THE UNIVERSITY OF CALGARY

The Ecotoxicology of Cadmium on a Freshwater Leech

by

Daniel Daly Wicklum

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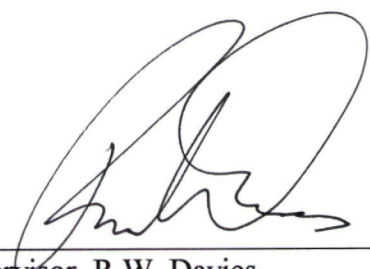
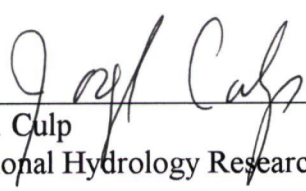
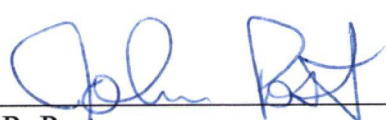
The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Ecotoxicology of Cadmium on a Freshwater Leech" submitted by Daniel Daly Wicklum in partial fulfillment of the requirements for the degree of Master of Science.



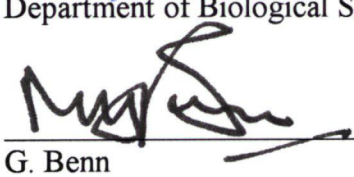
Supervisor, R.W. Davies
Department of Biological Sciences



J.M. Culp
National Hydrology Research Institute



J.R. Post
Department of Biological Sciences



G. Benn
Department of Chemistry

12 Dec. '94
Date

ABSTRACT

Acute toxicity tests showed that the cocoon was the most resistant developmental stage of Nephelopsis obscura when exposed to Cd stress. Survivorship of hatchlings maintained in clean water, but hatched from Cd exposed cocoons, decreased with increasing concentration. Biomass had a significant positive effect on survivorship to acute Cd toxicity.

Chronic bioenergetic tests showed no significant differences in survivorship, energy acquisition or allocation among the control (0), low (5) and medium (10 μg $Cd \cdot L^{-1}$) treatments. The high treatment (50 μg $Cd \cdot L^{-1}$) showed significant decreases in biomass, survivorship, ingestion and activity, and an initial increase in mucus production. No significant differences occurred among treatment or in the control for resting or active respiration, aerobic scope, assimilation efficiency and reproductive investment.

Preference-avoidance studies showed that large leeches (>450 mg) became toxically immobilized at lower Cd concentrations than small leeches (<200 mg).

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CHAPTER 1

GENERAL INTRODUCTION

INTRODUCTION

A primary focus of toxicological studies is to identify the immediate, and predict the long term, biological effects of toxicants. The discipline of aquatic toxicology deals primarily with acute (short-term) exposures to high levels of aquatic xenobiotics using endpoints at the biochemical, cellular or individual levels of biological organization. Although acute toxicity tests have many short-comings (eg. low ecological relevance, low predictive ability of toxicant induced effects at high levels of biological organization) (Geisy and Graney 1989), they are rapid, inexpensive and can be useful for establishing initial benchmark or relative toxic potentials of contaminants. As a result, acute tests are a logical place to begin to understand the effects of toxicants on aquatic ecosystems (Geisy and Graney 1989).

The newer discipline of ecotoxicology uses more ecologically relevant endpoints to predict effects of environmentally realistic concentrations and chronic (long-term) exposures to toxicants at the population and community levels. If an environmental factor illicits a change in energy acquisition or the patterns of energy allocation within an animal such that fitness is reduced from a defined baseline, then the factor is termed a stress (Grime 1989). That environmental factors can contribute to inter-individual differences in the manner in which organisms react to stresses emphasizes the importance of intra-individual processes in ecotoxicology. On a fundamental level it is of importance to understand to what extent processes *within* individuals as well an interactions *among* them, contribute to population changes (Metz and Diekmann 1986).

A model predicting population demographics using a physiological basis for population processes based on the potential relationship between short-term

physiological responses and long-term patterns in species distribution and abundance has been developed (Calow and Sibly 1990). The premise of this model is that physiological processes in animals have certain limits. Outside these limits, metabolism fails and thus distribution and abundance of the species is limited. Within these limits, metabolism is likely to vary and influence growth, reproduction and ultimately abundance.

Scope for Activity (SFA)(Fry 1947), the energy available for aerobic activities, is the difference between an animal's maximum metabolic rate and basal metabolic rate when the basal metabolic rate is that level of metabolism below which the animal cannot survive. SFA can be related to survival (S), such that after the costs of basal metabolism are met, S either increases or is not affected by excess energy (Calow and Sibly 1990). However, quantification of SFA is not practical as determination of basal metabolic rate, which excludes costs of growth, is often difficult. In practice, aerobic scope (AS) is often used as a surrogate of SFA, where AS is the difference between active respiration (Ra), the metabolic costs incurred by an animals while active, and resting respiration (Rm), the metabolic costs incurred by an animals while resting (which includes costs of maintenance, repair and growth). Initial attempts to establish the relationship between AS and S include the quantification of the preference-avoidance behavior of an organism to a toxicant. If an animal is able to detect, and has energy available to actively avoid a xenobiotic, then AS would have a positive effect on S.

Scope for Growth (SFG)(Warren and Davis 1967), the difference between absorbed energy and total metabolic costs, is the energy available for production and can, in theory, be related to the time to and between reproductive bouts (t) and reproductive effort (n). In the absence of reproductive effort, energy is allocated to somatic tissue at a definable rate, which can then be related to time to reproduction, and in the absence of somatic growth in mature animals, SFG can be related to reproductive effort. In theory, given the values of n, t, and S, computed from AS and SFG, predictions about changes

in population dynamics can be made. A preliminary step in this process of linking physiological processes to population dynamics is quantifying stress induced changes in energy acquisition and allocation patterns in test organisms.

Animals are often able to neutralize the effects of a stress by changing acquisition or allocation patterns of energy such that stress induced alterations in the animal's fitness are decreased or negated (Davenport 1985). This phenomenon is termed compensation. Compensatory or stress resisting mechanisms evolve as a result of selective pressure. However, as anthropogenic perturbations increase, organisms are being exposed to stresses that have not been present in the evolutionary history of the species. The result of this lack of historical selective pressure is that organisms have not evolved direct compensatory mechanisms for the new stresses. A stress resistance syndrome (SRS) has been identified that includes suites of traits that animals have evolved in response to natural stresses (Chapin et al. 1993). Whether or not animals react in a similar manner to stresses for which they have been selected for and stresses for which they have not, is emerging as a major issue in ecotoxicology.

This study is an ecotoxicological investigation of the effects of the heavy metal cadmium (Cd) on the freshwater leech Nephelopsis obscura Verrill (Erpobdellidae).

TEST SPECIES

Nephelopsis obscura was chosen as a test species because it occurs in lentic and lotic habitats, it is exposed to toxicants in both the water column and substrate (via physical contact with water and sediment, and through ingestion of prey items), is well studied (Davies 1991), easily maintained, and shows phenotypic plasticity in response to environmental conditions.

Nephelopsis obscura is a predatory leech and is the most abundant species of erpobdellid in Alberta and often the numerically dominant leech species in lentic

ecosystems (Davies et al. 1978, Davies et al. 1982, Davies 1991). A tactile predater, greater proportion of the diet of small N. obscura (<30 mg) consists of amphipods, copepods and cladocerans while large animals (>180 mg) prey primarily on chironomids and oligochaetes (Davies et al. 1978).

Eggs of N. obscura are deposited in cocoons attached to firm substrates (macrophytes or rocks). In the laboratory adults produced an average of 5.2 cocoons per individual and each cocoon hatchs an average of 2.97 young, giving a mean production of 15.4 young per adult (Davies and Everett 1977). In southern Alberta prairie sloughs, cocoons are laid in two distinct periods, May-June (early summer) and August-September (late summer). The growth of N. obscura is indeterminate therefore environmental conditions dictate how quickly the animals grow and reach reproductive maturity. Although size at reproduction is variable, the minimum size at reproduction is 150 mg (Davies and Everett 1977). Depending on the age at which breeding size is attained, the early summer cohort breeds after either 12 or 15 months, while the late summer cohort breeds after either 12 or 19 months . In the field, animals are primarily semelparous (Davies and Everett 1977), however Baird et al. (1986) showed that N. obscura have the genetic potential for iteroparity.

Nephelopsis obscura has been extensively studied in the laboratory and exhibits considerable phenotypic plasticity in its response to changes in environmental conditions. Respiration rates are plastic, increasing after both exposure to conditions mimicking overwintering stress (low temperatures, low oxygen concentrations) (Davies and Kalarani 1993), and increased ionic concentration (Linton et al. 1982), yet decreasing with increasing concentrations of residual chlorine (Osborne et al. 1980) and short-term hypoxia (Davies and Baird 1988). Growth rates decrease with overwintering stress (Dratnal et al. 1993) and experimentally manipulated dissolved oxygen regimes (10%, 300%, and alternating between 100% and 10% oxygen saturation)(Davies et al. 1992b),

but increase with oxygen super-saturation (alternating between 100% and 300% saturation)(Davies et al. 1992b). Feeding rates (Dratnal and Davies 1990), amount of energy allocated to storage (glycogen, triacylglycerols, total lipids) and rate of energy storage also increase after overwintering stresses (Dratnal et al. 1993).

CADMIUM

Cadmium was chosen as a stress because it a relatively easy toxicant to work with and is ecologically relevant. Both natural and anthropogenic activities result in the liberation of Cd into aquatic environments. Weathering and erosion of Cd-bearing rock can result in natural levels of Cd in freshwater systems from 0 to ~ 4.0 $\mu g\ Cd \cdot L^{-1}$ (Nriagu et al. 1981, Anderson et al. 1986, Hinch and Stephenson 1987, Lum 1987, Smith 1987, Stephenson and Mackie 1988, Alikhan et al. 1990, Allan and Ball 1990, Yan et al. 1990, Campbell and Evans 1991). Aquatic environments with anthropogenically elevated concentrations of Cd, most often resulting from mining activities or the disposal of industrial waste, have been found to contain up to 425 $\mu g\ Cd \cdot L^{-1}$ (Jackson 1978) and 508 $\mu g\ Cd \cdot L^{-1}$ (McFarlane et al. 1979) in areas of chronic discharge, and up to 1,000,000.0 $\mu g\ Cd \cdot L^{-1}$ in acute, spill situations (McKee and Wolf 1963).

Cadmium is a rare, naturally occurring metal in group IIb of the transition series in the Periodic Table of the Elements between zinc (Zn) and mercury (Hg). Two oxidation states are possible: the metallic and Cd 2+ states. Free metallic Cd is rarely found in nature but Cd does occur in abundance (up to 90 $mg \cdot kg^{-1}$) in association with sulphide ores of lead (Pb), copper (Cu), iron (Fe), Zn and Hg (Smith 1984). Although in its metallic form Cd is insoluble in water, many of its salts, including $CdCl_2$ are freely soluble. Table 1.0 lists the physical and chemical properties of Cd and $CdCl_2$.

All commercial production of Cd is coupled with the mining and purification of metallic Zn. Most recent estimates (1990) of global production of Cd metal put production at 21 800 tonnes (Hosken 1991). Canada is the fourth largest producer of metallic Cd in the world with an output in 1991 of 1860 tonnes (Koren 1992).

There are four main commercial uses of Cd: Nickel/Cd batteries, electroplating, pigments and plastic stabilizers. The major use of Cd in Canada is surface electroplating which accounts for (61-77%) of total consumption (Environment Canada 1987).

Cadmium can have adverse effects on aquatic invertebrates even at low concentrations. Helisoma anceps (Fuiji), an aquatic gastropod, exposed to water containing 10, 100, and 500 μg $Cd \cdot L^{-1}$ showed 33% mortality in 100 μg $Cd \cdot L^{-1}$ after 8 d and 50% mortality in 500 μg $Cd \cdot L^{-1}$ after 3 d (Dillaman 1980). Helmelraad et al. (1990) exposed Anodonta cygnea (Lea), a freshwater clam to 50 μg $Cd \cdot L^{-1}$ for 12 weeks and found a disruption in the function of organelles involved in the energy metabolism of resorptive kidney cells. In general, Cd is acutely toxic to freshwater invertebrates at concentrations ranging from 3.6 μg $Cd \cdot L^{-1}$ for Daphnia spp. (48 h EC_{50} using swimming behavior as an endpoint), to 450,000.0 μg $Cd \cdot L^{-1}$ for the caddisfly Agapetus fuscipes Curtis (24 h LC_{50})(Baird et al. 1991, McCahon et al. 1989). Adverse effects in freshwater invertebrates resulting from chronic Cd exposure have been observed from 0.17 μg $Cd \cdot L^{-1}$ (significant reproductive impairment in Daphnia magna Straus during 21 d test), to 840 μg $Cd \cdot L^{-1}$ (reproduction significantly reduced in the midge Polypedium spp. during 35 d test)(Biesinger and Christensen 1972, Hatakeyama 1987).

The only Cd toxicity tests using leeches species have been acute tests. Acute toxicity of Cd to leech species ranges from a Cd 96h LC_{50} of 480 μg $Cd \cdot L^{-1}$ for Glossiphonia complanata (L.) to a Cd 96h LC_{50} of 1000 μg $Cd \cdot L^{-1}$ for Erpobdella octoculata (L.) (Brown and Pascoe 1988).

Table 1.0. Physical and chemical properties of Cd and its most common salt $CdCl_2$ (from Aylett 1979, Smith 1984).

Property	Cd	$CdCl_2$
Density ($g \cdot cm^{-3}$ at 20 °C)	8.65	4.05
Melting Point (°C)	321	568
Boiling Point (°C)	765	960
Atomic Number	48	
Molecular Weight	112.4	183.32
Chemical Abstracts Service (CAS) Number	7440-43-9	10108-64-2
Physical State	solid	solid

GENERAL OBJECTIVES

1) Identify the relative sensitivities of different developmental stages of Nephelopsis obscura to Cd using acute toxicity tests (LC_{50} and EC_{50}), quantify post-exposure mortality and investigate the effect of body size of Nephelopsis obscura on acute Cd toxicity.

2) Quantify changes in patterns of energy acquisition and energy allocation in Nephelopsis obscura exposed to chronic, low levels of Cd.

3) Determine if there is a preference avoidance response by Nephelopsis obscura exposed to Cd.

This ecotoxicological research is a comprehensive analysis of the effects of Cd on N. obscura. It will provide data that will enable a Cd risk assessment to be performed on N. obscura for any freshwater system.

CHAPTER 2

ACUTE TOXICITY

INTRODUCTION

Acute aquatic toxicological bioassays were originally developed to provide relatively quick and inexpensive ways to assess the toxicity of waterborne compounds. The 96 h Median Lethal Concentration (96 h LC_{50}), the concentration of contaminant resulting in 50% mortality of test animals after 96 h of exposure, and the 96 h Median Effective Concentration (96 h EC_{50}), the concentration of contaminant which elicits a predefined response in 50% of tests animals after 96 h of exposure, have emerged as the standard toxicity tests in both research and regulatory contexts (Buikema et al. 1982). Acute toxicity tests are useful because they establish relative toxic potential and can be compared to, or calibrated with, observations in natural ecosystems (Geisy and Graney 1989). However, in order to fully understand the toxic effects of a contaminant on an organism, and for an estimate of acute toxicity to be more ecologically relevant, the relative sensitivities of different developmental stages of the test species, post-exposure mortality, and the effect of body size on toxicity should be investigated.

The embryonic, larval and early juvenile stages of fish are generally more susceptible to toxicants than later stages (McKim 1977, Middaugh and Dean 1977), and the data available for invertebrates suggests that in general, crustaceans (Hubuschman 1967, McLeese 1976, Green et al. 1986, Collyard et al. 1994), molluscs (Rehwoldt et al. 1973, Harrison et al. 1984) and insects (Sanders and Cope 1968, Clubb et al. 1975) exhibit higher susceptibility to toxicants at earlier lifestages. Previous studies have not identified the most sensitive developmental stage of any annelid species to a toxicant.

High mortality of organisms used in acute toxicity after the test is completed is a well documented phenomenon (Rand and Petrocelli 1985). There is a paucity of data however,

regarding mortality rates of animals hatched from eggs or cocoons exposed to toxicants. Beattie and Pascoe (1979) monitored mortality of rainbow trout, Oncorhynchus mykiss (Walbaum), alevins for 220 h after hatching from eggs that were exposed to up to 50.5 mg•Cd^{-1}, and reported that pretreatment of trout with Cd while in the egg stage increased survivorship when subsequently exposed to Cd. No studies quantify mortality of animals hatched from toxicant exposed eggs or cocoons.

When developing standardized toxicity tests, confounding variables must be controlled so inter-laboratory results can be compared. There is often a trend of decreased sensitivity to toxicants with increasing biomass in many animals (Green et al. 1986, Ringwood 1990, Collyard et al. 1994). Quantifying the relationship between body size and toxicity for any test species is important to determine if size is a potential confounding variable in toxicity tests.

The purpose of these experiments was to identify the most sensitive developmental stage of N. obscura exposed to Cd, quantify survivorship of animals hatched from Cd exposed cocoons and investigate the effect of biomass of N. obscura on acute Cd toxicity.

METHODS

COLLECTION SITE DESCRIPTION

Stephenson's Pond is a small (2.2ha), shallow (<2.5m) hypereutrophic prairie slough located 4.5 km west of Calgary, Alberta, Canada (114° 16' W, 51° 9' N) in the knob and kettle topography of the prairie-foothill transition zone (Legget 1961). The littoral zone is mostly soft mud and sand with some large rocks and gravel for substrate at the southern end of the pond. Potamogeton richardsonii (Benn.) and Myriophyllum exalbescnes (Fern.) are the most abundant macrophytes in summer. A hill on the western shore is covered with Salix spp. extending to the waterline and the remaining three shores are vegetated

with open grazed grassland. Stephenson's Pond has been described in detail by Davies and Reynoldson (1976) and Davies et al. (1987).

Hatching Success of Cocoons Exposed to Cd and Survivorship of Hatchlings

Cocoons, containing developing leech embryos, were collected from the field in June 1993, and examined under ×20 magnification to determine the number and developmental state of the embryos. The potential number of hatchlings were quantified and ten cocoons containing embryos of the same developmental state were placed into each test chamber of six geometrically increasing concentrations of Cd and a control. Treatments were performed in triplicate for N = 3. Cadmium solutions were prepared by diluting a stock solution of cadmium chloride crystals ($CdCl_2 \cdot 2.5H_2O$) dissolved in distilled water with control water. Control water was City of Calgary tap-water, declorinated by 48 h aeration with a Cd concentration below the detection limit of 2.0 μg $Cd \cdot L^{-1}$ and taken as 0.0 μg $Cd \cdot L^{-1}$. After 96 h all cocoons were removed from the Cd solutions, rinsed twice in distilled water, and maintained in standard conditions defined as a mixture of pond (20%) and declorinated tap water (80%), in a 12 h light :12 h dark photoperiod regime at 100% oxygen saturation and 20° C. Hatching success was quantified, recorded as number hatching/total potential hatching, and hatchlings maintained in standard conditions with an ad libitum ration of prey (Tubifiex tubifex Müeller or Lumbriculus spp.) for 3 $h \cdot d^{-1}$, three times each week for 12 weeks. Mortality was quantified daily. .

A non-linear, bounded, monotonic response function of the form:

$$\text{Hatching Success} = A + \frac{1 - A}{1 + e\,[\,B - C \times \log(\text{concentration Cd in } \mu g\, L^{-1})]}.$$

where, A = the background response, or rate of dying

B = a location parameter for the curve

C = a slope parameter for the curve

was iteratively fit using the quasi-newton estimation method to hatching success data (Wilkinson 1990).

A 96 h EC_{50}, using a reduction in hatching success by 50% as the endpoint, was calculated by probit analysis following Stephan (1977). The hypothesis that survivorship of hatchlings was not significantly affected by exposure to Cd during embryo development was tested with a Chi-square test for heterogeneity. A posteriori unplanned multiple comparisons were performed using 2×2 contingency tables using an adjusted critical value (Zar 1984). For all analyses P=0.05.

Effect of Body Size on Acute Toxicity

96 h LC_{50}s were obtained for animals of four mean body masses: 3.4 mg (SD ± 0.4, N=180), 77.6 mg (SD ± 8.3, N=180), 147.6 mg (SD ± 12.6, N=180) and 358.4 mg (SD ± 27.04, N=180), following a modified version of the Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout (Environment Canada 1990). Cocoons were collected in the field, hatched animals raised under standard conditions, and static tests conducted under standard conditions using 300 ml of Cd solution in 450 ml plastic containers. Each LC_{50} test included three replicates of five geometrically increasing solutions of Cd and a control, using 20% pond water and 80% declorinated water with 10 animals per replicate.

Water samples were removed from five test containers chosen at random at the beginning and at the end of each 96 h test period and analyzed for Cd concentration using a Perkin-Elmer Atomic Absorption Spectrophotometer (model 3300) fitted with an impact bead. All tested concentrations at the beginning of the test periods were within 10% of

the nominal values. Concentrations were within 13% of the nominal values at the end of the test periods except replicate # 1 and #2 for the highest concentration of Cd (4000.0 μg Cd•L^{-1}) used for the largest animals (358.4 mg, SD ± 27.04) which were 16 and 19% lower than nominal.

LC_{50} values were calculated by probit analysis following Stephan (1977). To provide a descriptive model of LC_{50} as a function of biomass, a least squares linear regression was computed using Systat (Wilkinson 1990).

RESULTS

Hatching Success of Cocoons Exposed to Cd and Survivorship of Hatchlings

The 96h Cd EC_{50} for cocoons was 832.6 μg Cd L^{-1} with 95% CI = 717.9 and 962.2 μg Cd L^{-1}. Table 2.0 presents the values of parameters A, B and C, of the bounded monotonic response function that were obtained when the curve that best fits the raw data was derived by iteration. Graphical representation of this function (Figure 2.0) shows that hatching success decreases with increasing Cd concentration. Apparent >100% hatching success for the control and the lowest Cd concentration is a result of underestimating the number of potential hatchlings.

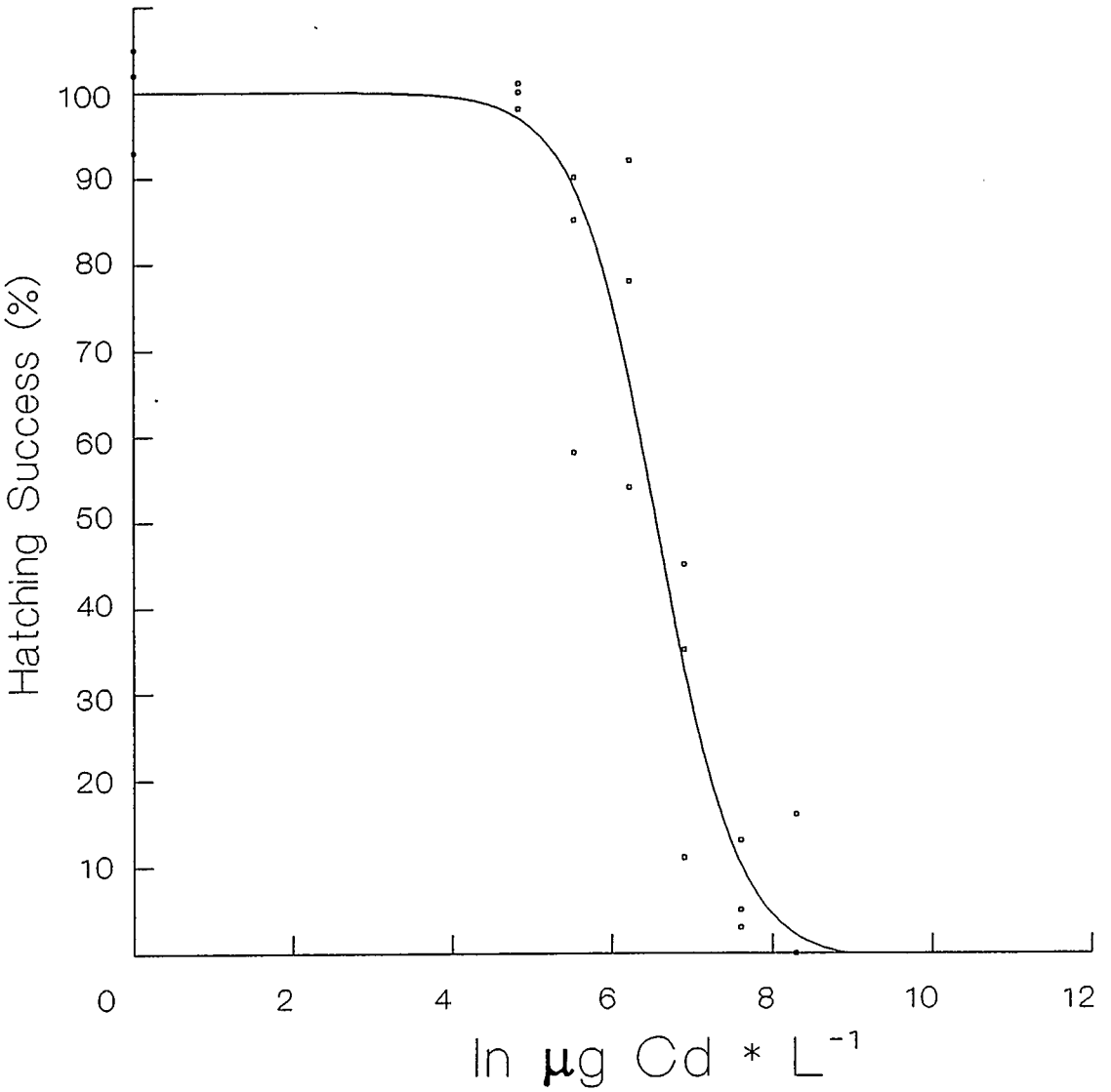
Cadmium had a highly significant effect on post-hatching survivorship (X^2 = 131.9, df=6)(Figure 2.1). All significant post-hatching mortality of animals from Cd exposed cocoons took place in the first week after hatching. A posteriori 2×2 contingency tables performed on ranked survivorships revealed no significant difference in survivorship among the control, 125, 250 and 500 μg Cd L^{-1}, a significant difference between 125 and 1000 μg Cd L^{-1}, and no significant difference among 1000, 2000, and 4000 μg Cd L^{-1}.

Table 2.0. Nonlinear curve-fitting by iterative estimations of the bounded monotonic response function parameters describing hatching success of Nephelopsis obscura as a function of Cd concentration ($R^2 = .911$).

Source	Sum-of-squares	df	Mean-square	F	P
Regression	9.872	3	3.291	205.69	<0.05
Residual	0.293	18	0.016		

Parameter	Estimate	SE
A	-0.009	0.086
B	-13.131	3.438
C	-2.001	0.542

Figure 2.0. Hatching success of Nephelopsis obscura cocoons as a function of Cd concentration. Line generated from response function model in Table 2.0 fit to raw data.


Hatching Success (%)
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
ln µg Cd * L^{-1}

Effect of Biomass on Median Lethal Concentration

A simple linear regression model was found to best represent the relationship between LC_{50} and biomass ($R^2 = 0.97$, F=408.98, df=1) (Figure 2.2). Table 2.1 presents the values of a least square regression analysis fit through the raw data. Resistance to acute Cd toxicity increased with increasing biomass.

DISCUSSION

Hatching Success of Cocoons Exposed to Cd

Leech embryos encased in a cocoon are more resistant to Cd toxicity than the other developmental stages tested. The 96h EC_{50} value for the embryos (832.6 μg Cd L^{-1}) was 3624.7% higher than the 96h LC_{50} value for hatchlings (22.97 μg Cd L^{-1}). Similarly, embryos for other aquatic species exhibit higher resistance to Cd toxicity than other lifestages. The LC_{50} of embryonic Asellus aquaticus (L.), a freshwater isopod, were up to 3302.0 % higher than newly hatched juveniles (LC_{50}s of 1750 μg Cd L^{-1} and 53 μg Cd L^{-1} respectfully) (Green et al. 1986). Eggs of the freshwater snail Biomphalaria glabrata (Say) are more resistant to Cd toxicity than adults (Ravera 1977), and eggs of the estuarine teleosts Fundulus heteroclitus (L.), Menidia menidia (L.), and Atlantic salmon, Salmo salar (L.), are more resistant to acute Cd toxicity than larval stages (Middaugh and Dean 1977, Rombough and Garside 1982).

The high resistance of encapsulated embryonic stages to toxicants may be due to two factors. Firstly, embryo capsules may provide a physical barrier between the Cd and the developing embryos. Beattie and Pascoe (1979) found that when rainbow trout eggs exposed to 22 mg Cd L^{-1} for 22 h were analyzed for Cd content, 98.0% of the Cd was

Figure 2.1. Post-hatching survivorship of Nephelopsis obscura hatched from Cd exposed cocoons as a function of time. Treatments with similar letters are not significantly different ($P<0.05$).

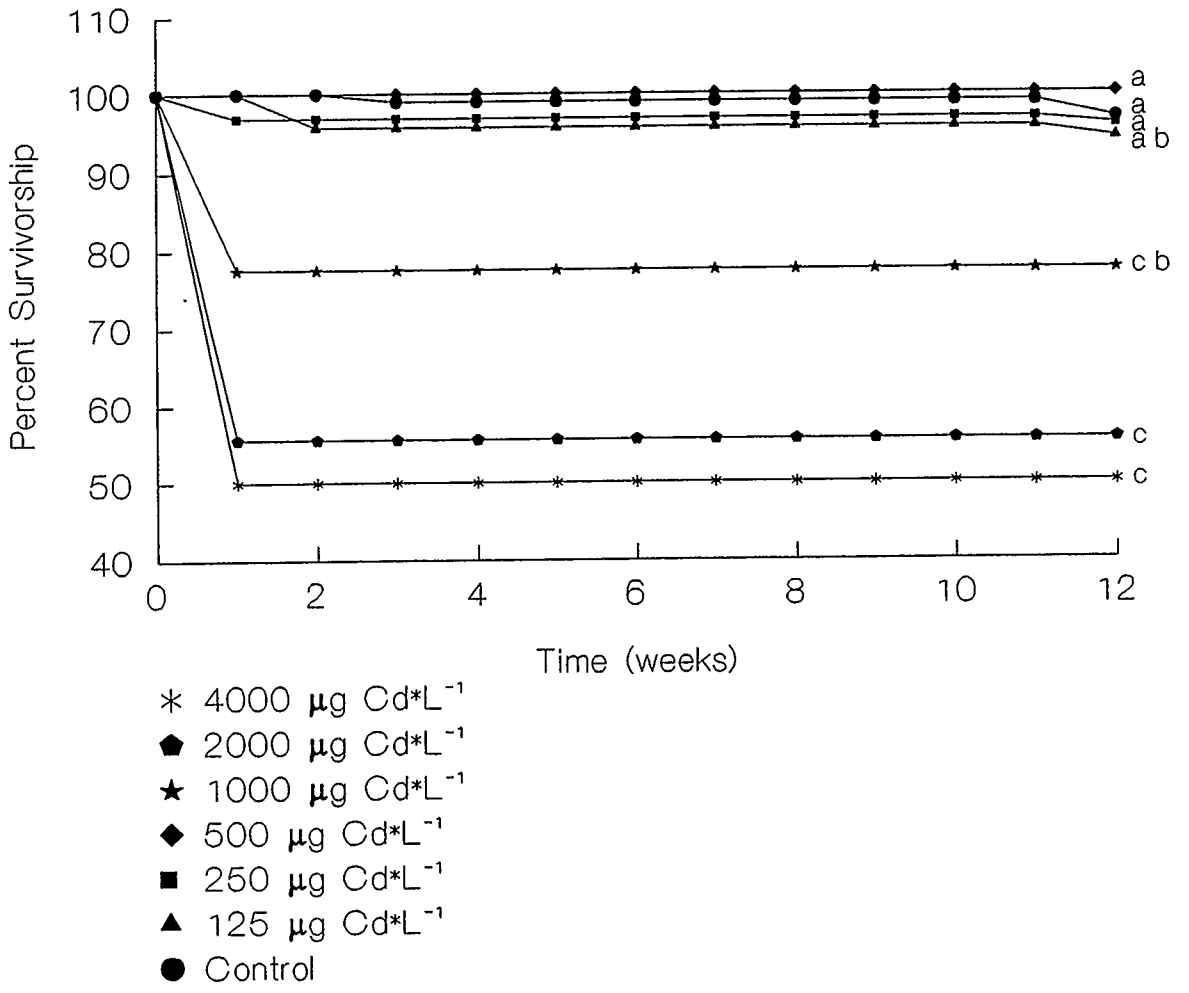

110
100
90
80
70
60
50
40
Percent Survivorship
0
2
4
6
8
10
12
Time (weeks)
a
a
a
a b
c b
c
c
* 4000 µg Cd*L-1
● 2000 µg Cd*L-1
★ 1000 µg Cd*L-1
◆ 500 µg Cd*L-1
■ 250 µg Cd*L-1
▲ 125 µg Cd*L-1
● Control

Figure 2.2. 96 h Cd LC_{50} for Nephelopsis obscura as a function of biomass (WWT). Regression (± 95% C.I.) generated from model in Table 2.1 fitted to raw data .

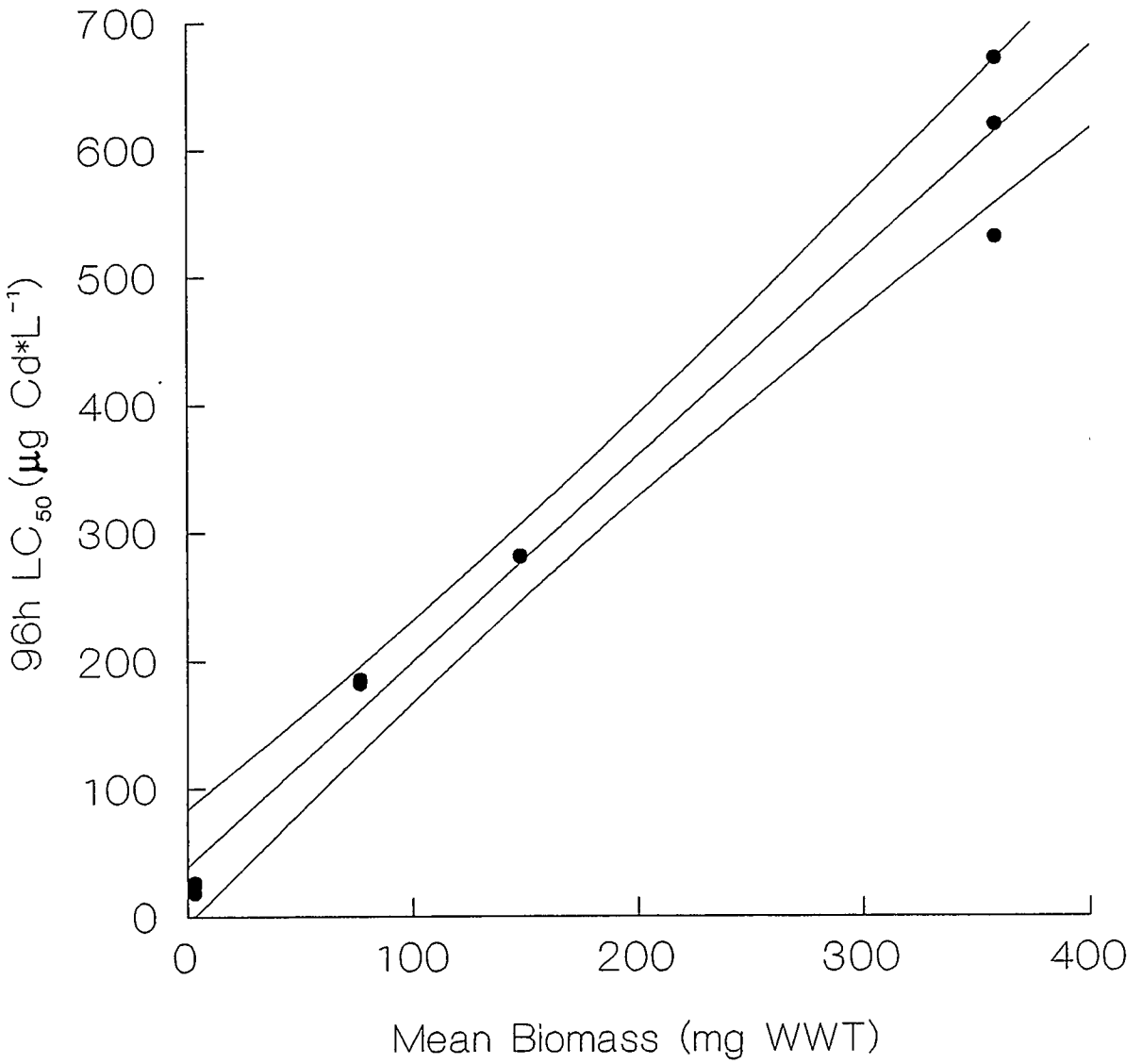

700
600
500
400
300
200
100
0
96h LC_{50} (µg $Cd*L^{-1}$)
0
100
200
300
400
Mean Biomass (mg WWT)

Table 2.1. Regression analysis of Cd LC_{50} of Nephelopsis obscura as a function of body mass ($R^2 = 0.97$).

Source	Sum-of-squares	df	Mean-square	F	P
Regression	542658.263	1	542658.263	408.98	<0.05
Residual	13269.414	10	1326.841		
Variables	**Coefficient**	**SE**	**df**	**t**	**P**
Constant	38.757	15.66	10	2.474	<0.05
Biomass	1.604	0.079	10	20.003	<0.05

associated with the chorion (egg membrane) while only 1.8% with the embryo and 0.2% with the yolk. Rosenthal and Sperling (1974) concluded from the results of their study that herring, Coregonus artedii Lesueur, egg capsules bind Cd, thus protecting the developing embryos. A similar phenomenon may be occurring with the leeches. Although the cocoon protects the embryos from toxicity, Figure 2.0 shows that there is a minimum threshold concentration of Cd ($\approx$ 90 μg Cd L^{-1}) below which the cocoon capsule can prevent Cd related mortality and a maximum threshold ($\approx$ 4915 μg Cd L^{-1}) above which the capacity to bind or adsorb metals is exceeded and embryo mortality is complete.

Secondly, developing leech embryos rely on yolk for energetic sustenance and are incapable of ingesting Cd, therefore any Cd entering the embryo does so through the surface. Ingested Cd has been found to be the dominant mechanism of accumulation for other invertebrate predators including water mites, Limnesia spp., caddisfly larvae Mystacides spp. and crab, Cancer spp. (Davies et al. 1981, Timmermans et al. 1992). Higher embryonic resistance to Cd may be partially related to the absence of ingested Cd.

Significant mortality of hatchlings exposed to Cd as embryos took place in the first post-hatching week (Figure 2.1) Hatchlings that emerged from cocoons exposed to 1000, 2000 and 4000 μg Cd L^{-1} were qualitatively less active and smaller than those from the other treatments, supporting the hypothesis that the protective function of the cocoon capsule is ineffective at high concentrations.

Effect of Biomass on Median Lethal Concentration

A significant positive effect of biomass on resistance to Cd toxicity was found (Figure 2.2). Similar findings have been reported elsewhere, where generally younger or smaller specimens of freshwater snails, Pysa gyrina Say, crustaceans, Asellus aquaticus, chironomids, Chironomus riparius (Meigen) and plecopterans, Acroneuria pacifica Banks, Arcynopteryx signata (Hagen) and Pteronarcella badia (Hagen), were more susceptible to

Cd toxicity (Clubb et al. 1975, Wier and Walter 1976, Green et al. 1986, Williams et al. 1986).

One factor that may contribute to this finding is the large surface area to body weight ratio for small organisms relative to large ones. However, phenomena related to scaling alone would not account for the linear increase in Cd resistance with biomass exhibited by N. obscura, since the surface areas of geometrically congruent objects do not increase directly with volume but as volume$^{2/3}$ (Peters 1983). The relationship between Cd resistance and biomass may be linear for the range of biomasses tested yet deviate from linearity outside this range, or some mechanism of Cd resistance may be present in large animals that is absent in small ones.

The variation in individual LC_{50} replicates was greater in the large animals compared to small animals. This could be due to larger differences in energy storage reserves that are used in stress resisting processes between large animals compared to small.

CHAPTER 3

BIOENERGETICS

INTRODUCTION

Energy is a common currency of ecological systems and is necessary for any biophysical process (Hall et al. 1992). Since energy is usually limited there is often competition for its use within an organism, resulting in physiological tradeoffs (Sibly and Calow 1986). These tradeoffs may be plastic in response to environmental change and developmental stage. For example, under slightly stressful conditions an organism may allocate more energy to stress resisting processes and less to reproduction, hedging that increased survival will maximize fitness over the long-term. However, under extremely stressful conditions, where death in the short term is highly probable, all available resources may be put into reproduction, again maximizing fitness (Tilman 1988). Because organisms must maintain a positive net energy balance in order to grow and reproduce, bioenergetic studies can provide a comprehensive and explicitly testable set of mechanisms for predicting where species will be found and for determining why they are not found in other locations (Hall et al. 1992).

These experiments test the effects of chronic (long-term) exposure for 15 weeks to four concentrations of Cd, 0, 5,10 and 50 $\mu g \cdot Cd^{-1}$, on the bioenergetics of N. obscura. These concentrations of Cd represent a range spanning natural background levels and levels associated with anthropogenic pollution.

The bioenergetic model used is based on that of Sibly and Calow (1986):

$$I - Fe = A = R + G + U \qquad (1)$$

Where I = energy ingested as prey , Fe = the energy content of faeces, A = the energy absorbed across the gut wall, R = energy used in respiration, G = energy allocated to growth, U = the energy value of excretory and secret0ory products.

Because of the technical difficulties in separating leech Fe and mucus (Mu)(Dratnal and Davies 1990), the energy contained in Fe and Mu are measured together. Thus, A, the energy absorbed across the gut wall, cannot be directly measured so assimilated energy (AE), the energy available for growth and metabolism other than Mu production is used in the modified equation (2). Energy lost in excretion by N. obscura is quantifiable, but comprises a minor component (<5.0%) of the total amount of energy acquired (Kalarani and Davies 1994), and, for convenience, is excluded.

Respiration is subdivided into two categories, resting (Rm) and active (Ra). Resting respiration is the metabolic cost incurred by an animal when attempting to maintain its physiologic state and includes costs of maintenance, repair, and growth. Active respiration is the metabolic cost incurred when the animal is active, which, following Davies and Kasserra (1989), is any motion resulting in the displacement of the entire body. Aerobic scope (AS) (Fry, 1947), the amount of oxygen available for aerobic metabolism above maintenance is a measure of the potential energy available for processes such a predator avoidance, foraging and growth, and is calculated as the difference between Ra and Rm.

As a result, the general equation (1) is modified for N. obscura to:

$$I - (Fe + Mu) = AE = (Rm + Ra) + G \qquad (2)$$

This study will quantify the allocation patterns of the variables in equation (2) in N. obscura exposed to 0, 5, 10 and 50 μg $Cd \cdot L^{-1}$ for 15 weeks. To further delineate the

allocation patterns of energy, separate experiments will quantify the relative production of mucus, and reproductive investment among treatments.

Because N. obscura exhibits plasticity in its response to different environments (references in Test Species Chapter 1), it is hypothesized that levels of energy acquisiton and allocation patterns will differ among Cd treatments. The objectives of these experiments are to quantify the effects in N. obscura of chronic exposure to Cd in terms of biomass production, mortality, assimilation efficiency, patterns of energy ingestion, allocation of energy to activity and respiration, aerobic scope, relative mucus production and relative reproductive investment.

METHODS

Collection

Nephelopsis obscura cocoons were collected from Stevenson's Pond in June 1993 and maintained in the laboratory at 20° C, 100% oxygen saturation and a 12 h:12 h light:dark photoperiod regime until they hatched. Hatchlings were reared under standard conditions (Chapter 2) with ad libitum prey until they reached approximately 200 mg.

Treatments

Two containers (each 4 L) per treatment, containing aerated solutions (2 L) of either 0 (control), 5 (low stress), 10 (medium stress), or 50 $\mu g \cdot Cd^{-1}$ (high stress) were prepared following the method presented in Chapter 2. In one container per treatment, termed the main treatment, five randomly selected animals (initial mean size 200 mg, range ± 50 mg) were maintained in standard conditions (Chapter 2) and used for quantifying changes in biomass over time. In the other container for each treatment, termed the stock treatment, 100 animals (mean size 200 mg, range ± 50 mg) were maintained under standard conditions and used for all other bioenergetic variable determinations and for quantifying

survival. This design was used so that biomass determinations could be made on exactly the same animals for each measurement. Since animals were expected to grow at different rates in different treatments and animals are known to exhibit biomass related survival differences when exposed to Cd (Chapter 2), randomly selecting animals from each treatment for biomass determination could give an apparent increase in mean biomass if small animals had a higher probability of death.

The difference in group size between the main (5) and stock (100) treatments was not expected to have a effect on growth as Smith (1994) showed that N. obscura maintained in group sizes of 5 to 100 for 18 weeks showed no differential growth rate after 18 weeks.

Because of the experimental design employed in this study there is the possibility that treatments effects are confounded by differences among tanks not related to Cd (psuedoreplication). Care was taken to minimize confounding effects by strictly controlling temperature, lighting, etc. and by changing water weekly.

All experiments were continued until the five animals in the main control treatment reached an asymptotic biomass.

Growth

Every second week leeches in the main treatments were blotted to remove excess water and weighed to the nearest mg wet weight (WWT), with the difference between two successive weights representing growth.

Survivorship

Survivorship was quantified daily in the stock treatments and dead leeches removed.

Ingestion

The apparent biomass of food ingested per meal was quantified by providing individual leeches, randomly selected from the stock treatments that had been and starved for 48 h, with about 150 mg T. tubifex or Lumbriculus spp. for 3 h and calculating the difference between the initial WWT and final WWT of prey available for consumption by the leech (N=3). To correct for potential errors due to biomass changes of prey during feeding periods, prey were also added to containers without leeches (controls), left for 3 h, and the difference between initial and final WWT of prey calculated. The actual biomass of prey ingested by each leech was calculated by correcting the apparent biomass ingested by the mean weight change of the prey species in the controls.

To convert ingestion into energy equivalents, biomass of ingested prey species was multiplied by energy conversion factors. The conversion factor for T. tubifex was 23.1 $KJ \cdot g^{-1}$ (Qian 1992), and the conversion factor for the Lumbriculus prey species, 20.7 $KJ \cdot g^{-1}$, was determined by bomb calorimetry. Four samples of prey species (approx. 150 mg) were sampled once a month, freeze dried to a constant weight and the mean calorific value (converted to J) determined using a Philipson microbomb calorimeter.

Assimilation Efficiency

After 2 weeks, and approximately every 3 weeks thereafter, leeches were selected from the stock treatments (three animals = one replicate, N = 5), weighed, fed a known biomass of food, and immediately placed in 500 ml of water filtered through a 0.45 µm filter. As the animals selected from each treatment were of similar biomass, the total biomass in each replicate did not differ significantly. After 48 h the animals were removed, and the water filtered with a dry, preweighed 0.45 µm filter. The filter containing Fe plus Mu was oven-dried at 40 C° until constant weight, weighed and the mass of Fe plus Mu produced determined by the difference between initial and final filter

weights. The mass of Fe plus Mu produced per mg food ingested was calculated and converted to an energetic equivalent using the conversion factor 21.0 J•mg $^{-1}$ (Qian 1992). Assimilation efficiency was calculated from:

$$AE = \frac{I - (Fe + Mu)}{I} \times 100$$

Where:

AE	=	Assimilation Efficiency
I	=	Ingested Energy
Fe	=	Energy contained in Faeces
Mu	=	Energy contained in Mucus

Respiration

After 2, 6, 10 and 13 weeks, respiratory costs were measured (N=5)directly using a computerized flow through respirometer (Davies et al. 1992a). All respiratory measurements were taken 48 h after feeding to avoid specific dynamic action (Kalarani and Davies 1994). Metabolic costs associated with growth, storage and repair were measured together as resting metabolism (Rm), respiratory costs incurred while active (Ra), and aerobic scope (AS), the difference between Ra and Rm, were all expressed in $\mu L\ O_2 \cdot h^{-1}$.

An additional experiment was conducted to remove the possible confounding effect of biomass on respiration by monitoring the respiratory responses (Rm, Ra and AS) of N. obscura exposed to Cd for approximately 3 weeks, during which time there was no significant growth differential between treatments.

Over-wintered animals were collected from Stevenson's Pond in May 1994 from the undersurfaces of rocks and acclimated to standard conditions (Chapter 2). Leeches of similar biomass (500 ± 85 mg) were randomly allocated (N = 5) to containers containing 0, 5, 10, 20, 40, 80, 160, 320 640 and 1240 $\mu g\ Cd \cdot L^{-1}$, and maintained under exactly the same conditions as in Chapter 3. Mortality and respiratory measurements of Rm and Ra were taken after 3, 11 and 18 d of exposure with AS calculated for each time period.

Mucus

After 1, 6, 9 and 11 weeks, a colorimetric technique was used to quantify the relative production of mucus by N. obscura. Five replicates, each consisting of three animals starved for 48 h to clear the gut of faeces, were rinsed in distilled water to remove clinging particles of mucus, and placed in 500 ml of treatment water. After 24 h the animals were removed and the water sonicated to evenly disperse the mucus. The colorimetric technique of Mantle and Allan (1978) was followed which involves the oxidation of polysaccharides using periodic acid and the binding of colored metabisulphite Schiff solution molecules to glycoproteins whose absorbance is then read at 555 nm.

Activity

After 2 weeks and approximately every 3 weeks thereafter, animals from the stock treatment (N=5), were starved for 48 h, placed in individual 100 cm^2 arenas containing the treatment concentrations of Cd, and acclimated for 1 h. Animals were then videotaped for the first 10 min of every h for a 24 h period. The amount of time the animals spent active, defined as any motion resulting in the total displacement of the body, was quantified and expressed as a percentage of the total time monitored (240 min)$\bullet d^{-1}$.

Reproductive Investment

When mortality in the high Cd stock treatment reached 50%, animals of similar biomass (550 mg WWT ± 70 mg, N = 5) were removed from each stock treatment and the surface area of reproductive tissue on a mid-longitudinal section quantified. Leeches were fixed in Bouin's fluid, histologically prepared following McManus and Mowry (1960) and stained in Harris haemotoxylin and eosin. Total reproductive tissue area (testisac area plus ovisac area) was determined under ×30 magnification using a gridded microscope eyepiece.

Statistical Analysis

After 13 weeks, the last week before 0% survivorship in the high Cd stock treatment, the hypothesis that biomass of N. obscura was not significantly effected by Cd was tested using a One-way ANOVA (Zar 1984).

At the completion of the experiment (week 15) the hypothesis that survivorship in the stock treatments was not significantly affected by exposure to Cd was tested with a Chi-square test for heterogeneity. A posteriori unplanned multiple comparisons were performed using individual 2 × 2 contingency tables using an adjusted critical value (Zar 1984).

The hypothesis that patterns of energy ingested, time spent active, energy expended as Rm, Ra and AS were not affected by Cd was tested with ANCOVA using Cd concentration as a main effect and biomass as the covariate (Zar 1984). To meet the assumption of linearily needed for ANCOVA, log transformations were performed where needed. Comparison of regression lines was performed over ranges of similar biomass.

For the supplemental respiration experiment a One-way ANOVA was used to test the hypothesis's that Cd had no effect on Rm, Ra and AS after 3 d of exposure. After 18 d of exposure a Two-way ANOVA was used to test the hypothesis that time, Cd or the interaction of these factors had no effect on Rm, Ra and AS. Empty cells, resulting from mortality were not included in the analysis after 18 d. Missing datum points resulting from mortality of an individual in a treatment were filled following Zar (1984).

The hypothesis that AE and Mu production of N. obscura were not significantly effected by Cd or time or their interaction was tested using a Two-way ANOVA.

A One-way ANOVA was used to test the hypothesis that Cd had no effect on reproductive energy as measured by total area of reproductive tissue.

A posteriori comparisons after an ANCOVA were made using multiple slope comparisons, and after an ANOVA using Tukey's tests (Zar 1984). All bioenergetic

statistical results were deemed either not significant (NS), significant ($P<0.05$) or highly significant ($P<0.01$). All statistical analyses were performed with Systat (Wilkinson 1990).

RESULTS

Growth

Cadmium had a highly significant effect on biomass production of N. obscura ($F=10.59$, df=3)(Figure 3.0). Animals in the high Cd water had highly significantly lower biomass than any other treatment after 13 weeks, while there was no significant difference among other treatments. Control, low and medium Cd stressed animals exhibited a sigmoidal growth curve, reaching an asymptotic biomass at 13 weeks, with no significant increase in biomass thereafter. Control animals reached a mean asymptotic biomass of 1664 mg at week 13 while animals reared in the low, medium and high concentrations of Cd reached 13-week mean asymptotic biomasses of 1379, 1497 and 518 mg respectively.

Survivorship

The effects of Cd on survivorship of N. obscura exposed to Cd was highly significant ($X^2=656.42$, df=3) (Figure 3.1). There was no significant difference in survivorship among the control, the low and the medium Cd treatments while the high Cd treatment was highly significantly different from all other treatments. The control, low and medium Cd treatments had survivorships of 94, 91, and 94% after 15 weeks while the high Cd treatment had 45% survivorship after 13 weeks and 0% after 14 weeks.

Ingestion

Cadmium had a highly significant effect on the pattern of food ingestion exhibited by N. obscura (slope $F=2.9$, df=3; elevation $F=18.47$, df=3)(Figure 3.2). Although the slopes

Figure 3.0. Mean biomass of Nephelopsis obscura exposed to 0, 5, 10 and 50 μg $Cd \cdot L^{-1}$ (mg WWT ± SE) versus time (weeks). Treatments with similar letters are not significantly different ($P<0.05$) at week 13.

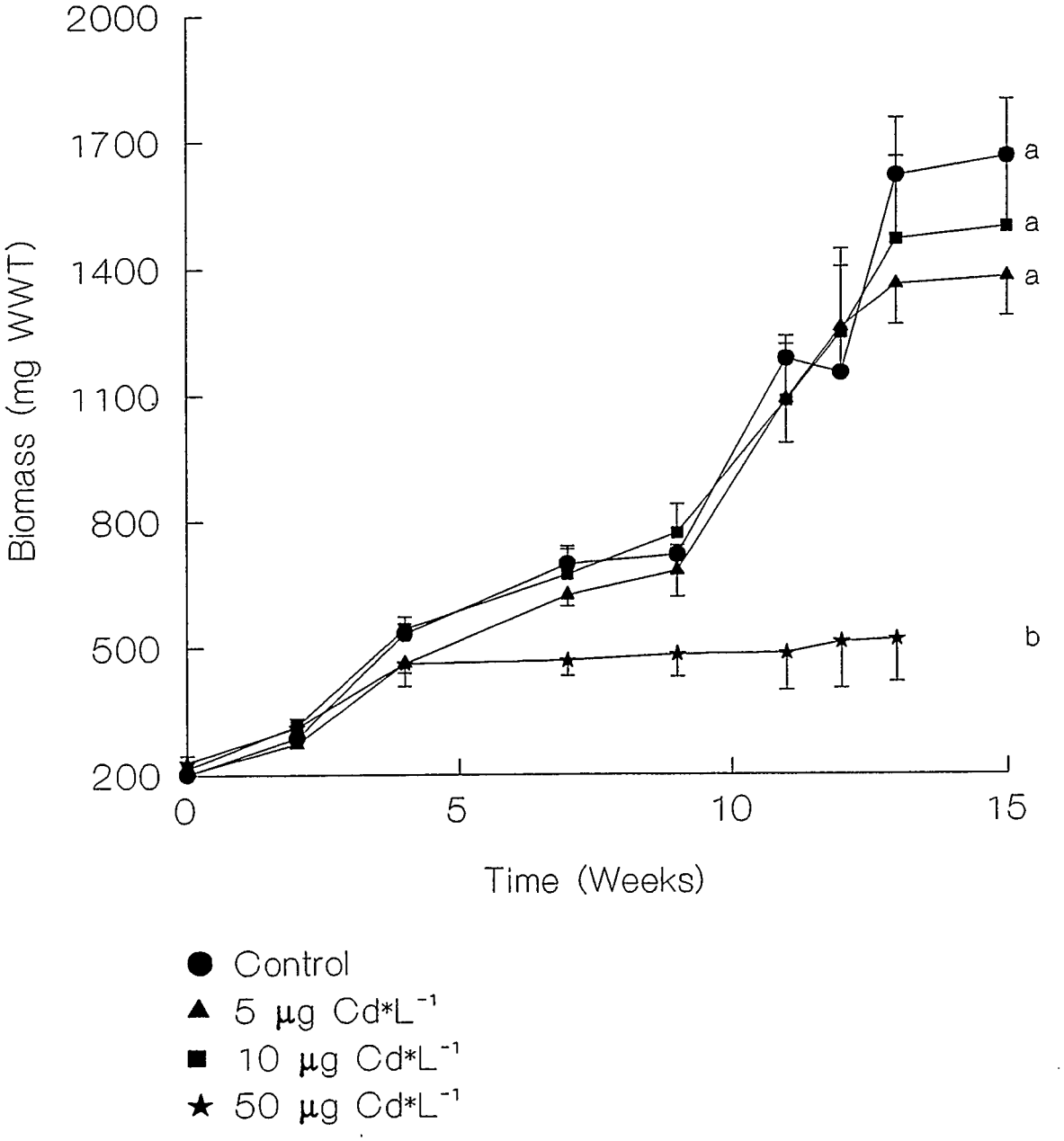

2000
1700
1400
1100
800
500
200
Biomass (mg WWT)
0
5
10
15
Time (Weeks)
a
a
a
b
Control
5 µg Cd*L⁻¹
10 µg Cd*L⁻¹
50 µg Cd*L⁻¹

Figure 3.1. Percent survivorship of Nephelopsis obscura exposed 0, 5, 10 and 50 $\mu g \cdot Cd^{-1}$ versus time (weeks). Treatments with similar letters are not significantly different ($P<0.05$).

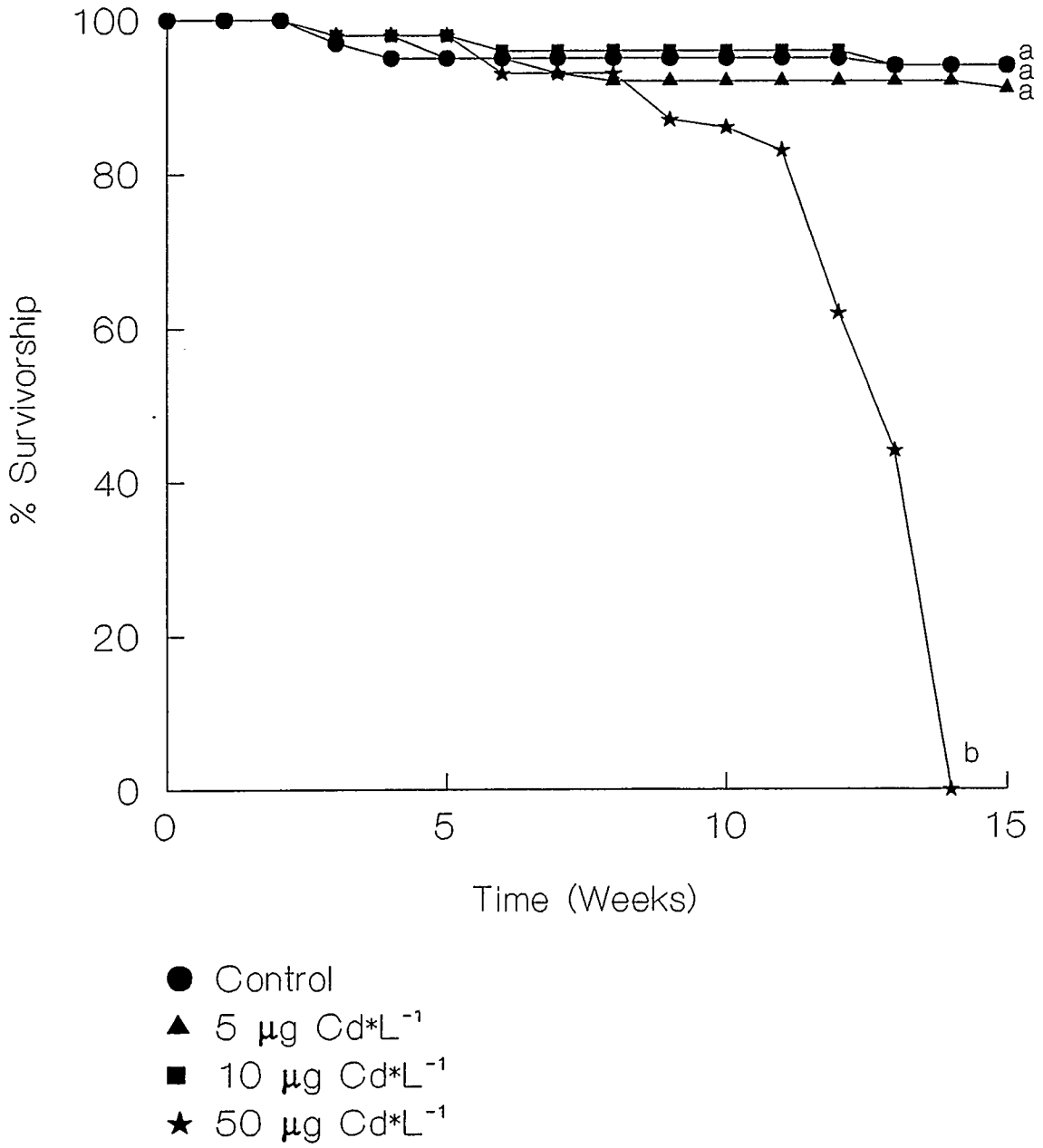
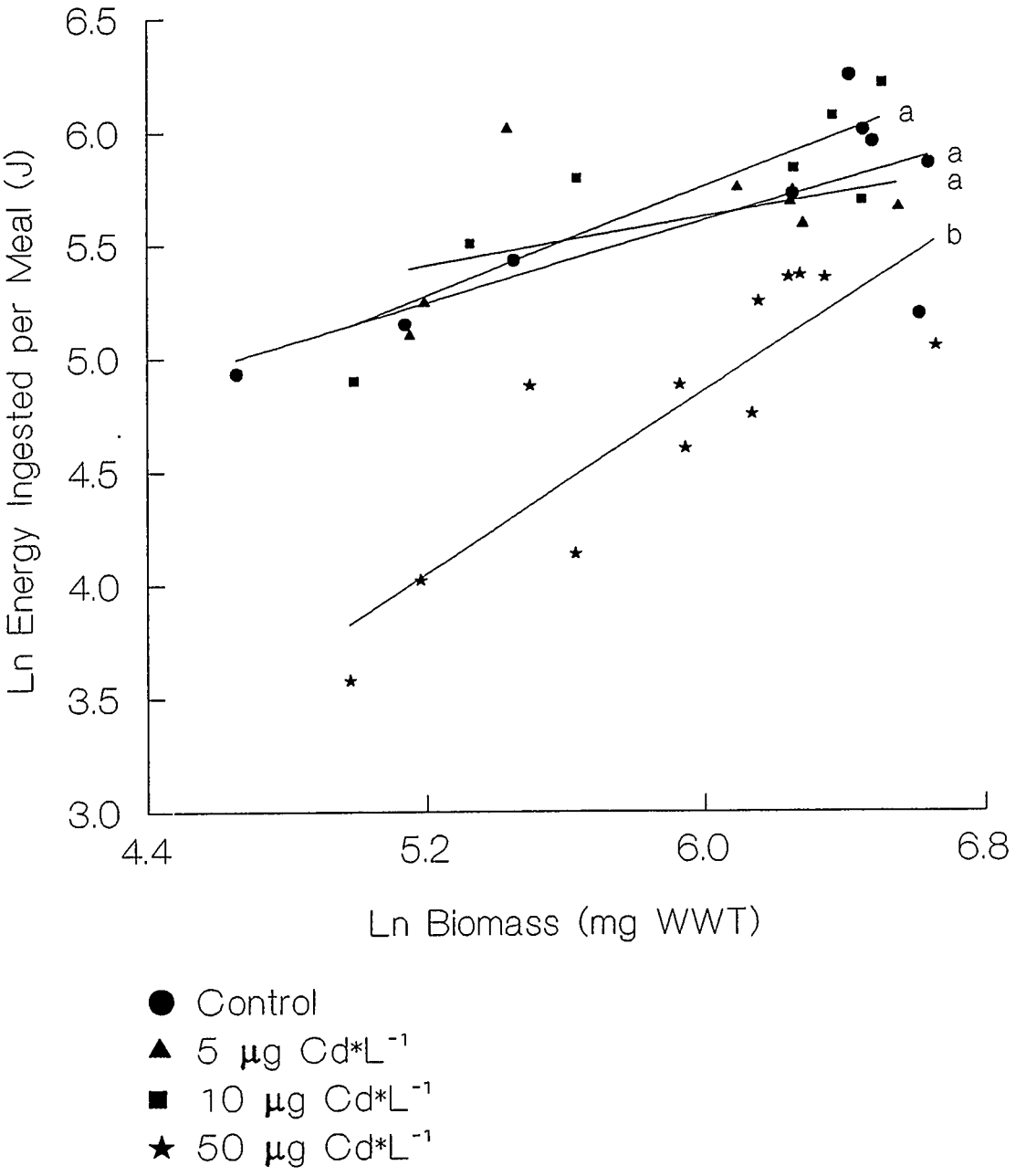

100
80
60
40
20
0
% Survivorship
0
5
10
15
Time (Weeks)
a
a
a
b
Control
5 µg Cd*L⁻¹
10 µg Cd*L⁻¹
50 µg Cd*L⁻¹

Figure 3.2. Linear regressions of ln of total energy ingested per meal (J) versus ln biomass (mg WWT) by Nephelopsis obscura exposed to 0, 5, 10 and 50 $\mu g \cdot Cd^{-1}$. Treatments with similar letters are not significantly different ($P<0.05$).


6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Ln Energy Ingested per Meal (J)
4.4
5.2
6.0
6.8
Ln Biomass (mg WWT)
a
a
a
b
Control
5 µg Cd*L-1
10 µg Cd*L-1
50 µg Cd*L-1

of lines regressed through the log of food ingested per meal were not significantly different among treatments, ingestion was highly significantly depressed in the high Cd treatment with the elevation of the regresssion highly significantly lower than the elevations for the other treatments. There was no significant difference between among elevations for the other treatments.

Assimilation Efficiency

Assimilation efficiency was not affected by Cd (F=0.059, df=3), time (F=0.104, df=1) or the interaction of these two factors (F=0.027, df=3)(Table 3.0). Assimilation efficiency ranged from 81.2% to 89.3%.

Respiration and Supplementary Respiration

Cadmium had no effect on Rm (slope F=0.184, df=3; elevation F=0.19, df=3), Ra (slope F=0.903, df=3, elevation F=0.03, df=3) or AS (slope F=1.118, df=3; elevation F=1.52, df=3)(Figure 3.3). All respiratory costs (Rm, Ra and AS) increased with increasing biomass but size specific respiratory costs decreased with increasing size.

In the supplemental respiration experiment mortality increased with Cd concentration and duration of exposure (Figure 3.4). After 3 d of exposure most treatments had 0% mortality but in 640 μg $Cd \cdot L^{-1}$ there was 20 % mortality. After 11 d there was 100 % mortality in 1280 and 640 μg $Cd \cdot L^{-1}$. After 18 d there was 100 % mortality in 320 μg $Cd \cdot L^{-1}$ but only 40% mortality in 160 μg $Cd \cdot L^{-1}$.

One-way ANOVA showed that Cd had no significant effect on Rm (F=1.435, df=9)(Table 3.1), Ra (F=0.808, df=9) or AS (F=0.571, df=9) after 3 d of exposure. Similarly, Two-way ANOVA showed that after 18 d of exposure, Cd had no effect on Rm (F=1.453, df=6), Ra (F=0.710, df=6) or AS (F=0.658, df=6). The interaction of Cd and time also had no effect on Rm (F=0.685, df=12), Ra (F=1.188, df=12), or AS (F=1.132,

Table 3.0. Mean assimilation efficiency of Nephelopsis obscura exposed to 0, 5, 10 and 50 μg Cd•L^{-1} after 2, 5, 8, and 13 weeks (% of ingested energy assimilated ± SE). NS= No significant difference (P<0.05).

Treatment	Week 2	5	8	13	ANOVA
Control	81.2 ± 4.74	89.3 ± 4.03	86.6 ± 11.2	86.2 ± 5.00	
5 μg Cd • L^{-1}	86.4 ± 3.93	88.6 ± 6.22	81.7 ± 4.38	88.5 ± 5.09	
10 μg Cd • L^{-1}	81.5 ± 4.52	87.0 ± 6.39	88.3 ± 6.66	82.5 ± 7.56	
50 μg Cd • L^{-1}	84.7 ± 6.44	83.0 ± 6.75	82.2 ± 8.86	85.5 ± 5.90	
					NS

Figure 3.3. Linear regressions of ln resting respiration (Rm), active respiration (Ra) and aerobic scope (AS) versus ln of biomass (mg WWT) for Nephelopsis obscura exposed to 0, 5, 10 and 50 μg $Cd \cdot L^{-1}$ (total μL $O_2 \cdot h^{-1}$). All treatments are not significantly different from each other ($P>0.05$).

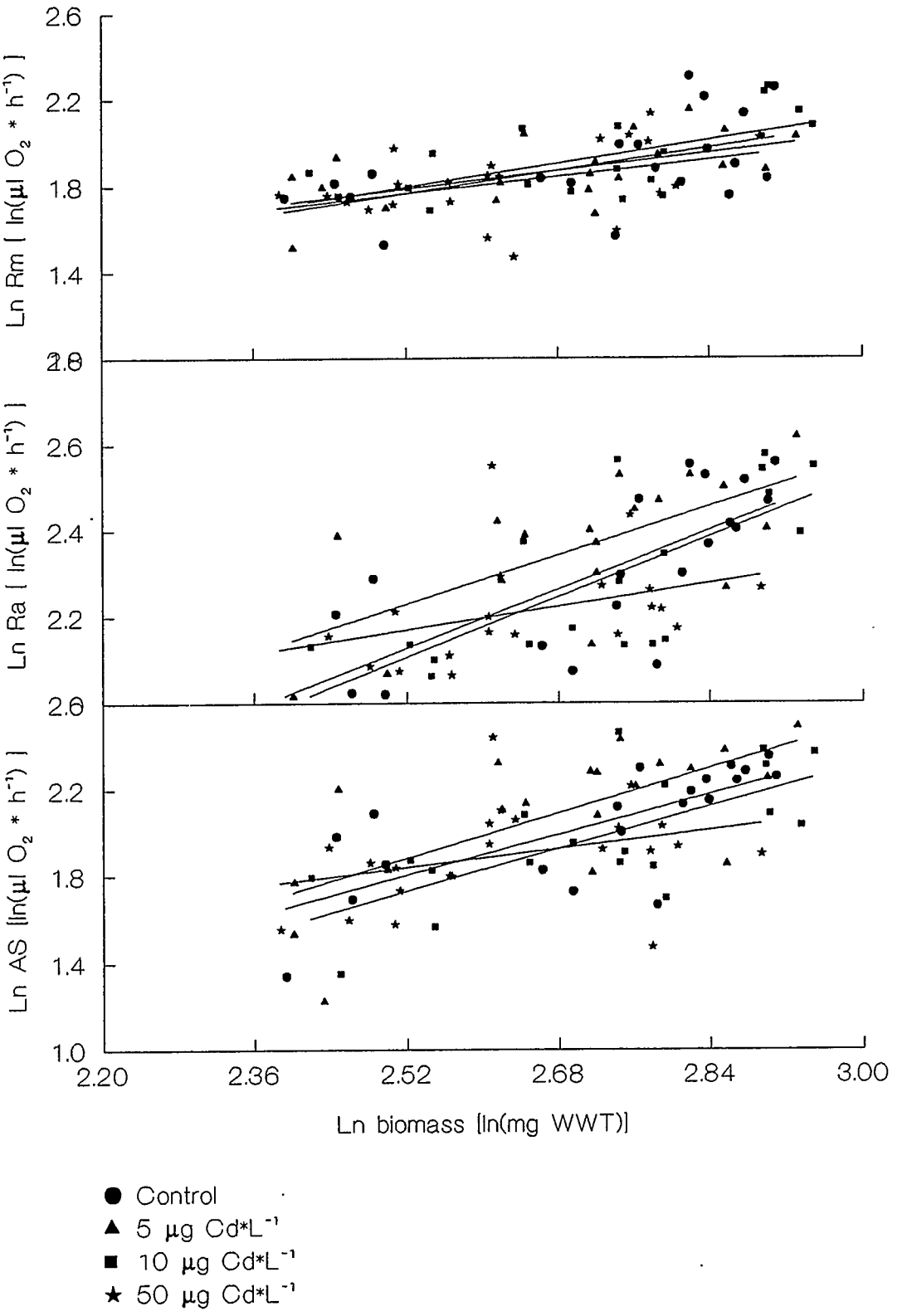

Ln Rm [ln(µl O2 * h-1)]
Ln Ra [ln(µl O2 * h-1)]
Ln AS [ln(µl O2 * h-1)]
2.6
2.2
1.8
1.4
2.8
2.4
2.0
1.0
2.20
2.36
2.52
2.68
2.84
3.00
Ln biomass [ln(mg WWT)]
Control
5 µg Cd*L-1
10 µg Cd*L-1
50 µg Cd*L-1

Figure 3.4. Percent mortality of Nephelopsis obscura exposed to 0, 5, 10, 20, 40, 80, 160, 320, 640 and 1280 μg $Cd \cdot L^{-1}$ after 3, 11 and 18 d.

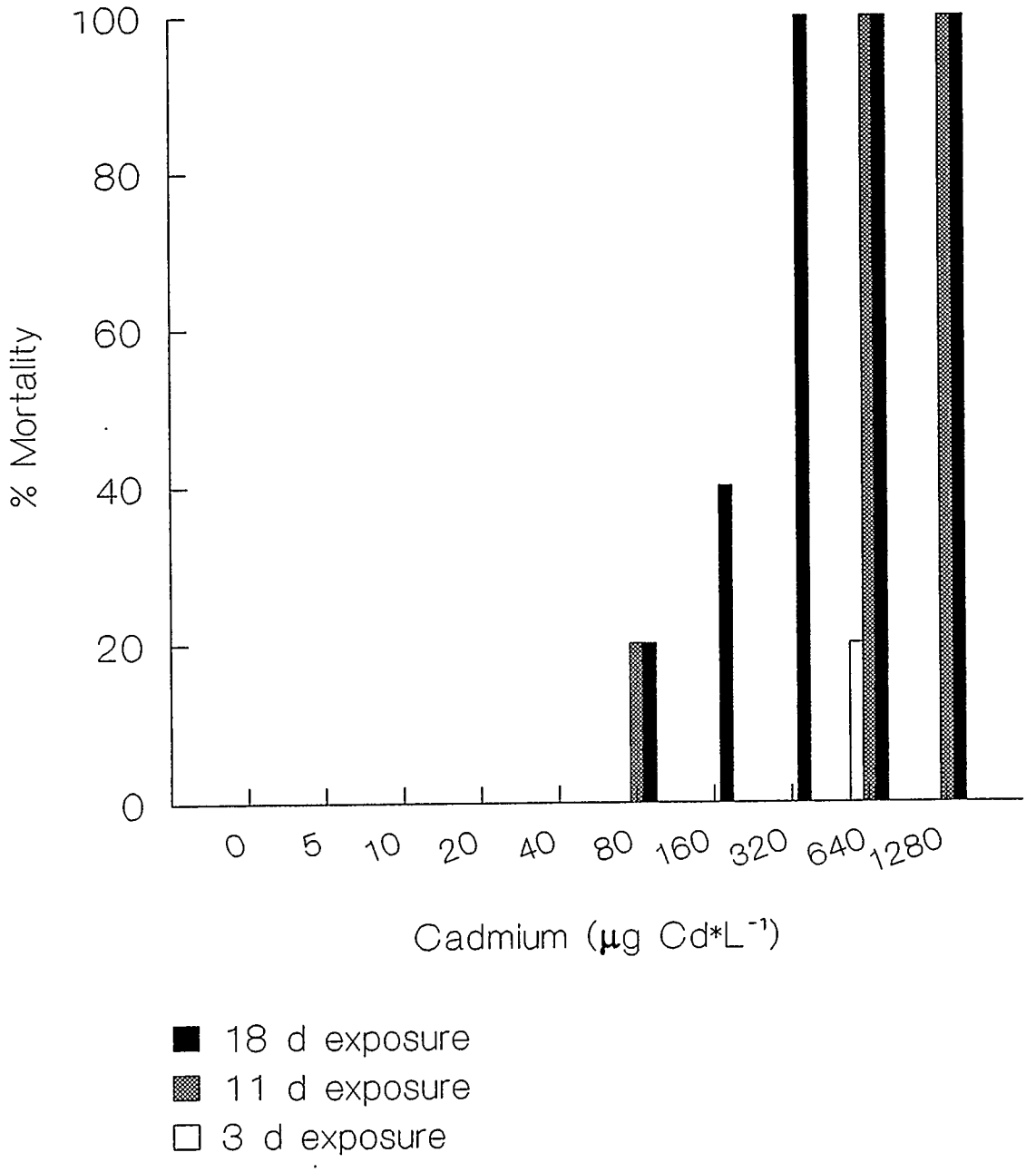

100
80
60
40
20
0
% Mortality
0
5
10
20
40
80
160
320
640
1280
Cadmium (µg Cd*L⁻¹)
18 d exposure
11 d exposure
3 d exposure

Table 3.1. Mean resting respiration, active respiration and aerobic scope of Nephelopsis obscura exposed to 0, 5, 10, 20, 40, 80, 160, 320, 640 and 1280 µg $Cd \cdot L^{-1}$ for 3, 11 and 18 d (µL O_2•mg WWT h^{-1} ± SE).

Resting Respiration

Day	Treatment									
	Control	5	10	20	40	80	160	320	640	1280
3	0.159 ± 0.023	0.125 ±0.019	0.136 ±0.022	0.133 ±0.013	0.165 ±0.013	0.163 ±0.015	0.132 ±0.012	0.145 ±0.012	0.142 ±0.016	0.151 ±0.022
11	0.133 ± 0.021	0.137 ±0.018	0.102 ±0.014	0.145 ±0.009	0.120 ±0.014	0.111 ±0.010	0.142 ±0.020	0.143 ±0.014		
18	0.122 ± 0.026	0.118 ±0.026	0.094 ±0.025	0.095 ±0.023	0.103 ±0.022	0.112 ±0.008	0.096 ±0.005			

Active Respiration

Day	Treatment									
	Control	5	10	20	40	80	160	320	640	1280
3	0.302 ±0.056	0.300 ±0.057	0.312 ±0.023	0.272 ±0.023	0.272 ±0.019	0.264 ±0.035	0.386 ±0.047	0.357 ±0.041	0.391 ±0.096	0.312 ±0.065
11	0.296 ±0.054	0.306 ±0.051	0.379 ±0.087	0.274 ±0.028	0.302 ±0.063	0.276 ±0.012	0.405 ±0.038	0.315 ±0.044		
18	0.331 ±0.026	0.314 ±0.044	0.282 ±0.072	0.299 ±0.042	0.254 ±0.063	0.233 ±0.039	0.140 ±0.019			

Aerobic Scope

Day	Treatment									
	Control	5	10	20	40	80	160	320	640	1280
3	0.176 ± 0.052	0.175 ±0.053	0.176 ±0.025	0.128 ±0.043	0.139 ±0.013	0.172 ±0.038	0.209 ±0.041	0.209 ±0.040	0.273 ±0.104	0.179 ±0.059
11	0.153 ±0.037	0.173 ±0.038	0.278 ±0.086	0.128 ±0.019	0.182 ±0.059	0.161 ±0.005	0.242 ±0.035	0.197 ±0.031		
18	0.209 ±0.021	0.196 ±0.031	0.188 ±0.055	0.207 ±0.044	0.158 ±0.043	0.156 ±0.044	0.172 ±0.033			

df=12). Time had a significant effect on Rm (F=6.143, df=2) but not on Ra (F=2.246, df=2) and AS (F=.0.562, df=2). Tukey's multiple comparisons showed that the significant time difference in Rm occurred between day 11 and 18.

Mucus

A Cd by time interaction had a significant effect on mucus production in N. obscura (F=3.449, df=3)(Figure 3.5). Mucus production in the high Cd water was significantly higher than the other treatments after 1 week of Cd exposure, while there was no significant difference among the other treatments. After 6 weeks there was no significant difference in mucus production among treatments.

Activity

Cadmium had a highly significant effect on activity time of N. obscura (slope F= 54.5, df=3; elevation F=26.6, df=3) (Figure 3.6). In the high Cd treatment, N. obscura was highly significantly less active than in the control, low and medium Cd treatments (different slope and elevation of regression lines), which were not significantly different from each other. In the control, low and medium Cd treatments N. obscura were active for 20% of the time monitored for the first 4 weeks, but activity dropped to about 6% between weeks 8 and 13. In the high Cd treatment activity was 2 - 4% of the time monitored for the 6 weeks monitored.

Reproductive Investment

There was no significant difference in reproductive investment, as surface area of reproductive tissue, between the control or any treatment nor among any treatment (F=0.467, df=19)(Figure 3.7). The mean area of reproductive tissues ranged from 25.98 mm^2 for the high Cd treatment to 28.20 mm^2 in the control.

Figure 3.5. Mean mucus production by Nephelopsis obscura exposed to 0, 5, 10 and 50 $\mu g \cdot Cd^{-1}$ (absorbance at 540 nm ± SE) versus time (weeks). Treatments with similar letters are not significantly different ($P<0.05$).

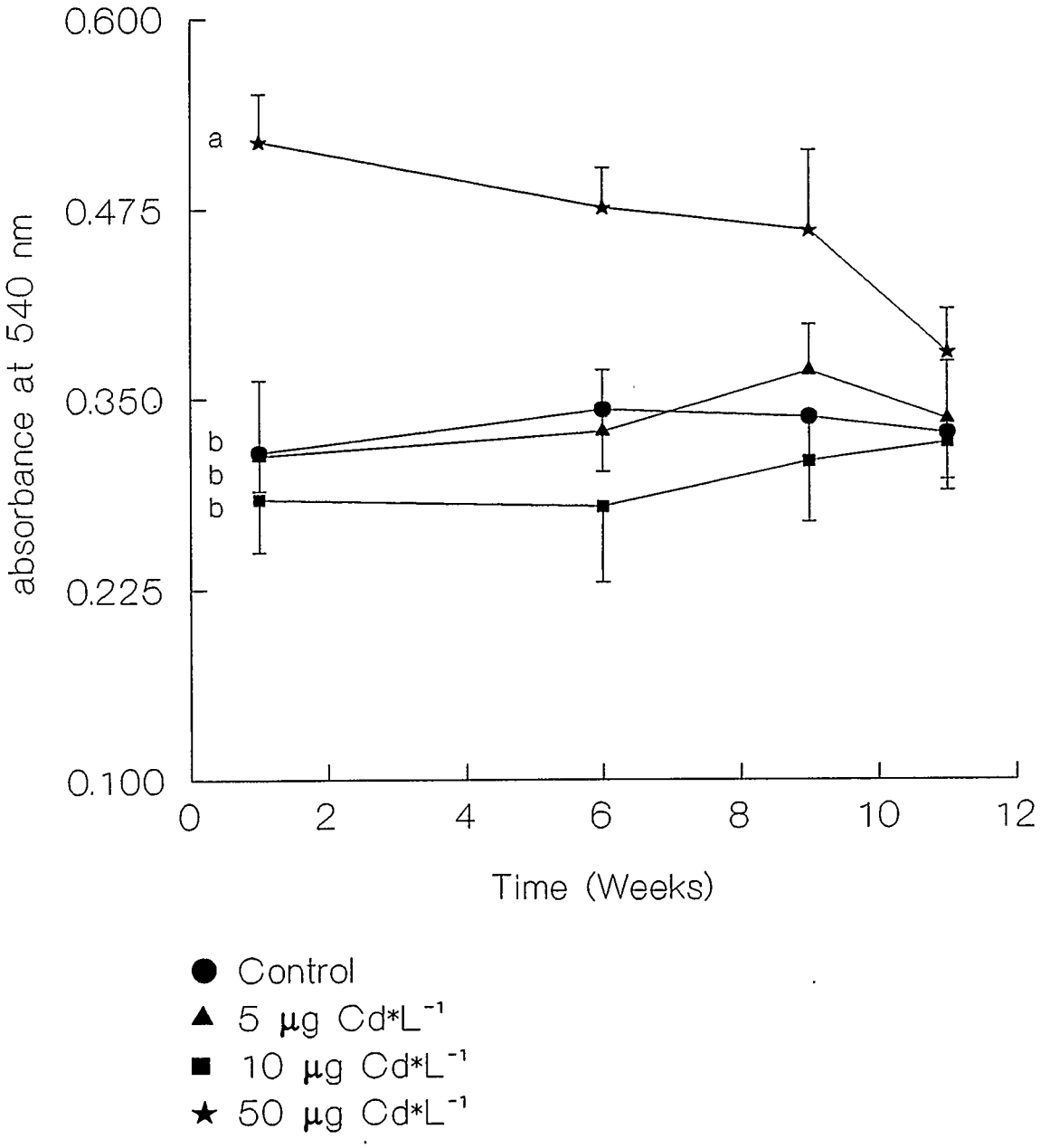

0.600
0.475
0.350
0.225
0.100
absorbance at 540 nm
a
b
b
b
0
2
4
6
8
10
12
Time (Weeks)
Control
5 µg Cd*L⁻¹
10 µg Cd*L⁻¹
50 µg Cd*L⁻¹

Figure 3.6. Linear regressions of biomass specific activity of Nephelopsis obscura exposed 0, 5, 10 and 50 μg $Cd \cdot L^{-1}$ (% time spent active). Treatments with similar letters are not significantly different ($P<0.05$).

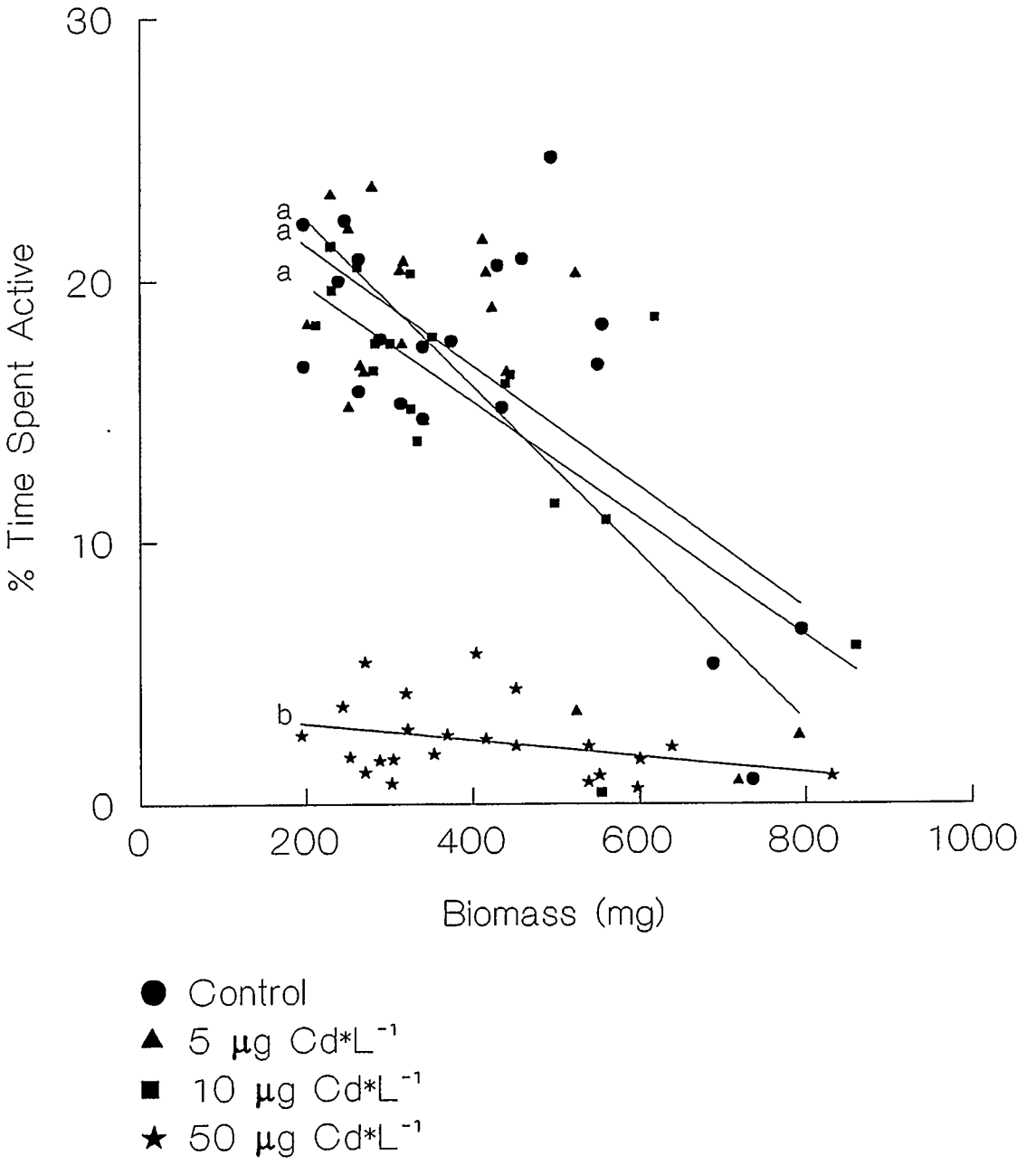
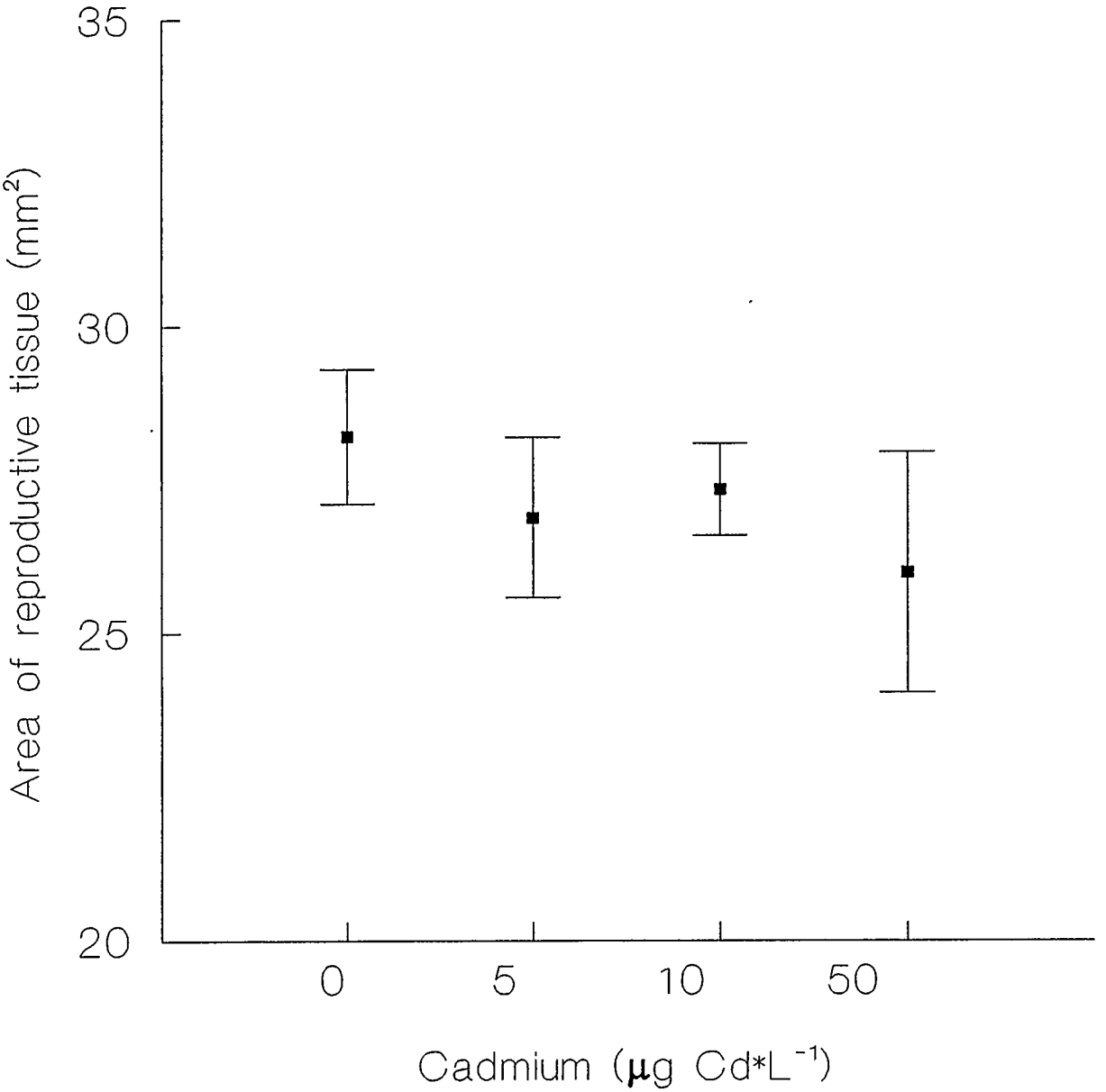

30
20
10
0
% Time Spent Active
a
a
a
b
0
200
400
600
800
1000
Biomass (mg)
Control
5 µg Cd*L⁻¹
10 µg Cd*L⁻¹
50 µg Cd*L⁻¹

Figure 3.7. Mean area of reproductive tissue of 550 mg Nephelopsis obscura exposed to 0, 5, 10 and 50 µg $Cd \cdot L^{-1}$ for 12 weeks ($mm^2 \pm SE$). All treatments are not significantly different from each other ($P>0.05$).


35
30
25
20
Area of reproductive tissue (mm²)
0
5
10
50
Cadmium (µg Cd*L⁻¹)

DISCUSSION

Growth

A decrease in growth of an animal, as measured by relative changes in biomass, results from less energy being allocated to production (somatic, reproductive and/or storage). A reduction in energy allocation to production could result from either no change in energy allocation pattern but less assimilated energy available, or no change in assimilated energy but a repriorization of energy to other physiological compartments at the expense of production. As there were no differences in energy ingested and assimilated and no differences in patterns of energy allocation between the control and the medium and low Cd treatments, differences in growth rates would not be predicted and were not observed. The decrease in ingestion, and the changes in energy allocation (e.g. increase in mucus production) of animals in the high Cd treatment indicates both contributed to the lower growth rate in the high Cd treatment.

Other studies have also reported a Cd induced decrease in growth rate. Heinis et al. (1990) found that decreases in ingestion resulted in lower biomasses of the chironomid larvae Glyptotendipes pallens (Meigen) exposed to 0.5 mg μg $Cd \cdot L^{-1}$ for 96 h. The growth of the gastropod Hydrobia ventrosa Montagu was significantly reduced by exposure to 100 or 200 μg $Cd \cdot L^{-1}$ for 3 weeks (Forbes 1991), and reduced growth and delayed development of third and fourth instar Chironomus riparius (Meigen) occured after exposure to 0.15 mg $Cd \cdot L^{-1}$ for 14 d (Pascoe et al. 1989). Ringwood (1992) similarly showed that exposure to 2 to 50 μg $Cd \cdot L^{-1}$ for 10 d resulted in a significantly decreased growth rate in the bivalve Isognomon californicum Pacas.

For the first 4 weeks of the experiment the growth rate of N. obscura in the high Cd treatment was similar to that in the medium, low and control treatments. Initially, increased mucus production in the high treatment may have protected N. obscura from the Cd solutions delaying direct toxic effects of Cd exposure.

Survivorship

Exposure to only the high Cd water resulted in a significant decrease in survivorship. Other studies have shown similar increases in mortality resulting from chronic Cd exposure (Borgmann et al. 1989, Glynn et al. 1992, Postma et al. 1994), however mechanisms of Cd toxicity are not well known. Cadmium toxicity may result from abnormal protein formation caused by Cd exposure (Jungmann et al. 1993), interference with epithelial ionic permeability processes (Hillyard et al. 1979, Takada and Hayashi 1980), an inhibitory effect on ATPases (Muino et al. 1990) or a reduction in O_2 availability (Hutcheson et al. 1985, Reddy et al. 1989). The relative importance of these toxic mechanisms may be species specific and change with Cd concentration and duration of exposure.

Ingestion

Ingestion decreased when N. obscura was exposed to high Cd water, and was unchanged in the medium and low Cd treatments compared to the control. Similarily, exposing the snail Hydrobia ulvae Montagu to 200 μg $Cd \cdot L^{-1}$ for 24 h resulted in significantly lower ingestion rates (Forbes and Depledge 1992). Heinis et al. (1990) reported that decreases in food uptake could be observed when Glyptotendipes pallens was exposed to 0.5 mg $Cd \cdot L^{-1}$ for 96 h and that food uptake ceased completely in Glyptotendipes pallens when exposed to 5.0 mg $Cd \cdot L^{-1}$ for 96 h. A decrease in pumping rate, and therefore water filtering rate, was exhibited in < 5 min by the mussel Mytilus edulis L. when exposed to 7.5 mg $Cd \cdot L^{-1}$ (Redpath and Davenport 1988). Cadmium depressed the feeding rate of the amphipod Gammarus pulex (L.) when exposed to 6 μg $Cd \cdot L^{-1}$ for 24 h (Brown and Pascoe 1989). Similar decreases in energy intake were reported for Gammarus pulex exposed to field concentrations of metallic industrial waste, quarry and pulp mill effluent, Zn and Fe (Maltby and Naylor 1990, Maltby et al. 1990a,

Maltby et al. 1990b, Crane and Maltby 1991, Maltby and Crane 1994). No reports of toxicant induced increases in energy acquisition have been reported.

Most studies that have found a metal induced decrease in ingestion have also found decreases in activity levels. Clearly in many animals ingestion and activity are linked. For example, pumping rate and volume of water filtered are correlated in filter feeders. A decrease in pumping activity results in less water being filtered which in turn causes a decrease in energy ingested. For active foragers like N. obscura a decrease in activity would result in a decreased probability of encountering prey items. In this study the toxic immobilization of N. obscura by Cd seems to be the mechanism by which levels of energy ingestion are reduced. Since it has been shown that size at maturity is the best correlate of fitness (Linton and Davies 1987) in N. obscura, changes in ingestion rates, and concomitant changes in growth rates and size at maturity, have clear ecological significance.

Assimilation Efficiency

Assimilation efficiency of N. obscura was not affected by Cd. Although Sibly and Calow (1986) suggest that animals may have the ability to increase gut retention times for food thereby increasing digestive efficiency, very few studies have quantified AE of animals exposed to toxicants. Forbes and Depledge (1992) found that the gastropod Hydrobia ulvae showed no changed in AE when exposed to 100 or 200 $\mu g\ Cd \cdot L^{-1}$.

The absense of change in AE by N. obscura means that the same proportion of ingested energy was lost as Fe plus Mu regardless of Cd concentration. However, the initial increase in Mu production determined colorimetrically in the high Cd treatment, should have increased energy lost as Fe plus Mu as determined by filtering treatment water. Because AE did not change, even though Mu production increased, this means that either the relative contribution of mucus to the Fe plus Mu measurement endpoint is

small or that Fe decreased. A decrease in Fe production would mean that AE actually increased. If Fe did not increase the technique employed to calculate AE is robust enough to accommodate increases in Mu that would incorrectly point to a decrease in AE.

Respiration

Cadmium had no significant effects on Rm, Ra or AS in N. obscura. The literature contains conflicting reports of the respiratory response of invertebrates exposed to toxicants. Capuzzo et al. (1976) and Capuzzo (1977) found significant reductions in O_2 consumption in larval lobster, Homarus americanus M. Edw., exposed to chlorine. Maltby et al. (1990a) found that respiration rate decreased in Gammarus pulex exposed to 0.04 - 0.19 mg unionized ammonia•L^{-1} for 24 h and Osbourne et al. 1980, found that both Erpobdella punctata Leidy and N. obscura, when exposed to total residual chlorine (TRC) for 1.5 h, displayed a decrease in respiration when exposed to low and high concentrations (0.7 mg and 3.64 mg TRC• L^{-1}) while medium concentrations (1.55-2.75 mg•L^{-1}), significantly elevated respiration. Daphnia magna exhibited a significantly elevated respiration rate when exposed to 5 µg Cd•L^{-1} and 50 µg dichloroaniline•L^{-1} for 48 h (Barber et al. 1990). Exposure to 0.3 - 0.7 mg Zn•L^{-1} and 0.125 - 1.0 mg 3-4 dichloroaniline•L^{-1} for 24 h was found to have no effect on respiration of Gammarus pulex (Maltby et al. 1990a, Naylor et al. 1989), and Gammarus pulex exposed to sewage treatment effluent and coal mine effluent for 6 d showed no change in respiration (Maltby et al. 1990b).

Many studies reporting a toxicant induced decrease in respiration do not distinguish between Rm and Ra but report only total respiration (Naylor et al. 1989, Barber et al. 1990, Maltby et al. 1990a, Maltby et al. 1990b, Naylor et al. 1989). If, as in this study, time of activity decreased, then the reported decrease in respiration could be due to

decreases in Ra and not Rm. Similarily, if activity increased with toxicant exposure, then total respiration would increase.

High mortality in the high Cd treatment indicates that Cd is severely stressing the animals. It has been hypothesized that in general terms there are metabolic costs of combating toxic stress. The removal or neutralization of a toxicant, and repair of cellular damage caused by xenobiotics, would be energetically costly (eg. costs can be incurred in the production of metallothioneins, proteins which chelate heavy metals thereby detoxifying the animal (Roesijadi 1992)), and should result in an increase in maintenance metabolism, a component of Rm (Calow 1991). However, Rm is not a single process and cannot be simply portrayed and understood because of its heterogeneous nature (Clarke 1993). Basal metabolism, that level of metabolism below which the animal cannot survive, cellular repair mechanisms, excretion, costs of mucus production, as well as costs of somatic and reproductive growth are all included in, and therefore measured together as Rm. No change in Rm does not necessarily mean physiological compensatory changes in response to a stress are not taking place, only that the sum of the energetic expenditures that make up Rm is constant. Changes in the relative contributions of these process to the sum might well be changing. Decreases in somatic and reproductive growth rates observed in N. obscura exposed to high Cd result in presumably a lower contribution of costs of tissue production to Rm, which means that if Rm does not change, other processes contributing to Rm are being increased. Removal or neutralization of Cd, the repair of cellular damage caused by Cd, and the costs of mucus production are the most likely to increase altough the relative contributions of these processes to Rm are not known. A partial shift to anaerobic respiratory pathways also provides an untested explanation of increased respiratory costs without an increase in Rm. As N. obscura is a facultative anaerobe which can survive 3 - 4 months of severe hypoxia/anoxia in the field during winter this is possible (Baird et al. 1987, Davies et al. 1987).

No change in Ra by N. obscura exposed to Cd means that activity became neither more nor less energetically costly with Cd exposure. When active, the same amount of energy was used by control animals and animals exposed to all concentrations of Cd. However, the total amount of energy respired while active, obtained by the product of time of activity and Ra, was significantly changed by Cd which affected the time spent in activity. Because Ra did not change with Cd concentration, the total energy expended while active when exposed to Cd is proportional to time of activity (Figure 3.5). As Cd concentration increased, time spent active decreased and therefore the total energy respired while active also decreased.

As AS is the difference between Rm and Ra, and neither Rm nor Ra changed with Cd exposure, AṠ was also not altered by Cd. As AS did not change with Cd, and there was therefore energy available for aerobic processes including activity in all treatments, yet time spent in activity decreased in high Cd, the conclusion is supported that a decrease in activity is a toxic effect.

In the supplementary respiration experiment high mortality in the high Cd treatments indicate animals were actutely stressed, yet there was no significant change in any respiratory variable in any treatment. This experiment confirms that when the effect of biomass on respiration is removed, either statistically or experimentally, Cd has no effect on respiration. The significant effect of time on Rm is due to the established phenomenon where larger animals have lower respiraton rates per unit biomass than small animals.

Mucus

Mucus production initially increased when N. obscura was exposed to high Cd as it did when N. obscura was exposed to chlorpyrifos, an organophosphate insecticide (Singhal et al. 1989) and increased salinity (Silva 1988). Osborne et al. (1980) hypothesized that increased mucus production by Erpobdella punctata and N. obscura, in response to

increased chlorine concentrations was a result of irritation of, or damage to, the epithelial tissue.

Mucus secretions increase in response to heavy metal stress in many animals. The bivalve Mytilus edilus increased mucus secretions when exposed to 40 μg $Zn \cdot L^{-1}$ (Hietanen et al. 1988), and Wilson et al. (1994), exposing Oncorhynchus mykiss to sublethal aluminum (38 μg $Al \cdot L^{-1}$) found a fourfold increase in mucus cell density after 5 d. Mueller et al. (1991) found a significant hypertrophy of mucous cells after 4 d exposure to 150 μg $Al \cdot L^{-1}$ and Handy et al. 1989 hypothesized that mucus production may function as a barrier to diffusion of heavy metals.

Initially mucus production was significantly increased in the high Cd treatment then subsequently dropped to the same level as the control, medium and low Cd treatments. When mucus production was elevated it may have acted as a physical barrier to Cd since initial growth rates in the high Cd treatment were similar to the other treatments. Growth rate fell in the high Cd treatment at about the same time as mucus production decreased to control levels and direct Cd toxicity probably occurred.

Increased mucus production could be a pathological result of Cd exposure. However, as N. obscura also secretes copious amount of mucus when stressed by chlorine, saline solution and physical handling (personal observation) it is most probably a general stress reaction. A genetically determined disposition to secrete mucus when stressed could be part of a SRS where suites of traits occur as adaptive evolutionary responses to stress. Suites of traits, evolved in response to natural stresses, may also be invoked when an animal is confronted with a novel, anthropogenic stress. As Cd has not been an historical selective pressure on N. obscura in Alberta (Monita 1994), any observed, apparently adaptive responses, induced by Cd exposure has probably evolved in response to other stresses.

Activity

Nephelopsis obscura exposed to high Cd exhibited a significant decrease in time spent in activity. Janssenn et al. (1993) also found significant reductions in the filtration rate and the swimming activity of the rotifer Brachionus calyciflorus (Pallas) after exposure to 12 and 20 μg $Cu \cdot L^{-1}$ for 30 min and 5 h respectively. Redpath and Davenport (1988) reported a decrease in water pumping rate in Mytilus edulis, after exposure for < 5 min to 7.5 mg $Cd \cdot L^{-1}$. The amphipod Diporeia spp. exhibited slower swimming rates when exposed to 25 g sea salt L^{-1} for 48 h (Gossiaux et al. 1992), and G. pallens, exposed to Cd for 96 h, exhibited decreases in activity at 2.5 mg $Cd \cdot L^{-1}$ (Heinis et al. 1990).

Decreased activity in response to a stress can either be a pathological response or an adaptive response. The discussion in Chapter 4 suggests that the effect of Cd on activity in N. obscura is a toxic response with Cd having a narcotizing or immobilizing effect on N. obscura. Decreased activity will decrease the rate of prey encounter rate by N. obscura, resulting in a reduced growth rate and probably decreased in fitness.

Reproductive Investment

Cadmium had no effect on reproductive investment in N. obscura as also shown by Wicklum et al. (1994) who found that Cd concentrations of 50 μg $Cd \cdot L^{-1}$ had no effect on the biomass of testisacs, ovisacs, and epidymis plus cornu, and no effect on the spermatozoa count•mg $testisac^{-1}$, and ova count•mg $ovisac^{-1}$. Selective pressures are constantly changing the type and magnitude of energetic tradeoffs between the amount of energy allocated to somatic and reproductive growth and survival mechanisms (Sibly and Calow 1989). As reproductive investment did not change with Cd exposure, the only physiological tradeoffs occurred between growth of somatic tissue and survival mechanisms. Mucus secretion, clearly a survival mechanism, increased, and growth of somatic tissue, as measured by changes in biomass, decreased. The compensatory,

evolutionarily derived stress resisting strategy adopted by N. obscura, as observed when exposed to Cd concentrations up to 50 μg $Cd \cdot L^{-1}$, is to maintain survival rate and reproductive effort and reduce energy allocated to somatic growth.

Statistics

As there were growth rate differentials between treatments, animals of similar biomass at a point in time may be a different age and therefore have been exposed to Cd for different lengths of time. This complicates the ANCOVAs where comparisons were made between treatments for similar ranges of biomass. Also, when animals were selected from treatments that had similar biomass, biomass was removed as a confounding effect but the ANOVA assumption of random selection was violated.

CHAPTER 4

PREFERENCE-AVOIDANCE

INTRODUCTION

The assessment of contaminant induced animal behavior is an ecologically relevant approach to evaluate the toxicity of xenobiotics (Doving 1991). A behavioral change may be the first response of an organism to a contaminant and represents a whole-animal response, based on the integration of underlying physiological processes (Hartwell et al. 1989, Heinis et al. 1990, McNicol and Scherer 1991). Aquatic environments are not homogeneous therefore an animal's behavior can potentially result in disproportionate amounts of time spent in optimal or suboptimal microhabitats, thereby altering the animal's toxicant exposure regime. As the amount of exposure to a toxicant usually dictates the magnitude of toxicant induced changes in growth, survival and reproduction, behavioral responses become a linkage between biophysical, biochemical and physiological events, and ultimate ecological manifestations (Scherer 1992).

The objective of a behavioral assay is to determine whether an animal can detect a toxicant and change its behavior on the basis of sensory information thereby altering the animal's toxicant exposure regime. Although any behavior that can be quantified has the potential to be used as an assessment endpoint, observation of preference-avoidance behaviors allow simple data collection and are ecologically relevant (Beitinger 1990). In preference-avoidance studies, an animal is placed in an experimental tank which contains a spatial gradient of toxicant concentrations. The amount of time an animal spends in different concentrations is quantified and statistically compared to determine if a disproportionate amount of time is spent in any one concentration of toxicant.

The preference-avoidance response of different fish species to Cd shows Oncorhynchus mykiss have an avoidance threshold of 50 $\mu g \cdot Cd\ L^{-1}$ (Black and Birge 1980), but golden shiners, Notemigonus crysoleucas (Mitchill), showed no significant response to Cd over a

range of concentrations from 5 to 68 μg•Cd L^{-1} (Hartwell et al. 1989) while lake whitefish, Coregonus clupeaformis (L.), could detect and respond to water containing Cd at concentrations as low as 0.2 μg•Cd L^{-1} (McNicol and Scherer 1993). Preference-avoidance experiments using invertebrates have not been carried not.

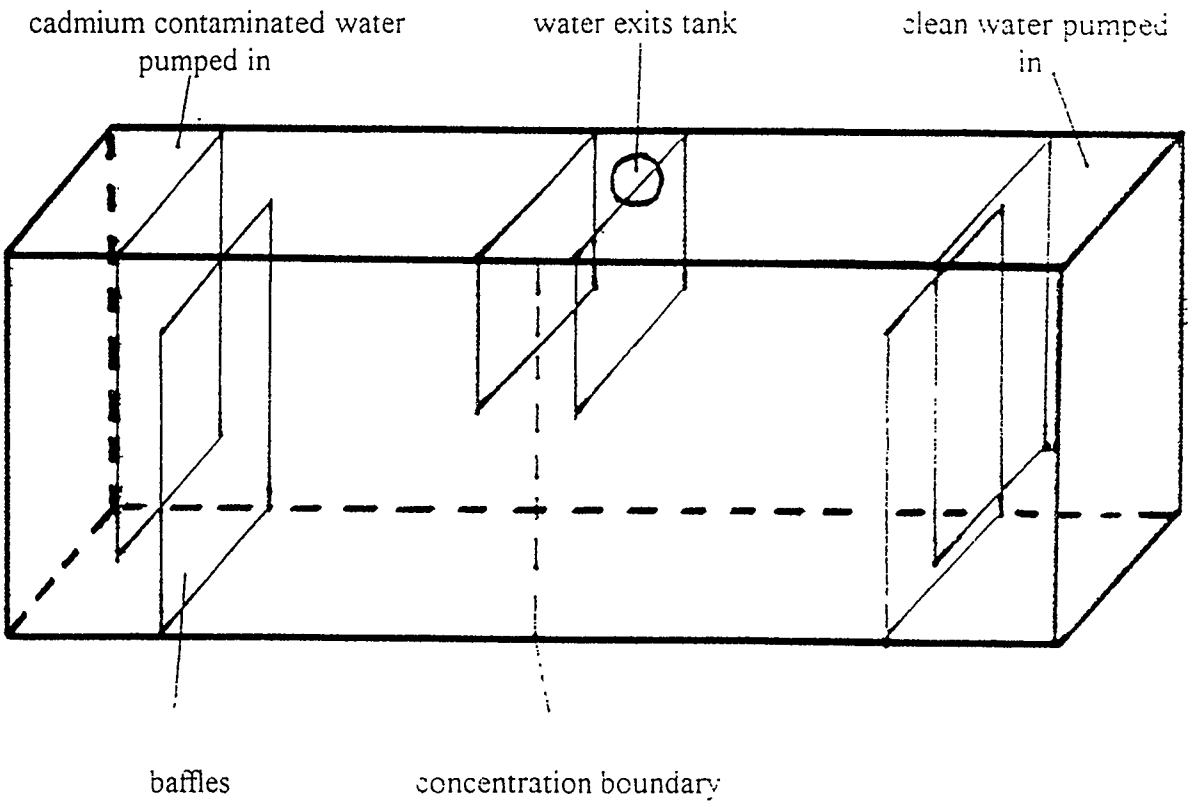
It has been shown (Chapter 2) that body size affects the acute toxicity of Cd to N. obscura . Although not generally addressed in the literature, different behavioral responses to toxicants exhibited by conspecifics of different sizes could result in changes in population demographics. Sullivan et al. (1978) found that fathead minnows, Pimephales promelas (Rafinesque), undergoing short-term (24 h) and long-term (21 d) Cd exposure prior to interacting with largemouth bass Micropterus salmoides (Lacepède) showed increased vulnerability to predation. Clearly toxicant induced differential predation pressures on different cohorts of a species will affect population structure.

The aims of this study were to determine if N. obscura exhibited a preference-avoidance response to the heavy metal Cd and, determine if body size affected this response.

METHODS

Nephelopsis obscura cocoons were collected in June 1993, hatched in the laboratory, and the hatchlings maintained as described in Chapter 2. The behavioral response of N. obscura to Cd was tested in a preference-avoidance chamber 45 cm long, 6 cm high and 4 cm wide (Figure 4.0). Water containing Cd (prepared as in Chapter 2) was pumped into one end of the tank, while control water (Chapter 2), was pumped into the opposite end by a dual action pump set at a flow rate of 1.0 mg•s^{-1}. Clean and contaminated water flowed toward the center of the tank resulting in a sharp and constant concentration boundary. Water was discharged through an outlet located at the middle of the tank. In order to visually assess the change in concentration boundary, initially water dyed with methyl blue was used in place of Cd contaminated water. To avoid biases produced by the

Figure 4.0. Preference-avoidance tank. Cadmium contaminated water is pumped into one end of the tank while clean water is pumped into the other end. A sharp, constant boundary occurs in the middle of the tank where the two flows of water meet and exit the tank. The baffles are designed to aid in maintaining the boundary by controlling water flow dynamics.


cadmium contaminated water pumped in
water exits tank
clean water pumped in
baffles
concentration boundary

apparatus, the side into which Cd contaminated water was pumped was changed after each replicate.

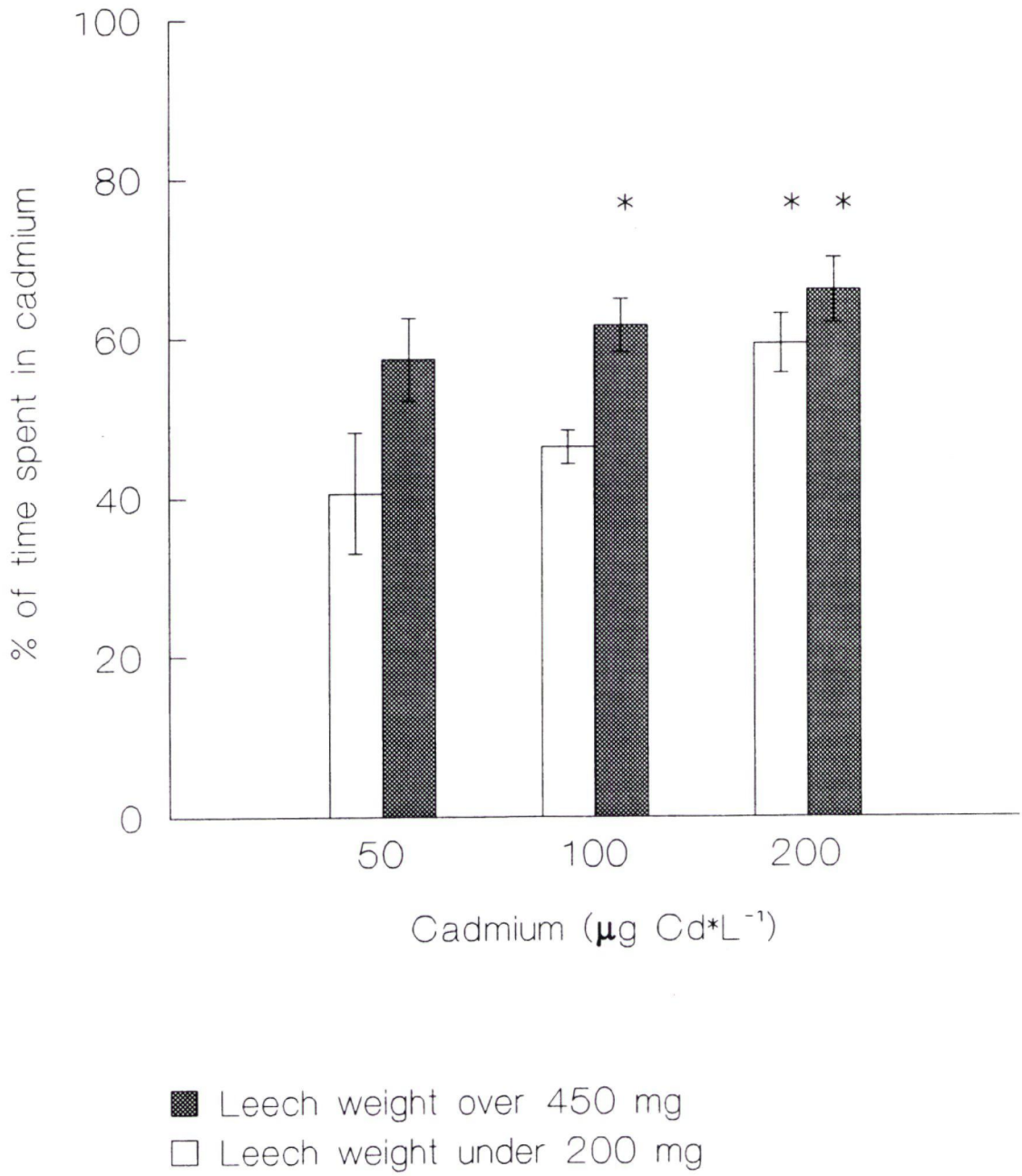
Nephelopsis obscura were divided into two groups, large (>450 mg) and small (<200 mg). The preference-avoidance response of these size classes were tested at 50, 100 and 200 μg•Cd L^{-1} versus clean water for 30 min (N=10). Leeches were acclimated for 15 min prior to trial initiation. To ensure that animals had been exposed to both test media, animals that did not cross the concentration boundary at least twice were excluded from the analysis. All trials were video-taped, and the time each animal spent in Cd contaminated water and control water quantified.

The amount of time spent in Cd contaminated water was tested against the amount of time spent in clean water using paired-sample t-tests ($P<0.05$) (Zar 1984).

RESULTS

Cadmium had a significant effect on the amount of time both large and small N. obscura spent in contaminated water (Figure 4.1). Small leeches spent statistically similar amounts of time in Cd water and clean water when tested at 50 and 100 μg Cd•L^{-1} ($t= 1.36$, $df=9$; $t=1.82$, $df=9$), but spent significantly more time in 200 μg Cd•L^{-1} Cd water compared to clean water ($t=2.55$, $df=9$). Large leeches spent statistically equal amounts of time in 50 μg•Cd L^{-1} water and clean water ($t=1.40$, $df=9$), however spent a significantly greater proportion of time in 100 and 200 μg•Cd L^{-1} water than in clean water ($t=3.60$, $df=9$; $t=4.09$, $df=9$).

Figure 4.1. Proportion of time spent in different concentrations of Cd contaminated water compared to clean water when tested in preference-avoidance chamber by large (>450 mg) and small (<200 mg) Nephelopsis obscura (% ± SE). Significant differences in time spent in Cd contaminated water indicated by * ($P<0.05$).


100
80
60
40
20
0
% of time spent in cadmium
*
* *
50
100
200
Cadmium (µg Cd*L⁻¹)
Leech weight over 450 mg
Leech weight under 200 mg

DISCUSSION

Small leeches spent statistically more time in water with a Cd concentration of 200 µg•Cd L^{-1} while large leeches spent more time in cadmium contaminated water at both 200 µg•Cd L^{-1} and 100 µg•Cd L^{-1}. There are two possible explanations for N. obscura spending significantly more time in Cd contaminated water. Firstly, the reaction could be a positive chemotaxis, or a true preference. In this case the animals must be equipped with sensory organs that can distinguish between the concentrations of Cd tested and remains in the Cd as a result of sensory information. Secondly, the reaction could be the result of a toxic effect. In this case the animals encounter the Cd, and become immobilized and are unable to leave the contaminated area.

Jones (1947) found that the ten-spined stickleback, Pygosteus pungitius (L.), exposed to 500 mg•Cd L^{-1} chloroform, 400 mg•Cd L^{-1} ethyl alcohol and 10 mg•$CuSO_4$ L^{-1} exhibited intoxication, causing animals to remain motionless on the bottom of the test chamber, so that percent time spent in the contaminant appeared to be a preference response. Black and Birge (1980) similarily found Oncorhynchus mykiss spending disproportionate amounts of time in solutions of 1 µg•Hg L^{-1} and 100 µg•Cu L^{-1}. In a separate study Oncorhynchus mykiss were found to spend disproportionate amounts of time in Cu solutions ranging from 300 to 390 µg•Cu L^{-1} (Giattina et al. 1982). It has been hypothesized that these data all reflect the immobilization of the test animal giving apparent preferences for the toxicants tested.

Examples of heavy metal induced decreases in activity have also been reported for invertebrates. Significant reductions in the filtration rate and swimming activity of the freshwater rotifer Brachionus calyciflous were observed after 2 h exposure to 20 µg•Cu L^{-1} and 5 h exposure to 12 µg•Cu L^{-1} respectively (Janssen et al. 1993). Fourth instar larvae of the midge Glyptotendipes pallens exposed to 2.5, 5.0 and 10.0 mg•Cd L^{-1} for 96 h displayed long periods of inactivity (Heinis et al. 1990). Tricopteran larvae,

Hydropsyche contubernalis Ross exposed to 12 μg•Cd L^{-1} for 24 h, spent less time trying to displace resident larvae than did larve in the control conditions (Vuori 1994). These examples, coupled with the decrease in activity of N. obscura exposed to 50 μg•Cd L^{-1} Cd (Chapter 3), indicate that the disproportionate amount of time N. obscura spent in Cd contaminated water is probably a result of a toxic effect and is not a preference resulting from sensory information.

Toxicant induced immobilization can result from two mechanisms. An animal can be genetically programmed to remain motionless when a stress in encountered. Alternatively, the toxicant could reduce the animal's physical ability for movement. A genetically determined disposition to remain motionless when stressed could be part of a SRS. Here a behavioral homeostatic mechanism would evolve if benefits of lowering activity levels when stressed outweigh the costs of immobility. Adopting a sit-and-wait strategy when a toxicant is encountered could allow an animal to priorize energy for stress resisting mechanisms at the expense of energy used for activity and may maximize the probability that water movement alone will remove the toxicant from the vicinity of the animal. However, this behavioral response to a toxicant would seem more appropriate in a lotic environment where water movement would have a higher probability of removing the stress. In a lentic environment such as Stevenson's Pond, where there is little water movement or replacement, a sit-and-wait strategy of toxicant avoidance would probably be maladaptive (Davenport 1985). Qualitative observations, where leeches in Cd water that were prodded remained motionless, supports the conclusion that the apparent preference of N. obscura for Cd contaminated water is a result of physical immobilization and is not a genetically determined behavior. Toxic effects of Cd may be expected to take place in the field because of N. obscura's inablility to avoid Cd contaminated areas as a result of immobilization.

As detailed in Chapter 1, available energy above that needed for basal metabolism would be expected to increase or not affect suvival (Calow and Sibly 1990). Since Cd immobilizes N. obscura, energy available above basal levels cannot be used for avoiding Cd contaminated areas, and AS would not affect survival via preference avoidance behavior.

Large N. obscura were more susceptible to Cd narcosis then small N. obscura, with more time spent in Cd concentrations of 100 μg•Cd L^{-1} and higher while small leeches only showed disproportionate amounts of time in Cd starting at 200 μg•Cd L^{-1}. Although smaller leeches were more susceptible to acute Cd toxicity then large leeches, behavioral responses are not necessarily indicative of traditional toxicological responses (eg. LC_{50}). Population dynamics of N. obscura could be disrupted because of size specific susceptibility to direct Cd toxicity, or from differential predation pressure resulting from size specific levels of narcosis and consequent susceptibility to attack.

These results identify the need for ecologically relevant endpoints in environmental risk assessment. Insights into toxicant induced behavioral changes, such as the preference-avoidance behavior of an animal, that may alter an animal's exposure regime to a toxicant is important for environmental risk assessment where risk is a function of probability of exposure and susceptibility to the toxicant.

The data presented here indicate that sublethal, ecologically relevant effects can be demonstrated in time spans normally utilized for traditional acute toxicity testing (Heinis et al. 1990). Acute behavior endpoints may also be used as correlates of ecologically relevant processes such as ingestion rate.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Summary

An ecotoxicological investigation of the effects of the heavy metal Cd was performed on the freshwater predatory leech Nephelopsis obscura using acute, chronic bioenergetic and preference-avoidance toxicity tests.

Acute toxicity tests showed that the developmental stage of N. obscura with embryos encased in a cocoon is the stage most resistant to acute Cd toxicity with a 96h EC_{50}, using reduction in hatching success as the endpoint, of 832.6 $\mu g\ Cd \cdot L^{-1}$. Survivorship of hatchlings maintained in clean water, but hatched from cocoons exposed to Cd, decreased with increasing Cd concentration. Biomass had a significant positive effect on resistance to acute Cd toxicity as 96h LC_{50}s ranged from 22.97 $\mu g\ Cd \cdot L^{-1}$ for ≈3.4 mg animals to 605.24 $\mu g\ Cd \cdot L^{-1}$ for ≈360 mg animals.

Chronic bioenergetic toxicity tests (15 week) using 0 (control), 5 (low), 10 (medium) and 50 (high) $\mu g\ Cd \cdot L^{-1}$, showed no significant differences in suvivorship, energy acquisition or allocation among the control, low and medium Cd treatments. The high Cd treatment showed significant decreases in biomass production, survivorship, energy acquisition and activity and an initial increase in mucus production. No differences were found among any treatment for, resting respiration, active respiration, aerobic scope, assimilation efficiency or reproductive investment.

Preference-avoidance studies showed that large N. obscura (>450) spent significantly more time in Cd contaminated water when tested against clean water starting at 100 $\mu g\ Cd \cdot L^{-1}$. In contrast small N. obscura (<200mg) spent significantly more time in Cd contaminated water versus clean water starting at 200 $\mu g\ Cd \cdot L^{-1}$. It was concluded that disproportionate time spent in Cd water was the result of a toxic narcotizing effect and was not a preference.

This study showed that 50 μg $Cd \cdot L^{-1}$ can have ecologically significant negative effects on N. obscura. Cadmium induced decreases in growth rate and survivorship would potentially decrease fitness in N. obscura. Toxic Cd induced effects may be expected to take place in the field because of the inability of N. obscura to avoid Cd contaminated areas as a result of immobilization. Population dynamics of N. obscura could be changed because of size specific susceptibility to direct Cd toxicity, or from differential predation pressure resulting from size specific levels of immobilization and consequent susceptibility to attack.

Scope for Growth

Bioenergetic modeling of organisms exposed to toxicants can be linked to population processes through scope for growth (SFG) (Chapter 1). All previous studies that calculated SFG (Maltby and Naylor 1990, Naylor et al. 1989, Maltby et al. 1990a, Maltby et al. 1990b) conducted experiments for short periods of time (6d) and SFG was calculated for each animal which can then be expressed as a treatment mean with variation. Short experiments simplifies the analysis as the confounding effect of differential growth of animals is removed . In this study the same animals were not used for determination of all bioenergetic variables. The manipulation of bioenergetic model components, determined from experimentation on different animals, does not provide an accurate SFG.

Stress Response Syndrome (SRS)

Whether animals react in a similar manner to stresses that have been a part of their evolutionary history compared to those that have not (SRS), is a fundamental question in ecotoxicology. The important stress responses of N. obscura involving mucus production,

activity, respiration and energy acquisition were examined for both naturally occuring and anthropogenic stresses.

Mucus production in N. obscura increases in response to both natural and anthropogenic stresses. Physical handling stress (personal observation), hyperoxia (Singal and Davies 1987), exposure to chlorpyrifos (Singhal et al. 1989b), chlorine (Osborne et al. 1980) and Cd (Chapter 3) all result in increased mucus production. Stress induced increased mucus secretion could have evolved to minimize the contact of natural toxicants (eg. hypoxic water) to the leech integument, and to discourage predator handling. Anthropogenic toxicants and handling also trigger increased mucus production as a stress resistance response.

Singhal and Davies (1987) found an initial increase in activity in N. obscura exposed to hyperoxic water followed by periods of inactivity, and Davies and Gates (1991) showed that changes in vertical distribution of N. obscura with changes in oxygen regime were behavioral adaptations to minimize oxygen stress. No behavioral changes were found in N. obscura exposed to chlorpyrifos (Singhal et al. 1989) while activity was found to increase with chlorine exposure (Osborne et al. 1987) and decrease with Cd exposure (Chapter 3). Behavioral responses seem to be stress specific and are probably dictated by the ability of N. obscura to detect the stress and the level of stress, where low levels may lead to an escape reaction while high levels may immobilize the animal.

This study showed no differences in Rm or Ra for N. obscura exposed to Cd (Chapter 3 and 4). The observed increase in activity levels was cited by Osborne et al. (1987) to explain the increase in total respiration in N. obscura exposed to chlorine. Because of the differences in techniques used to measure respiratory responses in animals exposed to toxic stress (closed versus flow-through respirometers) and differences in measurement variables (total respiration versus resting and active respiration) comparison of toxicant induced respirometric responses is difficult.

Levels of energy acquisition in N. obscura are correlated with prey density and probability of prey encounter (Dratnal et al. 1992). Davies and Gates (1991) postulated that an increase in feeding rate with an increase in dissolved O_2 saturation in N. obscura was a result of an increase in prey encounter rate, while this study explained Cd induced decreases in ingestion as a result of decreased activity and subsequent decrease in prey encounters (Chapter 3). Thus, levels of energy acquisition are correlated to levels of activity which were found to have no stress response pattern.

In conclusion, exposure to both natural and anthropogenic stresses elicits an increase in mucus production in N. obscura, while changes in behavior, respiration and energy acquisition are stress specific.

This research was a comprehensive investigation into the effects of Cd on N. obscura. It has yeilded data that will allow a risk assessment to be done on the effects of Cd on N. obscura in any freshwater system.

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