Bioaccumulation of methylmercury by aquatic insects and fish at the Experimental Lakes Area

96

by

Britt Dianne Hall

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

Master of Science
Department of Entomology
University of Manitoba
Winnipeg, Manitoba

ISBN 0-612-13165-3

Canada

Name _____

Dissertation Abstracts International and *Masters Abstracts International* are arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation or thesis. Enter the corresponding four-digit code in the spaces provided.

EL0L004

SUBJECT TERM

0329 UMI

SUBJECT CODE

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS
Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION
General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523
Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS
Language
- General 0679
- Ancient 0289
- Linguistics 0290
- Modern 0291

Literature
- General 0401
- Classical 0294
- Comparative 0295
- Medieval 0297
- Modern 0298
- African 0316
- American 0591
- Asian 0305
- Canadian (English) 0352
- Canadian (French) 0355
- English 0593
- Germanic 0311
- Latin American 0312
- Middle Eastern 0315
- Romance 0313
- Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY
Philosophy 0422
Religion
- General 0318
- Biblical Studies 0321
- Clergy 0319
- History of 0320
- Philosophy of 0322

Theology 0469

SOCIAL SCIENCES
American Studies 0323
Anthropology
- Archaeology 0324
- Cultural 0326
- Physical 0327

Business Administration
- General 0310
- Accounting 0272
- Banking 0770
- Management 0454
- Marketing 0338

Canadian Studies 0385
Economics
- General 0501
- Agricultural 0503
- Commerce-Business 0505
- Finance 0508
- History 0509
- Labor 0510
- Theory 0511

Folklore 0358
Geography 0366
Gerontology 0351
History
- General 0578
- Ancient 0579
- Medieval 0581
- Modern 0582
- Black 0328
- African 0331
- Asia, Australia and Oceania 0332
- Canadian 0334
- European 0335
- Latin American 0336
- Middle Eastern 0333
- United States 0337

History of Science 0585
Law 0398
Political Science
- General 0615
- International Law and Relations 0616
- Public Administration 0617

Recreation 0814
Social Work 0452
Sociology
- General 0626
- Criminology and Penology 0627
- Demography 0938
- Ethnic and Racial Studies 0631
- Individual and Family Studies 0628
- Industrial and Labor Relations 0629
- Public and Social Welfare 0630
- Social Structure and Development 0700
- Theory and Methods 0344

Transportation 0709
Urban and Regional Planning 0999
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES
Agriculture
- General 0473
- Agronomy 0285
- Animal Culture and Nutrition 0475
- Animal Pathology 0476
- Food Science and Technology 0359
- Forestry and Wildlife 0478
- Plant Culture 0479
- Plant Pathology 0480
- Plant Physiology 0817
- Range Management 0777
- Wood Technology 0746

Biology
- General 0306
- Anatomy 0287
- Biostatistics 0308
- Botany 0309
- Cell 0379
- Ecology 0329
- Entomology 0353
- Genetics 0369
- Limnology 0793
- Microbiology 0410
- Molecular 0307
- Neuroscience 0317
- Oceanography 0416
- Physiology 0433
- Radiation 0821
- Veterinary Science 0778
- Zoology 0472

Biophysics
- General 0786
- Medical 0760

EARTH SCIENCES
Biogeochemistry 0425
Geochemistry 0996
Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES
Environmental Sciences 0768
Health Sciences
- General 0566
- Audiology 0300
- Chemotherapy 0992
- Dentistry 0567
- Education 0350
- Hospital Management 0769
- Human Development 0758
- Immunology 0982
- Medicine and Surgery 0564
- Mental Health 0347
- Nursing 0569
- Nutrition 0570
- Obstetrics and Gynecology 0380
- Occupational Health and Therapy 0354
- Ophthalmology 0381
- Pathology 0571
- Pharmacology 0419
- Pharmacy 0572
- Physical Therapy 0382
- Public Health 0573
- Radiology 0574
- Recreation 0575
- Speech Pathology 0460
- Toxicology 0383

Home Economics 0386

PHYSICAL SCIENCES
Pure Sciences
Chemistry
- General 0485
- Agricultural 0749
- Analytical 0486
- Biochemistry 0487
- Inorganic 0488
- Nuclear 0738
- Organic 0490
- Pharmaceutical 0491
- Physical 0494
- Polymer 0495
- Radiation 0754

Mathematics 0405
Physics
- General 0605
- Acoustics 0986
- Astronomy and Astrophysics 0606
- Atmospheric Science 0608
- Atomic 0748
- Electronics and Electricity 0607
- Elementary Particles and High Energy 0798
- Fluid and Plasma 0759
- Molecular 0609
- Nuclear 0610
- Optics 0752
- Radiation 0756
- Solid State 0611

Statistics 0463

Applied Sciences
Applied Mechanics 0346
Computer Science 0984
Engineering
- General 0537
- Aerospace 0538
- Agricultural 0539
- Automotive 0540
- Biomedical 0541
- Chemical 0542
- Civil 0543
- Electronics and Electrical 0544
- Heat and Thermodynamics 0348
- Hydraulic 0545
- Industrial 0546
- Marine 0547
- Materials Science 0794
- Mechanical 0548
- Metallurgy 0743
- Mining 0551
- Nuclear 0552
- Packaging 0549
- Petroleum 0765
- Sanitary and Municipal 0554
- System Science 0790

Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY
General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

Nom _____

Dissertation Abstracts International est organisé en catégories de sujets. Veuillez s.v.p. choisir le sujet qui décrit le mieux votre thèse et inscrivez le code numérique approprié dans l'espace réservé ci-dessous.

_____ ☐☐☐ U·M·I

SUJET — CODE DE SUJET

Catégories par sujets

HUMANITÉS ET SCIENCES SOCIALES

COMMUNICATIONS ET LES ARTS
Architecture 0729
Beaux-arts 0357
Bibliothéconomie 0399
Cinéma 0900
Communication verbale 0459
Communications 0708
Danse 0378
Histoire de l'art 0377
Journalisme 0391
Musique 0413
Sciences de l'information 0723
Théâtre 0465

ÉDUCATION
Généralités 515
Administration 0514
Art 0273
Collèges communautaires 0275
Commerce 0688
Économie domestique 0278
Éducation permanente 0516
Éducation préscolaire 0518
Éducation sanitaire 0680
Enseignement agricole 0517
Enseignement bilingue et multiculturel 0282
Enseignement industriel 0521
Enseignement primaire. 0524
Enseignement professionnel 0747
Enseignement religieux 0527
Enseignement secondaire 0533
Enseignement spécial 0529
Enseignement supérieur 0745
Évaluation 0288
Finances 0277
Formation des enseignants 0530
Histoire de l'éducation 0520
Langues et littérature 0279
Lecture 0535
Mathématiques 0280
Musique 0522
Orientation et consultation 0519
Philosophie de l'éducation 0998
Physique 0523
Programmes d'études et enseignement 0727
Psychologie 0525
Sciences 0714
Sciences sociales 0534
Sociologie de l'éducation 0340
Technologie 0710

LANGUE, LITTÉRATURE ET LINGUISTIQUE
Langues
- Généralités 0679
- Anciennes 0289
- Linguistique 0290
- Modernes 0291

Littérature
- Généralités 0401
- Anciennes 0294
- Comparée 0295
- Médiévale 0297
- Moderne 0298
- Africaine 0316
- Américaine 0591
- Anglaise 0593
- Asiatique 0305
- Canadienne (Anglaise) 0352
- Canadienne (Française) 0355
- Germanique 0311
- Latino-américaine 0312
- Moyen-orientale 0315
- Romane 0313
- Slave et est-européenne 0314

PHILOSOPHIE, RELIGION ET THÉOLOGIE
Philosophie 0422
Religion
- Généralités 0318
- Clergé 0319
- Études bibliques 0321
- Histoire des religions 0320
- Philosophie de la religion 0322

Théologie 0469

SCIENCES SOCIALES
Anthropologie
- Archéologie 0324
- Culturelle 0326
- Physique 0327

Droit 0398
Économie
- Généralités 0501
- Commerce-Affaires 0505
- Économie agricole 0503
- Économie du travail 0510
- Finances 0508
- Histoire 0509
- Théorie 0511

Études américaines 0323
Études canadiennes 0385
Études féministes 0453
Folklore 0358
Géographie 0366
Gérontologie 0351
Gestion des affaires
- Généralités 0310
- Administration 0454
- Banques 0770
- Comptabilité 0272
- Marketing 0338

Histoire
- Histoire générale 0578
- Ancienne 0579
- Médiévale 0581
- Moderne 0582
- Histoire des noirs 0328
- Africaine 0331
- Canadienne 0334
- États-Unis 0337
- Européenne 0335
- Moyen-orientale 0333
- Latino-américaine 0336
- Asie, Australie et Océanie 0332

Histoire des sciences 0585
Loisirs 0814
Planification urbaine et régionale 0999
Science politique
- Généralités 0615
- Administration publique 0617
- Droit et relations internationales 0616

Sociologie
- Généralités 0626
- Aide et bien-être social 0630
- Criminologie et établissements pénitentiaires 0627
- Démographie 0938
- Études de l' individu et de la famille 0628
- Études des relations interethniques et des relations raciales 0631
- Structure et développement social 0700
- Théorie et méthodes. 0344
- Travail et relations industrielles 0629

Transports 0709
Travail social 0452

SCIENCES ET INGÉNIERIE

SCIENCES BIOLOGIQUES
Agriculture
- Généralités 0473
- Agronomie. 0285
- Alimentation et technologie alimentaire 0359
- Culture 0479
- Élevage et alimentation 0475
- Exploitation des pâturages 0777
- Pathologie animale 0476
- Pathologie végétale 0480
- Physiologie végétale 0817
- Sylviculture et faune 0478
- Technologie du bois 0746

Biologie
- Généralités 0306
- Anatomie 0287
- Biologie (Statistiques) 0308
- Biologie moléculaire 0307
- Botanique 0309
- Cellule 0379
- Écologie 0329
- Entomologie 0353
- Génétique 0369
- Limnologie 0793
- Microbiologie 0410
- Neurologie 0317
- Océanographie 0416
- Physiologie 0433
- Radiation 0821
- Science vétérinaire 0778
- Zoologie 0472

Biophysique
- Généralités 0786
- Medicale 0760

SCIENCES DE LA TERRE
Biogéochimie 0425
Géochimie 0996
Géodésie 0370
Géographie physique 0368
Géologie 0372
Géophysique 0373
Hydrologie 0388
Minéralogie 0411
Océanographie physique 0415
Paléobotanique 0345
Paléoécologie 0426
Paléontologie 0418
Paléozoologie 0985
Palynologie 0427

SCIENCES DE LA SANTÉ ET DE L'ENVIRONNEMENT
Économie domestique 0386
Sciences de l'environnement 0768
Sciences de la santé
- Généralités 0566
- Administration des hôpitaux 0769
- Alimentation et nutrition 0570
- Audiologie 0300
- Chimiothérapie 0992
- Dentisterie 0567
- Développement humain 0758
- Enseignement 0350
- Immunologie 0982
- Loisirs 0575
- Médecine du travail et thérapie 0354
- Médecine et chirurgie 0564
- Obstétrique et gynécologie 0380
- Ophtalmologie 0381
- Orthophonie 0460
- Pathologie 0571
- Pharmacie 0572
- Pharmacologie 0419
- Physiothérapie 0382
- Radiologie 0574
- Santé mentale 0347
- Santé publique 0573
- Soins infirmiers 0569
- Toxicologie 0383

SCIENCES PHYSIQUES
Sciences Pures
Chimie
- Généralités 0485
- Biochimie 487
- Chimie agricole 0749
- Chimie analytique 0486
- Chimie minérale 0488
- Chimie nucléaire 0738
- Chimie organique 0490
- Chimie pharmaceutique 0491
- Physique 0494
- PolymÈres 0495
- Radiation 0754

Mathématiques 0405
Physique
- Généralités 0605
- Acoustique 0986
- Astronomie et astrophysique 0606
- Électronique et électricité 0607
- Fluides et plasma 0759
- Météorologie 0608
- Optique 0752
- Particules (Physique nucléaire) 0798
- Physique atomique 0748
- Physique de l'état solide 0611
- Physique moléculaire 0609
- Physique nucléaire 0610
- Radiation 0756

Statistiques 0463

Sciences Appliqués Et Technologie
Informatique 0984
Ingénierie
- Généralités 0537
- Agricole 0539
- Automobile 0540
- Biomédicale 0541
- Chaleur et thermodynamique 0348
- Conditionnement (Emballage) 0549
- Génie aérospatial 0538
- Génie chimique 0542
- Génie civil 0543
- Génie électronique et électrique 0544
- Génie industriel 0546
- Génie mécanique 0548
- Génie nucléaire 0552
- Ingénierie des systèmes 0790
- Mécanique navale 0547
- Métallurgie 0743
- Science des matériaux 0794
- Technique du pétrole 0765
- Technique minière 0551
- Techniques sanitaires et municipales 0554
- Technologie hydraulique 0545

Mécanique appliquée 0346
Géotechnologie 0428
Matières plastiques (Technologie) 0795
Recherche opérationnelle 0796
Textiles et tissus (Technologie) 0794

PSYCHOLOGIE
Généralités 0621
Personnalité 0625
Psychobiologie 0349
Psychologie clinique 0622
Psychologie du comportement 0384
Psychologie du développement 0620
Psychologie expérimentale 0623
Psychologie industrielle 0624
Psychologie physiologique 0989
Psychologie sociale 0451
Psychométrie 0632

THE UNIVERSITY OF MANITOBA

FACUTY OF GRADUATE STUDIES

COPYRIGHT PERMISSION

BIOACCUMULATION OF METHYLMERCURY BY AQUATIC INSECTS AND FISH AT THE EXPERIMENTAL LAKES AREA

BY

BRITT DIANNE HALL

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

MASTER OF SCIENCE

Table of Contents

List of Tables

List of Figures

Preface

This thesis is presented in a paper-style format. Two manuscripts have resulted:

Hall, B.D., D.M. Rosenberg and A.P. Wiens. Bioaccumulation of methylmercury by aquatic insects in an experimental reservoir. Can. J. Fish. Aquatic Sci. (Submitted).

Hall, B.D., R.A. Bodaly, R.J.P. Fudge, J.W.M. Rudd and D.M. Rosenberg. Food as the dominant pathway of methylmercury uptake by fish. Water Air Soil Pollut. (In press).

Acknowledgments

I could not have hoped for a more rewarding graduate student experience and this is directly a result of my outstanding committee. Drs. David Rosenberg, Drew Bodaly and Terry Galloway gave me the guidance I required as a graduate student and the respect I craved as a scientist and colleague. However, what I most value is the enthusiasm that each of them possesses for their individual, and our mutual, interests. A special thanks to Dave for his superior editing of my work and for putting up with me when I inevitably broke any of the "Rosenberg Rules".

I believe that the Experimental Lakes Area Reservoir Project's greatest asset was its multidisciplinary nature. The results of my thesis stem from ideas that were developed with scientific input from Drs. John Rudd, Carol Kelly, Vince St. Louis, Mike Paterson, Diane Malley, Ray Hesslein, and Jack Klaverkamp. In addition, I received statistical and modelling input from Susan Kasian, Reed Harris and Dr. Brigitte de March. Thanks also to Dr. Lyle Lockhart for acting as the external examiner at my thesis defence. I thank these people for their input and because it was always a pleasure to interact with them and others at the Freshwater Institute.

I especially want to acknowledge many students and staff who worked at the Experimental Lakes Area because this work would not have been possible without their assistance. Their collective efforts in everything from sampling to re-floating the whaler from the bottom of Lake 240 made my work possible. This field assistance was provided to me by Bob Fudge, Allen Wiens, Pauline Gerrard, Sheena Majewski, Vince St. Louis, John Embury and Andrew Heyes. The ELA Chemistry Lab provided water chemistry analysis and Field Services enabled me to get to my field sites. Bob Hunt, Karen Scott and Rose Omole assisted in THg and MeHg analyses at the Freshwater Institute.

I treasure the friendship of the ELA people (too many to list names) with whom I shared the exciting (and frustrating!) moments of this research, but special thanks go to Shannon Harris for support, laughter and Jobson's Cove.

Many thanks are due to my wonderful family, to whom I dedicate this thesis, for their never-ending understanding and support. I especially thank Dr. John Rudd for showing

me pond water under the microscope for the first time many years ago and for the inspiration and enthusiasm that he has provided since.

Finally, I am very grateful to Manitoba Hydro for directly financing my work, and to the Department of Fisheries and Oceans, Hydro Quebec, Ontario Hydro and NSERC for financial support to ELARP.

Abstract

The general objective of my study was to develop a better understanding of the role of organisms of intermediate trophic levels (specifically aquatic insects) in the movement of mercury (Hg) to fish. This was achieved by focusing on the following two questions: 1. Do methylmercury (MeHg) concentrations in aquatic insects increase in response to flooding? and 2. Is ingestion of a high MeHg diet responsible for elevated Hg levels in fish?

Aquatic insects were collected from the shorelines of a wetland lake before and after experimental flooding and from nearby wetland and oligotrophic lakes at the Experimental Lakes Area (ELA) in northwestern Ontario. Insects were categorized into three functional feeding groups (FFGs): predators, predator/herbivores and collector/shredders and analyzed for MeHg and total mercury (THg) concentrations. Predators and predator/herbivores displayed an ~2 fold increase in MeHg concentrations after flooding, whereas collector/shredders concentrated MeHg to a lesser degree. The ratios of MeHg to THg were also examined and showed no statistically significant change in response to flooding. Trends in MeHg concentrations in aquatic insects from reservoirs and natural lakes in Finland and Québec were similar to the ELA reservoir.

A field experiment was conducted to determine the relative importance of food versus water in the uptake of MeHg by fish. Finescale dace (*Phoxinus neogaeus*) were held in enclosed pens floating in an undisturbed, oligotrophic lake. Fish were exposed to water with varying MeHg concentrations and fed zooplankton with either low or high concentrations of MeHg. Fish fed zooplankton with high concentrations of MeHg had significantly higher concentrations of THg in muscle after 32 days than fish fed zooplankton with low concentrations of MeHg. Fish fed zooplankton with low concentrations of MeHg had the same amount of Hg in their tissues as fish at the start of the experiment. Uptake from water was at most 15%. This is the first experiment done at natural levels of MeHg to confirm that food is the dominant pathway of MeHg bioaccumulation in fish.

Aquatic insects act as part of a food-web system that transfers MeHg from the physical environment to fish, and therefore increases in MeHg concentrations in aquatic

insects are an important consequence of reservoir creation. This study clarifies some of the aspects of the behaviour of MeHg in food webs; however, a large potential exists for study of MeHg dynamics in the lower food web.

General Introduction

Mercury as a global environmental problem.

The first indication that toxic organic mercury (Hg) compounds could biomagnify in aquatic food webs was the discovery of methylmercury (MeHg) as the primary cause of permanent damage to the nervous system of people eating fish contaminated by discharge from an industrial source into Minimata Bay, Japan (Kurland *et al.* 1960; reviewed in D'Itra and D'Itra 1977). This was the beginning of the study of Hg cycling in aquatic ecosystems, which continues to be a popular research topic because human-related activities release Hg to the environment in excess of natural inputs. In fact, 50-75% of total yearly input comes from anthropogenic sources (Fitzgerald 1995). Model simulations (Mason *et al.* 1994) predict that Hg in the atmosphere will continue to increase by 0.01 ng $m^{-3}yr^{-1}$ (0.6% yr^{-1}; Fitzgerald 1995). Point source contamination of Hg is now illegal in Canada, and rigid pollution standards have reduced, but not eliminated, emissions of Hg from North American and western European industries. The high mobility of Hg in the atmosphere (Fitzgerald and Clarkson 1991) has made Hg contamination of ecosystems a global environmental problem.

Biogeochemistry of Mercury

Ninety-five -100% of Hg in the atmosphere is gaseous Hg^0. Photocatalytic reactions oxidize Hg^0 to the dipositive mercuric ion, Hg^{+2} [Hg(II)] which is soluble in water and is removed from the atmosphere by wet or dry deposition (Winfrey and Rudd 1990). Once in the aquatic environment, Hg(II) may adsorb to inorganic and organic particulates, including dissolved organic carbon (DOC; Winfrey and Rudd 1990, Meili 1991). The most interesting form of Hg is the merthylmercuric ion (CH_3Hg^+) which is associated with thiol groups in proteins. It is bioaccumulated by aquatic animals and consumption of fish with sufficiently high concentrations of MeHg causes neurological damage in humans (Kurland *et al.* 1960). There is a small amount of MeHg in the atmosphere (0-5% of THg is MeHg, which may increase with altitude; Lindqvist, 1991) and, therefore in precipitation (St. Louis *et al.* 1995) and runoff (St. Louis *et al.* in press). However, the major source of MeHg is the process of *in situ* methylation by microbial organisms (most likely sulfate-reducing bacteria;

Gilmour and Henry 1991). The production of MeHg is reversible either by microbial demethylation (Robinson and Tuovinen 1984) or ultra-violet photodegredation (Sellers *et al.* in press). The removal of the organic group from MeHg returns Hg(II) ions to the system, which in turn are reduced back to Hg^0 and dissipated to the atmosphere as a gas (Winfrey and Rudd 1990). Environmental parameters such as pH (Gilmour and Henry 1991), DOC concentrations (Driscoll *et al.* 1994), temperature (Winfrey and Rudd 1990), concentrations of metal ions (eg. Fe, Cu, Al, Mn ions affect methylation: Matilainen *et al.* 1991; Se^{-2} affects fish uptake: Turner and Rudd 1983), and redox conditions (Compeau and Bartha 1984) will affect the biogeochemical cycle of Hg. However, it is difficult to distinguish among the effects of environmental variables.

Methyl Hg is only present in trace amounts (i.e. <0.05 ng L^{-1}) in unmanipulated aquatic systems. Recent developments in clean-sampling protocols and ultra-sensitive analytical techniques (Bloom 1989) have enabled collection and analysis of low-concentration samples. However, past difficulties in sampling and analysis of MeHg have forced investigators to focus on concentrations of THg (inorganic plus organic Hg compounds) and to use THg concentrations as a predictor of the more bioavailable and toxic MeHg concentrations. There may be no relationship between the two in some situations (Kelly *et al.* 1995), so although the literature on THg is quite diverse, the behaviour of MeHg in natural systems has only recently begun to be clarified.

The mercury problem in hydroelectric reservoirs

Fish with tissue Hg concentrations exceeding government guidelines (0.05 $\mu g\, g^{-1}$ wet weight in Canada) have been found in five different types of aquatic ecosystems: 1. those polluted by point sources (Slotton *et al.* 1995); 2. remote waterbodies receiving atmospheric inputs of Hg (Verta *et al.* 1986); 3. acidified aquatic ecosystems (Winfrey and Rudd 1990); 4. natural wetlands (St. Louis *et al.* 1994); and 5. reservoirs created by flooding large areas of land (Bodaly *et al.* 1984).

Over 20,000 km^2 of peatlands and uplands have been flooded in the creation of five major hydroelectric reservoirs in Canada (Rosenberg *et al.* 1987). The creation of reservoirs destroys habitat through flooding and water diversion (Rosenberg *et al.* 1995), releases

greenhouse gases (CH_4 and CO_2) through increased decomposition of organic material (Rudd *et al.* 1993) and contaminates fisheries with Hg (Bodaly *et al.* 1984), sometimes to the point that they can no longer be used as a source of food (Rosenberg *et al.* 1995).

The Experimental Lakes Area Reservoir Project (ELARP) was initiated to increase our understanding of the processes underlying two of the main effects of reservoir creation: 1. increased production of greenhouse gases resulting from decomposition of flooded peatlands, and 2. increased Hg concentrations in the fish from flooded systems. This multidisciplinary, whole-ecosystem manipulation was carried out at the Experimental Lakes Area (ELA) in northwestern Ontario (Figure 1). In 1993, a small wetland pond, Lake (L) 979, was flooded after 2 yr of pre-flood monitoring (Kelly *et al.* submitted). A second wetland pond, L632, was studied concurrently as a reference system. The ELARP is unique in its orientation to natural levels of MeHg, and is the first experimental reservoir study done before and after flooding of a peatland.

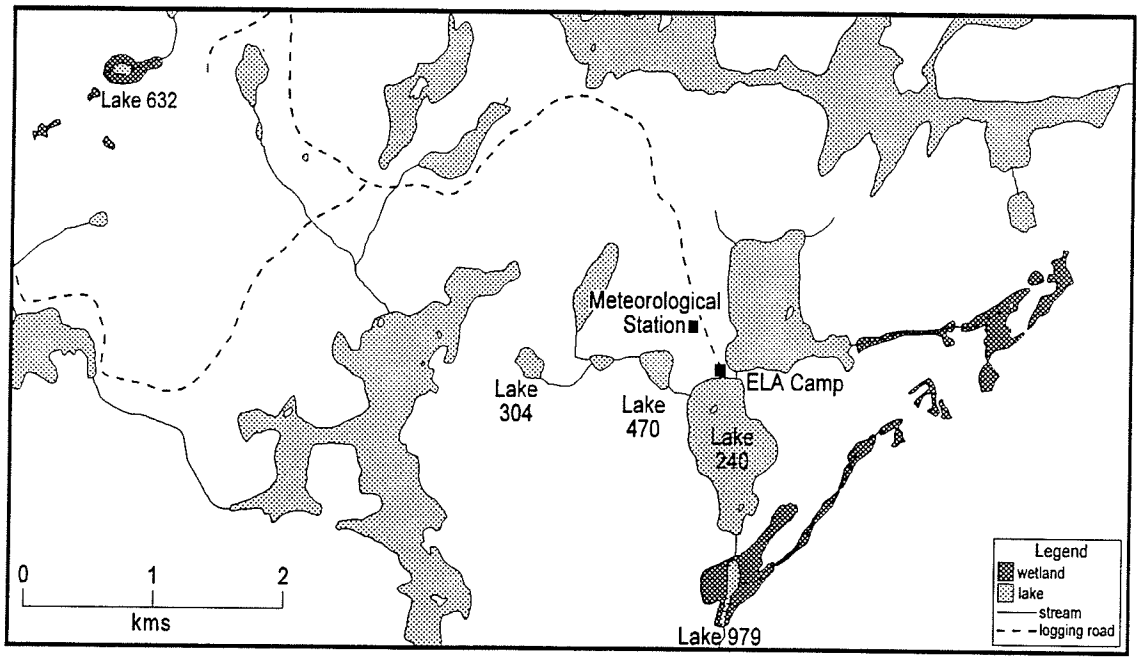


Figure 1. Relative location of lakes and wetlands used in this study (from St. Louis *et al.* in press).

Part of the ELARP's second objective is the assessment of the effects of flooding on MeHg concentrations in the aquatic food web. Zooplankton, aquatic insects, omnivorous fish (finescale dace; *Phoxinus neogaeus* Cope), piscivorous fish (northern pike; *Esox lucius* Linnaeus) and tree swallows (*Tachycineta bicolor* Linnaeus) were used as study organisms. My objective was to develop a better understanding of the role of organisms of intermediate trophic-levels, specifically aquatic insects, in the movement of Hg to fish. This objective was achieved by focusing on the following two questions: 1. Do MeHg concentrations in aquatic insects increase in response to flooding? and 2. Is ingestion of a diet containing high concentrations of MeHg responsible for elevated Hg levels in fish?

Methylmercury concentrations in aquatic insects in response to flooding

The objective of Chapter 1 is to describe changes in MeHg concentrations in aquatic insects resulting from creation of the experimental reservoir. The study is unique because it deals with MeHg concentrations in aquatic insects before and after experimental flooding. Mercury dynamics in aquatic insects have been examined previously either in laboratory studies using unnaturally high levels of Hg and MeHg or in field studies where only THg was measured (Table 1). It is difficult to extrapolate results of laboratory experiments using high concentrations of MeHg to field situations. Moreover, use of THg as a predictor of MeHg concentrations can be unreliable.

The null hypothesis explored in Chapter 1 is that concentrations of MeHg in aquatic insects will not increase in response to flooding. To test this hypothesis, aquatic insects were collected from the shorelines of the experimental reservoir (Lake 979) before and after flooding and from nearby unmanipulated lakes (Lakes 632 and 240; Figure 1). Insects were categorized into three functional feeding groups (FFGs): predators, predator/herbivores and collector/shredders. Results from the analysis of MeHg and THg concentrations in aquatic insects were used to explore four questions: 1. Does flooding cause an increase in MeHg concentrations in aquatic insects?; 2. Do FFGs accumulate MeHg differently?; 3. Is the ratio of MeHg to THg in aquatic insects constant? and 4. How do concentrations of MeHg in water, aquatic insects and fish compare? Results from this study were compared to those

Table 1. Summary of mercury studies on freshwater benthic insects. THg= total mercury; MeHg= methylmercury; d.w.= dry weight, w.w.= wet weight.

Type of study	Form of Hg	Ranges in concentrations in biota (ug/g)	Reference
Laboratory			
	THg	0.42-90 d.w.	Borgmann *et al.* 1993
		2200->4500 w.w.	Odin *et al.* 1994
		4.20-69.35 w.w.	Rossaro *et al.* 1986
		0.037-3.76 w.w.	Saouter *et al.* 1993
	MeHg	2.12-7.48 w.w.	Saouter *et al.* 1993
Field			
Natural aquatic systems			
	THg	0.03-0.93 d.w.	Albers and Camardese 1993
		0.09-0.56 w.w.	Armstrong and Hamilton 1973
		0.17-0.22 d.w.	Elwood *et al.* 1976
		0.065-0.088 w.w.	Hildebrand *et al.* 1976
		0.002-0.066 w.w.	Huckabee and Hildebrand 1974
		0.034-5.757 d.w.	Parkman and Meili 1993
		0.14-1.23 d.w.	Snyder and Hendricks 1995
		0.045-0.055 w.w.	Surma-Aho *et al.* 1986
		0.129-0.256 d.w.	Tremblay *et al*. in press
	MeHg	0.040-0.045 w.w.	Surma-Aho *et al.* 1986
		0.013-0.124 d.w.	Tremblay *et al.* in press
Impacted aquatic systems			
	THg	<0.10-0.41 w.w.	Albers and Camardese 1993[1]
		0.01-10.0 w.w.	Armstrong and Hamilton 1973[2]
		0.22-1.94 w.w.	Hildebrand *et al.* 1976[2]
		0.05-17.0 w.w.	Johnels *et al.* 1979[2]
		0.002-0.23 w.w.	Potter *et al.* 1975[3]
		0.02-0.472 w.w.	Surma-Aho *et al.* 1986[3]
		0.139-1.675 d.w.	Tremblay *et al.* in press[3]
	MeHg	0.047-0.186 w.w.	Surma-Aho *et al.* 1986[3]
		0.063-1.519 d.w.	Tremblay *et al.* in press[3]

[1]Acid system
[2]Point-source contamination
[3]Reservoirs

from two other investigations focusing on MeHg concentrations of insects in natural and impounded aquatic systems (Tremblay *et al.* in press, Surma-Aho *et al.* 1986).

The uptake of methylmercury by fish

The major pathway of MeHg to humans is through the consumption of fish, so most bioaccumulation information is fish-related. Most (90-98%) of the Hg in fish is MeHg (Spry and Wiener 1991), and can be obtained by uptake from food (biomagnification; Moriarty and Walker 1987) and uptake from water passing over the gills during respiration (bioconcentration; Barron 1990). The importance of these two routes of uptake has never been demonstrated in a field situation using natural concentrations of MeHg. Laboratory experiments using unnaturally high levels of MeHg in food and water have been used to provide information on this question, but these studies have not presented conclusive results. Model simulations using water concentrations of MeHg at the ng L^{-1} level (approximately the concentrations in natural waters), aspects of fish physiology and environmental factors predict that food pathways are most responsible for MeHg uptake in fish (Rodgers 1994, Harris and Snodgrass 1993). Studies examining the relationship between MeHg concentrations and ratios of stable-nitrogen isotopes also suggest that diet is the most important pathway of MeHg uptake in fish (Cabana *et al.* 1994, Kidd *et al.* 1995).

The results of a field experiment conducted to determine the relative importance of food and water to the uptake of MeHg by fish at natural concentrations of MeHg are presented in Chapter 2. Finescale dace were held in 2000-L enclosures containing water with varying concentrations of MeHg. Zooplankton with either low or high MeHg concentrations were added daily to each pen. Total Hg concentrations in fish tissue at the end of the experiment were compared to concentrations in fish sampled at the beginning of the experiment.

The importance of lower trophic animals in the bioaccumulation of MeHg in fish is demonstrated, providing an important piece of information in our understanding of MeHg cycling in flooded systems. The chapters are summarized and recommendations for future research topics are discussed in the final considerations and general summary.

Chapter 1: Bioaccumulation of methylmercury by aquatic insects in an experimental reservoir.[1]

Abstract. Aquatic insects were collected from the shorelines of a wetland pond before and after experimental flooding and from a nearby wetland and oligotrophic lakes. Insects were categorized into three functional feeding groups (FFGs): predators, predator/herbivores and collector/shredders. Results from the analysis of methylmercury (MeHg) and total mercury (THg) concentrations in aquatic insects were used to answer four questions: 1. Does flooding of a wetland pond cause an increase in MeHg concentrations in aquatic insects?; 2. Do FFGs differ from each other in how they accumulate MeHg?; 3. Is the ratio of MeHg to THg in aquatic insects constant? and 4. How do concentrations of MeHg in water, aquatic insects, and fish compare?

Predators and predator/herbivores displayed an ~2 fold increase in MeHg concentrations after flooding, whereas collector/shredders concentrated MeHg to a lesser degree. There was no statistically significant change in MeHg:THg in any FFG in response to flooding. Concentrations and % MeHg were similar between predators (mean MeHg = 190.5 ng g^{-1} d.w., mean % MeHg= 67%) and predator/herbivores (mean MeHg = 175.7 ng g^{-1} d.w., mean % MeHg= 69%), but were lower in collector/shredders (mean MeHg = 71.5 ng g^{-1} d.w., mean % MeHg= 46%). Trends in MeHg concentrations in aquatic insects from reservoirs and natural lakes in Finland and Québec are similar to ours.

Introduction

Recent development of analytical techniques sensitive enough to measure low levels of methylmercury (MeHg) in water (Bloom 1989) has led to improved understanding of the biogeochemical cycling of MeHg in aquatic environments such as natural wetlands (St. Louis *et al.* 1994), impounded wetlands (e.g. Kelly *et al.* submitted) and large-scale hydroelectric reservoirs (e.g. Dmytriw *et al.* 1995). Concentrations of neurotoxic MeHg in fish harvested from reservoirs often exceed Canadian mercury (Hg) concentration guidelines of 0.5 µg g^{-1} wet wt. (Bodaly *et al.* 1984, Morrison and Thérien 1995, Verdon *et al.* 1991,

[1] Submitted for publication to Canadian Journal of Fisheries and Aquatic Sciences.

Yingcharoen and Bodaly 1993), thus threatening the health of people who subsist on reservoir fisheries (Rosenberg *et al.* 1995).

The importance of dietary sources of MeHg to fish has been clarified by experiments in the field (Chapter 2), experiments in the laboratory (Rodgers and Beamish 1981, Phillips and Buhler 1978) and theoretical models (Rodgers 1994, Harris and Snodgrass 1993). It is apparent that MeHg concentrations in lower trophic levels are important in determining concentrations of MeHg in fish.

The objective of this study was to quantify changes in Hg concentrations in aquatic insects before and after the creation of an experimental reservoir. Mercury dynamics in aquatic insects have been previously examined primarily in laboratory studies using unnaturally high levels of Hg and MeHg (Saouter *et al.* 1993, Rossaro *et al.* 1986), or in field studies (Parkman and Meili 1993, Snyder and Hendricks 1995) that have measured only total Hg (THg). There are two studies that have dealt with MeHg concentrations in reservoirs (Surma-Aho *et al.* 1986; Tremblay *et al.* in press). These studies relied on comparisons of MeHg concentrations in aquatic insects from reservoirs to those from natural lakes. The study is unique because it addressed MeHg concentrations in aquatic insects before and after experimental flooding of a wetland.

This research was completed as part of the Experimental Lakes Area Reservoir Project (ELARP), a whole-ecosystem study designed to understand two problems associated with the decomposition of flooded organic matter due to reservoir creation: 1. increased MeHg concentrations in fish harvested from reservoirs; and 2. increased fluxes of greenhouse gases (CO_2 and CH_4) from reservoirs created over peatlands (Kelly *et al.* submitted).

The following questions were addressed: 1. Does flooding of a wetland pond cause an increase in MeHg concentrations in aquatic insects?; 2. Are there differences in MeHg accumulation and %MeHg among functional feeding groups (FFGs) (Merritt and Cummins 1984)?; 3. Is the ratio of MeHg to THg in aquatic insects constant in response to flooding? If so, the less difficult and cheaper analysis of THg concentrations could be used as an indication of MeHg concentrations and also offer direct comparisons to past studies, and 4. How do concentrations of MeHg in water, aquatic insects, and fish compare?

Site descriptions

Two wetlands at the Experimental Lakes Area (ELA) in northwestern Ontario were chosen as study sites. The experimentally manipulated wetland had a central pond, Lake (L) 979 (2.4 ha) with a main inflow originating from upstream, oligotrophic L240. The pond was surrounded by a 14.2 ha peatland. Lake 979 was impounded in early July of 1993 and 1994 by damming the outflow (Kelly *et al.* submitted). As a result, surface area of the pond increased to 16.7 ha and maximum depth increased from ~1 m to 2.3 m. To simulate operation of boreal hydroelectric reservoirs, L979 was drawn down by 1 m on 5 October, 1993 and 3 October, 1994. The reference wetland is a headwater system with a central pond, L632 (0.86 ha), and a surrounding peatland area (3.4 ha). See St. Louis *et al.* (in press) for detailed information regarding these wetlands.

Sampling program

A qualitative sampling program was established on both reference and treatment wetland ponds. Shoreline insects were sampled by sweeping with a triangular (32cmx34cmx32cm) net of mesh size 400 μm, in L979 before and after it was flooded (every 2 wk: May through Sept., 1992 and 1993; weekly: 2 June-22 June, 1993). Sampling was relocated after flooding away from the edge of the old pond to shallow areas over newly flooded peat. Samples were collected during the next field season starting on 18 May, 1994 before reflooding (May 26, 1994) and every 2 wk after reflooding until 1 September 1994. After drawdown in mid October 1994, another sample was taken from the former shoreline. Shoreline insects were collected every ~2 wk in the reference wetland from May to September of 1992-1994. Samples were also periodically taken in L240 (an upstream reference to L979) in 1993 and 1994 (10 May-24 Aug., 1993; 8-9 June, 1994).

Samples were sorted into major taxa in the field and were identified to lower taxa (usually genus) in the laboratory. Because we were concerned with the total body burden of MeHg potentially transferable to fish, insects were frozen shortly after sorting to avoid clearing of their guts.

Insects were classified into FFGs according to food type and feeding habit (Merritt and Cummins 1984). Three FFGs resulted: 1. predators: insects feeding on other

invertebrates; 2. collector/shredders: insects feeding on living plants or detritus: and 3. predator/herbivores: insects that feed on both other animals and plants or those that change their diet at different life stages. Stable N isotope ratios were then used to determine the trophic position of certain insects within FFGs because the ratio changes in a constant fashion with the transfer of organic matter through each trophic level (Peterson and Fry 1987). The ratio of $^{15}N/^{14}N$ in a small number of invertebrate samples from L979 was compared to the ratio in high quality N_2 reference gas (Hesslein and Ramlal 1993). Ratios of $\delta N^{15}/\delta N^{14}$ for collector/shredder samples were 0.75‰ and 1.79‰, which were much different than those from predators which ranged from 2.7‰ to 3.32‰, and predator/herbivores which ranged from 2.75‰to 3.65‰. Predators and predator/herbivores had the same nitrogen ratios. Presumably the two groups were feeding at the same trophic level, and therefore belong to the same group. However, FFGs are used throughout this paper to distinguish among different taxonomic groups (Table 2).

Mercury analysis

Specimens were rinsed in low-Hg water and blotted to remove excess water. Insects were placed individually into acid-washed plastic scintillation vials. A minimum of 0.05 g dry weight (d.w.) of sample was required for MeHg and THg analysis. When required to obtain sufficient biomass for analysis, insects were pooled with others of the same taxa on the same sampling date. Samples were freeze-dried and then ground using a glass mortar and pestle.

Organic Hg and THg were determined using cold vapour atomic absorption spectrophotometry (AAS) (Armstrong and Uthe 1971, Hendzel and Jamieson 1976), as described by Malley *et al.* (in press). Briefly, protein-bound organic Hg was released by homogenizing dried tissue in a solution of acidic NaBr (30% w/v) and $CuSO_4$ (2.5% w/v). A 3:2 mixture of methylene chloride and hexane was used to partition the resulting organic mercury bromide. An aliquot was digested overnight in a 4:1 mixture of H_2SO_4 and HNO_3, and oxidized Hg was converted to the elemental state with a $(NH_2OH)_2{\cdot}H_2SO_4$-$SnCl_2$-NaCl

Table 2. Insect taxa included in three FFGs: Collector/shredders, Predator/herbivores and Predators.

Functional Feeding Group		
Order Family	Genus species	Life Stage
Collector/Shredders		
Amphipoda	*Hyalella azteca*	Adults
Ephemeroptera	*Siphlonurus*	Nymphs
Trichoptera		
Limnephilidae		Larvae
Predator/Herbivores		
Trichoptera		
Polycentropodidae		Larvae
Phryganeidae		Larvae
Hemiptera		
Corixidae		Adults and Nymphs
Predators		
Odonata		
Aeshnidae		Nymphs
Corduliidae		Nymphs
Hemiptera		
Belostomatidae	*Lethocerus americanus*	Adults
Gerridae	*Gerris*	Adults and Nymphs
Notonectidae	*Notonecta*	Adults and Nymphs
Nepidae	*Ranatra*	Adults
Coleoptera		
Dytisicidae	*Dytiscus*	Adults
Gyrinidae	*Gyrinus*	Adults and Larvae
	Dineutus	Adults and Larvae

reducing solution. The Hg^0 was partitioned into air and determined by AAS. Reference material [National Research Council of Canada dogfish muscle (Dorm-1)] was analyzed along with samples; organic Hg determinations of the reference material were always within certified range (671-793 ng g^{-1} d.w.). Two samples analyzed for organic Hg in our lab were also analyzed at the ELA MeHg laboratory using distillation, ethylation and atomic fluorescence (Horvat *et al.* 1993, with modifications given in Moore *et al.* 1995). Concentrations of MeHg in these samples (452 ng g^{-1} d.w. and 180 ng g^{-1} d.w.) were within the range of those done by the AAS method (423 ng g^{-1} d.w. and 228 ng g^{-1} d.w.). These results confirmed that the organic Hg extracted by the AAS method was primarily MeHg (A. Heyes, McGill University, Montreal, PQ, pers. comm.). The high cost of the technique prohibited the analysis of more than two samples.

Two sources of variability were associated with MeHg determination: 1. analytical: which includes error associated with instrumentation and sample preparation (homogenization and digestion) and 2. natural variation in MeHg concentrations in insect populations. Error caused by analytical technology was determined by repetitive analysis of subsamples of the same extraction digest on the same day. Error caused by the preparation of samples was determined by analyzing different digests of a sample on the same day. Natural variation was determined by analyzing sets of samples of the same taxonomic group collected on the same date. Statistical variances (s^2) were calculated and the extent of each type of error was obtained by subtraction of the s^2 from the previous error. The coefficient of variation (CV) was calculated for all the samples for each kind of variation. Natural variation before subtraction of s^2 included all types of variation and is considered the overall variation. The average CVs resulting were as follows: analytical instruments: 2%; preparation of samples: 9%; and natural variation among individuals: 24%. No other reports exist for sources of error for the MeHg techniques used here. However, overall CVs of 40% are considered acceptable in most toxicological studies (Chapman 1991). Concentrations of MeHg and THg were not corrected for the overall error.

Statistical Analysis

The data were examined using analysis of variance (ANOVA; SYSTAT for DOS, Wilkinson 1990). When the null hypothesis was rejected at the 5% level, orthogonal contrasts were used to determine which main effects were significantly different. Details of statistical tests can be found in Appendix 1 and 2. ANOVA was used in the data analysis but caution should be used in interpreting the significance levels because of temporal pseudoreplication (Hurlbert 1984).

Question 1: Does flooding of a wetland pond cause an increase in MeHg concentrations in aquatic insects?

The effect of flooding on MeHg concentrations in insects was determined by comparing MeHg concentrations before and after periods of flooding. The lowest taxonomic levels possible (genus or family) were used and, for each taxon, individual one-way ANOVAs were performed using a combination of year, lake, and state of flooding as main effects. MeHg concentrations, %MeHg and the results of statistical analysis for all taxa are reported in Appendices 1 and 2.

Question 2: Are there differences in MeHg accumulation and %MeHg among FFGs?

Data within each FFG were organized by lake, year, and state of flooding, and the FFGs were used as the main effects in one-way ANOVAs to determine if 1. ratios were constant among FFGs, and 2. there were differences in MeHg concentration in the three FFGs.

Question 3: Is the ratio of MeHg to THg in aquatic insects constant response to flooding?

To determine the ratio of MeHg to THg, both MeHg and THg were analyzed on individual specimens when sufficient biomass was available. Ratios of MeHg to THg were calculated and lake, year, and state of flooding were used as the main effects in a one-way ANOVA to determine if ratios changed in response to flooding. The data were normally distributed, and therefore, not transformed.

Question 4: How do concentrations of MeHg in water, aquatic insects, and fish compare?

Concentrations of MeHg in the FFGs were compared to MeHg data in water (Kelly *et al.* submitted) and fish (Bodaly and Fudge 1994). This proportional increase in

MeHg concentrations above background levels over time was not tested statistically because different sampling intervals were used to collect the data.

Results

Does MeHg concentrations in aquatic insects increase after flooding of L979?

When examining the data at the genus or family level, MeHg concentrations tended to increase after flooding of L979, but most increases were not statistically significant because of the small numbers of samples for each taxon (see Appendices). When the insects were combined into FFGs, differences in MeHg concentrations before and after flooding and among FFGs became apparent.

Predators- MeHg concentrations in predators from L979 increased significantly (p value < 0.001, see Appendix 1) after flooding (Figure 2A) and was still elevated at the end of the study. Concentrations of MeHg were constant over time in the two reference systems (Figure 2A), except in L632 in 1992, and were similar to L979 preflooding conditions in 1993 (Figure 2A). In 1992, high numbers of Dytiscidae (*Dytiscus*) and Belostomatidae (*Lethocerus americanus* Leidy), with unusually high concentrations of MeHg accounted for the relatively high concentrations in both L979 (176.1 ng g^{-1} d.w.) and L632 (350.6 ng g^{-1} d.w.). If these genera are excluded, the 1992 averages in both L979 (83.8 ng g^{-1} d.w.) and L632 (103.1 ng g^{-1} d.w.) are similar to L979 before flooding in 1993. These genera were present in other years but in much lower numbers (Appendix 4).

Predator/herbivores- Predator/herbivores were not collected before flooding of L979 or from L240. MeHg concentrations in L979 after flooding were significantly higher than in L632 (Figure 2B; p values < 0.001, see Appendix 1).

Collector/shredders- The first significant increase in MeHg concentrations in collector/shredders in L979 occurred after reflooding in 1994 (Figure 2C; p values=0.005). Concentrations of MeHg in L632 in 1992 and 1993 were similar to those in L979 but also increased significantly in 1994, reaching levels similar to L979 after reflooding in 1994 (Figure 2C). Most (85%) of the collector/shredders in L632 in 1994 were late-instar *Siphlonurus*, that had relatively high MeHg concentrations. *Siphlonurus* were not present in L979.

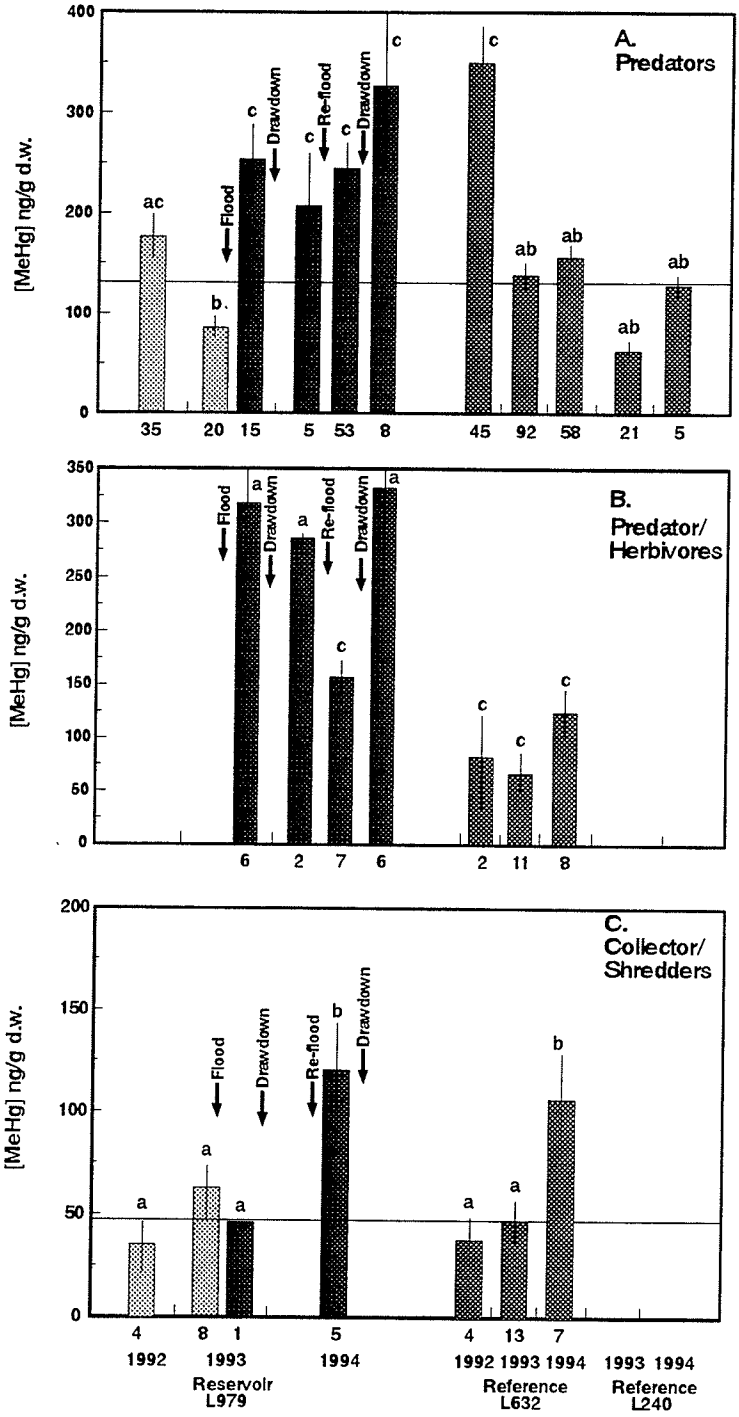


Figure 2. MeHg concentrations ± SE ng g^{-1} d.w. in aquatic insects: (A) Predators, (B) Predator/herbivores, (C) Collector/shredders. Numbers below bars are number of samples. Within each panel, bars with the same letter are not statistically different. Solid horizontal line represents average MeHg concentrations in preflood conditions (1992 and 1993), which are not available for (B). Light bars indicate preflood conditions, dark bars, flood conditions and medium bars, reference systems.

Do FFGs accumulate MeHg differently?

Ranges of average MeHg concentrations followed the order: predators (mean= 190.5 ng g^{-1} d.w., range= 19.0-715.5 ng g^{-1} d.w.) > predator/herbivores (mean= 175.7 ng g^{-1} d.w., range= 35.1-352.0 ng g^{-1} d.w.) > collector/shredders (mean= 71.5 ng g^{-1} d.w., range= 18.9-136.0 ng g^{-1} d.w.). There were significant differences between predators and collector/shredders ($p<0.001$ for tests see Appendices) and between predator/herbivores and collector/shredders ($p=0.003$). There was no difference between predators and predator/herbivores ($p=0.904$). When the MeHg:THg ratios were tested with FFGs as the main effect, the ANOVA and orthogonal contrast revealed a significant difference between predators and collector/shredders ($p=0.014$). There were no significant differences ($p=0.146$) in the percent MeHg in predators (average ratio=0.67) and predator/herbivores (average ratio=0.69), or in predator/herbivores and collector/shredders (average ratio=0.46).

Are the ratios of MeHg:THg in aquatic insects constant among lakes and before and after flooding?

MeHg:THg percentages for the three FFGs are shown in Figure 3. Total Hg concentrations are given in Appendix 5. Some ratios exceeded 1, which was a result of analytical variability in both MeHg and THg analysis. When the MeHg:THg ratios were tested across lake, year and state of flooding, no significant differences were observed ($p=0.098$). There was no significant difference in mean ratios of MeHg to THg among lakes or in response to flooding of L979.

How do concentrations of MeHg in water, aquatic insects, and fish compare?

Absolute MeHg concentrations increased from water through insects to fish that were held in pens in the wetland ponds (Figure 4). The proportional increases in MeHg concentrations over time are similar between predatory insects, water and fish, but dissimilar among fish, water and collector/shredders or predator/herbivores.

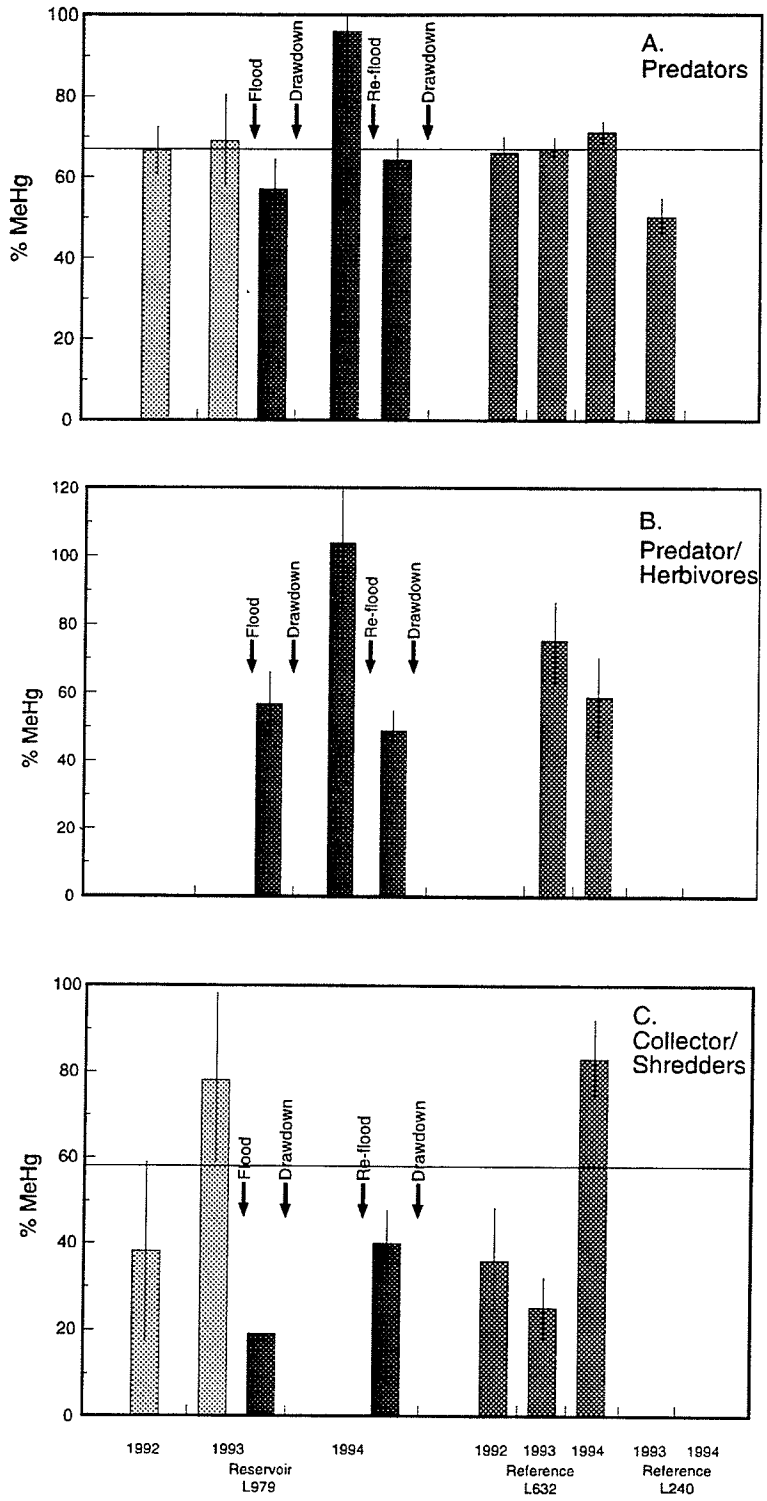


Figure 3. Percent of MeHg ± SE in (A) Predators, (B) Predator/herbivores and (C) Collector/shredders. Solid horizontal line represents average % MeHg in L979 preflood conditions (1992 and 1993), which is not available for (B). Hatching as in Figure 1.

Discussion

The effect of flooding on concentrations of MeHg in aquatic insects.

Summary of the ELA data

MeHg concentrations in aquatic insects increased in response to experimental flooding of the wetland. This increase was evident, but not always statistically significant, at the family or genus level (Appendix 1). There were statistically significant increases of MeHg in predators within a few months of flooding in 1993 (82.9 ng g^{-1} d.w. before flooding vs. 252.2 ng g^{-1} d.w. after flooding). Predator/herbivores in L979 after flooding had concentrations ~2 times higher than the reference L632, suggesting that this FFG also experienced increased MeHg concentrations in response to flooding. Concentrations in predators and predator/herbivores were elevated after flooding, indicating that effects of flooding last longer than one season, which is similar to observations of MeHg concentrations in fish from reservoirs (Anderson *et al.* 1995, Bodaly *et al.* 1984). This pattern of MeHg increase was not observed in collector/shredders, although the 1994 level differed significantly from previous years in L979 (Figure 3). Concentrations in collector/shredders were also high in L632 in 1994. In general, MeHg concentrations in predators from L240 and L632 were not significantly different from L979 before manipulation.

The life histories of the aquatic insects may be important in interpreting these results. For example, large numbers of belostomatids and *Dytiscus* beetles, raised the overall average of MeHg concentrations in 1992 in both the experimental reservoir and L632. These large insects may have higher MeHg concentrations than other insects because of differences in metabolism and exposure to bioavailable MeHg. There was no difference in the stable nitrogen isotope data for trophic levels of predators and predator/herbivores, which explains why accumulation of MeHg was not different among these two groups. However, the use of FFGs distinguished different taxa. When examining MeHg concentrations at the family or genus level MeHg concentrations vary. It may be more appropriate to use criteria such as details of the life history in addition to FFGs when addressing changes in MeHg concentrations.

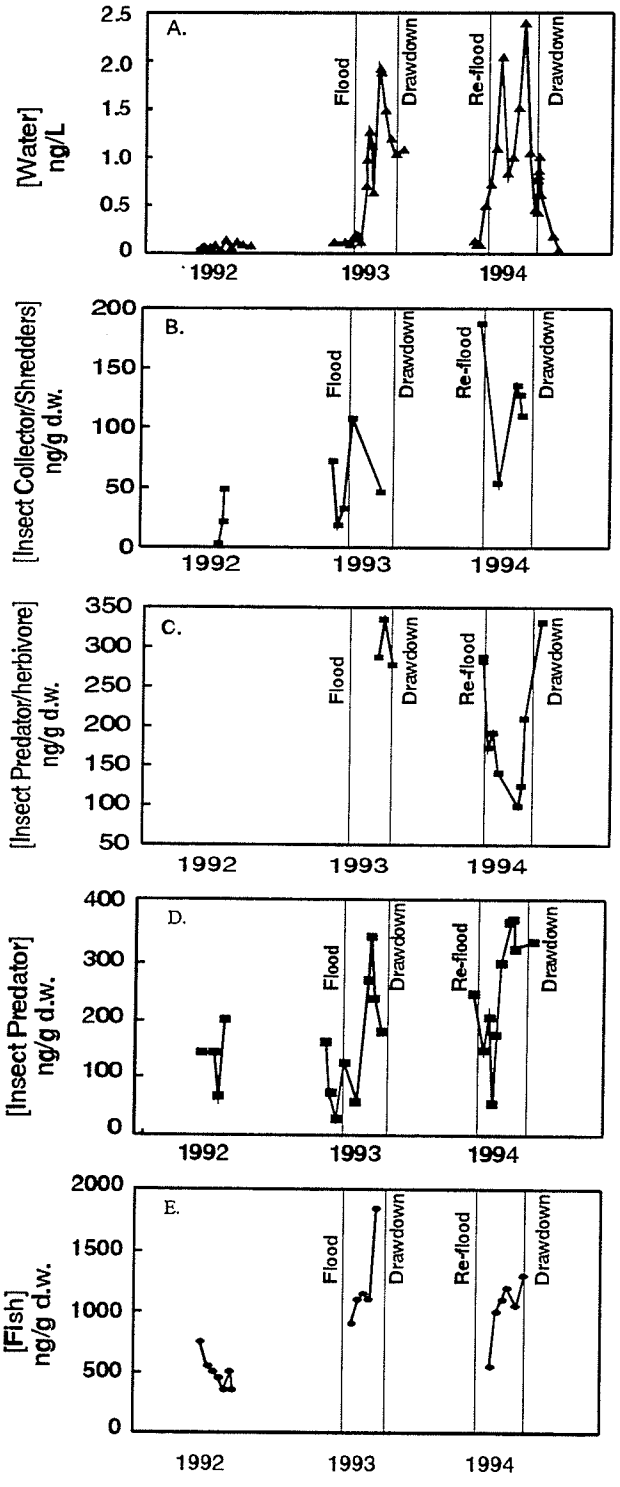


Figure 4. Concentrations of MeHg in water (A), Collector/shredders (B), Predator/herbivores (C), Predators (D), and fish (E) from Lake 979. Water data are from Kelly *et al.* (submitted); fish data are from Bodaly and Fudge (1994). Note scale differences.

Comparisons to other studies

Changes in MeHg concentrations in response to flooding by aquatic insects at the ELA can be compared to studies in Finland (Surma-Aho *et al.* 1986) and northern Québec, Canada (Tremblay *et al.* in press). In both of these studies, insects from large-scale reservoirs had higher concentrations of MeHg than those from natural lakes.

Surma-Aho *et al.* (1986) collected Odonata and Trichoptera, which were analyzed as one group, whereas Tremblay *et al.* (in press) analyzed individual taxa that were then grouped into FFGs similar to those used in this study. The results from all three studies can be compared by selecting data on ELA and Québec Odonata and Trichoptera, combining all data into either reservoir or unmanipulated lake categories and converting all MeHg concentrations to ng g^{-1} d. w. (Table 3). MeHg concentrations in Trichoptera and Odonata were ~2.5-3.0 times greater in reservoirs than in unmanipulated lakes (Table 3).

A more direct comparison can be made between the FFGs of Tremblay *et al.* (in press) and the present study. MeHg concentrations in collector/shredders from the 14 year-old Québec reservoir, LG-2, did not vary markedly from natural Duncan Lake, a similar result to our own (Figure 5). Predators and predator/herbivores in the Québec reservoir averaged 4 times more MeHg than those in Duncan lake (Tremblay *et al.* in press). MeHg concentrations in predators and predator/herbivores in flooded L979 were lower than in the LG-2 reservoir in Québec, but higher than in Duncan Lake (Figure 5). The similarity of MeHg concentrations in L979 and the Québec reservoir is remarkable considering the differences in surface area (1.67 km^2 vs 13672 km^2) and type of flooded land (peat vs podzolic soils and peat; Tremblay *et al.* in press). Moreover, insects from the Québec lake had similar MeHg concentrations to the ELA lakes used as references (L632, 240, and 979 before manipulation, Figure 5).

In both studies, FFGs accumulated MeHg to different degrees. Predators and predator/herbivores from ELA and from Duncan Lake in Québec had similar MeHg concentrations (~2 times higher than those in collector/shredders). In Québec reservoirs, MeHg concentrations in predators and predator/herbivores were 5 and 16 times greater, respectively, than in collector/shredders.

Table 3. Comparison of MeHg concentrations in Odonata and Trichoptera of three reservoir studies.

	MeHg concentration (ng/g d.w.)		
	Finland[1]	**Quebec**[2]	**ELA**[3]
Reservoir(s)	124.4	214.4	179.3
Lake(s)	51.6	62.3	83.3

note: [1]Values from Surma-Aho (1986) converted from mg/kg w.w. to ng g^{-1} d.w. (d.w./w.w. conversion factor=20%. [2]An average MeHg concentrations in insects taken from two reservoirs and one natural lake (Tremblay *et al.* in press). [3]An average MeHg concentrations in insects taken from flooded L979 (reservoir) and unmanipulated L979, L632 and L240.

MeHg:THg ratios

The ratio of MeHg:THg differed among FFGs. In our experimental reservoir, predators had significantly higher percentages of MeHg than collector/shredders. Neither group differed statistically from predator/herbivores. Insects from Québec reservoirs and Duncan Lake exhibited similar trends (Figure 6). In both the ELA and Québec studies, MeHg:THg ratios did not exhibit any statistically significant differences among lakes or in response to flooding. Because of the apparent lack of sensitivity of MeHg:THg ratios, MeHg concentrations should not be predicted from THg concentrations.

Bioaccumulation of MeHg along the aquatic insect food chain

The proportional increases in MeHg concentrations over time appears to be similar between water and predators but dissimilar between water and the the other FFGs. Peak water MeHg concentrations increased an average of 10 times in response to flooding (Kelly *et al.* submitted), but concentrations in predators exhibited maximum 3-fold increase. There may be time lags as the MeHg moves through the food compartments of the insects. The experimental reservoir is very complex and has not reached a steady state and changes in the

communities and environments of aquatic insects are far from complete. The move towards a steady state is continually disrupted in the drawdown/reflooding cycle.

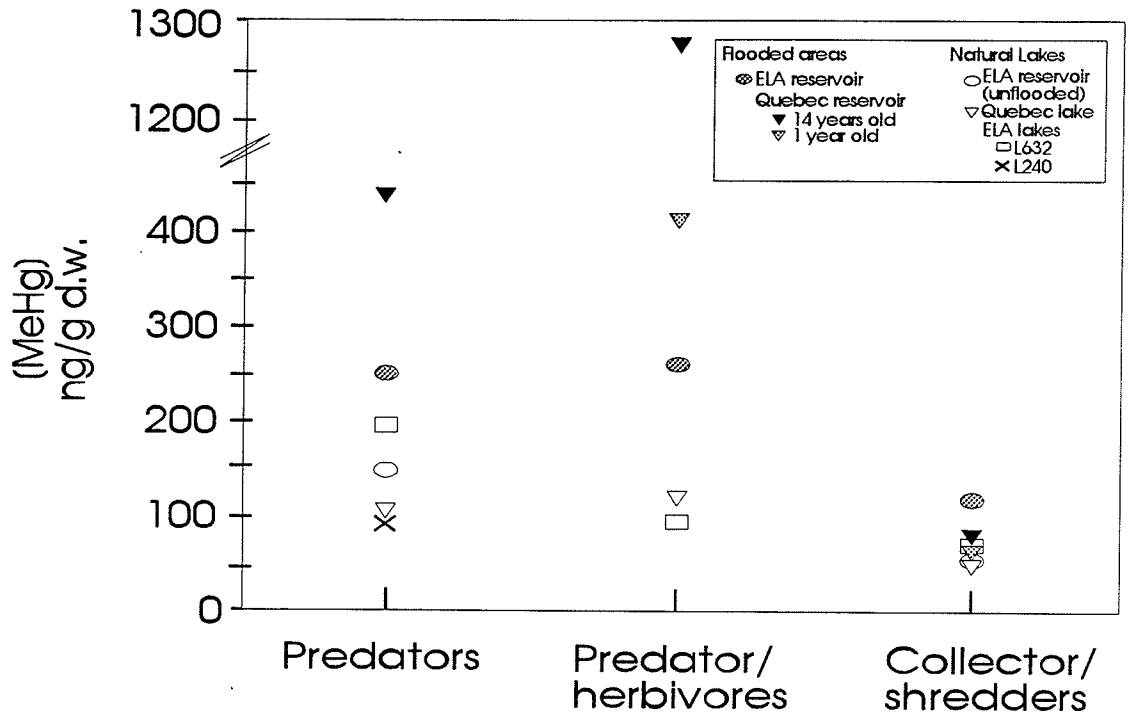


Figure 5. MeHg concentrations in aquatic insects from the Experimental Lakes Area flooding experiment (this study) and the Québec reservoir study (Tremblay *et al.* in press).

A number of hypotheses were considered to help clarify the relationships between the insect and water compartments and the lack of an obvious flooding response in collector/shredders. First, herbivores may be inefficient in the assimilation of MeHg (i.e. MeHg may be less easily taken up from plant material, detritus, or water than from animal tissues). Second, aquatic insects may be able to depurate MeHg, with some insects being more efficient than others. Third, FFGs are exposed to different carbon sources, and may experience different exposure to MeHg. Further research on the relative importance of different routes of uptake and depuration of MeHg by aquatic insects and tests of the importance of growth and metabolism in accumulation of MeHg by aquatic insects needs to be investigated in order to address these hypotheses.

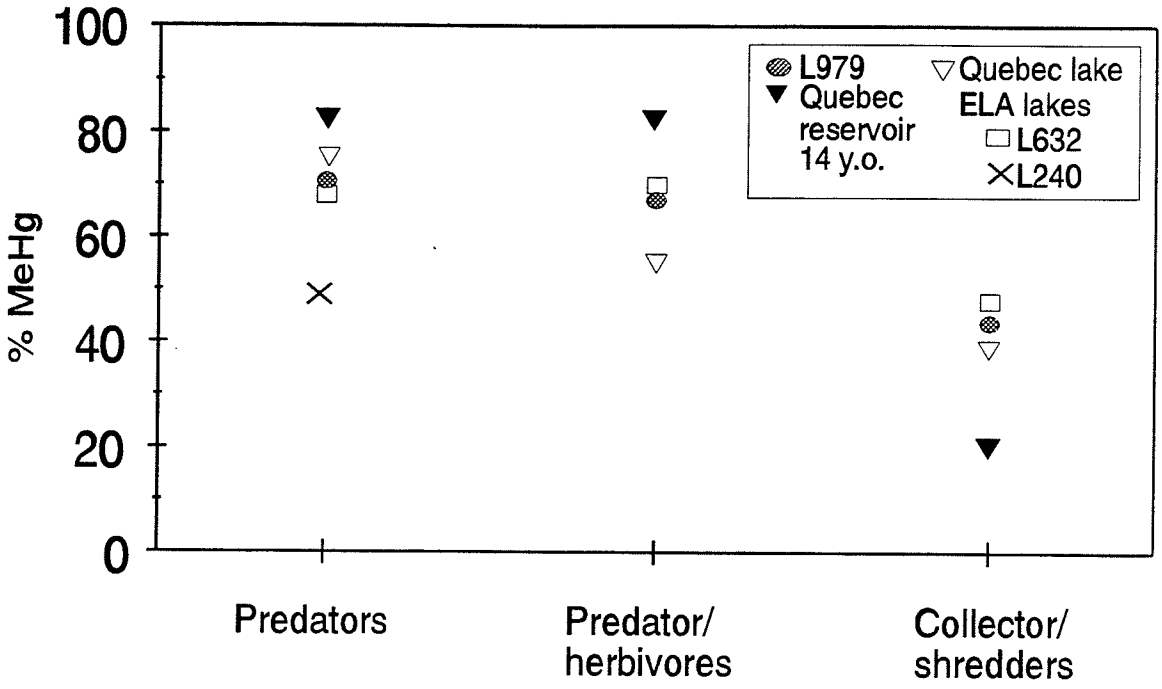


Figure 6. Comparison of percent methylmercury (MeHg) in insects in this study to Tremblay *et al.* (in press) (y.o.=years old).

The role of aquatic insects in bioaccumulation of MeHg in fish.

The results of Hg uptake experiment in Chapter 2 and model simulations of Rodgers (1994) and Harris and Snodgrass (1993), indicate that most Hg in fish comes from diet, so consistent increases in MeHg concentrations in aquatic insects in response to flooding can explain elevated Hg concentrations in fish in reservoirs. In our experimental reservoir, finescale dace (*Phoxinus neogaeus*) were held in pens to quantify changes in rates of MeHg uptake in response to flooding. The diet of these fish, as determined by gut content analysis, was primarily aquatic insects and zooplankton. Average rates of MeHg uptake after flooding in 1993 and 1994 were 0.73 and 0.64 μg Hg m^{-2} yr^{-1} respectively, compared to preflood rates of < 0.25 μg Hg m^{-2} yr^{-1} (R.A. Bodaly, Freshwater Institute, Winnipeg, MB, pers. comm.). This is equivalent to a 4-5 times increase in fish MeHg uptake in response to flooding (Kelly *et al.* submitted).

Conclusion

Aquatic insects act as part of a food web system that transfers MeHg from the physical environment to fish, and therefore increases in MeHg concentrations in aquatic insects are an important consequence of reservoir creation. The results from this study will be used to calibrate a predictive MeHg model that will help evaluate the changes in Hg concentrations in fish in response to flooding (R. Harris, Tetra Tech Ltd., pers. comm.). Further studies should explore effects of life history and behavior, e.g. life span, metabolism and growth, habitat selection and migration, on the bioaccumulation of MeHg in insects. As well, the importance of different uptake routes of MeHg must be established to help clarify how increases in MeHg concentrations in the physical environments of reservoirs lead to increased MeHg concentrations in insects, and eventually fish.

Acknowledgments

Assistance in the field and laboratory was provided by Pauline Gerrard, Rose Omole and Andrew Heyes. John Embury and Dr. Ray Hesslein provided samples for and analysis of stable isotopes. The manuscript was strengthened through early reviews and statistical advice by Susan Kasian and Dr. Micheal Paterson. Additional reviews by Drs. Diane Malley, Vince St. Louis, Terry Galloway, John Rudd, Lyle Lockhart and Drew Bodaly were much appreciated. Funding was provided by Manitoba Hydro and Department of Fisheries and Oceans.

Chapter 2: Food as the dominant pathway of methylmercury uptake by fish.[1]

Abstract. A field experiment was conducted to determine the degree to which fish accumulated methylmercury (MeHg) via their food or via passive uptake from water through the gills. Finescale dace (*Phoxinus neogaeus*) were held in 2000 L enclosed pens floating in an undisturbed, oligotrophic lake in northwestern Ontario. Fish were exposed to water containing either low (0.10-0.40 ng L^{-1}), intermediate (0.45-1.30 ng L^{-1}), or high (0.80-2.10 ng L^{-1}) concentrations of MeHg. Zooplankton with either low (0.16-0.18 µg g^{-1} d.w.) or high (0.28-0.76 µg g^{-1} d.w.) concentrations of MeHg were added daily to each pen. Fish fed zooplankton with high concentrations of MeHg had significantly higher concentrations of mercury in muscle after 32 days than fish fed zooplankton with low concentrations of MeHg (ANCOVA, $P < 0.0001$). Fish feeding on zooplankton with low concentrations of MeHg had the same amount of Hg in their tissues as fish at the start of the experiment. Uptake from water was at most 15%. This is the first experiment to confirm that food is the dominant pathway of MeHg bioaccumulation in fish at natural levels of MeHg.

Introduction

Threats to human health resulting from the consumption of fish containing high levels of methylmercury (MeHg) justify detailed studies of MeHg in natural aquatic ecosystems. Biomagnification of MeHg in aquatic food chains resulting in elevated concentrations of MeHg in fish tissue has been well documented (Bodaly *et al.* 1993, Cabana *et al.* 1994, Kidd *et al.* 1995, Spry and Wiener 1991). Fish with elevated MeHg concentrations in their tissues have been found in lakes with point sources of mercury (Hg) (Johnels *et al.* 1979), in remote lakes with only natural, watershed-derived and atmospheric inputs of Hg (Bodaly *et al.* 1993), in acid lakes (Winfrey and Rudd 1990) and in hydroelectric reservoirs (Bodaly *et al.* 1984).

Over 90% of the total Hg (THg) in fish tissue is MeHg (Spry and Wiener 1990). MeHg can be obtained by fish from food and from water as it passes over the gills during

[1] Accepted for publication by Water Air and Soil Pollution.

respiration. As well, MeHg can be produced within the fish's gastrointestinal tract (Rudd *et al.* 1980) and on the external slime layer (Jensen and Jernelöv 1969), but the amount of MeHg contributed to tissue concentrations by these processes has not been quantified and is assumed to be insignificant. Although the accumulation of MeHg from food and water may both be important, most researchers have assumed, without conclusive evidence, that food is the dominant pathway of MeHg uptake in fish (Jernelöv and Lann 1971, Phillips and Buhler 1978, Rodgers and Beamish 1981). Hg models predict that uptake via the gills is relatively small compared to that from food, based on aspects of fish physiology and environmental factors (Harris and Snodgrass 1993, Rodgers 1994).

This experimental field study was initiated to determine the relative importance of food and water to MeHg uptake in fish at natural concentrations of MeHg. This is the first study in which accumulation of MeHg at natural field levels has been examined, and presents strong evidence that food is the dominant pathway of MeHg uptake by fish.

Methods

Study design and clean techniques

The experimental design was a 2 x 2 factorial using food (zooplankton) and water with high and low concentrations of MeHg. Fish were held in 2000 L enclosed pens floating in Lake 240 (L240) at the Experimental Lakes Area in northwestern Ontario in the summer of 1993. The pens were constructed by fitting 2000 L impermeable nylon bags onto PVC frames equipped with floats and covered by window screening. Lake 240 was chosen because of its low MeHg water concentrations (average [MeHg]=0.09 ng L^{-1} from May to October, 1993; J.W.M. Rudd, unpublished data) to avoid contamination. Duplicate pens were randomly assigned to one of four water/food combinations (Figure 7).

Precautions were taken to prevent contamination of natural low levels of MeHg used in the experiment. A small amount of MeHg leached from the nylon pen material after soaking in lake water, so the 2000 L bags were acid washed and rinsed in low-MeHg L240 water prior to assembling the pens. Pumps and hoses used in water transfer were acid washed and tested as sources of contamination of MeHg. "Clean-hands dirty-hands" sampling protocols, as outlined in St. Louis *et al.* (1994), were followed for sampling water.

Zooplankton were collected using tow nets (400 µm mesh) and bottles rinsed in low MeHg water prior to each use. All equipment was stored in plastic bags in a designated clean shed. Samples of both zooplankton and water were taken regularly for MeHg analysis to confirm that contamination did not take place over the course of the experiment.

	High MeHg water L470	Low MeHg water L240
Low MeHg zooplankton L340	Low MeHg Food High MeHg water 2 pens 12 fish each	Low MeHg Food Low MeHg water 2 pens 12 fish each
High MeHg zooplankton L979	High MeHg Food High MeHg water 2 pens 12 fish each	High MeHg Food Low MeHg water 2 pens 12 fish each

Figure 7. Two-by-two factorial design of the uptake experiment (MeHg= methylmercury).

Water

Water from natural sources, consisting of either high or low MeHg concentrations, was used to fill the pens. Pens holding low MeHg water were filled directly from L240 using battery operated pumps and a 400 µm filter. High MeHg water (average [MeHg]=0.5 ng L^{-1}, June and July 1993) was taken from nearby Lake 470 (L470), a lake surrounded by wetlands, transferred to the pens using an acid washed PVC holding tank and added to the pens as above. Twenty percent of the water in each of the pens was changed three times a week. Water samples were not filtered prior to analysis; however, large particles were removed. Whole water was used for MeHg analysis because MeHg associated with particulates and/or dissolved organic carbon (DOC) exists in equilibrium with, and is therefore readily exchangeable with, water (Watras *et al.* 1994). Thus, DOC can be considered to be a reservoir of MeHg that is in flux with the surrounding physical environment and the biota (e.g. Driscoll *et al.* 1994). In addition, analysis of whole water ensured adherence to a natural situation. Samples were analyzed by Flett Research Ltd., Winnipeg, MB, using Bloom's

(1989) procedure as modified in Horvat *et al.* (1993; detection limits=0.01-0.02 ng L^{-1} at a blank level of 0.05-0.1 ng L^{-1}). Flett Research Ltd. successfully participated in the recent interlab comparison for MeHg analysis (Bloom *et al.* 1995).

An increase in water MeHg concentrations with the addition of high MeHg zooplankton resulted in the fish being exposed to water containing either low (0.10-0.40 ng L^{-1}), intermediate (0.45-1.30 ng L^{-1}), or high (0.80-2.10 ng L^{-1}) concentrations of MeHg (Figures 8, 9). The unexpected elevated MeHg concentrations in the water resulted from either leaching of MeHg during decomposition of dead zooplankton or equilibration of levels of MeHg in living zooplankton with the water.

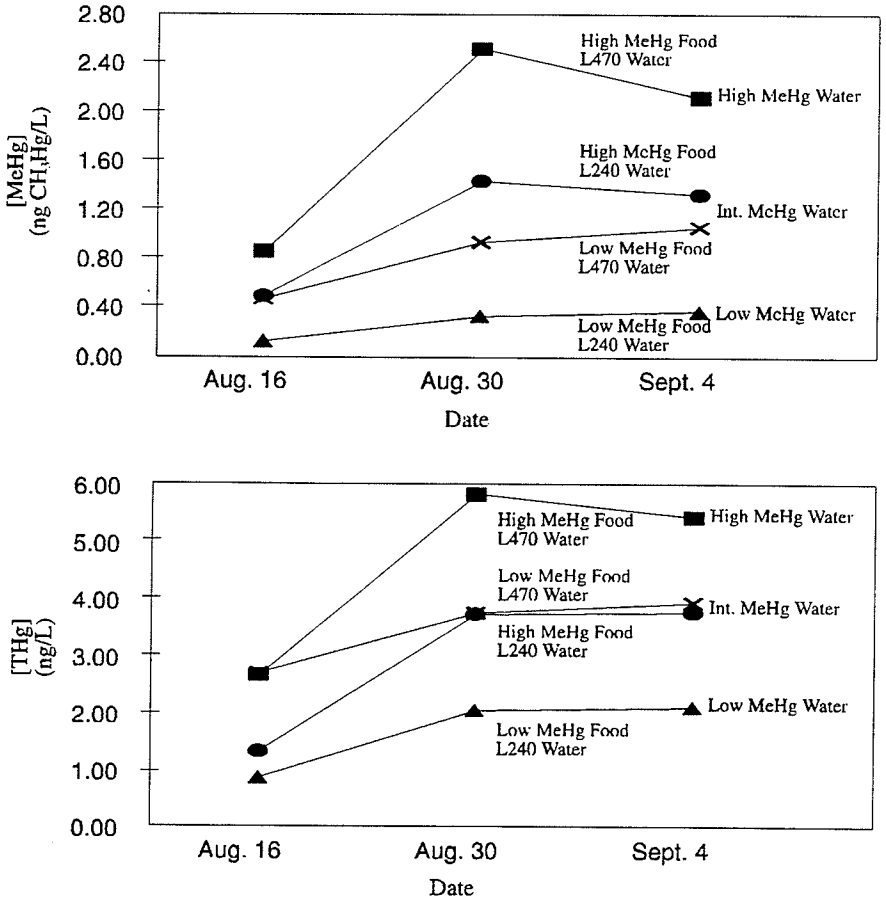


Figure 8. Concentrations of methylmercury (MeHg) and total mercury (THg) in the water held in the experimental pens. A sample was taken near the beginning, middle and end of the 32-day experiment (L= lake; Int.= intermediate).

	High MeHg water L470	Low MeHg water L240
Low MeHg zooplankton L340	Low MeHg Food Int. MeHg water 2 pens 8 and 11 fish	Low MeHg Food Low MeHg water 2 pens 11 and 12 fish
High MeHg zooplankton L979	High MeHg Food High MeHg water 2 pens 9 and 10 fish	High MeHg Food Int. MeHg water 2 pens 10 and 9 fish

Figure 9. Revised experimental design after zooplankton addition and consequent increase in water methylmercury (MeHg) concentrations (L=lake; Int.= intermediate).

Weekly water chemistry samples were taken from each pen and analyzed for DOC (OI Corporation model 700 Carbon Analyzer with calibration to glucose standard), pH (*in situ* measurements with an Orion Ross Sureflow pH electrode) and calcium (Ca^{+2}) concentrations (Stainton *et al.* 1977). Average error between replicate pens was 2.3% (range:0-11%). Temperature and oxygen (YSI oxygen probe) in the pens were monitored regularly and were similar to the levels in L240. Mid-day temperatures ranged from 20.6-22.3 °C (Appendix 6). Water held in experimental pens remained at or near oxygen saturation.

Food

Zooplankton was analyzed in the Experimental Lakes Area Reservoir Project (ELARP) Mercury Laboratory at the Freshwater Institute by atomic absorption spectrophotometry (AAS) after organic partitioning into hexane and methylene chloride (Malley *et al.*, in press). This method is used to measure all organic forms of Hg with a method detection limit of 10 ng Hg g^{-1}. We assumed that the organic Hg concentrations measured were all MeHg, although small amounts of dimethyl-mercury (DMHg) may have been present (Horvat *et al.* 1993). Zooplankton with either high (0.28-0.76 μg g^{-1} d.w.) or

low (0.16-0.18 μg g^{-1} d.w.) concentrations of MeHg were collected and added to each pen daily. Zooplankton with low concentrations of MeHg were collected from Lake 304 (L304), a small fishless lake. Lake 979 (L979), an experimentally flooded wetland pond, was the source of the high MeHg zooplankton. Zooplankton community structure differed in the two lakes so, to ensure fish were receiving similar amounts of sustenance, dry/wet weight relationships were determined weekly and used to calculate the quantity of live zooplankton added to each pen on a dry weight basis. On a given day, all pens received the same dry weight of zooplankton. Amount per day varied from 0.025-0.125 g d.w. per fish. Neither the growth of the fish (Table 4) nor mortality (Figures 7, 9) were related to the source of zooplankton fed to fish.

Fish

Finescale dace (Cyprinidae: *Phoxinus neogaeus*) were obtained from a commercial bait fisherman and transported to L240 in oxygen-saturated water in plastic bags. After acclimatization to pen temperature for 1/2 h, 12 fish were added to each pen. THg was determined on one fillet from each fish randomly selected from the initial stock at the start of the experiment and on all penned fish surviving at the end of the 32-day experiment. Cold vapour AAS was used (method detection limit of 10-25 ng Hg g^{-1}; Hendzel and Jamieson 1976, Armstrong and Uthe 1971). Reference material (National Research Council of Canada (NRCC) dogfish muscle, DORM-1) was analyzed coincidentally with experimental fish samples. Average concentration of DORM-1 material was 768.5±10% ng g^{-1} d.w., which was within the certified range of 724-862 ng g^{-1} d.w. To determine significance of differences of THg concentrations in fish from different treatments, an ANCOVA was performed on log-transformed fish THg concentrations. High and low MeHg zooplankton were the main effects and MeHg concentrations in water was the covariate. The two different food sources were used as a main effect in a one-way ANOVA to test the significance between any differences in fish weight.

Results and Discussion

Fish fed zooplankton with high concentrations of MeHg had significantly higher concentrations of Hg in muscle than fish fed zooplankton with low concentrations of MeHg

(ANCOVA, $p<0.0001$; Figure 10). The Hg concentrations of fish that fed on zooplankton with low concentrations of MeHg were not significantly different from those in fish at the start of the experiment. The significant increase in fish Hg concentrations in those fish eating high MeHg zooplankton is an indication that food was the dominant pathway of MeHg uptake by fish.. Differences between average Hg concentrations of fish from duplicate pens were not significant (one-way ANOVA, Table 4).

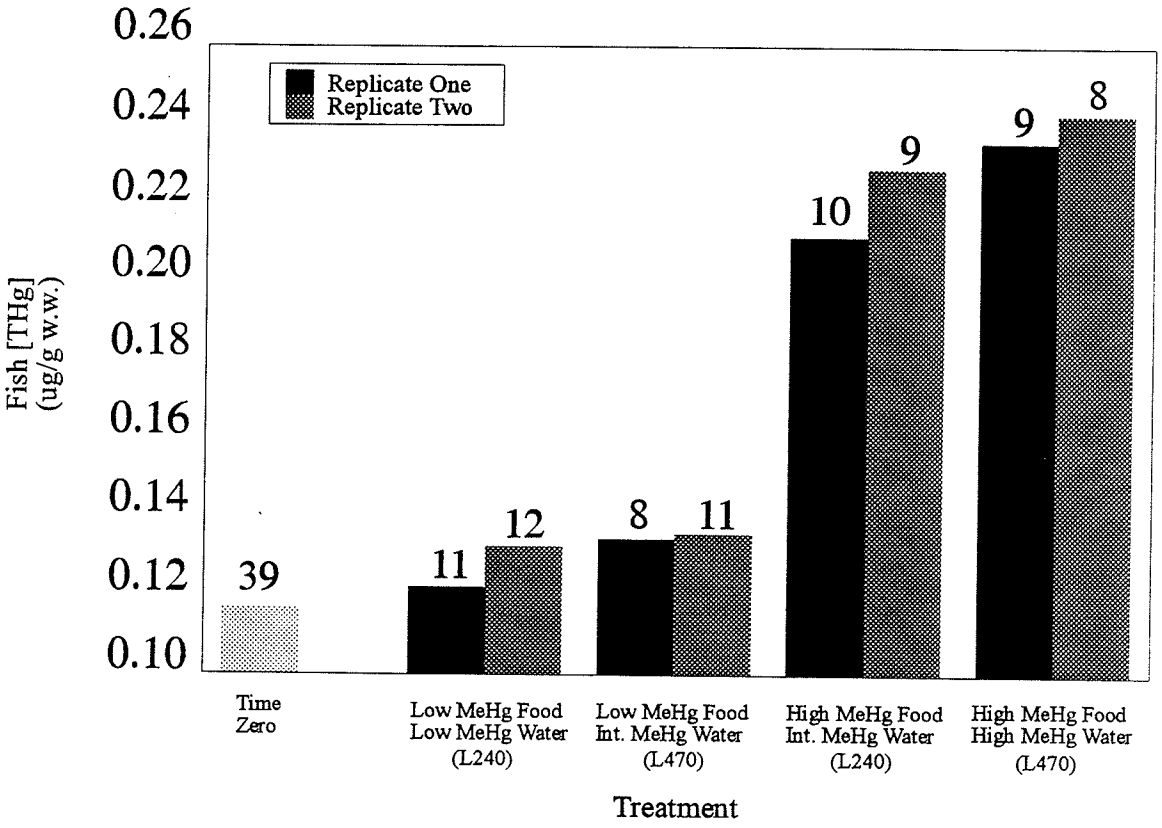


Figure 10. Total mercury (THg) in fish tissue at the beginning (Time Zero) and end of the experiment. Number of fish analyzed is shown above bars (Int.= intermediate).

The fish either maintained their weight or lost between 0.3 and 1.13 g over the course of the experiment (Table 4). However, weight loss was not dependent on the type of food fed to the fish (one-way ANOVA, $p=0.982$). The relatively small weight loss indicates significant feeding. Using parameters from the Wisconsin Bioenergetics model (Hewett and Johnson 1992) and the average ambient temperature of the water in the pens, the theoretical amount of food required for fish to experience the moderate weight loss over the course of

Table 4. Mercury (Hg) concentrations and weights of experimental finescale dace (±SEM). Roman numerals in column three are used in the text. (MeHg=methylmercury, Int.=intermediate).

Treatment	Hg Conc. ug/g wet wt. per pen	mean	Percent difference from time zero	Average initial weight (g)	Average final weight (g)
Time Zero	0.117 ± 0.009				5.08 ± 0.112
Low MeHg food, low MeHg water[1]	0.112 ± 0.011	(I) 0.123		4.77	4.08 ± 0.187
	0.133 ± 0.013		4.6 12.0	4.42	4.05 ± 0.234
Low MeHg food, int. MeHg water[2]	0.135 ± 0.015	(II) 0.136	13.3	5.08	4.34 ± 0.244
	0.136 ± 0.006		14.0	4.96	3.83 ± 0.192
High MeHg food, int. MeHg water[3]	0.212 ± 0.007	(III) 0.221	44.8	4.60	4.60 ± 0.111
	0.229 ± 0.015		48.9	4.98	4.53 ± 0.637
High MeHg food, high MeHg water[4]	0.236 ± 0.019	(IV) 0.240	50.4	4.73	4.06 ± 0.122
	0.243 ± 0.023		51.9	4.76	4.05 ± 0.246

note:

Results from ANOVAs testing differences between pens: [1] p=0.544, [2] p=0.932, [3] p=0.288, [4] p=0.827.

the experiment was determined. The model predicts that fish consumed 10.3 g of food over 32 days. This ration is equivalent to 0.34 g wet weight per day or ~7 % of a 5 g fish's body weight. During the experiment, a mean of 0.78 g w.w. (range: 0.25-1.25 g w.w., using a 10% wet weight/dry weight conversion factor) of zooplankton was fed to the fish each day, which is similar to the estimated consumption. Therefore the fish consumed most of the food added to the pens, and were eating enough food to assimilate Hg into body tissues.

There was a small but measurable uptake of MeHg from the water. Changes in fish MeHg concentrations attributable to uptake of MeHg from the water were equal to the difference in the mean concentrations of THg in fish tissue between fish fed the same food but held in water with different MeHg concentrations (Table 4, Col. 3: II-I=0.013 and IV-III=0.019 $\mu g\ g^{-1}$ THg). The resulting concentrations (0.013 and 0.019 $\mu g\ g^{-1}$) were relatively small compared to those attributable to uptake of MeHg from food. The latter were calculated by comparing the mean THg concentrations of fish fed high MeHg food to those fed low MeHg food and held in water with comparable MeHg concentrations (Table 4, Col. 3: III-I=0.098, III-II=0.085, and IV-II=0.104 $\mu g\ g^{-1}$ THg). Thus, direct absorption from the water may have been responsible for ~15% of the Hg uptake in fish muscle.

If elevated MeHg concentrations in water were a result of loss of MeHg from zooplankton into water, then MeHg concentrations in zooplankton may have decreased before consumption by fish. Thus, fish fed high MeHg zooplankton may have been exposed to less MeHg via food than was measured by analyzing zooplankton, and the estimated 85% uptake from diet would be conservative.

Although uptake of MeHg from water was small and relatively insignificant as compared to uptake from food, chemical differences between the two sources of water may have had an effect on the uptake of MeHg from water. This could have happened either by affecting gill permeability (Rogers and Beamish 1983) or by changing the amount of bioavailable (dissolved) MeHg (Watras *et al.* 1994). With respect to gill permeability, fish from waters with elevated Ca^{+2} concentrations (Rodgers and Beamish 1983) or high pH (Winfrey and Rudd 1990) tend to have lower tissue MeHg concentrations than fish from waters with low Ca^{+2} concentrations and low pH. Ca^{+2} concentrations and pH in this study

were examined to determine if differing chemical characteristics of water had any effect on uptake from water. If the chemical composition of high MeHg water was preventing MeHg uptake from water, then high MeHg water should have higher Ca^{+2} concentrations and higher pH than low MeHg water. However, high MeHg water had lower Ca^{+2} concentrations and lower pH than low MeHg water (Figure 11). Therefore, Ca^{+2} concentration and pH characteristics of water with high concentrations of MeHg are opposite to what would be expected if the chemical characteristics of the water source were confounding the results of the experiment.

A similar trend was observed for chloride (Cl^-) ions, which may enhance uptake of MeHg from water by forming membrane permeable MeHg complexes (MeHgCl; Boudou *et al.* 1983). Average Cl^- concentrations in low MeHg water and high MeHg water in 1993 were 0.327 and 0.134 mg L^{-1} (M.P. Stainton, unpublished data), respectively, opposite to the expected effect if uptake from water via MeHgCl complexes were observed.

DOC may also have affected MeHg uptake from water. Humic and fulvic acids bind MeHg to varying degrees depending on their concentrations and the pH of the water (Hintlemann *et al.* 1995). This binding decreases the free MeHg concentration and its uptake from water. The DOC concentrations in our experiments (650-1100 µm, Figure 11) were similar to the humic acid concentrations (830 µm) used in experiments of Hintlemann *et al.* (1995). Assuming that the relationship between pH and dissolved MeHg was the same in the ELA water as in Fawn Lake (Hintlemann *et al.* 1995), we estimated that in our experiment about 20% of the MeHg was free at pH 8.5 and about 25% at pH 6.5 (Figure 11). This small difference in percent free MeHg among our treatments would not have affected our interpretation of MeHg uptake from water. Although examination of the chemical characteristics of the water is important in evaluating the effects of water chemistry on MeHg uptake from water, water is contributing, at most, 15% of the Hg to fish. Thus, water chemistry is not an important determinant of MeHg bioaccumulation by fish.

This investigation supports conclusions obtained from past studies (Jernelöv and Lann 1971, Rodgers and Beamish 1981, Phillips and Buhler 1978), which were laboratory-based and done at unnaturally high MeHg concentrations. Also, these studies were done prior

to the development of clean-sampling procedures and ultra-sensitive analytical techniques for measurement of low concentrations of MeHg. In another group of studies with designs similar to the experiment described here (Rodgers and Beamish 1981, Phillips and Buhler 1978), most MeHg taken up by fish was from the diet, and water contributed ~10% of the MeHg assimilated by fish. In a factorial field experiment done by Parks *et al.* (1987), using

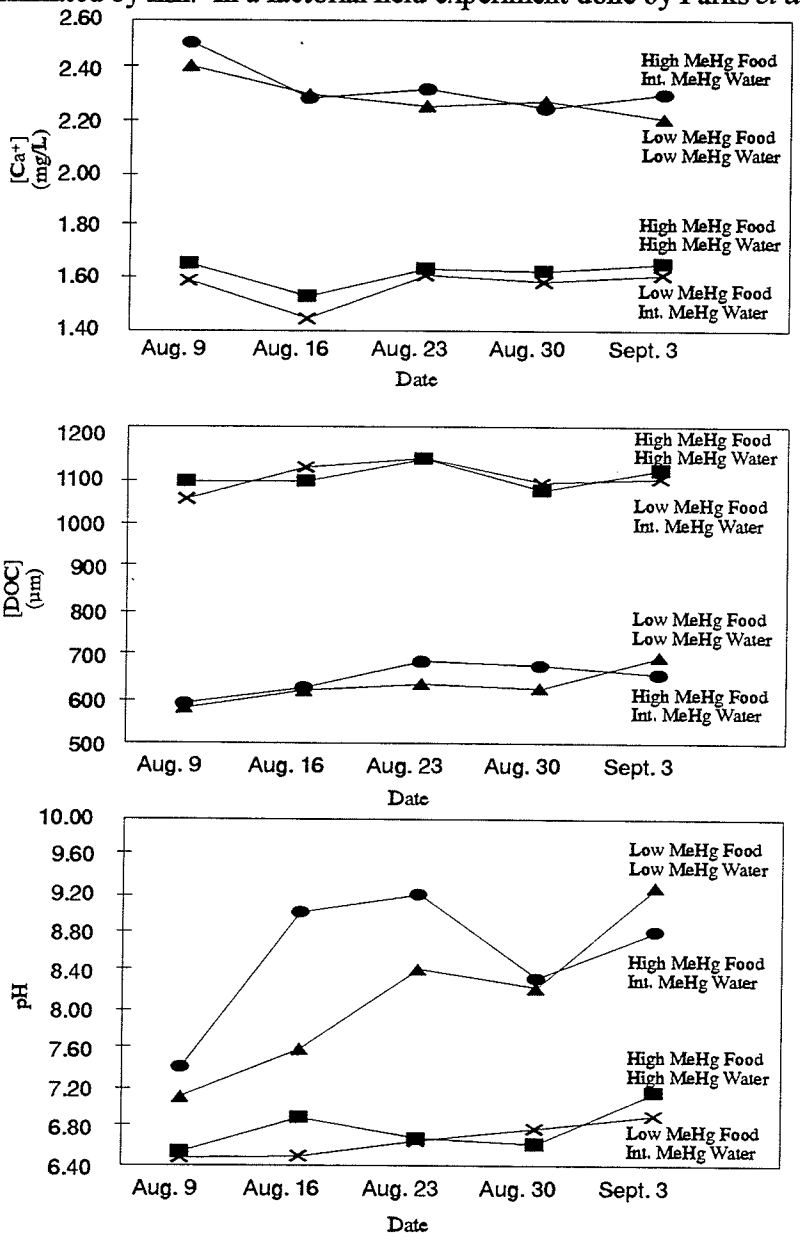


Figure 11. Average results of weekly water chemistry analysis from each pen (MeHg= methylmercury; Lake 470= rectangle and cross; Lake 240= oval and triangle; Int.= intermediate.)

crayfish in field situations (Hg-contaminated and -uncontaminated rivers) food was also important in MeHg uptake.

The results of this experiment also agree with predictions made from bioenergetic mercury models. For example, Rodgers (1994) conducted three simulations using yellow perch (*Perca flavescens* Mitchill) and lake trout (*Salvelinus namaycush* Walbaum) and reported that diet was responsible for a large proportion of MeHg uptake. Harris and Snodgrass (1993) predicted that food pathways were responsible for 90% of MeHg uptake in walleye (*Stizostedion vitreum* Mitchill) and yellow perch. Both of these models used MeHg concentrations at the ng L^{-1} level in water, which approximates concentrations in natural waters.

Conclusions

Food was the dominant pathway of MeHg uptake by planktivorous fish at natural concentrations of MeHg. Only ~15% of MeHg was taken up from the water, given the chemical characteristics and MeHg concentrations prevailing in the experiment. These results confirm theoretical modelling studies and indicate the need for increasing emphasis on food-chain factors affecting the transfer of MeHg to fish.

Acknowledgments

We would like to thank Sheena Majewski, John Embury, Vince St. Louis, Allen Wiens and Andrew Heyes for field assistance. Bob Hunt, Karen Scott and Rose Omole assisted in THg and MeHg analyses. The ELA Chemistry Lab provided water chemistry data. Brigitte de March and Susan Kasian provided statistical advice. Reed Harris of Tetra Tech provided modelling expertise. We thank Vince St. Louis, Michael Paterson, and Carol Kelly for advice on experimental design. Reviews were provided by Jack Klaverkamp and two anonymous reviewers. This study was supported financially by Manitoba Hydro and the Department of Fisheries and Oceans.

Final Considerations

The results presented and discussed in Chapters 1 and 2 generate additional issues pertaining to the design of the fish Hg-uptake experiment, ELARP and needs for future research on the biogeochemical cycling of MeHg. These are addressed in the following discussion.

Design of the fish Hg-feeding experiment

Pilot study

A pilot experiment was conducted in early summer 1993 to test the feasibility of the design of the fish Hg-uptake experiment described in Chapter 2. The basic design of the pilot experiment was a 2 x 2 factorial using the same water sources (L240 [MeHg] = 0.16 ng L^{-1}, L470 [MeHg] = 0.48 ng L^{-1}) as the final uptake experiment. High MeHg zooplankton were obtained from L632 (~0.1 μg g^{-1}; M. Paterson, Freshwater Institute, Winnipeg, MB, unpublished data) and low MeHg zooplankton from L240 (0.08 μg g^{-1}). The concentrations of MeHg in the test waters remained relatively constant over the course of the experiment. The results of the pilot experiment are presented in Appendix 8. There were no differences in the concentrations of THg in fish muscle among treatments.

Several reasons for this lack of differentiation in THg concentrations among treatments were identified and the methods for the next uptake experiment were adjusted to address these concerns. First, the fish were not receiving enough food, as evident by the greater weight loss in fish in the pilot experiment than those in the final uptake experiment. Therefore, the amount of food fed to the fish daily in the final uptake experiment was doubled. Second, the difference between the high and low MeHg zooplankton was not large enough to allow for a difference in accumulation over the course of the experiment. To rectify this, the source of high MeHg zooplankton was changed from L632 (~0.1 μg g^{-1}) to L979 (0.28-0.76 μg g^{-1}). Third, the length of the pilot experiment was too short, so it was increased from 27 to 32 days to allow fish longer exposure times to different treatments. Fourth, time-zero fish in the pilot

experiment had mean THg concentrations of 0.24 μg g^{-1}, double those of the fish used in the final uptake experiment (0.117 μg g^{-1}). Because of the high time-zero concentrations, the increase in Hg concentrations may not have been noticeable over the 27 d time course of the pilot experiment. Finally, the pilot experiment was run during a period of below-normal temperatures. Water and air temperatures during the final uptake experiment were average for that period in the summer.

Zooplankton vs. aquatic insects as food

The link between Chapters 1 and 2 may have been made stronger had we used aquatic insects rather than zooplankton as fish food. However, it would have been difficult, if not impossible to collect and quantify macroinvertebrate food. In addition, the experimental fish are gape limited, so the macroinvertebrates fed to them would have to be sorted to size. The use of zooplankton ensured that we would be able to collect sufficient fish food and that fish were able to eat all the food provided to them.

Replication and scale in ELARP

The only true replication of the insect bioaccumulation study would be the controlled flooding of an additional peatland pond. However, as with other whole-ecosystem experiments, the key is to demonstrate realistic effects. The ELARP has produced data that show dramatic changes in MeHg cycling within the experimental reservoir, and increases in the fluxes of CO_2 and CH_4 out of the reservoir (Kelly *et al.* submitted). These processes are also evident from other reservoir studies (e.g. Duchemin *et al.*, J.W.M. Rudd, Freshwater Institute, Winnipeg, MB,.pers. comm.)

Similarities in the dynamics of MeHg in both the small experimental and large hydroelectric reservoirs demonstrate that the understanding obtained from the ELARP can be applied to large-scale reservoirs. The use of a whole-ecosystem approach provides information on processes occurring in nature, an understanding that can be difficult to extrapolate from laboratory and mesocosm-based studies. It also allows the study of the interactions of different compartments within the aquatic ecosystem. The understanding gained by the use of whole-ecosystem manipulation studies transcends the problems associated with the inability to replicate and the comparison to large scale systems.

Future research needs

Continued examination of the MeHg dynamics of organisms at the lower end of the food web is important in interpreting MeHg concentrations in response to reservoir creation. Biological (age: Huckabee *et al.* 1979; metabolism: Rodgers and Beamish 1981) and physical/chemical (pH: Winfrey and Rudd 1990; temperature: Bodaly *et al.* 1993) factors affecting MeHg accumulation in fish are well understood. However, it is unclear how differences in environmental, ecological and physiological factors affect bioaccumulation of MeHg in aquatic insects.

Examples of the effects of environmental parameters on the accumulation of Hg by aquatic insects can be found in two studies done in the field and laboratory. Parkman and Meili (1993) concluded that THg concentrations in benthic insects were highest in acidic dystrophic lakes, less in neutral dystrophic and mesotrophic lakes, and lowest in insects from oligotrophic lakes. Total Hg concentrations in insects were negatively correlated with pH and positively correlated with colour (an indication of DOC concentrations). Insects living in anoxic sediments tended to have higher THg concentrations than those in oxic sediments.

The effects of temperature, pH and photoperiod on THg concentrations were examined by Odin *et al.* (1994) by exposing *Hexagenia rigida* (McDunnough) to MeHgCl-contaminated sediments. Total Hg concentrations in insects were positively correlated with temperature and pH. Although these two studies indicated that environmental conditions influenced the accumulation of MeHg by aquatic insects, it is important to do other studies using natural, rather than elevated, concentrations of MeHg, and to do analyses of MeHg in addition to THg concentrations.

Methyl Hg accumulation may differ among taxa, because of differences in ecological and physiological factors such as life cycles (e.g. voltinism, length of each life stage), ecological niches (e.g. habitat, feeding behaviour), and metabolic processes (e.g. growth rates, depuration of MeHg). For example, if *Lethocerus americanus* Leidy have a one-year life span, the amount of growth must be greater than that of smaller predatory insects of the same age. Greater growth will be a result of increased feeding and greater assimilation of carbon, which means greater exposure to MeHg. In fact, Parkman and Meili

(1993) found that THg concentrations in aquatic insects were correlated with body size, and concluded that this may be caused by large animals feeding on large prey.

Exposure to environmental MeHg may change during the course of the life cycle. Examination of insects of similar size but having different life cycles may provide insights into MeHg bioaccumulation. For example, insects that go through periods of dormancy may have different exposure to bioavailable MeHg than insects that do not. Insects with a high turnover might be more likely to respond quickly to changes in MeHg concentrations in water and/or food sources.

We also need to have a better understanding of the routes of MeHg uptake and depuration by aquatic insects. Saouter *et al.* (1993) concluded that MeHg uptake in Ephemeroptera depends on which compartment (sediment or water) is contaminated with high levels of MeHg. If the water contained high concentrations of MeHgCl, uptake occurred through the gills. In contaminated sediment, uptake was from the gut.

The relative importance of food and water as routes of uptake in aquatic insects needs to be addressed. A laboratory uptake experiment, similar to the L240 fish experiment, using large aquatic insects and natural concentrations of MeHg in food and water may be appropriate.

The pool of MeHg represented by benthic invertebrates and the loss of MeHg from aquatic ecosystems through the emergence of aquatic insects are important in flux calculations and are currently being explored in ELARP (D.M. Rosenberg, Freshwater Institute, Winnipeg, MB unpublished data). It would be interesting to quantify the total amount of MeHg leaving the system via drifting and emergence of all adult insects. These measurements would indicate the contribution of insects to the biogeochemistry of MeHg in terrestrial and downstream systems, and would also help clarify the role of aquatic insects in the transfer of MeHg from sediments to other lake compartments.

The duration of elevated levels of mercury methylation needs to be determined by intensive study of reservoirs of different ages. This would enable the prediction of the time required for MeHg concentrations in the physical and biological compartments to return to pre-impoundment levels.

The results of the ELARP are being used to calibrate a model that will predict changes in MeHg concentrations and fluxes in reservoirs (R. Harris, Tetra Tech Ltd., Oakville, ON pers. comm.). However, ELARP entailed the flooding of a peatland pond, whereas large-scale hydroelectric reservoirs flood both upland and wetland areas. Upland areas do not produce as much MeHg as wetland areas (St. Louis *et al.* 1995). The next step in understanding the effects of reservoir creation on MeHg cycling is the controlled experimental flooding of a purely upland area. Results from an upland-flooding experiment could contribute to calibrating a model that would more accurately predict MeHg concentrations based on the percent of peatland and upland areas flooded.

The main factor in the bioaccumulation of MeHg in fish is the production of bioavailable MeHg. Factors affecting the supply of MeHg via microbial methylation need to be examined fully at natural concentrations. Understanding the mechanisms behind the production and biogeochemical cycling of MeHg will help in planning reservoir mitigation strategies.

General Summary

This thesis had two objectives: 1. to quantify MeHg concentrations in aquatic insects in response to experimental flooding, and 2. to determine the dominant pathway of MeHg uptake by fish. Some aspects of MeHg accumulation by aquatic organisms were clarified; however, many details remain unclear. In this section, the results of my work are summarized.

Bioaccumulation of MeHg by aquatic insects in an experimental reservoir

This study is the first to examine MeHg concentrations in aquatic insects before and after experimentally flooding a wetland. There was a 2-3 fold increase in MeHg concentrations in predators and predator/herbivores in response to flooding. Collector/shredders also concentrated MeHg, but to a lesser degree. Increased MeHg concentrations in aquatic insects make more MeHg potentially available to fish. In addition, the amount of MeHg stored in the aquatic biota increases, so aquatic insects may play an important role in moving this stored MeHg within and out of the aquatic ecosystem.

Ecology is an important factor in the behaviour of MeHg in aquatic insects. Different types of insects accumulated MeHg to varying degrees. Concentrations and % MeHg were similarly high between predators (MeHg range= 19.0-715.5 ng g^{-1} d.w., % MeHg= 67%) and predator/herbivores (MeHg range= 35.1-352.0 ng g^{-1} d.w., % MeHg= 69%), but were lower in collector/shredders (MeHg range= 18.9-136.0 ng g^{-1} d.w., % MeHg= 46%).

Methyl Hg concentrations in aquatic insects in the newly flooded reservoir were elevated 2 yr after flooding. The new reservoir is not yet at a state of equilibrium and there is no indication of how long concentrations in insects will remain elevated. Methyl Hg concentrations in aquatic insects from the ELARP are being used as part of a predictive MeHg model that will evaluate changes in Hg concentrations in aquatic biota in response to flooding (R. Harris, Tetra Tech Ltd., Oakville ON, pers. comm.).

Food as the dominant pathway of MeHg uptake by fish

The feeding experiment using fish showed that at least 85% of the Hg in fish muscle is attributable to food, whereas water contributed at most 15%. These results support conclusions obtained from past studies (Jernöv and Lann 1971, Rodgers and Beamish 1981, Parks *et al.* 1987). Conclusions also agree with predictions made using bioenergetic mercury models (Rodgers 1994, Harris and Snodgrass 1993). This body of work illustrates the importance of the lower food web and shows that MeHg concentrations in the water and animals of lower trophic levels must be known to predict fish Hg levels in response to flooding.

Concluding remarks

Almost all freshwater fish depend at some point in their lives on aquatic invertebrates as food. Diet is the most important route of uptake of MeHg to fish, so an increase in concentrations of MeHg in aquatic insects means an increase in the MeHg available to fish.

Methyl Hg cycling in both natural and flooded ecosystems is complex and there are many aspects of its biogeochemistry that still require clarification. However, this study, together with work from other components of the ELARP, have increased our understanding of MeHg cycling in aquatic food chains of newly flooded ecosystems and may eventually contribute to reservoir mitigation strategies.

References

Albers, P.H. and M.B. Camardese. 1993. Effects of acidification on metal accumulation by aquatic plants and invertebrates. 1. Constructed wetlands. Environ. Tox. Chem. **12**:959-967.

Anderson, M.R., D.A. Scruton, U.P. Williams, and J.F. Payne. 1995. Mercury in fish in the Smallwood Reservoir, Labrador, twenty one years after impoundment. Water Air Soil Pollut. **80**:927-930.

Armstrong, F.A.J. and A.L. Hamilton. 1973. Pathways of mercury in a polluted northwestern Ontario lake. *In:* P.C Singer (*Ed.*) Trace metals and metal-organic interactions in natural waters. Ann Arbor Science Publishers, Ann Arbor, Michigan. p.131-156.

Armstrong, F.A.J. and J.F. Uthe. 1971. Semi-automated determination of mercury in animal tissue. At. Absorpt. Newsl. **10**:101-103.

Barron, M.G. 1990. Bioconcentration. Environ. Sci. Technol. **24**:1612-1618.

Bloom, N.S. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Can. J. Fish. Aquat. Sci. **46**:1131-1140.

Bloom, N.S., M. Horvat, and C.J. Watras. 1995. Results of the international aqueous mercury speciation intercomparison exercise. Water Air Soil Pollut. **80:**1257-1268.

Bodaly, R.A. and R.J.P Fudge. 1994. Uptake of mercury by fish in an experimental reservoir, northwestern Ontario. *In:* Proceedings of the Mercury as a Global Pollutant Conference. Whistler, Canada, July 10-14, 1994.

Bodaly, R.A., R.E. Hecky, and R.J.P. Fudge. 1984. Increases in fish mercury levels in lakes flooded by the Churchill River diversion, northern Manitoba. Can. J. Fish. Aquat. Sci. **41**:682-691.

Bodaly, R.A., J.W.M. Rudd, R.J.P. Fudge, and C.A. Kelly. 1993. Mercury concentrations in fish related to size of remote Canadian Shield lakes. Can. J. Fish. Aquat. Sci. **50**:980-987.

Borgmann, U., W.P. Norwood and C. Clarke. 1993. Accumulation, regulation and toxicity of copper, zinc, lead and mercury in *Hyalella azteca*. Hydrobiologia **259**:79-89.

Boudou, A., D. Georgescauld, and J.P. Desmazès. 1983. Ecotoxicological role of the membrane barriers in transport and bioaccumulation of mercury compounds. p. 117-

136 *In:* J.O. Nriagu (*Ed.*) Aquatic Toxicology. John Wiley and Sons Inc., New York, New York, 696 pp.

Cabana, G., A. Tremblay, J. Kalff, and J.B. Rasmussen. 1994. Pelagic food chain structure in Ontario lakes: a determinant of mercury levels in lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. **51**:381-389.

Chapman, P.M. 1991. Environmental quality criteria: What type should we be developing? Environ. Sci. Technol. **25**:1353-1359.

Compeau, G. and R. Bartha. 1984. Methylation and demethylation of mercury under controlled redox, pH, and salinity conditions. Appl. Environ. Microbiol. **48**:1203-1207.

Duchemin, E., M. Lucotte, R. Canuel, and A. Chamberland. 1995. Production of the greenhouse gases, CH_4 and CO_2, by hydroelectric reservoirs of the boreal region. Global Biogeochem. Cycles. **9**:529-540.

D'Itra, P.A. and F.W. D'Itra. 1977. Mercury contamination; a human tragedy. John Wiley and Sons, Inc. New York, New York, 311 pp.

Dmytriw, R., A. Mucci, M. Lucotte, and P. Pichet. 1995. The partitioning of mercury in the solid components of dry and flooded forest soils and sediments from a hydroelectric reservoir, Québec (Canada). Water Air Soil Pollut. **80**:1099-1103.

Driscoll, C.T., C. Yan, C.L. Schofield, R. Munson, and J. Holsapple. 1994. The chemistry and bioavailability of mercury in remote Adirondack lakes. Environ. Sci. Technol. **28**:136A-142A.

Elwood, J.W., S.G. Hildebrand, and J.J. Beauchamp. 1976. Contribution of gut contents to the concentration and body burden of elements in *Tipula* spp. from a spring-fed stream. J. Fish. Res. Bd. Can. **33**:1930-1938.

Fitzgerald, W.F. 1995. Is mercury increasing in the atmosphere? The need for an atmospheric mercury network (AMNET). Water Air Soil Pollut. **80**:245-254.

Fitzgerald, W.F. and T.W. Clarkson. 1991. Mercury and monomethylmercury. Present and future concerns. Environ. Health Perspect. **96**:159-166.

Gilmour, C.C. and E.A. Henry. 1991. Mercury methylation in aquatic systems affected by acid deposition. Environ. Pollut. **71**:131-169.

Harris, R.C. and W.J. Snodgrass. 1993. Bioenergenics simulations of mercury uptake and retention in walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*). Water Pollut. Res. J. Can. **28**:217-236.

Hendzel, M.R. and D.M. Jamieson. 1976. Determination of mercury in fish. Anal. Chem. **48**:926-928.

Hesslein, R.H. and P.S. Ramlal. 1993. Stable isotopes of sulfur, carbon, and nitrogen in biota of the Hinton Reach specific study area. Northern River Basins Study Project Report No. 22. Northern River Basins Study, Edmonton, Alberta. 17pp.

Hewett, S.W. and B.L. Johnson. 1992. Fish Bioenergetics Model 2. University of Wisconsin Sea Grant Institute WIS-SG-91-250.

Hildebrand, S.G., A.W. Andren, and J.W. Huckabee. 1976. Distribution and bioaccumulation of mercury in biotic and abiotic compartments of a contaminated river-reservoir system. *In:* R.W. Andrew, P.V. Hodson, and D.E. Konasewich (*Eds.*) Toxicity to biota of metal forms in natural water. Int. Jt. Comm. Great Lakes Res., Windsor, Ontario. p.211-232.

Hintelmann, H., P.M. Welbourn, and R.D. Evans. 1995. Binding of methylmercury compounds by humic and fulvic acids. Water Air Soil Pollut. **80**: 1031-1034.

Horvat, M., N.S. Bloom, and L. Liang. 1993. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples. Anal. Chem. Acta. **281**:135-152.

Huckabee, J.W., J.W. Elwood, and S.G. Hildebrand. 1979. Accumulation of mercury in freshwater biota. p. 277-302. *In:* J.O. Nriagu (*Ed.*) Aquatic Toxicology. John Wiley and Sons, Inc., New York, New York, 696 pp.

Huckabee, J.W. and S.G. Hildebrand. 1974. Background concentrations of mercury and methylmercury in unpolluted freshwater environments. *In:* Prim Cong. Int. Merc., Barcelona, Spain, May 6-10, 1974, p. 214-224.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. **54**:187-211.

Jensen, S. and A. Jernelöv. 1969. Biological methylation of mercury in aquatic organisms. Nature **223**:753-754.

Jernelöv, A. and H. Lann. 1971. Mercury accumulation in food chains. Oikos **22**:403-406.

Johnels, A., G. Tyler, and T. Westermark. 1979. A history of mercury levels in Swedish fauna. Ambio **8**:160-168.

Kelly, C.A., J.W.M. Rudd, R.A., Bodaly, N.R. Roulet, V.L. St. Louis, A. Heyes, T.R. Moore, S. Schiff, B. Warner, and B. Dyck. Effects of flooding on CO_2, CH_4 and

methyl mercury fluxes in an experimental reservoir. Environ. Sci. Technol. submitted.

Kelly, C.A., J.W.M. Rudd, V.L. St. Louis, and A. Heyes. 1995. Is total mercury concentration a good predictor of methyl mercury concentration in aquatic systems? Water Air Soil Pollut. **80**:715-724.

Kidd, K.A., R.H. Hesslein, R.J.P. Fudge, and K.A. Hallard. 1995. The influence of trophic level as measured by $\delta^{15}N$ on mercury concentrations in freshwater organisms. Water Air Soil Pollut. **80**:1011- 1015.

Kurland, L.T., S.N. Faro, and H. Siedler. 1960. Minimata disease: The outbreak of a neurological disorder in Minimata Bay, Japan, and its relationship to the ingestion of seafood contaminated by mercuric compounds. World Neurol. **1**:370.

Lindqvist, O. 1991. Mercury in the Swedish environment: physical/chemical forms of mercury. Water Air Soil Pollut. **55**:7-17.

Mason, R.P., W.F. Fitzgerald, and F.M.M. Morel. 1994. The biogeochemical cycling of elemental mercury: Anthropogenic influences. Geochemica Cosmochim. Acta. **58**:3191-3198.

Malley, D.F., A.R. Stewart, and B.D. Hall. Uptake of methylmercury by the floater mussel, *Pyganodon grandis* (Bivalvia, Unionidae) caged in a flooded wetland. Environ. Toxicol. Chem. in press.

Matilainen, T., M. Verta, M. Niemi, and A Uusi-Rauva. 1991. Specific rates of net methylmercury production in lake sediments. Water Air Soil Pollut. **56**:595-605.

Meili, M. 1991. Mercury in Boreal Forest Lake Ecosystems. Acta Universitatis Upsaliensis: Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science 336.

Merritt, R.W. and K.W. Cummins. 1984. An introduction to the aquatic insects of North America; second edition. Kandal/Hunt Publishing Co., Dubuque, Iowa.

Moore, T.R., J.L. Bubier, A. Heyes, and R.J. Flett. 1995. Methyl and total mercury in boreal wetland plants, Experimental Lakes Area, Northwestern Ontario. J. Environ. Qual. **24**:845-850.

Moriarty, F. and C.H. Walker. 1987. Bioaccumulation in food chains- a rational approach. Ecotoxicol. Environ. Safety **13**:208-215.

Morrison, K.A. and N. Thérien. 1995. Changes in mercury levels in lake whitefish (*Coregonus clupeaformis*) and northern pike (*Esox lucius*) in the LG-2 reservoir since flooding. Water Air Soil Pollut. **80**:819-828.

Odin, M. A. Feurtet-Mazel, F. Ribeyre, and A. Boudou. 1994. Actions and interactions of temperature, pH and photoperiod on mercury bioaccumulation by nymphs of the burrowing mayfly *Hexagenia rigida*, from the sediment contamination source. Environ. Tox. Chem. **13**:1291-1302.

Parkman, H. and M. Meili. 1993. Mercury in macroinvertebrates from Swedish forest lakes: influence of lake type, habitat, life cycle, and food quality. Can. J. Fish. Aquat. Sci. **50**:521-534.

Parks, J.W., J.A. Sutton, J.D. Hollinger, and D.D. Russell. 1987. Uptake of mercury by caged crayfish. Appl. Organomet. Chem. **2**:181-184.

Peterson, B.J. and B. Fry. 1987. Stable isotopes in ecosystems studies. Ann. Rev. Ecol. Syst. **18**:293-320.

Phillips, G.R. and D.R. Buhler. 1978. The relative contributions of methylmercury from food or water to rainbow trout (*Salmo gairdneri*) in a controlled laboratory environment. Trans. Am. Fish. Soc. **107**:853-861.

Potter, L., D. Kidd, and D. Stanford. 1975. Mercury levels in Lake Powell-Bioamplification of mercury in a man-made desert reservoir. Environ. Sci. Technol. **9**:41-46.

Robinson, J.B. and O.H. Tuovinen. 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical and genetic analysis. Microbiol. Rev. **48**:95-124.

Rodgers, D.W. 1994. You are what you eat and a little bit more: Bioenergenics-based models of methylmercury accumulation in fish revisited. p.427-439. *In:* C.J. Watras and J.W. Huckabee (*Eds.*) Mercury pollution: integration and synthesis. Lewis Publishers. Boca Raton, Florida. 727 pp.

Rodgers, D.W. and F.W.H. Beamish. 1981. Uptake of waterborne methylmercury by rainbow trout (*Salmo gairdneri*) in relation to oxygen consumption and methylmercury concentration. Can. J. Fish. Aquat. Sci. **38**:1309-1315.

Rodgers, D.W. and F.W.H. Beamish. 1983. Water quality modifies uptake of waterborne methylmercury by rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. **40**:824-828.

Rosenberg, D.M., R.A. Bodaly, R.E. Hecky, and R.W. Newbury. 1987. The environmental assessment of hydroelectric impoundments and diversions in

Canada. *In:* M.C. Healey and R.R. Wallace (*Eds.*) Canadian Aquatic Resources. Can. Bull. Fish. Aquat. Sci. **215**:71-104.

Rosenberg, D.M., R.A. Bodaly, and P.J. Usher. 1995. Environmental and social impacts of large scale hydroelectric development: who is listening? Global Environ. Change **5**:127-148.

Rossaro, B., G.F. Gaggino, and R. Marchetti. 1986. Accumulation of mercury in larvae and adult *Chironomus riparius* (Meigen). Bull. Environ. Contam. Toxicol. **37**:402-406.

Rudd, J.W.M., A. Furutani, and M.A. Turner. 1980. Mercury methylation by fish intestinal contents. Appl. Environ. Micro. **40**:777-782.

Rudd, J.W.M., R. Harris, C.A. Kelly, and R.E. Hecky. 1993. Are hydroelectric reservoirs significant sources of greenhouse gases? Ambio **22**:246-248.

Saouter, E., L. Hare, P.G.C. Campbell, A. Boudou, and F. Ribeyre. 1993. Mercury accumulation in the burrowing mayfly *Hexagenia rigida* (Ephemeroptera) exposed to CH_3HgCl or $HgCl_2$ in water and sediment. Water Res. **27**:1041-1048.

Sellers, P., C.A. Kelly, J.W.M. Rudd, and A.R. MacHutchon. Photodegradation of methylmercury in lakes. Nature. in press.

Slotton, D.G., J.E. Reuter, and C.R. Goldman. 1995. Mercury uptake patterns of biota in a seasonally anoxic northern California reservoir. Water Air Soil. Pollut. **80**:841-850.

Snyder, C.D. and A.C. Hendricks. 1995. Effect of seasonally changing feeding habits on whole animal mercury concentrations in *Hydropsyche morosa* (Trichoptera: Hydropsychidae). Hydrobiologia **299**:115-123.

Spry, D.J. and J.G. Wiener. 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. Environ. Pollut. **71**:243-304.

St. Louis, V., J.W.M. Rudd, C.A. Kelly, K.G., Beaty, N.S. Bloom, and R.J. Flett. 1994. Importance of wetlands as sources of methyl mercury to boreal forest ecosystems. Can. J. Fish. Aquat. Sci. **51**:1065-1076.

St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, and L.A. Barrie. 1995. Wet deposition of methyl mercury in northwestern Ontario compared to other geographic locations. Water Air Soil Pollut. **80**:405-414.

St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, K.G. Beaty, R.J. Flett, and N.T. Roulet. Production and loss of methylmercury and loss of total mercury from boreal

forest catchments containing different types of wetlands. Environ. Sci. Tech. in press.

Stainton, M.P., M.J. Capel, and F.A.J. Armstrong. 1977. Chemical analysis of fresh water. Fish. Mar. Serv. Misc. Spec. Publ. **2**, 180.

Surma-Aho, K., J. Paasivirta, S. Rekolainen, and M. Verta. 1986. Organic and inorganic mercury in the food chain of some lakes and reservoirs in Finland. Publ. Water Environ. Res. Inst. **65**:59-71. National Board of Waters, Helsinki, Finland.

Tremblay, A., M. Lucotte, and I. Rheault. Methyl mercury in a benthic web chain of two hydroelectric reservoirs and a natural lake of northern Québec (Canada). Water Air Soil Pollut. in press.

Turner, M.A. and J.W.M. Rudd. 1983. The English-Wabigoon River system: III. Selenium in lake enclosures: its geochemistry, bioaccumulation, and ability to reduce mercury bioaccumulation. Can. J. Fish. Aquat. Sci. **40**:2228-2240.

Verdon, R., D. Brouard, C. Demers, R. Lalumière, M. Laperle, and R. Schetagne. 1991. Mercury evolution (1978-1988) in fishes of the La Grande hydroelectric complex, Québec, Canada. Water Air Soil Pollut. **56**:405-417.

Verta, M., S. Rekolainen, J. Mannio, and K. Surma-Aho. 1986. The origin and level of mercury in Finnish forest lakes. Publ. Water Res. Int. Finland **65**:21-31.

Watras, C.J., N.S. Bloom, R.J.M. Hudson, S. Gherini, R. Munson, S.A. Claas, K.A. Morrison, J. Hurley, J.G. Wiener, W.F. Fitzgerald, R. Mason, G. Vandal, D. Powell, R. Rada, L. Rislov, M. Winfrey, J. Elder, D. Krabbenhoft, A.W. Andren, C. Babiarz, D.B. Porcella, and J.W. Huckabee. 1994. Sources and fates of mercury and methylmercury in Wisconsin lakes. p. 153-177. *In:* C.J. Watras and J.W. Huckabee (*Eds.*) Mercury pollution: integration and synthesis. Lewis Publishers. Boca Raton, Florida. 727 pp.

Wilkinson, L. 1990. SYSTAT: the system for statistics. SYSTAT Inc., Evanston, Ill. 822 pp.

Winfrey, M. R. and J.W.M. Rudd. 1990. Environmental factors affecting the formation of methylmercury in low pH lakes. Environ. Tox. Chem. 9:853-869.

Yingcharoen, D. and R.A. Bodaly. 1993. Elevated mercury levels in fish resulting from reservoir flooding in Thailand. Asian Fish. Sci. **6**:73-80.

Appendices

Appendix 1. Design and results of statistical tests using state of flooding, lake and year as main effects. Orthogonal contrasts and one-way ANOVAs were used to determine significant relationships among methylmercury (MeHg) concentrations in aquatic insects. When using orthogonal contrasts, values being compared are assigned numbers. The numbers of the groups being compared must sum to the same number but with a different sign. Groups of elements with the same sign are compared to groups of element of a different sign. If there is a statistically significant difference between the two groups, the p value will be <0.05. For example, under question 1, "Odonata, L979 preflood vs. natural lakes", a contrast was done between L979 (1992 and 1993 unflooded; 5+5=10) and both L632 (1992, 1993 and 1994) and L240 (1993 and 1994; -2+-2+-2+-2+-2=-10). The p value=0.571, so we conclude there is no significant difference in MeHg concentrations between unflooded L979 and natural lakes. Groups assigned 0 were not included in the contrast.

Appendix 2. Design and results of statistical tests using functional feeding group as the main effect. Statistical techniques described in Appendix 1 were used to determine if ratios and concentrations of methylmercury (MeHg) in functional feeding groups were significantly different.

Appendix 3. Percentages of methylmercury (MeHg) for insects sampled from the study lakes. The percent of total mercury (THg) that is MeHg in aquatic insects from Lakes 979, 632 and 240 are presented. Values are %MeHg means ± one standard error.

Appendix 4. Concentrations of methylmercury (MeHg) in insects sampled from the study lakes. The number of MeHg analyses done, the mean MeHg concentration ± one standard error and ranges of MeHg concentrations are presented for aquatic insects from Lakes 979, 632 and 240.

Appendix 5. Concentrations of total mercury (THg) in insects sampled from the study lakes. The number of THg analyses done, the THg concentration mean ± one standard error and ranges of THg concentrations are presented for aquatic insects from Lakes 979, 632 and 240.

Appendix 6. Total mercury (THg) concentrations (ng g^{-1} d.w.) in three functional feeding groups: predators, predator/herbivores, and collector/shredders. Solid horizontal line represents average THg concentrations in preflood conditions (1992 and 1993). These data which are not available for predator/herbivores. Light bars indicate preflood conditions, dark bars, flood conditions and medium bars, reference systems.

Appendix 1.

Design and results of statistical tests using state of flooding, lake and year as main effects. MeHg=methylmercury, THg=total mercury, nd=no data.

		Main Effect										
		Lake 979						Lake 632			Lake 240	
		1992	1993	1993	1994	1994	1994					
		pre-flood	pre-flood	flooded	drawndown	reflooded	drawndown	1992	1993	1994	1993	1994
Taxon	p-value											
Did MeHg concentrations in insects increase after flooding (Question 1)?												
Collector/Shredders												
Hyalella azteca												
ANOVA	0.192											
Limnephilidae												
ANOVA	0.916											
Predator/Herbivores												
Phryganeidae, Polycentropodidae												
ANOVA	0.007											
Contrast												
L979 drawdown vs. L632 and L979 reflood	0.004	nd	nd	nd	nd	-1	3	nd	-1	-1	nd	nd
L979 vs. L632	0.083	nd	nd	nd	nd	-1	-1	nd	1	1	nd	nd
Corixidae												
ANOVA	<0.001											
Contrast												
L979 vs. L632	0.001	nd	nd	3	3	3	3	-4	-4	-4	nd	nd
Predators												
Odonata												
ANOVA	<0.001											
Contrast												

L979 pre flood vs. L979 post flood	<0.001	-2	-2	1	1	1	1	0	0	0	0	0
L632 1993 vs. L632 1994	0.017	0	0	0	0	0	0	0	-1	1	0	0
L979 pre flood vs. natural lakes	0.571	5	5	0	0	0	0	-2	-2	-2	-2	-2
Lethocerus americanus												
ANOVA	0.035											
Contrast												
L979 pre flood vs. L632	0.851	-3	-3	nd	nd	nd	nd	2	2	2	nd	nd
L632 1992 vs. L632 1993 and 1994	0.006	0	0	nd	nd	nd	nd	-2	1	1	nd	nd
Gerris sp.												
ANOVA	0.046											
Contrast												
L979 pre flood vs. L979 post flood	0.160	nd	-2	1	nd	1	nd	nd	nd	nd	0	0
L979 1993 preflood vs. 1993 post flood	0.043	nd	-1	1	nd	0	nd	nd	nd	nd	0	0
L979 pre flood vs. L240	0.829	nd	-2	0	nd	0	nd	nd	nd	nd	1	1
Notonecta sp.												
ANOVA	0.013											
Contrast												
L979 pre flood vs. L979 post flood	0.057	nd	-4	1	1	1	1	0	0	0	nd	nd
L979 drawdown vs. rest	0.025	nd	1	1	1	1	-7	1	1	1	nd	nd
L979 pre flood vs. L632	0.338	nd	-3	0	0	0	0	1	1	1	nd	nd
Dytiscus sp.												
ANOVA	0.641											
Gyrinidae												
ANOVA	0.005											
Contrast												
L979 pre flood vs. L979 post flood	0.010	-1	-1	nd	1	1	nd	0	0	0	0	nd
L979 pre flood vs. natural lakes	0.090	2	2	nd	0	0	nd	-1	-1	-1	-1	nd

Did MeHg concentrations in FFGs increase after flooding (Question 1)?												
Total Predators												
ANOVA	<0.001											
Contrast												
L979 1993 preflood vs. 1993	0.001	0	-1	1	0	0	0	0	0	0	0	0
L979 preflood vs. 1994 L979	<0.001	0	3	0	-1	-1	-1	0	0	0	0	0
L240 1993 vs. L240 1994	0.580	0	0	0	0	0	0	0	0	0	-1	1
L632 1993 vs. L632 1994	0.338	0	0	0	0	0	0	0	-1	1	0	0
L632 1992 vs. L632 1993 and	<0.001	0	0	0	0	0	0	-2	1	1	0	0
L979 preflood vs. L632 1993 and 1994 and L240	0.274	0	-4	0	0	0	0	0	1	1	1	1
L632 1992 vs. L632 1993 and 1994 and L240	<0.001	5	0	0	0	0	0	5	-2	-2	-2	-2
L979 1992 vs. L979 post flood and L632 1992	0.960	2	0	-1	-1	-1	-1	2	0	0	0	0
Total Predator/Herbivores												
ANOVA	<0.001											
Contrast												
L979 post flood vs. L632	<0.001	nd	nd	3	3	3	3	-4	-4	-4	nd	nd
L979 reflood vs. L979 flooded and dranwdown	0.001	nd	nd	-1	-1	3	-1	0	0	0	nd	nd
L979 reflooded vs. L632	0.198	nd	nd	0	0	3	0	-1	-1	-1	nd	nd
Total Collector/Shredders												
ANOVA	0.005											
Contrast												
L979 preflood 1993 vs. L979 post flood 1994	0.787	0	-1	1	nd	0	nd	0	0	0	nd	nd
L979 preflood vs. L979 reflooded	0.015	0	-1	0	nd	1	nd	0	0	0	nd	nd
L632 1994 vs. L632 1992 and 1993	0.001	0	0	0	nd	0	nd	-1	-1	2	nd	nd
L979 reflooded vs. L632 1994	0.748	0	0	0	nd	1	nd	0	0	-1	nd	nd

Did MeHg:THg change in response to flooding (Question 2)?	
All samples	
ANOVA	0.098

Appendix 2

Design and results of statistical tests using functional feeding group as the main effect. Abbreviations as in Appendix 1.

		Main Effect		
	p-value	Collector/shredders	Predator/herbivores	Predators
Is there a difference in MeHg concentrations among FFGs?				
ANOVA	<0.001			
Contrasts				
Collector/shredders vs. predators	<0.001	1	0	-1
Collector/shredders vs. predator/herbivores	0.003	1	-1	0
Predator/herbivores vs. predators	0.904	0	-1	1
Is there a difference in MeHg:THg ratios among FFGs?				
ANOVA	0.049			
Contrasts				
Collector/shredders vs. predators	0.014	1	0	-1
Collector/shredders vs. predator/herbivores	0.146	1	-1	0
Predator/herbivores vs. predators	0.920	0	-1	1

Appendix 3.

Percentages of methylmercury (MeHg) ± one standard error for insects sampled from Lakes 979, 632 and 240.

		Lake 979						Lake 632			Lake 240	
		1992 pre-flood	1993 pre-flood	1993 flooded	1994 drawndown	1994 reflooded	1994 drawndown	1992	1993	1994	1993	1994
Taxon	Life Stage											
Collector/Shredders												
Amphipoda												
Hyalella azteca	Adults		121 ± 18			46 ± 6		24	22 ± 13			
Ephemeroptera												
Siphlonurus sp.	Nymphs							60		88 ± 9		
Trichoptera												
Limnephilidae	Larvae	38 ± 21	30 ± 5	19				22	28 ± 10			
Total Collector/Shredders		38 ± 21	78 ± 19	19		40 ± 7		36 ± 12	25 ± 7	83 ± 9		
Predator/Herbivores												
Trichoptera Phryganeidae, Polycentropodidae	Larvae								107			
Hemiptera												
Corixidae	Adults and Nymphs			54 ± 11	104 ± 16	49 ± 6			98	49 ± 6		
Total Predator/Herbivores				54 ± 11	104 ± 16	49 ± 6			75 ± 13	59 ± 12		
Predators												
Odonata												
Aeshnidae	Nymphs	71 ± 6	65 ± 11	69 ± 15		53 ± 20		58	65 ± 5	82 ± 13		
Corduliidae	Nymphs		30			68 ± 25		73	67 ± 10	76 ± 6		
Other Odonata	Nymphs											
Total Odonata		72 ± 6	56 ± 8	57 ± 18	73	66 ± 11		66 ± 7	65 ± 5	74 ± 6	34 ± 9	
Hemiptera												

Belostomatidae												
Lethocerus americanus	Adults	59 ± 14	86				58 ± 3	84 ± 9	61 ± 12			
Gerridae												
Gerris sp.	Adults and Nymphs		110			68 ± 7		59 ± 23		65 ± 3	57 ± 6	
Notonectidae												
Notonecta sp.	Adults and Nymphs		18	54 ± 7	83	65 ± 8		54 ± 9	73 ± 4			
Coleoptera												
Dytisicidae												
Dytiscus sp.	Adults	50 ± 4	103	71	69	62 ± 26	57	70 ± 6	65 ± 10			
Gyrinidae												
Gyrinus sp.	Adults	30 ± 0.4	59 ± 12		69	53 ± 7		63 ± 2	83 ± 3			
Dineutus sp.	Adults	39	32 ± 4						53			
Total Gyrinidae		43 ± 9	58 ± 10		69	53 ± 7	76 ± 9	63 ± 2	76 ± 8			
All Predators		67 ± 6	69 ± 12	57 ± 7	96 ± 23	64 ± 5	66 ± 6	67 ± 3	71 ± 3	50 ± 5		
Predators excluding *L. americanus* and *Dytiscus sp.*		75 ± 10					42 ± 18					

Appendix 4

Concentrations of methylmercury (MeHg) in insects sampled from Lakes 979, 632 and 240. Top number= no. of analyses; second number=MeHg ± one SE ng/g d.w.; third number= range of MeHg.

		Lake 979						Lake 632			Lake 240	
		1992 pre-flood	1993 pre-flood	1993 flooded	1994 drawndown	1994 reflooded	1994 drawndown	1992	1993	1994	1993	1994
Taxon	Life Stage											
Collector/Shredders												
Amphipoda												
Hyalella azteca	Adults	1 67.7	2 78.2 ± 12 65.9 - 90.5			5 128.4 ± 21 54.3 - 188.0		1 31.0	6 57.4 ± 21 11.7 - 127	1 18.9		
Ephemeroptera												
Siphlonurus sp.	Nymphs		1 87.1					2 48.3 ± 23 25.2 - 71.3		6 136.3 ± 9 108 - 168.8		
Trichoptera												
Limnephilidae	Larvae	3 24.7 ± 13 3.4 - 49.0	5 47.7 ± 16 19.0 - 107.8	1 46.8				1 26.4	7 37.6 ± 15 1.3 - 125			
Predator/Herbivores												
Trichoptera												
Phryganeidae, Polycentropodidae	Larvae					1 86.0	5 351.5 ± 94 126.4 - 694	1 35.1	8 32.7 ± 5 9.0 - 51.1			
Hemiptera												
Corixidae	Adults and Nymphs			6 318.1 ± 33 222 - 462.8	2 285.4 ± 3 282.6 - 288.3	6 157.1 ± 17 99.6 - 211	1 233.7	1 121.0	3 155.8 ± 22 113 - 184	8 124.2 ± 22 36.9 - 218.1		
Predators												
Odonata												
Aeshnidae	Nymphs	10 107.3 ± 17 60.1 - 197	4 79.8 ± 17 49.7 - 121.4	2 191.3 ± 13 178.0 - 204.5		5 169.4 ± 41 19.4 - 235	3 344.7 ± 154 132.9 - 645	2 135.6 ± 20 116 - 155.7	32 77.3 ± 6 34.6 - 182.0	6 185.8 ± 21 140 - 266.6		
Corduliidae	Nymphs		1 59.4		1 200.3	5 179.0 ± 26 136.6 - 278	4 216.0 ± 38 133.2 - 283	1 95.7	18 87.3 ± 8 48.4 - 140	13 140.0 ± 22 60.0 - 361.8		
Other Odonata	Nymphs	2 88.2 ± 0.1 88.1 - 88.2	3 79.3 ± 5 69.6 - 87.5	3 283.1 ± 141 11.4 - 484.3	1 84.7	5 195.7 ± 51 56.2 - 360			11 72.6 ± 9 7.6 - 116	9 86.2 ± 18 21.2 - 173.7		
Total Odonata		12 104.1 ± 15 60.1 - 197	8 77.1 ± 9 49.7 - 121.4	5 246.4 ± 81 11.4 - 484.3	2 142.5 ± 58 84.7 - 200.3	15 181.3 ± 22 19.3 - 360	7 271.2 ± 67 132.9 - 645	3 122.3 ± 33 95.7 - 155.7	61 79.1 ± 4 7.6 - 182.0	28 132.5 ± 14 21.2 - 361.8	8 52.6 ± 11 14.2 - 112.1	3 112.9 ± 10 93.7 - 129.3
Hemiptera												
Belostomatidae												

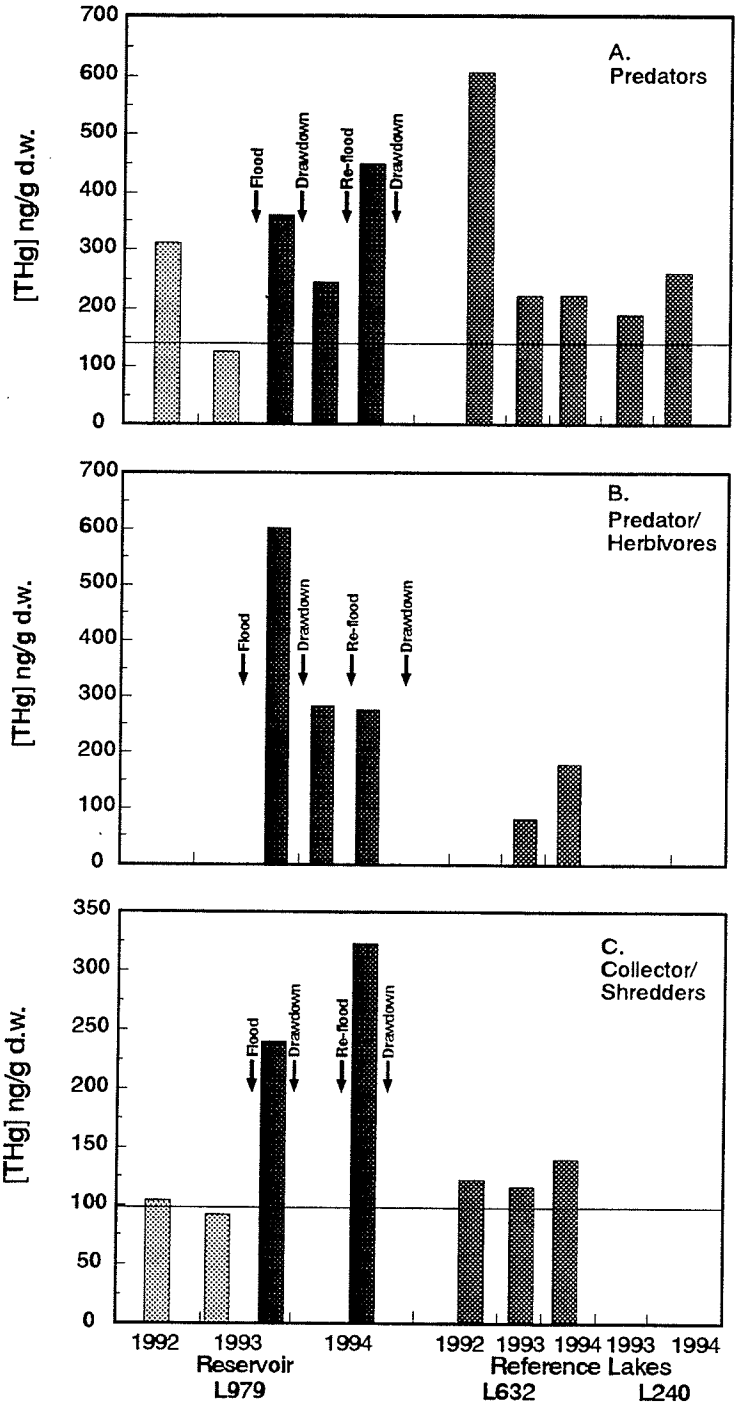
Lethocerus americanus	Adults	3 499.3 ± 28 443 - 531		1 107.7										32 429.2 ± 43 71.9 - 1023		7 310.6 ± 74 140 - 600.0		4 93.5 ± 5 86.1 - 109.6			
Gerridae																					
Gerris sp.	Adults and Nymphs			1 108.6		3 404.9 ± 118 201 - 610.7			2 180.6 ± 104 76.6 - 285						2 142.2 ± 47 94.7 - 190				6 118.6 ± 7 86.5 - 136.3		2 151.0 ± 12 140 - 162.5
Notonectidae																					
Notonecta sp.	Adults and Nymphs			2 159.6 ± 134 25.9 - 293.4		5 260.6 ± 28 184 - 336.0		1 331.4	15 442.7 ± 60 86.3 - 846		1 715.5			1 430.2	7 182.3 ± 25 65.7 - 265			10 235.0 ± 50 96 - 612.6			
Nepidae																					
Ranatra sp.	Adults													2 334.4 ± 1 333.0 - 335.8		1 233.7		2 365.5 ± 137 229 - 502.2			
Coleoptera																					
Dytisicidae																					
Dytiscus sp.	Adults	15 216.1 ± 19 50.3 - 332.6		1 118.2		1 188		1 337.8	3 169.2 ± 62 60.9 - 272					3 196.0 ± 105 10.4 - 375.5	11 205 ± 33 96.5 - 487			9 156 ± 30 30.2 - 281.6			
Gyrinidae																					
Gyrinus sp.	Adults	2 19.0 ± 6 13.1 - 24.5		2 34.6 ± 9 25.5 - 43.7				1 84.4	7 106.0 ± 12 59.1 - 147						2 55.0 ± 17 38.5 - 71.6			3 64.7 ± 10 46.0 - 78.5			
Dineutus sp.	Adults	1 29.6		2 39.8 ± 4 35.6 - 44					1 169.1									1 172.7			
Total Gyrinidae		5 35.1 ± 11 13.1 - 78.6		6 55.4 ± 13 25.5 - 107.9				1 84.4	8 113.9 ± 13 59.1 - 169					4 68.0 ± 15 44.5 - 107.2	2 55.0 ± 17 38.5 - 71.6			4 91.7 ± 28 46.0 - 172.7		8 57.0 ± 6 32.8 - 82.9	
Others[1]				1 54.7		1 53.8			10 196.0 ± 40 50.1 - 377.0						1 307.0			1 88.1			

[1]Other predators include Gyrinidae larvae, Dytisidae adults and larvae not including *Dytiscus sp.*

Concentrations of total mercury (THg) in insects sampled from Lakes 979, 632 and 240. Top number= no. of analyses; second number=THg ± one SE ng/g d.w.; third number= range of THg.

		Lake 979						Lake 632			Lake 240	
		1992 pre-flood	1993 pre-flood	1993 flooded	1994 drawndown	1994 reflooded	1994 drawndown	1992	1993	1994	1993	1994
Taxon	Life Stage											
Collector/Shredders												
Amphipoda												
Hyalella azteca	Adults		3 71 ± 6 64 - 82			3 301 ± 47 253 - 394		1 128	3 119 ± 19 82 - 146	2 116 ± 51 65 - 167		
Ephemeroptera												
Siphlonurus sp.	Nymphs							1 118		5 167 ± 18 117 - 206		
Trichoptera												
Limnephilidae	Larvae	2 104 ± 21 83 - 125	7 103 ± 13 67 - 149	1 240				1 120	6 216 ± 117 45 - 788			
Predator/Herbivores												
Trichoptera												
Phryganeidae, Polycentropodidae	Larvae					1 386			4 102 ± 31 65 - 194			
Hemiptera												
Corixidae	Adults and Nymphs			5 621 ± 76 409 - 867	2 281 ± 40 240 - 321	6 275 ± 47 78 - 406			1 188	4 178 ± 72 83 - 389		
Predators												
Odonata												
Aeshnidae	Nymphs	9 158 ± 22 71 - 246	4 109 ± 14 67 - 124	2 285 ± 82 213 - 378		6 421 ± 78 190 - 748		2 159 ± 40 119 - 198	30 133 ± 15 54 - 490	6 215 ± 45 27 - 347		
Cordulilidae	Nymphs		1 195	1 286		4 298 ± 68 107 - 427		2 120 ± 11 109 - 131	14 123 ± 11 59 - 196	14 183 ± 27 116 - 497		
Other Odonata	Nymphs										9 184 ± 26 103 - 448	
Total Odonata		10 154 ± 20	7 133 ± 15	6 292 ± 50	1 116	14 349 ± 48		4 139 ± 20	55 135 ± 11	24 188 ± 55	9 184 ± 26	

		71 - 246		67 - 195		155 - 426				103 - 748		109 - 198		54 - 490		27 - 497		103 - 448			
Hemiptera																					
Belostomatidae																					
Lethocerus americanus	Adults	3 922 ± 164 607 - 1157		1 126		1 157						31 750 ± 78 157 - 1912		7 376 ± 91 154 - 863		4 168 ± 26 115 - 240					
Gerridae																					
Gerris sp.	Adults and Nymphs					2 260 ± 106 154 - 365				2 287 ± 184 102 - 471				2 248 ± 16 232 - 264				5 176 ± 5 160 - 191		2 261 ± 12 249 - 273	
Notonectidae																					
Notonecta sp.	Adults and Nymphs			1 144		4 510 ± 30 440 - 568		1 399		15 729 ± 89 141 - 1249				7 326 ± 54 228 - 628		9 305 ± 63 151 - 731					
Nepidae																					
Ranatra sp.	Adults											2 334 ± 1 333 - 336		1 234		2 366 ± 137 229 - 502					
Coleoptera																					
Dytisicidae																					
Dytiscus sp.	Adults	9 506 ± 35 364 - 651		1 115		1 262		1 488		3 338 ± 80 251 - 499		1 574		10 329 ± 65 132 - 845		7 265 ± 46 125 - 419					
Gyrinidae																					
Gyrinus sp.	Adults	2 65 ± 21 44 - 85		2 58 ± 3 55 - 62				1 122		8 222 ± 22 158 - 309				2 88 ± 29 59 - 117		3 78 ± 11 58 - 98					
Dineutus sp.	Adults	1 76		2 124 ± 3 121 - 128						1 223						1 325					
Total Gyrinidae		5 77 ± 9 44 - 101		6 100 ± 16 55 - 150				1 122		9 223 ± 20 158 - 309		3 83 ± 16 61 - 115		2 88 ± 29 59 - 117		4 140 ± 62 58 - 325		8 122 ± 22 46 - 218			

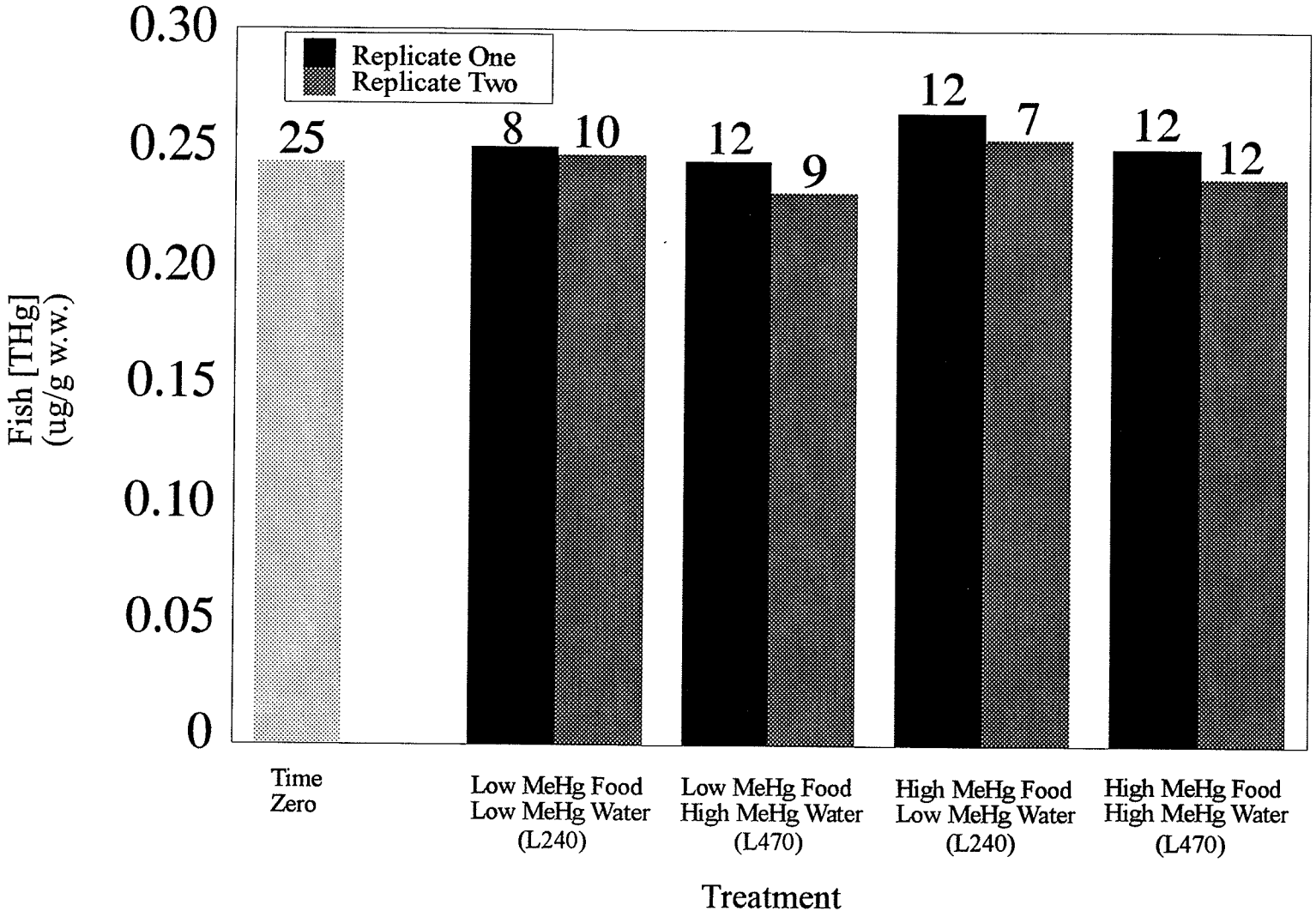


Appendix 6. Total mercury (THg) concentrations in ng g^{-1} d.w. in (A) predators, (B) predator/herbivores, and (C) collector/shredders. Solid horizontal line represents average THg concentrations in preflood conditions (1992 and 1993); these data are not available for predator/herbivores. Light bars indicate preflood conditions, dark bars, flood conditions and medium bars, reference systems.

Appendix 7

Instantaneous water temperatures (°C) in experimental pens in L240.

	10-Aug-93	17-Aug-93	23-Aug-93	Pen #
Low MeHg food, low MeHg water	20.6	20.8	22.0	1
	20.7	20.8	22.1	5
Low MeHg food, int. MeHg water	20.7	20.7	22.0	2
	20.8	20.6	22.4	7
High MeHg food, int. MeHg water	20.6	20.8	22.0	3
	20.6	20.6	22.0	8
High MeHg food, high MeHg water	20.6	20.7	22.2	4
	20.8	20.8	22.0	6



Appendix 8. Results from the pilot mercury uptake experiment in Lake 240. Total mercury (THg) in fish tissue at the beginning (Time Zero) and end of the experiment. Numbers of fish analyzed are shown above bars.