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Problems of Aquatic Toxicology, Biotesting and Water Quality Management

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Problems of Aquatic Toxicology, Biotesting and Water Quality Management

Proceedings of USA-Russia Symposium, Borok, Jaroslavl Oblast, July 21-23, 1992

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Foreword

For more than two decades, cooperation and exchange of scientific information under the USA-(former) USSR Agreement on Cooperation in the Field of Environmental Protection has helped both countries in their efforts to control environmental pollution. These efforts are pursued in recognition of the international nature of the problem: pollution knows no boundaries.

Three projects are addressed under the joint Agreement's Working Group on Cooperation in the Area of Water Pollution Prevention. These are Project 02.02-11 "Planning and Management of Water Quality in River Basins," Project 02.02-12 "Protection and Management of Water Quality in Lakes and Estuaries," and Project 02.02-13 "Effect of Pollutants on Aquatic Organisms and Ecosystems: Development of Water Quality Criteria."

Over the years, scientific delegations and individual scientists have traveled to each other's countries to visit scientific institutions, perform joint research, and exchange technical information. This Proceedings presents the papers that were presented at the symposium held in Borok, Jaroslavl Oblast, in July 1992.

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Abstract

Sixteen papers delivered at the international symposium entitled "Problems of Aquatic Toxicology, Biotesting and Water Quality Management" are presented. The impact of permethrin on zooplankton in high latitude ponds is examined, and an aquatic food chain model for simulating toxicant impact on zooplankton population dynamics in a water body is presented. Problems related to monitoring and modeling pollution from agricultural watersheds are described, and a water quality planning model for agricultural watersheds (Answers-2000) is presented. The effects of metals on fish are investigated in studies of mercury content and ultrastructure of gills and scales of fish in Russian lakes that are polluted by atmospheric deposition and of beryllium damage to gills in three freshwater fish species. In another study, the effect of heavy metals and chlorpyrifos on a continuous-flow mesocosm is examined. An investigation of relationships between waterborne tetrachloroguaiacol uptake and oxygen consumption by fishes is described. Management issues are addressed in reports on a Green Bay/Fox River (Wisconsin) mass balance study, on an effort to restore aquatic resources to the lower Missouri River, and on a project to improve habitat in riverine lakes through hydrologic modification. A classification scheme for wetlands in Russia is introduced.

The use of simulation models to investigate water quality in the lower Don River is described. Complex anthropogenic factors that influence the ecological state of the upper Volga River are addressed; physiological and biochemical indicators of contaminant stress in aquatic organisms of large river systems are identified. Attention is directed to the work of N.V. Lazarev in the early development of structure-activity relationships for environmental toxicology, leading to modern approaches to assessing contaminant effects on man and on organisms in the environment.

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SIMULATING TOXICANT IMPACT ON ZOOPLANKTON POPULATION DYNAMICS IN A WATERBODY

by

Y. M. Plis[1] and R. R. Lassiter[2]

ABSTRACT

In the past 20 years models have become useful tools for studying processes in waterbodies. We developed an aquatic food chain model. It was used to represent zooplankton population dynamics in a waterbody, through seasonal changes in water temperature and growth dynamics.

INTRODUCTION

Over the last 20 years, ecosystem models have become effective tools for investigating the complex physical, chemical, and biological processes in waterbodies. Most such models have represented aquatic organisms in all ecological roles simply as scalar biomass concentrations. Their approach assumes that differences among individuals in a population can be ignored. This approach is acceptable if aquatic ecosystem models are used for investigation of the processes of eutrophication in waterbodies, when the primary interest is in exploration of the dependence of plankton biomass changes on enrichment of a waterbody by nutrients. However, greater resolution of the intrapopulation structure is needed if one is to understand population dynamics in other situations. For example, competition for resources; predator avoidance; and, especially, ability to withstand severe environmental stress by toxicant impact depend on an individual's physiological state.

Models of physiologically structured populations were discussed in Metz and Diekmann (1986). Physiologically structured models that include body composition elements as variables of their individual model component have been proposed and used by Hallam et al. (1988a, b, 1990a, b). Such equations are often referred to as "McKendrick-von Foerster" equations, a general form of which is

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$$\frac{\partial P(t, \vec{a})}{\partial t} = -\sum_i \frac{\partial}{\partial a_i}[g_i(t, P)P(t, \vec{a})] - M(t, \vec{a}, P)P(t, \vec{a}) \quad (1)$$

where P is the population density function, t is time, $\vec{a}$ is a vector of individual physiological properties (age, masses of organism body components, or contained substances), g_i is the growth rate for the ith physiological property, and M is the mortality rate function.

In this work, we combined equation 1 with equations from the individual Daphnia model of Hallam et al. (1990a), equations from the model of toxicant assimilation by zooplankton of Hallam et al. (1988a), and transport equations derived by Plis (1981) using a curvilinear coordinate system. The combined model has been used to represent zooplankton population dynamics in a waterbody whose environment varies in time and space. These variations include seasonal changes in water temperature and growth dynamic of the phytoplankton that is used as food by zooplankton--all in a spatially variable field of water currents.

Hence, toxicant effects on temporal-spatial variations of the zooplankton population will be estimated as a result of the solution of four related problems: 1) transport of dissolved and solid components by water currents and turbulent diffusion; 2) ecosystem interaction of different trophic levels of the model--zooplankton population, phytoplankton, and nutrient; 3) assimilation of toxicant by phytoplankton and zooplankton organisms; and 4) population zooplankton dynamic and growth of zooplankton individuals under the conditions of lethal and sublethal toxicant impacts.

COORDINATE SYSTEM AND TRANSPORT MODEL

The transport model uses curvilinear coordinates representing the spatial boundaries of waterbodies as coordinate surfaces. These coordinates offer the advantage of working with a simple waterbody shape and of using a mesh with the optimal numbers of nodes for approximating the waterbody area with an acceptable level of accuracy. The procedure for calculating curvilinear coordinates was described by Plis (1992).

The transport equation for a shallow water body has the following form

$$\frac{\partial C}{\partial t} + \frac{1}{Jh}\left[\frac{\partial}{\partial \xi}(JhS^{\xi}C) + \frac{\partial}{\partial \eta}(JhS^{\eta}C)\right] + v_g^{\sigma}C =$$
$$\frac{1}{Jh}\left[\frac{\partial}{\partial \xi}\left(hD_L\frac{\partial C}{\partial \xi}\right) + \frac{\partial}{\partial \eta}\left(hD_L\frac{\partial C}{\partial \eta}\right)\right] + F + Q, \quad (2)$$
$$S^{\xi} = \int_0^1 v^{\xi}d\sigma, \quad S^{\eta} = \int_0^1 v^{\eta}d\sigma,$$

where C is the matrix-column, elements of which are the dependent variables of the model; F is the matrix-column of functions of the chemo-biological interactions of model components; Q is the matrix-column of external sources of model components; $J=\partial(x, y) / \partial(\xi, \eta)$ is the Jacobian of the transformation from Cartesian coordinates (x, y) to curvilinear (ξ, η); v^{ξ} and v^{η}

are the contravariant components of current velocity; v_g^σ is the component of sediment velocity; D_L is the horizontal coefficient of turbulent diffusion; and S^ξ and S^η are components of the full stream vector.

The system of equations of the Ekman theory of marine currents expressed in the curvilinear coordinate system was used for calculation of current components, S^ξ and S^η.

MODEL FOR DYNAMICS OF AN INDIVIDUAL DAPHNID

A mathematical model describing life history and bioenergetics of an individual daphnid, along with tables of parameters, was presented by Hallam et al. (1990a). Following this model, an individual daphnid is represented as consisting of two body components--structure and lipid. Its dynamics are represented by the rates of change of these two components, as controlled by energy supply and demand. Input of structure and lipid from food balanced against the demands of maintenance, carapace formation, work, and reproduction determines the net rate of change of the body components. Reproduction and carapace formation, in contrast to the other demands, are computed on a discrete time interval. In general, we follow this model closely, including the use of most of its parameters.

To use the model in a dynamic situation including seasonal dynamics and a wide range of food densities, however, we had to make some alterations. Specifically, we added the representation of the influence of water temperature on growth and mortality and a limitation on maximum size of the protein and lipid compartments.

MODEL FOR PHYTOPLANKTON DYNAMICS

Phytoplankton is primary food for daphnids, and in this model phytoplankton dynamics depend on availability of mineral nutrients, on changes in temperature, and on consumption by zooplankton. Our nutrient-phytoplankton model is structured only with respect to space and is the simplest that we could conceive to provide dynamics to support a physiologically and spatially structured population of zooplankton.

MODEL OF TOXICANT ASSIMILATION BY ZOOPLANKTON AND PHYTOPLANKTON

We assumed that the dissolved toxicant is assimilated by individuals of the population through two mechanisms. The first is penetration across the surface of the organism's body by diffusion. The second is toxicant accumulation from food. In accordance with our assumptions, the food for zooplankton is phytoplankton. It is assumed that processes of toxicant accumulation by phytoplankton can be described using an equilibrium hypothesis. Removal of toxicants from the zooplankton organism takes place with nonassimilated food and through diffusive exchange across the body surface of the organism.

We assume here that the toxicant does not impact on phytoplankton life processes. In relation to zooplankton organisms, however, the toxicant slows down processes of

accumulation and consumption of energy in accordance with theoretical suppositions described below. If the toxicant concentration in the liquid phase of the organism exceeds values equal to the lethal concentration (LC_{50}), it is assumed this organism will die.

It was assumed also that the distribution of toxicant in the waterbody depends only on conditions of transport by currents and turbulent diffusion, and the location and intensity of external sources of pollution. Biochemical and physical processes of toxicant transformation in a waterbody have not been taken into account in the present work.

The equation of the toxicant balance in the individual organism of Daphnia M. (Hallam et al. 1988a) was used as the basis of the model of toxicant assimilation. This equation was modified to take into account losses of toxicant caused by reproductive processes. The equation can be written as

$$\frac{dm_T}{dt} = SK_w (C_w - C_a) + FC_F - EC_E - R, \tag{3}$$

where m_T is the mass of toxicant accumulated into an individual organism, mg; K_w is the coefficient of conductivity across organism body surface, mm/day; S is the effective surface area of the organism body, permeable for chemical, mm^2; C_w and C_a are the concentrations of toxicant in the ambient water and in the aqueous phase of the organism, mg/L; F is the mass of food eaten per unit time, mg/day; E is mass of material defecated per unit time; C_F and C_E are concentrations of toxicant in the food and in the feces; R is mass lost for reproduction per unit time, mg/day.

Using the assumption of internal equilibrium between concentrations of the toxicant assimilated in the three basic phases of the organism (aqueous, lipid, and structural [nonlipid organic]), we can represent the concentration of toxicant in the aqueous phase C_a, in accordance with Hallam et al. (1988a) and Burns et al. (1990) by the expression

$$C_a = \frac{m_T}{m_L(\delta_2\delta^{-1} + K_L + K_S\,\delta_2)}, \tag{4}$$

where $m_T = (m_aC_a + m_LC_L + m_SC_S)$; m_a, m_L, and m_S are mass of aqueous phase, mass of lipid, and mass of structure, respectively, in the body of the individual; $\delta_2 = m_S/m_L$; $\delta = m_S/m_a$; K_L and K_S are thermodynamic partition coefficients of the toxicant between the lipid and the water, and the structure and the water, respectively; C_L and C_S are concentrations of toxicant assimilated in lipid and structure phases of the organism, respectively.

In the framework of the present model, the assimilation of a toxicant by phytoplankton was taken into account using the assumption about equilibrium between ambient toxicant concentration and toxicant concentration in phytoplankton biomass.

$$C_L^P = K_L C_P\, f_L, \quad C_S^P = K_S\, C_P\, f_S\,, \tag{5}$$

where C_P is the phytoplankton biomass, mg Chlorophyll *a*/L; f_L and f_S are fractions of the lipid and the structure of the phytoplankton biomass.

For term R (equation 3) we assume that the loss of individual mass during the reproductive process is accompanied proportionally by the loss of the toxicant mass accumulated in the parent organism's body, or

$$m_T^e = m_T\, m_L^{-1}\, m_L^e, \tag{6}$$

where, m_T^e and m_L^e are mass of toxicant and mass of lipid, respectively, in one newborn; And R becomes

$$R = P_n\, m_T^e, \tag{7}$$

where P_n is the number of newborn in the one birth event of one organism of zooplankton.

MODEL OF TOXICANT EFFECTS ON AN INDIVIDUAL

The problem of modeling lethal toxicant effects on Daphnia M. population dynamics was discussed by Hallam et al. (1990b). In their model, the amount of chemical in the aqueous phase of the organism was compared with the LC_{50} determined on the basis of qualitative structure-activity relationships (QSARs) that connect chemical property, e.g., the octanol-water partition coefficient(K_{O_W}) , with mortality of the individual. We assumed that if

$$\log(C_a) \geq -0.8 - \log(K_{O_W}) \tag{8}$$

the individual will die.

The principal difference between lethal and sublethal effects, which was accepted as the initial assumption in this paper, is that the sublethal reaction of the individual is a monotonically increasing function of the internal concentration of the toxicant, C_a. This function has a lower bound, where the sublethal effect is not observed, and an upper bound, where the lethality occurs. Lassiter (1990) proposed a model for the effect on organisms exposed to a sublethal toxicant concentration, C_w. With prolonged exposure, the organism's internal aqueous concentration, C_a, approaches C_w at a rate controlled by the organism's exchange mechanisms (gill, gut), and by its body composition and circulation.

NUMERICAL REALIZATION OF THE MODEL

The scheme of component-by-component splitting (Marchuk 1982) was used for the numerical solution of the system of quasilinear equation 2. This method reduces a complicated problem to a simpler one when the initial differential operator of the system is positive-semidefinite and can be represented as a sum of positive-semidefinite simpler operators.

Besides a formal numerical scheme for solution of the system of equations, other simplifications were made in the interest of computational feasibility. Although these simplifications undoubtedly reduced the fine-scale resolution and variance, they were made subject to conservation constraints so that mass and numerical density (number of individuals per unit volume of water) of the population were preserved.

Initially, the population density function is a function of 6 variables

$$P = P\,(a,\ t,\ m_1,\ m_2,\ m_3,\ m_4), \tag{9}$$

where a is the age of the individual organism; m_1, m_2, m_3, and m_4 are the masses of individual lipid, structure, nonlabile structure, and assimilated toxicant, respectively. The following assumptions were made to simplify equation 1. The daphnid population was shared by I physiological clusters distinguished, as in Hallam et al. (1990a), by different ratios of maximal filtering rate to organism length squared. Each of the I physiological clusters was shared by N age groups. All individuals of any age group of a physiological cluster are physiologically and morphologically identical. This is accomplished first by an assumption about average properties of the first age group. The first age group of a cluster consists of offspring from all reproductive age groups of that physiological cluster. Parents are distinguished, in addition to age, by lipid, by structural (labile and nonlabile) body composition, and by assimilated toxicant. Hence, by the model of Hallam et al. (1988b, 1990b), they produce offspring that also vary somewhat. Our assumption is that these differences in the offspring (i.e., the first age group) are inconsequential, and hence, we average these properties to derive their values for the entire age group of the physiological cluster. Additionally, populations are mixed by spatial movement. Here we assume that the properties of each age group of a physiological cluster at a spatial point in a waterbody are the weight averages of the remaining residents and the recruits from neighboring spatial points.

As a result of the foregoing assumptions, population density and individual mass characteristics for each ith physiological cluster can be simplified to

$$p^i = P^i\,(a,t);\quad m_j^i = m_j^i\,(a,\ t),\qquad j = 1,\ldots,4 \tag{10}$$

This, together with assumed functions describing zooplankton population mortality, permits equation 1 to be transformed to

$$\frac{\partial P^i}{\partial t} = \frac{\partial P^i}{\partial a} - (M_a^i + M_p^i)P^i, \tag{11}$$

$$P^i(O,t) = \int \beta(a,\ t,\ m_1^i,\ m_2^i,\ m_3^i,\ m_4^i)P^i(a,\ t)\,da,\qquad i = 1,\ 2,\ldots,I$$

where M_a^i and M_p^i are the age dependent and density dependent mortality, respectively; β is the birth function that represents the number of neonates born to an individual of age a with mass characteristics m_1, m_2, m_3, and m_4 at time t (Hallam et al. 1990a).

NUMERICAL EXPERIMENTS

To explore the behavior of this model, we conducted numerical simulation of seasonal dynamics of the nutrient-toxicant phytoplankton-daphnid system in a hypothetical, shallow waterbody with arbitrary shapes for the lateral boundaries and with current velocities low enough that they did not dominate ecological processes in determining concentrations of nutrients or densities of phytoplankton and zooplankton. We assumed a time-invariant distribution of current velocities during a simulated year. Zooplankton population was assumed to consist of only one physiological cluster ($I = 1$) consisting of 50 age groups ($N = 50$) with the age group interval being 1 day.

Although our computations relate to a hypothetical situation, we selected values of physical properties of the hypothetical system to be well within the range of real physical systems. We discuss the results of our simulations of the hypothetical situation for two purposes: first, to show the behavior of the Daphnia population model in the context of a spatially and temporally variable system; and second, to give a concrete description of the nature of some of the results that can be expected from a spatially and physiologically distributed aquatic food chain model.

As we simulated the seasonal dynamics of the zooplankton population in one hypothetical waterbody, various spatio-temporal distributions of population characteristics were obtained. These characteristics included numerical densities, population biomass, ratios of the population lipid to population biomass, and distributions of densities and mass characteristics among age groups. We noticed strong correlations between these characteristics and the spatio-temporal variations of phytoplankton biomass and concentration of toxicant in the waterbody. We found that the distribution of individual lipid content is the most sensitive indicator of environmental stress. For an individual organism, lipid fluctuates more in response to environmental stress fluctuations than does body structure or total biomass. The next most important characteristic for a daphnid population is the distribution of age at first reproduction, which depends on the distribution of lipid and nonlipid mass content. It was shown that the inhibiting influence of toxicants on activity and reproduction decreases with increasing individual lipid content.

CONCLUSIONS

A spatially- and physiologically-structured aquatic food chain model was developed. It represents the transport of both dissolved and passively suspended components by water currents and turbulent diffusion. The model also represents the chemical-biological reactions along a food chain, including chemical, nutrient, phytoplankton, toxicant, and age- and mass-structured zooplankton populations. The structured population is represented by an extended McKendrick-von Foerster equation that requires an individual model at the physiological level of resolution for each of the dimensions of the population model (age, lipid, structure, nonlabile structure, and toxicant body burden).

A curvilinear coordinate system also was employed to improve computational efficiency in comparison with an ordinary Cartesian coordinate system. This approach improves computational efficiency by permitting the waterbody to be represented in the

transformed coordinates as a simple rectangular shape, and to be approximated by a grid with the optimal number of nodes for numerical approximation at an acceptable level of accuracy. The algorithm for numerical solution of the model equations was developed using the method of component-by-component splitting.

The example of the model application to a polluted lake showed opportunities to obtain additional comparative information for decision makers about the behavior and life conditions of populations in clean and polluted areas of waterbodies. The success of this and other attempts depends on existing biologically and ecologically realistic individual based models.

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GREEN BAY/FOX RIVER MASS BALANCE STUDY--MANAGEMENT IMPLICATIONS

by

Lyman F. Wible[1], John G. Konrad[2], and Jeffrey Steur[3]

ABSTRACT

In 1986, the Green Bay/Fox River Mass Balance Project was initiated to demonstrate the feasibility of the mass balance concept as a water quality management tool. The project monitored and refined mathematical models which represented the inputs, transformations, and outputs of four toxicants in the waters of Green Bay, Wisconsin. Green Bay is an embayment of Lake Michigan and historically has received significant pollutant loads from the industries and municipalities along the Lower Fox River. The toxicants of concern for the Green Bay/Fox River mass balance study are polychlorinated biphenyls (PCBs) at the congener level, dieldrin, lead and cadmium. Existing hydrodynamic models, water/sediment models, and a food chain model were coupled to estimate the pollutant body burden in target fish species, mainly carp, brown trout, and walleye. Preliminary results of the modeling and the implications of the model results are presented. Project management experiences and lessons learned from conducting a large multi-participant project also are discussed.

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INTRODUCTION

This paper presents some of the preliminary results from the Green Bay/Fox River Mass Balance Project, which is an analysis of the sources, sinks, pathways, and fates of four pollutants in the Green Bay and Fox River aquatic systems. Green Bay is a part of Lake Michigan, one of the Great Lakes, and is the largest freshwater estuary in the world. The Bay is 4,520 km^2 (1,640 mi^2) in areal extent, with an average depth of 3-5 m (10-15 feet) in the southwestern end and 15 m (49 feet) in the northeastern end with a maximum depth of 54 m (177 feet). Figures 1 and 2 depict the location of Green Bay and the Fox River. The Fox River is the major tributary to the Bay, and extends 63 km (39 mi) from Lake Winnebago to the river mouth at Green Bay. The City of Green Bay and the other communities along the Lower Fox River are home to 270,000 residents, or 6% of the population of the State. This area includes 20 pulp and paper mills discharging either directly to the Fox River or indirectly via municipal sewage treatment plants, and is generally considered the largest concentration of pulp and paper mills in the world.

Because the project is just entering its final stages, all results are not yet available. Some of the preliminary results are presented in this paper. Additional results will be reported by project investigators in future papers. The status and general findings for the entire study area are included in this paper, but the focus is primarily on the lower Fox River, for which the earliest project results have become available.

The Green Bay/Fox River Mass Balance Study began in 1986 after more than one yr of dialogue among the various government and private organizations with an interest in these waters. The study has involved more than 20 major organizations and laboratories of the U.S. Environmental Protection Agency. The participating organizations are listed in Table 1. The direct expenditures in this project have totalled more than $10,000,000 with substantial indirect expenditures by the participants and other supporting organizations through their efforts to support, assist, and make this historic study effective.

During the first 2 yr, 1986 and 1987, project participants developed monitoring and quality assurance programs and began field testing analytical and monitoring methods. In 1988 the field work was initially tested and sampling started with a shakedown cruise on October 1988. Intensive field work was initiated in the spring of 1989 and continued through May 1990. From 1990 to 1992, samples were analyzed, data interpreted, and modeling was conducted. By October 1992 the modeling is scheduled for completion, while the management analyses using these sophisticated models may be expected to extend for many additional years. A final report on the project is due at the end of calendar year 1992.

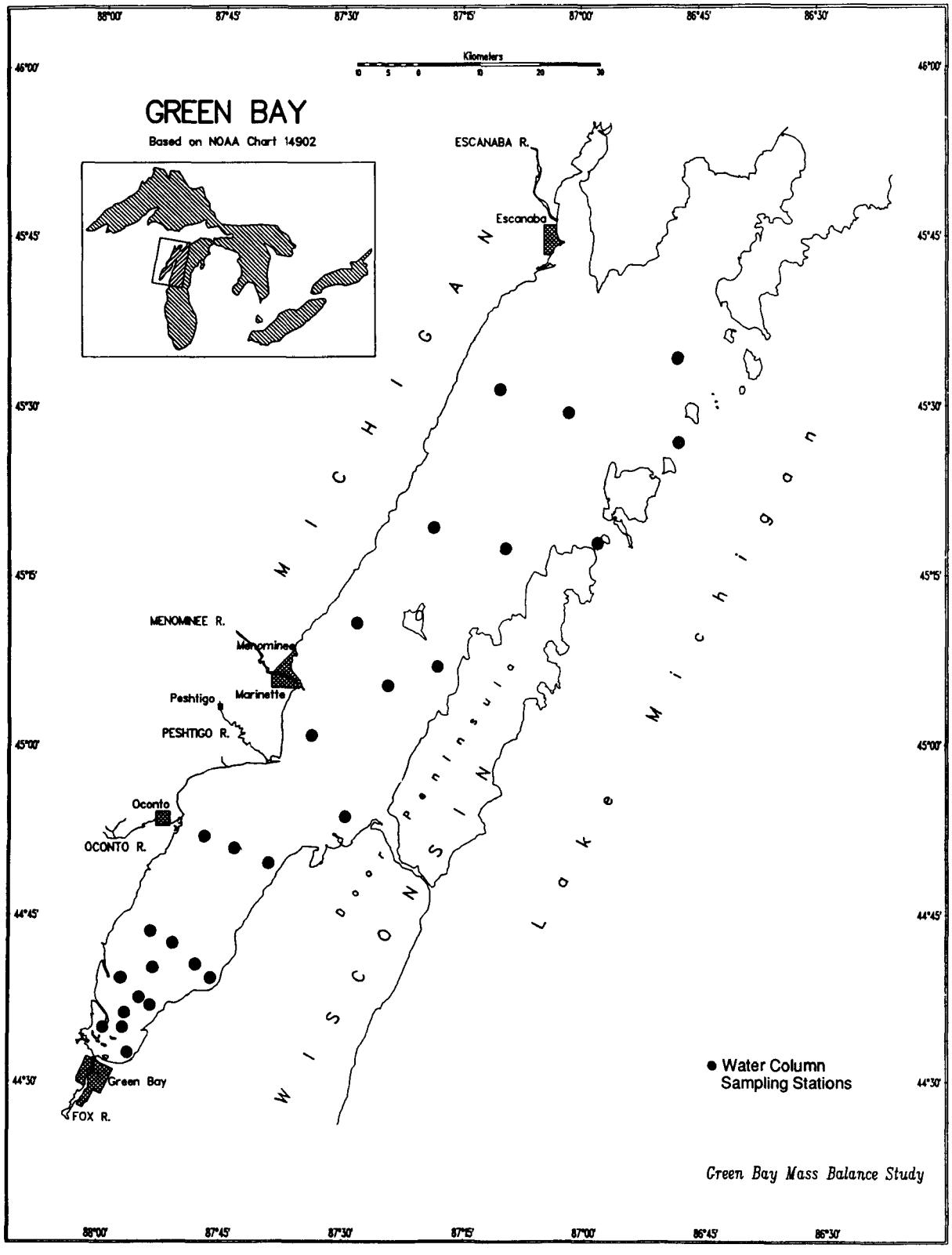

Figure 1. Location of Green Bay and water column sampling stations.

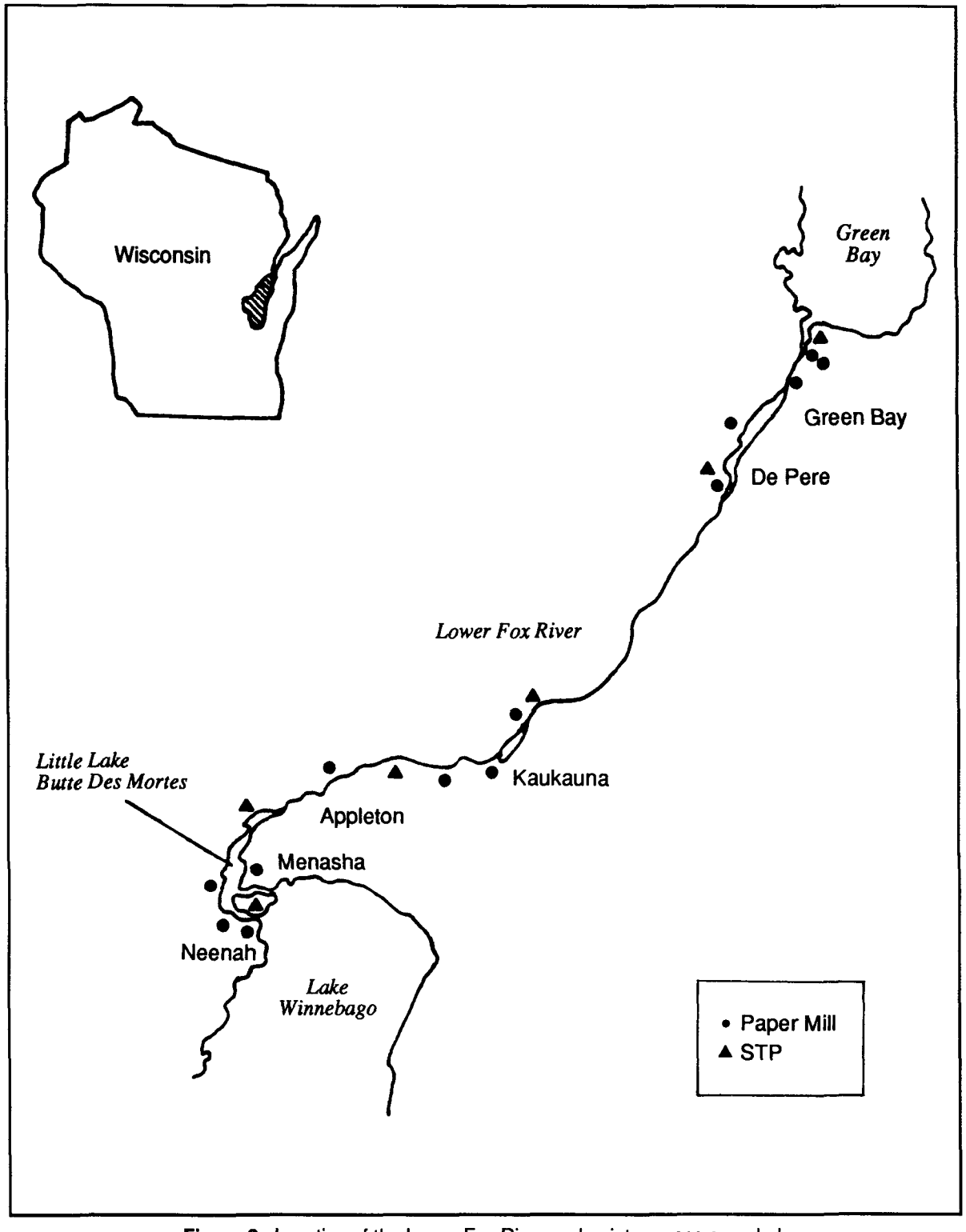

Figure 2. Location of the Lower Fox River and point sources sampled.

TABLE 1. MAJOR COMPONENTS OF THE GREEN BAY/FOX RIVER MASS BALANCE PROJECT AND RESPONSIBLE ORGANIZATIONS

Major Components	Responsible Organization
-Fox River transport modeling (above DePere) -Point source monitoring -Urban nonpoint source estimates -Ground water impacts -Abandoned landfill impacts -Fish collection and processing -Project management -Lower Fox River deep core sediment samples	Wisconsin Department of Natural Resources - Madison and Green Bay, WI
-Conventional parameter analyses (Tributaries and Fox River above DePere) -PCB analyses in Fox River sediment and Fox River water samples above DePere	Wisconsin State Laboratory of Hygiene - Madison, WI
-Green Bay water column monitoring -Project management -Conventional parameter analyses (Green Bay and Lower Fox River)	U.S. Environmental Protection Agency - Great Lakes National Program Office-Chicago, IL
-Modeling coordination -Fish and other biota analyses -Lower Fox River transport modeling (below DePere)	U.S. Environmental Protection Agency - Large Lakes Research Station-Grosse Ile, MI
-Water column analyses -Analysis of plankton samples	U.S. Environmental Protection Agency - Environmental Research Laboratory-Duluth, MN
-Food chain modeling -Statistical integration	Manhattan College - New York, NY
-Green Bay mass balance modeling	University of Notre Dame - South Bend, IN State University of New York-Buffalo, NY Clarkson University - Potsdam, NY Limno-Tech, Inc. - Ann Arbor, MI Youngstown State University - Youngstown, OH
-Physical characterization of Fox River bottom sediments -Bottom sediment resuspension and flux	University of California-Santa Barbara, CA
-Desorption kinetics -Sedimentation and volatilization rates	University of Wisconsin-Madison, WI University of Wisconsin-Milwaukee, WI Wisconsin Sea Grant Program - Madison, WI
-Water volume transport -Sediment flux in Green Bay	National Oceanic and Atmospheric Administration - Ann Arbor, MI
-Atmospheric monitoring -Atmospheric deposition	Illinois State Water Survey - Champaign, IL DePaul University - Chicago, IL University of Minnesota-Minneapolis, MN
-Quality assurance/quality control -Bioaccuumulation of PCB in phytoplankton	University of Minnesota-Minneapolis, MN
-Local area coordination	University of Wisconsin-Green Bay, WI
-Tributary monitoring -Sediment mapping	U.S. Geological Survey - Madison, WI
-Data management	Computer Sciences Corporation - Grosse Ile, MI

ENVIRONMENTAL PROBLEMS IN GREEN BAY

Eutrophication problems in the Great Lakes have focused remedial programs on reduction of phosphorus and other conventional pollutants. Current research on environmental problems in the Great Lakes indicates persistent toxic substances are a major problem (1). Green Bay, as a major arm of Lake Michigan, constitutes an important subsystem of the whole lake. The predominant water quality problems in the waters of Green Bay have been historically associated with the Green Bay metropolitan area and the discharge of the Fox River. Green Bay and the Fox River have a long history of water quality problems derived from the contributions of pulp and paper mills, municipal sanitary wastes, and other sources (2).

From the 1930s to the 1970s dissolved oxygen conditions grew worse. The biochemical oxygen demand (BOD) load discharged to the Fox River steadily increased because of paper industry growth and -- to a lesser extent -- population growth. The results of pollution abatement efforts since 1972 have been excellent and have shown dramatic reductions in BOD and suspended solids. The discharge of conventional pollutants (BOD) to the Fox River has been reduced by more than 95%, from 375,000 pounds/day in 1971 to 17,400 pounds/day in 1984 (2,3). The biological response in the aquatic system has been equally dramatic. The waters of the Lower Fox River and Green Bay, largely unused from the 1930s to the 1970s for recreation or fishing, have become a major attraction and economic asset. A highly productive walleye fishery has returned, land uses are changing, marinas and shoreland development are flourishing, and commercial and sport fishing as well as recreational boating have increased beyond any expectations at the time of the cleanup. Community pride in the accomplishments is evident.

In spite of these achievements, monitoring programs using improved analytical precision and information on the recovering fisheries and wildlife have indicated that health advisories are prudent to guide the public concerning human consumption of fish and wildlife. Accordingly, the past decade has been a time of increased public sensitivity and awareness to governmental warnings on the potential health hazards of food from these waters.

In 1981, the International Joint Commission (IJC) identified 39 Areas of Concern (increased to 42 in 1985 and to 43 in 1991) in the Great Lakes Basin. State and local governments began to develop Remedial Action Plans (RAPs) in 1986 to address these problem areas. Annex 2 of the 1987 Protocol to the 1978 Great Lakes Water Quality Agreement formalized the review and approval of RAPs by the IJC. The persistent environmental problems in Green Bay, coupled with the IJC's call for RAPs, prompted the development of the RAP for the lower portions of Green Bay and the Fox River.

Supported by major public participation during its development, the Lower Green Bay RAP was completed in 1988. The Lower Green Bay RAP was the first prepared in the Great Lakes Basin and first to receive the approval of the IJC. While public support has been strong, public tolerance for scientific uncertainty has been fairly limited, with local officials and citizens alike demanding as much scientific insight as possible. Fortunately, an extensive database and understanding of the ecosystem of Green Bay was available from previous research studies. For these reasons the Green Bay Mass Balance Project was seen as a very positive and scientifically credible addition to the RAP process.

THE MASS BALANCE PROJECT STUDY DESIGN

As discussed by Fink and Wise (1), the intent of the mass balance concept is to evaluate the sources, transport, and fate of contaminants in the study area. The approach requires that the quantities of contaminants entering the system, less quantities stored, transformed, or degraded within the system, must equal the quantities leaving the system. Toxicants of concern in the Green Bay/Fox River mass balance study are Polychlorinated Biphenyls (PCBs) at the congener level, dieldrin, lead, and cadmium. These toxicants were chosen for their ecological significance (PCBs) or as representatives of classes of contaminants (pesticides, methylating metals, and inorganic metals).

The initial mass balance study area was limited to the waters of Green Bay with the shoreline serving as the boundaries for the project. The Wisconsin Department of Natural Resources believed that because the lower Fox River played such an integral role in the water quality dynamics of the shoreline waters and open waters of the Bay, this portion of the river should be included in the study area. For this reason, a major effort to model the transport of PCBs in the 51.5 km (32 mi) portion of the lower Fox River from Lake Winnebago to the last dam (located at DePere, Wisconsin) was also developed. The overall project objectives are

- Quantify and map the presence of selected pollutants within the aquatic system and the environmental compartments of the system.
- Identify the loads of these pollutants moving into and through the system.
- Identify the sources of these pollutants.
- Identify the water/sediment/food chain interactions within these aquatic systems.
- Provide a tool for the analysis of alternative management scenarios (addressing the sources of toxic substances and existing accumulations or inventories of the substances).

Because of the extensive field work involved in the project and the need to coordinate the efforts of a large number of different organizations, project leaders worked almost 2 yr to develop the study design and evaluate appropriate field and

laboratory procedures. We targeted the field work to the characterization of the inputs, the outputs, and the internal interchange mechanisms for transfer of pollutants from one environmental compartment to another inside the study boundary. Figures 1 and 2 show the study area and the location of the surface water column sampling stations and point sources. For this study it was necessary to sample the full range of inputs. We conducted sampling and determined pollutant loads for 5 tributaries, 19 point sources (including 6 municipal wastewater treatment plants and 13 pulp or paper mills), 3 atmospheric deposition stations, 12 storm sewers, 4 abandoned dumps or landfills, 60 groundwater locations, and 375 river sediment locations. Sampling was conducted to characterize the mass of pollutants in the open Bay at 27 water column sites and at 167 bay sediment sites. The water column sites were sampled 8 times during 1988-90. Sedimentation rates and volatilization from the water column were characterized by special rate experiments. Internal recycling and storage studies were conducted for water column interactions with sediments in Green Bay. Biota studies were conducted throughout the project by collecting phytoplankton and zooplankton at each of the water column sites. More than 3500 fish were combined into more than 685 samples covering various food chains including forage base and several age classes of 3 top predators. The final Study Plan for the project was adopted in April 1989 (4).

All of these data were available for modeling applications. Existing hydrodynamic models, water/sediment models, and food chain models were refined and calibrated. As illustrated in Figure 3, the physical and chemical models were coupled with the food chain models to estimate the pollutant body burden in target fish species, mainly carp, brown trout, and walleye. We constructed models to provide calibration, verification, and prediction of the conditions in the water, sediment, and the biota under varying combinations of regulatory and remedial action plans. Both short-term and long-term predictive capability were developed.

The sampling efforts were particularly successful and have resulted in an unparalleled database available for both current and future researchers. Laboratory quality was assured through Quality Assurance/Quality Control (QA/QC) protocols. The ability of several laboratories to process samples on the specified schedules was an issue that required continual oversight by project managers. The quality of laboratory data along with the ability to provide low-level analyses in a timely way were issues that required several modifications in the project scope and time schedules. We accomplished these modifications through a project management structure that involved all of the project participants. The resulting data could support far broader research in the future as the full scope and detail of this database are made available to potential investigators beyond those already included in the project.

In addition to the development and testing of the modeling component, project managers conducted important and necessary activities for the management of the project at both the technical and administrative levels. Among these activities was the identification of the appropriate types of public involvement to assure that the high level of public interest was not threatened by a lack of understanding of the project.

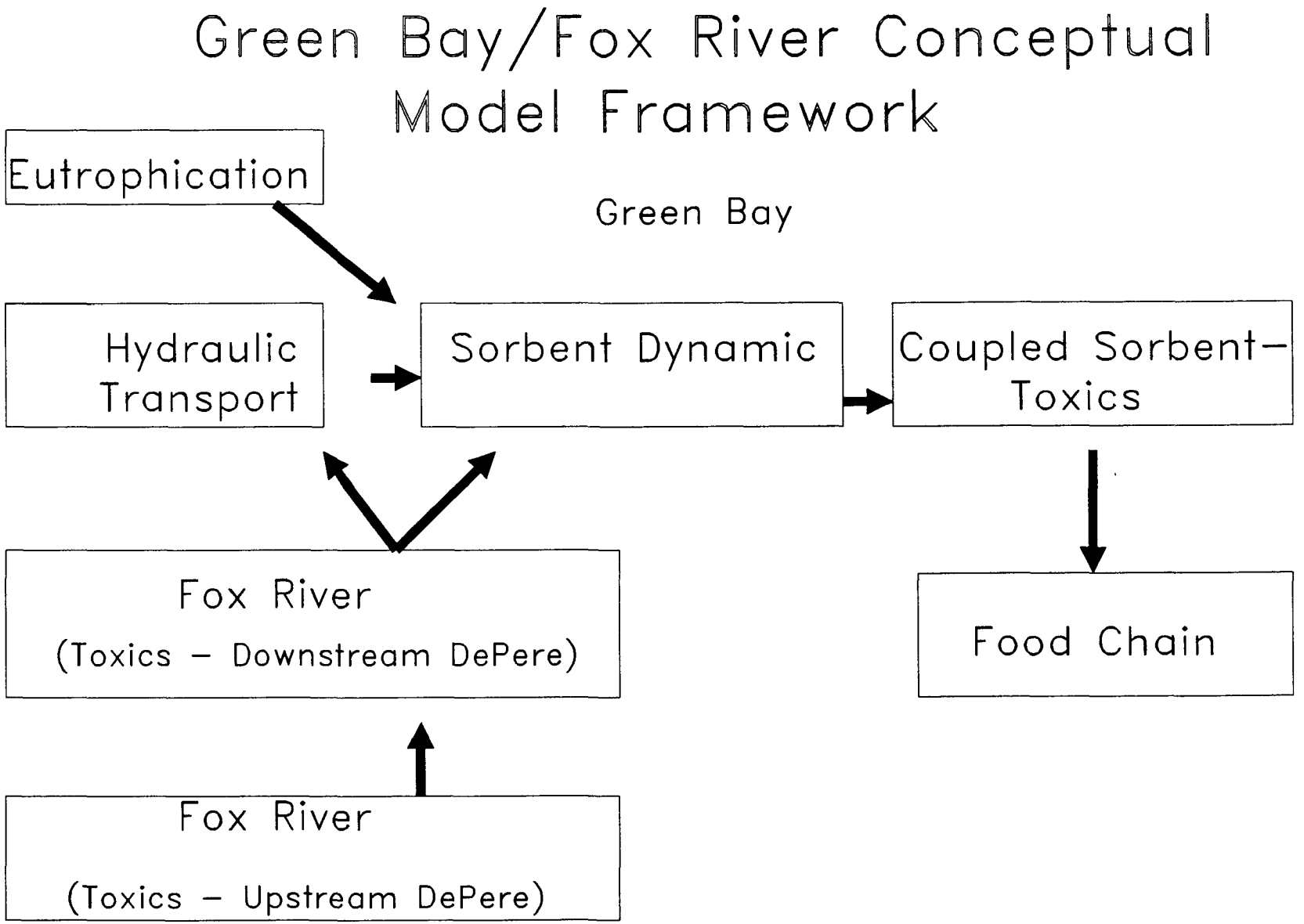

Figure 3. Green Bay/Fox River mass balance simulation models

RESULTS TO DATE

Because all modeling will not be completed until late 1992, this paper can present only preliminary results. Even the preliminary results are very useful for management purposes. Some of the earliest and most useful information is derived simply from the mapping and mass estimates for contaminants in the sediments.

PCB RESULTS

PCBs have been measured, mapped, and modeled in Green Bay and the Lower Fox River. We determined the depth to which PCBs are found in the Green Bay and Fox River sediments from the analyses of sediment cores. From these data it is possible to calculate the total mass of PCBs in the sediments. Preliminary calculations using these sediment cores indicate that the mass of PCBs in the system could be as high as 63,000 kg. Of this total, about 14,000 kg or 22% is in the bay (A. Andren, personal communication, 1992), with the remaining 78% estimated to be in the Fox River system (49,000 kg). The greatest portion of this mass of PCBs resides below the DePere Dam (45,000 kg) in the estuary-like, lowest reach of the Fox River. The surveys and inventories have identified the proportions of the PCBs that are coming from the various sources and moving into various compartments for the Lower Fox River. These initial mass balance estimates indicate that sediments are a substantial source (more than 98%) of the annual PCB load to the river above DePere (Figure 4), and that the river is the major source (67%) to the Bay itself, with point sources comprising no more than 3 to 11% of the load to the Bay depending on the assumptions made in using detection or less-than-detection values to compute point source loads (5). In addition, the early analyses seem to indicate that the waters of Green Bay may be a source of PCBs to the atmosphere (especially for lower-chlorinated congeners) under many conditions, rather than serving as a sink, as might have been anticipated. The volatilization of PCBs from the water column probably contributes more mass to the atmosphere than does atmospheric wet or dry deposition to the Bay. More complete project results are required before this conclusion can be firmly or unconditionally validated.

As shown in Figures 5-7, the bottom sediment PCB flux to the water column for model segments from Neenah to DePere is determined by flow characteristics (quantity and channel configuration), sediment PCB content, and to a lesser degree by total suspended solids. This later factor is illustrated by the several orders of magnitude difference in the summer and winter daily sediment fluxes. In the summer, the suspended solids loads are increased because of algae and resuspension of bottom sediments. These water column-sediment interactions show how the various deposits of PCBs in the bottom sediments act as either sources or sinks under the typical range of

Fox River (upstream DePere Dam)

3/12/89 to 3/11/90 (preliminary)

PCB Water Column Flux

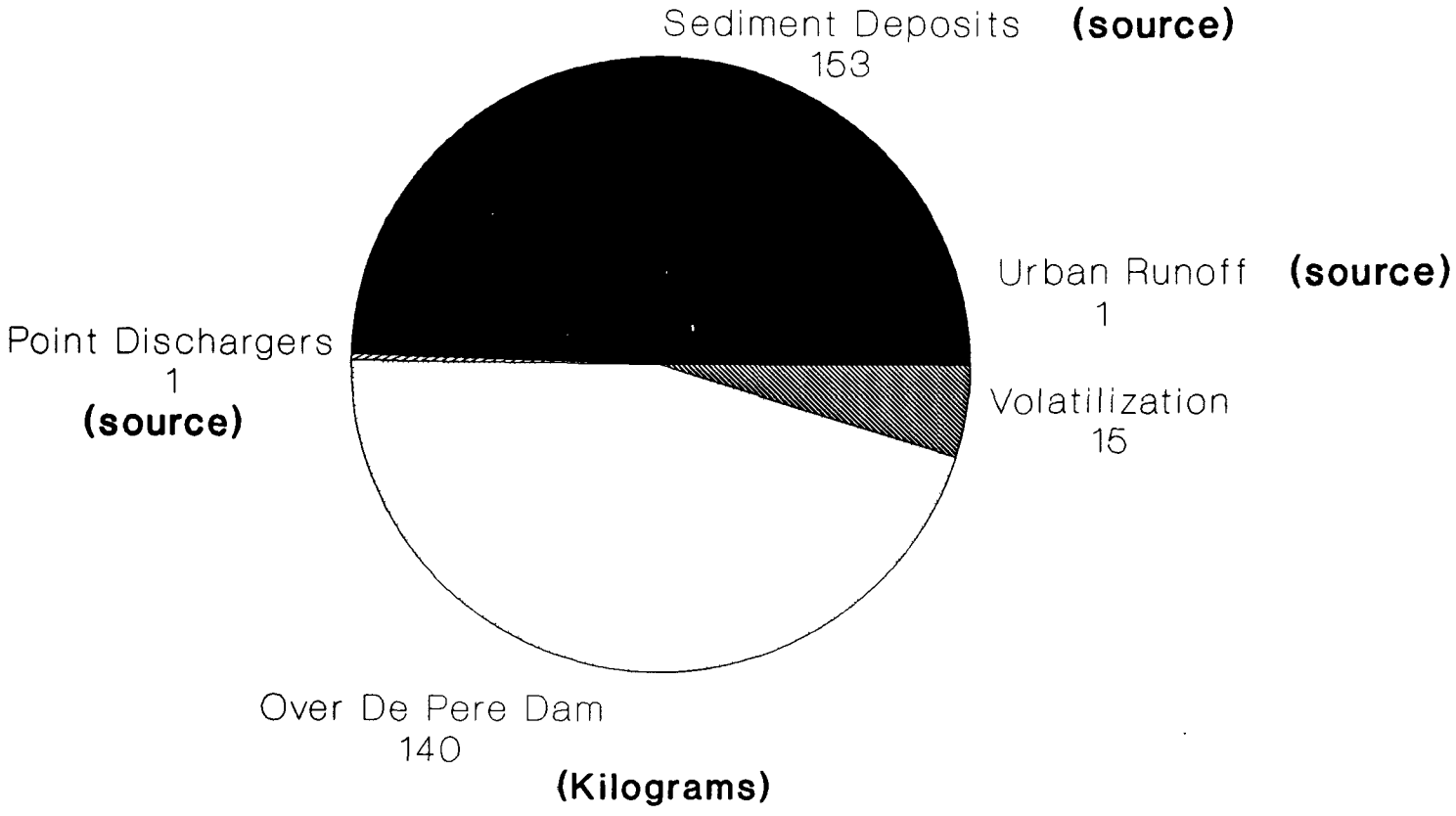

Figure 4. Sources and sinks of PCBs in Fox River

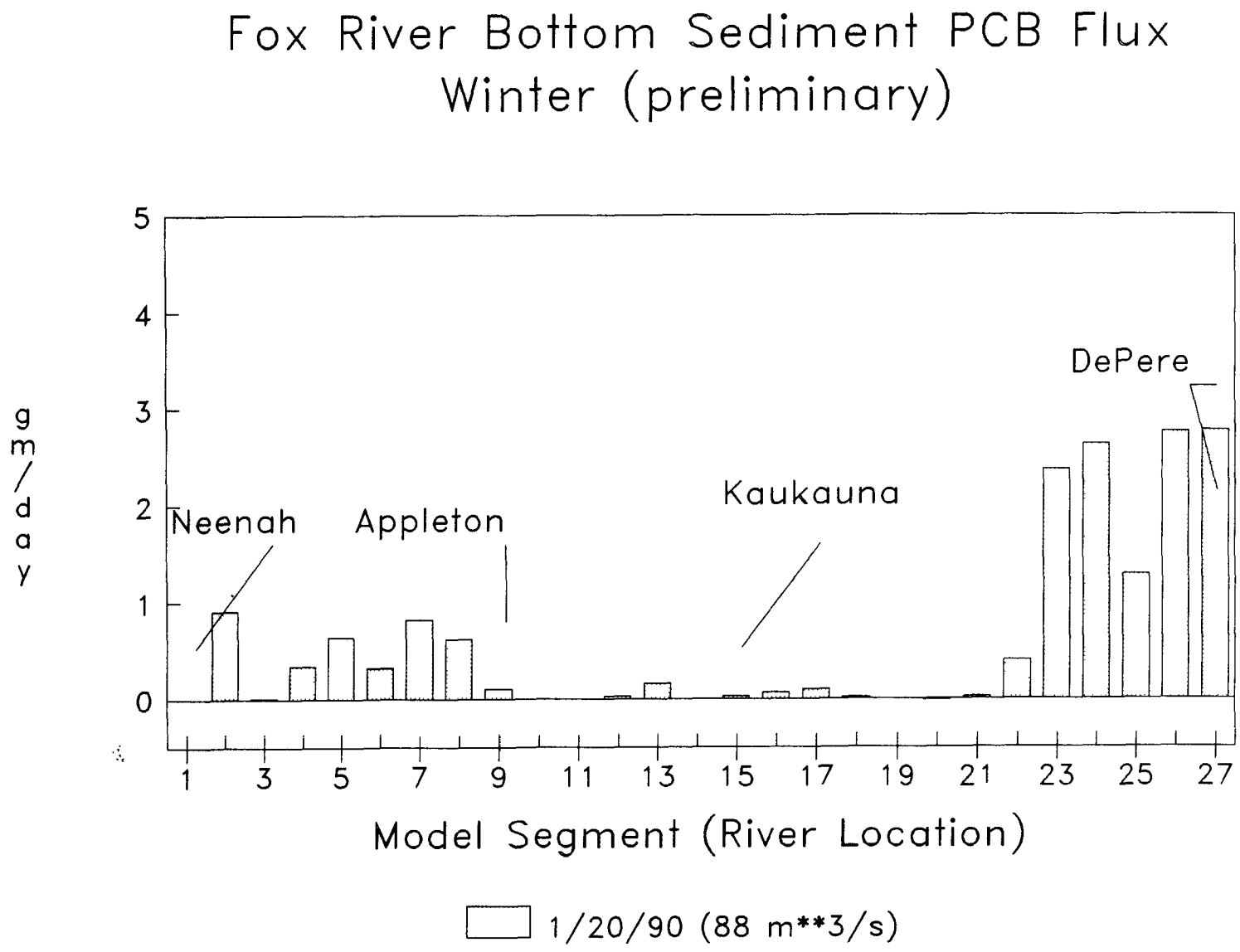

Figure 5. PCB flux from bottom sediment under winter flow

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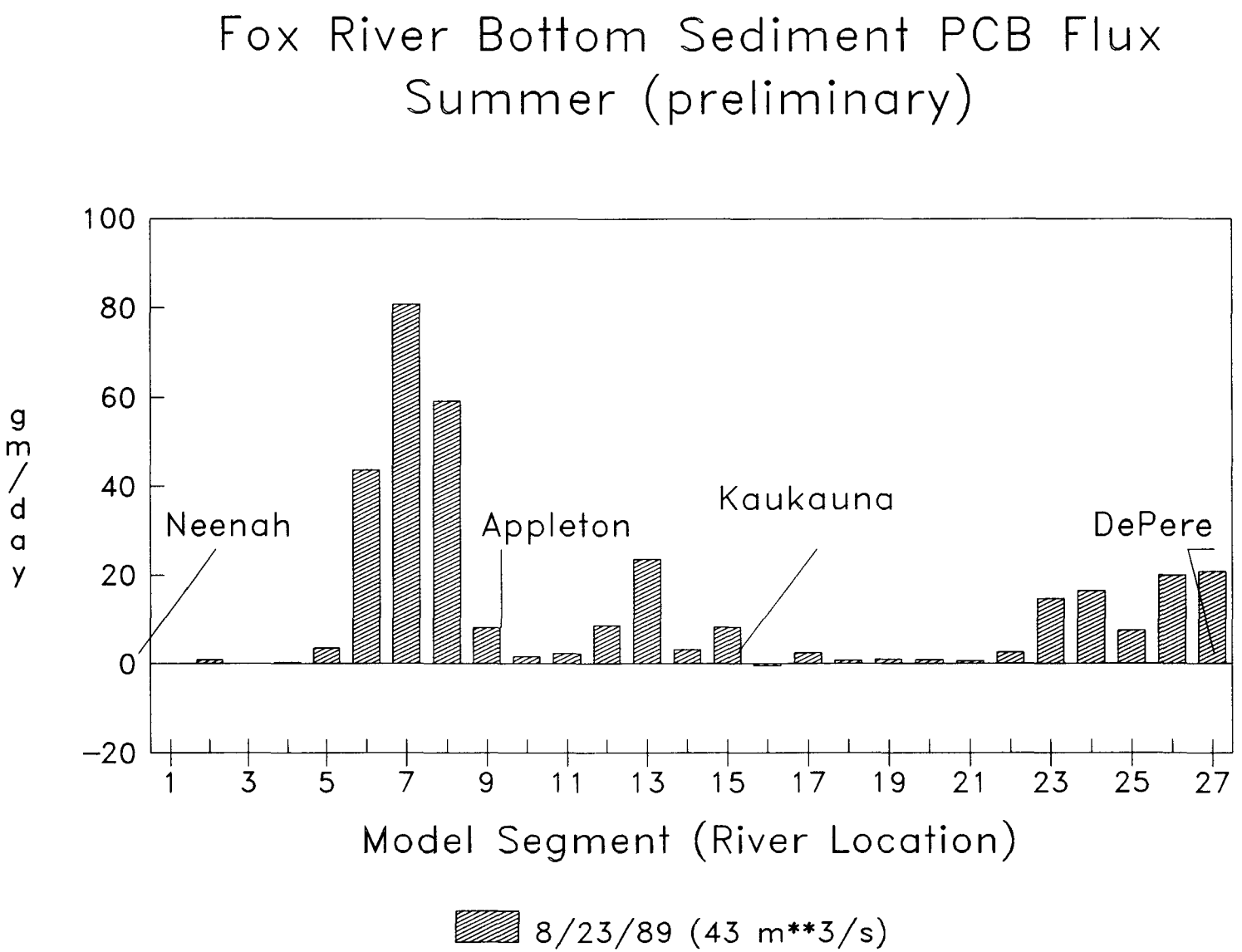

209foxrv Figure 6. PCB flux from bottom sediment under summer flow

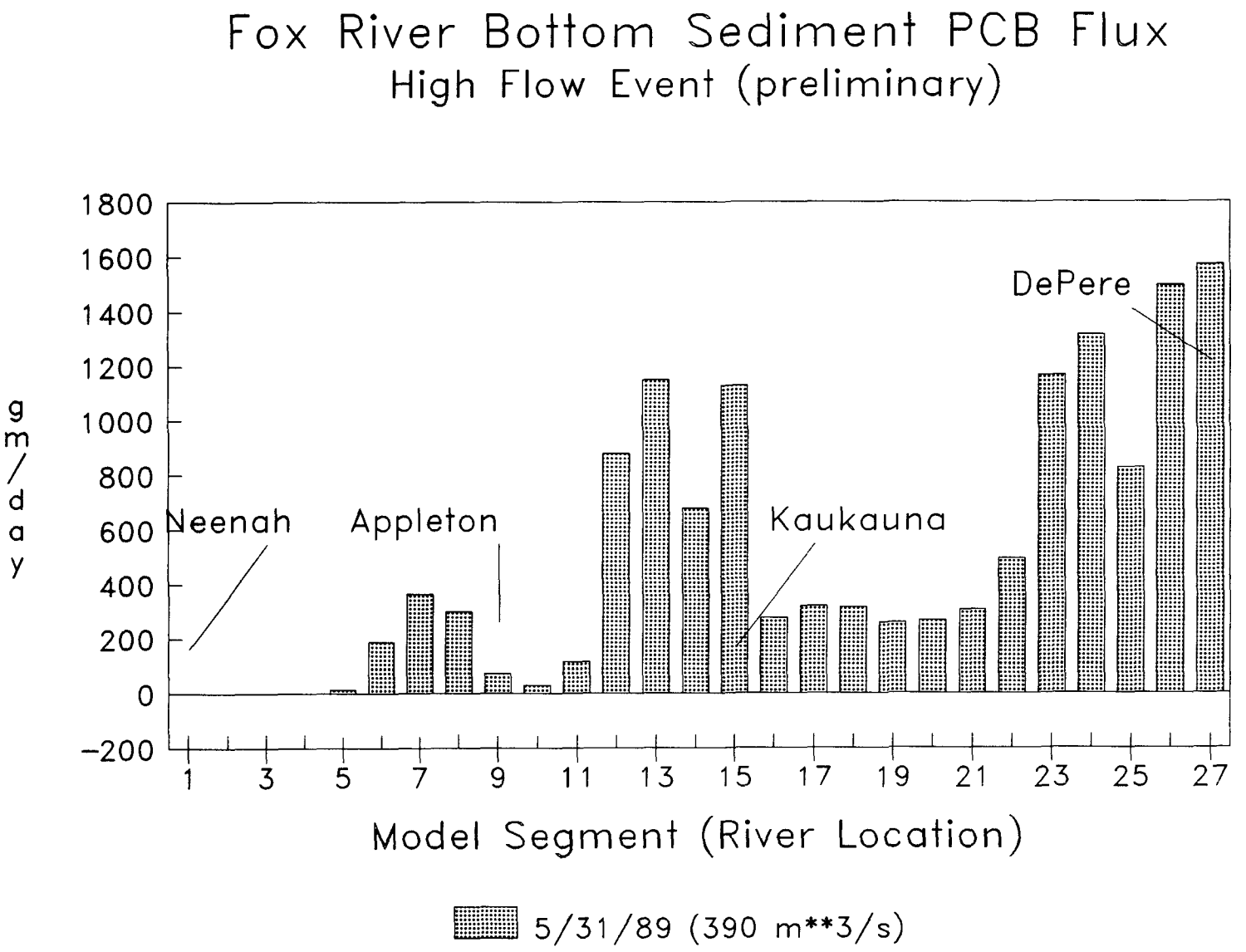

Figure 7. PCB flux from bottom sediment with high flow

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flow and hydrodynamic conditions. The models further indicate that these sources and sinks can change their behavior significantly under other flow conditions (Figure 7).

We used depth and areal extent estimates for the Fox River to quantify and map the distribution of PCBs in the sediments. The Little Lake Butte des Morts river reach is shown in Figure 8. This reach is the location of some logical first targets of opportunity for remediation of the contaminated sediments. Specifically Deposit A, the most upstream-known PCB deposit, accounts for an estimated 18.4% of the PCB mass in the bottom sediments above the DePere Dam. This deposit provides an opportunity for PCB remediation by possible removal or other means of deactivating this important source.

At the lower end of the river (below the DePere Dam) the PCB transport model indicates that a serious threat exists if the sediments were to be flushed from their current location under high flow conditions. Field transport of PCB contaminants (currently stored in the river sediment) into the open waters of Green Bay would virtually quadruple the amount of PCB available to the biota of the Bay. This increased load would constitute a major increase in PCB availability in the waters of Lake Michigan, which ultimately receive the outflow from Green Bay. The review of historic events and dating of the sediments indicate that such a flow event may have occurred as recently as 1960, and remains quite possible in the future. Figure 9 identifies the major flow events in the period from 1960 to 1990 and correlates with the sediment stratigraphy data for the major zones of deposition in the lower Fox River.

The levels of accuracy found in these studies and models have interacted very effectively in the early results. For example, the sediment survey data are sufficiently precise and the models sufficiently accurate to correlate well with the seiche effects on the lower end of the river. The simulated hydrodynamics indicate sediment deposition along the northern shoreline and actually upstream (because of the seiche effects) of historic discharges of PCBs. This condition was confirmed by field samples. In addition, the presence and the congeners of PCBs in the sediment stratigraphy correlate well with the general knowledge of the historic wastewater discharge loads.

The PCB transport model has already been used to identify the effects of alternative remedial measures especially on Deposit A, but also for others. As decisions are made on Deposit A, the effects and possible remedial actions at the downstream deposits can be considered. These modeling scenarios will be especially useful in understanding the role of major events involving high flows, seiche effects, historic periods of high rates of PCB discharge, and evaluating the effects of potential remedial measures.

Management options that have been analyzed to date for Deposit A are summarized in Table 2. These options demonstrate the value of the models used in decision making. The remediation of major sediment deposits may include inactivation

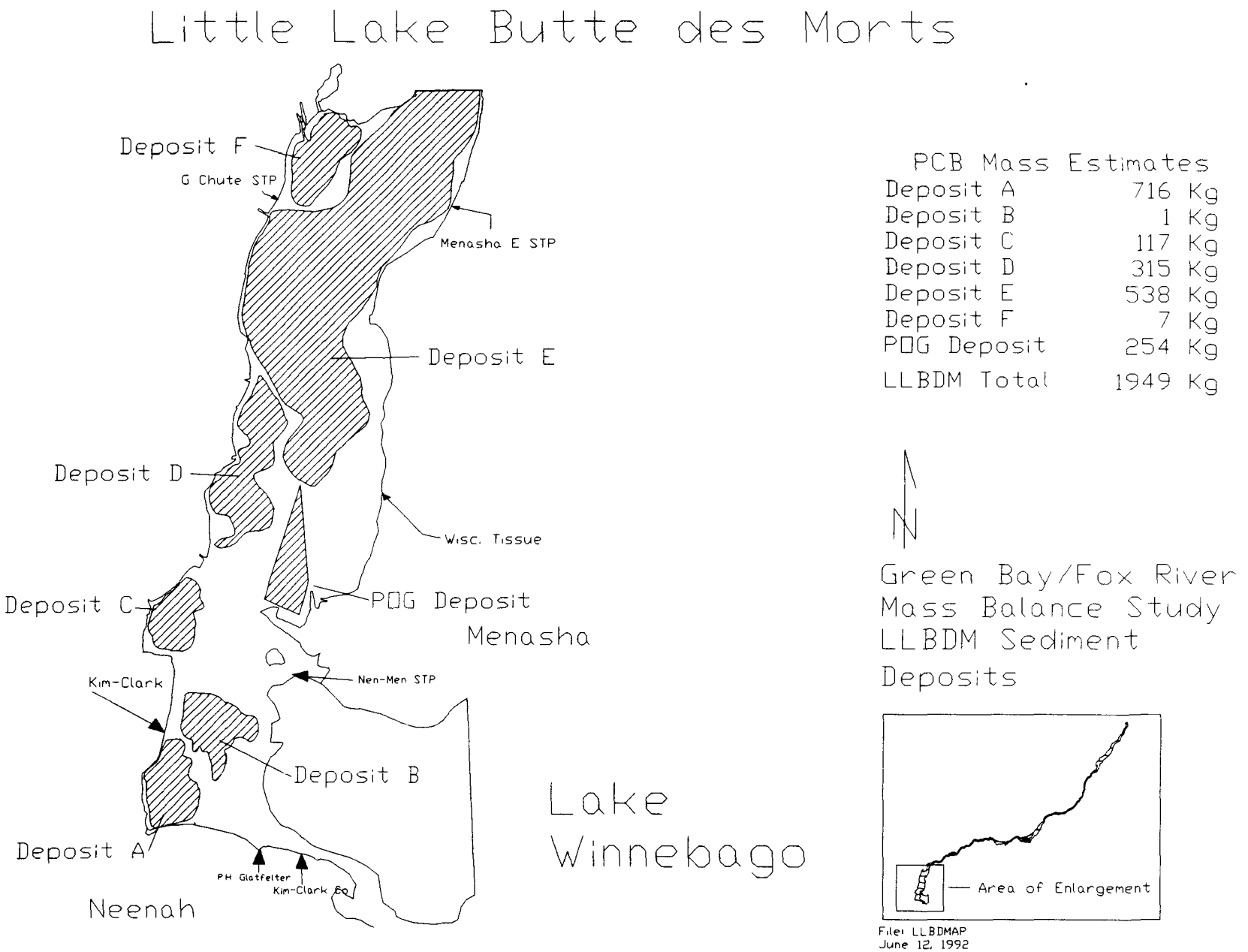

Figure 8. Locations and masses of PCB deposits in a portion of the Lower Fox River

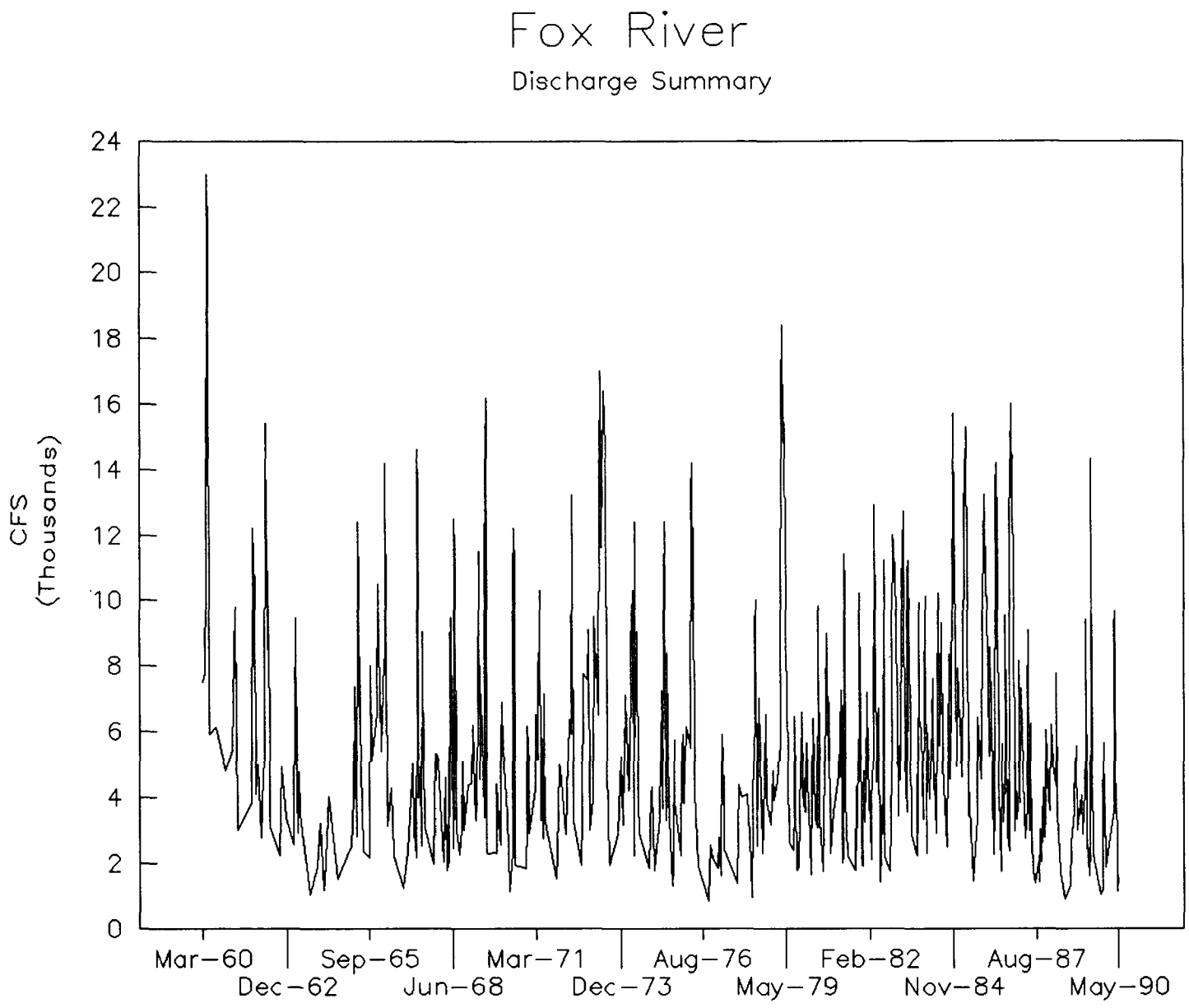

Figure 9. Fox River high flow events

or isolation as well as removal. Under the current schedule, remedial projects for Deposit A may be initiated as early as the Fall of 1993.

TABLE 2. OPTIONS SELECTED FOR FURTHER CONSIDERATION FOR THE REMEDIATION OF DEPOSIT A IN LITTLE LAKE BUTTE DES MORTS

Option	Estimated Cost ($1,000,000)	Estimated Time (months)
Inlake Confined Disposal Facility	3.5	28
Upland Temporary Cell	7.2 - 13.3	32 - 36
Upland Confined Disposal Facility	2.7 - 10.2	29 - 33
Pretreatment With On-site Thermal Destruction	15.2 - 17.3	9
Pretreatment using P.H. Glatfelter Paper Company Combustor	4.9 - 6.5	14

The linkage of PCB transport models to food chain models is of special importance to public health protection and general public interest and support. For these mechanisms it is possible to predict fish contamination levels and impacts on other aquatic organisms.

Modification or control of sediment loads from the tributaries must also be considered as a way to change the sediment transport characteristics of the river. Changes in tributary loads could result in increased deposition or scour at various sites. Finally, the general management of flow rates could provide an important tool. The river is highly managed by the presence of 14 dams and the inflow to the river is subject to management of the storage capacity of Lake Winnebago. An evaluation of the feasibility for control of peak flows to avoid scour and resuspension of PCB-laden sediments will be made. In addition, perhaps other flow management regimes could result in the transport of selected sediment deposits from some of the less manageable sites into some of the most manageable deposits--those deposits that are most easily subject to dredging, inactivation, or other sediment management techniques.

The PCB transport model provides useful predictions in characterizing the changes in the downstream load after reductions in individual deposits. Deposits A-F (Figure 8) in Little Lake Butte des Morts account for approximately 50% of the PCB mass in the bottom sediments above the DePere Dam. The effect of the removal of all of these contaminated sediment deposits is shown in Figure 10.

In the larger context, we will conduct simulations on the entire Fox River/Green Bay system to evaluate management options from no action to extreme conditions such as high flow events under catastrophic storm events. Table 3 identifies some of the management scenarios being considered.

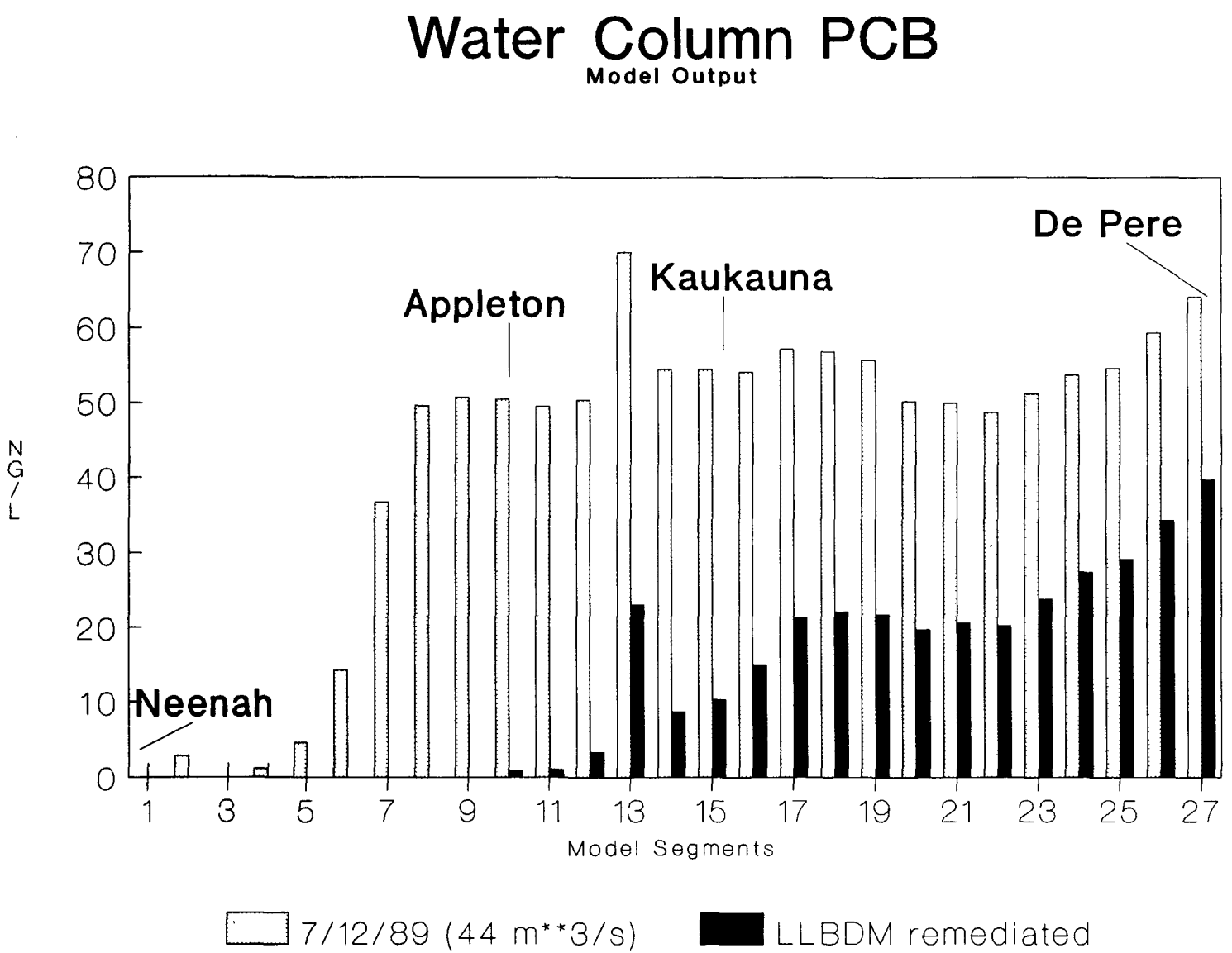

Figure 10. Fox River PCB concentrations before and after remediation of sediments in LLBDM

TABLE 3. MANAGEMENT SCENARIOS FOR WHICH THE GREEN BAY/FOX RIVER MASS BALANCE MODEL WILL BE RUN

Scenario	Description
1	No action run for a 25-year prediction period.
2	Major event loading from a maximum scour event similar to 1960.
3	Remediation of all areas above DePere that can be practicably removed.
4	Extension of Scenario 3 with all areas below DePere Dam remediated.
5	A 25-year projection for the Bay under 25, 50, and 75% reductions in the Fox River PCB loadings upstream of the DePere Dam.
6	Peak flow reduction for the Fox River by control of the Lake Winnebago outlet dams.
7	A 25-year projection on the impact of phosphorus load reductions due to nonpoint source controls.

DIELDRIN RESULTS

The development of a mass balance for Dieldrin was hampered by analytical problems and by the low levels of Dieldrin that were found in the study area. At the present, the development of mass balance models for Dieldrin are not anticipated.

CADMIUM AND LEAD RESULTS

Because of the low concentrations and the lack of a concentration gradient, a mass balance for cadmium could not be accurately developed. Without accurate quantitative data on the presence and mass of cadmium transported in the system, it is not possible to develop a useful model. Accordingly, the study of cadmium was discontinued until some future time when more sensitive analytical methods are available.

Time constraints and the difficulty in providing low-level lead concentration analyses have delayed the development of a mass balance model for lead. However, as would be expected, the Green Bay urban area is the major source of lead to the Fox River/Green Bay system. As lead additives are eliminated from gasoline, lead loading from the urban area should decrease. The environmental effects of the current loads is unknown at this time.

QUALITY ASSURANCE/QUALITY CONTROL CONSIDERATIONS

Any report on this project would be incomplete without at least a brief note concerning the management of QA/QC and the critical importance of adequate and accurate laboratory facilities. This project involved the use of 6 different laboratories for the analysis of PCB congeners. At the beginning of the project there were concerns that laboratory capacity and quality were inadequate for such an intensive project. These problems were overcome by extensive commitment of existing laboratory capacity

and the development of additional capacity outside of that which could be provided by established laboratories. Timely and accurate data were made available, although often requiring major project management efforts several times during the project period. In addition, the QA/QC protocols developed for this project were essential for the credibility of the laboratory data critical to the technical success of the project (6). Table 4 summarizes the QA/QC approach used. The appointment of a Quality Assurance Coordinator and a Data Manager were extremely beneficial to the maintenance of data credibility.

TABLE 4. COMPONENTS OF THE GREEN BAY/FOX RIVER QUALITY ASSURANCE/QUALITY CONTROL PLAN

Component	Comments
Field Blanks	Sampling container, material, solvent, or apparatus is taken into the field and handled in the same manner as it would be for actual sample collection. One field blank for every 9 field samples.
Duplicate Samples	One duplicate sample is collected for every 9 samples.
Duplicate Analyses	A single sample is split and both halves are analyzed separately. One duplicate analyses is performed for every 9 samples.
Procedural Blank	Solvent and extraction apparatus are subjected to the same analytical procedures as samples. A procedural blank is included with every sample set.
Matrix Spike	A known amount of the performance standard is added to a sample matrix previously shown to be uncontaminated at a concentration similar to sample concentrations. Matrix spikes are included 10% of the time.
LOQ Verification	A sample matrix previously shown to be uncontaminated is spiked with the performance standard at a concentration 2x-5x the target LOQ of the smallest peak in the performance standard. This QA/QC sample is included with every 20 analyses of samples expected to have low concentrations.
Surrogate Standard	A known quantity of a compound that is similar to the analyte of interest, but not found in the environment is added to all samples and blanks at the beginning of the analytical procedure to monitor recoveries of the analyte of interest.
Internal Standard	A compound similar to the analyte of interest, but not found in the environment is added to all samples, blanks and standards just prior to instrumental analysis to quantitate the analytes of interest.
Comparability	All laboratories participating in data generation must analyze a series of blindly coded QA samples supplied by the QA Coordinator. The QA requirements of the comparison must be met prior to any sample analyses by the laboratory.
Data Assurance/Management	All data sets must be submitted to the QA Coordinator along with all supporting QA/QC documentation. The QA Coordinator must approve the quality of the data set before it can be released for use by the Data Manager.

OTHER SIGNIFICANT RESULTS

Additional results anticipated from this effort are summarized below.

A. Evaluation of the level of detail required for modeling to support management decisions envisioned, and a basis for evaluating the management value and costs of less intensive monitoring and modeling efforts.

B. Contribution of new scientific methods that have been developed during the study and as part of the study (e.g., the study served as an active field test for the design and evaluation of sampling techniques and analytical methodologies for low concentrations of PCBs and metals).

C. Development of shared insights among decision makers, politicians and scientists concerning the alternative and selective management solutions. This linkage is illustrated by the multi-dimensional video-tape (of modeled sediment transport) produced by Dr. Wilbert Lick at the University of California-Santa Barbara.

D. Strengthening and increased visibility of the Remedial Action Plan for Green Bay and the Fox River, with resultant public support for financial and regulatory actions necessary.

E. Contribution to a strategy for sediment cleanup and specific engineering design for remedial measures -- and thus development of a prototype for cleanups of the areas of concern around the Great Lakes.

F. Development of other scientific results as this extensive and unparalleled data set is reviewed and known by other researchers.

IMPLICATIONS FOR WATER QUALITY MANAGEMENT

Many important lessons have been learned. Only a few can be identified in this brief paper but the following are some of the earliest noted.

A. Development of a detailed, documented project study design is essential, including pre-planning field, laboratory and modeling needs to meet the desired project objectives. Even a dress-rehearsal sampling cruise contributed to problem prevention by early identification of problems and solutions, which otherwise could have jeopardized the coordination of the overall project field sample collection.

B. Quality assurance and quality control for both laboratory and field operations are critical to the success of a mass balance project of this type. Our experience indicates that without such an effort, the data would not provide comparability and consistency in the database. It is essential that we assure compatible

sampling and laboratory analyses of conventional parameters -- especially important for total suspended sediment/solids.

C. Database management is critical if diverse and physically separated analysts and simulation modelers are to work together with confidence and full ability to communicate.

D. Sediment is an active and critically important feature of such contaminated systems. The sediment reflects the results of pollutant contributions from pulp and paper mills, publicly owned treatment works, combined sewer overflows, rural stormwater runoff from agriculture, urban stormwater runoff from acres of active daily use, as well as raw materials and waste storage areas.

E. Sediment dynamics are widely variable and can best be understood with detailed studies.

F. Sediment modeling is invaluable to decision making. This point is critical for administrators, managers, and politicians to understand. The time and cost of conducting such sediment studies and developing modeling tools would be a difficult reality for decision makers to accept. This result, however, is a clear consensus of the experienced staff who have participated in this project. Wisconsin Department of Natural Resources' staff have been seasoned by 25 years of pollution abatement programs, and are veterans of major successes in abatement of both conventional and toxic pollutants from point sources. This same staff has pioneered the modeling and abatement of nonpoint source pollution since 1978. These staff members have already found the sediment modeling tools to be of greater use than were the conventional water column pollutant modeling tools. Simulations of dissolved oxygen and biochemical oxygen demand were especially important tools in water quality successes achieved to date, but staff indicate that sediment modeling will be of even greater value and will lead to even greater savings and more intelligent decision making.

G. The sediment, hydrodynamic, water quality, and food chain models are all critically linked submodels of the overall environmental system and represent the major components of an effective water quality management program. As these models are linked, our insights and understanding of the system have increased dramatically. The conceptual interaction of these subsystems has been made much more real to the analysts and decision makers than could have been otherwise possible.

H. The costs of mass balance studies are justified even at present costs. At a total cost of over $10,000,000, the mass balance project is very expensive. By contrast, however, the initial estimated costs of sediment cleanup for the Fox River/Green Bay area are far higher. The Green Bay RAP of 1988 estimated that sediment clean up costs could run as high as $640,459,000. As the first sediment cleanup

experiences occur, it is evident that these mass balance models have great value for development of strategies, support of implementation, setting priorities, and for design of sampling and monitoring systems. These tools are also of great value for analyzing and evaluating the results of the monitoring before, during, and after implementation of remedial actions.

CONCLUSION

State of the art advances may be expected to reduce the costs of mass balance analyses in the future. Studies such as these are costly. Based on this project, it seems there could be savings in the frequency, locations, precision, and even types of testing undertaken. However, this project indicates that the study costs would be justified, even without the cost-savings that may come with future technologies.

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PROBLEMS RELATED TO MONITORING AND MODELING POLLUTION FROM AGRICULTURAL WATERSHEDS

by

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ABSTRACT

Pollution from agricultural activities has only recently been acknowledged as a significant danger to waterbodies. Agricultural nonpoint source pollution is undoubtedly a major pollution source in the Ukraine and the other republics of the former Soviet Union. We review methods of evaluating agricultural pollution problems and relate these to conditions in the Ukraine. Models are one good evaluation method, and ANSWERS (areal nonpoint source watershed environment response simulation) is the most accurate of presently available models.

INTRODUCTION

Scientists have been aware of agricultural pollution for several decades, but since the pollution was generally out of sight, it was not treated as a significant danger to waterbodies until recently. As agricultural production intensity and use of mineral fertilizers and pesticides increased, negative environmental consequences of modern agriculture became more apparent. These consequences of poor agricultural production practices include: loss of fertile top soil, growth of ravines and gullies, sedimentation of waterbodies, loss of nutrients from top soil, excessive loadings of nitrogen and phosphorus to waterbodies with resulting eutrophication problems, and movement of pesticides and their metabolites to waterbodies where they negatively impact aquatic organisms. In the United States, the Environmental Protection Agency has identified agricultural nonpoint source pollution as a major source of pollution in rivers, lakes, reservoirs, and coastal waters (Novotny and Chesters 1989). In the Ukraine and other republics of the former Soviet Union, agricultural nonpoint source pollution is identified as a problem. The objective of this paper is to discuss some of the

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experiences we have had in attempting to assess agricultural nonpoint source pollution problems on experimental watersheds in the Ukraine. Recommendations on use and development of water quality planning models for nonpoint source pollution control are also made.

Influences on the mass of nutrient and pesticide loadings to waterbodies are both natural and anthropogenic. Important factors are: climatic and hydrogeological conditions; hydraulic and physical properties of watershed soils; distance between source areas and receiving waters; chemical application rates, formulation, and methods; degradability of agricultural chemicals; etc.

DISCUSSION

Several methods are available for quantitative evaluation of soil loss from agricultural areas. Shwebs and Formovanie (1974) reported four: 1) mark evaluation, 2) comparative, 3) empirical dependencies, and 4) hydromechanical. Additional methods include conceptual, physical, and mathematical models (Kuchment et al. 1983). The empirical dependency and hydromechanical methods are the most widely practiced. Their development is the result of accumulation and summarization of great volumes of experimental observations. Examples of these approaches include the Universal Soil Loss Equation (USLE; Gudzon 1974) and the methods of Shwebs and Formovanie (1974), Surmach (1976), and Mirtshulava (1970). These methods have been improved over time, but their application in practice is still limited because they do not estimate sediment loadings to receiving waters. Use of these methods alone leads to significant overestimation of sediment loadings to receiving waters (Novotny and Chesters 1989). Empirical methods are inherently limited because their application conforms to the database from which they were derived. Unlike conceptual and physically based models, empirical models cannot describe important processes affecting pollutant movement such as sediment detachment, transport, and deposition in upland areas and channels. Neither can they deal with the transport and fate of sediment-bound, dissolved, and colloidal pollutants in ephemeral channels, streams, and receiving waters.

Effective management of pollutant loss from agricultural areas requires complex, physically based models that simulate the significant processes affecting sediment and chemical loss from agricultural catchments. These models must also consider effects of the spatial distribution of catchment parameters, and are often termed distributed parameter models. Such physically based, distributed parameter models require four major submodels: 1) a geometric model representing catchment topography and drainage patterns (mathematical image of the catchment area), 2) a hydraulic model to simulate water movement through the drainage network of the catchment, 3) an erosion and sediment transport model, and 4) a chemical transport and fate model (nitrogen, phosphorus, and pesticides).

Physically based models are much more demanding in terms of data requirements and mathematical calculations than empirical models. In fact, solution of the equations involved usually requires computers. Data requirements for physically based, distributed parameter models were so demanding that, prior to development and use of geographic information systems, they were rarely used. While these models are more demanding of the

user, the value of improved sediment and chemical loss predictions often offsets additional costs of using them.

To accurately simulate chemical transport and fate in and from catchments, one must first successfully simulate erosion and sediment transport, which is in turn dependent on successful simulation of surface runoff by a hydraulic model. The hydraulic model is dependent on the geometric model. Let us first consider the geometric or mathematical image model that seeks to represent catchment topography. Distortions or errors in the geometric model will introduce errors in the hydraulic model, which will then introduce errors into the erosion and subsequent chemical transport models. These errors will be magnified as they pass from one model to the next. Catchment topography can be represented by a variety of geometric models, as shown in Fig. 1.

The simplest geometric model, kinematic cascade (Fig. 1a), represents the catchment as a series of overland flow planes of varying slope (Kibler and Woolhiser 1970). This simple model can be expanded to include channels. Simple geometrical models composed of such planes and channels include the Wooding water catchment approach (Fig. 1b) and the Cherry (1976) approach (Fig. 1c). Kuchment et al. (1983) represented the catchment area as a system of triangular and quadrilateral shaped planes and channels that represent catchment topography and ephemeral and perennial drainageways (Fig. 1d).

Kolpak and Lysenko (1982) developed a geometric model that represents catchment topography as a collection of triangular plains or cells of varying sizes that approximate catchment relief in the best quadratic approximation (Fig. 1e); channels are also represented. Complex watersheds may require hundreds of triangular cells and channels to accurately reflect site topography, while simple catchments may require only a single cell. Flowpaths from any plain to the catchment outlet are a function of cell slope direction and are determined automatically by the model. Machmeier and Larson (1968) developed a method of discretizing watersheds into discrete subwatersheds, which in turn are divided into elementary slope areas as depicted in Fig. 1f.

Beasley (1978) represented catchments as a system of square elements or overland flow plains ranging from 1/3 to 4 ha in size (Fig. 1g). Concentrated flow through catchment drainageways is modeled with rectangular cross-section channel elements of specified slope, width, and hydraulic roughness. The model requires that all surface runoff exit the watershed from a single outlet cell and that the channel network be continuous. In an approach similar to Beasley (1978), Rumyantseva et al. (1986) represented watersheds as systems of square cells, each with an area of 10 km^2 (Fig. 1h). For each cell, the steepness and direction of waterflow into the river system are determined.

Other geometric model approaches have been presented, but those discussed above are the most commonly used and accepted. In our opinion, the combination of the spline-function method and the method of Kuchment et al. (1983) with full automation of computations on a personal computer is the best approach to follow in geometric modeling in the long term. This approach could be used to identify hydraulically active areas as proposed by Machmeier and Larson (1968) and to determine flowpaths from upland source areas to the watershed outlet (Kolpak and Lysenko 1982). Whatever the geometric approach, development of a practical agricultural nonpoint source planning model requires

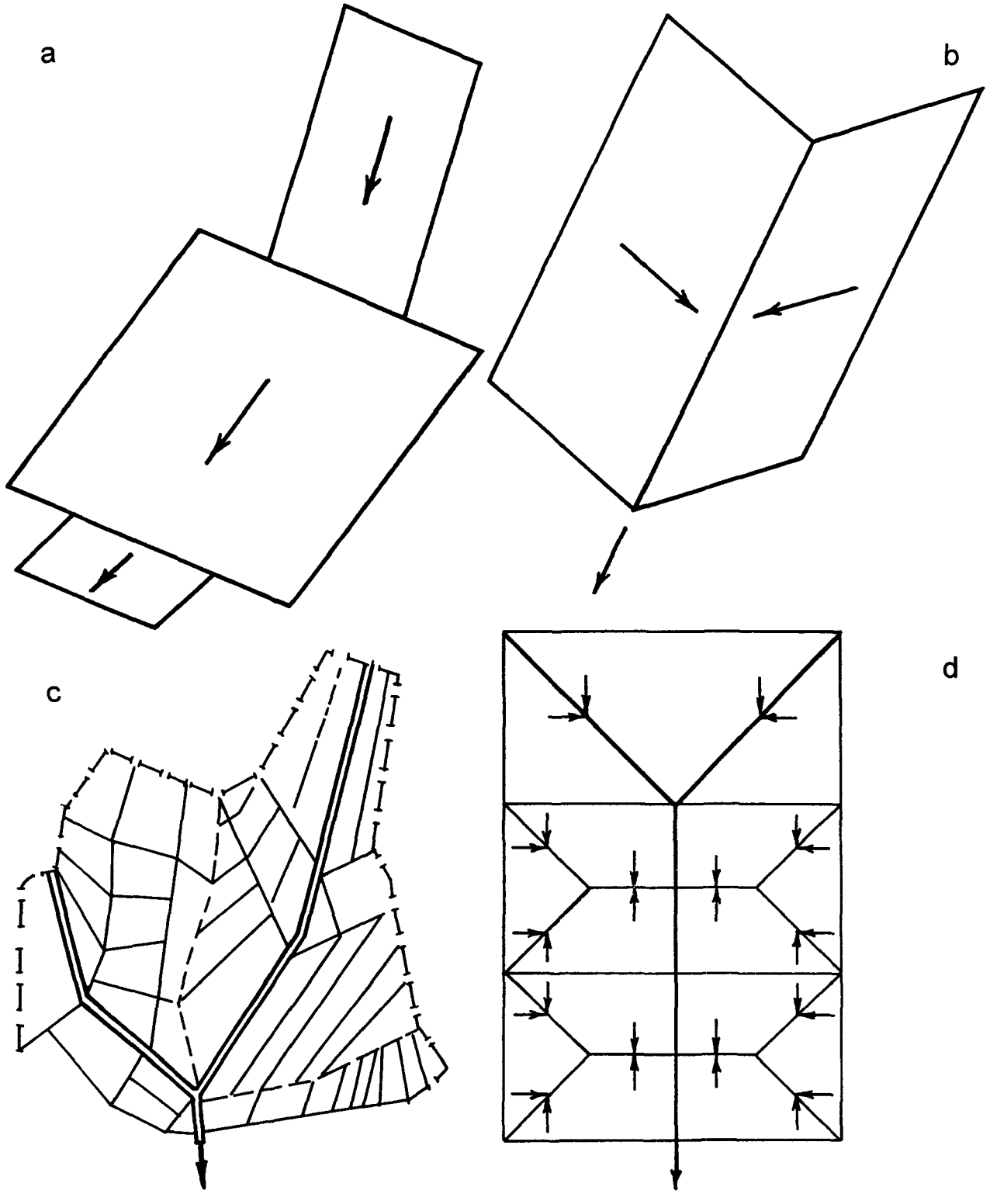

Fig. 1. Approaches to the approximation of watershed relief: a) kinematic cascade (Kibler and Woolhiser 1970) and b) Wooding water catchment area, c) Cherry (1976), d) Kuchment et al. (1983), e) Kolpak and Lysenko (1982), f) Machmeier and Larson (1968), g) Beasley (1978), and h) Rumyantseva et al. (1986).

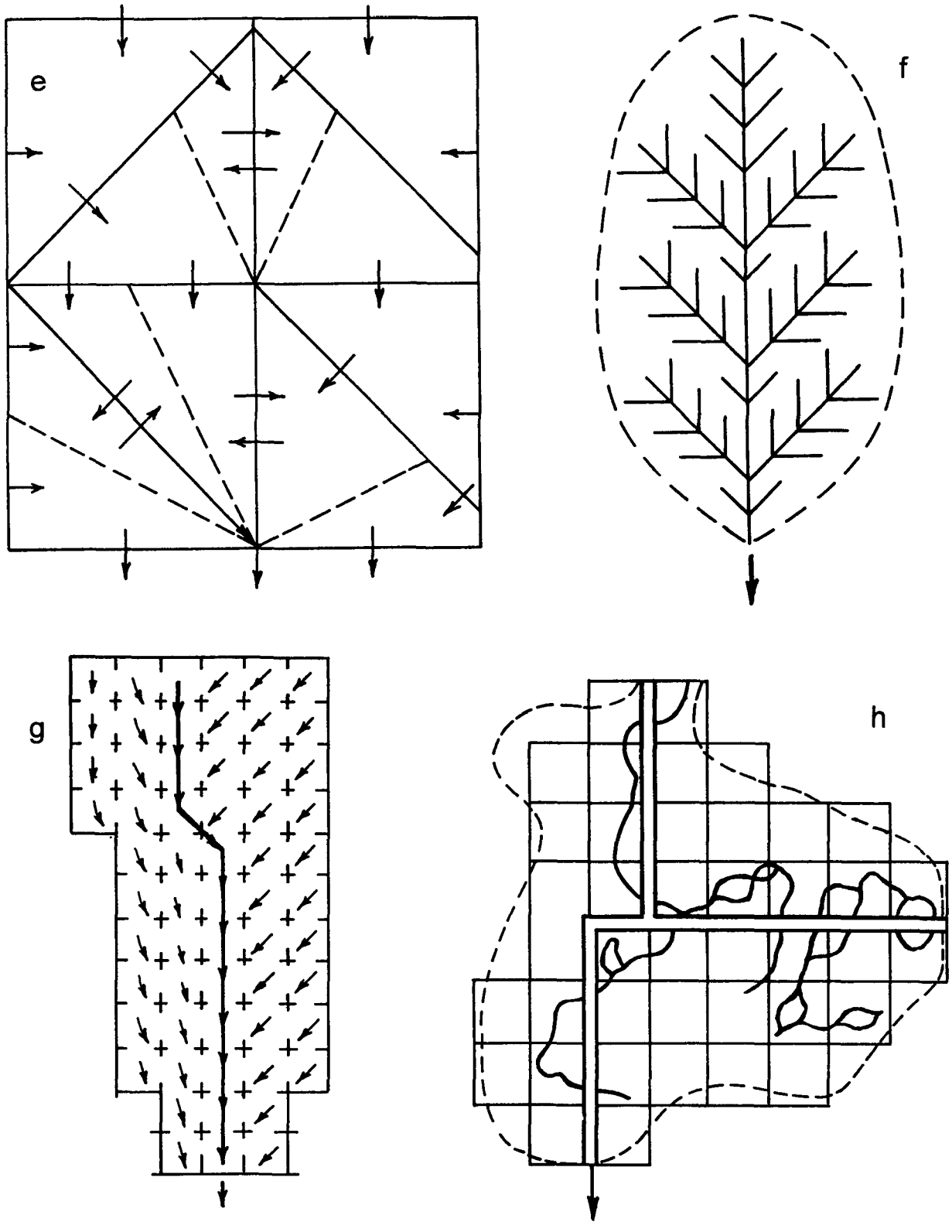

Fig. 1 (continued).

coupling a geometric model with models describing surface runoff; erosion; sediment transport; and dissolved and sediment-bound nitrogen, phosphorus, and pesticides transport from upland areas. The creation of a user-friendly program interface to facilitate data input and interpretation of model output is also essential.

At present, we believe that ANSWERS (Beasley 1978) is the most suitable model currently available for water quality planning in agricultural areas. The ANSWERS model was developed in the U.S. in 1977 for modeling runoff and sediment loss for short periods (1-2 days) from upland watersheds with complex topography, soils, and landuse. Since then, ANSWERS has been modified to simulate particle size distribution of transported sediment and nutrient transport.

ANSWERS is a distributed parameter watershed model; this means it can consider spatial effects of topography, soils, and landuse variations on watershed response. This allows the model to be used to study effects of changes in small areas of the watershed on overall watershed response. ANSWERS represents a watershed as a system of square overland flow cells and a channel network. Each of these model elements is described in terms of its physical and chemical characteristics. Important parameters described for each element include: 1) break point rainfall data; 2) surface and subsurface soil parameters; 3) channel parameters, if a channel is present; 4) surface conditions; 5) element slope and flow direction; 6) agrochemical characteristics; and 7) landuse and management. Additional information on the ANSWERS model and its data requirements is given in a companion paper in these proceedings "ANSWERS-2000: A Model for Agricultural Pollution Control Planning in the USA and the Ukraine" and elsewhere (Beasley and Huggins 1982, 1991).

Although the ANSWERS model has been tested and verified in the U.S., Europe, and Australia, it has never been tested for Ukrainian soil, climate, and agricultural conditions. The present study, therefore, was undertaken to provide data for testing the model under Ukrainian conditions.

In our opinion, there has been insufficient attention to model testing and validation issues in the countries of the CIS. This has often resulted in models that poorly represent the physical systems simulated. For example, Golosov et al. (1990) used the USLE equation as modified by Larinov (1984), to quantify erosion from small to medium scale catchments. Largescale maps of soil loss from these areas were then produced without model verification or investigation of the sensitivity of the model to input parameters.

In the CIS (GOST 1984) and the U.S. (Barch et al. 1989), procedures have been proposed to improve and assess the quality of model input data with respect to: 1) precision of measurements--the quality that reflects the repeatability or closeness of repeated measurements; 2) accuracy of measurements--reflecting the closeness of measurement results to the true value of the parameter measured; 3) reproducibility (comparability)--the closeness and level of comparability of measurements performed by different researchers at different times and locations in measuring the same parameter; 4) representativeness--the degree to which measured data taken from a limited number of samples of the system reflects true system values; and 5) completeness--the quantity of data obtained in the process of performing investigations in comparison with the quality of data under ideal conditions.

Alternative agricultural pollution management scenarios can be evaluated through either mathematical modeling or monitoring. If modeling is used, limited monitoring may also be required for model verification and calibration. This is particularly important in regions where the models have not been previously used. Surface runoff monitoring on representative catchment areas enables collection of data on the volume and quality of surface runoff for various precipitation and landuse conditions that can be used for model verification purposes. In addition, these data may be necessary for model calibration of both physically controlled and uncontrolled model relationships. Once the model is calibrated and successfully verified for a particular region, the model can be used to assess losses of sediment and agricultural chemicals in surface runoff from smaller or larger catchments with similar climatic, soils, topographic, and management conditions without additional monitoring studies. Development of mathematical models includes creation of a comprehensive list of parameters required by the model. Designing systems to collect these data often results in improved monitoring systems.

Main stages in the design of an effective monitoring system are: detailed definition of purposes and scope of the proposed monitoring system; definition of required accuracy of measured parameters; selection of a monitoring site; collection of preliminary technical data on the site; and development of protocols for observations, field sampling and measurements, laboratory procedures, and data processing. Details of the application of this step by step approach in designing a watershed monitoring system are reported elsewhere (D. B. Beasley, G. J. Platonova, T. A. Dillaha, V. Z. Kolpak and Y. M. Plis, unpublished ms., Biological and Agricultural Engineering Department, North Carolina State University, Raleigh; and G. J. Platonova and V. Z. Kolpak, unpublished report, Ukrainian Scientific Center for Protection of Waters, Kharkov). These reports give special attention to criteria for monitoring site selection and for the collection of input data to which the ANSWERS model is the most sensitive.

The monitoring system design program was tested during development and implementation of a surface runoff monitoring system on two experimental watersheds in the forest-steppe zone of the Ukraine. The specific watersheds were located on the Martovoe State Farm in the Boikov Yar River watershed that drains to the Pechenezhskoye water reservoir, a primary water supply reservoir for the Kharkov region. The larger monitored watershed had an area of 586 ha and the smaller one, 2.4 ha.

As a result of field and office investigations, the representativeness of the monitored Martovoe catchments for the Left Bank Forest-Steppe region of the Ukraine was determined. Topographic, soil, and agrochemical surveys were conducted to develop geometric models of the catchments and monitoring protocols for obtaining soil and agrochemical information.

Since commercial equipment was not available in the CIS for monitoring and sampling surface runoff, we were forced to design and construct required monitoring systems including: flumes, water level recorders of the "Valdai" type, semiautomatic discrete water samplers, and a 1-m diameter Coshocton wheel (Brakensiek et al. 1979) with accumulation tank for flow proportional sampling of runoff. All samplers, accumulation tanks, and flumes were constructed from stainless steel to minimize pesticide adsorption problems. The monitoring system has been in operation for 3 years and has been essentially failure-free.

The most complicated aspect of the monitoring system was the characterization of soils in monitored watersheds. A soil sampling program had to be developed that would supply data required by the ANSWERS model, as well characterize temporal and spatial variations in soil properties in the watersheds. The soil sampling protocol includes full descriptions of methodologies for field determination of soil parameters; sampling and laboratory procedures; and quality assurance procedures specifying the required number of samples, replications, frequency, and spatial distributions of samples (G. J. Platonova and V. Z. Kolpak, unpublished report).

The monitoring system has been adequate for collecting data required by ANSWERS, with the exception of soil infiltration parameters. Infiltration parameters were collected with double ring infiltrometers. Differences in measured infiltration have varied by orders of magnitude with small temporal and spatial changes in sampling. Alternative methods of determining infiltration parameters, such as adding runoff to the upper end of a slope at a known rate and measuring the runoff rate at intermediate points down the slope (Nazarov 1981, Shemyakin et al. 1990), should also be considered. These methods, however, will probably result in variations in infiltration parameters equal to or larger than the double ring method.

A monitoring system must also accurately record landuse and management factors for each field or farm management unit. Required types of data include: field identification; crop species; tillage and other cultural management practices; plant growth development dates and cover conditions; dates of all tillage and management activities; and dates, methods, and rates of agrochemical applications.

Our experience has shown that the physically based input data required for the ANSWERS model can be obtained on experimental watersheds in the Ukraine. The ANSWERS User's Manual (Beasley and Huggins 1991) was found to provide reasonable approximations of soil, cover, and landuse parameters required by the model for conditions in the chernozem zone, European territory, of the former Soviet Union and the Ukraine. Work by Roshkovan (1988) and Shwebs and Formovanie (1974) was useful in estimating potential interception, surface roughness as a function of tillage and soil cover, and erodibility of Ukrainian soils. Applicability of the USLE to the European territory of the former Soviet Union was not experimentally investigated in the present study. However, other scientists (Larinov 1984, Kiryukhina and Pacukevich 1989) have adapted the USLE soil erodibility nomograph by developing correlations between particle size analysis procedures used in the U.S. and in the former Soviet Union. We, therefore, assume that USLE parameters required by the ANSWERS model can be reasonably estimated for Ukrainian conditions.

CONCLUSIONS

Further enhancement of ANSWERS is necessary before it can be used as a practical planning model. First of all, it is currently a research model and, as such, it is not very user friendly. For ANSWERS to be useful for routine planning purposes, it will require a user-friendly program interface. The creation of an interactive interface for data input and display of output would facilitate model use, and extend use to many more users. Additional work must also be done to identify sources of information required to determine model parameters

related to soil nutrient dynamics (soil sorption-desorption kinetics, mineralization rates, nitrification-denitrification rates, etc.). Inclusion of routines for simulating pesticides fate and transport are also needed. Finally, ANSWERS needs to be converted into a continuous simulation model to better represent processes affecting long-term sediment and agrochemical loss. Development of the user-friendly program interface is the key task for the near term. Additional and expanded monitoring studies are also needed to accumulate data needed for model development, testing, and verification.

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ANSWERS-2000: A WATER QUALITY PLANNING MODEL FOR AGRICULTURAL WATERSHEDS

by

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ABSTRACT

This paper describes ANSWERS-2000, a water quality planning model under joint development in the USA and the Commonwealth of Independent States (CIS). ANSWERS-2000 is being developed from the ANSWERS model, an event-oriented, deterministic, distributed parameter, water quality model developed for agricultural nonpoint source pollution control planning in the USA. The current version of the ANSWERS model simulates surface runoff and sediment yield as a function of particle size. ANSWERS-2000 will expand the model to include phosphorus and nitrogen transport (dissolved and sediment-bound); long-term, continuous simulation; a user-friendly interface to facilitate data file preparation and control model execution and output; and possibly a pesticide transport model.

This work is part of Project 02.02.11 "River Basin Water Quality Planning and Management" of the joint USA-USSR Agreement on Cooperation in the Field of Environmental Protection. In the USA, support is being provided by the U.S. Environmental Protection Agency, Virginia Polytechnic Institute and State University, and North Carolina State University. In the CIS, the Ukrainian Scientific Center for Protection of Waters (USCPW) is supporting this work.

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INTRODUCTION

Water quality models are indispensable tools for studying processes that influence water and chemical movement from agricultural watersheds. They are also used by planners to assess the impacts of proposed best management practices (BMPs) before installing the BMPs. Widely known and used models, developed specifically for BMP evaluation in agricultural watersheds, include CREAMS (1), GLEAMS (2), AGNPS (3), and ANSWERS (4). These models use different approaches to simulate the complicated processes of hydrology, erosion, and chemical transport, and all of them have limitations.

CREAMS and GLEAMS, for example, are long-term, continuous models describing sediment, nutrient, and pesticide transport, but they are only applicable to field-sized areas with uniform soils and cropping. Their sediment and chemical transport algorithms are state-of-the-art, but the commonly used versions of the models base their runoff calculations on the curve number method that poorly represents runoff. AGNPS is an event-oriented, watershed-scale, distributed-parameter model describing runoff, sediment, nutrient, and COD losses from agricultural watersheds. AGNPS has a sophisticated user-friendly interface that facilitates data file creation and interpretation of model output, but its runoff calculations are limited because they are based on the curve number method. Nutrient transport algorithms are similar to those found in CREAMS.

The current version of the ANSWERS model has significant limitations also. It is event-oriented and cannot predict long-term average annual losses; it has nutrient transport versions, but they have not been widely tested and verified; it uses an infiltration equation whose parameters are difficult to define; and data file creation is tedious and not very user friendly. In spite of these limitations, ANSWERS offers an excellent platform from which a state-of-the-art agricultural nonpoint source pollution control model can be constructed. Advantages of ANSWERS, compared with the other models, include; free public access to the model source code, a modular design facilitating incorporation of new model components, a state-of-the-art surface runoff model, and a data file structure that is compatible with geographic information systems. This paper describes the proposed changes that are being made to the ANSWERS model to facilitate its use for agricultural nonpoint source pollution control planning in the USA and the CIS

THE ANSWERS MODEL

ANSWERS (Areal Nonpoint Source Watershed Response Simulation) was developed in the late 1970s to address questions concerning the water quality impacts of agricultural activities (4). The model was developed as part of the Great Lakes Program in the midwestern USA. Most of the data used in developing and validating the model is characteristic of the soils and conditions of this region. The earliest version of the model (5) simulated surface water hydrology only. Beasley and Huggins (6) added sediment detachment and transport to the model. The model was updated by Dillaha and Beasley (7) to allow simulation of sediment detachment and transport for different soil particle size classes. Dissolved and sediment-bound phosphorus (8) and nitrogen (9) transport versions of the model have also been developed, but they have not been documented well or tested rigorously.

ANSWERS is intended for use on ungaged watersheds and uses physically based parameters to reduce or eliminate the need for calibration. The ANSWERS model also can simulate changes in management practices and is thus a valuable tool in selecting among different management practice alternatives. It also allows the identification of subareas within watersheds that are responsible for proportionally more off-site pollution than the watershed as a whole. Identification of these critical subareas allows water quality planners to focus limited pollution control resources more cost effectively.

The model divides watersheds into discrete square cells (up to 2000 cells per watershed) with uniform cropping, precipitation, slope, soil type, and management practices. This allows the model to consider the effects of spatial differences in watershed properties on watershed response. The hydrology component simulates runoff, infiltration, interception, surface storage, and subsurface drainage. Infiltration is computed using Holtan's equation (10). Detachment of soil particles by raindrop impact and overland flow is computed using relationships based on studies by Meyer and Wishmeier (11). A modification of Yalin's equation (12) is used to compute the transport of different size sediment particles.

One of the major limitations of the current version of ANSWERS is data collection and data preparation. About seven parameters must be entered for each cell. This task can be tremendous for large watersheds. For very large watersheds, there is a dilemma: should one select large cell sizes (>1 hectare) to reduce the amount of input data and computer time required to run the model; or should cell size be reduced to increase homogeneity within cells?

The ANSWERS model has been tested and verified on watersheds in Illinois, Indiana, Iowa, Ohio, Oklahoma, Texas, Virginia, Pennsylvania, and Canada. However, researchers have found that ANSWERS does not work as well with European and Australian soils and cropping systems. As a result, these researchers added different infiltration and erosion algorithms to the model to account for differences in soil characteristics. These changes have personalized the model for the local conditions.

Originally, the purpose of the joint research described in this paper was to determine the suitability of the ANSWERS model for nonpoint source pollution control planning in agricultural areas of the Chernozem zone of the western CIS. Early work on the model in the Ukraine identified limitations of the model. Consequently development of the new version of the model, ANSWERS-2000, was initiated to improve the model in the Ukraine and elsewhere.

ANSWERS-2000

ANSWERS-2000 is currently under development and a prototype version of the model should be available for release and testing in August 1993. Major differences between ANSWERS-2000 and ANSWERS include: (a) replacing the Holtan infiltration model with the Green-Ampt infiltration model; (b) incorporating dissolved and sediment-bound P and N transport into the model; (c) use of leaf area index data to represent crop growth and the addition of an evapotranspiration model to allow long-term simulation; and (d) development of a user-friendly program shell to facilitate data file creation and to control program execution and output. The proposed changes are summarized below.

INFILTRATION MODEL

The ANSWERS model currently uses a modified version of Holtan's infiltration equation (13):

$$FMAX = FC + A\left(\frac{PIV}{TP}\right)^{P} \tag{1}$$

where FMAX = infiltration rate with surface inundated (cm/hr); FC = steady state infiltration rate (cm/hr); A = maximum infiltration rate in excess of FC (cm/hr); TP = total porosity within the control depth (cm); PIV = air remaining in control depth before saturation (cm); and P = an empirical coefficient.

The use of this equation is limited because of the dependency of the infiltration rate on the control zone depth (14). This fictitious control zone has no physical meaning and there are no uniform rules concerning the choice of the appropriate control zone depth. For example, the ANSWERS user manual suggests a control zone depth of one-half the A horizon or plow depth while the FESHM model suggests a control zone depth of the entire A horizon. Furthermore, this dependency of the infiltration rate upon the control zone depth defies hydraulic principles (14). This equation is difficult to use because A and P are best determined by calibration.

The Green-Ampt infiltration equation (Green and Ampt, 1911) was chosen to replace Holtan's equation for three major reasons. It is a physically based approach, it is computationally efficient, and its parameters can be determined from readily available soil and vegetal cover information. The Green-Ampt equation can be expressed as

$$K_e = F - N_s \ln\left(1 + \frac{F}{N_s}\right) \tag{2}$$

where K_e = effective saturated hydraulic conductivity (cm/hr); F = cumulative infiltration (cm), and N_s = effective matrix potential (cm). Infiltration rate is determined by differentiating Eq. 2.

PHOSPHORUS MODEL

Sediment-Bound Phosphorus Model

The sediment-bound P transport model assumes that sediment-bound P transport is directly proportional to sediment transport. The sediment transport model simulates detachment, transport, and deposition of sediment. The conservation of mass of P for each particle size class and each cell can be expressed as

$$P_i - P_o = \frac{dP}{dt} \tag{3}$$

where P_i = sediment-bound P inflow from surrounding cells and soil detachment within the cell (kg/s); P_o = sediment-bound P outflow to adjacent cells and P loss due to deposition within the cell (kg/s); P = sediment-bound P in transit (kg); and dt = model time step (s).

Equation 3 is solved at each time step for each particle size class separately. The initial P content of the soil is distributed among the different particle size classes according to the specific area of the particles, such that

$$PSSA = \frac{POSOIL \times 10^{-9}}{SSAT} \tag{4}$$

where PSSA = P content of the soil (kg/m^2); POSOIL = P content of the original soil (μg-P/g-soil); and SSAT = specific surface area of the original soil (m^2/g-soil).

The concentration of P for each particle class, PO_i, is

$$PO_i = SSA_i \, PSSA \tag{5}$$

where SSA_i is the specific surface area for particle size class i (m^2).

The basic assumptions of the sediment-bound P model are

1. The sediment transport model accurately simulates sediment transport.
2. Sediment-bound P is distributed between the soil particle size classes in proportion to the specific surface area of the individual particle size classes.
3. There is no redistribution of P between different particle size classes during or after a storm.

Dissolved Phosphorus Model Development

An extraction function (16) is used to describe the release of dissolved P from surface soil to runoff:

$$P_d = K_{ext} * t^{\alpha} * WS^{\beta} * P_{ext} \tag{6}$$

where P_d = cumulative P desorbed (μg-P/g-soil); P_{ext}= initial extractable soil P level (μg-P/g-soil); t = time of contact (minutes); WS = water to soil ratio (cm^3/g); and K_{ext}, α, β = empirical soil type specific constants.

The water to soil ratio is defined as the ratio of the rainfall volume falling during the time increment and the mass of suspended sediment. Phosphorus is desorbed from the mixing zone or effective depth of interaction (EDI), which is defined as the depth of soil at the soil surface that interacts with surface runoff. The amount of P desorbed during the time increment can be expressed as

$$\frac{dP_d}{dt} = \frac{C \; I}{EDI \; DX^2 \; \rho_b} \tag{7}$$

where C = concentration of dissolved P in runoff (μg/L); EDI = effective depth of interaction (cm); I = rainfall volume during time increment (L); DX^2 = area of the element (cm^2); and ρ_b = soil bulk density (g/cm^3).

The concentration of dissolved P can be derived from Eqs. 6 and 7 as

If runoff occurs, then the loss of dissolved P is the product of the runoff

$$C = \frac{\alpha K_{ex} t^{\alpha - 1} WS^{\beta} P_{ex} EDI DX^2 \rho_b}{I} \quad (8)$$

at the end of the time increment and the average concentration of dissolved P in the cell. The average concentration of dissolved P in the cell is the volume weighted P concentration due to dissolved P inflow from neighboring cells and storage and extraction from the given cell.

At the end of each storm, a P balance is computed and an average soil P concentration is determined. This average P concentration is used as the initial soil P concentration at the beginning of the next storm event.

The assumptions made for the dissolved P model are

1. Precipitation and living and decomposing plant material are negligible sources of P.
2. P desorption is diffusion controlled.

Phosphorus Uptake by Plants

Plants absorb available forms of inorganic dissolved P. The following equation (17), developed for describing N uptake by plants, is also used to describe P uptake:

$$UP = AU \frac{ET}{SW} \quad (9)$$

where UP = plant uptake of nutrient by plants (kg/ha); AU = available nutrient concentration (kg/ha); ET = evapotranspiration (cm); and SW = average soil water content (cm).

Phosphorus uptake is assumed to occur uniformly throughout the rooting depth. If the root depth is larger than the average depth to the wetting front (depth to which infiltrated water has penetrated), nutrient uptake is reduced to

$$UP_r = UP \frac{L}{R_d} \quad (10)$$

where UP_r = reduced amount of P uptake (kg/ha); L = average depth to the wetting front as predicted by the Green-Ampt infiltration model (m); and R_d = rooting depth (m). If the root depth is less than the average depth to the wetting front, uptake is distributed uniformly down to a depth equal to L. The nutrient uptake by the plant model will be used between storms to estimate soil phosphorus levels at the beginning of runoff events.

NITROGEN MODEL

The proposed model simulates the transport of the following forms of nitrogen: dissolved nitrate and ammonium, and sediment-bound ammonium and organic N. Nitrogen processes modeled include mineralization, nitrification, and adsorption/desorption of ammonium.

Mineralization

Mineralization, or conversion of organic N to nitrate between storms, is simulated as (18)

$$MIN = POTM \frac{AWC}{FC} (1 - e^{-\Delta t\, TK}) \tag{11}$$

where MIN = mineralized N (kg/ha); TK = temperature coefficient; AWC = average volumetric water content (mm^3/mm^3); Δt = time increment (days); FC = field capacity (mm^3/mm^3); and POTM = potentially mineralizable N in the soil (kg/ha).

The transformation of NH_4^+ to NO_3^- through nitrification between storms is

$$DNI = NO_3 * (1 - e^{(DKT * (DT - 0.5))}) \tag{12}$$

where DNI = amount of denitrification (kg/ha); NO_3 = nitrate in the surface zone (kg/ha); DT = duration of drainage since the last storm (days); and DKT = temperature adjusted rate constant.

Sediment-Bound Nitrogen Model

The sediment-bound N model considers ammonia and organic N separately. Transport of each sediment-bound N form is simulated with the same equations used by the sediment-bound P model except that the ammonia and organic N are the species being simulated.

Dissolved Nitrogen Model Development

Nitrate Transport--

The nitrate transport model assumes that all nitrate in the effective depth of interaction is available for transport. Thus nitrate transport from a cell is the product of the total amount of nitrate in a cell, times the volume of outflow from the cell, divided by the total volume of water in the cell.

Ammonium Transport--

Dissolved ammonium transport is more complex because of the dynamic equilibrium existing between the dissolved and attached forms of NH_4^+. The Freundlich equilibrium isotherm (19) is used to express the equilibrium between the two phases of NH_4^+

$$S_e = K\, C_e^{1/n} \tag{13}$$

where S_e = concentration of ammonium adsorbed by soil (μg/g-soil); C_e = concentration of dissolved ammonium (mg/L); and K and n = constants.

Plant Uptake of Nitrogen

The predominant form of N available is NO_3^- because under most soil conditions NH_4^+-N is rapidly transformed to NO_3^--N (nitrification). Ammonium becomes the major form of available N to plant under conditions that are not

favorable to the nitrification process (20). In the proposed model, only the uptake of NO_3^- is considered. Plant uptake of N is simulated with the same equations used to model P uptake.

EVAPOTRANSPIRATION MODEL

Ritchie's method (21) will be used to estimate evapotranspiration (ET). Incorporating ET is a necessary step in making ANSWERS-2000 a continuous model because ET depletes the soil moisture that establishes infiltration at the beginning of each storm.

The Ritchie's approach was selected because it uses the leaf area index as a parameter. This approach allows ET to be simulated as a function of plant surface condition and cover. Transpiration from plants and soil evaporation will be simulated separately because parts of watersheds may be vegetated while some others may be bare. The ANSWERS model does not divide the soil into layers, and the only layer used is that defined by the soil surface and the average wetting front depth. Evapotranspiration will remove water from the zone defined by the average wetting front depth. The soil moisture will be assumed to be uniform to the average wetting front depth.

Evapotranspiration will be computed on a daily basis between storms for each combination of plant cover and soil type. The fraction of potential ET (FETP) transpired is determined by

$$FETP = \frac{ETP}{1 - e^{-3.065}} \left(1 - e^{-3.065\, L/RZ}\right) \qquad (14)$$

where RZ is the root zone depth (cm) and ETP is the potential ET predicted by the Ritchie model. If the water requirement for ET exceeds the amount of water available (water above residual content), ET will be reduced accordingly.

USER-FRIENDLY INTERFACE

A user-friendly interface is also being developed for ANSWERS-2000. The interface or shell has three principal functions: (1) input and editing of required data; (2) control of model execution; and (3) control of model output.

The shell will assist in creating and entering data required by the model. It will first assist in creating the required basic elemental data file containing topographic, soils, landuse, and channel information required by the basic model that simulates surface runoff and sediment transport. The shell will eventually include a link with a public domain geographic information system to facilitate entry and manipulation of elevation, soils, landuse, and channel network data. Help screens will be provided to guide the user through the creation of the database.

The shell will then request information on the parameters to be simulated, whether the model is to be run for a single event or in continuous simulation mode. The shell will then query the user for the data required for the type of simulation desired. For example, if only P losses are required from a single design storm, then the shell will build the data file required for a single event simulation and for running the P model only.

Another portion of the shell will graphically display the watershed, after the basic elemental data file has been created, to enable the user to specify the points in the watershed at which output is desired.

Finally, the shell will include a graphical output section to assist in interpretation of the model's output and to identify cells with proportionally higher sediment and nutrient losses. The shell will allow the user to adjust the parameters of cells with high pollutant losses to simulate alternative BMPs and then run the model again. This process can be repeated until a system of BMPs is identified which results in acceptable pollutant losses. Model output will be assessable by the geographic information system used in creating the original watershed database.

MODEL TESTING AND VERIFICATION

The model will initially be tested on four watersheds in the USA and the Ukraine. The two Ukrainian watersheds are agricultural and representative of the Chernozem region of the western CIS. The watersheds are located on the Martovoe State Farm near Kharkov. One watershed is 586 ha and the other is 2.4 ha. Both field and vegetable crops are grown in the watersheds and cropping rotations are fairly complex. These watersheds are described in another symposium paper by Kolpak and Platonova.

In the USA, the model will be tested on the Owl Run and Nomini Creek watersheds in Virginia. These watersheds are predominately agricultural and have been monitored since 1986. The Owl Run watershed is in the Piedmont region, while the Nomini Creek watershed is in the Coastal Plain. Runoff and precipitation are monitored continuously from these 1200-ha watersheds.

SUMMARY AND CONCLUSIONS

ANSWERS-2000, a physically based, distributed-parameter, continuous water quality model is currently under development in the USA and CIS. The model is intended for evaluating the effects of agriculture and best management practices on water quality. The model will initially simulate surface runoff, sediment transport, and dissolved and sediment-bound nitrogen and phosphorus losses. The model will be interfaced with a geographic information system and user friendly shell to facilitate data file creation, model execution and interpretation of model output. A preliminary version of the model is scheduled for release in August 1993 for testing.

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RELATIONSHIPS BETWEEN WATERBORNE TETRACHLOROGUAIACOL UPTAKE AND OXYGEN CONSUMPTION BY FISHES

by

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ABSTRACT

The objective of the studies reported here was to determine whether there is a correlation between oxygen consumption by fishes and bioconcentration of tetrachloroguaiacol (TCG). Two species of fishes were tested--largescale suckers (*Catostomus macrocheilus*) and rainbow trout. Test fish were exposed to known TCG water concentration under controlled ventilation conditions using a modified Brett-type respirometer where oxygen consumption could be dynamically measured. Individual whole body TCG concentrations were determined by Ni^{63} electron capture gas chromatography. Lipid levels were also determined.

INTRODUCTION

The presence of chloroguaiacols and other chlorinated phenols in Kraft pulp mill bleach effluent was reported as early as 1975 (Leach and Thakore, 1975) where 3,4,5-trichloroguaiacol and 3,4,5,6-tetrachloroguaiacol (TCG) were isolated from effluents of paper manufacturing facilities in northwestern Canada. Static toxicity tests on these compounds using juvenile rainbow trout (*Ocorhynchus mykiss*) indicated that both compounds were toxic with 96-hour LC-50

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measuring at 0.32 mg/liter TCG. Guaiacol (2-methoxyphenol) is a naturally occurring product derived from lignin cleavage (Dence 1971) during paper production. Once cleaved, chlorination occurs during bleaching and eventually TCG and other chlorinated toxic byproducts are discharged with the caustic effluent, including resin acids (Leach and Thakore 1973).

Chlorinated guaiacols and phenols were also detected in pulp mill effluents located on the shores of the Baltic Sea where accumulation of these components in rainbow trout were measured by exposing test fish to caustic extraction effluent from milling operations (Landrer 1977). Significant uptake of at least 3 different chlorinated phenols: 2,3,6-trichlorophenol, trichloroguaiacol, and TCG was observed.

Swedish investigators (Renberg 1980) measured bioaccumulation (BCF) and toxicity (96-hour LC-50) of TCG to bleaks (*Alburnus alburnus*) native to the brackish seawater located in the vicinity of Kraft pulp plants along the Baltic Sea and also observed toxic effects and reproductive degeneration in crustacea (*Nitocra spinipes*) exposed to TCG.

Taste-impaired fish collected near Kraft pulp mill operations in Finland were found to have elevated concentrations of chlorinated veratroles and anisoles including TCG (Paasivirta 1987).

Bioconcentration factors (BCF) of TCG and other chlorinated phenols present in pulp and paper mill effluent were measured in subadult (mean weight 140 g) rainbow trout (Niimi 1990). These fish were exposed to TCG at waterborne concentrations of 1 μg/liter . Equilibrium was reached in 2 days with fish averaging 189 μg/g (log BCF = 2.26). Log P for TCG was also determined at 4.28. Niimi stated that these kinetics are consistent with residue levels reported in fish collected from waters receiving mill effluent where water borne uptake appears to be the primary mode of accumulation. Dietary exposure was also evaluated and it was found that TCG is poorly absorbed, and has a half-life of less than 2 days.

The objective of the studies reported here was to determine if there is a correlation between oxygen consumption by fishes and bioconcentration of TCG. Two species of fishes were tested: largescale suckers (*Catostomus macrocheilus*) and rainbow trout. Test fish were exposed to known TCG water concentrations under controlled ventilation conditions using a modified Brett-type respirometer (Gehrke et al. 1990) where oxygen consumption could be dynamically measured. Individual whole body TCG concentrations were determine by Ni^{63} electron capture gas chromatography. Lipid levels were also determined.

METHODS AND MATERIALS

Fish exposure - respirometer protocol

Largescale suckers (400 - 900 g) used in this study were wild fish collected from Cultass Lake located in British Columbia, Canada. Large (340 - 490 g) and small (4.6 - 5.8 g) rainbow trout used in these tests were procured from fish farms in British Columbia. All

fish were held in aquaria prior to testing at the Department of Zoology, University of British Columbia, Vancouver, British Columbia. At the time of test, individual large fish or groups of small fish were introduced into the respirometer and allowed to accumulate at the test water temperature for at least 1 hour prior to dosing. During this time, the fish were swum at low swimming speeds, < 1 body length per second (Bl/s). All fish were exposed to the same initial TCG water concentration of 200 μg/liter by the addition of 26 mg TCG (in 67 ml acetone) to the water at the start of the test. Once toxicant was added, the system was sealed and the water flow adjusted to a predetermined speed controlling ventilation. Five minutes after dose addition, flow was stopped and the system opened for 2 -3 minutes so that water samples could be collected for subsequent TCG analysis. The system was again sealed, the test continued, and oxygen depletion measurements initiated and continued throughout the duration of the test. All fish were them swum for a 2-hour period, at the end of which water samples were again taken for analysis. The fish were removed from the respirometer, rinsed briefly with tap water and sacrificed. Each fish was weighed and measured and then placed in a labelled plastic bag. All chemical analyses on both fish and water samples were performed at Fisheries Bioassay Laboratory, Montana State University, Bozeman, Montana. After each test, samples were either shipped by overnight commercial air carrier to Bozeman, packed with dry ice, or stored at -80°C until shipment. Once received in Bozeman, samples were also stored on dry ice until analyses.

All fish used in the study were exposed to TCG at two different ventilation rates. For tests involving largescale suckers, five fish were individually placed in the respirometer and exposed to TCG at a low ventilation rate where the water temperature was 9°C. A second set of five fish were subjected to the same regime, but a higher ventilation rate was induced by using a water temperature of 18°C. These two temperatures were used to achieve different oxygen consumption rates by largescale suckers rather than dissimilar swimming speeds because these fish were unable to swim at speeds much above 1 Bl/s. For the tests with rainbow trout, different ventilation rates were achieved using various swimming speeds. Five large rainbow trout were individually exposed to toxicant at a low ventilation rate with swimming speeds of 1.3 - 1.4 Bl/s. Then a second group of five fish were tested at a higher ventilation rate using swimming speeds of 1.7 - 1.9 Bl/s. In all these tests the respirometer water temperature was maintained at 7°C. Two groups of small rainbow trout were tested; one group (n=27) was swum at rates of 2.1 - 2.5 Bl/s, the second (n=26) from 3.2 - 3.9 Bl/s. The respirometer water temperature during these tests was held at 5°C.

TCG analysis - Fish

Fish preparation, surrogate addition, and extraction - Each large fish was partially thawed, cut into several pieces and then ground using a conventional kitchen meat grinder. The ground sample was further mixed to achieve as homogeneous a tissue sample as might be possible. This homogenate was placed in a labelled plastic bag and stored on dry ice or in a freezer at -80°C until analysis for TCG and lipid content. TCG analyses were performed in duplicate on portions of the homogenate taken from different parts of the sample, and on different days. At the time of extraction, weighed aliqouts (ca. 5 g) of homogenate was placed in square wide mouth extraction bottle. Small fish were weighed and then diced into small

pieces while still frozen. These pieces were placed into a tared square-shaped extraction bottle and the weight recorded. This weight was used as the fish weight when calculating final TCG concentrations. Each sample, either homogenate aliquot or diced small fish, was acidified by the addition of 2.0 ml 18N sulfuric acid followed by thorough mixing. Surrogate (pentachlorophenol, 10 μg) was then added to each sample followed by 80 ml extraction solvent (1:1 acetone:methylene chloride). Extraction was achieved by blending this mixture for 30 seconds using a Polytron™ tissue homogenizer. The extract was decanted through anhydrous sodium sulfate into a 250-ml volumetric flask. The residue extracted again, both extracts were combined, and the volume adjusted to 250 ml with additional extraction solvent.

Extract methylation and cleanup - Aliquots (5 ml) of extract were taken to near dryness with the aid of a gentle stream of dry nitrogen and low heat. The residue was immediately methylated (≥ 30 minutes) using ca. 3 ml ethereal diazomethane solution (Pressley 1982). Excess diazomethane was removed by passing a gentle stream of nitrogen over the sample and then the entire contents quantitatively transferred to a 11 x 300 mm glass column filled with 5 g florisil (60-80 mesh) topped with 1-2 cm anhydrous sodium sulfate. These prepared columns were stored at 130°C for at least 24 hours prior to use. Just before sample addition, each column was rinsed with several milliliters of 5% methyl-*t*-butyl ether/hexane solution (elution solvent). Methylated TCG and surrogate were eluted with 40 ml elution solvent and collected in a 100-ml volumetric flask. Hexachlorobenzene internal standard solution (1.0 ml 880 μg/liter) was added and the contents diluted to volume. Required dilutions were made with internal standard solution at 8.8 μg/liter. Controls and matrix spike samples (controls to which 10 μg of TCG and surrogate had been added) were treated similarly.

Ni^{63} electron capture gas chromatographic analysis - TCG and surrogate concentrations were determined by gas chromatographic analysis using a Varian model 6000 gas chromatograph equipped with a Ni^{63} electron capture detector, autosampler and data system. The column used was 180 cm x 2 mm i.d. glass column packed with 3% Carbowax-20M on 100-120 mesh Supelcoport™. This column was operated at 160°C. Complete resolution of internal standard, TCG, and surrogate was accomplished under these conditions. Retention times of hexachlorobenzene, surrogate, and TCG were 7.6, 10.0, and 11.4 minutes. Nitrogen carrier gas was purified nitrogen at 25 ml/minute. Detector and inlet temperatures were set at 300 and 200°C, respectively. Linear regression parameters from calibration curves were used to quantitate toxicant concentrations. Calibration standard concentrations ranged from 0.5-10 μg/liters. All samples and standards were injected in duplicate. TCG levels were not corrected for surrogate recoveries.

TCG analysis - Water

Two milliliters of each 5-minute respirometer water sample, and 5.0 ml of each 2-hour sample, were placed in 100-ml square-shaped volumetric flasks containing 0.5 μg surrogate, ca. 50 ml double distilled water, 2 ml 18N sulfuric acid, 10 ml 1:1 methyl-*t*-butyl ether/hexane, and a magnetic stir. Extraction proceeded by mixing the flask contents on a magnetic stirrer for ≥30 minutes. Extraction solvent was then forced up into the neck of the flask by addition of more water, and 1.0 ml of this solvent transferred to a 10-ml volumetric

flask. Diazomethane solution (1-2 ml) was added and the solution let stand for at least 30 minutes. Excess diazomethane was removed, internal standard was added (1.0 ml at 88 μg/liter) and the contents diluted to volume with hexane. TCB and surrogate concentrations were determined by gas chromatography as previously described. Method blanks and TCG (0.5 μg) fortified blanks were analyzed along with the water samples.

TCG method validation - Fish

To ensure that quantitative recovery of TCG could be accomplished at concentrations anticipated to be observed in the exposed fish, different amounts of TCG (5, 10, 20, 50, and 100 μg) were added to each of a series of samples of small rainbow trout taken from the same pool of trout used in these tests. These fortified samples represented a TCG concentration range in the fish from ca. 1 - 24 μg/g. Surrogate (10 μg) was added and then these samples were taken through the same procedure used to analyze the small trout exposed to TCG in the respirometer. TCG and surrogate recoveries were calculated.

Lipid analysis -

Total lipids were determined (Folch 1957) in duplicate on each large fish tissue homogenate. Lipid concentrations were also determined in the six small rainbow trout from each of the two tests previously described. This procedure involved blending 5-g aliquots of frozen tissue homogenate or diced small whole fish (previously weighed) to a powder in a small stainless steel blender, to which dry ice and anhydrous sodium sulfate were also added. Lipids were extracted by blending this powder two times for 3 min each with 100 ml 2:1 chloroform:methanol. Two extractions were used. The combined extracts were purified by washing with aqueous potassium chloride solution (0.88 % w/v). Layers were allowed to separate overnight and the aqueous layer discarded. The organic layer containing the lipids was vacuum filtered through a 0.2-μ Nylon-66 membrane and made to a 250 ml volume. A 25-ml aliquot was added to a tared beaker and solvent was removed using a gentle stream of nitrogen and low heat. The beaker and lipid residue was vacuum dried, weighed, and percent lipid calculated.

RESULTS

TCG concentrations (wet weight basis) in largescale suckers ranged from 2.24 - 8.64 μg/g (Table 1). When expressing this toxicant loading in terms of oxygen consumption, the rate of uptake in these fishes varied from 5.94 - 23.99 μg TCG/ mg O_2/hour and averaged 12.43. Concentrations of TCG in large rainbow trout measured 6.76 - 13.01 μg/g with uptake rates calculated at from 8.49 - 12.90 (mean 10.17). Small rainbow trout had TCG concentrations ranging from 13.74 - 21.97 μg/g with corresponding uptake rates of 9.51 - 20.15 μg TCG/mg O_2/hour (mean 13.31).

Duplicate TCG values measured in the homogenate samples agreed within 10% for all except three samples where differences were noted as high as 16%. Surrogate recoveries in these tissue homogenates ranged from 84.5 - 119%. TCG recoveries from fortified control

Table 1. Test summary

DOSE µg/l	5min-TCG µg/l	H2O T °C	FISH nb	FISH WT g	LIPID %	SPEED Bl/s	MO2 mg/h	Σ MO2 mg	TCG µg/g	Σ TCG µg	RATE µ/mg/h
Largescale suckers											
215	244	9.15	SFU-1	668	7.96	1.13	56.79	113.58	3.52	2351	10.35
208	230	9.19	SFU-2	931	9.23	1.09	53.99	107.98	4.26	3966	18.36
223	220	9.20	SFU-3	745	8.19	1.07	50.86	101.72	6.55	4880	23.99
208	211	9.23	SFU-4	780	4.47	1.12	34.85	69.70	2.48	1934	13.88
215	210	9.31	SFU-9	685	9.78	1.11	64.55	129.10	2.24	1534	5.94
200	189	17.97	SFU-5	553	10.83	1.01	71.88	143.76	4.10	2267	7.89
215	214	18.09	SFU-6	868	11.22	1.07	123.69	247.38	6.70	5816	11.75
208	194	17.94	SFU-7	582	3.21	1.12	87.04	174.08	8.64	5028	14.44
215	196	18.08	SFU-8	850	7.84	1.08	118.68	237.36	5.68	4828	10.17
215	203	17.97	SFU-10	403	8.06	0.99	47.55	95.10	3.57	1439	7.56
mean				707	8.08						12.43
Large rainbow trout											
261	224	7.10	223	464	6.25	1.40	75.2	150.4	8.36	3879	12.90
261	227	7.08	225	493	7.67	1.30	102.0	204.0	7.03	3466	8.49
261	230	7.15	226	487	7.02	1.32	93.3	186.6	6.76	3292	8.82
261	240	7.06	231R	360	9.13	1.29	84.1	168.2	10.08	3629	10.79
258	239	7.06	232	382	7.36	1.26	104.0	208.0	10.86	4149	9.97
261	223	7.16	224	392	7.42	1.88	106.0	212.0	10.88	4265	10.06
261	227	7.09	227R	446	9.64	1.71	104.0	208.0	11.46	5111	12.29
258	229	7.13	228	367	9.52	1.95	133.0	266.0	13.01	4775	8.97
258	234	7.08	229	344	8.21	1.90	108.0	216.0	12.06	4149	9.60
255	229	7.17	230	360	6.14	1.90	92.6	185.2	10.11	3640	9.83
mean				410	7.84						10.17
Small rainbow trout											
256	253	5.00	a-1	4.74	(1)	2.41	1.27	2.54	17.09	81.01	15.95
"	"	"	a-3	4.66	"	2.44	1.27	2.54	21.97	102.38	20.15
"	"	"	a-9	5.40	"	2.29	1.46	2.92	15.78	85.21	14.59
"	"	"	a-13	5.68	"	2.23	1.54	3.08	15.77	89.57	14.54
"	"	"	a-18	4.90	"	2.38	1.33	2.66	18.55	90.90	17.09
"	"	"	a-20	5.22	"	2.35	1.40	2.80	13.74	71.72	12.81
"	"	"	a-24	4.80	"	2.38	1.30	2.60	17.27	82.90	15.94
"	"	"	a-27	5.39	"	2.29	1.46	2.92	15.98	86.13	14.75
259	256	4.90	b-8	4.83	"	3.48	1.88	3.76	19.98	96.50	12.83
"	"	"	b-10	5.32	"	3.44	1.99	3.98	14.23	75.70	9.51
"	"	"	b-12	5.66	"	3.35	2.13	4.26	17.70	100.18	11.76
"	"	"	b-15	5.78	"	3.48	2.13	4.26	16.59	95.89	11.25
"	"	"	b-20	5.38	"	3.31	2.02	4.04	16.26	87.48	10.83
"	"	"	b-21	5.69	"	3.40	2.09	4.18	15.49	88.14	10.54
"	"	"	b-22	5.82	"	3.35	2.17	4.34	15.91	92.60	10.67
"	"	"	b-24	5.62	"	3.31	2.09	4.18	14.42	81.04	9.69
mean				5.31							13.31

(1) TCG analysis required used of the entire fish so lipid levels could not be measured on these fish.
Lipid concentrations were measured on 6 additional fish from each test group (range 3.44 - 7.57%, mean = 5.75).

fish homogenates varied from 84.5 - 107%. No TCG was detected in any of the control fish analyzed. Surrogate recoveries from the small rainbow trout tested ranged from 86.9 - 108%. Recoveries of TCG in untreated small rainbow trout varied from 86.4 - 108%.

TCG concentrations in the 5-minute respirometer water samples collected during each test ranged from 189 - 256 μg/liter indicating proper toxicant addition at the start of test. Surrogate recoveries from these samples ranged from 89.7 - 115%. TCG recoveries from fortified control water samples and method blanks varied from 95.6 - 109%. No TCG was detected in the control water sample or in the method blanks analyzed.

Lipid concentrations in largescale suckers varied from 3.21 - 11.22% total body weight, and averaged 8.08%. Lipid concentrations in large rainbow trout ranged from 6.19 - 9.64% (mean 7.84%). Variance in duplicate analysis of the tissue homogenate samples was never greater than 10%. Lipid concentrations in small rainbow trout lipid values ranged from 3.44 - 7.57%, averaging 5.75%.

Recoveries of TCG in the control fish fortified at various levels ranged from 86.4 - 102% with a mean calculated at 94.12 (Table 2). Linear regression analysis comparing analytical values to fortification concentrations indicated quantitative recovery over the concentration range tested (Figure 1):

$$Y_{analytical} = 1.02X_{fortification} - 0.297 \qquad (r^2 = 0.9974)$$

Surrogate recoveries in these samples ranged from 88.3 - 105%.

DISCUSSION

Mean TCG uptake rates of the three groups of fishes tested were very similar. The small rainbow trout absorbed the greatest amount of toxicant at 13.31 μg TCG/mg O_2/hour, followed by the largescale suckers at 12.43, and then the large rainbow trout at 10.17. When the uptake rates of all the fishes tested are evaluated as a single group, the mean uptake (n=36) is 12.93 μg TCG/mg O_2/hour with a 95% confidence interval of $\pm$ 2.53. These values imply that all the fishes essentially absorbed TCG at about the same rate and indicates a direct correlation between waterborne TCG uptake (at a given water concentration) and oxygen consumption by the fish. This relationship is shown graphically in Figure 2. More data are required to develop a predictive absorption model. Tests should involve medium size rainbow trout, medium and small largescale suckers, and then other species of fishes.

Lipid concentrations in these fishes were also very similar, especially in the larger fishes. However, even though lipid concentrations were similar, the mean TCG concentration in the largescale suckers was slightly less than half that of the larger rainbow trout, but at the same time their mean oxygen consumption rate was approximately 70 % of that of the larger rainbow trout (Table 1). This indicates the important role of oxygen consumption rates in rates of toxicant bioconcentration. When uptake rates of these two groups of larger fishes were tested for significance using the Mann-Whitney (Wilcoxon) nonparametric rank sum test,

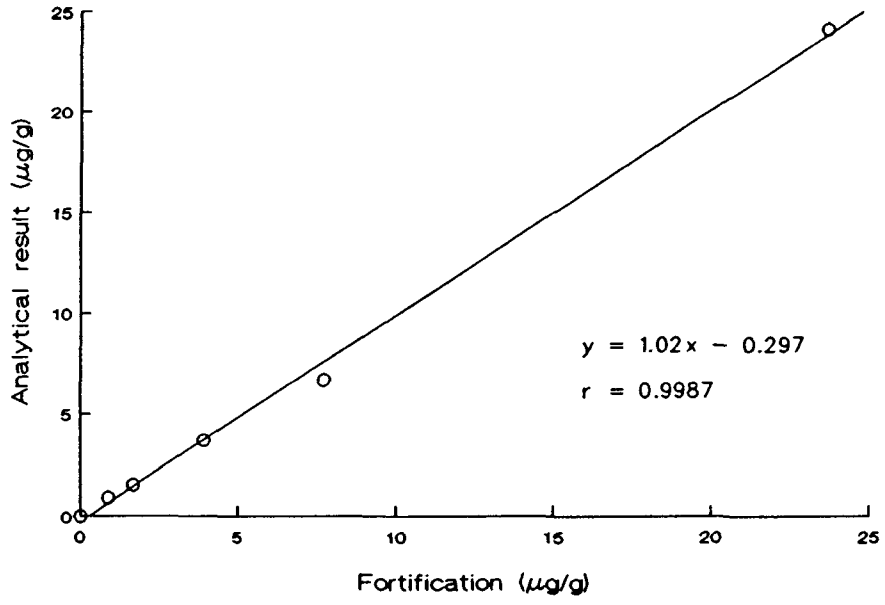

Figure 1. TCG recovery from fortified rainbow trout, *method validation*

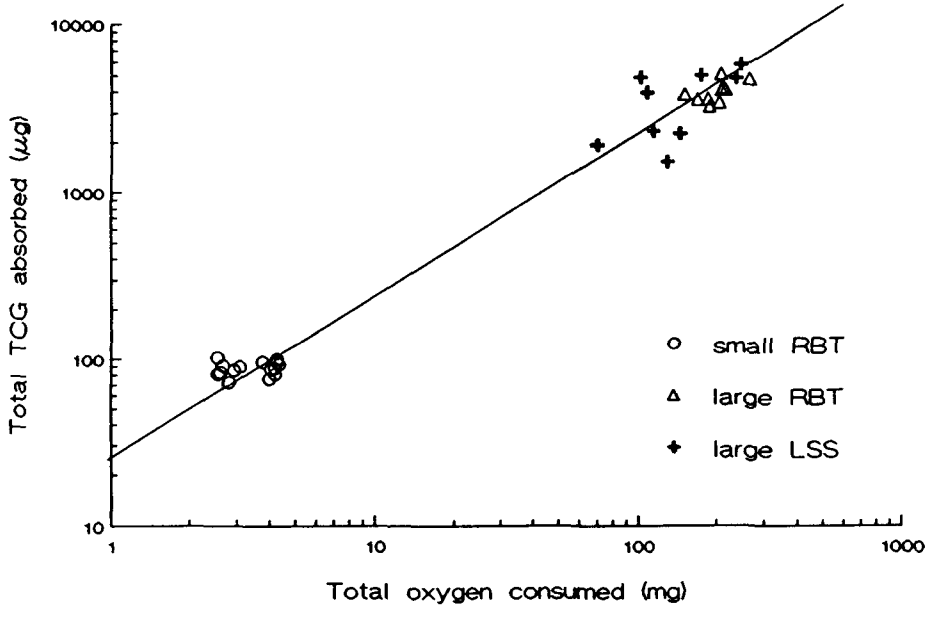

Figure 2. TCG concentration vs oxygen consumed, largemouth suckers and rainbow trout.

Table 2. TCG fish analysis - method validation.

FISH	WEIGHT (g)	µg TCG ADDED	µg/g (THEORETICAL)	µg/g (ANALYTICAL)	% RECOVERY
C-14	6.22	0	0	0	-
C-11	5.57	5	0.898	0.892	99.3
C-10	5.90	10	1.69	1.51	89.3
C-18	5.09	20	3.93	3.68	93.6
C- 5	6.48	50	7.72	6.67	86.4
C-15	4.22	100	23.7	24.2	102

no differences could by measured. This may not be a valid test with so few numbers; however, one-way analysis of Variance (ANOVA) applied to all three groups of uptake rate data suggested that the differences in the means of the groups was no more significant than the variability within each group.

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AN INVESTIGATION OF THE LOWER DON RIVER WATER QUALITY USING SIMULATION MODELS

by

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ABSTRACT

This paper describes a hydrodynamic and water quality modeling effort on the Don River in Russia. The waterway is one of the largest rivers of Russia and has been adversely impacted by development; its lower part in particular is severely impacted by anthropogenic processes. Don River basin is an intensively exploited region with respect to agricultural and industrial activities. Waters of the Lower Don system are diverted for municipal and industrial uses, as well as for irrigation of agricultural fields. Preliminary results from water samples collected in the summer of 1990 indicated that conventional pollutants and synthetic organic materials are the major contaminants of concern. The developed linked model of the system consists of a river hydraulics and sediment transport code (RIVMOD), its modified version for handling branched systems (RIVNET), and the water quality modeling package (WASP4). Simulation results indicate that detailed morphometric characteristics are needed at the Don mouth for better model performance. Hydrodynamic and water quality data should also be collected on the main branches of the Don at its mouth on a concurrent basis in order to be useful for modeling purposes. To determine the best management alternative for water quality improvement, watershed models should be used to delineate the nonpoint source loading patterns. For these purposes, information on water uptake from the river and wastewater loading to the river are also required. Although evaluation of model performance is difficult due to inadequacy of information and uncertainty about representativeness of available data, nevertheless results are useful for designing data collection networks for long-term water management and further simulation studies.

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INTRODUCTION

Among the large rivers of Russia, the Don--and in particular its lower part--is severely affected by anthropogenic impacts. Runoff and irrigation return waters from fields, discharges from industrial plants along the river, and discharges from municipalities adversely impact the quality of Don waters. Although there are heavy industrial complexes in the river basin, preliminary data from water samples showed that conventional pollutants and synthetic organic materials are the major contaminants of concern. For water quality modeling, information about hydrological regime, transport and transformation of contaminants carried to the river, and river ecosystem characteristics are required. Such data and associated simulation results from modeling can then provide necessary inputs for water management alternatives that are environmentally and ecologically sound and economically feasible. To this end, hydrodynamic and water quality modeling of the Lower Don River system can shed some light on the processes active in the waterway and provide insights that could be helpful in selection of water quality management alternatives.

In this paper only the stretch of the Don River from Razdorskaya to Taganrog Gulf is considered in the simulations. Analysis of data collected by the Hydrochemical Institute in Russia, and the Environmental Protection Agency's Athens Environmental Research Laboratory (AERL) in the U.S., showed that the main water quality problems of the Lower Don system are associated with processes of eutrophication, contamination by organic matter, and pesticides. Besides, high sediment load and turbidity may essentially influence the aquatic ecosystem and hence self-purification processes.

PHYSICAL SETTING

The Don region covers an area of about 323,000 km^2. The Don watershed consists of three different physiographic zones: mixed forest, partially wooded steppe, and steppe. This region is part of the Don River watershed, supplying much of its annual flow. The Don River is situated between 44 and 54°N latitude and 37-45°E longitude. The maximum north-south spread is 650 km, and the east-west spread is about 160 km. The Don is a relatively large river, approximately 1870 km long and is fed by a watershed with an area of about 422,000 km^2. The river's headwaters are on the east side of the Middle Russian Hills. The Don flows into the Taganrog Gulf on the Azov Sea. In the upper parts, the river flows through a narrow valley and its channel has high right banks. In these parts the river also intercepts many ravines and has a number of shoals, which are formed by the sediment transport from tributaries and inflows from ravines. The river channel is very winding and often meanders in the flat sections.

In the middle section the river valley is significantly wider, though the river channel runs close to the right side of the valley with elevated banks. At the end of the Don's middle section the natural flow is altered by a hydropower and water supply dam behind which is the Tsimlyansk Reservoir. Downstream from the dam the river valley is quite wide with lateral stretches up to 30 km. Water depth in the river channel at the thalweg can be as much as 20 m. The river estuary is situated downstream from the city of Rostov-On-Don. The estuary covers an area of approximately 340 km^2. In the lower reach of the river more tributaries discharge into the Don.

Anthropogenic activity plays a significant role in transformation of natural features of the Don River. The river basin is one of the most exploited regions of the country with respect to industry and agriculture. Ecological problems such as soil plugging, forest cutting, and soil erosion are widespread. In addition to the Tsimlyansk Reservoir on the Don, a number of reservoirs also are in place on the Don's tributaries, as well as hydraulic engineering structures such as dams and irrigation channels. Almost 7500 km^2 of agricultural fields are irrigated in the Don River basin. The Don River and its tributaries are sources of water supply for many large industrial complexes. The stretch of the Don River from the Tsimlyansk Reservoir to the Taganrog Gulf is referred to as the Lower Don River. The area of the watershed in the lower part is over 160,000 km^2; the length is about 313 km. The river in the lower part meanders through the valley with a spread of usually about 11-12 km, though it can reach up to 22-25 km. The river width varies from 400 to 600 m in the lower part. Average water depth during low-water regime is in the range of 4-6 m in the main channel, and decreases to 0.7 m in shoal areas.

HYDROLOGY OF THE LOWER DON RIVER SYSTEM

The Lower Don region is situated in a semiarid climate zone with characteristics of which are irregular precipitation and highly variable surface and ground waterflow. The main source of water is precipitation. Average total runoff is estimated to be about 30 mm per year of which 20 mm is produced by surface runoff and the rest from ground water runoff as interflow or baseflow. Direct surface runoff to the river from precipitation produces 75% of the flow of which snow accounts for about 67%. The rainfall component of surface runoff is no more than 8% of whole river flow. Hence, the majority of the river discharge takes place in spring during snowmelt. Overall, summer rains and winter snow characterize the hydrology of the Lower Don River system and contribute most of the annual flow. The hydrochemical composition of the Lower Don River is influenced by many factors. The primary chemical characteristic and biological features are defined by the waters behind the Tsimlyansk Reservoir. As water flows downstream, its chemical composition transforms significantly. This transformation is due to loading of the dissolved matter which is anthropogenic and of natural origin. Yereschukova et al. (1992) give more details on the river system.

WATER QUALITY CONSTITUENTS

SEDIMENT WASHOFF AND ITS CHARACTERISTICS

Most sediment washoff to the river occurs during spring high flows. Suspended sediment distribution across the channel cross-section is both temporally and spatially very heterogeneous. Turbidity increases in the middle sections of the river and near the bed. Surface soil layers in the watershed of the Lower Don River have a highly impermeable crust. This feature causes rapid accumulation of water from rainfall, since very little water can infiltrate the soil crust. Runoff carries with it all the accumulated fine materials from the soil surface. The concentrated flow can harness enough energy to induce erosive power to the point of gully erosion and heavy sediment load to the river system.

During spring more than 70% of annual solid loads are transported to coastal waters. The annual load of suspended matter is close to 1.6 million tonnes in the Lower Don. About 40% of these materials are accumulated in the river channel; only 1 million tonnes are transported to the coastal-sea areas (Yereschukova et al. 1992). The composition of suspended materials in the lower part of the river may provide some hint concerning conventional water quality parameters. The transported sediments consist of mineral matter, detritus and live bacteria, phytoplankton, and zooplankton. Analysis of transported materials showed that during spring 1984 solid load comprised 24.3% and live organisms and minerals 75.5% of the total load. The water column saturation with mineral load has negative effect on the biota of the Lower Don. Phytoplankton is a source of significant biomass; composition of different species is sufficiently stable. During spring much of the phytoplankton consists of diatoms (61-93% of total biomass). Also represented are the greens and goldens which are about 2-29% and 0.08-39%, respectively; bluegreens do not play a very significant role (1-17% of total biomass). Quantitative analysis indicates that phytoplankton distribution is very uneven. Total biomass changes from 0.5 to 6.8 mg/L, with an average value of 3.0 mg/L.

DISSOLVED OXYGEN REGIME AND OTHER CONTAMINANTS

The dissolved oxygen regime of the river is fairly stable. Oxygen content has never decreased below critical level (i.e., 5 mg/L). At the same time, in Tsimlyansk Reservoir, during intensive growth of blue-green algae anoxic conditions may prevail and the oxygen concentration in the bottom layers can go down to 1 mg/L. Concentrations of heavy metals, phenols, and synthetic surfactants are generally stable within the study area and slightly exceed maximum allowable concentration value. Concentrations of chloro-organic pesticides such as DDT, lindane, and their metabolites are also measured for routine monitoring purposes. These pesticides were detected in more than 50% of the samples for all locations. Maximum concentrations of DDT may reach dozens of micrograms per liter, while lindane concentrations of several hundred parts per billion are not uncommon. Determinations of concentrations for trace organic pollutants showed that detectable concentrations were predominantly in zones of direct influence from major point sources of pollution. Most of these pollutants are biodegradable, so they may have only local toxic effects.

NUTRIENTS

Part of the chain in the nutrient recycling process is dissolved organic matter. The amount of dissolved organic matter is measured by the amount of oxygen needed for oxidation of the organic load or the biochemical oxygen demand. The concentration of dissolved organic matter is highly variable. In 1984 it ranged from 4.32 up to 15.52 mg O_2/L. The organic load distribution along the river is uneven. There is a small increase in the amount of dissolved organic matter from Tsimlyansk Dam to the mouth of the Don (6.08 to 7.2-11.52 mg O_2/L). This trend is disturbed only at the mouth of tributaries Sal and Manuch.

There is a clearer trend in distribution of organic matter in the Don across the river section. Higher concentrations have been observed at the right bank of the river. The reason is that more sources of anthropogenic pollution are situated on the right bank of the river. The nutrient load to the Lower Don River system changes unevenly during the year

and from 1 year to the next. Nutrients come to lower parts of the Don River from upstream tributaries and lateral point and nonpoint sources. The average load through the Rasdorskaya cross-section is presented in Table 1.

Sources of the phosphorus supply to the Lower Don are varied. Part of it is due to decay of dead organisms; another part is supplied through bottom erosion processes. A significant portion of phosphorus load can be attributed to return water from agricultural fields, stock farms, and municipal wastewater treatment plants. During spring the mean concentration is about 0.009 mg/L (with a range of 0.006-0.012). In spring, concentrations of phosphorus in the Lower Don River are at background levels. In summer, concentrations rise to very high levels, sometimes up to 0.3 mg/L, and the waterbody shows signs of low level phosphorus contamination. In autumn, phosphorus concentrations decline to 0.09 mg/L.

TABLE 1. NUTRIENT LOADS TO THE LOWER DON RIVER SYSTEM (IN METRIC TONNES PER YEAR)

Month	NO_3	NO_2	NH_4	P	Si
January	830	74	400	8	4
February	1120	88	590	29	5
March	1770	223	85	60	8
April	2550	78	1670	105	10
May	3630	147	1480	76	10
June	3060	162	60	53	6
July	1010	42	250	41	4
August	1090	60	360	50	7
September	1860	217	290	50	5
October	1450	32	320	46	7
November	3080	70	460	59	8
December	1770	26	280	25	5

WATER QUALITY PROBLEMS IN THE LOWER DON SYSTEM

Based on data from observations, water quality problems of the Lower Don can be characterized only in general terms. For example, the high hydrocarbon level can be explained by intensive use of the river as a waterway for navigation and transportation. Petroleum products, such as gasoline and diesel fuel, may leak from small boats and ships used for transport and recreational purposes and from underground storage tanks in the vicinity of the river; these are considered major sources of hydrocarbon contamination. In addition, more hydrocarbon loadings may be added to the river from urban runoff. Partially treated wastewater from domestic sources through sewage treatment plants or by direct discharge may be contributing to high nitrite concentrations. Detectible pesticide concentrations can be interpreted as a result of intensive application on crops and other plants in the surrounding watersheds.

THE MODELING FRAMEWORK

The above brief description of characteristics of the Lower Don River system shows that, in order to get a feel for the enumerated problems, models that can describe the hydrodynamic, sediment transport, and water quality processes in branched river systems are needed. The most appropriate models for this kind of study are those with dynamic riverine water quality simulation. However, most available models did not have the capability for sediment transport or handling branched systems. Because of familiarity and previous experience we used the WASP4 water quality modeling package (Ambrose et al. 1988) in association with a river hydraulic and sediment transport model, RIVMOD (Hosseinipour and Martin 1991). The water quality model of the system is based on mass balance in a compartment of a waterbody. Hydrodynamic parameters for the water quality model were supplied by a modified version of RIVMOD for branched systems called RIVNET. The model is based on the one-dimensional equations of continuity and momentum for a branching or channel-junction computations network. The resulting unsteady hydrodynamic information from RIVNET are averaged over large time intervals and stored for later use by the water quality component of the simulation package. Simulations were conducted for spring high flows, considering branches and creeks flowing into the system.

WATER QUALITY SIMULATIONS

Hydrochemical processes play an important role in mixing and transport of chemical and biological compounds in natural waterbodies. To simulate hydrochemical and hydrobiological processes in rivers, discharges, velocities, and depths are needed. Therefore, water quality modeling of the Lower Don River system was started with hydrodynamic simulation. At the upstream of this part of the river, daily discharge and stage are available, but these data belong to the period before 1987. Besides, short-term variations in flow may not be significant in long-term simulations. Thus, average values of discharge for the 10-day period were chosen as a boundary value. Downstream boundary values for discharge or depth can be specified only approximately because very little data on the branches at the mouth of the Don River exist. Lack of precise information induces increasing uncertainty about modeling results. To estimate the influence of errors with regard to boundary conditions on results of simulation, an uncertainty analysis was conducted (Yereschukova et al. 1992). To compare results of different experiments, the measure of deviations between base runs and solutions with modified input data sets was introduced as follows:

$$\Delta=[\frac{1}{N}\sum_{i=1}^{N} X_b(t_i)-\frac{1}{N}\sum_{i=1}^{N} X(t_i)] \qquad (1)$$

where

Δ	= absolute measure of error influence
$X_b(t_i)$	= the value of characteristic at time t_i, which is obtained from the simulation with the basic input data
$X(t_i)$	= the value of characteristic at time t_i, which is obtained from the simulation with modified input data
N	= the number of time steps during the simulation.

The relative measure is a more representative estimator of uncertainty. The relative measure can be calculated by the following formula (Jorgensen 1986):

$$\acute{o} = \frac{\dfrac{\Delta}{\dfrac{1}{N}\sum_{i=1}^{N} X_b(t_i)}}{\dfrac{\Delta p}{p}} 100\% \qquad (2)$$

where

ó = the relative measure of changes in %

Δp/p = the relative change in the parameter value (here in the boundary value).

The above formulas were applied to estimate the change in water distribution between branches, which are caused by a change in boundary conditions. Such information is very important in identification of water quality parameters for modeling. To provide accurate simulation of the hydrodynamic regime at the mouth of the Don River, downstream boundary conditions have to be specified as accurately as possible, and no less accurately than upstream boundary conditions. This accuracy is even more important for simulation of low flow events. The last cross-section where discharge is measured is near Razdorskaya. The discharge does not change significantly along the river channel; the high discharge period coincided with the beginning of the study, and after that their deviations from average flow do not exceed 15% of mean value. Hence, it is possible to use constant discharge for water quality simulation. An example of results of discharge simulations from May to October 1983 are presented in Fig. 1.

As stressed above, one of the main water quality problems in the Lower Don River system is eutrophication. Simulations could help to detect crucial parameters affecting the river ecosystem. Once those parameters are determined, the trophic level of this waterbody can be estimated more precisely. Therefore, the model used for this purpose has to contain the stated variables at least for the description of algal biomass and organic and inorganic nutrients. During preliminary analysis of available water quality data, we found that the data are not adequate for model calibration. However, calibration of the model, even with incomplete data, could be useful for exploration of the main stated variables and other parameters of interest. As a whole, simulation results can hardly be used for testing different management strategies for water quality improvement in the region. However, simulations can help determine areas that need more research and plan for data collection and further investigations.

Eutrophication simulation was conducted using the EUTRO4 module of the WASP4 modeling package. This package allows several choices for eutrophication modeling. Considering the fact that adequate data were not available to describe dynamics of different phytoplankton groups, the following stated variables were included in the model: biomass of total phytoplankton, organic nitrogen and phosphorus, and inorganic phosphorus and nitrogen constituents (including ammonium and nitrate). Although diatoms constitute a large part of spring phytoplankton and silicon is the nutrient that affects their dynamics, this nutrient cycle is not taken into consideration because it is not taken up by the other groups of algae.

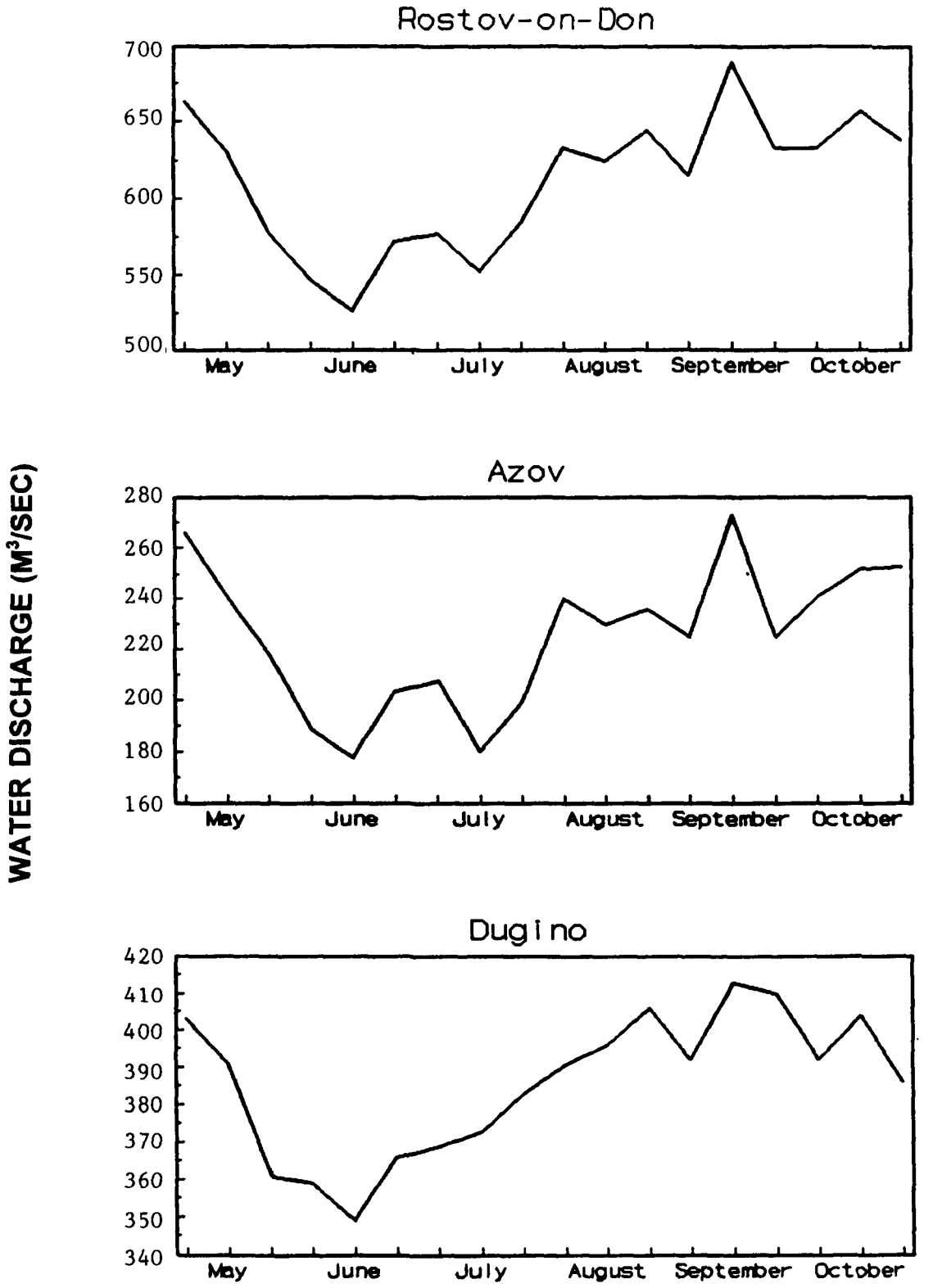

Fig. 1. Water discharges in the Don mouth.

Implicitly, the water quality model assumes that silicon is never depleted enough to limit diatom growth. Water temperature and light intensity significantly affect phytoplankton dynamics. These parameters are included in the model as forcing functions.

SENSITIVITY ANALYSIS

Sensitivity analysis is an important step in environmental transport and fate modeling, especially in situations where data are incomplete and/or unreliable. Such analysis allows researchers to look into the most crucial parameters of the model and get a feel for their impact on the overall analysis. To quantify the influence of parameter changes on dynamics of the stated variables, equation 2 was applied. Values were varied $\pm 10\%$ of their base values. The analysis was conducted according to a 1-factor plan. Maximum values of relative changes based on equation 2 are presented in Table 2.

TABLE 2. MODEL SENSITIVITY DUE TO PARAMETER VARIATIONS. PARAMETERS AFFECTING PHYTOPLANKTON DYNAMICS ARE MAXIMUM GROWTH RATE (G), RATES OF DECOMPOSITION OF ORGANIC PHOSPHORUS AND NITROGEN (K_p, K_N), MORTALITY RATE (M), LIGHT SATURATION COEFFICIENT (L), AND CARBON-TO-CHLOROPHYLL RATIO (r)

Variable	G	K_p	K_N	M	L	r
Phytoplankton	0.0850	0.0014	0.0000	0.0220	0.9444	0.9991
Ammonia	0.1641	0.0018	0.3643	0.3800	0.1810	0.1178
Nitrate	0.0237	0.0006	0.0601	0.0592	0.0259	0.0283
Organic nitrogen	0.0438	0.0005	0.0000	0.0029	0.0484	0.0340
Inorganic phosphorus	0.0248	0.0616	0.0000	0.0010	0.0272	0.0185
Organic phosphorus	0.0041	0.3633	0.0000	0.0219	0.0045	0.0729

It should be noted that the model is structurally stable in the domain of parameter values in Table 2, because the values of criterion do not exceed 1.0. Since phytoplankton dynamics are most sensitive to variations in carbon-to-chlorophyll ratio, it would be useful to determine more precisely the species composition of algae and their elemental composition. Among factors affecting phytoplankton dynamics, light intensity plays the most important role and is probably the most limiting factor on this ecosystem. Nitrogen influence on phytoplankton dynamics is not very significant, in part because its concentrations in Lower Don waters are significantly higher than its half-saturation value. Changes in phosphorus dynamics do not affect significantly the algae biomass, because its concentrations are not significantly below half-saturation values.

Effect of waterflow on the modeled ecosystem was investigated. Flow monitoring data and hydrodynamic simulations indicate that the discharge does not fall below 400 m^3/sec in the Lower Don system during the growing season. At times the discharge may reach as high as 1800 m^3/sec in the beginning of spring (e.g., 1979), but after a short time it decreases to about 500-600 m^3/sec. A discharge of 600 m^3/sec was chosen as the baseflow. The model

was run with the same input data set, except discharges were changed to 400 and 1000 m^3/sec (see Fig. 2). It appeared that the maximum concentration of phytoplankton biomass does not change significantly with flow. Values were close to those at the upstream boundary. Even increasing travel time when discharge is low did not cause intensive blooming of phytoplankton. We attempted to estimate the influence of flow redistribution between branches on phytoplankton dynamics at the mouth of the Don River. As described above, significant changes in distribution of flow can be caused by errors in specifying hydrodynamic boundary conditions. During water quality simulations, however, only slight deviations in biomass (2% of maximum value) were discovered. It should be noted that only part of the Don River mouth was modeled, and travel time along the branches did not increase sufficiently to allow phytoplankton to bloom.

Observations indicate that phytoplankton distribution is very uneven along and across the river. Near the banks, concentrations of phytoplankton biomass may be very high. Simulation of this phenomenon with a 1-D model is difficult and because hydrological and hydrodynamic data are sparse, 2-D modeling is not feasible. It is possible, nevertheless, to estimate probable effect of phytoplankton spots on average phytoplankton concentrations on this reach of the river under certain assumptions. It was assumed that the five upstream segments of this part of the Don River are connected with shallow pools, where concentrations of phytoplankton reach their maximum values of nearly 6-7 mg/L. Phytoplankton from these pools may be transported to the main channel by dispersion. Simulations including this extra load of phytoplankton did not result in significantly higher phytoplankton biomass concentrations in the main channel. Maximum concentrations increased only about 7%. Hence, a major part of phytoplankton biomass comes from upstream, and is not significantly augmented by phytoplankton that may disperse into the main river channel from shallow waters near the banks.

MODEL TESTS

Hydrologic data on water stage applicable in modeling are available only on the segment of the river from Razdorskaya to Rostov-on-Don. Therefore, simulations were conducted for this part of the river. Hydrodynamic model parameters were identified for July 1-10, 1979, and the model was run to simulate the system for this period. In other tests, the model was run with an input data set corresponding to February 5-15 and September 1-10. Modeling results compared well with observed data; the difference between simulated and measured values of stages did not exceed 5%. The period of simulation, corresponding to March 1-10, 1979, seemed to be a good start. During this time of year snow starts to melt on the watershed of the Lower Don River system, and lateral inflows are a significant part of the river total flow. Simulations were conducted with zero lateral inflows to the segments, because the real values of lateral inflows were unknown. As a result, simulated values of stages were less than observed values by about 30% in the middle segments of the river.

Sediment transport simulation was conducted with hypothetical data because of lack of real data. The model was run for data corresponding with particle sizes from 0.07 to 0.20 mm. Results of simulation showed that the river capacity for sediment transport is very uneven from segment to segment. The capacity is high for the marginal segments, and lower in the middle portion of the river. Results conform with the fact that shoaling is proceeding

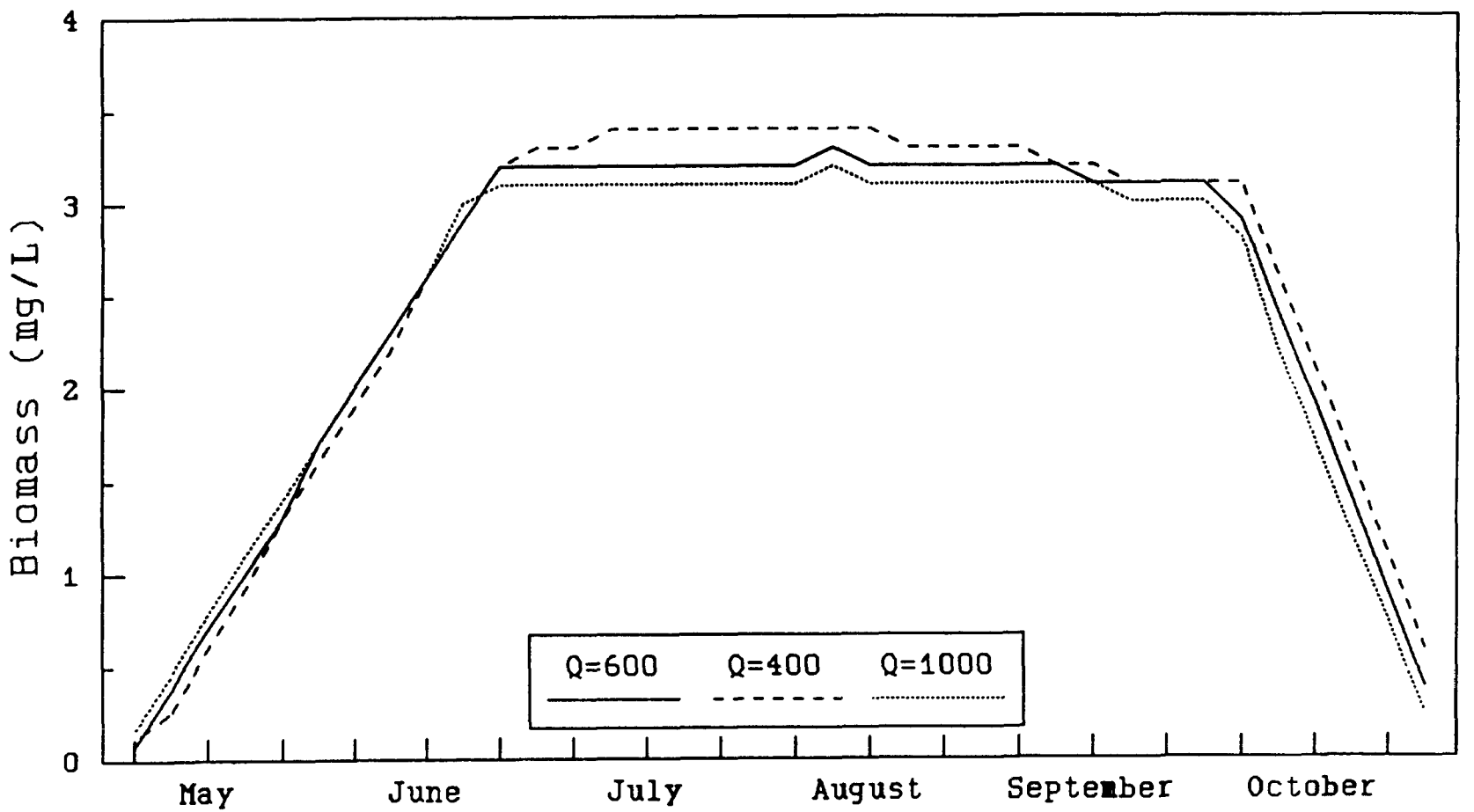

Fig. 2. The water flow influence on the phytoplankton biomass near Rostov-on-Don.

very intensively in the Lower Don River system. Sediment transport capacity depends on the size of the particles. Sand, with diameter of particles about 0.07 mm, is transported with the discharge from 0.002 up to 0.036 m^3/sec. For particles with a diameter of 0.20 mm, the discharges are significantly less and vary from 0.004 up to 0.005 m^3/sec. Sediment discharges in shallower branches are higher than the deeper ones by about 2.5 times.

CONCLUSIONS

Modeling results and experimentation with simulations on the Lower Don River system led to come conclusions that are helpful in planning future investigations. First of all, more detailed morphometric information on the mouth of the Don River is needed. Additional information is required concerning channel segment lengths, depth, widths, and bottom and surface slopes of branches and creeks in the delta and particularly near the junction of the Don with inflowing and outgoing branches at the mouth. Discharges and/or water stages need to be measured at the junctions as well. Also, stage or discharge near the Taganrog Gulf have to be measured with the same frequency as those at the upstream of the Lower Don River system for boundary condition specification.

Simulation of eutrophication processes showed that the main factor controlling phytoplankton dynamics is the river discharge. The river flow is sufficient to inhibit intensive phytoplankton blooms in the portion of river from Razdorskaya to the Azov Sea. According to the data, most phytoplankton biomass passing through this reach comes from upstream. These primary water quality simulations allow us to draw only general conclusions about some features of the ecosystem. To estimate the eutrophication level at the Don River mouth, the stretch of river from Dugino to the Taganrog Gulf has to be studied in more detail. For this purpose, information about the segment should be collected as well as about the whole Lower Don River system. For a comprehensive study, the data collection plan should include gathering information about phytoplankton dynamics and nutrient cycle with at least 2 week frequency and at 10 km intervals along the river. Quantitative information about phytoplankton species composition can also be very useful. Additional study of macrophyte distribution and their role in the eutrophication process would be very helpful in simulating phytoplankton and nutrient dynamics more accurately.

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EARLY DEVELOPMENT OF STRUCTURE-ACTIVITY RELATIONSHIPS FOR ENVIRONMENTAL TOXICOLOGY IN RUSSIA

Robert L. Lipnick[1], Vladimir A. Filov[2], and
Rosemarie C. Russo[3]

ABSTRACT

Quantitative structure-activity relationships have proved to be an essential tool in assessing the risks to humans and the environment from exposure to industrial organic chemicals. One of the pioneers in the application of structure-activity relationships to toxicology was Nikolai Vasilyevich Lazarev (1895-1974) at the Leningrad Institute of Industrial Hygiene and Worker Safety. Among his accomplishments was the recognition that toxicity could be limited by water solubility, a finding that has been applied decades later by the U.S. Environmental Protection Agency in defining an appropriate set of tests for a chemical under review. His pioneering work, then, presaged modern approaches in drug design and the assessment of toxicological effects to man and to organisms in the environment.

INTRODUCTION

Of increasing international concern is our ability to identify those untested industrial organic chemicals likely to have the potential to produce adverse effects to human health and to the environment. Even with active international cooperation and data sharing, resources are not likely to be adequate in the foreseeable future to test all industrial chemicals for all possible effects. For this reason, environmental regulatory agencies need scientifically defensible methods for setting testing priorities.

Ideally, one would like to be able to write the structure of an organic compound and use this information to predict its spectrum of possible or likely

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toxicological effects. Such approaches--referred to as quantitative structure-activity relationships (QSAR)--have been used for some time in pharmacology, and are becoming of increasing importance in toxicology.

Under Section 5 of the Toxic Substances Control Act, the U.S. Environmental Protection Agency is authorized to asssess, prior to their manufacture, what risks new industrial chemicals may pose to human health and the environment. Under this legislation, however, the chemical manufacturer is not required to provide EPA with any data other than that in its files at the time of the submission. EPA scientists must then determine if a case can be developed as to why testing needs to be performed prior to introduction of the chemical into the marketplace. A parallel chemical selection and review process exists for chemicals already in commercial production. Structure-activity relationships have provided an essential tool to EPA in meeting these statutory requirements, for the more than 20,000 cases thus far reviewed.

In 1975 (WHO, 1979), an international symposium *Methods Used in the USSR for Establishing Biologically Safe Levels of Toxic Substances* was held in Moscow, sponsored by the World Health Organization. Part of the Symposium was devoted to estimations of toxicity from physicochemical properties and chemical structure, which made reference to the pioneering work of Nikolai Vasilyevich Lazarev (1895-1974) (Fig. 1) in Leningrad. Lazarev's contributions have been extensively cited in a Russian toxicology textbook (available in English) by four of his students (Filov, et al., 1979).

Fig. 1 Portrait of Nikolai Vasilyevich Lazarev (courtesy of V.A. Filov).

LAZAREV'S PIONEERING STUDIES

DEVELOPMENT OF INDUSTRIAL HYGIENE STANDARDS

Lazarev's application of structure-activity relationships to toxicology provided the foundation for the development of industrial hygiene standards in the Soviet Union (Lipnick and Filov, 1992). While leader of a group of toxicologists at the "Krasny Treugolnik" (Red Triangle), Lazarev became interested in developing generalizations about the toxicity of simple nonelectrolytes based upon his work on petroleum hydrocarbons (Lazarev, 1931). He continued this work at the Leningrad Institute of Industrial Hygiene and Worker Safety, where in 1932 he began serving as head of the toxicology laboratory. He and his co-workers studied the toxicity of over 200 industrial chemicals at this Institute from 1941. During these studies, they also discovered systematic relationships between the chemicals' potency and certain physicochemical properties. These relationships were documented in a number of books (Lazarev and Astakhantsev, 1933; Lazarev, 1938, 1940, 1941, 1944, and 1958).

LAZAREV'S DATABASE

In an effort to investigate the interrelationship of various physicochemical properties such as water solubility, partition coefficient, boiling point, and molar refractivity to one another, Lazarev assembled an extensive set of data from the literature for his analyses. In his 1944 monograph (Lazarev, 1944) *Nonelectrolytes*, subtitled *Quantitative Classification of Biological, Physical, and Chemical Properties* (Fig. 2), he used these data to develop a systematic approach for the classification of nonelectrolytes.

GROUP NUMBER

Lazarev confirmed the earlier discovery made independently by Meyer (Lipnick, 1989a) and Overton (Lipnick, 1986; Overton, 1991) that the partition coefficient between water and an immiscible organic phase provides the most useful property for describing the biological activity of simple nonelectrolytes. For the sake of simplicity, Lazarev proposed the use of a classification system involving nine groups. He chose the olive oil/water partition coefficient as the basis for his physicochemical classification of nonelectrolytes. Compounds belonging to group 1 (the most hydrophilic compounds), were termed *weak nonelectrolytes*. This group includes compounds for which log $K_{oil/water}$ falls within the range of -3 to -2. In Lazarev's group number system, the most hydrophobic compounds were classified as group 9. These were termed *strong nonelectrolytes* and have log $K_{oil/water}$ values within the range 5 to 6.

Fig. 3 shows a plot of the relationship between Lazarev's Group Number and chain length for nine different homologous series of compounds. Lazarev found this type of systematic behavior very useful for predicting the Group Number for compounds for which measured values were not available, and which is analogous to the π- and fragment constant systems for estimating partition coefficient from chemical structure (Hansch and Leo, 1979; Rekker, 1977; Rekker and Mannhold, 1992). Once he was able to establish relationships between Group Number and biological

МЕДИКО-САНИТАРНОЕ УПРАВЛЕНИЕ ВМФ

ВОЕННО-МОРСКАЯ МЕДИЦИНСКАЯ АКАДЕМИЯ

Полковник мед. службы
проф. Н. В. ЛАЗАРЕВ

НЕЭЛЕКТРОЛИТЫ

ОПЫТ БИОЛОГО-ФИЗИКО-ХИМИЧЕСКОЙ ИХ СИСТЕМАТИКИ

ИЗДАНИЕ
ВОЕННО-МОРСКОЙ МЕДИЦИНСКОЙ АКАДЕМИИ
ЛЕНИНГРАД 1944

Fig. 2 Title page of *Neelektrolity* (courtesy of the Library of Congress).

transport and response, this method of estimating partition coefficients became critical for the application of predictive methods in toxicology.

USE OF A LOG-LOG SCALE

Lazarev investigated relationships between partition coefficient and biological activity graphically using log-log plots. Such plots provided the means to compare data over nine orders of magnitude, and presaged the use of logarithmic transformations in the development of extrathermodynamic linear free energy relationships (Leffler and Grunwald, 1963) and quantitative structure-activity relationships (Hansch and Leo, 1979).

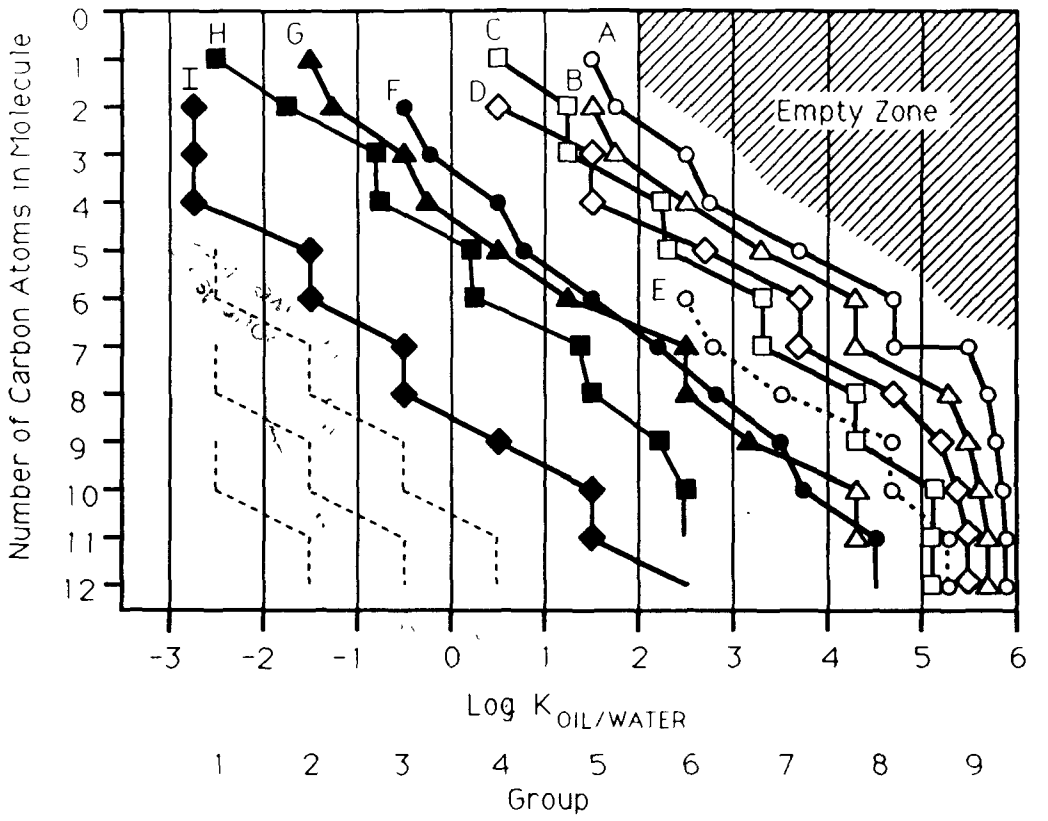

Fig. 3 Relationship between Lazarev's Group Number and chain length for nine different homologous series of compounds (adapted from Fig. 4, Lazarev, 1944).

SOLUBILITY IN WATER

A typical plot from *Neelektrolity* is shown in Fig. 4, depicting the relationship between water solubility and oil/water partition coefficient. The data have been presented as partition coefficient ranges defined by Lazarev's Group Number with the lowest and highest water solu-bilities within each of these Groups represented by open and closed circles, respectively. Fig. 5 is a similar plot, with data on compounds whose molecular weight is less than 100 Daltons. In this case, the correlation is much better[4]. In contrast, Lazarev found that the relationship between group number and log $K_{water/gas}$ to be much poorer. He interpreted this finding in terms of the fact that the product ($K_{water/gas} \cdot K_{oil/water}$) equals $K_{oil/gas}$, which changes little with increase in $K_{oil/water}$.

[4]Based upon current knowledge, the better correlation with lower molecular weight compounds appears to be due mostly to the fact that these are mostly liquids at room temperature. A better correlation between partition coefficient and water solubility of solids requires a melting point term to account for the enthalpy of fusion (Banerjee *et al.*, 1980).

CORRELATION OF GROUP NUMBER WITH TOXICITY

Fig. 6 shows a log-log plot of ocular irritation and tongue irritation versus group number. In each case there is an increase (i.e., lower concentrations of test substance are required to produce a standard toxicological response) in irritant action with increasing group number. Similarly, Fig. 7 presents a log-log plot of hypnotic dose of urea derivatives as a function of log $K_{hexane/water}$.

Lazarev was able to demonstrate similar graphical relationships between partition coefficient or water solubility for various other effects such as arrest of isolated frog heart, contraction in isolated segments of frog heart ventricle, paralysis of isolated rabbit intestine, narcosis in tadpoles and small fishes, and concentration in mammalian blood associated with changes in reflex time, narcosis, respiratory failure, or death.

EFFECT OF METABOLISM ON ELIMINATION

Lazarev recognized that an important function of metabolism was to increase the rate of elimination of foreign substances by decreasing their lipophilicity, as

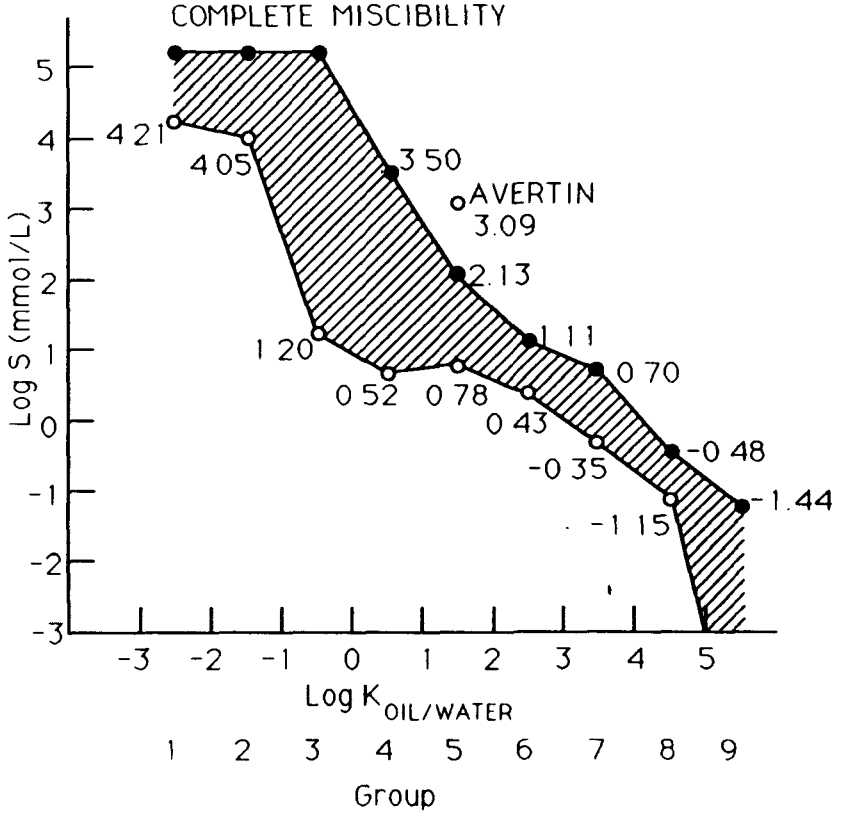

Fig. 4 Relationship between water solubility and oil/water partition coefficient (adapted from Fig. 10, Lazarev, 1944).

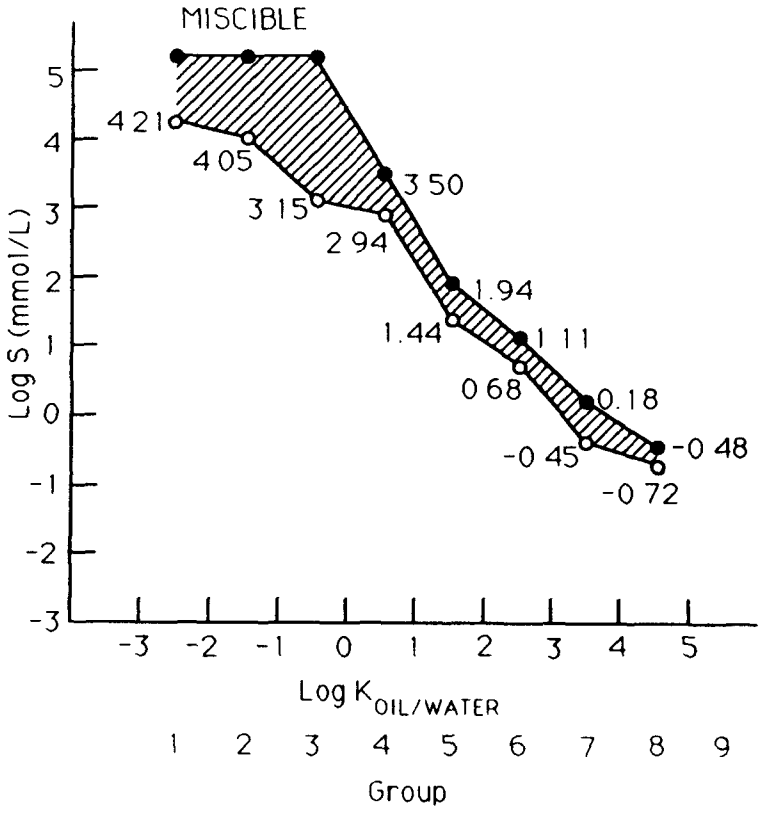

Fig. 5 Relationship between water solubility and oil/water partition coefficient for compounds with molecular weight less than 100 Daltons (adapted from Fig. 12, Lazarev, 1944).

illustrated in Fig. 8. Thus, benzene upon its biotransformation to phenol and then to hydroquinone changes from group 6 to group 2. Similarly, metabolism of ethylene glycol to oxalic acid produces a change from group 2 to group 1.

MATHEMATICAL PREDICTIVE MODELS

From his data base and log-log plots, Lazarev derived mathematical models relating partition coefficient to physicochemical properties, *in vitro* effects, and *in vivo* effects.

Physicochemical properties

$\log K_{benzene/water} = 1.05 \log K_{oil/water} + 0.32$

$\log S = -0.89 \log K_{oil/water} + 3.15$, where S = Solubility in water (mmol/L).

***In Vitro* effects**

$\log C_{hemolysis} = -0.62 \log K_{oil/water} + 2.45$, where C = Effect concentration (mmol/L).

***In Vivo* effects**

$\log C_{tadpole\ narcosis} = -0.69 \log K_{oil/water} + 1.06$, where C = Effect concentration (mmol/L).

$\log C_{tadpole\ narcosis} = 0.72 \log S - 1.04$, where C = Effect concentration (mmol/L).

$\log C_{ocular\ irritation} = 0.72 \log S - 0.60$, where C = Effect concentration (mmol/L).

Although these models were apparently not derived using regression analysis, they serve the same function as that served today by certain statistically-derived QSAR models in predictive toxicology (Lipnick 1990).

WATER SOLUBILITY CUTOFF

Lazarev, like Overton, recognized that toxicity could be limited by water solubility. Lazarev appears to be the first to have represented this phenomenon graphically, and to have predicted the cutoff point by solving the two log-log

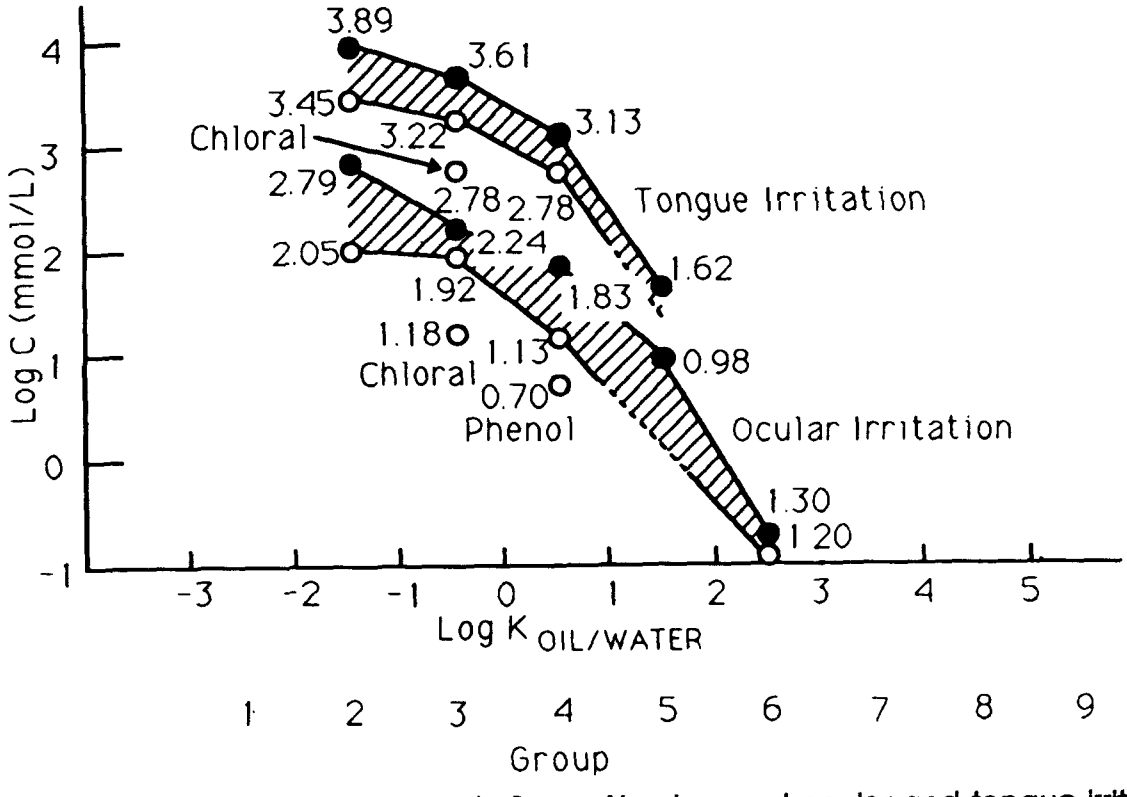

Fig. 6 Relationship between Lazarev's Group Number and ocular and tongue irritation (adapted from Fig. 57, Lazarev, 1944).

functions of partition coefficient.[5] A graph showing this cutoff for *in vitro* hemolysis is depicted in Fig. 9.

USE OF THE WATER SOLUBILITY CUTOFF IN EPA ENVIRONMENTAL ASSESSMENTS

The solubility-based cutoff in toxicity discussed above has been applied in a practical way in defining an appropriate set of tests for a chemical under review. For example, for simple nonelectrolytes acting by a non-specific mechanism such as the ones Lazarev used in his models, the concentration C (moles/L) producing narcosis in the tadpole *Rana temporaria* can be described by the following QSAR model (Lipnick, 1989b), expressed as the Hansch convention (reciprocal of biological activity):

$$\log (1/C) = 0.909 \log P + 0.727, \text{ where } P = \text{octanol/water partition coefficient.}$$

As an example of the use of this relationship and the solubility cutoff criteria discussed previously, experimental toxicity tests show that phenanthrene produces tadpole narcosis at 2 mg/L, which compares well with the QSAR prediction of 2.78 mg/L. Thus, for phenanthrene, the toxicity effect occurs close to, but does not exceed, the water solubility (Lipnick, 1989b):

Log P (octanol/water)[6] = 4.49
Melting point = 97.5 to 98.5°C
Water solubility = 3.3 mg/L
Molecular weight = 178.22

Similarly, Lazarev's oil-water correlation predicts effects at 1.5 mg/L; his solubility correlation at 0.9 mg/L. Therefore, no solubility cutoff for phenanthrene should have been expected.

On the other hand, anthraquinone shows no effect at saturation for the same test. This should not be surprising since with a melting point of 286° and log P (octanol/water) value of 2.72, the predicted water solubility is 0.051 mg/L, which is greatly exceeded by the predicted toxic concentration from the above QSAR of 132 mg/L.

Log P (octanol/water) = 2.72
Melting point = 286°C
Water solubility (est.) = 0.051 mg/L
Molecular weight = 208.22

Therefore, the solubility cutoff should be anticipated for this chemical.

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[5]The position of this cutoff would shift to lower group numbers as a function of increasing melting point for liquid solutes (Lipnick, 1990).

[6]Calculated using the CLOGP computer program, Version 3.3 (Leo and Weininger, 1985)

CONCLUSION

Nikolai Vasilyevich Lazarev was clearly a scientist ahead of his time. The methods that he pioneered were not widely known outside of Russia, but presaged modern approaches in drug design and the assessment of toxicological effects to man and to organisms in the environment.

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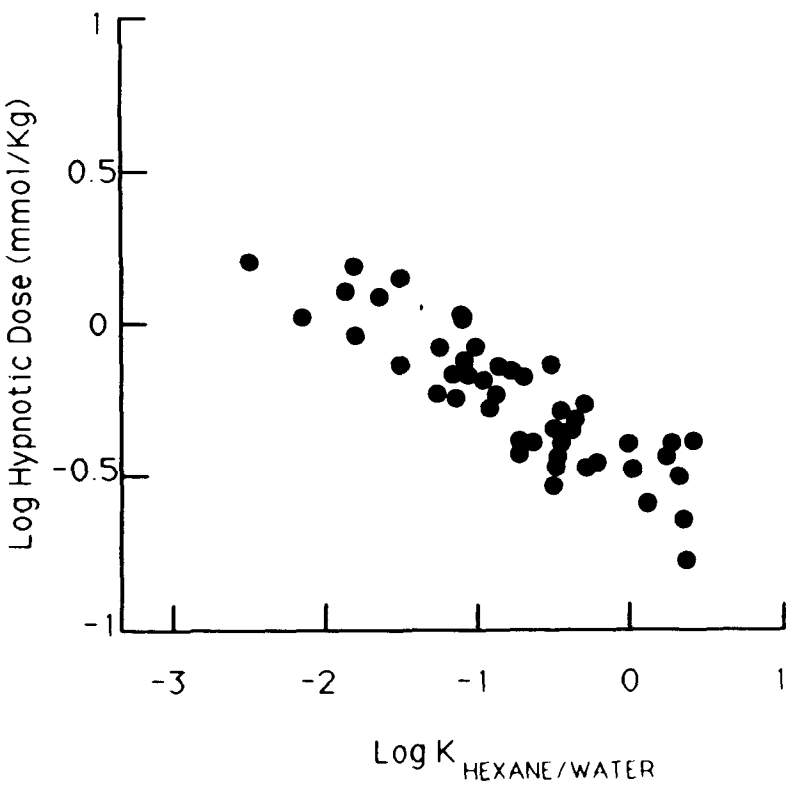

Fig. 7 Relationship between intraperitoneal hypnotic dose of urea derivatives to white mice and hexane/water partition coefficient (adapted from Fig. 74, Lazarev, 1944).

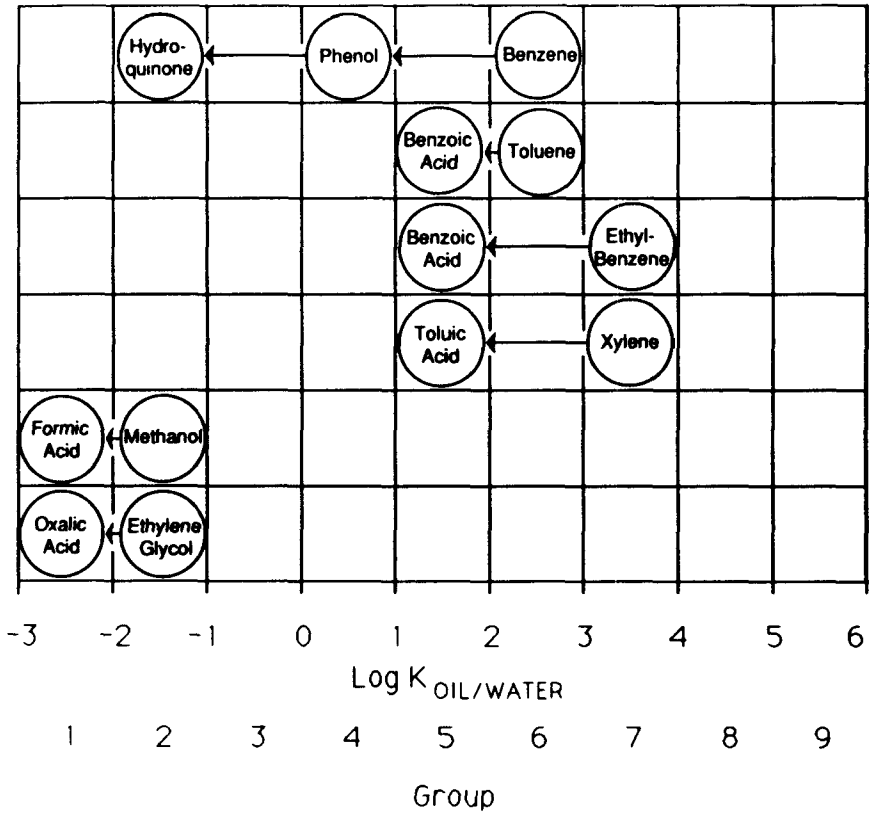

Fig. 8 Effect of metabolism on decreasing lipophilicity for benzene, toluene, ethylbenzene, xylene, methanol, and ethylene glycol (adapted from Fig. 75, Lazarev, 1944).

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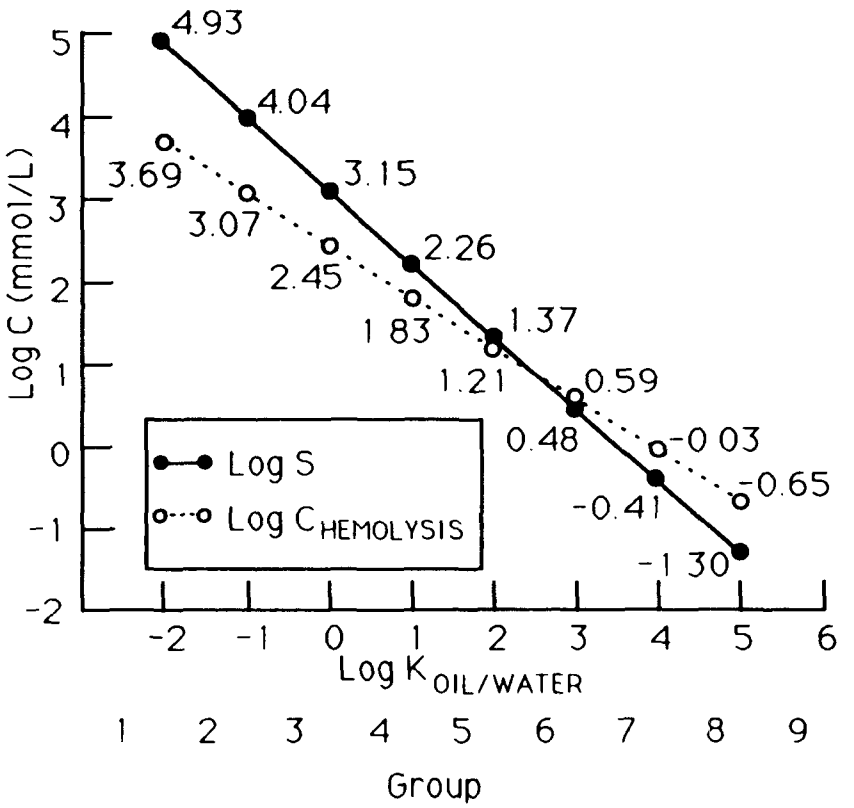

Fig. 9 Relationship between water solubility (solid circles and solid line) and *in vitro* hemolysis (open circles and dashed line) as a function of oil/water partition coefficient. The point of intersection represents the highest partition coefficient or lowest water solubility at which hemolysis is not water solubility limited (adapted from Fig. 64, Lazarev, 1944).

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MERCURY CONTENT AND ULTRASTRUCTURE OF GILLS AND SCALES OF FISH FROM LAKES IN NORTH AND NORTHWESTERN RUSSIA THAT ARE POLLUTED BY ATMOSPHERIC DEPOSITION

Terry Haines[a], Victor Komov[b], Charles Jagoe[c], and Victoria Matey[b]

ABSTRACT

Field studies were conducted of lakes in Darwin National Reserve in 1989, and in Karelia in 1991. Darwin National Reserve is 300 km north of Moscow at the west end of Rybinsk Reservoir. The terrain is generally flat and consists of thick sandy till covered with hardwood forest. Karelia is north of St. Petersburg and consists of hilly terrain underlain with precambrian bedrock. In each case, remote lakes were visited and sampled for water and fish, primarily perch *Perca fluviatilis*. Water samples were analyzed for pH, color, specific conductance, and major cations and anions. Fish were weighed and measured, dorsal muscle tissue was collected and analyzed for mercury, and gills and scales were sampled for examination by light and electron microscopy. In both locations acidic lakes (acid neutralizing capacity <0) were common. Acidic lakes were both clear and colored and the dominant anion in both types was sulfate, indicating that the lakes were acidic because of atmospheric deposition of strong acids and not because of organic acids. Mercury content of fish was increased in acidic and in colored lakes. Mercury appears to enter the lakes by atmospheric deposition, as there are no local sources. Organic acids are believed to increase mercury bioavailability in lakes by transporting mercury from terrestrial regions and possibly contributing to methylation. Also, mercury methylation is probably enhanced in acidic lakes, increasing bioavailability. Gills of perch from acidic lakes had thicker lamellar epithelia and more ion-transporting cells than those collected in circumneutral lakes. The density of microridges on gill surfaces was reduced in perch from acidic lakes. These differences in gill structure serve to decrease gill surface area, increase diffusion distances, increase active ionic influx, and represent responses that allow perch to inhabit acidic waters. Scales of perch from acidic lakes had little or none of the pattern of ridges at the foci observed in scales of perch from higher pH lakes. This probably results from development and growth in low pH, low Ca water, and may be a useful indicator of stressful acidic conditions during early life stages.

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INTRODUCTION

Atmospheric transport and deposition of contaminants is a world-wide problem. The importance of atmospheric transport in contamination of aquatic ecosystems was first documented for emission of acidic gases such as sulfur oxides and nitrogen oxides and the deposition of strong acids, which resulted in water acidification and loss of fish populations (Schindler 1988). This process is also important in the mercury contamination of fish in waters of low acid neutralizing capacity (ANC) in northern Europe and North America (Lindqvist *et al.* 1991; Spry and Wiener 1991). Further, lake acidification increases mercury concentration in fish (Grieb *et al.* 1990; Häkanson *et al.* 1990; McMurtry *et al.* 1989).

Atmospheric deposition of sulfur compounds in Europe has been estimated using a model developed by the Cooperative Programme for Monitoring and Evaluation of the Long Range Transmission of Air Pollutants in Europe (EMEP). The results of this model give a deposition rate of 800-1,640 eq S/ha per year for northern Russia, which is comparable to or exceeds deposition rates for regions in Scandinavia where lake acidification and fish mercury accumulation has occurred (Chadwick and Kuylenstierna 1991). In undertaking this study, our objectives were to confirm the previously reported presence of clear-water and brown-water acidic lakes in two areas of Russia to determine whether fish inhabiting these lakes contained elevated levels of mercury, and to determine whether these fish exhibited symptoms of damage from acidity.

METHODS

This study was conducted in two phases. In May and June 1989 we sampled lakes in Darwin National Reserve, Yaroslavl Region, and in June 1991 we sampled lakes in the Karelia Autonomous Republic (Figure 1). Darwin National Reserve is a protected natural area created in 1943, located at the western end of Rybinsk Reservoir. It is characterized by thick tills and sandy soils (Ager 1980), and the lakes are primarily precipitation-dominated seepage lakes. In Karelia we visited lakes in the vicinity of Suoyarvi, which were primarily highly colored lakes, and lakes in the vicinity of Kondopoga, which were primarily clear-water lakes. Both areas were underlain by granitic bedrock covered with thin soil (Ager 1980), and the lakes were primarily drainage lakes. Most lakes were in forested catchments with no dwellings or roads. An exception was Suoyarvi Lake, which has a settlement at the southern end.

At each lake, a water sample was collected by immersion of cleaned polyethylene bottles just below the surface near the point of maximum depth. Bottles were tightly capped, held in the dark, and within a few hours analyzed for pH (Orion model SA210 meter equipped with a Ross combination electrode), ANC (acid neutralizing capacity, measured by inflection point titration), and true color (visual comparison with platinum-cobalt standards). The remaining water sample from each lake was refrigerated and later analyzed for major cations and anions. Calcium, magnesium, and sodium were determined by flame atomic absorption spectrophotometry, chloride, fluoride, sulfate, and nitrate by ion chromatography, and total aluminum by graphite furnace atomic absorption spectrophotometry.

Perch (*Perca fluviatilis*) were collected by angling from various points around the shoreline of each lake, except for Suoyarvi Lake, where fish were captured in a gill net set overnight. The second and third gill arches were dissected from each fish and fixed in either 0.1 M cacodylate buffer, pH 7.3, containing 2.5% glutaraldehyde, or in 0.1 M HEPES buffer, pH 7.4, containing 1% glutaraldehyde, 4% formaldehyde, and 5% sucrose. Fish were then placed in plastic bags and frozen (-5°C) within a few hours, and remained frozen until they were analyzed for mercury. Fish and fixed gill tissue were transported to the Institute for Biology of Inland Waters, Borok, in 1989, and the Institute for Research on Northern Fishes, Petrozavodsk, in 1991.

At the laboratories, the fish were thawed, weighed to the nearest gram, and measured (total length) to the nearest millimeter. Scales were removed from the left side of the fish adjacent to the dorsal fin and placed in paper envelopes for future ultrastructure determination. Fish were

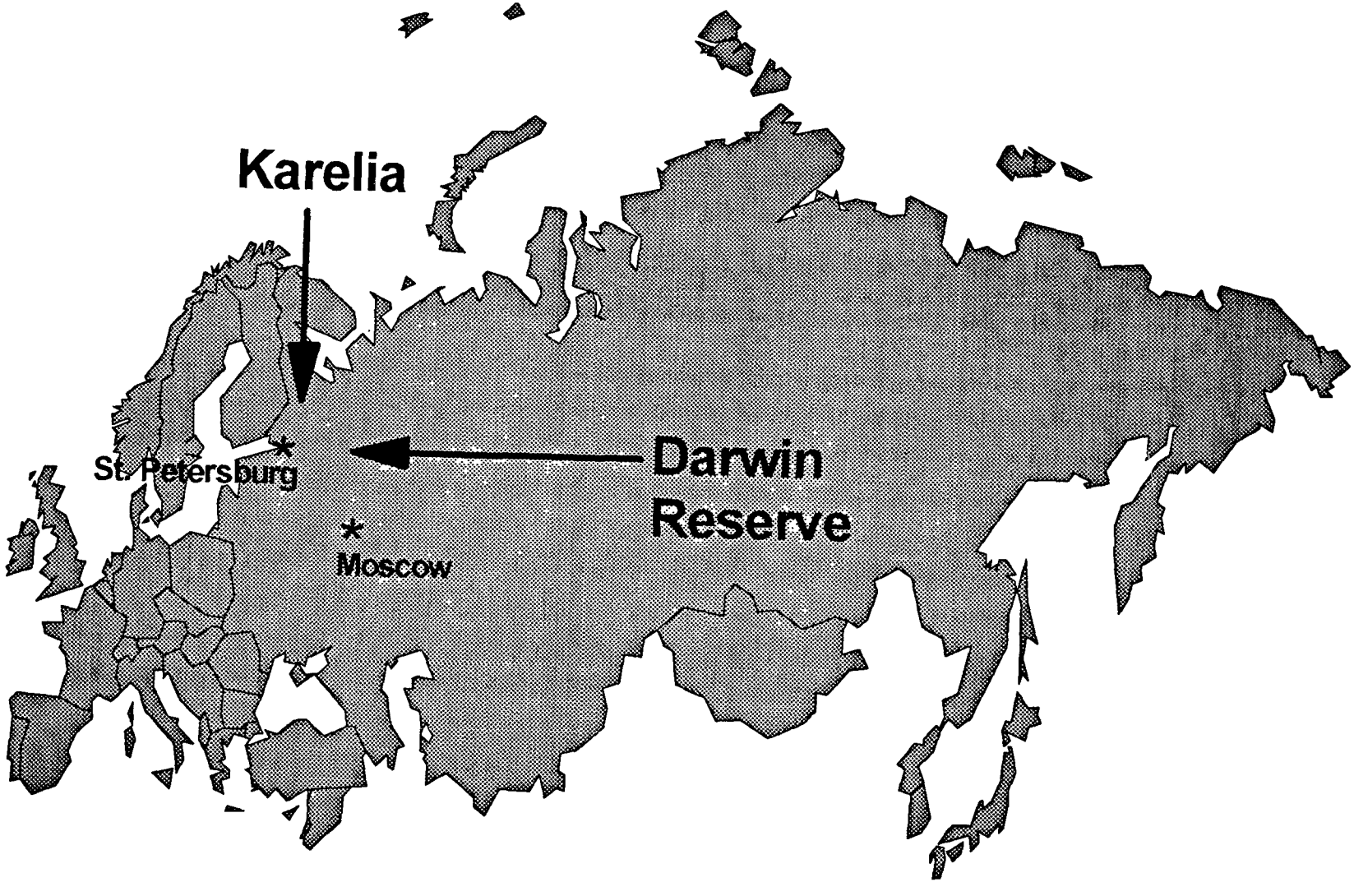

Figure 1. Map of Europe showing the location of the two study sites.

placed on an acid-washed plexiglass plate and a 2- to 3-g sample of skeletal muscle was dissected from the left side of the fish, adjacent to the dorsal fin and extending from the rib cage to the center of the dorsal surface. All dissecting instruments were stainless steel, and all instruments and glassware were cleaned with 10% nitric acid and rinsed with distilled water.

Moisture content of muscle tissue was determined by placing a portion of the tissue in a dried, tared glass dish and weighing before and after drying at 105°C. The remaining tissue, typically 1 to 2 g, was placed in a tared glass beaker, weighed, and digested in a 1:1 mixture of nitric acid and hydrogen peroxide (FAO/SIDA 1983).

Fish were processed in batches of 5 to 10. Two fish from each batch were randomly selected for use in determination of accuracy and precision of mercury determination. For these fish, the tissue was divided into two nearly equal portions. A known amount of mercury standard solution was added to one portion of tissue, and all portions were digested and analyzed. A sample of National Institute of Science and Technology (NIST) tuna reference material and a reagent blank was included with each batch of samples. After digestion, solutions were cooled and diluted to 25 ml with distilled water, and 1- to 2-ml portions were analyzed for total mercury with a gold film analyzer (Arizona Instrument Co., Jerome, Arizona USA). Each digestate was analyzed in duplicate. Mercury in all samples exceeded our calculated detection limit of 5 ng. The recovery of mercury from spiked samples averaged 95.4% (range 90-102%). The percent difference between replicate samples averaged 2.9% (range 1.7-5.2%). The measured mercury content of NIST tuna averaged 0.93 μg/g (range 0.88-0.96 μg/g), and all analyses were within the certified range for this material.

Gill tissue was postfixed in buffered OsO_4 for two hours and dehydrated through a graded ethanol series into absolute acetone. Whole gill arches were critical point dried under liquid CO_2, sputter-coated with gold, and examined using a JEOL JSM-25S electron microscope operated at 15 KV. Scales were first placed in glass vials with 5% NaOH for 6-12 h, then hydrolyzed in a saturated solution of $K_2Cr_2O_7$ in 10% NaOH for 1-7 d. The scales were then washed in distilled water until all yellow color was removed, placed in 3:1 mixture of absolute ethanol and acetone and then in absolute acetone for 10-15 min each, air dried, mounted on stubs, and coated with gold and examined in the same manner as were gills.

RESULTS

The lakes surveyed varied in pH from 4.6 to 8.1 and in color from 3 to 188 Hazen (Figure 2, Table 1). Drainage and seepage lakes were relatively uniformly distributed over the range of color values, but all seepage lakes surveyed were acidic (pH $\leq$5.0). The lakes in Darwin Reserve were predominately seepage lakes whereas the lakes in Karelia were predominately drainage lakes.

The fish collected ranged in weight from 8 to 515 g, and in mercury content from 0.06 to 3.04 μg/g wet weight (0.76-17.9 μg/g dry weight) (Table 2). Omitting two unusually large fish from Tyomnoye Lake reduces the maximum weight to 196 g and the maximum mercury content to 1.03 μg/g wet weight (6.87 μg/g dry weight). Fish mercury content was correlated with fish size, especially weight (R^2=0.32, p=0.0001); therefore the least square mean mercury concentration, which represents fish mercury concentration normalized by fish weight, was computed by analysis of covariance using weight as the covariate.

Plotting fish mercury concentration against lake pH and color (Figure 3) indicates that fish from high pH lakes have relatively low mercury content regardless of lake color but in low pH lakes fish mercury content increases with lake color. Accordingly, stepwise regression analysis was performed using the non-intercorrelated variables pH, color, and sulfate, with the data stratified by lake drainage type. The results (Table 3) indicate that color is more important in the regression than is pH for seepage lakes, and that the relation between fish mercury and lake pH is not significant in the absence of color data for drainage lakes. The inclusion of lake sulfate did not significantly improve the regression for seepage lakes.

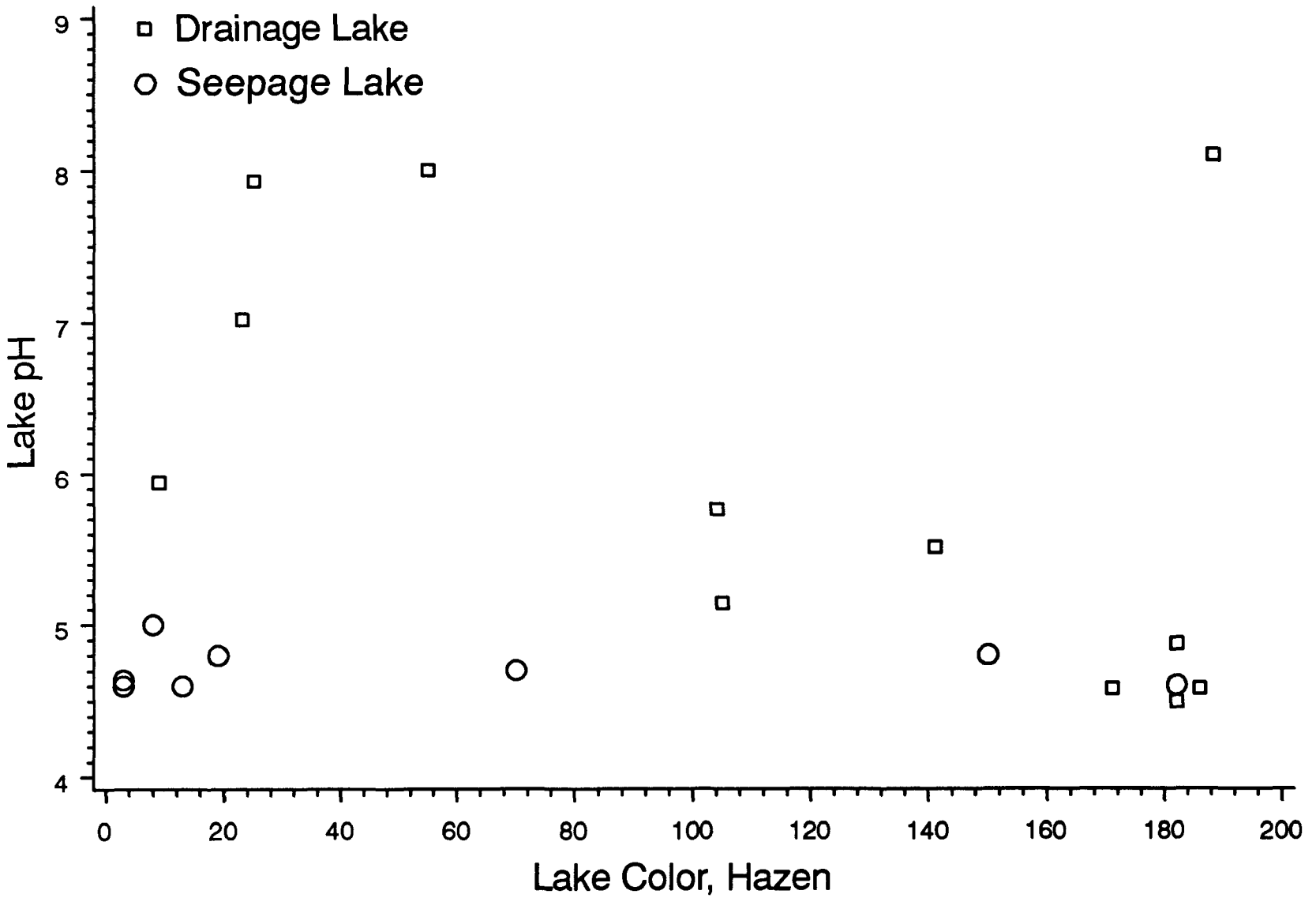

Figure 2. Plot of lake pH versus lake color for all lakes, by drainage type.

TABLE 1. PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE STUDY LAKES.

Lakes	Area (ha)	Depth (m)	Drainage Type	Color (Hazen)	pH	ANC (μeq/l)	SO_4 (μeq/l)
Darwin Lakes							
Dorojiv	200	3	Seepage	13	4.6	-41	71
Dubrovskoye	20	2	Seepage	182	4.6	-50	64
Hotavets	160	3	Drainage	188	8.1	227	32
Motykino	2	4	Seepage	19	4.8	-38	49
Rybinsk	395,000	30	Impoundment	55	8.0	1,369	420
Tyomnoye	20	2	Seepage	70	4.7	-53	52
Uteshkovo	5	3	Seepage	150	4.8	-33	29
Karelia Lakes							
Blue Lamba	307	4	Seepage	3	4.6	-17	67
Chuchyarvi	112	5	Seepage	8	5.0	-9	48
Grushna Lamba	3	5	Seepage	3	4.6	-19	48
Ilyakalkenyarvi	104	6	Drainage	171	4.6	-25	54
Kabozero	210	3	Drainage	141	5.5	13	60
Lamba Vegarous	7	4	Drainage	182	4.5	-39	75
Leukunyarvi	-	-	Drainage	182	4.9	-9	68
Sargozero	200	4	Drainage	25	7.9	252	29
Suoyarvi	6,070	5	Drainage	104	5.8	23	83
Uros	426	3	Drainage	9	5.9	16	72
Vegarousyarvi	1,880	5	Drainage	105	5.1	-2	49
Venderskoye	998	6	Drainage	23	7.0	167	44
Vuontelenyarvi	394	3	Drainage	186	4.6	-24	68
Mean Values							
Drainage Lakes[a]	1,045	4.2	Drainage	120	5.8	56	58
Seepage Lakes	84	3.5	Seepage	56	4.7	-33	53

[a]Rybinsk omitted.

TABLE 2. MEAN AND RANGE OF FISH WEIGHT, MOISTURE CONTENT, AND MERCURY CONCENTRATION IN FISH, AND LEAST SQUARE MEAN MERCURY CONCENTRATION IN FISH FROM THE STUDY LAKES.

			Mercury, μg/g wet weight	
Lake	Weight, g Mean (Range)	Moisture, % Mean (Range)	Arithmetic Mean (Range)	Least Square Mean
Blue Lamba	22 (18-28)	85.6 (81.1-89.7)	0.34 (0.26-0.44)	0.41
Chuchyarvi	59 (18-133)	90.6 (83.2-93.9)	0.10 (0.08-0.13)	0.08
Dorojiv	62 (31-94)	80.9 (78.0-86.2)	0.50 (0.36-0.71)	0.47
Dubrovskoye	27 (23-30)	83.6 (77.8-88.1)	0.64 (0.56-0.68)	0.71
Grushna Lamba	38 (28-48)	81.5 (76.9-86.0)	0.30 (0.20-0.43)	0.33
Hotavets	62 (37-150)	88.6 (80.4-93.8)	0.11 (0.08-0.16)	0.09
Ilyakalkenyarvi	35 (20-78)	82.4 (72.0-94.2)	0.28 (0.19-0.36)	0.31
Kabozero	35 (24-47)	81.5 (61.7-87.2)	0.31 (0.22-0.39)	0.34
Lamba Vegarous	55 (28-80)	80.5 (75.9-85.1)	0.40 (0.30-0.52)	0.39
Leukunyarvi	30 (8-54)	88.2 (82.8-96.3)	0.21 (0.17-0.24)	0.25
Motykino	60 (43-75)	81.4 (79.3-87.9)	0.57 (0.43-0.97)	0.54
Rybinsk	86 (59-163)	80.8 (78.1-82.6)	0.19 (0.08-0.45)	0.11
Sargozero	18 (8-27)	85.2 (75.0-89.6)	0.12 (0.09-0.18)	0.19
Suoyarvi	98 (53-131)	81.5 (79.6-83.6)	0.29 (0.19-0.38)	0.18
Tyomnoye	155 (50-515)	79.6 (74.6-83.4)	1.06 (0.45-3.04)	0.56

Uros	19 (14-21)	83.7 (76.1-90.3)	0.12 (0.06-0.18)	0.19
Uteshkovo	45 (18-98)	80.4 (75.7-85.8)	0.78 (0.54-0.97)	0.80
Vegarousyarvi	26 (17-45)	79.5 (76.9-82.8)	0.34 (0.20-0.44)	0.41
Venderskoye	41 (12-107)	79.8 (72.9-81.0)	0.15 (0.10-0.24)	0.17
Vuontelenyarvi	43 (16-196)	84.5 (76.9-93.0)	0.53 (0.32-1.03)	0.54

TABLE 3. STATISTICALLY SIGNIFICANT ($P \leq 0.05$) REGRESSION EQUATIONS, CORRELATION COEFFICIENTS, AND PROBABILITY FOR STEPWISE REGRESSION OF LAKE PHYSICAL AND CHEMICAL VARIABLES ON FISH MERCURY CONTENT. ANALYSES ARE PRESENTED FOR ALL LAKES, AND STRATIFIED BY DRAINAGE TYPE.

Lake Type	Number of Variables	Equation	R^2	P
All	1	-0.0347 pH + 0.317	0.46	0.001
	2	-0.0336 pH + 0.000144 color + 0.298	0.49	0.0035
	3	-0.0331 pH + 0.000143 color -0.000019 sulfate + 0.297	0.50	0.0119
Seepage	1	0.000711 color + 0.128	0.56	0.0332
	2	0.000671 color - 0.173 pH + 0.946	0.69	0.05
Drainage	2	-0.0260 pH + 0.000030 color + 0.252	0.68	0.0058
	3	-0.0256 pH + 0.0000284 color -0.0000167 sulfate + 0.251	0.68	0.0216

The structure of the gills of perch has been previously described by Matey (1984). Primary lamellae or filaments project in two parallel rows from the gill arch, and each primary lamella supports two rows of secondary lamellae (Figure 4a). The epithelium covering the gill consists primarily of thin, squamous respiratory cells, along with mucous cells and chloride (ion transporting) cells, underlain by undifferentiated cells. Most chloride cells occur in the primary lamellar epithelium, especially in the spaces between secondary lamellae. Normally, the surfaces

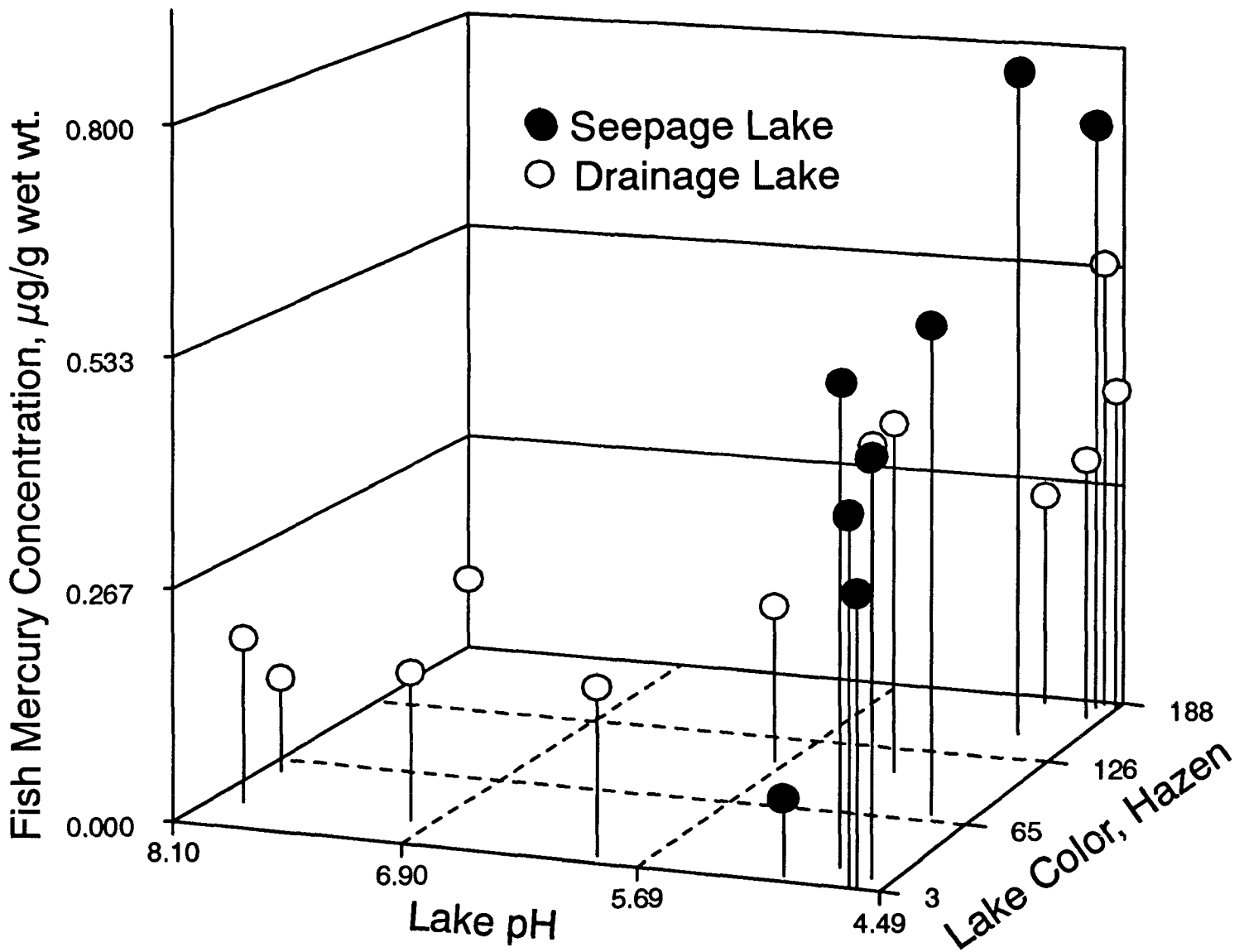

Figure 3. Three-dimensional plot of least square mean fish mercury content versus lake pH and color, by lake drainage type.

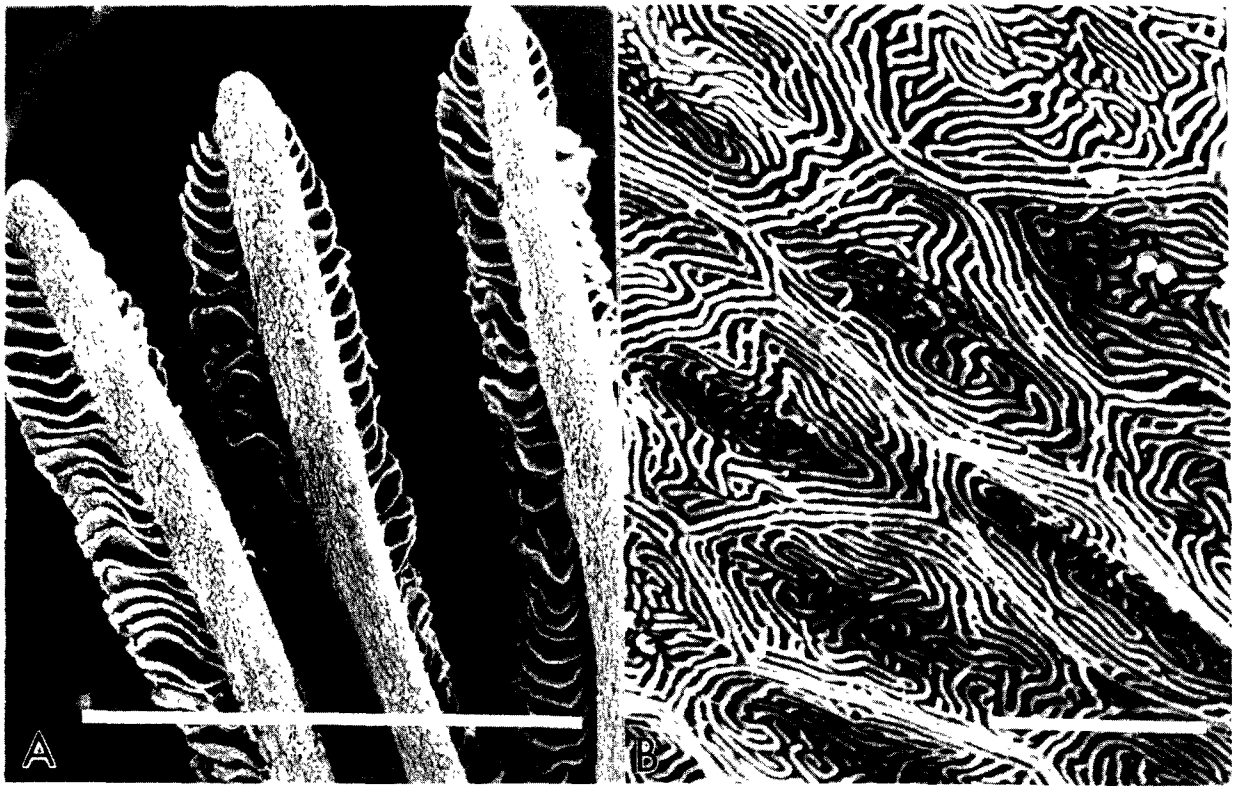

Figure 4. Scanning electron micrographs of normal gill structure. A. Gill filaments with secondary lamellae. Bar= 1 mm. B. Epithelial surface. Bar= 10 μm.

of the respiratory cells are covered by a well-developed system of microridges, which may serve to anchor mucus, increase surface area, or create micro-turbulence (Figure 4b). This structural arrangement is found in most teleost fishes (Hughes 1984; Laurent 1984).

For the Darwin Reserve lakes, all fish examined from Hotavets Lake (pH 8.1) had normal, well-formed gills. The primary lamellar epithelia contained pavement cells with well-formed microridges and few chloride cells (Figure 5a), and secondary lamellae were thin and regular (Figure 5b). Perch from the more acidic lakes (Dubrovskoye, Dorojiv, Motykino, and Uteshkovo lakes) all had alterations in gill structure. Chloride cell numbers were greatly increased along the primary lamellae (Figure 5c). Some chloride cells had apical crypts (Figure 5d), which were not observed in fish from the higher pH lake. Chloride cells were also present on many secondary lamellae (Figure 5e). Mucus secretion was elevated, as evidenced by increased numbers of mucus droplets and secretory pores associated with mucous cell activity (Figure 5f). The density of microridges on the gill epithelia was reduced in fish from the acid lakes compared with fish from the higher pH lake (Figure 5g; compare with Figure 4b).

For the highly colored lakes in Karelia, fish from lakes of pH <5.5 had thickened and swollen secondary lamellae (Figure 6a). This phenomenon was especially severe in fish from Leukunyarve, Vegarousyarvi, and Lamba near Vegarous lakes, where epithelial hyperplasia produced regions of fused secondary lamellae (Figure 6b). These fish also often had locally swollen or evaginated regions on the primary lamellae (Figure 6c), although these abnormalities were not observed in fish from Ilyakalkenyarvi Lake, which had a pH of 4.6. Chloride cell number on primary lamellae appeared to increase with decreasing pH, and was greatly elevated in fish from Vuontelenyarvi, Ilyakalkenyarvi, and Lamba near Vegarous lakes (Figure 6d). In perch from lakes with pH below 5.1, chloride cells were also abundant on secondary lamellar epithelia (Figure 6e).

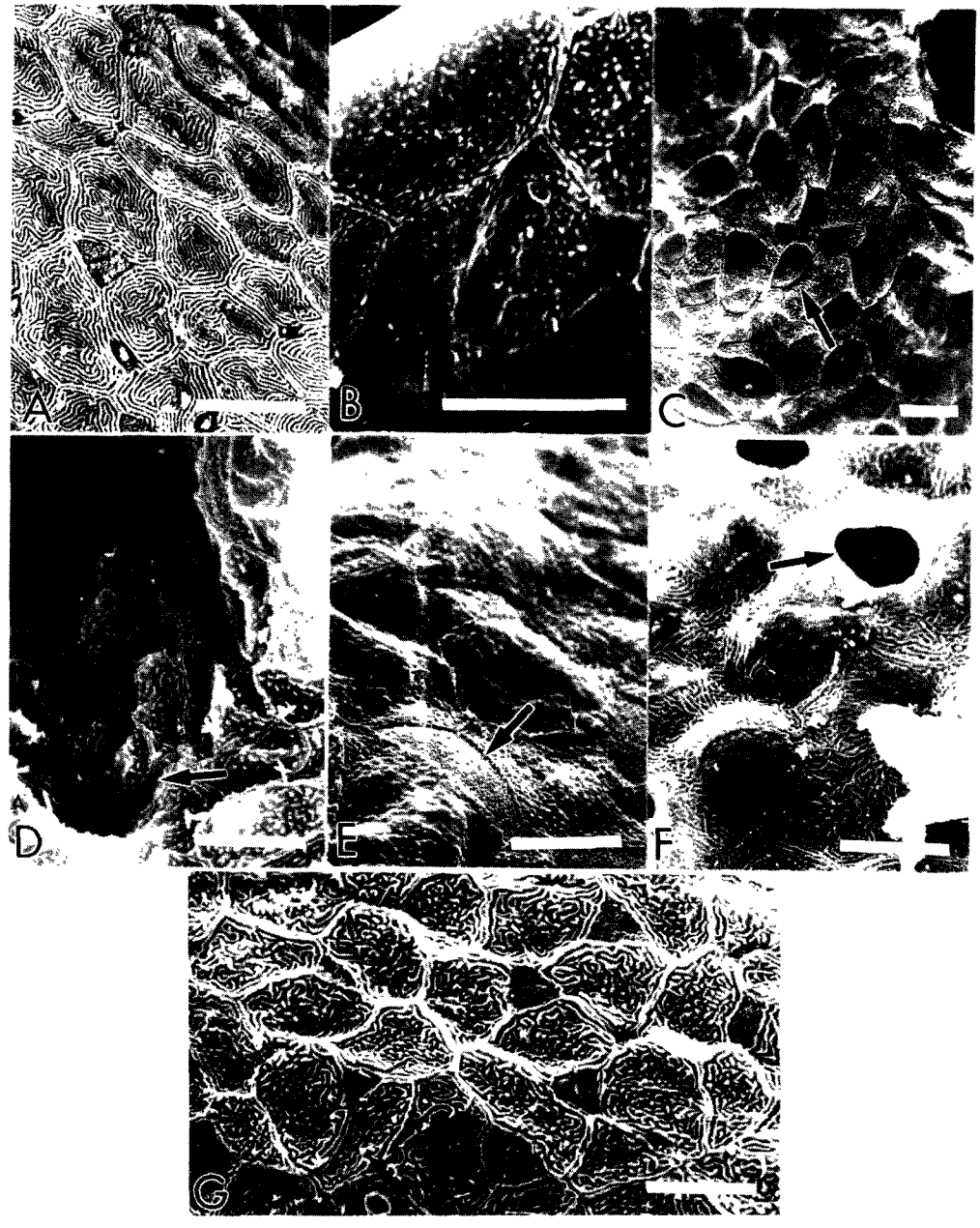

Figure 5. Scanning electron micrographs of gills from Darwin Reserve fish. Bar=10 μm in all cases. A. Primary lamellar epithelium of a fish from a high pH lake, showing respiratory pavement cells and a lack of chloride cells. B. Secondary lamellar epithelium of a fish from the same lake as A. C. Primary lamellar epithelium of a fish from an acidic lake showing numerous chloride cells (arrow). D. Chloride cell with an apical crypt (arrow). E. Secondary lamellar epithelium of a fish from an acidic lake showing numerous chloride cells (arow). F. Secondary lamellar epithelium of a fish from an acidic lake showing numerous secretory pores (arrow). G. Primary lamellar epithelium of a fish from an acidic lake showing reduced microridge density.

Figure 6. Scanning electron micrographs of gills of fish from colored, acidic Karelian lakes. A. Swollen and thickened secondary lamellae (arrow). Bar=100 μm. B. Epithelial hyperplasia (arrow). Bar=1 mm. C. Primary lamelar epithelial swelling (arrow). Bar=100 μm. D. Primary lamellar epithelium with numerous chloride cells (arrow). Bar=100 μm. E. Secondary lamellar epithelium with numerous chloride cells (arrow). Bar=10 μ.

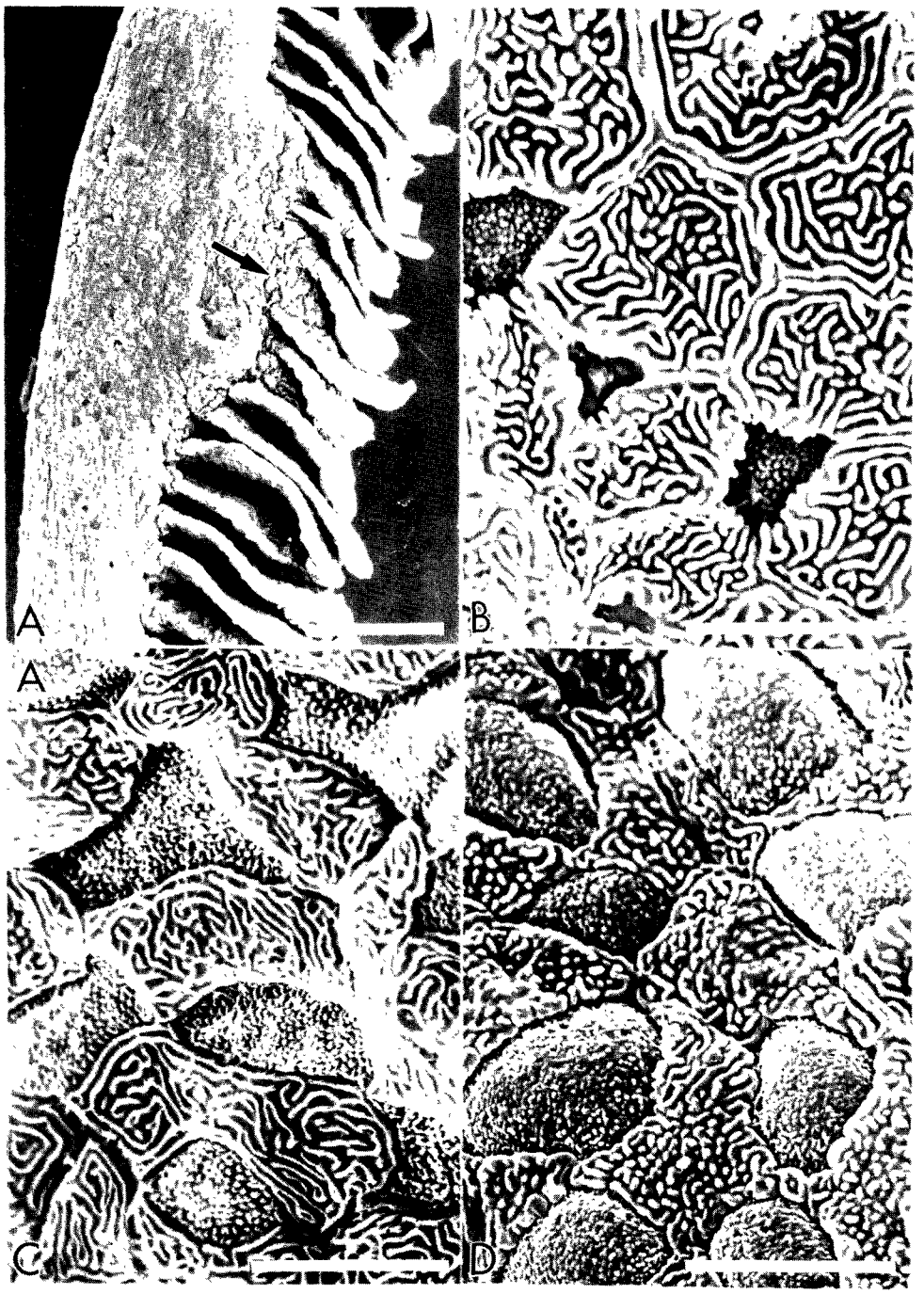

Figure 7. Scanning electron micrographs of gills of fish from Karelian lakes. A. Epithelial hyperplasia leading to secondary lamellar fusion (arrow) in a fish from a colored, acidic lake. Bar=100 μm. B. Cell surface microridge density in a fish from a high pH lake. Bar=10 μm. C. Cell surface microridge density in a fish from a low pH, clear lake. Bar=10 μm. D. Cell surface microridge density in a fish from a low pH, colored lake. Bar=10 μ.

Alterations in gill structure associated with low pH appeared less severe in fish from clear-water lakes in Karelia than in those from colored-water lakes. Epithelial hyperplasia leading to secondary lamellar fusions was observed only in fish from Chuchyarvi, pH 5.0 (Figure 7a). Secondary lamellae were somewhat swollen and thickened in fish from the lower pH clear-water lakes. Chloride cell numbers were increased on both the primary and secondary lamellae in fish from lakes with pH <5.9. Compared with perch from lakes of higher pH (Figure 7b), cell surface microridge density was slightly decreased in fish from the most acidic clear-water lakes (Figure 7c). In contrast, fish from low pH, colored-water lakes had substantially reduced microridge patterns, with the typical labyrinth appearance replaced by shorter, smaller surface ridges (Figure 7d).

Perch have typical ctenoid scales (Figure 8a). The scales of perch from Hotavets (pH 8.1) were characterized by a focus consisting of a number of microridges creating a complex pattern (Figure 8b). These microridges were lacking in the scales of the fish from lakes in Darwin Reserve with pH 4.4-4.8 (Figures 8c, 8d).

DISCUSSION

The areas of Russia where we surveyed lakes receive relatively high levels of sulfur deposition. Karelia receives 420-800 eq/ha/yr, comparable to southern Finland, Sweden, and Norway, and Yaroslavl region receives 800-1640 eq/ha/yr, comparable to northern Germany (Chadwick and Kuylenstierna 1991). The critical loading of acidity is estimated at 200 eq/ha/yr or less for Karelia and 200-500 eq/ha/yr for Yaroslavl region, and deposition is estimated to exceed critical loading by 500-1000 eq/ha per year in Karelia, but only 200 eq/ha per year or less in Yaroslavl region (Hettelingh *et al.* 1991). Consequently, we found no acidic drainage lakes in Darwin Reserve. The seepage lakes were precipitation-dominated as reflected in the low ion content, and were not influenced by the chemistry of the soils or surficial material. The acidity of the seepage lakes in Darwin Reserve reflects the acidity of the precipitation they receive. In Karelia, both drainage and seepage lakes were acidic. The situation here is similar to that in Finland, Sweden, and southern Norway, where acidic precipitation falling on a landscape consisting of resistant bedrock and thin, nutrient-poor soils has resulted in the acidification of thousands of lakes, with resultant biological effects (Rosseland *et al.* 1986).

The mercury content of perch from undisturbed lakes in Russia is comparable to that reported for this species from similar lakes in Sweden and Finland, disregarding the two large fish from Tyomnoye lake, which had unusually high mercury concentrations. Mercury concentrations were reported to be 0.2 to 2.0 μg/g dry weight for "small" perch and 0.6 to 6.0 μg/g dry weight for "large" perch in Swedish forest lakes (Lindqvist *et al.* 1991). Mercury content of perch muscle tissue in 11 Swedish lakes of pH 5.2-6.2 was between 0.04 and 0.29 μg/g wet weight (Paulsson and Lundbergh 1991). In Finland, mercury concentrations between 0.03-0.53 μg/g in perch from circumneutral lakes and 0.15-0.63 in fish from acidic lakes have been noted (Verta *et al.* 1990). The larger fish from the most acidic lakes in our study approached or exceeded mercury concentrations believed harmful to fish consumers (Wiener 1987; Grant 1991).

The association of elevated mercury content in fish with low water pH has been widely reported (Lindqvist *et al.* 1991; Spry and Wiener 1991). Our results confirm these findings and extend them to two regions of Russia not previously investigated. However, the phenomenon is much more complex than a simple water acidity-fish mercury relation. An extensive study of mercury accumulation by fish in Swedish forest lakes led to the conclusion that concentrations were determined by the bioavailability of mercury to lower trophic levels, and that lake humicity, productivity, and acidity controlled bioavailability (Lindqvist *et al.* 1991). Our results suggest that acidity and humicity are important factors in affecting fish mercury content in Russian lakes (we did not investigate productivity). We found low concentrations of mercury in fish from high pH lakes regardless of color, which suggests that acidity is a necessary but not sufficient factor in mercury accumulation by fish in these lakes.

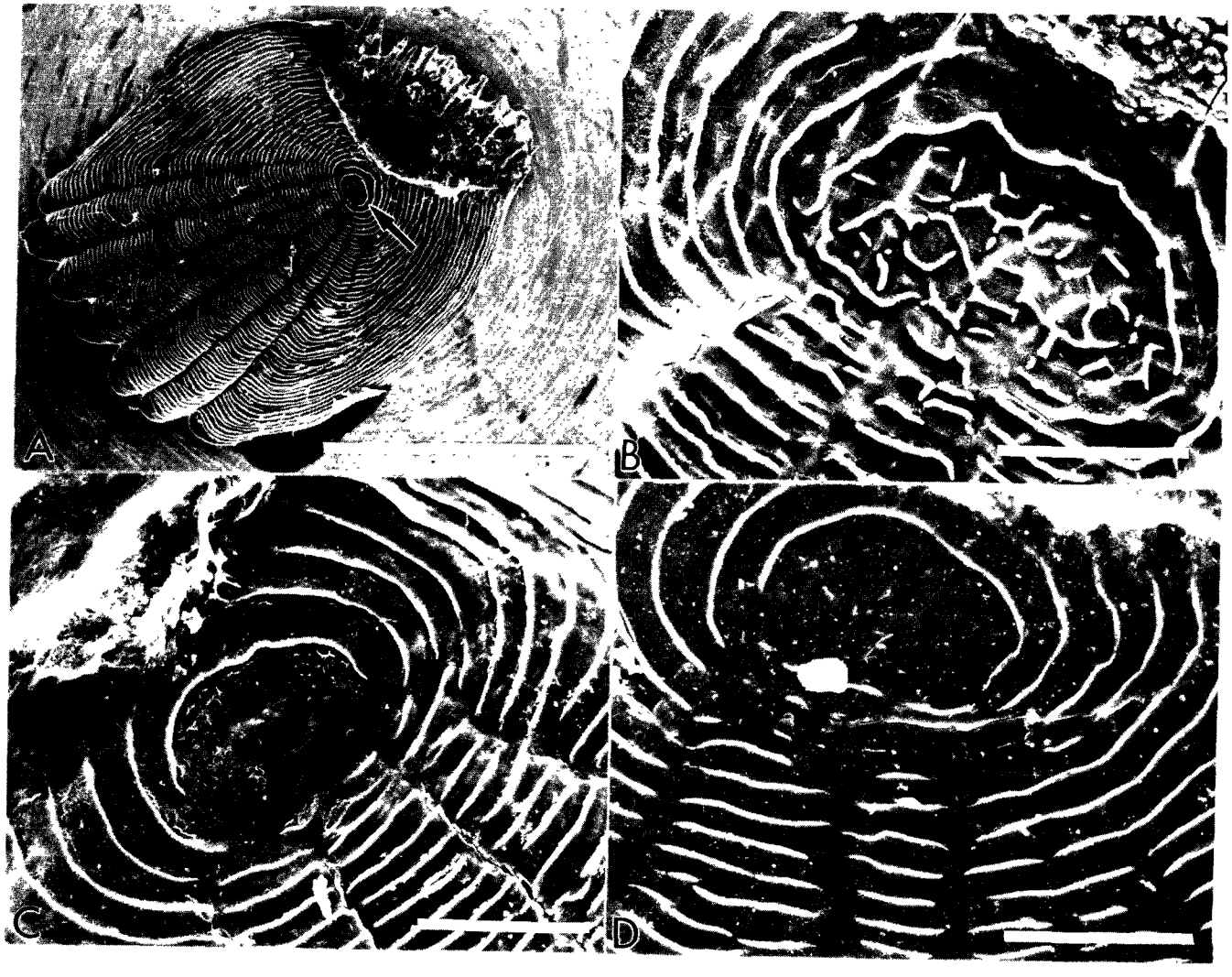

Figure 8. Scanning electron micrographs of scales from Darwin Reserve fish. A. View of entire scale. Bar=1 mm. B. Focus of scale from Hotavets Lake (pH8.1) fish showing normal ridge pattern. Bar=100 μm. C. Focus of scale from Motykino Lake (pH 4.8) fish showing lack of normal microridges. Bar=100 μm. D. Focus of scale from Tyomnoye Lake (pH 4.4) fish showing lack of normal microridges. Bar=100 μm.

Humic matter was found to control the solubility and watershed export of mercury deposited in precipitation in Sweden and Canada (Iverfeldt and Johansson 1988; Mierle and Ingram 1991). Further, water concentrations of mercury were highly correlated with water color (Meili *et al.* 1991). Most of the mercury in clearwater lakes was deposited in the sediment whereas most of the mercury in humic lakes was retained in the water column (Meili 1991). Thus humic matter may affect fish mercury content by affecting the mercury loading to a lake and by retaining mercury in the water column where it is available for uptake in biota.

Alterations in gill morphology have been reported in a number of fish species in response to water acidification (Jagoe and Haines 1983; Matey 1984; Evans *et al.* 1988; Tietge *et al.* 1988). Most studies have examined effects after laboratory exposure; relatively few studies have been conducted on wild fish from chronically acidic environments. Chevalier *et al.* (1985) reported gill abnormalities associated with acidification in brook trout (*Salvelinus fontinalis*) collected from acidic lakes, and Leino *et al.* (1987) found changes in the gill epithelium of two species of cyprinids collected from an experimentally acidified lake. Our results expand these observations to perch inhabiting acidified environments in Russia, and allow comparisons from colored and clear acidic waters.

Exposure to low pH water causes osmoregulatory and ionoregulatory disturbances in fish (McDonald 1983), primarily because of increased passive efflux of sodium. The changes in gill morphology we observed may represent adaptation of acclimation to minimize this effect. The thickening of the epithelium would lengthen the diffusion pathway by which ion losses occur, and increasing numbers of chloride cells would allow increased active uptake of ions from the environment. Perch are known to be relatively acid tolerant and are frequently the only species of fish inhabiting acidic lakes (Rask and Tuunainen 1990), including the ones we studied. Perhaps these changes in gill morphology allow this species to survive at low pH.

Ctenoid fish scales consist of two layers: an upper, ridged osseous layer with cteni and a lower fibrillary plate (Fouda 1979). The focus is formed early in the life of the individual (Sire 1986) and does not change appreciably after formation. Thus the morphology of this structure reflects environmental conditions early in the life of the fish. The loss of microridges from the scale focus in fish from acidic lakes may reflect disruption of calcium metabolism in these fish. Steingraeber and Gingerich (1991) found that brook trout exposed to pH 5 water had reduced body calcium, and Reader *et al.* (1989) found a similar effect for brown trout (*Salmo trutta*) at pH 4.5. Majewski *et al.* (1990) found reduced bone calcium concentration in adult Atlantic salmon (*Salmo salar*) held in a river of pH 4.7-5.2. Therefore, scale structure may be a sensitive measure of physiological stress from water acidity in fish. Because scale structure remains unchanged after formation, examination of scales of older fish could be used as an indicator of water chemistry at the time the scale was formed.

SUMMARY AND CONCLUSIONS

We surveyed 20 lakes in two regions of Russia to assess lake acidity, and to determine the effects of water acidity on fish mercury content and gill and scale ultrastructure. The lakes surveyed ranged in pH from 4.5 to 8.1 and in color from 3 to 188 Hazen. The mercury content of fish ranged from 0.06 to 3.04 μg/g wet weight, which was comparable to that reported for this species from forest lakes in Sweden and Finland. Inasmuch as these were all remote lakes with no local sources of pollution, atmospheric deposition is presumed to be the source of both acidity and mercury. Lake acidity and color, or humic content, were the major lake characteristics related to fish mercury content. Fish from high pH lakes were low in mercury regardless of other lake characteristics. Fish from low pH lakes varied widely in mercury content, with fish from colored lakes having higher concentrations than those from clear lakes. Regressions including lake pH and color, separated by drainage type, explained about 70% of the variance in fish mercury content. Acidity and color may affect fish mercury content by regulating loading and bioavailability of mercury to lower trophic levels in these lakes.

Gills and scales of fish from acidic lakes were morphologically different from those of fish from circumneutral lakes. In acidic lakes, fish gills had thickened secondary lamellae, increased numbers of chloride cells, apical crypts in some chloride cells, increased mucous production, and decreased microridge density. These changes were more severe in highly colored acidic lakes than in clear-water lakes. Scales of fish from acidic lakes lacked microridges in the focus. The gill abnormalities are all changes that would tend to reduce the effects of ion loss, which is the major physiological effect of water acidity to fish. The scale abnormality may result from disrupted calcium metabolism in acidic lakes.

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MORTALITY AND GILL DAMAGE FROM BERYLLIUM IN ACIDIC WATER: A COMPARISON OF ACUTE AND CHRONIC RESPONSES IN THREE FRESHWATER FISH SPECIES

by

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ABSTRACT

Increased concentrations of toxic trace metals such as Aluminum (Al) are found in waters acidified by acid deposition, and elevated Beryllium (Be) levels occur in some acidic waters in eastern Europe. Beryllium and Al are chemically similar, suggesting that their toxic effects may be similar as well. We exposed fry of roach and two species of perch to Be in soft water (calcium [Ca] = 2 mg/L) at two levels of pH (4.5 and 5.5) using static and flow-through bioassays. In acute toxicity tests with *Perca fluviatilis*, 10 µg/L or more Be at pH 5.5 produced gill abnormalities including chloride cell hyperplasia and hypertrophy, increased mucous production, and hyperplasia of the primary lamellar epithelium. At high concentrations, we observed fusion and loss of secondary lamellae, progressing to fusion of adjacent primary lamellae. Less gill damage occurred at pH 4.5, but mortality was much higher at low Be concentrations. Roach were killed only when Be was >50 µg/L, regardless of pH. Roach gills were damaged by 50 µg/L Be or more at both pH 4.5 and 5.5. With chronic exposure, similar abnormalities were caused in *P. flavescens* gills by 6.25 µg/L or more Be regardless of pH. The different responses observed may represent interspecies variation, but were probably influenced by small differences in age among species. Concentrations of Be similar to those reported in some polluted waters produce gill pathologies indicative of ionoregulatory stress. The effects of Be and Al are analogous, but Be is toxic at lower concentrations.

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INTRODUCTION

The concentration of Be in most surface waters is very low. These levels reflect both the relative insolubility of $BeOH_2$ at circumneutral pH (Baes and Mesmer 1976) and the overall scarcity of Be in the earth's crust (Reeves 1986). Although the average Be content of soils is low, some soils and soft coals are enriched in Be (Wilber 1980) in portions of eastern Europe (Vesely et al. 1989).

Acid deposition increases the mobility of many trace metals (Norton 1982), resulting in elevated concentrations of toxic trace metals such as Al in surface waters (Cronan and Schofield 1979). Dickson (1980) showed that cadmium, magnesium, zinc, and lead levels were elevated in some acid waters, and these trace elements were apparently mobilized by acid deposition. Vesely et al. (1989) reported elevated Be concentrations in acidified waters in eastern Europe and suggested that Be was mobilized by acid deposition. Both Be and Al have comparable speciation properties in water (Baes and Mesmer 1976, Vesely et al. 1989). Given the analogous geochemical properties of Be and Al, Be may also be an important toxic agent in some acidic waters.

Few studies have examined toxic effects of Be on fish. Slonim (1973) showed that relatively high concentrations of Be were toxic to guppies *Lebistes reticulatis* at circumneutral pH in hard water (400 mg/L Ca). Slonim and Slonim (1973) found that Be was more toxic to guppies as Ca levels decreased, but even the lowest Ca concentrations they tested were much higher than those present in most acidic waters. In the very dilute waters typical of acidified lakes and streams, Be would be much more toxic than previously suspected. Fish gills are damaged by Al (Karlsson-Norrgren et al. 1986, Jagoe et al. 1987, Evans et al. 1988, Tietge et al. 1988), which also causes ionoregulatory disturbances (Witters 1986, Booth et al. 1988). By analogy, similar effects might result from Be exposure.

MATERIALS AND METHODS

We performed acute toxicity bioassays at the Institute for the Biology of Inland Waters in Borok, Russia, in June 1989 to evaluate the toxicity of Be in low pH water and to determine effects of Be on gill structure (Jagoe et al. 1992). European perch (*P. fluviatilis*) and roach (*Rutilus rutilus*) from the Rybinsk Reservoir were allowed to spawn in artificial ponds, and fry (perch 0.08-0.15 g and roach 0.7-0.9 g) were collected and acclimated to reconstituted soft water for 72 hours. This water simulated the ionic composition of low-alkalinity lakes in the region (Haines et al. 1992), and was prepared by adding salts to distilled water (final concentrations in mg/L: 2 Ca, 1 sodium, 0.25 potassium, 0.25 magnesium, 1 SO_4, and 2.6 chlorine).

Fish were exposed to Be at two levels of pH in 2 L polyethylene aquaria (n = 10 fish/aquarium), two replicates per treatment, at room temperature (20-22°C). Dilute sulfuric acid was added to the reconstituted soft water to obtain a pH of 4.5 or 5.5, and a $BeSO_4$ solution (nominal concentration 1 mg/mL, measured concentration 0.907 mg/mL, SD = 0.12, N = 3) was added to yield final nominal concentrations of 0, 10, 25, 50, 100 or 150 µg/L Be. Controls (pH 7.0) received neither acid nor Be. Acute exposures were performed sequentially, with perch tested first, followed by roach. Dead fry were counted and removed

at 24-hour intervals, and pH recorded and adjusted if necessary. The cumulative number of fish killed by each treatment after 96 hours exposure were compared by analysis of variance. Separate analyses were conducted for each species and pH level. When significantly different levels of mortality were found among the Be treatments, those treatments which differed significantly from the control were identified by Dunnetts' t-test (SAS 1988).

All fish surviving 96 hours were collected and fixed. During the experiments, some moribund fry were removed, scored as dead, and fixed to obtain samples for microscopy from treatments exhibiting high mortality. Whole fry were fixed in 1% glutaraldehyde and 4% formaldehyde in 0.1 m HEPES buffer, pH 7.4. Only fish that were alive when fixed were used for microscopic examination.

To better understand the consequences of chronic exposure to low levels of Be on fish, we exposed juvenile yellow perch (*P. flavescens*) at pH 4.5 and 5.5 for 30 days in May and June 1990 in laboratory experiments at the Midwest Science Center in Columbia, Missouri. Perch (8-10 months old, average weight 1.5 g) were obtained from the National Biological Survey, National Fisheries Research Center in La Crosse, Wisconsin. Exposures were conducted at 20°C in reconstituted soft water using a flow-through proportional diluter system as previously described (Cleveland et al. 1986). Test water contained 2 mg/L Ca, and was very similar in composition to the water used in acute toxicity experiments conducted in Russia in 1989. The water was acidified to pH 4.5 or 5.5 using a mixture of sulfuric and nitric acid, and Be was added (as $BeCl_2$) to nominal concentrations of 0, 6.25, 12.5, 25, or 50 µg/L. Fish were exposed in 77-L aquaria, and test solutions were renewed at the rate of about 8 L/hour. Control treatments (pH 6.9-7.1) received neither acid nor Be. Fish were fed a commercial fish diet ad libitum three times daily. In each treatment, pH was determined daily; oxygen, alkalinity, and conductivity were determined twice each week; and Be was measured weekly. Initially, 40 fish were placed in each treatment, and 5 were removed from each after 5, 15, and 30 days of exposure and fixed for microscopic examination as described above.

Fish from both sets of experiments were prepared for scanning electron microscopy (SEM) using the same protocols. After at least 24 hours of fixation, the samples were treated with 1% OsO_4 for 1 or 2 hours to increase specimen conductivity, dehydrated using a graded ethanol or ethanol-acetone series, then critical-point dried. European perch and roach fry were dried whole, mounted on aluminum specimen stubs, and the opercula removed using fine-tipped forceps to expose the branchial baskets. For yellow perch, gill arches were removed and dried individually. The specimens were spatter-coated with gold or a gold-palladium mixture, and examined by SEM at accelerating voltages of 5 or 15 kV.

RESULTS AND DISCUSSION

ACUTE TOXICITY

No European perch or roach held at pH 7.0 without Be died during the acute toxicity experiments. Exposure to pH 5.5 and Be $\leq$50 µg/L did not kill any fish within 96 hours (Figs. 1a and 2a). Mortality of perch fry exposed for 96 hours to 100 or 150 µg/L Be was significantly higher than mortality of perch fry exposed to lower concentrations at pH 5.5

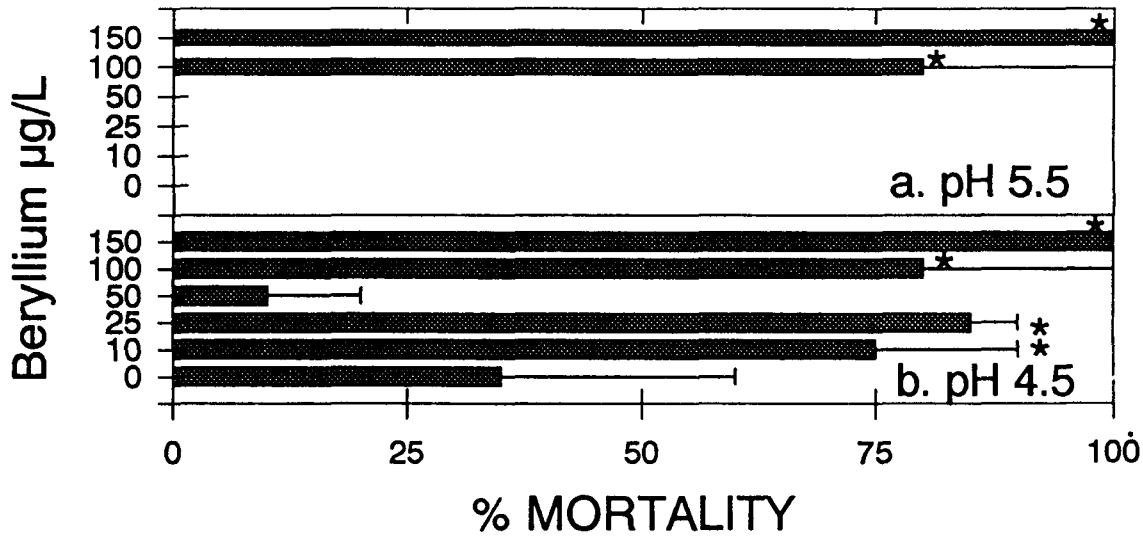

Fig. 1. Mortality of European perch fry exposed to beryllium (Be) at pH 4.5 and 5.5. Values represent the means of two replicates ± standard error. Asterisks indicate significant differences ($p < 0.05$) compared to the control (pH 7.2, 0 µg/L Be) treatment.

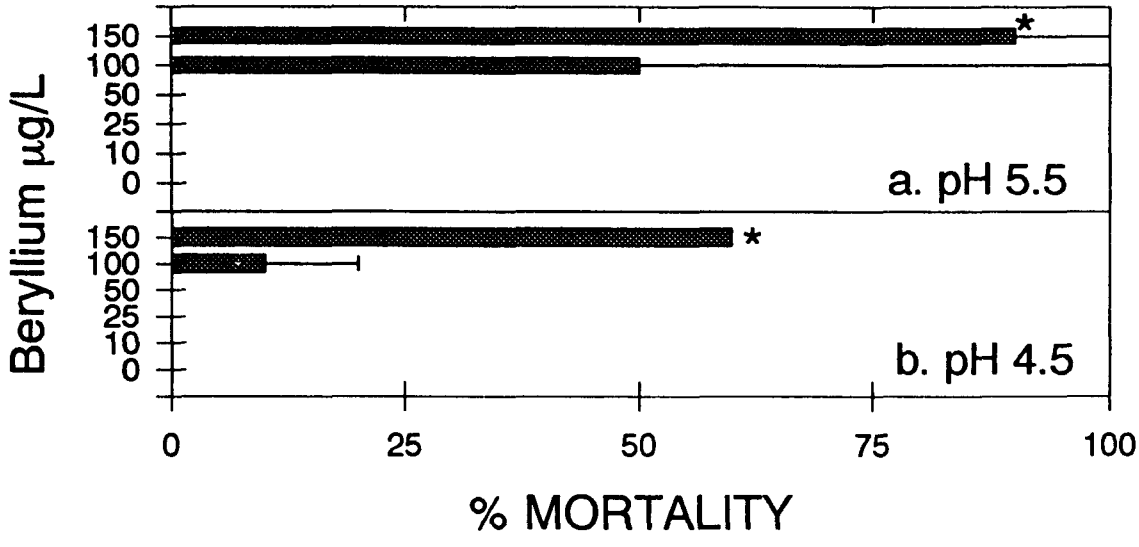

Fig. 2. Mortality of roach fry exposed to beryllium (Be) at pH 4.5 and 5.5. Values represent the means of two replicates ± standard error. Asterisks indicate significant differences ($p < 0.05$) compared to the control (pH 7.2, 0 µg/L Be) treatment.

($p < 0.05$; Fig. 1a). Similarly, roach fry were killed by exposure to 100 and 150 µg/L Be at pH 5.5 (Fig. 2a) for 96 hours. Because of variation among the replicates, at pH 5.5 only mortality at 150 µg/L Be was significantly greater than control mortality in roach ($p < 0.05$).

At pH 4.5, mortality of European perch was lowest in the 0 and 50 µg/L Be treatments (Fig. 1b), which did not differ significantly from each other or the control treatments (pH 7.0, no Be). All other Be concentrations tested at pH 4.5 caused significantly higher mortality of European perch fry after 96 hours ($p < 0.05$). Roach were less sensitive to Be at low pH than the perch. Roach fry were killed only at the highest Be concentrations (100 and 150 µg/L) at pH 4.5, and mortality was significantly greater than control mortality ($p < 0.05$) only at the highest concentration tested.

In European perch, mortality was similar at both levels of pH tested at Be >50 µg/L. Lower concentrations of Be (≤25 µg/L) were more toxic to European perch at pH 4.5 than at pH 5.5. Apparently the pH 4.5 treatment without Be was also stressful to European perch, as over 25% of the fish in this treatment died. The reduction in mortality observed in 50 µg/L Be, pH 4.5, could have resulted from a dilution error; however, this situation is unlikely because reduced toxicity occurred in both replicates and the water in these was prepared independently. A similar response has been observed in Al exposures; survival and body ion content of brook trout (*Salvelinus fontinalis*) fry at low pH were reduced at high and low Al levels, but were enhanced at moderate levels (Wood et al. 1990). Polyvalent Al species present at low pH may stabilize membranes at intermediate concentrations (Baker and Schofield 1982, Wood et al. 1988a) and Be may function in a similar manner.

Dissolved Ca clearly influences Al toxicity at low pH (Brown 1983, Wood et al. 1990), probably by modifying gill epithelial permeability. Previous work on Be toxicity (Slonim 1973, Slonim and Slonim 1973) showed that guppies in hard water (400 mg/L Ca) at circumneutral pH had a 96-hour median tolerance limit for Be of 27 mg/L (Slonim 1973). Toxicity increased as dissolved Ca concentration decreased; the 96-hour median tolerance limit for guppies was about 0.2 mg/L Be when Ca = 22 mg/L. In acidified waters, even less Ca is typically present than used in these previous studies. Our studies yielded 96-hour LC_{50}s of approximately 80 µg/L for European perch fry and 100 µg/L for roach fry at pH 5.5 when Ca = 2 mg/L.

GILL DAMAGE DUE TO ACUTE EXPOSURE

Both juvenile perch and roach held in pH 7.0 water without Be had well-formed gills with no visible abnormalities (Fig. 3a, b). Secondary lamellae appeared regular in size and configuration, and individual cells covered with microridges were observed, with little mucous visible at higher magnification (Fig. 3c). No gross morphological changes were observed at pH 5.5 in the absence of Be in gills of European perch (Fig. 4a) or roach (Fig. 4b). Surface microridges appeared somewhat less abundant, but no swelling or mucous accumulation was evident (Fig. 4c). Exposure to pH 4.5 without Be also had little effect on the gross morphology of European perch gills (Fig. 4d) or roach gills (Fig. 4e), although we observed effects typical of low pH stress, such as greater chloride cell numbers. Swelling of mucous cells indicative of increased mucous production, and many chloride cells with apical crypts were visible at higher magnification (Fig. 4f).

Fig. 3. Scanning electron micrographs of gills at pH 7.0 with no beryllium: a) European perch, scale bar = 100 μm; b) roach, scale bar = 1000 μm; and c) European perch, scale bar = 100 μm.

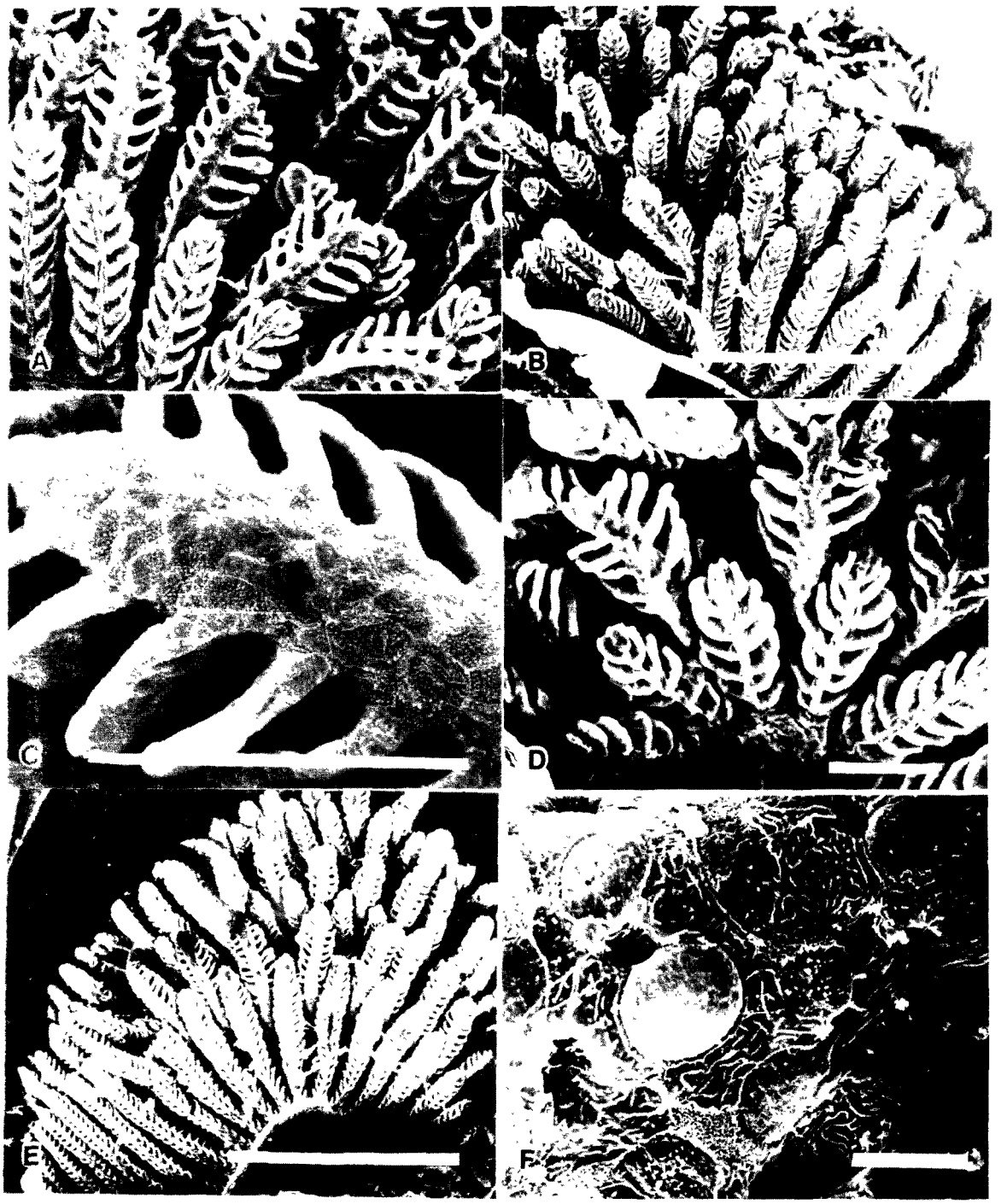

Fig. 4. Scanning electron micrographs of gills of fry exposed to low pH (5.5) without beryllium (Be): a) European perch, scale bar = 100 μm; b) roach, scale bar = 1000 μm; c) European perch, scale bar = 100 μm; d) European perch, scale bar = 100 μm; e) roach, scale bar = 1000 μm; and f) European perch, scale bar = 10 μm.

In European perch, exposure to as little as 10 µg/L Be at pH 5.5 caused changes in gill morphology. Chloride cell apical crypts were present in perch exposed to 10 µg/L Be (Fig. 5a), but not in perch exposed to pH 5.5 without Be. We also found some swelling of epithelial cells and a reduction in microridges on cell surfaces. Microridge loss and swelling of primary lamellar epithelial cells were more marked in European perch after 96 hours exposure to 25 µg/L Be, pH 5.5. Exposure to 50 µg/L Be at pH 5.5 caused hyperplasia of the primary lamellar epithelium in European perch, which filled in the spaces between secondary lamellae, causing the secondary lamellae to appear shorter and less distinct (Fig. 5b). This hyperplasia became more severe at higher Be concentrations; above 100 µg/L Be, massive epithelial hyperplasia fused adjacent primary lamellae in perch gills into a continuous mass (Fig. 5c). Low Be concentrations produced few effects on roach gills at pH 5.5. At pH 5.5 with 50 µg/L Be, primary and secondary lamellae of roach thickened, the density of microridges on the surfaces of epithelial cells was markedly reduced, and increased numbers of enlarged mucous cells appeared on primary lamellae (Fig. 5d). Gill abnormalities became more severe at higher Be concentrations. Mucous cells were enlarged in gills of roach exposed to 100 µg/L Be, pH 5.5, and many secretory pores were visible, indicating increased mucous secretion (Fig. 5e). After 96 hours exposure to 150 µg/L Be at pH 5.5, hyperplasia of both the primary and secondary lamellar epithelia was pronounced in roach (Fig. 5f), resulting in gills appearing greatly thickened and clubbed. Fusion of adjacent primary lamellae, as found in European perch fry, was not observed in roach.

At pH 4.5, high mortality occurred in European perch, but the effects of Be on gill structure were less striking than at pH 5.5. European perch exposed to 50 µg/L Be at pH 4.5 for 96 hours exhibited swelling of the primary lamellar epithelia, some loss of microridges, and increased mucous secretion (Fig. 6a). After exposure to Be at concentrations of 100 µg/L or more at pH 4.5, areas devoid of microridges were visible (Fig. 6b) and hyperplasia caused fusion of adjacent secondary lamellae in a number of regions (Fig. 6c). In this species, the hyperplasia at pH 4.5 was much less severe than at pH 5.5.

In roach exposed to Be concentrations of 50 µg/L or higher at pH 4.5, epithelial hyperplasia occurred. Primary lamellae became enlarged, with few surface microridges visible (Fig. 6d). At 100 µg/L Be, pH 4.5, hyperplasia was severe in roach gills; many secondary lamellae fused as the interlamellar spaces filled in (Fig. 6e). At 150 µg/L Be, pH 4.5, numerous secondary lamellae were lost in roach gills (Fig. 6f), and epithelial hyperplasia resulted in thickened, club-like primary lamellae. Many chloride cell apical crypts and swollen mucous cells were seen. Although fewer roach than European perch died at pH 4.5, gill abnormalities we observed were more severe in the roach at this pH.

Conversely, Be at pH 4.5 killed most European perch, although they exhibited fewer gill abnormalities. Most of the deaths occurred quickly, within 48-72 hours of exposure. The secondary lamellae of European perch dying at high Be concentrations were extremely swollen and irregular, with increased amounts of mucous. This observation suggests Be at the low pH damaged epithelial cells and destroyed their function as a water- and ion-tight barrier. Disruption of this barrier function likely produced rapid, severe ionoregulatory stress that killed the fry before compensatory mechanisms could occur, such as epithelial hyperplasia to increase both chloride cell numbers and the thickness of the diffusion pathway.

Fig. 5. Scanning electron micrographs of gills of fry exposed to beryllium (BE) at pH 5.5: a) European perch, 10 μg/L Be, scale bar = 10 μm; b) European perch, 50 μg/L Be, scale bar = 10 μm; c) European perch, 100 μg/L Be, scale bar = 100 μm; d) roach, 50 μg/L Be, scale bar = 100 μm; e) roach, 100 μg/L Be, scale bar = 100 μm; and f) roach, 150 μg/L Be, scale bar = 100 μm.

Fig. 6. Scanning electron micrographs of gills of fry exposed to beryllium (Be) at pH 4.5: a) European perch, 50 μg/L Be, scale bar = 10 μm; b) European perch, 100 μg/L Be, scale bar = 10 μm; c) European perch, 150 μg/L Be, scale bar = 100 μm; d) roach, 50 μg/L Be, scale bar = 100 μm; e) roach, 100 μg/L Be, scale bar = 100 μm; and f) roach, 150 μg/L Be, scale bar = 100 μm.

EFFECTS OF CHRONIC EXPOSURE

Survival of yellow perch was not affected by exposure to Be at concentrations up to 50 µg/L at either pH level tested. During the first week of exposure, hyperactivity and abnormal schooling behavior were observed in yellow perch exposed to 50 µg/L Be at both pH 4.5 and 5.5, but these effects disappeared as exposure continued.

The gross morphology of gills was not noticeably different among yellow perch exposed for 30 days to pH 7.0 (Fig. 7a), pH 5.5 (Fig. 7b), or pH 4.5 (Fig. 7c) without Be. Both primary and secondary lamellae were distinct and normal in appearance. Mucous droplets were more abundant after exposure to the lower levels of pH. Numerous chloride cells were present on primary lamellae at all levels of pH, probably resulting from prolonged exposure to relatively soft water. Laurent et al. (1985) and Leino et al. (1987) have also reported chloride cell proliferation in fish held in very soft water.

In contrast, 30 days exposure to Be produced gill abnormalities, even at the lowest concentration tested. Compared to controls (pH 7.0, no Be; Fig. 8a), the secondary lamellae of yellow perch exposed to 6.25 µg/L Be at pH 5.5 (Fig. 8b) were swollen and wrinkled, and increased numbers of chloride cells were present in the secondary lamellar epithelia. The gills of yellow perch exposed to 6.25 µg/L Be at pH 4.5 were coated with mucous that obscured much surface detail, and epithelial hyperplasia had reduced the spaces between adjacent secondary lamellae, making them shorter and less distinct (Fig. 8c). This shortening and loss of secondary lamellae also occurred at 6.25 µg/L Be at pH 5.5, and was most noticeable near the tips of the primary lamellae (Fig. 8d). At both levels of pH, 6.25 µg/L Be caused loss of cell surface microridges (Fig. 8e) and a noticeable increase in chloride cell surface area. We also observed an unusually high number of mucous cell secretory pores, along with bulging, convex chloride cell apices in yellow perch exposed to 6.25 µg/L Be at pH 4.5 (Fig. 8f).

As in acute exposure experiments, gill abnormalities in yellow perch became more severe at higher Be concentrations. Thirty days exposure to 25 µg/L Be at pH 5.5 caused more severe epithelial hyperplasia, causing secondary lamellae to thicken and grow together (Fig. 9a). This hyperplasia resulted in increased numbers of chloride cells as well (Fig. 9b). Hyperplasia leading to secondary lamellar loss and primary lamellar thickening also occurred at 25 µg/L Be at pH 4.5 (Fig. 9c). Abnormalities were not obviously more severe at the highest Be level tested (50 µg/L) than at 25 µg/L Be. Secondary lamellae were also shortened, thickened, and fused by exposure to 50 µg/L Be for 30 days at pH 5.5 (Fig. 9d). Exposure to the 50 µg/L Be at pH 4.5 caused similar effects on secondary lamellae (Fig. 9e, f), and surface microridges were sparse with considerable mucous visible (Fig. 9f).

The first and fourth gill arches in young yellow perch always had a row of noticeably shortened primary lamellae. In fish exposed to pH 7.0 with no Be, the morphology of these short primary lamellae was very similar to the adjacent longer ones (Fig. 10a). Exposure to pH 4.5 without Be for 6 days did not affect the morphology of these shortened lamellae (Fig. 10b), but they were severely damaged by the presence of Be (Fig. 10c). To our knowledge, this report is the first of the presence of these short filaments in the species. Perhaps these primary lamellae are rapidly growing and developing in young yellow perch, as their size and structure is similar to that reported in other young fish (Morgan 1974, El-Fiky et al. 1987),

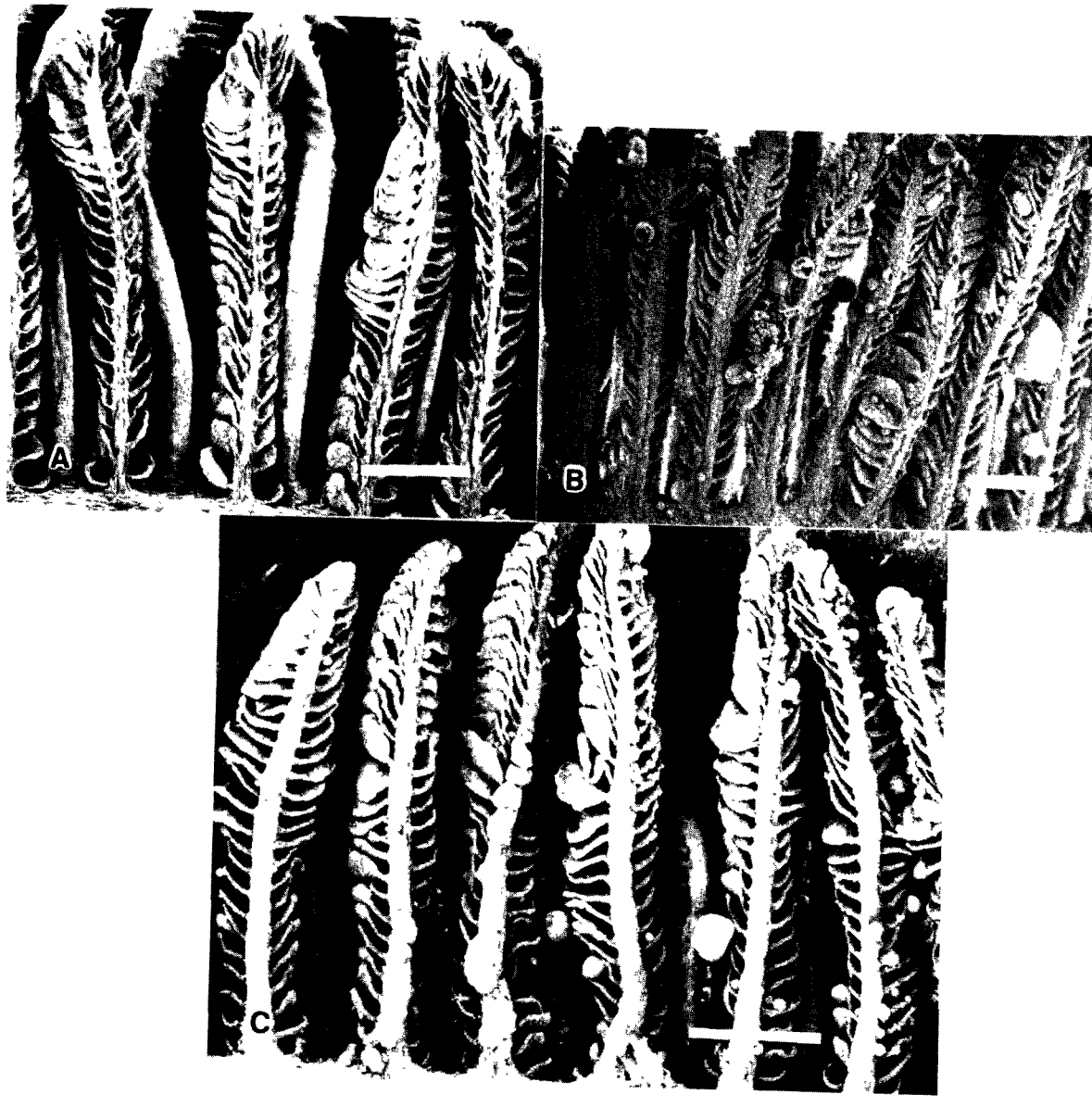

Fig. 7. Scanning electron micrographs of gills of yellow perch fry held at various levels of pH without beryllium (Be); scale bars = 100 μm: a) pH 7.0, b) pH 5.5, and c) pH 4.5.

Fig. 8. Scanning electron micrographs of gills of yellow perch fry: a) pH 7.0, no beryllium (Be), scale bar = 10 μm; b) pH 5.5, 6.25 μg/L Be, scale bar = 10 μm; c) pH 4.5, 6.25 μg/L Be, scale bar = 10 μm; d) pH 5.5, 6.25 μg/L Be; scale bar = 100 μm; e) pH 5.5, 6.25 μg/L Be, scale bar = 10 μm; and f) pH 4.5, 6.25 μg/L Be, scale bar = 10 μm.

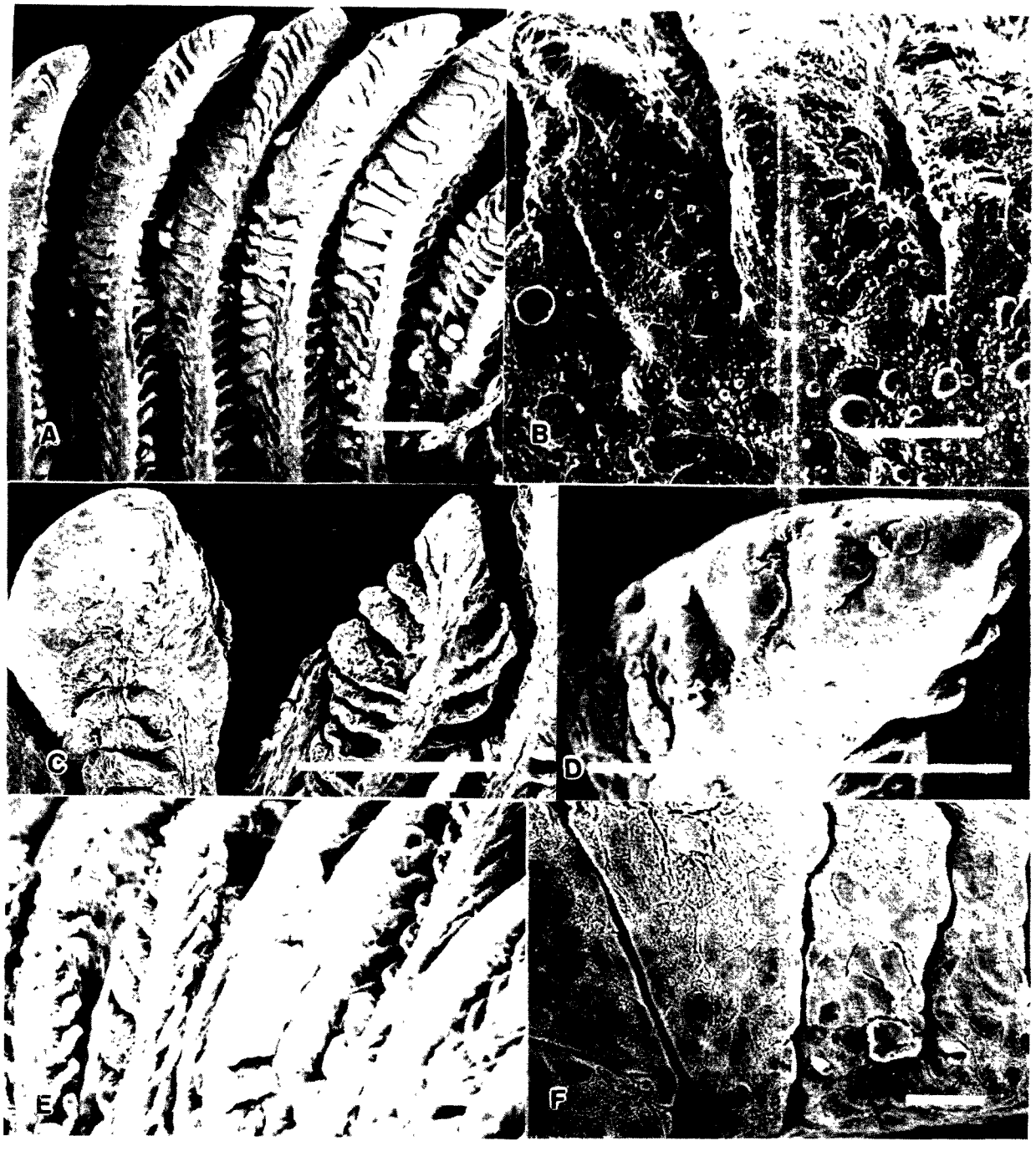

Fig. 9. Scanning electron micrographs of gills of yellow perch fry: a) pH 5.5, 25 μg/L beryllium (Be), scale bar = 100 μm; b) pH 5.5, 25 μg/L Be, scale bar = 10 μm; c) pH 4.5, 25 μg/L Be, scale bar = 100 μm; d) pH 5.5, 50 μg/L Be, scale bar = 100 μm; e) pH 4.5, 50 μg/L Be, scale bar = 100 μm; and f) pH 4.5, 50 μg/L Be, scale bar = 10 μm.

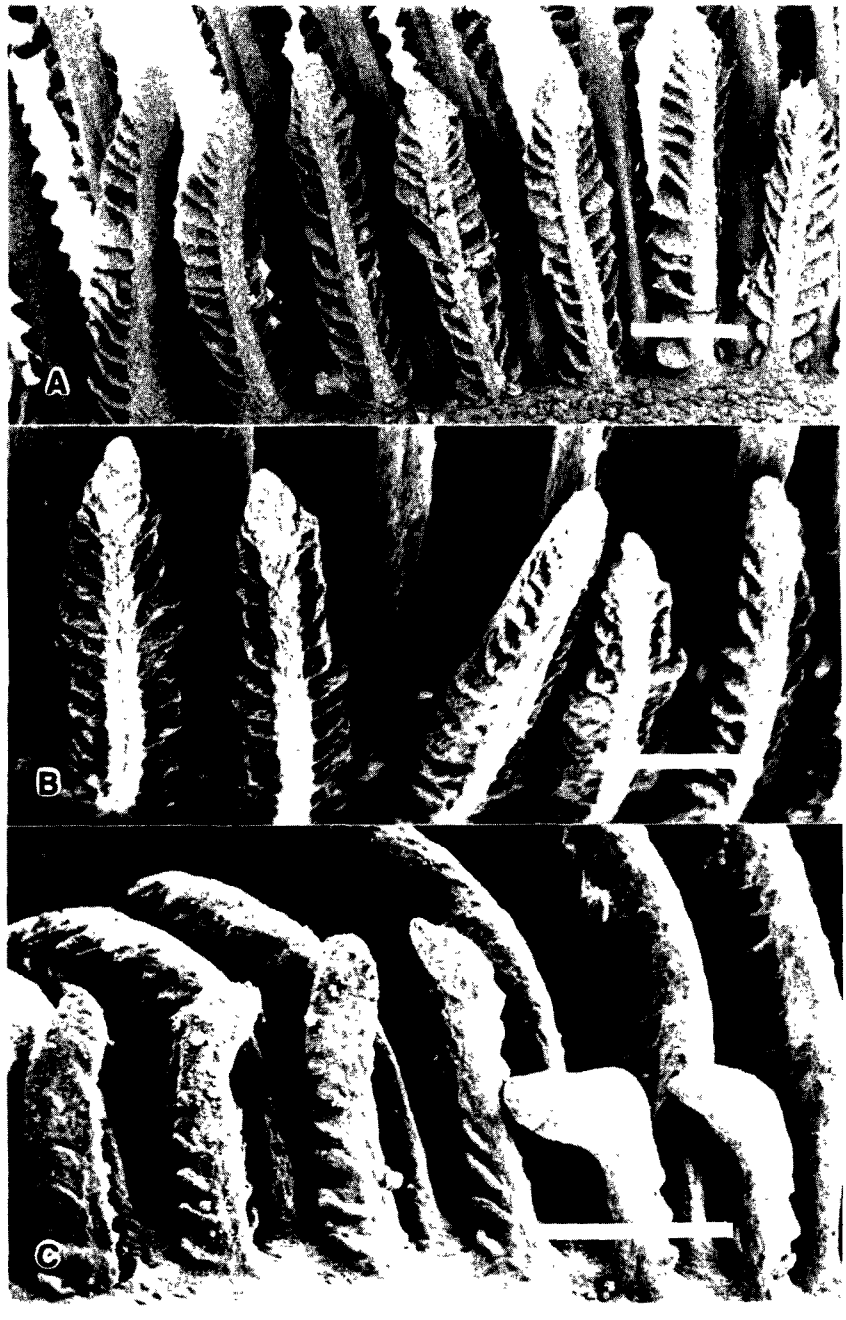

Fig. 10. Scanning electron micrographs of the gills of yellow perch fry showing the shortened row of primary lamellae on the first and fourth gill arches; scale bars = 100 μm: a) pH 7.0, no beryllium (Be), 30 days exposure; b) pH 4.5, no Be, 6 days exposure; and c) pH 4.5, 25 μg/L Be, 6 days exposure.

although these authors do not mention large differences in size among adjacent rows of lamellae. If this situation is true, the rapidly growing structures may be especially susceptible to pollutant damage, as we observed for Be, and may also be a very sensitive indicator of pollutant-induced stress.

COMPARISON BETWEEN SPECIES AND pH LEVELS

The three fish species tested seemed to respond differently to Be. These results may reflect interspecies differences; for example, roach differs from perch in sensitivity to low pH (Johansson and Milbrink 1976). A more thorough comparison of interspecies sensitivity must consider differences in size and age of fish tested. European perch fry were somewhat smaller than roach fry, and both were younger than yellow perch fry. Newly hatched fish and those just beginning to feed are especially sensitive to low pH and metal-induced stress (Peterson et al. 1982). As fish grow, they become more resistant. Thus, the difference in size and age among the species tested makes evaluation of true interspecies differences in sensitivity difficult.

Both Be and Al speciation are pH dependent. Calculation of equilibrium species distributions from thermodynamic data indicated that the major species present in both the acute and chronic toxicity experiments were monomeric Be^{2+} and $BeOH^{+}$, depending on pH (Garrels and Christ 1965, Smith and Martell 1976). Be^{2+} accounts for 88% of the total Be present at pH 4.5, while at pH 5.5, 56% would be in the form $BeOH^{+}$. In European perch, the most striking abnormalities in gills occurred at pH 5.5, when most Be present was in the hydroxide form. In roach and yellow perch, abnormalities occurred at both levels of pH. Aluminum-induced gill abnormalities also occur both at pH levels where AlOH species dominate (Karlsson-Norrgren et al. 1986, Jagoe et al. 1987) and lower pH levels where Al^{3+} becomes important (Evans et al. 1988, Tietge et al. 1988).

COMPARISON OF EFFECTS OF BE AND AL

Aluminum at low pH causes gill abnormalities, including hyperplasia resulting in epithelial thickening, increased mucous production, and changes in chloride cell and mucous cell number and structure (Evans et al. 1988, Jagoe 1988, Tietge et al. 1988). Epithelial hyperplasia, partly due to increased production of chloride cells, can cause fusion and disappearance of primary and secondary lamellae: this greatly reduces the functional surface of the gill (Karlsson-Norrgren et al. 1986, Jagoe et al. 1987). Gill physiological processes known to be affected by Al include ion regulation (Witters 1986, Booth et al. 1988) and gas exchange (Wood et al. 1988b, Playle et al. 1989). Beryllium causes the same gill abnormalities, which strongly suggests that the same physiological processes are affected by the two metals.

The altered chloride cell morphology we observed suggests that Be interferes with ion regulation, because chloride cells are responsible for ion uptake in freshwater fish (Laurent et al. 1985). Also, exposure to Be causes apical crypts to develop in chloride cells. Chloride cell apical crypts normally occur only in seawater-adapted fish (Foskett and Schelley 1982), but also occur in freshwater fish experiencing ionoregulatory disturbances caused by low pH

(Leino and McCormick 1984) and Al (Jagoe et al. 1987). Increased chloride cell production may represent a compensatory response to offset ionic losses if initial exposure to the Be is not acutely lethal. However, such hyperplasia involves a trade-off; in exchange for increased ionic uptake sites, the functional surface area for gas exchange is greatly decreased.

CONCLUSIONS

Vesely et al. (1989) found Be concentration was highly correlated with decreased pH in acidified waters in Czechoslovakia. In the waters they sampled, Be >5 µg/L was found at 27 sites, at concentrations as high as 58 µg/L. This study demonstrates that these Be concentrations would be harmful to fishes, and likely cause gill abnormalities or damage. Our results also suggest that the effects of Be and Al are analogous. In some areas where Be is abundant in soils or because of extensive use of lignite coal usage (Wilber 1980), Be may be an important toxicant whose behavior and effects are still poorly understood.

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IMPACT OF PERMETHRIN ON ZOOPLANKTON IN HIGH LATITUDE EXPERIMENTAL PONDS

by

Thomas W. La Point[1], O.D. Zhavoronkova[2] and D.F. Pavlov[2]

ABSTRACT

This study evaluated the effect of a range of concentrations (0.5-50 ug liter^{-1}) of permethrin on experimental pond zooplankton in northern USSR. Each pond was dosed once and the experiment was unreplicated. Samples were collected from each pond prior to, 7 days and 11 days after sampling. High sampling variances precluded measuring statistically significant differences among the ponds, with the exception of rotifers. Certain rotifer species appear to be highly sensitive to pyrethroid insecticides. The value of this descriptive study is in outlining future studies of the response of planktonic organisms to one-time or pulsed dosing of chemicals in experimental ecosystems. The frequency and duration of exposure needs to be taken into account relative to the generation time and reproductive capacity of the organisms under study.

INTRODUCTION

A critical consideration in measuring the effect of pesticides on non-target aquatic species is integrating exposure and effect, relative to life history characteristics of the exposed organisms. Trophic status, including nutrient concentration, suspended particulate, and

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dissolved and particulate organic matter affects chemical bioavailability in surface waters (Kaiser 1980, McCarthy & Bartell 1988). Morpho-edaphic components of the environment (depth, slope, current, physical-chemical nature of the substrate) also exert a strong influence on the nature of the biota in aquatic environments (Minshall 1988, Johnson *et al.* 1993). Biotic response to a contaminant depends upon life history characteristics of the individual species (body size, reproductive potential, and habitat and food requirements) (Barnthouse *et al.* 1987, Barnthouse *et al.* 1990, La Point 1994). Further, consideration must be made for conservatism, as little is known of inter-specific interactions in planktonic food webs. Most of the work to date has dealt with effects on zooplankton (Liber *et al.* 1994, DeNoyelles *et al.* 1994), benthos (Fairchild *et al.* 1992, Lozano *et al.* 1992, Giddings *et al.* 1994) and fishes (Fairchild *et al.* 1994, Barnthouse *et al.* 1987). Planktonic and benthic fauna are important in that they form the base of food webs in natural ecosystems and are chiefly responsible for decomposition of particulate and in influencing the overall secondary production within aquatic ecosystems. How these not-target groups respond to pesticides will influence rates of decomposition, primary production, nutrient cycling, and effects on organisms higher in the food chain (Dewey and DeNoyelles 1994).

Increasingly, more information is being collected on micro fauna, particularly the zooplankton. However, one group of micro invertebrates for which there is very little information is that of water mites, *Hydracarina*. *Hydracarina* are widely distributed all over the world, except the Antarctic region, and inhabit almost all types of water bodies (Gledhill 1985). The population density of water mites may reach extremely high levels (Tuzovshiy 1972). The nymphs and imagoes of water mites are mainly predators and play a great role in the functioning of aquatic ecosystems (Ten Winkel and Davids 1985, Kutikova 1977). At the same time, this group of Chelicerata is comparatively poorly studied in most aspects of aquatic ecology and particularly in aquatic ecotoxicology. The number of papers or reports dealing with the influence of various toxic substances on water mites is comparatively small and the conclusions made in these papers are often contradictory (Alexeev 1971, Nair *et al.* 1977, Stephenson 1982). For example, V.A. Alexeev (1986) stated that *Hydracarina* are generally resistant to a variety of toxicants. On the other hand, it has been shown that they are very sensitive to toxic action of pesticides and may be useful for the purposes of bioindication (Nair *et al.* 1977).

Synthetic pyrethroids are becoming widely used in agricultural regions across the world. This class of insecticide is characterized by low water solubility, high lipophilicity, high toxicity to fish and invertebrates, and relatively low toxicity to mammals. Because synthetic pyrethroids are widely used within the USA and Russia, it is important that the effect of these chemicals on non-target aquatic species and ecosystems be studied. The research described in this paper was conducted at the experimental ponds of the Institute of Biology of Inland Waters (IBIW), Russian Academy of Sciences, Borok, Jaroslavl Region, Russia. Permethrin (3-phenoxybenzyl (1RS)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) was used as the commercial product Pounce, manufactured by FMC, Inc. Pounce has 38.47% active ingredient, 50.7% xylene, and 10.8% related reaction and inert products.

MATERIALS AND METHODS

DESCRIPTION OF STUDY PONDS

The IBIW is located on the Rybynsk Reservoir in the Jaroslavl Region of Russia, roughly 330 km northeast of Moscow at 58 degrees N latitude. The experimental ponds at the IBIW have a surface area of 800 m^2, volume of 400 m^3, and average depth of 50 cm. The ponds are characteristic of high-latitude, oligotrophic ponds (Table 1).

Five ponds were used in this experiment: control, acetone control, and three ponds treated with permethrin to achieve concentrations of 0.5, 5.0 and 50.0 ug $liter^{-1}$ (pond numbers 1, 2, 3, 4, and 5, respectively). Acetone was used as the carrier for permethrin; 1 liter was dosed into each pond with the permethrin by sub-surface application (at 15 to 20 cm depth) using a hand-held sprayer. The concentration of permethrin in 1-liter acetone was 0.52 ml, 5.2 ml, and 52.0 ml to achieve the 0.5, 5.0 and 50.0 ug $liter^{-1}$ concentrations, respectively. The chemical was mixed into the ponds by transects taken across the surface in a boat. Ponds were treated once, on May 25, 1989.

RESIDUE ANALYSIS

Permethrin samples were intended to be analyzed by gas chromatography following collection and transport to the USA. Permethrin samples were collected on days 1 and 11 following dosing. Collected water samples were extracted onto C-18 packed glass columns, frozen, and carried to the National Fisheries Contaminant Research Center in Columbia, Missouri, for analysis. Unfortunately, the samples were not allowed into the country; hence, results are in terms of nominal permethrin concentrations.

Table 1. Water chemistry of Russian Academy of Sciences Institute (IBIW) ponds at Sunuga, Borok, USSR.

Pond No.	Control	Acetone Control	0.5 µg/l	5.0 µg/l	50.0 µg/l
Total suspended particulates, mg/l	1.0	1.9	1.9	5.5	6.0
Total inorganic phosphorus, µg/l	27.5	6.0	5.0	8.0	8.0
NO_2-N, µg/l	0.7	0	0	0	0
NO_3-N, µg/l	10.0	2.0	2.6	4.0	4.0
Total N, mg/l	0.81	0.70	0.58	0.78	0.73
Biological oxygen demand, mg O_2/l	1.3	1.3	1.1	1.7	2.5
Total organic carbon, mg/l	11.6	11.1	14.0	12.2	11.9
Total suspended particulate carbon, mg/l	0.6	0.3	nd	1.1	1.2

SAMPLING PROCEDURE FOR POND MICROBIOTA

Pond microbiota sampled included bacteria, protozoa, algae, and zooplankton. During the 1-month study, samples were taken 5 times from each pond (except the control pond, where samples were taken 4 times). We sampled once prior to treatment and at 4-day intervals subsequent to dosing. Sediments were collected from each pond and subsampled for chlorophyll *a*, heterotrophic activity, and percentage organic matter.

Bacterial density was determined using epifluorescence microscopy and direct counts on membrane filters stained with erythrosin stain. Heterotrophic flagellates were quantified using epifluorescence microscopy with primulin stain. Rotifera were live-counted in Sedgewick-Rafter cells. Zooplankton were sampled using a 10-liter bucket following standardized techniques for small, shallow water bodies (Metodika izutchenia 1976). In each pond, ten 10-liter zooplankton samples were collected along a transect from the shore to the center for a total of 100 L water per sample. Each 100-liter sample was concentrated using a Juday net with brass screen (No. 73) to volume of 30 ml and preserved in 4 % formalin. In the laboratory, two subsamples of either 0.5, 1, or 2 ml (depending on visual density of zooplankters) were pipetted into Bogorov's counting chamber and zooplankters counted and identified using a binocular microscope.

To collect water mites, the water volume filtered for sampling was approximately 4.25 m^3 per sample. In all experimental ponds, except in the control, 3 samples were collected during the study period: before dosing, after 7 days, and after 11 days. In the control pond, the samples were collected before dosing and after 11 days.

Collected water mites were preserved immediately with Oudemans fixation liquid. The identification of water mites' taxonomic status was conducted in accordance with the classification of B.A. Wainstein (1980). Water mite species were identified using I.I. Sokolov's keyword book (1940).

DATA ANALYSIS

For species identification, we used Manuilova (1964), Kutikova (1970), Bening (1941) and Opredelitel presnovodnykh bespozvonotcnhykh (1977). For quantifying uncommon zooplankton species, the entire sample was enumerated. All zooplankters were divided into five groups: Cladocera, Copepoda, Rotatoria, copepod nauplii and copepod copepodites, and numerical analyses were performed on these groups.

The pond treatments were unreplicated. Comparisons were drawn from responses over time for the various taxonomic groups. Data were transformed by logarithms or arc-sine, as appropriate, to minimize correlations between the mean and variance of samples.

RESULTS

Before the permethrin application, planktonic communities differed among the ponds (Tables 2-4). At this time, Cladocerans dominated the zooplankton in all ponds studied; however, density of Cladocera, as well

Table 2. Density of common cladoceran species (individuals per liter pond water).

SPECIES	Density				
	sample day[1]				
	0	1	6	10	15
Control Pond					
Bosmina longirostris	3.6	3.6	12.5	-	31.2
Ceriodaphnia sp.	144.3	20.4	42.5	-	np[1]
Diaphanosoma brachiurum	0.6	3.0	7.0	-	19.0
Polyphemus pediculus	304.8	558.0	6.0	-	np
Simocephalus vetulus	6.3	1.2	14.5	-	np
Total density	465.0	600.0	98.0	-	63.6
Number of species	10	9	9	-	6
Acetone Control					
Bosmina longirostris	172.5	40.0	3.5	2.6	161.5
Ceriodaphnia sp.	139.6	3.7	3.0	105.9	26.0
Polyphemus pediculus	3.5	2.7	2.6	0.1	4.0
Scapholeberis mucronata	4.0	0.6	np	19.7	36.5
Total density	423.5	54.3	13.2	134.3	238.5
Number of species	10	10	7	9	9
0.5 µg/l					
Bosmina longirostris	0.3	43.5	101.5	0.5	3.0
Ceriodaphnia sp.	30.2	275.5	2.5	40.0	2.0
Polyphemus pediculus	40.3	8.0	36.6	14.1	3.0
Simocephalus vetulus	0.3	13.5	np	6.0	9.5
Total density	83.3	367.0	142.8	66.6	45.0
Number of species	11	9	6	9	12
5.0 µg/l					
Bosmina longirostris	np	0.9	112.0	42.0	69.0
Ceriodaphnia sp.	110.4	0.1	2.5	3.0	2.0
Diaphanosoma brachiurum	18.5	np	np	np	np
Polyphemus pediculus	6.0	np	3.5	np	7.5
Sida crystallina	4.8	np	np	1.0	0.5
Total density	144.3	1.0	122.5	47.3	89.5
Number of species	9	7	6	6	5
50.0 µg/l					
Bosmina longirostris	54.9	1.2	7.0	2.0	15.6
Ceriodaphnia sp.	307.8	np	np	np	np
Polyphemus pediculus	190.2	4.2	np	np	np
Total density	655.8	6.6	7.0	2.0	15.6
Number of species	10	4	1	1	1

[1]Days following pond dosing.
[2]np = not present.

Table 3. Density (individuals per liter pond water) of common copepod species and of nauplii and copepodites.

SPECIES	Density				
	sample day[1]				
	0	1	6	10	15
Control Pond					
Cyclops sp.	0.6	0.6	np[2]	-	9.6
Eudiptomus gracilis	0.6	np	0.1	-	np
Heterocope appendiculata	np	1.8	0.1	-	np
Mesocyclops leuckarti	0.2	1.2	1.5	-	14.4
Paracyclops fimbriatus	np	np	np	-	7.2
Nauplii	np	np	7.0		464.4
Copepodites	0.1	6.0	15.5	-	538.8
Total density	1.8	3.6	1.7	-	32.4
Number of species	8	3	3	-	4
Acetone Control					
Acanthocyclops viridis	7.0	0.3	0.1	np	np
Eudiaptomus gracilis	3.6	1.2	0.8	np	np
E. graciloides	np	10.5	1.0	21.0	np
Heterocope apendiculata	4.0	6.3	0.3	7.0	np
Mesocyclops leuckarti	4.0	np	0.1	0.5	2.5
Nauplii	np	0.5	4.0	3.0	121.0
Copepodites	7.4	2.4	5.0	1.5	57.0
Total density	26.7	18.3	2.3	28.5	2.5
Number of species	5	4	5	3	1
0.5 µg/l					
Eudiaptomus gracilis	1.7	2.0	0.2	0.2	np
E. graciloides	np	1.0	np	np	np
Heterocope appendiculata	0.3	0.2	2.0	0.6	np
Mesocyclops leuckarti	np	2.0	np	3.5	10.0
Nauplii	np	4.5	12.0	14.5	8.0
Copepodites	1.2	1.1	18.0	11.5	14.0
Total density	2.4	5.4	2.2	4.4	10.0
Number of species	3	7	2	4	1
5.0 µg/l					
Eudiaptomus graciloides	np	0.6	1.0	0.3	np
Heterocope	0.3	1.0	1.5	0.1	np
Mesocyclops leuckarti	np	5.5	3.0	6.3	36.5
Nauplii	0.6	7.0	13.0	29.0	45.0
Copepodites	11.4	5.5	12.5	18.0	84.5
Total density	20.0	8.2	5.5	7.6	40.0
Number of species	5	6	3	7	4
50.0 µg/l					
Acanthocyclops viridis	3.0	nd	nd	nd	nd
Cyclops strenuus	13.2	nd	nd	nd	nd
Mesocyclops leuckarti	nd	nd	nd	u[3]	9.6
Nauplii	nd	2.4	1.0	3.0	9.6
Copepodites	9.6	0.6	1.0	3.0	39.6
Total density	16.2	0	0	0	9.6
Number of species	3	0	0	1	1

[1]Days following pond dosing.
[2]np = not present.
[3]u = single organism

Table 4. Density (individuals per liter pond water) of common Rotifera species.

SPECIES	Density				
	sample day[1]				
	0	1	6	10	15
Control Pond					
Asplanchna priodonta	np	6.0	10.0	-	12.0
Brachiounus angularis	np	np	35.0	-	200.4
B. calcyciflorus	np	0.6	13.0	-	1.2
B. diversicornis	np	np	5.5	-	202.8
Filinia longiseta	np	3.6	5.5	-	194.4
Keratella quadrata	np	4.2	21.0	-	1.2
Synchaeta sp.	0.3	np	np	-	np
Trichocerca cylindrica	np	np	4.5	-	327.6
Total density	0.3	18.6	169.0	-	1348.2
Number of species	1	8	19		18
Acetone Control					
Brachiounus angularis	1.0	1.5	1.0	np	32.0
B. calcyciflorus	1.0	2.0	1.0	1.0	np
Euchlanis dilatata	np	0.5	np	1.5	20.5
F. longiseta	np	np	1.0	np	35.0
Filinia mayer	4.0	2.0	1.5	2.0	np
K. cochlearis	2.0	2.0	0.5	3.0	1.0
Keratella quadrata	4.0	7.0	11.5	11.5	1.5
Total density	14.0	17.5	20.0	23.5	117.5
Number of species	7	8	11	10	11
0.5 µg/l					
Asplanchna priodonta	0.3	1.0	79.0	1.5	0.5
Euchlanis dilatata	np	np	np	8.0	8.0
Filinia longiseta	np	4.0	27.0	np	1.0
F. mayer	np	np	190.0	5.0	np
K. cochlearis	np	np	107.5	2.5	np
Keratella quadrata	0.3	6.5	342.0	53.0	3.0
Lecane luna	np	np	3.0	11.5	5.0
Total density	1.2	16.5	1008.5	94.0	21.0
Number of species	4	7	18	15	11
5.0 µg/l					
Asplanchna priodonta	1.5	2.5	71.0	44.0	np
Brachiounus calcyciflorus	np	6.5	66.0	157.0	np
Conochilus sp.	0.2	33.0	1.5	2.0	np
Euchlanis dilatata	nd	1.5	nd	64.0	23.0
Keratella quadrata	1.5	19.0	215.5	120.0	18.5
Total density	2.7	80.0	603.0	592.0	64.5
Number of species	3	15	21	21	11

Table 4. (continued)

SPECIES	Density				
	sample day[1]				
	0	1	6	10	15
50.0 ug/l					
Asplanchna priodonta	226.8	139.2	30.0	8.0	48.0
Brachiounus calcyciflorus	np	0.6	66.5	9.0	32.4
Euchlanis dilatata	np	1.8	2.5	8.0	99.6
F. longiseta	np	np	18.0	6.0	296.4
Filinia *mayer*	np	np	np	35.0	np
K. cochlearis	np	np	11.0	28.0	12.0
Keratella quadrata	2.7	0.6	87.5	50.0	9.6
Lecane luna	2.4	np	4.0	6.0	164.5
Total density	236.1	144.0	283.5	206.3	786.7
Number of species	5	7	18	18	17

[1]Days following pond dosing.
[2]np = not present.

as dominant species from other orders, differed among ponds (Figure 1, Table 2). The lowest initial density of Cladocera was in pond 3 (83.3 individuals per liter), while the highest density was in pond 5 (655.8 individuals per liter). *Polyphemus pediculus* and *Ceriodaphnia* sp. dominated populations in ponds 1,3, and 5. *Bosmina longirostris* and *Ceriodaphnia* sp. Were dominant in pond 2, and *Ceriodaphnia* sp. and *Diaphanosoma brachiurum* in pond 4 (Table 2).

Initial copepod density was much less than that of the Cladocera (range 1.8 to 26.7 individuals per liter, Table 3). Copepod density was greatest in pond 2 and least in pond 1 (Figure 2, Table 3). Before treatment, copepod nauplii were found only in pond 4 and their density was very low, less than 1 nauplius per liter. Copepod copepodites were present in all ponds, but at very low densities (Table 3).

Initially, the highest rotifer density was in pond 5 (Table 4). However, this stemmed from one dominant rotifer species, *Asplanchna priodonta*, which, at 226.8 individuals per liter, was very high compared to the densities of all other rotifers in all ponds. Typically, total rotifer density was much lower, not exceeding 14.0 individuals per liter. Among the other rotifers, *Keratella quadrata* was dominant in ponds 2-4, while in the control pond, the only species present at this time was *Synchaeta* sp.

Density dynamics of zooplankton during the post-treatment period are shown in Figures 1-4. Quantitative and qualitative changes occurred in all ponds. By the end of the second week, cladoceran densities were reduced in all ponds; however, their response dynamics were different (Figure 1). In the control and 0.5 ug/l permethrin ponds, cladoceran

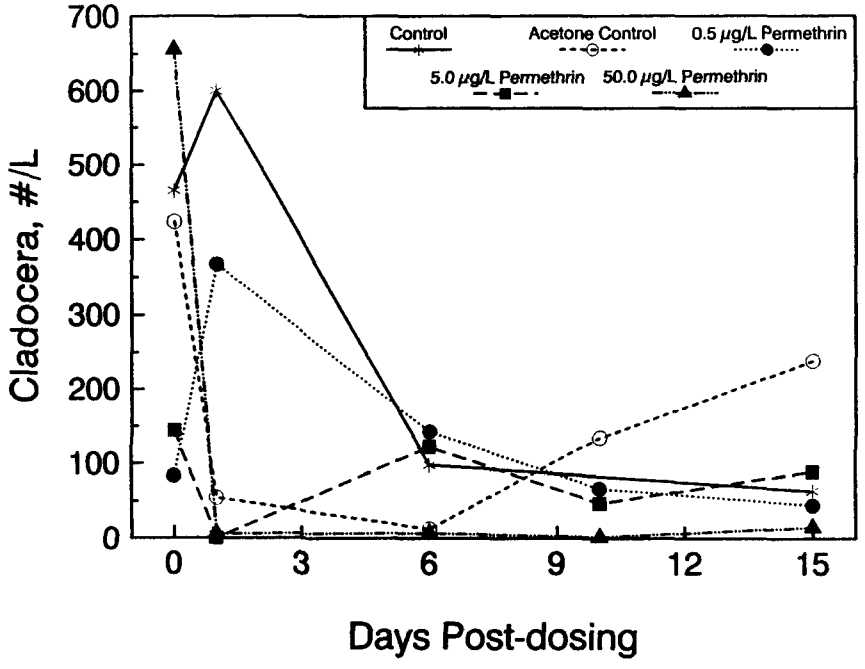

Figure 1. Number of cladocera per liter in IBIW experimental ponds dosed with permethrin.

density initially increased, gradually decreasing to less than pre-treatment densities. In these ponds, the initially codominant species, *Ceriodaphnia* sp. and *Polyphemus pediculus*, were replaced by *Bosmina longirostris* (in the control pond) and *Simocephalus vetulus* (in the 0.5 ug/l pond).

In ponds treated with 5.0 and 50.0 ug liter^{-1} permethrin (ponds 4 and 5, respectively), and in the control plus acetone pond (pond 2), cladoceran densities decreased dramatically beginning on day 1. After day 3, however, cladoceran density increased in ponds 2 and 4, but not in 5. The only cladoceran species that survived in pond 5 was *Bosmina longirostris*. This species became the most common cladoceran at the end of the observation period in the acetone control and the 5.0 ug liter^{-1} permethrin ponds (Table 1).

Density dynamics of early life-stage copepods were generally similar among the ponds (Figure 4). However, there were marked numerical differences among the populations. In the control pond, copepod nauplii and copepodites reached densities as high as 464.4 and 538.8 individuals per liter, respectively (Figures 3 and 4 respectively). In all other ponds, and especially in the 50.0 ug liter^{-1} permethrin pond, their densities were much less (Table 3). However, after day 15, there was a distinct increase in copepodites and nauplii. This phenomenon has been described by Solomon *et al.* (1980) and Kaushik *et al.* (1985). The copepodites and nauplii may show recovery as the permethrin concentrations in the ponds decrease.

By the end of the observation period, *Mesocyclops leuckarti* was the most common copepod species in all ponds. However, it was the only copepod species in the acetone control, 0.5 ug liter^{-1}, and 50.0 ug liter^{-1} permethrin ponds, while in the control and 5.0 ug liter^{-1} permethrin ponds, each copepod population consisted of four species. But, in fact, the copepod community was less complex in the 5 ug liter^{-1}, because *M. Leuckarti* was accompanied only by single individuals of *Cyclops* sp., *Acanthocyclops* sp., and *Ectocyclops* sp., while in the control pond *Cyclops* sp. and *Paracyclops fimbriatus* were codominant with *M. leuckarti* (Table 3).

Rotifers increased in both the control and acetone control ponds (Figure 5); however, there was an order of magnitude difference in density between the two ponds at the end of the experiment (Table 4). In the control pond, four rotifer species were codominant: *Filinia longiseta, Brachionus angularis, M. diversicornis* and *Trichocerca cylindrica*, while in the acetone control pond, *F. Longiseta, B.* angularis, and *Euchlanis dilatata* dominated the population.

Rotifer density in the 0.5 ug liter^{-1} permethrin ponds peaked 4 days after permethrin application and then decreased to pre-treatment

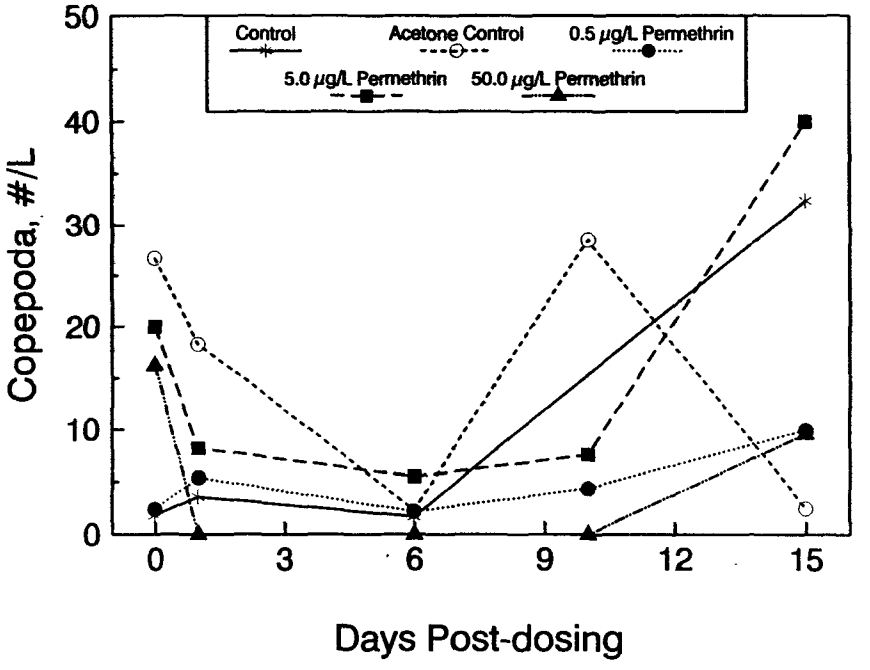

Figure 2. Number of copepods per liter in IBIW experimental ponds dosed with permethrin.

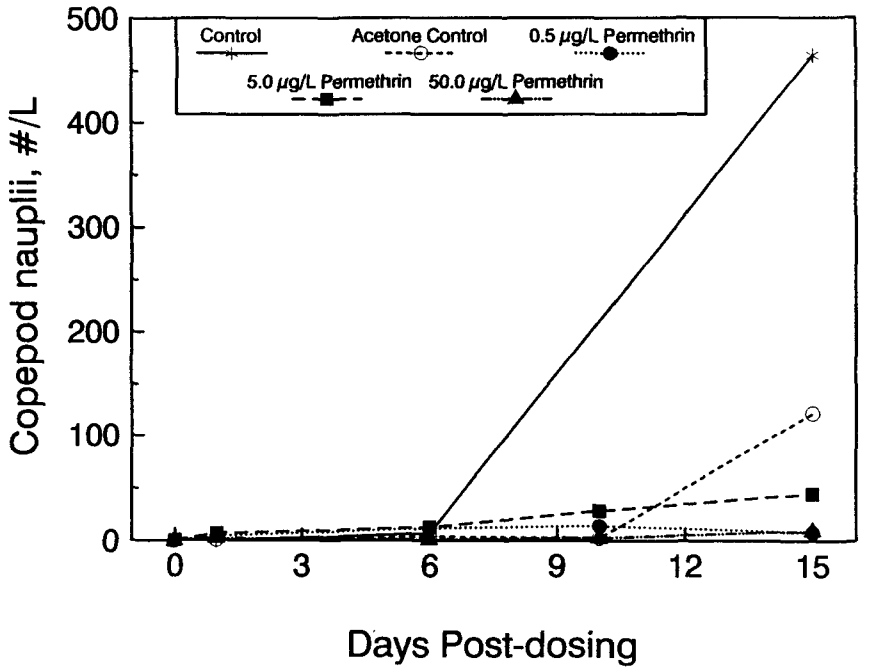

Figure 3. Number of copepod nauplii per liter in IBIW experimental ponds dosed with permethrin.

levels. The peaks in density were primarily a function of the increased number of *Keratella quadrata* in both ponds, *K.* cochlearia and *Filinia mayer* in pond 3, and *Asplanchna priodonta* and *Brachionas calyciflorus* in pond 4 (Figure 4). By the end of the experiment, *K. quadrata* and *Euchlanis dilatata* codominated the rotifer populations in both ponds, but their densities were low. In pond 5, 1 day after permethrin was applied, total rotifer density was slightly decreased. However, the rotifers gradually increased and reached a density of 786.7 individuals per liter. The dominant species at the beginning of the experiment in pond 5, *Asplanchna priodonta*, became much less abundant than *Filina longiseta* and *Euchlanis dilatata* by day 15. Interestingly, the rotifer population in pond 5 consisted of 17 species, which was nearly that of the control pond (with 18 species) (Table 4).

Water mites of *Eylais* and *Piona* genera were dominant in all the experimental ponds. The list of water mites collected from the IBIW ponds is provided in Table 5. Water mites of other species were found only in single exemplars. Before permethrin was added to any ponds, the greatest density of water mites of *Eylais* was found in pond 3 and for *Piona*, the greatest density was in pond 4. During the experimental period, the number of *Eylais* increased in ponds 1 (control) and 2 (control plus acetone) by 26% and 300%, respectively.

After permethrin was added, the number of water mites of both genera decreased in all treated ponds (except for the *Piona* mites in

pond 3). In pond 3, after 7 days, the number of *Eylais* decreased 32.5% compared to pre-treatment levels and decreased by 29.1% after 11 days post-treatment. In pond 4, density decreased to 20.4% of original at 7 days and thereafter. The *Piona* in pond 3 increased in number, but this increase was very slight. We assume these mite densities, in fact, did not change after adding permethrin at a concentration of 0.5 ug liter^{-1}. In pond 4, the decline in number of water mites of this genus was much more pronounced. Seven days after treatment, the number was only 27.5%; 11 days after treatment only 14.5% compared to pre-treatment period. In pond 5, no water mites of either *Eylais* and *Piona*, as well as other genera, were found after pond dosing.

DISCUSSION

Published laboratory and field experiments have demonstrated that synthetic pyrethroids exert a toxic effect on zooplankton, especially crustaceans (Day 1989, Crossland 1982, Kaushik *et al.* 1984, Perevoznikov *et al.* 1984). Permethrin concentrations as low as 1.0 ug liter^{-1} and less were referred as acutely toxic for the variety of crustaceans (Day 1989). However, exposures simulating natural conditions help to complete a full evaluation of the effects of pyrethroid insecticides on natural planktonic communities.

Our pond experiments have shown that at a nominal permethrin concentration of 0.5 ug liter^{-1}, acutely toxic for a variety of aquatic invertebrates when tested in laboratory conditions, did not produce a marked direct effect even on species shown to be sensitive to a variety of toxicants, such as *Ceriodaphnia* (Winner 1988). Furthermore, while

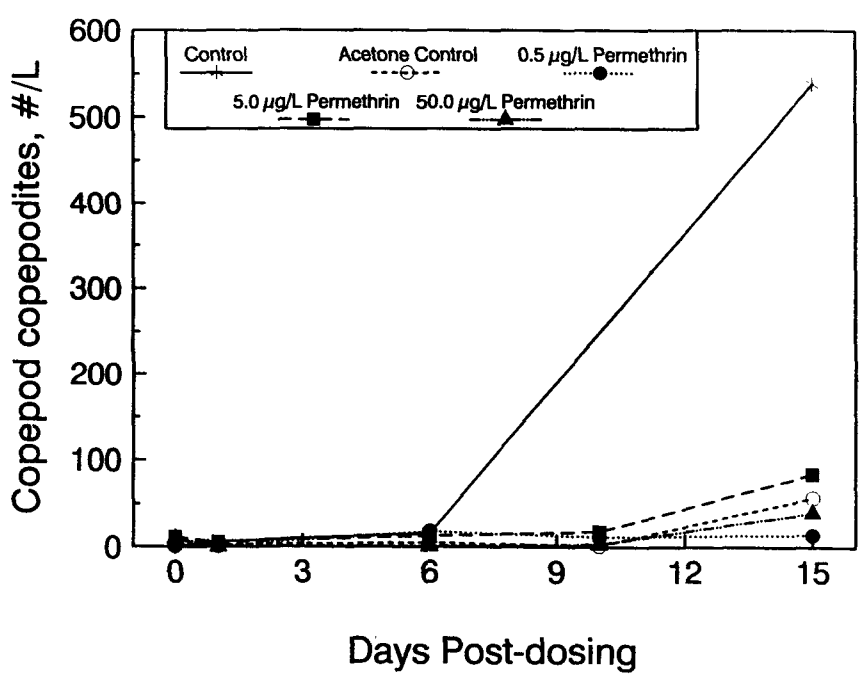

Figure 4. Number of copepod copepodites per liter in IBIW experimental ponds dosed with permethrin.

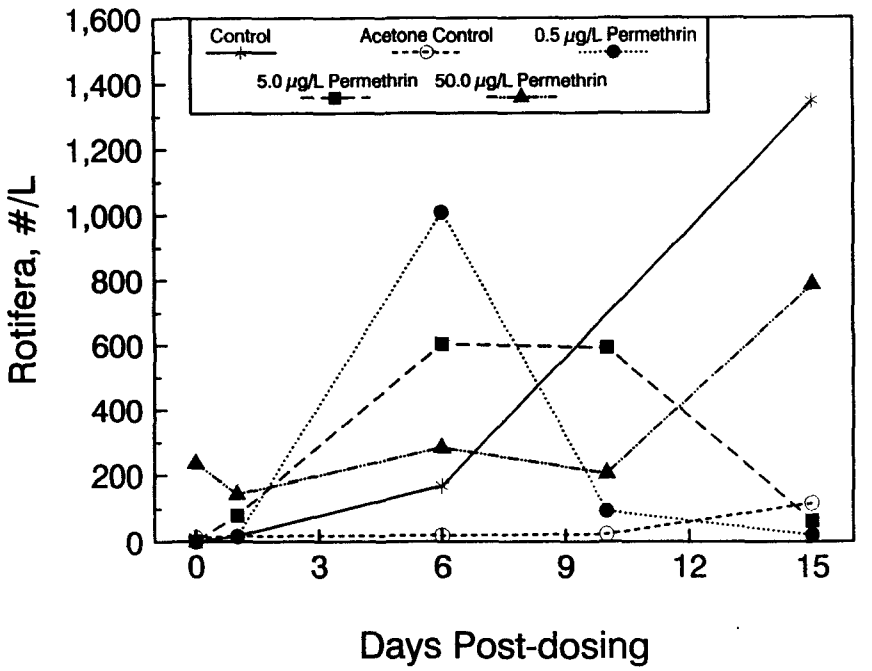

Figure 5. Number of rotifers per liter in IBIW experimental ponds dosed with permethrin.

5.0 ug liter^{-1} permethrin led to a dramatic decrease in density of cladocerans and copepods, the effects were less than would be expected on the basis of laboratory-measured responses.

Our results indicate that, in the control and acetone-control ponds, the density of *Eylais* water mites increased during the observation period. In the permethrin-treated ponds at concentrations of 0.5 and 5.0 ug liter^{-1}, their density decreased (Figure 1). On the other hand, *Piona* density decreased both in controls and in the 5.0 ug liter^{-1} permethrin-treated pond; however, in the treated pond, the effect was more pronounced. The concentration of 0.5 ug liter^{-1} seems to have no effect on this genus of mites (Figure 2). *Eylais* mites seem to be more susceptible to the permethrin than the *Piona*. However, at the permethrin concentration of 50.0 ug liter^{-1}, all *Eylais* and *Piona* water mites suffered complete mortality.

Hence, results of this study demonstrate the susceptibility of certain water mites to permethrin under field conditions. This is in good accordance with published results on the toxicity of insecticides to water mites (Nair *et al.* Stephenson *et al.* 1986, Crossland and Wolff 1985). However, our results are quite different from the results of investigations on the effects of industrial pollutants on aquatic mites (Alexeev 1986). Evidence exists to indicate that the primary reason for the elimination of water mites was the direct toxic effect of permethrin. That the elimination of this planktonic invertebrate was due to an elimination of more sensitive prey zooplankters seems unlikely because the mites have been shown to survive more than 15 to 20 days when starved (Zhavoronkova, unpubl.).

Table 5. Acari present in Sunuga ponds.

FAMILY	SPECIES
Hydrachnidae	*Hydrachna sp.*
Eylaidae	*Eylais mulleri Koenike, 1897; E. extendens O.F. Mull., 1776; E. hamata Koenike, 1897; E. koenikei, 1903*
Hydriphantidae	*Hydryphantes sp., Hydrodroma sp.*
Limnesiidae	*Limnesia sp.*
Hygrobatidae	*Piona coccinea (C.L. Koch), 1836; P. nodata (O.F. Muller), P. variabilis (C.L. Koch), 1836; Hydrochoreutes sp., Acercus sp.*
Arrhenuridae	*Arrhenurus sp.*

Our experiment was performed in mature ponds, all with extensive macrophyte growth. The biomass of submerged higher aquatic plants was high, as was the concentration of suspended solids. The propensity of permethrin to adsorb quickly onto solid surfaces (e.g., suspended solids, particulate organic matter, etc.) is well known (Muir *et al.*, Sharom and Solomon 1979).

The IBIW ponds are relatively productive. High ecosystem productivity, with associated high plant surface area and concentration of particulate organic material, lessened the bioavailability of pentachlorophenol in a mesocosm study (Robinson-Wilson *et al.* 1983). Functional redundancy in the macrophyte community within mesocosms also lessens the potential for measuring ecological damage (Fairchild *et al.* 1994). Empirical laboratory results (McCarthy and Bartell 1988) show that for periphyton, macrophytes and detritivorous fish, there is a decrease in toxic effects of phenol to daphnia. The lessened toxicity was directly related to increased particulate organic matter (POM). However, for phytoplankton , zooplankton, benthic invertebrates and omnivorous fish, greater reduction in toxic effects corresponded to increasing dissolved organic material (DOM) in the system. Bacterial and zooplanktonic growth was dramatically enhanced as a function of available organic material and, presumably, greater surface area for microbial activity.

Higher levels of primary production corresponded to lower pentacholorophenol (PCP) residues and toxicity to fish in a pond-mesocosm study (Robinson-Wilson *et al.* 1983). Yet, lower PCP toxicity could not be ascribed solely to an increase in primary production. Their results indicated that the larger surface area available within

the macrophyte-dominated ponds contributed to an active epiphytic community which degraded the PCP. In a similar manner, the adsorption of permethrin onto plant surfaces and suspended solids in these ponds was apparently high and may explain the less pronounced direct toxic effect of the 0.5 and 5.0 ug liter^{-1}, permethrin concentrations, and the greater susceptibility of active filter-feeding cladocerans (*Polyphemus pediculua, Sida crystallina, Diaphanosoma brachiurum*) and copepods (*Eudiaptomus gracioloides*), while predatory copepods such as *Mesocyclops leuckarti* appeared to be much less susceptible to the insecticide. Among the active filter-feeding zooplankters, such as *Polyphemus pediculus, Daphnia longispina, Sida crystallina, Eudiaptomus gracilis,* and *E. Graciloides*, are the most sensitive to the toxic effects of permethrin. Of the zooplankton collected in our experiment, Rotifera were the most resistant to permethrin. This agrees well with previous results reported by Kaushik *et al.* (1985). The reasons for such resistance, as well as factors influencing rotifer population dynamics as a function of permethrin, were discussed by these authors in detail.

The reasons for the noted changes in zooplankton density following acetone application are not clear. The decline in Cladocera no doubt stems from unmeasured pond-to-pond differences. Such pond-to-pond differences have recently been documented in a separate study (Hellenbrant 1994). Different rates of decline and growth in zooplankton as a result of permethrin exposure were observed by Kaushik *et al.* (1985). They note zooplankton numerically respond to an insecticide in two ways, direct reduction from acute toxicity and indirect numerical responses attributed to release from predatory or competitive pressures.

There is evidence that the recovery of zooplankton populations in mesocosms is a function of their relatively short generation times, the number of times they are exposed, and the persistence of the pesticide in the water column (Fairchild *et al.* 1991). In that study, zooplankton responded much more dramatically to pulsed addition (6 dosing periods over 12 weeks) of esfenvalerate than to similar concentratioins dosed twice over the same period. There was a minimal effect on cladocerans in ponds dosed one time at 0.1 mg liter^{-1} carbaryl (Hanazato and Yasuno 1990). In ponds dosed twice, the cladocerans were able to recover within to 15 days. However, in ponds dosed 10 times over a period of 20 days, each time at the 0.1 mg liter^{-1} concentration, the cladocerans failed to recover.

Recovery of copepod densities after permethrin application in this experiment occurred after 2 weeks, even in the highest concentration. The major source of recovery was the appearance of the neonatal individuals (Figures 3 and 4). High resistance of eggs of these copepods to a variety of the environmental stressors has been noted by previous investigators (Makrushin 1984). It is important to note that another route of recovery for planktonic populations exists, that of immigration of smaller or younger forms into the experimental ponds. This remains an uncontrolled variable in similar ponds and mesocosm studies.

The effect of pesticides on aquatic organisms needs to be studied relative to the potential for recovery and recolonization of the species. An important aspect of disturbance on communities is the rate

at which the habitat becomes less suitable in relation to the requirements of the organisms (Southwood 1988). In effect, the number of dosing intervals and the concentration of the pesticide become points on disturbance and adversity axes. Over sufficient time, disturbance and periods of adversity have a strong influence on the biotic structure of communities. Incorporating such factors into future mesocosm studies will allow a study of species dynamics relative to the degree and frequency of disturbances.

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THE EFFECT OF HEAVY METALS AND CHLORPYRIFOS, SEPARATELY AND IN COMBINATION, ON A CONTINUOUS FLOW MESOCOSM AQUATIC SYSTEM

by

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ABSTRACT

Pollutants selected for testing were heavy metals and the pesticide chlorpyrifos. A flow-through aquatic system was found to be useful to test biological effects of these toxicants separately and in combination. The continuous flow is more natural, as pollutants are usually being continuously added to a waterbody. This ecosystem-level testing is seen as the only strategy available to obtain ecologically relevant information. A good fit of predicted and observed effects shows that mathematical modeling can be applied to such systems.

INTRODUCTION

Test systems at different levels of biological order can be used to identify potential impacts of pollutants in an attempt to predict possible damage that would occur in natural ecosystems. Ecosystem-level testing is advocated as the only strategy available to obtain ecologically relevant information, and a variety of model ecosystems are used in studies of aquatic pollution (Andrushkaitis et al. 1989, Lundgren 1985).

This research was carried out by the Experimental Ecology Laboratory of the Institute for the Biology of Inland Waters, Russian Academy of Science, as a part of the joint USA and USSR project on the evaluation of techniques used to predict effects of environmental pollutants. In 1986, the Environmental Research Laboratory at Duluth, Minnesota, performed studies on effects of chlorpyrifos on a natural aquatic system using littoral enclosures. We developed a flow-through mesocosm test system using 1.5 m^3 tanks designed to measure toxicant effects on main ecosystem structure variables. The rationale behind this test system is that it is large enough to include larger organisms, such as fish, and it is more easily

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controlled and monitored than bigger systems such as ponds. The continuous flow regime was chosen because flow-through systems tend to approach a steady state depending on system inputs and, therefore, are easier to control and to model mathematically. The other factor which contributed to the decision to work with this system was to compare different approaches to the same problem used in the USA and USSR. Since many contaminants contribute to the pollution of natural aquatic systems, it was of interest to look at effects of the combination of some pollutants. Heavy metals and the pesticide chlorpyrifos were selected because of the high priority status given these substances in both countries.

METHODS

Twenty-four fiberglass tanks half filled with water were placed into a pond and served as a thermostat. The experiment was conducted at natural illumination and temperature. The tanks were filled to a volume of 1.5 m^3 with pond water containing natural plankton assemblages, and a 5 cm thick layer of bottom sediments taken from the same pond was added to each tank. The pond water first passed through plankton gauze net (mesh size 80 μm) and was then continuously pumped into the tanks at a dilution rate of 0.33/day. The same kind of net was installed at the outlet of each tank to prevent washout of organisms bigger than 80 μm.

The experimental design consisted of eight treatment tanks, each with three replicates. Treatment tanks (the first four tanks were without fish) were as follows: 1 = chlorpyrifos (Ch), 2 = heavy metals (HM), 3 = chlorpyrifos and heavy metals (Ch + HM), 4 = control (C); the second four, tanks 5-8, received the same treatments but with fish (+ F). The HM included zinc, cadmium, copper, and mercury in concentrations of 150.0, 15.0, 7.5, and 1.5 μg/L, respectively. The mixture of the HM was continuously supplied to the tanks by a diluter designed by G. A. Vinogradov (Vinogradov and Tagunov 1989) to provide the above concentrations in the total inflow. These concentrations were selected taking into account our mesocosm experiments of 1989 which revealed different crustacean species' sensitivity to the heavy metals used in this research. Chlorpyrifos was added manually once a week to attain a concentration in the tank of 0.1 μg/L. This concentration was chosen on the basis of the results of preliminary 10-day experiments with zooplankton communities, which showed that this chemical causes statistically significant changes in the abundances of crustaceans without elimination of any of the zooplankton groups. Fish treatments initially contained 10 bream (*Abramis brama*) underyearlings (each weighing 0.4 g on the average) per tank. According to preliminary 10-day experiments, such a density of fish exerted the same influence on zooplankton as the toxicants.

To measure effects of the toxicants, we monitored weekly what we believe to be principal structural ecosystem variables. They included phytoplankton (chlorophyll *a* concentration), bacterioplankton (number of cells), and zooplankton (number and size structure of crustaceans and rotifers). Concentrations of principal ions, nutrients, dissolved oxygen, and organic matter were determined to provide information about general conditions under which the experiments were performed. No significant differences were found in these parameters among treatments and over the time period. Average values are given in Tables 1-4. Fish studies included determinations of linear and mass growth of fish and their resistance to flow.

TABLE 1. CONTENTS OF PRINCIPAL IONS IN THE MESOCOSM WATER, MMOL/L

Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO^{-}	SO^{--}	Cl^{-}	pH
0.43	1.04	0.21	0.10	1.70	0.22	0.15	6.9-9.0

TABLE 2. CONCENTRATIONS OF NUTRIENTS (MG/L), DISSOLVED ORGANIC MATTER (DOM, MG/L), AND DISSOLVED OXYGEN (DO, MG/L) IN THE MESOCOSM WATER

Nitrate	Nitrite	Ammonium	P miner	P total	DOM	DO max*	DO min*
50	1.0	30	20	40	10	9.0-12.8	6.8-9.0

*DO max measured at 1 pm; DO min measured at 5 am.

TABLE 3. TEMPERATURE REGIME IN MESOCOSMS

	25/07	02/08	09/08	16/08	23/08	30/08	12/09
T min	16.7	15.8	18.3	17.2	18.0	10.1	9.6
T max	20.6	19.3	23.7	20.8	17.3	13.2	11.3

TABLE 4. AVERAGE MEASURED CONCENTRATIONS OF HEAVY METALS IN THE CONTROL AND HEAVY METAL TREATMENTS, MG/L (MEANS AND STANDARD ERRORS)

Control	Heavy metal treatments			
Zn	Zn	Cd	Cu	Hg
14.9 1.9	103.4 17.2	- *	- *	< 1

*Below analytical detection level.

To analyze experimental results, we used mostly multivariate statistics (Dillon and Goldstein 1984). Differences among treatments and effects were assessed using 2-way ANOVA with repeated measures (Vanni 1986). To predict major changes in the principal ecosystem living components in response to the influence of some anthropogenous pollutants (such as heavy metals and pesticides), a mathematical model was developed.

MODEL DESCRIPTION

Because of the lack of space, only some principal aspects of the model are described here. The model includes the usual components of trophic levels found in any ecosystem: higher aquatic plants, planktonic and periphytic (or benthic) algae and bacteria, herbivorous and carnivorous zooplankton, and fish. Dead organic matter is represented by detritus (particulate matter), dead plants, and fish.

For the purpose of ecosystem simulation, various biotic components have been subdivided into groups, based upon size, trophic function, and sensitivity to a toxicant. For instance, copepods are divided into two trophic groups, herbivorous and carnivorous. However, to assess their sensitivity to the toxicant, they are classified as one group. Further, algae are divided into two size classes to measure growth rates and consumption by herbivores; however, they are combined into one group to assess their sensitivities to the toxicant. Herbivorous copepods and cladocera are lumped into one group of "herbivorous crustacea," but they are split into two groups according to their sensitivity to toxicants (i.e., cladocera are generally much more sensitive to toxicants than are copepods). This generalization is carried through into the model equations (Baudouin and Scoppa 1974, Anderson 1982, Kaushik et al. 1985).

Mathematically, the model is essentially a system of differential equations. These are mass balance equations describing the flow of one chemical element through various trophic levels in aquatic systems. It is nitrogen in the model, but could be applied to any other biostructural element without changing the model structure. The model is based upon the following assumptions: growth rates of the organisms are functions of food substrate concentrations which follow generally accepted Michaelis-Menten kinetics. Equations for the living components are Verhulst-Pearl equations, whereby the natural mortality of the organisms is proportional to the square of their biomass. A limiting substrate for the planktonic and periphytic algae and bacteria is assumed to be mineral nitrogen dissolved in the water. Higher plants are assumed to take their nitrogen from a source external to (not part of) the system (e.g., deeper layers of the bottom sediments). However, the material is added to the system during lysis of dead bodies; therefore, this assumption is valid for only rooted submersed vegetation.

As for benthic and periphytic organisms, their carrying capacities are assumed to be functions of the area available for their settling, i.e., surfaces of bottom sediments, higher plants, and side walls (or banks) of the containers.

Generally, the equations describe biomass growth, food consumption (including grazing and predation), natural mortality, and the effect of chemical toxicity to these biotic

components. Coefficients of feeding selectivity are incorporated using the entire diet, with proportions established based upon percentages in the diet.

At this initial stage, the model is meant to describe changes over relatively short time periods, usually used in experimental studies (not more than 2 months). For these shorter time periods, seasonal periodicity in the organisms' reproduction, and changes in the light and temperature regime can be neglected. The model will serve to describe batch (static) and flow-through systems.

Model parameters used in simulations were taken from the literature (Eppley et al. 1969, Buhr and Miller 1983, Kokova 1982, Kruychkova 1974, Zaika 1972, 1983). It should be noted, however, that cited literature sources do not usually contain the needed parameters; the latter had to be recovered from the quantitative data provided in the sources. With these parameters, the model predicted correctly (qualitatively and in many cases, quantitatively) the effects of factors applied in the experiments. After experimental data were obtained, the model was calibrated to achieve a better fit of predicted and observed effects. Tables below contain model simulation data after such calibration.

RESULTS AND DISCUSSION

Here we present the results of observations of the main ecosystem variables over a period of 1 month (from July 27 until August 27) during which time toxicants were applied to the experimental mesocosms. The period of recovery that followed is not considered. The most pronounced changes due to toxicant application occurred in the zooplankton community.

The natural seasonal course of zooplankton, as observed in the control mesocosms, was characterized by an increase in *Cladocera* from 25 to 55% of total zooplankton abundance. At the same time, *Copepoda* decreased from 72 to 38%. The abundance of *Rotifera* only slightly increased during the observation period (from 3 to approximately 15%).

Effects of the toxicants, separately or in combination, are shown in Tables 5-9. They were calculated as differences between the means of treatments and corresponding controls, so that positive values mean an increase and negative values a decrease compared to the control. Fish are also considered as a factor which influences other ecosystem variables.

Since biomasses of the mesocosm living components were not estimated and the model provided data in terms of biomass, we do not compare here the time courses of the components in the model and the experiments. Still, we can assess effects of factors within the model and the experiments and compare these effects. Comparisons are valid provided there are no significant differences in the size structure of the compared populations among treatments, so that linear relationships between biomass and number may be assumed. This was not the case with crustaceans; therefore, model values of their biomasses were converted into organism numbers for assessments of the effects, taking into account the size structure of their populations.

TABLE 5. EFFECTS (PERCENT OF CONTROL) OF INDIVIDUAL FACTORS UPON MAIN ECOSYSTEM VARIABLES. UPPER LINE FOR EACH CELL REPRESENTS EXPERIMENTAL DATA, LOWER LINE = COMPUTER SIMULATION, SIGN. 1 = SIGNIFICANCE LEVEL

Ecosystem variables	Heavy metals		Chlorpyrifos		Fish	
	Effect	Sign. 1	Effect	Sign. 1	Effect	Sign. 1
Cladocera	-44.76	<0.01	-53.24	<0.01	-44.26	<0.01
abundance	-36.30	-	-57.54	-	-50.25	-
Copepoda	16.78	0.07	33.32	<0.01	53.34	<0.01
abundance	11.39	-	21.65	-	49.29	-
Rotifera	-42.66	<0.01	37.31	<0.01	75.36	<0.01
abundance	-29.22	-	11.34	-	73.16	-
Algae	-4.25	0.35	24.29	<0.05	60.04	<0.01
abundance	3.94	-	4.43	-	13.57	-
Bacteria	9.24	0.25	12.60	0.14	43.80	<0.01
abundance	8.33	-	13.41	-	27.26	-
Number of species						
Cladocera	-5.64	0.20	-26.15	<0.01	-6.64	<0.05
Copepoda	-1.87	0.35	-18.44	<0.01	-5.88	0.08
Rotifera	-11.70	<0.05	-3.02	0.31	24.46	<0.01

Analysis of the whole body of data (Tables 5-7) has shown that the direct individual effect of heavy metals and chlorpyrifos is a reduction in abundance of the most sensitive species due to acute toxicity. This was indicated by an overall decline in abundance of cladocerans. These crustaceans have been found to be the species most sensitive to toxicants in many other studies (Baudouin and Scoppa 1974, Hurlbert 1975, Kaushik et al. 1985). In our experiments, only three species persisted in the toxicant treatments. These were *Ceriodaphnia quadrangula, Bosmona longirostris*, and *Daphnia longispina*.

Judging by a reduction in the number of species, both types of pollutants are directly toxic to copepods but to a lesser extent than to cladocerans. On the whole, chlorpyrifos appeared to be more toxic than the heavy metals as seen by the greater reduction in cladoceran abundance and a greater and highly statistically significant reduction in species richness of both groups of crustaceans. The mathematical model used here showed a decrease in the biomass of copepods exposed to both toxicants. In real experiments, however, abundance (number of individuals) of the copepods significantly increased. An analysis of the size structure of the copepod population revealed that elimination of larger species and specimens occurred, and the ratio of large to small individuals suffered an almost 5-fold decrease. One of the events was complete elimination of the adults of *Mesocyclops crassus* and *M. oithonoides*. The copepod population consisted mostly of nauplii and copepodites rather than adults, probably because the toxicants induced some

hindrances to growth or moulting. It is only after model biomass data were converted into organism numbers that a good fit of the observed and predicted effects of factors was obtained.

As seen from Table 5, the mixture of heavy metals in the concentrations used is probably directly toxic to rotifers because the effect is great and statistically significant. In the literature (Moore and Ramamoorthy 1984), rotifers have been noted to be among the organisms most sensitive to heavy metals. The toxic effect of chlorpyrifos is insignificant judging by a decrease in species richness and by the fact that their abundance did not diminish.

Both types of toxicants apparently were not directly toxic to algae and bacteria, as negative effects are small and statistically insignificant. Concentrations of toxicants used in the experiments were below acute toxicity and growth inhibition concentrations reported in the literature for algae and bacteria (Hurlbert et al. 1972, Roberts and Miller 1970, Maly and Ruber 1983, Stratton et al. 1980, Moore and Ramamoorthy 1984).

TABLE 6. EFFECTS (PERCENT OF CONTROL) OF A COMBINATION OF FACTORS UPON MAIN ECOSYSTEM VARIABLES. UPPER LINE FOR EACH CELL REPRESENTS EXPERIMENTAL DATA, LOWER LINE = COMPUTER SIMULATION, SIGN. 1 = SIGNIFICANCE LEVEL, INTER. = INTERACTION

Ecosystem variables	Heavy metals + chlorpyrifos				Heavy metals + fish			
	Effect	Sign. 1	Inter.	Sign. 1	Effect	Sign. 1	Inter.	Sign. 1
Cladocera	-72.05	<0.01	25.85	0.27	-61.99	<0.01	22.93	0.29
abundance	-75.21	-	18.63	-	-73.09	-	13.46	-
Copepoda	54.21	<0.01	4.12	>0.05	86.36	<0.01	16.24	0.35
abundance	35.52	-	2.47	-	67.41	-	6.72	-
Rotifera	-22.35	<0.01	16.99	0.07	0.68	>0.50	-32.03	0.47
abundance	-21.34	-	-3.46	-	12.12	-	-31.81	-
Algae	18.20	0.11	-2.14	0.26	63.22	<0.01	7.43	<0.01
abundance	8.57	-	0.89	-	18.53	-	1.04	-
Bacteria	-4.91	0.36	-1.50	>0.50	56.81	<0.05	3.71	>0.50
abundance	23.77	-	1.33	-	39.72	-	4.11	-
Number of species								
Cladocera	-30.36	<0.01	1.43	0.25	-10.78	0.09	0.50	0.46
Copepoda	-19.49	<0.01	0.82	0.30	-7.63	0.16	0.13	>0.50
Rotifera	-14.33	<0.05	0.39	0.46	10.58	0.15	-2.17	0.35

TABLE 7. EFFECTS (PERCENT OF CONTROL) OF A COMBINATION OF FACTORS UPON MAIN ECOSYSTEM VARIABLES. UPPER LINE FOR EACH CELL REPRESENTS EXPERIMENTAL DATA, LOWER LINE = COMPUTER SIMULATION, SIGN. 1 = SIGNIFICANCE LEVEL, INTER. = INTERACTION

Ecosystem variables	Chlorpyrifos + fish				Heavy metals + chlorpyrifos + fish			
	Effect	Sign. 1	Inter.	Sign. 1	Effect	Sign. 1	Inter.	Sign. 1
Cladocera	-72.63	<0.01	24.86	0.27	-83.75	<0.01	58.41	0.11
abundance	-81.52	-	26.28	-	-88.20	-	55.89	-
Copepoda	103.92	<0.01	17.26	0.21	136.72	<0.01	33.28	0.16
abundance	81.13	-	10.18	-	123.43	-	41.07	-
Rotifera	91.19	<0.01	-21.48	<0.01	-11.38	0.42	-81.39	0.06
abundance	85.57	-	-31.81	-	35.18	-	-20.09	-
Algae	94.32	<0.01	9.69	<0.01	78.72	<0.01	-1.66	<0.01
abundance	19.46	-	1.45	-	25.74	-	3.79	-
Bacteria	24.98	0.28	-23.40	>0.50	17.05	0.23	-23.40	>0.50
abundance	48.49	-	7.79	-	70.69	-	21.67	-
Number of species								
Cladocera	-30.63	<0.01	1.16	0.25	-39.11	<0.01	-1.68	0.16
Copepoda	-22.66	<0.01	1.67	0.10	-21.13	<0.01	5.08	<0.05
Rotifera	19.46	<0.05	-1.97	0.28	-1.94	0.41	-11.67	0.48

When fish are considered as another factor (actually predation by fish), the effect is, superficially, similar to effects of the toxicants; although some principal differences emerge. Fish presence caused almost the same magnitude of decline in cladoceran abundance and in the biomass of copepods as did toxicants, but their effect on crustacean population size structure was more dramatic. Fish predation appeared to be much more size selective than toxicity: the ratio of small cladoceran forms to larger ones in the toxicant treatments increased only slightly over the control ratio, while in the fish treatment, the large forms were near complete extinction. As for copepods, this ratio in the presence of fish was almost twice as great as in toxicant treatments. On the whole, the main effect of fish is selective elimination of large crustacean forms.

Concerning direct effects of fish on rotifers, algae, or bacteria, it seems from the information stated, that fish did not feed on any of them, as their diet consisted mostly of crustaceans.

Several indirect effects of factors applied here were observed. Indirect or secondary effects may be described as changes in ecosystem variables resulting from changes caused by direct toxicity to some most sensitive populations (or by predation in case of fish). The

decline in the cladoceran population due to toxicant effects caused a significant increase in the number of algae (mostly small forms as the model showed), which comprise the main food item for herbivorous copepods and for immature stages of the predators (Wylie and Currie 1991). This led to release of these forms of copepods (and rotifers in the case of chlorpyrifos) from competition pressure and to an increase in their abundance. The model and experimental observations showed an increase in small herbivorous copepods; predators changed very little. Correlation analysis of the whole body of experimental data revealed a negative relationship between abundances of herbivorous copepods and cladocerans ($r = -0.82$, $p < 0.05$), which supports the paradigm of their competition.

Secondary effects of fish addition also included increases in abundances of algae, bacteria, rotifers, and copepods. These effects were even more pronounced than those of the toxicants because in addition to cladocerans, fish also greatly reduced large copepods which are mostly predatory. Therefore, rotifers and herbivorous copepods were released not only from competition but also from predation. Both invertebrate predation and competition have been found to be very important in determining freshwater zooplankton community structure (Vanni 1988, Soto and Hurlbert 1991) and contribute to the scarcity of small

TABLE 8. EFFECTS (PERCENT OF CONTROL) OF INDIVIDUAL FACTORS AND THEIR COMBINATION UPON MAIN ECOSYSTEM VARIABLES WITHOUT FISH. UPPER LINE FOR EACH CELL REPRESENTS EXPERIMENTAL DATA, LOWER LINE = COMPUTER SIMULATION, SIGN. 1 = SIGNIFICANCE LEVEL, INTER. = INTERACTION

Ecosystem variables	Heavy metals		Chlorpyrifos		Heavy metals + chlorpyrifos			
	Effect	Sign. 1	Effect	Sign. 1	Effect	Sign. 1	Inter.	Sign. 1
Cladocera	-51.78	<0.01	-55.82	<0.01	-75.81	<0.01	31.79	0.28
abundance	-35.38	-	-58.02	-	-75.92	-	17.48	-
Copepoda	36.78	<0.05	35.18	<0.05	76.55	<0.01	11.59	>0.50
abundance	13.15	-	28.71	-	46.71	-	4.85	-
Rotifera	-8.20	0.31	46.29	<0.01	15.24	<0.05	19.25	0.17
abundance	-25.34	-	38.14	-	3.70	-	-9.44	-
Algae	32.44	0.09	13.14	0.26	43.77	0.10	-1.80	0.34
abundance	7.34	-	11.78	-	20.15	-	1.02	-
Bacteria	7.99	0.36	-17.63	0.19	-10.90	0.32	-1.26	>0.50
abundance	18.44	-	33.33	-	60.34	-	8.57	-
Number of species								
Cladocera	-8.82	0.21	-24.32	<0.01	-30.17	<0.01	2.98	0.16
Copepoda	-2.41	0.38	-23.02	<0.01	-24.65	<0.01	0.78	0.42
Rotifera	1.46	>0.50	-14.77	0.09	-12.90	0.20	0.40	>0.50

TABLE 9. EFFECTS (PERCENT OF CONTROL) OF INDIVIDUAL FACTORS AND THEIR COMBINATION UPON MAIN ECOSYSTEM VARIABLES WITH FISH. UPPER LINE FOR EACH CELL REPRESENTS EXPERIMENTAL DATA, LOWER LINE = COMPUTER SIMULATION, SIGN. 1 = SIGNIFICANCE LEVEL, INTER. = INTERACTION

Ecosystem variables	Heavy metals		Chlorpyrifos		Heavy metals + chlorpyrifos			
	Effect	Sign. 1	Effect	Sign. 1	Effect	Sign. 1	Inter.	Sign. 1
Cladocera	-30.03	0.12	-48.35	<0.01	-73.62	<0.01	4.75	0.37
abundance	-38.40	-	-46.42	-	-63.62	-	21.19	-
Copepoda	5.46	0.38	31.06	<0.05	37.92	<0.05	1.39	>0.50
abundance	10.24	-	23.66	-	34.81	-	0.91	-
Rotifera	-57.28	<0.01	13.98	<0.01	4.69	>0.50	-38.01	0.17
abundance	-31.79	0	-2.70	-	-33.63	-	0.68	-
Algae	-21.82	<0.05	32.39	<0.01	3.40	0.43	-7.16	0.35
abundance	1.04	-	-1.62	-	-0.59	-	-0.02	-
Bacteria	10.12	0.32	-9.02	0.39	0.21	>0.50	-0.89	>0.50
abundance	1.03	-	0.00	-	1.03	-	0.00	-
Number of species								
Cladocera	-2.15	>0.50	-28.04	<0.01	-30.57	<0.01	0.39	-0.50
Copepoda	-1.29	>0.50	-13.31	<0.05	-13.85	0.06	0.75	0.48
Rotifera	-21.09	<0.01	7.55	0.19	-15.56	0.07	-2.01	0.29

plankton in fishless habitats. Therefore, the indirect effects of fish in our experiments consisted in the removal of invertebrate predators and large herbivores, thus securing the proliferation of small planktonic organisms. The model also showed an increase of protozoans which were not observed in the experiments.

As far as the experimental design including treatments where more than one factor was present at a time, it was possible to assess the combined effect of factors and to find out if the factors interacted with each other. Interactions were calculated as differences between observed effects of two or three factors present in a given treatment and the sum of the individual effects of these factors taken from other treatments. This sum was considered to be equal to the additive effect. Therefore, if the interaction effect coincides by its sign (plus or minus) with the observed combined effect of factors, the interaction is synergistic; if not, it is antagonistic. As seen from Tables 2 and 3, a combination of factors in most cases caused purely additive effects; interactions of factors were small in magnitude and statistically insignificant. Only some interactions involving fish and observed through the effects on rotifers and algae were large and significant, but these are indirect secondary effects, so these interactions may not be interactions of the factors themselves but just reflections of interactions of the ecosystem living components.

To see whether an ecosystem with one vertebrate predator on the top differs in responses to toxicants from an ecosystem without such a predator, we divided the experimental data into two, one with fish and another without fish, and performed the same kind of analysis separately on each half. The results are presented in Tables 4 and 5.

It can be seen from the tables that, in the presence of fish, effects of both toxicants on crustaceans were damped down, the effect of heavy metals on rotifers increased, and the effect of chlorpyrifos diminished. It seems that there is no regularity in this pattern, but the following reasoning may serve as a probable explanation. If a living population is well developed and flourishing, then negative effects on it are pronounced, the positive ones are barely observed, and vice versa. Cladocerans are well developed without fish, so adverse effects of the toxicants were manifested better than with fish. Copepods attained high numbers in the presence of fish; therefore, the stimulatory effect of toxicants on their abundance was smoother. Rotifers flourished in the presence of fish so the negative effect of heavy metals on them was amplified, and the positive effect of chlorpyrifos was diminished. Of course, these explanations should be taken cautiously because the fish presence imparted great variability to experimental results and rendered many values statistically insignificant. As for effects on algae and bacteria, at the present stage of the investigation they are hard to interpret.

When fish are considered as another ecosystem component, the effects of toxicants on their principal characteristics can be determined. As seen in Fig. 1, heavy metals alone and in combination with chlorpyrifos, each induced almost 2-fold decreases in linear growth and weight of fish. The greatest negative effect was caused by the combination of heavy metals and the pesticide. In view of this, the significant stimulation of fish growth by 25% compared to the control that was observed in the chlorpyrifos treatment is of great interest.

Investigations of resistance of fish to waterflow, reflecting general physiological fitness of fish (Pavlov and Fomin 1978), showed that the underyearlings which had been exposed to chlorpyrifos manifested the greatest resistance to flow (Fig. 2). These were, on the average, 18% more resistant than those in the control. In the heavy metals treatment, they showed 5% lower resistance, and in the treatment heavy metals + chlorpyrifos, their resistance was reduced by 20%. After 30 days of recovery from toxicants, the length and mass of fish exposed to combined effects of chlorpyrifos and metals was greater than in control fish. The largest were fish from the chlorpyrifos treatment, although differences in size and weight were, on the whole, statistically insignificant.

If we judged the whole ecosystem by fish conditions, it would seem that the ecosystem flourished when exposed to chlorpyrifos since the top link of the trophic chain was in a better state than in clear water. This misleading evidence shows that the whole ecosystem testing involving many organisms belonging to different trophic levels is more appropriate to assess toxicant effects.

CONCLUSIONS

In general, results presented here agree well with those obtained by the Environmental Research Laboratory, especially concerning major effects of toxicants on the

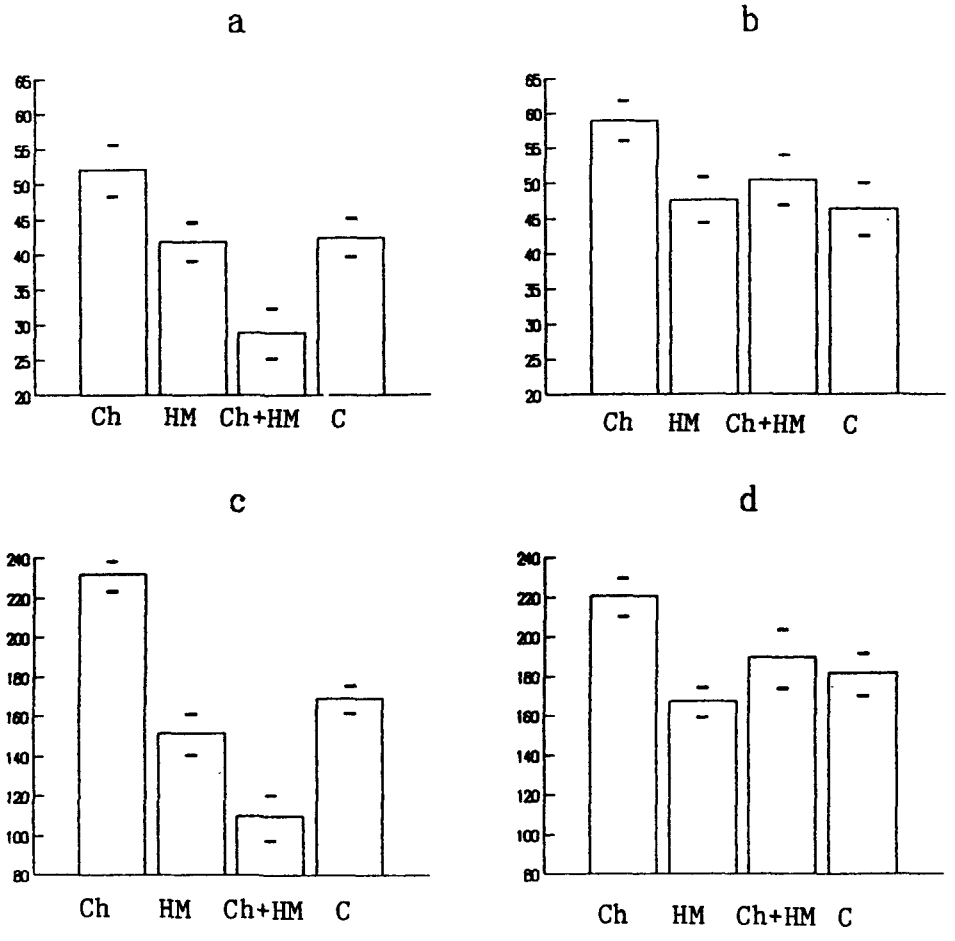

Fig. 1. Growth in mass and length of bream underyearlings. Experimental treatments: Ch = chlorpyrifos, HM = heavy metals, Ch + HM = chlorpyrifos + heavy metals, C = control; a) increases in length after 30 days of exposure, b) increases in length after 30 days of recovery, c) increases in mass after 30 days of exposure, and d) increases in mass after 30 days of recovery.

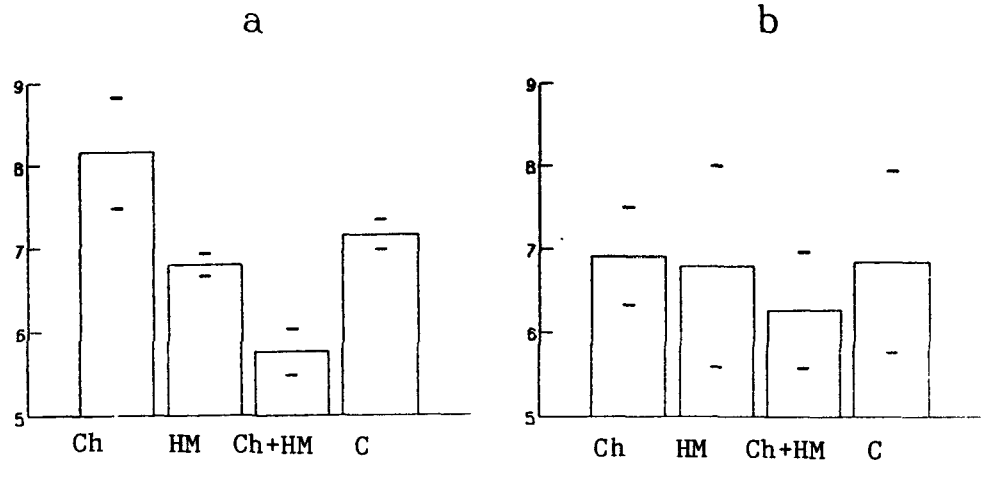

Fig. 2. Resistance of the fish to flow. Index of resistance of fish to flow expressed as V/L, where V is velocity of the flow (cm/sec) and L is length of the fish in sm; a) resistance of bream to flow after 30 days of exposure and b) resistance of bream to flow after 30 days of recovery.

zooplankton community (U.S. EPA 1986). Earlier, similar effects were shown in the studies of Moscow University using static mesocosms (Filenko 1984). Results demonstrate the flow-through mesocosm protocol's utility to test biological effects of different kinds of toxicants used separately or in combination. In some aspects, the continuous system is better than stationary systems, as it allows easier simulations of natural conditions, whereby pollutants are usually being continuously added to a waterbody. A good fit of the predicted and observed effects of anthropogenous factors shows that mathematical modeling can be successfully applied to such systems.

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BIOCHEMICAL AND PHYSIOLOGICAL INDICATORS OF CONTAMINANT STRESS IN AQUATIC ORGANISMS OF LARGE RIVER SYSTEMS

by

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ABSTRACT

Examination of the biochemical and physiological responses of organisms to xenobiotic chemicals has been an area of scientific investigation for many years. Historically, these techniques have been used in the development and screening of natural and synthetic pharmaceuticals, biocides, and other biologically active chemicals and in elucidating their modes of action. However, only recently have these techniques been applied as indicators of the nature and extent of chemical pollution of aquatic ecosystems. Today, research on the effects of chemical contaminants at the molecular level of biological organization is one of the most rapidly developing and intensively studied areas in the field of environmental toxicology. In this paper, we discuss the relationship of biochemical and physiologic responses to effects at higher levels of biological organization, examine general categories and provide specific examples of techniques that are presently being applied to aquatic systems, and provide recommendations for implementation of biochemical and physiological techniques in aquatic contaminant assessment programs.

INTRODUCTION

Water, soil, and sediments serve as the ultimate sinks for most chemicals produced and used by man. General sources of anthropogenic chemical contaminants are from industrial, agricultural, and urban uses. These uses result in both point and nonpoint source pollution of our environment. Large rivers are of particular concern because they receive and integrate pollutants from man's activities and land use practices over large geographical regions. Large rivers are the direct recipients of municipal and industrial effluents and additionally receive contaminant loadings from tributaries and agricultural practices in their watersheds.

These multiple sources of contaminant input result in complex mixtures of contaminants being present in the water and sediments of large river systems. Unfortunately, most of our knowledge and understanding of the effects of chemical contaminants in aquatic organisms is based upon the effects of single compounds tested in the laboratory. Much less is known about the effects of complex environmental mixtures. Field studies can document the correlation of population-level effects with contaminant exposure, but the complexity of environmental variables and multiple contaminant exposure makes it difficult to establish cause and effect relationships with specific chemical pollutants. Environmental scientists are presently focusing more effort on

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expanding our understanding of the effects of pollutants at the molecular level to help discriminate among the various environmental stressors affecting aquatic populations and communities.

Management practices for aquatic resources and regulatory practices regarding contaminant discharge are primarily directed at the population level. However, biochemical and physiological measures of contaminant stress have received increased attention in recent years due to their rapid response, sensitivity, and potential to provide a further level of discrimination in the environmental risk assessment process. In this paper, we discuss the relationship between contaminant effects at the biochemical level and those at higher levels of biological organization, examine general categories and specific examples of techniques that can be applied to aquatic systems, and provide recommendations for integration of biochemical and physiological measures of contaminant stress with other environmental risk assessment procedures.

RELATIONSHIP OF BIOCHEMICAL AND PHYSIOLOGICAL STRESS TO EFFECTS AT HIGHER LEVELS OF BIOLOGICAL ORGANIZATION

A basic premise in the field of toxicology is that to elicit a toxic response, a chemical must first reach and interact with its biomolecular site of action. This response on a molecular level may then be followed by a tissue response, an organ response, a whole animal response, and finally a population or community response, but it must occur first at the biomolecular level.

Each level possesses an inherent resiliency, or assimilative capacity, that allows it to maintain homeostasis. To provide a continuum of response, the magnitude of effect at each level of organization must be great enough to exceed the assimilative capacity of the next higher level. Ultimately, for a contaminant to elicit a response at the population or community level, it must exceed the capacity of all lower levels to absorb or mitigate the insult.

These relationships and interdependencies provide both promise and challenge to the use of biochemical and physiological responses as indicators of aquatic community health. First, it is apparent that biochemical changes must be the first and most sensitive biological responses that occur following contaminant exposure. Thus, bioindicators may be useful as early warning indicators of contaminant problems. Alternatively, because the continuum may be broken at subsequent levels of biological organization, effects at the biochemical level may not be ecologically significant. Thus, the challenge facing the environmental scientist is to develop an understanding of the magnitude of effect at the biochemical level that is required to elicit an ecologically significant response at higher levels of organization.

Toxicants interact with organisms on the molecular level in a variety of ways that can challenge the maintenance of homeostasis. Metals can bind to various proteins disrupting membrane integrity, ion transport, and cellular metabolism. For example, mercury can bind to sulfhydryl groups on structural proteins (Luckey and Venugopal 1977), thereby disrupting membrane integrity. Copper can interfere with ion transport by affecting gill ATPase activity, reducing the ability of coho salmon (*Oncorhynchus kisutch*) to adapt to seawater (Lorz and McPherson 1976). Lead can deactivate δ-aminolevulinic acid dehydratase (ALAD), disrupting red blood cell metabolism.

Organic contaminants also interact with organisms on the molecular level by disrupting membrane integrity and function, displacing endogenous substrates of enzymes, or by diminishing energy reserves through increased demands upon detoxification mechanisms. For

example, pentachlorophenol uncouples oxidative phosphorylation by interacting with the mitochondrial membrane (Moreland 1980). Organophosphates and carbamates can displace acetylcholine and inhibit its hydrolytic cleavage by acetylcholinesterase (ACHe), thereby affecting nerve transmission (O'Brien 1976). Widdows and Donkin (1991) report a variety of ways that toxicants can diminish the energy reserves of an organism to the detriment of survival.

To cause effects at higher levels of biological organization, the effects at the molecular level mentioned above must be of sufficient severity to challenge the organism's ability to maintain homeostasis. Organisms invite a variety of mechanisms for dealing with toxicants. In some cases, they may be able to detect and avoid the toxicant (Folmar 1976). They may sequester the toxicant (e.g., through induction of metal-binding proteins, such as metallothioneins), or they may metabolically alter the compound to a less toxic form that may be more readily eliminated (e.g., oxidative metabolism of pyrethroid insecticides). Alternatively, they may simply repair the cellular damage caused by the toxicant. Each of these mechanisms incurs some physiologic cost on the part of the individual organism (Calow 1991). When the organism's ability to absorb or mitigate the toxic insult is exceeded, stress will be applied to the higher levels of organization such as the population or community.

At present, a wide range of biochemical and physiological parameters are known to be responsive to chemical exposure. However, our understanding of how these responses are quantitatively linked to effects at the aquatic population and community levels is much more limited. Relatively few of the measurable biochemical and physiological responses are specific to certain chemical classes (e.g., ACHe inhibition by organophosphates and carbamates; ALAD inhibition by lead). The majority of responses are sensitive to a wide variety of chemical compounds and other environmental stressors. Thus, with a few exceptions, the majority of biochemical and physiological measures are more useful as *general indicators* of organism health rather than *specific indicators* of specific chemical exposure. It is important to recognize the range of sensitivity of the indicators we use in aquatic contaminant risk assessment, because our goal is to establish cause and effect between contaminant exposure and responses at the population and community level. Responses that are general indicators of organism health must be used in concert with other measures of chemical exposure, such as chemical residue analysis. Responses that are more specific indicators of individual chemical exposure must be calibrated to quantify the continuum of response from the biochemical to the population and community level.

Within the broad range of measurable biochemical and physiological parameters, a further distinction can be made between *regulated parameters* and *regulatory mechanisms*. Regulated parameters are usually maintained within relatively narrow ranges to maintain physiological function. Contaminant exposure that displaces these parameters beyond the tolerated range will usually result in acute injury or death (Zachariassen et al. 1991). However, the regulatory mechanisms that govern these parameters may fluctuate much more widely before significant injury or death occurs in an attempt to maintain the optimal range, and may be more responsive and sensitive indicators of contaminant insult (Zachariassen et al. 1991). For example, the level of ATP present in an organism is a highly regulated parameter. ATP levels can be maintained at the expense of other endogenous high-energy phosphate compounds. Aunaas et al. (1991) demonstrated that sublethal exposure of the blue mussel (*Mytilus edulis*) to contaminants generally resulted in larger fluctuations of components of the phosphate pool other than ATP. Thus, the regulatory mechanism (the phosphate pool) was more responsive than the regulated parameter (ATP).

The range of sensitivity, level of variability, and relationship to responses at higher levels of biological organization are all important parameters for consideration in selecting biochemical and physiological indicators for application in aquatic contaminant risk assessment programs.

CATEGORIES AND EXAMPLES OF TECHNIQUES

Biochemical and physiological indicators of contaminant stress can be categorized in a variety of ways--general versus specific sensitivity to compounds, regulatory versus regulated parameters, indicators of exposure versus indicators of effect, or by category of biochemical and physiological function. In this section, we will identify general categories of biochemical and physiological function and discuss representative examples of indicator techniques for each category that are presently being used or show promise for further development.

OSMOREGULATION

A variety of inorganic and organic environmental pollutants affect osmoregulation in fishes. For this reason, many osmoregulatory stress indicators are more useful as indicators of general organism health rather than diagnostic tools for identification of specific pollutants.

Plasma ion concentrations are responsive to heavy metal exposure. Sublethal exposure to copper causes transient decreases in plasma chloride concentrations in brown bullheads (*Ictalurus nebulosus*) and brook trout (*Salvelinus fontinalis*); however, the levels return to normal after about 3 weeks even during continuous exposure, indicating the presence of a compensatory mechanism for maintenance of homeostasis (Christensen et al. 1972, McKim et al, 1970). Zinc and cadmium also affect plasma ion concentrations (Lewis and Lewis 1971, McCarty and Houston 1976).

Gill ATPase activity in fishes is also sensitive to chemical exposure. This measure of osmoregulatory ability is affected by a wide range of environmental pollutants including copper (Lorz and McPherson 1976), PCBs (Davis et al. 1972), and a variety of chlorinated pesticides (Davis et al. 1972). Gill ATPase activity has direct ecological relevance in that it is intimately linked to the migratory success of anadromous fish species (Epstein et al. 1967, Zaugg and Wagner 1973).

Histological and histochemical examination of gill tissue has also been used successfully to demonstrate the effects of osmoregulatory stressors. As with ATPase activity, a wide variety of environmental contaminants can cause histological lesions in fish gill. Aluminum (Jagoe et al. 1987), copper (Baker 1969), mercury (Wobeser 1975), sodium arsenite (Gilderhaus 1966), and zinc (Skidmore and Tovell 1972) are some of the metals that cause gill lesions in fishes. A wide variety of organic compounds also cause histological lesions in fish gill. Endrin (Eller 1971), heptachlor (Andrews et al. 1966), methoxychlor (Lakota et al 1978), mirex (Van Valin et al. 1968), naphthalene (DiMichele and Taylor 1978), and phenol (Mitrovic et al. 1968) are only a few of the organic contaminants that cause gill lesions.

METAL SEQUESTRATION AND REGULATION

Metallothioneins are a class of metal-binding proteins found in a wide variety of organisms (Roesijadi 1992) that are inducible after exposure to Cd, Cu, Hg, and Zn (Noel-Lambot et al. 1978). Metallothioneins are thought to reduce the toxicity of heavy metal exposure by sequestration (Hamilton and Mehrle 1986), with toxicity occurring only after its binding capacity is

exceeded (Brown and parsons 1978). Hepatic metallothionein levels in feral fishes from lakes contaminated with Cu, Zn and Cd are closely correlated to metals exposure (Roch et al. 1982). The inducible nature of metallothioneins suggests that they represent a regulatory mechanism, and as such provide a useful indicator of exposure to certain metals. Other metal-binding proteins such as ferritin (Fe) and copper-chelatin (Cu) have been less thoroughly studied in aquatic organisms.

OXIDATIVE METABOLISM

Oxidative metabolism serves three important functions in eukaryotic organisms that provide useful biochemical indicators of stress. First, oxidative metabolism plays a central role in catabolic energy production. The resultant storage currency is adenylate, and its utility as a biochemical indicator of contaminant stress is discussed in the next section.

Secondly, oxidative metabolism is associated with the reduction of oxygen radicals and the maintenance of oxidative homeostasis within the cell. This critical function also lends itself for use as a biochemical indicator of stress in aquatic organisms. Oxygen radicals are formed as intermediates of cytosolic enzymes, electron transfer systems, and by activated cells of the immune system. Inducible prophylactic enzymes such as superoxide dismutase, catalase, and glutathione peroxidase serve a vital role in protecting the cell from oxidative stress and are useful indicators of contaminant stress in aquatic organisms (DiGiulio et al. 1989). Additionally, the pools (and relative ratios) of reducing equivalents (e.g., glutathione, α-tocopherol, and ascorbate) that are required to maintain oxidative homeostasis are useful indicators of contaminant stress. These antioxidant defense mechanisms of the cell are sensitive to compounds that block or inhibit electron flow and, in particular, those that can undergo cyclic reactions (redox cycling) to produce oxygen radicals. These important classes of environmental contaminants include paraquat, diquat, and various amines.

The third, and probably the most thoroughly studied, function of oxidative metabolism is xenobiotic metabolism. Xenobiotic metabolism associated with the cytochrome P450 monooxygenases (MO) has been reviewed for use in biomonitoring by Payne et al. (1987), who concluded that induction of MO could be a sensitive biochemical indicator of exposure in many aquatic organisms. Induction of MO activity is caused by a wide variety of chemical contaminants (e.g., PAHs, PCBs, PCDDs, and PCDFs). The MO system plays a central role in the detoxification of these and many other hydrophobic compounds largely by facilitating their elimination. Additionally, MOs are highly conserved phylogenetically. All of these characteristics enhance the potential utility of MO activity as a biochemical indicator of contaminant stress. However, its use in feral organisms has been limited because of the confounding factors of age, sex, reproductive status, diet, disease, and general health conditions that can all greatly affect the MO response to xenobiotics (Neal 1980). A thorough understanding of the effects caused by these variables is lacking in most species of fish and wildlife. Even if these relationships were understood in aquatic species, it is often difficult to obtain such information on feral organisms. Thus, MO activity in this context offers a more qualitative than quantitative measure of contaminant exposure. However, successful use of MO activity as a quantitative bioindicator of contaminant stress in the field has been accomplished using caged fish studies (Lindstrom-Seppa and Oikari 1990) and by using developing embryos (Hoffman et al. 1987). In both of these cases, the organismal and environmental factors affecting MO activity could be controlled or monitored during the period of exposure.

Another alternative to limiting the confounding factors that influence MO activity is the use of *in vitro* systems where the conditions are tightly controlled. In these assays, the environmental contaminant mixture is brought to the laboratory to expose the system, as opposed to taking the system (organisms) to the environment for exposure. An example of such an *in vitro* model system for MO activity is the H4IIE hepatoma cell bioassay (Tillitt et al. 1991). In this bioassay, environmental contaminants are extracted from the species of concern and serial dilutions are used to dose the H4IIE cells. After a period of incubation, ethoxyresorufin-o-deethylase (EROD) activity is measured in the cells. The MO response of the H4IIE cells is highly correlated to the effects observed in the whole organism (Safe 1987). Therefore, the MO response *in vitro* can integrate the interactions of complex mixtures of environmental contaminants at the cellular/biochemical level and serve as a quantitative measure of both exposure and effect in the whole organism from which the contaminants were extracted. Recent studies have demonstrated the ability of the H4IIE bioassay to be predictive of contaminant responses at higher levels of biological organization, such as egg mortality rates in PCB-exposed populations of fish-eating birds (Tillitt et al. 1992).

MAINTENANCE OF ENERGY STATUS

Adenylate energy charge is a term developed by Atkinson (1968) to describe the dynamic equilibrium between the various components of the cellular energy system--ATP, ADP, and AMP. It is calculated as (ATP + 0.5 X ADP)/(ATP + ADP + AMP) and its value reflects the energy status of the organism. Adenylate energy charge is sensitive to a variety of environmental factors such as food availability and temperature (Moal et al. 1991), season (Giesy and Dickson 1981), salinity (Ivanovici 1980), and pollutants (Verschraegen et al. 1985). Because ATP levels are regulated within relatively narrow limits in many organisms, Aunaas et al. (1991) have proposed the use of a phosphorus index--which reflects the status of a larger phosphorus pool--as a more sensitive indicator of energy status. The broad spectrum of environmental stressors to which energy status is sensitive indicates that this biochemical parameter may be most useful as a general indicator of organism health, rather than a specific indicator of contaminant exposure.

REPRODUCTION

Another category of biochemical and physiological function that is sensitive to contaminant exposure is reproduction. The complexity of the reproductive process in aquatic organisms and the confounding influence of behavior, nutritional status, seasonality, and other variables makes it difficult to ascertain the effect of contaminants on the reproductive success of natural populations. However, a number of biochemical reproductive parameters have been investigated in the laboratory in terms of their sensitivity to contaminant exposure. Vitellogenin is the major yolk protein in salmonids and many other species. It is produced in the liver and transported to the ovaries in the blood. Blood levels of vitellogenin are elevated during ovary maturation. Vitellogenin levels in brook trout and rainbow trout are affected by low pH exposure (Tam et al. 1987, Parker and McKeown 1987). Reproductive endocrine function in fishes has also been studied to a limited extent in terms of the effects of contaminants on steroid hormone levels. Thomas (1990) demonstrated an increase in plasma hormone levels in Atlantic croaker (*Micropogonias undulatus*) after exposure to lead, benzo(a)pyrene, or Arochlor 1254; vitellogenin levels were similarly affected by cadmium and PCBs.

NEUROTRANSMISSION

One of the more specific biochemical indicators of contaminant exposure is measurement of the neurotransmitter acetylcholinesterase (ACHe). Organophosphates and carbamates are specific inhibitors of ACHe activity (O'Brien 1976), although other physiological factors can influence activity levels. It should be noted that measurement of brain activity levels represents primarily true acetylcholinesterase whereas plasma levels generally reflect both acetylcholinesterase and butyryl(pseudo)cholinesterase (O'Brien 1976). Depressed ACHe activity is associated with a variety of behavioral responses in fishes (Symons 1973) and it has been measured in feral fishes from polluted waters (Williams and Sova 1966). However, brain activity levels resulting in death of fishes can be quite variable (Weiss 1961).

INTERACTIONS WITH GENETIC MATERIAL

The genotoxic effects of contaminants are receiving increased interest in the area of environmental contaminant assessment. A variety of techniques are becoming available for use in aquatic systems. Detection of the presence of DNA adducts using ^{32}p-postlabeling is one method of assessing exposure to contaminants that are capable of interacting with genetic material. Varanasi et al. (1989) used this method to correlate DNA adducts in livers of English sole (*Parophrys vetulus*) and winter flounder (*Pseudopleuronectes americanus* from Pugent Sound, with exposure to PAHs in sediments. Another method that shows promise is the use of the DNA alkaline unwinding assay. Toxic chemicals that interact with genetic material can cause strand breaks in DNA. The rate of unwinding of the DNA double helix when placed in an alkaline solution is a measure of DNA integrity that has been shown to be related to exposure of fish to genotoxic chemicals (Shugart 1988).

IMMUNOLOGY

A wide range of environmental pollutants affect the immune system of fishes (Zeeman and Brindley 1981). Immunosuppression can result in increased susceptibility of fishes to disease (Sinderman 1979). Contaminant-reduced immunocompetence of fishes has been a subject of study in both feral fishes from polluted environments (Warinner et al. 1988), and laboratory studies with single compounds (Walczak et al. 1987) and complex mixtures (Secombes et al. 1991). Various approaches to assessment of immune status have been used. These include differential white blood cell counts (Gardner and Yevich 1970), serum protein levels and ratios (Smith et al. 1976), agglutination titer (Roales and Perlmutter 1977), phagocytosis (Secombes et al. 1991), and disease susceptibility (Hansen et al. 1971). As with many other biochemical and physiological indicators, measures of immunocompetence are more useful as indicators of general organism health rather than a diagnostic indicator of specific contaminant exposure.

RECOMMENDATIONS FOR IMPLEMENTATION

In this brief overview, we have had the opportunity to focus on only a minute fraction of the biochemical and physiological response of aquatic organisms that have been measured in relation to contaminant exposure. Of the literally hundreds of measurable biochemical and physiological responses of organisms, most have been studied at least to some extent in relation to exposure to one chemical or another. The wide variety of species of aquatic organisms and the wide variety of chemicals that pose a threat as environmental pollutants provides an extremely extensive and complex matrix of possible combinations of exposure and response for study.

Relatively few biochemical and physiological responses of aquatic organisms are specific to individual chemicals (e.g., ALAD); several others are sensitive to general chemical classes (e.g., acetylcholinesterase activity; EROD activity). These *specific* indicators may be very useful in environmental assessments where sources and types of contaminants are unknown, by directing evaluation efforts toward particular chemicals. The sensitivity of these tests may also make them useful for documenting the fringes of environmental disturbance or areas of subtle contaminant exposure when the types of contaminants present in a system are known. Their use, coupled with supportive analytical chemistry, can provide important information for establishing cause and effect relationships and interpreting observed effects at higher levels of biological organization.

The majority of biochemical and physiological responses of aquatic organisms are sensitive to broad ranges of chemical contaminants and other environmental stressors. For this reason, these techniques can provide sensitive measures of organism health, but are not diagnostic for specific chemical exposure. These *general* indicators are most effectively applied in environmental contaminant assessments in concert with other measures of contaminant effects and exposure. The use of general biochemical indicators should be coupled with chemical analysis, on-site and *in-situ* toxicity assays, infaunal surveys, and other classical contaminant assessment procedures to identify the nature and extent of the contaminant problem. As with specific indicators of contaminant exposure, these general indicators of organism health may provide additional sensitivity in identifying the fringes of environmental disturbance and areas of subtle contaminant exposure. However, supporting information for establishing cause and effect is extremely important when general indicator techniques are applied in environmental contaminant assessments.

In summary, measurement of the biochemical and physiological responses of aquatic organisms to toxic chemicals has provided extensive insight into the modes of action of environmental contaminants. However, their use in the identification and monitoring of the nature and extent of aquatic pollution is a relatively new application. Their rapid response and sensitivity provides potential for adding a further level of discrimination in the environmental risk assessment process, particularly in marginally impacted areas. Proper application of these techniques requires a thorough understanding of their specificity in regard to the types of environmental stressors to which they are sensitive, and a quantitative understanding of the linkages between effects at various levels of biological organization. For many biochemical and physiological indicators, these are topics of current and future work.

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CLASSIFICATION OF WETLANDS OF RUSSIA

by

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ABSTRACT

The term "wetland," which is new for the Russian scientific literature, describes a diversity of zones lying between typical terrestrial habitats and deep water ones. There is no unified classification of wet habitats in Russia. In order to classify Russian wetlands, we used the well-known publication, "Classification of Wetlands in the United States," because of its ecological comprehensiveness and simplicity.

INTRODUCTION

Habitats at the water and land interface are complex structures in which we observe the interaction of water, air, soil, bottom sediments, and living organisms. These habitats are quite common at marine coasts, lake and river shores, relief depressions, and ground water outlets. In Russia we use a whole set of terms designating such ecosystems. They may be terms which are quite common, such as wet meadows, bogs, swamps, and coasts or local specific terms such as laid, alas, plav, tugai, zaimizche, kardash, iskar, urem, grass visk, sogr, saz, ryam, ols, vatt, badaran, etc. Sometimes, one term is understood differently in different areas of Russia. For instance, the term "laid" is used for designation of the meadows at marine coasts; lakes in Vasugany, Siberia; and wet depressions in the tundra.

These wet areas may be attributed both to terrestrial and aquatic ecosystems which are diverse and specific. From the biogeochemical point of view these areas, when compared to deep water habitats, are characterized by lower and more variable spacial pH values, lower concentrations of dissolved oxygen, extreme reducing conditions, a higher probability of photodegradation and biodegradation, chelation and formation of organic and organomineral complexes, and higher sulfide concentrations. Hydrology is the controlling factor in all of these wet areas, in spite of their diversity. All these ecosystems are periodically flooded or saturated with water. In accordance with Polynov classification, these habitats are attributed to "aqual" and "superaqual" elementary landscapes (biogenosis).

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Wetland, which is a new term in Russian scientific literature, describes a diversity of zones lying between typical terrestrial habitats and deep water ones.

The U.S. Fish and Wildlife Service (USFWS) developed and published the classification of U.S. wetlands entitled "Classification of Wetlands and Deepwater Habitats of the United States" (Cowardin et al. 1979). This classification is not the only one in use in the U.S., but its ecological comprehensiveness and simplicity has provided an excellent classification tool. Because of this, we believe it would have an excellent possibility for classifying Russian wetlands.

The USFWS system standardizes wetland classification and provides the basis for a wetland inventory. There is no unified classification of wet habitats in Russia. The first project undertaken by American and Russian specialists was to evaluate the USFWS classification in Russia and determine its applicability to Russian wetlands. These investigations were carried out in the framework of Cooperative American-Russian Project 02.02.14. "Influence of Pollutants on Wetland Ecosystems" in accordance with an Intergovernmental Agreement in the Field of Environmental Protection.

Below is a brief review of the application of the USFWS wetland classification system used in Russia. This is preliminary and the work will be continued. Some details of USFWS classification are omitted purposefully to underline the principles of classification.

BRIEF ANALYSIS OF THE USFWS CLASSIFICATION OF WETLANDS

Wetland is understood as a wet habitat periodically flooded or saturated with water. The USFWS defines a wetland as transitional between terrestrial and deepwater ecosystems with the water table at the surface or close to the ground surface. To classify an environment as a wetland it must possess at least one of the three following features: 1) the area should be inhabited by hydrophytes, at least periodically; 2) the surface substrate is not continuously drained, and hydromorphic soils are formed; and 3) soils are saturated with water or flooded during some part of the yearly growing season, i.e., the hydrological regime may be variable--from permanent flooding or saturation to occasional water at the land surface.

According to USFWS classification of hydrophytic plants, hydrophytes are plants inhabiting environments characterized by recurring anaerobic conditions or close to anaerobic conditions because of high moisture content. The substrate of wetlands may not necessarily be a hydromorphic soil, e.g., sand and gravel beaches, but at least for some period during the vegetation growing season the substrate must be saturated or flooded.

Because of the absence of a distinct interface between dry and wet habitats, there may not be indisputable determinations of wetland boundaries. We have to use the term wetland even though it is somewhat artificial from a scientific point of view. In Russian scientific literature, much less definite terms are used to describe these areas, i.e., wet boggy areas, which cannot be taken as synonymous with the term wetland.

Only definite biogenoses may be attributed to wetlands. This is illustrated by Table 1. However, the basic features of wetlands differ in different climatic zones of Russia. In all climatic zones there are plants and animals that cannot survive saturated or flooded soils.

Wetland spatial boundaries also differ. If marine, delta, riverine, and lacustrine wetlands along moisture gradients (from deepwater to terrestrial habitats) are considered, wetland boundaries will be determined by minimum and maximum water levels during the vegetation growing season. One of the conventional boundaries of riverine and lacustrine wetlands is the distribution of emergent vascular plants (for lacustrine wetlands this is the 2 m depth zone). Beyond this is the deepwater habitat typical of aquatic ecosystems. The boundaries between terrestrial ecosystems and wetlands are the areas in which hydrophytes are replaced by mesophytes and xerophytes. There are also signs of variation in hydrological regime indicating occasional flooding or soil saturation.

Table 2 presents USFWS classification with the hierarchical structure of wetlands at three groups: systems, subsystems, and classes. Experience with the USFWS classification system in Russia (Caucasus, southern part of the country; Rostov region, forest steppe zone of Voronezh; Kursk regions, taiga of the European part of the country; and basins of the Olekma and Amga rivers in Yakutia, Siberia) indicated that at the levels of systems, subsystems, and classes, the system can be applied for classification of Russian wetlands. No wetlands endemic to Russia could not be classified at the level of class or higher. Some Russian wetlands did not appear to meet the classification criteria at the level of subclass, so it may be necessary to develop specific subclasses for Russian wetlands.

TABLE 1. BASIC FEATURES OF WETLANDS*

	With hydrophytes		Without hydrophytes	
Wetland system	With hydric soils	Without hydric soils	With hydric soils	Without hydric soils
Marine	Rocky shore		Sand beaches	Salt marsh
Delta		Plavni		
Riverine	Aquatic bed	Phragmites, Scirpus, Carex, Salix and others	Temporal streams	
Lacustrine	Phragmites	Carex, Salix, Phragmites, Scirpus and others		Man-made pool banks
Palustrine		Peat bogs		

*Absence of wetland terms in some columns does not mean there are no wetlands with such features.

TABLE 2. CLASSIFICATION OF RUSSIAN WETLANDS AT LARGE TAXONOMIC GROUP LEVELS

Systems	Subsystems	Classes
Marine	Subtidal	Rock bottom; unconsolidated bottom; aquatic bed
	Tidal	Aquatic bed; rocky shore; unconsolidated shore
Delta	Subtidal	Rock bottom; unconsolidated bottom; aquatic bed
	Tidal	Aquatic bed; stream bed; unconsolidated shore; emergent wetland; shrub wetland; forested wetland
Riverine	Tidal	Rocky bottom; unconsolidated bottom; aquatic bed; rocky shore; unconsolidated shore; emergent wetland
	Lower perennial	Rock bottom; unconsolidated bottom; aquatic bed; rocky shore; emergent wetland
	Upper perennial	Rock bottom; unconsolidated bottom; rocky shore; unconsolidated shore
	Intermittent	Stream bed
Lacustrine	Limnetic	Rock bottom; unconsolidated bottom; aquatic bed
	Littoral	Rock bottom; unconsolidated bottom; aquatic bed; rocky shore; unconsolidated shore; emergent wetland
Palustrine		Rock bottom; unconsolidated bottom; aquatic bed; unconsolidated shore; moss-lichen wetland; emergent wetland; shrub wetland; forested wetland

The structure of the higher levels of the USFWS classification system, presented in Table 2, are unified and may be used for description of different wetlands. This could not be achieved using common geographic and geochemical systems for classification of landscapes.

Table 2 also shows that the highest taxonomic level in the USFWS classification scheme is system. This is determined as a complex of wetlands exposed to the influence of similar hydrological, geomorphological, and biogeochemical factors. According to the USFWS classification there are five wetland types at the system level: marine, delta, riverine, lacustrine, and palustrine. These systems, with the exception of palustrine, are subdivided into subsystems based on hydrological and geomorphological factors. As a result, marine and delta systems possess two similar subsystems: 1) located below the level of the tidal zone and those flooded permanently and 2) located above the level of the tidal zone and occasionally flooded and exposed.

The riverine system is subdivided into four subsystems. These are enumerated from the mouth to the head of the river: 1) tidal, exposed to the influence of tides and wind induced tides; 2) lower perennial with features typical of plains rivers; 3) upper perennial with features typical of mountain rivers; and 4) intermittent, flooded occasionally during a year.

The Lacustrine system also comprises two subsystems: 1) limnetic and 2) littoral subsystem.

Subsystems of wetlands are divided into classes based on life forms and plant cover. While identifying classes of wetlands, two additional factors should be taken into consideration: 1) structure of the substrate cover if there are no soils and 2) water chemical composition. These factors will largely determine vegetative composition. In case plant cover is less than 30% of the wetland area, this wetland may be included in one of the following five classes: 1) rock bottom, 2) rocky shore, 3) unconsolidated bottom, 4) unconsolidated shore, and 5) stream bed.

Where plant cover occupies greater than 30% of the wetland area, the wetland may be included in the following five classes: 1) aquatic bed, 2) moss-lichen, 3) emergent vascular plants, 4) shrub, and 5) forested.

These classes are subdivided into subclasses (Table 3) identified by detailed analysis of the life forms of plants and a sublayer of the soil. The following subclasses were isolated in Russia, based primarily on the plant character and life forms of dominant plants: 1) persistent, 2) nonpersistent, 3) emergent, 4) moss, 5) lichen, 6) broad-leaved deciduous, 7) broad-leaved evergreen, 8) needle-leaved evergreen, 9) deciduous, and 10) dead.

On the basis of the substrate sublayer, classes are divided into the following subclasses: 1) bedrock, 2) rubble, 3) cobble-gravel, 4) mud, 5) sand, and 6) organic matter (detritus).

Subclasses are further subdivided into dominance types, determined by dominant plant and animal species. The dominance types describe individual features of each wetland and the number of dominant types is indefinite.

Four types of modifiers are used to more accurately identify characteristics of wetlands: 1) water regime, 2) water chemical composition, 3) soil, and 4) special.

Water regime modifiers describe flood conditions or the degree of wetland saturation. In this case they are divided into two basic groups: 1) subtidal, intermittently exposed, permanently flooded, and intermittently flooded; and 2) permanently flooded, intermittently exposed, semipermanently flooded, seasonally flooded, and artificially flooded.

Some delta and riverine wetlands are exposed to the influence of tidal and nontidal hydrologic regimes. It is difficult to determine flooding patterns and saturation levels for such wetlands.

Water chemical composition modifiers consist of two parameters: mineralization and pH. These are divided into two groups: 1) marine and delta wetlands exposed to tidal and

wind induced influence and 2) riverine, lacustrine, and palustrine wetlands not exposed to tidal and wind induced influence.

Soil modifiers are developed for two groups of soils: those rich in organic matter (more than 20% in a 40 cm layer) and mineral soils.

The following specific modifiers describe anthropogenic and zoogenic impact on wetlands: excavated, impounded, ditched, partially drained, farmed, and artificial.

TABLE 3. CLASSES AND CORRESPONDING SUBCLASSES OF RUSSIAN WETLANDS.

Number	Classes	Subclasses
1	Rock bottom	Bedrock, rubble
2	Unconsolidated bottom	Cobble-gravel, mud, sand, organic matter
3	Aquatic bed	Algal, aquatic moss, rooted vascular, floating vascular
4	Streambed	Bedrock, rubble, cobble-gravel, sand, mud, organic matter
5	Rocky shore	Bedrock, rubble
6	Unconsolidated shore	Cobble-gravel, moss-lichen
7	Moss-lichen	Moss, lichen, moss-lichen
8	Emergent vascular plants	Persistent, nonpersistent
9	Shrub	Broad-leaved deciduous, broad-leaved evergreen, needle-leaved evergreen, dead
10	Forested	Broad-leaved deciduous, broad-leaved evergreen, dead

WETLANDS OF THE AMGA RIVER BASIN, YAKUTIA

APPLICATION OF THE USFWS CLASSIFICATION SYSTEM

To compare characteristics of wetland habitats using the USFWS wetland classification scheme and the physico-geographic description common in Russia, we chose the Amga River Valley. This river flows through the Olekma Preserve, Yakutia.

In July 1991 a 30-day field survey was carried out by the Laboratory of Ecological Investigations to study the wetlands of this area.

Within the Olekma Preserve, the Amga River has two distinct sections. The first extends from the headwaters to the inflow of the river Olobochor. In this section the Amga River consists of many branches. There is also a chain of islands along the river. Along the shore are sand-rubble ridges, and the bed consists of a shallow pebble bottom. The second section extends from the point of the Olobochor River inflow to the boundary of Olekma Preserve. During this transit, the Amga River Valley becomes wider and the river meanders extensively. Many of the meanders are eventually cut off from the river and form bogs within the floodplain. The area located in front of the wide floodplain terrace contains many bogs.

Ecological conditions of the Amga River section correspond to the criteria of the upper perennial subsystem of the USFWS classification. In the lower section, there are some features of the low perennial subsystem: flow current velocity in the pools decrease and the proportion of fine fractions in sediment sublayer increases. In small bays, with the local name "kuria," the sediment sublayer is mud with organic matter. Some river tributaries flooded in the period of peak flow may be classified intermittent.

Elements of the Amga River such as stream bottom, banks, and pools may be classified as riverine wetlands of the upper perennial subsystem: class = unconsolidated bottom; subclass = cobble-gravel (sometimes subclass = sand or mud); water regime is permanently flooded, fresh water.

Elements of the Amga River floodplain composition such as bichevnik (local Russian term), that is pebble beach, levees, spits, beaches, summits, and tails of the islands can be attributed to riverine wetlands of the upper perennial subsystem: class = unconsolidated shore; subclass = cobble-gravel (sometimes, subclass = sand or mud); water regime is seasonally flooded, temporarily flooded, and intermittently flooded, freshwater.

The section of the Hatyn River at the point of inflow to the Amga River possesses features of the lower perennial subsystem. The wetlands at the mouth of the Hatyn River are class = unconsolidated bottom; subclass = mud.

Palustrine wetlands were found at the floodplain of the Olobochor River. They were classified as wetlands of class = shrubs; subclass = broad-leaved deciduous; dominant genus = willows (*Salix*); water regime is semipermanently flooded, fresh waters. Upstream of the Olobochor River class = moss-lichen wetlands; subclass = mosses, possessing saturated water regime.

In the vicinity of the point Hatyn, there is a hillocky bog that was classified as class = emergent; subclass = persistent. In all cases the wetland water was fresh.

Wet habitats were not limited to the riverbed or floodplain of the Amga River. In the historical records of Olekma Preserve, other wet areas are mentioned: stream mouths and willow thickets in the valleys, intermittently exposed beds of the streams, needle-leaved deciduous and broad-leaved deciduous forests located at sedge sphagnum palustrine wetlands, shrub wetlands, spring wetlands, etc. This list does not include all wet habitats of the area.

Thus, the USFWS Classification tested at the Amga River, Yakutia, allowed us to place the wet habitats in a hierarchical structure and to describe them with unified terms. Though the definition of wetlands is sometimes rather long, USFWS classification is still convenient and may be easily used in Russia if you understand the principles of its structure. This classification does not need any transformation above the level of subclass. It may, however, be necessary to include additional subclasses describing wetlands endemic for Russia (Euro-Asia).

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ECOLOGICAL STATE OF THE UPPER VOLGA RIVER AS INFLUENCED BY COMPLEX ANTHROPOGENIC FACTORS

by

N. P. Smirnov and A. I. Kopylov[1]

ABSTRACT

Ever increasing degradation of wetland and aquatic habitat has caused great urgency to maintain biological diversity and protect the gene pools of plant and animal populations native to the Volga River. Observations point to a sharp decrease in biological diversity and loss of species--for instance, many species of aquatic and littoral plants are on the verge of extinction. There is need to identify the degree of anthropogenic disruption of structural-functional components of the ecosystem, its ability to restore itself to the original condition, and potential methods for remediation.

INTRODUCTION

The Volga River--3530 km long--is the largest river in Europe and the sixth largest in the world. In general, the Volga is divided into three parts: Upper, Middle, and Lower. The dam of Rybinskoye Reservoir, near the town of Rybinsk, is considered the boundary between the Upper and Middle Volga. The Volzhskaya Hydro-electro Power Station and dam separates the Middle from the Lower part of the Volga River.

The Upper Volga, about 800 km long, includes the Verhnevolzhskoye, Ivankovskoye, Uglichskoye, and Rybinskoye reservoirs. All the reservoirs, except the Verhnevolzhskoye, are about 50 years old and differ significantly in morphometric characteristics (Table 1).

Today, flora and fauna of the Upper Volga are relatively well studied, due mainly to investigations of the Institute of Biology of Inland Waters (IBIW), Russian Academy of Science (Table 2). There are 1018 species and varieties of algae and 342 species of higher aquatic plants. The total number of invertebrate and fish species found in reservoirs of the Upper Volga is 2288, including 62 species that are considered rare. Insects, nematodes, and

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TABLE 1. PRIMARY MORPHOMETRIC CHARACTERISTICS OF RESERVOIRS ON THE UPPER VOLGA RIVER

Reservoir	Volume (km^3)	Area (km^3)	Length (km)	Depth Mean (m)	Depth Maximum (m)	Dates of creation and reconstruction
Upper Volga	794	179	92	4.4	16.1	1843 (1943-47)
Ivankovo	1,120	327	120	3.4	19.0	1937
Uglich	1,245	249	143	5.0	23.2	1940
Rybinsk	25,420	4,550	250	5.6	30.4	1941-47

TABLE 2. FLORA AND FAUNA OF THE UPPER VOLGA RIVER

General groupings	Species numbers
Algae	1018
Vascular plants	342
Protozoa	139
Spongia	2
Coelenterata	1
Platyhelminthes	240
Nemathelminthes	610
Acanthocephala	7
Annelida	79
Mollusca	64
Tentaculata	9
Arthropoda	720
Chordata	32

rotifers are the most rich in species. In total, there are more than 3000 species of autotrophic and heterotrophic eukaryotic organisms in the Upper Volga basin.

Regular observations of floral and faunal species composition over many years can be a sensitive indicator, or monitoring tool, to evaluate the state of the ecosystem. Available data suggest a sharp decrease in biological diversity and an irreparable loss of species and even biocoenosis in the region. Also, the role of invading species from northern waterbodies seems to be decreasing. Siltation of flooded channels resulted in the disappearance of

mysids and gammarids from some areas. Changes in hydrophilic flora have been observed, and many species of aquatic and littoral plants are on the verge of extinction.

At the same time, unusual species or those previously exotic to the region are appearing in the reservoirs, or entire drainage of the Upper Volga. For example, the Caspian polychaeta (*Hypania invalida*), common in southern reservoirs, was found in Ivankovskoye Reservoir in 1986. The ostracod (*Stenocirpis malcolmsoni*)--a typical inhabitant of European botanical gardens and heated basins--has also been discovered in the same reservoir. It is interesting that uncommon parasites are appearing in the area. Mass infections of cyprinid fry (roach, *Rutilus rutilus*; and white bream, *Blicca bjoerkna*) by the ectoparasite infusorium (*Ambiphrya ameiruri*) has been noted in the Rybinskoye Reservoir. This infusorium was brought to Europe in the 1980s along with its host--the American catfish (*Ictalurus punctatus*)--and passed on to other native species in fish farms of the country. Invasion of the ectoparasites into the Upper Volga is apparently linked to conditions favorable for reproduction of the infusorium (high concentrations of dissolved organic matter and abundant plankton microflora), which occur in some areas. These are the primary foods for this infusorium. The ectoparasites are distributed evenly on the skin epithelium and sometimes cover 60% of the surface.

Significant changes in the species composition of plankton and benthic communities are primarily the result of eutrophication and pollution from anthropogenic sources in the Upper Volga basin. Anthropogenic loads on waterbodies have greatly increased within the last decade. Primary anthropogenic sources include industrial and municipal wastes released into waterbodies, air pollution, more intensive use of the Volga basin for transportation and recreation purposes, industrial construction along the banks of the river and it's basin, dredging for construction purposes, and intensive use of biological resources.

This manuscript presents just a small part of the information on the effects of anthropogenic eutrophication and anthropogenic pollution of the Upper Volga reservoirs gained by scientists of the IBIW.

EUTROPHICATION OF THE UPPER VOLGA RIVER

Reservoirs are unique aquatic ecosystems where biological succession is a complicated and infinitely long process. The succession, or aging, of reservoir ecosystems differs markedly from that of lakes. Anthropogenic factors affect reservoirs from the moment of their completion and play a lead role in reservoir ecosystem dynamics.

Trophic level changes in different stages of reservoir succession are caused by a variety of factors and are not always associated with anthropogenic enrichment by biogenes. Therefore, eutrophication of reservoirs is not always caused by anthropogenic factors. Thus, it is more correct to consider changes in the structural and functional characteristics of aquatic communities to be the result of a combination of natural and anthropomorphic eutrophication. Ivankovskoye Reservoir is a good example to illustrate this combined eutrophication process. In addition to diatoms, pyrrophytic and blue-green algae begin to dominate in phytoplankton communities, and can be used as indicators of eutrophic conditions. Before 1970, the blue-green algae biomass had not exceeded 7 g/m^3, but within

the last decade it has reached hundreds of grams per cubic meter. Intensive increases in these algal species groups have become characteristic of all reservoirs in the region. In comparison to the 1950s, total biomass of plankton has increased by 2.0-6.6 times. Data on phytoplankton primary production from the 1980s shows that production in the photosynthetic zone has increased by 1.2-6.5 times during the same period.

Along with phytoplankton, total numbers and biomass of zooplankton increased significantly over the last few years. The number of species that are indicative of eutrophication has increased sharply as has filter feeding zooplankton. Also, ratios between taxonomic groups have changed.

The present state of eutrophication in Ivankovskoye Reservoir is characterized by increases in annual macrophyte production, and percent area of coverage by emergent plants. Simultaneously, the process of endogenous succession is resulting in increased dominance of plants typical of bogs. The appearance of herbaceous floating mats followed by replacement with brushy and woody plants is common for this type of endogenous succession. The following scheme illustrates the change in plant communities in general: 1) emergent plants; 2) emergent plants and floating mats; 3) floating mats and islands; and, finally, 4) floating islands with individual willow shrubs and trees, and birch and fir forest. At the willow development stage, shallow waters are converted into boggy soil, over a period of 30-40 years. The Ivankovskoye Reservoir has been in existence for 40 years, and due to endogenic succession, floating mats now cover 14% (13 km^2) of littoral area, and 3% (11 km^3) of the total area of the reservoir has been turned into boggy soil (Table 3).

TABLE 3. HIGHER AQUATIC PLANTS OF IVANKOVSKOYE RESERVOIR

Observation	1957	1973	1980
Area of vegetation (km^2)	55	79	83
Degree of overgrowth (%)	17	24	28
Annual production of organic matter (higher water plants, thousands of tons)	25	54	68

POLLUTION OF THE UPPER VOLGA RESERVOIRS

Effects of discharge of large amounts of industrial and municipal wastewater have been studied in the northern part of the Sheksna Reach of the Rybinskoye Reservoir (near the town of Cherepovets). Major pollutants include oil products, polychlorinated biphenyls (PCBs), di- and polyaromatic hydrocarbons (PAHs), heavy metals, and others. According to 1990 data, taken from some locations, the concentration of oil exceeded the maximum

permissible concentration by four times, and the concentration of PAHs by 150 times (Table 4).

The pollution of bottom sediments by PAHs presents a serious hazard. The concentrations, extrapolated from benzopyrene, for the majority of samples were 25-53 mg/g of dry weight, which exceeds by 3-4 orders of magnitude the background concentration in unpolluted areas. Bottom sediments and fish also contained a number of genotoxic substances which are known to cause mutagenic effects. The portions of Skeksna Reach receiving these wastes should be considered toxic to aquatic resources. During summer, the length of this pollution zone is about 7 km. As oxidation/reduction transformations of the various pollutants occur at this time, the toxic environment expands into a zone of intensified eutrophication that extends for 20-25 km downstream.

Ecological impacts of pollutants can be observed at the organism, population, and community levels. The disturbance of some physiological functions, changes in behavior, reduced growth rates, and increased morbidity and mortality are caused by direct poisoning and decreased tolerance to different stresses. Aquatic organisms in toxic zones accumulate these pollutants (Table 5). According to 1990 data, the concentrations of persistent and accumulable PCBs were higher in invertebrates than in bottom sediments. Fish containing PCB residues were found in areas of the reservoir considerable distances away from the zone directly polluted by this substance. In addition, we have observed a number of moribund mollusks (*Unio* sp.) with different stages of liver degeneration and glandular cancer.

Fish captured in the polluted sections of Skeksna Reach were found to have a 60-90% incidence of immune system deficiency, whereas fish taken from habitats remote from Cherepovets had an incidence of 0-30%. The livers and tissues associated with the immune system of immuno-deficient fish showed severe cirrhosis and had little natural immunity to disease.

Crayfish (*Bythotrephes longimanus*) collected in the reach were moribund, and examination showed severe degeneration of the intestinal epithelium and abnormal

TABLE 4. POLLUTANTS IDENTIFIED IN WATER AND BOTTOM SEDIMENTS IN THE NORTHERN PART OF THE SHEKSNA REACH (RYBINSK RESERVOIR, NEAR CHEREPOVETS 1990-91)

Pollutants	Water (μg/L)	Bottom sediments (mg/g dry weight)
Polychlorinated biphenyls	Trace = 0.29	Trace = 0.60
Oil products:		
Hydrocarbons	0.05-0.19	0.56- 0.62
Resinous components	0.00-0.03	0.44- 2.65
Polyaromatic hydrocarbons	0.10-0.64	3.33-49.73

development of the placental structure leading to the brood pouch. This can lead to mortality of parthenogenetic embryos developing in the pouch. As a result, crayfish reproduction and population levels have declined, and its distribution has narrowed. In the near future, we expect crayfish to disappear altogether.

At the population level, pollution causes changes in number and biomass, birth and mortality, and in dimensional structure and dynamics in aquatic organisms. The concentrations of saprophytic bacteria, as well as specialized groups of microorganisms which oxidize phenol, naphthalene, oil, and other organic pollutants, increases sharply in polluted waters. The presence of these toxicants in water has an especially negative impact on large crayfish with prolonged life cycles (*Bythotrephes* sp., *Heteroscope* sp., *Eudiaptomos* sp.). Genetic and population-level analyses have shown considerable disturbance in the genetic structure of fish inhabiting areas receiving industrial wastewaters. These genetic changes are similar to those observed in the zones of radioactive pollution at the Chernobyl Nuclear Station.

TABLE 5. RESIDUES OF POLLUTANTS IN TISSUES OF FISH AND BENTHIC INVERTEBRATES IN THE NORTHERN PART OF THE SHEKSNA REACH (RYBINSKOYE RESERVOIR, NEAR CHEREPOVETS, 1990-91)

Organism	Tissues	PCB* (mg/kg)	PAC+OPC† (mg/kg)	Oil products (mg/kg)
		FISH		
White bream	Muscle	0.00-0.53	3.60-39.4	14.465
	Liver	0.45-5.41	0.40-23.3	10-140
Pike perch (*Lucioperca volgensis*)	Muscle	0.00-0.49	15.4	17.0
	Liver	0.77-1.76	20.0	17.0
Blue bream (*Abramis ballerus*)	Muscle	0.25		
	Liver	0.87		
		INVERTEBRATES		
Dreissena	Whole organism	0.20-1.40	9.0-37.0	39.0-733.0
Chironomidae	Whole organism	1.15-0.67		
Oligochaetes	Whole organism	0.00-0.42		
Viviparus (pulmonate snails)	Whole organism	0.14-1.32		

*PCB = polychlorinated biphenyl.
†PAC = Polyaromatic hydrocarbon; OPC = oxidized polynuclear compound.

At the ecosystem level, pollution affects the structure and function of plankton and benthic communities. Areas, such as the Sheksna Reach, receiving heavy loads of industrial and municipal wastes from Cherepovets, characteristically have a low index of species diversity and a low quantity and biomass of phytoplankton and zooplankton (Table 6). Trophic food chains are shortened in these aquatic communities and the number of predators has declined sharply. In spite of extremely high concentrations of biogenic elements, the intensity of photosynthesis in these areas is low, which is probably related to low water transparency and toxic effects of wastewaters. The significant increase of bacterial production over primary production is the likely result of the intensive destruction of microflora and microfauna that feed on bacteria, such as rotifers, etc. Such increases in development of planktonic bacteria in comparison to phytoplankton is related to the input of large amounts of anthropogenic organic matter. These relationships are demonstrated by ratios of primary production to total heterotrophic decay (Table 6). South of the Cherepovets area, toxic effects of pollution decrease and eutrophication occurs: photosynthesis increases sharply and total biomass of the plankton community increases. An increase in species diversity is also observed, but the role of predators in the total biomass of zooplankton remains insignificant.

Pollution of bottom sediments greatly influences the structure and function of benthic communities. Monitoring of microbiological processes taking place in the bottom sediments

TABLE 6. STRUCTURAL/FUNCTIONAL CHARACTERISTICS OF PLANKTON COMMUNITIES IN THE POLLUTED SHEKSNA REACH AND TWO DOWNSTREAM ZONES OF THE RYBINSKOYE RESERVOIR, SUMMER 1989

		Downstream zones	
Parameters	Sheksna Reach*	Eutrophic	Relatively clean
Zooplankton (Shannon Index)†	1.4-1.7	3.9	2.0-2.5
Total plankton biomass	7.1	17.0	3.8
Ratio, biomass of predator zooplankton to prey (%)	22.2	14.0	84.7
Photosynthesis (mg carbon [C]/M^3/hour)	0.94	2.21	0.77
Bacterial production (mg C/m^3/hour)	2.97	2.10	1.20
Ratio, primary production to total heterotrophic decay	0.45-0.73	0.44-1.51	2.24-3.73

*Data from 1986-88.
†Index of biodiversity.

of the toxic zone can be used as an indicator of the adverse effects of long-term chronic pollution, such as that caused by wastes discharged in the Cherepovets area. The specific activity of dark assimilation of C^{14} tagged carbon dioxide in sediments collected in the polluted zone was higher than in sediments from other regions of the reservoir (Table 7). These results suggest that certain microflora in sediments function actively to break down the different pollutants in spite of their low abundance. In contrast to less polluted regions, there is a sharp decrease in aerobic processes and an increase in anaerobic methane-forming and sulfate-reducing microflora. Thus, the pollution-induced destruction of the aerobic bacterial community, and development of a stable anaerobic community has seriously inhibited natural self-purification processes in the sediments, and further, has caused secondary pollution in the form of anaerobic decay products in downstream waters.

Anthropogenically caused changes in the Sheksna Reach of the Volga have been caused by heavy industrial discharges, and the situation can be considered an ecological disaster. It is obvious that the return of this ecosystem to its original condition through self-renewal is impossible without compulsory remediation. The development of local zones with varying degrees of adverse changes in their structural-functional ecosystem components can be found near other large industrial centers. In addition, the riverine ecosystems downstream of these polluted zones can be considered "at ecological risk" due to increased eutrophication. However, it is possible to return these ecosystems to their original condition naturally by remediating upstream pollution.

TABLE 7. FUNCTIONAL CHARACTERISTICS OF BENTHIC MICROFLORA IN THE POLLUTED SHEKSNA REACH AND TWO DOWNSTREAM ZONES IN THE RYBINSKOYE RESERVOIR, SUMMER 1986-89

		Downstream zones	
Parameters	Sheksna Reach	Eutrophic	Relatively clean
Dark assimilation	2.7	2.0	1.8
Specific activity per 1 x 10^9 cells of bacteria	1.7	0.5	0.2
Aerobic decay, mg C/m^2/day	87	203	180
Methane transformation, mL/dm^3/day			
Formation	2.5	0.06	-
Oxidation	1.6	0.01	-
Intensity of sulfate reduction, mg S/kg/day	41.1	3.1	1.1

CONCLUSIONS

With a growing trend of wetland and aquatic habitat degradation, there is even greater urgency to maintain the biological diversity and protection of gene pools of aquatic plant and animal populations native to the Volga River. The present situation requires enhancement of research to estimate the tolerance (resistance) of biological systems and frequency of their anthropogenic-caused changes. Complex ecological investigations of areas under the influence of industrial-municipal wastes have special significance because they permit us to identify the degree of anthropogenic disruption of structural-functional components of the ecosystem, its ability to restore itself to the original condition, and to evaluate potential methods of remediation.

RESTORING AQUATIC RESOURCES TO THE LOWER MISSOURI RIVER: ISSUES AND INITIATIVES

by

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ABSTRACT

Large, low-gradient rivers, such as the Volga and the lower Mississippi, are dependent on the river-floodplain linkage and periodic flood pulse for their integrity. The Missouri River is the longest river in the United States and in presettlement times exhibited extensive channel migration and high turbidity. The flora and fauna of the river-floodplain complex reflected this physical dynamic equilibrium. Presently, about one-third of the river has been channelized, one-third impounded, and the hydrograph of the remaining one-third severely altered. Additionally, agricultural, industrial, and urban development in the river basin has degraded water quality. Physical and chemical consequences of these alterations are reviewed as well as impacts on biological structure and function, including commercial and sport fishery resources. Restoration of the Missouri River necessitates a return of the ecosystem to an approximation of its former condition, including recreating both structural and functional components. Objectives to attain this goal include reestablishment of a semblance of the precontrol hydrograph and sediment regime, restoration of some of the precontrol channel structural diversity and the river-floodplain linkage, reestablishment and enhancement of native Missouri River fishes and their migrations, reduction of major point and nonpoint sources of pollution, and restoration of native terrestrial and wetland plant communities along the river channel and floodplain. Several recent initiatives are summarized that attempt to resolve competing watershed interests and address one or more of these objectives. These initiatives are the Mississippi Interstate Cooperative Resource Agreement, the Cooperative Interjurisdictional Rivers Fisheries Resources Act of 1992, the Missouri River Initiative (MOR-CARE), and the Missouri River Fish and Wildlife Migration Project. Successful restoration of the Missouri or any river-riparian ecosystem necessitates a holistic perspective at the landscape scale of the river basin.

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INTRODUCTION

Most large rivers in developed countries have been severely influenced by human alteration (Petts 1984, Davies and Walker 1986, Hynes 1989) and the Missouri River is no exception. Significant Anglo intervention began after the Louisiana Purchase, when in 1804 Lewis and Clark were commissioned by the federal government to find a road to the west for economic development (Keenlyne 1988). Subsequently, the Missouri River became the first great highway for exploitation and settlement of the American west. The Missouri River today, like the Volga, has been so radically altered by damming, channelization, and pollution that its fundamental aquatic character and processes no longer approximate natural conditions. Our goal is to share with our Russian colleagues existing information on the lower Missouri River. To accomplish this goal we have four objectives: 1) review the theoretical framework for perceiving large river-floodplain ecosystems and natural versus man-induced disturbance, 2) briefly describe the lower Missouri River ecosystem, 3) summarize major alterations to it and their effects on the biota, and 4) conclude by recommending restoration approaches and reviewing current restoration efforts. We believe that despite obvious differences between the Missouri and Volga rivers they share the fundamental similarities of all large rivers. Moreover, both rivers have experienced comparable alterations (Hesse et al. 1989a, Pavlov and Ya Vilenkin 1989). Collaborative research is essential to achieve our mutual goals of understanding and restoration of both systems. Exchange of information is the first step to facilitate collaborative research (Biette et al. 1989) and to develop the knowledge or approaches needed to restore and manage the aquatic communities of these large rivers.

RIVER-FLOODPLAIN INTERACTIONS IN LARGE RIVERS

Disturbance and recovery of large rivers cannot be understood without a conceptual framework of their normal behavior. Streams and rivers exist in a state of dynamic equilibrium (National Research Council 1992). Local physical features are naturally created, change through time, and eventually disappear, while the overall pattern (e.g., riffle-pool sequence, meandering) remains constant at large spatial and long temporal scales. This dynamic equilibrium in the physical system creates a corresponding dynamic equilibrium in the biological system.

Contemporary perceptions of the structural and functional properties of lotic waters are largely expressed in two paradigms: the River Continuum Concept (RCC, Vannote et al. 1980, Minshall et al. 1985) and the resource spiraling concept (Webster and Patten 1979, Newbold et al. 1981, Elwood et al. 1983). The RCC says that a continuous gradient of physical conditions and resources exists from a river's headwaters to its mouth. The stream's physical features provide much of the habitat templet for stream community structure and function. River networks are viewed as longitudinally connected systems of ordered biotic

assemblages, forming a temporal continuum of synchronized species replacements. Ecosystem level processes in downstream reaches are linked to those upstream through processing inefficiencies or leakage so that upstream energy loss becomes downstream energy gain. Consequently, there is a trade-off between maximizing nutrient and energy use within a reach via retention mechanisms that minimize downstream energy loss and the dependency on this material to drive downstream processes.

Within this framework a storage-cycle-release phenomenon termed resource spiraling (rather than recycling) becomes apparent because of the unidirectional flow of water and continuous transport of materials in lotic ecosystems (Webster and Patten 1979, Elwood et al. 1983). Efficiency of utilization of nutrients and organic carbon within a reach is associated with the tightness and magnitude of the spirals. Physical retention, microbial activity, and macroinvertebrate processing are important activities for defining the tightness of resource spiraling and preventing rapid throughput of materials (Minshall et al. 1985).

The RCC and resource spiraling concepts were developed largely from small temperate streams, and their usefulness as generalized paradigms for large rivers has been questioned (Davies and Walker 1986, Statzner and Higler 1985, Welcomme 1985, Cuffney 1988, Junk et al. 1989, Sedell et al. 1989). A more relevant framework has now emerged in which to test and clarify concepts about the structure and function of large floodplain river ecosystems (Dodge 1989). This complementary perspective is termed the flood pulse concept (Junk et al. 1989). Junk et al. postulate that the bulk of aquatic biomass in many unaltered large floodplain rivers is derived directly or indirectly from production within the floodplain and not from downstream transport or organic matter produced elsewhere in the basin. Whereas longitudinal linkages in small to moderate sized streams are the basis for the continuum aspect within the RCC, lateral exchange between the floodplain and river channel and nutrient recycling within the floodplain have a more direct impact on the biota and biological activity in large rivers. Whereas downstream losses of organic matter in small streams are reduced primarily by instream structure (e.g., pools, debris dams), geomorphic features within the lateral floodplain (e.g., sloughs, side channels, backwaters) are largely responsible for retention of organic matter and nutrients in large low-gradient rivers. The foundation of the flood pulse concept is that seasonal pulsing of flood flows onto the floodplain is the driving force controlling the river-floodplain complex (Junk et al. 1989, Welcomme et al. 1989, Sparks et al. 1990, Bayley 1991, Schlosser 1991).

While contributions of organic matter from floodplains may be quantitatively smaller than from upstream sources, they may be nutritionally of higher quality. Fremling et al. (1989) postulate that organic matter from tributary sources consists largely of dissolved humic acids or refractory particles by the time it is delivered to the main stem Mississippi River. The more nutritious fractions have been utilized or retained by upstream communities. They conclude that local sources of primary production, largely from within the floodplain, are responsible for the high fish production observed in large floodplain rivers.

Floodplain wetlands are regarded as among the most productive ecosystems in the world (Lieth and Whittaker 1975, Brinson et al. 1981). In situ primary production is high, and effective retention mechanisms contribute to efficient internal recycling of most carbon and nutrients (Junk et al. 1989). Although nutrient and organic matter losses from the floodplain complex to the river channel may be small relative to internal inputs within the floodplain, leakage from the floodplain to the river during the annual flood pulse is the principal source of

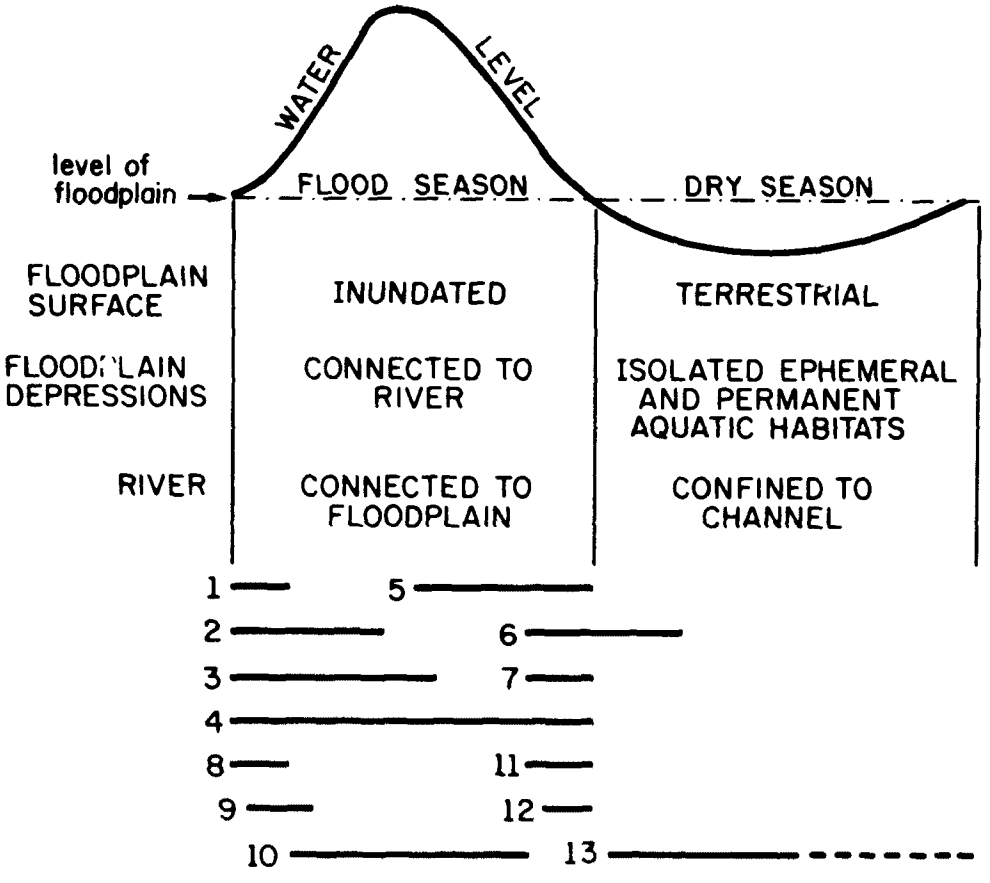

Fig. 1. Idealized changes in water level over an annual cycle for a riverine floodplain. Numbered horizontal bars indicate characteristic annual periodicity patterns for some major interactions as follows: 1) nutrients released as floodplain surface is flooded; 2) nutrient subsidy from river; 3) rapid growth of aquatic plants and invertebrates on floodplain; 4) major period of detrital processing on floodplain; 5) DOM and FPOM exported to river; 6) maximum plankton production in floodplain depressions; 7) drift of plankton, benthos and macrophytes to river; 8) fishes enter floodplain from river and fishes that survived dry season in floodplain depressions move to floodplain surface; 9) major period of fish spawning on floodplain; 10) period of maximum fish growth; 11) fishes move from floodplain to river; 12) heavy fish predation losses at mouth of drainage channels; 13) high mortality of fishes stranded in floodplain depressions. (Source: Ward 1989.)

these materials to the main channel in unaltered rivers (Mulholland 1981, Cuffney 1988, Grubaugh and Anderson 1989, Junk et al. 1989, Ward 1989, Sparks et al. 1990).

Fishes capitalize on this highly productive floodplain environment for feeding, spawning, nurseries, and as refuge from adverse river conditions (Fig. 1). Indeed, floodplain wetlands are considered the essential component responsible for the high fish production recorded in large, low gradient-rivers (Welcomme 1985, Ward 1989). Risotto and Turner

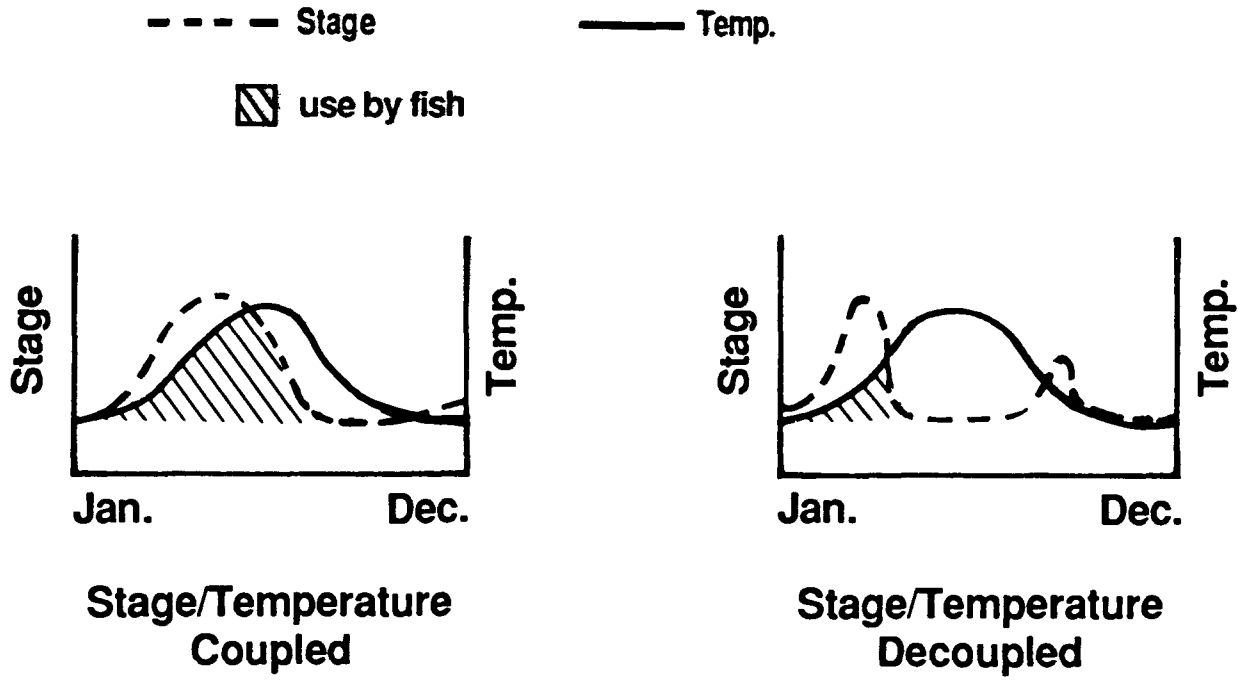

Fig. 2. Hypothetical examples of river stage and temperature relationships for a large temperate river floodplain. A) Shows an extended spring flood that, in combination with a normal temperature regime, provides good conditions for fish using floodplain habitats. B) Shows a short-duration, early flood that is decoupled from temperature. This restricts productivity of fish and invertebrates in the floodplain, but permits greater productivity of macrophytes. (Sources: Junk et al. 1989, Sparks et al. 1990.)

(1985) showed that variation in commercial fish harvest in the Mississippi River Basin was positively associated with acreage of bottomland hardwoods in the basin floodplain. This benefit of the floodplain and the flood pulse to aquatic productivity of large rivers has been termed the flood pulse advantage by Bayley (1991). He defines the flood pulse advantage as the hypothesized increase in multispecies fish yield over that which would result from the same water surface area with no flood pulse (i.e., from a system with constant water level). He further argues that particularly strong year classes of fish tend to result from gradually increasing water levels accompanied by a high amplitude flood of long duration. Ideal conditions for spring spawners in temperate rivers occur during years in which the flood and water temperature rise are coupled (Junk et al. 1989). Alterations that decouple temperature from river stage restrict invertebrate and fish productivity in the floodplain (Fig. 2, Sparks et al. 1990). These ideas that rivers and their floodplains are so intimately linked that they should be understood, managed and restored as integral parts of a single system make up the foremost integrative concept of restoration efforts (National Research Council 1992).

NATURAL AND ANTHROPOGENIC DISTURBANCE

Disturbance in lotic waters has been defined as any unpredictable, discrete event that disrupts structure or function at the ecosystem, community, or population level (Resh et al. 1988). Lack of predictability is an important component of this definition. Resh et al. (1988) consider disturbances as events characterized by a frequency (rate of occurrence of events) and intensity (physical force of event per time) that are outside a predictable range (see Poff [1992] for an alternative perspective). Periodic flood pulses of large rivers are predictable events under this definition. Indeed, the periodic flood pulse is critical to maintenance of aquatic populations, communities, and ecosystem processes. From this perspective, floods are not disturbances, unless so amplified, reduced, or mistimed that they fall outside the long-term pattern (Sparks et al. 1990). Large floods are not disruptive events in the long term, as they contribute to the dynamic equilibrium of the system. Such flood events can reset late successional stages to earlier stages, thereby increasing habitat and species diversity (Sparks et al. 1990). Sand islands are an example of such an ephemeral early successional habitat in the Missouri River. Grace (1985) reported 46 species, or two-thirds of the total lower Missouri River fish fauna, utilized this habitat. Also, two federally listed birds, the least tern (Sterna antillarum) and piping plover (Charadrius melodus) nest primarily on sand islands.

Man has isolated rivers from their floodplains by draining and filling wetlands, channelizing river segments, constructing levees to contain flood flows within the main channel, and constructing mainstream dams and impoundments to reduce downstream flooding and regulate flow (Petts 1984, Brookes 1988, Ward and Stanford 1989, Bayley 1991). These activities have drastically affected aquatic communities and processes and severed the river-floodplain linkage. Channelization and damming, together with agricultural, municipal, and industrial pollution, constitute the major man-induced disturbances to the integrity of the world's large river ecosystems. We will briefly describe the nominal state of the Missouri River and then summarize effects of these disturbances.

MISSOURI RIVER ECOSYSTEM

The Missouri River's present southeasterly diagonal course across the midcontinent of the United States (U.S.) traces the southern limits of Pleistocene glaciation (Fig. 3). It is the longest river in the U.S., 3,768 km, with a drainage basin encompassing about 1,327 thousand km^2 or about one-sixth of the continental U.S. Four physiographic provinces comprise its drainage basin: 142 thousand km^2 of the Rocky Mountains in the west, 932 thousand km^2 of the Great Plains in the center of the basin, 228 thousand km^2 of Central Lowlands in the north lower basin, and 24.5 thousand km^2 of the Interior Highlands in the south lower basin (Slizeski et al. 1982, Robison 1986). River slope varies from about 38 m km^{-1} in the Rocky Mountains to an average of 0.17 m km^{-1} in the Great Plains and Central Lowlands (USACE 1985). A prominent feature of the Missouri River's drainage pattern is that most major tributaries in the upper and middle portions of the basin enter on the right bank, flowing to the east or northeast.

Climate of the basin is controlled by three air circulation patterns: one originating in the Gulf of Mexico, another in the northern Pacific Ocean, and the third in the northern polar

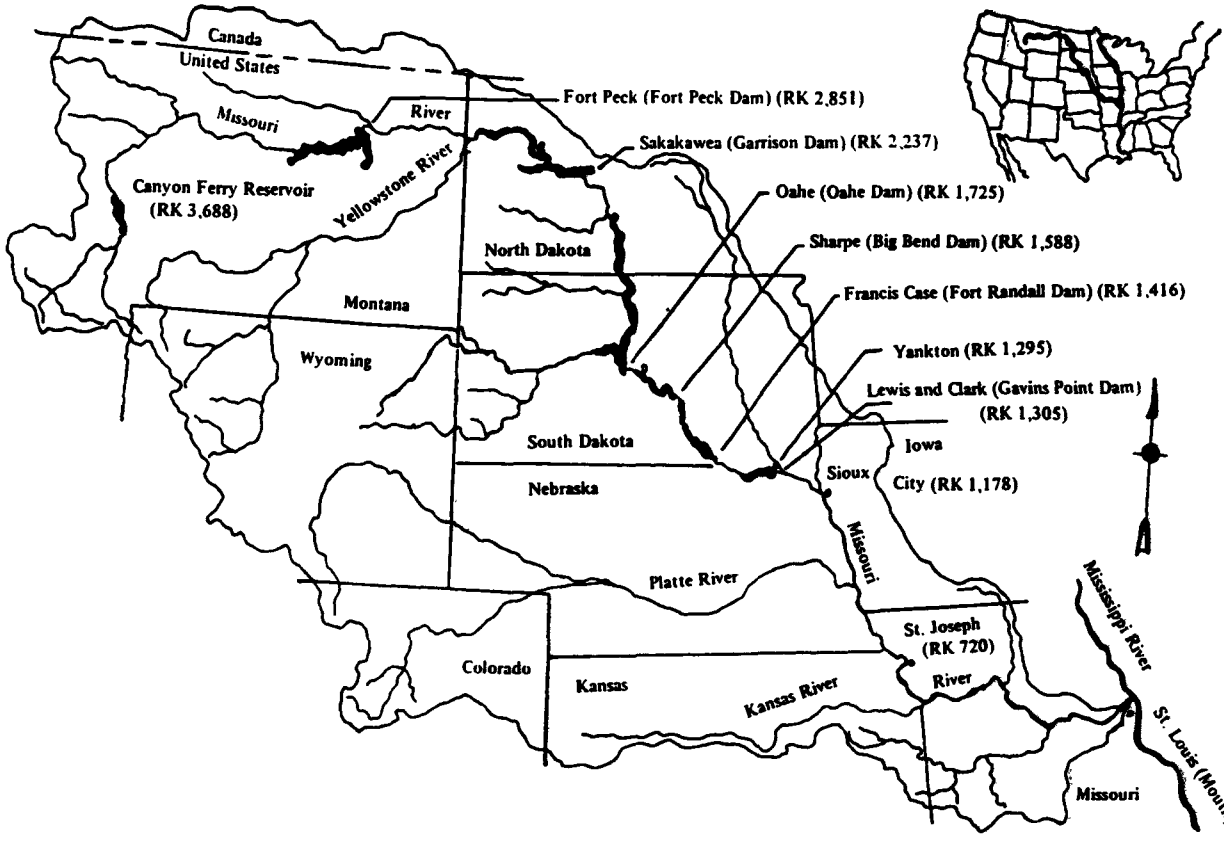

Fig. 3. The Missouri River Basin showing most of the civil works projects completed by the U.S. Army Corps of Engineers and U.S. Bureau of Reclamation. RK represents river kilometer. (Source: Hesse et al. 1989a.)

region (USACE 1985). The freeze-free season ranges from fewer than 40 days in the Rocky Mountains to more than 120 days in the Interior Highlands (Hesse et al. 1989a). The drainage basin is generally arid and subject to seasonal and long-term droughts due to the dominance of the Great Plains physiographic region. Average annual precipitation ranges from more than 80 cm in the Rocky Mountains, to about 45 cm in the Great Plains, and more than 90 cm in the Interior Highlands (Hesse et al. 1989a).

Annual Missouri River discharge to the Mississippi River is about 7.0×10^{10} m^3. Two seasonal periods of flooding occurred prior to impoundment (Fig. 4). The first, or March rise, was caused by snow melt in the Great Plains and break-up of ice in the main channel and tributaries. The second, or June rise, was produced by runoff from snow melt in the Rocky Mountains and rainfall throughout the basin.

In presettlement times the Missouri River was one of the most turbid river systems in North America, earning it the nickname Big Muddy. The magnitude of the Missouri River's sediment load can best be illustrated by its influence on the Mississippi River. Average turbidity of the Mississippi River upstream of the preimpounded Missouri River was reported

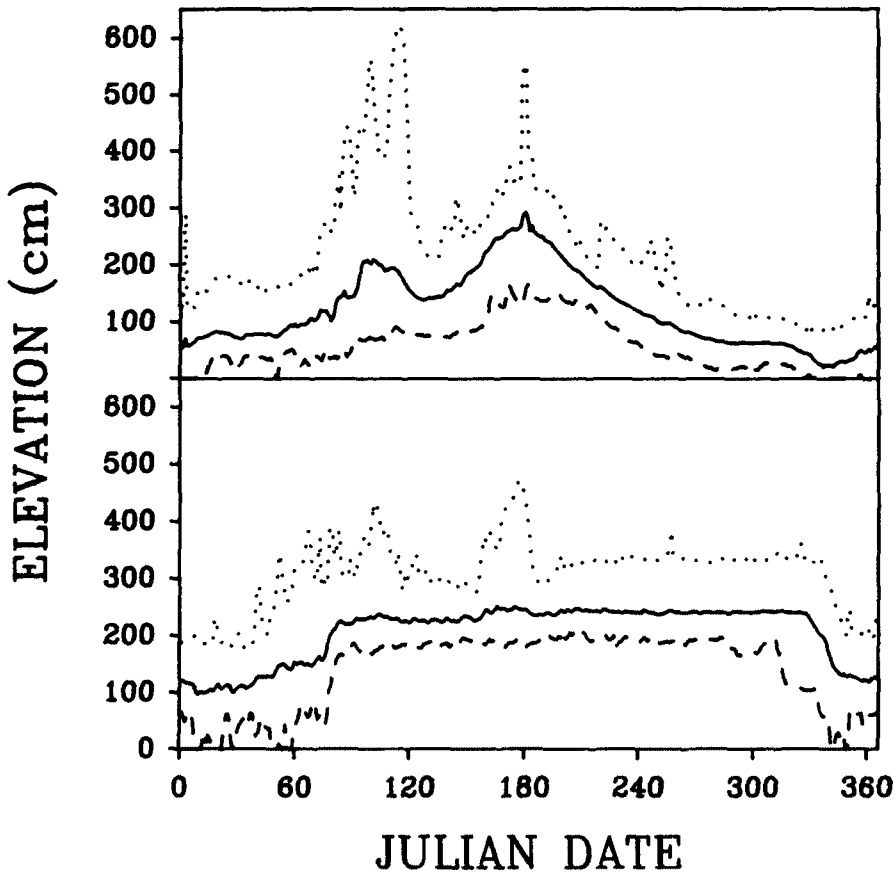

Fig. 4. Mean (solid line), maximum (dotted line) and minimum (dashed line) stages of the pre- (upper panel) and post-regulated (lower panel) Missouri River at Omaha, Nebraska (Rkm 1107). Pre-regulated stages are based on averages of daily stage recordings between 1880 and 1899 and post-regulated stages are based on averages between 1966 and 1985. (Modified from Hesse and Mestl 1992.)

by Platner (1946) as 300 ppm, whereas below the mouth of the Missouri River the average increased to 1,800 ppm.

The Missouri River's course through highly erodible soils resulted in major changes in channel configuration during flooding. During normal flows the channel was characterized by continuous bank erosion, a braided shifting configuration, and numerous sand islands and bars. Extensive channel migration in the lower river resulted in a floodplain width of 2.4 to 27.4 km, averaging 8.1 km (Hesse et al. 1989a). For example, about one-third of the floodplain of the lower Missouri River was reworked by the river between 1879 and 1930 (Schmudde 1963).

Erosional and depositional characteristics of this dynamic equilibrium resulted in a range of serial forest communities in the Missouri River floodplain (Bragg and Tatschl 1977). Recently deposited and exposed sandbars are rapidly colonized by willows *(Salix* spp.) and succeeded by cottonwood *(Populus deltoides)*, which dominates the canopy for up to 30

TABLE 1. SELECTED CHRONOLOGY OF SIGNIFICANT EVENTS IN THE HISTORY OF LOWER MISSOURI RIVER DEVELOPMENT (PRINCIPAL SOURCES: FUNK AND ROBINSON 1974, FORD 1982, HESSE 1987, HESSE ET AL. 1982, 1989A, BENSON 1988, SCHMULBACH ET AL. 1992)

Date	Event
1803	Acquisition of basin to U.S. from France through Louisiana Purchase.
1804-06	Captains M. Lewis and W. Clark expedition of Missouri River from mouth at St Louis, Missouri, to origin in Montana.
1819	First steamboat travel on Missouri River.
1829	First commercial steamboat barge line: St. Louis to Leavenworth, Kansas--Steamboat era begins.
1832	Snag removal authorized under Act of Congress.
1838	2,245 large trees removed from river channel and 1,700 overhanging trees cut from bank in 619 km of river upstream from St Louis.
1867-68	Major C. W. Howell Survey and Report on Improvement of Missouri River.
1869	Peak of steamboat era; 47 steamboats deliver about 9,000 mt of cargo to Ft. Benton, Montana, 3,540 km upstream from St. Louis.
1881	Lt. Col. C. R. Suter Report detailing long-range plans for aiding navigation on river.
1884	Missouri River Commission established by Congress to improve navigation of river by contracting its width, stabilizing channel location, protecting banks from erosion, and snag removal.
1885-1910	Snag removal systematic and intensive; 17,676 snags, 69 drift piles, and 6,073 overhanging bankline trees removed in 866 km of river in 1901 alone.
1902	Repeal of Act establishing Missouri River Commission. Railroads dominate freight traffic--Steamboat era ends.
1902	Congress enacts Reclamation Act of 1902 (PL 57-161) to survey, construct, and maintain irrigation works in arid lands of the western United States. Start of reservoir development planning.
1902-12	No maintenance of Commission structures--most wash out.
1910	Increase in typhoid deaths in towns along Missouri River.
1912	Congress authorizes 1.8 m deep, 61 m wide channel from Kansas City, KS to St. Louis, Missouri (PL 62-241).

(continued)

Table 1. (continued)

Date	Event
1912-17	Active dike and revetment construction to stabilize channel.
1913	U.S. Public Health Service (USPHS) report identifies sewage pollution in river as a major factor in typhoid deaths.
1917-33	Maintenance of channel structures, active period of levee construction.
1920-58	Records and studies of water suppliers and USPHS confirm bacterial contamination. Treatment by most water suppliers does not meet USPHS standards.
1925	PL 68-585 authorizes 200 ft wide channel, Kansas City, Missouri, to mouth.
1927	Extension of 1.8 m deep channel to Sioux City, IA (PL 70-560).
1934	Passage of Fish and Wildlife Coordination Act (PL 73-121) requiring that fish and wildlife receive equal consideration to other purposes of federal planning in federally funded or approved water development projects.
1936	Passage of Flood Control Act (PL 74-738) to develop "works of improvement" on more than 50 major river throughout the U.S.
1937	Construction completed on the first mainstem dam and impoundment on Missouri River, Ft. Peck Dam and Reservoir, Montana, to supply water for river navigation.
1944	Flood Control Act of 1944 (PL 78-534) authorized Pick-Sloan Plan to construct 6 dams on mainstem of Missouri River. Missouri River Bank Stabilization and Navigation Project authorized for flood control, bank stabilization, land reclamation, hydropower generation, and development and maintenance of navigation channel.
1945	Rivers and Harbors Act (PL 79-14) passed, provided a 2.7-m deep, 91.4-m wide navigation channel from St. Louis to Sioux City.
1946	Fish and Wildlife Coordination Act of 1946 (PL 79-732) passed, required federal agencies to construct water projects with a view to preventing loss of and damage to wildlife resources.
1946-55	5 additional dams and reservoirs constructed on Missouri River. See Table 4 for details.
1956	Federal Clean Water Act (PL 84-660) passage strengthens water quality regulations.
1958	Fish and Wildlife Coordination Act of 1958 (PL 85-624) required the cost of water project modifications or land acquisition earlier required under PL 79-732 to prevent loss or damage to wildlife be included as part of the project costs.

(continued)

Table 1. (continued)

Date	Event
1960-	Replacement of permeable pile dikes with impermeable rock dikes.
1960-70	Construction of primary wastewater treatment facilities for major discharges on lower river.
1964	Fish kill in Missouri River extending more than 161 km downstream from Kansas City, Missouri.
1965	Federal Water Project Restoration act (PL 89-72) required non-federal public agencies to administer fish, wildlife, and recreation on project lands and pay one-half of costs allocated to these resources.
1969	Flavor tests reveal unacceptable tastes in fishes from several locations in Missouri River. PCB levels in common carp pose potential threat.
1969	Federal Water Pollution Control Authority, and later U.S. Environmental Protection Agency (USEPA), establishes downstream minimum daily average flow requirements to maintain federally approved water quality standards.
1970-71	25% of fishes sampled from a bay in Lake Oahe, South Dakota, contained unsafe levels of methylmercury. Source of mercury was mining operations on a tributary stream.
1970-74	PCBs, aldrin, and dieldrin levels in fishes at Hermann, Missouri, pose potential health threat.
1971	USEPA study reveals levels of Salmonella, fecal coliform bacteria, and viruses in Missouri River present a potential hazard for drinking water or recreation.
1972	Federal Water Pollution Control Act of 1972 (PL 92-500) passed requiring USEPA to establish national effluent and toxic discharge standards.
1972-88	Construction of secondary wastewater treatment facilities for most major dischargers to lower river.
1973	Endangered Species Act (PL 93-205) passed requiring U.S. Fish and Wildlife Service to list species threatened or endangered with extinction; authorizes programs for their recovery; prohibits authorization of federal projects that jeopardize listed species or their habitats. See Table 6 for Federally listed Missouri River biota.
1975-80	U.S. Army Corps of Engineers (USACE) constructs environmental notches in 1,306 wing dikes from Sioux City to St. Louis to create fish habitat on downstream side of dike.
1976-78	PCBs, aldrin, dieldrin, and chlordane residues in fishes exceed safe limits.

(continued)

Table 1. (continued)

Date	Event
1976	Toxic Substances Control Act (PL 94-469) passed phasing out use of PCBs and restricting use of chlordane.
1977	Clean Water Act of 1977 (PL 95-217) established national effluent standards for water pollutants; required cities to implement secondary sewage treatment and provided federal grants to aid construction; set target date for discharge elimination of 129 priority pollutants; required USEPA permit for point source discharge of pollutants.
1978	240 km of free-flowing Missouri River in Montana and 93 km below Gavins Point Dam incorporated into National Wild and Scenic Rivers System.
1980	37 common industrial solvents (13 metals, 23 organic compounds, and cyanide) detected in St. Louis water treatment plants.
1984-86	Chlordane levels in fish flesh from lower Missouri River reported to exceed safe limits for consumption.
1986	Water Resources Development Act (PL 99-662) authorizes USACE to mitigate aquatic and terrestrial habitat losses from past projects.
1987-	Missouri Department of Health advisories issued warning against consumption of specific commercial fish species from areas of Missouri River due to toxic contamination.
1988	Missouri River Natural Resources Committee (MRNRC) established to promote preservation, wise utilization, and enhancement of natural and recreational resources of Missouri River.
1988-90	Major drought in Missouri River Basin. Water shortage precipitates conflict over water allocations for navigation versus recreation. USACE initiates Master Manual Review and updates to develop and evaluate alternative water management operations for mainstem reservoir system.
1989	Mississippi Interstate Cooperative Resource Agreement (MICRA) formed by various entities in the Mississippi River Basin (see text).
1989-	More stringent discharge permit limitations for toxic metals and organics imposed on wastewater treatment facilities of major cities along lower Missouri River, Missouri.
1990-	Upper Missouri River Basin states (Montana, N. and S. Dakota) sue USACE claiming reservoir operation should consider upstream recreation needs in addition to lower river navigation needs for water releases.
1990	Missouri River Initiative (Missouri River--Conserving a River Ecosystem, MOR-CARE) formed to facilitate cooperation among governmental, Tribal, and private parties for optimal recovery of natural resource values and environmental health of Missouri River ecosystem (see text).

(continued)

Table 1. (continued)

Date	Event
1990	Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (PL 101-646) passed to prevent and control infestations of coastal inland waters by zebra mussel and other nonindigenous aquatic nuisance species.
1991	USACE mitigation projects begin on lower Missouri River, include land purchases in floodplain and construction to enhance aquatic resources (see text).
1991	A 63 km section from Ft. Randall Dam to headwaters of Lewis and Clark Lake, including 40 km of lower Niobrara River and 13 km of Verdegie Creek, designated a National Recreation River
1991	Missouri Department of Conservation publishes Big River Fisheries 10 year Strategic Plan.
1992	Introduction of Gunderson Bill (H.R. 4169) to establish a Council on Interjurisdictional Rivers Fisheries and to provide funds to MICRA to conduct a comprehensive study of the status, management, research, and restoration needs of fisheries of Mississippi River drainage basin (see text).
1992	Closure of commercial fishing for all catfish species in lower Missouri River (see text).

years. Box elder *(Acer negundo),* silver maple *(Acer saccharinum),* red mulberry *(Morus rubra),* and American elm *(Ulmus americana)* replace cottonwood as an intermediate serial stage. Mature floodplain forests contain several species of oaks *(Quercus* spp.) and hickories *(Carya* spp.), plus hackberry *(Celtis occidentalis),* American elm, black walnut *(Juglans nigra),* green ash *(Fraxinus pennsylvanica),* sycamore *(Plantanus occidentalis),* basswood *(Tilia americana),* and--almost exclusively in old growth forests--pawpaw *(Asimina triloba)* (Weaver 1960, Bragg and Tatschl 1977). Little light penetrates the dense canopy of mature Missouri River floodplain forests resulting in an understory dominated by climbing vines (Weaver 1960), including poison ivy *(Rhus radicans),* Virginia creeper *(Partenocissus quinquefolia),* and wild grapes *(Vitis* spp.).

Studies of the structural and functional biology of the Missouri River abound. Sowards and Maxwell (1985) list more than 600 Missouri River references and Hesse et al. (1982, 1989a) provide comprehensive reviews of phytoplankton, periphyton, invertebrates, fishes, and energy dynamics of the river and its mainstem reservoirs. Many of these studies treat how biota and processes have been influenced by river alteration, and are referenced in Table 1. Lower Missouri River fish and fisheries are treated in a separate section.

ALTERATIONS TO THE MISSOURI RIVER ECOSYSTEM

Modifications to the integrity of the natural Missouri River-floodplain ecosystem have been immense and ongoing for more than 150 years (Table 1). Presently, 35% (1,316 km) of the river's length is impounded, 32% (1,212 km) is channelized or stabilized, and the

remaining 33% (1,241 km) is free-flowing (Schmulbach et al. 1992). Major civil works projects included channelization, channel maintenance, and impoundment and reservoir operation. Total cost for construction, operation, and maintenance of civil works projects through 1984 was $5.9 billion dollars (Table 2, Hesse 1987). Agricultural, industrial, and urban development within the basin also significantly modified the Missouri River and produced extensive water pollution (Table 1).

CHANNELIZATION

Abundant large woody debris (snags) in the river channel, fluctuating water levels, and extensive channel migration made early Missouri River navigation perilous. Modifications of the river to facilitate navigation included snag removal; channel dredging; and construction and maintenance of dikes, revetments, and levees. Stabilizing a river channel contrasts sharply with the concept of dynamic equilibrium discussed earlier. Stabilized channels are static. They lack the successional pattern and periodic disturbance events that maintain physical habitat diversity. Consequently, structure and function of the biological system also become stabilized. Funk and Robinson (1974) described how channelization and associated activities were accomplished in the lower Missouri River and we summarize its chronology in Table 1. Presently all of the Missouri River from Sioux City, Iowa, to its mouth at St. Louis, Missouri, is channelized. Even during flooding only about 10% of the original floodplain is inundated, as high agricultural levees confine the river to a width of 183-335 m (Schmulbach et al. 1992). Impacts of snag removal and channelization have been numerous and severe on

TABLE 2. SUMMARY STATISTICS FOR CIVIL WORKS PROJECTS IN THE MISSOURI RIVER BASIN THROUGH 1984. CONSTRUCTION COSTS ARE ACTUAL DOLLARS SPENT, AS ARE OPERATIONS AND MAINTENANCE ON CORPS OF ENGINEERS PROJECTS, BUT OPERATIONS AND MAINTENANCE HAD TO BE ESTIMATED FOR BUREAU OF RECLAMATION PROJECTS. THE DOLLARS DEPICTED IN THIS TABLE ARE CONSIDERED CONSERVATIVE ESTIMATES. (SOURCE: HESSE 1987)

		Millions of dollars		
Project type	Numbers of projects	Construction cost	Operations and maintenance	Total costs
Channel	19	561.5	369.0	930.5
Levee	2	92.2	NA	92.2
Reservoir	77	3948.1	921.7	869.8
Other construction	NA	235.0	1.6	236.6
Federal recreation	19	21.8	NA	21.8
Total	117	4858.6	1292.3	150.9

TABLE 3. SUMMARY OF EFFECTS OF RIVER CHANNELIZATION (C), INCLUDING SNAG REMOVAL, AND CONSTRUCTION OF DIKES, REVETMENTS, AND LEVEES; CONSTRUCTION AND OPERATION OF MAINSTREAM DAMS (D), AND BOTH TYPES OF ALTERATIONS (CD) ON THE LOWER MISSOURI RIVER ECOSYSTEM

Physical

C Changes in channel geomorphology:
8% reduction in channel length
27% reduction in bank-to-bank channel area
50% reduction in original surface area of river
98% reduction in surface area of islands
89% reduction in number of islands
97% reduction in area of sandbars

resulting in reduction in channel diversity through loss of side channels, backwaters, islands, and meandering (Funk and Robinson 1974, Hesse et al. 1988).

C Change in physical substrate from dominance of silt, sand, and wood to rock rip-rap.

C Increased water depth and velocity in main channel.

D Pre- versus post-impoundment declines in suspended sediment loads at Omaha, Nebraska, and St. Louis, Missouri, of 175 to 25 and 250 to 125 million tonnes per year (Schmulbach et al. 1992).

D Reduction in river sediment load resulting in channel bed degradation including channel deepening, increased bank erosion, and drainage of remnant backwaters downstream from dams (Hesse et al. 1988, 1989a, b).

D Silt-clay fraction of suspended sediment load reduced by 50%, but sand fraction increased 260%, following closure of Gavins Point Dam in 1954 (Slizeski et al. 1982).

D Reduction in turbidity resulting in increased light penetration (Morris et al. 1968, Pflieger and Grace 1987).

D Modification of natural flow regime by evening maximum and minimum discharges and eliminating periodic flood pulse.

D Reduction in annual temperature range.

CD Loss of periodic flooding and floodplain connectivity.

Chemical

C Higher water velocities reduce travel time for dissolved ions, nutrients, and contaminants.

(continued)

Table 3. (continued).

Chemical (continued)

D Increase in dissolved oxygen concentrations below mainstem dams (Morris et al. 1968).

D Higher postimpoundment summer flows for navigation dilute impacts of point source discharged pollutants (Ford 1982).

D Reductions in nitrogen and phosphorus concentrations downstream from reservoirs and changes in spiraling patterns (Ward and Stanford 1983, Schmulbach et al. 1992).

Biological

C Decline in habitat richness resulted in presumed decrease in diversity of periphytic algae (Farrell and Tesar 1982).

C Elimination of plankton and invertebrates produced in standing water chutes and sloughs with loss of these habitats (Whitley and Campbell 1974).

C Loss of instream snag habitat and functions of organic matter retention and substrate for invertebrates and fishes (Benke et al. 1985).

C Greater standing crop of benthic invertebrates in main stream of unchannelized versus channelized river sections (Berner 1951, Morris et al. 1968, Nord and Schmulbach 1973).

C Smaller standing crops of benthic invertebrates in chutes and mud banks of unchannelized versus channelized sections (Morris et al. 1968).

C Larger standing crop of drift in unchannelized than channelized sections of river and little similarity between drift and benthos (Morris et al. 1968, Modde and Schmulbach 1973).

C 67% reduction in benthic area suitable for invertebrate colonization (Morris et al. 1968).

C 54% decline in benthic invertebrate production from all unchannelized habitats of Missouri River downstream from mainstem dams between 1963 and 1980 and 74% decrease in production in chute/backwater habitats (Mestl and Hesse 1992).

C Loss of river-floodplain connection for fish migration, spawning and rearing.

C Reduction in microhabitats resulting in decreased abundance of fish species in channelized versus unchannelized section of river in Nebraska (Schmulbach et al. 1975).

C Higher standing crop of sportfishes in unchannelized sections of river in Nebraska compared with channelized sections attributed to more backwater habitat and greater habitat diversity (Groen and Schmulbach 1978).

(continued)

Table 3. (continued)

Biological (continued)

C	Loss of nesting habitat for sandbar/sand island birds leading to drastic population declines (e.g., Sterna albifrons, Charadrius melodus).
D	Elimination of riparian forests and stream channels in areas flooded by reservoirs, totaling more than one-third entire length of Missouri River (Hesse et al. 1988).
D	Entrainment of fluvial particulate organic matter in reservoirs.
D	Temperature induced shifts in periphyton and phytoplankton community structure, particularly below dams (Farrell and Tesar 1982, Reetz 1982).
D	Increase in periphyton primary production below dams (Ward and Stanford 1983).
D	Increased relative importance of phytoplankton biomass and primary production compared with upstream allochthonous inputs.
D	Increase in diversity and density of zooplankton community in river downstream from reservoirs (Repsys and Rogers 1982).
D	Changes in standing crop and diversity, and shifts in functional feeding groups of benthic macroinvertebrates in river downstream from reservoirs (Ward and Stanford 1979).
D	Alteration of emergence cues, egg-hatching, diapause-breaking, and maturation of aquatic insects due to thermal modifications below reservoirs (Ward and Stanford 1979, Petts 1984).
D	Blockage of riverine fish migration.
D	Inundation of floodplain fish spawning and nursery habitats.
D	Development of extensive sportfisheries in reservoirs and tailwaters (Hesse et al. 1989a).
CD	Near elimination of natural riparian community (Hesse et al. 1988, 1989a, b). Changes reported: -41% deciduous vegetation -12% grasslands -39% wetlands
CD	25% decrease in post-dam tree growth in North Dakota floodplain compared with pre-dam period related to absence of annual soil profile saturation, lowering of water table in spring to reduce downstream flooding (Reiley and Johnson 1982), and lack of nutrient silt deposition (Burgess et al. 1973).

(continued)

Table 3. (continued)

Biological (continued)

CD	Increasing proportion of mature forest to other successional stages in remaining floodplain (Bragg and Tatschl 1977).
CD	80% decline in organic carbon load of post-control Missouri River to Mississippi River compared with pre-control (Hesse et al. 1988).
CD	Loss of major floodplain habitat types reduced populations of associated flora and fauna (Clapp 1977).
CD	Decreases in endemic large river fishes (e.g., Scaphirhynchus albus, Polydon spathula, Cycleptus elongatus, Hybopsis gracilis) and increases in pelagic planktivores (e.g., Dorosoma cepedianum, Alosa chrysochloris) and sight-feeding carnivores (e.g., Morone chrysops, Lepomis macrochirus) (Pflieger and Grace 1987, Hesse et al. 1992).
CD	Population declines of 11 native Missouri River Basin biota leading to listing as Federally threatened or endangered (Table 7).
CD	As much as an 80% decline in commercial fishery in Nebraska and 97% decline in tailwater recreational fishery below Gavins Point Dam (Hesse and Mestl 1992).
CD	Decline in legal-sized catfishes in Missouri River, Missouri, attributed in part to increased susceptibility to exploitation due to lost habitat diversity (Funk and Robinson 1974, Robinson 1992).
CD	Introduction and establishment of non-native fishes and invertebrates (e.g., Oncorhynchus spp., Osmerus mordax, Mysis relicta). See Table 6 for list of introduced fishes.

Social

D	Hydroelectric power generation of over 2.2 Gw, sales totaling $1.5 billion from 1943-86 (Sveum 1988).
D	Development of major reservoir-based recreation and associated commercial services, supported spending of $65 million in 1988 (General Accounting Office 1992).
CD	Commercial navigation industry transports about 2 million tonnes goods, producing gross revenues of $17 million in 1988 (General Accounting Office 1992).
CD	Water supply provided to 40 cities (3.2 million people), 21 power plants, and 2 chemical manufacturers in lower Missouri River (General Accounting Office 1992).
CD	4,000% increase in area of agricultural land use (Hesse et al. 1988).
CD	95% of protected floodplain now in agricultural, urban, and industrial uses (Hesse et al. 1989b).

the physical, chemical, and biological structure and function of the Missouri River and its floodplain (Table 3). The most damaging of these alterations to aquatic communities has been the near complete isolation of the river from its floodplain, subsequent loss of floodplain habitat, a drastic reduction in area and diversity of river channel habitats, and an increase in main channel flow velocity. See Brookes (1988) for a further review of the general physical and biological impacts of river channelization.

DAM CONSTRUCTION AND OPERATION

Widespread flooding during the war years of 1942-44 was the impetus for passage of the 1944 Flood Control Act to construct a six-dam mainstem Missouri River flood control system (Keenlyne 1988). Called the Pick-Sloan Plan, it would "...provide for the most efficient utilization of waters of the Missouri River Basin for all purposes including irrigation, navigation, power, domestic and sanitary purposes, wildlife, and recreation" (House Report No. 475 1944).

The last project, Big Bend, was completed in 1963 yielding a total storage capacity for the six reservoirs of 91.5 km^3, the largest of any system in the U.S. (Table 4). Other large storage reservoirs, more than 1,300 smaller impoundments, and farm ponds also have been built on the Missouri River mainstem and tributaries (Schmulbach et al. 1992). Sveum (1988) summarized the controversial operating history of the six mainstem reservoirs for their designed multiple uses.

Impacts of mainstem regulation on downstream lotic ecosystems are numerous and well documented (Ward and Stanford 1979, 1983, Lillehammer and Saltveit 1984, Petts 1984, Davies and Walker 1986, Dodge 1989, National Research Council 1992). Reduction in suspended sediment loads and turbidity in the lower Missouri River has been one of the most obvious results of upstream impoundment (Morris et al. 1968, Whitley and Campbell 1974, Ford 1982, Slizeski et al. 1982, Schmulbach et al. 1992).

Average annual suspended loads decreased between 67 and 99% among various lower river cities (Table 5) and mean annual turbidity at the mouth of the Missouri River above St. Louis, Missouri, decreased fourfold from the 1930s to the 1970s (Fig. 5). Changes in particle sizes of suspended sediment, periphyton growth, and fish functional feeding groups have all been associated with reductions in suspended load and increased water clarity (Table 3). Concurrently, sediment retention by mainstem reservoirs has increased the erosive power of water discharged from dams. The river's bed 8.3 km downstream from Gavins Point Dam downcut 2.3 m between 1929 and 1980 and degradation continues to occur at least 346 km downstream to the mouth of the Platte River (Hesse et al. 1989a).

Alteration of the natural hydrograph has undoubtedly been the most significant impact of dam construction to the lower Missouri River and constitutes a major disturbance (sensu Resh et al. 1988) to the system. Bimodal March and June discharge maxima evident prior to impoundment have been replaced by a flat hydrograph for the April through November navigation season (Hesse and Mestl 1992, Fig. 4). Present water management has also reduced flushing flows or flows that exceed bank-full discharge (Hesse and Mestl 1992). Bank-full discharge is responsible for maintaining channel configuration and substrate composition, and is the discharge above which the floodplain is inundated in floodplain rivers (Stalnaker et al. 1989). Earlier we discussed the importance of the flood pulse to the integrity

TABLE 4. CHARACTERISTICS OF MAIN-STEM MISSOURI RIVER RESERVOIRS IN THE PICK-SLOAN PLAN. (SOURCES: SVEUM 1988, SCHMULBACH ET AL. 1992)

Dam (date closed)	Location (Rkm)*	Reservoir	Length (km)	Total volume (km^3)	Mean annual discharge (km^3/yr)	Drainage area (10^3 km^2)	Annual energy output (Kwh x 10^6)†
Fort Peck (1937)	2851	Fort Peck	216	23.30	7.8	148.9	1043
Garrison (1953)	2237	Sakakawea	286	29.50	21.3	320.9	2354
Oahe (1958)	1725	Oahe	372	28.80	22.8	160.8	2694
Big Bend (1963)	1588	Sharpe	129	2.34	19.4	13.2	1001
Fort Randall (1952)	1416	Francis Case	172	6.90	13.8	36.8	1745
Gavins Point (1955)	1305	Lewis and Clark	40	0.62	15.6	41.4	700

*Rkm = river kilometers; distance along the thalweg upstream of convergence of Missouri and Mississippi rivers.

†Kwh = kilowatt hours.

TABLE 5. AVERAGE ANNUAL SUSPENDED SEDIMENT LOAD (MILLION METRIC TONNES) IN THE LOWER MISSOURI RIVER, MODIFIED FROM FORD (1982)

Location	River (km)	Sediment load <1953	Sediment load >1955	Percent change
Yankton, South Dakota	1305	125.0	1.3	-99
Sioux City, Iowa	1178		10.7	
Omaha, Nebraska	1107	148.6	25.9	-83
Nebraska City, Nebraska	904		42.7	
St. Joseph, Missouri	727	233.3	52.3	-78
Kansas City, Missouri	579	215.9	71.7	-67
Boonville, Missouri	290	317.5		
Hermann, Missouri	161	295.9	91.4	-69

of large river ecosystems. Hesse and Mestl (1992) calculated bank-full discharge (the maximum instantaneous flow with a recurrence interval of about 1.5 years, Stalnaker et al. 1989) for the Missouri River between 1929 and 1948 as 3,115 m^3/sec. This discharge occurred in 15 of 24 mostly predam years (1929-1952), but in only 2 of 33 years following closure of mainstem dams (1954-1986). Channelization and impoundment of the Missouri River have effectively decoupled the lower river from its floodplain and disrupted the annual flood pulse. This disruption has resulted in widespread and severe disturbance to the physical, chemical and biological character of the lower river (Table 3).

WATER POLLUTION

Settlement of the Missouri River floodplain was accompanied by discharge of a variety of pollutants into the river. As early as 1909 increases in typhoid deaths within towns along the lower Missouri River prompted investigations into the sources of river water pollution (Ford 1982). Significant water pollution events in the lower Missouri River include contamination from municipal, industrial, and agricultural sources (Table 1).

Organic pollution from untreated human sewage, the meat packing industry, and stockyards produces bacterial and viral contamination of drinking water supplies, sludge deposition, and low dissolved oxygen concentrations resulting in fish kills (Ford 1982). Major industrial pollutants in the lower Missouri River include petroleum wastes, heavy metals (primarily mercury), and PCBs from urban industries and the processing of mined ores. Petroleum contamination has resulted in reports of off flavors in fishes (Ford 1982), and PCB

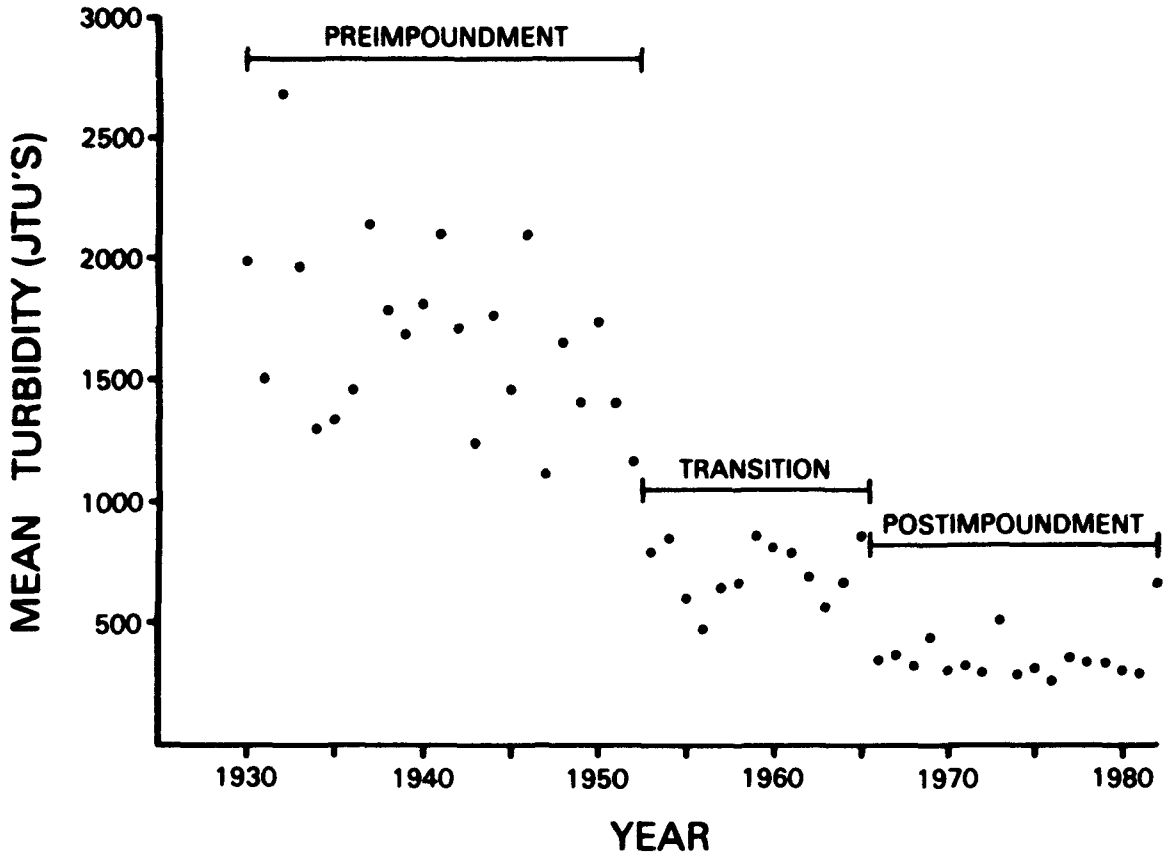

Fig. 5. Mean annual turbidities (JTUs) of the Missouri River determined from daily measurements at the St. Louis water-treatment facility. (Source: Pflieger and Grace 1987.)

concentrations in fish tissue have routinely exceeded allowable federal standards (Ford 1982, Bush and Grace 1989). Levels of PCBs in fishes from the Missouri reach of the Missouri River, have been declining since their manufacture and use was discontinued in 1979 (Bush and Grace 1989). Methylation of mercury, its bioaccumulation, and biomagnification in Missouri River tributaries, the mainstem river, and reservoirs, has created severe environmental hazards to fish and fish-eating birds and poses a potential threat to human health (Hesse et al. 1975, Schmulbach et al. 1992).

Concentrations of the agricultural pesticides dieldrin and DDT occasionally violated lower Missouri River water quality standards during the 1970s (Ford 1982). Violations of federal standards for dieldrin in fish were reported during the late 1970s (Ford 1982), but were below advisory levels by the mid 1980s (Bush and Grace 1989). Chlordane is a pesticide formerly used extensively in the Midwest for termite control in houses. High concentrations in fish tissue resulted in public health advisories against consumption of selected species and from specific river reaches in Missouri and Nebraska (Bush and Grace 1989, Christiansen et al. 1991). Chlordane use was banned in 1988 and fewer fish are currently exceeding unsafe levels (Bush and Grace 1989). More extensive reviews of these and other contaminants in

the lower Missouri River can be found in Ford (1982) and Schmulbach et al. (1992). Water quality legislation and enforcement requiring permits for point source pollution discharge to rivers, use of the best available technology to control toxic pollutants, and improved wastewater treatment for municipalities have done much to improve water quality in the lower Missouri River (See Table 1 for a summary of important environmental legislation).

FISHES AND FISHERY RESOURCES OF THE LOWER MISSOURI RIVER

The Mississippi River Basin, of which the Missouri River is a major tributary, supports the richest freshwater fish fauna in North America, about 260 species (Robison 1986). Freshwater dispersants make up about 88% of these species (Moyle and Cech 1988), predominantly Cyprinids (30%), Percids (26%), Centrarchids (7%), Catostomids (9%), and Ictalurids (5%); while diadromous (7%) and freshwater representatives of marine families (5%) are poorly represented. The Mississippi River Basin was also uniquely important in North America as a center of fish evolution, as a refuge during times of glaciation from which species have been able to reoccupy water vacated during glacial advances and as a refuge of ancient fish faunas (Moyle and Cech 1988). The archaic families Acipenseridae, Polyodontidae, Lepisosteidae, and Hiodontidae are all extant in the Missouri River Basin.

Hesse et al. (1989a) reviewed the fishes and fisheries of the entire Missouri River Basin. We will, therefore, concentrate on selected aspects of the lower basin. Ninety-one fish species have been reported from the mainstem Missouri River, Missouri (Table 6, Grace and Pflieger 1989). Surveys made at approximately 20-year intervals from 1940 to 1983 in the Missouri reach were analyzed by Pflieger and Grace (1987) and show an increase in the number of species collected and substantial changes in their relative abundances. Species reported to become established or more abundant were mostly pelagic planktivores and sight-feeding carnivores: skipjack herring, gizzard shad, white bass, bluegill, white crappie, emerald shiner, and red shiner (see Table 6 for scientific names). These shifts appear related to decreased turbidity (Fig. 5) and changes in the flow regime following impoundment.

Fishes that declined over the same period included common carp, river carpsucker, bigmouth buffalo, and two endemic large river species--pallid sturgeon and flathead chub. The pallid sturgeon was never reported as abundant throughout its range in the Missouri-Mississippi River Basin (Kallemeyn 1983). However, dangerously low populations and hybridization with shovelnose sturgeon (Carlson et al. 1985) posed such threats to the species survival that it was listed in 1990 as endangered under the Endangered Species Act (Table 7, 16 U.S.C. 1531). Habitat alteration and destruction due to dam construction and channelization, as summarized in Table 3, are cited as major factors responsible for this species' decline (Deacon et al. 1979, Kallemeyn 1983, Pallid Sturgeon Recovery Team 1992).

Reported declines in numbers of other mainstem Missouri River fishes in the Nebraska reach include several large species: paddlefish, sauger, flathead catfish, and blue sucker; in addition to several chub species: flathead, speckled, sturgeon, sicklefin, and silver (Hesse and Mestl 1992). Similar reductions in populations of small fishes were reported by Pflieger and Grace (1987) in the uppermost sections of the Missouri reach, but populations in lowermost sections of the Missouri reach appear stable or increasing. Populations of two silvery minnows (western silvery minnow and plains minnow), which typically occur in backwater habitats, have also declined throughout the lower Missouri River because of habitat loss (Pflieger and Grace 1987, Hesse and Mestl 1992).

TABLE 6. FISH FAMILIES AND SPECIES OF THE MISSOURI RIVER, MISSOURI, INCLUDING PRESENT FEDERAL (CANDIDATE FOR LISTING: C2, THREATENED: FT, ENDANGERED: FE) AND MISSOURI (WATCH LIST: WL, RARE: R, ENDANGERED: SE) STATUS AND IF INTRODUCED (I) TO THE BASIN. (SOURCES: GRACE AND PFLIEGER 1989, MISSOURI DEPARTMENT OF CONSERVATION 1991)

Family and species	Status
Petromyzontidae	
Chestnut lamprey, Ichthyomyzon castaneus	
Acipenseridae	
Lake sturgeon, Acipenser fulvescens	C2, SE
Shovelnose sturgeon, Scaphirhynchus platorynchus	
Pallid sturgeon, Scaphirhynchus albus	FE, SE
Polyodontidae	
Paddlefish, Polyodon spathula	WL
Lepisosteidae	
Shortnose gar, Lepisosteus platostomus	
Longnose gar, Lepisosteus osseus	
Anguillidae	
American eel, Anguilla rostrata	
Clupeidae	
Skipjack herring, Alosa chrysochloris	
Alabama shad, Alosa alabamae	R
Gizzard shad, Dorosoma cepedianum	
Hiodontidae	
Goldeye, Hiodon alosoides	
Mooneye, Hiodon tergisus	R
Osmeridae	
Rainbow smelt, Osmerus mordax	I
Esocidae	
Northern pike, Esox lucius	
Cyprinidae	
Common carp, Cyprinus carpio	I
Grass carp, Ctenopharyngodon idella	I
Bighead carp, Hypophthalmichthys nobilis	I
Silver carp, Hypophthalmichthys molitrix	I
Golden shiner, Notemigonus crysoleucas	
Creek chub, Semotilus atromaculatus	
Silver chub, Hybopsis storeriana	

(continued)

TABLE 6. (continued)

Family and species	Status
Cyprinidae (continued)	
Gravel chub, Hybopsis x-punctata	
Speckled chub, Hybopsis aestivalis	SE
	Flathead chub, Hybopsis gracilis
Sturgeon chub, Hybopsis gelida	C2, R
Sicklefin chub, Hybopsis meeki	C2, R
Suckermouth minnow, Phenacobius mirabilis	
Emerald shiner, Notropis atherinoides	
Rosyface shiner, Notropis rubellus	
Western redfin shiner, Notropis umbratilis umbratilis	
Silverband shiner, Notropis shumardi	
Common shiner, Notropis cornutus	
Striped shiner, Notropis chrysocephalus	
River shiner, Notropis blennius	
Bigeye shiner, Notropis boops	
Bigmouth shiner, Notropis dorsalis	
Spotfin shiner, Notropis spilopterus	
Red shiner, Notropis lutrensis	
Sand shiner, Notropis stramineus	
Channel mimic shiner, Notropis wickliffi	
Ghost shiner, Notropis buchanani	WL
Brassy minnow, Hybognathus hankinsoni	R
Western silvery minnow, Hybognathus argyritis	
Plains minnow, Hybognathus placitus	
Bluntnose minnow, Pimephales notatus	
Fathead minnow, Pimephales promelas	
Central stoneroller, Campostoma anomalum	
Largescale stoneroller, Campostoma oligolepis	
Catostomidae	
Blue sucker, Cycleptus elongatus	C2, WL
Bigmouth buffalo, Ictiobus cyprinellus	
Black buffalo, Ictiobus niger	
Smallmouth buffalo, Ictiobus bubalus	
River carpsucker, Carpiodes carpio	
Highfin carpsucker, Carpiodes velifer	R
Quillback, Carpiodes cyprinus	
White sucker, Catostomus commersoni	
Northern hog sucker, Hypentelium nigricans	
Golden redhorse, Moxostoma erythrurum	
Shorthead redhorse, Moxostoma macrolepidotum	
Ictaluridae	
Black bullhead, Ictalurus melas	
Yellow bullhead, Ictalurus natalis	

(continued)

TABLE 6. (continued)

Family and species	Status
Ictaluridae (continued)	
Channel catfish, Ictalurus punctatus	
Blue catfish, Ictalurus furcatus	
Tadpole madtom, Noturus gyrinus	
Freckled madtom, Noturus nocturnus	
Stonecat, Noturus flavus	
Flathead catfish, Pylodictis olivaris	
Gadidae	
Burbot, Lota lota	
Cyprinodontidae	
Blackstripe topminnow, Fundulus notatus	
Poeciliidae	
Mosquitofish, Gambusia affinis	
Atherinidae	
Brook silverside, Labidesthes sicculus	
Percichthyidae	
White bass, Morone chrysops	I
Striped bass, Morone saxatilis	I
Hybrid striper, Morone chrysops x Morone saxatilis	I
Centrarchidae	
Spotted bass, Micropterus punctulatus	
Smallmouth bass, Micropterus dolomieu	
Largemouth bass, Micropterus salmoides	
Warmouth, Lepomis gulosus	
Green sunfish, Lepomis cyanellus	
Orangespotted sunfish, Lepomis humilis	
Longear sunfish, Lepomis megalotis	
Bluegill, Lepomis macrochirus	
Rock bass, Ambloplites rupestris	
White crappie, Pomoxis annularis	
Black crappie, Pomoxis nigromaculatus	
Percidae	
Walleye, Stizostedion vitreum vitreum	
Sauger, Stizostedion canadense	
Slenderhead darter, Percina phoxocephala	
Ozark logperch, Percina caprodes fulvitaenia	
Johnny darter, Etheostoma nigrum	
Sciaenidae	
Freshwater drum, Aplodinotus grunniens	

Enrichment of lower Missouri River fish species diversity during the past 40 years appears due largely to accidental (e.g., Asian carps) and intentional (e.g, rainbow smelt, Morone spp.) introductions (Table 6), and an increased frequency of species in the mainstem that are stragglers from tributaries (Pflieger and Grace 1987). Regulation of the river appears to have reduced environmental constraints on these species.

COMMERCIAL AND RECREATIONAL FISHERY IN THE MISSOURI REACH

Data have been compiled since 1945 by the Missouri Department of Conservation (Robinson 1992) for the Missouri River, Missouri, on the number of licensed commercial fishers (determined from permit sales), weight of their reported catches, and amount and type of gear used. Numbers of commercial fishers gradually decreased from 1948 to 1963, remained fairly stable through 1969 and then increased to a peak of 1,039 in 1982 (Fig. 6). Causes for these fluctuations are unknown. However, the subsequent decline in permit sales

TABLE 7. FEDERALLY LISTED CANDIDATE (C1, C2, C3), THREATENED (T) AND ENDANGERED (E) SPECIES ENDEMIC TO THE MISSOURI RIVER FLOODPLAIN MISSOURI. (SOURCE: WHITMORE AND KEENLYNE 1990)

Species	Status
Plants	
Western prairie fringed orchid, Platanthera praeclara	T
Insects	
American burying beetle (Nicrophorous americanus)	E
Regal fritillary butterfly (Speyeria idalia)	C2
Six-banded longhorn beetle (Dryobius sexnotatus)	C2
Fish	
Listings included in Table 6	
Reptiles	
Alligator snapping turtle (Macroclemys temminckii)	C2
Birds	
Interior least tern (Sterna antillarum)	E
Piping plover (Charadrius melodus)	T
Whooping crane (Grus americana)	E
Bald eagle, Haliaeetus leucocephalus	E
Peregrine falcon, Falco peregrinus	E
Swainson's hawk (Buteo swainsoni)	C3
Eskimo curlew (Numenius borealis)	E
Migrant loggerhead shrike (Lanius migrans)	C2
Swallow-tailed kite (Elanoides forficatus)	C3
Mammals	
Gray bat (Myotis grisescens)	E
Indiana bat (Myotis sodalis)	E

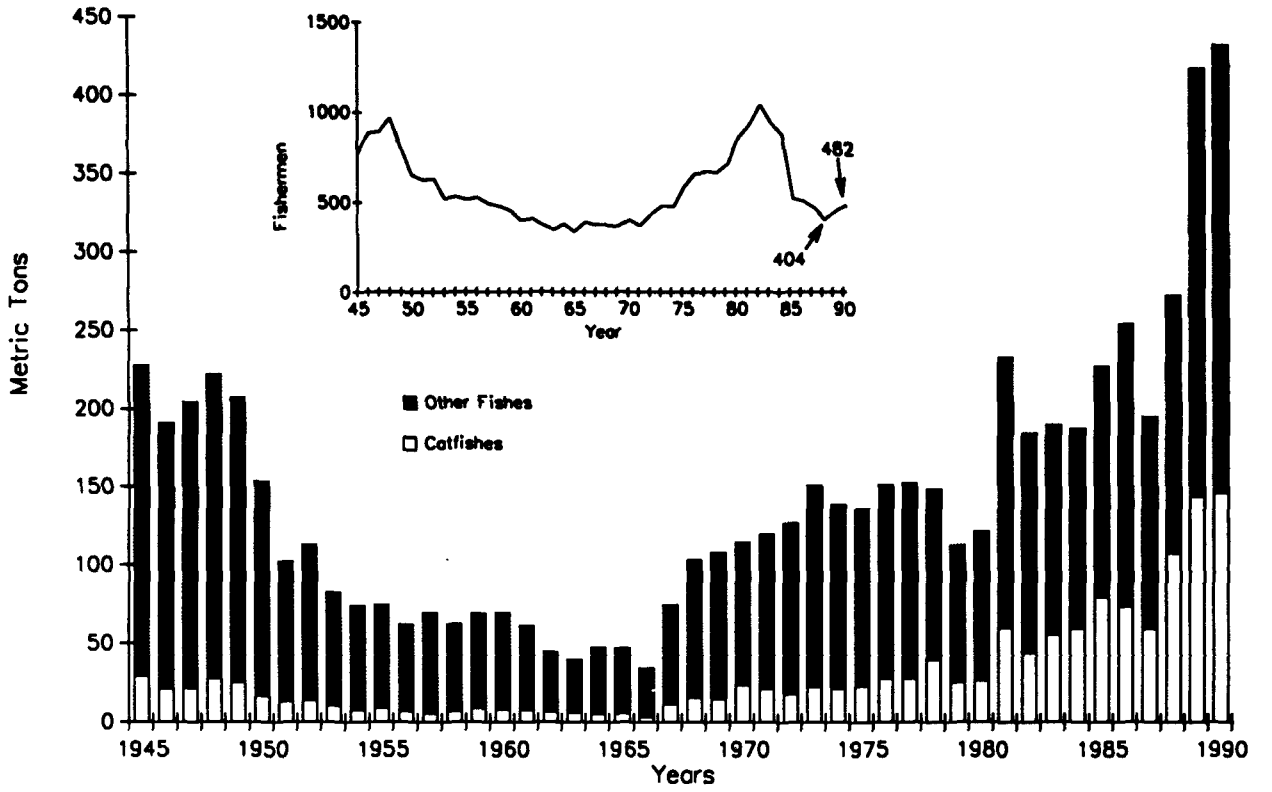

Fig. 6. Number of commercial fishers from the Missouri River, Missouri, and their reported harvest of all fish species and catfish species, 1945-1990.

from 1982 to 1988 may be due to increased commercial fishing fees implemented in 1984, and health advisories issued from 1987 to 1990 warning against consumption of Missouri River fishes (Robinson 1992). Total harvest first peaked in 1945 at 228 mt, then declined gradually to 35 mt in 1966, paralleling the decrease in numbers of fishers (Fig. 6). Methods of estimating annual harvest changed in 1967, yielding a more accurate but higher reported harvest. Methods of estimating harvest have been constant since 1975 and harvest has generally increased since then, with 1989 and 1990 being the highest years recorded (Fig. 6). These record harvests occurred despite the dramatic decline in numbers of fishers observed in the mid 1980s. Reasons for these high catches vary, but include low river water resulting from a basin-wide drought, increased vulnerability to gear, more accurate reporting of catch, and lack of concern over successive health warnings (Robinson 1992). The most important species in the commercial catch are common carp, buffalo fishes, and catfishes. Additional species contributing to the commercial harvest include: freshwater drum, carpsuckers, paddlefish, and shovelnose sturgeon.

Catfish harvest, particularly channel catfish, has increased dramatically during the past 10 years (Fig. 6). Concomitantly, there has been a continuous decline in the proportion of

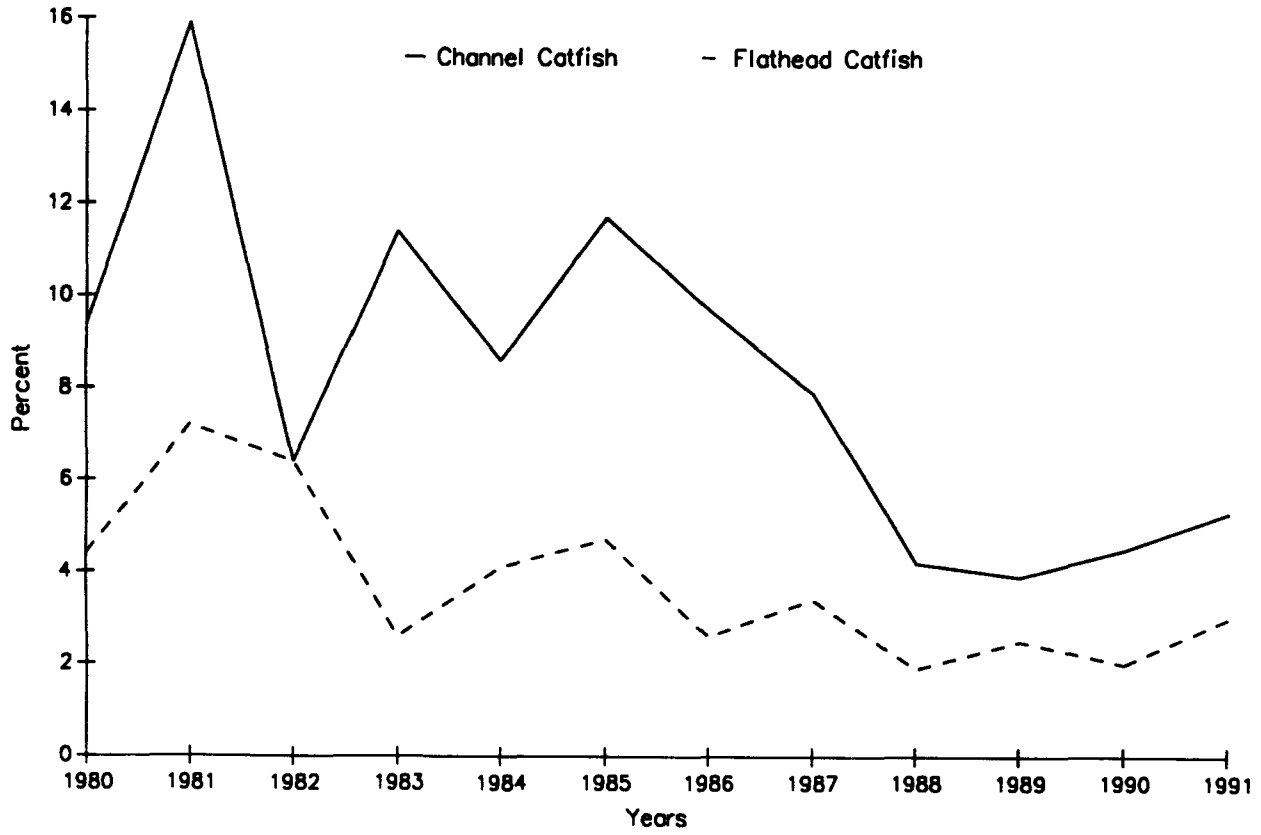

Fig. 7. Percentage of legal length (≥381 mm total length) channel and flathead catfishes captured by 2.5 cm mesh hoopnets (channel catfish) and electrofishing (flathead catfish) from Missouri River, Missouri, August-November 1990.

legal (≥381 mm total length) channel and flathead catfishes in research harvests from Missouri (Fig. 7) and other states bordering the lower Missouri River. Record high commercial catfish catches and a shift in population size structure to sublegal lengths implies overharvest. Consequently, all states bordering the lower Missouri River have recently prohibited commercial catfish harvest.

An additional impetus for closure of the lower Missouri River commercial catfish fishery was that most of the commercial harvest was captured by very few fishers while Missouri River recreational fishing is ranked as the number one public activity on the river (Weithman and Fleener 1988). Analysis of the number of commercial fishers and their reported harvest shows that 84% reported total annual catfish catches of less than 230 kg, while only 3% reported catches over 2000 kg. Nearly 50 and 70% percent of the total 1990 Missouri reach commercial catfish catch of 132,120 kg was reported by 10 and 25 fishers, respectively (Fig. 8). Weithman and Fleener (1988) estimated recreational fishing on the Missouri reach to be 86,000 days per year (96 visits/km or 3.1 visits/ha) from 1983 to 1985. Seventy percent of this effort was for catfishes, contributing 57% of the total catch (212,000 fish) and 69% of the total

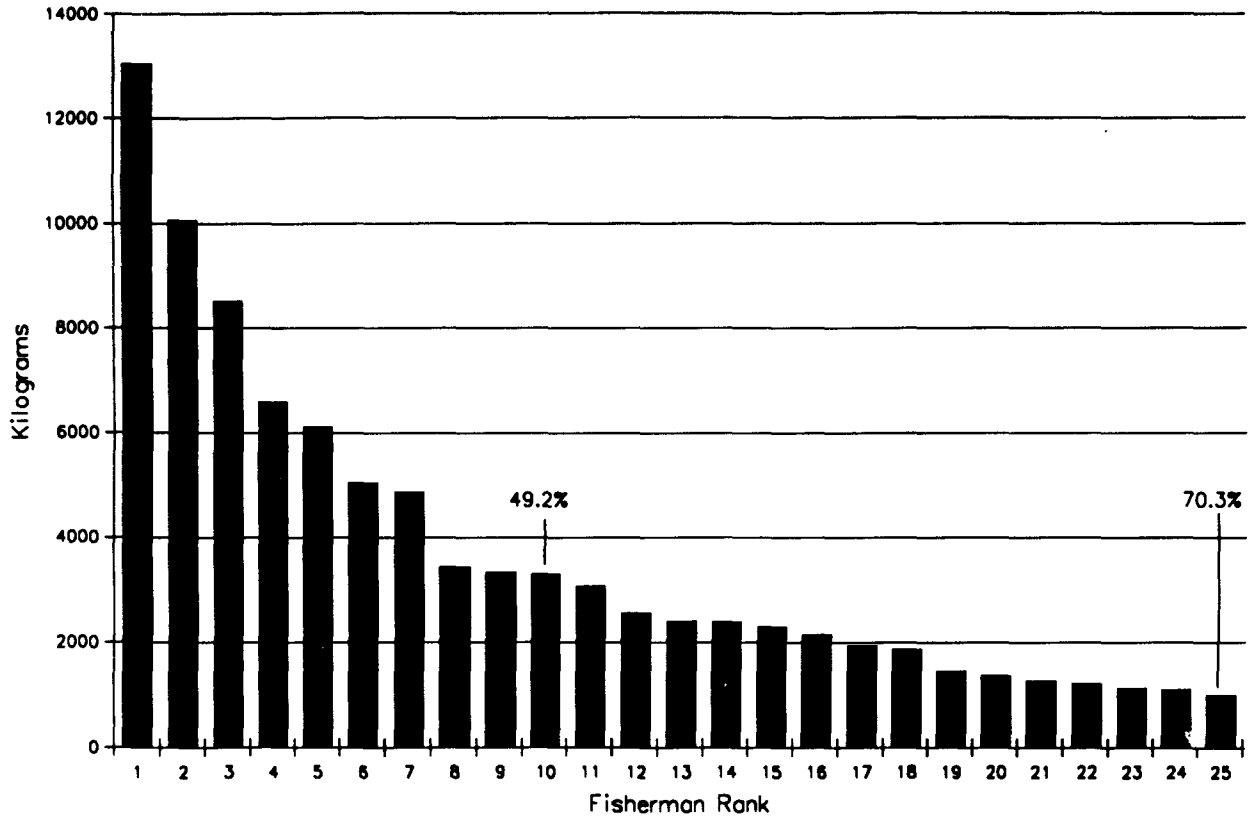

Fig. 8. Rank of the top 25 fishers and their reported total catch of all catfish species from Missouri River, Missouri, 1990.

fish harvested by the recreational fishery. Net annual economic benefits of recreation were estimated at $1.9 million and recreational fishing at $660,000 on the Missouri reach. These totals compare with an estimated net wholesale value of the commercial catch for all species from the Missouri River of $128,000 in 1987 (Robinson 1989). Future recreational worth of the Missouri River is perceived by resource professionals as the highest of Missouri's six watersheds (Bachant et al. 1982). Closure of the lower Missouri River commercial catfish fishery should allocate a greater proportion of this resource to a larger number of citizens and yield greater economic benefit to the area.

RESTORATION OF THE LOWER MISSOURI RIVER

Restoration is defined by the National Research Council (1992) as the return of an ecosystem to a close approximation of its condition prior to disturbance. This definition necessitates that both ecosystem structure and function be recreated. Merely recreating the form without the functions, or the functions in an artificial configuration with little resemblance to the natural river, does not constitute restoration (National Research Council 1992). Restoration must be conducted as a holistic process at the landscape scale of the river basin.

It cannot be achieved through isolated manipulation of individual structural or functional elements or river segments.

The essence of a fluvial system is the dynamic equilibrium of the physical system, which in turn establishes a dynamic equilibrium in the biological system (National Research Council 1992). The goal of fluvial restoration, therefore, is reestablishment of this dynamic equilibrium. The National Research Council's (1992) report on Restoration of Aquatic Ecosystems lists four general objectives for fluvial restoration that can be applied to the Missouri, Volga, or any large river. These objectives are:

1. Restore the natural water and sediment regime. Time scales of daily-to-seasonal variations in water and sediment loads and the annual-to-decadal patterns of floods and droughts constitute this regime.

2. Restore the natural channel geometry, if restoration of the water and sediment regime does not.

3. Restore the natural riparian plant community, if the natural plant community does not restore itself upon completion of objectives 1 and 2.

4. Restore native aquatic plants and animals if they do not recolonize on their own.

Restoring the natural water and sediment regimes and the dynamic equilibrium of the Missouri and Volga rivers is a tremendous challenge, given their size, present state of alteration, competing uses for water, existing water and channel control structures, and floodplain development. However, as acknowledged by the National Research Council (1992), fluvial restorations are exercises in approximation, and restoration of the Missouri and Volga rivers will only be successful through compromise among competing interests.

If restoration of the Missouri River is to succeed, the four broad objectives listed above must be realized. We believe this goal can be accomplished by the groups described below through coordinating management of all significant ecological elements at a comprehensive river basin scale, termed integrated aquatic ecosystem restoration (National Research Council 1992). Restoration should include both nonstructural methods (that do not involve physical alteration or building of structures) as well as traditional structural techniques (National Research Council 1992), and follow specific approaches recommended by Hesse et al. (1989a, 1989b), Hesse and Mestl (1992), and Hesse et al. (1992). We summarize these approaches as six Missouri River restoration objectives and recommend a range of strategies to attain them.

Objective 1. Reestablish a semblance of the precontrol natural hydrograph

Strategies--

1.1. Hesse and Mestl (1992) suggest timing reservoir releases to emulate the precontrol hydrologic cycle as a daily percentage of total annual discharge (Fig. 9). They contend this approach would recreate the timing of the historic March and June rises while minimizing flooding and incorporate flexibility for drought or wet years.

1.2. Controlled flooding is necessary to reestablish the river-floodplain linkage and maintain habitat diversity. True river restoration must take discharge dynamism into account by allowing enough spatial and temporal scope for flooding to occur (National Research Council 1992). The capability for controlled flooding through reservoir releases currently exists.

1.3. Deauthorize irrigation projects of the Pick-Sloan Plan. Water supply in the Missouri River Basin is insufficient to meet the extensive development envisioned by this plan and poses the greatest threat to current water uses (Lord et al. 1975).

Objective 2. Reestablish a semblance of the precontrol sediment regime

Strategies--

2.1. Reduce sediment entrapment in reservoirs. Hesse et al. (1989a) and Singh and Durgunoglu (1991) suggest methods to bypass sediment through reservoirs, which if implemented or modified for Missouri River reservoirs would benefit the reservoirs by improving water quality, increasing hydropower potential, and increasing reservoir storage life. Sediment bypass would benefit the river by reducing downstream channel bed degradation, providing material for restoration of natural channel geometry and habitats, contributing sediment and organic matter to downstream reaches, and favoring native turbid-water fishes over sight-feeding carnivores.

Objective 3. Restore some of the precontrol channel structural diversity and the river-floodplain linkage

Strategies--

3.1. Accomplish Objectives 1 and 2. This will do much to meet Objective 3.

3.2. Implement a variety of nonstructural floodplain management methods that promote floodplain restoration, including:

3.2.1. Congressional establishment of U.S. Army Corps of Engineers' reservoir operating priorities based on economic, environmental, social, and other benefits to be derived from all authorized project purposes (General Accounting Office 1992);

3.2.2. Adoption of regulatory floodway zones (Brookes 1988), purchase of easements to prevent construction in the floodplain, increase land acquisition by willing purchase, buy out of drainage or levee districts, easements, and fee-title acquisition;

3.2.3. Support changes in flood damage insurance policies to discourage continued urban and industrial floodplain development;

3.2.4. Reduce public financial support for private sector economic gain by allocating maintenance costs of channel regulatory structures to direct beneficiaries (i.e., navigation companies and drainage and levee districts).

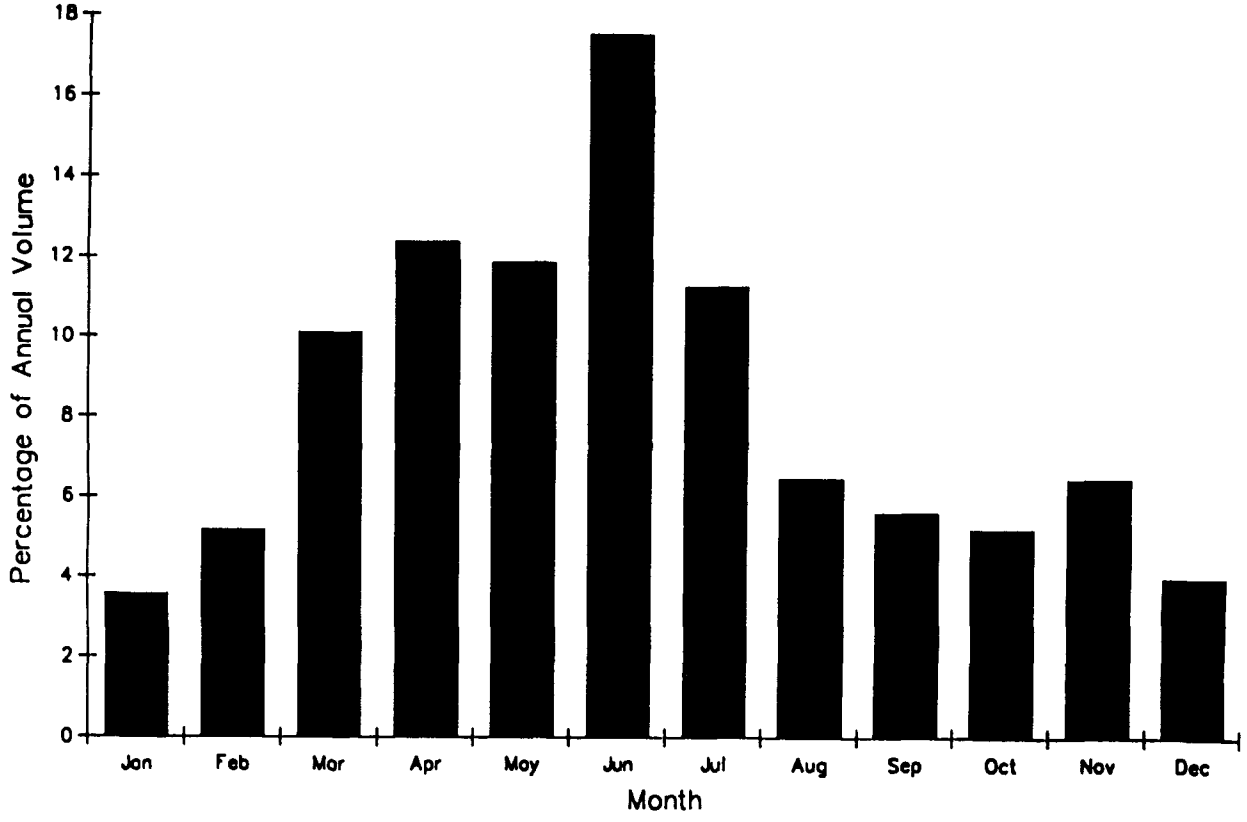

Fig. 9. Percentage of annual discharge for the Missouri River at Boonville, Missouri (Rkm 317), by month for the preregulation years 1929-1948. This is an example of the natural hydrograph approach Hesse and Mestl (1992) advocate to restore the natural water regime of the lower Missouri River.

3.3. Structural techniques for channel restoration should stress low-cost, low maintenance soft engineering (recreation of the natural river channel geometry using fluvial geomorphic principles and locally available materials) over traditional hard hydraulic engineering approaches which use concrete, rip-rap, and other imported materials and have high maintenance costs. See Brookes (1988) and National Research Council (1992) for additional structural techniques.

3.4. Expand the Missouri River Mitigation Project to include the entire length of existing river channel and mainstem and tributary impoundments, and have it incorporate an integrated aquatic ecosystem approach to mitigation.

Objective 4. Reestablish and enhance native Missouri River fishes and their migrations

Strategies--

4.1. Construct fish bypasses or elevators at selected dam barriers.

4.2. Modify reservoir releases of water to provide precontrol thermal cues including synchronization of river stage-temperature relationships.

4.3. Enhance the present species-centered recovery efforts by incorporating fish guild/community and habitat based approaches.

4.4. Implement a more equitable balance between management for river recreational fisheries and native species restoration.

4.5. Implement more restrictive commercial and sport fishery harvest regulations where populations of native fishes show declining trends (e.g., sauger, blue sucker, and other species in Nebraska, Hesse et al. 1992).

Objective 5. Reduce or eliminate major point and nonpoint sources of pollution

Strategies--

5.1. Improve enforcement of existing point-source water quality regulations. Establish long-term goal of upgrading water quality of the lower Missouri River to meet criteria for whole-body contact.

5.2. Continue to expand governmental support for agricultural land use practices that reduce soil erosion and crop overproduction and conserve or enhance wetlands (e.g., Conservation Reserve and Wetlands Reserve programs).

5.3. Incorporate a multi-level water quality restoration strategy, including elements of isolation, removal, transfer, and dilution through space and time (Herricks and Osborne 1985).

Objective 6. Reestablish native terrestrial and wetland plant communities along the river channel and floodplain, including native prairie, wet meadow, bottomland hardwood forests, sandbar, and sand island successional plant communities and vegetated islands (Hesse et al. 1989a)

Strategies--

6.1. Remnants of native plant communities exist and should become reestablished upon achievement of Objectives 1-3.

RESTORATION INITIATIVES AFFECTING THE MISSOURI RIVER

After decades of degradation, there is now a flurry of restoration activity for Missouri River Basin natural resources. Current initiatives, strategies, and action plans, if implemented, have the potential to greatly enhance lower Missouri River aquatic resources. We summarize several of these to illustrate how U.S. governmental and administrative processes operate in the area of environmental management. Our attention as ecologists is often focused on the biological details of our disciplines. However, we must be equally cognizant of the policy

aspects of river management, because it is here that the decisions are made and the money allocated which enable restoration to occur.

Mississippi Interstate Cooperative Resource Agreement (MICRA)

Large river drainage basins seldom exist within a single political boundary; rather, they typically are interjurisdictional. Interjurisdictional rivers are defined as crossing or common to two or more state, provincial, country, etc., boundaries and coming under the shared jurisdiction of two or more governmental entities. Restoration of interjurisdictional rivers is hampered by the multiplicity of authorities responsible for their management. The MICRA is an example of an attempt at a unified approach to restoration of the Mississippi River and its over 90 interjurisdictional tributaries. Twenty-eight State conservation departments having fisheries jurisdiction in the Mississippi River drainage system, plus the American Fisheries Society and U.S. Fish and Wildlife Service (USFWS), have invited other federal, nonfederal, tribal, and private entities to band together, "To assess the Mississippi River drainage fishery resources and habitat requirements to protect, maintain, and enhance interstate fisheries in the basin" (Rasmussen 1991). The Mission of MICRA is to "Improve the conservation, development, management, and utilization of interjurisdictional fishery resources in the Mississippi River Basin through improved coordination and communication among the responsible management entities" (Rasmussen 1991). MICRA is managed by an interagency Steering Committee composed of personnel from member states and entities. They completed a Comprehensive Strategic Plan in August 1991 (Rasmussen 1991) detailing step-by-step goals and objectives to complete MICRA's mission. Goals set by MICRA are presented here as an example of a river basin-wide restoration effort.

A. Develop a formal framework and secure funding for basin-wide networking and coordination mechanisms that complement existing and emerging administrative entities.

B. Develop public information and education programs to disseminate information that supports fishery resource management in the Mississippi River Basin.

C. Develop an information management program based on standardized methods for collecting and reporting fishery resource data basin-wide.

D. Determine and document the socio-economic value of fishery resources and related recreation.

E. Improve communication and coordination among entities responsible for fisheries resources management in the Mississippi River Basin.

F. Periodically identify and prioritize issues of concern in the Mississippi River Basin for coordinated research that supports cooperative resource management.

G. Identify and coordinate fishery management programs to address species and habitat concerns from an ecosystem perspective.

H. Develop compatible regulations and policies for fishery management to achieve interstate consensus on allocation of fishery resources.

I. Develop protocols, policies, and regulations for disease control, introduction of exotics, maintenance of genetic integrity, and maintenance and enhancement of indigenous species.

J. Preserve, protect, and restore fishery habitats basin-wide.

MICRA is seeking federal funding to accomplish these goals through the Cooperative Interjurisdictional Rivers Fisheries Resources Act of 1992.

Cooperative Interjurisdictional Rivers Fisheries Resources Act of 1992 (H.R. 4169)

A nationwide consensus is emerging that construction and maintenance of waterway developments are responsible for much of the decline of large river natural resources in the U.S, and the public has become increasingly aware of the need for restoration of the river-riparian ecosystem (National Research Council 1992). Moreover, they recognize that without demonstrable changes in current management strategies there will be further loss of large-river fisheries and reduced opportunities for recreational, commercial, subsistence, and aesthetic uses of our river-floodplain ecosystems. Several programs have been proposed or are currently underway, including those described herein, to resolve conflicts among management strategies. The Cooperative Interjurisdictional Rivers Fisheries Resources Act of 1992 was introduced to the U.S. Congress to improve coordination, cooperation, research, and information sharing at the national level on the variety of present programs to conserve fisheries resources of major U.S. interjurisdictional rivers.

If approved, this bill will fund and establish for 3 years a Council on Interjurisdictional Rivers Fisheries (Council) and authorize a pilot test of MICRA. Funds requested include $1 million per year to support Council activities and $2 million per year for MICRA. Membership on the Council would include high-level representatives of federal and state agencies with interests in fisheries resources on interjurisdictional rivers. The Council is to develop strategies on the management of interjurisdictional rivers fisheries. These strategies would include a) listing the 10 highest-priority interjurisdictional rivers in need of cooperative fisheries management and b) development of comprehensive fishery strategic plans for the five highest-priority interjurisdictional rivers identified in a).

The pilot test of MICRA would include nine tasks. Briefly these tasks are

1. Identification and description of each of the river ecosystems in the Mississippi River Basin and their associated fishery resources and habitat.

2. Identification and description of impacts of, and mitigation for, water and waterway development projects on fishery resources.

3. Analysis of existing data on regional depletion of important fish stocks and the potential for their restoration.

4. Identification of major information gaps and technological needs to improve the cooperative management of interjurisdictional fisheries resources.

5. A comprehensive study of the status, management, research, and restoration needs of the interjurisdictional fisheries of the Mississippi River Basin.

6. Development of recommendations for MICRA participants to undertake cooperative management and research projects.

7. Development of plans and projects for restoration and enhancement of depleted fish stocks and their habitats.

8. Evaluation of MICRA and the merits of extending such a program to other river basins in the U.S.

9. Estimates of funds required to implement 6, 7, and 8.

MISSOURI RIVER INITIATIVE

A specific example of a Mississippi River subbasin effort to address management strategies is the USFWS's proposed Missouri River Partnership or MOR-CARE (Missouri River--Conserving a River Ecosystem). The goal of MOR-CARE would be similar to MICRA's, but is restricted to the Missouri River Basin. MOR-CARE has four draft objectives (Brabander 1992):

1. To facilitate establishment and coordination of an operational Missouri River environmental research, management, restoration, and enhancement program involving federal, state, tribal, and local governments and public interest groups.

2. To coordinate the preparation, facilitation, and implementation of a comprehensive Action Plan for the management, restoration, and enhancement of fish, wildlife, and related environmental and recreational resources within the Missouri River ecosystem in concert with existing and future navigation, flood control, and water supply needs.

3. To develop and implement plans for providing fish and wildlife resource-based recreational opportunities for the people of the Missouri River ecosystem.

4. To establish a functional outreach program to involve and exchange information with the public concerning problems, opportunities, and resource management and restoration needs in the Missouri River ecosystem.

MOR-CARE would operate through a Steering Committee organized similarly to MICRA. This Steering Committee would identify resource management problems and information needs and establish Working Groups to develop information and alternatives for problem solution. Products of the Working Groups would be incorporated by the Steering Committee into a Missouri River Plan of Action. This Plan would include a Cooperative Agreement for signature by MOR-CARE partners indicating all would agree to utilize their resources to accomplish the goals of the Plan.

MISSOURI RIVER FISH AND WILDLIFE MITIGATION

The National Research Council (1992) defines mitigation as, "...actions taken to avoid, reduce, or compensate for the effects of environmental damage." Possible mitigation actions include restoration, enhancement, creation, or replacement of damaged ecosystems.

Channelization of the Missouri River directly eliminated 40,591 ha of aquatic habitat and 151,479 ha of wetlands and terrestrial habitat from the river and its floodplain from Sioux City, Iowa, to St. Louis, Missouri (Hesse et al. 1989b). The initial attempt to mitigate for these habitat losses was Section 601 (a) of the Water Resources Development Act of 1986 (PL 99-662), which authorized the Missouri River Fish and Wildlife Mitigation Project within the states affected by river channelization (Missouri, Kansas, Iowa and Nebraska).

Estimated cost of the Missouri River Mitigation Project between 1990 and 1999 is $67.7 million. Funds are to be allocated among four main project elements (USACE 1992): 1) 48% of requested funds will be for habitat development of 12,100 ha of newly acquired nonpublic lands and 7,365 ha of existing public lands; 2) 44% will be for acquisition of the nonpublic lands to provide aquatic and terrestrial habitat for fish and wildlife; 3) 5% will be for planning, engineering, and design of the project, including 0.4% for baseline evaluation and monitoring; and 4) 3% will be for construction management.

The Missouri River Mitigation Project will acquire 6.3% of the habitat lost in the original floodplain (Hesse et al. 1989b), a small beginning toward restoration of the Missouri River ecosystem. The authorization plan recognizes that restoration of the Missouri River is a long-term goal rather than short-term objective (Hesse et al. 1989b). This acknowledgment is important because this first mitigation step displays a piecemeal strategy, an example of, "...the isolated manipulation of individual elements approach," (National Research Council 1992) and therefore insufficient by itself to achieve restoration.

CONCLUSIONS

Fewer than 200 years have elapsed since Lewis and Clark encountered the abundant natural resources of the Missouri River Basin. Europeans have a much longer history of river modification than we in the U.S. Consequently they also are more familiar with river restoration and we should look to them for guidance (Petts 1984, Brookes 1988, Dodge 1989). The days of the truly Big Muddy are history. Missouri River restoration should be directed toward reestablishment of a basin-wide dynamic equilibrium and maintenance of natural functions and characteristics, albeit in a more limited scope.

Missouri River mitigation does not yet embody this holistic view toward restoration of the structural and functional attributes of the unaltered river on the landscape scale of the entire basin. This is not to say that current small-scale restoration efforts are ineffective. However, success in recreating a self-sustaining Missouri River ecosystem is more probable if individual mitigation and restoration projects are planned within the context of the basin landscape. Decisions about restoration and management of aquatic resources should not be made on a small-scale, short-term, site-by-site basis, but should be made to promote the long-term sustainability of all aquatic resources (National Research Council 1992). The task

of such groups as the Council on Interjurisdictional Rivers Fisheries, MICRA and MOR-CARE's Steering Committee will be to provide this perspective.

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HYDROLOGIC MODIFICATION TO IMPROVE HABITAT IN RIVERINE LAKES: MANAGEMENT OBJECTIVES, EXPERIMENTAL APPROACH, AND INITIAL CONDITIONS

by

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ABSTRACT

The Finger Lakes habitat-rehabilitation project is intended to improve physical and chemical conditions for fish in six connected backwater lakes in Navigation Pool 5 of the upper Missouri River. The primary management objective is to improve water temperature, dissolved oxygen concentration, and current velocity during winter for bluegills, *Lepomis macrochirus*, and black crappies, *Pomoxis nigromaculatus*, two of the primary sport fishes in the lakes. The lakes will be hydrologically altered by installing culverts to introduce controlled flows of oxygenated water into four lakes, and an existing unregulated culvert on a fifth lake will be equipped with a control gate to regulate inflow. These habitat modifications constitute a manipulative field experiment that will compare pre-project (1991 to summer 1993) and post-project (fall 1993 to 1996) conditions in the lakes, including hydrology, chemistry, rooted vegetation, and fish and macroinvertebrate communities. Initial data indicate that the Finger Lakes differ in water chemistry, hydrology, and macrophyte abundance. Macroinvertebrate communities also differed among lakes; species diversity was highest in lakes with dense aquatic macrophytes. The system seems to support a single fish community, although some species concentrated in individual lakes at different times. The introduction of similar flows into five of the lakes will probably reduce the existing physical and chemical differences among lakes. However, our ability to predict the effects of hydrologic modification on fish populations is limited by uncertainties concerning both the interactions of temperature, oxygen, and current in winter and the biological responses of primary and secondary producers. Results from this study should provide guidance for similar habitat-rehabilitation projects in large rivers.

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INTRODUCTION

Large rivers are diverse, dynamic ecosystems that are heavily affected by human activities (Hynes 1989). Yet information on the structure and function of large riverine ecosystems, needed to establish effective management and rehabilitation policies, is scant (Regier et al. 1989; Ward and Stanford 1989). In addition, few of the potential management approaches for large rivers have been critically evaluated (Petts et al. 1989).

The Mississippi River is the largest river in North America and one of the world's major rivers in size, physical diversity, and biotic productivity (Fremling and Claflin 1984; Fremling et al. 1989). The upper Mississippi River extends 1078 km from St. Anthony Falls, Minnesota, to the confluence with the Ohio River. In the 1930s, the upper Mississippi River was divided by a series of locks and dams into 29 navigation pools to enhance commercial navigation (a navigation pool is the reach between two consecutive locks and dams). The upstream portions of many navigation pools contain multiple channels characteristic of the unimpounded river, whereas the downstream reaches are similar to reservoirs. This impounded river contains large areas of diverse habitats that support economically important sport fisheries (Fremling et al. 1989).

Man's activities have adversely affected the quality of the riverine environment. Much of the watershed of the upper Mississippi is intensively cultivated, and many tributary streams deliver substantial loads of nutrients, pesticides, and sediment from eroding farmland. This sediment accumulates in quiescent riverine lakes and backwaters and in impounded areas behind dams (McHenry et al. 1984) -- all important habitats for fish. Substantial loadings of point-source pollutants are discharged into the river from bordering industrialized metropolitan areas. For example, organic pollution depleted dissolved oxygen in 100 km of river below metropolitan Minneapolis-St. Paul, Minnesota, for several decades (Fremling 1964; Fremling and Johnson 1990). Water quality in this reach improved since the late 1970s, because of better treatment of wastewater in the metropolitan area. Yet the river is still a nutrient-enriched, eutrophic system, where fish are occasionally affected by oxygen depletion in both winter and summer (Bodensteiner and Lewis 1992).

Federal and state resource-management agencies have initiated several rehabilitation and enhancement projects to improve certain degraded riverine habitats for fish and wildlife as one component of the Environmental Management Program for the Upper Mississippi River System. In this paper, we describe the Finger Lakes Project, a habitat-rehabilitation project intended to improve water quality for fish in a series of backwater lakes. We present the management objectives of the project and outline the experimental approach used to evaluate the limnologic, population, and community responses to hydrologic modification of the lakes. Last, we summarize baseline information on the initial physical and chemical conditions and biotic communities (rooted vegetation, fish, and benthic macroinvertebrates) in the lakes during summer and fall 1991.

MANAGEMENT OBJECTIVES

The Finger Lakes system near Kellogg, Minnesota, contains six connected lakes (Clear, Lower Peterson, Schmokers, Third, Second, and First) in Navigation Pool 5 of the upper Mississippi River, just below the dike at Lock and Dam 4 (Figure 1). Some of these shallow,

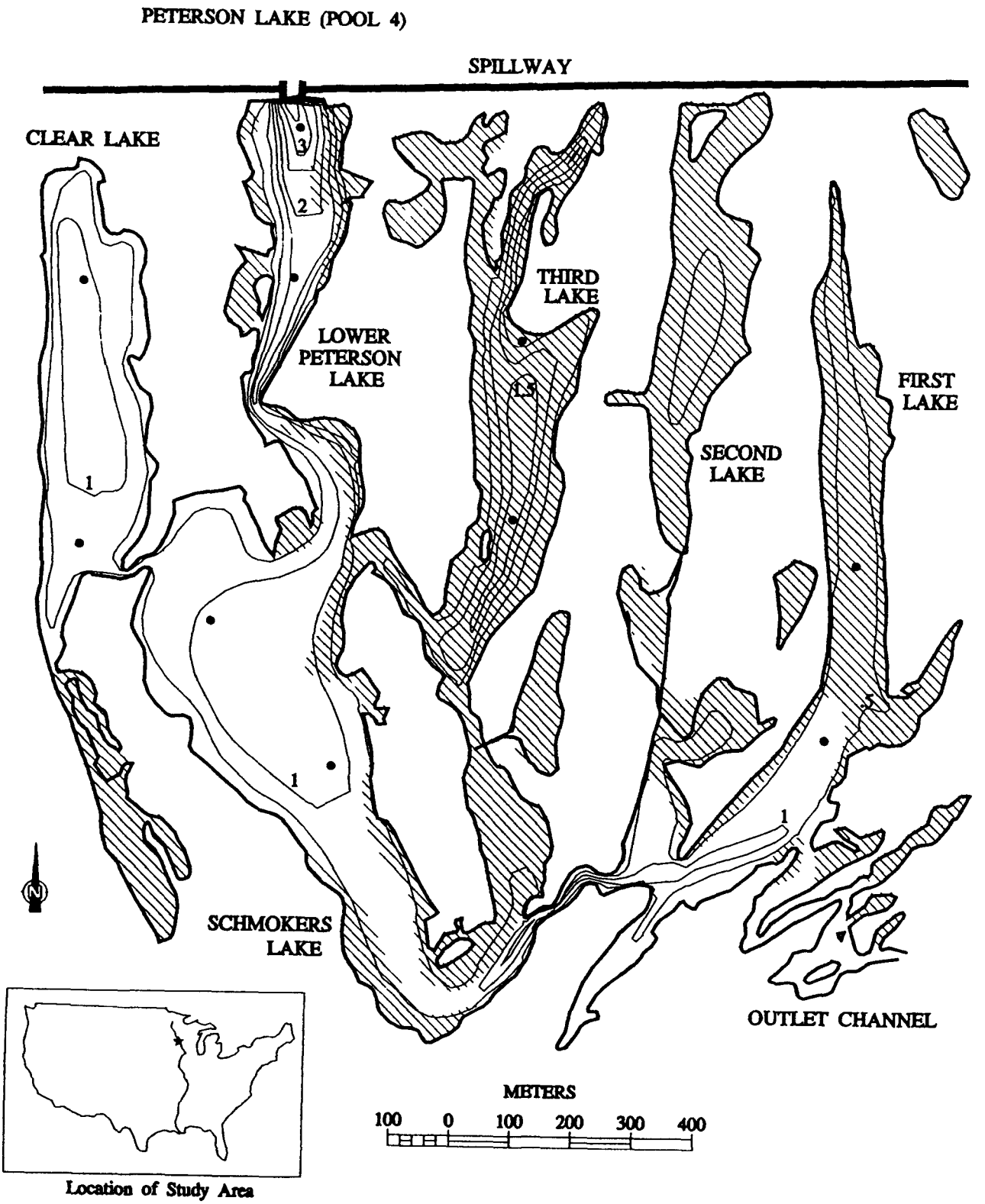

FIGURE 1. Map of the Finger Lakes study area in Pool 5 of the upper Mississippi River near Kellogg, Minnesota, USA (star on inset map), at an elevation of 201.2 m above mean sea level, showing depth contours (0.5-m intervals), water sampling stations (●), and distribution of aquatic macrophytes (cross-hatched areas) during summer 1991.

eutrophic lakes have periodically undergone oxygen depletion in winter and summer (Grunwald 1977), which reduces their suitability for fish. A culvert through the dike allows flow into Lower Peterson Lake from Upper Peterson Lake, which is part of the impoundment behind Lock and Dam 4. A mean flow of 2.3 m^3/s through the culvert provides sufficient dissolved oxygen to Lower Peterson and Schmokers lakes for fish, but reduces water temperatures in winter to near 0°C. Many sport fishes in the upper Mississippi River are intolerant of such low temperatures, particularly in the presence of flow (Sheehan et al. 1990). At normal pool elevation, all six lakes drain through a common outlet to the main channel of the Mississippi River (Figure 1).

The primary management objective of the Finger Lakes Project is to provide suitable water temperature, dissolved oxygen concentration, and current velocity in winter for bluegills and black crappies the two primary sport fishes in the lakes. To achieve these objectives, the culvert on Lower Peterson Lake will be equipped with a control gate to regulate flow, and culverts will be installed to introduce and regulate flow into Clear, First, Second, and Third lakes.

The principal objectives of our research are to determine (1) the response of the Finger Lakes ecosystem to these hydrologic modifications, and (2) the flow rates that will optimize winter temperature, dissolved oxygen concentration, and current velocity in these lakes for bluegills and black crappies. We consider the habitat modifications to be a manipulative field experiment that will entail comparisons of the pre-project and post-project status of lake hydrology, lake chemistry, rooted vegetation, fishes, and benthic macroinvertebrates. The study began in 1991 and will continue through 1996. Results from this study should provide guidance for other habitat-rehabilitation projects on the river.

EXPERIMENTAL APPROACH

HYDROLOGIC MODIFICATION

Three new culverts will be installed through the dike of Lock and Dam 4 in 1992 and will become operational by summer 1993. Two culverts, each 0.9 m in diameter, will provide flow to Clear and Third lakes. A third culvert, 1.2 m in diameter, will provide flow to both Second and First lakes through a junction box. Gates for controlling flow will be installed on the inlets of each new culvert, on the existing culvert in Lower Peterson Lake, and on the junction box.

Operation of the culverts and determination of their flow rates will be based on the oxygen deficit of each lake. Preliminary estimates, based on lake morphometries and limited chemical data, indicated that flow rates averaging about 0.8 m^3/s for Lower Peterson Lake and 0.2 to 0.3 m^3/s for other lakes should maintain dissolved oxygen concentrations of at least 5 mg/L in winter (U.S. Army Corps of Engineers, St. Paul, Minnesota, unpublished data). Total inflow through all culverts would average about 1.7 m^3/s for the entire system, which is less than the 2.3 m^3/s that now flows into Lower Peterson Lake.

PHYSICOCHEMICAL

Annual water budgets will be modeled with the following mass-balance equation:

(1)	culvert inflow	+	groundwater inflow	+	dike seepage	=	channel outflow	+	evaporation.

Inflow and outflow will be monitored every 2 weeks from August 1991 through September 1996. Evaporative losses will be estimated from weather data. The sum of groundwater inflow and seepage through the dike will be estimated by difference.

We will determine flow patterns and water exchange rates within the system from the movement of a fluorescent dye injected into the water. Dye studies will be conducted before the installation of the culverts (winter 1991-1992) and again during culvert operation (winter 1994-1995) under conditions of similar river discharge and water elevation.

We will sample water at two stations in each lake (Figure 1), at weekly intervals during ice-free periods, and less frequently during ice cover. At each station, Secchi disk transparency will be measured, and depth profiles of temperature, dissolved oxygen, pH, and conductivity will be determined in situ. Water samples collected at depths of 0.1 m below the surface and 0.1 m above the sediment-water interface at each station will be analyzed for alkalinity, seston dry mass, particulate organic matter, dissolved organic carbon, total and dissolved nitrogen, ammonia-nitrogen, total and dissolved phosphorus, soluble-reactive phosphorus, dissolved iron, dissolved manganese, chlorophyll, and phaeopigments.

VEGETATION

We will survey and map aquatic vegetation annually with a combination of transect sampling, visual surveys, and aerial photography. Transects will be established at 50-m intervals across each lake. At 10-m intervals along each transect, plants will be sampled with a plant rake to estimate the relative density of plant species within a 1-m radius. In the area between transects, vegetation beds larger than 5 m in diameter will be visually surveyed to determine species composition and bed size. Aerial photographs (scale 1:15,000) taken in August of each year will be used to map the extent of vegetation beds identified in field surveys.

MACROINVERTEBRATES

Macroinvertebrates will be sampled from Clear, Lower Peterson, and Third lakes to determine taxonomic composition, density, and biomass. Sampling will be conducted twice annually, in April or May (coinciding with minimum macrophyte abundance) and in August (coinciding with maximum macrophyte abundance). In each lake, a maximum of 15 sample sites will be randomly selected in each of three habitats: benthic substrate in non-vegetated areas, benthic substrate under vegetation, and vegetation. Three samples will be collected at each site. Substrates will be sampled with a ponar grab (0.0225 m^2) and washed through a 595-μm mesh sieve. Macroinvertebrates associated with vegetation will be sampled by

cutting a standard length of non-branching stem (50 cm for Nuphar, Nymphaea, and Nelumbo; 25 cm for other plant species) from the uppermost portion of five plants of the predominant species at a site. All samples will be preserved in 10% formalin.

FISH

We will describe the fish community of the lakes and the population biology of three target species (bluegill, black crappie, and largemouth bass Micropterus salmoides) before and after culvert operation. Population and community characteristics will be assessed in relation to habitat features both within lakes and among lakes. Second Lake will not be routinely studied because access to the lake is restricted for both fish and investigators.

Standardized sampling will be done in each lake with fyke nets and electrofishing during spring, summer, and fall (Nielsen and Johnson 1983) to determine community composition and the relative abundance of fish species. Target species will be measured and weighed, and scale samples for age estimation and back-calculation of growth will be taken in spring. Diets of each target species will be described by analyzing stomach contents from fish captured seasonally. Age 1 and older fish of each target species will be fin-clipped for mark-recapture population estimates. Age 2 and older fish of each target species (approximated by total lengths of at least 150 mm for bluegills, 170 mm for black crappies, and 200 mm for largemouth bass) will be tagged with individually numbered anchor tags. Anglers will be surveyed in winter to estimate fishing effort and harvest. The survival rates of target species will be estimated directly from spring and fall mark-recapture studies and also from the return rate of tagged fish (Ricker 1975; Burnham et al. 1987).

Movements of fish within the Finger Lakes will be determined in part by the locations of recaptured, tagged fish caught by anglers and during our sampling. Fyke nets will also be set in the outlet channel during spring, summer, and fall to determine rates of immigration and emigration for the system.

The effect of habitat modification on the movement of bluegills and black crappies will be further examined in two radio-telemetry studies -- both in winter --one before and one after installation of the culverts. Radio transmitters will be implanted in 20 bluegills and 20 black crappies during each study. Fish will be located at least weekly, and dissolved oxygen concentration, temperature, and current velocity will be measured at each fish's location.

DATA MANAGEMENT

We will determine spatial coordinates based on the Universal Transverse Mercator System for all sample points and transects, and will develop Geographic Information System (GIS) data bases for both the pre- and post-modification periods. These data bases can be combined to determine the distribution of habitat features over time. GIS maps will be developed to examine both spatial and temporal changes in habitat and fish populations. These spatial analyses will complement traditional statistical analyses, and will help to identify spatial patterns and the relations between changes in habitats and biota.

INITIAL CONDITIONS

Data from the first sampling period of this study were collected in late summer and fall in 1991 and are summarized herein.

MORPHOMETRY

A bathymetric survey conducted in July 1991 provided morphometric information for the lakes (Table 1). The total surface area of the Finger Lakes system is 100 ha; individual lakes range in area from 8 to 19 ha. Lower Peterson Lake is generally deeper (mean depth 1.2 m; maximum, 4.3 m near the culvert) than the other lakes, whose mean depths ranged from 0.6 to 0.9 m and whose maximum depths ranged from about 1 to 2 m. The only shoreline development in the system consists of 25 houses and a boat ramp, all located on the western shore of Clear Lake.

HYDROLOGY

At present, the hydrology of the Finger Lakes depends primarily on the inflow through the culvert into Lower Peterson Lake and on outflow through the outlet channel. Pool elevations in Upper Peterson Lake are nearly constant at 203.1 m above mean sea level, except during spring floods, and have never exceeded 206.8 m, the level needed to overflow the dike. Therefore, the flow rate through the culvert is primarily controlled by the elevation of the Mississippi River in the tailwater below Lock and Dam 4, which is determined by discharge through the dam. Inflow through the culvert remains at about 2.3 m^3/s at dam-discharge rates up to 1,700 m^3/s. Higher dam-discharge rates raise tailwater elevations, reducing flow through the culvert to a minimum of about 1 m^3/s. Discharges through Lock and Dam 4 exceed 1,700 m^3/s only about 10% of the time, primarily during spring floods; thus, flow through the culvert does not vary widely during the year.

During late summer and fall 1991, the volume and surface elevation of the Finger Lakes was closely correlated with elevation of the Lock and Dam 4 tailwaters (Figure 2A) and was regulated primarily by flow through the outlet channel. Inflow through the culvert and outflow through the outlet channel were similar (Figure 2B) except in mid-September, when increased discharge through Lock and Dam 4 raised the tailwater; this increase reversed the direction of flow through the outlet channel (negative value in Figure 2B), increasing the volume of the lakes. A rapid decrease in tailwater elevation in late September produced large outflows, rapidly decreasing lake volume.

PHYSICOCHEMICAL

Mean physicochemical characteristics varied among lakes during late summer and fall 1991 (Table 2). We present only data collected from 0.1 m below the surface because values from 0.1 m below the surface and 0.1 m above the sediment-water interface were similar within lakes, except as noted.

TABLE 1. MORPHOLOGY OF THE FINGER LAKES, MINNESOTA, AT A WATER SURFACE ELEVATION OF 201.2 M ABOVE MEAN SEA LEVEL.

Lake	Mean depth (m)	Maximum depth (m)	Surface area (ha)	Volume (1000 m^3)
Clear	0.8	1.4	10	84
Lower Peterson	1.2	4.3	8	94
Schmokers	0.9	1.3	19	164
Third	0.6	2.0	15	95
First	0.6	1.1	10	56
Outlet channel (including bays)	0.6	1.7	8	49
Total system			100[a]	542

[a]Includes 30 ha of surface area in isolated lakes, contiguous ponds, and wetlands.

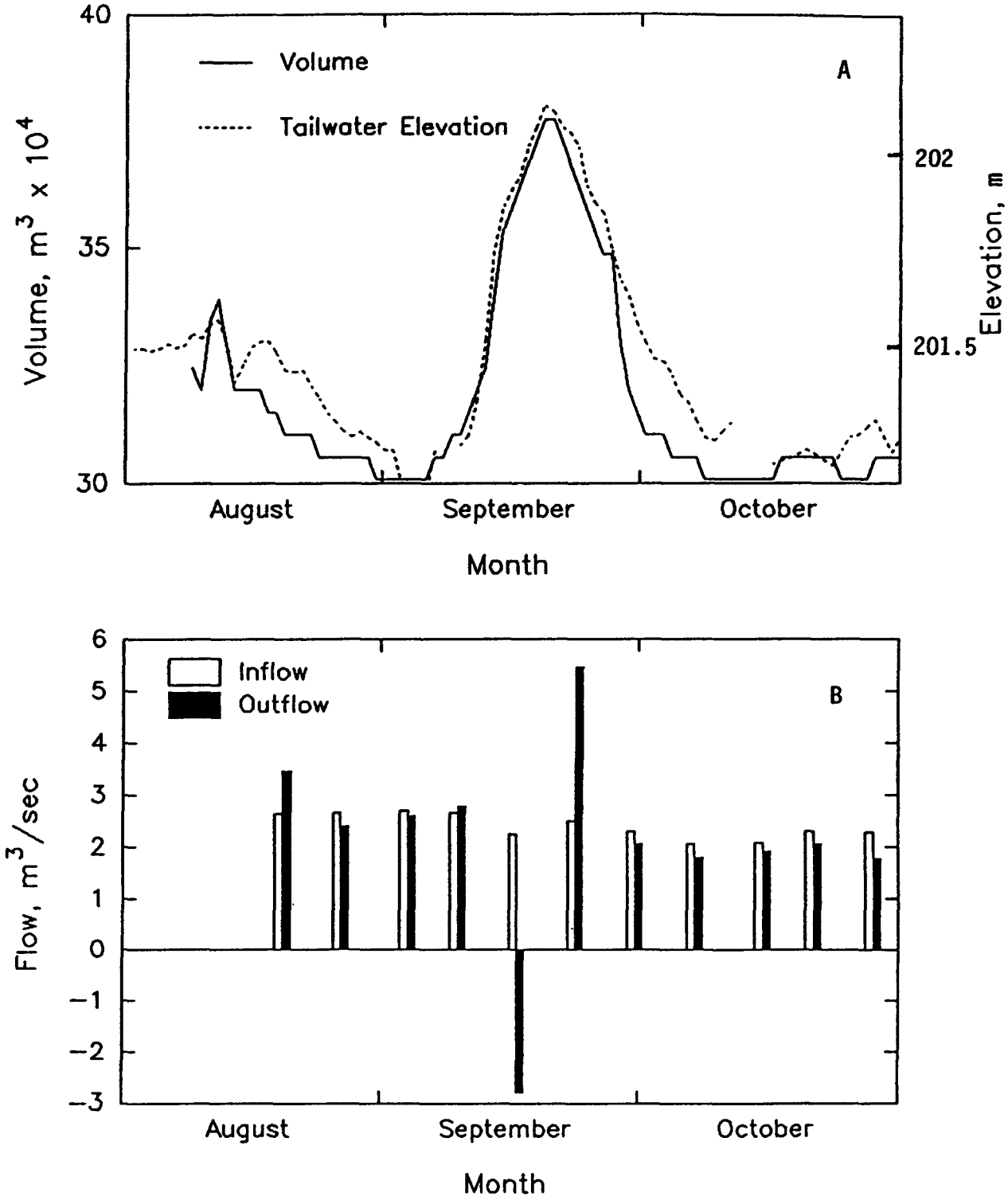

FIGURE 2. Hydrologic characteristics of the Finger Lakes, Minnesota, during August through October 1991. (A) Volume of the Finger Lakes and elevation (meters above mean sea level) of the tailwaters of Lock and Dam 4. (B) Inflow from the culvert in Lower Peterson Lake and outflow through the Finger Lakes outlet channel.

TABLE 2. MEAN VALUES (RANGE IN PARENTHESES) FOR SELECTED PHYSICOCHEMICAL CHARACTERISTICS OF THE FINGER LAKES, MINNESOTA, MEASURED OR SAMPLED AT 0.1 M BELOW THE WATER SURFACE. MEANS WERE CALCULATED FROM DATA FROM TWO SAMPLING LOCATIONS PER LAKE ON 10 SAMPLING DATES DURING AUGUST THROUGH OCTOBER, 1991 (N = 20 PER LAKE).

Variable	Lake				
	Clear	Lower Peterson	Schmokers	Third	First
Temperature, °C	15.0 (6.4-24.6)	16.3 (9.0-24.6)	16.3 (8.1-25.3)	15.4 (7.8-24.4)	16.6 (7.9-27.1)
Dissolved oxygen, mg/L	8.9 (5.5-12.4)	9.0 (6.4-11.8)	9.3 (6.8-11.9)	7.3 (4.4-10.6)	10.6 (7.3-17.8)
Conductivity, μS/cm	303 (282-341)	467 (399-521)	467 (395-561)	392 (367-452)	432 (366-519)
pH	7.8 (7.2-8.5)	8.0 (7.6-8.4)	8.0 (7.8-8.5)	7.4 (7.1-7.8)	8.1 (7.7-8.9)
Alkalinity, mg/L (as $CaCO_3$)	143 (133-150)	173 (153-189)	171 (151-184)	194 (177-208)	173 (154-181)
Seston, mg/L	39.6 (11.7-71.9)	12.0 (7.0-18.7)	21.8 (6.9-58.7)	10.4 (4.6-22.2)	31.9 (8.3-64.6)
Particulate organic matter, mg/L	13.4 (9.1-21.3)	3.1 (0.7-5.2)	4.9 (2.5-15.2)	4.5 (1.4-13.2)	7.9 (2.7-18.9)
Dissolved organic carbon, mg/L	19.9 (8.0-29.2)	25.0 (17.9-30.1)	25.1 (14.6-29.3)	21.3 (13.0-30.9)	24.3 (11.3-30.1)
Ammonia-nitrogen, mg/L	0.08 (0.01-0.69)	0.05 (0.01-0.2)	0.05 (0.01-0.11)	0.18 (0.01-0.5)	0.07 (0.01-0.41)
Chlorophyll, mg/m^3	79.0 (32.1-150)	13.9 (5.6-33.2)	20.0 (10.7-45.4)	26.0 (2.1-76.2)	43.4 (14.4-114)

Mean water temperatures were similar among lakes, which indicates that inflow to Lower Peterson Lake had little effect on temperature during summer and fall 1991. Conductivity, pH, alkalinity, and dissolved organic carbon were similar among Lower Peterson, Schmokers, and First lakes, but differed from values in Clear and Third lakes. These data suggest that flow from the culvert into Lower Peterson Lake moved directly through Schmokers Lake to the system outflow, with little water entering Third and Clear lakes. Conductivity, alkalinity, and temperature were lowest in Clear Lake, probably because of the influence of groundwater inputs. Mean and minimum dissolved oxygen concentrations were lowest in Third Lake, the only lake that exhibited temperature and oxygen stratification during summer 1991. Minimum dissolved oxygen concentrations approached zero in the hypolimnion of Third Lake, and were associated with high concentrations of ammonia-nitrogen. Lower Peterson Lake, the deepest lake, did not stratify, probably because of mixing caused by inflow from the culvert.

Mean concentrations of seston, particulate organic matter, and chlorophyll were highest in Clear Lake, which suggests higher primary productivity than in the other lakes (Table 2). In contrast, concentrations of these biogenic components were generally lowest in Lower Peterson Lake, but progressively higher in Schmokers and First lakes. These data indicate that phytoplankton production increases from the inflow to the outflow and suggest that there was a net export of biogenic matter from the system.

VEGETATION

Eleven species of submersed and floating-leaved aquatic vegetation were found in the Finger Lakes (Table 3). Based on our samples and on aerial photographs taken in 1991, Third Lake was the most heavily vegetated and Clear Lake the most sparsely vegetated of the Finger Lakes (Figure 1). Nelumbo was the most common macrophyte in Clear Lake, whereas some combination of Ceratophyllum, Myriophyllum, and Nymphaea dominated in the other lakes. Plant beds generally occurred in strips near shorelines. In First and Third lakes, the upper third of each lake and most protected bays contained dense beds of Ceratophyllum and often contained the free-floating macrophyte, duckweed (Lemna spp.). Beds of Myriophyllum existed offshore in Schmokers Lake, and small beds of Nelumbo were found in Lower Peterson, Schmokers, and Clear lakes. The other six plant species were rare.

MACROINVERTEBRATES

We collected 276 samples of macroinvertebrates from three of the Finger Lakes in August 1991. Based on initial analyses of about 10% of the samples from each lake and habitat type, the benthos were dominated by oligochaetes, followed by chironomids, and in Third Lake, by the amphipod Hyalella azteca (Table 4). Amphipods and chironomids were the dominant macroinvertebrates on vegetation. Odonate larvae, primarily Enallagma spp., were common on vegetation but rare elsewhere. Bivalve molluscs were common in sediments from non-vegetated areas of Lower Peterson Lake. Diversity of macroinvertebrates was highest in Third Lake, which had 26 taxa (Table 4). Densities of macroinvertebrates in vegetated areas (benthic plus plant-associated) were higher than in

TABLE 3. FREQUENCY OF OCCURRENCE (PERCENT OF SITES CONTAINING THE SPECIES) OF SUBMERSED AND FLOATING-LEAFED AQUATIC PLANTS IN THE FINGER LAKES, MINNESOTA, IN AUGUST 1991. "P" INDICATES THAT THE PLANT WAS PRESENT IN THE LAKE, BUT NOT SAMPLED ON TRANSECTS.

Species	Lake				
	Clear	Lower Peterson	Schmokers	Third	First
		Submersed plants			
Ceratophyllum demersum	0	23	16	92	45
Vallisneria americana	0	10	0	0	0
Myriophyllum sp.	0	20	38	0	7
Elodea canadensis	0	4	0	2	1
Najas flexilis	0	0	P	0	0
Potamogeton americanus	0	P	3	P	4
Potamogeton zosteriformis	0	4	0	0	0
Potamogeton crispus	0	0	0	0	2
Heteranthera dubia	0	0	3	0	0
		Floating-leafed plants			
Nelumbo lutea	P	11	P	0	0
Nymphaea tuberosa	P	25	9	38	9
Nuphar luteum	0	P	0	P	0
Lemna spp.	0	0	0	P	P

TABLE 4. TAXONOMIC LIST AND RELATIVE PERCENTAGE BY NUMBER OF MACROINVERTEBRATES IN THREE HABITAT TYPES (V = ON VEGETATION, S = SUBSTRATES UNDER VEGETATION, N = SUBSTRATES IN NON-VEGETATED AREAS) IN CLEAR, LOWER PETERSON, AND THIRD LAKES IN AUGUST 1991.

Macroinvertebrate taxon	Clear Lake			Lower Peterson Lake			Third Lake		
	V	S	N	V	S	N	V	S	N
Class Oligochaeta	0	91	95	5	48	32	12	69	53
Class Crustacea									
Order Isopoda									
Caecidotea	0	0	0	0	3	0	1	6	0
Order Amphipoda									
Hyalella azteca	0	0	0	43	2	0	41	2	37
Class Arachnoidea									
Hygrobates	0	0	0	0	0	4	0	0	0
Limnesia	0	1	1	0	1	0	0	1	0
Neumania	0	0	0	0	0	0	0	2	2
Class Bivalvia									
Family Sphaeriidae	0	0	0	0	0	18	0	2	0
Family Unionidae	0	0	0	0	0	7	0	0	0
Class Insecta									
Order Diptera									
Family Chironomidae	100	4	0	37	38	29	24	12	4
Family Ceratopogonidae	0	3	1	1	0	7	0	0	0
Family Psychodidae	0	0	0	0	0	0	0	1	0
Family Chaoboridae									
Chaoborus	0	0	2	0	0	0	0	1	1
Order Odonata									
Suborder Zygoptera	0	0	0	0	1	0	1	1	0
Enallagma	0	0	0	11	0	0	7	0	0
Order Coleoptera	0	0	0	1	2	0	1	0	0
Order Ephemeroptera									
Caenis	0	0	1	3	3	4	2	0	1
Miscellaneous taxa	0	2	0	0	3	0	11	4	2
Number of samples	1	2	10	3	2	2	4	4	3
Mean number per sample	8	69	34	59	93	14	73	44	70

non-vegetated areas. All macroinvertebrate communities were dominated by small organisms, which suggests intensive, size-selective predation by fish.

FISH

The fish assemblage of the Finger Lakes (Table 5) was typical of Upper Mississippi River backwaters. In summer, bluegills and black crappies dominated the catch in all lakes, followed by catostomids. Catch rates for bluegills and black crappies were highest in Lower Peterson Lake. In fall, catch rates increased in most lakes because age-0 fish were large enough by then to be caught in fyke nets (Table 5). Bluegills and black crappies were concentrated in Third and First lakes. Catches in Clear, Lower Peterson, and Schmokers lakes were dominated by young-of-the-year white bass Morone chrysops and gizzard shad Dorosoma cepedianum.

These data agree with population estimates for age-1 and older fish in fall 1991, which showed that numbers and densities of bluegills and black crappies were greatest in Third Lake (Table 6). Largemouth bass, the primary piscivore in the system, are less vulnerable to fyke nets, but population estimates indicated that largemouth bass were concentrated in Lower Peterson Lake (Table 6).

Tag returns from anglers indicated that fish moved freely within the Finger Lakes system, and that few fish left the system. Through 31 March 1992, 49% of the 125 bluegill tags and 58% of the 73 black crappie tags received from anglers were from fish recaptured in a lake other than that where they were tagged. Only two bluegills and four black crappies, representing only 3% of all tag returns, were recaptured outside the Finger Lakes system. These initial data suggest that the Finger Lakes system forms a spatial boundary for populations of bluegill and black crappie, but that these populations are not confined to individual lakes within the system.

Preliminary analysis of stomach contents from fish sampled in fall 1991 showed that both bluegills and black crappies fed mainly on macroinvertebrates, mostly amphipods and dipterans. Zooplankton were important in the diets of young-of-the-year fishes. Adult black crappies occasionally ate fish, primarily gizzard shad and Lepomis spp. Largemouth bass consumed mostly gizzard shad, Lepomis spp., and darters Etheostoma, but smaller bass also ate macroinvertebrates.

CONCLUSIONS AND UNCERTAINTIES

Initial data indicate that the Finger Lakes differ in water chemistry, hydrology, and macrophyte abundance. Clear Lake had the highest chlorophyll concentrations, probably received groundwater inputs with lower conductivity, and had few aquatic macrophytes. Lower Peterson and Schmokers lakes, which were heavily influenced by inflow from the culvert, were both low in chlorophyll and particulate organic matter, had high conductivity, and had moderate production of aquatic macrophytes. Third Lake, which was intermediate in physicochemical characteristics, contained dense aquatic macrophytes, and exhibited oxygen depletion during summer. The upper portion of First Lake was similar to Third Lake, but the lower portion, near the system outlet, was influenced by flow from the culvert and

TABLE 5. CATCH PER NET DAY, TOTAL NUMBER OF SPECIES, AND TOTAL NUMBER OF FISH CAUGHT IN FYKE NETS, AND NUMBER OF DAYS OF NETTING FOR THE FINGER LAKES, MINNESOTA, IN 1991.

	Summer 1991						Fall 1991					
Taxa	Clear Lake	Lower Peterson Lake	Third Lake	First Lake	Schmokers Lake	All lakes combined	Clear Lake	Lower Peterson Lake	Third Lake	First Lake	Schmokers Lake	All lakes combined
Dorosoma cepedianum	0.9	<0.1	0.1	0.8	0.8	0.5	16.5	2.5	0.3	2.1	9.2	5.4
Esox lucius	1.3	0.2	0.1	0.8	0.9	0.6	0.6	0.5	0.1	0.4	0.3	0.4
Cyprinus carpio	0.4	1.1	0.7	0.4	0.8	0.6	0.4	0.8	0.1	0.4	0.6	0.4
Catostomidae[a]	5.5	2.5	1.2	3.2	2.2	2.8	3.2	1.5	3.0	1.7	2.0	2.4
Morone chrysops	0.1	0.2	0	0.1	0.3	0.1	9.5	8.7	0.2	8.9	9.2	6.4
Lepomis macrochirus	3.9	9.1	6.0	5.6	4.6	5.6	4.5	2.2	46.5	14.4	1.9	18.0
Other *Lepomis* spp.[b]	0.2	0.4	0.8	<0.1	0.1	0.3	<0.1	<0.1	4.6	0.6	0	1.5
Pomoxis nigromaculatus	5.1	19.4	2.8	5.1	11.0	8.2	2.5	2.2	14.8	3.5	1.0	6.1
Pomoxis annularis	1.0	0.1	<0.1	0.5	0.8	0.4	1.6	0.1	0.2	0.4	0.4	0.5
Perca flavescens	0.5	1.8	1.9	0.8	1.5	1.2	0.8	1.9	3.3	0.4	0.2	1.6
Aplodinotus grunniens	1.7	0.4	0.1	0.7	0.2	0.6	1.7	3.2	<0.1	0.6	1.7	1.3
Other species[c]	0.8	0.4	1.0	1.3	0.8	0.8	1.6	0.7	1.0	0.9	1.1	1.0
Number of species	27	27	18	25	27	33	25	24	21	24	23	33
Number of fish caught	767	1279	525	696	766	4033	1204	683	3265	959	583	6694
Days of netting	36	36	36	36	32	184	28	28	44	28	21	149
Total catch per net day	21.3	35.5	14.6	19.3	23.9	21.9	43.0	24.4	74.2	34.2	27.8	44.9

[a]Includes *Carpiodes carpio*, *C. cyprinus*, *Catostomus commersoni*, *Ictiobus bubalus*, *I. cyprinellus*, *Minytrema melanops*, *Moxostoma anisurum*, *M. carinatum*, *M. erythrurum*, and *M. macrolepidotum*.

[b]Includes *Lepomis cyanellus*, *L. gibbosus*, and *L. humilis*.

[c]Includes *Lepisosteous osseus*, *L. platostomus*, *Amia calva*, *Notemigonus crysoleucas*, *Ameiurus melas*, *A. natalis*, *A. nebulosus*, *Ictalurus punctatus*, *Pylodictis olivaris*, *Ambloplites rupestris*, *Micropterus salmoides*, *Stizostedion canadense*, and *S. vitreum*.

TABLE 6. POPULATION ESTIMATES AND 95% CONFIDENCE INTERVALS (IN PARENTHESES), CALCULATED BY THE MODIFIED SCHNABEL FORMULA (RICKER 1975), FOR BLUEGILLS AND BLACK CRAPPIES EXCEEDING 120 MM IN TOTAL LENGTH AND FOR LARGEMOUTH BASS EXCEEDING 150 MM IN TOTAL LENGTH IN THE FINGER LAKES IN OCTOBER 1991. A DASH (--) INDICATES THAT TOO FEW FISH WERE RECAPTURED TO CALCULATE A POPULATION ESTIMATE. THERE WERE TOO FEW RECAPTURES IN SCHMOKERS LAKE TO CALCULATE POPULATION ESTIMATES FOR ANY SPECIES.

	Lake				
Species	Clear	Lower Peterson	Third	First	Total system
Bluegill	2,247	--	39,493	3,751	28,649
	(1,806-2,796)		(27,794-53,646)	(2,948-4,771)	(24,910-32,965)
Black crappie	471	517	6,148	1,029	7,411
	(336-701)	(277-1,058)	(4,419-8,895)	(618-1,824)	(5,983-9,167)
Largemouth bass	475	1,650	560	--	2,803
	(338-691)	(1,190-2,230)	(300-2,246)		(2,266-3,463)

exhibited characteristics similar to Schmokers Lake. Macroinvertebrate communities differed among lakes, with aquatic macrophytes providing an important substrate for colonization. The macroinvertebrate community was most diverse in Third Lake, where macrophytes were most dense, but was composed almost exclusively of chironomids and oligochaetes in Clear Lake, where macrophytes were sparse. The Finger Lakes system appears to have a single fish community, although some species concentrate in individual lakes at different times.

The extensive data base collected during the pre-culvert studies should allow us to estimate optimal temperature, dissolved oxygen concentration, and current velocity, and to then re-estimate the inflows needed to achieve optimal conditions in each lake. Although the estimated inflows may change, the flow regime after culvert installation will presumably involve similar, low rates of inflow to each lake. Lower Peterson and Schmokers lakes will have reduced flows relative to present conditions, whereas flows will increase in the other lakes. Total inflow to the system should be near or below present levels.

Responses of the system to modification in the flow regime are presently difficult to predict, particularly for the fish populations. Fish may respond to changes in habitat, trophic dynamics, or species interactions through altered survival, growth, reproduction, or emigration. Changes in any of these fish-population variables could affect the relative abundance of species and the community composition of fishes.

The introduction of similar, low flows into each lake will probably reduce the physicochemical differences among lakes that existed in 1991. Inflow may eliminate anoxia in summer and increase available habitat for fish and macroinvertebrates. Winter oxygen concentrations can be increased by introduced flows, improving habitat for aquatic biota, but this could also decrease water temperature and increase current velocity, which could be stressful to certain fishes -- including our target species (Sheehan et al. 1990; Bodensteiner and Lewis 1992). Consequently, the flow rates needed to create optimal winter conditions for bluegills and black crappies must be accurately determined.

Increased flows could affect the production of macrophytes in various ways. Physical disturbance caused by flows may eliminate macrophytes in some areas, particularly near the mouths of the culverts. Changes in transparency can modify the depth of the photic zone, but it is difficult to predict how increased flow will alter transparency. Increased inflow may reduce transparency by increasing the concentration of suspended solids or by stimulating phytoplankton production. Conversely, increased flow may increase transparency by flushing phytoplankton, suspended solids, and non-rooted aquatic plants, such as duckweed and Ceratophyllum, from the system. Changes in vegetation abundance may affect spawning and nursery habitat for many fish species.

Fish populations in the Finger Lakes may be indirectly affected by trophic changes. The food of target fishes seems to consist primarily of macroinvertebrates. Increased oxygen concentrations in winter may improve the over-winter survival of macroinvertebrates, thereby increasing the food supply for target fishes. Population densities of macroinvertebrates seem strongly related to the abundance of aquatic plants; thus, changes in aquatic macrophytes could greatly influence the food chain supporting key fishes.

Our ability to predict the effects of hydrologic modification on fish populations is limited by uncertainties in the physical responses of the Finger Lakes system, particularly the

interactions of temperature, dissolved oxygen, and current velocity in winter, and by uncertainties in the responses of primary and secondary producers. The ability to regulate flows should allow us to reduce undesirable responses and to determine appropriate inflows for each lake that will optimize the quality of habitat across the system as a whole.

The response of this system to a modified hydraulic regime could not be predicted from controlled laboratory experiments because of the size and complexity of the system. Questions about systems of this size can best be answered when management programs are treated as large-scale field experiments whose results are carefully evaluated (Walters 1986). Results from this project should yield hypotheses concerning the responses of backwater systems to modified flows which can be used to develop new management strategies for similar ecosystems.

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