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TITLE

MERCURY IN THE AQUATIC ENVIRONMENT: A SUMMARY OF RESEARCH CARRIED OUT BY THE FRESHWATER INSTITUTE 1970-1971

EDITED BY

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P R E F A C E

Canadians can rightfully boast of the infinite variety and beauty of their vast country so generously endowed with open countryside, forests, and seemingly endless miles of rivers, lakes and ocean coastline. The fact that our boundaries encompass far more of the world's supply of these environmental amenities than might be justified on a per capita basis has contributed to our complacency regarding pollution. However, recent awareness of the hazards and detrimental effects of man's abuse of his environment has stemmed from a number of events. Perhaps the most important of these was the discovery that some of our waterways and their living resources are contaminated with toxic mercury compounds. The resultant crisis posed a tremendous challenge to a great many scientists in Canada and the same problem was soon recognized in the United States. The reports that follow record the commendable efforts and accomplishments of scientists at the F.R.B. Freshwater Institute at Winnipeg in meeting this challenge.

A few remarks about mercury and the situation prior to the Canadian mercury crisis may be of interest. First of all, mercury or "quicksilver" is a natural constituent of the earth's crust and exposed deposits of "cinnabar", the red sulphide of mercury, are visible in certain parts of the Canadian Rockies. Secondly, the toxic properties of mercury and its compounds have been known for centuries. Quicksilver

itself is very toxic, particularly in the vaporous state; nevertheless, the unusual liquid can be safely handled and is utilized in a number of household commodities. Consequently, the discharge of quicksilver in the process of manufacturing chlorine or in purifying gold and silver was not considered a real threat. Being exceedingly heavy and already in the elemental state, it would readily settle into sediments and stay put. However, what was not known until relatively recently is that when mercury and its compounds become deposited in an aquatic environment, they are converted biologically to the most toxic form, methyl mercury. This in essence, is what has created the alarm.

Methyl mercury again is not new to chemists inasmuch as it has been used for years as a fungicide in the treatment of seeds. Its exceedingly toxic properties have been recorded since a number of human deaths have resulted from the use and manufacture of methyl mercury and its compounds. On the other hand, we now know that fish and shellfish can accumulate methyl mercury seemingly without ill effects. However, if humans, birds, or wildlife consume quantities of mercury-contaminated fish, severe illness and even death can result as happened at Minamata Bay, Japan. In this instance, it was some time before methyl mercury in highly contaminated fish was established as the cause of "Minamata Disease". Also, the wholesale use of methyl mercury seed dressings in Sweden was claimed to be responsible for drastic reductions in bird populations.

With this as background, and the fact that most of the mercury found in fish is in the methyl form, it is not

difficult to appreciate the great concern in the minds of Canadian officials when it was announced in November of 1969 that fish from the South Saskatchewan River had been found to contain up to 10 ppm mercury. It was only a matter of hours until the Freshwater Institute was formally requested to launch a detailed investigation into mercury in Canadian fish.

When you have read the reports herein, you will readily understand why I am indeed proud to have been associated with those responsible.

May, 1972

E. Graham Bligh

MERCURY CONTAMINATION IN FISH FROM CANADIAN WATERS

by

K.C. Tam and F.A.J. Armstrong

Mercury contamination of fish in water systems in Sweden was found in the 1960's and in Canada Fimreite (1968) presented evidence of a foreseeable problem with mercury.

This problem became urgent after the announcement on 27 November 1969, by Wobeser and his colleagues at the University of Saskatchewan, that fish from the South Saskatchewan River contained up to 10 parts per million of mercury. This river is part of a large system with several lakes (including Lake Winnipeg) which support important commercial fisheries. For the protection of the public the Department of Fisheries immediately detained all stocks of fish believed to come from this river system, and an inspection scheme was set up. Until June 1970 this was at the Freshwater Institute in Winnipeg. Analytical methods were developed there and a programme of research initiated.

The Food and Drug Directorate of the Department of National Health and Welfare set a limit of 0.5 ppm (mg mercury per Kg wet

weight) which is enforced under a section of the Food and Drug Act and Regulations. Fish with mercury concentrations above this level may not be offered for sale. This limit is in use also in the United States. It is subject to revision, and is believed to have a safety-factor of 10-100 against the development of signs of neurological disease in men. It is one half of the limit in use in Sweden and in Germany. Fish consumption in Canada is estimated to be about 18 g per capita per day, which is considerably less than in many European and Asiatic countries. The 0.5 ppm limit is applied to fish exported from Canada, whatever its destination.

As part of the initial inspection scheme and of the continuing research program at the Freshwater Institute, a national survey was started, in cooperation with various Federal and Provincial Government departments. Most species of food fishes from a great many locations were analysed. It was found that contamination by mercury varied with the position of the fish in the food-chain and on geographical location. Mercury levels are highest in predatory fish, as, in freshwater, in pike and walleye, or in the ocean in tuna and swordfish. Table 1 shows mercury levels in fish from Lake Winnipeg to illustrate this point.

Contaminated waters were located in this survey. In the light of Swedish experience chlorine-alkali plants were suspect. These plants, if they use the electrolytic process with mercury cathodes, are known, unless special precautions are taken, to

discharge mercury and its salts in their liquid effluent. Most of the mercury contamination of fish in Canadian inland waters can be related to all but one of the 14 chlorine-alkali plants in the country the location of which is shown in Figure 1. These plants are stated to have consumed some 100,000 Kg of mercury in 1969 out of a total Canadian consumption of about 140,000 Kg. Their discharges are now strictly regulated.

Some lakes have been found in which, although there is no obvious source of man-made pollution, mercury levels in fish exceed the 0.5 ppm limit. It should be stated at once, however, that the concentrations found are seldom much higher than 1 ppm, and no nowhere near as high as those which can sometimes be found downstream of a chlorine-alkali plant. Table 2 shows waters in which fish have low mercury content, and Table 3 those waters in which they contain more than the statutory limit of 0.5 ppm. These analyses were of dorsal muscle and were all done in 1970 and 1971. For freshwater fish they are, where possible, of either Northern pike (Esox lucius) or of walleye (Stizostedion vitreum) which usually show the highest,and similar, levels of mercury when a variety of fishes from a given water body is analysed. Table 4 shows areas where mercury contamination of fish can be related to the existence of chlorine-alkali plants. Table 5 shows areas where fish may contain more than 0.5 ppm mercury, but where there is no obvious source of mercury pollution. There is reason to believe that there may be natural occurrences of mercury, often in association with metal sulphide ore deposits, in perhaps one

half of these areas, or more. The various parts of the Gazetteer of Canada listed in the reference section may be used to identify the lakes and rivers listed.

These tables are by no means exhaustive. Further information is to be found in the publications of Bligh (1970, 1971a, b) and (for the Province of Ontario only) in an anonymous booklet issued by the Ontario Department of Lands and Forests for the guidance of anglers. Much valuable information has been compiled and is in course of preparation for publication by the Inspection Branch of the Fisheries Service.

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Table 1. Mercury levels in fish from Lake Winnipeg

Species	No. of Samples	Mean	Max	Min
Burbot (*Lota lota*)	95	0.33	0.88	0.07
Carp (*Cyprinus carpio*)	8	0.38	0.51	0.23
Cisco (*Coregonus artedii*)	97	0.19	0.88	0.03
Freshwater Drum (*Aplodinotus grunniens*)	73	0.40	3.59	0.10
Goldeye (*Hiodon alosoides*)	4	0.32	0.40	0.24
Lake Whitefish (*Coregonus clupeaformis*)	48	0.05	0.20	0.01
Mooneye (*Hiodon tergisus*)	6	0.16	0.09	0.28
Northern Pike (*Esox lucius*)	145	0.41	1.27	0.14
Sauger (*Stizostedion canadense*)	140	0.51	0.99	0.14
Walleye (*Stizostedion vitreum*)	189	0.35	0.76	0.13
White sucker (*Catostomus commersoni*)	147	0.11	0.55	0.02
Yellow Perch (*Perca flavescens*)	127	0.48	1.14	0.07

Table 2. Canadian waters containing fish with less than 0.5 ppm mercury (wet weight) in muscle tissue.

British Columbia

Burrard Inlet (Cb)
Cheakamus River (RT)
Cypress Creek, West Vancouver (Cb)
Fraser River Flats (SF)
Hecate Strait (Ro, SF)
Lajoie Lake (RT)
Mamquam River (CS)
Okanagan Lake (RT)
Prince Rupert (He)
Squamish River (DV, RT)
Stuart Lake (DV)
Tezzeron Lake (LT)
Tofino (Cb)

Saskatchewan

Churchill River
Cree Lake (LT)
Deschambault Lake
Dillon Lake
Dore Lake
Frobisher Lake
Lake Athabasca
Manawan Lake
McLennan Lake
Nemeiben Lake
Pipestone Lake
Reindeer Lake
Turner Lake
Wasekamio Lake
Wollaston Lake
Wood Lake

Alberta

Athabasca River at Jasper (DV)
Athabasca River at Whitecourt
Bangs Lake
Battle River at North of Ponoka
Blackett Lake
Bow River [Go]
Lac la Biche
Lac St. Anne
Chip Lake
Cold Lake
Isle Lake
Kehiwin Lake
Kinnaird Lake
Peace River and Wabasca River
Pigeon Lake
Pine Lake
Seibert Lake
Sylvan Lake
Wabamun Lake
Wabasca Lake
Whitefish Lake

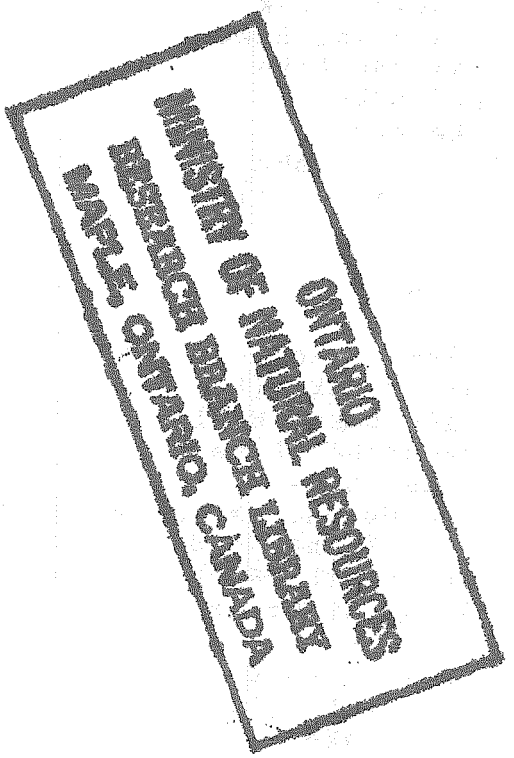

Table 2. (cont'd)

Manitoba

Athapapuskow Lake
Atkinson Lake
Baldock Lake
Barrington Lake
Bell Lake
Betula Lake
Brereton Lake
Buckland Lake (LT)
Childs Lake
Churchill River
Clearwater Lake
Cockeram Lake
Colen Lake
Cormorant Lake
Dauphin Lake
Dunphy Lake
Falcon Lake
Gauer Lake
Gods Lake
Goldsand Lake
Heming Lake
High Hill Lake
Island Lake
Kisseynew Lake
Kississing Lake
Kistigan Lake
Knee Lake
Lake Audy
Lake Manitoba
Lake St. Martin
Lake Winnipegosis
Landing Lake
Long Lake, Manigotagan River
Molson Lake
Moose Lake, North of Cedar Lake
Moose Lake, Northwest Angle Forest Reserve
Moose Lake, West of Washaw Bay
Oak Lake
Pakwa Lake
Partridge Crop Lake
Pelican Lake
Pikwitonei Lake
Rat river at St. Malo (Ch)
Seller Lake
Setting Lake
Silsby Lake
Steeprock Lake
Stevenson Lake
Stupart Lake
Utik Lake
Vandekerckhove Lake
Walker Lake
Waterhen Lake
Wellman Lake
Wells Lake
Whitemud River and Willow Bend Creek
Whiteshell Lake
Wintering Lake
Wuskwatin Lake

Ontario

Atikwa Lake
Caviar Lake (LT)
Eagle Lake, Eagle River
Favourable Lake
Kakagi Lake
Lake Huron, except at south end
Lake of the Woods at Gohere Bay, Regina Bay and Witch Bay
Lake of the Woods at Lot Nos. 5,7,8,9,13,15,20,20,22 and 28
North Spirit Lake
Ord Lake
Perrault Lake
Roddy Lake (LT)
Rowan Lake
Thunder Lake, east of Dryden
Trout Lake

Table 2. (cont'd)

Quebec

Chaleur Bay (AC, AH)
Gulf of St. Lawrence (AC, Lo)
St. Lawrence River, down from Ile d'Orleans except near mouth of Saguenay River (Cm, He, Re)

Atlantic Provinces

Bathurst, N.B. (Lo)
Charlo River, N.B. (AM)
Lacquet River, N.B. (Cb)
St. Croix River, near St. Stephen, N.B. (Fa)
Picton, N.S. [Lo]
Strait of Canso, N.S. (Cu)
Burin, NFLD (AC)
Cape Bonavista, NFLD (AC)
Grand Bank, NFLD (AC)
St. Anthony, NFLD (AC)
St. John, NFLD (AC)
Twillingate Islands (AC)

Northwest Territories

Baker Lake (LT)
Grandin Lake (LT)
Great Slave Lake
Lac la Martre (LT)
MacKay Lake (LT)
Merkley Lake (LT)
Mosquito Lake (LT)
Rebesca Lake (LT)
Tathlina Lake
Tronka Chua Lake

The species code used in Tables 2 and 3

AC = Atlantic Cod (Gadus morhua)

AH = Atlantic Halibut (Hippoglossus hippoglossus)

AM = Atlantic Mackerel (Scomber scombrus)

AW = Atlantic Wolffish (Anarhichas lupus)

BF = Buffalo Fish (Ictiobus spp.)

Cb = Crab (Cancer spp.)

Ch = Crayfish (Orconectes virilis)

Cm = Clam (Mya arenaria)

CS = Chinook Salmon (Oncorhynchus tshawytscha)

Cu = Cunner (Tautogolabrus adspersus)

DV = Dolly Varden (Salvenlinus malma)

Fa - Fallfish (Semotilus corporalis)

Go = Goldeye (Hiodon alosoides)

He = Herring (Clupea harengus)

Lo = Lobster (Homarus americanus)

LT = Lake Trout (Salvelinus namaycush)

Re = Redfish (Sebastes marinus)

Ro = Rockfish (Sebastes spp.)

RT = Rainbow Trout (Salmo gairdneri)

Sa = Sauger (Stizostedion canadense)

SF = Starry Flounder (Platichthys stellatus)

Table 3. Canadian waters containing fish with more than 0.5 ppm mercury (wet weight) in muscle tissue

British Columbia

Dogfish, and tuna from most locations
Carpenter Lake (DV)
Howe Sound near Squamish (Cb, Rd, SF)
Pinchi Lake (LT)

Alberta

Athabasca River, downstream Fort Assiniboine
Athabasca River and Pembina River
Chickenhill Lake
Freeman Lake
Gull Lake
Lac la Nonne
McLeod River and Wolf Creek
Moore Lake
Moose Lake
North Saskatchewan River, starting from mouth of Rose Creek
Red Deer River, starting from Sunde (Go, Sa)
South Saskatchewan River, starting from Bow Island
Ware Creek and Three Point Creek (RT)
Wolf Lake, Sand River

Saskatchewan

Last Mountain Lake (BF)
Qu'Appelle River, starting from outflow of Last Mountain Lake
Murray Lake
North Saskatchewan River
Saskatchewan River
South Saskatchewan River

Manitoba

Aikens Lake
Assiniboine River
Caddy Lake, Whiteshell Provincial Park
Carrot River
Cauchon Lake
Clear Lake, Riding Mountain National Park
Drunken Lake, Minago River
Family Lake
Fishing Lake

Manitoba (cont'd)

Harrop Lake
Lake St. George
Lake Winnipeg
Long Lake, Riding Mountain National Park
McGavock Lake
Moar Lake
Nelson Lake
Oiseau River
Red River
Sasaginnigak Lake
Saskatchewan River
Smokey Lake
Souris River
Washahigan Lake
Whitefish Lake
William Lake
Winnipeg River

Ontario

Bell Lake, English River Tributary
Bemaji Lake
Black Sturgeon Lake
Caribou Lake, Northwest of Lake Nipigon
Cliff Lake
Cobham Lake
Deer Lake
Detroit River
Dryberry Lake
Eagle Lake, Oiseau River
English River starting from Oak Lake
Hornby Lake, south of Sandy Lake
Indian Lake, English River Tributary
Lac Seul
Lake Abitibi
Lake Erie
Lake Huron, at south end
Lake Nipigon
Lake Nipissing
Lake Ontario
Lake St. Clair
Lake Superior, near Marathon
Lake Superior, near Thunder Bay
Lake of the Woods, at Clearwater Bay, Echo Bay and Splitrock Bay
Lake of the Woods, at Lot Nos. 1,2,3,14,29,31,41,47 and 51
Long Dog Lake
Lower Manitou Lake
McCrea Lake
Miniss Lake
Mojikit Lake
Ottawa River
Ozhiski Lake
Pelicanpouch Lake
Pickle Lake, near Central Patricia
Rainy Lake

Table 3 (cont'd)

Ontario

Red Lake
St. Clair River
St. Lawrence River
St. Mary's River
Sandy Lake
Savant Lake
Stout Lake
Sydney Lake
Wabigoon Lake
Wabigoon River, starting from Wabigoon Lake
Winnipeg River

Quebec

Lac Champlain
Lac Chibougamau
Lac Dore
Lac Evans
Lac au Goeland
Lac Opemisca
Lac Ouescapis
Lac Poncheville
Lac Rocher
Lac Waswanipi
Ottawa River
Riviere Chateauguay
Riviere du Nord
Riviere Richelieu
Riviere Yamaska
St. Lawrence River, down to Ile d'Orleans
St. Lawrence River, near mouth of Saguenay River
Saguenay River, starting from Arvida

Atlantic Provinces

Swordfish and tuna from most locations
Passamaquoddy Bay, N.B. (AW)
Restigouche River, near Dalhousie, N.B. (Cm)

Northwest Territories

Gordon Lake (LT)
Hjalmar Lake (LT)
Kaminak Lake (LT)
Kaminuriak Lake (LT)
Merkham Lake (LT)
Nonacho Lake (LT)
North Henik Lake (LT)
Talston Lake (LT)

Table 4. Mercury contaminated areas where known mercury-cell chlor-alkali plants may be sources of pollution.

British Columbia

Howe Sound near Squamish

Saskatchewan

Saskatchewan River
South Saskatchewan River, starting from Saskatoon

Manitoba

Lake Winnipeg
Saskatchewan River
Winnipeg River

Ontario

Detroit River
English River starting from mouth of Wabigoon River
Lake Erie
Lake Huron, at south end
Lake Ontario
Lake St. Clair
Lake Superior, near Marathon
Lake Superior, near Thunder Bay
St. Clair River
St. Lawrence River
Wabigoon River, starting from Dryden
Winnipeg River starting from mouth of English River

Quebec

Saguenay River, starting from Arvida
St. Lawrence River, down to Ile d'Orleans
St. Lawrence River, near mouth of Saguenay River

Atlantic Provinces

Restigouche River, near Dalhousie, N.B.

Table 5. Mercury contaminated areas where no evident source of pollution exists

Alberta

Athabasca River, downstream Fort Assiniboine
Athabasca River and Pembina River
Chickenhill Lake
Freeman Lake
Gull Lake
Lac la Nonne
McLeod River and Wolf Creek
Moore Lake
Moose Lake
North Saskatchewan River, from mouth of Rose Creek to Edmonton
North Saskatchewan River, further downstream from Edmonton
Red Deer River, starting from Sun
South Saskatchewan River, starting from Bow Island
Ware Creek and Three Point Creek
Wolf Lake, Sand River

Saskatchewan

Last Mountain Lake
Qu'Appelle River, starting from outflow of Last Mountain Lake
Murray Lake
North Saskatchewan River down to Prince Albert
South Saskatchewan River, down to Saskatoon

Manitoba

Aikens Lake
Assiniboine River
Caddy Lake, Whiteshell Provincial Park
Carrot River
Cauchon Lake
Clear Lake, Riding Mountain National Park
Drunken Lake, Minago River
Family Lake
Fishing Lake
Harrop Lake
Lake St. George
Long Lake, Riding Mountain National Park
McGavock Lake
Moar Lake
Nelson River
Oiseau River
Red River
Sasaginnigak Lake
Smokey Lake
Souris River
Washahigan Lake
Whitefish Lake
William Lake

Table 5. (cont'd)

Ontario

Bell Lake, English River Tributary
Bemaji Lake
Black Sturgeon Lake
Caribou Lake, Northwest of Lake Nipigon
Cliff Lake
Cobham Lake
Deer Lake
Dryberry Lake
Eagle Lake, Oiseau River
Hornby Lake, south of Sandy Lake
Indian Lake, English River Tributary
Lac Seul
Lake Abitibi
Lake Nipigon
Lake Nipissing
Lake of Woods, at Clearwater Bay, Echo Bay and Splitrock Bay
Lake of Woods, at Lot Nos. 1,2,3,14,29,31,34,41,47 and 51
Log Dog Lake
Lower Manitou Lake
McCrea Lake
Miniss Lake
Mojikit Lake
Ozhiski Lake
Pelicanpouch Lake
Pickle Lake, near central Patricia
Rainy Lake
Red Lake
Sandy Lake
Savant Lake
Sydney Lake
Wabigoon Lake
Winnipeg River, down to mouth of English River except near Kenora

Quebec

Lac Champlain
Lac Chibougamau
Lac Dore
Lac E ans
Lac au Goeland
Lac Opemisca
Lac Ouescapis
Lac Poncheville
Lac Rocher
Lac Waswinipi
Ottawa River
Riviere Chateauguay
Riviere du Nord
Riviere Richelieu
Riviere Yamaska

Table 5 (cont'd)

Atlantic Provinces

Pasamaquoddy Bay, N.B.

Northwest Territories

Gordon Lake
Hjalmar Lake
Kaminak Lake
Kaminuriak Lake
Merkham Lake
Nonacho Lake
North Henik Lake
Talston Lake

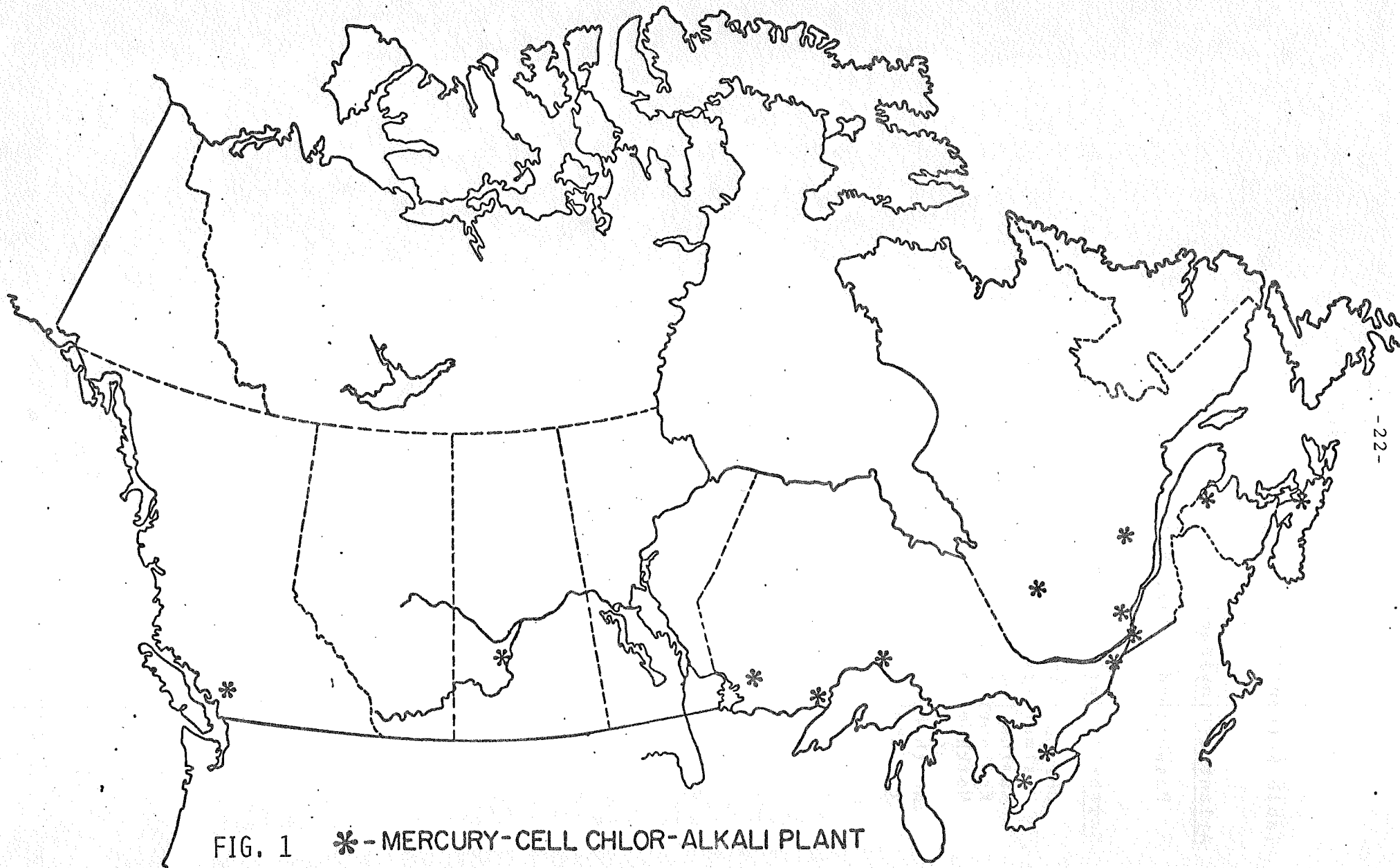

FIG. 1 *-MERCURY-CELL CHLOR-ALKALI PLANT

LENGTH-WEIGHT-MERCURY CONTENT RELATIONSHIPS IN VARIOUS GROUPS OF FISHES

by

D.P. Scott

Early in the investigations of mercury contamination of commercial species of fishes, data from some samples seemed to indicate that there might be a positive relationship between size of fish and degree of contamination, ie. larger fish of a particular species sample seemed to have more mercury than small specimens. The possibility was suggested that selective fishing for those individuals smaller than a certain critical size might permit limited restoration of some of the closed fisheries. To that end, a statistical investigation was undertaken to try to answer the following questions:

1. Is the apparent relationship between fish size and mercury content sufficiently tight within groups to permit reasonably accurate predictions of one variable from the other?
2. Are the relationship parameters highly diverse from species to species and area to area, requiring individual investigations and control limits for each commercial population?

would undoubtedly improve predictions.

2. Relationship parameters are also highly diverse, ranging in slope from -1.2, indicating quite a substantial loss of mercury as growth proceeds (as opposed to dilution, which would yield an approximate slope of -0.33), to as high as +3.30, indicating absorption and retention of mercury at a rate equivalent to over the cube of the weight increase (i.e. double the fish weight, and the mercury content would be over 8 times as high). General levels are also highly diverse, and one may conclude that each population must be treated separately.

3. Predictions based on the criterion of 95% of individuals containing less than 0.5 ppm total mercury showed considerable promise, although all species from the highly contaminated areas such as Clay Lake, etc., showed either no solution or ridiculously small values. However, some populations in which the average sample may have a mean value of mercury higher than 0.5 shown quite acceptable length limits to permit size-selective fishing.

Further analyses are in process to determine effects of condition factor, age, etc., on the relationships, and more samples will be analyzed to determine whether the relationships can be improved.

3. Are the sizes below which at least 95% of the individuals of a particular population contain less than 0.5 ppm mercury (the presently accepted limit) reasonable from a commercial viewpoint?

Fifty-three samples of eleven species of fish from a number of areas were obtained by gill netting. Fork lengths and total weights were taken and total mercury content obtained by the method of Armstrong and Uthe (1971). Simple and multiple regression analyses were carried out on log-transformed data, and various analyses of covariance and multiple covariance applied for between-groups comparisons. From these, predictions were made to show maximum permissible size at which 95% of individuals would have at most 0.5 ppm total mercury.

The results may be summarized as follows:

1. There is an enormous diversity in goodness-of-fit of a linear regression on the log-transformed data, ranging from approximately 0.00 correlation to as high as 0.93, the latter being enormously significant. Of the 53 samples, there were 31 with significant positive relationships of mercury content with length, 14 non-significant positive relationships, seven non-significant negative relationships, and only one significant negative relationhip. The answer to question 1 is, therefore, a qualified yes, depending on area and species, as indicated by these samples. Further sampling with larger sample sizes and ranges

Literature Cited

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A SURVEY OF MERCURY LEVELS IN THE BIOTA OF A MERCURY-CONTAMINATED RIVER SYSTEM IN NORTH-WESTERN ONTARIO

by

A. L. HAMILTON

Introduction

When Wobeser et al. (1970) first reported unacceptably high levels of mercury in freshwater fish from Canadian waters there was almost no information available on mercury levels in other freshwater organisms. Johnels et al. (1967) had included some invertebrate organisms in their search for indicators of mercury pollution in Sweden and another Swedish worker (Hannerz, 1970) had conducted a number of experiments on the accumulation of mercury by freshwater organisms. Matida and Kumada (1969) had reported on the distribution of mercury in water, bottom mud, and aquatic organisms from marine, estuarine and river habitats in Japan, however, there were apparently no published North American surveys of mercury levels in invertebrate organisms.

The objectives of this survey were: to determine the levels of mercury in invertebrates and small vertebrates from a mercury contaminated ecosystem; to attempt to relate differences in mercury concentrations to the life histories of the organisms involved; to identify organisms that

might be suitable as indicators of mercury pollution. Most of the organisms were collected in the vicinity of Clay Lake, Ontario since previous work (Bligh, 1970) had shown that fish from this lake had very high concentrations of mercury. Clay Lake is the first significant widening of the Wabigoon River downstream of a chlor-alkali plant located at Dryden, Ontario. The lake has a surface area of 3,000 hectares, a mean depth of approximately 8 meters and the theoretical renewing time is approximately 50 days (Armstrong, Metner and Capel 1972).

Materials and Methods

An initial survey of the biota in Clay Lake was conducted in June, 1970. Benthic invertebrates living in the deeper parts of the lake were collected with an Ekman dredge and the mud samples were then sieved through a 400 micron mesh. Individual organisms were picked out with forceps and sorted into taxonomic groups. Littoral invertebrates were collected with dip nets and sienes and then sorted into taxonomic groups. Planktonic organisms including small minnows, were collected with a towed plankton net (apertures 73 microns) and then put through a series of graded sieves to sort the organisms into size categories. Crayfish were collected in August and September of 1970 and throughout the summer of 1971. Most of the latter were taken with minnow traps baited with raw meat. The crayfish collected in 1970 were dissected and several organs analyzed separately while in 1971 the specimens were measured and then the abdominal muscle was removed and stored for later analysis. All samples were frozen

for storage and later assayed for mercury following the procedure outlined by Armstrong and Uthe 1971. All values presented in this report are expressed as parts per million (ppm) on a wet weight basis.

Results

The initial survey of food chain organisms indicated that some taxa accumulated much more mercury than others. Organisms living and/or feeding in the water column had only about 1/10 the concentration of mercury as organisms that lived in or attached to the bottom substrate (Fig. 1). It also appeared that organisms feeding on phytoplankton, zooplankton or attached algae had only about 1/10 as much mercury as detritus feeders, omnivores or taxa that fed primarily on other benthic invertebrates (Fig. 2).

The crayfish Orconectes virilis (Hagen) contained substantially higher concentrations of mercury than any other invertebrate collected. The 1970 adult specimens had concentrations of approximately 10 ppm in the abdominal muscle with concentrations in other organs ranging from 0.9 to 6.0 ppm (Fig. 3). The claw muscle of one of these specimens was analyzed for methyl mercury and the result was within 10 per cent of the total mercury value.

Analysis of a large sample of crayfish collected in June, July and August, 1971 from the bay adjacent to the North Star Lodge indicated a definite relationship between body weight and mercury concentration in abdominal muscle (Fig. 4).

A survey of mercury concentrations in the abdominal muscles of

crayfish collected in August, 1972 from the Wabigoon, Eagle, English and Winnipeg Rivers clearly indicated that there was a major source of mercury pollution originating in the vicinity of Dryden, Ontario (Fig. 5). Four samples (each consisting of 4 individuals) collected in the Wabigoon River above Dryden, Ontario averaged between 0.18 and 0.27 ppm on a wet weight basis. Five similar samples from the Eagle River which flows into the Wabigoon about mid-way between Dryden and Clay Lake yielded similar values (0.09 to 0.27 ppm). In contrast 13 samples from Clay Lake and the Wabigoon River immediately above and below the lake yielded values between 1.43 and 7.36 ppm. A single sample from the English River downstream from its junction with the Wabigoon River had a value of 1.34 ppm. Values for eight samples taken in the Winnipeg River varied from 0.12 to 0.56 ppm.

Discussion

Results of this investigation have demonstrated that organisms in a mercury-contaminated freshwater ecosystem accumulate very different concentrations of mercury. Undoubtedly many factors are involved, however these differences seem to be generally related to both food habits and habitat selection. In this survey it was apparent that organisms living in bottom sediments or attached to littoral vegetation or the water surface had much higher mercury concentrations than organisms living and/or feeding in the water column. This does not correspond with the findings of Matida and Kumada (1969) who found that in a marine habitat the mercury content of planktonic micro-organisms was extremely high (up to 279 ppm on a dry weight

basis). In contrast, zooplankton organisms collected in Clay Lake never had concentrations greater than 0.1 ppm or a wet weight basis.

Mercury concentration also appeared to be related to food selection and in most cases omnivorous organisms and organisms feeding on detritus or bottom dwelling invertebrates had much higher mercury concentrations than either herbivorous organisms or organisms feeding on zooplankton (Fig. 2). The most conspicuous exceptions were the samples of dragon fly larvae and caddis fly larvae where the mercury concentrations were much lower than expected. Both samples were collected within 200 meters of a large, unpolluted stream flowing into the Wabigoon system and consequently the specimens may have been recent arrivals to Clay Lake. Hannerz (1968) concluded that there was no general connection between mercury concentration in invertebrates and their trophic level although he did find that predaceous insect larvae, such as dragon flies and Sialis did accumulate more mercury than organisms feeding on detritus or decaying plant material. He concluded that several factors including metabolic rate and feeding habits were responsible for differences in rates of mercury accumulation.

A series of experiments designed to provide some indication of the factors affecting the uptake and elimination of mercury by selected fresh-water organisms was conducted in 1971 (Hamilton, 1972). These experiments which were designed to simulate conditions in Clay Lake indicated that mercury uptake via the food chain was probably much more important than direct uptake from either water or sediment.

The more detailed survey of mercury concentrations in the crayfish Orconectes virilis showed that mercury accumulates in some parts of the

body more than others with the highest levels recorded being found in abdominal muscle. Similarily Johnels et al. (1967) found that the mercury concentration in the abdominal muscle of a Swedish crayfish (Astacus fluviatilis) was approximately 5 times as high as that in the carapace and about twice that in the liver. The little evidence that is available indicates that the mercury is concentrated in the methylated form. There also appears to be a definite age-mercury concentration relationship in this species with the highest concentrations being found in older specimens. A complicating factor is that mercury levels in the crayfish from this part of Clay Lake appear to be dropping. As indicated, levels in abdominal muscle of adult crayfish averaged 10 ppm wet weight in specimens collected in 1970. The mean value for a large collection of similar-sized specimens collected in May of 1971 was 6.6 ppm. As indicated in Figure 4 none of the values (pooled samples of abdominal muscles from 4 specimens) collected in June, July and August exceeded 6 ppm. Consequently the relatively low concentrations of mercury in the abdominal muscles of smaller crayfish may be partly a reflection of improving conditions. The smaller (and younger) specimens were 0+ and 1+ individuals who would have spent their entire lives in an environment that was apparently receiving substantially less mercury than in previous years. In contrast the older specimens (2+ and 3+) would have spent the earlier parts of their lives in an environment that apparently was receiving a greater load of mercury. The evidence indicating that mercury levels are dropping in Clay Lake is certainly not conclusive, but it has interesting implications and additional surveys will be conducted in 1972 and 1973 to establish whether or not this trend continues.

The survey of crayfish from different parts of the Wabigoon, Eagle, English and Winnipeg River systems indicates that mercury concentrations in the vicinity of Clay Lake are approximately 20 times as high as those found above Dryden or in the Eagle River. The pattern certainly suggests that the major source is in the immediate vicinity of Dryden where a chlor-alkali plant has been in operation.

Present indications are that the crayfish Orconectes virilis is a very useful indicator of mercury pollution. This species clearly concentrates mercury to very high levels and it is large enough so that a single specimen provides a tissue sample suitable for analysis. In addition individuals are much less migratory than the fish species usually used as indicators and comparisons with the material reported on by Bligh (1970) indicate that mercury levels in crayfish abdominal muscle are very similar to levels in the muscle of northern pike (Esox lucius) and yellow walleye (Stizostedion vitreum). The species of crayfish is also abundant, widely distributed and specimens are easily collected.

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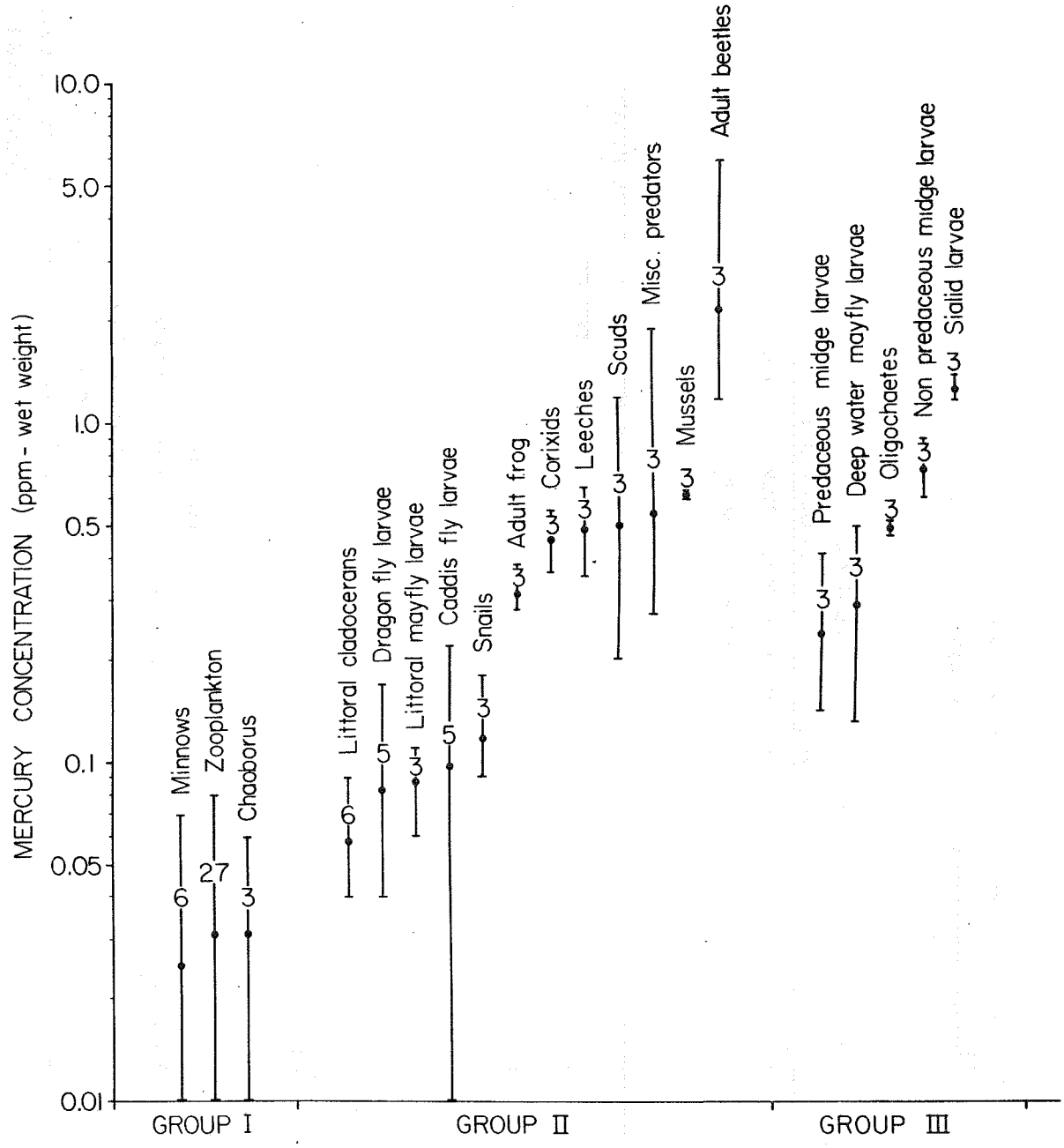

Figure 1. The relationship between mercury concentration and habitat for organisms collected in Clay Lake, Ontario during June, 1970. Group I-organisms living and/or feeding primarily in the water column; Group II-organisms living in the shallow littoral part of the lake in association with substrate, surface or aquatic macrophytes; Group III-organisms living in the profundal sediments. Numbers indicate sample size while the lines and points refer to the range and geometric mean respectively.

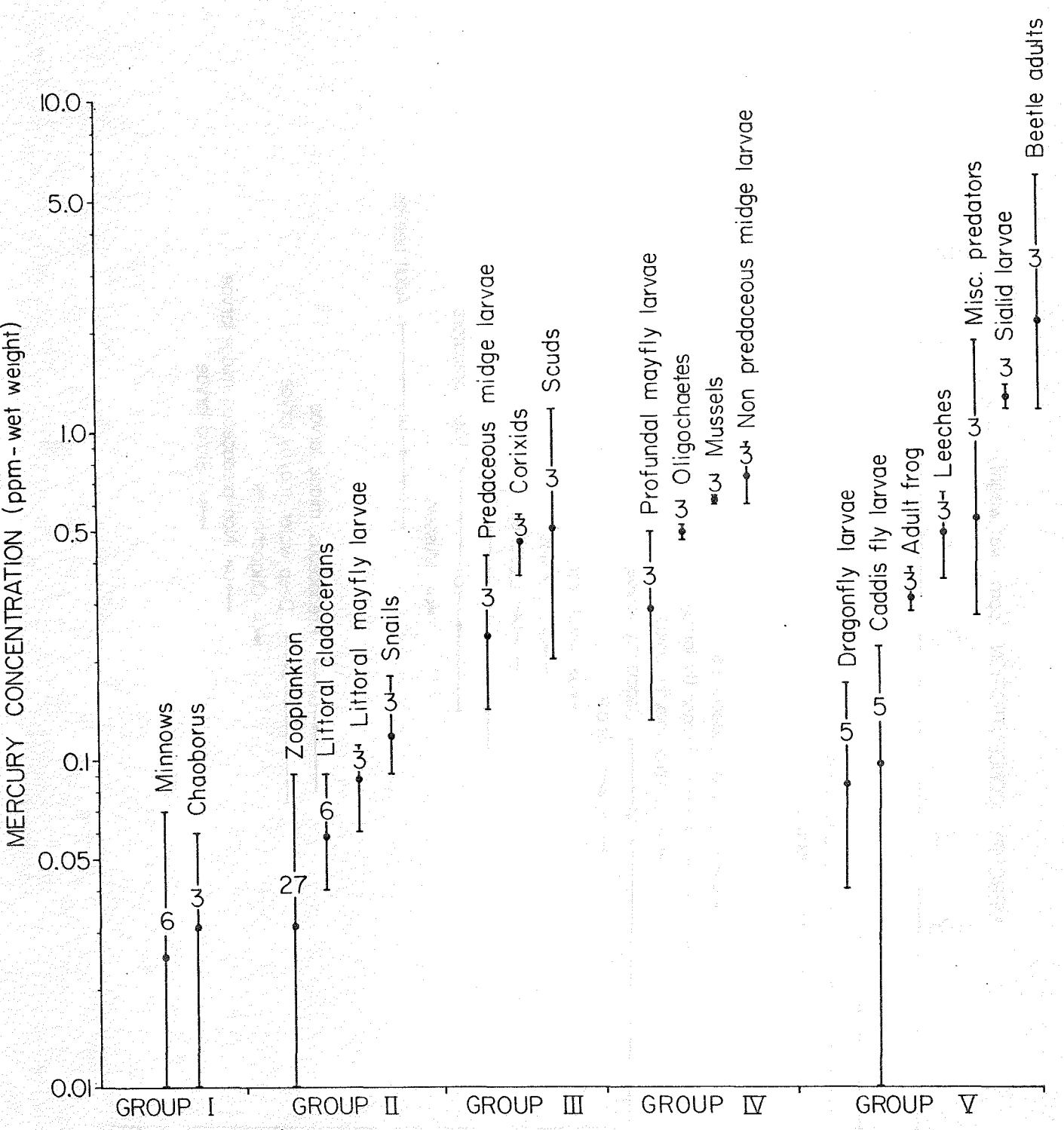

Figure 2. The relationship between mercury concentration and food selection for organisms collected in Clay Lake, Ontario, June 1970. Group I - organisms feeding primarily on zooplankton; Group II - organisms feeding primarily on phytoplankton or periphyton; Group III - omnivorous organisms; Group IV - detritus feeders; Group V - organisms that are predaceous on other benthic invertebrates. Numbers indicate sample size while the lines and points refer to the range and geometric mean respectively.

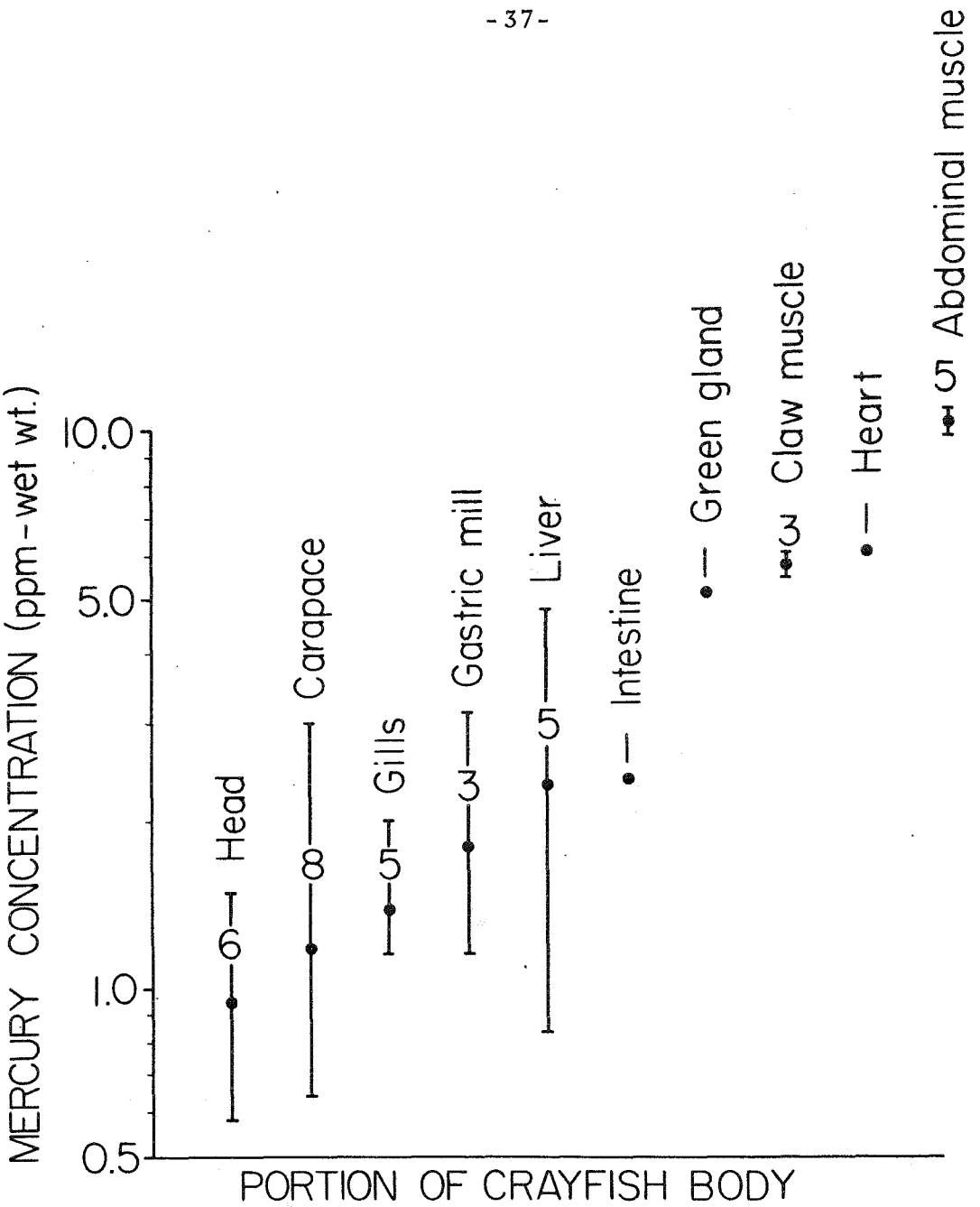

Figure 3. Mercury concentrations in different body parts of mature crayfish (Orconectes virilis) collected in Clay Lake, Ontario during summer, 1970. Numbers indicate sample size while the lines and points refer to the range and geometric means respectively.

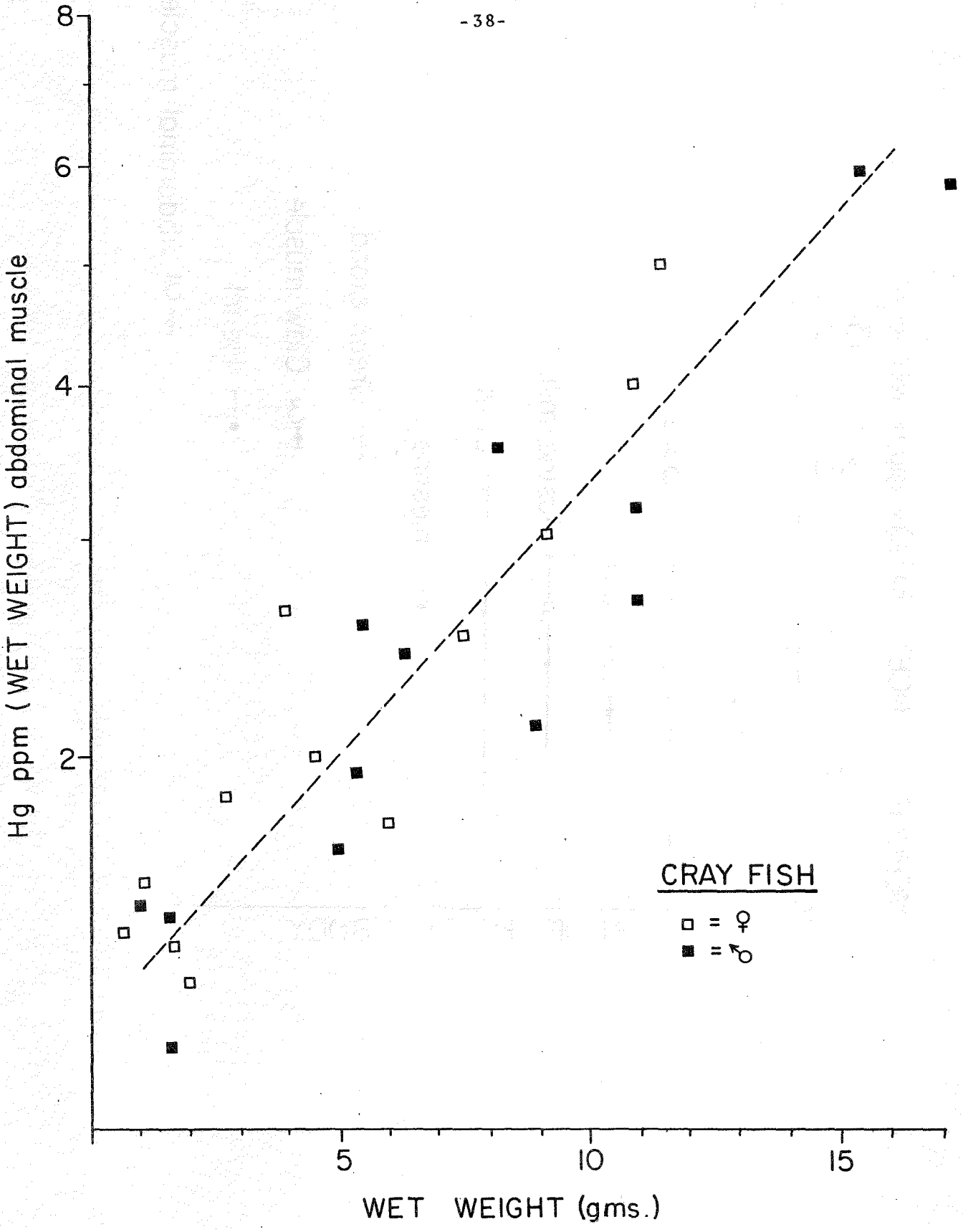

Figure 4. Relationship between body weight and concentration of mercury in abdominal muscle for crayfish (Orconectes virilis) collected during June, July and August 1971 in the North Star Bay at Clay Lake, Ontario. Each point represents a pooled sample consisting of 4 specimens.

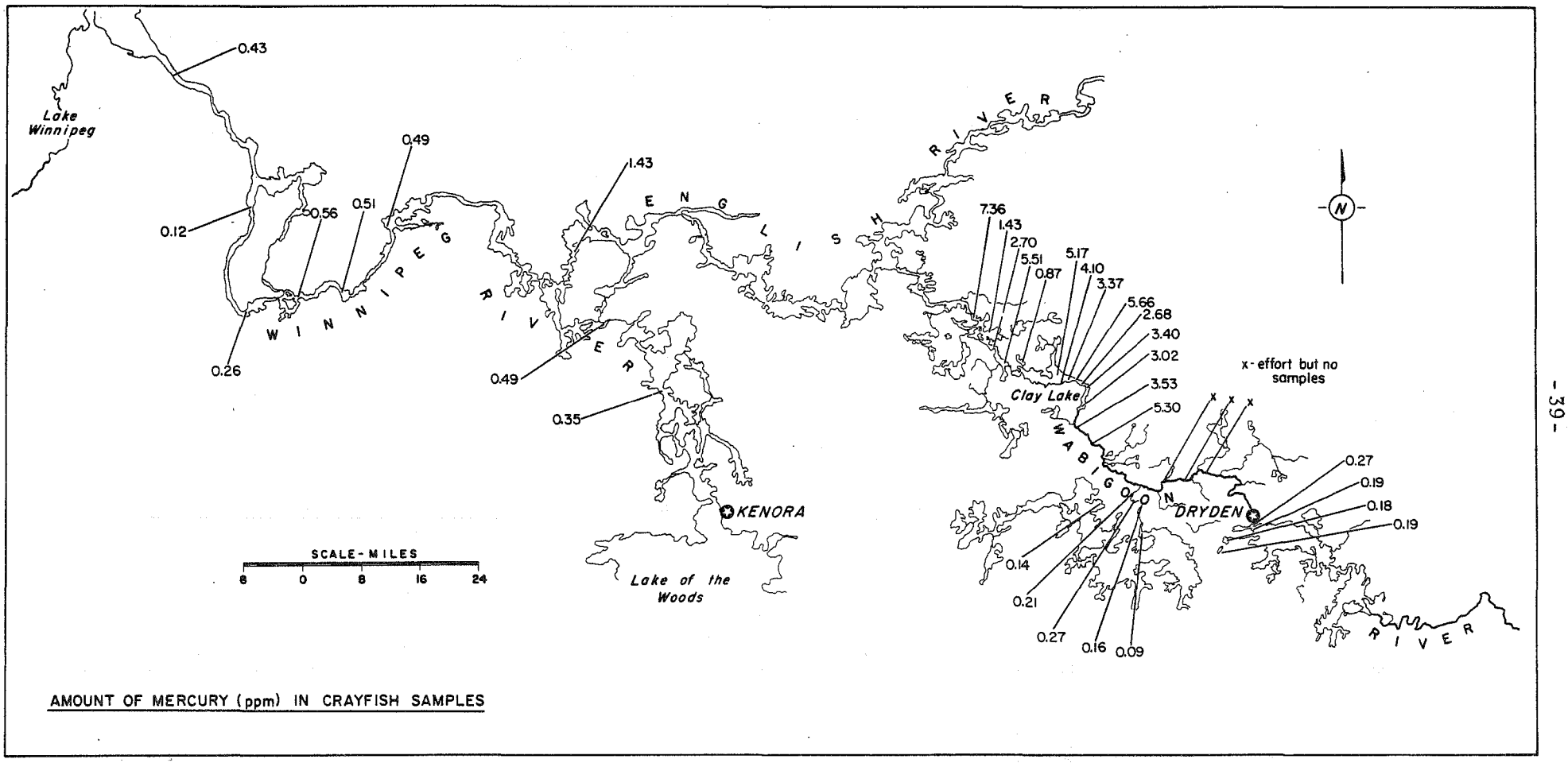

Figure 5. Concentrations of mercury in the abdominal muscle of mature crayfish (Orconectes virilis) collected in the Wabigoon, English, and Winnipeg River systems during August 1971. Each value represents the arithmetic mean of the values found for 4 individual specimens.

TOTAL MERCURY AND METHYLMERCURY IN FISH ORGANS

by

A.R. Kenney

Total mercury concentration and distribution ratios for some tissues of several fish species taken from Clay Lake are shown in Table 1. Distribution ratios show that mercury accumulates in some tissues more than others, highest concentrations being observed in the blood of whitefish and cisco and kidney, liver, spleen and heart tissues of all species. Specific tissue ratios are relatively constant within a species in a lake, however differences exist among species.

Whole blood of several fish species was analyzed for methylmercury. Methylmercury values averaged 89 ± 6, 99 ± 7 and 92 ± 2 percent of the total mercury content for northern pike, whitefish and cisco respectively. Erythrocyte mercury accounted for some 90 percent of the whole blood mercury of all species studied and most of this was associated with hemoglobin. Also 95-100 percent of the erythrocyte mercury was present as methylmercury.

Total mercury levels in bile of several northern pike, whitefish and cisco ranged from 0.26 - 3.17, 0.08 - 0.60 and 0.07 - 0.39 ug/g respectively. Methylmercury accounted for all

of the mercury present in the bile of these fish.

While methylmercury was the predominant form of mercury in all tissues studied, there is evidence that the percent concentration of methylmercury varies in similar tissues of different fish species. Determinations on several fish indicated that methylmercury accounts for more than 90 percent of the total mercury in pike, kidney and liver, while similar whitefish tissues had methylmercury values ranging from 71-85 and 80-85 percent respectively.

Total mercury concentrations, in several tissues of northern pike taken from three lakes, are shown in Table 2. While the observed differences in tissue ratios between lakes are not significant, they tend to indicate a constant but different tissue distribution in pike from the three lakes. Similar tissue distribution differences were observed for several species of fish taken from these lakes. However, it should be noted that among species, differences similar to those of Clay Lake fish were observed for the same species in the other lakes.

In November 1970 northern pike heavily contaminated with methylmercury were captured from Clay Lake and released in Heming Lake. (J.F. Uthe, this report) Total mercury levels in recaptured fish were calculated as a product of muscle biopsy level times total weight, a method that gave a good estimate on starved fish. A Clay Lake male gave 3,690 ug Hg by calculation and 3,770 ug Hg by homogenizing the whole fish.

Total mercury distribution and percent methylmercury in some

tissues from Clay Lake and transplanted northern pike are shown in Table 3. The tissue ratios were relatively similar and indicate few differences in tissue mercury distribution in fish from Clay Lake and those transplanted to Heming Lake. The greatest change was observed for lens ratios, however, the significance of this difference has not been resolved. While the methylmercury values of transplanted fish are slightly lower in most cases, there was no large change in tissue distribution and methylmercury remained the predominent form in all tissues studied.

Table 1. Total mercury concentration in some tissues of several species of fish taken from Clay Lake.

Species		Total mercury (ppm - wet weight basis) White Muscle	Red Muscle	Blood	Liver	Kidney	Heart	Brain	Spleen
Northern Pike	1	7.28	7.21(1.0)	2.20(0.3)	9.01(1.6)	17.3(2.4)	7.73(1.1)	5.22(0.7)	7.86(1.1)
Northern Pike	2	9.80	9.80(1.0)	2.54(0.3)	20.4 (0.9)	28.9(2.9)	11.5(1.2)	7.14(0.7)	11.3 (1.2)
Northern Pike	3	15.8	15.7 (1.0)	4.04(0.3)	21.3 (1.4)	25.6(1.6)	---	---	16.6 (1.1)
Northern Pike	4	8.98	8.99(1.0)	---	11.2 (1.3)	20.1(2.3)	13.4(1.5)	8.20(0.9)	10.4 (1.2)
Walleye	1	13.0	11.0 (0.8)	*---	13.6 (1.0)	14.0(1.1)	9.12(0.7)	11.2 (0.9)	12.1 (0.9)
Walleye	2	14.7	12.0 (0.8)	---	17.4 (1.2)	20.3(1.4)	16.8 (1.1)	13.4 (0.9)	15.6 (1.1)
Whitefish	1	4.98	3.96(0.8)	10.9 (2.2)	15.4 (3.1)	10.4(2.1)	---	---	---
Whitefish	2	12.9	13.0 (1.0)	34.8 (2.7)	32.6 (2.5)	24.8(1.9)	21.9 (1.7)	18.0 (1.4)	29.6 (2.3)
Whitefish	3	0.66	0.63(0.9)	---	1.78(3.7)	1.56(2.8)	1.00(1.5)	0.72(1.1)	1.32(2.0)
Whitefish	4	1.31	1.00(0.7)	4.40(3.4)	4.86(2.7)	3.66(2.4)	---	---	---
Burbot		12.8	17.8 (1.3)	---	20.0 (1.7)	22.6 (1.8)	31.0 (2.4)	9.60(0.8)	30.0 (2.3)
Cisco	1	5.90	5.79(0.9)	*---	12.0 (2.0)	25.6 (2.6)	15.7 (2.6)	5.02(0.8)	39.4 (5.9)
	2	7.84	8.41(1.1)	*---	26.0 (3.3)	18.6 (2.4)	13.7 (1.6)	8.04(1.0)	30.8 (3.9)

* Total blood mercury levels were determined for several Cisco. Blood valves of 19.7, 11.0 and 18.2 ppm were obtained for fish containing 4.34, 1.86 and 3.78 ppm respectively in their white muscle. A blood value of 2.44 ppm was obtained for a walleye which had a white muscle level of 11.8 ppm.

() Value in parenthesis represents the ratio of tissue mercury concentration/white muscle mercury concentration.

Table 2. Total mercury concentration in several tissues of Northern Pike taken from three lakes, expressed as a ratio of total mercury tissue/total mercury muscle.

	Clay Lake		Cross Lake		Lake Winnipeg	
Tissue	Mean Ratio	S.D.	Mean Ratio	S.D.	Mean Ratio	S.D.
Red Muscle	1.01	0.08	0.88	0.07	0.90	0.06
Liver	1.57	0.42	0.85	0.04	0.46	0.04
Kidney	2.16	0.77	0.91	0.01	0.69	0.09
Heart	1.22	0.17	1.09	0.16	0.71	0.02
Brain	0.74	0.10	0.35	0.05	0.08	0.03
Spleen	1.11	0.08	0.34*	--	--	--

Clay Lake values were determined from tissues of five fish. The Cross Lake and Lake Winnipeg values, with one exception, were determined on two fish from each lake.

* One sample.

Table 3. Total mercury distribution and percent methylmercury in tissues of Northern Pike

Tissue	Clay Lake			Transplanted from Clay to Heming		
	Number of Samples	Mean* Ratio	Percent MeHg** in Tissue	Number of Samples	Mean* Ratio	Percent MeHg** in Tissue
White Muscle	5	1.00	105	12	1.00	100
Red Muscle	5	1.01	98	12	0.99	98
Liver	5	1.57	97	12	1.50	82
Kidney	5	2.16	104	12	1.64	94
Spleen	5	1.11	91	10	0.86	92
Stomach	5	0.75	102	12	0.92	80
Intestine	5	1.49	97	11	1.32	85
Gills	5	0.52	102	12	0.46	89
Skin	5	0.30	111	7	0.35	85
Ovary	4	0.39	99	3	0.37	92
Testis	2	0.59	93	4	0.62	102***
Heart	5	1.22	63	8	1.04	82
Scales	5	0.26	61	7	0.54	83
Lens	5	2.42	112	3	5.77	94
Blood	4	0.22	89	3	0.27	89***
Bile	3	0.12	102	3	0.17	103
Brain	5	0.74	87	12	0.66	63

* Total mercury distribution is presented as a mean ratio of tissue mercury concentration/white muscle mercury concentration.

** Methylmercury percentages are means of determinations for 2-5 individuals except as noted.

***One Sample.

MERCURY IN SEDIMENTS AND WATERS OF CLAY LAKE NORTHWESTERN ONTARIO

by

F.A.J. Armstrong, D. Metner and M.J. Capel

The Wabigoon River - Clay Lake - English River - Winnipeg River system (Figure 1) in western Ontario and eastern Manitoba appears to be one of the waterways in Canada most heavily contaminated with mercury. Hamilton (1972) has found high levels of the metal in crayfish in this system, decreasing from about 5 ppm (in abdominal muscle) just downstream of Dryden Ontario to less than 1 ppm near the mouth of the Winnipeg River on Lake Winnipeg, a distance of about 250 miles. In Clay Lake, about 50 miles downstream of Dryden, fish have been shown to contain up to 16 ppm of mercury (Bligh 1970).

The source of this mercury is believed to be a chlorine-alkali plant at Dryden, of capacity 11,000 - 12,000 tons of chlorine per year, which has been in operation since 1962. Since early in 1970 the effluent of this plant has been regulated, and discharges reduced to 0.007 lb (3 g) mercury per ton of chlorine produced. The discharge before this has been started to be 0.21 (95g) mercury per ton of chlorine.

Calculation shows that during the 8 years of unregulated operation from 1962 to 1969, the total discharge of mercury in the waste waters from this plant may have amounted to 20,000 lb (9,000 - 11,000 Kg).

The work reported here was done in order to measure the distribution of mercury in the sediment in Clay Lake and to estimate the total quantity present. It is part of a continuing study of the mercury pollution of the lake and of the effects upon it of control measures.

Description of Clay Lake

The lake at 50°03'N 93°30'W probably takes its name from the deposits of clay in the area. Deposits of gently undulating clay lake sediments with rock outcrops are found throughout the Wabigoon River drainage area (Zoltai 1965) and give the river and Clay Lake high turbidity. The lake is shown in outline in Figure 2; it has an area of about 7,400 acres (3,000 HA), and maximum depth about 75 feet (24 m). The volume estimated from a bathymetric map prepared by the Ontario Department of Lands and Forests is about $2.4 \times 10^8 m^3$, from which the mean depth is about 25 feet (8m). The 12 year (to 1965) mean inflow from the Wabigoon River is 1580 cfs (56 m^3/sec) (German 1969), so that turnover time of the water in the lake is about 50 days.

Sampling

As a preliminary to the main sampling program, sediment samples were taken with a Mackereth piston corer at the positions in the lake marked with the letters A, B, K through N in Figure 2. Sections of these cores were analysed for mercury in order to determine the variation of mercury content with depth, together with cores at positions C through J, which were taken later with an Ekman dredge as described below. The analyses are shown in Table 1. High concentrations were found in the uppermost 4 to 6 cm, and below 6 cm concentration were mostly less than 1 µg/g. It was estimated from these analyses that mercury in the uppermost 6 cm accounted for 92-95% of the total above a background level of about 0.1 µg/g, and it seemed sufficient for survey purposes, to have analyses of the sediments to this depth only.

To collect samples for the main survey therefore, the Mackereth sampler was set aside and an Ekman dredge was used to bring up hemi-cylindrical portions of the top 10 cm of sediment. From these portions an undisturbed hemi-cylindrical core representing the uppermost 6 cm was removed with a plastic tube of 4 cm diameter. This portion was placed in a polyurethlene envelope, labelled, and as soon as possible frozen and stored at -20 C until analysed.

During July and August 1971 27 transects of the lake were made, and a total of 283 samples collected.

Water samples were collected with a Van Dorn 1 liter

sampler, and transferred to polyurethlene bottles. No preservative was added to samples for estimation of quantity of suspended matter and its analysis for mercury. For analysis of total mercury water samples were preserved by addition of 1 cc sulphuric acid (36N) per liter.

Chemical analysis

Sediment samples were air-dried for 2-3 days, usually in a current of air in a fume hood, pulverized and screened. Small portions were extracted with aquaregia, and mercury in the extracts determined by flameless atomic absorption spectrophotometry. The extraction procedure was that used in the Dow Chemical Company procedure (Anon 1970) for muds and sediments, and the AAS procedure and instrumentation were those in use for mercury determinations in this Institute (Armstrong and Uthe 1971). Several checks of the accuracy of this method have been made (Tables 2, 3 & 4).

A number of sediment samples was also analysed for carbon and nitrogen content using a Carlo Erba Elemental Analyzer Model 1102, and for iron by an absorptiometric method using 1:10 phenanthroline (Armstrong 1957). Some samples were subjected to particle size analysis by the pipette method (Jackson 1956). Suspended matter in water samples was separated by filtering about 300 cc on Millipore® matched weight monitor filters of porosity 0.8 µm. The filters were air-dried and-weighed to obtain the weight of suspended matter, and then the

filter and adhering material was digested with nitric and sulphuric acids and permanganate and the mercury content determined. For this determination the water samples were not acidified, and were analysed within 24 hours of collection in order to minimise change on storage. In the first set of such samples the filtrate was then analysed by the method of Chau and Saitoh (1970) for dissolved mercury. In a later experiment samples were acidified when collected, and the suspended matter filtered off but not analysed. The filtrates were analysed for mercury after being irradiated for 12 h with UV radiation to decompose any organic mercury compounds which may have been present (Goulden and Afghan 1970).

Results

The horizontal distribution of mercury in the sediments of the lake is shown in Figure 2. Concentrations vary from about 0.1 µg Hg/g at the mouth of the Wabigoon River at the eastern end, and at the margins and around islands (that is to say, where river scour or wave action have removed fine material leaving sand and gravel) to 7.8 µg/g in the deeper part of the eastern basin. The mean value for the samples analysed is 3.1 µg/g.

The total amount of mercury in the lake sediments has been estimated, from the areas and mean values within the contours, to be about 4,400 lb (2000 Kg). As discussed above, this value may be underestimated by 5-10%, since the analyses are of only the top 6 cm of sediment.

Results of particle size analysis are shown in Tables 5 and 6. Table 5 includes mercury content of the inorganic fractions, and shows that the clay fraction is highest in mercury content. To measure further the relationship between the amount of fine material and mercury concentration, the analyses reported in Table 6 were done. There is a weak positive correlation ($r = 0.23$) between mercury concentration and silt content, which is not significant ($P > 0.1$). There is a slightly stronger one ($r = 0.49$) between mercury concentration and clay content, which is highly significant ($0.03 > P > 0.02$).

25 samples with a range of mercury concentrations from 0.14 to 7.83 µg/g were analysed for carbon, nitrogen and iron content. The results are shown in Table 7. Since these sediments are free of carbonate, the carbon and nitrogen content is a measure of the amount of organic matter present. There are highly significant positive correlations between total mercury and carbon ($r = 0.69$ $P < 0.001$) nitrogen ($r = 0.56$ $P = 0.01$) and iron ($r = 0.671$ $P < 0.001$). The first two of these correlations may be the result only of the known affinity of mercury compounds for organic matter. The correlation of mercury and iron may result from a co-precipitation phenomenon.

We have measured the quantity of suspended matter in the water and its mercury content in a series of samples from positions eastward from a point about 2 miles up the Wabigoon River to the narrows near point G in Figure 2. The results are given in Table 8. The quantity of suspended material de-

creases along the lake as suspended material settles out. It is possible to make a rough estimate of the rate of sedimentation at the time these samples were taken, from the difference in the amounts of suspended material at the inlet and outlet ends of the lake. If these are, respectively 20 and 7 mg/l and if the volume of $2.4 \times 10^8 m^3$ is passed through the lake in 50 days, it may be calculated that the mean sedimentation rate for the lake is 0.6 - 0.7 mm/year.

Some analyses of water and suspended matter from positions near the centres of the eastern and central basin are shown in Table 9. The deeper samples at each position were from about 1 m from the bottom. Sampling was toward the end of winter when river flow through the lake was probably at a minimum and when the water column could be expected to be thermally stable. In the central basin suspended matter was in higher concentration near the bottom, which indicates that it was settling out. It is seen that mercury in suspension is a substantial fraction (11-44%) of the total.

Discussion

Elevated mercury concentrations are found in the topmost 5-6 cm of the sediments, a much greater depth that follows from the estimated rate of deposition during 8 years, which is probably 5-6 mm only. However, Jernelow (1971) has shown that fish and clams can cause mixing of surface sediment down to a depth of several centimetres, and such mixing has probably occurred in Clay Lake, having thereby diluted the deposited

material with about ten times its volume of earlier sediment. This implies that the newly deposited material may have had a mercury content of about 30 µg/g. It is not possible at present to check this, nor to say whether the mercury-rich layer will deepen with time as the mixing process continues.

In October 1971, the suspended matter in the lake water contained about 3 µg Hg/g, which is about one tenth of the concentration (if our supposition is valid) in material deposited when the effluent from the chlorine-alkali plant was uncontrolled. Nevertheless it is close to the mean concentration in the top part of the sediment, and on settling out cannot greatly change this concentration.

The origin of this mercury in suspension must be sought in the river, and it is possible that previously deposited material is still being washed out. It is, however, instructive to calculate the concentrations in water and in suspended matter which will result from the controlled discharge at the Dryden plant of 0.007 lb (3 g) of mercury per ton of chlorine manufactured. This mercury discharge would be about 100 g per day and with a river flow (at Clay Lake) of 56 m^3 per second represents a concentration in the water of 0.02 µg/litre or in the suspended matter (at 20 mg/l) of about 1 µg Hg/g if all mercury was absorbed.* These concentrations

* At the earlier unregulated discharge of 0.21 lb mercury per ton of chlorine the corresponding concentrations would be 0.6 g/l and about 30 µg Hg/g, in quite remarkable agreement with our supposition about the concentration of mercury in sediment deposited before control.

are not too greatly different from what we have measured. They offer little hope that there can be any rapid improvement in the condition of the lake, even with this degree of control of the effluents. For any improvement it is of course necessary that existing deposits should be covered by sediment of low mercury content. Several centimeters depth may be required (Jernelov 1971), because of disturbance by animals, and such an accumulation might take some centuries if our estimate of the sedimentation rate in the lake is correct. Moreover, improvement is dependent on the newly deposited material having a sufficiently low mercury content that it is itself harmless, and this can hardly be said of material with mercury content 1 µg/g, which can be expected in the Wabigoon River at the present time.

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Table 1. Variation with depth of mercury content of sediment cores from Clay Lake (µg Hg/g, dry weight basis)

Depth (cm)	A	B	C	D	E	F	G	H	J	K	L	M	N
0-2	8.42	8.30	0.95	6.53	6.34	6.65	5.17	2.38	7.50				
2-3	8.04	6.38	0.91	7.98	6.31	7.25	4.27	2.59	7.32				
3-4	7.14	4.48	0.62	8.55	6.42	7.81	3.55	2.07	6.96	5.92	6.40	4.58	6.04
4-5	5.52	1.58	0.43	6.57	6.12	7.81	3.43	1.61	4.10				
5-6	5.52	0.20		4.05	3.61	6.34	1.06	0.92	0.32				
6-7	3.93	0.20		2.32	1.15	2.42	0.24	0.88	<0.01	1.79	2.29	0.17	0.16
7-8	0.66	0.16				0.14	0.27	0.13	<0.01	0.57	0.65	0.06	0.08
8-9	-	0.18								0.42	0.22	0.06	<0.01
9-10		-										<0.01	<0.01
10-11	0.04	0.10											
20-22		0.06											
30-32		0.02											
42-44		0.05											

Table 2. Analysis of sediment samples before and after drying (µg Hg/g dry weight). Means of 3 analyses.

Sample No.	Wet Sample	Dry Sample
1	6.67	6.74
2	4.60	4.61
3	4.58	4.36
4	0.92	1.23

Table 3. Recovery of mercury added to wet sediment (means of 4 analyses)

Compound	Mercury added µg Hg/g dry wt.	Mercury found µg Hg/g dry wt.	Recovery %
$HgCl_2$	0	0.04	-
	0.484	0.51	97
	0.971	1.00	99
	1.884	1.88	98
$CH_3Hg\ Cl$	0	0.08	-
	0.563	0.68	107
	1.083	1.19	102
	2.237	2.11	91

Table 4. Interlaboratory comparison analyses of sediment samples (µg Hg/g, dry weight)

Sample No.	1	2	3
Mean of all laboratories	0.117	0.102	75.3
SD	±0.06	±0.06	±10.1
N	12	11	12
Freshwater Institute	0.14	0.08	77
SD	±0.017	±0.01	± 2.3
N	6	6	3

Table 5. Particle size analysis of sediment samples from locations P R and S in Clay Lake

Location	Inorganic material	Sand 2mm - 50μ		Silt 50 - 2μ		Clay <2μ	
	%	% of inorganic	μg Hg/g	% of inorganic	μg Hg/g	% of inorganic	μg Hg/g
P	97	73	<0.01	20	0.51	7	5.93
R	90	0	-	39	1.07	61	6.10
S	89	0	-	10	1.20	90	4.78

Table 6. Particle size analysis of sediments from Clay Lake

Location	Sample No.	Inorganic material %	Sand 2mm-50μ % of inorganic	Silt 50-2μ % of inorganic	Clay <2μ % of inorganic	Mercury content μg Hg/g
E basin	3 SN 4	95	80	14	6	1.10
	5 SN 4	92	<1	49	50	5.92
	7 SN 8	92	<1	36	64	4.92
	16	95	<1	39	61	4.5
	18	92	<1	46	53	3.3
	24	94	<1	34	66	4.2
	8 SN 7	93	<1	34	66	6.18
	8	97	0	38	62	6.98
	11	94	<1	45	55	4.00
	14	96	0	33	67	4.98
	23	95	<1	34	66	3.46
Central basin	11 NS 3	95	<1	28	72	4.66
	14 SN 3	95	<1	24	76	3.1
	13	95	<1	16	84	6.3
	19	92	<1	23	77	4.95
	16 SN 5	94	0	17	83	4.8
	12	95	<1	16	84	6.3
	20 SN 4	94	0	17	83	5.23
	9	95	1	38	61	1.29
W. Basin	22 NS 6	96	1	24	75	2.5
	25 SN 4	95	51	14	35	0.95
	29 SN 7	90	3	28	69	2.9
	30 WE 5	89	1	23	76	2.5

Table 7. Analysis of sediments from Clay Lake (Dry weight basis)

Location	Sample No.	Carbon %	Nitrogen %	Iron %	Mercury µg/g
E. Basin	2 SN 2	0.8	0.07	0.91	0.21
"	3 SN 4	1.3	0.09	0.67	1.10
"	4 SN 4	2.7	0.15	2.56	5.57
"	5 SN 6	4.2	0.27	3.34	4.93
"	7 SN 7	4.3	0.31	4.17	7.34
"	8 SN 6	4.6	0.35	4.20	7.83
"	12	2.7	0.19	4.42	4.23
"	25	3.1	0.28	4.50	3.36
"	9 SN 4	0.6	0.10	1.19	0.52
"	9	3.7	0.30	4.64	2.68
Central basin	11 WS 13	3.9	0.37	4.79	2.37
	12 NS 2	1.8	0.16	1.56	0.88
"	6	3.3	0.24	4.64	5.41
"	15 NS 6	3.9	0.26	4.52	4.43
"	16	4.2	0.27	4.95	6.21
"	16 SN 11	4.7	0.36	5.32	5.30
"	18 SN 6	4.6	0.34	5.06	6.00
"	10	3.7	0.29	5.53	3.99
"	20 SN 10	3.9	0.32	4.35	1.51
W. Basin	24 SN 1	0.9	0.12	0.71	0.19
"	6	4.1	0.31	4.74	2.55
"	26 SN 3	0.6	0.07	2.41	0.14
"	28 SN 5	4.8	0.37	4.68	1.98
"	8	4.9	0.38	5.13	3.06
River	R4	1.2	0.09	1.18	1.41

Table 8. Suspended material in water of Wabigoon River and Clay Lake. 19 October 1971.

Location		Suspended matter (mg/l)	µg Hg/g
Wabigoon River	1	12.5	4.4
"	2	14.7	2.5
"	3	21.0	2.5
"	4	18.0	2.0
"	5	19.5	4.1
E. basin	6	20.5	3.4
"	7	16.2	2.3
Central basin	8	7.7	2.9
"	9	8.7	2.1
"	10	6.4	5.8
W. basin	11	6.9	3.2
"	12	7.2	3.6

Table 9. Analysis of water and suspended matter from Clay Lake (filtration on Millipore® membrane 0.8 µ A.P.D.)

Location	Date	Depth	Suspended matter mg/l	Suspended matter µg Hg/g	Hg in solution µg/l	Hg in suspension µg/l	Total Hg µg/l
E basin	9 Feb 1972	0m	10.8	3.10	0.11	0.03	0.14[1]
		5m	9.9	2.34	0.05	0.02	0.07[1]
	18 Feb 1972	0m	10.1	7.0	0.09[2]	0.07	0.16
		5m	9.7	4.3	0.13[2]	0.04	0.17
Central basin	9 Feb 1972	0m	6.6	1.56	0.05	0.01	0.06[1]
		17m	16.1	-	0.05	-	-
	18 Feb 1972	0m	7.0	1.7	0.08[2]	0.01	0.09
		17m	15.5	5.7	0.03[2]	0.09	0.12

[1] By addition [2] By difference

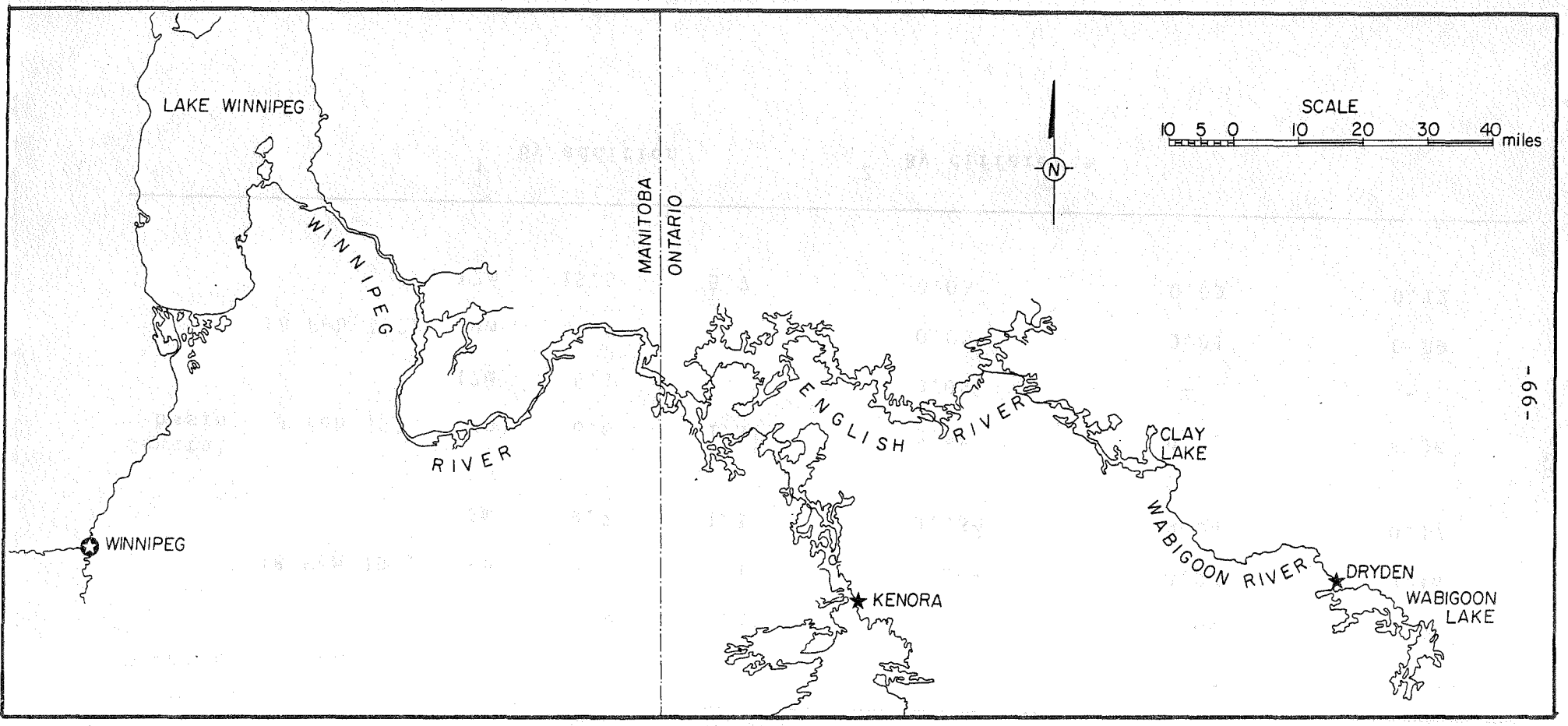

Figure 1. Location of Dryden Ontario, Wabigoon River. Clay Lake, English River and Winnipeg River.

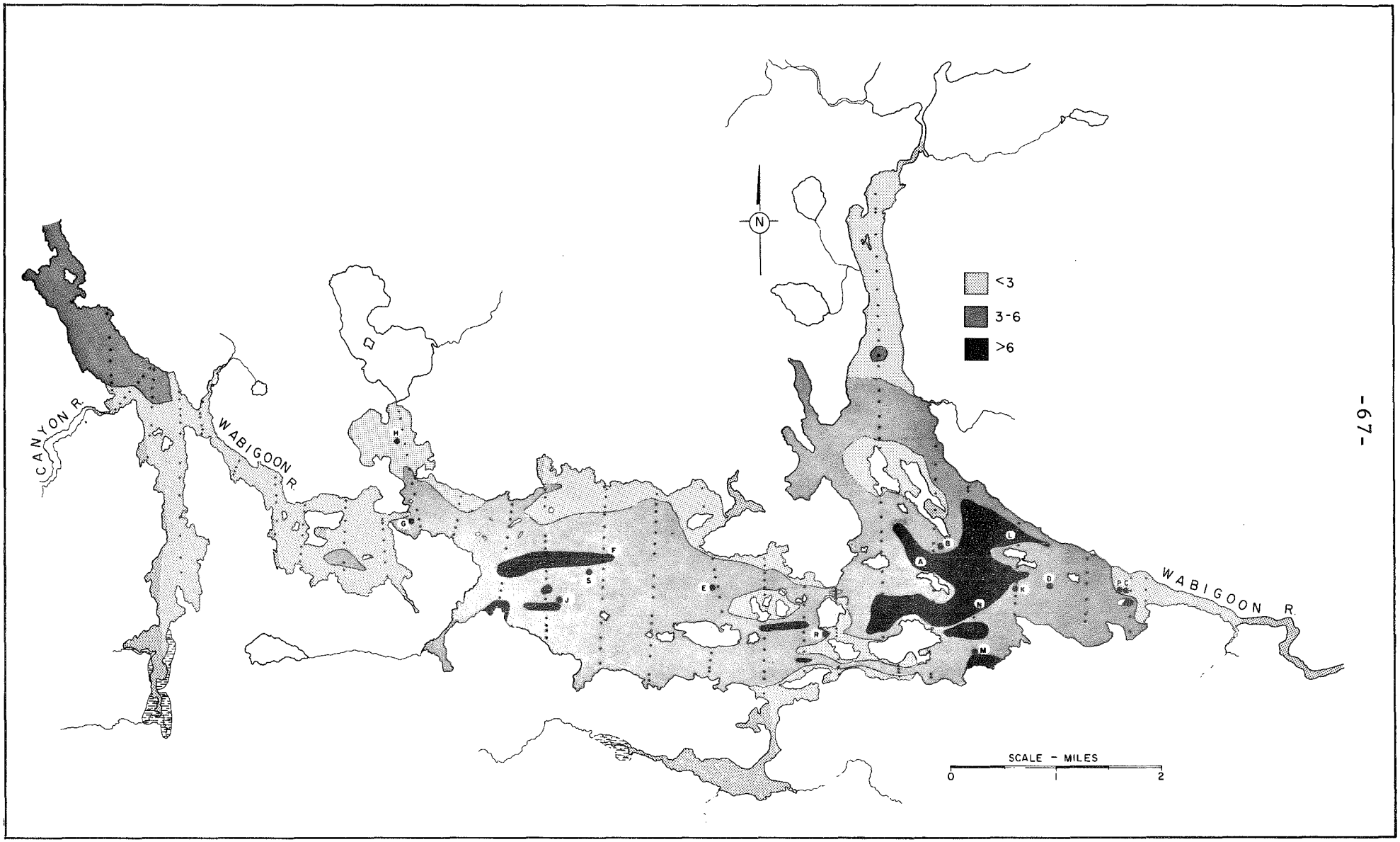

Figure 2. Concentration of mercury (µg Hg/g dry weight) in sediments of Clay Lake N.W. Ontario.

MERCURY UPTAKE IN CAGED RAINBOW TROUT IN THE SASKATCHEWAN RIVER

by

J.F. Uthe, F.M. Atton* and L. Royer*

Caged Trout Experiment 1969-1970

Shortly after the discovery of mercury pollution in fish caught downstream of Saskatoon caged rainbow trout (Salmo gairdneri) were placed in the South Saskatchewan River under the ice at various locations upstream and downstream of the city. Cages were constructed out of plywood with a large mesh door and mesh vents on the side. The dimensions of the cage were 2' x 2' x 4'. Each cage held approximately 75 fish at the beginning of each experiment. Fish of approximately the same size were used. Six fish were taken at each sampling period. Kidneys and livers were each pooled and homogenized. The dressed headless carcasses, were also pooled and homogenized. The total mercury content of each homogenate was then determined. (Armstrong and Uthe, 1971). During the first year (1970) three experiments were carried out; a winter (Jan through March), a summer (June through August) and a fall (September through November) one. A second summer experiment was carried out during the

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summer of 1971. In 1971 a cylindrical cage with an open vane cover at one end and a net at the other was used due to difficulties encountered with the earlier design.

Location of Cages

The various sites used during the course of this experiment are shown in Fig. 1. The blow-up inset of the map illustrates the area of major discharges; one from the sewage system of the city, the other from a chemical complex located on the banks of the river a few hundred yards downstream of the sewer outfall. The flow in this section of the river is controlled by Gardiner Dam and was subject to marked fluctuations in flow in an unpredictable manner. The river is heavily sand-barred and thus cage locations had to be chosen so as to avoid severe silting and still allow for reasonable accessibility. One cage was located (coded as IPCO) between the city sewer outfall and the chemical complex outfall and successfully maintained. Attempts to hold cages in a location just downstream of the chemical plant outfall (coded as Sutherland) met with very limited success since fish in these sites showed extremely poor survival. That is was probably a result of discharges from the city and chemical complex since one cage, lost upstream of the city in the fall of 1971 was recovered in the summer of 1972 with live trout still in it.

Results

No mercury increase was found in the carcass during the winter experiment. The liver and kidney each showed a small rise then fall and the overall result is that uptake is extremely limited during the winter. It is still possible for methylation and release of mercury to be occurring in the river bottoms but that fish do not pick it up due to the overall cold depression of their metabolism. During June and especially July the mercury levels in all tissues rose dramatically in the fish downstream of the chemical complex then appeared to level of. A few new fish were placed in these cages at the end of July and after one month did not show such a great rise. This is suggestive of the occurrence of a short period of rapid release of mercury into a form available for pickup. During the month of June there was a great deal of water being passed through the river. During July and August there was much less flow. I would suggest that the spring runoff disturbs the riverbottom and scours the bed and distributes a fresh layer of mercury laden sediment downstream of the deposit. The warm temperatures of June and July plus the extreme nutrient conditions probably rapidly mobilize this mercury and then the system enters a period of mild mobilization until the next cycle.

A fresh experiment initiated the end of August did not show marked mercury accumulation in fish downstream of the plant. There was a small accumulation of mercury but the levels did not greatly exceed those in fish upstream of the plant. This adds to the belief that uptake of mercury by fish is most rapid during

the first months of summer.

The summer experiment was repeated in 1971 to attempt to determine how long mercury deposits in the river bed just downstream of the chemical complex would continue to contaminate fish. The results of this experiment indicate that even after one year of flushing the beds were still able to contaminate trout to about the same level as the preceeding year (Clarkboro ferry levels). In the 1970 experiment a cage placed downstream of the chemical complex showed little uptake of mercury although apparently positioned just downstream of the mercury deposits. This cage was located on the opposite side of the river although out near the middle. Due to lamellar flow it is probable that mercury flowing downstream from the mercury deposits stayed near the west side of the river. Further evidence for this phenomena was obtained in 1971 from cages placed on each side of the river at Clarkboro Ferry. The cage on the west side of the river (equivalent to the same side as the mercury deposits) showed marked uptake while the cage on the east side (equivalent to the Sutherland cage side) showed very little uptake. All of the above values are for total mercury content. Methylmercury levels were determined for the carcass samples from the 1970 summer experiment. (Uthe, Solomon and Grift, 1972). These results show that, like fish naturally contaminated, the bulk of the mercury in the carcass is present as methylmercury.

References

1. Armstrong, F.A.J. and J.F. Uthe. Atomic Absorption Newsletter 10, 101 (1971).

2. Uthe, J.F., J. Solomon and N. Grift. J of the AOAC (1972) in press.

Table 1. South Saskatchewan River Cage Experiment-Winter 1970
- Stocked mid-January 1970
- values are expressed in parts per million (ppm) total mercury on a wet weight basis.

	Carcass				Liver				Kidney			
Control	0.07				0.09				0.07			
Sampling Date	29/1	25/2	11/3	26/3	29/1	25/2	11/3	26/3	29/1	25/2	11/3	26/3
Diefenbaker Lake		0.05	0.06	0.05		0.08	0.05	0.08		0.19	0.11	0.19
Pike Lake	0.05	0.05	0.07		0.09	0.05	0.05		0.12	0.15		
Clarkboro Ferry	0.06	0.06	0.06	0.05	0.15	0.21	0.07	0.07	0.28	0.32	0.19	0.23
Hague Ferry	0.05	0.05	0.06	0.05	0.08	0.12	0.06	0.08	0.22	0.22	0.16	0.17

Table 2. South Saskatchewan River Cage Experiment - Summer 1970
- values are in ppm total mercury
- stocked mid June 1970

Control	Carcass 0.02					Liver 0.08					Kidney 0.20			
Sampling Date	6/7	20/7	4/8	17/8	27/8	7/7	20/7	4/8	17/8	27/8	20/7	4/8	17/8	27/8
Diefenbaker Lake	0.05 (0.08)[B]	0.12 (0.13)	0.22 (0.11)	0.21 (0.11)	-	0.01*	0.15	0.35	0.32	-	0.36	0.50	0.53	-
Pike Lake	0.03 (0.08)	0.15 (0.07)	0.16 (0.16)	0.20 (0.12)	0.09 (0.08)	0.01*	0.27	0.24	0.27	0.27	0.51	0.30	0.52	0.69
Sutherland	0.04 (0.04)	0.12 (0.06)	0.32 (0.16)	0.14 (0.11)	0.09 (0.07)	0.02*	0.20	0.28	0.25	0.33	0.34	0.62	0.43	0.29
Clarkboro Ferry	0.18 (0.20)	0.35 (0.25)	0.57 (0.54)	0.56 (0.44)	0.27[A] (0.22)	0.02*	0.57	1.11	1.26	-	0.94	1.37	1.16	0.61[A]
Hague Ferry	0.20 (0.20)	0.26 (0.24)	0.49 (0.39)	0.37 (0.33)	0.29[A] (0.16)	0.08*	0.65	0.87	0.97	-	0.70	1.02	0.77	0.61[A]
Tobin Lake	-	0.06 (0.04)	0.13 (0.10)	0.18 (0.08)	-	-	0.14	0.23	0.28		0.17	0.22	0.34	

* liver and kidney combined
A- fish in cases one month
B- methylmercury (as Hg) in sample

Table 3. South Saskatchewan River Cage Experiment - Fall 1970
- Stocked Sept. 9. Sampled every two weeks
- Values are in ppm total mercury

	Date of First Sample	Carcass 0.02 Sample N 1	2	3	4	5	Liver 0.08 1	2	3	4	5	Kidney 0.20 1	2	3	4	5
Deifenbaker Lake	19/9	0.05	0.10	0.15	0.11	0.08	0.07	0.12	0.19	0.14	0.14	0.11	0.18	0.23	0.14	0.15
Pike Lake	27/9	0.07	0.14	0.13	0.10	0.14	0.15	0.19	0.18	0.20	0.18	0.12	0.39	0.32	0.19	0.24
IPCO	27/9	0.10	0.14	0.14	0.14	0.22	0.19	0.32	0.28	0.26	0.23	0.24	0.39	0.33	0.39	0.54*
Clarkboro Ferry	27/9	0.17	0.21	0.25	0.22	-	0.33	0.51	0.48	0.40	-	0.27	0.46	0.60	0.37	-
Hague Ferry	27/9	0.12	0.26	0.29	0.26	-	0.37	0.50	0.48	0.40	-	0.28	0.58	0.56	0.34	-
Tobin Lake	26/9	0.16	0.24	0.15	0.12	0.15	0.18	0.34	0.22	0.23	0.19	0.25	0.41	0.27	0.20	0.24

- Cage lost

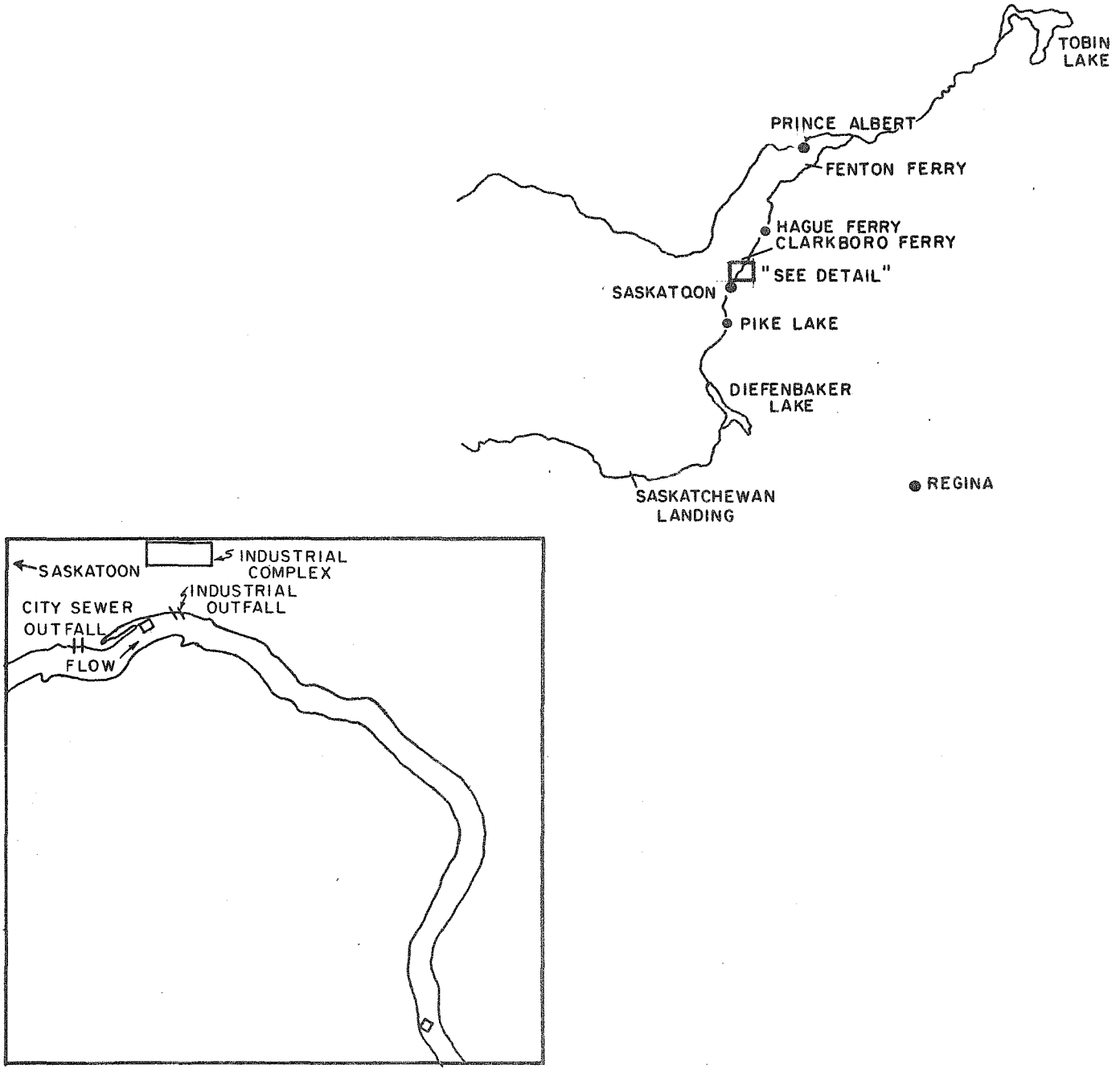
* single determination

Table 4. South Saskatchewan River Experiment - Summer 1971
- values are in ppm total mercury
- sampled every two weeks

Control		Carcass 0.06(0.10)			Liver 0.19(0.49)			Kidney 0.11(0.16)		
	Stocked	(1)	(2)	(3)	1	2	3	1	2	3
Pike Lake	15/6	0.08	0.07	-	0.06	0.08	-	0.16	0.18	-
IPCO	15/6	0.15	0.08	-	0.15	-	-	0.20	0.35	-
CLARKBORO FERRY										
East	15/6	0.07	0.09	0.10	0.10	0.12	0.17	0.18	0.24	0.19
West	15/6	0.10	0.22	0.44	0.30	0.34	0.85	0.03	0.34	1.01
Hague Ferry	15/6	0.17	-	-	0.29	-	-	0.44	-	-
Fenton Ferry	15/6	0.12	N.S.[1]	0.13	0.38	N.S.	0.36	0.44	N.S.	0.53

[1] No sample

Figure 1. Location of cages in the South Saskatchewan River.



ELIMINATION OF MERCURY FROM CONTAMINATED NORTHERN PIKE PLACED IN A CLEAN LAKE

by

J.F. Uthe

Northern Pike (Esox lucius) were captured by gill-netting in Clay Lake, Ontario (50°03' N; 93°30' W) and released in Heming Lake, Manitoba (54°53' N; 101°07' W). The white muscle mercury level was determined prior to release of each animal by removal of a small piece of white muscle by needle biopsy (Uthe, 1971). At this time the fish was tagged and its length and weight recorded. This part of the experiment was carried out during mid-October 1970. During the next year fish were recaptured at four different times and the level of mercury present in the white muscle redetermined. Heming Lake, the site of a field station of the Freshwater Institute, was chosen because pike native to this lake has only background levels of mercury in their muscle(∿0.2 ppm). The average level of the white muscle was 8 ppm at the time of release. Over the period of the year during which this study was carried out the concentration of mercury in the white muscle decreased. This decrease however was caused by at least two factors - elimination of the mercury from the fish and dilution due to growth. During the

year, particularly during the summer the fish grew a significant amount. Even with the fish being unable to eliminate any mercury from its body whatsoever the concentration would drop due to the growth of the animal. It is improbable that no mercury whatsoever would be eliminated. In order to determine the elimination rate it was assumed that the total weight of the fish represented white muscle from a concentration of mercury viewpoint. The validity of this assumption is the finding that irregardless of absolute level the mercury concentration between white muscle and other body organs is approximately constant. (A. Kenney, this report). Furthermore it was assumed that the organ weight ratios to white muscle were approximately constant since all fish were adult and with the exception of the last sampling period did not show marked weight change . The absolute and gross concentrations of mercury present in the fish from each sample period were calculated and compared with the corresponding figure calculated from the white muscle mercury values obtained by biopsy prior to release.

Two values were then calculated, one the gross part per million (µg/g) level of the total recapture sample and the equivalent value at the release time, and the total amount (µg) of mercury in the recapture sample and the equivalent value at release time.

The change in the total amount of mercury is a reflection of the elimination of mercury only, i.e. the dilution effect is not shown by this calculation. The elimination rate is quite slow, amounting to less than 30% for the year. This gives

an elimination rate of 50% every two years, i.e. 50% of the mercury present at the start of any period will be eliminated during the next two years.

The drop in the parts per million level of mercury reflects both elimination of mercury from the animal and the dilution effect due to growth. The magnitude of change in the part per million drop is considerably larger than the drop in total µg of mercury during the summer period when the fish were undergoing rapid growth. These fish were probably undergoing about maximum growth for the conditions under which they were held in Heming Lake. The pike as they were taken out of Clay Lake were emaciated with little body fat. This is apparently due to the presence of a difficult predation situation brought on by the very muddy water of Clay Lake. Mercury probably did not cause the emaciation since the pike recaptured at Heming Lake showed no evidence of emaciation although their level of mercury was still quite high (∿3 ppm or ∿10X background levels). The average size of the transplanted pike exceeded that of the native pike population by quite a bit. These fish should readily become the uppermost member of the aqueous food chain and probably would only receive clean food. We are therefore probably looking at the maximum possible dilution effect and the drop of approximately 70% in one year is maximal while the 30% drop in total body burden of mercury is probably minimal. In the situation where the mercury discharge has been stopped the pike will probably eliminate mercury as the transplanted fish

but will probably not show the fast growth found in the transplants. On top of this these fish will not be feeding solely on foodstuffs with only background levels of mercury and will have a higher intake of mercury. Such fish could show a much lower gross body elimination rate of mercury than even the 30% minimal rate of the Heming Lake fish. During the winter (Dec/70 - April/71) common sucker (Catostomus commersoni) from Clay Lake were held in aquaria using municipal water ($\sim 3^{\circ}C$). During this period the fish were biopsied, weighed and their white muscle mercury level determined (Table 2). The animals lost a slight bit of weight $\sim$5%) but no significant difference was noticed in mercury content.

Reference

1. Uthe, J.F. J. Fish. Res. Bd. Canada 28, 1203 (1971).

Table 1. Mercury recovered from transplanted Northern Pike (Values are for total mercury)

Sample Date	Elapsed Time (months)	Number	Original Weight(g)	Capture Weight(g)	% Weight Change	Original Gross ppm	Recapture Gross ppm (% of original)	Original Mercury (µg)	Recapture Mercury(µg) (% of Orig)
June/71	3	9	9,560	9,370	-2	7.0	7.47(106)	67200	70,100 (105)
April/71	6	18	16,160	16,210	+0.3	8.7	7.24(83)	140,800	117,200 (83)
July/71	9	17	18,500	22,040	+6	7.9	5.45(69)	146,000	120,300 (82)
Oct/71	12	5	3,920	8,360	+113	8.32	2.75(33)	32,700	23,100 (71)

Table 2. Mercury Recovered from common suckers held in aquaria at ∿3°C

Sample Date	Elapsed time(weeks)	No. of fish	Gross weight(gm)	Total Hg(µg)	Gross ppm Hg
Dec. 15/70	0	11	11080	38707	3.50
Jan. 26/71	6	11	10990	36800	3.21
March 10/71	12	11	10890	40100	4.03
April 27/71	18	11	10460	39801	3.81

UPTAKE OF MERCURY BY GUPPIES (*Lebistes reticulatus*) FROM MERCURY CONTAMINATED SEDIMENTS

by

D.C. Gillespie

Guppies (*L. reticulatus*) were exposed in aquaria to aerobic and anaerobic sediments to which various forms of mercury were added and to sediments from two sites of industrial pollution. Whole body levels of total mercury by atomic absorption analyses (Armstrong and Uthe, 1971) in these fish were used as a measure of mobilization of mercury from these sediments. Spot checks for methyl mercury were carried out at intervals during the tests. Under aerobic there was little mobilization of mercury from sediments to which mercuric chloride and sulphide had been added at 50 ppm level (dry weight basis) but mercury concentrations rose rapidly in fish exposed to sediments containing metallic mercury. The proportion of methylmercury in fish reached maximum of 40% for metallic mercury, 40% for mercuric chloride and 45% for mercuric sulphide. In anaerobic sediments total mobilization was low and only mercuric chloride was significantly methylated (40% of total in the fish).

Both mobilization and methylation were proportionately greater in industrial sediments with a low mercury content than in sediments with a higher mercury content. Enrichment of sediments with lignosulphonate showed no real effects aerobically, possibly because of the chelating effects of the preparation used. Anaerobically lignosulphonate stimulated methylation of mercury.

The levels of methylmercury found in these experiments are consistent with data from nature.

References

Armstrong, F.A.J. and J.F. Uthe. Atomic Absorption Newsletter 10,110 (1971).

Balle, C.A. et al., Science 172, 951 (1971).

Table 1. Total and methyl mercury content of guppies exposed to sediments with added mercury.*

Sediment	Days Exposure	Series	ppm mercury Methyl	Total
Delta Marsh	28	1	0.1	1.0
(Control)	125	1	0.15	0.7
(0.1 ppm)	45	2	0.1	0.4
Metallic	28	1	0.1	4.8
Mercury	125	1	0.14	2.9
(50 ppm)	45	2	0.34	1.4
	88	2	0.92	2.7
	70	An	0.1	0.65
Mercuric	97	1	0.46	1.0
Chloride	35	2	0.34	0.8
(50 ppm)	70	An	0.23	0.59
Mercuric	28	1	0.1	1.0
sulphide	125	1	0.4	0.9
(50 ppm)	35	2	0.32	0.7
	77	2	0.24	(1.35)
	70	An	0.09	0.81

* Figures in parentheses indicate total mercury level at the last date for which it was determined; An-data from experiment using anaerobic sediments; series - refers to first and second additions of fish; total mercury values from the average of five fish; methyl mercury values from single large fish or two fish pooled to give enough material for analysis.

Table 2. Total and mercury content of guppies exposed to sediments from the St. Clair River.*

Sediment	Days Exposure	Series	ppm Mercury Methyl	ppm Mercury Total
St. Clair	35	1	0.3	0.7
low (9 ppm)	45	2	0.4	0.79
	60	2	0.3	0.72
	88	2	0.71	0.98
St. Clair	84	1	0.39	(1.2)
medium (40 ppm)	45	2	0.08	0.6
	88	2	1.31	(1.3)
St. Clair	45	2	0.23	1.8
high (120 ppm)	88	2	0.82	1.97
	70	An	0.06	0.69
St. Clair	84	1	0.15	(2.8)
high (120 ppm)	45	2	0.07	1.2
with lignosulphonate	88	2	0.3	1.49
	150	2	0.65	1.75
	70	An	0.31	0.60

* Figures in parentheses indicate total mercury level at the last date for which it was determined;An-data from experiment using anaerobic sediments;series - refers to first and second additions of fish;total mercury values from the average of five fish;methyl mercury values from single large fish or two fish pooled to give enough material for analysis.

Table 3. Total and methyl mercury content of guppies exposed to sediments from the Saskatchewan River and to cinnabar ore.*

Sediment	Days Exposure	Series	ppm mercury Methyl	Total
	28	1	0.1	2.5
Saskatchewan R.	125	1	0.6	2.0
high (60 ppm)	35	2	0.49	1.9
	77	2	0.32	(1.6)
Saskatchewan R.				
low (7 ppm)	74	1	2.43	2.1
plus	45	2	0.63	0.7
plus				
lignosulphonate				
Lake 227	126	1	0.15	0.7
(control)	140	1	0.92	0.8
(0.15 ppm)	45	2	0.11	0.35
Cinnabar	45	2	0.09	110
	89	2	0.28	32

* Figures in parentheses indicate total mercury level at the last date for which it was determined;An-data from experiment using anaerobic sediments;series - refers to first and second additions of fish;total mercury values from the average of five fish;methyl mercury values from single large fish or two fish pooled to give enough material for analysis.

AQUARIUM EXPERIMENT ON THE UPTAKE OF MERCURY BY THE CHIRONOMID, *Chironomus tentans* Fabricius

by

A.L. Hamilton

Introduction

The purpose of this experiment was to establish whether or not the aquatic midge *Chironomus tentans*, accumulated mercury and if so whether the rate of accumulation was influenced by the form of mercury present in the environment. This particular test organism is easily cultured in the laboratory and it is often very abundant in shallow eutrophic ponds and lakes in Canada. In addition related species belonging to the same genus are often extremely important as food items for various freshwater fishes including such commercially important species as the whitefish, *Coregonus clupeaformis*.

Materials and Methods

Eighteen eight-liter aquaria were used as test containers. Each was initially filled to a level of 3 liters with distilled

water. Fifty grams of dry Delta marsh sediments[1] were then mixed into all but one of the aquaria. After the sediments had been allowed to settle for one day an additional 3 liters of culture medium was carefully added to each aquaria. This medium consisted of distilled water that had been treated with a mixture of 30 per cent ground up alfalfa and 70 per cent wheat hearts added at a rate of approximately 0.3 grams/liter.day for a five-day period. The mercury concentration in this mixture was assayed at 0.04 ppm. The aquaria were spiked with mercury compounds as follows: Methylmercuric chloride, and phenylmercuric acetate were added directly to the water of six of the aquaria in quantities equivalent to 0.05, 0.5 and 5.0 ppm mercury per gram of sediment. Similarly mercuric chloride was added to three of the aquaria at levels of 0.5, 5.0 and 50.0 ppm mercury per gram of sediment. The metallic mercury and mercuric sulphide were mixed directly into the sediment using a sonicator to ensure homogenous distribution of the mercury within the sample. Levels used were 0.5, 5.0 and 50.0 ppm mercury per gram of sediment. This left two "control" aquaria with unspiked sediments and a third aquarium without sediments or added mercury.

Newly hatched larvae of Chironomus tentans were introduced into each of the aquaria where they were left to complete their life cycle. Each aquarium was equipped with an airstone to maintain oxygen levels and a nylon mesh cover to prevent the

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[1] Mercury concentration in these sediments was assayed at 0.1 ppm on a dry weight basis.

emerging adults from escaping. Each culture was fed 0.5 grams of the alfalfa wheat hearts mixture/day. Emerging adults were collected with an aspirator, killed by freezing and then air dried. The pooled sample was then analyzed for total mercury following the procedure outlined by Armstrong and Uthe 1971.

Results and Discussion

Results showed that aquaria spiked with all forms of mercury excepting mercuric sulphide yielded adult Chironomus tentans with elevated levels of mercury (Fig. 1). Uptake was most rapid in aquaria receiving methylmercuric chloride and phenylmercuric acetate, the two organic forms of mercury. The lack of uptake in aquaria receiving mercuric sulphide (Cinnabar) is interesting since this form of mercury is a very widespread, naturally occurring mineral.

References

1. Armstrong, F.A.J. and J.F. Uthe. Atomic Absorption Newsletter 10, 101 (1971).

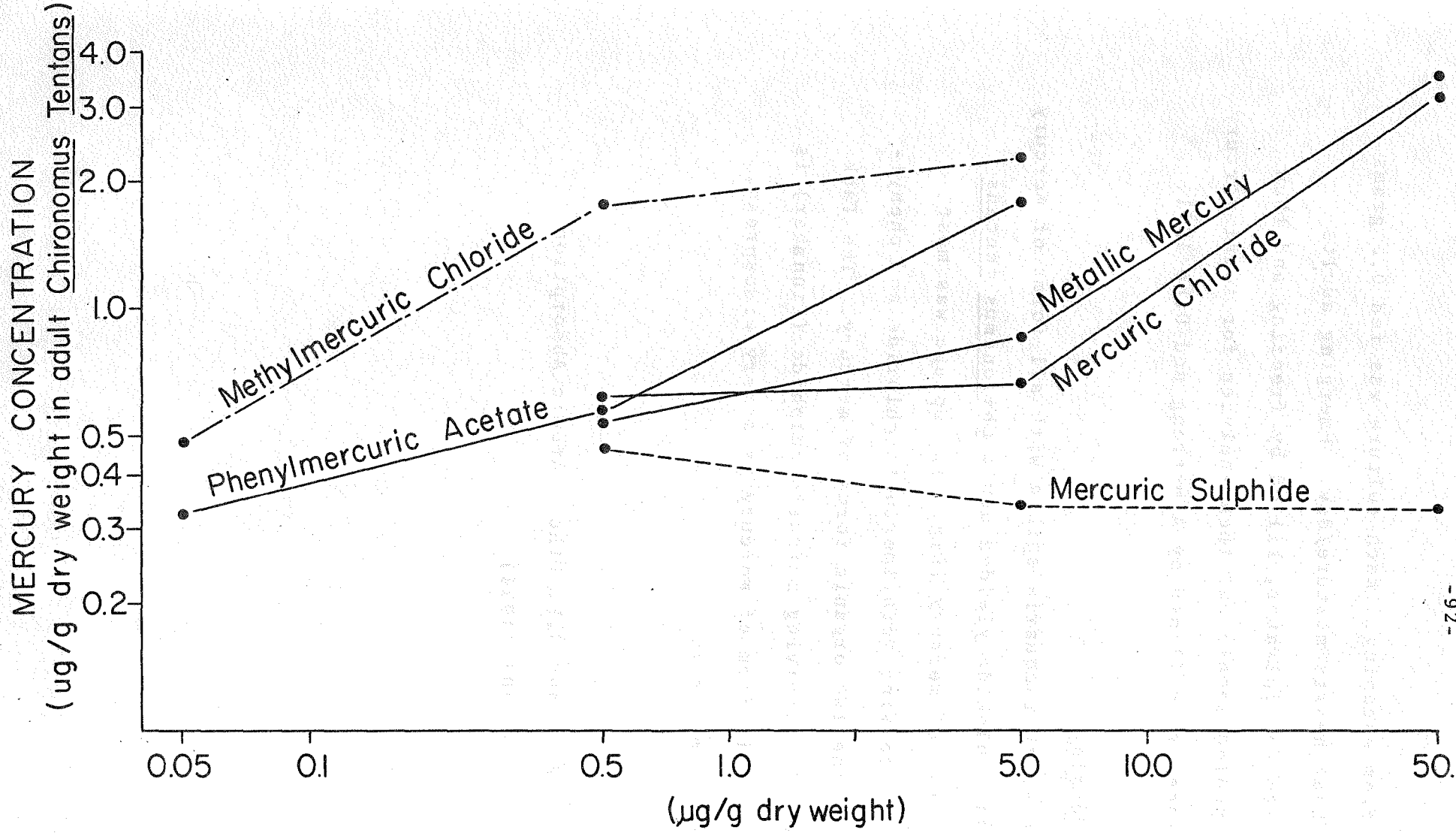

Figure 1. Mercury concentration in adult Chironomus tentans reared in aquaria containing sediments spiked with metallic mercury, mercuric chloride, mercuric sulphide, methylmercuric chloride and phenylmercuric acetate.

POND EXPERIMENTS ON THE UPTAKE AND ELIMINATION OF MERCURY BY SELECTED FRESHWATER ORGANISMS

by

A.L. Hamilton

Introduction

Mercury in aquatic ecosystems tends to accumulate in certain components of the biological community and although the precise mechanisms for this process are not well understood it is recognized that more than one mechanism is involved. There is also a theoretical basis for suspecting that either natural or man-made chelating agents might affect these mechanisms. The first objective of this series of experiments was to estimate the relative importance of water, sediment and food as sources of mercury contamination in a series of ponds set up with sources of mercury corresponding approximately to those found in a mercury-contaminated lake. The second objective was to determine whether or not the chelating agents nitrilotriacetic acid (NTA), ethylenediaminetetracetic acid (EDTA), and penta-sodium tripolyphosphate (TPP) had any effect on the rates of mercury uptake or elimination.

Materials and Methods

Twenty-one polyethylene-lined swimming pools were used as holding containers. They were 10 feet (3.048 meters) in diameter and 30 inches (76.2 cm) high with an outflow located at a height of 24" (61.0 cm). Each therefore, contained a volume of approximately 4,450 liters. Thirteen of the tanks were located beside Clay Lake, Ontario and water from this mercury-contaminated lake was pumped through each of the tanks at a rate of 5 liters/minute. This represents a theoretical renewal time of approximately 15 hours. The other eight tanks were located beside Rawson Lake at the Fisheries Research Board of Canada field station situated approximately 30 miles southeast of Kenora, Ontario. Clean water from this unpolluted lake was pumped through these tanks at the same 5 liters/minute rate.

Approximately 5 cm of sediments were added to each tank. "Clean" sandy sediments were collected from a depth of about 0.5 meters in Rawson Lake. Analysis of these sediments indicated an average mercury concentration of .02 ppm dry weight. "Contaminated" sediments were collected at a depth of 0.5 meters from a sandy beach in Clay Lake. Each tank receiving contaminated sediments was also "spiked" with about 20 liters of soft profundal sediments from the central part of Clay Lake. Average mercury concentration in these combined sediments was 0.11 ppm. The ponds treated with contaminated food received ground up Clay Lake ciscoe (Leucicthyes sp.) at a rate of 40 grams (wet weight) per day. Ground up ciscoe from northern Manitoba was added at the same rate to ponds receiving clean

food. Mercury concentrations in these two foods were 4.97 and 0.13 ppm (wet weight) respectively.

"Clean" adult crayfish, Orconectes virilis Hagen, and mussels, Arondonta grandis, were collected from the St. Malo reservoir approximately 50 miles south of Winnipeg, Manitoba. Initial mercury levels in abdominal muscle of crayfish and the foot muscle of the mussels averaged 0.17 and 0.06 ppm respectively on a wet weight basis. "Clean" rainbow trout (Salmo gairdnerii Richardson),were hatchery stock obtained from Thunder Bay, Ontario (they were 7.0 to 12.5 cm in fork-length and averaged 0.06 ppm mercury in back muscle). "Contaminated" crayfish collected in Clay Lake, Ontario had an average of 6.56 ppm mercury in abdominal muscle.

Eleven of the ponds were used for chelate experiments. Drip bottles fastened to the edge of these ponds were partially filled with chelate solutions and the drip rate was adjusted so that chelate additions averaged 0.5, 1.0 or 5.0 mg per liter of water entering the ponds. As indicated in Table 1 NTA, EDTA and TPP were each added to three of the ponds at the 1.0 mg/liter rate. In addition 0.5 mg/liter NTA was added to one pond and 5.0 mg/liter NTA was added to a second pond. The rate of addition was not entirely constant since the drip bottles characteristically dripped faster during the initial hours and the rate decreased as the bottles emptied. However, any solution remaining in the bottles at the end of the 24 hour period was added to the ponds so that the daily addition was always similar.

All test populations were sampled after 4, 10, 19, 33 and 53 days' exposure and the crayfish and mussel populations were also sampled on day 83. In most cases 4 crayfish, 3 rainbow trout and 2 mussels were collected on each sampling date. All mercury determinations were made following the method described by Armstrong and Uthe 1971.

Results and Discussion

Statistical analysis of the results are not yet completed; however, some of the major conclusions are obvious. At the termination of the experiments organisms in ponds receiving the contaminated ciscoe invariably contained much higher mercury concentrations than organisms fed clean ciscoe (Table 1). It is also clear that the Clay Lake water contained enough mercury to produce mercury concentrations approximately 2 to 5 times as high as that in organisms in the "clean" environment (for example compare pond 3C with ponds 1R and 3R). It is also apparent that initially clean crayfish and trout did accumulate some mercury in even the "clean" environment. This may indicate that the "clean" ciscoes used as food did in fact contain significantly more mercury than food the trout and crayfish had encountered previously. Crayfish and trout concentrated mercury much faster than mussels and there was no indication that contaminated crayfish eliminated a significant quantity of mercury when placed in a "Clean" environment.

As summarized in Table 1 there is no clear indication that

any of the chelates had any real influence on either the uptake or elimination of mercury. The average concentrations of mercury in the trout from ponds treated with chelates are illustrated in Figures 1 to 3. It appears that the accumulation rate was independent of chelate additions, however, in ponds receiving clean food the trout from ponds treated with the 5.0 mg/l NTA addition and the 1.0 mg/l TPP addition are somewhat above the control and it is possible that there is statistical significance. The rates of mercury accumulation in crayfish were certainly not related to chelate additions (Figures 4-6). Similarly mercury concentrations in contaminated crayfish placed in a clean environment were apparently not influenced by the three chelates tested (Figure 7). In fact there is no indication that contaminated crayfish eliminated any mercury during the 83-day test period. Mercury concentrations did drop slightly (from an average of 6.56 to an average of 5.13 ppm) over the period, however, new, less contaminated, tissue produced during the experiment may simply have diluted the mercury. In other words the total amount of mercury per specimen could well be essentially identical to what it was at the beginning of the experiment.

There appears to be a fundamental difference in the trout curves (Figures 1-3) and the crayfish curves (Figures 4-7). In every case the mercury concentrations in trout increased very apidly at first and then tended to level off. When the values e plotted on semilogarithmic graph paper there is a pronounced ge in slope. In contrast similar plots of the values for

crayfish produce what is essentially a straight line and there is no indication that the rate of mercury accumulation is levelling off. This could mean that the trout reach an equilibrium level where the rate of mercury elimination equals the rate of mercury uptake. In contrast it is apparent that under the experimental conditions crayfish never did reach an equilibrium level. The uptake and elimination curves both suggest that crayfish are essentially unable to excrete even small amounts of mercury. It could be postulated that the differences in the curves for trout and crayfish are in fact a reflection of basic physiological differences between fish and crustaceans.

Some caution is required when extrapolating these experimental results to the actual situation in Clay Lake; however, certainly these results do provide evidence that the concentration of mercury as it moves through the food chain is probably one very important mechanism. It is also probable that the very dense population of highly contaminated crayfish in Clay Lake constitute a significant proportion of the food of burbot, northern pike and yellow walleye. This may be a partial explanation for the extremely high mercury concentrations in the predatory fish from Clay Lake.

References

1. Armstrong, F.A.J. and J.F. Uthe. Atomic Absorption Newsletter 10, 101 (1971).

Table 1. Mercury concentrations (ppm - wet weight) in test organisms at the completion of experiments. Values are the minimum and maximum concentrations with the number of specimens in brackets. Crayfish, mussels and rainbow trout were exposed for 83, 83 and 53 days respectively. Initial concentrations of mercury were: clean crayfish 0.15-0.18 (8); contaminated crayfish 3.72-11.8 (32); rainbow trout 0.04-0.11 (11) and mussels 0.04-0.08 (12).

Pond No.	Sediment	Water	Food		Clean crayfish		Contaminate crayfish		Trout		Mussels	
1R & 3R	clean	clean	clean	-	0.35-0.55	(3)	3.76-10.25	(4)	0.12-0.19	(2)	0.05-0.06	(2)
6R	clean	clean	clean	1.0 mg/l NTA	-		2.77-6.63	(3)	-		-	
7R	clean	clean	clean	1.0 mg/l EDTA	-		3.90-6.89	(4)	-		-	
8R	clean	clean	clean	1.0 mg/l TPP	-		4.00-6.10	(4)	-		-	
2R	Cont.	clean	clean	-	0.33	(1)	2.74-5.04	(3)	0.34-0.40	(2)	0.04-0.10	(2)
3C	clean	cont.	clean	-	1.00-1.17	(2)	3.18-5.69	(3)	0.62-0.70	(3)	0.14-0.19	(2)
2C	cont.	cont.	clean	-	0.94-1.36	(3)	5.64-7.20	(4)	0.62-0.96	(3)	0.15-0.28	(2)
6C/3	cont.	cont.	clean	0.5 mg/l NTA	0.88-1.30	(3)	-		0.76-0.88	(3)	0.09-0.23	(2)
6C/2	cont.	cont.	clean	1.0 mg/l NTA	0.66-1.46	(3)	-		0.40-0.55	(3)	0.29-0.32	(2)
6C/4	cont.	cont.	clean	5.0 mg/l NTA	1.26-2.09	(4)	-		1.08-1.36	(3)	0.07-0.18	(2)
7C/2	cont.	cont.	clean	1.0 mg/l EDTA	0.85-1.28	(3)	-		0.44-0.48	(3)	0.16-0.33	(2)
8C/2	cont.	cont.	clean	1.0 mg/l TPP	0.90-1.07	(3)	-		0.94-1.17	(3)	0.07-0.14	(2)
5R	clean	clean	cont.	-	7.95-8.70	(2)	14.2-19.9	(3)	10.4 -12.5	(2)	0.82-1.37	(2)
4R	cont.	clean	cont.	-	5.41-5.48[1]	(2)	15.3-17.0	(3)	8.56-10.4	(2)	0.46-0.50	(2)
5C	clean	cont.	cont.	-	17.2 -20.7	(3)	14.9-25.8	(4)	9.28-11.4	(3)	0.31-0.43	(3)
1C & 4C	cont.	cont.	cont.	-	12.4-15.4	(3)	19.6-24.0	(4)	8.58-11.3	(3)	0.45-0.83	(3)
6C/1	cont.	cont.	cont.	1.0 mg/l NTA	-		16.2-23.4	(4)	7.90-10.7	(3)	-	
7C/1	cont.	cont.	cont.	1.0 mg/l EDTA	-		17.2-19.7	(3)	6.60-9.82	(2)	-	
8C/1	cont.	cont.	cont.	1.0 mg/l TPP	-		15.2-23.6	(4)	3.76-6.78[2]	(2)	-	

1. Final sample taken on day 53 rather than day 83.
2. Final sample taken on day 33 rather than 53.

Pond Environment

Clean food
Contaminated water
Contaminated sediment

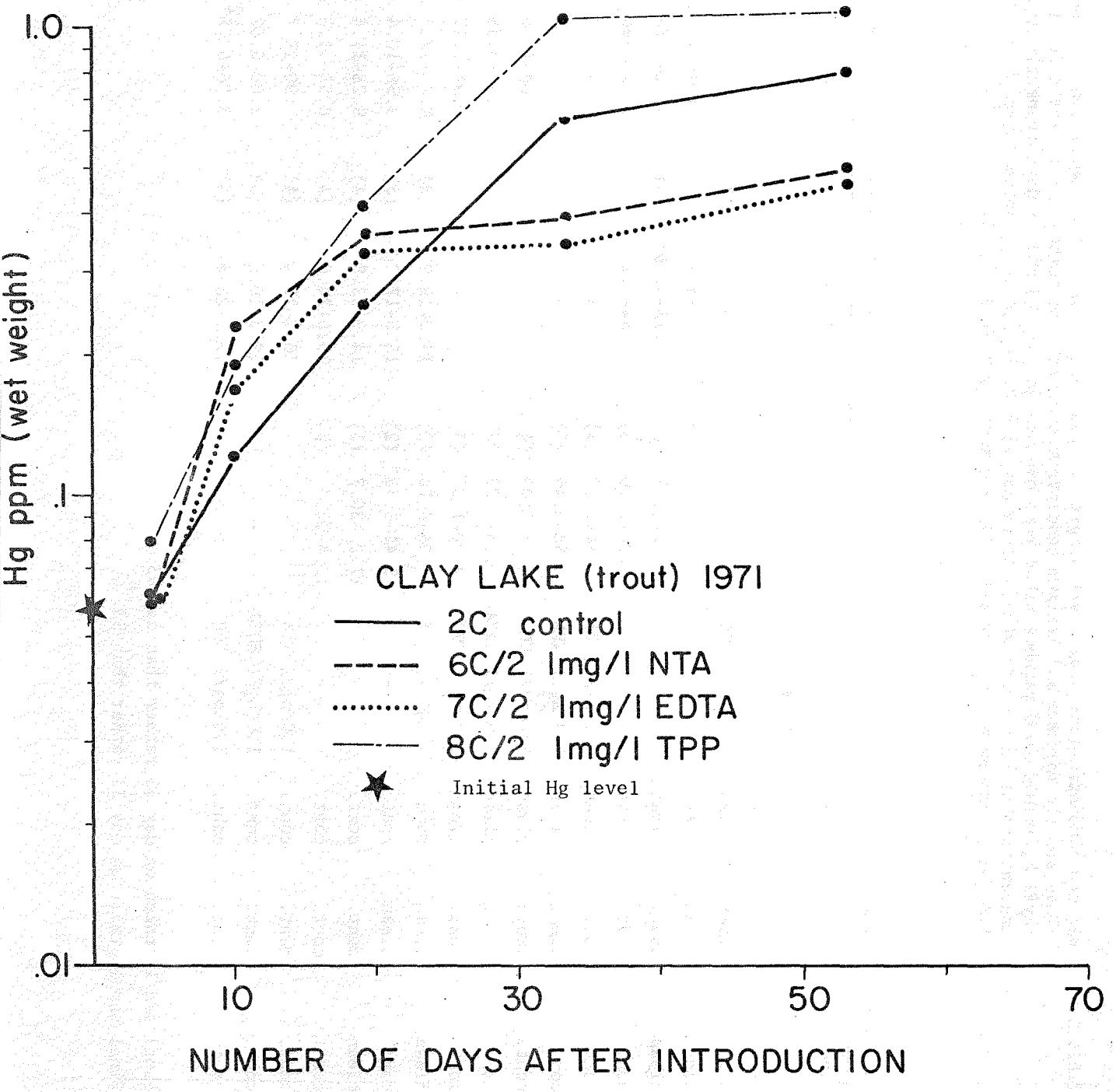

Figure 1. The effect of three chelates on the rate of mercury uptake by back muscle of rainbow trout kept in ponds containing contaminated water, contaminated sediment and clean food.

Clean food
Contaminated water
Contaminated sediment

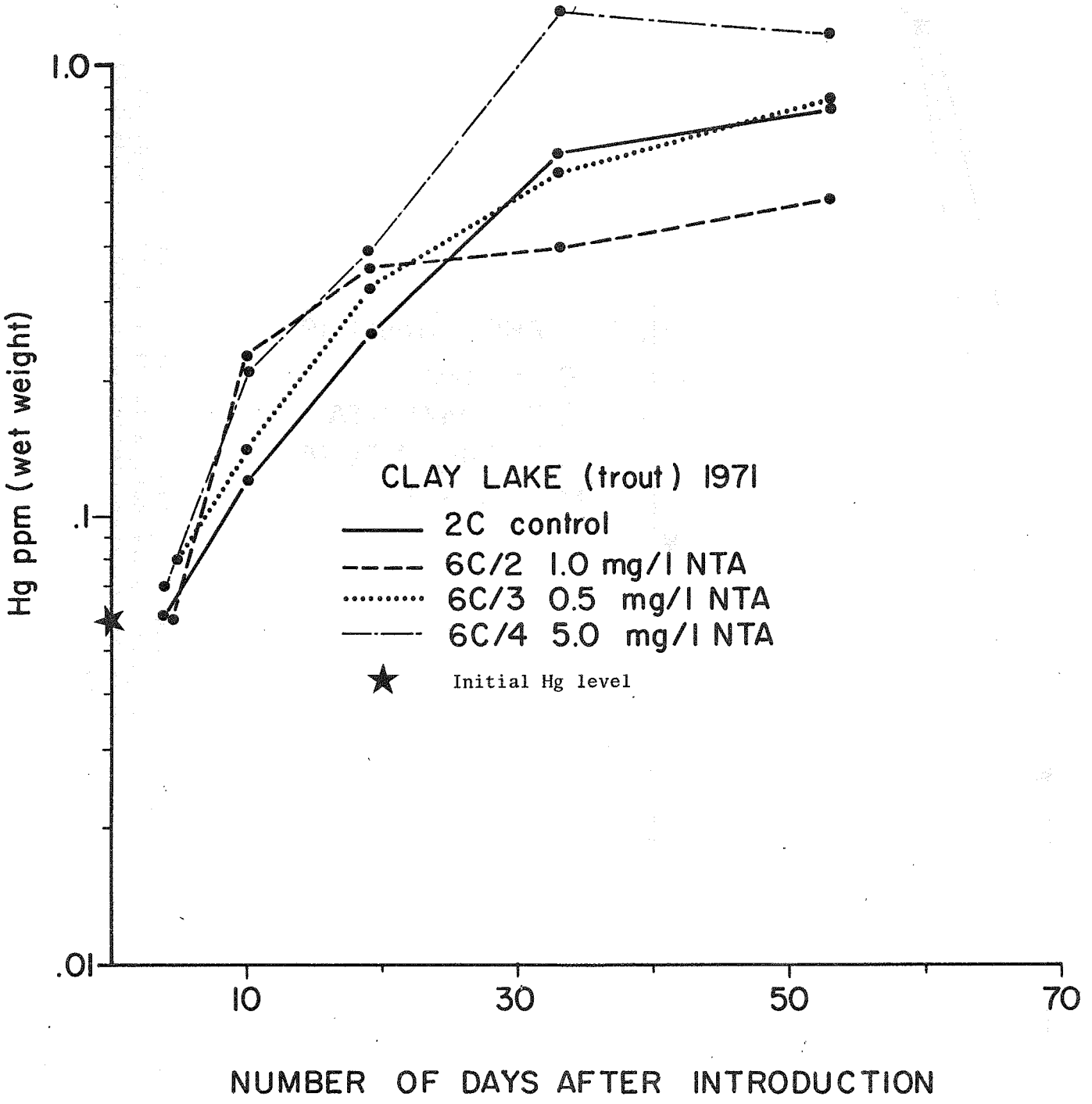

Figure 2. The effect of three concentrations of NTA on the rate of mercury uptake by back muscle of rainbow trout kept in ponds containing contaminated water, contaminated sediment and clean food.

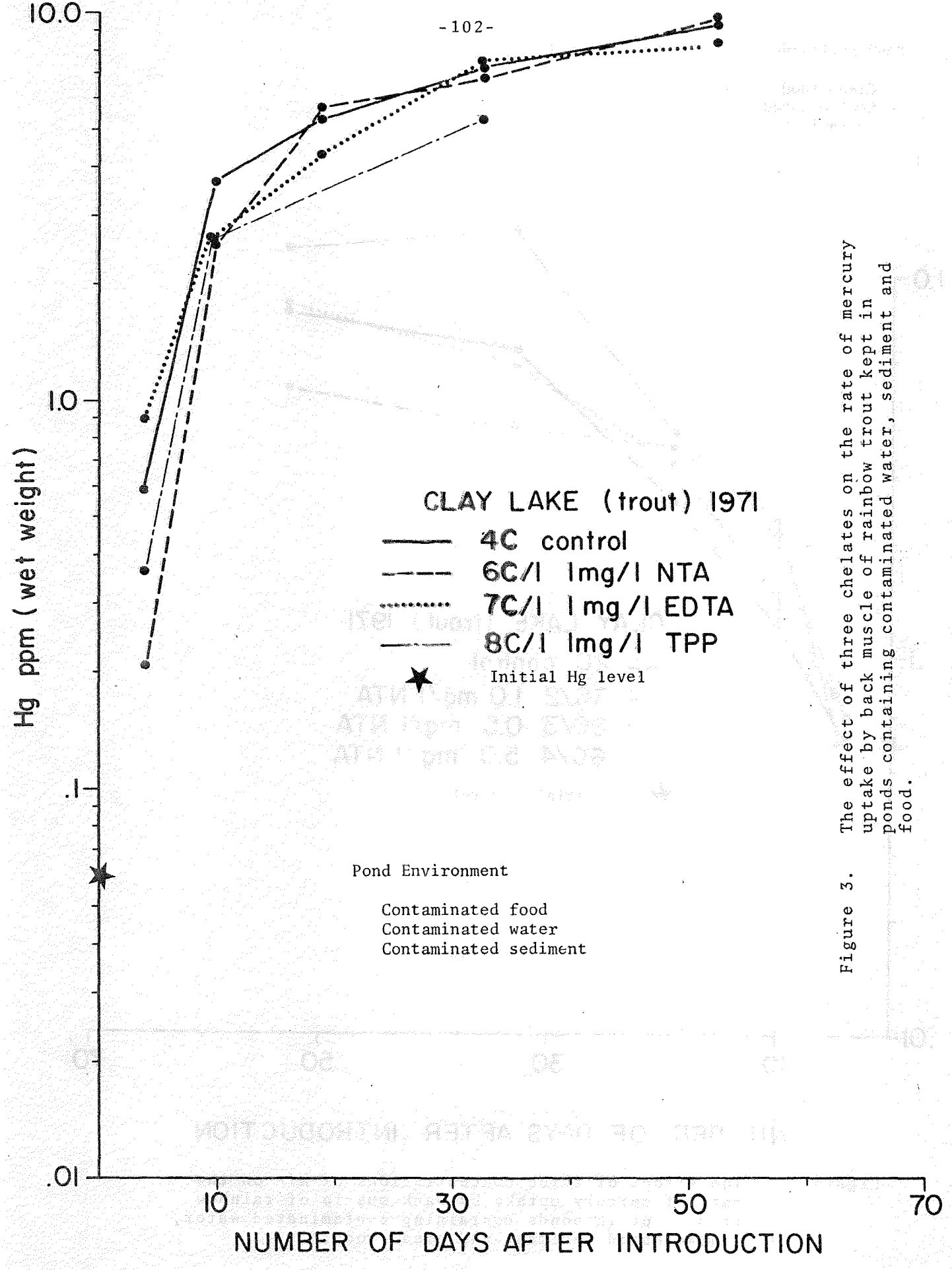

Figure 3. The effect of three chelates on the rate of mercury uptake by back muscle of rainbow trout kept in ponds containing contaminated water, sediment and food.

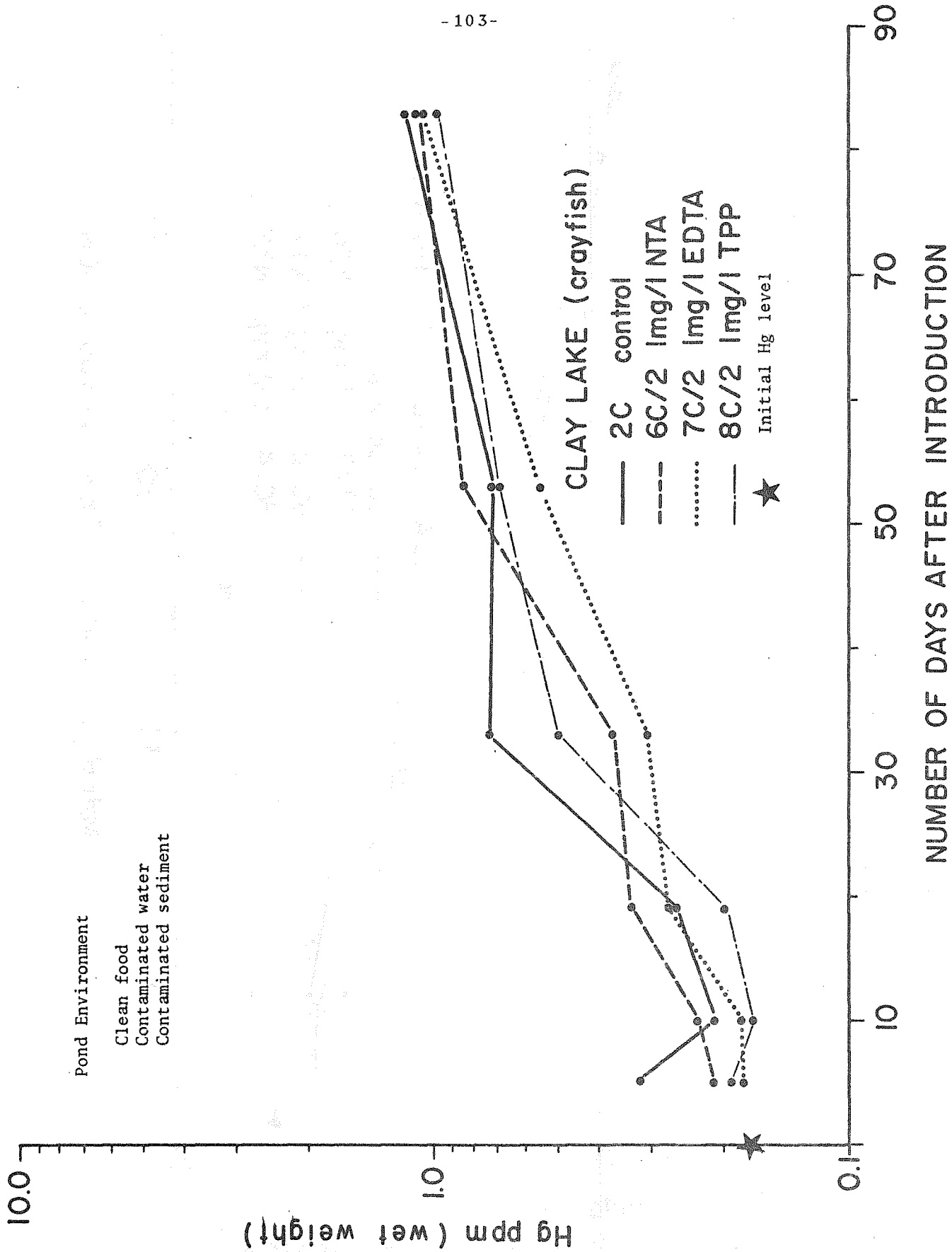

Figure 4. The effect of three chelates on the rate of mercury uptake by abdominal muscle of crayfish kept in ponds containing contaminated water, contaminated sediments and clean food.

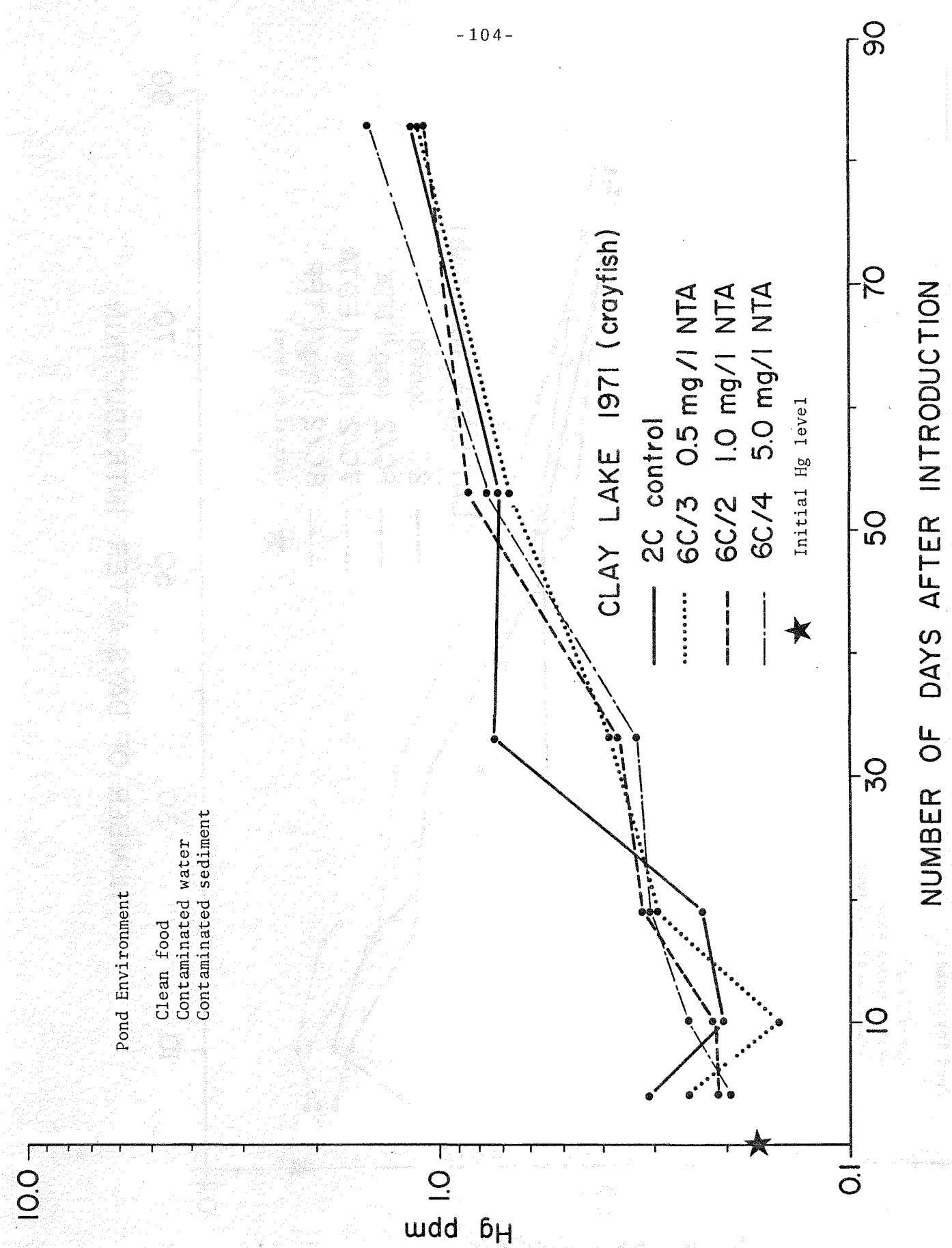

Figure 5. The effect of three concentrations of NTA on the rate of mercury uptake by abdominal muscle of crayfish kept in ponds containing contaminated water, contaminated sediment and clean food.

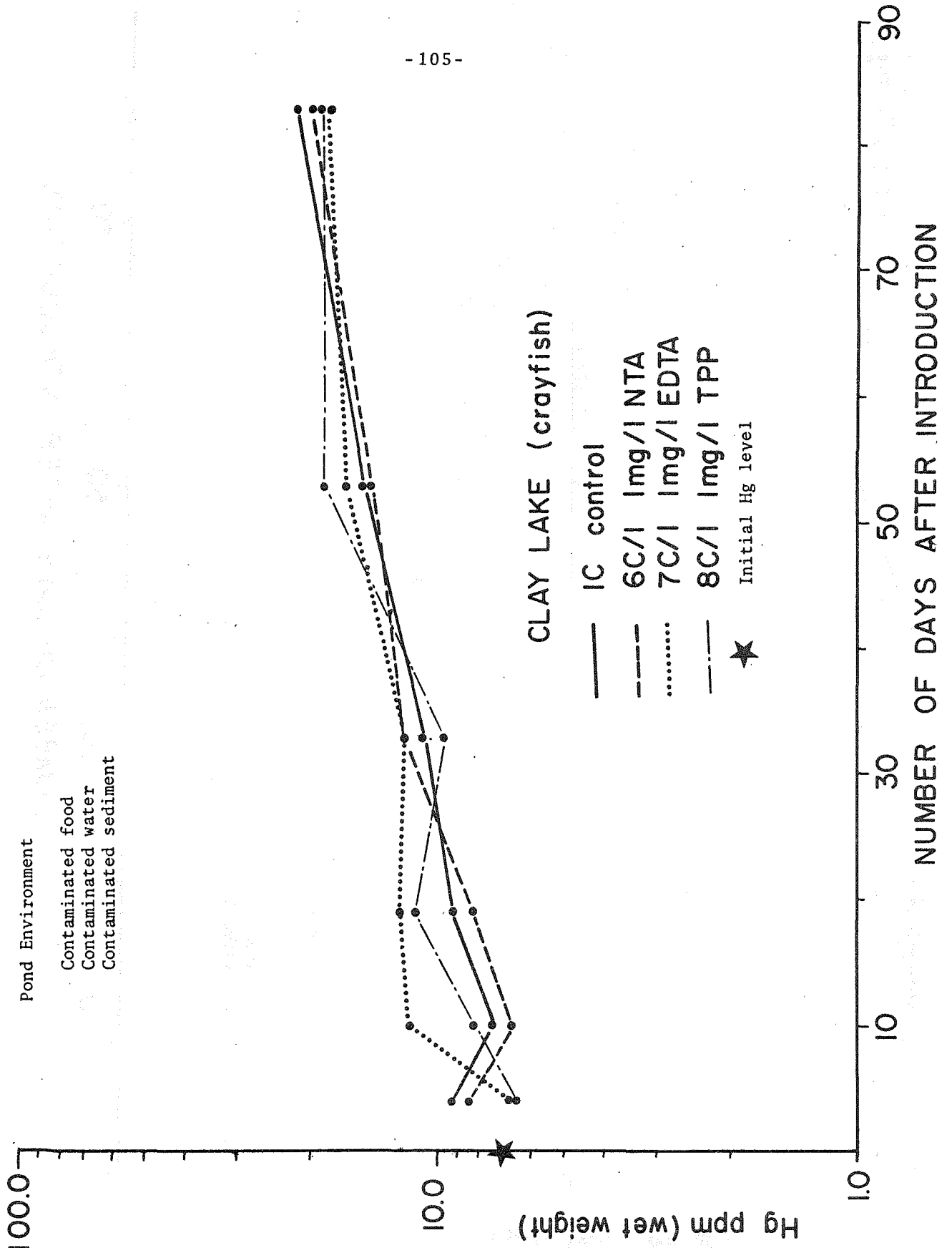

Figure 6. The effect of three chelates on the rate of mercury uptake by abdominal muscle of mercury-contaminated crayfish kept in ponds containing contaminated water, sediment and food.

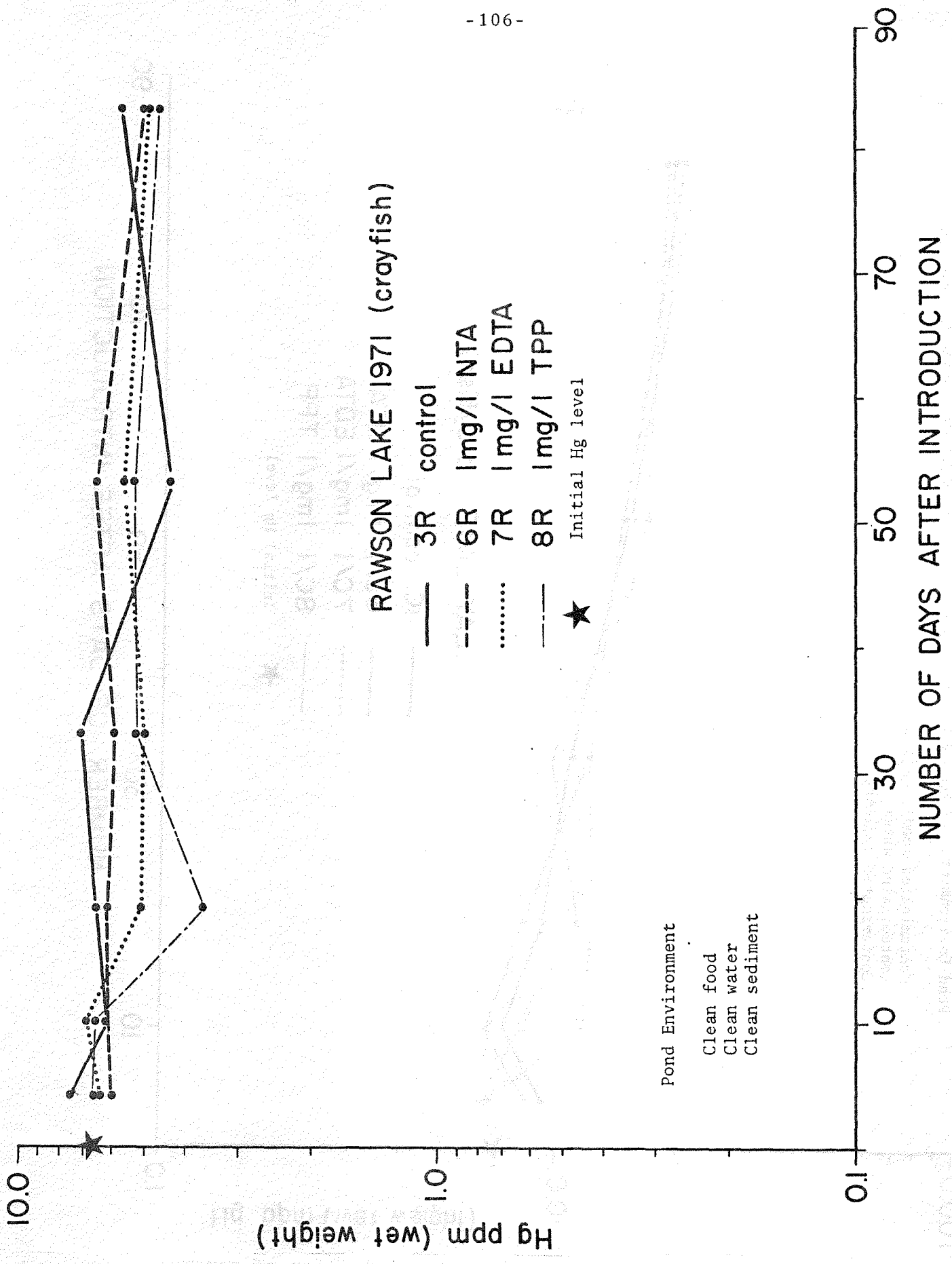

Figure 7. The effect of three chelates on mercury concentrations in the abdominal muscle of mercury contaminated crayfish kept in a clean pond environment.

THE EFFECTS OF CHELATING AGENTS ON THE MOBILIZATION OF MERCURY IN AN AQUARIUM ECOSYSTEM

by

A.L. Hamilton, D. Gillespie
J. Barica and G.P. McRae

Introduction

The purpose of this experiment was to determine whether or not levels of nitrilotriacetic acid (NTA), sodium tripolyphosphate (STP) or ethylenediaminetetracetic acid (EDTA) that were likely to be found in the aquatic environment either enhanced or retarded the uptake of mercury and other heavy metals by aquatic organisms in a mercury contaminated aquarium. The effects of these chelates on the mobilization of iron, manganese, copper, lead, zinc and chromium are reported in elsewhere (Barica et al). The major reason for conducting this experiment at this time was the U.S. Surgeon General's press release of December 18, 1970. In this release it was suggested that high concentrations of one strong chelator, Nitrilotriacetic acid (NTA), could increase the incidence of

deformities in rats subjected to high concentrations of mercury. Since NTA was under active consideration as a potential substitute for the phosphates in detergents it was imperative to evaluate its role in the mobilization of mercury in aquatic ecosystems. Large quantities of the other two chelates, EDTA and STP, were already entering freshwater ecosystems.

Materials and Methods

Ten aquaria each of 10 gallon capacity were used as test containers. Two hundred grams of dry Clay Lake sediments were added to each aquaria. These sediments were from a mercury contaminated lake and averaged about 1.0 ppm mercury on a dry weight basis. Five of the tanks were filled with tap water, the other five were filled with a 50-50 mixture of tap water and culture medium. This medium consisted of distilled water to which a mixture of 70% alfalfa and 30% wheat hearts was added at a rate of 0.3 grams/liter day for a 5-day period. The tanks were aerated for several days and then approximately 1000 newly hatched midge larvae (Chironomus tentans Fabr.) were added to the 5 aquaria containing the culture medium. Forty five guppies (Lebistes (Poecilia) reticulatus) were added to each of the 5 aquaria containing only tap water and Clay Lake sediments. The aquaria were paired so that each test organism was exposed to a closed system receiving the following weekly doses of chelates: NTA at 0.2 and 1.0 mg/liter; EDTA at 1.0 mg/liter and STP at 1.0 mg/liter, plus a control that received no chelate additions. Water samples were taken before each addition to follow the

residual levels of NTA, EDTA and STP in the guppy aquaria. Methods of analysis are outlined in Barica et al., MS. Individual heaters were used to hold the water temperature at 20°C. pH remained steady at 7.4-7.8 and oxygen levels were maintained at near saturation levels by aeration. A nylon mesh cover was placed on each chironomid aquaria to prevent adults from escaping and mariotte bottles filled with distilled water were used to maintain water levels in the aquaria. The Chironomus tentans cultures were each fed 4-5 grams of the alfalfa-wheat hearts mixture per week and the guppies were fed a maintenance ration of tetramin staple flake food. Adult chironomids were collected after they emerged, frozen to kill them, air-dried and the pooled samples were submitted for analyses at one, two or three-week intervals. Guppy samples were taken at one or two week intervals for wet weight mercury determinations (Armstrong and Uthe, 1971). Each sample consisted of five individuals analyzed separately. The chironomid and guppy experiment were continued for 115 and 75 days respectively.

Results and Discussion

Mercury concentrations in adult chironomids emerging from all aquaria increased until approximately day 60 when levels stabilized (Fig. 1). At this temperature (20°C) it ordinarily takes somewhere between 20 and 40 days for this species to complete its life cycle, hence after approximately day 40 all emerging adults would have spent all their immature

stages in the test aquaria. There is some indication that the EDTA additions did enhance the rate of mercury uptake; however, considerably more data would be required to verify effects as minor as those indicated here. There was no indication that the other chelates contributed at all to the rate of mercury uptake. In fact on the final day of the experiment the mercury concentration in adults emerging from the control aquarium was slightly higher than that in adults emerging from the aquaria receiving these chelates.

Mercury concentrations in the guppies increased from 0.08 ppm wet weight to between 0.31 and 0.48 ppm during the 75 day period (Fig. 2). There was some indication that the high NTA (1.0 mg/l) additions and the TPP additions slightly enhanced the uptake of mercury; however, considerably more data would be required to substantiate or refute this possible trend.

Analysis of samples from the guppy aquaria demonstrated that NTA, and STP were both breaking down rather quickly (Fig. 3). By the end of the experiment almost all of these compounds had disappeared during the 7-day sampling interval. In contrast EDTA levels continued to build up and by the end of the experiment the concentration was approximately 3 times the weekly dose. The 1.0 mg/l additions of chelates are very high and in practical terms it is likely that levels in receiving waters would rarely, if ever, be this high. Consequently these experiments though not conclusive, do indicate that the chelates tested are likely to have very little effect on rates of mercury mobilization in freshwater ecosystems.

References

1. Armstrong, F.A.J. and J.F. Uthe. Atomic Absorption Newsletter 10, 101 (1970).

2. Barica, J., M.P. Stainton and A.L. Hamilton. M.S. Mobilization of some heavy metals in water and animal tissue by NTA, EDTA and TPP.

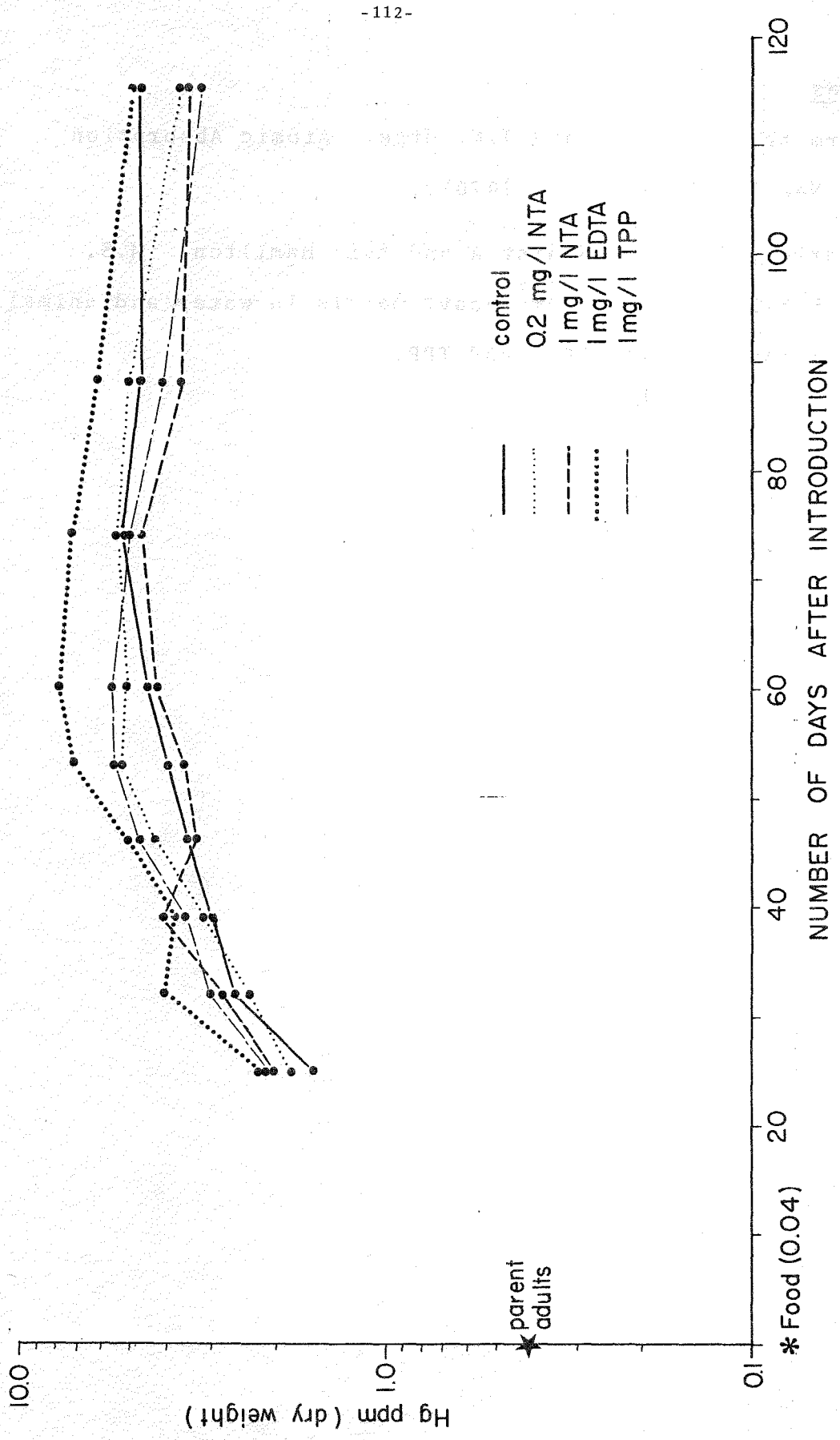

Figure 1. The effect of NTA, EDTA and TPP on the mercury concentrations in adult Chironomus tentans reared in aquaria containing contaminated Clay Lake sediments.

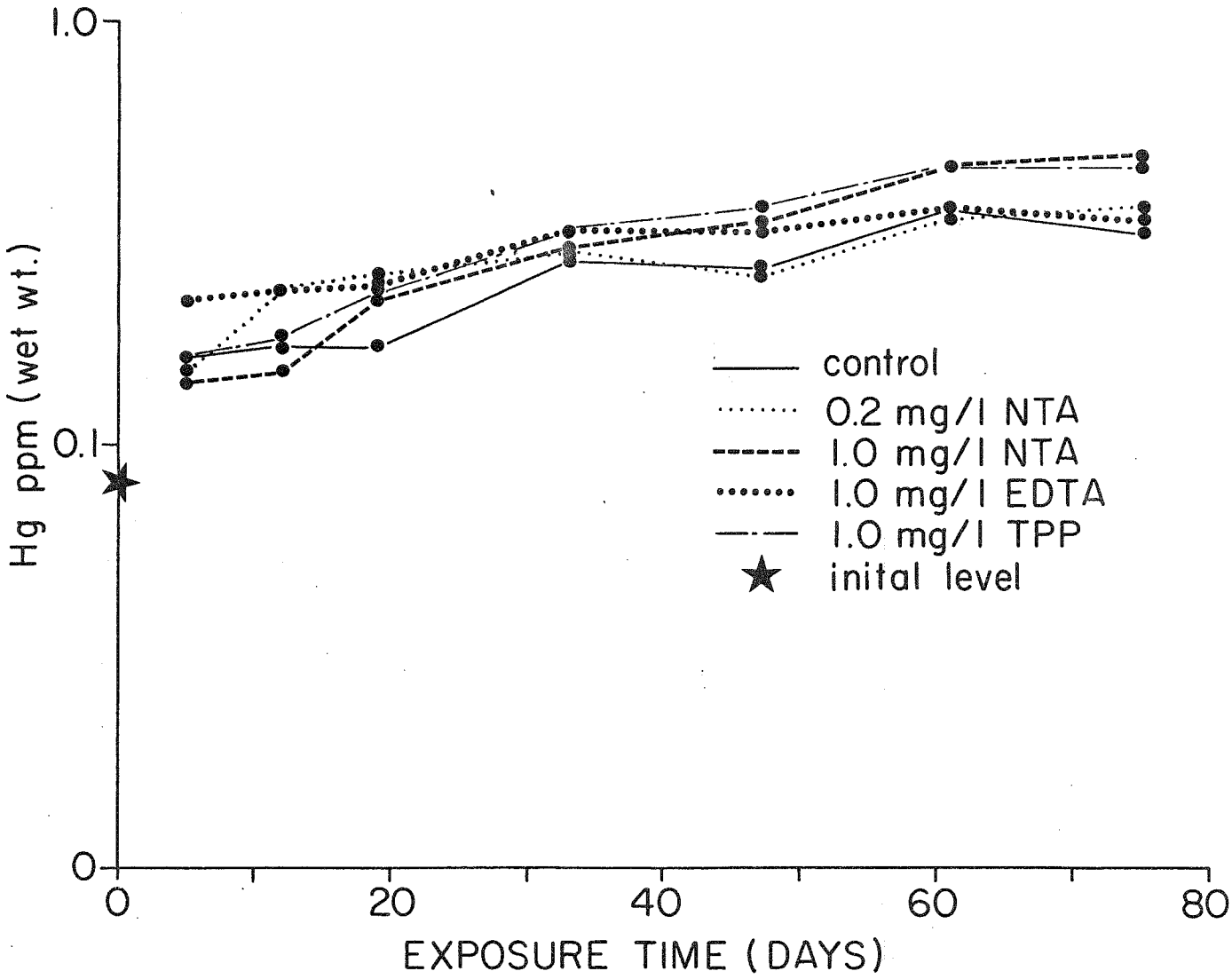

Figure 2. The effect of NTA, EDTA and TPP on the mercury concentrations in guppies reared in aquaria contaminated Clay Lake sediments.

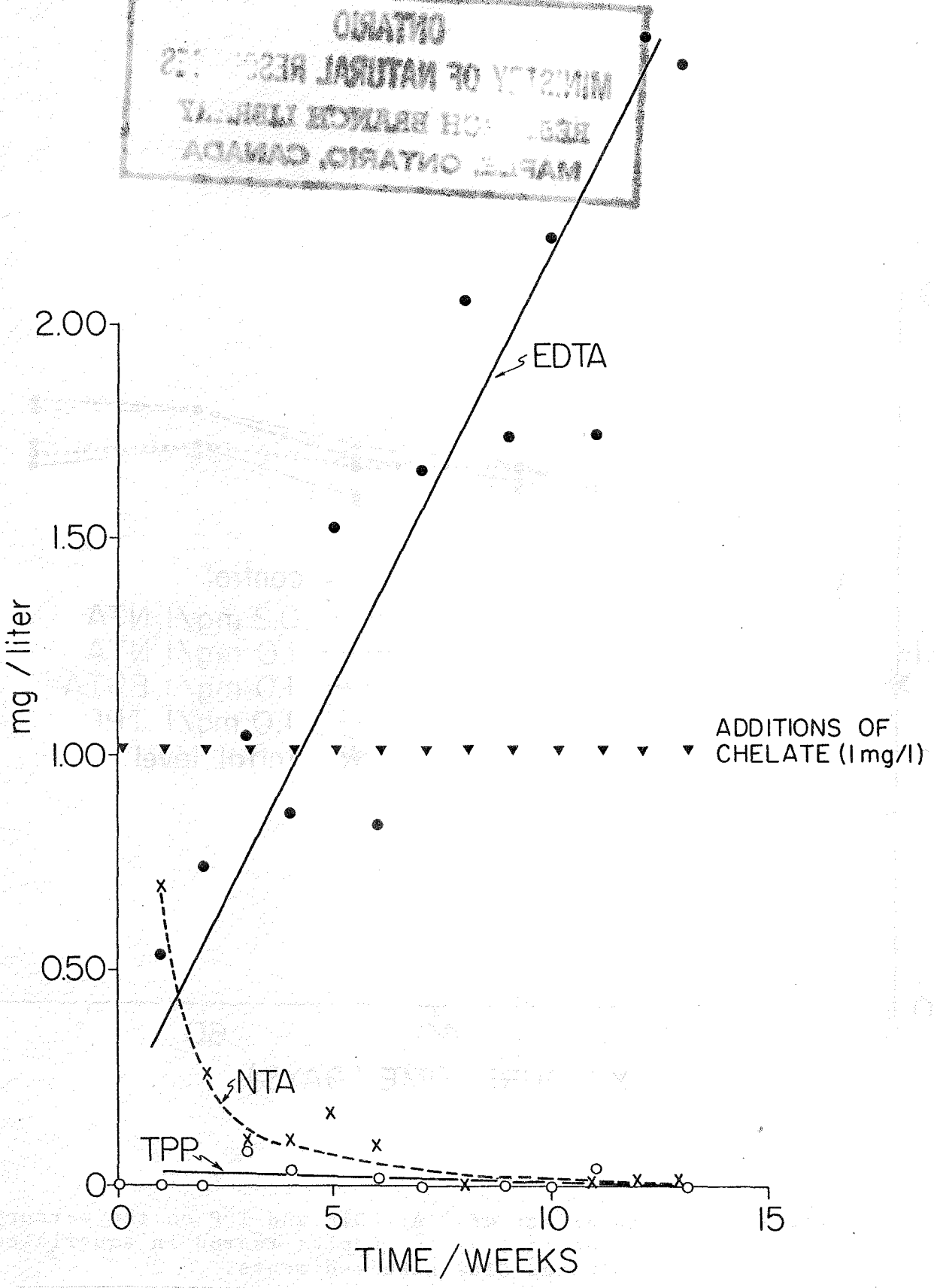

Figure 3. Residual concentrations of NTA, EDTA and TPP in the guppy aquaria sampled seven days after each weekly chelate addition.

THE EFFECT OF TEMPERATURE ON MERCURY CONTENT IN DRESSED RAINBOW TROUT (*Salmo gairdneri*) PRE-EXPOSED TO METHYL MERCURIC CHLORIDE

by

J.C. MacLeod and E. Pessah

Summary:

Rainbow trout were exposed in a continuous flow system to concentrations of methylmercuric chloride to achieve levels of 3-5 mg Hg per Kg in the fish. One group of fish were exposed to 0.016 mg Hg/l for 4 days at 15 C and another group at 0.032 mg Hg/l for 2 days at 6 C. Exposure times and toxicant concentrations were sufficiently low that exposed fish showed no visible stress symptoms.

Exposed and non-exposed (control) fish were then transferred to temperature controlled tanks supplied with fresh, dechlorinated city tap water. Dressed fish (head and entrails removed) were sampled at regular intervals to monitor the mercury levels. Fish were fed a commercial dry food which was found to contain 0.10 mg Hg/Kg. This amount of mercury in the diet was not considered significant since control fish fed on this diet showed no increase in Hg levels in their flesh through-

out the experiment. Total mercury was analysed with the atomic absorption method (Armstrong and Uthe, 1971),by the Analytical Chemistry Group at the Freshwater Institute. Over 90% of the mercury found in the fish was in the methyl form as determined in several samples using a gas chromatographic method (Uthe et al., 1972).

Mercury concentrations in fish held at 15 C showed an initial upward shift 2 days after exposure terminated (Fig. 1). This was likely caused by the "redistribution" of mercury from gill and entrails (not included in the mercury measurement) to the muscle tissues (Rucker and Amend, 1969). The maximum average concentration observed in the flesh was 4.3 ± 0.5 mg/Kg[1]; this fell to 0.48 ± 0.1 mg/Kg after 205 days. The apparent biological half life of mercury in dressed fish was approximately 40 days. The 0.5 ppm (mg/kg) "safe" mercury concentration level was reached after 205 days.

At 6 C the mercury "redistribution period" was almost 9 times longer than at 15 C (Fig. 2). A maximum of 5.4 ± 0.5 mg Hg/Kg was reached 17 days after exposure ceased, and decreased to 2.75 ± 1.0 mg Hg/Kg after 168 days. Biological half-life at 6 C was about 168 days.

Growth varied with temperature. At 15 C treated fish grew from an estimated size of 5.0 g at day 1 to an average size of 25.6 ± 6.6 g in 100 days, while at 6 C treated fish grew from 5.1 ± 1.5 G at day 1 to an average of 11.5 ± 5.4 g after 106 days

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[1] ± Figures indicate 95% confidence interval of the mean.

(Table 1). There was no significant difference in growth between control and treatment fish at either temperature.

Though decreases in mercury concentrations in dressed fish were observed at both temperatures, much of the decreases could be attributed to growth of the experimental animals. In the absence of any new contamination sources the addition of body tissues (growth) reduces mercury concentration by dilution. When the absolute amount of mercury in the dressed fish was calculated an apparent increase from 0.013 to 0.016 mg and from 0.009 to 0.022 mg was noted in fish held at 15 C and 6 C respectively (Table 1). Though the values are not significantly different there is a consistent increase within each group. This apparent increase in absolute mercury content likely results from continuing of mercury shifts from entrails to other body tissues.

These data do not demonstrate any actual elimination of mercury by the fish but do demonstrate the importance of growth in reducing mercury concentration in the dressed portion of the fish. A major factor influencing growth is temperature. Fish held at 15 C grew much faster than those held at 6 C, therefore the mercury concentration decreased more rapidly in fish held at the higher temperature. Temperature and the annual growth rates of fish would thus appear to be important factors in determining when mercury concentrations in contaminated fish will fall within "safe"limits.

REFERENCES CITED

Armstrong, F.A.J. and J.F. Uthe. Atomic Absorpt. News. 10; 101-103. (1971).

Rucker, R.R. and D.F. Amend. Prog. Fish. Cult. 31: 197 (1969).

Uthe, J.F., J. Solomon and N. Grift. J. of the AOAC, in press.

Table 1. Mercury content in rainbow trout at various times following exposure to methyl mercuric chloride at 15C and 6C. Mercury levels are for dressed fish (head and entrails removed). Bracketed numbers indicate 95% confidence intervals of the means.

Experimental Group	Day	Sample Size	Fish Size (g)	Mean Hg Conc. (mg/Kg)	Absolute Hg Content ($mgx10^{-3}$)
15C Treated	1	15	5.0[1]	2.96(±0.5)	12.7
	3	10	-	4.34(±0.5)	-
	5	14	-	3.48(±0.4)	-
	9	13	-	3.12(±0.4)	-
	17	14	-	2.79(±0.4)	-
	33	14	6.4(±1.2)	2.45(±0.3)	11.7(±1.9)
	72	14	12.5(±2.2)	1.45(±0.1)	10.9(±2.2)
	101	12	25.6(±6.6)	1.00(±0.2)	15.7(±4.3)
	142	12	36.9(±14.6)	0.71(±0.2)	15.7(±5.5)
	206	12	48.3(±21.3)	0.48(±0.1)	16.1(±9.4)
15C Control	1	15	5.0[1]	0.13	0.6
	3	10	-	0.15	-
	5	14	-	0.13	-
	9	14	-	0.15	-
	17	14	-	0.12	-
	33	14	8.6(±1.9)	0.13	0.5(±0.1)
	72	14	13.2(±3.7)	0.10	0.8(±0.2)

[1] Extrapolated from growth curve

...2/

Table 1. (cont'd)

	101	8	17.4(±8.4)	0.10	1.2(±0.5)
	142	12	38.4(±11.4)	0.09	1.6(±0.6)
	206[2]	6	19.1(±12.1)	0.09	1.1(±0.8)
6C Treated	1	12	5.1(±1.5)	3.44(±0.5)	9.4(±2.1)
	5	11		4.85(±0.5	
	11	12		5.19(±0.6)	
	18	12		5.44(±0.5)	
	35	14	5.7(±1.5)	5.40(±0.4)	17.6(±4.4)
	70	12	5.6(±0.8)	4.61(±0.3)	14.8(±1.9)
	107	12	11.5(±5.4)	3.35(±0.6)	19.8(±5.8)
	169	6	15.3(±11.3)	2.75(±1.0)	21.9(±12.2)
6C Control	1	12	4.0(±0.8)	0.14	0.3(±0.1)
	5	12		0.14	
	11	12		0.09	
	18	10		0.11	

[2] Represents a different group of the same population

....3/

Table 1 (cont'd)

35	14	5.3(±1.1)	0.14	0.4(±0.1)
70	12	7.1(±2.4)	0.14	0.5(±0.3)
107	12	6.1(±1.8)	0.15	0.4(±0.1)
169	6	16.2(±10.7)	0.11	0.9(±0.4)

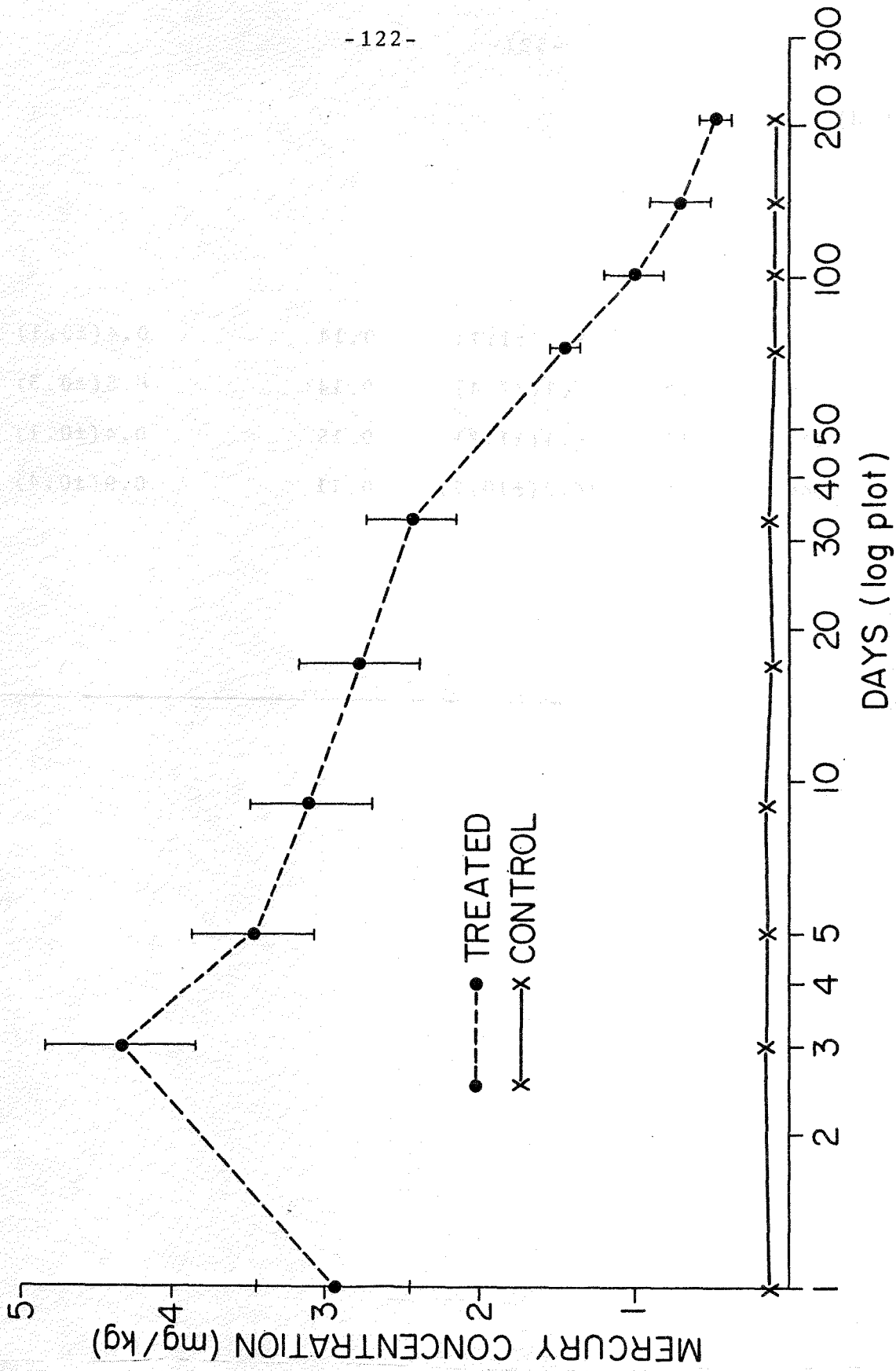

Fig. 1 Mercury concentrations in rainbow trout held at 15C and sampled at various times after pre-exposure to methyl mercuric chloride. Each point represents the mean of 8 to 15 fish. Vertical bars represent the 95% confidence intervals of the means. Mercury concentrations measured in the dressed fish (head and entrails removed).

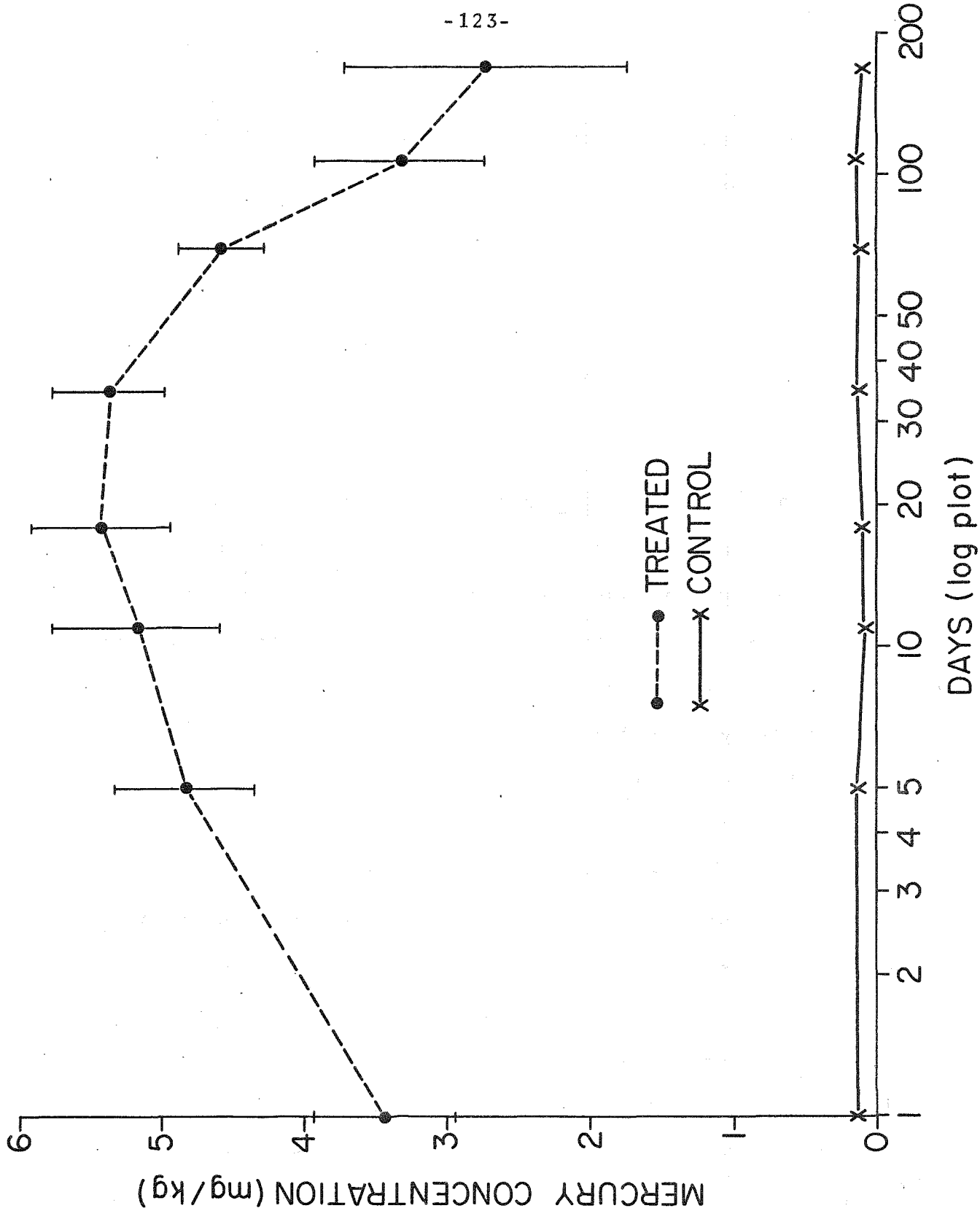

Fig. 2 Mercury concentrations in rainbow trout held at 6C and sampled at various times after pre-exposure to methyl mercuric chloride. Each point represents the mean of 12 to 14 fish. Vertical bars represent the 95% confidence intervals of the means. Mercury concentrations measured in the dressed fish (head and entrails removed).

TEMPERATURE EFFECTS ON MERCURY TOXICITY IN RAINBOW TROUT FINGERLINGS (Salmo Gairdneri)

by

J.C. MacLeod and E. Pessah

Rainbow trout (Salmo gairdneri) were exposed to various concentrations of mercury in 96 hour continuous-flow bioassays at various temperatures. Acute toxicity, mercury residue accumulation in tissues, and effects on active metabolism were assessed.

Hatchery reared rainbow fingerlings, ranging in weight from 9 to 28 grams, were acclimated for at least 2 months to 5, 10 and 20 C (± 1 C^{o}).

The approximate 96 hr TL50 values (concentrations that killed 50% of the fish in 96 hrs) for mercuric chloride at 5, 10 and 20 C were 0.40, 0.28 and 0.22 mg Hg/liter respectively (Table 1, Fig. 1). The organic mercurial phenyl mercuric acetate (PMA), was approximately 11 times more toxic, having a 96-hr TL50 of 0.025 mg Hg/liter at 10 C.

In mercuric chloride the velocity of mortality showed a linear relationship with temperature (Fig. 2). For 1.0 mg

Hg/liter the equation is

$$V = 0.011 + 0.005t$$

and for 0.5 mg Hg/liter is

$$V = 0.002 + 0.0023t$$

where V = velocity of mortality (1/time to death of 50% of the individuals) and

t = temperature in ^{o}C.

Increased temperature greatly increased the acute toxicity of mercuric chloride and exerted a greater effect when mercury concentrations in the water were high. Also, the calculated Q_{10} values at 5-10 C were almost double the Q_{10} values between 15 and 20 C. This means that at a given toxic mercury concentration, temperature changes had a much larger effect on velocity of mortality at lower than at higher temperatures. These observations suggest that normal seasonal temperature variations are an important factor influencing the tolerance of fish to toxic levels of mercury in nature.

Total uptake of mercury in the dressed portion of the fish (with head, fins and internal organs removed) was directly related to the concentration in the water (Fig. 3). The fish exhibited an ability for high biological magnification of mercury, accumulating levels many times those found in the water. After exposure to 0.22 mg Hg/1, as mercuric chloride, the maximum 4-day accumulation was about 2.8 mg Hg/Kg of flesh. These levels in the water killed a portion of the exposed fish in 96 hours.

Accumulation rate of mercury in fish flesh was also

directly related to temperature. For 4 days exposure to a mercuric concentration of 0.1 mg Hg/1 in the water, biological magnification factors in the fish flesh were 4X, 10X and 22X at 5, 10 and 20 C respectively (Table 2). The organic mercurial, PMA, produced much higher magnification factors varying from 80 to 100X at 10°C.

Elimination of mercury proceeded at a slow rate (Fig. 4). There was a 2 to 4 week delay before reductions occurred in the dressed portion of the fish. This delay is probably attributable to transference from internal organs to the muscle.

There was a significant correlation between mercury concentration in the water and metabolic rate at both 10 C and 20 C, with higher concentrations of mercury acting to depress active metabolism (Table 3). Equations of the general form $y = mX+b$, where y = active metabolism and X = mercury concentration in the water, were used to calculate active metabolism values for 2 selected sublethal concentrations at each temperature (Table 3). Using these calculated values, curves were constructed describing the combined effect of temperature and mercury on active metabolism (Fig. 5). The interaction is such that higher temperatures, while acting to increase active metabolism, also potentiate the depressant action of mercuric chloride. Any depression in active metabolism resulting from the action of a pollutant can be interpreted as biological stress on the organism and reflects damage to one or more vital systems.

In nature normal seasonal temperature changes in lakes

and rivers would be expected to markedly influence mercury toxicity and residue accumulation in the tissues. These effects can be correlated directly with the influence of temperature on the fishes active metabolic rate.

Table 1. Acute toxicity of mercuric chloride to rainbow trout in 96-hr tests. Concentrations were measured as mg Hg/1. At 5 C and 10 C, 15 fish were exposed at each concentration; at 20 C, 10 fish were exposed at each concentration. 95% confidence limits are in brackets.

Temperature C	Hg conc. mg/1	Av. wt. fish (g)	% dead at 96 hr	Estimated Time to 50% mort. TD50 (hr)	Estimated 48-hr TL50 (mg Hg/1)	Estimated 96-hr TL50 (mg Hg/1)
5	0.00	15.5	0.0	-		
	0.10	11.2	0.0	-		
	0.25	10.8	0.0	-	0.65	0.40
	0.49	9.5	93.4	77.5(73.2-83.8)		
	0.95	9.1	100.0	26.5(23.7-30.0)		
	2.00	15.3	100.0	12.0(10.9-13.2)		
10	0.00	19.6	0.0	-		
	0.07	18.8	0.0	-		
	0.19	21.3	0.0	-	0.45	0.28
	0.37	15.2	100.0	51.7(47.0-56.8)		
	0.71	19.1	100.0	26.7(23.9-29.7)		
	1.50	13.2	100.0	10.3(8.3-12.9)		
20	0.00	23.1	0.0	-		
	0.05	27.8	0.0	-		
	0.12	20.0	10.0	-	0.30	0.22
	0.24	24.9	10.0	-		
	0.48	23.2	60.0	23.3(18.5-29.4)		
	1.00	18.5	100.0	9.0(7.3-11.1)		

Table 2. Biological magnification factors for mercury accumulating in trout exposed to different concentrations of mercuric chloride for 4 days, and at different temperatures. For PMA the exposure period was 7 days and therefore the Hg accumulation for 4 days was estimated by interpolation.

Chemical Compound	Conc. in water (mg Hg/l)	Magnification Factors (conc. in tissues ÷ conc. in water) 5 C	10 C	20 C
$HgCl_2$	0.05	5.0	12.0	26.0
$HgCl_2$	0.10	4.0	10.0	22.0
$HgCl_2$	0.20	3.8	8.5	14.0
PMA	0.005	-	100	-
PMA	0.010	-	90	-
PMA	0.020	-	80	-

Table 3. Relationship between active metabolism of rainbow trout and mercury concentration in the water for 3 temperatures. Fish were exposed for 96 hours to mercuric chloride concentrations. All metabolism tests were of 15 min duration in 3.1 respirometers containing fresh water. Each replicate was based on 4 fish per respirometer except at 20 C where 3 fish per respirometer were used. Active metabolism measured as mgO_2/Kg/hour.

	5 C			10 C			20 C				
Hg Concentration (mg/1)	0.00	0.10	0.25	0.00	0.07	0.19	0.00	0.05	0.12	0.24	0.48
Mean Active Metabolism($\overline{X}$)	383	402	356	441	434	402	726	645	708	528	390
Standard Error ($S_{\overline{X}}$)	13	13	9	10	20	12	85	-	76	49	-
No. of replicates (n)	4	4	4	4	3	3	3	2	3	3	1
Correlation Coefficient(r)	-0.46 (n.s.)			-0.61*			-0.71**				
Regression Equation [Y=metab. in mgO_2/Kg/hr, X=Hg conc. in mg/liter]	Y = -123X + 395			Y = -208X + 443			Y = -721X + 730				
Average fish weight (gm)	9.3			17.2			26.5				
Average O_2 conc in respirometer (mg/l)	7.8			6.8			7.0				

n.s. = non significant at 0.05 level
* = significant at 0.05 level
** =significant at 0.01 level

Table 4. Comparison of active metabolic rates of salmonid species as determined by various methods and authors.

Active metabolism in mg O_2/Kg of fish/hr							
Author	Graham, 1949	Job, 1955	Basu, 1959	Gibson and Fry, 1954	Brett, 1964	Dickson and Kramer, 1971	Present Study
Species	*S. fontinalis*	*S. fontinalis*	*S. fontinalis*	*S. namaycush*	*O. nerka*	*S. gairdneri*	*S. gairdneri*
Fish Size(gm)	16-62	10-30	164	16-38	50	137-249	10-30
Respirometer Type	A	A	B	A	C	D	E
5°C	240	240	-	-	510	380	380
10°C	350	240	330	300	620	470	440
15°C	500	320	390	440	880	580	-
20°C	530	350	330	400	840	570	730

Key to Respirometer Type

A Fry and Hart (1948) rotating chamber, no electric stimulus
B Basu (1959) rotating annular chamber, with electric stimulus
C Brett (1964) tunnel respirometer, with electric stimulus
D Dickson and Kramer (1971) "Blaska" tunnel respirometer, with electric stimulus
E Present study - flask with magnetic stirrer, no electric stimulus

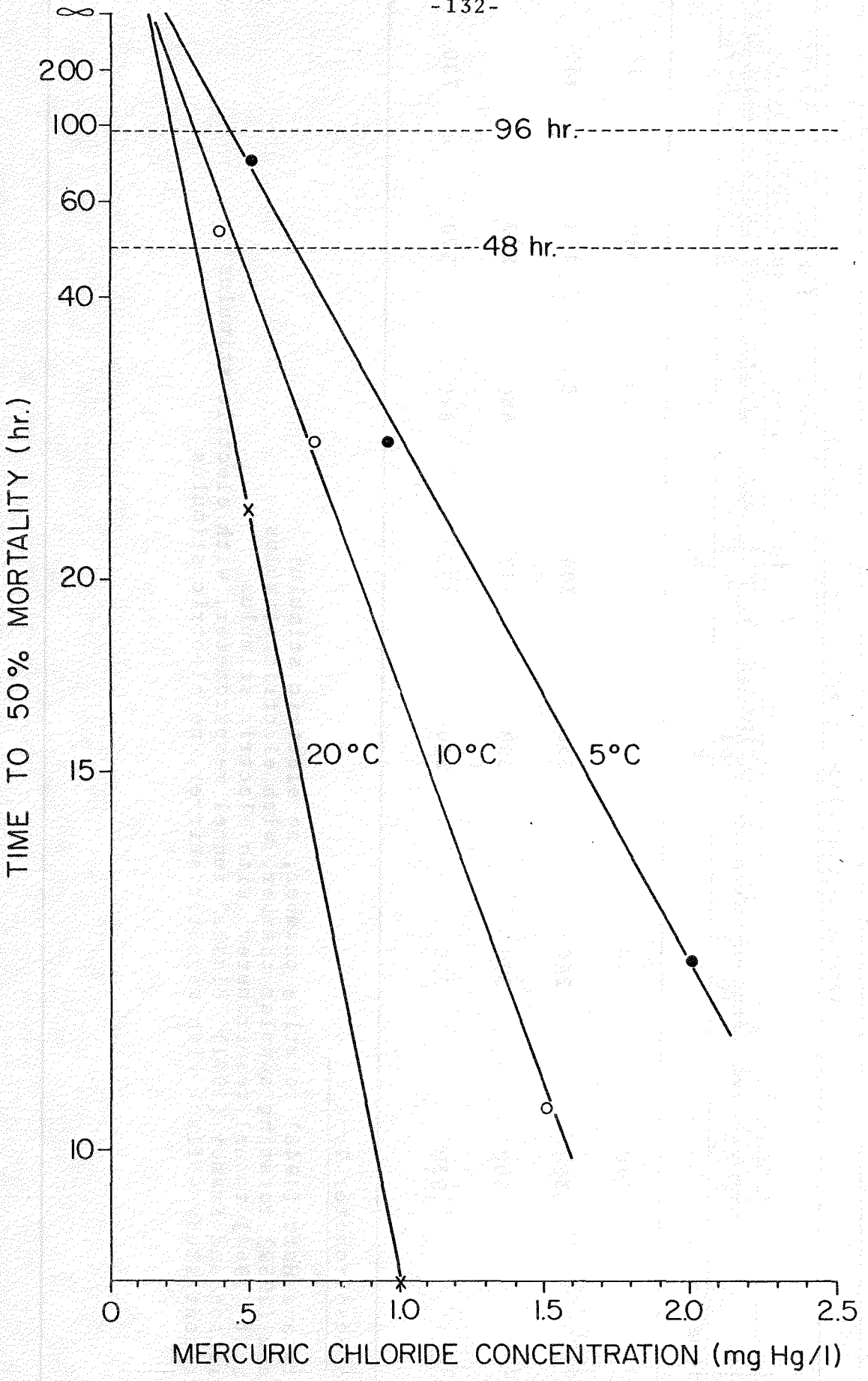

Figure 1 Toxicity curves for rainbow trout exposed to various concentrations of mercuric chloride at 5, 10 and 20 C for 96 hours. Vertical axis is on a hyperbolic (reciprocal) scale.

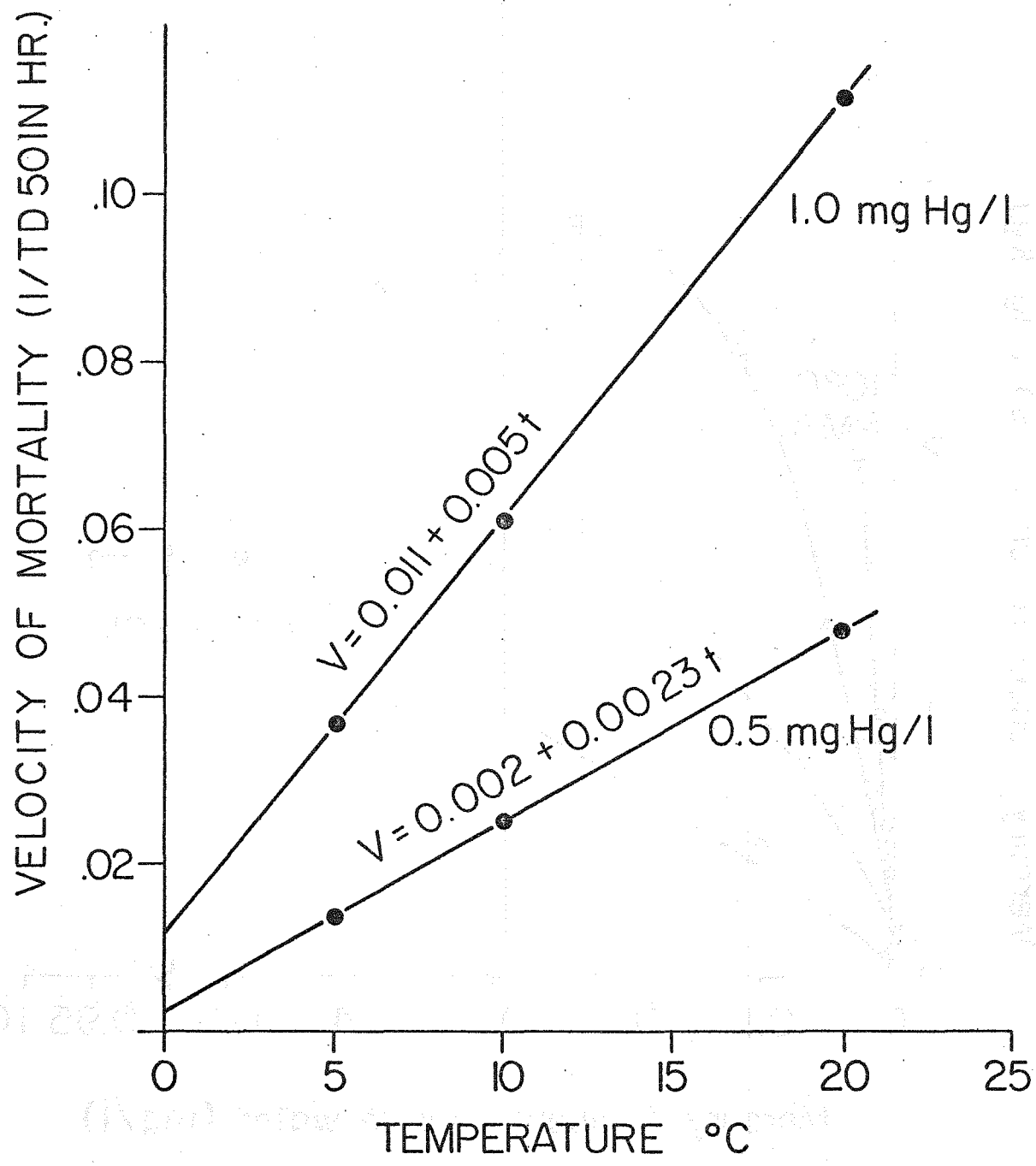

Figure 2 Relationship of velocity of mortality (V) to temperature in concentrations of mercuric chloride of 0.5 and 1.0 mg Hg/1. Data points are derived from the toxicity curves in Figure 1.

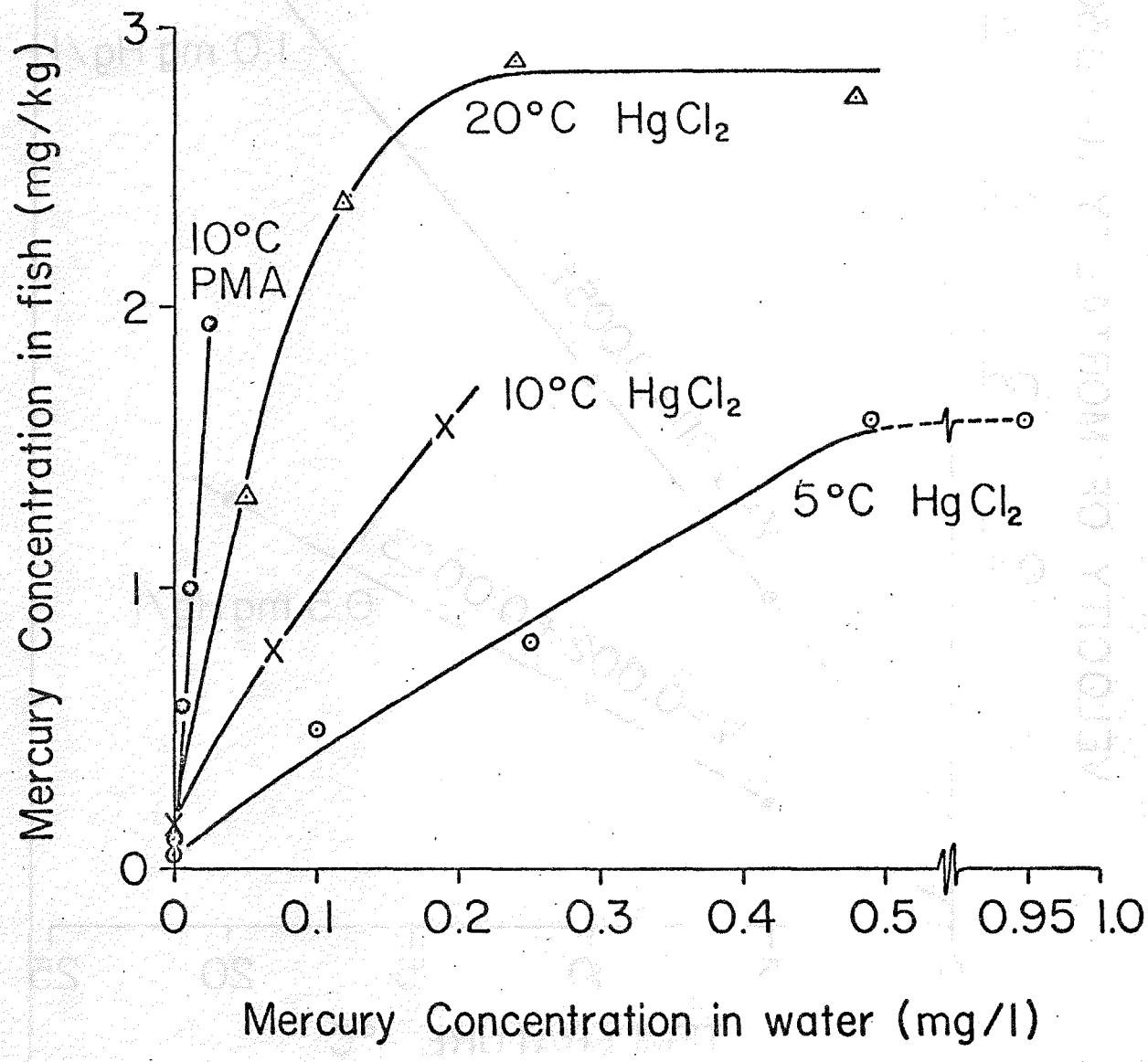

Figure 3 Mercury concentration accumulating in rainbow trout exposed for 4 days to various concentrations of mercuric chloride or PMA and at different temperatures. (Data for PMA is interpolated from measurements made after 7 days exposure. All concentrations are in the dressed portion of the fish with head, fins and internal organs removed.)

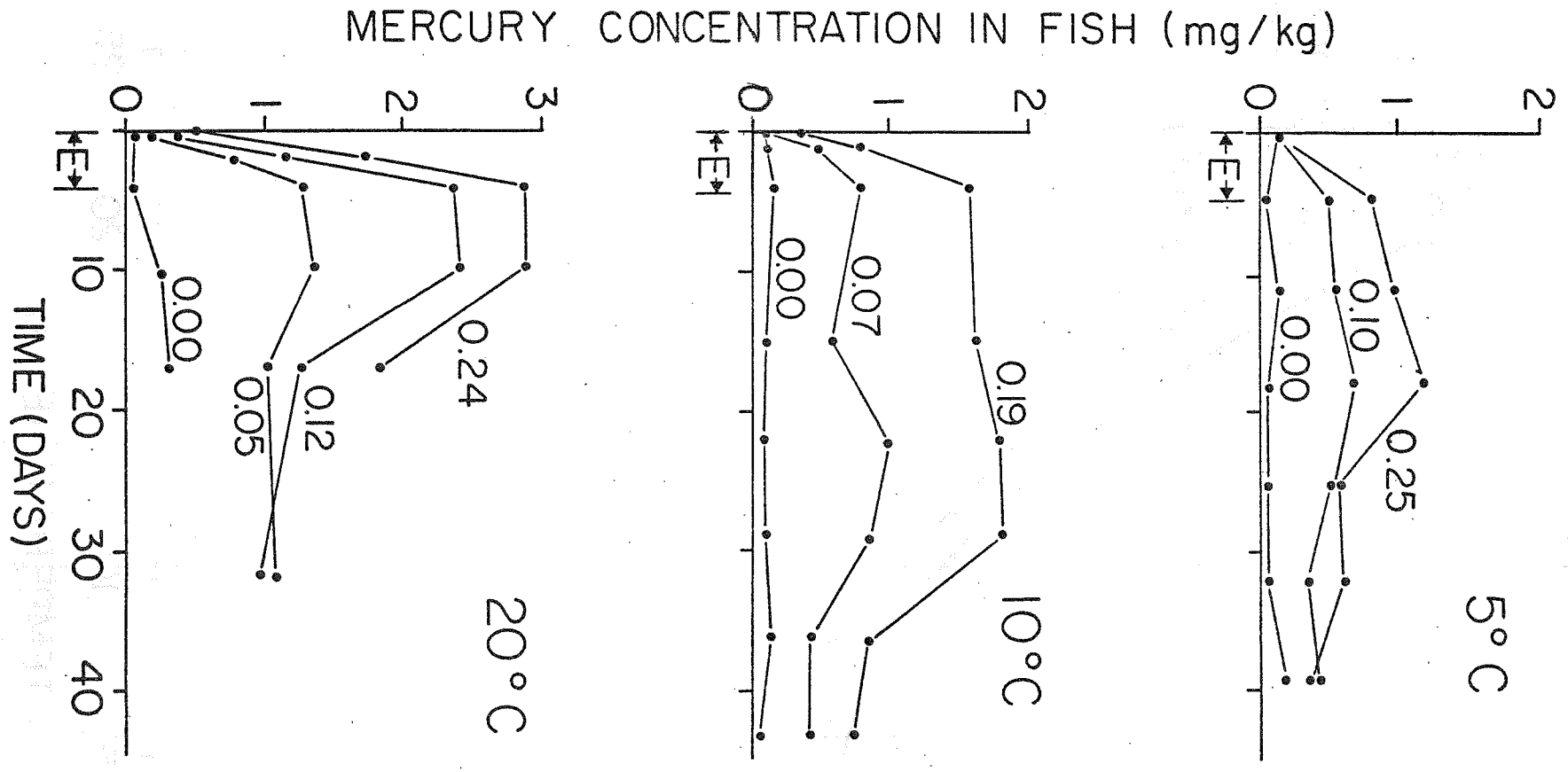

Figure 4 Changes in mercury concentration in trout exposed to various concentrations of mercuric chloride and then held in fresh running water at 5, 10 and 20 C. Each point represents a sample of 2 fish. "E" indicates the 96 hr exposure period. Numbers on curves represent the exposure concentration of mercuric chloride in the water as mg Hg/l.

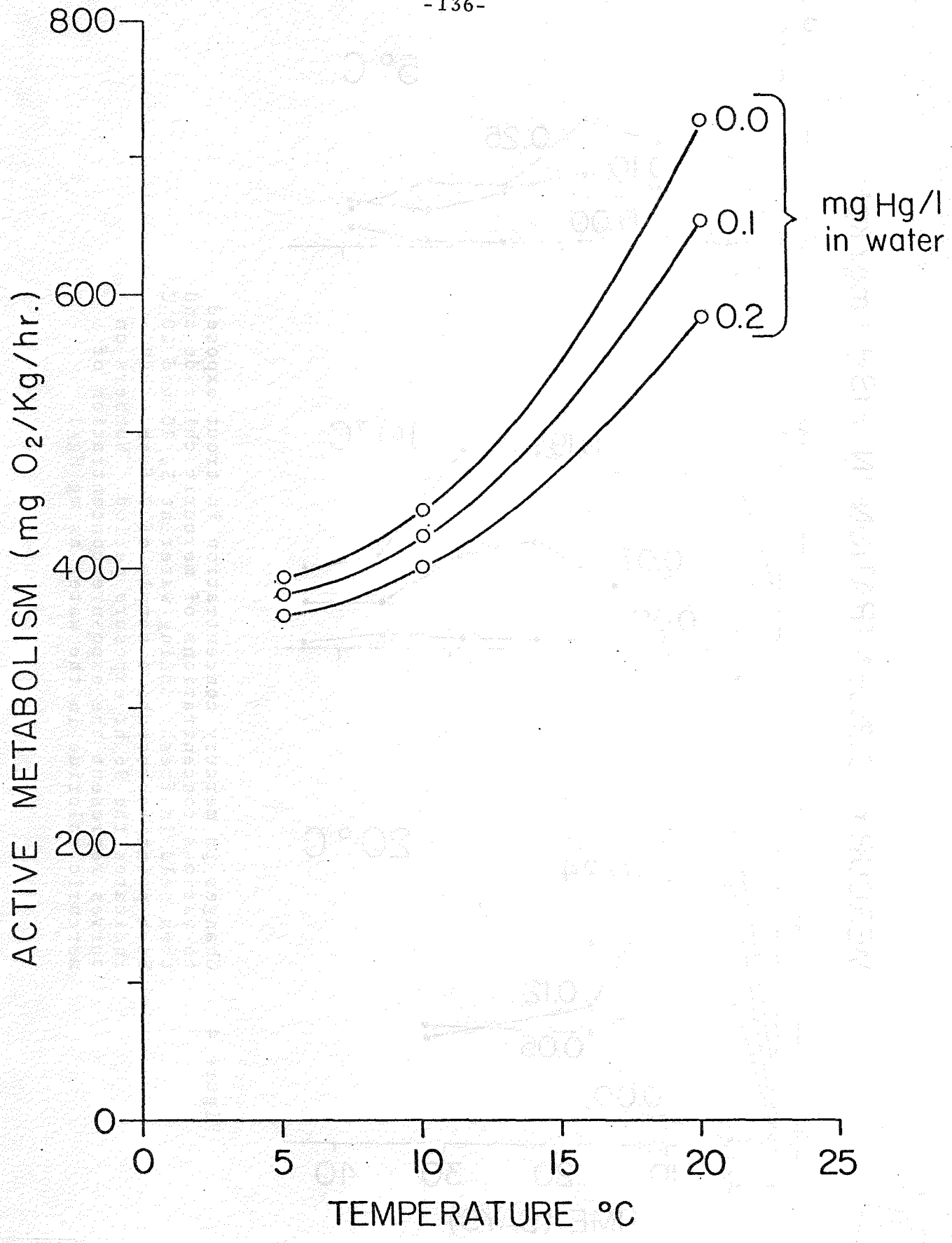

Figure 5 Effect of temperature on the active metabolism of rainbow trout for selected concentrations of mercuric chloride (measured as mg Hg/l). Points are calculated from regression lines in Table 3.

SUBLETHAL EFFECT OF MERCURY ON THE OLFACTORY RESPONSE IN RAINBOW TROUT

by

T. Hara

Much information on the distribution and transformation of mercury, and its lethal level on certain fish species have been accumulated by the existing chemical and bioassay techniques. However, little information has been available on the sublethal effect of mercury and its mechanism of action, which are equally important to the functional activity of fish. One of the more important biological activities is the sensory process.

Sensory receptors are the immediate detectors of environmental chemical stimuli. Of many sensory activities, the sense of smell or olfaction plays an important role in fish behaviour such as procurement of food, defense against predators and orientation. Particularly in salmonid species which have extremely acute sense of smell, the cue by which they return to their home stream is believed to be an olfactory one. Impingement of effective stimuli upon the receptor causes nerve impulse generation. These nerve impulses are conveyed into the central nervous system, where they are variously integrated. They are then

transformed into efferent motor nerve impulses, which finally cause a behavioral pattern. Interference of the effective stimuli or damage of the receptor sites by existence of water-borne substances can cause impairment of nerve impulse generation, which in turn may lead to abnormal behavioral pattern.

Spontaneous electrical potentials or electroencephalographic activities (EEG) are recorded from the olfactory bulk of anaesthesized and restrained rainbow trout through bipolar electrodes placed on its surface. When the noses of fish are infused or stimulated with water containing odorous substances (human hand rinse, food extracts or amino acids are extremely effective stimulant), the spontaneous background activity is immediately interrupted by an oscillatory potential response that is terminated by rinsing.

Treatment of the nasal cavity with 10^{-4} (27 ppm) solution of $HgCl_2$, which is non-stimulating or even slightly inhibitory by itself, for 10 seconds blocked almost completely the responses to the stimulants used. The effect of a single treatment with mercury on the bulbar electrical response seems to be reversible; the response recovered to its original level in about 30 minutes. The mercuric ion is known to tie up the sulphydryl group of protein. The blocking effect observed here may be due to interference with either receptor sites or enzymatic components of the receptor membranes.

The effect of longer treatment of fish with mercury was also tested by continuous introduction of the appropriate solution into a fish holding tank at the final concentration of (0.1 ppm)

for up to 3 days. In the treated fish, olfactory responses were reduced to 20 to 25% of those of the control fish. The fact that a longer period is necessary for eliciting responses in Hg^{++}- treated fish suggests a dual nature of its action. More detailed analyses of the threshold level of action of mercury, recovery from, and its mechanism are underway. However, above results clearly indicate the sense of smell of rainbow trout can be easily impaired by the presence of a low level of mercury ion in their environment.

Experiments with Pacific salmon smolts suggest more differential effects of mercury, reflecting their ecological and physiological diversities.

HISTOPATHOLOGY OF MERCURY - CONTAMINATED PIKE

by

B.R. Hobden

Northern Pike taken from Clay Lake, Ontario have been shown to contain extremely high concentration of mercury. From this source, a number of contaminated fish were transplanted into Heming Lake, Manitoba, a lake essentially free from the effects of mercury pollution. After a period of one year, these transplanted fish were recovered, along with a sampling of the natural Heming Lake and the natural (non-transplanted) Clay Lake stocks. The three groups of samples were prepared and examined for pathological condition using appropriate histological techniques and optical microscopy.

Tissue samples from the Heming Lake and transplanted Clay Lake stocks were obtained in the field from freshly-killed specimens and were fixed immediately in 10% neutral formalin. Samples from Clay Lake stock were obtained from fish transported live to the Freshwater Institute and were fixed in both 10% neutral formalin and Bouin's fluid. Liver, kidney, spleen and gill were sampled in all cases. Following fixation, the tissues

were embedded in paraffin and cut into 10μ thick sections which were then stained using the standard hematoxylin and eosin method. When mounted, the prepared tissues were observed under bright-field microscopy for micro-anatomical anomalies.

None of the preparations yielded any evidence to suggest that the presence of mercury had had an effect, deleterious or otherwise, upon the micro-structure of any of the four tissues considered. Advanced fatty degeneration in the livers of the natural Heming Lake stock was noted as was the considerable fat deposition in the livers of the Clay Lake transplants. The natural Clay Lake stock showed virtually none of this condition and it would appear to be indicative of the different nutritional opportunities existing in the two lakes rather than the presence or absence of contamination by mercury.

STUDIES ON THE BLOOD CHEMISTRY OF NORTHERN PIKE (Esox lucius) FROM A MERCURY CONTAMINATED LAKE AND A NON-CONTAMINATED ONE

by

W.L. Lockhart

Studies on blood chemistry of northern pike (Esox lucius) were begun in an effort to determine whether mercury had a significant effect on chemical constituents of blood serum. Blood samples were taken from fish transplanted from Clay Lake to Heming Lake (J.F. Uthe, this report) as well as from fish native to both lakes and analyzed by a medical laboratory. Analyses are shown in Table 1 and it is evident that several differences existed between pike in the two lakes. In general the transplanted fish had values which had moved in the direction of the Heming population. It is not possible to establish any cause and effect relationships between mercury and other measurements from these date, although neither can they be excluded.

Pike from Clay Lake appeared visibly emaciated and dissection revealed no visceral adipose tissue in contrast to both Heming and transplanted fish in which fat was abundant. Similarly histological examination revealed that fat storage in liver tissue was minimal in Clay Lake but abundant in those from Heming and in the transplants (B. Hobden, this report). Low levels of

blood glucose, total protein, and alkaline phosphatase have been described in cases of human malnutrition and low Clay Lake values in these parameters may be due to their undernourised state. Highest levels of mercury were found in eye lens tissues both in Clay Lake and in the transplants but no evidence for structural damage to eyes has been found by light microscopy. Very preliminary observations have suggested that red blood cells from Clay Lake and transplanted fish are less easily broken by osmotic shock than those from Heming Lake. In addition muscle potassium levels appeared to decrease with increasing mercury but both these observations will require further work before they can be accepted.

Cortisol (see table) is of greatest interest because ability to produce this hormone is a good indicator of ability to respond to situations of stress. Clay Lake values were depressed compared to transplants and these in turn were depressed compared to Heming fish. Clay Lake fish and even transplants may well be impaired in stress response. When Heming and transplanted fish were confined together in a submerged cage, the Heming fish proved difficult to catch since they readily avoided the net, however, transplants were caught easily, usually on the first or second attempt.

In summary it appears that the environment provided in Clay Lake does have undesirable effects on the condition of adult northern pike, but that fish can recover from these effects. It has not been possible to distinguish whether any effects are related to mercury or to other ecological factors.

Table 1. Serum values for Northern pike.

	Inorganic Phosphate (mg % P)	Glucose (mg %)	BUN (mg %)	Uric acid (mg %)	Total Protein (g %)	Albumin (g %)	Bilirubin (mg %)	Alkaline phosphatase (mU/ml)	Cortisol (ug %)
Clay Lake									
mean	11.0	115	3.4	2.2	3.9	0.22	0.36	51	25.0
S.D.	2.4	74	1.8	1.1	0.7	0.11	0.35	23	15.6
range	7.2-	20-	2.0-	0.4-	2.4-	0.10-	0.10-	14-	8-
	17.2	360	7.0	5.2	5.4	0.45	0.11	130	64
Heming Lake									
mean	8.4	280	2.4	2.6	4.8	0.14	0.32	92	96.3
S.D.	1.2	152	1.4	1.2	0.9	0.08	0.22	19	41.2
range	5.5	25-	1.0-	1.4-	3.5-	0 -	0.15-	48-	56-
	9.8	550	5.0	7.0	7.7	0.38	0.95	140	148
Transplants									
mean	6.6	435	2.3	1.7	4.5	0.15	0.23	64	54.0
S.D.	1.5	167	0.5	0.3	0.7	0.04	0.06	8.0	19.4
range	5.4	195-	2.0-	1.5-	3.8-	0.10-	0.16-	57-	32-
	8.8	550	3.0	2.2	5.5	0.18	0.29	74	74
t' (for difference between Clay and Heming means)	4.95**	2.45*	2.47*	0.91	3.58**	2.71*	0.58	5.95**	4.13**

* P<.05

** p<.01

COMPARATIVE HEMATOLOGY OF PIKE (Esox lucius) FROM A MERCURY CONTAMINATED AND A NON-MERCURY CONTAMINATED SOURCE

by

E. Pessah and C.C. Merry[1]

Hematological measurements have long been used in clinical evaluation of general health and in the identification of specific disorders, e.g. heavy metal poisoning in humans. Recently, more attention has been focused on the application of this clinical method to evaluate the general health of fish (Elliot, Fowler and Burrows, 1966) and in studying responses of fish to toxic material (McKim and Benoit, 1971). The purpose of this study was to determine the possible effects of mercury on various blood parameters in pike (Esox lucius) from Clay Lake, in the Winnipeg river watershed which is mercury contaminated by chlor-alkali effluent and from Whitemud River, a non-contaminated water.

Pike from Whitemud R. (49 samples) and Clay L. (54 samples) were gill netted between July 27 and August 5, 1971.

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Assistant Professor of Medicine, University of Manitoba

Blood samples were collected by caudal vein puncture in EDTA using Vacutainers (trade mark) within 2 to 5 minutes after the fish were removed from the water. Blood samples were analysed using a Coulter Counter Model S (trade mark) at the Winnipeg General Hospital. Total cell count, and mean cell volume (MCV) were measured. Haemoglobin (Hb) was estimated separately by cyanmethaemoglobin method. The Hematocrit (hct), was calculated in the automated system and the mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated manually.

The spleen and a section of dorsal muscle just posterior to the head were removed for mercury analysis. Mercury analyses were carried out by the Chemistry Group at the Freshwater Institute using the method of Armstrong and Uthe (1971). Weight, fork length, age and sex data were also collected from each fish.

Sex ratios were 2:1 (males dominant) in Clay L. and 1:1 in Whitemud R. samples. Pike from Clay L. were leaner in appearance and contained markedly less adipose tissue in the mesenteries of the body cavity than did pike from Whitemud R.

Average muscle mercury values were 12.8 (range = 4.2 - 20.3) mg Hg/Kg from Clay Lake Pike and 0.12 (range - 0.02-0.25) mg Hg/Kg from Whitemud R. pike. Males and females showed no differences in muscle mercury within either populations. Average mercury levels in spleen tissues were lower than in muscle tissues by 75% in the Whitemud R. samples and 22% in Clay L. samples.

Whitemud R. fish showed a positive correlation ($P \leq 0.05$) of muscle mercury concentration with age, but not with Log_e length or Log_e weight for males ($r = 0.515$) and females ($r = 0.514$). In Clay L. males showed a positive correlation between muscle mercury and age ($p \leq 0.01$; $r = 0.428$), Log_e weight ($p \leq 0.05$; $r = 0.332$) and Log_e length ($p = 0.01$; $r = 0.475$); females showed none of these correlations.

Clay L. males showed a negative correlation between muscle mercury concentration and the Hct and MCV (at $p \leq 0.05$). None of the other correlations were significant (at $p \leq 0.05$).

The results indicate that the measured blood parameters of pike from the two bodies of water are different. In humans an increase in MCV almost invariably indicates the onset of an anaemia, usually due to some disturbance of deoxyribonucleic acid (D.N.A.) synthesis.

An increase in M.C.V. in fish may or may not indicate a similar disturbance in D.N.A. synthesis. In humans however an increase in MCV is normally accompanied by a decrease in Hct and Hb. This decrease is not observed between the populations but the relationships within the individual populations has not been determined at present.

The negative correlation of muscle mercury with the MCV and Hct in Clay L. males suggests that mercury has some effect on cell kinetics. However, a direct cause and effect relationship between mercury and a change in blood parameters should not be assumed without further experimental evidence.

References

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Elliot, J.W., L.G. Flower and R.E. Burrows. U.S. Bur. Int. Fish and Wildl. Technical paper no. 8., 21 pp (1966).

McKim, J.M. and D.A. Benoit. J. Fish. Res. Bd. Canada 28, 655 (1971).

Table 1. Comparison of the blood parameters of Pike from Clay L. and Whitemud R.

	Whitemud R. 49 samples $\bar{X}$ (± S.D.)	Clay L. 54 samples $\bar{X}$ (± S.D.)
* Corpuscular Count ($x10^6$)[1]	1.54 ± 0.24	1.57 ± 0.27
Range	(1.20 - 2.15)	(1.00 - 2.04)
* Hemoglobin (gm %)	4.9 ± 0.7	5.1 ± 0.9
Range	(3.8 - 6.7)	(2.9 - 6.4)
** Hematocrit (%)	27.0 ± 4.4	31.8 ± 5.9
Range	(19.6 ±37.2)	(18.4 -43.9)
** Mean cell volume (u^3)	176 ± 12	201 ± 17
Range	(150 - 203)	(152 - 224)
* Mean cell hemoglobin (10^{-12}g)	32.3 ± 3.0	32.2 ± 2.9
Range	(26.5 - 40.2)	(19.3 - 38.3)
** Mean corpuscular hemoglobin concentration[2] (%)	18.4 ± 1.7	16.0 ± 2.0
Range	(14.8 - 21.5)	(10.0 - 19.9)

[1] This data to be further analysed to differentiate red and white cell counts. Value is in cells/mm^3.

[2] Hgb ÷ Hct.

* $p > 0.005$ using a two-tailed t test.

** $p \leq 0.005$, using a two-tailed t test

Hematocrit, mean cell volume and mean corpuscular hemoglobin concentration of the two populations were significantly different.

Table 2. Correlation coefficients for comparisons of blood parameters with muscle mercury concent ration in pike from Clay L. and Whitemud R.

Blood parameter		Clay Lake		Whitemud R.	
		Male	Female	Male	Female
		r = correlation coefficient)			
Corpuscular Count	($x10^6$)	-0.329^{ns}	0.329^{ns}	-0.169^{ns}	-0.358^{ns}
Hgb	(g %)	-0.199^{ns}	0.149^{ns}	-0.013^{ns}	-0.138^{ns}
Hct	(%)	-0.345^{*}	0.226^{ns}	-0.052^{ns}	-0.233^{ns}
MCV	(u^3)	-0.338^{*}	-0.176^{ns}	0.356^{ns}	0.171^{ns}
MCH	(10^{-12}g)	0.262^{ns}	-0.309^{ns}	0.238^{ns}	0.352^{ns}
MCHC	(%)	0.207^{ns}	-0.116^{ns}	0.049^{n-}	0.172^{ns}

* $p \leq 0.05$

n.s. $p > 0.05$

DETERMINATION OF TOTAL MERCURY IN FISH

by

A. Lutz

At the present time there are numerous references pertaining to the determination of mercury. These are based on many different techniques; colorimetry, dithizone titration, pyrolysis, X-ray fluorescence, isotope exchange, neutron activation and atomic absorption. None has achieved as much widespread use as flameless atomic absorption spectrophotometry. This is the method of choice because of its precision, sensitivity, reproducibility and speed of analysis.

Initially the method as developed by Uthe, Armstrong and Stainton (1970) was used, later to be modified to a semi-automated procedure (Armstrong and Uthe, 1971).

The present method consists of weighing on a recording balance 0.1 - 0.4 gram sample in duplicate into calibrated digestion tubes. Five millilitres of a sulfuric: nitric acid (4:1) mixture is added and the sample is incubated at 68^{o}C in a shaking water bath for two hours. After dissolution of samples

the tubes are cooled in an ice bath and fifteen millilitres of 6% $KMnO_4$ are added while shaking. Complete oxidation is achieved by letting the samples sit overnight at room temperature. There was some doubt expressed by other laboratories using our previous method as to the completeness of digestion of allowing only thirty minutes after addition of permanganate Table I. There is approximately 75% completion after initial addition of permanganate; no noticeable difference between overnight and thirty minutes. However, overnight digestion is used since it is convenient. The digestion procedure has been found to be adequate for various fish tissue, hair, animal tissue and aquatic vegetation.

The manganic precipitates are redissolved by the dropwise addition of 30% hydrogen peroxide until clear using a Vortex-mixer. Excess of hydrogen peroxide is to be avoided. The samples are made up to 25 millilitre volume with distilled water and aliquots are transferred to cups on the turntable of a Technicon sampler. Following its release from the sample on mixing with reductant in the manifold, the mercury vapour is monitored at 253.7 nm on the Perkin Elmer 403 atomic absorption spectrophotometer.

Should the mercury value be high (noticed by the off scale reading on the chart paper)the sample is appropriately diluted with 20% sulfuric acid. Low results are obtained on aqueous dilutions.

Blanks and mercuric chloride standards are carried through the procedure in parallel with the samples. As a control measure,

check samples are carried through the procedure in quadruplicate. The check sample consists of a homogenized fish muscle tissue and is frozen in small portions (20 gram) so that a sample may be carried through the procedure daily. A new portion is taken from the freezer storage at weekly intervals. This check sample provides an indication of the daily operations of weighing digestion, oxidation and standardization.

The relative standard deviation of the method as shown by the daily use of the check was 3.7%. (Mercury value 0.81 ± 0.03 ppm. N = 61).

The Freshwater Institute has participated with other laboratories throughout Canada and the United States in a number of check programs. Laboratories included various digestion methods, pyrolysis followed by amalgamation in gold or silver followed by flameless atomic absorption spectrophotometry and neutron activation. Table III lists a comparison of our mercury results with the mean value obtained of the various check programs carried out by other laboratories.

References

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Armstrong, F.A.J. and J.F. Uthe. 1971. Atomic Absorption Newsletter Vol 10, #5 101-103. 1971.

Table 1.

Time of $KMnO_4$ oxidation	A(ppm Hg)	B(ppm Hg)	Recovery of 200 ng CH_3-Hg spike
0	5.64±0.76	0.49±0.03	75±9%
30 minutes	6.95±0.21	0.53±0.02	113±1%
overnight	6.76±0.02	0.54±0.02	107±6%

Table 2. Recovery of added mercury.
Semi automated Flameless Atomic Absorption

Methyl mercury(added ng)	% Recovery
100	94
200	95
300	100
400	93
Mercuric chloride (ng Hg)	
100	98
200	96
300	97
400	92

Table 3. Check Sample Program

Program		Number Participating laboratories	mean Hg(ppm)	Freshwater Institute Hg/ppm
OWRC	D_1	17	0.17±.03	0.19±.01
	D_2	19	0.79±0.13	0.86±.02
	D_3	19	2.40±.29	2.68±.16
Kodak		18	2.02±.08	2.05±.11
Fisheries	A	15	0.71±.04	0.64±0.03
Service	B	13	0.60±.07	0.54±0.04
	C	12	0.24±.04	0.21±0.01
	D	11	2.45±.17	2.34±0.09

TOTAL MERCURY DETERMINATION IN FISH - THE RESULTS OF A CHECK SAMPLE STUDY

by

J.F. Uthe

Three samples of ground, frozen fish were distributed to 29 laboratories for analysis of mercury content. The samples were unspiked, i.e. no mercury was added to them and had approximately 0.05, 0.5 and 5 ppm total mercury in them. Of the laboratories reporting the majority of them (19) employed acid digestion followed by flameless atomic absorption methods of analysis. Six laboratories used neutron activation methodology; two used dithizone methods and two the fast direct pyrolysis procedure. The results obtained are shown in Table 1. Precision figures were only submitted by about one-half of the laboratories and ranged between 5-10%. A corrected mean was calculated after elimination of results from four laboratories. These laboratories were dropped because of the presence of one grossly abberant result or no result. An interlaboratory precision figure was calculated and used to calculate the coefficient of variation. This figure was much larger for the sample with the lowest mercury content, not unexpectedly,since the level of

of mercury in this sample was, in a number of cases below the lower limit of quantitative detection for a number of laboratories. The other two coefficients of variation were the same, an unexpected finding since it had been envisioned that due to the high percentage of fat in check sample C and the known effect of undigested fat on mercury volatilization that laboratories would have more trouble with this sample than with the low-fat check sample A.

Comparison (Table 2) of the combined results by flameless atomic absorption spectroscopy with those obtained by the flame method (t-test) showed that the latter method gave just significantly lower results and poorer precision than the flameless methods. Differences between neutron activation results and those of flameless atomic absorption values were probably insignificant. Further statistical analysis showed that most laboratories had a tendency to yield results consistently higher or lower than the mean rather than random results.

References

Uthe, J.F., F.A.J. Armstrong and K.C. Tam. Journal of the A.O.A.C. 54 866 (1971).

Table 1. Concentration of mercury reported.

Lab.	Method[a]	Check Sample, ppm A	B	C
A	FAA	1.46	0.04	4.53
B	FAA	1.18	0.18	5.21
C	FAA	1.80	0.10	5.10
D	FAA	1.34	0.11	3.73
E	FAA	1.7	<0.1	4.6
F	FAA	1.26	0.11	5.4
G	FAA	1.16	0.04	2.87
H	FAA	1.04	<0.05	4.12
I	FAA	1.47	0.10	3.76
J	FAA	1.45	0.08	4.55
K	FAA	1.43	0.21	5.38
L	FAA	1.39	0.06	4.14
M	FAA	0.93	<0.05	2.8
N	FAA	1.35	0.03	3.84
O	NA	1.34	0.04	4.25
P	NA	1.77	0.12	4.56
Q	NA	1.29	0.07	4.06
R	NA	1.5	0.18	4.6
S	NA	1.4	0.05	-
T	NA	0.95	0.19	2.83
U	AA	1.80	0.05	5.40
V	AA	1.21	0.06	3.57
W	AA	1.40	0.10	4.0
X	AA	0.72	0.06	2.26
Y	AA	1.31	0.49	3.35
Z	pyrolysis	1.52	0.04	7.20
AA	pyrolysis	0.47	0.10	2.0
AB	dithizone (AOAC)	1.31	0.05	3.98
AC	dithizone titration	0.095	<0.03	0.088
	Corrected mean[b]	1.34±0.26	0.12±0.10	4.12±0.84
	Coeff. of var[c]	19	83	20

[a] FAA = flameless atomic absorption; NA = neutron activation, destructive; AA = flame atomic absorption.

[b] Excluding Laboratories S, A, AA, and AC.

[c] Coefficient of variation = (standard deviation/mean) x 100

Table 2. Comparison of 3 methods of analysis used by participating laboratories[a]

Sample	Check Sample, Mean Values ± Std Dev. A	B	C
FAA	1.36±0.23(14)[b]	0.10±0.055(11)	4.28±0.77(14)
AA	1.29±0.38(5)	---	3.72±1.2(5)
NA	1.37±0.26(6)	0.11±0.06(6)	4.06±0.67(5)

	Check Sample	"t" Test Significance
FAA vs. NA	A	0.9 <P
FAA vs. NA	C	0.6 <P <0.7
FAA vs. AA	A	0.6 <P <0.7
FAA vs. AA	C	0.2 <P <0.3

[a] See footnote a, Table 1.

[b] Value in parentheses is the number of determinations, i.e., laboratories.

DETERMINATION OF METHYLMERCURY IN A VARIETY OF AQUATIC FAUNA

by

J.F. Uthe

Swedish studies (Westöö, 1966) showed that the bulk of mercury present in mercury contaminated fish from Swedish waters is present as methylmercury ($CH_3 Hg^+$-). Methylmercury ingestion has been shown to cause a serious neurological disease (Minimata disease) and concern has been expressed that total mercury determinations currently used by regulatory agencies are not sufficient by themselves and methylmercury analyses should be carried out as well.

We have been carrying out methylmercury analyses using a modified Swedish method (Boveng 1970). The tissue was homogenized with 0.667M copper sulfate and 1M sodium bromide in the presence of 1.33N sulfuric acid to solubilize the methylmercury as methylmercuric bromide. The methylmercuric bromide was partitioned into toluene, then into ethanolic sodium thiosulfate (0.005M in ∿50% ethanol) and finally into benzene as methylmercuric iodide. The amount of methylmercuric iodide present in the final benzene extract was determined by gas-liquid chromatography. The total

mercury content of the tissue was determined by flameless atomic absorption following wet acid digestion according to the method of Armstrong and Uthe (1971).

Comparisons between the levels of total mercury and methylmercury values for a variety of aquatic fauna are shown in Table 1. It is obvious that, keeping in mind the inherent variation in both analytical methods (5-7%) mercury in freshwater fish muscle exists as methylmercury. In marine fish tuna there is probably a small component or non-methylmercury while swordfish is again essentially all methylmercury. Muscle tissue from mink and seal, two fish predators, also only had methylmercury but the livers from both animals had only a minor portion of their total mercury present as methylmercury. Since their source of mercury is their diet fish which are essentially all methylmercury this finding is strongly suggestive of the present in these two mammals of an enzyme system capable of removing the methyl group.

Duck breast muscle showed varying percentages of their total mercury as methylmercury. This may be a reflection of varying types of mercurials in their diet.

References

1. Westöö, G. Acta Chim. Scand. 20, 2131 (1970).
2. Bouveng, H. Swedish Air & Water Pollution Inst. Publication C7Z (1970).
3. Armstrong, F.A.J. and J.F. Uthe. Atomic Absorption Newsletter 10 101 (1971).

Species and Tissue	Methylmercury (expressed as mercury)	Total Mercury	% as methylmercury
Northern Pike White muscle	0.20	0.20	100
	1.67	1.67	100
	12.7	13.1	97
	17.6	17.1	100
Yellow Walleye White muscle	0.62	0.61	100
	1.16	1.15	100
Rainbow trout White muscle	0.18	0.21	86
	0.56	0.57	98
Whitefish White muscle	0.02	0.02	100
	1.89	3.05	95
Swordfish White muscle	0.75	0.74	100
	1.34	1.38	97
Albacore Tuna White muscle	0.34	0.38	90
	0.49	0.54	91
Yellowfin Tuna White muscle	0.31	0.35	90
	0.51	0.61	83
Skipjack Tuna White muscle	0.09	0.12	75
	0.25	0.29	86
Tullibee White muscle	0.56	0.57	98
Dogfish White muscle	0.9	1.00	90
Mink muscle	0.26	0.29	89
Seal muscle	1.67	1.80	93
Mink liver			10, 41, 92
Seal liver	2.65	38.7	<1
Common Merganser Duck breast muscle	18.6	18.4	100
Hooded Merganser Duck breast muscle	17.8	17.6	100
Bluewing Teal Duck breast muscle	7.44	10.4	72
Mallard breast muscle	0.11	0.16	69
breast muscle	11.4	12.1	95
American Goldeneye breast muscle	12.3	16.8	73
	14.9	18.4	81