Bioavailability of Sediment-Associated Contaminants to Aquatic Invertebrates in Littoral Mesocosms

by

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BIOAVAILABILITY OF SEDIMENT-ASSOCIATED CONTAMINANTS TO AQUATIC INVERTEBRATES IN LITTORAL MESOCOSMS

BY

ROBERT S. CURRIE

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

The bioavailability of cadmium and tritiated 2,3,7,8-tetrachlorodibenzofuran (TCDF) was studied in littoral mesocosms, in two lakes, at the Experimental Lakes Area in northwestern Ontario. The bioavailability of cadmium was determined in four mesocosms and in lake 382, which was receiving experimental additions of cadmium. Two of the mesocosms were treated with nutrients to increase their productivity. Concentrations of cadmium in the water column of the two enriched mesocosms were higher than in the unenriched enclosures. The bioavailability of cadmium to aquatic invertebrates, described as biota sediment accumulation factors (BSAFs) or bioaccumulation factors (BAFs), was unaffected by the nutrient additions. However, a trend for increased bioaccumulation of cadmium in the enriched enclosures was observed in zooplankton and larval Chironomidae but not in floater mussels (Anodonta grandis grandis), crayfish (Orconectes virilis), emerging insects or benthic mayflies. The results suggested that mussels and zooplankton received the majority of their contaminant load from the water column either as dissolved or particle-ingested Cd, whereas crayfish and benthos obtained Cd from the sediment either as food or via adsorption.

The bioavailability of TCDF from sediment in littoral mesocosms, decreased in 1991 relative to 1989 in mussels and emerging Diptera, whereas it remained the same for crayfish and zooplankton and increased in burrowing mayfly (Hexagenia limbata) adults. BSAFs were consistently higher for TCDF in all organisms at the beginning of the uptake experiments relative to later in the experiment. This suggested that bioavailability was high because of resuspension of sediment-associated TCDF.

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Special recognition must go to my friends in the Department of Entomology and at the Freshwater Institute. They supplied many sources of comfort and entertainment over the past years, for this I thank you sincerely.

Lastly, I will forever be grateful to Karen Scott for her part in keeping me sane over the past three years as I sometimes struggled with the meaning of this project.

A lake is the landscape's most beautiful and expressive feature. It is earth's eye, looking into which the beholder measures the depth of his own nature.

Henry David Thoreau

Nature has no voice of its own, so some of us must try to speak for it.

Hugh Cochrane

To my family, especially my father and mother, who have supported me to greater lengths than parents should have to.

LIST OF ABBREVIATIONS

^{3}H	Tritium
AVS	Acid volatile sulfide
BB	Body burden
BAF	Bioaccumulation factor
BSAF	Biota sediment accumulation factor
Cd	Cadmium
CEPA	Canadian Environmental Protection Act
DPM	Disintegrations per minute
ELA	Experimental Lakes Area
FAA	Flame Atomic Absorption Spectrophotometry
GFAA	Graphite Furnace Atomic Absorption Spectrophotometry
H_2O_2	Hydrogen peroxide
H_3PO_4	Phosphoric acid
KB	Kajak-Brinkhurst Corer
L304	Lake 304, Experimental Lakes Area
L375	Lake 375, Experimental Lakes Area
L382	Lake 382, Experimental Lakes Area
L468	Lake 468, Experimental Lakes Area
LSC	Liquid Scintillation Counting
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
Ni	Nickel
Mg	Magnesium
OCDD	1,2,3,4,6,7,8,9-octachlorodibenzodioxin
PCDF	Polychlorinated dibenzofurans

TCDD	Tetrachlorodibenzo-*p*-dioxin
TCDF	2,3,7,8-tetrachlorodibenzofuran
$t_{½}$	half-life
USEPA	United States Environmental Protection Agency

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CHAPTER I

General Introduction

Cadmium (Cd) is naturally present in the environment in some common rock types, most importantly sphalerite (Bewers et al. 1987) and greenockite (Ravera 1984), and is released naturally into the atmosphere during volcanic eruptions, forest fires, wind blown dust and leaching of rocks (Ravera 1984). Anthropogenic emissions of Cd have significantly increased the concentrations of Cd in aquatic systems from preindustrial levels by a factor of 20 (Lantzy and Mackenzie 1979) but these emissions are usually only of local importance (Ravera 1984). Anthropogenic sources include mining and smelting, phosphate fertilizers, incinerator waste, and coal and oil combustion (Ravera 1984). The primary source of Cd in the emissions results from the mining of zinc ores. These ores contain small amounts of Cd which is released during the smelting operation. The major industrial use of Cd is electroplating, which protects steel surfaces from corrosion. Cd is also used as a pigment in plastics, a stabilizer in PVC resins, and for protection from ultraviolet radiation. The Ni/Cd battery manufacturing process is a growing application for cadmium (Totsch 1990).

Cd has no known beneficial biological use and is considered to be one of the most toxic of metals (Dressing et al. 1982, Ravera 1984). Wong (1987) and Eisler (1985) have collated some of the literature on the toxicity of Cd to invertebrates. The data demonstrate that ambient water concentrations as low as 0.7 to 55 μg/L may cause high mortality (Thorp et al. 1979), reduced growth (Eisler 1985, Wong 1987), inhibited

reproduction (Biesinger and Christensen 1972, Pascoe et al. 1989), and decreased emergence (Pascoe et al. 1989) in various invertebrate species. Freshwater invertebrates show varying degrees of sensitivity: generally crustaceans are the most sensitive, followed by gastropods, and finally insects (Wong 1987).

Because anthropogenic emissions have significantly increased the concentrations of Cd in aquatic systems and toxicological data show low levels of tolerance, Cd has been designated a high priority contaminant by the Canadian Environmental Protection Act (CEPA, Environment Canada 1989).

Tetrachlorinated dibenzofuran (TCDF), also on the CEPA priority substances list, belongs to a group of highly toxic compounds collectively termed polychlorinated dibenzofurans (PCDFs). PCDFs, unlike Cd, have never been produced intentionally and, like Cd, serve no useful biological purpose (Fiedler, et al. 1990). All sources of PCDFs are anthropogenic in nature; the primary sources include the combustion of municipal, hospital and hazardous wastes, and sewage sludge; fires containing polychlorinated biphenyls (PCBs) or polyvinylchlorides (PVCs); and scrap metal recycling (Fiedler et al. (1990), Rappe (1987), Marklund et al. 1986). PCDFs are also produced in large quantities in various industrial processes, especially bleached kraft pulp and paper, metallurgical processes (eg. Mg or Ni refining; Oehme et al. 1989) and in the manufacture of flame-retardant plastics (Fiedler et al. 1990).

TCDF has not been studied to the same extent as many other contaminants, so toxicological and environmental data are limited. Morrissey and Schwetz (1989) contend that the toxic effects of PCDFs are

similar to other aromatic hydrocarbons, especially 2,3,7,8-tetrachlorodibenzodioxin (TCDD). 2,3,7,8-TCDD is one of the most toxic compounds known to man and 2,3,7,8-TCDF is considered to be only slightly less toxic (McConnell and Moore 1979). Twenty-eight day LC_{50} for 2,3,7,8-TCDD in fathead minnows are <63 ng/L; exposures as short as 24 h to only 82 ng/L result in >90 % mortality within 60 d (Adams et al. 1986). Significant mortality occurs in rainbow trout within 14 days at exposure to TCDF of 3.93 and 8.78 ng/L (Mehrle et al. 1988). Morrissey and Schwetz (1989) and Eisler (1986) list mortality, carcinogenesis, teratogenesis, mutagenesis, and reproductive effects as possible consequences for both terrestrial and aquatic vertebrates. Invertebrates are comparatively resistant to TCDD; for example. there are no adverse effects on growth, reproduction or food consumption in daphnids and snails during exposure to solutions containing 2.4 to 4.2 ng/L TCDD (Yockim et al. 1978). Although invertebrates are comparatively resistant to TCDD and TCDF, current Canadian guidelines indicate that ambient water levels of 2,3,7,8-TCDD should not exceed 10 picograms/liter (pg/L) (Boddington et al. 1990). However, the ability of these compounds to bioaccumulate (Eisler 1986) and biomagnify may result in toxic concentrations reaching more intolerant organisms.

Cd is particle reactive (K_d =~10^7 L/ng, Lum 1987) and TCDF is lipophilic (Log K_{ow}= 6.53, Sijm et al. 1989) so they quickly partition into the sediment after entering an aquatic system. Concentrations of Cd in the sediment of some Canadian lakes range from non-detectable to 56 μg/g and in the water column from <2 to 542 ng/L (Table 1.1). Concentrations of 2,3,7,8-TCDF in sediment of Canadian waterways near

Table 1.1. Cadmium concentrations in the sediment and water column of selected Canadian lakes.

Location	Concentration		Reference	
	sediment µg/g	water ng/L	sediment	water
Lake Superior	2.2	19	Kemp et al. 1978	Poldoski and Glass 1978
Lake Michigan	0.9	27	Cahill and Shimp 1984	Muhlbaier et al. 1982
Lake Huron	2.0	16	Kemp et al. 1978	Rossmann 1982
Lake Ontario	5.1	50	Kemp and Thomas 1976	Nriagu et al. 1981
Lake Erie	3.6	98	Kemp and Thomas 1976	Rossmann 1984
Clearwater Lake, ON	-[1]	542	-	Stephenson and Mackie 1988a
Plastic Lake, ON	-	62	-	"
Mackie Lake, ON	-	<2	-	"
Hamell Lake, MB	56	-	Harrison and Klaverkamp 1990	-
Cleaver Lake, MB	7	-	"	-
Lakes in Central Saskatchewan	<1	-	"	-

[1] Dash signifies no information.

pulp and paper mills range from non-detectable to 3,179 pg/g (Table 1.2, Trudel 1991), but remain undetectable in Canadian drinking water because of the hydrophobic nature of TCDF (Boddington et al. 1990).

There is considerable evidence that contaminants such as trace metals and chlorinated hydrocarbons are adsorbed by sediments and suspended matter in aquatic systems (Adams 1987a, Allan 1986, Hart 1982, Knezovich et al. 1987). The interaction of contaminants with these naturally occurring sorbents affects not only the distribution of the contaminant in the environment (McCarthy and Black 1988), but also contaminant availability to both pelagic and benthic organisms (Knezovich et al. 1987). Sediments and particulates were once considered to be sinks for contaminants because of this binding ability (McCarthy and Black 1988). In fact, sediment may serve more as a source than a sink, especially for benthic organisms that spend most or all their life cycles in close association with the sediments (Knezovich et al. 1987).

The extent of bioavailability of pollutants in sediment depends greatly on factors associated with the contaminant, but it also depends on characteristics of the sediment. Sediments are a complex mixture of clays, silica, organic matter, metal oxides (silica, manganese, aluminium, and iron), carbonates, sulfides, and minerals (Hart 1982). The presence of interstitial water controls density and influences bioavailability of contaminants (Landrum et al. 1987). The degree to which these sediment characteristics influence bioavailability varies for individual contaminants.

Table 1.2. Range of concentrations of 2,3,7,8-tetrachlorodibenzofuran in bed sediment near pulp and paper mills using chlorine bleach (Trudel 1991).

Location	N	Sediment Concentration pg/g (dry wt.)
Howe Sound, BC	1	3179
Terrace Bay, ON	3	15-730
Thurso, PQ	1	5
Grande Prairie, AB	2	32-36

Sediment-bound contaminants may remain bioavailable long after emissions of toxic pollutants have been decreased or eliminated. Therefore, it is important to examine interactions between the sediment and water compartments that may affect the bioavailability of contaminants to aquatic organisms, and to determine the length of time sediment contaminants are bioavailable.

Taylor et al. (1991) hypothesised that the bioavailability of organic contaminants in eutrophic lakes was lower than that in oligotrophic lakes. They observed a biomass dilution (i.e. a lower concentration of contaminant in a larger biomass of zooplankton relative to a smaller biomass of zooplankton) in eutrophic lakes relative to oligotrophic lakes. Taylor et al. (1991) proposed that this was caused by increased production of zooplankton and subsequent dilution of available organic contaminants. McCarthy and Bartell (1988) also suggested that organisms inhabiting the same ecological niches in oligotrophic and eutrophic lakes would be exposed to different concentrations of contaminants because of differences in the quantity of sorptive sites which would affect bioavailability by binding with contaminants. Larsson et al. (1992) found that predatory fish in lakes with high productivity had significantly lower concentrations of persistent organic pollutants than fish in less productive lakes. This was due in some part to increased growth rates of fish in productive lakes, and partly because of the increased amount of humic and phytoplankton material in productive lakes that was present to sorb pollutants and render them unavailable to fish.

Landrum (1989) found that as contact time with sediment increased,

contaminants became less available to amphipods. This observation was attributed to sorption of contaminants into less bioavailable sediment compartments, removal of ingestible material containing contaminants through the "packaging of faecal material", and changes in organism behaviour (Landrum and Robbins 1990).

The primary objective of this thesis was to compare the bioavailability of Cd to aquatic invertebrates in littoral nutrient-enriched and unenriched mesocosms, and in a whole lake that was receiving experimental Cd additions to the epilimnion. The present study and the ongoing experiment in L382 also allowed for the comparison of bioavailability of Cd from a predominately sediment source (the mesocosms) to its bioavailability from a predominately water-based source (Lake 382).

A secondary objective was to determine the effects of ageing of sediments on the bioavailability of TCDF to aquatic invertebrates as seen by Landrum (1989) in amphipods.

TCDF is potentially a good surrogate for many other hydrophobic organochlorines such as TCDD, while Cd is an important heavy metal with properties that are similar to other metals. The results of this study should therefore be broadly applicable to a range of similar organic and inorganic sediment contaminants

CHAPTER II

Factors Affecting Bioaccumulation of Cadmium in Aquatic Invertebrates.

The amount of Cd that is accumulated by any organism is a balance between influx and efflux (Connell 1988, Luoma 1983). Aquatic organisms can accumulate Cd directly from water or indirectly from food, sediment, and detrital particles and subsequently translocate it through the body by active and passive transport mechanisms (Knezovich et al. 1987, Ray 1984). However, the amount that is accumulated is regulated by bioavailability from various sources. For metals, bioavailability is affected by speciation, i.e. its physical-chemical form (O'Donnell et al. 1985, Hart 1981, Taylor 1983).

A number of factors affect speciation. The presence and quantity of dissolved and particulate organic matter in the water column and organic matter in the sediment are important (Hart 1982, Breteler and Saksa 1985, Allan 1986, Luoma 1983). The quantity of these components in a lentic system also affects the trophic status of the system, which affects the bioavailability of organic contaminants to biota (McCarthy and Bartell 1988, Taylor et al. 1991). A eutrophic system should have less bioavailable contaminant than an oligotrophic lake because the additional organic carbon and particulates present should complex with contaminants and render them unavailable or less available to biota. This hypothesis may also apply to metals (McCarthy and Bartell 1988). For example, Stephenson and Mackie (1988b) found that dissolved organic carbon content, total cadmium and water hardness were significantly correlated with cadmium concentrations in the amphipod Hyalella azteca

in southern Ontario lakes. They suggested that dissolved organic carbon may form complexes with free cadmium ions and reduce their concentration in solution, which would subsequently reduce bioavailability and Cd concentrations in H. azteca. Breteler and Saksa (1985) found that the accumulation of Cd in Mytilus edulis and Modiolus demissus depended primarily on percent total sediment organic matter. High concentrations of organic matter in sediment may affect contaminant bioavailability, depending on the route of exposure for an organism. High organic matter in the sediment may increase contaminant exposure for infaunal organisms (Knezovich et al. 1987, Harvey and Luoma 1985), but decrease exposure for pelagic organisms such as zooplankton (Taylor et al. 1991). Thus, the quantity of organic matter in a system will affect the bioavailability of many contaminants, but the degree to which it affects biota is tempered by an organism's niche in the aquatic system.

The objective of this study was to compare the bioavailability of Cd to aquatic invertebrates in littoral nutrient-enriched and unenriched mesocosms and in a whole lake that was receiving experimental Cd additions to the epilimnion. The mesocosms excluded the enclosed water and sediment from the Cd additions. Two hypotheses were tested: (1) that nutrient additions would affect the bioavailability of sediment-associated Cd to aquatic invertebrates and (2) that bioavailability of Cd from a predominately sediment source (the mesocosms) would differ from that of a primarily water based source (Lake 382), depending on the niches and feeding habits of the aquatic organisms involved.

2. MATERIALS AND METHODS

2.1. Study Site

Beginning on June 23, 1987 and continuing every ice-free season until 1993, Cd as $CdCl_2 2H_2O$, and its radioactive tracer ^{109}Cd, have been added to the epilimnion of Lake 382 (L382) at the Experimental Lakes Area (ELA) in northwestern Ontario (93°47'30"W, 49°44'30"N). This experiment was intended to do the following: (1) test the adequacy of the Canadian water quality guideline for the protection of aquatic biota, (2) determine adverse effects of Cd on a lake ecosystem, and (3) determine any bioindicators that may be used to serve as "early warning indicators of... ecological damage" (Malley et al. 1989).

In 1991 and 1992, Cd concentrations in the epilimnion were maintained as close as possible to the Canadian water quality guideline of 200 ng/L (Reeder et al. 1979). Total amounts of 2143 g and 1117 g of Cd, were added to the lake in 1991 and 1992 respectively (D. Malley pers. comm., Freshwater Institute, Winnipeg, MB). As a result of these additions, Cd concentrations in the sediment of the lake have increased approximately four to seven times (D. Malley pers. comm., M. Stephenson Atomic Energy Canada Ltd. Pinawa MB. pers. comm.) and water concentrations have varied from background levels (approx. <10 ng/L) to 200 ng/L over the year (Malley et al. 1990, B. Hunt pers. comm., Freshwater Institute, Winnipeg, MB,). Biota in L382 have shown no population or reproductive effects (Malley et al. 1989, Malley and Chang 1991, Harrison et al. 1990), but Cd concentrations in long-lived organisms (e.g. the floater mussel, Anodonta grandis grandis (Say), and

lake trout, Salvelinus namaycush Walbaum) have increased continuously since the experiment began (Malley et al. 1990).

2.2. Enclosures

Between May 9 and 14, 1991, four 5-m diameter x 2-m deep enclosures were placed in the littoral zone of the south bay of L382 (Fig. 2.1). The enclosures were constructed of nylon-reinforced polyethylene (Curry Industries, Winnipeg, MB) that was supported by a tubular aluminium frame. The enclosures floated on the surface of the water using Styrofoam™ blocks sewn into the top edge. The bottom edge of the enclosures contained a five-cm diameter plastic pipe that helped maintain shape and allowed for a secure fit to the sediment. The bottom edge was sandbagged to the bottom sediments to seal off the enclosures from the water column. This isolated the water and sediments within the enclosures from the new additions of Cd that was added in 1991 and 1992 to the whole lake. The enclosures were placed at approximately 1.75 m depth, and were allowed to equilibrate for 14 d before the nutrient additions commenced. The mesocosms were randomly labelled C1, C2, N1 and N2 for controls and nutrient treated enclosures respectively (Fig. 2.1).

2.3. Sampling Outline

The sampling schedules for 1991 and 1992 are shown in Table 2.1. Specific sample sizes and methods for the various matrices are described in greater detail in subsequent sections.

Table 2.1. Sampling schedule for L382 nutrient-enriched and unenriched mesocosms and lake sites

Sample	Month of 1991					Month of 1992					
	6	7	8	9	10	5	6	7	8	9	10
Water Nutrient sampling + additions	Every second week					Every second week					
[Cd]	X[1]	X				X		X		XX	X
Tritium								X	X		
Sediment Physical[2]		X		X							
[Cd]	X		X		X	X		X		X	
AVS[3]								X		X	
Porewater	X		X		X	X		X		X	
Mussels			X	X	X		X	X	X	X	X
Depuration									XXX	XX	X
Crayfish Exp.1			X	X	X	X		XX	X		
Exp.2								XX	X		
Zooplankton		X	X	X	X	X		X		X	
Benthos		X	X		X	X		X		X	
Emergence	X	X				X		X			X

[1] "X" indicates that samples were taken; the total number of "Xs" equals the number of sample days in that month.

[2]Physical characteristics of the sediment, including % sand, silt, clay, organic matter, carbon and nitrogen.

[3]Acid volatile sulfide analysis.

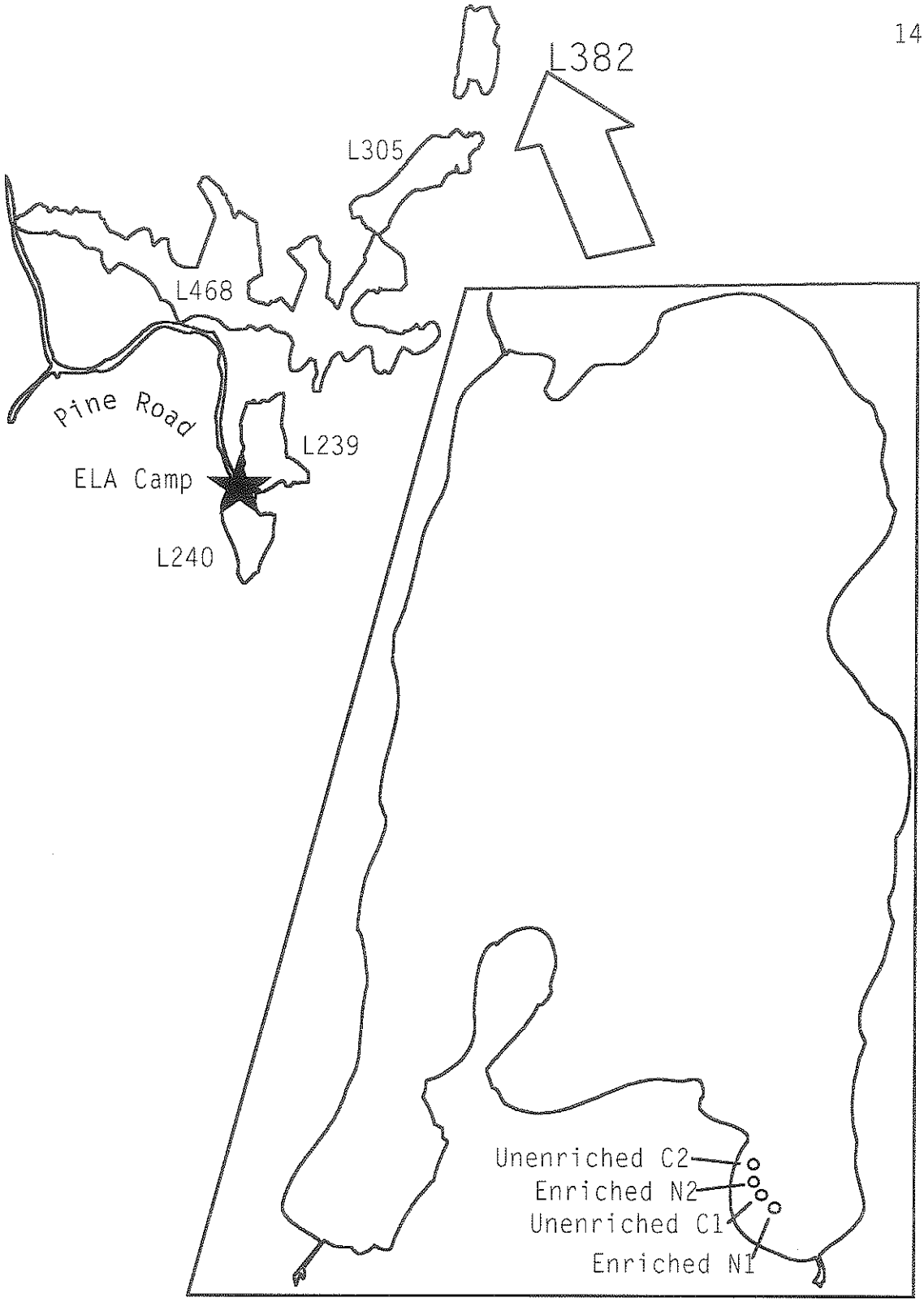

Fig. 2.1. Schematic diagram of Lake 382 at the Experimental Lakes Area, northwestern Ontario.

2.4. Nutrient Additions

On June 5 1991, nutrients at a molar ratio of approximately 10:1 nitrogen:phosphorus were added to two of the four enclosures as a solution form. The nitrogen source was a sodium nitrate fertilizer (NPK ratio 13:0:0, Prairechem, Winnipeg, MB) and the phosphorus source was reagent grade phosphoric acid (H_3PO_4) (J.T. Baker, Phillipsburg, N.J., USA). The nutrient solutions were added to the enclosures weekly until September 15, 1991, additions were recommenced on May 14 1992, and continued weekly until October 15 1992. Approximately 56 g of $NaNO_3$ and 1 mL of H_3PO_4 were added in 1991 and 89.6 g and 1.6 mL in 1992 in each of the enriched mesocosms.

The sodium nitrate fertilizer was analyzed for Cd content by dissolving one g of dry fertilizer in deionized, distilled water and diluting the solution to 100 mL. The Cd analysis is described in detail in section 2.8.1.

2.5. Water Sampling

2.5.1 Nutrients

Water was collected in two L Nalgene® bottles (Nalge Company, Rochester, New York) every two wk (Table 2.1) from each enclosure and from the lake itself. The analysis of dissolved and particulate carbon, nitrogen, and phosphorus, pH and chlorophyll *a* concentrations was performed by the ELA chemistry laboratory using analytical techniques described in Stainton *et al*. (1977).

2.5.2. Cadmium

Water concentrations of Cd were determined by two different methods. In 1991, water samples were to be sampled and analyzed for Cd as a regular component of the water chemistry analysis. However, the samples were accidentally discarded, so alternate data were obtained from M. Holoka (Freshwater Institute, Winnipeg, MB) who was determining Cd concentrations in various particle sizes in the water column of the mesocosms. The Cd data used came from water filtered through a 142 mm Nucleopore® polycarbonate filter with three μm pore sizes.

In 1992, Cd concentrations in the water column of the mesocosms and the lake were determined by taking duplicate, whole, unfiltered water samples using pre-acid-washed 60 mL Nalgene® bottles. The Cd analysis is described in detail in section 2.8.1.

2.5.3 Tritium Leak Test for Mesocosms

To ensure the mesocosms were not leaking in the late stages of the experiment, 2 mL of ^{3}H-H_2O (specific activity 5 mCi/mL, New England Nuclear, Boston, MA) was added to the mesocosms and tracked over the course of two months in 1992. On each sample day (Table 2.1), five mL of water were pipetted from the water column directly into 20 mL scintillation vials (in duplicate), diluted with 12 mL of scintillation fluor (Atomlight, New England), and assayed by liquid scintillation counting (LSC) on a Beckman Model 7500 liquid scintillation counter (Beckman Instruments, Irvine, CA). The loss of ^{3}H-H_2O was then measured over a 30-d period and the decline in ^{3}H concentration was compared between mesocosms using linear regression analysis (SAS Proc Reg).

2.6. Sediment Samples

2.6.1. Physical Characteristics

Duplicate sediment samples were collected with a five-cm internal diameter Kajak-Brinkhurst (KB) corer in 1991 (Table 2.1) from the mesocosms and lake to determine percent sand, silt, clay, total carbon, organic matter and nitrogen. The samples were stored in polyethylene bags at -40°C until analysis by Northwest Labs (Winnipeg, MB) using established methods described in MSS (1978).

2.6.2. Cadmium Concentrations

Duplicate sediment cores (0-2 cm layers) were obtained on each sample day (Table 2.1) using the KB corer to determine Cd concentrations in sediment. Water was siphoned off the core, the sediment extruded and the 0 to 2 cm layer removed and stored in polyethylene bags at -40°C until analysis. The sediment was freeze dried on a Lab Con Co. Freeze Dry 5 (Fisher Scientific Co., Winnipeg MB) at -68 to -75°C and a pressure of 0.5 to 1 Pa for at least 120 h.

Concentrations of Cd in pore water were determined by centrifuging the top two cm of duplicate sediment cores in pre-acid-washed 250 mL Nalgene® centrifuge tubes at 4000 RPM for 30 min. The supernatant was siphoned off and stored in pre-acid-washed Nalgene® bottles and acidified to 0.5 % with nitric acid, 24 h before analysis. Cadmium analysis is described in detail in section 2.8.2.1.

2.6.3. Acid Volatile Sulfide (AVS) Analysis

Selected sediment samples were subsampled to determine AVS

concentrations before 1992 sediments were analyzed for Cd concentrations. The AVS method is described in detail in Section 2.8.2.2.

2.7. Biota

2.7.1. Bioaccumulation of Cd in Anodonta grandis grandis (Say)

Floater mussels, A. grandis grandis, were collected from ELA L104, by snorkel and mask in one to three m of water, placed in a cooler, and transported to L382. At L382, the mussels were placed into closed-bottom, plastic-mesh cages constructed with a wooden frame (50 cm x 50 cm x 35 cm) and lowered to the sediment in the enclosures and in the lake itself. Approximately 34 mussels were placed into each mesocosm and in the lake in two cages at the beginning of each experiment (June 20, 1991 and June 10, 1992). Twenty mussels were kept to determine background concentrations of Cd, and to confirm species identification. Six mussels were sampled from each enclosure and the lake on each sample day (Table 2.1) and stored at -40°C in polyethylene bags until analysis. The length, width and height of each mussel was measured to the nearest 0.05 mm using callipers (Huebner et al. 1990), and the soft tissues removed and weighed after blotting dry. Cadmium analysis is described in section 2.8.3.1.

2.7.2. Depuration of Cd From A. grandis grandis

To determine the depuration of Cd from mussels, 60 mussels were placed into two separate cages in L382 itself in 1992. After 63 d the mussels were removed from L382 and placed into cages in L468, an

undisturbed lake. Five mussels were sampled after 0, 5, 13, 23, 34, and 56 d (Table 2.1). These mussels were analyzed in the same manner as described above.

2.7.3. Bioaccumulation of Cd in Orconectes virilis (Hagen)

Freshwater crayfish O. virilis were collected from ELA L468. Minnow traps baited with beef liver were left in the water overnight. The following morning the traps were retrieved and the male crayfish removed and transported to L382. Three crayfish were placed into each 30 cm x 40 cm plastic-mesh cage with three rocks obtained from the shoreline of L382. Six cages were situated in each enclosure as well as in the lake itself. Approximately 20 crayfish were kept to determine background concentrations of Cd and to confirm species identification. Three or four crayfish were sampled on each sample day (Table 2.1), placed in polyethylene bags and stored at -40°C until analysis. The carapace of each crayfish was measured to the nearest 0.05 mm using callipers and the whole organism weighed after blotting dry. The Cd analysis method is described in detail in section 2.8.3.1.

2.7.4. Accumulation of Cd in Zooplankton (>200 μm)

A 30-cm diameter Wisconsin net (mesh size 200 μm) was pulled vertically through the top 1 to 1.5 m of each enclosure and through the lake to collect zooplankton. Duplicate samples were taken on each sample day (Table 2.1). Each sample was rinsed where it was collected and stored in pre-acid-washed Nalgene® bottles at -40°C until analysis. Samples were thawed and then filtered through Nitex® screening to remove

the water. The samples were allowed to dry overnight and then were weighed. Samples were pooled within treatments if less than six to ten mg of material was obtained. Methods for the analysis of Cd are described in section 2.8.3.2.

A subsample of the collected zooplankton was taken from the enclosures and identified by M. Patterson (Freshwater Institute Winnipeg, MB) during the July 1992 sample day.

2.7.5. Bioaccumulation in Chironomidae and Hexagenia limbata (Serville) Nymphs

Benthic invertebrates were obtained on each sample day (Table 2.1), using a 15 cm x 15 cm Ekman grab (Wildlife Supply Co., Saginaw, MI). Organisms and sediment were sieved through a 400-μm mesh and immediately hand sorted into pre-acid-washed glass scintillation vials and stored at -40°C until analysis. The organisms were then blotted dry and weighed wet. Approximately 10 to 15 mg of wet tissue was digested to ensure accurate Cd results. Methods for the determination of Cd are described in section 2.8.3.2.

2.7.6. Cadmium in Emerging Insects

Insects emerging from the mesocosms were sampled using three submerged emergence traps (Davies 1980) set at even intervals across the mesocosm. In the lake a transect of nine submerged emergence traps was used. On each sample day (Table 2.1) collected insects were sorted into acid-washed vials and stored at -40°C until analysis. Organisms were thawed, blotted dry and weighed wet. Methods for determining Cd

concentrations are described in section 2.8.3.2.

2.8. Chemical Analysis of Samples

2.8.1 Water

Water and the $NaNO_3$ fertilizer were analyzed by graphite atomic absorption spectrophotometry (GFAA) using a Varian GTA-95, (Varian Instruments, Georgetown, Ont.) 24 h after acidification with nitric acid to 0.5 %. Ten μL of phosphoric acid modifier was injected with the sample to stabilize and sharpen the peak of the Cd absorbance. Instrumental parameters can be found in Appendix 1A.

2.8.2. Sediment

2.8.2.1 Cadmium

Duplicate samples of approximately 0.5 g of dry sediment were digested in Teflon® beakers. The analysis involved a 15 min digestion at 100°C in an aqua regia mixture of 1:3 volumetric ratio of concentrated Bakers nitric acid:concentrated hydrochloric acid. The digest was diluted to 100 mL and stored in Nalgene® bottles. Recovery of PACS-1 reference marine sediment (National Research Council of Canada, Ottawa, Canada) using this digest was well within specifications at 2.37 ± 0.19 μg/g (n=8) (product specification is 2.38 ± 0.2 μg/g dry wt.).

Cadmium concentrations in the sediment were also determined from a subset of sediments during AVS analysis of the sediment. Simultaneously extracted metals (SEM) were generated from a cold acid digest during the AVS analysis. Twenty mL of cold 6M HCl was added to a sediment-water

slurry in duplicate AVS reaction flasks and the mixture was stirred continuously for approximately one h. The slurry was filtered through a 0.2-μm membrane filter and the supernatant stored in Nalgene® bottles.

Cadmium in digested sediment, the SEM and porewater samples was determined by GFAA (Varian GTA-95, Georgetown, ON). Instrument parameters can be found in Appendix 1A. Five μL of phosphoric acid modifier was injected with the sample onto the graphite tube.

2.8.2.2. Acid Volatile Sulfide

In 1992, sediment was also analyzed for AVS using the colorimetric method of the United States Environmental protection Agency (USEPA) guidelines (Allen et al. 1991). Duplicate samples of approximately 10 to 15 g of wet sediment were used for each analysis. Sulfide from sediment was generated by addition of deaerated, cold 6.0 M HCl under a nitrogen atmosphere to prevent oxidation of sulfide to sulfate. Sulfide was trapped in two NaOH (Fisher Scientific, Nepean, ON) traps and was analyzed by UV spectrophotometry (Pharmacia Ultraspec II, LKB Biochrome, England) after addition of mixed diamine reagent to develop color.

2.8.3. Biota Analysis

2.8.3.1. Mussels and Crayfish

The dried mussel and crayfish tissues were weighed after freeze drying, ground up, and homogenized as well as possible. Duplicate samples of approximately 0.5 to 0.6 g were then digested in a 4:1 volumetric ratio of concentrated nitric acid:concentrated perchloric acid at 140°C for three h followed by eight h at 200°C. The resulting

digest was diluted to 25 mL with deionized, distilled water and analyzed using flame atomic absorption (FAA) spectrophotometry using an air-acetylene flame with background correction (Varian AA-20 and Varian AA-975, Varian Instruments Georgetown, Ont.). Where necessary, low concentrations of Cd were analyzed after extraction with 5 % diethyl dithioaminocarbamate (DDDC) in butyl acetate (Dutton 1991). Recovery of Cd from Dolt-1 reference material (National Research Council Canada, Ottawa, ON) using this method yielded 4.22 ± 0.20 μg/g (n=20). Product specification for Dolt-1 is 4.18 ± 0.28 μg/g; therefore the analysis was well within the defined range. All glassware was washed in Fisher's nitric acid (Fisher Science, Nepean, ON) and rinsed with deionized, distilled water before use. Instrument parameters can be found in Appendix 1B.

2.8.3.2. Zooplankton, Benthic Invertebrates and Emerging Insects

Zooplankton, benthic invertebrates, and emerging insects were combined with 2.5 mL concentrated nitric acid in pre-acid-washed digestion tubes and twice heated to dryness. The mixture was then oxidized by addition of one mL of 50 % H_2O_2 (Fisher Science, Nepean, ON) followed by drying. The remaining ash was dissolved in 250 μL of concentrated nitric acid and diluted to 12.5 mL with deionized water (Malley et al. 1989). Cadmium concentration was measured by GFAA (Varian GTA-95). Recovery of citrus leaves reference material (National Institute of Standards and Technology, Washington DC) was 0.024 ± 0.013 (n=17) which was within product specifications of 0.03 ± 0.01 μg/g. Instrument parameters can be found in Appendix 1A.

2.9. Data Analysis

Bioaccumulation factors (BAF) for some biota were determined using:

$$BAF = C_b/C_w \quad [2.1]$$

where BAF is the bioaccumulation factor, C_b is the concentration in biota (μg/kg dry wt.) and C_w is the concentration of Cd in water (μg/L). Because water from 1991 was not analyzed for Cd during the same periods as the biota were sampled it was only possible to compute accurate BAFs for 1992 when consistent water data were available.

Body burdens of Cd in biota were calculated using:

$$BB = (C_b)(DW) \quad [2.2]$$

Where BB is the body burden, and DW is the dry weight of the organism.

Biota sediment accumulation factors (BSAF) for biota were calculated using:

$$BSAF = C_b/C_s \quad [2.3]$$

where BSAF is the biota sediment accumulation factor, and C_s is the sediment concentration on a dry weight basis. To determine BSAFs for organisms that were analyzed on a wet weight basis (Chironomidae and H. limbata larvae and adults), a wet to dry weight ratio of ten was assumed (Dermott and Paterson 1974). Concentrations in the organisms were converted before determining the BSAF.

The half life ($t_{½}$) of Cd in the mussels was estimated by linear regression (SAS Proc Reg, SAS Institute 1989) assuming a first order kinetic rate model using the equation:

$$t_{½} = \ln 2 / k_2 \quad [2.4]$$

where k_2 is the depuration rate or slope of $\ln C_b$ vs. time (t).

The slopes of regression lines for the loss of tritium in the mesocosms were compared using the equation:

$$t\ value = \frac{(slope\ 1 - slope\ 2)}{(Sqrt[(RRSS\#1 + RRSS\#2\ /\ n1\text{-}2 + n2\text{-}2)(1/RSX\#1 + 1/RSX\#2)]}$$

where t-value at α=0.05, slope 1 and slope 2 are the slopes of the line of the concentration of tritium vs. time, Sqrt is the square root, RRSS #1 and #2 is the standard error of the Y estimate squared for slope 1 and 2, and RSX#1 and #2 are the standard errors of the regression coefficients squared.

2.10. Statistical Analysis

The concentration of Cd in the different compartments or biotic samples was compared ($p<0.05$) between treatments using Procedure GLM of SAS (SAS Institute 1989) and Tukey's mean test. Where necessary, the data were log transformed because of non-homogeneity of variance before statistical analysis was done.

3. RESULTS

3.1. Water

3.1.1. Impact of Nutrient Additions on Productivity of Mesocosms

The nutrient additions had little effect on the amount of chlorophyll a in the mesocosms in 1991 (Fig. 2.2a). From July 2 through July 29 the two nutrient-enriched mesocosms, N1 and N2, had slightly more chlorophyll a [3.03 ± 0.26 and 2.73 ± 0.14 μg/L (mean ± SD) respectively] than the controls, C1 and C2 (2.12 ± 0.14 and 2.41 ± 0.15

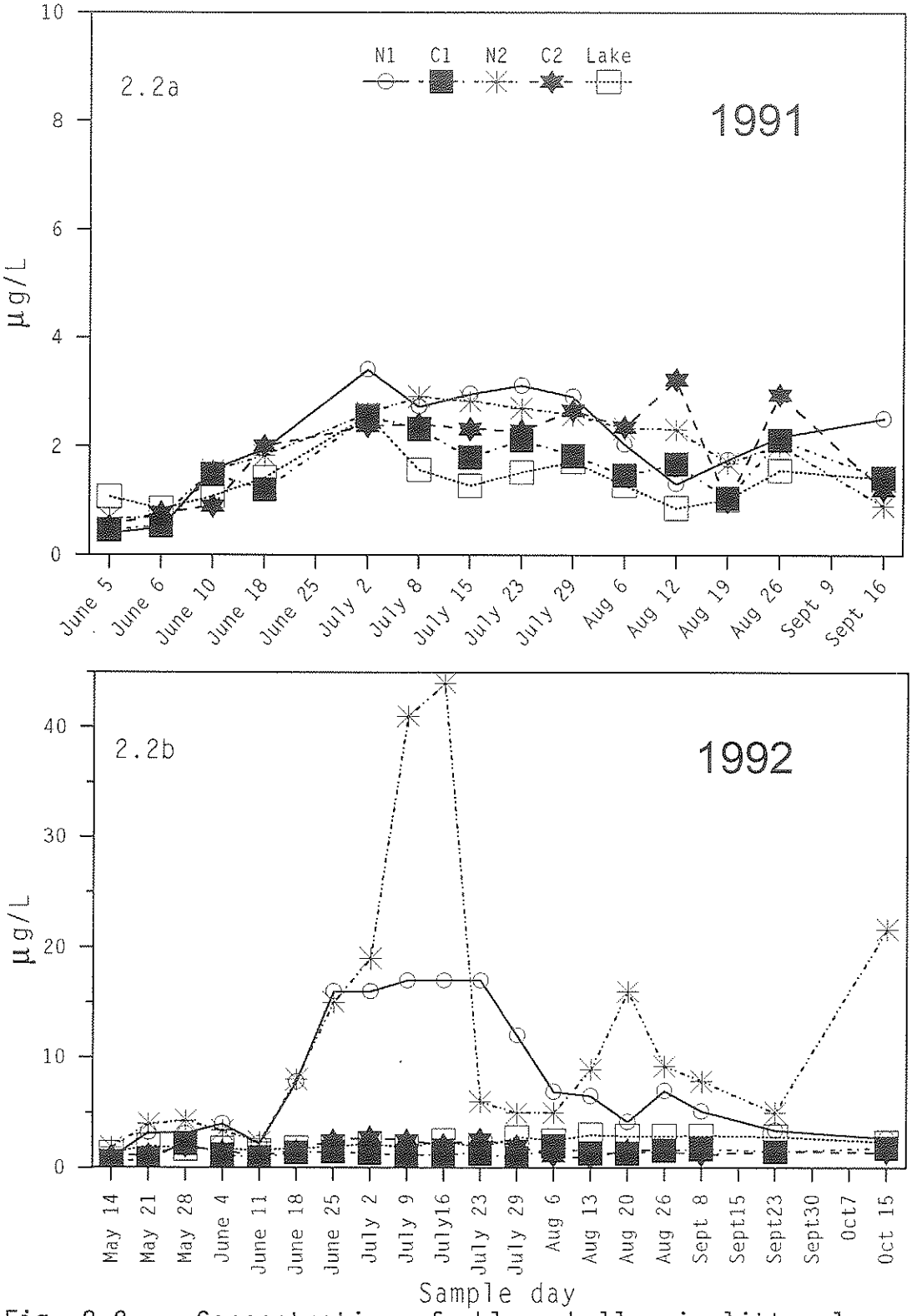

Fig. 2.2. Concentration of chlorophyll a in littoral mesocosms and Lake 382, Experimental Lakes Area. N1 and N2 equal nutrient enriched, C1 and C2 equal unenriched mesocosms.

μg/L respectively) during the same time period.

Nutrient additions had a greater effect on chlorophyll *a* production in 1992 than 1991 (Fig 2.2b). On June 11, 28 d after the nutrient additions began, both enriched mesocosms began to show elevated chlorophyll production. N1 maintained a maximum concentration of 17 μg/L from June 25 until July 23 when it started to decrease gradually until the last sampling day in October. N2 showed the same trend but reached a maximum concentration of 44 μg/L on July 16. Concentrations then dropped and fluctuated between 5 and 20 μg/L until the end of the experiment in October. Over the course of the 1992 field season, the enriched mesocosms had mean (± SD) chlorophyll *a* concentrations of 8.9 ± 5.82 and 11.9 ± 12.2 μg/L (N1 and N2, respectively) whereas the unenriched mesocosms had mean levels of 1.35 ± 0.41 and 1.69 ± 0.58 μg/L (C1 and C2, respectively). Over all of 1992, the nutrient-enriched mesocosms had five to eight times higher levels of chlorophyll *a* than the unenriched mesocosms.

The $NaNO_3$ fertilizer contained approximately 3.74 μg/g of Cd. Therefore, in 1991 and 1992, approximately 2.09 μg and 3.34 μg of Cd respectively, were added to each nutrient-enriched enclosure.

3.1.2. Cadmium Concentrations in Water

The enriched mesocosms had much greater concentrations of Cd in the filtered water than the unenriched mesocosms in 1991 (73-99 vs. 15-22 ng/L, respectively) (Fig. 2.3). The lake itself had concentrations two to nine times higher than the mesocosms because of the new additions of Cd to the lake during this time.

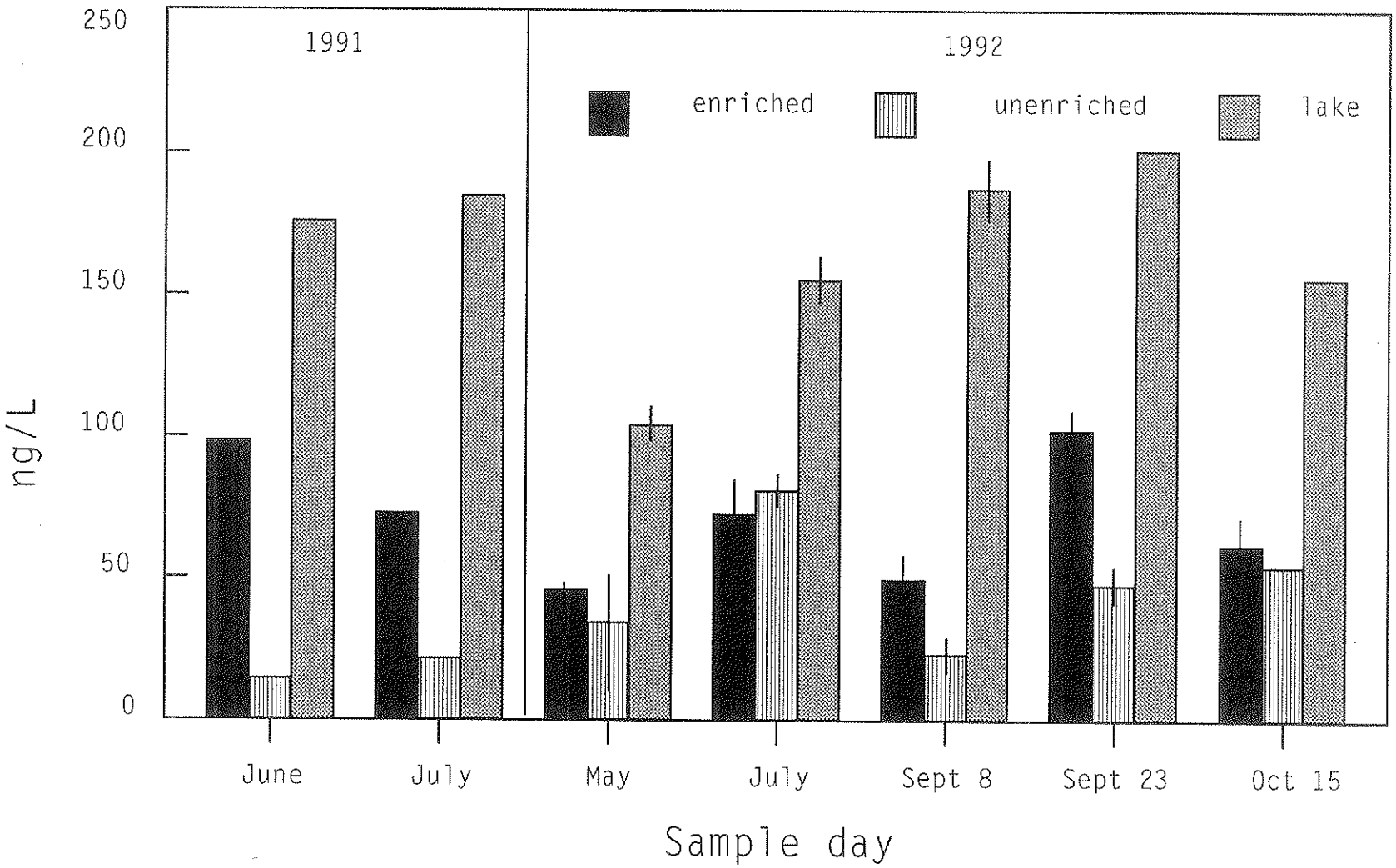

Fig. 2.3. Concentration of cadmium in the water column of littoral mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992. Error bars equal +/- 1 SD.

In 1992, the enriched mesocosms usually had higher concentrations of Cd than the unenriched mesocosms in unfiltered water, but the differences were not as pronounced as in 1991. Cd concentrations in the enriched mesocosms ranged from 49.0 to 103 ng/L compared to 14.6 to 81.0 ng/L in the unenriched mesocosms.

3.1.3. Tritium Loss

The mesocosms lost ^{3}H at a relatively constant rate during the late stages of the experiment (Appendix 2). Significant differences occurred in only two comparisons: C1 vs. N2, and N1 vs. N2 (Table 2.2). Both C1 and N1 lost ^{3}H faster than the other mesocosms, but these rates were only significantly different from N2 which had the slowest rate of loss of ^{3}H (Table 2.2).

3.2. Sediment

3.2.1. Physical Characteristics

Bottom sediments of mesocosms and the lake were 90.4 to 94.4 % sand, 2 to 6 % silt and 3.6 to 5.7 % clay (Table 2.3). Sediments had 0.55 to 1.05 % total organic carbon, 0.98 to 1.87 % organic matter and 0.11 to 0.16 % nitrogen.

3.2.2. Concentration of Cadmium in Sediment

Cadmium in the sediments ranged from 0.30 to 0.59 μg/g dry weight in 1991 and 0.27 to 0.57 in 1992 and showed no treatment effect (Fig. 2.4). These concentrations were approximately 380 to 1000x greater than in the porewater (see below). The SEM analysis yielded sediment concent-

Table 2.2. Comparison of slopes of the loss of tritium from littoral mesocosms in 1992.

Treatment Comparison	Slope 1 (DPM/day)[1]	Slope 2	t value	Significance[2] at P<0.05
C1 vs. C2	-3.66089	-2.93778	-0.881	ns
C1 vs. N1	-3.66089	-3.24675	-0.635	ns
C1 vs. N2	-3.66089	-1.60158	-9.575	s
C2 vs. N1	-2.93778	-3.24675	0.302	ns
C2 vs. N2	-2.93778	-1.60158	-1.634	ns
N1 vs. N2	-3.24675	-1.60158	-2.538	s

[1] Linear regression of the loss in concentration of tritium vs. time.
[2] Students t-test; s=significant, ns= not significant.

Table 2.3. Sediment (0-2 cm) characterization of mesocosms and Lake 382, Experimental Lakes Area, in June 1991 and August 1991.

Sample	Particle Size Analysis (%)			Texture	% Total OC[1]	% OM[2]	% N[3]
	Sand	Silt	Clay				
June 6							
N1	92.4	3.0	4.6	Sand	0.56	0.99	0.12
C1	91.4	3.0	5.6	Sand	0.64	1.15	0.13
N2	91.4	5.0	3.6	Sand	0.61	1.09	0.13
C2	92.4	4.0	3.6	Sand	0.73	1.29	0.13
Lake	90.4	6.0	3.6	Sand	0.61	1.08	0.14
Aug.12							
N1	93.4	2.0	4.6	Sand	0.63	1.12	0.12
C1	93.4	2.0	4.6	Sand	0.98	1.74	0.16
N2	91.2	3.1	5.7	Sand	1.05	1.87	0.12
C2	93.2	2.4	4.4	Sand	0.75	1.33	0.13
Lake	94.4	2.0	3.6	Sand	0.55	0.98	0.11

[1] Organic Carbon [2] Organic Matter [3] Nitrogen

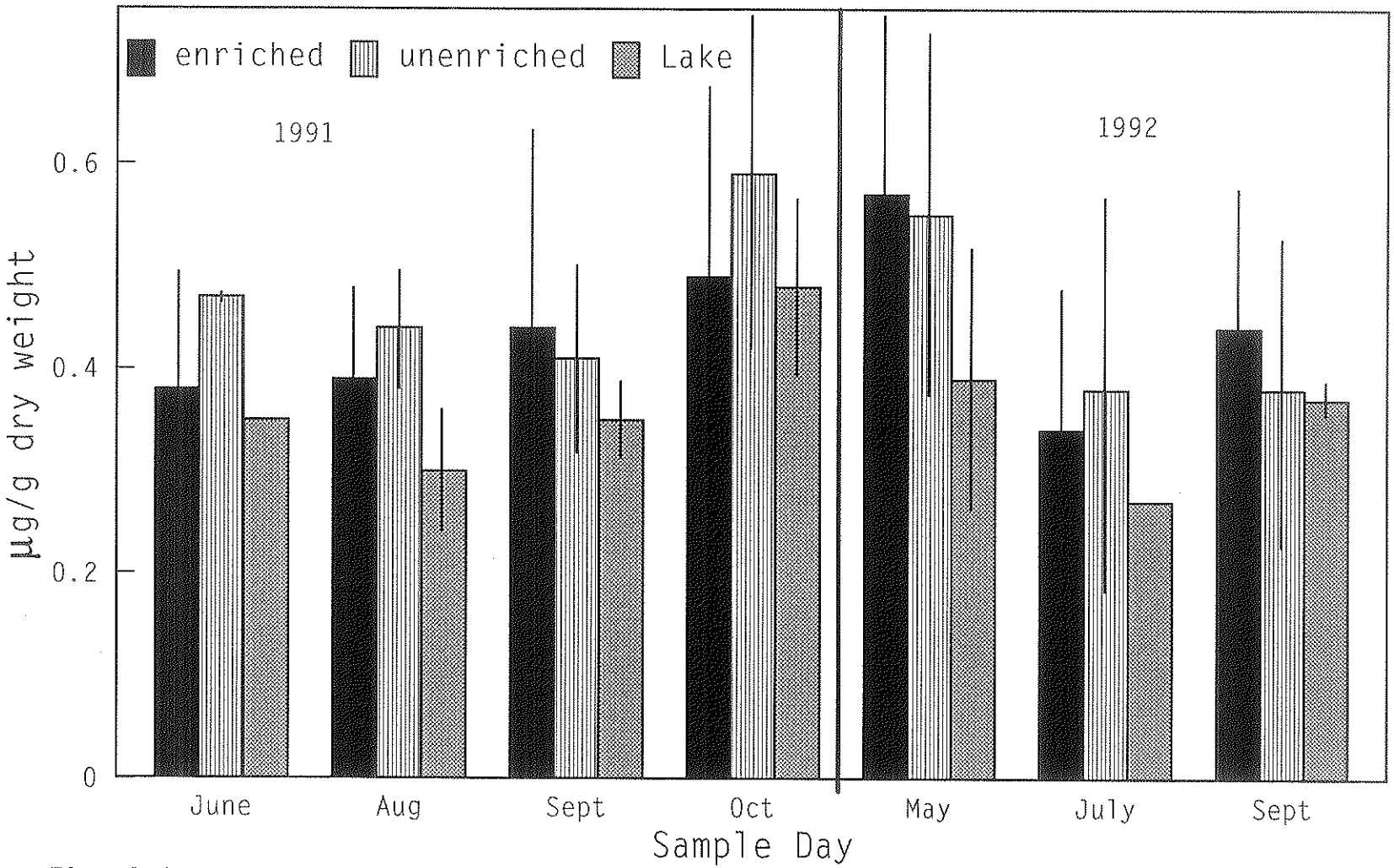

Fig. 2.4. Concentration of cadmium in sediment from littoral mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992. Each bar represents 2 to 4 samples. Error bars equal +/-1 SD.

rations in 1992 of 0.33 to 0.56 μg/g, which were similar to the results obtained with the aqua regia digestion process.

3.2.3. Concentration of Cadmium in Porewater

The concentration of Cd in the pore water was 2 to 48 times higher than in the water column of the mesocosms and in the lake. There was no effect or trend of nutrient enrichment on porewater Cd concentrations (Fig. 2.5). On three of the six sampling dates, Cd in lake pore water was higher than in the mesocosms by a factor of two to three; otherwise, the concentrations were similar. Results in August 1991 showed a significant increase in Cd concentrations in all of the mesocosms relative to the other sampling days so this result may be erroneous.

3.2.4. Acid Volatile Sulfide in Sediment

AVS concentrations in the sediment were extremely low, ranging from 5.7×10^{-3} to 6.16×10^{-2} μmoles/g dry sediment (Table 2.4). There were no treatment effects over the duration of 1992, although this conclusion was based on only two sample dates. There was substantial variation between duplicates for the first two samples; variation decreased with analytical experience (Table 2.4).

3.3. Bioaccumulation of Cadmium in Biota

3.3.1. *Anodonta grandis grandis*

Floater mussels had background Cd levels of 1.1 to 1.3 μg/g dry weight (n=19). The mussels required approximately 88 d to reach steady state concentrations in the mesocosms and in the lake in 1991 but did

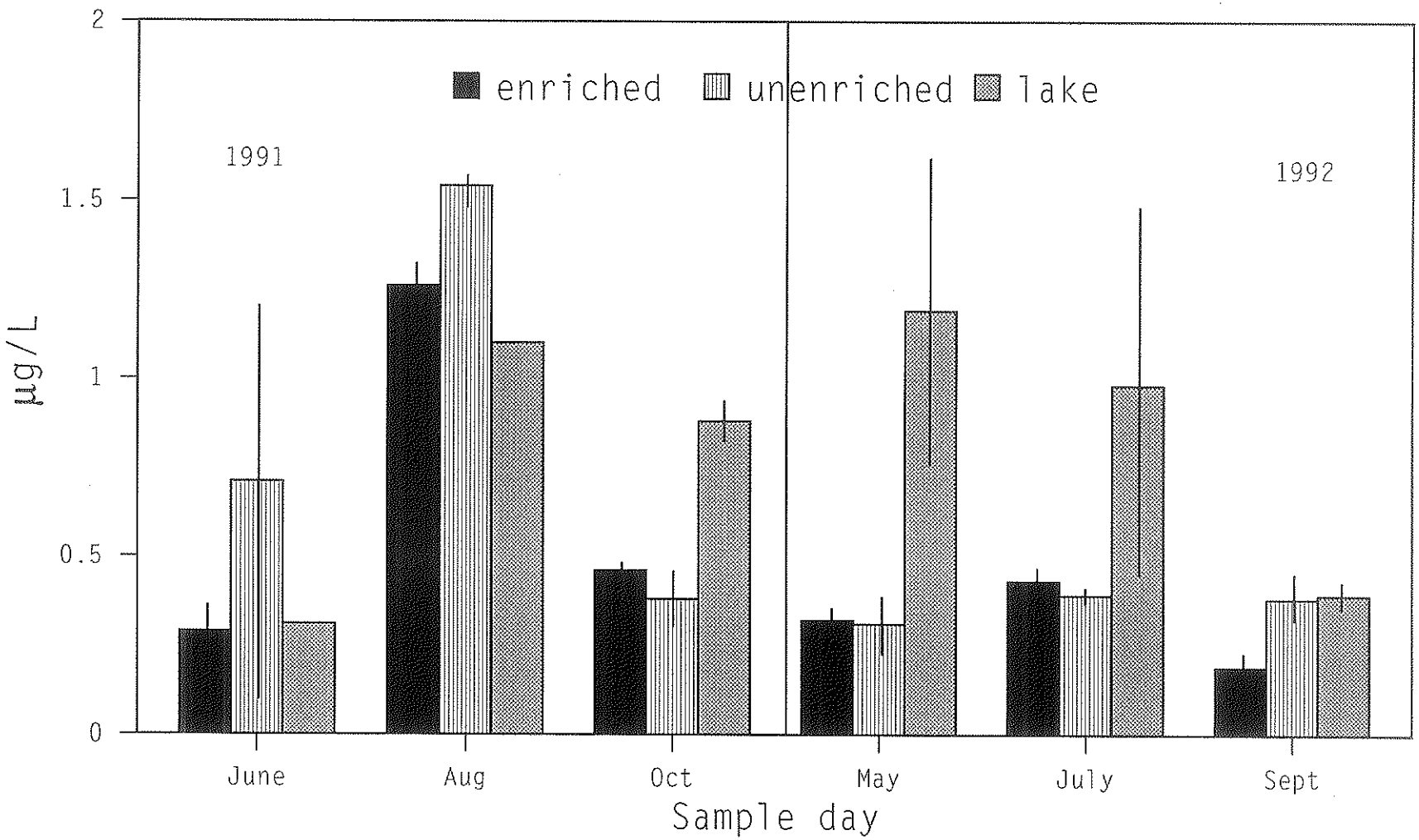

Fig. 2.5. Concentration of cadmium in pore water of sediments from littoral mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992. Error bars equal +/- 1 SD. Each bar represent 1 to 4 samples.

Table 2.4. Summary of acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) analyses on sediment from littoral mesocosms in lake 382, Experimental Lakes Area, 1992.

Analysis number	Treatment	Sample Day	AVS μmol/g ($\times 10^{-2}$)	CV[1] %	[Cd]/[AVS] ratio
1	C2	July 14	1.04 ± 0.75[2]	72.1	0.35 ± 0.08[2]
2	N2	July 14	0.76 ± 0.23	30.3	0.44 ± 0.23
3	N1	July 14	6.16 ± 0.75	12.2	0.05 ± 0.004
4	C1	July 14	0.57 ± 0.06	9.9	0.68 ± 0.01
5	N2	Sept.11	3.23 ± 0.27	8.3	0.15 ± 0.01
6	N1	Sept.11	0.94 ± 0.09	9.0	0.35 ± 0.01

[1] Coefficient of Variation = (SD/Mean) x100

[2] Mean ± SE (n=2)

not appear to reach steady state in 1992 after 126 d of exposure (Fig. 2.6). However, the body burden data (Eq. 2.2) showed that a steady state may have been reached on day 61 in 1992 in the mesocosms and in the lake (Fig. 2.7).

Terminal mean concentrations in 1991 were 7.70 ± 0.79, 8.17 ± 1.16 and 41 μg/g and in 1992, 6.16 ± 1.37, 5.74 ± 1.13 and 22.4 ± 3.19 μg/g dry weight in the unenriched and enriched mesocosms and in the lake, respectively (Fig. 2.6). These concentrations represent a four- to five-times increase in Cd accumulation in mussels exposed to Cd in the lake relative to in the mesocosms.

Bioaccumulation of Cd was not affected significantly by the nutrient additions and increased productivity in the enriched mesocosms (Fig. 2.6). There was a trend for lower bioaccumulation in the enriched mesocosms relative to the unenriched mesocosms in 1992, but mean BAFs were not significantly different ($p<0.05$) (Table 2.5).

3.3.2. Depuration in A. grandis grandis

There was no decline in Cd concentrations in the mussels over the 56 d of clearance (Fig. 2.8a). The slope of the depuration curve (ln C_b vs. t) was not significantly different from zero and in fact had a positive slope with the equation $y= 2.71 + 2.88 \times 10^{-3}x$ ($r^2= 0.04$). However, regression of the ln body burden of Cd against t showed that mussels may have had a slight loss of Cd over time ($y= 3.13 - 4.88 \times 10^{-4}x$, $r^2=0.001$), although the slope was not significantly different from zero ($p<0.05$).

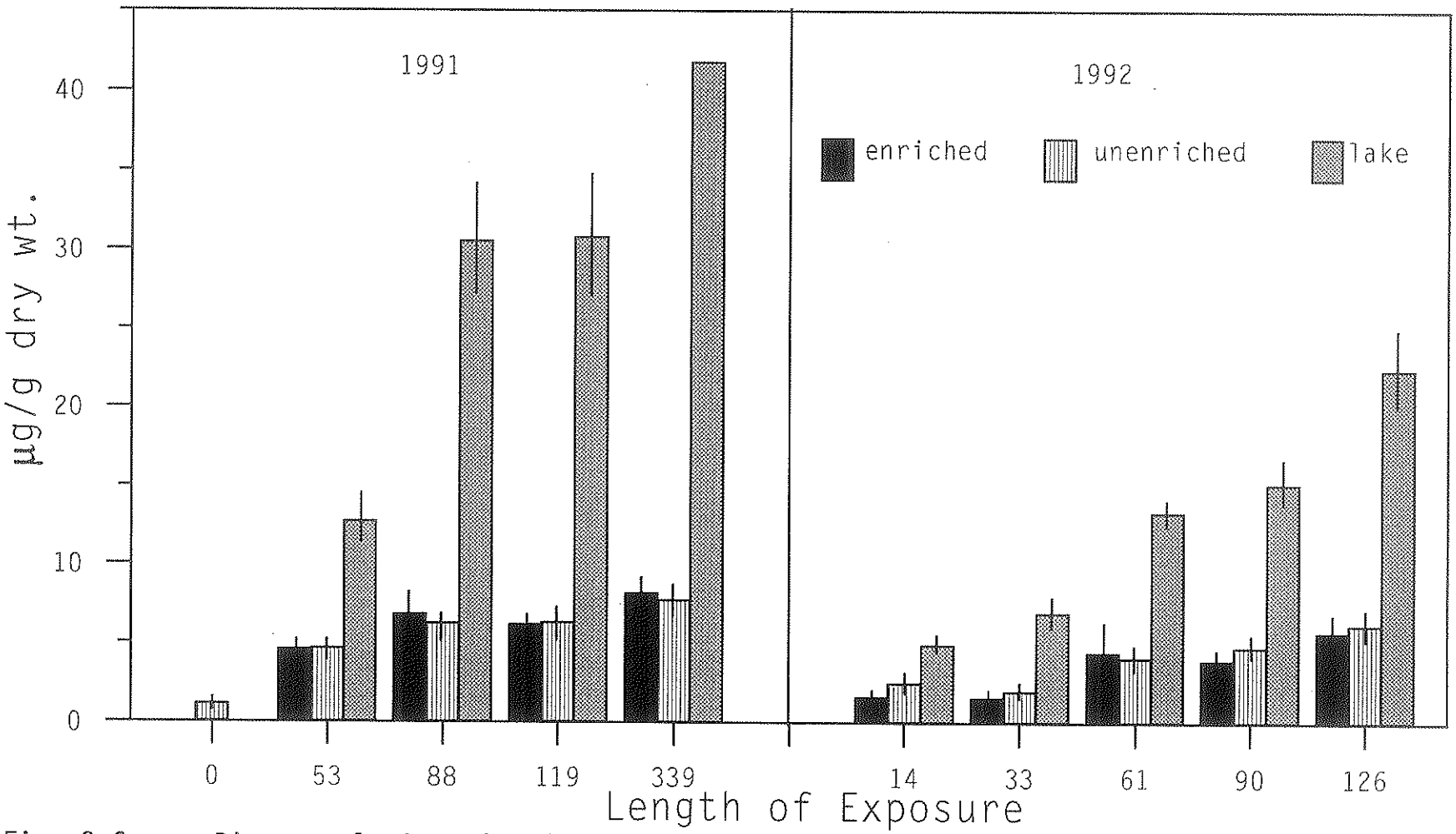

Fig. 2.6 . Bioaccumulation of cadmium in the floater mussel, Anodonta grandis grandis, (Say), in littoral mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992. Error bars equal +/- 1 SD. Each bar represents means of 12 in the mesocosms and 6 in the lake in 1991 and 6 in the mesocosms and 3 in the lake in 1992.

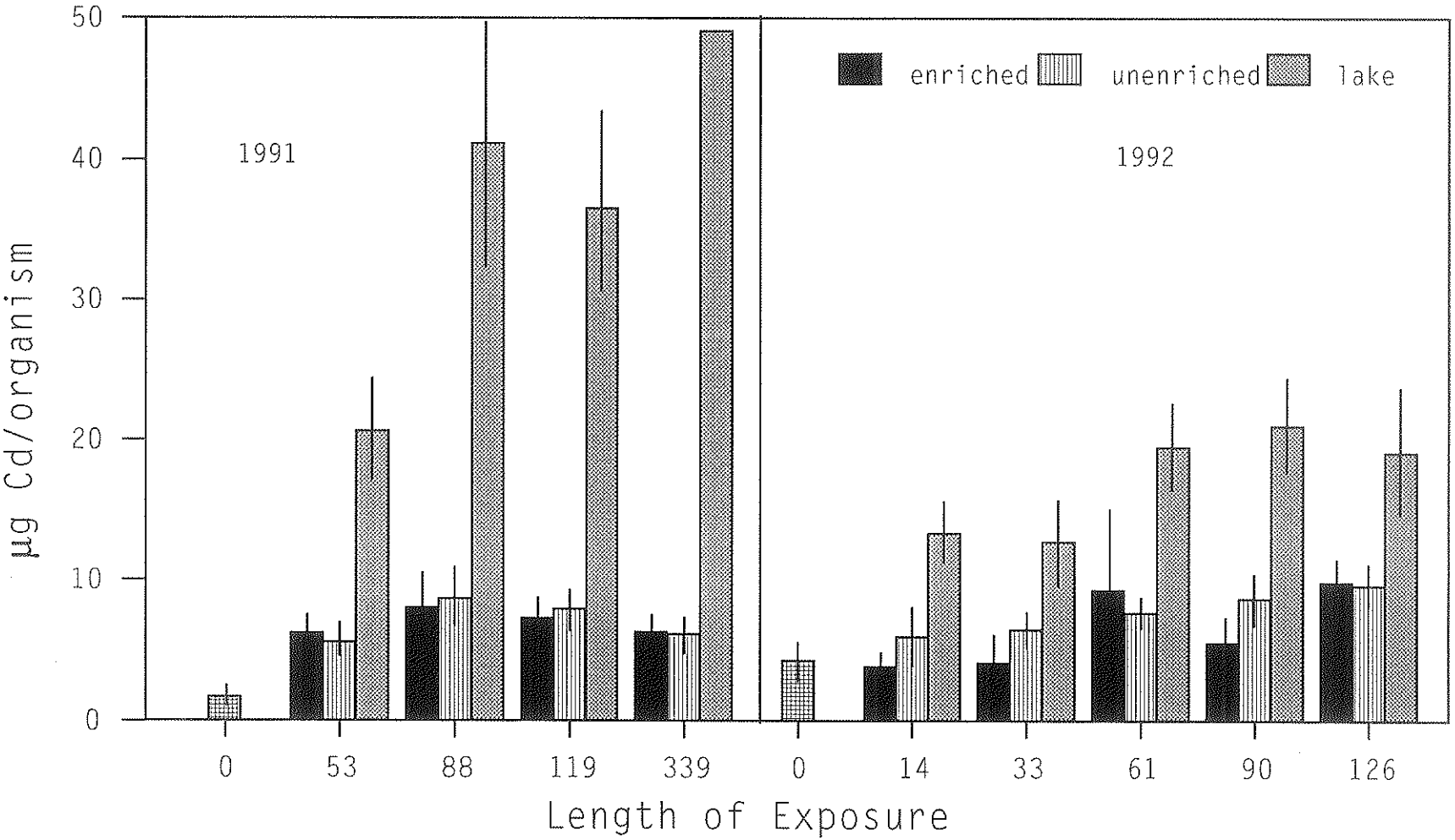

Fig. 2.7. Body burdens of cadmium in the floater mussel, Anodonta grandis grandis, (Say) from littoral mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992. Error bars equal +/- 1 SD. Each bar represents means of 12 in the mesocosms and 6 in the lake in 1991 and 6 in the mesocosms and 3 in the lake in 1992.

Table 2.5. Bioaccumulation factors[1] ($x10^{-3}$) for caged Anodonta grandis grandis from littoral mesocosms and Lake 382, Experimental Lakes Area in 1992.

	Treatment		
Sample Day	Enriched[2]	Unenriched	Lake[3]
14	35.0	72.9	47.1
33	21.7	24.6	44.6
61	70.7	78.2	77.6
90	79.6	204.4	80.5
126	92.7	81.5	144
Mean (±SD)	59.9 ± 30.2	92.3 ± 66.7	78.6 ± 39.9

[1] See equation 2.1.

[2] Calculated from the mean concentration of six organisms on each sample day

[3] Calculated from the mean concentration of three organisms on each sample day.

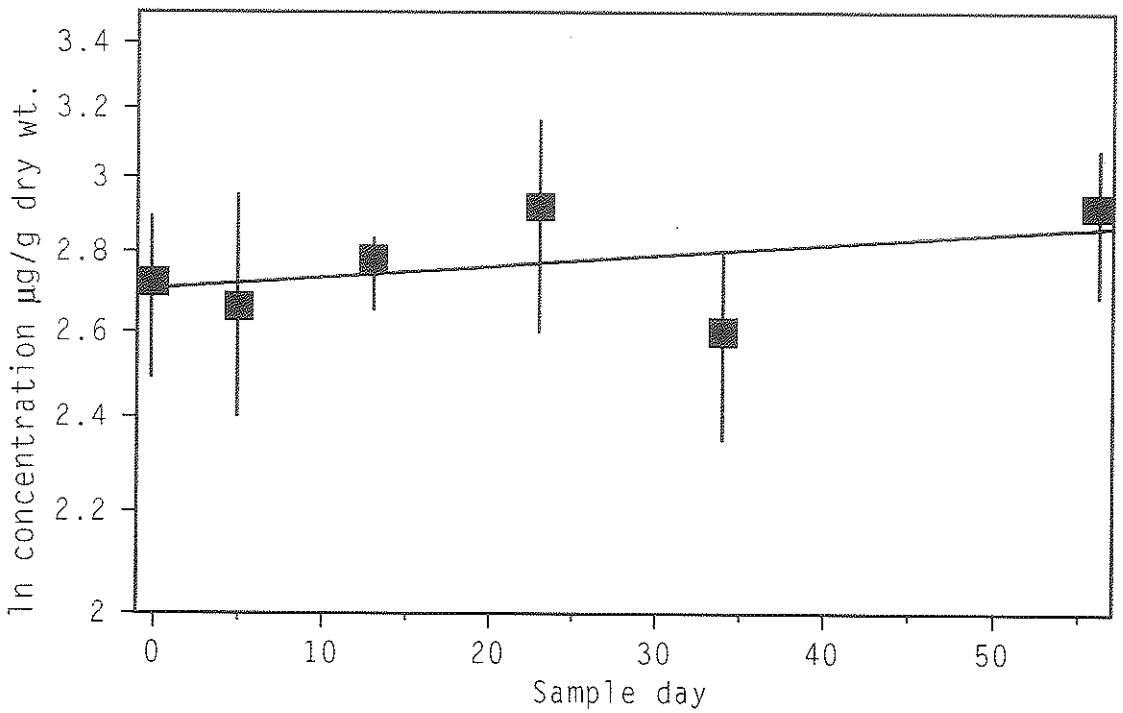

Fig. 2.8a. Depuration of cadmium from the floater mussel, Anodonta grandis grandis (Say), using concentration in organisms. Each point represents mean of 6.

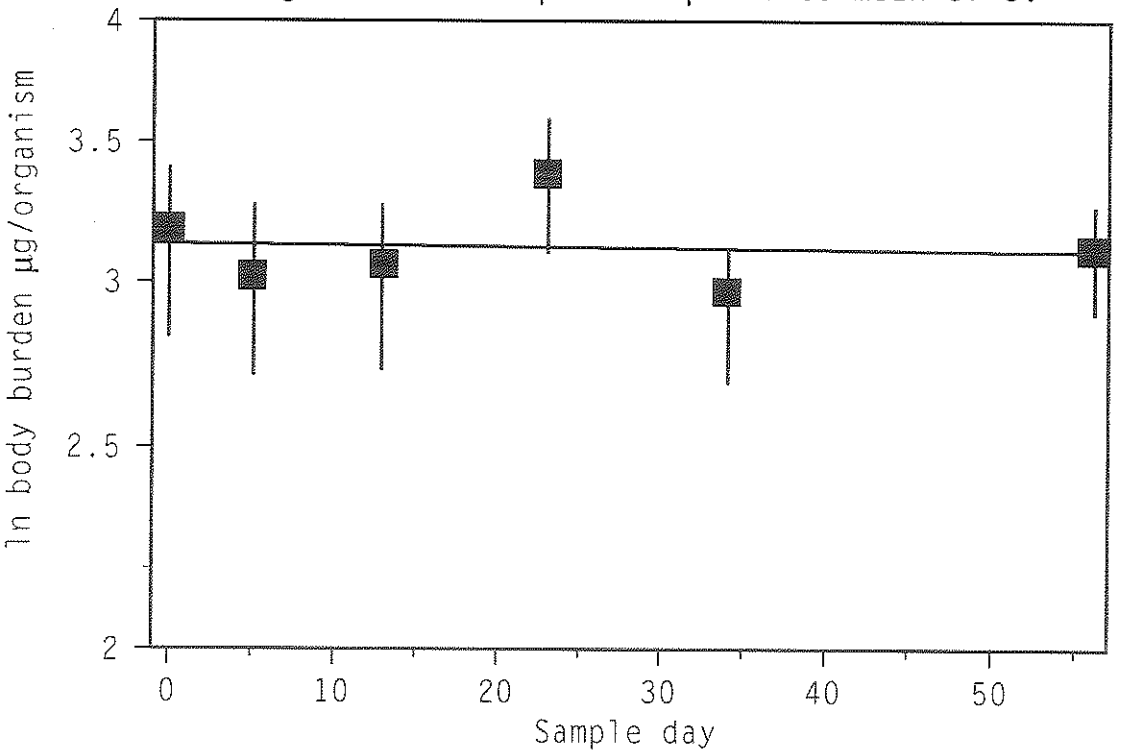

Fig. 2.8b. Depuration of cadmium from the floater mussel Anodonta grandis grandis (Say) using body burdens in organisms. Each bar represent means of 6 organisms.

3.3.3. Orconectes virilis

Background Cd concentrations in crayfish ranged from 0.37 to 0.67 μg/g dry weight (n=20). In 1991, there was no consistent effect of nutrient addition on the bioavailability of Cd to crayfish (Fig. 2.9). There were no significant differences in mean concentrations in experiment 1 and 2 in 1992 (Fig. 2.10).

Crayfish showed the same general pattern as the mussels: higher accumulation of Cd in the organisms from the lake relative to the mesocosms. However, in both 1991 and 1992, only one organism was removed from the lake on the last sample day.

Crayfish accumulated only 12 to 33 % of the Cd concentrations found in the mussels (Table 2.5), thus crayfish BAFs in 1992 were lower than those for mussels (Table 2.6).

3.3.4. Zooplankton (>200 μm)

The zooplankton sampled were mostly copepods. They accumulated approximately the same amount of Cd as the mussels (3.0 - 59.9 μg/g, Fig. 2.11). Zooplankton in the enriched mesocosms accumulated more Cd than those in the unenriched mesocosms except for the October 1991 and September 1992 sample days, but the differences were not significant ($p<0.05$). However, the zooplankton sample weights in July (1.0 mg) and September (0.8 mg) 1992 may have been too small; little confidence can be placed in the analysis of these samples because precision of analysis decreases with sample size (B. Hunt, pers. comm.).

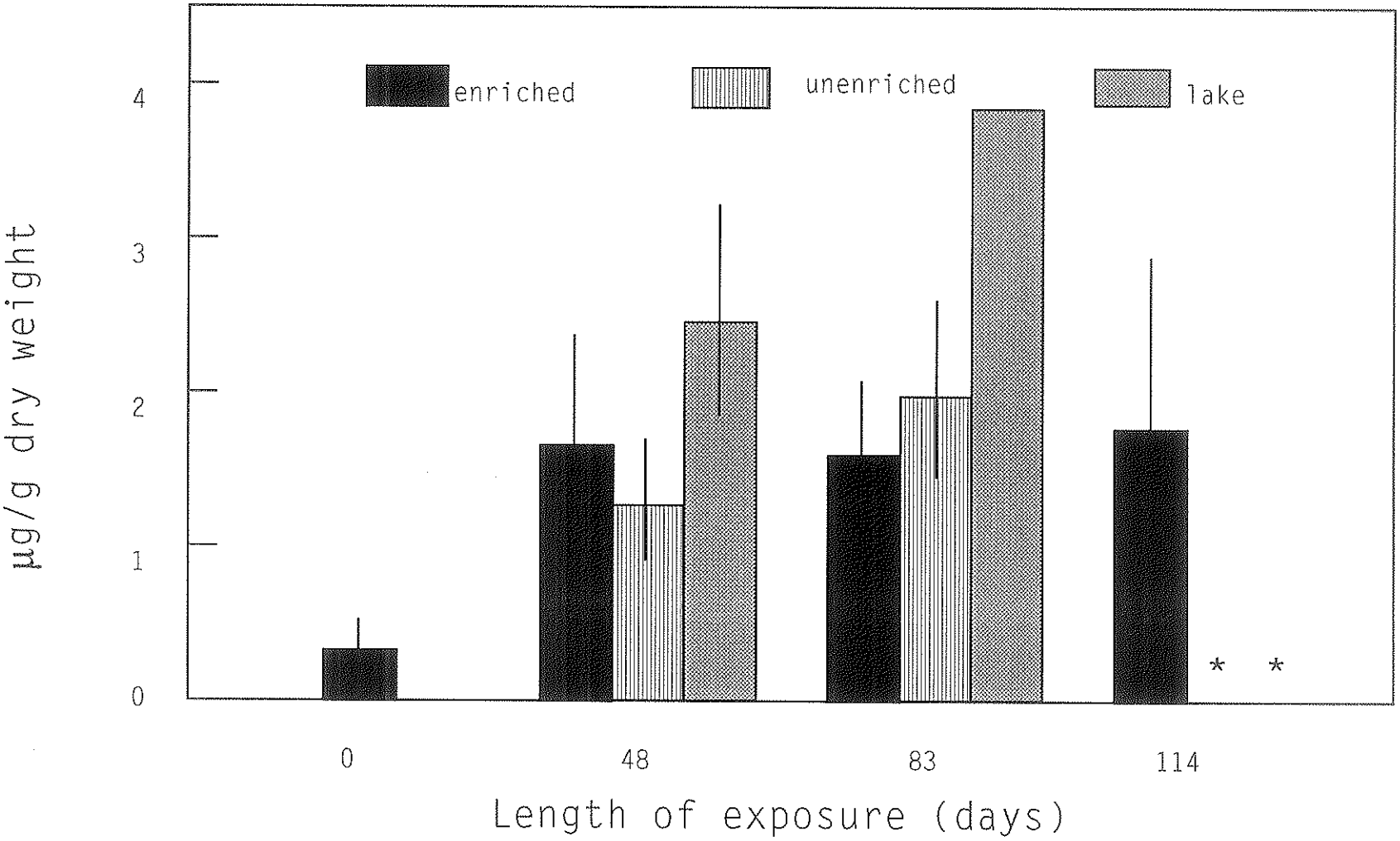

Fig. 2.9. Bioaccumulation of cadmium in crayfish Orconectes virilis (Hagen) from littoral mesocosms and Lake 382, Experimental Lakes Area, 1991. * indicates no sample. Error bars equal +/- 1 SD. Each bar represents between 1-8 organisms.

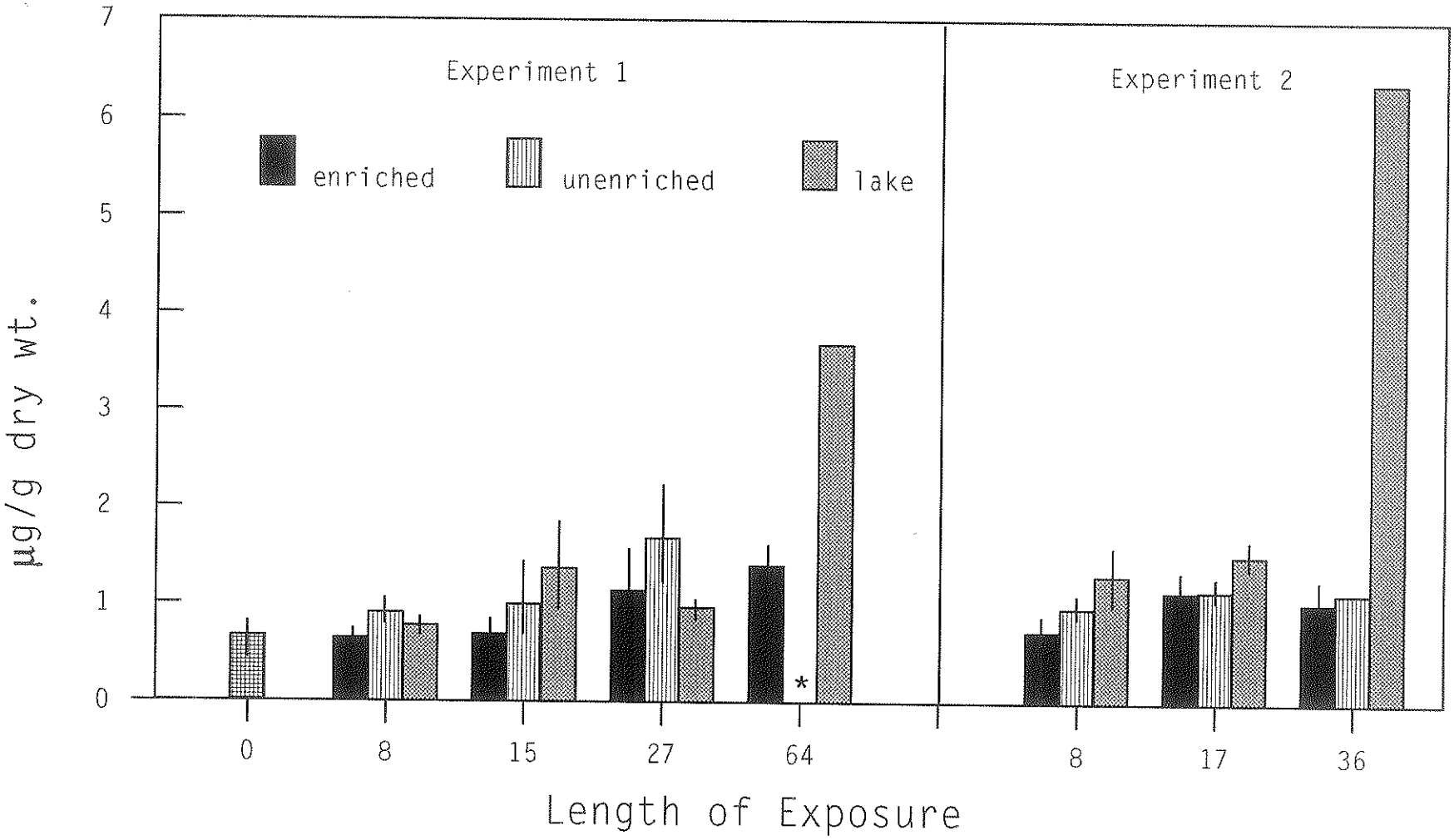

Fig. 2.10. Bioaccumulation of cadmium by the freshwater crayfish Orconectes virilis (Hagen) in littoral mesocosms and Lake 382, Experimental Lakes Area, 1992. * indicates no sample. Each bar represents betweeen 1 and 8 organisms. Error bars equal +/- 1 SD.

Table 2.6. Bioaccumulation factors[1] (x 10^{-3}) for caged crayfish (*Orconectes virilis*) from littoral mesocosms and Lake 382, Experimental Lakes Area, 1992.

		Treatment		
Expt.	Sample day	Enriched	Unenriched	Lake
1	8	14.2	26.5	7.5
	15	9.5	12.3	8.8
	27	15.7	20.7	6.2
	64	23.2	NS[2]	21.5
	Mean ± SD	15.7 ± 5.7	19.8 ± 7.1	11 ± 7.1
2	8	10.1	11.9	8.4
	17	15.7	14.3	9.7
	36	16.9	21.7	37.2
	Mean ± SD	14.2 ± 3.6	15.9 ± 5.1	18.4 ± 16.3

[1] See equation 2.1.

[2] No sample

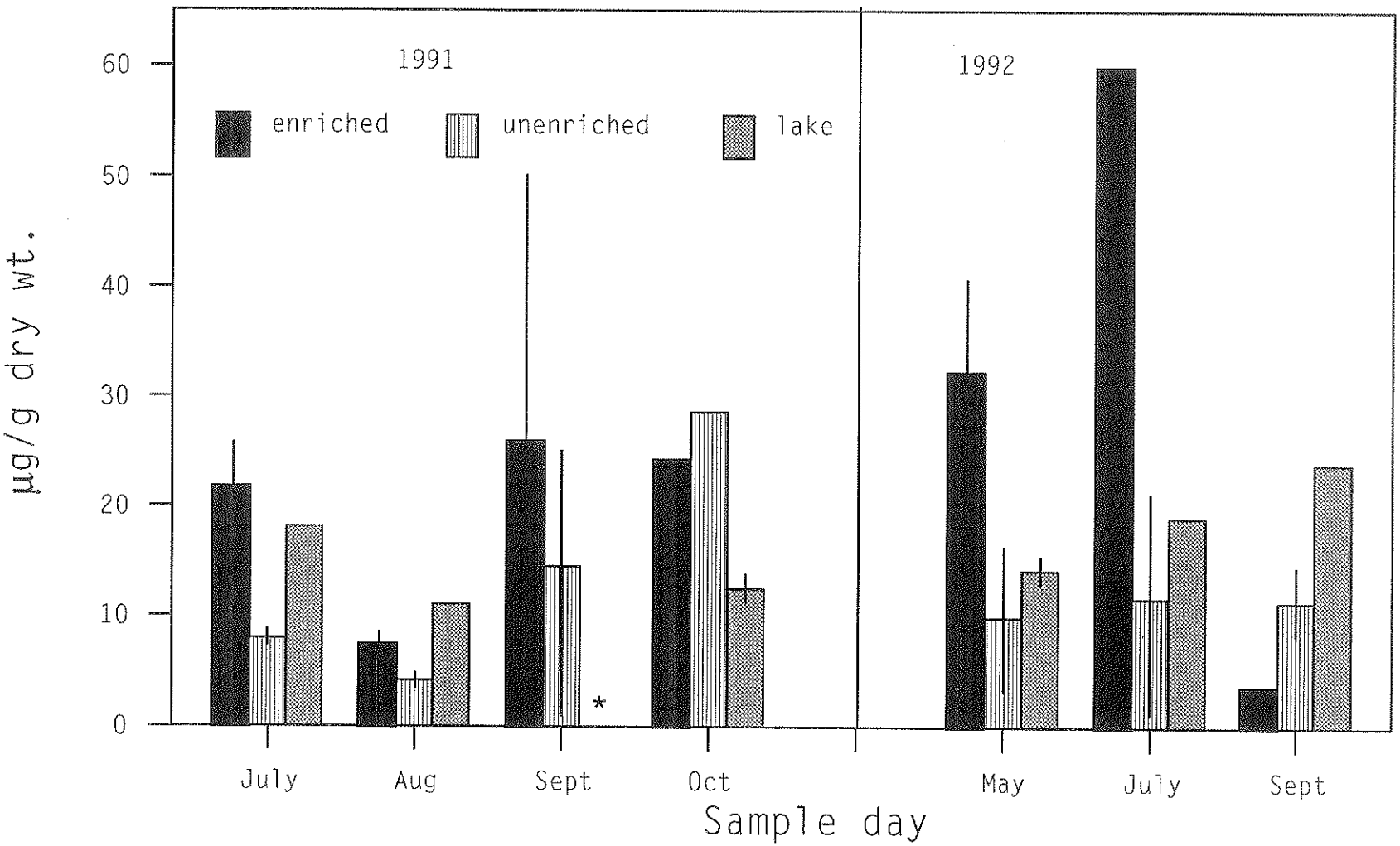

Fig. 2.11. Bioaccumulation of cadmium by zooplankton in littoral mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992. Error bars equal +/- 1 SD. Each bar represents 1 to 2 samples. * indicates sample was pooled with October.

BAFs for zooplankton in 1992 are shown in Table 2.7. BAFs are generally higher but within the same range as for mussels (Table 2.5), and much higher than for crayfish (Table 2.6).

3.3.5. Chironomidae and Hexagenia limbata Nymphs

In 1991 and 1992, larval chironomids in the enriched mesocosms consistently accumulated more Cd relative to the unenriched mesocosms. However, mean concentrations were not significantly different ($p<0.05$) at any sampling time. Bioaccumulation ranged from 0.59 to 1.78 μg/g wet weight (Fig. 2.12) in 1991, with corresponding BSAFs between 10 and 46 (Table 2.8). Cadmium concentrations ranged from 1.2 to 2.6 μg/g in 1992 with BSAFs between 22 and 91 (Table 2.8).

Nutrient additions had no consistent effect on the bioaccumulation of Cd in mayfly larvae, H. limbata. Larval mayflies accumulated Cd in the range of 0.1 to 0.7 μg/g wet weight (Fig. 2.13), with corresponding BSAFs of 2 to 14 (Table 2.8). There were no data for H. limbata larvae in 1992 because of a lack of organisms.

3.3.6. Emerging Insects

In 1991, emerging Diptera (mostly Chironomidae) had Cd concentrations ranging from 0.18 to 4.58 μg/g wet weight. There were no effects or trends attributable to the nutrients in the enriched mesocosms (Fig. 2.14). Diptera in the lake and mesocosms accumulated similar amounts of Cd, 0.49 to 1.76 and 0.18 to 4.58 μg/g wet weight, respectively.

In 1992, emerging Diptera were rare; therefore, only two sample

Table 2.7. Bioaccumulation factors[1] (x 10^{-3}) for zooplankton from littoral mesocosms and Lake 382, Experimental Lakes Area, 1992.

Sample Day	Enriched	Unenriched	Lake
May	705	286	136
July	59.9[2]	143	122
September	3.6[2]	487	127
Mean ± SD	256 ± 390	305 ± 173	128 ± 7.1

[1]See equation 2.1.

[2]Estimated Cd concentration due to low sample weights.

Table 2.8. Biota sediment accumulation factors (BSAFs) for invertebrates collected from the sediment of mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992.

Taxon	BSAF		
	Enriched	Unenriched	Lake
Hexagenia limbata 1991			
July	7.6	9.6	7.1
August	8.7	2.2	11.7
October	13.7	NS[1]	7.8
Mean ± SD	10 ± 3.3	5.9 ± 5.2	7.8 ± 3.6
Chironomidae 1991			
July	NS	NS	38.9
August	45.6	17.1	NS
October	10	28	NS
Mean ± SD	27.8 ± 25.2	22.6 ± 7.7	38.9
Chironomidae 1992 May	40.7	22.4	58.7
July	76.8	52.4	90.7
September	49.8	36.1	31.9
Mean ± SD	55.8 ± 18.8	36.9 ± 15	60.4 ± 29.4

[1] No sample collections were available.

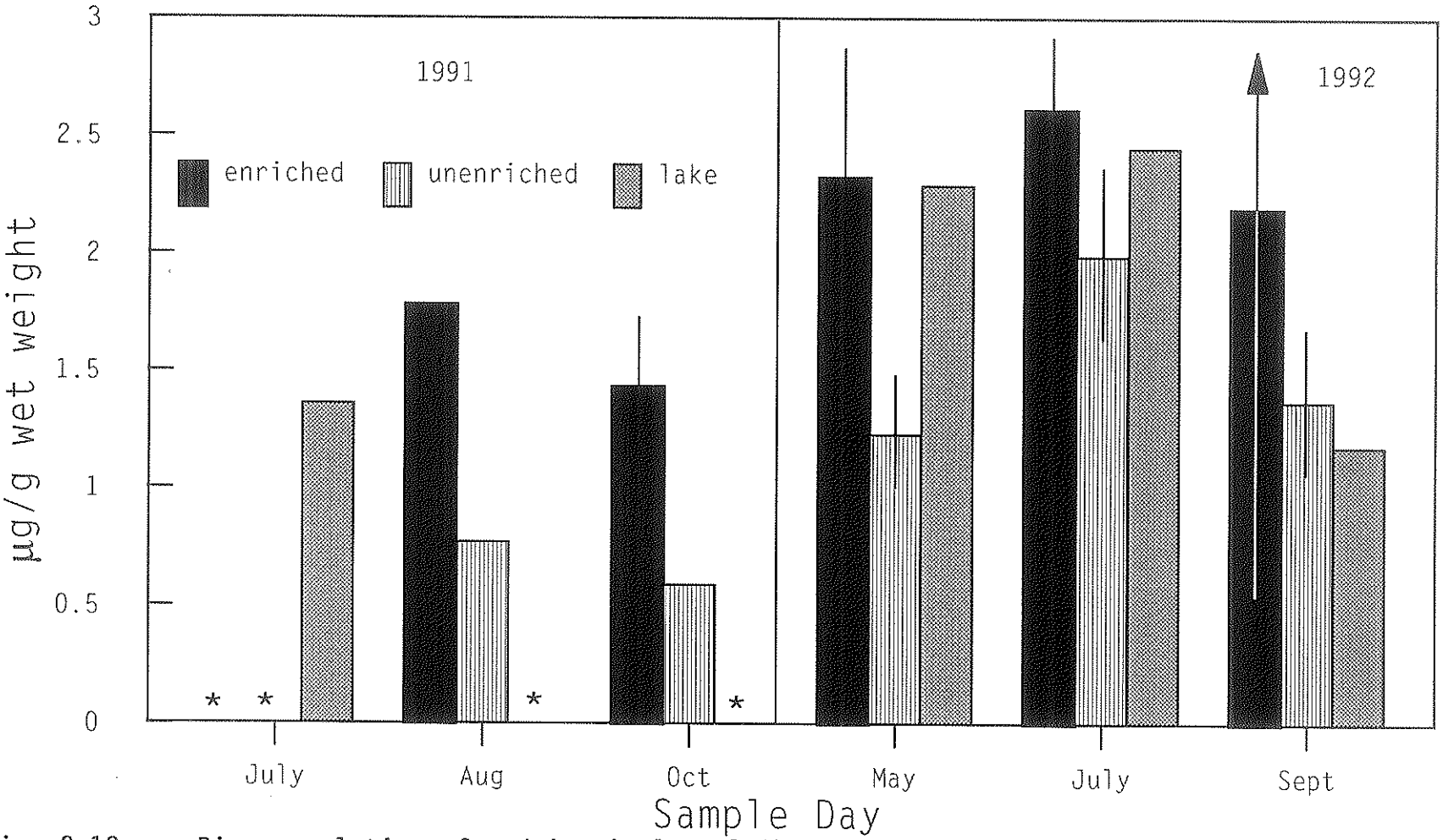

Fig. 2.12. Bioaccumulation of cadmium in larval Chironomidae from sediment in littoral mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992. Error bars equal +/- 1 SD. Arrow indicates SD bar continues above graph. * indicates no sample. Each bar represents 1 to 2 sets of pooled organisms.

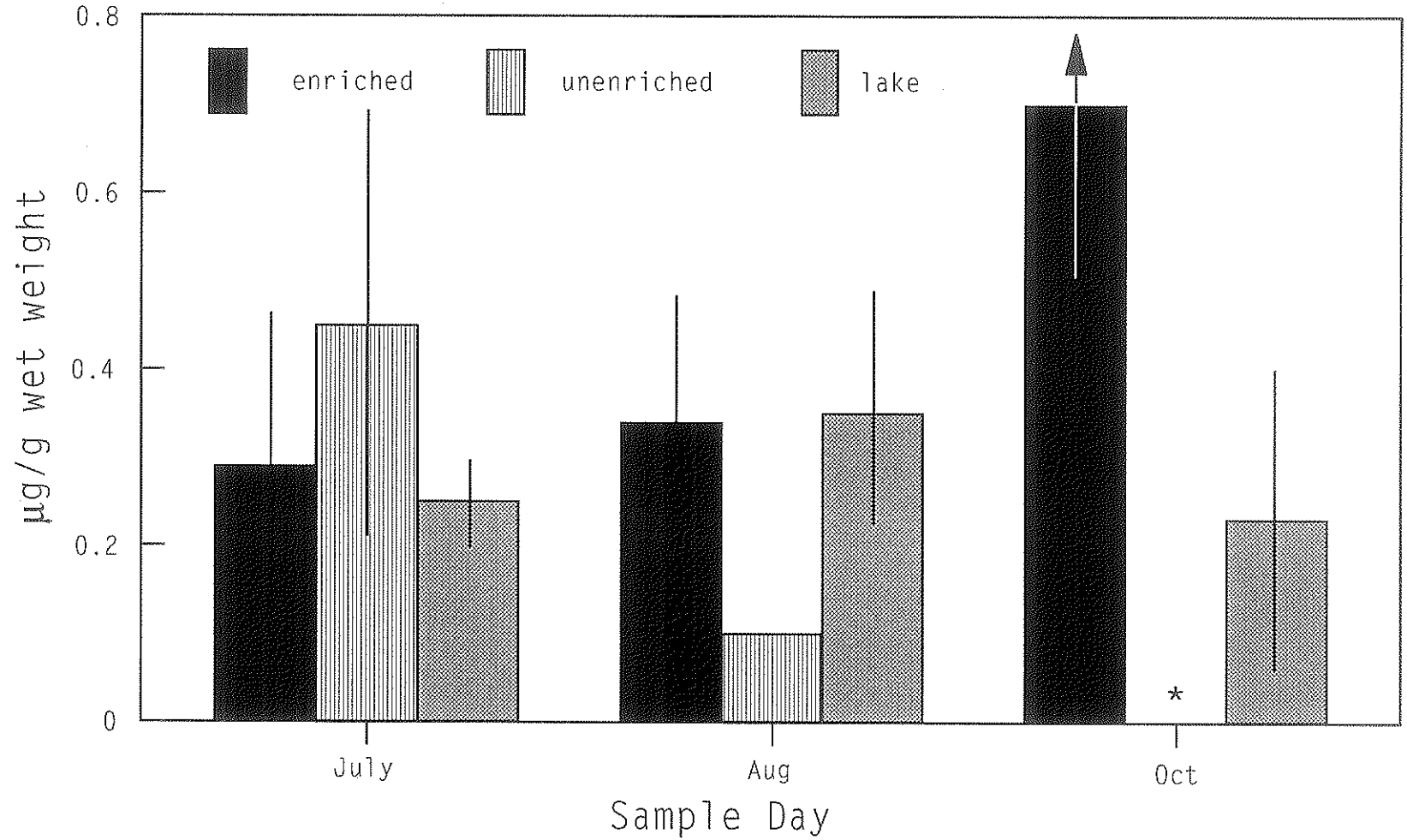

Fig. 2.13. Bioaccumulation of cadmium in larval Hexagenia limbata from sediment in littoral mesocosms and Lake 382, Experimental Lakes Area, 1991. Error bars equal +/- 1 SD. Arrow indicates SD bar continues above graph. * indicates no sample. Each bar represents between 1 and 11 organisms.

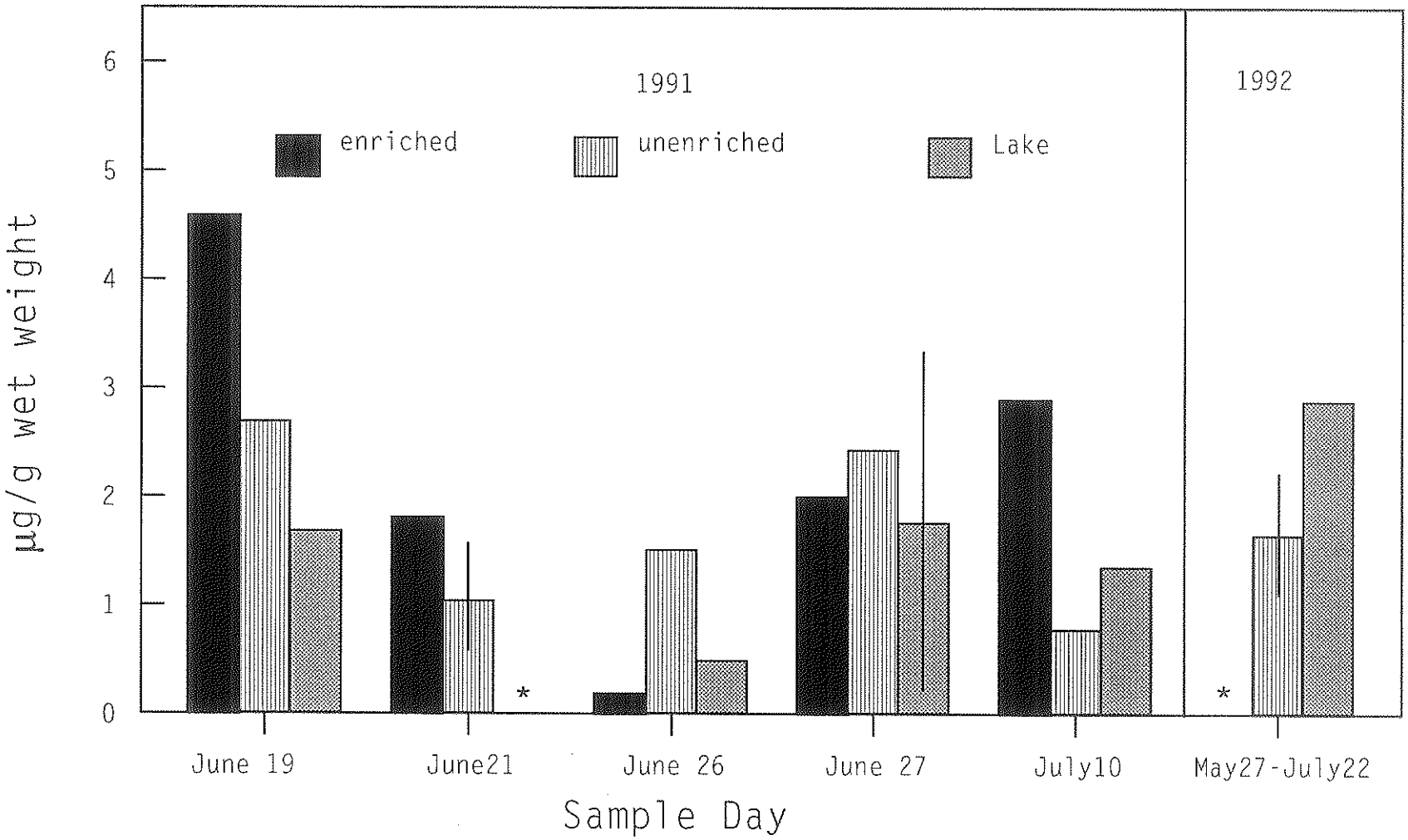

Fig. 2.14. Concentration of cadmium in emerging Diptera from littoral mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992. * indicates no sample. Each bar represents sample sizes of 1-3 sets of pooled organisms. Error bars equal +/- 1 SD.

days were available. When the data were pooled the levels were much the same as 1991 (1.28 to 2.88 μg/g wet weight; Fig. 2.14).

Emerging H. limbata in 1991 had concentrations of Cd ranging from 0.07 to 0.72 μg/g wet weight in the mesocosms. Over all sample days, the organisms from all mesocosms accumulated the same amount of Cd (Fig. 2.15).

In 1992, the concentrations were quite similar (0.38 to 0.66 μg/g wet weight). Emerging Hexagenia from the lake had just under half of the Cd as organisms emerging from the mesocosms (Fig. 2.15).

4. DISCUSSION

4.1. Water

4.1.1. Nutrient Additions

The amount of chlorophyll a in the enriched mesocosms in 1992 is within the range generally associated with mesotrophic or eutrophic lakes (3-11 and 3-78 μg/L, respectively; Wetzel 1983). Levels in the unenriched mesocosms and the lake are more closely associated with oligotrophic lakes (0.3-4.5 μg/L, Wetzel 1983). Although a portion of the increase in chlorophyll a observed in 1992, relative to 1991, may be attributable to the increased nutrient additions, it is also possible that sediment adsorption of the nutrients took place to a greater extent in 1991 than 1992. Schindler et al. (1971) found that 62 % of added phosphorus and 44 % of added nitrogen was lost to the sediment during the first year of nutrient additions to L227 at the ELA.

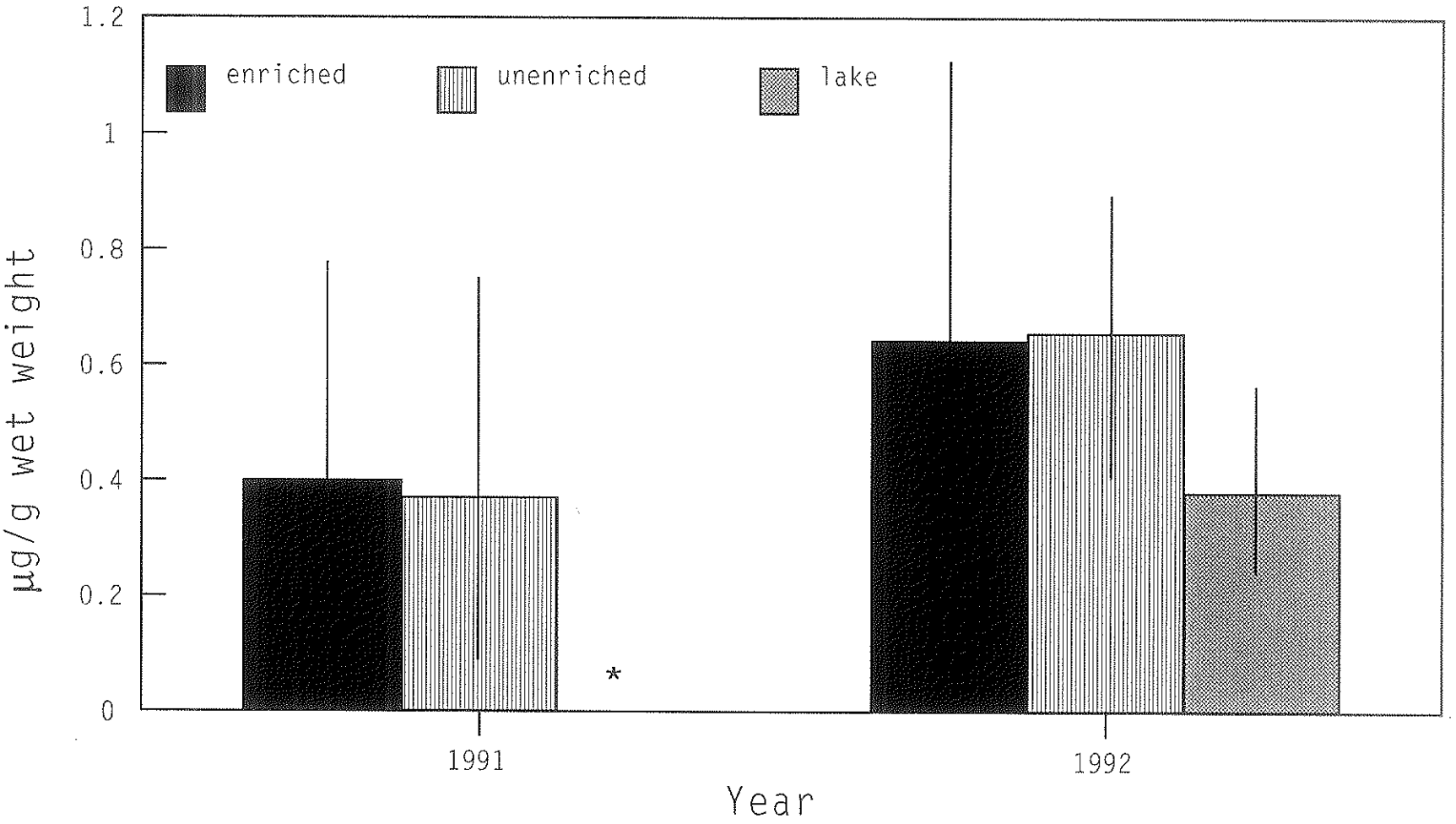

Fig. 2.15. Concentrations of cadmium in emerging Hexagenia limbata from littoral mesocosms and Lake 382, Experimental Lakes Area, 1991 and 1992. * indicates no sample. In 1991 bars represent means of 17 and 25 organisms from unenriched and enriched mesocosms, respectively. In 1992 bars are means of 3-6 organisms. Error bars equal +/- 1 SD.

4.1.2. Cadmium Concentrations in Water

Concentrations of Cd in the water column of the mesocosms are within the same range as those found in central Ontario lakes (<2 - 120 ng/L; Stephenson and Mackie 1988a) and in many European lakes (Laxen 1984, Borg and Andersson 1984). Less than 100 ng/L is considered to be uncontaminated (Stephenson and Mackie 1988a, Laxen 1984). Therefore, the mesocosms are not considered to be contaminated and the lake is considered to be only slightly contaminated. Concentrations in "remote" lakes in central Ontario range from non-detectable (<2 ng/L) to 13 ng/L (Stephenson and Mackie 1988a), so concentrations seen in L382 are more significant relative to these remote lakes.

Nutrient additions may have increased the amount of Cd in the water column of the enriched mesocosms relative to the unenriched mesocosms (Fig. 2.3). Because there was an increase in the amount of particulates in the water column in the form of chlorophyll _a_ (Fig. 2.2a,2.2b), phytoplankton (L. Hendzel pers. comm.) and bacteria (M. Holoka per. comm.) available binding sites for Cd were increased. Particulate and dissolved organic matter in the water column will enhance the apparent solubilities of contaminants in water (Hart 1982, Servos 1988). Thus, concentrations will appear to increase in unfiltered water with high DOM and POM. However, the bioavailability of the complexed-Cd from the water column of the enriched mesocosms may be decreased relative to the unenriched mesocosms.

The trace amounts of Cd that were present in the $NaNO_3$ fertilizer were not a factor in increasing Cd concentrations in the water column of the enriched mesocosms. If the mesocosms were approximately 34000 L,

then the total amount of fertilizer-associated Cd would account for less than 62 and 98 pg/L of additional Cd to the enriched enclosures (1991 and 1992 respectively). This is, on average, less than 0.12 % of the Cd present in the water column and would be below the detection limit of the GFAA.

4.2. Sediment

Aquatic sediments are the ultimate sink for trace metals from both natural and anthropogenic sources (Luoma 1983, McCarthy and Black 1988). However, these sediment-bound metals remain bioavailable to organisms, especially to benthic organisms, which spend most or all of their life cycle in close association with the sediments (Knezovich et al. 1987). The bioavailability of these metals depends on factors such as the geochemical characteristics of the sediment (e.g. organic carbon content, mineral content: Bendall Young et al. 1992, Hart 1982) and associated components such as interstitial water.

The nutrient additions did not affect the concentrations of Cd in the sediment of the enriched mesocosms. A decrease in sediment concentrations, thus bioavailability, would have taken longer than the two-year duration of this study. Increased sedimentation of seston may decrease concentrations of Cd in the sediment by dilution, increase organic matter content of the sediment (Thomas 1984), and increase sulfide content (Wetzel 1983, DiToro et al. 1990). A combination of these factors may have resulted in decreased Cd concentrations and bioavailability in the sediment (Hart 1982, Allan 1986).

Cadmium concentrations in the sediment of the enclosures and the

littoral sediments from the lake itself are similar to concentrations seen in unimpacted lakes in both Manitoba and Ontario. Stephenson and Mackie (1988a) reported levels of 0.01 to 2.51 μg/g dry weight in littoral sediments from small lakes in central Ontario. Harrison and Klaverkamp (1990) reported quantities ranging from <1 to 56 μg/g dry weight in northern Manitoba, with highest levels in lakes closest to, and downwind of, the Hudson Bay Mining and Smelting Co. in Flin Flon, MB. Lake 375 at ELA has only received Cd through atmospheric deposition and has levels of approximately 0.1 to 0.16 μg/g dry wt. (R. Currie unpublished data).

4.2.1. Pore Water

Contaminants in the pore water of sediment are thought to be more important for bioavailability and toxicity, than contaminants found in the bulk sediment (DiToro et al. 1990, Landrum 1989, Kemp and Swartz 1988, Swartz et al. 1985). Porewater contaminants are an especially important route of exposure for infaunal organisms because direct exposure to concentrated contaminants is possible (Swartz et al. 1985).

The partitioning of Cd into interstitial water is affected by the grain size of the sediment, the organic content of both the water column and the sediment (Landrum et al. 1987) and the AVS content (DiToro et al. 1990). These factors will affect concentrations, toxicity and bioavailability of contaminants in interstitial water and can lead to higher contaminant concentrations than found in the water column (Fowler et al. 1978, Knezovich et al. 1987) In this study, the concentration of Cd in the porewater was 2 to 48 times higher than in the water column.

The high levels of Cd in the porewater may be caused by increased dissolved organic matter content in the interstitial water, which would subsequently increase binding sites and concentrations. However, no determinations of the portion of Cd associated with DOC were made so the bioavailability of DOC-associated Cd could not be determined.

Nutrient additions did not affect concentrations of Cd in the pore water of the enriched mesocosms perhaps because of the short duration of the experiment. Over time, increased particulate matter or seston in the enriched mesocosms would be expected to cause changes in the partitioning of Cd from the water column and sediments to the pore water. Increased organic matter associated with the sediments could complex Cd and prevent diffusion of Cd into the sediments and pore water. Alternatively, increased POM and DOM could increase porewater concentrations because of entrainment of Cd-associated particles into the pore water (Hart 1982). Because the experiment was of a short duration the conclusive effect of nutrient additions on Cd porewater concentrations is unclear.

4.2.2. Acid Volatile Sulfide

If the Cd:AVS ratio in sediment is less than or equal to one, toxicity to organisms is not observed, ratios of greater than one are associated with lethality of Cd (Carlson et al. 1991, DiToro et al. 1990). In contrast, ratios of Cu:AVS are unimportant when determining toxicity to amphipods and can result in a conservative estimate of the acute toxicity of a sediment (Ankley et al. 1993). Because Cd:AVS ratios

were less than one in this study (Table 2.4) the Cd would appear not to be toxic to the various organisms within the mesocosms and the lake (DiToro et al. 1990, Carlson et al. 1991), however, Cd still remains bioavailable.

The concentration of AVS found in the littoral sediments of the mesocosms and L382 are quite low ($5.7x\ 10^{-3}$ to 6.16×10^{-2} μmol/g dry sediment) in comparison to levels reported by DiToro et al. (1990) in the Great Lakes (0.31 - 112 μmol/g dry sediment). The low values observed in this study are most likely caused by the low concentrations of organic carbon found in the sediment (Table 2.3), which would result in less sulfide formation. Carlson et al. (1991) found that sediment with 10.6, 1.5 and 1.8 % total organic carbon contained respectively, 42 ± 8, 8.8 ± 1.4 and 3.6 ± 1.6 μmol of AVS/g dry sediment. Other measurements of AVS in L382 have also shown that levels are low in the littoral sediments, ranging from non-detectable to 2 μmol/g dry sediment but levels increase in hypolimnetic sediments, ranging from 1 to 4 μmol/g dry sediment (C. Baron, Freshwater Institute, unpublished data).

4.3. Bioaccumulation of Cadmium in Biota

For the purposes of this discussion the terms "water route" and "sediment route" need to be defined in the context that they will be used. "Water route" will refer to any Cd accumulated via the water, which includes: (1) particle-associated Cd taken up as a result of filter feeding and (2) simple passive uptake via gills and adsorption to the exoskeleton of the organism. "Sediment route" refers to any Cd accumulated via the sediment which include: (1) particle-associated Cd

accumulated from the sediment as food and (2) passive uptake via gills and adsorption to the exoskeleton as a result of contact with pore water and sediment-associated Cd. It is impossible to break down the bioaccumulation routes further in this study.

4.3.1. *Anodonta grandis grandis*

Mussels caged in L382 accumulated concentrations of Cd during each year of the study that were similar to levels in natural populations of mussels during the first year of Cd additions to L382 (1987). Malley *et al*. (1989) found total Cd concentrations in soft tissues ranging from 18.6 to 27.2 μg/g dry weight over an exposure period of 120 d. In this study, concentrations in the mussels caged in the lake after 119 d exposure (1991), averaged 30.9 ± 4.26 μg/g dry weight and after 126 d (1992), 22.4 ± 3.19 μg/g dry weight. The similarity in results may indicate that the mussels in this study were not stressed significantly when placed into cages. Stress would be expected to produce increased filtration rates and therefore higher Cd accumulation than observed by Malley *et al*. (1989).

The concentrations of Cd seen in mussels in this study resemble concentrations reported in other lakes in North America and Europe. Average Cd concentrations in *Elliptio complanata* in small southern Ontario lakes, ranged from 0.5 to 7.5 μg/g dry weight (Campbell and Evans 1991). Luten *et al*. (1986) found Cd levels in *Mytilus edulis* of approximately 8.3 μg/g dry weight in a "polluted" estuary in the Netherlands.

Lower accumulation of Cd observed in 1992 in mussels caged in L382

may be a result of decreased Cd additions to the lake in 1992 (1117 g Cd in 1992; 2143 g and 211 mCi ^{109}Cd in 1991), but may also have been a result of lower water temperatures observed in 1992 than 1991. In 1991, the average epilimnion water temperature over the ice-free season was approximately 17.3 °C; in 1992 it dropped to 14.2 °C (D. Cruikshank, Freshwater Institute, Winnipeg, MB, unpublished data). Decreased filtration rates brought about by lower water temperatures may have lowered bioaccumulation by the mussels (Burky 1983, De Bruin and Davids 1970, Jenner 1991). Although biota accumulate increased amounts of Cd when exposed to higher concentrations of Cd (McCracken 1987, Luoma 1983), the fairly constant concentration of Cd in the water column in 1991 and 1992 make the water temperature decrease the most plausible explanation for the decreased accumulation seen in 1992.

This study tested the hypothesis that nutrient additions would affect the bioavailability of Cd to biota. BAFs for mussels indicated a slight decrease in bioavailability of Cd in the enriched mesocosms relative to the unenriched and lake organisms (Table 2.5), although these differences were not significant ($p<0.05$; t-test). The trend for a decrease in BAFs in mussels from the enriched mesocosms may have been caused by increased bacteria and algae (described as chlorophyll *a*; Fig. 2.2b), in the water column of the enriched mesocosms. Reduced bioavailability of Cd to mussels or other bivalves in the presence of POM, DOM and other ligands that may be present in both the water column and sediment has been reported in a number of studies (Campbell and Evans 1987, 1991, Breteler and Saksa 1985, Graney *et al*. 1984). Hemelraad *et al*. (1986), Elder and Collins (1991), Graney *et al*. 1984,

and Ray and McLeese (1987) show Cd BAFs ranging from 1700 to 10,000,000, which is within the same range observed in this study.

The low elimination rate obtained in this study indicates that Cd is firmly incorporated in the mussels. The regression line was not significantly different from zero thus no loss of Cd was detected in the mussels after 56 d of clearance. Literature values of $t_{½}$ for Cd in mussels are limited but the present results are in agreement with the sparse literature available. For example, the half life of Cd in M. edulis has been estimated to be either as short as 14 to 29 d (Ray 1984) or lengthy where no loss of Cd was measurable (Luten et al. 1986). The $t_{½}$ of Cd in oysters ranges from 30-85 d (Denton et al. 1981). Diane Malley (pers. comm.) has observed a step wise accumulation rate from year to year in L382 mussels with no loss of Cd over the winter months when Cd was not added to the water column, which also implies a long half life. However, the mussels in her studies are natural L382 populations so they remain exposed to Cd in the sediment and water during the winter months when Cd additions are discontinued.

The long $t_{½}$ of Cd in mussel tissues is likely a result of the storage mechanism (Jenner et al. 1991, George and Coombs 1977). In most organisms, cadmium is stored in the form of metallothionein, metallothionein-like proteins or membrane-limited vesicles, or all of these mechanisms (Ray and McLeese 1987). Cd is most likely stored in the mussel tissues as a Cd-thioneine complex (George and Coombs 1977), i.e. a metallothionein or sulfur-containing protein (Talbot and Magee 1978). This essentially isolates the Cd and immobilizes it from interfering with cellular mechanisms (Ray and McCleese 1987). The

detoxification process contributes to the long retention time of Cd in mussels.

Accumulation of Cd by mussels in the mesocosms was lower than in the lake itself because the mesocosms were sealed, essentially isolating their interiors from any new additions of Cd to the lake. In fact, concentrations of Cd in the water column of the lake were two to six times higher than in the mesocosms; however, sediment concentrations were similar. Because of higher concentrations of Cd in the lake water, mussels accumulated four to five times more Cd than the mussels inside the mesocosms (Fig. 2.6.). This result indicates that, for mussels, the water route of exposure is more important than the sediment route.

4.3.2. O. virilis

Initial concentrations of Cd in crayfish from uncontaminated L468 (0.37 - 0.67 μg/g) were similar to background levels of Cd in other ELA crayfish (0.10 - 0.22 μg/g dry wt.; France 1987). There were no significant differences between the accumulation of Cd in the enriched and unenriched treatments; however, there were slightly lower BAFs in crayfish from the enriched mesocosms relative to the unenriched on every sample day (Table 2.6). As discussed in the case of the mussels, this may indicate that the nutrients had a small effect on the bioavailability of Cd.

The omnivorous habits of crayfish bring them into contact with Cd from various sources. Crayfish consume detritus, decaying plants and animals in their natural habitat (Crocker and Barr 1968). Because crayfish are not filter feeders they are not exposed to particle-bound

Cd in the water column like A. grandis grandis. Consequently, maximum accumulation of Cd in crayfish caged in the lake was only 3.9 µg/g dry wt. in 1991 and 6.5 µg/g in 1992, compared to levels in mussels of 41 and 22.4 µg/g in 1991 and 1992 (Figs. 2.6, 2.9 and 2.10). Levels in the mussels were approximately three to ten times greater than the crayfish.

The main Cd source to crayfish in this study appeared to be the sediment, either as sediment-exposed benthos or through adsorption to the exoskeleton and gills. The early stages of all experiments showed that the concentrations of Cd in crayfish from the lake were similar to those in crayfish from the mesocosms, which suggests that crayfish received more Cd from sediment and only a portion of their contaminant load from the water column. Giesy et al. (1980) found that Cd in Procambarus acutus acutus was taken up via food and directly from water in an additive manner. However, Anderson and Brower (1978) found that O. virilis accumulated the greatest amount of Cd in the gills, suggesting that the water route (adsorption) was more important than food. Longer term experiments may have resolved which source is predominant, but crayfish do not respond well to confinement (Stinson and Eaton 1983, Segstro 1991), especially the small cage size used in this study. Release of crayfish directly into the mesocosms would be more natural, but would present problems such as predation, cannibalism and increased bioturbation.

4.3.3. Zooplankton

There appeared to be greater accumulation of Cd by zooplankton in the enriched mesocosms than in the unenriched mesocosms (Fig. 2.11).

However, there were no statistically significant differences in Cd concentrations between the two treatments on any sample day ($p<0.05$) because of the wide range of BAFs observed (Table 2.7). The increased accumulation of Cd in zooplankton from the enriched mesocosms may, in part, be caused by higher concentrations of Cd in the water column of the enriched mesocosms than in the unenriched ones. However, concentrations of Cd in the water column of the lake itself were higher than in the enriched mesocosms, but lake zooplankton usually had lower concentrations of Cd than in the enriched mesocosms and higher concentrations than in the unenriched mesocosms. Therefore, the nutrient additions apparently increased the bioavailability of Cd on almost every sample day in respect to the unenriched mesocosms and the lake. An increase in bioavailability in productive systems contradicts the hypothesis of Taylor et al. (1991) that increased productivity should decrease concentrations of organic contaminants in zooplankton.

This contradiction may be caused by the low biomass of zooplankton >200 μm in size in the enriched mesocosms (Table 2.9). There is conflicting evidence on the effects of dissolved humic material on the bioavailability of Cd to zooplankton. Dissolved humic materials constitute a major portion of DOM in water, sediment, and soils (Stackhouse and Benson 1989). Humic acids have been shown to decrease (Poldoski 1979, Sedlacek et al. 1983) or have no effect (Winner 1984, 1986) on the bioaccumulation of Cd by various zooplankton species.

Increased bioavailability in the enriched mesocosms may be caused by an increase in chlorophyll a concentrations, bacteria and algae. Copepods, the predominant zooplankton, are typically filter feeders.

Table 2.9. Zooplankton (>200 μm) biomass sampled from L382 enclosures in 1991 and 1992.

Year	Treatment	Biomass[1] (mg)
1991	enriched	14.9 ± 6.2
	unenriched	25.3 ± 10.0
1992	enriched	4.38 ± 3.99
	unenriched	15.1 ± 10.7

[1] Mean ± SD of all biomass obtained from each sample day in specified treatment.

They consume mainly phytoplankton and diatoms in sizes ranging from 1.5 to 22 μm (Barnes 1980). There was an increased mass of these particle sizes, specifically <3 μm in the enriched mesocosms (M. Holoka pers. comm.); therefore, copepods in the enriched mesocosms may have accumulated high Cd concentrations by feeding on these particles. Although Hart (1977, in McCracken 1987) and Parker et al.(1982) found limited transfer of Cd from algae to zooplankton, Carney et al. (1986), and Benayoun et al. (1974) concluded that dietary Cd was probably important in long term bioaccumulation and retention of Cd.

The importance of the water route of exposure (specifically particle-mediated accumulation) for zooplankton is clear by comparing the relative amounts of Cd accumulated by the zooplankton, mussels and crayfish. It is no coincidence that the two filter-feeding organisms had similar terminal concentrations of Cd (zooplankton: mean range 3.0-60 μg/g; mussels mean range: 5.7-42 μg/g), whereas the omnivirous crayfish had much lower terminal concentrations (mean range: 1.13-6.37 μg/g).

4.3.4. Benthic Organisms

Concentrations of Cd in chironomids (range: 0.6-2.6 μg/g wet weight or 6-26 μg/g dry wt., assuming a wet/dry ratio of 10) are generally higher than those observed by Bendall Young and Harvey (1988) for south-central Ontario lakes (4.6 to 6.5 μg/g dry weight). Mean BSAFs were between 22 and 61 in this study (Table 2.8), which are higher than the BSAFs of 1.8-3.5 calculated from Bendall Young and Harvey (1988). The higher BSAF values obtained in this study may be caused by

an inaccurate wet:dry conversion ratio. If a ratio of 10 underestimates dry weight, then dry-weight Cd concentrations would increase and so would BSAFs.

Cd was more bioavailable to chironomid larvae in the enriched mesocosms relative to the unenriched mesocosms in both years but the differences were not significant (Fig. 2.12). Thus, nutrient additions may have increased the bioavailability of Cd in some manner relative to the unenriched mesocosms. This was surprising because nutrient additions should have increased concentrations of organic matter in the enriched mesocosms and, thereby, decreased the bioavailability of Cd to the chironomids, as observed by Athalye and Gokhale (1991) with oligochaetes.

The apparent greater bioavailability in the enriched mesocosms relative to the unenriched ones may be due to higher Cd water column concentrations in the enriched mesocosms relative to the unenriched mesocosms, as explained above for the zooplankton, or increased sedimentation of Cd-rich seston to the sediment. Because chironomids are infaunal organisms, and porewater concentrations were similar the latter explanation would be more plausible for exposure within the mesocosms. However, because Cd concentrations in chironomids from the lake and enriched mesocosms were similar this suggests that higher porewater concentrations in lake sediments are responsible for Cd uptake in lake chironomids.

Bioaccumulation in Chironomidae does not appear to be explained by one specific route. The sediment should be a major source of Cd to chironomids (Pascoe et al. 1990, Seidman et al. 1986). If this was the

case, chironomids in all mesocosms and the lake should have similar concentrations because sediment concentrations of Cd are similar. If overlying water was the primary source of Cd, then the chironomids should have Cd concentrations in the following descending order: lake > enriched mesocosms > unenriched mesocosms. If porewater was the source, then lake organisms should have higher concentrations than both treated and untreated mesocosms over most sample days, if the bioavailability of the pore water Cd is equal between treatments. None of these possibilities are supported by the data from this study; therefore, multiple pathways for accumulation of Cd (i.e. Cd-rich seston and pore water) by chironomids seems likely.

Hexagenia limbata larvae do not show any consistent trend caused by the nutrient additions (Fig. 2.13). Mayflies from the lake and enriched mesocosms had similar Cd levels over the course of 1991, except in late October, indicating that exposure conditions were similar relative to the unenriched mesocosms. However, the primary source of Cd to H. limbata in the lake and unenriched mesocosms is as unclear as for the chironomids discussed above. Saouter et al. (1993) found that H. rigida accumulated significantly more inorganic Hg when exposed to water compared to sediment, suggesting that the water route was more important for accumulation of inorganic Hg. However, it is difficult to make any conclusions regarding the relative importance of pathways because of the small amount of data available in this study.

Sediment or pore water should be more important than water-column sources for the accumulation of Cd by benthic infaunal organisms such as Chironomidae and H. limbata because of the proximity of Cd to the

organisms. However, Cd concentrations in the sediment and pore water do not compare well with concentrations seen in the chironomids or H. limbata in either the lake or the mesocosms in this study. A combination of Cd sources for the accumulation of Cd to mayflies, as discussed above for the Chironomidae, may also apply. However, because the relative amounts of Cd accumulated by the chironomids and mayflies in the lake and mesocosms are similar, and no large increase is observable in the organisms from the lake (as for the mussels discussed previously), the sediment appears to be the most likely route.

4.3.5. Emerging Insects

Neither emerging chironomids nor emerging H. limbata showed clear trends during this study. In 1992, there was a trend for increased Cd accumulation in adult mayflies from the enclosures relative to the lake, but this difference was not significant ($p<0.05$). Mean concentrations of Cd for mayflies are similar for larval and adult stages (Figs. 2.13 and 2.15). Emerging mayflies do not feed, and would only be exposed to Cd in the water column during their emergence, however, the period of exposure would be short.

Cadmium concentrations in chironomid adults in south-central Ontario lakes averaged 3.06 ± 1.5 μg/g dry wt. (Bendall Young and Harvey 1988), a figure which is generally lower than observed in this study (range: 1.8 - 45.8 μg/g dry wt.). However, the concentration of Cd in the sediment of their study lakes was 1.4 to 3.5 μg/g dry wt., which is approximately 4.7 to 5.8 times higher than in the mesocosms and the sediment of the littoral zone of L382. Therefore, the lower Cd

concentration in chironomids of their study is surprising. The discrepancy may be a result of the weighing method used in this study. The organisms were blotted dry before weighing, which may actually remove more water from the organism than assumed. The conversion ratio of 10:1 (wet/dry) may then result in higher concentrations of Cd in the organisms.

5. CONCLUSIONS

Nutrient additions to the mesocosms increased Cd concentrations in the water column, probably because of increased particulate matter in the water column in the form of bacteria and phytoplankton. Few other differences were detected in Cd concentrations in the other compartments sampled. If the nutrient additions had continued for a longer time more differences may have resulted. Productivity would have increased and caused higher sedimentation rates and subsequent changes to the sediment and the sediment/water interface (Thomas 1987). Increased organic carbon content in the water column and the sediment affects the bioavailability of Cd to various biota by complexing and reducing the bioavailable fraction (Hart 1982, Allan 1986). However, in the present study there was insufficient time for this to occur.

This study demonstrated the presence of two different groups of organisms. Each group accumulated similar amounts of Cd on a per gram basis and, consequently, had similar BSAFs and BAFs. The first group, mussels and zooplankton, obtained the majority of their contaminant load

from the water column, probably either as dissolved Cd or through the ingestion of Cd contaminated particulates; sediment sources were secondary. The second group, crayfish and benthos obtained their contaminant load mainly from the sediment, possibly through feeding or via contact with sediment particles or porewater; water column sources were secondary.

CHAPTER III

Bioavailability of 2,3,7,8-tetrachlorodibenzofuran to Aquatic Invertebrates From Contaminated, Aged Sediments

Polychlorinated dibenzofurans (PCDFs) are a family of toxic, highly lipophilic, chlorinated hydrocarbons that are widely distributed in freshwater, estuarine and marine sediments, and biota at concentrations often exceeding that of the chlorinated dioxins (Fielder et al. 1990, Rappe et al. 1987). PCDFs are released into the environment from pesticide use, chlorophenol wood preservatives, combustion sources, and bleached kraft pulp and paper production.

In an aquatic environment, PCDFs quickly partition out of the water and into dissolved and particulate organic matter in the water column and, most importantly, to the sediment. Consequently, the sediment of aquatic systems becomes a sink for these compounds (McCarthy and Black 1988, Knezovich et al. 1987, Adams 1987a). However, in general, sediment-associated contaminants are still bioavailable to organisms, especially benthos that spend most or all of their life cycle in close association with the sediment (Knezovich et al, 1987).

The bioavailability of sediment-associated contaminants is affected by factors such as the amount of organic matter present (Adams 1987a, Knezovich et al. 1987), the concentration of contaminant in the pore water (Landrum et al. 1987, Knezovich et al. 1987), and the length of time the contaminant has been present in the sediment, i.e. contact time of the contaminant with the sediment (Landrum and Robbins 1990,

Landrum 1989). Contaminants may become less bioavailable with increasing age or contact time with sediment because of sorption into less bioavailable sediment compartments, through the removal of ingestible material containing contaminants by "packaging of faecal material" (Landrum and Robbins 1990), and by changes in organism behaviour which may alter accumulation of contaminants from the sediment (Landrum and Robbins 1990).

The initial objective of this study was to determine the impact of nutrient additions and subsequent increased trophic status on the bioavailability of TCDF to aquatic invertebrates. However, a number of the enclosures (which had been installed previously in 1989; Fairchild et al. 1992) suffered damage during the spring ice melt of 1991 and further irreparable damage mid-way through the 1991 field season by a wind storm. This resulted in the free exchange of water between some of the mesocosms and the lake so dilution of nutrient additions occurred. Because the mesocosms were no longer sealed from the lake the experimental hypothesis was revised so that the bioavailability of TCDF to aquatic invertebrates, from aged sediments, was studied instead.

2. MATERIALS AND METHODS

2.1. Lake 375 History

In the summer of 1989, six mesocosms were placed into the littoral zone of lake 375 (L375) (Fig. 3.1) of the Experimental Lakes Area (ELA) to determine the fate and bioavailability of ^{3}H-2,3,7,8 tetrachlorodibenzofuran (TCDF) (Fairchild et al. 1992). A total of 10.4

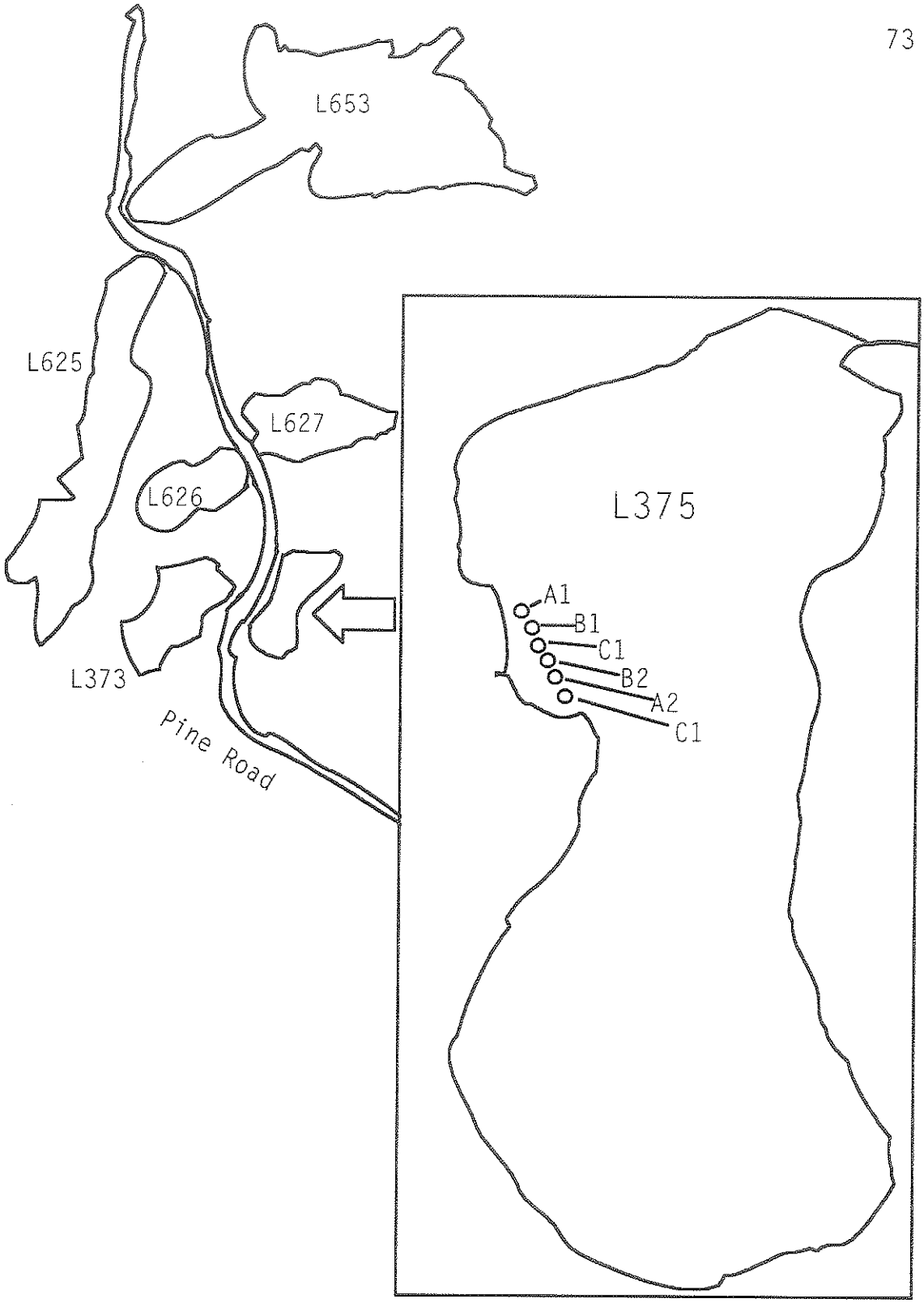

Fig. 3.1. Schematic diagram of Lake 375, showing littoral mesocosms, at the Experimental Lakes Area, northwestern Ontario.

μg of TCDF was added to each of four mesocosms, and the remaining two mesocosms were used as controls (Fairchild et al. 1992). TCDF bioavailability studies were subsequently conducted in the mesocosms (Fairchild et al. 1992, this study).

2.2. Sampling Outline

The 1991 sampling schedule for this study is shown in Table 3.1. Details are described in subsequent sections.

2.3. Chemical

Tritiated 2,3,7,8-tetrachloro[4,6-^{3}H]dibenzofuran (TCDF), having a specific activity of 140 dpm/pg and 95.5 % radiochemical purity (Chemsyn Science Laboratories, Lexena, KS), was added to the mesocosms on June 26, 1989 (Fairchild et al. 1992). The lone impurity was 2,3,7-T_3CDF (Muir et al. 1992a). The TCDF (dissolved in acetone) was mixed with small quantities of L375 surficial sediment. The acetone was evaporated and the sediment/TCDF mixture was combined with two L of lake water and added to the mesocosms in the propeller wash of an electric outboard motor (Fairchild et al. 1992.)

2.4. Sediment

Duplicate sediment cores were collected using a five cm diameter KB corer tube on each sample day (Table 3.1). The water was siphoned off the core tubes, the sediments extruded, and the top two-cm layer was collected for analysis. Sediments were frozen in glass 250 mL jars within a few hours of sampling and stored at -40°C until analysis.

Table 3.1. Sampling schedule for Lake 375, Experimental Lakes Area, 1991.

Sample	Month in 1991				
	June	July	August	September	October
Sediment [TCDF]			X[1]	X	X
Pore water [TCDF]			X		X
A. grandis grandis			X	X	X
O. virilis			X	X	X
Zooplankton		X	X	X	X
Emerging Insects	XXXXX	XX	XX	X	

[1] "X" indicates that samples were taken; the total number of "Xs" equals the number of sample days per month.

Chemical analysis is described in section 2.9.1.

2.5. Pore Water

Concentration of TCDF in pore water was determined from the top two cm of duplicate sediment cores. These were sliced off and stored in acetone-washed 250 mL stainless steel centrifuge tubes. The samples were then analyzed as soon as possible upon returning to the laboratory. Chemical analysis is described in section 2.9.2.

2.6. Bioaccumulation of TCDF In Biota

2.6.1. Anodonta grandis grandis (Say)

Floater mussels, A. grandis grandis, were collected from ELA L104, by snorkel and mask in one to three m of water, placed in a cooler, and transported to L375. At L375, the mussels were placed into closed-bottom mesh cages constructed with a wooden frame (50 cm x 50 cm x 35 cm) and lowered to the sediment in the enclosures. Approximately 34 mussels were placed into two cages in each mesocosm at the beginning of the experiment, June 21, 1991. Six mussels were removed on each sample day (Table 3.1) and stored at -40°C in polyethylene bags until analysis. The length, width, and height of each mussel was measured to the nearest 0.05 mm using callipers (Huebner et al. 1990). The soft tissues were removed from each shell, blotted dry, weighed, and weighed after lyophilization. Wet:dry ratios were calculated from the two weight measurements. Chemical analysis is described in section 2.9.3.1.

2.6.2. *Orconectes virilis* (Hagen)

Freshwater crayfish *O. virilis* were collected from ELA L468. Minnow traps baited with beef liver were left in the water overnight. The following morning the traps were retrieved and the male crayfish removed and transported to L375. Three crayfish were placed into each 30 cm x 40 cm plastic mesh cage with three rocks obtained from the shoreline of L375. Six cages were situated in each enclosure starting on June 27, 1991. Approximately four crayfish were sampled on each sample day (Table 3.1), placed in polyethylene bags, and stored at -40°C until analysis. The carapace of each crayfish was measured to the nearest 0.05 mm using callipers. The whole organism was weighed after blotting dry and after freeze drying. Wet:dry ratios were calculated from the weight measurements. Chemical analysis is described in section 2.9.3.1.

2.6.3. Zooplankton (>200 μm)

A 30-cm diameter Wisconsin net (mesh size 200 μm) was pulled vertically through the top 1 to 1.5 m of each enclosure to collect zooplankton. Duplicate samples were taken on each sample day (Table 3.1). The sample was rinsed in the lake and stored in acetone-washed 250 mL glass jars at -40°C until analysis. Chemical analysis is described in section 2.9.3.2.

2.6.4. Emerging Insects

Emerging insects from the mesocosms were sampled using three submerged emergence traps (Davies 1980) set at even intervals across the

mesocosm. On each sample day (Table 3.1), collected insects were sorted into vials as members of the order Diptera or Hexagenia limbata and stored at -40°C until analysis. Organisms were thawed, blotted dry, and weighed prior to analysis. Chemical analysis is described in section 2.9.3.2.

2.7. Chemical Analyses

2.7.1. Sediment

Sediments were freeze-dried, thoroughly mixed, and an eight to ten g portion refluxed in 1:1 hexane:acetone (Caledon, Georgetown, ON) for 24 h. The resulting slurry was filtered through a Whatman GFC filter, rotoevaporated and reduced to 0.5 mL under nitrogen. Duplicate 50 μL aliquots of the extract were diluted with 12 mL of scintillation fluor (Atomlight®, New England) and analyzed for TCDF using a Beckman Model 7500 liquid scintillation counter (Beckman Instruments, Irvine, CA).

To determine the unextractable portion of TCDF in the sediments, the refluxed sediment was air-dried for 24 h to remove any trace of solvents. Duplicate samples of 0.2 to 0.3 g of sediment were oxidized on a Packard model 306 sample oxidizer (Canberra Packard Instruments, Mississauga, Ont.). Tritium from the sediments was trapped and diluted in Monophase® (Canberra Packard Instruments, Mississauga, Ont.) scintillation fluor and assayed by liquid scintillation counting (LSC). A total TCDF concentration (pg/g) was calculated by summing the extractable and unextractable concentrations. Extraction efficiency for this procedure was calculated by dividing the unextractable concentration by the sum of the unextractable and extractable

concentrations and multiplying by 100.

2.7.2. Pore Water

TCDF in pore water was determined by centrifuging the top two cm of a sediment core in stainless steel centrifuge tubes at 4000 RPM for 30 min. The supernatant was removed via pipette and placed into 25 mL Corex tubes and spun at 10 000 RPM for 20 min using a Sorvall Superspeed RC2-B centrifuge (Newton, Conn.). Aliquots of the pore water were counted directly (LSC) by diluting in 12 mL of Atomlight® fluor and after injection into a reverse-phase cartridge (C_{18} SepPak, Waters Scientific, Milford, MA). This provided measurements of total and DOC-bound TCDF respectively (Landrum et al. 1984).

2.7.3. Biota

2.7.3.1. Mussels and Crayfish

The freeze-dried tissues of mussels and crayfish were extracted in 10, 15 or 20 mL of toluene (J.T. Baker Chemical Co. Phillipsburg, N.J.), depending on sample weight, using a model PT 10-35 Polytron homogenizer (Brinkman Instruments (Canada) Ltd., Rexdale, ON) at high speed. The residue was transferred to a centrifuge tube and spun at 2000 RPM for 15 min. Duplicate aliquots of one to two mL of solvent were assayed by LSC. The supernatant was removed from the centrifuge tubes, stored in glass centrifuge tubes and the unextractable TCDF from the tissue determined by oxidizing approximately two g of air-dried tissue and analyzing by LSC. The unextractable and extractable measurements were summed to provide a total concentration of TCDF.

The amount of lipid in the mussel and crayfish tissue was determined gravimetrically by evaporating duplicate 0.5 mL aliquots of the toluene extract to constant weight. The amount of lipid was determined by subtraction of the pan weight and concentration of TCDF was then calculated on a lipid basis.

2.7.3.2. Zooplankton and Emerging Insects

Zooplankton samples were thawed and filtered through Nitex® screening to remove water from the sample. The samples were allowed to dry overnight and were weighed. Emerging insects were blotted dry and counted. If less than six to ten mg of either zooplankton or emerging insects were obtained, samples were pooled within treatments. Samples were oxidized and subsequently analyzed by LSC.

2.8. Calculation of TCDF Concentrations

Because tritium has a half-life of 12.3 y, the specific activity of the TCDF tracer decreased from 146 dpm/pg in 1988 to 116 dpm/pg in July 1992 when analysis of samples was completed. All results were corrected to the original specific activity of the tritium tracer.

2.9. Data Analysis

Biota sediment accumulation factors (BSAF) were calculated by:

$$BSAF = C_b/C_s \qquad [1]$$

where C_b is the lipid-normalized concentration in the biota on a dry weight basis (pg/g) and C_s is the organic-carbon-normalized sediment concentration (pg/g). The dry weight lipid content was used to reduce

variability; wet:dry weight ratios are given in the results to enable comparisons to published data.

Bioaccumulation factors (BAFs) for zooplankton were calculated using:

$$BAF = C_b/C_w \quad [2]$$

where C_b is the concentration in biota (ng/kg) and C_w is the concentration in the water column (ng/L).

3. RESULTS

Because there were significant differences ($p<0.05$, t-test) in bioaccumulation of TCDF in the biota between mesocosms it was impossible to describe all the mesocosms as one treatment. Therefore, each mesocosm is described as a separate treatment in the Results and Discussion sections.

3.1. Sediment

The extraction procedure was approximately 64.4 ± 12.4 % efficient (n= 23). Sediment in all enclosures had mean TCDF concentrations from 7.7 to 29.6 pg/g dry wt. (Fig. 3.2).

The sediment in L375 had approximately 1 % organic carbon on a dry weight basis (Muir et al. 1992a). Therefore, mean concentrations of TCDF on an organic carbon basis ranged from 770 to 2960 pg/g.

3.2. Pore water

Concentrations of TCDF in the pore water ranged from 17.9 to 40.4

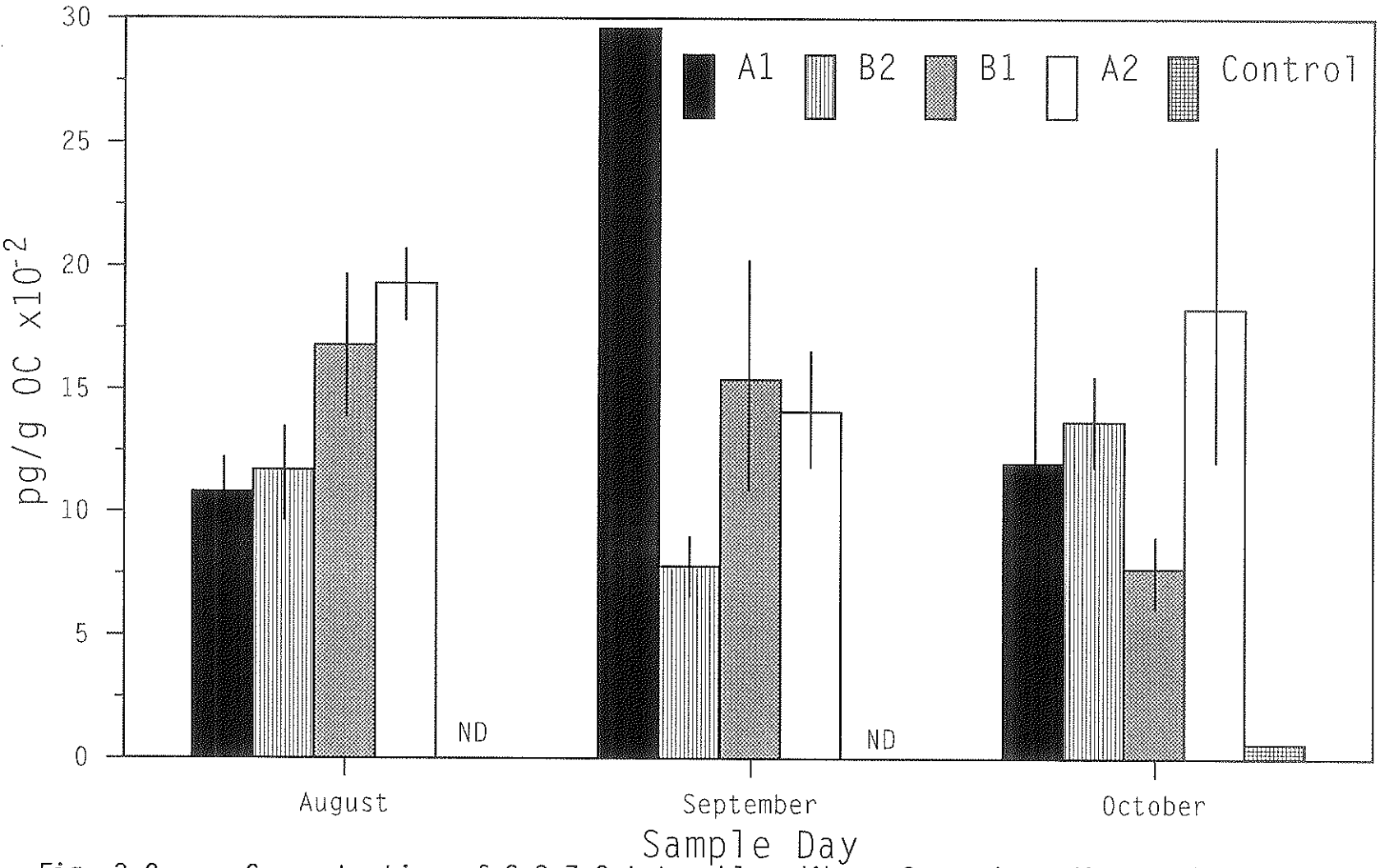

Fig. 3.2. Concentration of 2,3,7,8-tetrachlorodibenzofuran in sediment of littoral mesocosms in Lake 375, Experimental Lakes Area, 1991. Error bars equal +/- 1SD. Each bar represents means of 2, except September A1 N=1. ND=not detectable.

Table 3.2. Concentrations of 2,3,7,8-tetrachlorodibenzofuran in pore water of littoral mesocosms, Lake 375, Experimental Lakes Area, 1991.

Sample Day	Treatment	Supernatant pg/L	Sep-Pak[1] pg/L	% In Solution	% On DOC
August 14	A1	40.4	37.9	6.2	93.8
	B2	29.6	35.6	0	100
	B1	19.5	26.1	0	100
	A2	32.1	12.2	61.9	38.0
	Mean ± SD	30.4 ± 8.61	27.9 ± 11.7	17.0 ± 30.1	82.9 ± 30.1
October 26	A1	19.4	16.1	17.2	82.8
	B2	19.7	61.7	0	100
	B1	35.3	30.9	12.6	87.4
	A2	17.9	3.7	79.5	20.5
	Mean ± SD	23.1 ± 8.19	28.1 ± 25.0	27.3 ± 35.5	72.7 ± 35.5

[1]pg/L through Sep-Pak.

pg/L (Table 3.2). Approximately 72.7 to 82.9 % of this was associated with DOC and 17.0 to 27.3 % was in solution.

3.3. Bioaccumulation of TCDF In Biota

3.3.1. A. grandis grandis

The extraction efficiency of ^{3}H-TCDF using toluene averaged 63.3 ± 25.6 % (n=36). The mussels had higher accumulations of TCDF in A1 and B1 than in the two other enclosures. Organisms had the lowest bioaccumulation of TCDF in A2. These patterns were consistent throughout the experiment (Fig. 3.3).

Highest levels of TCDF were found in mussels on the first sample day (mean range 43.1-169 pg/g lipid dry wt.); levels subsequently declined (mean range 1.5-56.4 pg/g lipid dry wt.). The mussels had a mean wet to dry wt. ratio of 27.0 ± 6.82 :1 (n=59). BSAFs for the mussels ranged from 1.1 $x10^{-3}$ to 0.12 over the course of the experiment and decreased with time (Table 3.3).

3.3.2. O. virilis

The extraction efficiency of ^{3}H-TCDF using toluene averaged 86.3 ± 7.9 % (n=26) for the crayfish. The crayfish showed the same general trend of bioaccumulation as the mussels: a high initial accumulation of TCDF followed by a decline in the latter stages of the experiment (Fig. 3.4). Concentrations ranged from 87.6 to 954 pg/g lipid dry wt. over the experiment (Table 3.4). On a lipid basis, the crayfish accumulated approximately 5 to 58 times more TCDF than the mussels; consequently, BSAFs for the crayfish are higher relative to the mussels (range from

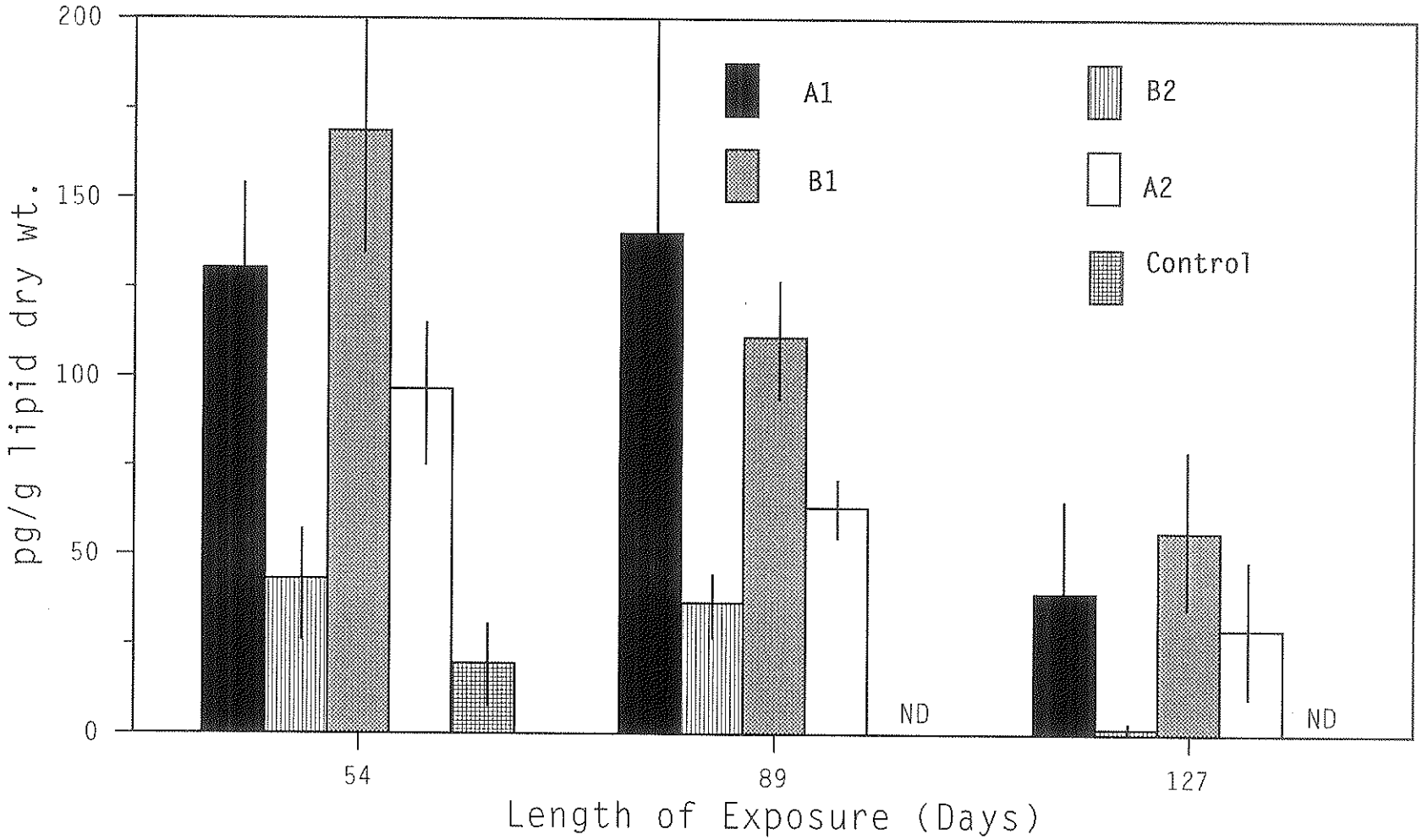

Fig. 3.3. Lipid normalized bioaccumulation of 2,3,7,8-tetrachlorodibenzofuran in Anodonta grandis grandis (Say) from littoral mesocosms in Lake 375, Experimental Lakes Area, 1991. Each bar represent means of 3 organisms. Error Bars equal +/- 1SD. ND=non-detectable.

Table 3.3. Biota sediment accumulation factors (BSAFs) for A. grandis grandis from littoral mesocosms, Lake 375, Experimental Lakes Area, 1991.

Exposure Length (Days)	Treatment	[Sediment] (pg/g OC dry wt[1])	[Mussel] (pg/g lipid dry wt[1])	BSAF
54	A1	1080	130	0.12
	B2	1170	43.1	0.04
	B1	1680	169	0.1
	A2	1930	96.5	0.05
89	A1	2960	140	0.05
	B2	777	36.5	0.05
	B1	1540	111	0.07
	A2	1410	63.2	0.05
127	A1	1200	39.2	0.03
	B2	1380	1.5	1.1×10^{-3}
	B1	770	56.4	0.07
	A2	1830	29.2	0.02

[1] Concentrations of TCDF were determined on a lipid weight when organisms were dry so that wet weight variations were avoided.

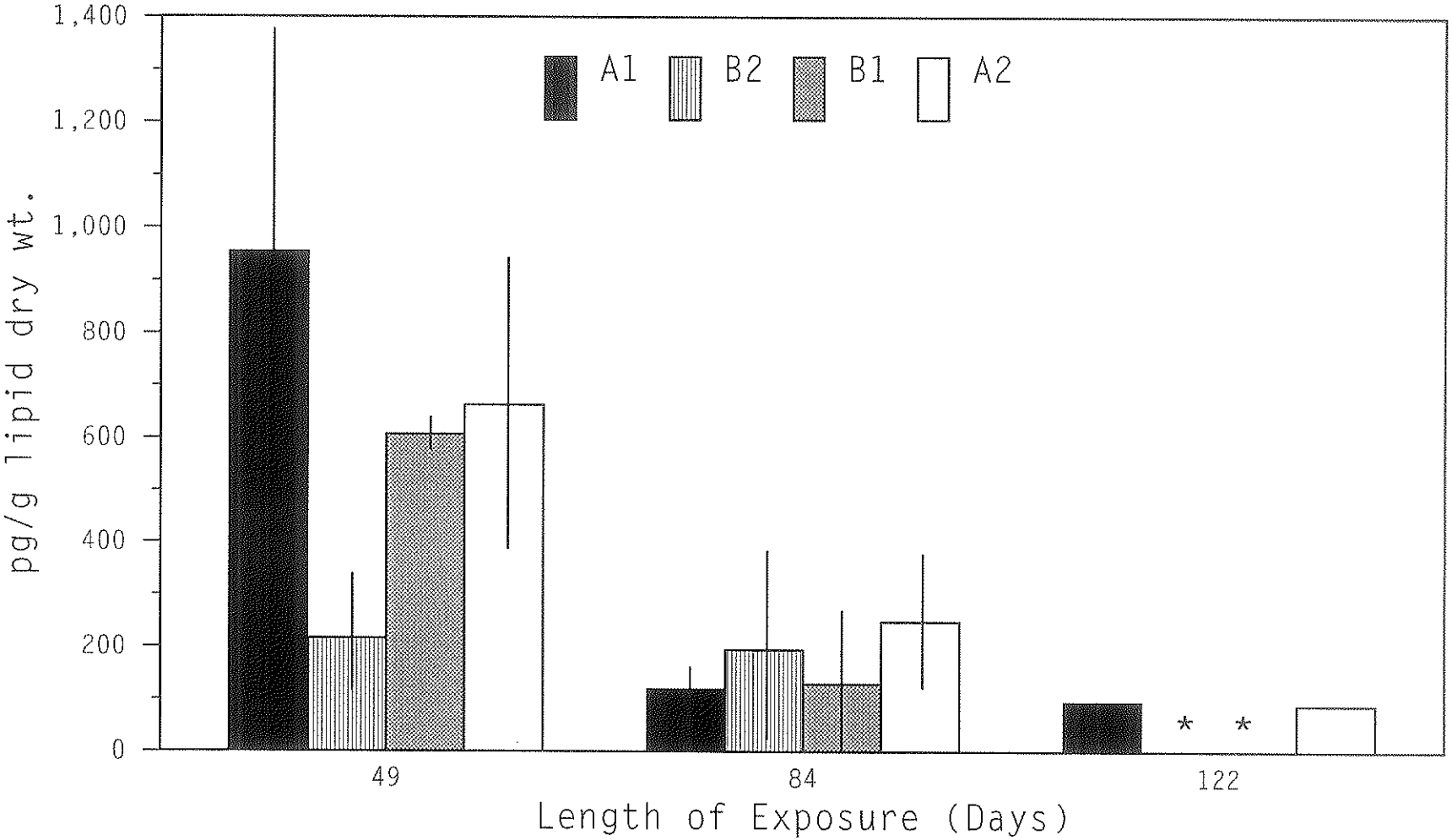

Fig. 3.4. Lipid normalized bioaccumulation of 2,3,7,8-tetrachlorodibenzofuran in O.virilis (Hagen) from littoral mesocosms in Lake 375, Experimental Lakes Area, 1991. * indicates no sample. Error bars equal +/- 1 SD. Controls remained undetectable.

Table 3.4. Biota sediment accumulation factors (BSAF) for crayfish from littoral mesocosms, Lake 375 , Experimental Lakes Area, 1991.

Exposure Length (Days)	Treatment	[Sediment] (pg/g OC dry wt[1])	[Crayfish] (pg/g lipid dry wt[1])	BSAF	Sample size
49	A1	1080	954	0.89	4
	B2	1170	216	0.18	4
	B1	1680	606	0.36	3
	A2	1930	663	0.34	3
84	A1	2960	119	0.04	3
	B2	780	194	0.25	2
	B1	1540	129	0.08	1
	A2	1410	248	0.18	3
122	A1	1200	93.8	0.08	1
	B2	1380	NS[2]	-	
	B1	770	NS	-	
	A2	1830	87.6	0.05	1
			mean ± SD	0.25 ± 0.25	

[1] Concentrations of TCDF were determined on a lipid weight when organisms were dry so that wet weight variations were avoided.

[2] No sample

0.04 to 0.89 Table 3.4) and decrease with time.

The crayfish had wet to dry wt. ratios averaging 6.05 ± 1.08 :1 (n=29) over the course of the experiment.

3.3.3. Zooplankton (>200 μm)

Low sample weights meant that only one sample from each enclosure on each sample day could be analyzed. The zooplankton showed the same pattern as the mussels and crayfish; declining concentrations after the first sample day. Lipid was not determined; however, zooplankton from other ELA lakes have approximately 25.8 ± 2.43 % lipid on a dry wt basis (D.C.G. Muir and M. Loewen, unpublished data). Using this percentage, the zooplankton had concentrations ranging from non-detectable (<1 pg/g) to 383 pg/g lipid dry wt. (Fig. 3.5). Mesocosm A2 consistently had the highest concentrations of TCDF, whereas B2 consistently had no detectable TCDF in zooplankton.

3.3.4. Emerging Insects

There were few emerging insects from the enclosures in 1991. Concentrations in the samples collected ranged from non-detectable (<1 pg/g) to 35.5 pg/g wet wt in Diptera (mostly Chironomidae) and 8.1 to 39.7 pg/g wet wt in H. limbata. Lipid concentration in chironomids is approximately 23 % on a dry wt basis (Gardner et al. 1985). H. limbata adults average 4.72 ± 2.34% (wet wt. basis; W.L. Fairchild, Gulf Fisheries Centre, Moncton NB., unpublished data). Assuming a wet:dry ratio of 10 for both organisms (Dermott and Paterson 1974) yields 47.2 ±

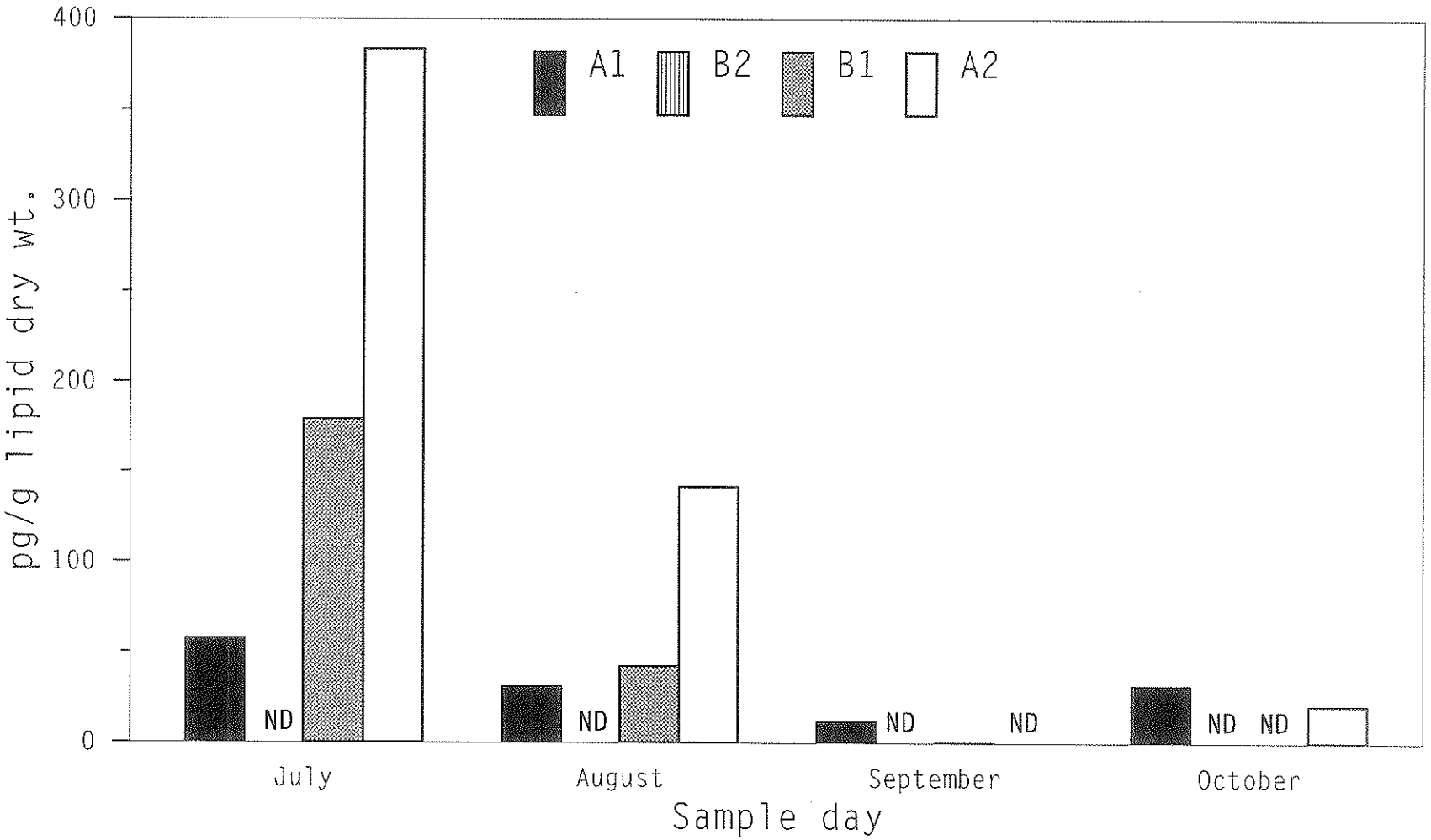

Fig. 3.5. Lipid normalized bioaccumulation of 2,3,7,8-tetrachlorodibenzofuran in zooplankton from littoral mesocosms, Lake 375, Experimental Lakes Area, 1991. ND = not detectable. Each bar represents one set of pooled zooplankton. Control levels were non-detectable during experiment.

Table 3.5. Concentrations of 2,3,7,8-tetrachlorodibenzofuran and biota sediment accumulation factors (BSAFs) for emerging insects from littoral mesocosms in Lake 375, Experimental Lakes Area, 1991.

Organism Type	Sample Day	Treatment	pg/g lipid[1]	BSAF
Diptera[2]	June 12	B2	152	0.12
	June 12	B2	926	0.47
	June 19	A1	ND[3]	-
	June 25	A1	1540	1.43
	July 10	B1	1260	0.74
		Mean ± SD	970 ± 600	0.69 ± 0.55
H. limbata[4]	June 12	A1	842	0.78
	June 28	A1	527	0.48
	June 28	A1	489	0.45
	July 13	B2	171	0.15
		Mean ± SD	507 ± 274	0.47 ± 0.26

[1] Dry wt. basis.

[2] Diptera were pooled to obtain sufficient sample weight for oxidation.

[3] Non-detectable.

[4] Data shown are individual organisms.

23.4% lipid on a dry weight basis for H. limbata. Converting TCDF concentrations to a lipid basis gives the values in Table 3.5. The mean BSAF for Diptera (or chironomids) was 0.69 ± 0.55, whereas the mean BSAF for H. limbata was 0.47 ± 0.26 (Table 3.5).

4. DISCUSSION

4.1. Sediment

Concentrations of TCDF in sediment in 1989 averaged 19.4 ± 1.9 pg/g dry wt. (n=16) over all sample days and treatments (Fairchild et al. 1992). The mean concentration (corrected for unextractable TCDF) in this study was 14.1 ± 5.9 pg/g dry wt (n=23), which was significantly lower (t-test $p<0.05$) from 1989. This result was expected because of burial of sediments by sedimentation, loss of bottom sediment as a result of damages sustained in the spring ice melt and removal of TCDF via insect emergence (Fairchild et al. 1992). There was a greater decline of TCDF concentrations between 1989 and 1991 if only the extractable portion of TCDF is considered (9.4 ± 4.6 pg/g dry wt.; n=23). This suggests that TCDF was more strongly bound or irreversibly bound to the unextractable portion of the sediment in 1991 relative to 1989.

Segstro (1991) observed decreased concentrations of 1,3,6,8-tetrachlorodibenzodioxin (TCDD) and 1,2,3,4,6,7,8,9-octachloro-dibenzodioxin (OCDD) in the sediment of littoral mesocosms in L304 at the ELA after five years. The loss was attributed to diffusion into the water column, export by emerging insects and burial by sedimentation.

The top of Segstro's (1991) mesocosms were cut off approximately 1.5 m below the surface of the water, so diffusion of compounds into the water column may have occurred. In this study, the mesocosms were initially above the water line so diffusion of TCDF into the water column was contained within the mesocosm and would eventually return to the sediment. Therefore, loss of TCDF would involve those mechanisms discussed above. However, water loss from the mesocosms may have occurred after a piece of the Styrofoam™ collar was lost from two of the enclosures, although loss of TCDF would be minimal because of its hydrophobic nature.

4.1.2. Pore Water

Concentrations of TCDF in pore water decreased slightly from 1989 to 1991. Twenty-one and 120 days after the TCDF spike in 1989, the concentrations averaged 39 ± 9 and 45 ± 11 pg/L in the pore water (Muir et al. 1992a). By August 1991, the concentration had declined to 30.4 ± 8.6 pg/L and in October the concentration was 23.1 ± 8.2 pg/L (Table 3.2). This result suggests decreased movement of the readily exchangeable fraction of TCDF into the pore water from the sediment. The percent found in solution and on DOC did not change, however, averaging 78.2 % on DOC and 21.8 % in solution in 1989 (Muir et al. 1992a) and between 72.7 and 82.9 % on DOC and 17 to 27.3 % in solution in 1991. This result was expected because the organic carbon partition coefficient (K_{oc}) for TCDF should remain constant; therefore, the relative amounts in solution and on DOC would also remain constant at equilibrium (DiToro et al. 1991). Bioavailability of the TCDF in the pore water was likely low because DOC-bound contaminants are generally unavailable for

accumulation by organisms (Servos et al. 1989, DiToro et al. 1991).

4.2. Bioaccumulation of TCDF in Biota

4.2.1. A. grandis grandis

The accumulation pattern of TCDF in whole mussels was not described by a classic uptake curve. In such a curve, concentrations in organisms increase until a steady state is reached and the uptake rate equals the depuration rate (Hawker and Connell 1985). Mussels had accumulated the greatest amount of TCDF after 54 d of exposure, from which point depuration took place until the mussels reached low terminal concentrations. The same pattern of accumulation occurred immediately after the addition of TCDF to the mesocosms in 1989 (W.L. Fairchild, Gulf Fisheries Centre, Moncton NB. unpublished data), and was attributed to the mussels filtering out the TCDF-contaminated particles used to spike the enclosures (Muir et al. 1992b). After these particles had settled to the sediment, the mussels could no longer accumulate the same amount of TCDF, so concentrations decreased (W.L. Fairchild unpublished data). Concentrations of TCDF in the water column in 1989 dropped from 70 to 110 pg/L (6 h after addition) to <5 pg/L after 60 d. The half-life of TCDF is approximately seven d in A. grandis grandis (W.L. Fairchild and R.S. Currie, unpublished data), so the mussels would lose TCDF quickly if water concentrations decreased.

The high TCDF accumulations (and BSAFs) seen in this study on the first sample day may have been caused by disruption and resuspension of the sediments within the mesocosms as a result of repairs done after spring ice out. Mesocosms A1 and B1 were moved during this time and had

to be repositioned, whereas only minor repair was required for C1, B2 and A2. Highest accumulation of TCDF in the mussels occurred in the enclosures that were disturbed most, A1 and B1, and the lowest accumulation occurred in the enclosures that were disturbed the least (B2 and A2). This result suggests that resuspension of sediment in the spring of the 1991 field season caused increased bioavailability of the TCDF. Sediment resuspension was also suggested to explain the accumulation pattern of TCDD in mussels in ELA L304 (Segstro 1991). Increases in contaminant bioavailability may be caused by both increased dissolved concentrations and particulate concentrations in the water column.

High accumulation of TCDF in the early, rather than the latter stages of the experiment may also have been caused by stress of transporting the organisms to the experimental site. Stress would have increased filtration rates which may have resulted in an initially high accumulation of TCDF; depuration would lower levels in the latter stages of the experiment.

The BSAFs for mussels in this study are, on average approximately 372-fold lower than those reported in 1989 for mussels (Table 3.6), which suggests that the bioavailability of TCDF has significantly decreased since 1989. However, Muir et al. (1992b) calculated BSAFs within 21 d of the TCDF addition, so the organisms were exposed to higher water concentrations and more contaminated particulates than in this study. After 120 d (1989), BSAFs dropped to 0.36 (Muir et al (1992a), which is still approximately 7.2 times higher than this study. Mussels exposed to TCDD and OCDD that had been present in sediment for

Table 3.6. Comparison of biota sediment accumulation factors (BSAFs) for TCDF and TCDD from littoral mesocosm experiments at the Experimental Lakes Area.

Reference	Compound studied	Organism[1]				
		Mussels	Crayfish	Mayfly Adults	Diptera adults	Diptera nymphs
Muir et al. (1992a, b)	TCDF	0.36 - 18.6	0.18 - 24.6	-	-	1.08
Fairchild et al. (1992)	TCDF	-	-	-	5.2[2]	-
This study	TCDF	0.05	0.25	0.47	0.69	-
Segstro (1991)	TCDD	0.22	0.85	-	-	-

[1] Mean BSAF presented.

[2] Calculated from wet wt. concentrations.

1484 to 1499 d yielded slightly higher BSAFs (Segstro 1991).

4.2.2. *O. virilis*

Crayfish showed a similar pattern as mussels: high accumulation of TCDF initially and a subsequent decrease in the latter stages of the experiment. Resuspensed sediment at the beginning of the experiment also may have been the cause of the initial high levels.

Crayfish had much higher lipid-based concentrations of TCDF than the mussels because of the low amount of lipid found in crayfish relative to the mussels (mean 1.72 ± 0.96 % vs 7.9 ± 2.4 %, respectively). As a result, crayfish had much higher BSAFs than the mussels. Crayfish in this study had mean BSAFs that were approximately 0.25 (n=10) (Table 3.6) over the course of the experiment, which suggests that the bioavailability of TCDF from aged sediments (663 -783 d post addition) has not decreased since 120 d after the original TCDF addition to the mesocosms in 1989. This similarity in BSAFs from year to year may be caused by the close association crayfish have to the sediment relative to the mussels. A slightly higher range of BSAFs for crayfish exposed to TCDD in aged, contaminated sediments was reported by Segstro (1991; Table 3.6.).

4.2.3. Zooplankton

Zooplankton showed the same trend as the mussels and crayfish; high concentrations of TCDF in the initial stages of the experiment and a subsequent loss or depuration (Fig. 3.5). Sediment-resuspension probably also caused the high initial levels.

The amount of TCDF accumulated by the zooplankton was lower than in the first 21 d of exposure in 1989. In 1989, zooplankton accumulated 9520 ± 5080 (n=8) pg/g dry wt lipid over the first three d followed by a constant downward trend [42.2 ± 58.1 (n=2) pg/g dry wt lipid] after 120 d (W. Fairchild unpublished data).

In this study, BAFs on the last sample day (October) ranged from 0 to 1788 using a mean water concentration of 14.5 pg/L (Friesen and Fairchild (1992). BAFs from 1989 after 120 d were 8440 but water concentrations were only <5 pg/L (Muir et al. 1992b). The higher BAFs calculated for 1989 are a result of the lower water concentration used to determine them.

Zooplankton typically receive the majority of their contaminant load from the water column either through adsorption of dissolved contaminant or through ingestion of contaminated, suspended particulates (Adams 1987b, Hall et al. 1986, Chapter II). Water concentrations of TCDF were too low in 1991 for the accumulation of significant amounts of TCDF by the zooplankton.

4.2.4. Emerging Insects

Diptera adults (mostly Chironomidae) accumulated much less TCDF in 1991 than in 1989. In 1989, emerging Diptera contained approximately 228 ± 16 pg/g wet wt. of TCDF in the first 60 d (Fairchild et al. 1992), compared to 17.8 ± 15.6 pg/g wet wt. in this study, which represents a 13-fold decrease in TCDF concentrations.

A BSAF of 5.2 for 1989 was obtained from Fairchild et al. (1992) (x 10 to convert mean wet wt. to dry wt. and then divide by a lipid

concentration of 23 % and a sediment concentration of 1900 pg/g OC dry wt; Muir et al. 1992b). The 1989 BSAF is approximately 7.5 times larger than in 1991 (Table 3.6), which suggests that the bioavailability of TCDF to Diptera has decreased. TCDF may be rendered less available because ingestible-sized particles are repackaged as larger faecal material in aged sediments, which the larval Diptera may not have been capable of processing (Landrum 1989). TCDF may also have been less available to the organisms in the sediment because of the incorporation of TCDF into the sediment matrix (Landrum and Robbins 1990). The extractable portion of TCDF decreased in the sediments from 1989 to 1991; therefore, greater incorporation into sediment is very likely.

No data are available for H. limbata adults from 1989 because the experiment did not start until after their emergence period was over. However, organisms from these mesocosms were collected by W. Fairchild (Freshwater Institute, Winnipeg, MB) in 1990. Concentrations of TCDF in these H. limbata ranged from undetectable to 798 pg/g lipid dry wt. (n=9). Concentrations in this study ranged from 171 to 841 pg/g lipid dry wt (Table 3.5). Mean BSAFs were 0.11 (n=9) for 1990 and 0.47 (n=4) for 1991, which represents an increase of 4.3 times between the two years. The mean value for 1990 was strongly influenced by the existence of non-detectable levels; removal of these from the analysis (because of low sample weights) generates a mean BSAF of 0.24 (n=5), which still demonstrates that bioavailability of TCDF to H. limbata has increased from 1990 to 1991. Because H. limbata are non-selective detritus feeders, they ingest large amounts of silt along with organic detritus (Walker 1968) which may have exposed them to similar concentrations of

TCDF in the sediment in 1990 and 1991. These two years are only one year apart, therefore, may not be long enough to observe differences in bioavailability of TCDF in these organisms relative to the two years for chironomids.

5. CONCLUSIONS

BSAFs decreased from 1989 to 1991 in mussels by 7.2 to 372 times, in emerging diptera by 7.5 times, showed no change in crayfish and zooplankton, and increased 1.9 to 4.3 times (1990 data) in adult H. limbata. Therefore, TCDF was still bioavailable in 1991 to some organisms but was less available to other organisms relative to 1989.

Decreases in bioavailability may be a result of decreased concentrations in the water column and the incorporation of TCDF into less available sediment matrices. Truly dissolved organic contaminants are considered to be the most bioavailable form to organisms (Adams 1987a, Connell 1988); water concentrations were extremely low in 1991, (7-22 pg/L, Friesen and Fairchild 1992). Whether decreased bioavailability in this study was caused by decreased water concentrations or increased contact time (i.e. more TCDF incorporated into the sediment with longer contact time) is unclear.

Explanations for seeing no change or increased bioavailability of TCDF includes both feeding habits of organisms and concentrations of TCDF in the exposure medium of most importance for each organism. Crayfish and H. limbata occupy the sediment/water interface and the sediment itself, so these organisms were exposed to similar

concentrations in 1991 as in 1989 and 1990. Zooplankton were exposed to similar water concentrations in the latter parts of 1989 and 1991, so they accumulated similar amounts as well.

Overall, the experimental organisms still accumulated detectable levels of TCDF from the sediment, so it must be concluded that TCDF in aged sediment-associated contaminants can still remain bioavailable to organisms. Segstro (1991) also found that TCDD and OCDD were still bioavailable to organisms after five years. It is important to consider bioavailability of contaminants from aged sediments when the lag period that may occur if emissions of a particular compound are decreased.

Sediment disturbance or resuspension was a factor affecting the bioavailability of TCDF. In this study, all organisms had increased concentrations of TCDF in the early stages of the experiment after disturbance of the mesocosms. This finding is important when considering dredged material from contaminated harbours or bays (e.g. Seelye et al. 1982, Rice and White 1987). It may be better to leave the contaminants in situ and wait for burial and ageing of the sediments to decrease the bioavailability of contaminants naturally. This is a lengthy process but sediment disturbance may actually increase exposure for extended periods of time.

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APPENDIX 1

A Instrument parameters for determination of Cd using Graphite Atomic Absorption Spectrophotometry

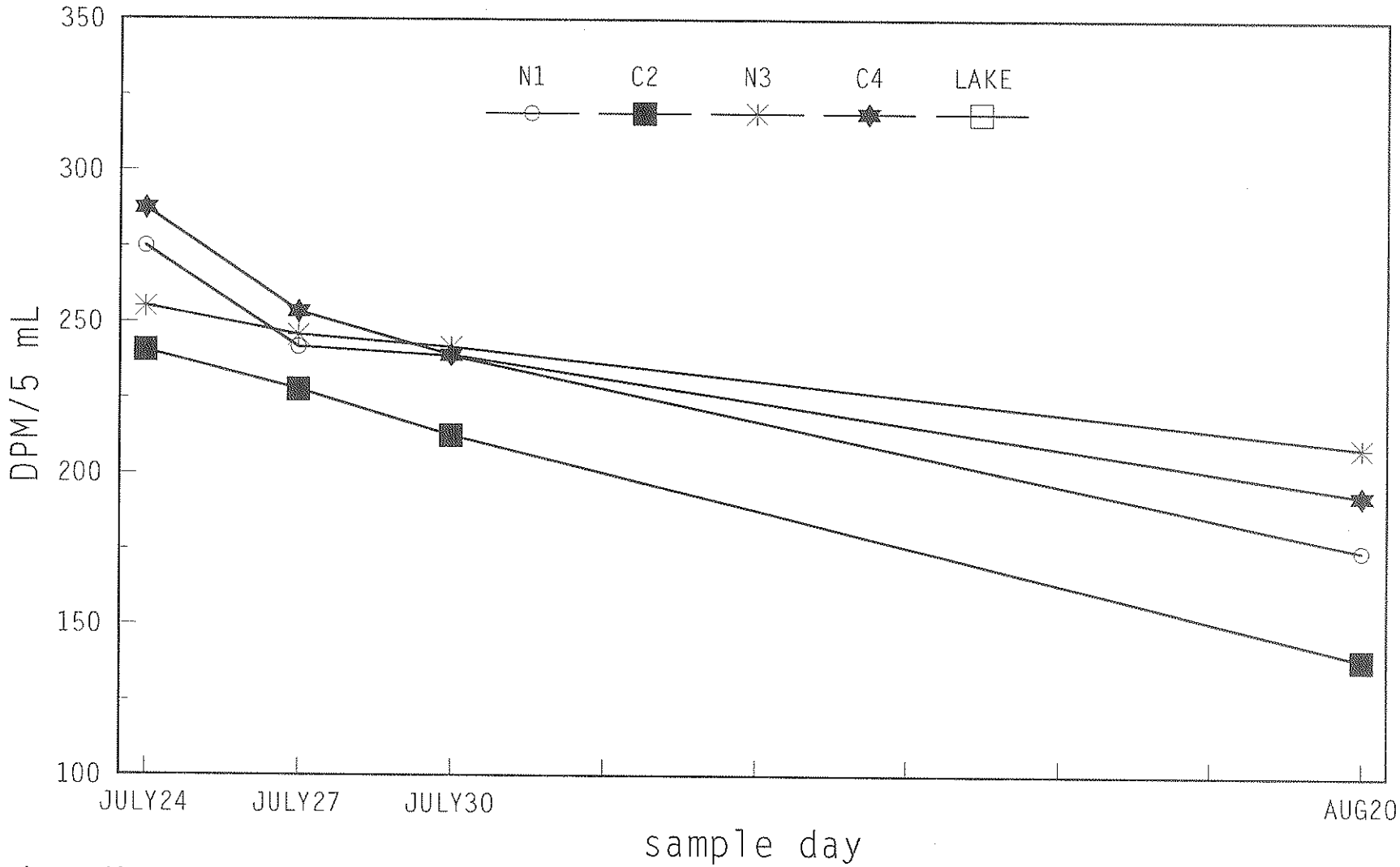
Furnace Operating Parameters:

Step #	Temperature °C	Time Sec.	Gas flow	Gas type	Read Command
1	75	10	3.0	NORMAL	OFF
2	90	20	3.0	NORMAL	OFF
3	120	5.0	3.0	NORMAL	OFF
4	150	25	3.0	NORMAL	OFF
5	150	5.0	3.0	NORMAL	OFF
6	350	5.0	3.0	NORMAL	OFF
7	350	35	3.0	NORMAL	OFF
8	350	5.0	0	NORMAL	OFF
9	2000	1.0	0	NORMAL	ON
10	2000	3.5	0	NORMAL	ON
11	2000	1.5	3.0	NORMAL	OFF
12	2000	3.0	3.0	NORMAL	OFF

APPENDIX 1 (CONTINUED)

B Instrument parameters for determination of Cd using Flame Atomic Absorption Spectrophotometry

Lamp Current (mA)	3
Sample Introduction	Manual
Delay Time (sec)	3
Measurement Time (sec)	4
Replicates	2
Background Correction	On
Expansion Factor	10



Appendix 2. Loss of tritium from littoral mesocosms in Lake 382, Experimental Lakes Area, 1992