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# COMPARISON OF LEECHES AND MUSSELS AS BIOMONITORS FOR CHLOROPHENOL POLLUTION

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ABSTRACT

Leeches were previously shown to have unusually high bioaccumulation capacities for chlorophenols (CPs) and have, therefore, been recommended as biomonitors for CP pollution. To determine whether leeches could survive on-site exposures in cages and to compare leeches and mussels as biomonitors for CPs, two field studies were conducted. CP residues in leeches (Nephelopsis obscura) exposed for three weeks above and below a pulp mill complex on the Rainy River, Ontario, were compared with concentrations in resident mussels, while leeches and mussels (Elliptio complanata) were directly compared by exposing them simultaneously in cages near a wood preserving plant on Thunder Bay Harbour. Survival of caged leeches in the Rainy River was excellent (77-87%), provided conductivity/alkalinity was within the tolerance range of the species. Leeches as far as 100 km downstream of the mills accumulated elevated levels of CPs (21-121 ng/g vs. 4-5 ng/g pre-exposure), with proportions of the various congeners reflecting those in mill effluent. Concentrations of CPs in mussels (<0.5-3.5 ng/g) were 1-2 orders of magnitude lower than in leeches. Leeches in Thunder Bay Harbour accumulated four CPs, with concentrations of the dominant pentachlorophenol (PCP) ranging from 817-5300 ng/g near the plant to 275-375 ng/g at 100 m, 55-85 ng/g at 300 m and <50 ng/g at 600 m beyond the discharges. In contrast, CPs were never detected in mussels from any site. Bioassays conducted on harbour sediment using fathead minnows (Pimephales promelas),

burrowing mayflies (Hexagenia limbata) and leeches demonstrated that uptake and toxicity decreased with increasing distance from the plant. At all sites, PCP residues were similar in field- and lab-exposed leeches. This study demonstrated that leeches are far superior to mussels as biomonitors for CPs, and that leeches can be successfully caged and used in routine monitoring where CP pollution is suspected.

RESUME

Des études ayant révélé que les sangsues sont des bio-accumulateurs de chlorophénols (CP) particulièrement efficaces, on a recommandé d'utiliser ces animaux comme bio-indicateurs de la pollution par les CP. On a fait deux études sur le terrain pour déterminer si des sangsues placées dans une cage peuvent survivre à l'exposition en milieu pollué et pour les comparer aux moules comme bio-indicateurs. On a déterminé la concentration de résidus de CP dans les tissus de sangsues (Nephelopsis obscura) laissées 3 semaines dans la rivière Rainy (Ontario) en aval et en amont d'une usine de pâte et papier et on l'a comparée à celle mesurée chez des moules trouvées sur les lieux; on a aussi comparé les concentrations mesurées chez des sangsues et des moules (Elliptis complanata) placées dans des cages immergées près d'une usine de traitement du bois au port de Thunder Bay. Le taux de survie était excellent chez les sangsues placées dans la rivière Rainy (77-87 %) lorsque la conductivité et l'alcalinité se situaient entre les limites de tolérance de l'espèce. Même chez les sangsues immergées à 100 km en aval des usines on a mesuré une concentration élevée de CP (4-5 ng/g avant exposition, 21-121 ng/g après) et les proportions des divers congénères détectés correspondaient à celles mesurées dans les effluents industriels. Les concentrations de CP mesurées chez les moules (moins de 0,5 - 3,5 ng/g) étaient inférieures d'un ou deux ordres de grandeur aux valeurs déterminées pour les sangsues. Chez les sangsues immergées à Thunder Bay, on a trouvé 4 types de CP; la concentration de pentachlorophénol (PCP), le plus abondant, allait de 817-5 300 ng/g, près de l'usine, à 275-375 ng/g, à 100 m, 55-85 ng/g, à 300 m, et moins de 50 ng/g à 600 m des points de déversement. Par contre, on n'a détecté aucun CP chez les moules prélevées aux mêmes endroits. On a étudié les sédiments du port en faisant des épreuves biologiques avec des têtes-de-boules (Pimephales promelas), des éphémères (Hexagenia limbata) et des sangsues; on a

constaté que l'absorption et la toxicité diminuent plus on s'éloigne de l'usine. Dans tous les emplacements, les résidus de PCP étaient les mêmes chez les sangsues exposées en conditions réelles que chez celles exposées en laboratoire. Cette étude montre que les sangsues sont de bien meilleurs bio-indicateurs de CP que les moules, qu'elles survivent bien en cages immergées et qu'elles peuvent servir pour la surveillance de routine dans les cas présumés de pollution par PC.

## MANAGEMENT PERSPECTIVE

The results of earlier research have shown that leeches have excellent potential as screening organisms for chlorophenols in aquatic environments because of their high bioconcentrations capacities, slow elimination rates and inability to degrade these compounds. The purpose of the present study was to determine whether biomonitoring with leeches could be successfully applied to real environmental problems, in this case to assessing the impact of industrial point sources of chlorophenols on their receiving waters. We found that leeches could be successfully caged for on-site exposures of up to three weeks and that they accumulated chlorophenol concentrations that were 1-2 orders of magnitude higher than those in mussels, which are the most commonly used benthic biomonitors. Concentrations of chlorophenols were elevated above pre-exposure levels in leeches caged over 100 km downstream of a pulp mill complex on the Rainy River, even though chlorophenols could not be detected in water or suspended solids. Tissue residues of chlorophenols in caged leeches also provided information on the origin, distribution and bioavailability of chlorophenols discharged from a wood preserving plant on Thunder Bay Harbour. As a result of the success of these field trials, the Ontario Ministry of the Environment's Water Resources Branch has begun to use leeches in routine monitoring wherever chlorophenol pollution is suspected. This represents a transfer of technology from this Institute to a provincial agency.

PERSPECTIVE GESTION

Des travaux antérieurs ont révélé que les sangsues ont beaucoup de potentiel comme organismes filtrants de chlorophénol en milieu aquatique parce que ces animaux ont un grand pouvoir de bioconcentration, qu'ils n'excrètent pas rapidement ces composés et qu'ils ne peuvent les décomposer. On a entrepris l'étude décrite ici afin de déterminer si la surveillance d'un milieu au moyen de sangsues comme bio-indicateurs peut s'appliquer à des problèmes écologiques réels, en l'occurrence, l'évaluation des effets des chlorophénols dans les eaux recevant des rejets industriels de sources ponctuelles. On a constaté qu'il est possible de laisser jusqu'à 3 semaines des sangsues dans des cages immergées sur les lieux : la concentration de chlorophénol qui s'accumule dans leurs tissus est supérieure d'un ou deux ordres de grandeur à celle atteinte chez les moules, bio-indicateurs benthiques les plus employés. La concentration de chlorophénols était plus élevée après exposition chez des sangsues immergées dans la rivière Rainy à plus de 100 km en aval d'une usine de pâte et papier et ce, même si l'on ne pouvait détecter ces composés dans l'eau et dans les sédiments en suspension. L'analyse des résidus présents dans les tissus des sangsues a aussi apporté des renseignements sur l'origine, la distribution et la biodisponibilité des chlorophénols rejetés par une usine de traitement du bois du port de Thunder Baie. Ces essais en conditions réelles étant concluants, la Direction des ressources en eau du ministère de l'Environnement de l'Ontario a commencé à se servir de sangsues comme bio-indicateurs pour les opérations de surveillance de routine dans tous les cas où l'on soupçonnait la présence de chlorophénols. Il s'agit là d'un transfert technologique entre l'Institut et un organisme provincial.

# INTRODUCTION

Unionid mussels bioconcentrate a wide range of pollutants and are frequently used as biomonitors for metals (Manly and George 1977; Tessier et al. 1984), industrial organic chemicals (Kauss and Hamdy 1985; Pugsley et al. 1985) and pesticides (Miller et al. 1966; Bedford et al. 1968; Leard et al. 1980) in freshwater environments. One of their main advantages is their ability to survive on-site exposures in cages for weeks or even months, which allows experimental conditions to be controlled and is of obvious benefit when studying areas not normally inhabited by mussels. There is some evidence, however, that mussels may not be suitable biomonitors for chlorophenols (CPs). Caged mussels exposed to effluent from a wood preserving plant on the Kaministikwia River in northwestern Ontario accumulated no traces of CPs, even though concentrations were measurable in both water and caged fish (Ontario Ministry of the Environment, unpublished data).

Leeches have excellent potential as screening organisms for CPs in freshwater systems, mainly because of their unusually high bioconcentration capacities for these compounds. In earlier work, Metcalfe et al. (1984) found that leeches from an industrially polluted creek accumulated CP concentrations that were up to two orders of magnitude higher than those in fish, tadpoles, crayfish, molluscs and a variety of aquatic insects. In a survey for the occurrence of CPs in several New Brunswick rivers 12 chlorophenol congeners were detected in resident leeches but only three in river water (Metcalfe et al. 1985), indicating that leeches were the more

sensitive monitoring medium. Jacob (1986) was the first to attempt caging leeches, successfully using Haemopsis marmorata (F. Hirudinidae) in one-week field exposures as biomonitors for CPs in the Fraser River Estuary.

The purpose of the present study was to compare leeches and mussels as biomonitors for CP pollution originating from two industrial point sources. The distribution of CPs in the Rainy River above and below two pulp and paper mills and in Thunder Bay Harbour adjacent to a wood preserving plant were assessed using residues accumulated by caged leeches and resident mussels (Rainy River study) or caged leeches and caged mussels (Thunder Bay study). Laboratory bioassays were also conducted on contaminated sediment from the harbour to determine its toxicity and to compare CP bioaccumulation under field and laboratory conditions. Procedures for conducting field studies with caged leeches are not well established; therefore, this study contributed to the development of an appropriate protocol for these organisms.

## MATERIALS AND METHODS

### Study Locations

Two of the main sources of entry of CPs into aquatic environments are pulp and paper mills and wood preserving plants. In addition, CPs can be generated as a result of aqueous chlorination of organic compounds during the treatment of municipal sewage (Jones 1981). Two locations in

northwestern Ontario were selected for study, both influenced by an industrial point source of CP pollution.

The first study was conducted on the Rainy River, which flows for approximately 130 km from Rainy Lake to Lake of the Woods (Fig. 1). The main sources of CPs are two kraft pulp mills located directly across the river from each other in Fort Frances, Ontario and International Falls, Minnesota. Minor sources are the municipal wastewater treatment plants for the towns of Fort Frances, International Falls, Emo, Rainy River and Baudette, which serve a total population of 20,000. The mills, however, account for about 95% of the loadings of chlorinated effluents to the river in terms of discharge volume, total suspended solids and BOD (International Rainy River Water Pollution Board 1985). The focus of our second study was a wood preserving plant on Lake Superior in Thunder Bay (Fig. 2). The facility applies creosote, pentachlorophenol (PCP) and chromated copper arsenate, under pressure and heat, to lumber intended for outdoor use. Approximately 700,000 L of 5% PCP solution and 8,500,000 L of 50% creosote were used in 1983 (Bérard and Tseng 1986).

## Rainy River Study

Collection, Transportation and Caging of Leeches - Approximately 600 specimens of the "bait leech", Nephelopsis obscura (F. Erpobdellidae), were purchased from a bait shop in Sudbury. Their origin was natural lake populations in Wisconsin or North Dakota. Specimens were transported to the field sites in bioassay bags containing 5 L of water. The bags were

given a head of oxygen, which was renewed daily, and were stored at ambient temperature. With the exception of a few injured animals, no mortality occurred during the week's field activities.

Leech cages were cylindrical in shape, 35 cm long x 15 cm in diameter, and were constructed of 10 x 10 mesh stainless steel wire cloth having a wire diameter of 0.064 cm and an opening of 0.191 cm. The overlapping edges were woven together with stainless steel wire, and the ends were capped with polyethylene plugs which had been pre-soaked in a cleaning solution to remove any soluble organic compounds. The plugs were secured with stainless steel gear clamps. A third clamp, with a smaller clamp attached to it, was placed around the middle of the cage. A line of quarter-inch nylon rope, strung through the smaller clamp, was used to tether the cages in the field. Our design was based on a cage described by Jacob (1986). Leeches were unable to escape from these cages in laboratory aquaria.

Experimental Design and Procedure - In early August 1986, cages containing 30 leeches (total biomass per cage approx. 90 g; average weight 2.8 g) were set at 17 sites along the river, in Rainy Lake and Lake of the Woods, and in the two major American tributaries (Fig. 1). Cages were attached by rope to either a concrete block or a navigation marker, and they rested on the substrate. Water temperature was about 18°C throughout the system. Leeches were exposed on-site for three weeks.

When the cages were retrieved, a dive search for resident mussels was conducted. Lampsilis radiata siliquoidea (Subf. Lampsilinae) were found at sites 4, 18, 28, 29, 31 and 32, while Andonta grandis (Subf. Anodontinae) occurred at sites 4, 31 and 32. Resident leeches, Haemopsis grandis (F. Hirudinidae), were also collected from sites 1 and 3 in Rainy Lake. Net-spinning caddisfly larvae of the genera Hydropsyche (F. Hydropsychidae) and Neureclipsis (F. Polycentropodidae) were found attached to the leech cages at sites 7, 8, 10, 13, 16 and 17 downstream and within 5 km of the mills. Proportionately more Neureclipsis were found on the cages nearest to the mills.

Samples of biota to be analyzed for CP residues were wrapped in prefired (450°C) aluminum foil, sealed in plastic bags and frozen on dry ice at the time of collection. The following samples were prepared: duplicate composite samples of 8-11 leeches weighing 18-30 g from each site where they survived; background samples of 15 and 25 leeches weighing 38 and 82 g, respectively; composite samples of two to four specimens of each species of resident mussel, weighing 51-79 g, from each site; three resident leeches from site 1 weighing 25 g and one leech from site 3 weighing 4 g; two composite samples of caddisfly larvae from sites 7, 8 and 17 below the American mill (122 specimens weighing 7 g) and sites 10, 13 and 16 below the Canadian mill (112 specimens weighing 6 g).

Samples of river water from sites 3, 18 and 32 and the final effluents from both mills were also analyzed for dissolved and particle-bound CPs. Water or effluent was clarified on site using a stainless steel Westfalia separator. A March 5C-MD submersible magnetic drive pump was operated at a

rate of 6 L/min to supply water to the centrifuge using a Teflon transfer line. One litre samples of clarified water were acidified to pH 2.0 with sulphuric acid, then stored at 4°C until analyzed. Suspended solids were removed from the centrifuge bowls by scraping the walls with a Teflon spatula. The slurry was then pressure-filtered through a 5 µm Teflon filter and the solids transferred to a pre-cleaned glass jar where they were stored frozen until analyzed.

Analytical Methods - Biota samples were partially thawed, weighed, ground with prefired (450°C) anhydrous sodium sulphate and Soxhlet extracted with dichloromethane for three hours. The extracts were evaporated to 2 mL under dry nitrogen, and lipids were removed by gel permeation chromatography using a 55/45 mixture of hexane/dichloromethane. The lipid-free sample was then extracted three times with 40, 30 and 30 mL of 0.1M potassium carbonate. To derivatize the phenols, 1 mL of acetic anhydride and 10 mL of hexane were added to the aqueous fraction and the mixture was shaken for 1 hr. Finally, the hexane extracts were eluted through a micro-column of 5% deactivated silica gel with a 95/5 solution of hexane/acetone, evaporated to an appropriate volume and analyzed for 21 CPs on a 30m DB-5 capillary column by GC-MSD (HP5890/5970).

Suspended solids from the two pulp mill effluents were thawed and homogenized. Twenty gram subsamples were prepared and analyzed as described for biota except that no GPC clean-up was needed, and the aqueous fraction was filtered through Celite to remove suspended material prior to acetylation.

Suspended solids from the three river water samples were prepared according to a method developed by Lee et al. (1987). It differed from the above-described procedure in that the 20 g subsample was first acidified to pH 1.0 with sulphuric acid, then "wet" extracted with acetone-hexane for 20 hr in a Soxhlet extractor. The extract was base-partitioned with 2% potassium bicarbonate solution, and the phenols derivatized with acetic anhydride while being extracted into petroleum ether. Clean-up and analysis were as described for effluent solids. The major difference between the two methods is that the initial acidification step was omitted during preparation of the effluent solids samples. Chlorophenols tend to bind to the organic material in sediments in the undissociated form (Xie 1983). Extraction with an organic solvent will efficiently remove the CPs, provided that the pH of the sediment is acidic or neutral (M.E. Fox, National Water Research Institute, personal communication). As whole effluent from both mills is neutral or slightly acidic (International Rainy River Water Pollution Board 1985, 1987), acidification of these samples was not considered necessary. It is possible, however, that CP extraction efficiencies were slightly higher for the river water suspended solids samples. Analytical detection limits for all of the above samples were 0.5 ng/g wet weight for all CP compounds.

Clarified water samples were analyzed for CPs according to the method of Lee et al. (1984). They were extracted with dichloromethane and base-partitioned with 2% potassium bicarbonate. Further preparation and analysis were as previously described for river water suspended solids samples.

Thunder Bay Harbour Study

Field Exposures of Caged Leeches and Mussels - Ten sites were selected for study (Fig. 2). Sites 1, 9 and 10 were in the immediate vicinity of the western, central and eastern discharges of the plant, respectively. Sites 8 and 16 were 100-150 m offshore, sites 7, 22 and 27 were 300-350 m offshore, site 24 was 600 m from the discharges and the control site was approximately 3 km from the plant. Leeches (N. obscura) were obtained from a bait shop in Toronto which was supplied by the same wholesaler as the Sudbury bait shop where leeches were purchased for the Rainy River study. Mussels (Elliptio complanata, Subf. Ambleminae), aged 9-11 years and ranging from 65-72 mm in valve length, were collected from Balsam Lake in central Ontario. They were transported in lined 20 L bioassay buckets, under oxygen, at a density of 50/bucket. Leeches were transported in the same way at a density of 125/bucket. All animals were held at 4°C prior to exposure. In early June 1987, cages containing 40 leeches (total biomass per cage approx. 55 g; average weight 1.4 g) were set alongside cages of 5 mussels (total biomass per cage approx. 40 g; average weight 8 g) at each of the 10 study sites. Mussel cages were constructed of 1.25 cm mesh galvanized poultry netting and measured 30 x 45 cm; leech cages have already been described. Water temperature in the harbour was approximately 9°C. The animals were exposed on-site for three weeks after which three composite samples of four leeches each and one individual mussel from each site were selected for analysis. At site 8, three additional mussels were taken. Samples of biota were wrapped in hexane-rinsed aluminum foil, sealed in plastic bags and stored on ice for 4-6 hr prior to freezing.

Laboratory Sediment Bioassays - Sediment bioassays were conducted in October 1986. Sediment was collected by ponar dredge from the six sites indicated in Fig. 2. Unfortunately, site 1 could not be included because only creosote-soaked gravel was present. Samples were shipped to the laboratory in buckets lined with bioassay bags, and were stored at 4°C until tested.

Bioassays were conducted on fathead minnows (Pimephales promelas), burrowing mayflies (Hexagenia limbata) and leeches (N. obscura). The minnows were 4-month-old juveniles cultured in the laboratory from stock obtained from the USEPA Laboratory in Duluth, Minnesota. Mayflies (second-year nymphs) were collected from Georgian Bay in Lake Huron and maintained in the lab for a short period prior to testing. Leeches were purchased from the Toronto bait shop which also supplied the specimens for the field study. Bioassays were performed in 1.5 L Mason jars. A 200 mL sediment sample was placed in the jar, topped with 800 mL dechlorinated tap water and allowed to settle for 24 hr. The system was then aerated and the test organisms introduced. Tested were conducted separately and in triplicate for each organism, using 5 minnows, 5 mayflies or 3 leeches per jar. Due to a shortage of specimens, tests on sediment from sites 9 and 10 could not be replicated for mayflies or leeches. Bioassays were run for 10 days. Mortality was recorded daily and dead organisms were removed and discarded. After 10 days exposure, surviving animals from each treatment (site/species) were composited, wrapped in hexane-rinsed aluminum foil, sealed in plastic bags and stored frozen until analysis for CPs.

Analytical Methods - Samples of field-exposed biota were weighed and homogenized using a sonification process. The samples were then digested with HCl and extracted with dichloromethane. Lipids were removed and the lipid-free extracts were dried through sodium sulphate and concentrated on a rotary evaporator. Extracts were derivatized using diazomethane, subjected to a Florisil clean-up, concentrated on a vortex evaporator and analyzed for six CPs using a capillary column gas chromatograph with an electron capture detector. Detection limits for this method were 100 ng/g wet weight for 2,3,4-TCP (TCP = trichlorophenol) and 50 ng/g for all other compounds.

Samples of laboratory-exposed biota were weighed and homogenized. An aliquot was digested overnight by gentle agitation with hydrochloric acid. The sample was then extracted with a 25/75 mixture of dichloromethane/ hexane. The extract was transferred to a gel permeation column and eluted with a solution of 45/55 dichloromethane/hexane to remove the lipids. The lipid-free extract was concentrated, derivatized with diazomethane and subjected to a Florisil clean-up. The resulting extract was evaporated to an appropriate volume and analyzed for six CPs by dual capillary column/dual ECD gas chromatography. Method detection limits were 5 ng/g wet weight for 2,3,4,5-TECP (TECP = tetrachlorophenol) and PCP, and 10 ng/g for the remaining compounds.

# RESULTS

## Rainy River Study

At 11 of the 17 caged leech sites, no leeches survived the three-week exposure period. At the three sites above the mills (1, 4 and 5) leeches had apparently been dead for some time, as only traces of tissue were left in the cages. At the six sites downstream and within 5 km of the mills (7, 8, 10, 13, 16 and 17), all leeches were dead and covered in fungus but had not completely decomposed. At sites 26 and 28 (mid-river), all leeches were dead, but there was no evidence of fungus or decomposition, which suggests they had been dead only a few days. Survival at the remaining six sites was excellent, ranging from 77-87%. These sites included four in the lower river (33, 34, 31 and 32), one in the Big Fork River (24) and one in the Baudette River (30).

Prior to setting the cages in the river, it was noted that many of the leeches were clitellate, that is, in reproductive condition. During the exposure period, many leeches deposited cocoons on the end plugs of their cages. Numerous cocoons were seen at sites 5, 7, 8, 10, 13, 26, 31, and 33, with lesser numbers at the remaining sites except for sites 1 and 4 where none were found.

Concentrations of CPs in clarified water and suspended solids from sites 3, 18 and 32 and both final mill effluents are presented in Table 1. The Canadian mill effluent contained much higher concentrations of total CPs (6.09 µg/L in water; 353.9 ng/g in suspended solids) than the American mill effluent (0.25 µg/L in water; 5.8 ng/g in solids). Dominant congeners in the dissolved and particle-bound phases of both effluents were 2,4,6-TCP, 2,3,4,6-TECP and PCP, accounting for 73-100% of the total CPs. Suspended solids from the Canadian effluent also contained a significant proportion of 2,4-DCP (19%; DCPs = dichlorophenol). Traces of 4-CP (CP = monochlorophenol) and 2,4,6-TCP in clarified water and PCP in suspended solids were found in river water both upstream and 5 km downstream of the mills, whereas only minute traces of 4-CP were found in water from Lake of the Woods.

Figure 3 compares the concentrations of total CPs accumulated by caged and resident leeches with concentrations in resident mussels. No data are shown for caddisfly larvae because they did not contain detectable residues, even though they were collected from sites located within 5 km of the mills. Concentrations of CPs in mussels were very low, ranging from <0.5 to 3.5 ng/g. The highest value was for L.r. siliquoidea collected from site 18, 5 km below the mills. Both species of mussels were collected from sites 4, 31 and 32, but the L.r. siliquoidea specimens from sites 4 and 32 were lost in a freezer malfunction; therefore, interspecific comparisons were not possible. Concentrations of total CPs in leeches prior to exposure were approximately 5 ng/g, which is similar to concentrations in resident leeches from Rainy Lake. After three weeks exposure,

concentrations in leeches increased to 21-121 ng/g at the four sites in the lower river, but decreased to less than 2 ng/g in the Big Fork River and to about 3 ng/g in the Baudette River.

Figure 4 compares relative proportions of the various CPs in leeches with proportions in effluent from the major point source - the Canadian mill. For clarity, only congeners accounting for over 5% of the total CPs are included. Profiles for caged leeches from the four sites in the lower Rainy River closely resembled the pattern for mill effluents, while those for caged leeches from the two American tributaries did not. Rainy Lake residents contained mainly PCP and some 2,3,4,6-TECP. The pattern in pre-exposure leeches was unique, with 4-CP, 2,4-DCP, 2,4,6-DCP and PCP present in almost equal portions. In Fig. 5, proportions of CPs in resident mussels are compared with the effluent profile. The pattern for L.r. siliquoidea collected 5 km below the mills resembled this profile. However, specimens collected farther downstream contained proportionately more of the lower chlorinated compounds (mono- and dichlorophenols), as did A. grandis from all sites. Pentachlorophenol was never detected in mussels, yet this compound was a dominant congener in caged and resident leeches.

## Thunder Bay Harbour Study

All caged mussels survived the three-week exposures in Thunder Bay Harbour, but with one exception did not accumulate detectable concentrations of any CP compound at any site. The exception was a mussel from site 8, which

contained 60 ng/g 2,4,5-TCP, 85 ng/g 2,3,4,5-TECP and 930 ng/g PCP. Three additional mussels from this site were analyzed individually and found to contain no traces of CPs. We therefore suspect that the original sample had been contaminated or mislabelled during preparation.

Caged leeches suffered some mortality at all sites, not exceeding 38% except at site 10 where 45% were dead and the rest moribund. It was also observed that specimens at site 1 lacked vigour. Unlike mussels, leeches did bioaccumulate CPs (Fig. 6); in decreasing order of importance, PCP, 2,3,4,5-TECP, 2,4,5-TCP and 2,4,6-TCP were identified in leech tissues. Concentrations of PCP ranged from 817 to 5300 ng/g at sites 1, 9 and 10, decreased to 275-375 at sites 8 and 16, decreased further to 55-85 ng/g at sites 7, 22 and 27, and were undetectable (<50 ng/g) at sites 24 and the control. Concentrations of 2,3,4,5-TECP and 2,4,5-TCP were 63-427 ng/g and 57-90 ng/g, respectively, in leeches caged near the discharges. Unlike PCP, however, these compounds did not persist into the harbour. A different distribution pattern was observed for 2,4,6-TCP. Concentrations of this isomer were similar at all sites (<50-70 ng/g) including the control. Leeches did not accumulate 2,3,4-TCP or 2,3,5,6-TECP at any site. All values reported above are averages for three composite samples of leeches. Variability was low, with standard deviation generally 10-20% of the mean. For the purpose of calculating averages, all values < 50 ng/g were considered to be equal to 50 ng/g.

All minnows, mayflies and leeches survived 10 d exposures in the laboratory to sediment from sites 7, 8 and the control. Two of 15 mayflies and one of 9 leeches died after exposure to sediment from site 24, and all

but one organism died after exposure to sediment from sites 9 and 10. The survivor was a leech from site 9. Concentrations of tri- and tetrachlorophenols in the tissues of surviving animals were near or below the detection limit for minnows and mayflies from all sites (Table 2). In leeches, concentrations of 2,4,5-TCP and the tetrachlorophenols were elevated only at site 9, the central discharge, while concentrations of 2,4,6-TCP were similar at all sites. Concentrations of PCP in leeches dropped off in a transect from the central discharge into the harbour. A similar trend was suggested by the minnow and mayfly data. Wherever data for all three test organisms were available, i.e., sites 8, 7, 24 and the control, leeches were shown to accumulate the highest concentrations of 2,4,6-TCP and PCP.

## DISCUSSION

The results of the Rainy River study demonstrated that field studies using caged leeches are feasible. Excellent survival rates were observed at 6 of the 17 sites, which indicates that the handling procedures and cage design were suitable and the exposure period appropriate. The cause of high mortality at the remaining 11 sites is believed to be related to water quality, and this will be discussed in detail below.

The Canadian pulp mill is obviously the major source of CPs to the Rainy River. Concentrations of total CPs in clarified water and suspended solids were 25X and 60X higher, respectively, than in effluent from the American mill. Assuming these samples are representative; actual daily

contributions to the river would be about 3700 g total CPs for the Canadian mill and only 50 g for the American mill, based on discharge volumes of 70 x $10^6$L/d and 124 x $10^6$L/d and suspended solids loadings of 9300 kg/d and 3272 kg/d, respectively, in 1986 (International Rainy River Water Pollution Board 1987). Concentrations of CPs in river water and suspended solids both upstream and 5 km downstream of the mills were similar, suggesting that the impact zone of the pulp mill complex extended no farther than 5 km.

Concentrations of CPs in caged leeches increased above background levels in animals exposed in the lower Rainy River, but decreased in those exposed in the Big Fork and Baudette Rivers. Although the latter site was just below the town of Baudette's sewage treatment plant, concentrations in leeches were very low. Site 34 in the Rainy River was also below a sewage treatment plant (for the town of Rainy River), yet concentrations in leeches here were no higher than in those upstream of the plant. For these reasons, local discharges did not appear to be responsible for the elevated levels of CPs in leeches in the lower Rainy River. Rather, this contamination is most likely attributed to the pulp mills located over 100 km upstream. Resident mussels collected throughout the Rainy River accumulated CP concentrations that were 1-2 orders of magnitude lower than those in caged leeches from the lower river. Mussels have been previously shown to have lower bioconcentration factors (BCFs) for CPs than leeches. Metcalfe *et al*. (1988) reported BCFs of 1200-4200 for 2,4,6-TCP, 4000-5500 for 2,3,4,6-TECP and 3000-4300 for PCP in leeches from a contaminated creek, whereas BCFs reported in the literature for bivalves exposed to

similar levels of contamination were 60 and 170 for 2,3,4,6-TECP and PCP, respectively, in the marine mussel Mytilus edulis (Folke et al. 1983) and 64 for 2,4,6-TCP in the freshwater mussel Anodonta piscinalis (Paasivirta et al. 1985). Chlorophenols are eliminated rapidly by most aquatic organisms, including mussels. A half life of 2-3 d has been reported for PCP in the marine mussel, M. edulis (Ernst 1979). In contrast, half lives for these compounds in leeches are generally longer than one month (Metcalfe et al. 1988). Assuming a 30 d half life for total CPs in leeches, and given a 4-5 ng/g background concentration in pre-exposure leeches, the 2-3 ng/g concentrations observed in leeches from the Big Fork and Baudette Rivers are consistent with residues expected after 3-4 weeks depuration in clean water. While the elimination of CPs by leeches is very slow, uptake is known to be rapid. Jacob (1986) exposed the leech H. marmorata to 10 µg/L concentrations of 5 CPs in the laboratory for one week and observed that equilibrium conditions were achieved within 4-5 d at temperatures of 4 and 12°C, although not within 7 d at 22°C. This combination of rapid uptake and slow elimination is probably due to the absence in these primitive organisms of enzyme systems capable of conjugating CPs and will, of course, result in very high BCFs for these compounds in leeches.

Relative proportions of the various CP isomers in pre-exposure leeches were dramatically altered after 3-week exposures in the field, presumably in response to local conditions. For example, profiles in caged leeches from all sites in the lower Rainy River closely reflected the pattern for effluent from the Canadian mill, which is the major source of CPs to the

river. Profiles for caged leeches from the American tributaries and resident leeches from Rainy Lake, however, were more characteristic of locations remote from point sources of CPs. These specimens contained high proportions of PCP, which is ubiquitous in the environment (Jones 1981), and which was shown to be the major isomer in suspended solids from Rainy Lake. In contrast to leeches, profiles for mussels from Rainy Lake were essentially the same as those for mussels collected downstream of the mills. While leeches accumulated mainly 2,4,6-TCP, 2,3,4,6-TECP and PCP, mussels contained mainly di- and trichlorophenols, very little 2,3,4,6-TECP and no PCP. Paasivirta et al. (1985), in their study on a CP-contaminated lake chain in Finland, also observed that mussels and other filter-feeding benthic invertebrates selectively accumulated the more water soluble compounds such as 2,4-DCP and 3,4-DCP.

The pattern of mortality observed among caged leeches in the Rainy River does not implicate the pulp mills as the cause, because 100% mortality occurred at all sites above the mills and 50-80 km downstream in addition to the 6 sites within 5 km of the outfalls. Instead, it suggests that differences in water quality between the upper and lower reaches of the river and between the main river and its tributaries are more likely to be responsible. To determine which factors may be involved, water quality data for the study area (Table 3) were compared with the environmental requirements of the test species from the literature.

N. obscura is a cold-water stenotherm occurring at temperatures of 4-15°C in Alberta (Linton et al. 1983a), below 16°C in Michigan (Kopenski 1972) and below 24°C in Colorado (Herrmann 1970a). It is tolerant of pH

fluctuations between 6.3-9.4 in Colorado (Herrmann 1970a) and 7.0-7.5 in Michigan (Klemm 1972). Herrmann (1970b) reported a tolerance range of 11.8-654.4 mg/L total dissolved solids (TDS) for N. obscura, which is both narrow and low. In laboratory experiments, Reynoldson and Davies (1980) observed optimal survival of this species at conductivities ranging from 1584-5554 µmhos/cm, and high mortality below 84 µmhos/cm. Most leech species are most abundant at alkalinities above 60 mg/L, while few are present below 18 mg/L (Sawyer 1974). Water quality conditions in the Rainy River were apparently suboptimal for N. obscura. Temperatures throughout the system were near the upper tolerance limit for the species. Conductivity, alkalinity and TDS were at or below its lower tolerance limit in the upper river, although conditions were more favourable in the lower river and the tributaries. Although these differences appear minor, they may have been critical considering the life stage of the test specimens. In Minnesota, N. obscura has a 24 month life cycle (Peterson 1983). Clitellum development requires a body weight of 1.20 g, and mature leeches die shortly after depositing cocoons. They are clitellate in June-July and exhibit a lack of normal vigour and poor body condition by July-August when reproduction is complete or nearing completion. Test specimens, which were obtained from lakes in Wisconsin or North Dakota in early August, were in reproductive condition (clitellate; body weight of 1.5-4.0 g). Several studies have reported narrower tolerance ranges for both temperature (Linton et al. 1983a) and dissolved salts (Reynoldson and Davies 1980) in post-reproductive individuals. It is therefore hypothesized that because the animals were already under stress due to high water temperatures and

their reproductive activities, the slightly lower values for conductivity, alkalinity and TDS in the upper Rainy River were responsible for the high mortality observed at these sites.

Cocoon production by caged leeches at various sites demonstrated an interesting phenomenon. Although environmental conditions were less than ideal, cocoons were deposited - frequently in large numbers - at most sites throughout the river. Linton et al. (1983b) demonstrated that reproduction, once it has begun, will continue even under suboptimal conditions because the adults have a limited life span once committed to this activity. As the test leeches were apparently well into this phase of their life cycle when obtained from the bait shop, this explains why cocoons were found at most sites. The reason why no cocoons were found at sites 1 and 4 may be that conditions were almost immediately lethal to the adults.

Several recommendations for conducting successful field studies with caged leeches, particularly N. obscura, arose from the Rainy River study. First of all, specimens in reproductive condition should be avoided, as they are nearing the end of their life cycle and natural mortality rates will be high. Furthermore, they are more sensitive to sub-optimal environmental conditions during this phase. Secondly, studies should be conducted at a time of year when water temperatures are cooler and, therefore, more favourable. Finally, the concentration of dissolved salts in the water is a primary limiting factor for leeches (Herrmann 1970a) and should be given prior consideration. Applying what was learned to the study on Thunder Bay Harbour, smaller, prereproductive leeches were used

and the study was conducted in early June when water temperature (9°C) was more acceptable to the test species. Conductivity and alkalinity throughout the harbour were also above the minimum requirements for N. obscura, at about 110 µmhos/cm and 45 mg/L, respectively.

In the Rainy River study, the biomonitoring potential of caged leeches was compared with that of resident mussels. The Thunder Bay Harbour field study provided a more direct comparison of these two invertebrates, because both organisms were caged and exposed under identical field conditions. Nevertheless, the findings of both studies were similar. Caged mussels did not bioaccumulate CPs above detection limits at any site in Thunder Bay Harbour; therefore, residues in mussels provided no information on the distribution of CPs in the receiving waters adjacent to the wood preserving plant. Leeches, however, accumulated four CP congeners in concentrations ranging from <50 to 5300 ng/g. From the patterns of residues in leech tissues, the extent of contamination originating from the plant could be determined, the plant was implicated as the source of 2,4,5-TCP, 2,3,5,6-TECP, 2,3,4,5-TECP and PCP (but not 2,4,6-TCP) to the harbour, PCP was identified as the major and most persistent congener and the relative loadings of the three plant discharges could be distinguished from one another.

Leeches were apparently more sensitive than mussels to contaminants discharged from the plant, as they suffered some mortality at all field sites. Field conditions may have been more stressful to leeches than lab conditions, however, as mortality in the lab occurred only after exposure to sediment from the most contaminated sites. Leeches exposed to sediment

from site 9 in the lab accumulated higher concentrations of 2,4,6-TCP, 2,4,5-TCP, 2,3,4,5-TECP, and 2,3,5,6-TECP than leeches exposed in the field, i.e., 120 vs. 57 ng/g, 5780 vs. 57 ng/g, 843 vs. 63 ng/g and 220 vs. <50 ng/g, respectively, although concentrations of PCP were similar (1080 vs. 817 ng/g). This suggests that leeches were in closer contact with contaminated sediment in the lab. In most other aspects, results of the field and laboratory studies correlated well. Concentrations of 2,4,6-TCP accumulated by leeches, mayflies and minnows in the lab and by caged leeches in the field showed no site to site variations, implying that this compound is ubiquitous in the area and is not associated with the plant's discharges. In leeches, concentrations of 2,4,5-TCP, 2,3,4,5-TECP and 2,3,5,6-TECP were elevated only in specimens from sites 1, 9 and 10 in the field and site 9 in the laboratory, which indicates that these contaminants do not persist beyond the immediate vicinity of the discharges. A comparison of PCP bioaccumulation under field and lab conditions is presented in Table 4. At all sites, PCP residues were similar in both field and lab-exposed leeches. The data for all organisms showed a trend towards decreasing uptake and decreasing toxicity in a transect away from the discharges. This does not necessarily identify PCP or CPs in general as the cause of toxicity. The analysis of selected organic and inorganic toxicants in sediments near the plant revealed high concentrations of CPs (approximately 5000 ng/g dry weight), chlorobenzenes (90 ng/g), DDE (25 ng/g), octachlorostyrene (15 ng/g), As (25 µg/g), Cr (100 µg/g), Cu (80 µg/g), and Zn (350 µg/g) (Ontario Ministry of the Environment, Northwestern Region, unpublished data). The Ontario Ministry of the

Environment's criteria for open-water disposal of dredged materials (Thomas and Mudroch 1978) were exceeded for the four metals. Sediments are also highly contaminated with oil and grease. Samples collected in 1984 contained 7600-80,000 µg/g oil and grease (Bérard and Tseng 1986), greatly exceeding OME's dredging guideline of 1500 µg/g.

## CONCLUSIONS

These studies on the Rainy River and Thunder Bay Harbour have shown that environmental monitoring with caged leeches is feasible, provided the life history of the test species is considered and its specific environmental requirements are accommodated. Leeches in reproductive condition should be avoided, as their poor condition and lowered resistance will lead to high mortality. Concentrations of dissolved salts and temperature appear to be critical water quality factors affecting survival. Incompatibility between test organism and study area could be avoided by recruiting local species, if they are available in sufficient numbers.

Caged leeches exhibited some mortality after exposure in Thunder Bay Harbour, while mussels subjected to identical conditions did not. Leeches may simply be less hardy than mussels, which would be a drawback to their use in field studies, or they may have been more sensitive to the specific toxicants discharged from the wood preserving plant.

Leeches were shown to have much higher bioconcentration capacities for CPs than resident mussels in the Rainy River and caged mussels in Thunder Bay Harbour. There was also evidence that leeches accumulate higher

concentrations of CPs than net-spinning caddisfly larvae, burrowing mayflies and fathead minnows. Leeches may therefore be the best available screening organism for CPs in aquatic environments.

Over a three-week exposure period in the field, leeches appeared capable of "adjusting" their body burdens to reflect the concentrations and relative proportions of CPs in the environment to which they were introduced. Exposures of shorter duration may be adequate, however, as 10 days were sufficient to demonstrate site-to-site differences in CP uptake from contaminated sediment and, according to Jacob (1986), equilibrium with water-borne CPs may be reached within a week. Because leeches eliminate CPs very slowly, any residues present in their tissues initially could persist and lead to erroneously high final concentrations. Screening the stock of test organisms for CPs prior to exposure, to be sure they are contaminant-free, is therefore strongly recommended.

The origin, distribution and persistence (with respect to bioavailability) of CPs in Thunder Bay Harbour could be determined from residues of CPs accumulated by caged leeches. Results of laboratory sediment bioassays supported the main findings of the field study, and also demonstrated that toxicity increased with increasing sediment contamination. When used together, bioaccumulation and toxicity studies can provide a powerful bioassessment tool for determining the impact of point sources of pollution on their receiving waters.

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Table 1. Concentrations of chlorophenols in clarified water (µg/L) and suspended solids (ng/g wet weight) from mill effluents and river water samples (no value = not detectable).

| Chlorophenol isomer | Canadian mill effluent | | American mill effluent | | Site 3 | | Site 18 | | Site 32 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Water | Solids | Water | Solids | Water | Solids | Water | Solids | Water | Solids |
| 2-CP | 0.015 | 1.3 | | | | | | | | |
| 4-CP | 0.014 | 1.2 | 0.011 | | 0.011 | | 0.011 | | 0.006 | |
| 2,6-DCP | 0.056 | 8.8 | | | | | | | | |
| 3METHYL-4CP | | 1.5 | | | | | | | | |
| 2,4-DCP | 0.162 | 65.8 | 0.021 | | | | | | | |
| 3,5-DCP | 0.015 | 2.9 | | | | | | | | |
| 3,4-DCP | | 1.6 | | | | | | | | |
| 2,4,6-TCP | 4.299 | 208.0 | 0.145 | 2.5 | 0.030 | | 0.050 | | | |
| 2,4,5-TCP | | 3.6 | | | | | | | | |
| 2,3,4-TCP | | 2.0 | | | | | | | | |
| 3,4,5-TCP | | 3.9 | | | | | | | | |
| 2,3,4,6-TECP | 1.064 | 39.7 | 0.041 | 1.8 | | | | | | |
| 2,3,4,5-TECP | | 1.3 | | | | | | | | |
| PCP | 0.465 | 12.3 | 0.028 | 1.5 | | 0.9 | | 1.1 | | |
| TOTAL | 6.090 | 353.9 | 0.246 | 5.8 | 0.044 | 0.9 | 0.061 | 1.1 | 0.006 | |

Table 2. Concentrations of chlorophenols (ng/g wet weight) in organisms surviving the sediment bioassays.

| Site | 2,4,6-TCP | 2,4,5-TCP | 2,3,4-TCP | 2,3,5,6-TECP | 2,3,4,5-TECP | PCP |
|---|---|---|---|---|---|---|
| | | | Leeches | | | |
| 9 | 120 | 5780 | 20 | 220 | 843 | 1080 |
| 8 | 140 | <10 | 20 | 30 | <5 | 102 |
| 7 | 120 | 60 | <10 | 50 | 6 | 92 |
| 24 | 110 | 30 | 20 | 30 | 8 | 42 |
| Control | 180 | 50 | 50 | 40 | 13 | 79 |
| | | | Mayflies | | | |
| 9 | -* | - | - | - | - | - |
| 8 | 30 | 30 | <10 | 20 | <5 | 68 |
| 7 | 40 | 30 | <10 | 20 | <5 | 31 |
| 24 | 30 | 50 | 20 | 30 | 8 | 66 |
| Control | 30 | 10 | 10 | 20 | 6 | 20 |
| | | | Minnows | | | |
| 9 | - | - | - | - | - | - |
| 8 | 20 | <10 | <10 | 10 | <5 | 61 |
| 7 | <10 | 10 | <10 | <10 | <5 | 13 |
| 24 | 30 | 20 | <10 | 20 | <5 | 42 |
| Control | 30 | 30 | <10 | 10 | 9 | 23 |

*dead, no sample

Table 3. Rainy River water quality data for August, 1986 (Range for summer months in brackets).

| Location | % survival of caged leeches | Temp (°C) | pH | Conductivity (µmhos/cm) | TDS (mg/L) | Alkalinity (mg/L $CaCO_3$) |
|---|---|---|---|---|---|---|
| Rainy Lake | 0 | 21.0 | 7.2 | - (47- 51) | - | - |
| Above mills | 0 | 21.0 | 7.0 | 49 (47- 51) | 39 (39-73) | 19 (19) |
| 15 km below mills | N* | 22.5 | 7.7 | 68 (54- 68) | 52 (50-75) | 24 (18-24) |
| Above Emo | N* | 22.0 | 6.9 | 72 (61- 72) | 36 (36-65) | 25 (22-30) |
| Below Emo | 0 | 21.5 | 7.0 | 72 (61- 72) | 50 (50-70) | 25 (22-28) |
| Above Rainy River | 83 | 23.0 | 6.9 | 84 (71- 84) | 62 (62-80) | 31 (29-33) |
| Below Rainy River | 87 | 22.0 | 6.9 | 84 (71- 84) | 62 (62-85) | 31 (28-31) |
| Big Fork River | 87 | 23.0 | 8.0 | - (140-210) | - | - |
| Baudette River | 80 | 22.0 | 7.3 | - (120-180) | - | - |

*N = no cage set
No value = no data available

Table 4. Bioaccumulation of pentachlorophenol (ng/g wet weight) from Thunder Bay Harbour sediment under field and laboratory conditions (no value = not tested).

| | Field | Laboratory | | |
|---|---|---|---|---|
| Site | Caged Leeches | Leeches | Mayflies | Minnows |
| 1 | 5300 | -- | -- | -- |
| 10 | 1267 | DEAD | DEAD | DEAD |
| 9 | 817 | 1080 | DEAD | DEAD |
| 8 | 373 | 102 | 68 | 61 |
| 7 | 83 | 92 | 31 | 13 |
| 24 | <50 | 42 | 66 | 42 |
| Control | <50 | 79 | 20 | 23 |

## FIGURE CAPTIONS

FIG. 1. Rainy River study sites (● = caged leeches; ○ = resident leeches; △ = resident mussels; □ = resident caddisfly larvae).

FIG. 2. Locations of leech and mussel cages in Thunder Bay Harbour (sediment bioassay sites circled).

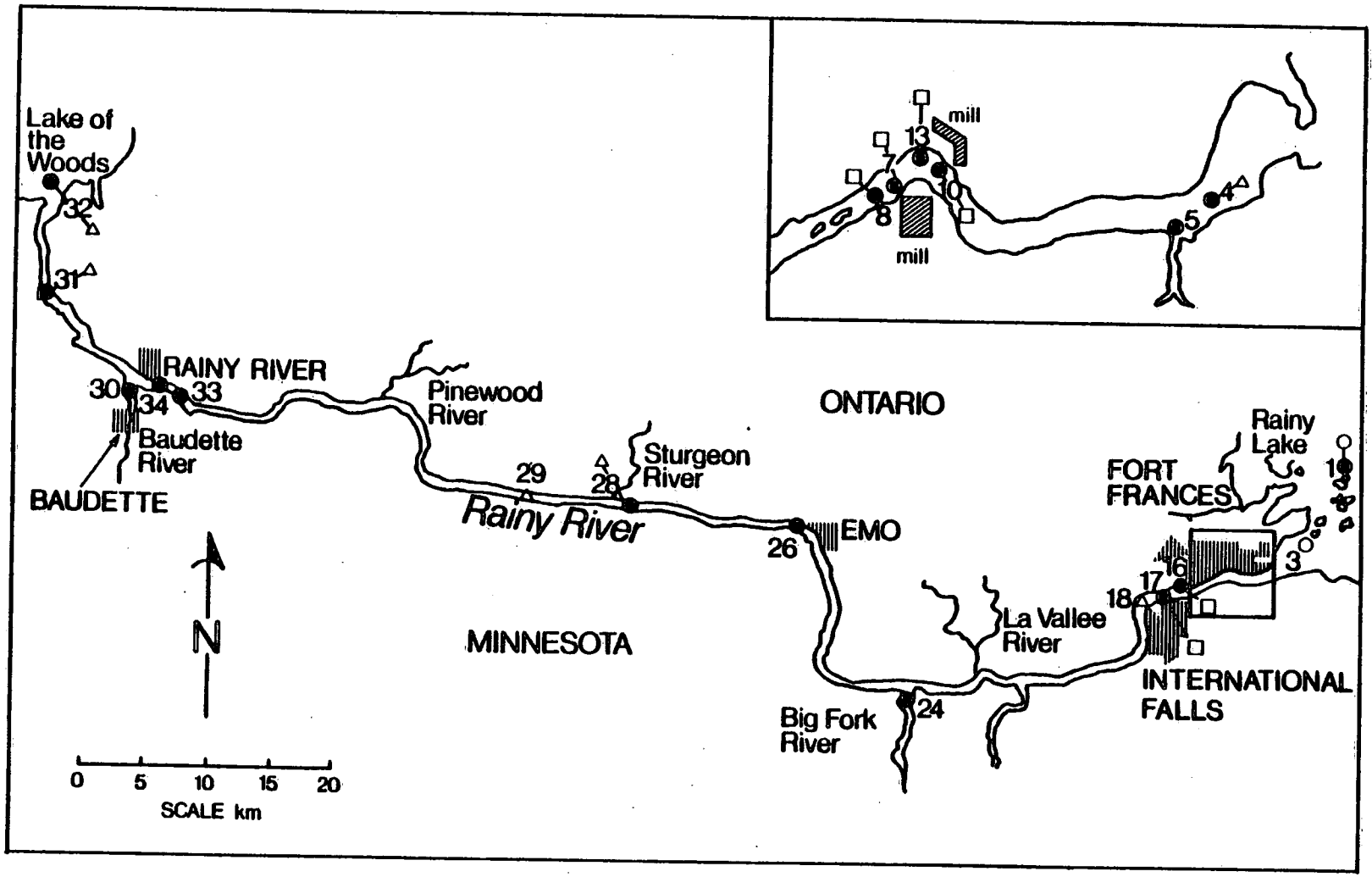
FIG. 3. Concentrations of total chlorophenols (ng/g wet weight) in leeches (numbers in lower portion of figure) and mussels (numbers in upper portion) in the Rainy River (* = resident leeches).

FIG. 4. Relative proportions of chlorophenol congeners in leeches and in pulp mill effluent. (For effluent, dark and light bars represent suspended solids and clarified water, respectively.)

FIG. 5. Relative proportions of chlorophenol congeners in mussels and in pulp mill effluent. (Legend as per Fig. 4.)

FIG. 6. Concentrations of four chlorophenol congeners (ng/g wet weight) in caged leeches from Thunder Bay Harbour.

RUNNING TITLE: Leeches and Mussels as Biomonitors for Chlorophenols


Lake of the Woods
32
31
RAINY RIVER
30
34
33
Baudette River
BAUDETTE
Pinewood River
29
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Sturgeon River
Rainy River
ONTARIO
MINNESOTA
26
EMO
Rainy Lake
FORT FRANCES
19
3
16
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La Vallee River
INTERNATIONAL FALLS
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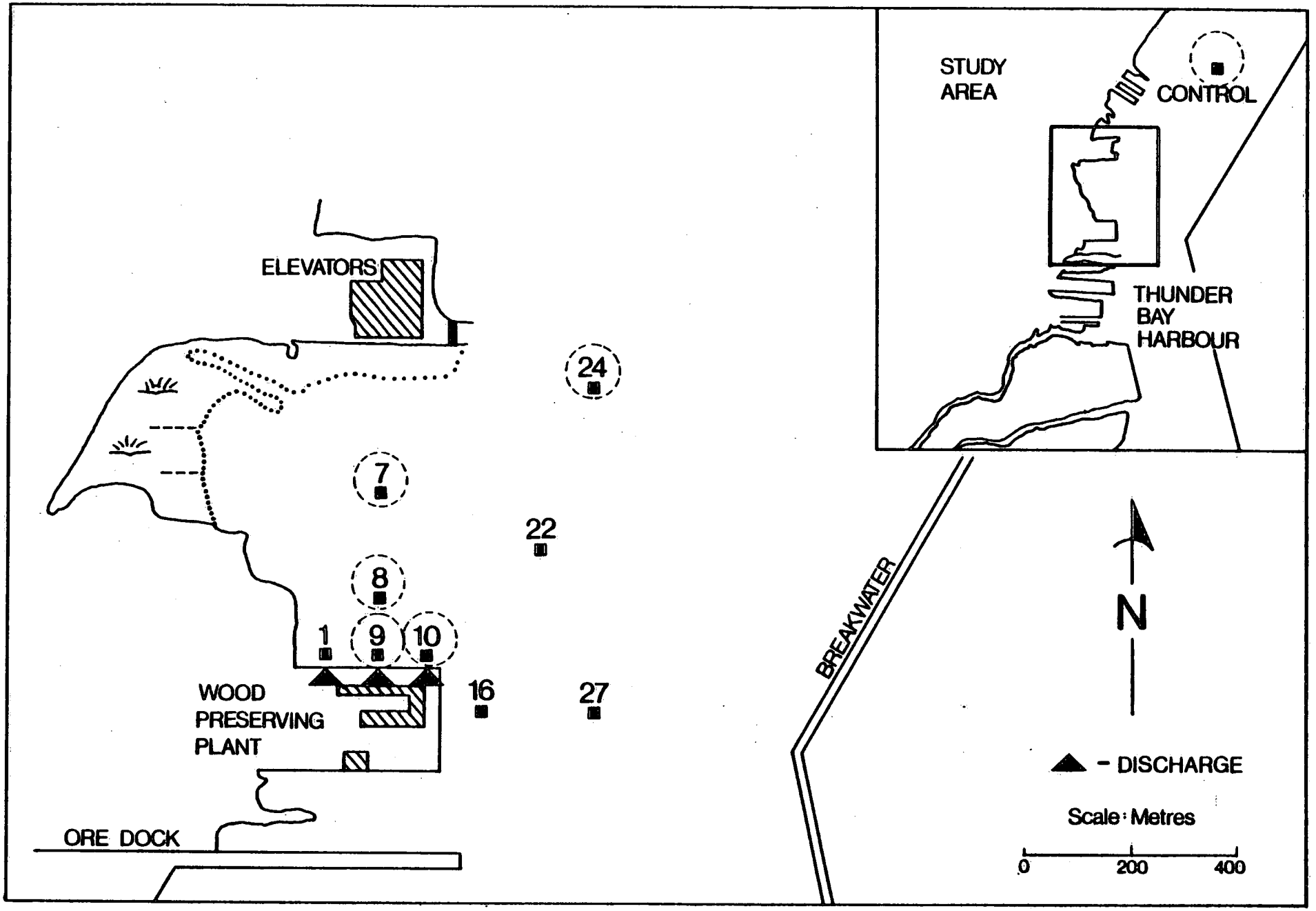
Figure 1


STUDY AREA
CONTROL
THUNDER BAY HARBOUR
ELEVATORS
24
7
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WOOD PRESERVING PLANT
ORE DOCK
BREAKWATER
N
- DISCHARGE
Scale: Metres
0
200
400

Figure 2

Figure 3

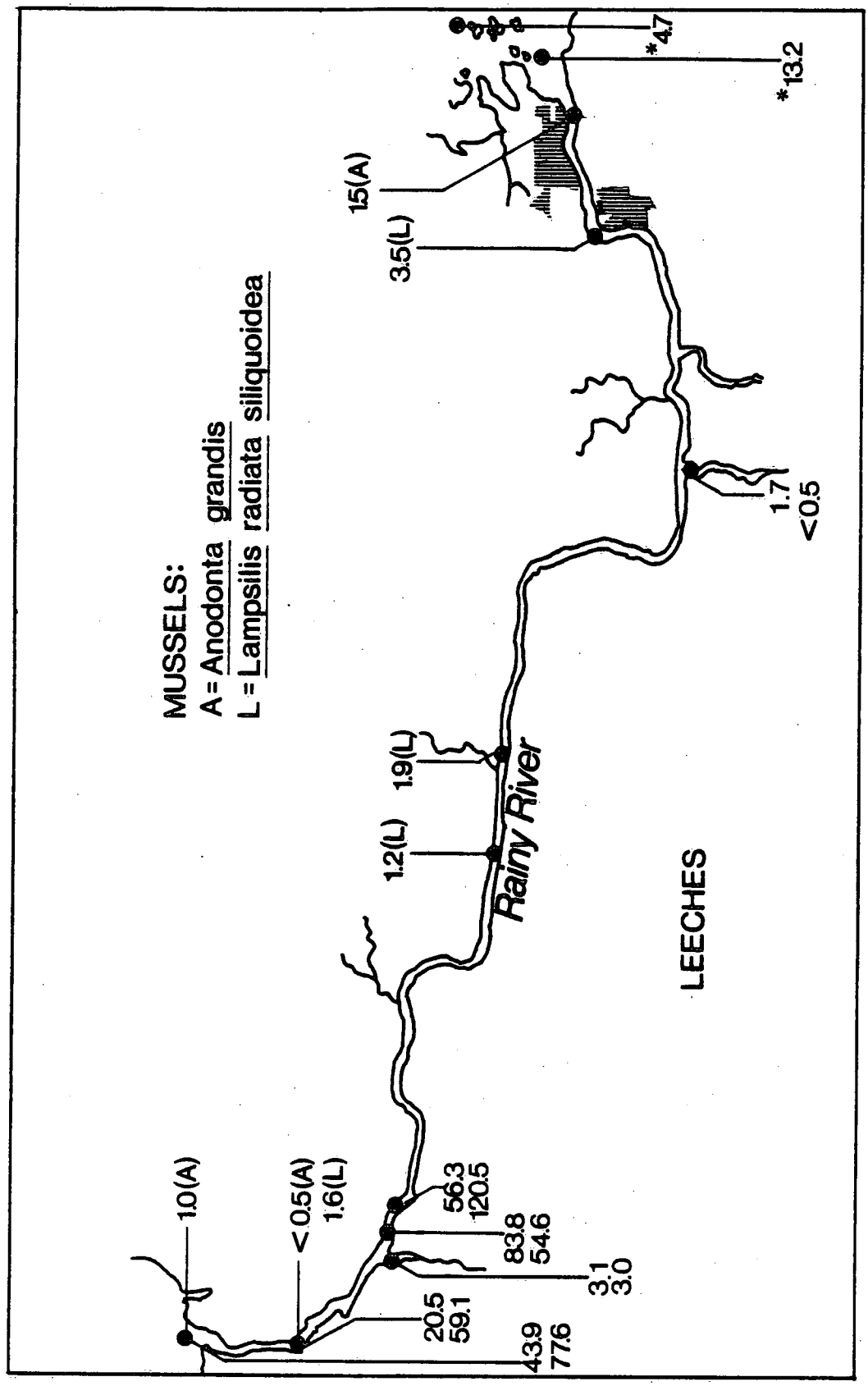
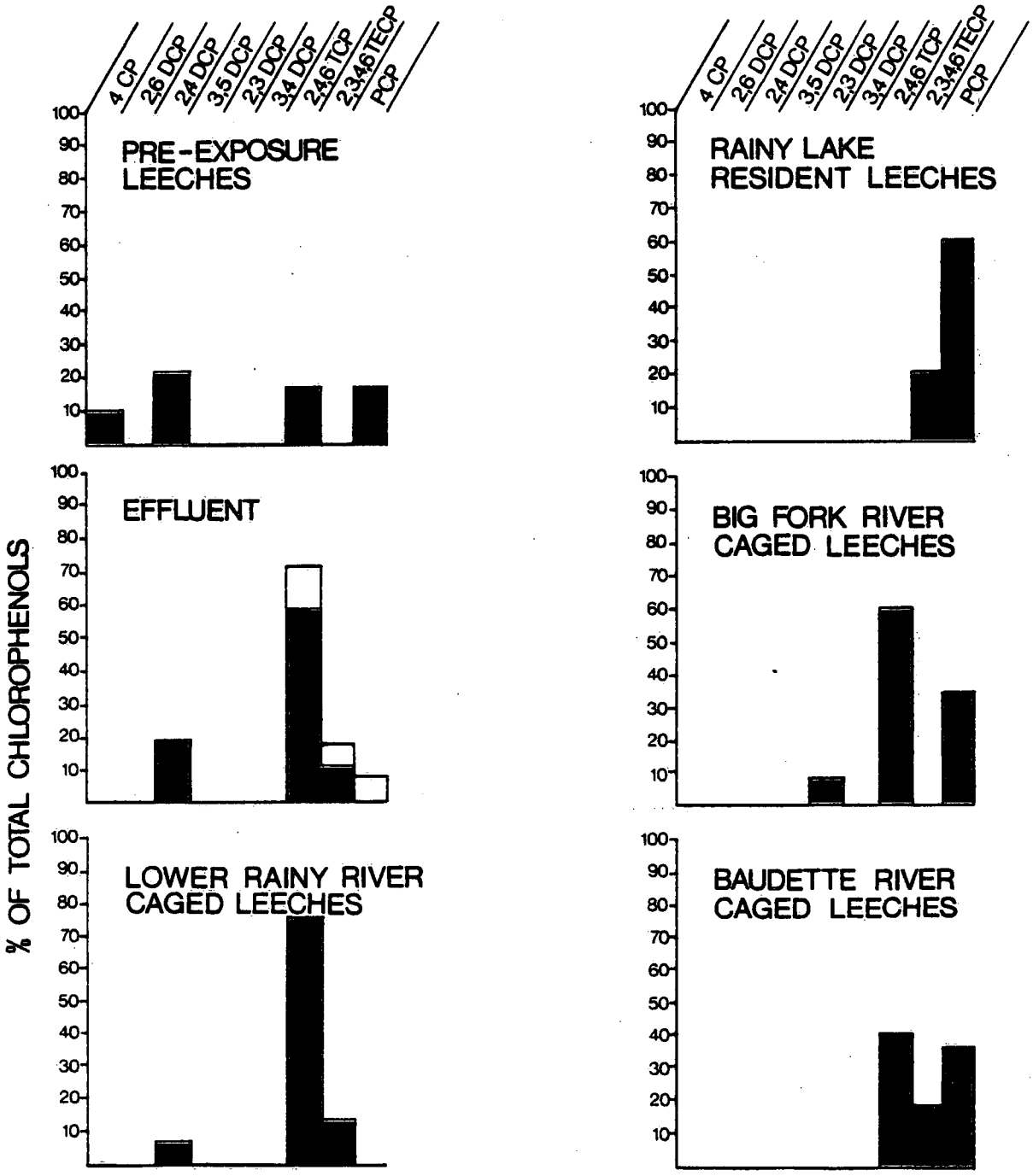

MUSSELS:
A = Anodonta grandis
L = Lampsilis radiata siliquoidea
LEECHES
Rainy River
1.0(A)
<0.5(A)
1.6(L)
20.5
59.1
43.9
77.6
56.3
120.5
83.8
54.6
3.1
3.0
1.2(L)
1.9(L)
15(A)
3.5(L)
*4.7
*13.2
1.7
<0.5

Figure 4


% OF TOTAL CHLOROPHENOLS
4 CP
26 DCP
24 DCP
35 DCP
23 DCP
34 DCP
246 TCP
2346TECP
PCP
PRE-EXPOSURE LEECHES
RAINY LAKE RESIDENT LEECHES
EFFLUENT
BIG FORK RIVER CAGED LEECHES
LOWER RAINY RIVER CAGED LEECHES
BAUDETTE RIVER CAGED LEECHES
100
90
80
70
60
50
40
30
20
10

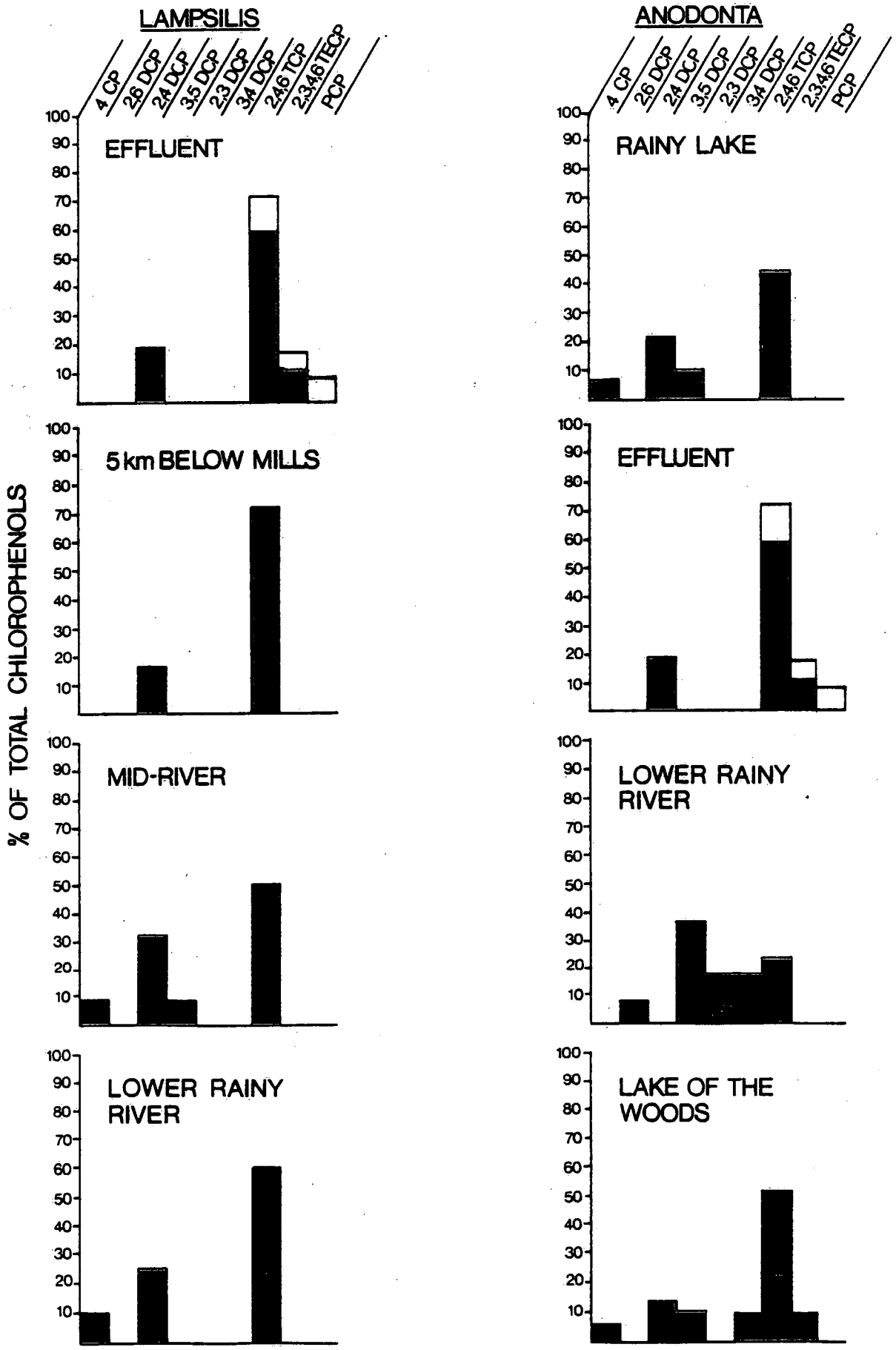
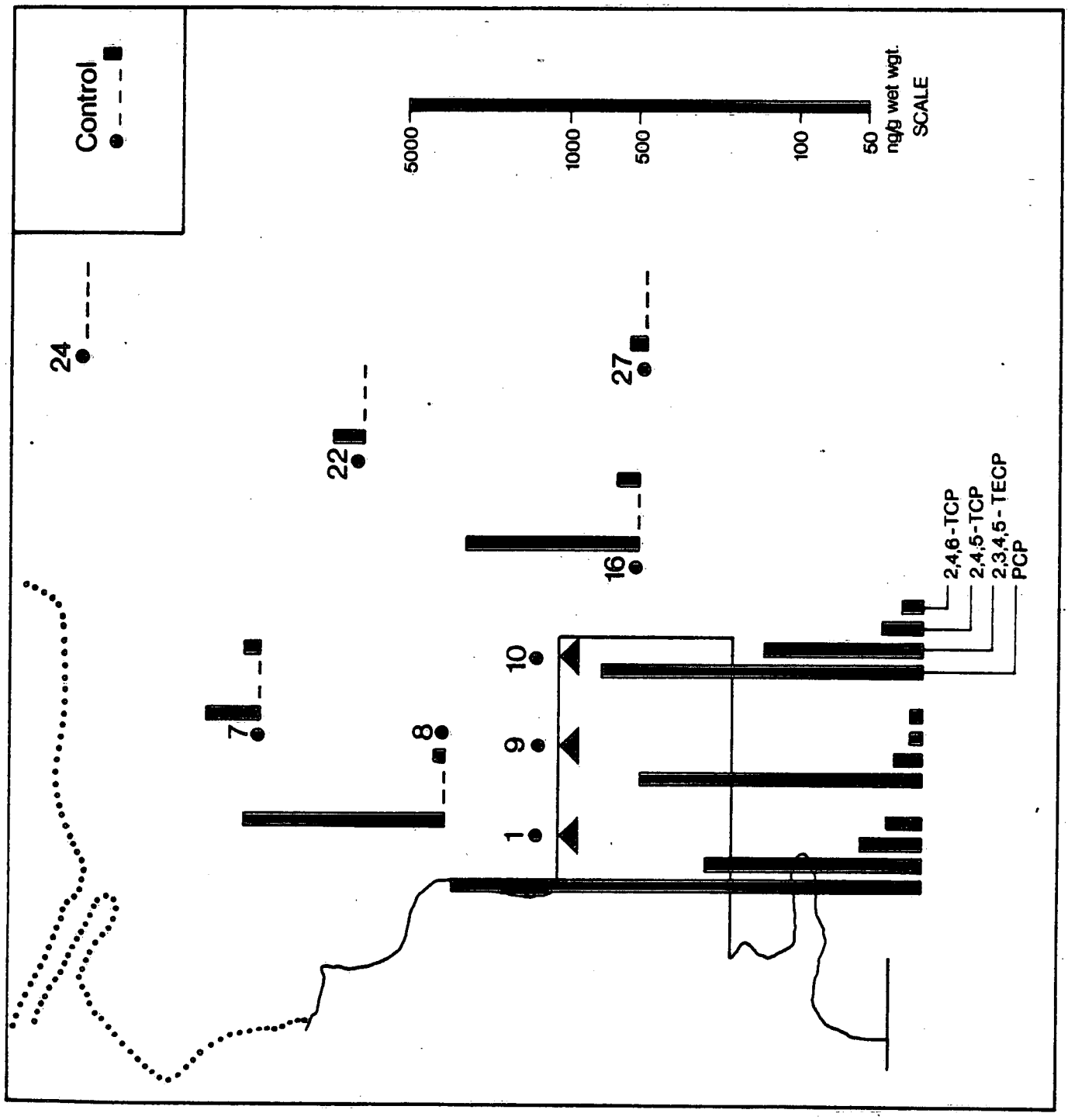

LAMPSILIS
ANODONTA
4 CP
2,6 DCP
2,4 DCP
3,5 DCP
2,3 DCP
3,4 DCP
2,4,6 TCP
2,3,4,6 TECP
PCP
% OF TOTAL CHLOROPHENOLS
EFFLUENT
RAINY LAKE
5 km BELOW MILLS
EFFLUENT
MID-RIVER
LOWER RAINY RIVER
LOWER RAINY RIVER
LAKE OF THE WOODS

Figure 6


Control
5000
1000
500
100
50
ng/g wet wgt.
SCALE
24
22
27
16
7
8
10
9
1
2,4,6-TCP
2,4,5-TCP
2,3,4,5-TECP
PCP