture 3-2.



11b. Culture 3-3.



11c. Culture 3-4.



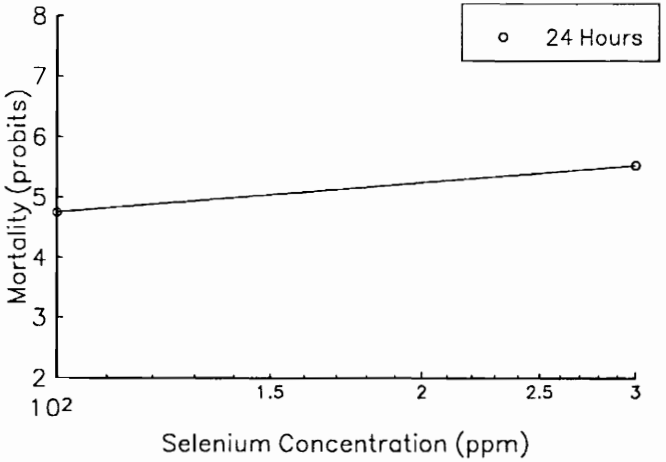
11d. Culture 1-6.

Figure 11. Body burden vs. larval dry mass for whole and eviscerated fourth instar larvae with regression curves.

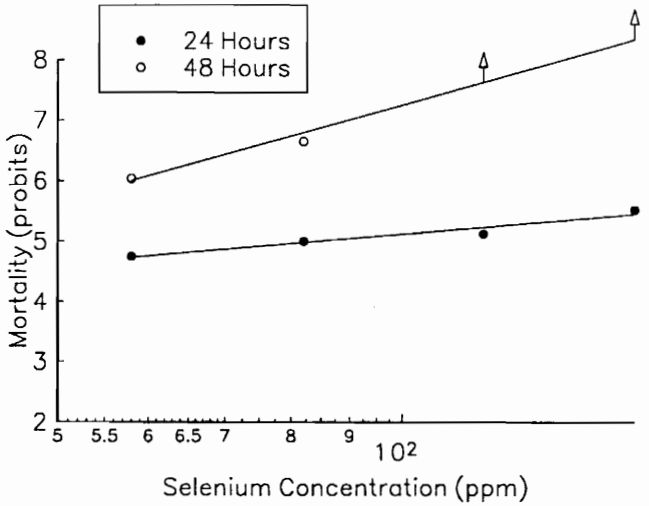
Table 8. Maximum bioconcentration factor (MBCF) and maximum bioaccumulation factor (MBAF) equations and MBCF's and MBAF's for 0.15 mg fourth instar larvae.

Culture	Equation		Estimate for 0.15 mg larva	
	MBCF	MBAF	MBCF	MBAF
1-2	Y=983+128.5/X	Y=0.242+0.0317/X	1240	0.3054
2-2	Y=1089+509.4/X	Y=0.153+0.0835/X	2107.8	0.32
1-4	Y=314.3+160.5/X	Y=0.074+0.379/X	635.3	0.832
2-2	Y=4900+650/X	Y=0.754+0.1/X	6200	0.954
2-4	Y=240.5+1120/X	Y=0.0147+0.0684/X	2480.5	0.1515
1-5	Y=381.8+406.5/X	Y=0.0415+0.0443/X	1194.8	0.1301
1-6	Y=2423.6+637.1/X	Y=0.2878+0.0757/X	3697.8	0.4392
3-2	Y=1153.1+48.5/X	Y=2.905+0.1222/X	1250.1	3.1494
3-3	Y=497+138.3/X	Y=1.299+0.3615/X	7736	2.022
3-4	Y=109.2+146.1/X	Y=1.837+2.4576/X	401.4	6.7522

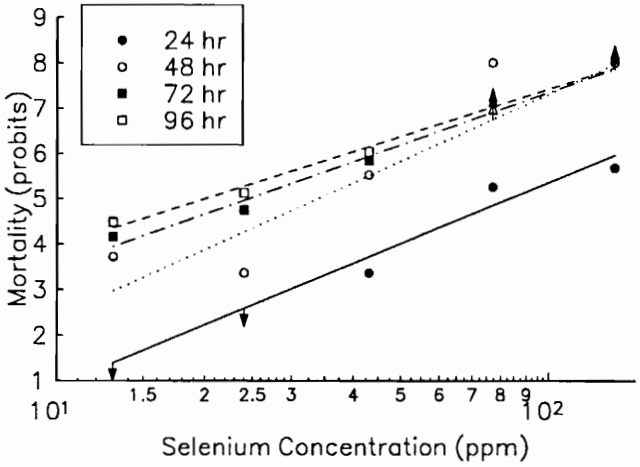
with the exception of test 4 as discussed below. Of the group 2 cultures, culture 2-3, which had the second lowest adsorbed and dissolved selenium concentrations, had the highest LC_{50}'s (first test 120 hr LC_{50}=15.6 ppm, second test 120 hr LC_{50}=3.6 ppm) in both tests. Cultures 3-1 and 3-3 performed similarly to culture 2-3 (3-1 120 hr LC_{50}=4.1, 3-3 120 hr LC_{50}=1.2). Cultures 3-4 and 3-5 developed a red film on the substrate surface. This coloration was also very evident in the toxicity tests. Figure 13 shows a series of test solutions after 24 hours. The substrate in most of the dishes was covered in a red film, and the water with higher selenium concentrations had a beige cast. The red film may have been elemental selenium which had precipitated (Zieve, et. al., 1985). The concentrations in the second test were too high to generate LC_{50}'s for elapsed times greater than 24 hours, so the test was repeated. Because the 24 hour LC_{50}'s for tests 2 and 3 are very close, it was thought that concentrations for subsequent tests should span from 15 to around 100 ppm. Test four was a range finding test using larvae from a culture which had been spiked for about six weeks to a dissolved selenium concentration of 3 ppm. The dose-response graph is shown in Figure 14. The plots



12a. First test.



12b. Second test.



12c. Third test.

Figure 12. Dose-response graphs of range-finding tests.

Table 9. Selenium concentrations in culture water and substrate.

Culture group-number	Date	Substrate concentration (µg/g)	Water concentration (µg/l)
1-2	8/2/90	81	21
1-3		88	22
1-4		254	69
1-5		173	121
1-6		106	63
1-7		----	534
1-8		397	341
2-2	8/2/90	71	10
2-3		----	22
2-4		99	24
2-5		159	80
2-6		246	1692
2-2	9/8/90	----	67
2-2	9/27/90	12	9
2-2	1/30/91	13	21
2-3		66	39
1-5		157	170
1-6		118	143
3-2	4/15/91	40	100
3-3		115	300
3-4		65	1100

Table 10. Lethal concentrations and confidence limits from acute toxicity tests.

Test Description	Date	Time (hr)	LC_{50} (ppm)$_2$	95% C. L.$_1$ Upper	Lower
First range-finding test on culture 1-1.	12/15/89	24	143.0	*	*
Second range-finding test on culture 1-1.	2/2/90	24	39.0	65.5	23.5 (85)
		48	37.5	50.9	0.9 (75)
			65.6 (90)	91.O	42.1 (75)
Third range-finding test on culture 1-1.	3/11/90	24	83.4	355.7	41.1
		48	34.0	46.0	24.8
		72	24.6	35.1	15.5
		96	20.1	29.3	10.3
Test on culture 1-8.	6/2/90	See Figure 5b .			
First test of acclimation to sediment-adsorbed selenium.	1/19/91				
Culture 2-1		24	39.0	88.4	17.7h
		48	30.1	30.1	30.1
		72	18.3	26.4	12.6
		96	13.4	27.0	6.0h
		120	6.7	13.2	0.5
Culture 2-2		24	18.4	128.0	1.6h
		48	8.1	13.9	2.5
		72	7.8	13.4	2.5
		96	6.7	9.4	3.7
		120	5.8	8.1	2.7
Culture 2-3		24	29.5	39.8	22.0
		48sk	16.2	21.9	12.1
		72	19.2	25.3	14.6
		96	16.6	22.2	12.5
		120	15.6	56.6	4.2
Culture 1-6		24	26.3	37.4	17.9
		48	95% mortality at 20 mg/l		
Culture 1-7		24	2.1	8.2	<0.1 (50)
		48	100% mortality at 20 mg/l		

Table 10 continued.

Test Description	Date	Time (hr)	LC_{50} (ppm)$_2$	95% C. L.$_1$ Upper	Lower
Second test of acclimation to sediment-adsorbed selenium.	1/30/91				
Culture 2-1		24	78.4	2.2E[4]	34.3
			19.8 (20)	82.4	12.0
		48	11.8	26.2	5.7
		72	7.5	12.5	2.1
		96	3.5	5.9	0.5
			5.1 (60)	7.9	1.3
		120	1.3	3.1	<0.1
			3.5 (70)	6.0	0.4
Culture 2-2		24	4.1	7.4	0.1
		48	0.3	1.3	<0.1
			3.9 (80)	7.0	0.2
		72	0.2	1.2	<0.1
			8.2 (90)	23.1	0.8
		96	<0.1	<0.1	<0.1
			2.2 (90)	4.9	<0.1
		120	<0.1	0.3	<0.1
			2.5 (90)	5.1	<0.1
Culture 2-3		24	38.2	224.3	23.6
			21.2 (30)	50.4	14.5
		48	10.0	32.4	1.8
		72	7.2	8.9	5.5
		96	5.3	6.4	3.9
		120	3.6	4.8	1.5
			4.2 (60)	5.5	2.2
Culture 1-6		24	25.6	48.0	19.0
		48	8.1	13.5	3.1h
		72	4.5	7.3	0.4
		96	3.7	5.3	<0.1
			4.3 (60)	6.1	<0.1
		120	3.8	4.4	1.7
			4.1 (60)	4.8	2.3
Culture 1-7		24	22.6	60.2	15.2
		48	5.0	8.0	<0.1h
		72	4.3	5.8	0.7h
		96	4.0	5.8	<0.1h
		120	4.0	5.8	<0.1h

Table 10 continued.

Test Description	Date	Time (hr)	LC_{50} (ppm)$_2$	95% C. L.$_1$ Upper	Lower
Test of acclimation to dissolved selenium.	4/15/91				
Culture 3-1		24	36.2	4.0E[11]	13.0
		48	9.6	12.6	7.1
		72	7.1	9.4	5.0
		96	5.2	7.3	3.0
		120	4.1	5.7	2.3
Culture 3-2		24	56.6	88.9	44.0
		48	29.9	70.5	13.2
		72	26.2	30.9	22.3
		96	23.4	29.8	18.4
		120	19.7	28.3	13.6
Culture 3-3		24	56.2	5863.8	28.1h
		48	25.4	68.2	10.7h
		72	14.8	31.1	3.8h
		96	6.7	14.0	<0.1h
		120	0.1	1.2	<0.1h
			2.1 (80)	7.0	<0.1h
Culture 3-5		24	No mortality		
		48	89.7	2877.8	60.3 (90)
			64.2 (30)	273.0	39.4 (90)
		72	64.7	335.8	37.3
		96	30.7	69.4	17.9
		120	13.6	35.0	0.5h

1-Alternate confidence limits are listed in parentheses.

2-If the LC_{50} was extrapolated, the concentration causing the tenth percent kill nearest to 50 is listed in addition to the LC_{50}. The percentage kill at the listed concentration is in parentheses.

*-Zero residual error degrees of freedom.

h-Heterogeneous data. Confidence limits calculated after multiplying all variances by heterogeneity factor.

of 24, 48 and 72 hour dose-responses are bizarre. At the lowest concentrations, toxicity increased, but then decreased over the next two concentrations. Toxicity then increased again as concentration increased starting at the fifth concentration. No LC_{50}'s were calculated for this test because these data severely violate the assumption of monotonically increasing dose-response and do not fit the probit model. However, this test shows a general acclimation to selenium because a lower percentage of organisms died at a higher concentration in this test than in test 3. The lower response peak for 72 hours is at 34 ppm, where 30% of test organisms died, where the 72 hour LC_{50} in test 3 is 25.5 ppm.

In test 5, there was 100% mortality after 48 hours at 40 ppm for all test groups. In the tests using the larvae from cultures 1-6 and 1-7, there was 100% mortality in all but the 20 ppm dish, so no LC_x could be estimated. These data indicate that sensitivity of the control culture had increased since test 3 was performed. Dose-response plots for this test are shown in Figure 15. The 24 hr LC_{50}'s ranged from 17.4 in culture 2-2 to 32.5 in culture 2-1, so the larvae from the selenium exposed cultures were more sensitive to selenium than were larvae from the control culture. The stock culture showed an increase in sensitivity to selenium, because the 96 hour LC_{50} in test three was 20.3 ppm, while in test 5 it was 4.7 ppm. Because test 5 had so few partial kills, test 6 was performed on these cultures 2 weeks later with a different series of concentrations.

Test 6 showed that sensitivity to selenium did not decrease in larvae which had been cultured in selenium-contaminated substrate. Dose-responses from this test are shown in Figure 16. In this test, the culture 2-1 larvae had the highest LC_{50}'s, while the larvae from cultures 2-2, 2-3, 1-6, and 1-7 had very similar LC_{50}'s. The larvae from culture 2-2 showed a much greater sensitivity to selenium than any other larvae. The 120 hour LC_{50}'s for cultures

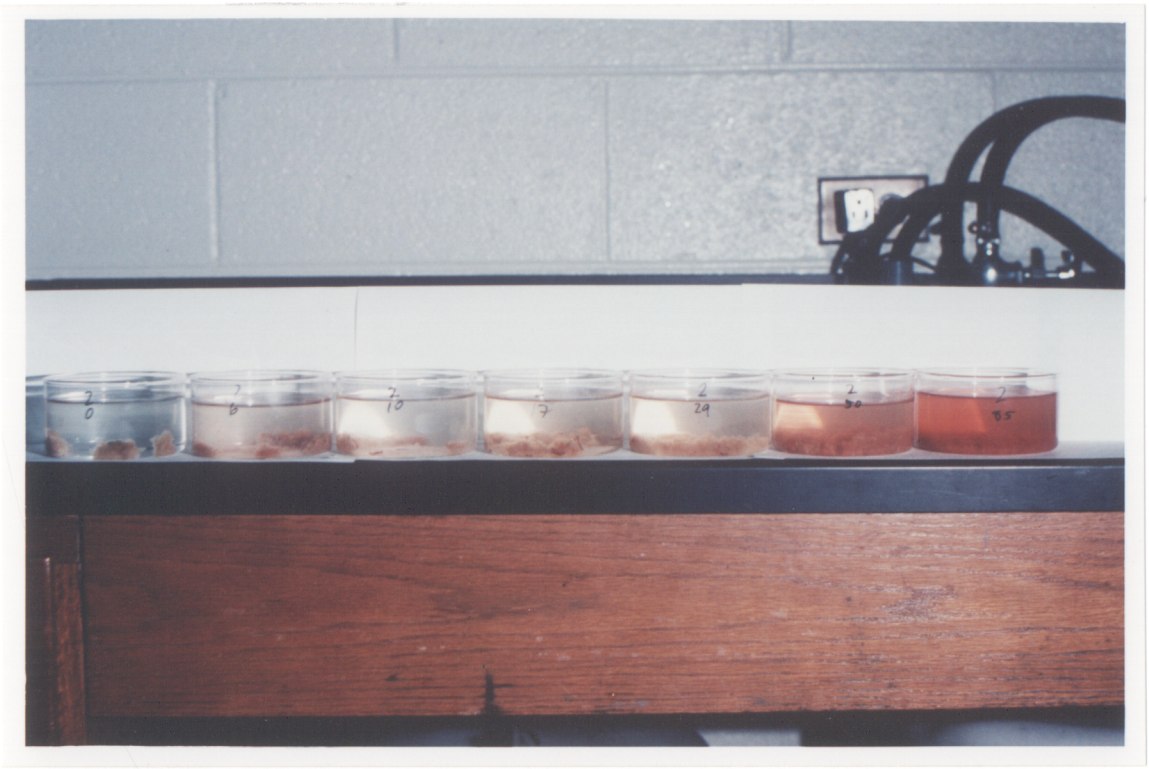


Figure 13. Selenium toxicity test solutions after 24 hours. Red color is thought to be precipitated elemental selenium.

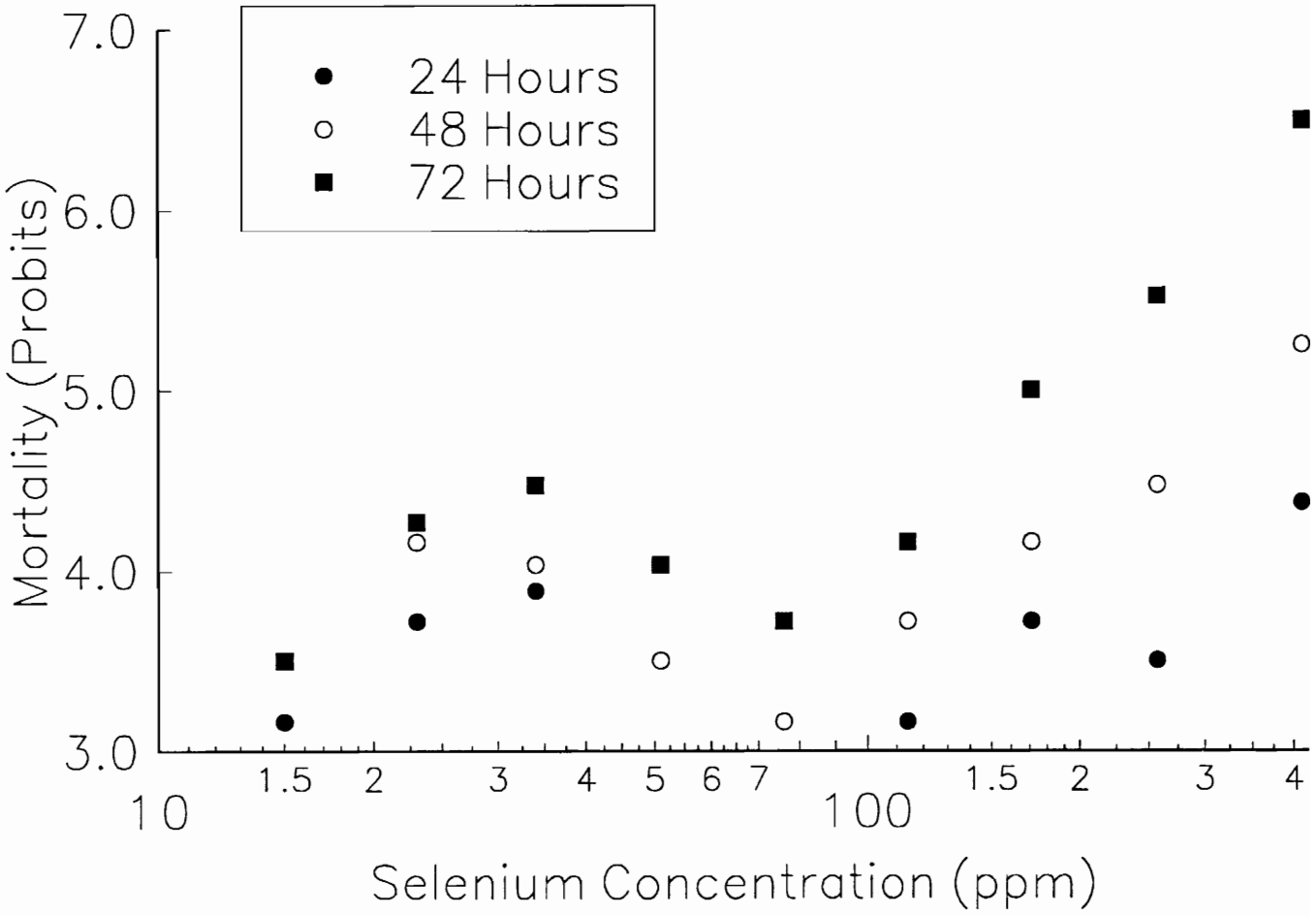


Figure 14. Dose-response graph of a culture exposed to 3 ppm Se pulses for six weeks.

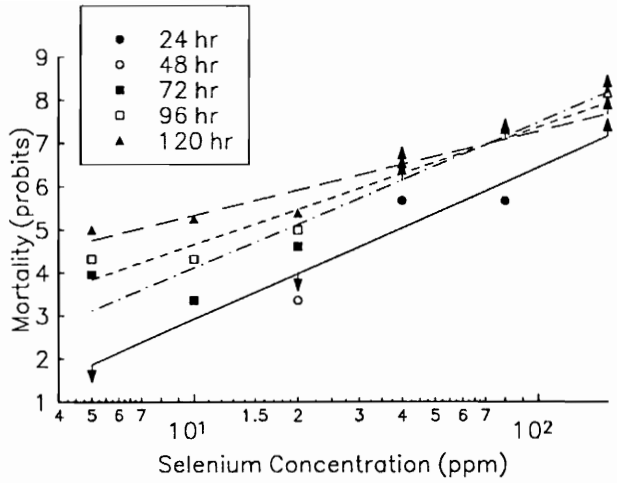
2-1, 2-3, 1-6, and 1-7 ranged from 5.5 to 4.1 mg/l with confidence limits close to ±3mg/l, so sensitivity to selenium was about the same for larvae from these cultures. Culture 2-1 larvae had the lowest 24 hour LC_{50}, and mortality was so high by 48 hours that only an LC_{80} could be calculated.

Test 7 showed that larvae cultured in selenium-contaminated water were less sensitive to selenium than larvae from an uncontaminated culture, or from a culture with elevated substrate selenium only. Figure 17 shows the selenium concentrations in the four dissolved selenium cultures for the 52 days preceding the toxicity test 7. Dose-responses from this test are shown in Figure 18.

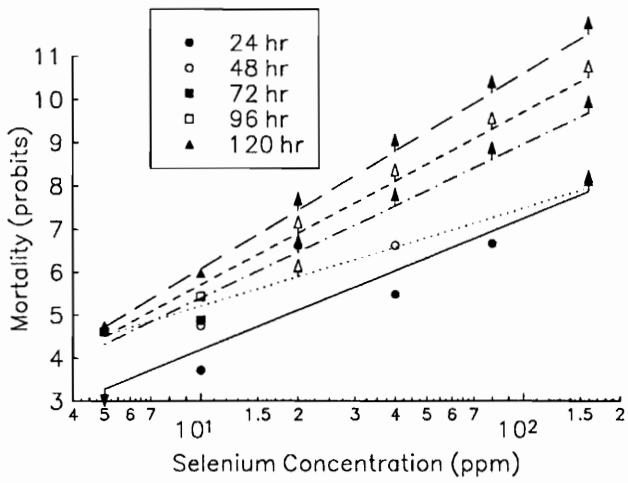
Multiple linear regression of target dissolved selenium concentrations, exposure times and their interaction against LC_{50}'s showed that all factors were significant determinants of LC_{50} ($P < 0.1$) and the slope and Y intercept are significantly different from zero. The relationship is

$$Y=43.5-0.3*X_1+25*X_2-0.2*X_1*X_2,\ r^2=0.6733,\ P=0.005 \qquad (12)$$

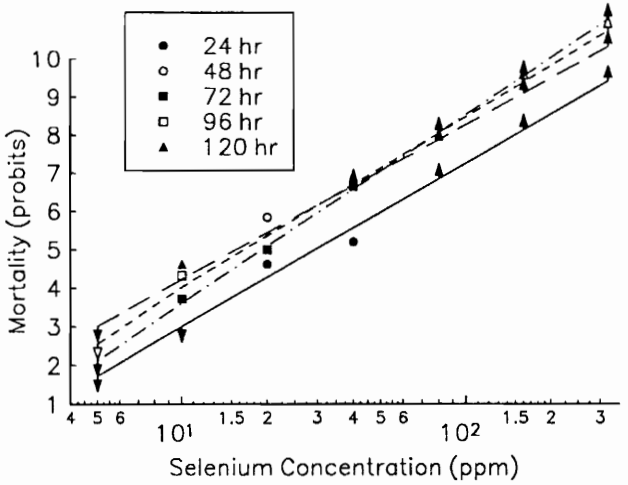
where Y is LC_{50} in ppm, X_1 is exposure time in hours, and X_2 is target dissolved selenium concentration. Correlations between variables, a regression summary and analysis of variance table are shown in Table 11. According to this regression, LC_{50} is reduced by increasing exposure time. Holding time constant and increasing previous concentration exposure will increase the LC_{50}, but the interaction term reduces the rate at which LC_{50} increases as the previous exposure concentration increases. The interaction term increases the rate of LC_{50} decrease as exposure time increases. The standardized slope estimates indicate that previous exposure concentration (b = 1.84) has a greater effect on LC_{50} than does exposure time



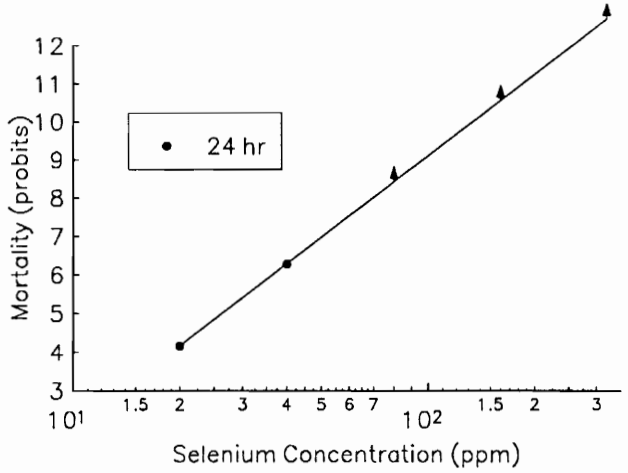
15a. Culture 2-1.



15b. Culture 2-2.

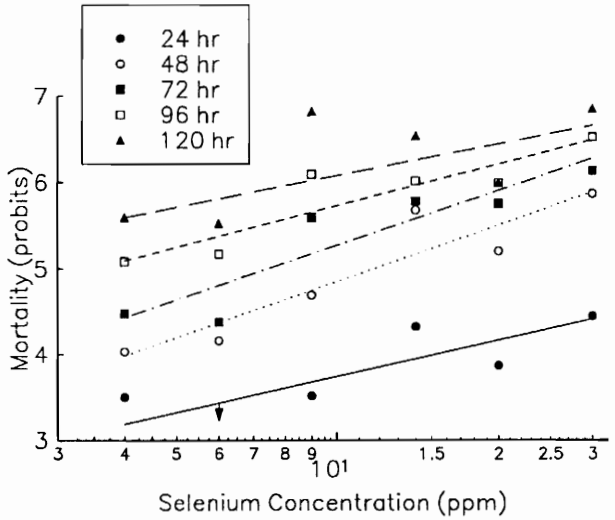


15c. Culture 2-3.

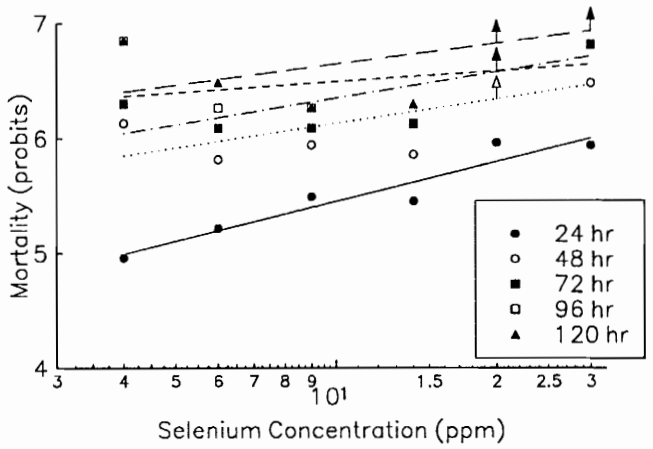


15d. Culture 1-6.

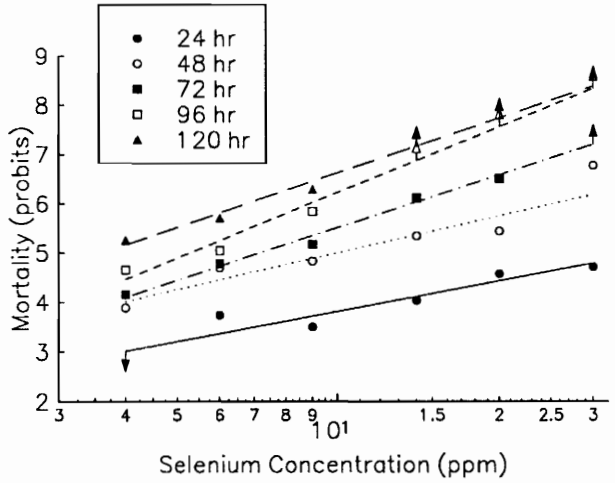
Figure 15. Dose-response graphs and probit regressions for first test of acclimation to sediment-adsorbed selenium.



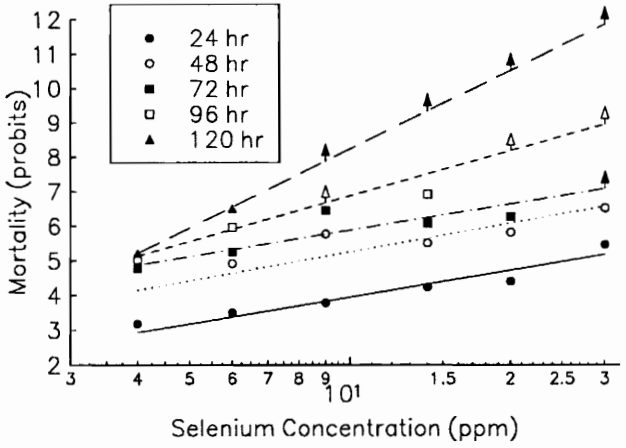
16a. Culture 2-1.



16b. Culture 2-2.

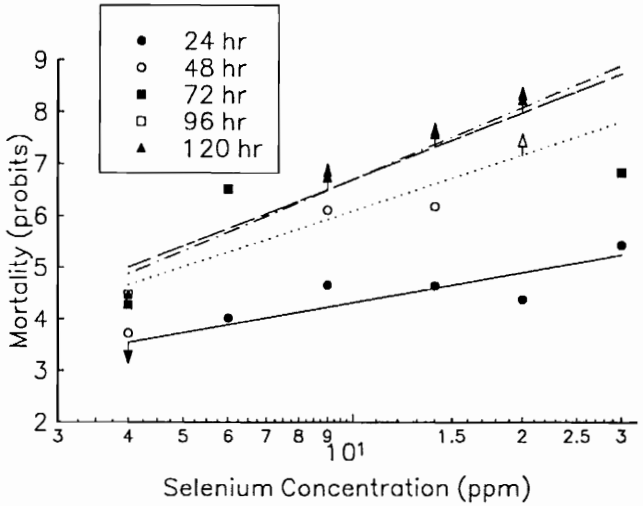


16c. Culture 2-3.



16d. Culture 1-6.

Figure 16. Dose-response graphs and probit regression lines for second test of acclimation to sediment-adsorbed selenium.



16e. Culture 1-7.

Figure 16, continued.

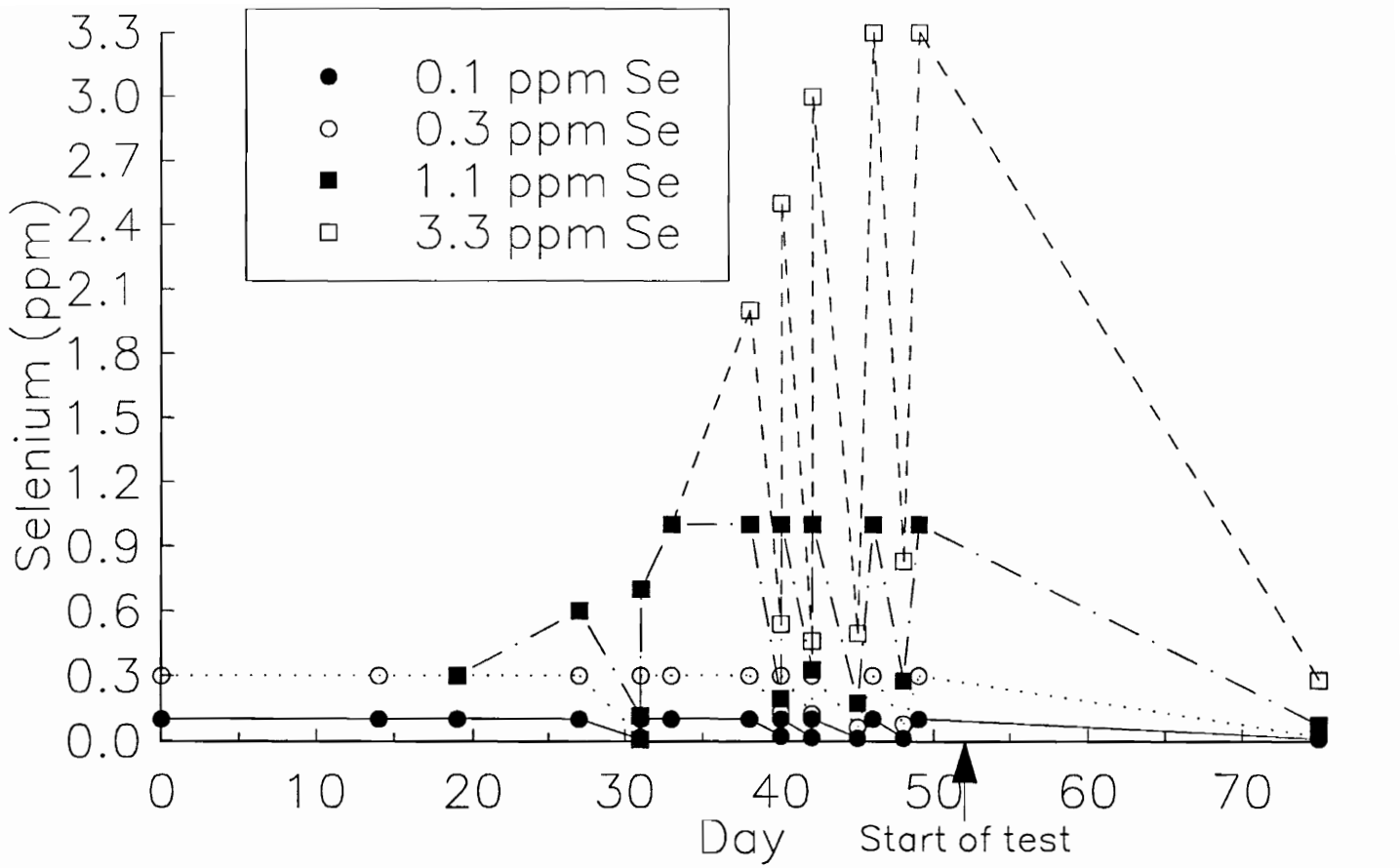
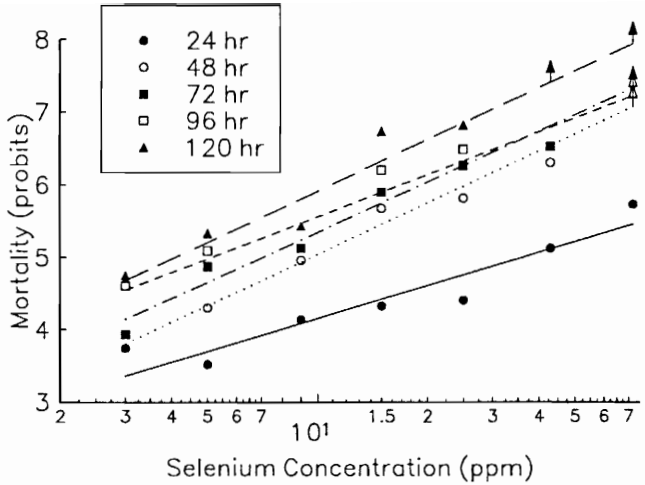
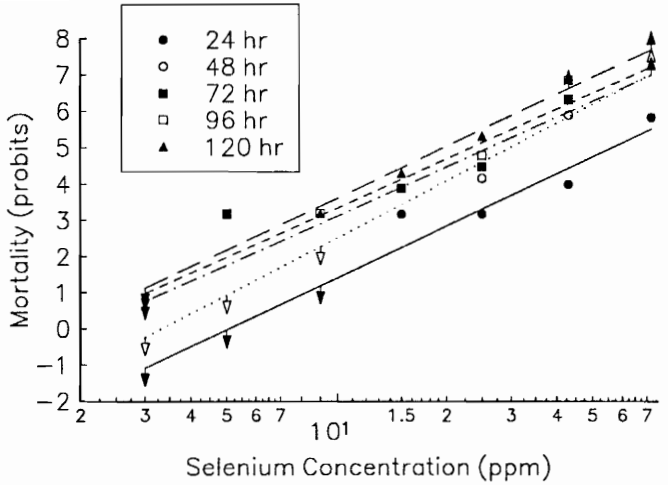


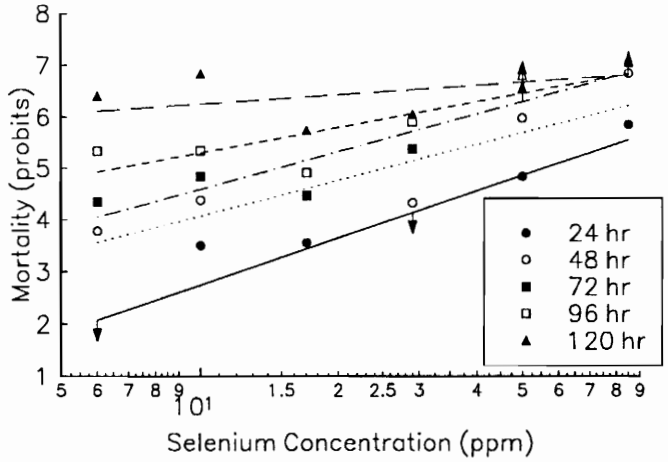
Figure 17. Dissolved selenium concentrations in group 3 cultures before and after test of acclimation to dissolved selenium.



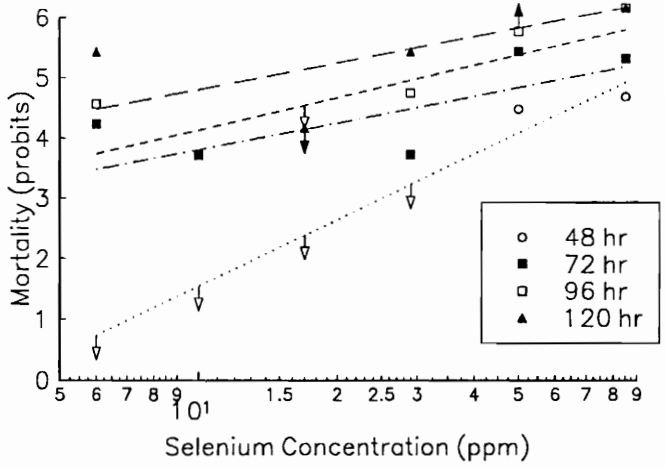
18a. Culture 3-1.



18b. Culture 3-2.



18c. Culture 3-3.



18d. Culture 3-5.

Figure 18. Dose-response graphs and probit regression lines for test of acclimation to dissolved selenium.

Table 11. Correlations, regression summary, and analysis of variance table for regression of target dissolved selenium concentration, exposure time, and their interaction against LC_{50} for data from toxicity test 7.

Correlations between variables

	Time	Previous exposure	Interaction	LC_{50}
Time	1.0000	0.3615	0.4364	-0.6267
Previous exposure	0.3615	1.0000	0.9729	0.1707
Interaction	0.4364	0.9729	1.0000	0.0320
LC_{50}	-0.6267	0.1707	0.0320	1.0000

Regression Summary

	Estimates				r^2	
Variable	Parameter	Standardized	S. E.	P	Sequential	Simple
Y Intercept (a)	43.51	0.0000	7.57	0.0001		
Time	-0.33	-0.6476	0.10	0.0081	0.3927	0.3927
Previous exposure	24.66	1.8440	10.46	0.0379	0.5742	0.0291
Interaction	-0.20	-1.4793	0.11	0.0951	0.6733	0.0010

Analysis of variance

Source	df	Sums of Squares	Mean Squares	F-Ratio	P
Regression	3	2964.853	988.2842	7.56	0.005
Residual	11	1438.925	130.8114		
Total	14	4403.777	314.5555		

H_0: The slope of the regression line is zero. H_A: The slope of the regression line is different from zero.

(b = -0.65). The interaction term (b = -1.48) is more important than exposure time, but less important than previous exposure concentration in determining LC_{50}.

DISCUSSION

Acute Toxicity Test Method Development

Larval density had no effect on chironomid growth and survival at densities below 50 larvae per 200 ml water. This was shown by the lack of correlation between initial density and final weight and between initial density and survival in crowding experiments 1 and 2 (Figure 2). In crowding experiment 3, the average larval weights and proportions surviving in flasks with initial densities of 50 were the highest of any of the other groups, which means factors besides density were controlling growth and survival at low density. There was a density-dependent reduction in weight at higher initial larval densities. In crowding experiment 3, the larvae in the flasks initially containing 300 larvae had significantly lower dry weights and there was a higher percentage of third instars. At high densities, from equation 7, an increase in mortality of 10% per 100 additional larvae was predicted. The turbidity and sewage odor of the water indicated organic enrichment. Although *C. riparius* larval density can be very high in sewage sludge, it may require nutrients not found in Tetra Growth Food, and the Tetra may decompose differently than sludge. The differences in conditions in the flasks and in a wastewater treatment clarifier are certainly not restricted to food. Repeating this experiment with higher initial larval densities and supplying sewage sludge as food instead of Tetra would determine whether this density-dependent decrease in weight was an artifact of using Tetra Growth Food or if it occurs with other foods.

There were significant differences in total survival between trials in the survival experiment. Test conditions were identical between trials one and two, and between trials three and four. Differences in total survival between groups are very likely real differences, and not artifacts of test conditions, since there were no significant differences in survival

between replicates within tests. These chironomids came from thriving cultures, and based on the densities in the cultures, were judged to be in good shape. However, it is evident that culture condition cannot be used as an indicator of individual health. This points to factors outside test conditions as variables affecting survival.

The larvae in the fourth test had the highest survival, and like the larvae in the third test, they were from a culture which was less than a month old, so the substrate and water were free from high amounts of accumulated waste. The larvae in tests one and two were from a culture that was about one year old, having been started in January, 1989. However, the larvae in the second test had higher survival than larvae in the third test, but lower survival than larvae in the fourth test. This eliminates the elapsed time since the last water or substrate renewal as a measure of culture condition.

The decrease in development time from test 2 to test 3 resulted from the difference in rearing methods. However, the decrease in development time from test three to test four may be attributable to a slight increase in temperature in the laboratory. Temperature in the laboratory fluctuated between 21 and 24°C throughout the year, but daytime temperatures were between 23 and 24°C during test four, and were between 21 and 23°C during test three. The relationship between development time and temperature has not been quantified, but temperature is the most likely explanation in this case.

The best explanation for the missing, unaccounted for larvae in the survival experiments is consumption of dead larvae by other larvae. Considering the extremely high survival in the fourth test, it appears that cannibalism did not occur. In the crowding experiments, no density-dependent cannibalism was indicated, because survival was not correlated with initial

larval density, and there was no tendency for larval densities to approach a certain level over time.

Larvae gave tube-building energetic priority over growth and development. This shows that they have an obligate relationship to the substrate, and that their occurrence in the field may be determined primarily by whether or not the substrate composition is suitable for them. Transferring the larvae to new culture vessels daily resulted in lower dry mass at the end of the observation and increased development time. It also suggests a tube is more important in determining reproductive success than growth rate or development rate.

Test protocols for pelagic organisms cannot be blindly applied to *C. riparius*. Using 50 larvae per 200 ml solution as a known non-stressful loading, and estimating that mature larvae can reach 1 mg dry mass, test organism loading should not exceed 1 mg dry mass per 4 ml test solution, or 1 larva per 4 ml. Static-renewal testing recommended by USEPA (1985, 1989) could not involve disturbing sediment. The technician is cautioned by USEPA (1985, 1989) to minimize handling and disturbing of organisms, but more important for *C. riparius* is to avoid disturbing the test sediment. Test solution renewal must be accomplished by siphoning or decanting most of the old solution and refilling the test vessel slowly to avoid disturbing the sediment. Sediment toxicity tests could not involve sediment replacement.

The control group mortality limits set by USEPA (1985, 1989) of no more than 10% for acute tests and 20% for chronic tests are not realistic for *C. riparius*. As shown in Figure 3, survival percentages and rates changed with larval age, and were different between tests. USEPA (1985) set mortality maxima to ensure that test organisms were "sufficiently healthy", a phrase which was not defined. A consequence of a mortality maximum which was not stated

is that it guarantees a minimum detection power, since probit analysis used for mortality data assumes a binomial distribution (Finney, 1978). For example, if all control and test group organisms die, the dose-response slope parameter estimate will be zero and the position parameter will be infinity, so limited information on substance toxicity is gained. Likewise, maximum detection power is provided by a test in which no control organisms die. For *C. riparius*, the most compelling argument for limits on maximum control mortality is to preserve the detection power of the test. The argument for assuring organism health may be valid, but other life-history parameters may be better indicators of organism health, which is an area that needs further investigation.

To help standardize culture conditions, in hopes of reducing variability in test organism sensitivity, values for some environmental parameters are prescribed by USEPA (1985,1989). These include temperature, photoperiod, light intensity and wavelength, feeding schedule, aeration and culture water. Optimal values of these parameters for *C. riparius* are not known, but since their values were held constant between tests, it is suggested that other factors varied, causing sensitivity to selenium to change. Until these factors are identified and parameterized, and optimal values are found for all parameters, the results of *C. riparius* toxicity tests should be taken with caution.

Larval Selenium Content and Body Burden

Second, third and fourth instar larvae of *C. riparius* accumulated selenium at constant rates as weight increased at all environmental selenium concentrations regardless of whether the selenium was predominantly substrate-adsorbed (Figure 8) or dissolved (Figure 9). Rates of selenium accumulation for different instars were not significantly different. Regression failed to find statistically significant evidence for dependence of selenium content on larval dry

mass of second and third instars, but this was probably a type II error because of the small sample sizes. With one exception, weight was not a determinant of selenium content when sample size was five or less, according to tests of significance of regression slopes. Second and third instar sample sizes were generally 5 or less, so selenium content is probably dependent on body mass also in smaller instars. Accumulation rates in younger instars were higher, except in culture 3-2, where they were slightly lower.

On the surface, it appears that there is a vertical asymptote for the body burden regressions so that body burdens are decreasing from infinity. Obviously, an egg cannot be composed entirely of selenium, but that is concluded if the regressions are extrapolated beyond the range of measured values. In order for body burdens to not approach infinity as dry mass decreases, the regression line for the relationship between metal content and body mass must pass through the origin. The fourth, third and second instar equations had *Y* intercepts which were significantly different from zero, but the equations for second and third instars came closer to passing through the origin than did regression lines for the fourth instars, primarily because the slopes of the regressions for smaller instars were greater. Since the appropriateness of separate regressions has been established for second, third and fourth instars, it is reasonable to expect that this is also true for eggs and first instars. The *Y* intercept for first instars is probably low, but positive, and the slope of the line is probably much greater than for any other larval instar, and, to avoid having eggs composed entirely of selenium, it is necessary that the *Y* intercept for eggs be zero.

The model suggested by these data is a set of intersecting linear functions, where the earliest developmental stage has the highest slope and a *Y* intercept through the origin, and each succeeding stage has a lower slope and higher *Y* intercept, with all slopes being positive,

as shown in Figure 19a. The body burden model is a set of functions where the earliest stage has a constant body burden which serves as an upper bound, each succeeding stage has a higher slope parameter estimate and a lower constant, with all slope parameter estimates being positive, as shown in Figure 19b. Because these are functions of the reciprocal of mass, it must be remembered that a positive slope parameter estimate produces a curve which decreases at a decreasing rate as mass increases, and that the constant does not equal the Y intercept because there is no Y intercept, since the Y-axis is an asymptote.

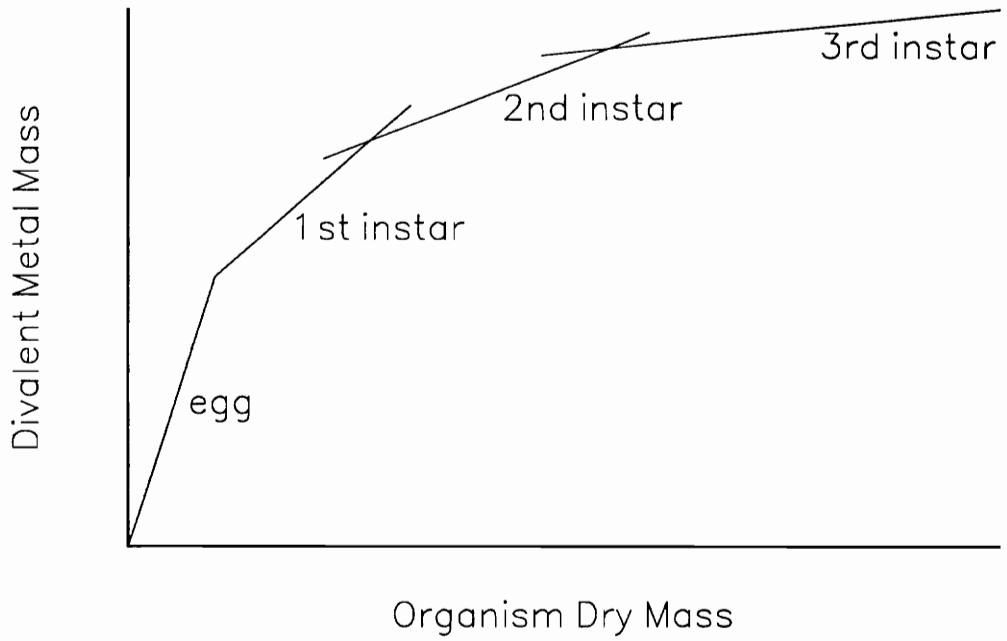
Generally, the data sets follow the multiple linear function model. An exception is culture 2-2, shown in Figure 8a, where the third and second instars had higher selenium contents and Y intercepts than fourth instars. However, the body burden data fit the model (Figure 9a). Another exception is culture 3-2 (Figure 10a), where the same thing happened with the third instars, and the fourth instars had a negative Y intercept. Again, the body burden data fit the model. Both exceptions were probably due to small third instar sample sizes. The negative Y intercept was not different from zero at 0.05, and was possibly due to an outlying selenium content in a large larva.

Two alternatives to multiple linear functions are the quadratic model

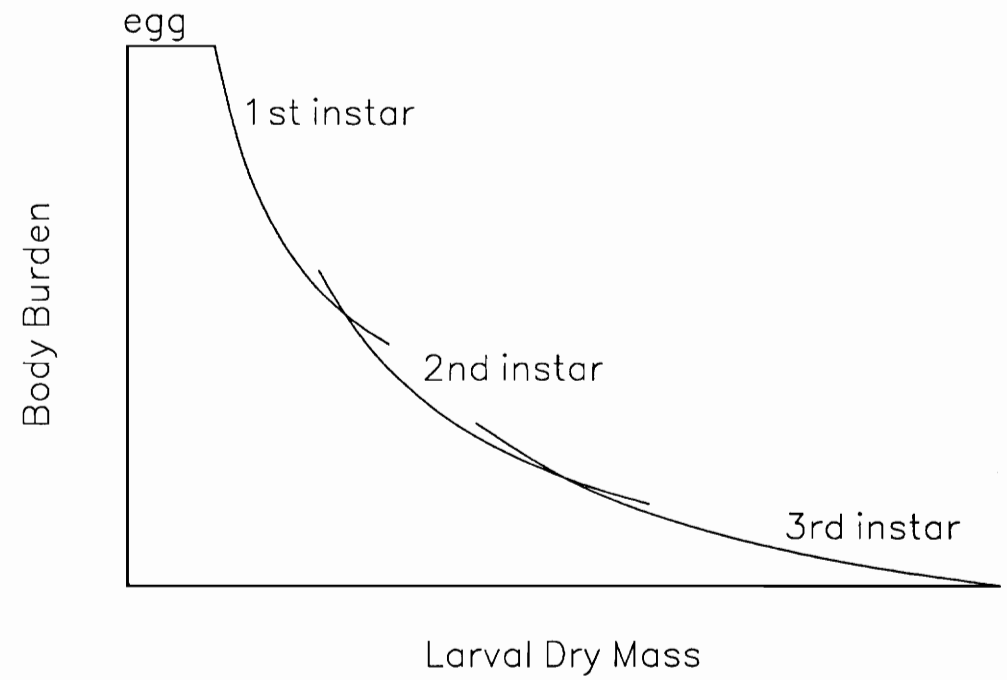
$$Y=\alpha+\beta_1*X+\beta_2*X^2$$

and the power model

$$Y=e^{\alpha+\beta*X}$$



19a. Hypothetical divalent metal mass vs. organism dry mass.



19b. Hypothetical body burden vs. organism dry mass.

Figure 19. Graphical representation of multiple linear function hypothesis of metal accumulation.

The quadratic model fits a section of a parabola to the data, while the power model fits a curve with a steadily increasing or decreasing slope. Both models were rejected because they did not improve the significance of the regression or the amount of variance explained beyond what the linear functions did, and had poorer fit to data like that in Figure 8a, where different instars were clustered. Both models also have theoretical problems. One is that acceptance of either would mean rejecting a linear relationship between metal content and dry mass. Another is that to make biological sense, they need to pass through the origin. This can be accomplished by setting α to zero, but this further reduced fit in some cases. The power model requires that the rate of metal accumulation change with respect to mass, not just with respect to developmental stage, which is allowed by the multiple linear function model. A feature of life history that a single function cannot model is the overlapping of ranges of dry masses by different instars. A good example of this is figure 8c, where the second and third instars cover about the same range of dry masses. Although there is a mean weight at which each ecdysis occurs, there will be a distribution around this mean. The multiple linear function model allows this to be represented by overlapping the ranges of the functions.

Bioaccumulation and Bioconcentration

The regressions expressed by Eqs. 9 and 10 show that dissolved selenium concentration was a highly significant factor affecting selenium content and body burden. Regression showed that adsorbed selenium was not a significant factor, so bioconcentration was the predominant route of selenium assimilation. Coefficients in the maximum bioconcentration factor (MBCF) equations, a measure of the increase in selenium body burden compared to dissolved selenium, were always 3 or more orders of magnitude higher than the coefficients in the maximum bioaccumulation factor (MBAF) equations. Although the importance of dissolved selenium in determining body burdens has been established, the differences in the

MBCF and MBAF equation coefficients show that a little dissolved selenium causes a huge increase in body burden compared to adsorbed selenium. However, assimilation is not the only source of selenium. Three modes of association of selenium to the organism are adsorption to the cuticle, gut-content association, and assimilation into the body. Routes of assimilation are parental transfer, bioaccumulation and bioconcentration. The multiple linear function model was evaluated against these modes and routes.

One prediction of the multiple linear function model which is untested for *C. riparius* is that selenium concentration decreases with each successive developmental stage, so selenium is most concentrated in the eggs. Since the eggs hatched in several days and are generally closed to their environment, it seems more likely that selenium would come from the parent and not from the environment. Selenium has been shown to accumulate in the gonads of fish (Baumann and Gillespie, 1986) and to be transferred from females to their progeny (Gillespie and Baumann, 1986; Woock, et. al., 1987), so survival and condition of fry may be a biomarker of selenium biomagnification in fish (Pyron and Beitinger, 1989). Schultz and Hermanutz (1990) showed that a wet mass body burden of 3.91 μg/g in fathead minnows resulting from transfer caused edema and lordosis, that selenium concentrations in ovaries were similar to those in embryos, and that fish embryos did not accumulate significant amounts of selenium from water. During egg formation, selenium in the parent material for the eggs is probably distributed homogeneously, so the linear relationship for metal content and mass holds, because selenium is added to the egg material in constant proportion to the total mass added. Therefore, it would be expected that if measurements were made on eggs in all stages of formation, regression would yield an equation with a Y intercept not significantly different from zero at a very low α level. This would mean that the body burden of eggs would be constant with respect to mass.

After hatching, the model predicts that total selenium content will increase linearly within each instar as weight increases. Data from this study were used to deduce this part of the model. However, mode of association is not addressed. Smock (1983a) reported that 64% to 88% of the body burden of Co, Cr, Fe, Sb and Sc in organisms which eat sediment was gut-content associated. He analyzed gut content and body separately. Gut tissue was not analyzed. Smock (1983b) attributed negative exponential decreases in body burdens of the divalent cations cobalt, chromium, iron, antimony and scandium to cation adsorption on the surface of the organism and gut-content association. *Stenonema modestum* (Banks) (Ephemeroptera: Heptageniidae) reduced their body burdens of Co, Cr, Sb, Sc, Fe and Mn after being held in clean stream water for 72 hours and allowing their guts to clear. To differentiate between metal adsorption and assimilation, Smock (1983b) recommended comparing metal content of nymphs before and after molts or before and after emergence. Using pre- and post- emergence metal content data for *Stenacron interpunctatum* and results from Smock (1983a), he concluded that 52% of associated Cr was gut-content associated, 33% was surface adsorbed and 15% was assimilated. Like selenium, all of these elements except antimony can have a 2^+ valence state. Adsorption to the chironomid cuticle is also a major route of absorption of DDE. Larval surface area was a highly significant factor which determined the amount of DDE accumulated from water (Derr and Zabik, 1974).

While my evisceration data cannot be used to differentiate between adsorbed and assimilated selenium, they strongly indicate that selenium was homogeneously distributed between the gut and muscle/cuticle portion. Figures 10a, 10b and 10d show that parameter estimates of regressions of selenium content on dry mass for whole and eviscerated fourth instar larvae were similar, and slopes for eviscerated larvae were not consistently higher or lower than those for whole larvae. Had selenium been more concentrated in the muscle/cuticle

portion, the selenium contents of the eviscerated larvae would have shifted to the left off the regression line for the whole larve. However Figure 10 shows that the selenium contents moved down the whole larva regression line.

An explanation that deserves examination is that selenium was concentrated both on the cuticle and in the gut, and that the selenium appeared to be homogeneously distributed because average weight of the eviscerated larvae was roughly half that of the whole larvae, so removing half the mass and half the selenium would make the eviscerated larvae follow the regression for the whole larvae. When the Y intercept (α_c) is greater than zero for a metal content on mass regression, carbon is being assimilated at a greater rate over time than metal. When the Y intercept is zero, assimilation rates are equal, and when the Y intercept is negative, metal is being assimilated faster than carbon. In Figures 10, the trend is for eviscerated Y intercept to be less than whole Y intercept, with similar slopes. Also, sediment Se was much lower than body burden in group 3 cultures (Figure 10), but it was equal to or higher than body burden in group 1 and 2 cultures (Figure 9), so body burden appears to be independent of substrate-adsorbed selenium concentration, which was confirmed by regression analysis (Eqs. 10 and 11). Considering that a left-shift would indicate higher selenium concentration on the cuticle, where a down-shift would indicate high gut selenium, gut-content associated selenium had no detectable effect on total body burden. Eliminating this as a selenium sink makes it unlikely that selenium adsorbed to the cuticle is much more concentrated than assimilated selenium.

Smock (1983a) found that organisms he classified as sediment dependent (those that ate sediment) had higher body burdens of Cr, Fe, Sb and Sc than organisms in other feeding categories. Similarly, Guthrie and Cherry (1976) found that metal concentrations were higher

in sediment-eating organisms than in other bottom trophic level feeding groups. However, this study showed that dissolved selenium concentrations had much more influence on selenium content and body burden than adsorbed selenium. An explanation which reconciles these apparently contradictory results is that the sediment-eating organisms, which are also sediment dwellers, were absorbing most of their metals from interstitial water. Many sites with contaminated sediments receive continuous low-level metal additions to the water, and the metals become adsorbed to the sediment. The elevated metal concentrations in the sediments then establish an equilibrium with the interstitial water and maintain higher dissolved metal concentrations there.

It was suggested by Smock (1983b) that the negative reciprocal relationship between body burden of antimony and mass occurred in that study in part because smaller organisms ingested smaller particles, which are more metal enriched. A larger particle would have a lower surface-to-volume ratio, and thus proportionately less surface for a metal to adsorb to. This would result in ingestion of particles with a lower concentration of metal, and would result in more fixed carbon ingestion and mass gain per unit of metal ingested. Like antimony, dissolved selenium was expected to be present as a negatively charged oxy-anion, selenite. This mechanism did not appear to exist in this study, primarily because dissolved selenium concentration was much more important than substrate-adsorbed selenium concentration in determining the rate of selenium accumulation and selenium content at a specific weight. Most metals are considered to be more bioavailable when dissolved than when adsorbed, and this study confirmed this rule for selenium and *C. riparius* larvae, and it is concluded that bioconcentration was the primary, if not virtually exclusive, route of selenium uptake, and assimilation was the predominant mode of association.

A factor which may have had great importance in eliminating other modes of association and bioaccumulation was the looseness of the substrate and continuous aeration of the water. Under oxidizing conditions, selenate and selenite were the predominant forms in Hyco Reservoir sediments, and solubility was maximized. At pH 7.5, selenium was found to be more soluble (Masscheleyn, et. al., 1991). Although sediment pH and redox were not measured, water pH remained near 8.3, and the aquaria were continuously aerated and the substrate remained loose, so it is reasonable to assume that dissolved selenium existed as selenite and selenate, and that solubility was maximized. However, adsorbed selenium concentrations were often 3 to 4 orders of magnitude higher than dissolved concentrations (Table 9), so even under these conditions, selenium had a high affinity for the substrate. Smock (1983a, 1983b) did not report sediment pH and redox, but it would be expected that sediments in the Haw and New Hope rivers in central North Carolina, where his study was performed, would be less oxidized than the substrate used in this study, and that pH and alkalinity would be lower. The solubilities of the metals he studied were not addressed, so the possibility that surface adsorption and bioaccumulation may be more significant for selenium under more reducing conditions and at lower pH remains open.

Since elevated selenium in aquatic ecosystems is a recent occurrence, it is unlikely that any aquatic organisms have had to cope with them for any evolutionarily significant time. A selenium regulation mechanism, if it exists, may be focused on retaining selenium ions instead of trying to excrete them. Because selenium is usually far more concentrated in sediment than in water, the gut will most likely be exposed to excess selenium, and will be the system most in need of a selenium regulation mechanism. Because low concentrations of dissolved selenium have a much greater effect on larval selenium content than higher adsorbed concentrations, it appears that the gut either can regulate selenium uptake, or absorbs selenium or its

compounds very inefficiently. Inefficient absorption would probably not be selected against because *C. riparius* rarely encounter adsorbed selenium concentrations low enough to cause selenium deficiency.

Acute Toxicity and Acclimation

C. riparius larvae readily acclimated to dissolved selenium when reared in high-selenium water. They did not acclimate when reared in lower-selenium water and high-selenium substrate, although selenium concentrations in the substrates were often between 3 and 4 orders of magnitude higher than dissolved selenium concentrations. Larvae in the group 3 cultures had much higher body burdens than larvae in other cultures, which clearly indicated that selenium entered the larvae from the water. A similar decrease in tolerance to copper by rainbow trout after a pre-test low level exposure was demonstrated by Dixon and Sprague (1981). This phenomenon was attributed to an increase in body burden that did not induce the detoxification system. The difference in acclimation between the group 3 and other larvae indicates that the selenium associated with the non-group 3 larvae did not induce acclimation. During the toxicity tests, mortality occurred because their body burdens increased to a lethal body burden. The question that arises is, does the median lethal body burden (LB_{50}) change as a result of long-term exposure to selenium? The fact that group 3 larvae acclimated and had much higher body burdens than the other larvae means that LB_{50} increased. This also introduces the idea of a detoxifying body burden. This is a body burden that triggers the detoxification system, causing acclimation via an improved ability to regulate the body burden.

Quite by accident, the anomalous results from test 4, shown in Figure 14, show a situation that might be explained by the detoxifying body burden hypothesis. There is an initial increase in mortality as concentration increases, followed by a decrease, finally followed

by an increase. All larve in this culture were expected to have elevated body burdens, although they were not measured. According to the detoxifying body burden hypothesis, the larvae in the lower concentrations had sub-detoxifying body burdens, and demonstrated the expected dose-response. The decline in mortality seen in the 51 and 76 ppm Se solutions suggests that the exposure was high enough to result in a detoxifying body burden, which enabled the larvae to survive the higher selenium exposure. The second increase in mortality beginning in the 114 ppm Se solution resulted from a body burden which was higher than the detoxification enzymes could cope with. Larvae in this test were from culture, 1-8, which was an early selenium-spiked culture, which had experienced brief slugs of 4.0 ppm dissolved selenium. The culture died out after several more spikings. Unfortunately, selenium contents of larve from this culture were not measured, so the detoxifying body burden was not quantified. Even if it were, it should not be regarded as a constant, since Se sensitivity varied among control cultures. A ready objection to this explanation is that if there were such a thing as a detoxifying body burden and it affected mortality in toxicity tests, we would expect more toxicity tests to show dose-response curves like the one for test 4. This should be especially true for other tests in this study performed on larvae from selenium-contaminated cultures. There is no easy answer to this objection. The best approach is to leave the possibility open and do further research.

Table 10 shows that 48-hour LC_{50}'s for uncontaminated cultures varied from 37.5 ppm to 9.6 ppm. This range is large enough to indicate that the sensitivity of *C. riparius* to selenium changes for unknown reasons. Ninety-six-hour LC_{50}'s among control cultures ranged from 20.1 ppm Se in the third range-finding test to 3.5 ppm Se in culture 2-1, tested 1/30/91. The LC_{50} for culture 2-1 was extrapolated beyond the range of data, but the LC_{60} was 5.1 ppm, so the estimate is not wildly unreasonable. In examining the tabulated LC_{50}'s in Table 10, there is

an apparent tendency for larvae from the lower selenium cultures to be more sensitive to selenium than either the controls or larvae from the higher selenium cultures. The exception is group 3 cultures, indicating that these larvae had detoxifying body burdens. The high mortality seen in cultures 1-6 and 1-7 in the 1/19/91 test resulted from the higher selenium concentrations used to test these cultures. In both test series, culture 2-3 represented a lower threshold of acclimation. Selenium contents of larvae from this culture are shown in Figure 8b. Although body burdens in larvae from the higher selenium cultures were higher, body burdens in larvae from culture 2-2 were not clearly lower. This, of course, does not support the hypothesis that larvae from culture 2-3 had detoxifying body burdens. There was an overall increase in sensitivity to selenium from the first to the second test series. In the both tests, 120-hr LC_{50}'s for cultures 2-1 and 2-2 were close, but 24-hr LC_{50}'s were higher in culture 2-1, suggesting that culture 2-1 larvae took more time to reach lethal body burdens. Clearly, acclimation was greatest in culture 3-5 larvae, which had chronically toxic body burdens, which were the highest of any larvae. However, their LC_{50} was much lower than that for culture 1-8. A difference was that 1-8 was exposed to monthly spikes, while culture 3-5 was spiked every few days before the test, as shown in Figure 17. A possibility that arises is that the culture 1-8 larvae attained high body burdens after the spikes, but then reduced them, requiring maximum use of the detoxification system. When the test was performed, it is possible that the body burdens were rather low, but that the detoxification systems were easily induced, while the culture 3-5 larvae already had higher body burdens to deal with.

Acclimation was defined as a change in the estimate of a biological parameter with respect to pre-test exposure toxin concentration. In culture 3-5, there was no mortality after 48 hours, but the adults failed to reproduce. This indicates that it is possible for an organism to decrease its sensitivity to a specific toxic effect. In this study, the biological parameter used

as an indicator of acclimation was death of half of a larval sample, which was estimated with an LC_{50}. Although no quantitative estimates were made of sub-lethal effects, larvae in culture 3-3 acclimated to the lethal effects of selenium without acclimating to some sublethal effects. The physiological basis for this is that the mode of action of a toxin in causing death can be different than the mode of action for causing a change in some other biological parameter, such as growth or reproduction.

Evaluation of National Surface Water Criterion for Selenium

The lowest 96-hour LC_{50} was <0.1 ppm Se, which was for larvae from culture 2-2 tested on 1/30/91. The LC_{90} for these test was 2.2 ppm Se. The second lowest LC_{50} was 3.5 ppm Se, for larvae from culture 2-1 tested on 1/30/91. Although the lowest LC_{50} is below detection limits, judging from other data it is unlikely the LC_{50} was below 20 ppb, the criterion maximum concentration (CMC) for selenium, so it appears to be adequate to protect *C. riparius* from acute selenium toxicity. The highest exposure that a culture survived was the pulsed exposure of 1.1 ppm Se to culture 3-4. The chronic endpoint used here can be criticized because sub-lethal effects to individuals were not considered, and it is unusual for the chronic effect concentration to be higher than the acute effect concentration. In any case, considering the fact that substrate and water selenium concentrations were always above the criterion continuous concentration (CCC) of 5.0 ppb (Table 9), the CCC is also adequate to protect for toxicity.

According to the inverse prediction using equation 9, a selenium body burden of 15 ppm in a 0.15 mg larva would be caused by 11.3 ppb dissolved selenium, which is about twice the CCC. But, using data from culture 2-2 (Fig. 8a), the body burden in 0.15 mg larvae associated with a 2 ppb dissolved selenium and 13 ppm sediment continuous exposure was about 45 ppm.

This apparent discrepancy results from the regression including much higher dissolved selenium concentrations and the regression not being forced through the origin. Because the intercept was greater than zero, body burdens in larvae from cultures with low dissolved selenium concentrations were underestimated. Reproductive failure in bluegills was caused by food containing 15 ppm Se (Coyle, et. al., 1993). While biomagnification factors for selenium have been shown to be less than 1, BMF's may be influenced by the size of the prey, since smaller prey have higher body burdens. This effect may be attenuated by a tendency for BMF's to decrease as food selenium concentration increases (Besser, et. al., 1993). Considering this, these data support Coyle, et. al.,'s (1993) conclusion that the CCC may not be protective enough in all ecosystems. In contrast Adams and Johnson (1981) estimated that not exceeding 52 ppb dissolved selenium would protect most aquatic species. This number was derived from toxicity tests performed on pelagic organisms, and took into account bioconcentration factors, but did not use information on bioaccumulation, and was designed to protect *Hyalella azteca* from chronic effects. Considering the oxidizing conditions in this study, selenium in this study may have been much more mobile, soluble and bioavailable than selenium in most other systems. More research is needed to determine if the selenium criteria should be parameterized to include sediment and water pH and redox potential as factors, as has been done with hardness for other metals. Quantifying the risk based on predator feeding habits and mean or median prey size is beyond the scope of this study, and would produce a result which should not be used for decision making. These data also indicate that water quality standards alone may not be protective. In this study, as in most cases of selenium contamination, selenium enters the water and then adsorbs to the sediments or substrate. Safe dissolved selenium concentrations, then, may depend on substrate concentrations, and criterion development should focus on setting water and substrate levels which are jointly safe.

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Academy of Science second place for presentation of research (high school).

Master of Science in Ecology, Biology Department, December, 1995.
Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Assistantships

- Sampled and keyed benthic macroinvertebrates in a receiving creek for Allied Chemical Company.
- Monitored water chemistry and analyzed metal and anion concentrations in artifical wetlands used to treat acid mine drainage.
- Taught freshwater ecology laboratory one semester.
- Taught freshman biology laboratories three semesters.
- Taught parasitology laboratory one semester.
- Taught human anatomy and physiology laboratory one semester.

Services

Zoology seminar coordinating committee.
Departmental computer committee.
Graduate student PC consultant.

EMPLOYMENT

Aquatic Toxicologist. (South Carolina Department of Health and Environmental Control, May, 1991 to present) Conducted toxicity tests, experimented with new test methods, researched and implemented use of non-linear regression to analyze toxicity data, developed NPDES permit language, reviewed TRE plans and reports, reviewed site-specific permit limit study plans, participated in 1993 Toxicity Control Strategy writing committee, developed toxicity data tracking system in Foxpro 2.6, provided technical support on use of PCS to track toxicity data. EQC and WPC employee of the month, February, 1992.

Loggerhead turtle conservation program. (Kiawah Island Company, South Carolina, May, 1985 to September, 1985) Checked beach nightly for loggerhead turtle emergences and conducted nest conservation according to guidelines.

Crowder's Mountain State Park. (King's Mountain, North Carolina, June, 1987 to November, 1987) Constructed natural resource exhibits and helped with education programs. Supervised community service workers and planned and supervised implementation of grounds improvement projects.

GRANTS

$301.00 from the Graduate Research Development Project, The Graduate School, Virginia Polytechnic Institute and State University. Matched by biology department.

PROFESSIONAL ORGANIZATIONS

Society of Environmental Toxicology and Chemistry.

SEMINARS

The Use of *Chironomus riparius* as a Toxicity Test Organism. 1990. Zoology Seminar Laboratory, Biology Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

The Use of *Chironomus riparius* (Diptera : Chironomidae) in Benthic Toxicity Tests and Its Response to Selenium. 1995. Master of Science Thesis Defense Seminar, Biology Department, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

PRESENTATIONS

Beaty, Jr., T. V. 1981. Development of an Efficient Method of Producing Ethanol from the Jerusalem Artichoke, or Girasole (*Helianthus tuberosus*). South Carolina Junior Academy of Science Annual Meeting, Columbia, South Carolina.

Beaty, Jr., T. V. and A. C. Hendricks. 1990. The Use of *Chironomus riparius* in Benthic Toxicity Tests. Society of Environmental Toxicology and Chemistry 11th Annual Meeting, Arlington, Virginia.

Beaty, Jr., T. V. and A. C. Hendricks. 1991. Accumulation of Selenium by Sediments and *Chironomus riparius* (Diptera : Chironomidae). North American Benthological Society 39th Annual Meeting, Blacksburg, Virginia.

Beaty, Jr., T. V. 1992. The Use of 0% and 100% Kill Data Points as Censored Data Points in Probit Analysis. Society of Environmental Toxicology and Chemistry 13th Annual Meeting, Cincinnati, Ohio. Reprinted in Carolinas SETAC Vignette. 1993. 3(1):4-11.

Beaty, Jr., T. V. 1994. Optimally Designed Toxicity Tests: One Replicate per Dilution. Society of Environmental Toxicology and Chemistry 15th Annual Meeting, Denver, Colorado.

TECHNICAL REPORTS

Beaty, V. 1991. Guidelines for Conducting Toxicity Reduction Evaluations (TRE's) in South Carolina. South Carolina Department of Health and Environmental Control. Columbia, SC.

Beaty, V. 1994. Standard Operating Procedures for Toxicity Testing. South Carolina Department of Health and Environmental Control. Columbia, SC.