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Ecological Studies of Fish Near a Coal-Fired Generating Station and Related Laboratory Studies

Wisconsin Power Plant Impact Study

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ECOLOGICAL STUDIES OF FISH NEAR A COAL-FIRED GENERATING STATION AND RELATED LABORATORY STUDIES

Wisconsin Power Plant Impact Study

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FOREWORD

The U.S. Environmental Protection Agency (EPA) was established to coordinate our country's efforts toward improving and defending the quality of the environment. These efforts depend greatly on research to monitor environmental change and to determine health standards.

One project the EPA is supporting through its Environmental Research Laboratory in Duluth, Minnesota, is the study "The Impacts of Coal-Fired Power Plants on the Environment." The Columbia Generating Station, a coal-fired power plant near Portage, Wisconsin, has been the focus of all field observations. This interdisciplinary study is conducted by the Environmental Monitoring and Data Acquisition Group of the Institute for Environmental Studies at the University of Wisconsin-Madison and involves investigators from many departments at that same university. Several utilities and state agencies also are cooperating in the study: Wisconsin Power and Light Company, Madison Gas and Electric Company, Wisconsin Public Service Corporation, Wisconsin Public Service Commission, and Wisconsin Department of Natural Resources.

Reports from the study will appear as a series within the EPA Ecological Research Series. The topics will treat chemical constituents, chemical transport mechanisms, biological effects, social and economic effects, and integration and synthesis.

The Columbia Generating Station has caused changes in nearby wetlands. Since the area has a diverse fish community, the fish-monitoring group of the Columbia Generating Station impact study has been studying the effects of habitat loss and habitat degradation on fish. This report assesses the station's effects on fish reproduction and documents research on the use of temperature preference to detect sublethal concentrations of zinc in bluegills (*Lepomis macrochirus*) and zinc selection for tolerance over four generations of flagfish (*Jordanella floridae*).

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ABSTRACT

Construction of a coal-fired electric generating station on wetlands adjacent to the Wisconsin River has permanently altered about one-half of the original 1,104-ha site. Change in the remaining wetlands continues as a result of waste heat and ashpit effluent produced by the station. Leakage of warm water from the 203-ha cooling lake is causing a shift in the wetlands from shallow to deep-water marsh. Coal-combustion byproducts enter the wetlands from the station's ashpit drain. Since this area was known to have a diverse fish community and to be a spawning ground for Wisconsin River game fish, we studied the effects of this habitat loss and degradation on fish populations. In laboratory experiments we investigated the use of temperature preference and activity as a sublethal bioassay. In selection experiments we examined the potential of fish to evolve metal-tolerant populations in chronically contaminated environments.

Three years of netting documented the continued use of this area by spawning fish despite extensive habitat alterations. An inventory of potential spawning marshes along the Wisconsin River between the dams at Wisconsin Dells and Prairie du Sac showed that the station site still contained 22.0% of the deep-water sedge meadow and 0.8% of the shallow-water sedge meadow likely to be used by spawning game fish. Construction of the power plant resulted in a loss of 18% of the shallow water sedge meadow formerly available in this section of the Wisconsin River. Loss of deep water sedge meadow was negligible. *In situ* and laboratory experiments showed that the ashpit effluent was not acutely toxic to eggs or larvae of northern pike (Esox lucius), although some reduction in hatchability was attributed to the flocculent precipitate found in the ashpit drain. Analysis of population structures of northern pike showed a weak year-class for fish hatched in the first post-operational year. Further monitoring will be needed to determine if the reduction was due to the generating station or to natural factors.

A bioassay utilizing temperature preference and activity proved no more sensitive than bioassay methods used by previous investigators. Bluegills (Lepomis macrochirus), thermoregulating in a temporal gradient, tended to increase activity and decrease preferred temperature after exposure to 2.5 mg/liter zinc. Neither change was statistically significant, however, and both factors returned to normal levels within 2 days.

A population of flagfish (Jordanella floridae) selected for zinc tolerance was more resistant than the control population for the first two generations but not after three generations. The failure of continued selection to produce increasing zinc tolerance may have been caused by inbreeding depression or by cumulative carry-over effects of zinc passed

from mother to offspring through the egg cytoplasm. The discrepancy between laboratory-selection experiments and field observations of fish living in chronically metal-contaminated environments is discussed.

This report was prepared with the cooperation of faculty and graduate students at the Limnology Laboratory at the University of Wisconsin-Madison.

Most of the funding for the research reported here was provided by the U.S. Environmental Protection Agency (EPA). Funds were also granted by the University of Wisconsin-Madison, Wisconsin Power and Light Company, Madison Gas and Electric Company, Wisconsin Public Service Corporation, and Wisconsin Public Service Commission. This report was submitted in fullfillment of Grant No. R803971 by the Environmental Monitoring and Data Acquisition Group, Institute for Environmental Studies, University of Wisconsin-Madison, under the partial sponsorship of the EPA. The report covers the period of July 1975-78 and work was completed as of April 1979.

CONTENTS

FIGURES

TABLES

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Researchers responsible for the three major sections of this report, under the direction of Dr. John J. Magnuson, were Frank J. Rahel, Michael J. Talbot, and Anne M. Forbes (Effects of the Columbia Generating Station on Fish Spawning); Frank J. Rahel (Selection for Zinc Tolerance Over Four Generations of Flagfish); and Patricia A. Medvick (Use of Temperature Preference and Activity as a Sublethal Bioassay for the Toxic Effects of Zinc to the Bluegill). The literature reviewed in the appendices were prepared by Anne M. Forbes, Walter A. Gauthier, Dorothy M. Harrell, and Frank J. Rahel.

SECTION 1

INTRODUCTION

Coal-fired electric generating stations play an important role in energy production in the United States and are likely to increase in importance as other fossil fuels become scarce (Gordon 1978). Increased coal use has serious environmental consequences, and the changes resulting from power-plant construction and operation are now receiving considerable attention nationally (Glass 1978). Impact studies have been conducted to predict and, we hope, to mitigate the negative impacts of coal-fired generating stations on both terrestrial and aquatic ecosystems. One of these stations, the Columbia Generating Station near Portage, Wis., has been the focus of an extensive 3-yr study funded by the U.S. Environmental Protection Agency (EPA). As part of this multidisciplinary effort, our research has sought to assess the effects of the station on the local fish populations. The study has three components: (1) a field study to determine the importance of the generating station site as a spawning ground for fish and to assess the station's effects on fish reproduction; (2) a laboratory study to determine if fish populations can rapidly evolve tolerance to trace-element contaminants released by coal-fired generating stations; and (3) a laboratory study to determine whether a bioassay utilizing temperature preference and activity could detect changes in fish behavior after exposure to sublethal levels of trace elements.

The Columbia Generating Station is located on wetlands near the Wisconsin River in south-central Wisconsin (Figure 1). The 1,104-ha site contains a wide range of plant and animal communities and includes aquatic, wetland, and forested areas. The site is bordered by Duck Creek on the north, Rocky Run Creek on the south, and the Wisconsin River on the west. Of the original acreage, one-half has been altered permanently by the installation of a 203-ha cooling lake, 28-ha ash basin, coal-handling facilities, and various other structures. The station has two power-generating units, Columbia I and Columbia II. Construction of Columbia I, a 527-MW unit with a 152-m boiler chimney equipped with two hot-side electrostatic precipitators, began in 1971; operation began in April 1975. Cooling water for the unit is recycled continuously through the cooling lake. Columbia II, a unit of similar size but with a 198-m stack and sulfur-removal scrubbers, began operation in March 1978. Cooling towers were built to remove excess heat from Columbia II to minimize further thermal loading to the cooling lake. Fly ash and bottom ash produced during operation of Columbia I are pumped as a slurry into the ashpit where the ash particles settle out in a series of lagoons. The water is then pumped to the ashpit drain and eventually reaches Rocky Run Creek. Columbia II adds bottom ash to the ashpit; all fly ash from Columbia II is disposed of dry.

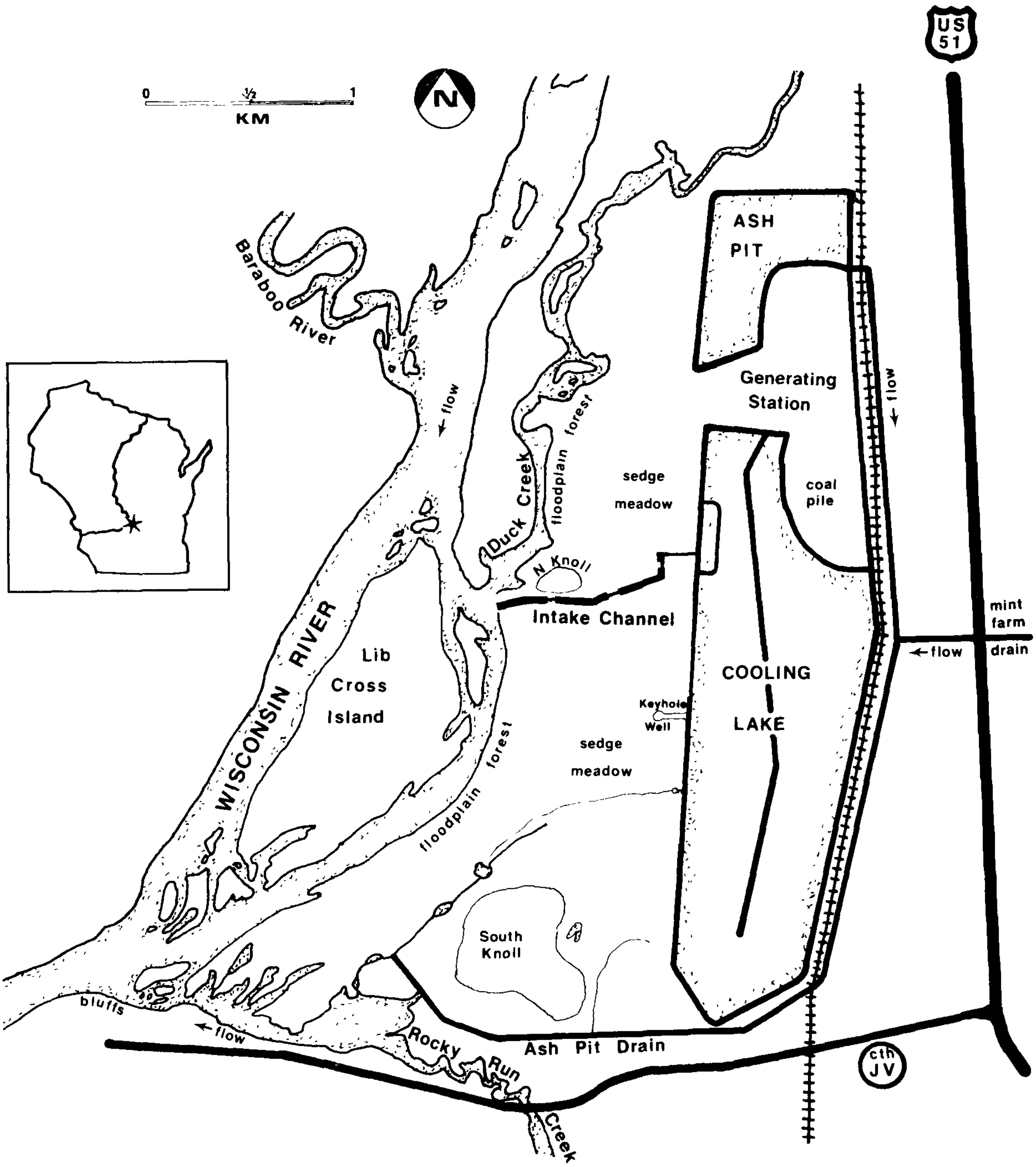


Figure 1. Map of the study site at Columbia Generating Station.

A diverse fish community lives in the remaining wetlands, in creeks bordering the site on the north and south, and in the Wisconsin River west of the site. Wetlands on the station site are spawning and nursery grounds for important game fish, including northern pike (Esox lucius), muskellunge (Esox masquinongy), and walleye (Stizostedion vitreum vitreum) (Ives and Besadny 1973). Four factors related to construction and operation of the generating station could potentially affect resident fish populations: habitat loss, habitat alteration, cooling lake intake, and acid precipitation.

About 446 ha of the original 1,104-ha site have been altered by construction of the facility. Much of the habitat lost, including land used for the cooling lake, was formerly sedge meadow, hence ideal spawning ground for game fish (Priegel and Krohn 1975). Since fish that use these spawning grounds are part of the Wisconsin River population, negative effects on the station site could affect the Wisconsin River fishery. The magnitude of these effects would depend on the availability of alternate spawning sites and the possible involvement of unique homing stocks.

Habitat alterations in the remaining wetlands continue because of increased ground-water discharge and waste heat from the cooling lake (Bedford 1977). Before construction of the facility, the upland sloped gradually to the flood plain sedge meadow and the Wisconsin River. Ground-water flow of 1 ft^3/s from adjacent uplands maintained the sedge meadow water levels. The flow varied seasonally, being high during spring and lower during the summer. The cooling lake established a 9-ft hydrostatic head above water levels in the adjacent wetlands and drastically altered the natural ground-water pattern (Figure 2). Now warm water from the cooling lake at a flow of 4 ft^3/s seeps west into the sedge meadow. The seepage of warm ground water into the rooting zone of plants and the associated rise in water level resulted in dramatic vegetation changes. The area west of the cooling lake, formerly dominated by perennial sedges, is being transformed into a community dominated by annuals and hydrophytic perennials such as cattails. Sedges offer dense mats of vegetation for spring spawning fish, but the annuals and hydrophytic perennials generally die down each winter and provide little suitable spawning substrate.

The warm-water seepage into the meadow west of the cooling lake is masked by volumes of Wisconsin River flood water in spring and does not directly affect spawning fish or eggs. However, the ashpit drain (east and south of the coolng lake) also receives cooling lake seepage and remains several degrees warmer even in the spring.

Habitat alterations in the wetlands south of the site along Rocky Run Creek are also caused by effluent from the ashpit. Metal oxides that compose the major reactive portions of the ash cause the pH of the ashpit water to rise 10 to 11 (Andren et al. 1977). Since Wisconsin water quality standards prohibit the release of water at a pH above 8, sulfuric acid is added before the ash effluent is discharged. The addition of acid causes the precipitation of elements such as barium and aluminum into a floc that coats the bottom of the ashpit drain and flows into Rocky Run Creek. Thus,

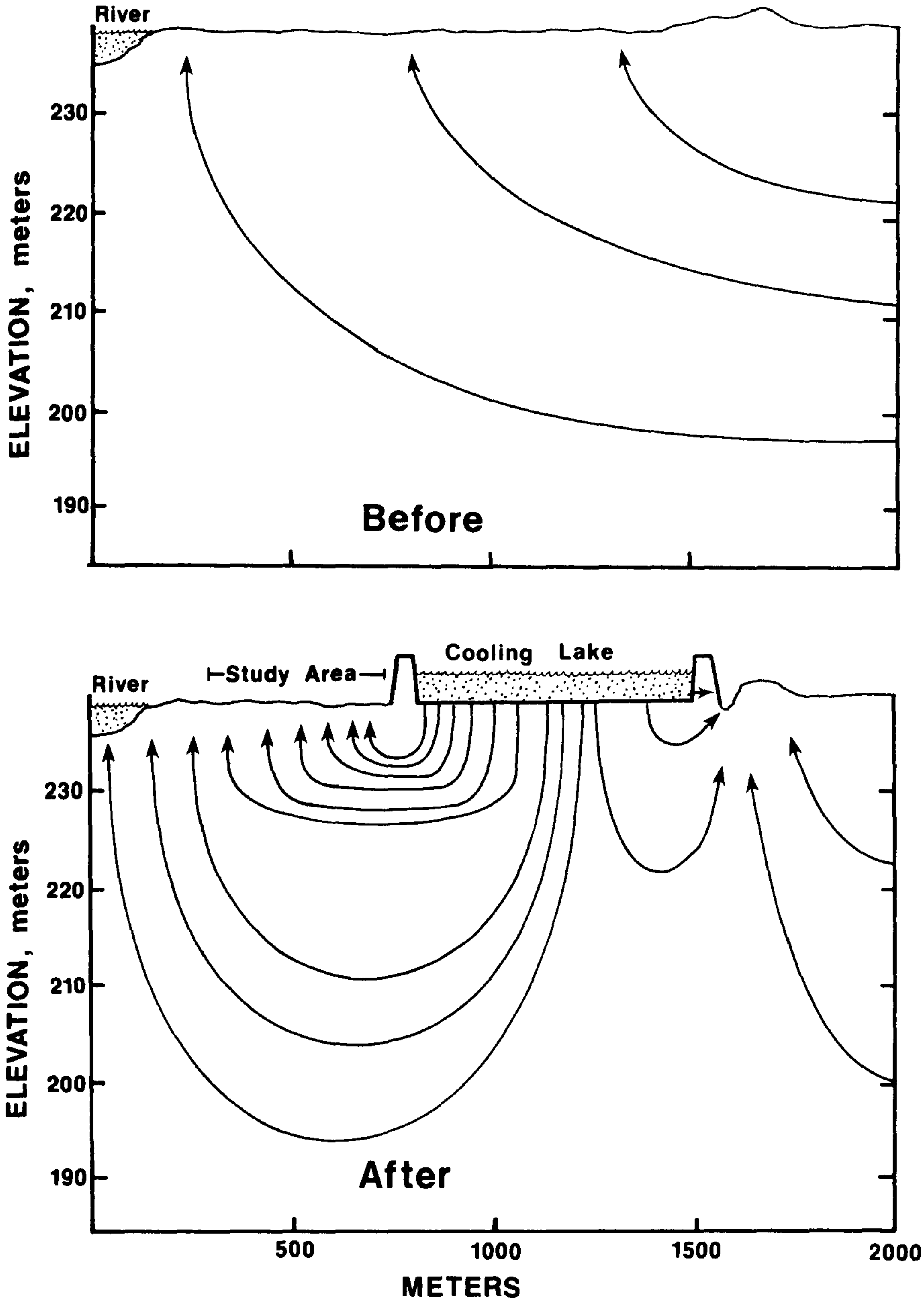


Figure 2. Ground-water flows before and after construction of the Columbia cooling lake. Arrows represent integrated flows 1 m^3/min, normal to the east-west cross section along the length of the cooling lake (Andrews and Anderson 1980).

dissolved and particulate trace elements, fly-ash particles, and perhaps organic contaminants in the ash effluent continually enter these streams, which then flow into the extensive Rocky Run wetland area. Many fish species, including the sensitive early life-history stages of important game fish, live in these waters and are exposed to the effluent.

Although the generating station recycles cooling water through the cooling lake, evaporative losses are made up by using Wisconsin River water. Since water and any organisms withdrawn from the river are not returned, the plant is analogous to a predator. Fish loss due to impingement and entrainment depends on the volume of water removed, the patchiness of fish distribution, and the ability of fish to avoid entrainment. A 1-yr study of egg and larval fish entrainment and juvenile and adult fish impingement at the Columbia site (Swanson Environmental, Inc. 1977) reported insignificant numbers of fish losses. The total river flow removed by the intake water at Columbia presently averages 0.3%, with a maximum of 1.08%. As long as the Columbia intake continues to remove a small percentage of the river flow, we expect no measurable effects of entrainment on the river system. An exception might occur when organism distribution is patchy near the intake, and a significant portion of one year-class (e.g., walleye larvae) is entrained. Aside from acting as a predator by removing organisms from the Wisconsin River, the usual types of entrainment effects (mechanical, toxic, and thermal) do not apply to the Columbia station, since organisms and water are not returned to the river.

Loss of fish populations due to lake acidification is related directly to acid precipitation from fossil-fuel combustion (Gorham 1976). The likelihood of waters undergoing acidification depends on the edaphic characteristics of their drainage basins and the intensity of acid input. Waters near the Columbia station site have a high buffering capacity because the drainage basin is calcareous. Although the problem has not been studied directly at the Columbia site, predictions based on the extensive literature indicate little potential for acid precipitation damage to the aquatic systems near the Columbia station.

DESIGN OF THE FISH STUDY

The impacts of the four above factors on fish populations were investigated through both the field and laboratory components of this study, as well as by reference to existing literature. Some of the concerns are site-specific; others are more general and therefore applicable to other locations. Overall conclusions are presented in Section 2.

Section 3 documents the site-specific effort to determine the importance of the generating station as a spawning ground for northern pike, muskellunge, and walleye. Spring sampling of these populations yielded information on the use of the marsh for spawning as well as on year-class strengths related to both natural and artifical changes. The effect of the ashpit effluent on fish reproduction is discussed in Section 3, as is the relative importance of the spawning marsh to the total Wisconsin River fishery.

Sections 4 and 5 concern questions that may arise at other generating sites. Section 4 describes the methods and results of the metal-tolerance study, in which flagfish were bred in the laboratory for resistance to zinc. Section 5 deals with the use of temperature preference and activity as a bioassay to detect subtle changes in fish behavior after exposure to sublethal metal levels.

Appendices include reviews of the literature on entrainment, acid rain, and fly-ash disposal.

SECTION 2

CONCLUSIONS

The major factors affecting fish at the Columbia site are habitat loss and habitat modification. Construction of the Columbia Generating Station eliminated approximately 18% of the shallow-water sedge meadow located between the Prairie du Sac and Wisconsin Dells dams. This habitat, when inundated by spring floods, is utilized by spawning northern pike and muskellunge. The station site currently contains about 22% of the deep-water sedge meadow and 0.8% of the shallow-water sedge meadow likely to be used by spawning northern pike in this section of the Wisconsin River. Tagging efforts indicated that northern pike from as far away as Lake Wisconsin (17 km downstream) migrate to the Rocky Run wetlands to spawn. However, these wetlands continue to be affected by effluent from the ashpit drain and by underground seepage of warm water from the cooling lake.

Northern pike, muskellunge, and walleye spawned in areas affected by ashpit effluent; in fact, northern pike were apparently attracted to the ashpit drain because of warmer spring water temperatures and higher current speeds.

The presence of ripe and spent northern pike adult spawners in 1976-78 indicated that spawning occurred in areas affected by the ashpit effluent. The presence of newly hatched fry in 1976 indicates that reproduction was successful.

Analysis of northern pike year-class strengths suggested that the 1976 year-class (the first year-class affected by the plant's operation) may be reduced. Further monitoring of population structure is warranted since year-classes hatched after the station began operation are now reaching maturity and should be returning to spawn.

In a laboratory bioassay, hatching success of pike eggs incubated in unfiltered ashpit drain water was lower than for eggs raised in filtered ashpit drain water; therefore, the flocculent precipitate found in ashpit drain water appears to hinder pike egg development. When the flocculent precipitate was removed by filtering, eggs hatched equally well in ashpit drain water and Rocky Run Creek water.

Fry hatched in the ashpit drain contained elevated levels of only one element, sodium, compared to fry hatched at other locations in the marsh. Acute toxicity due to trace-element bioaccumulation is unlikely to be a problem for fish eggs or newly hatched fry in the ashpit drain.

In the metal-tolerance study the selected population had a higher resistance to zinc after the first two generations, but did not differ from the unselected population in the third generation. Possible explanations for the failure of selection to continually increase zinc tolerance include inbreeding depression and carry-over effects passed from mother to offspring through the egg cytoplasm. After 2 to 8 weeks recovery time, flagfish which showed a zinc exposure lethal to the majority of the population, reproduced as successfully as unexposed fish.

The temperature preference and activity apparatus tested in the sublethal zinc bioassay is no more sensitive than bioassay methods used by previous investigators.

Potential damage to Wisconsin River fish and invertebrate populations from entrainment or impingement on water intake structures appears minimal.

Acid precipitation is not considered a potential problem for aquatic ecosystems at the Columbia site because of the high hydrogen-ion buffering capacity resulting from the calcareous nature of the drainage basin.

SECTION 3

EFFECTS OF THE COLUMBIA GENERATING STATION ON FISH SPAWNING

INTRODUCTION

Construction of a coal-fired electric generating station on a flood plain of the Wisconsin River (Columbia County, Wis.) has resulted in alteration of an important fish-spawning habitat. The station site formerly contained extensive wetland areas, particularly during spring floods when many fish species migrate to such areas to spawn on inundated vegetation. Among those species known to have used the Columbia Generating Station site for spawning are northern pike (Esox lucius), muskellunge (Esox masquinongy), and walleye (Stizostedion vitreum vitreum). Construction of the station permanently altered about one-half of the original 1,104 ha; much of the wetland affected was sedge meadow, an ideal spawning habitat for these fish. Portions of the remaining wetlands and Rocky Run Creek have undergone physical and chemical changes that may also influence fish reproduction. Given these considerations we undertook a site-specific study to determine both the importance of the station site as a spawning ground for Wisconsin River game fish and the effect of the power plant on the reproductive success of these fish. Our study involved the following: (1) netting on the site to discover which areas were important spawning grounds; (2) both *in situ* and laboratory bioassays to assess the effects of ashpit effluent on hatching and larval survival; (3) aging of scale samples to relate year-class strengths to both natural and artificial changes in the environment; and (4) use of infrared aerial photography to compare the spawning habitat at the station site to the total spawning habitat available in this section of the Wisconsin River.

One of the most obvious effects of the generating station is the introduction of ashpit effluent into potential spawning areas. This effluent is pumped from the ashpit settling basins into the ashpit drain. The ashpit drain joins a creek that flows through portions of the wetlands adjoining the site and then enters Rocky Run Creek 1.5 km above its mouth at the Wisconsin River (Figure 1). The effluent waters contain elevated levels of some trace elements and other coal-combustion by products (Andren et al. 1980, Helmke et al., Unpublished). Beginning in January 1977 sodium bicarbonate was routinely added to the pulverized coal to increase the efficiency of the electrostatic precipitators. This treatment resulted in increased conductivity in the ashpit drain and Rocky Run Creek and, in fact, served as a useful tool for measuring ash-effluent concentration downstream from the generating station. The water upstream of the ash effluent in the mint drain and in Rocky Run Creek is usually higher in alkalinity, hardness, and pH and lower in turbidity (Magnuson et al. 1980).

Invertebrate populations in the ashpit drain decreased in abundance and species composition after generating station operation began (Magnuson et al. 1980). Schoenfield (1978) demonstrated a tenfold increase in chromium and barium concentrations in several taxa of these ashpit-drain invertebrates (especially Asellus and Hyalella) since the start of station operation. The ashpit drain itself has a very sparse resident fish population (ictalurids and some cyprinids) probably as a result of inhospitable chemical and physical conditions as well as a lack of food organisms. Studies of another ashpit-drain system also documented a depauperate invertebrate fauna and the presence of only one fish species (Gambusia affinis) (Cherry et al. 1976).

The Rocky Run Creek area has a diverse fish community and is an important site for northern pike, muskellunge, and walleye. These species spawn on flooded wetlands where the newly hatched larvae remain for up to several months before emigrating to nearby rivers or lakes. A preoperational impact statement completed by the Wisconsin Department of Natural Resources (Ives and Besadny 1973) documented the occurrence of 47 fish species on or near the station site (Table 1). Included was one species listed as threatend in Wisconsin, the mud darter (Etheostoma aspirgene). Eggs and fry of both walleye and northern pike were also collected on the station site during the preoperational study.

METHODS

We conducted extensive fyke netting on flooded wetlands accessible from Rocky Run Creek from 1976 to 1978 to determine which areas were important spawning grounds, to see if fish bypassed the station site to spawn, and to assess year-class strengths of northern pike, the predominant species. By tagging fish we hoped to document the areas from which fish traveled to reach the spawning grounds and to determine whether homing to a certain site was recurring in successive years.

After discovering that pike were spawning in areas receiving ashpit effluent, we compared reproductive success in those areas with reproductive success in nearby unaffected control areas. Collection of pike fry in the field to study the natural hatch was unsuccessful; hence, we conducted both an *in situ* and a laboratory bioassay to assess the effects of ashpit effluent on egg hatching and larval survival. Water from both affected and unaffected areas was used, and survival was monitored daily. Northern pike fry hatched in various locations in the marsh were analyzed for trace elements to assess uptake in areas affected by the ashpit effluent.

After spawning, fish return to a 63-km section of the Wisconsin River bordered upstream by a dam at Wisconsin Dells and downstream by a dam at Prairie du Sac which forms Lake Wisconsin. During spring floods in this stretch of the river, many other backwater areas are formed that could serve as pike spawning grounds. The relative importance of spawning habitat on the plant site to the total spawning habitat available in this section of the river was determined by infrared aerial photography. Photo-interpretation allowed us to classify wetlands into various types according to their value as pike-spawning grounds and to calculate the acreage of each

TABLE 1. FISH SPECIES AT THE COLUMBIA GENERATING STATION SITE

Common name	Scientific name	Collection sites[a]	Reference[b]
Bigmouth buffalo	Ictiobus cyprinellus	C	2
Black bullhead	Ictalurus melas	B,C,D	1,2
Black crappie	Pomoxis nigromaculatus	A,B,C,D	1,2
Blacknose shiner	Notropis heterolepis	B	1
Blackstripe topminnow	Fundulus notatus	B	1
Bluegill	Lepomis macrochirus	A,B,C,D	1,2
Bluntnose minnow	Pimephales notatus	B	1
Bowfin	Amia calva	A,B,D	1,2
Brook silversides	Labidesthes sicculus	B	1
Brown bullhead	Ictalurus nebulosus	B,D	1
Brown trout	Salmos trutta	C	2
Bullhead minnow	Pimephales vigilax	B	1
Carp	Cyprinus carpio	A,B,C,D	1,2
Central mudminnow	Umbra limi	B	1
Channel catfish	Ictalurus punctatus	A,B	1
Chestnut lamprey	Ichthyomyzon castaneus	C	2
Emerald shiner	Notropis atherinoides	B	1
Fathead minnow	Pimephales promelas	B	1
Flathead catfish	Pylocictis olivaris	A	1,2
Freshwater drum	Aplodinotus grunniens	A,B,C,D	1,2
Golden redhorse	Moxostoma erythrurum	B,D	1
Golden shiner	Notemigonus chrysoleucas	B,D	1
Grass pickerel	Exox americanus vermiculatus	B	1
Green sunfish	Lepomis cyanellus	B	1
Johnny darter	Etheostoma nigrum	B	1
Lake sturgeon	Acipenser fulvescens	A	1
Largemouth bass	Micropterus salmoides	B	1,2
Mooneye	Hiodon tergisus	A	1
Mud darter	Etheostoma aspirgene	B	1
Muskellunge	Esox masquinongy	A,B,C	1,2

(continued)

TABLE 1 (continued)

Common name	Scientific name	Collection sites[a]	Reference[b]
Northern pike	Esox lucius	A,B,D	1,2
Pirate perch	Aphredoderus sayanus	B,C	1,2
Pumpkinseed	Lepomis gibbosus	A,B,D	1,2
Quillback	Carpiodes cyprinus	B	1
Rainbow trout	Salmo gairdneri	C	2
Redhorse	Moxostoma sp.	C	2
Red shiner	Notropis lutrensis	B	1
Rock bass	Ambloplites rupestris	A,B,D	1,2
Sand shiner	Notropis stramineus	B	1
Sauger	Stizostedion canadense	A,B,C,D	1,2
Shovelnose sturgeon	Scaphirhynchus platorynchus	A	1
Smallmouth bass	Micropterus dolomieui	A,B	1
Smallmouth buffalo	Ictiobus bubalus	C	1
Spotfin shiner	Notropis spilopterus	B	1
Spotted sucker	Minytrema melanops	B	1,2
Tadpole madtom	Noturus gyrinus	B	1
Walleye	Stizostedion vitreum vitreum	A,B,C,D	1,2
White bass	Morone chrysops	A,B	1,2
White crappie	Pomoxis annularis	A,B,D	1,2
White sucker	Catostomus commersoni	B,D	1,2
Yellow bullhead	Ictalurus natalis	A,B,D	1,2
Yellow perch	Perca flavescens	A,B,C,D	1,2

[a]Collection sites include: A--Wisconsin River, B--Duck Creek, C--Rocky Run Creek, and D--On-site flooded areas.
[b]List compiled by investigators from (1) the Wisconsin Department of Natural Resources and (2) the University of Wisconsin-Madison.

type. By comparing various watersheds we could determine what percentage of the total available pike spawning habitat in that section of the Wisconsin River was being influenced by the generating station.

Scale samples from pike, muskellunge, and walleye were aged in an effort to relate year-class strengths to both natural and man-caused changes in the environment. The introduction into spawning areas of the ashpit effluent, which had a complex and largely unknown chemical make-up, was one such change. Construction activities, which began in January 1971, destroyed a large portion of the sedge meadow and undoubtedly influenced spring flood patterns. Studies have shown that flooded vegetation, preferably dense mats of sedges (*Carex* sp.), is necessary for successful pike and muskellunge reproduction (McCarraher and Thomas 1972, Priegel and Krohn 1975). Since the generating station began operation, seepage under the cooling lake dikes has modified water temperatures and vegetation types in the adjacent wetlands (Bedford 1977). The general trend has been toward replacement of shallow-water marsh dominated by the sedge (*Carex lacustris*) and other perennials to deep water dominated by annuals and hydrophytic perennials such as arrowhead (*Sagittaria* sp.) and cattail (*Typha* sp.). These changes reduced the amount of densely matted vegetation available for spring spawning fish. Finally, one natural factor that strongly influences reproductive success is the timing and extent of spring flood levels. Johnson (1956) found a direct correlation between high spring water levels followed by a small decline in levels during egg incubation and production of a strong northern pike year-class.

With the above considerations in mind, our overall goal was to determine the importance of the generating station site as a spawning ground for spring-spawning game fish and to assess the plant's effects on the reproductive success of these fish. More specific information on methods is included in the following sections.

Survey of Spawning Grounds

To sample fish species that spawn on the site, fyke-net sites were established at five strategic locations (Figure 3) and checked regularly from ice-out in late February through the end of the spawning season in late April. All fish captured during upstream movements were released on the upstream side of each net. A description of each site and the years that the nets were worked follows.

Net 1, worked between 1976 and 1978, was located on Rocky Run Creek at the bridge on County Trunk Highway JV. This site, upstream from the plant, was unaffected by construction or operation of the generating station. This net completely crossed the river channel, except for peak floods, and caught fish that passed by the station site. Water depths ranged from 1.5 to 3 m, depending on flood stage, and the current was generally strong. Spawning habitat was available immediately above the net when the creek flooded surrounding marshland.

Net 2, worked between 1976 and 1978, was located in Rocky Run Creek downstream of its confluence with the ashpit drain. The net was placed in a

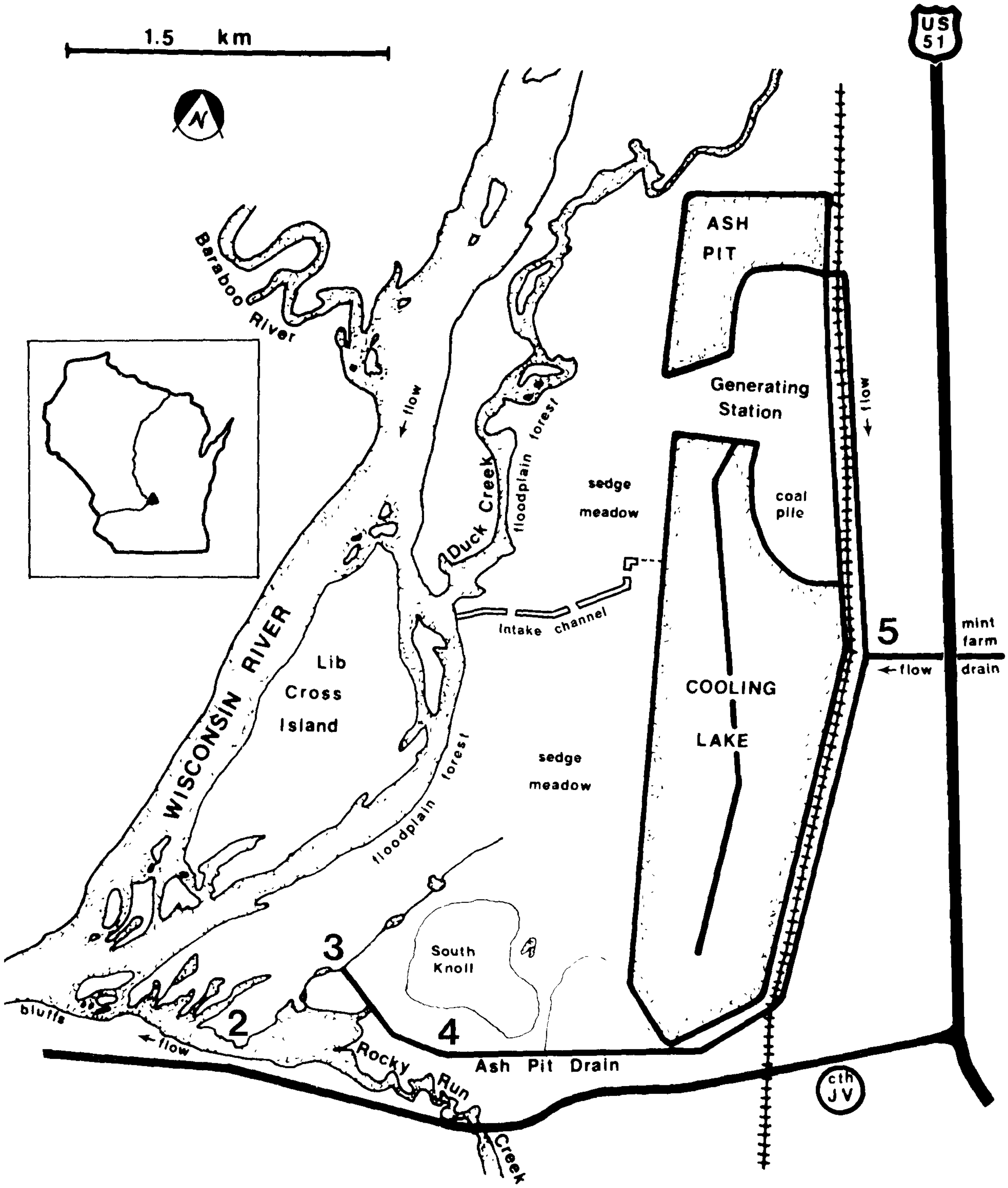


Figure 3. Location of fyke nets in spawning marshes on the Columbia site. (▲ on insert shows the location of the Columbia Generating Station in south-central Wisconsin.)

channel between two old bridge abutments and, except for peak flood periods, captured most fish entering the marsh system. Water depth ranged from 1.5 to 3 m depending on flood stage, and the current was moderate. Fish passing this point might spawn in the habitat immediately above the net, or they might proceed to spawning areas near nets 1, 3, or 4. Ashpit effluent was well mixed with Rocky Run water by the time it reached net 2.

Net 3, worked between 1976 and 1978, was located at the northern end of the Rocky Run Creek backwaters and in the main current channel leading into the sedge meadow near the cooling lake. This net caught fish that migrated into wetlands beyond those affected by ashpit effluent. Passage around the net was often possible when the entire area was flooded, and generally water levels dropped sharply at this site after the spring floods. Although spawning habitat was available near the net, it was mainly located upstream in flooded sedge meadows.

Net 4, worked between 1976 and 1978, was located in the ashpit drain above its junction with Rocky Run Creek in a diked channel that caught most of the fish migrating upstream. This net caught fish entering spawning areas most affected by ashpit effluent. The current was moderate and water levels were generally 0.5 to 2 m. Unlike net site 3, this site retained a flow of water throughout the year. Abundant spawning habitat was available below and above this net. Clumps of the precipitated floc described earlier were often found floating downstream, and finer floc particles could be observed in the water column.

Net 5, worked in 1978, was located above the confluence of the mint and ashpit drains, and was used to determine if fish would migrate all the way up the ashpit drain to reach spawning ground unaffected by ashpit effluent. It completely sealed off a small channel that was usually less than 1 m deep but contained water throughout the year.

The Rocky Run slough areas were electroshocked in 1976 and 1977 to determine if many fish were avoiding the nets, but still utilizing the site for spawning. Areas downstream from net 2 were shocked also to determine their importance as spawning grounds. Because of low water levels and technical problems, we found that our fyke nets were a more effective method of collecting fish.

Northern pike, muskellunge, and walleyes were measured, sexed, and fitted with a monel tag, from the National Band and Tag Co., inserted into their preopercular bone. The tag carried an identification number as well as the label "University of Wisconsin-Madison." To determine the distribution of these fish during the nonspawning season, we posted notices at various boat landings and fishing areas offering a reward to anglers who returned tags from fish they caught.

To estimate spawning success on the plant site, we set fry traps in 1976 and 1977 in areas corresponding to net sites 2, 3, and 4. The traps were generally ineffective, as were light traps tried in 1978. After study of the natural hatch proved unfeasible, we conducted field and laboratory bioassays to assess the effect of the ashpit effluent on northern pike egg and fry survival.

Effects of Ashpit Effluent on Reproductive Success

After documenting the use of areas receiving ashpit effluent as spawning grounds in 1976, we conducted an *in situ* bioassay in 1977 to determine if the effluent was affecting survival of eggs and larvae of northern pike, the predominant game species in the area. Fertilized eggs were obtained from the Wild Rose Fish Hatchery of the Wisconsin Department of Natural Resources. Eyed eggs were incubated in the main stream channel at three locations: (1) in the ashpit drain; (2) above the plant site in Rocky Run Creek; and (3) downstream Rocky Run Creek after ashpit effluent water was mixed well with creek water. These locations corresponded to net sites 4, 1, and 2, respectively.

Ten incubation bottles, each containing approximately 250 eggs, were anchored at each location. Incubation bottles were 1-gal plastic containers with plastic screen on the sides and bottom, current deflectors to maintain water exchange, and floats to keep them at the surface. Each day we emptied accumulated silt, counted live eggs and larvae, and observed the stage of egg development. Dead eggs were removed to minimize disease. Water temperatures at each station were recorded continuously with a Ryan Instruments temperature recorder, and relative siltation loads were estimated from silt deposition in graduated cylinders over 24-h periods. Dissolved oxygen, conductivity, water current speeds, pH, alkalinity, and EDTA hardness were measured regularly with standard techniques (American Public Health Association et al. 1975). Survival was calculated daily as the percentage of stocked eggs still as eggs or fry. The experiment was repeated with newly hatched pike larvae, also obtained from the Wisconsin Department of Natural Resources.

Northern pike fry that hatched in our incubation bottles were analyzed for 20 trace-element concentrations by neutron activation. The analyses were performed by the Trace Elements Subproject under the direction of Dr. Phillip Helmke (Helmke, unpublished). Fry hatched in areas affected by the ashpit effluent were compared with fry from unaffected waters. Since the ashpit effluent was known to contain elevated levels of certain trace elements, we felt it important to document any biological accumulation by this sensitive life-history stage.

At all three locations, the incubation bottles accumulated silt, but the problem was greatest at the Rocky Run stations where the daily accumulation of fine dark silt often completely covered the eggs. Although sediment also accumulated in the ashpit drain bottles, most of it consisted of the semibuoyant chemical floc. Ashpit drain eggs remained amber in color, whereas Rocky Run eggs were coated with brown silt. In nature, eggs are spawned over a mat of sedge grasses and do not become buried by sediments. Therefore, we repeated the experiments in 1978 under controlled laboratory conditions where siltation effects could be eliminated.

Egg cups made from polyinylchloride (PVC) piping covered at the bottom with plastic screen were used to hatch eggs in a laboratory chamber maintained at 12°C. Ten cups of approximately 100 eggs each were incubated in individual 500-ml beakers containing ashpit drain water, Rocky Run water, or the naturally occurring mixture. A similar set of egg cups was exposed to water from these same sources that had been passed through a 3-µm filter to eliminate sediment or floc, or both. Each lot of eggs was given a fresh exchange of their respective test water daily, and dead eggs and fry were removed. The experiment proceeded until all eggs either hatched or died.

Year-Class Strengths

Northern pike, muskellunge, and walleye were aged by counting annuli on their scales (Williams 1955). Scales were collected from above the lateral line below the dorsal fin. After cleansing in a solution of 0.1 *N* NaOH, impressions of the scales were made on cellulose-acetate slides. These impressions were then projected onto a screen, and the annuli were counted. Year-class strengths were then related to factors such as water levels or site construction activities that might have influenced reproductive success.

Importance of the Station Site to the Wisconsin River Fishery

An inventory of potential pike spawning areas in the section of the Wisconsin River bordered by the Wisconsin Dells and Prairie du Sac dams was prepared by manual photo-interpretation of 70-mm color infrared transparencies taken from an altitude of 11,000 ft. A series of 180 images along 12 flight lines was taken during low water levels on 9 September 1977 at wetland at a scale of 1:62,500. Flights took place in the fall when maximum vegetation was exposed.

The first step in the analytical process was preparation of a base map on which to trace the photo-interpretations. Because the photoscale approximated that of the standard U.S. Geological Survey (USGS) (15-min) topographic quadrangle maps, transparent mylar copies of the USGS maps were used as base maps. Each photograph was placed under the transparent base map, and vegetation areas were transferred to the base map.

An experienced photo-interpreter identified the resources on the imagery. Ground checking of mapped wetland classes showed an excellent correlation between photo-interpretation and actual vegetation types. From the infrared photographs the following wetland classifications were delineated:

1) Deep-water sedge meadow. The deep-water sedge meadows consist of lake sedge (Carex lacustris), bullrush (Scirpus spp.), and bluejoint grass (Calamagrostis canadensis and C. inexpansa); some colonies of cattail (Typha sp.) are mixed in. Other floating and emergent aquatic macrophytes that often occur in deep-water sedge meadows include arrowhead (Sagittaria spp.) and burreed (Sparganium eurycarpum). A deep-water sedge meadow is characterized by inundation throughout the year.

2) Shallow-water sedge meadow. This category is dominated largely by either tussock sedge (Carex stricta) or bluejoint grass. Forbs such as aster (Eupatorium spp.) are occasionally prominent. Cattail is fairly common, as is reed canary grass (Phalaris arundinacea). Shallow-water sedge meadow, as the name implies, has a drier moisture regime than deep-water sedge meadow. For long periods during the growing season the water table is somewhat below the surface.

3) Emergents and deep-water floating macrophytes. This category includes deep-water species floating or barely emerging above the surface. Floating mats of duckweed (Lemna spp.) frequently cover stagnant open water between other vegetation. Large mats of arrowhead (Sagittaria spp.), water lily (Nymphaea spp.) and (Nuphar spp.), or pondweed (Potamogeton spp.) often compose this vegetation type, which is often associated with the borders of open bodies of water.

4) Cattail. Monospecific stands of cattail are separated on the maps if large enough to outline.

5) Reed canary grass. This densely grown monospecific vegetation type often occurs along water courses or in regularly shaped plantations.

6) Bluejoint grass. This species seldom occurs in large pure stands and is an indication of a fairly undisturbed wetland community.

7) Drained wetland. A growth of weedy forbs and shrubs often indicates ditching and desiccated conditions. Typically, remnants of the original vegetation type are found under the weeds.

8) Shrub carr and swamp. Dogwood (Cornus stolonifera), spiraea (Spiraea alba), or willow (Salix interior) are the most common constituents of shrub carr. Lowland tree species of willow, silver maple (Acer saccharinum), aspen (Populus tremuloides), or green ash (Fraxinus pennsylvanica) are commonly interspersed or occur as solid stands of lowland forest.

9) Mixed vegetation. Vegetation complexes too detailed to separate by individual boundaries were combined and labeled by predominant types. For example, complexes of shrub-cattail-sedge meadow were fairly common.

Areas for each vegetation class were quantified bu using a Hewlett Packard Model 9107A digitizer and calculator. Comparison of pike spawning areas (viz., deep- and shallow-water sedge meadows) on the plant site with other potential spawning areas allowed us to draw some conclusions about the importance of the plant site to the Wisconsin River fishery.

RESULTS

Survey of Spawning Grounds

Our fyke nets caught a total of 21 species of fish over a 3-yr period (Appendix A). Northern pike was the dominant game fish, but walleye, muskellunge, and largemouth bass were caught along with many carp and various catostomid species. The average yearly catch (Figure 4) was largest at the entrance to the Rocky Run slough area (net 2) and smallest in upper Rocky Run (net 1). No fish were caught in the mint drain (net 5). The low number of fish caught at nets 1 and 5 indicates that most fish entering the system remain on the station site to spawn. Furthermore, our catches in the ashpit drain (net 4) were larger than those at the access point to the sedge meadow (net 3), indicating that many northern pike move into the ashpit drain during spawning migrations.

Water temperatures at each net location indicated that ashpit drain water was consistently warmer by several degrees centigrade than water draining the sedge meadow adjacent to the cooling lake (Table 2). The greater number of northern pike spawning near the ashpit drain may be attributed to the pike's affinity for warmer water currents during migration (Johnson 1956, Franklin and Smith 1963). Fish entering the Rocky Run slough through main current channels encounter an intersection where cooler water flowing through net 3 mixes with warmer water originating in the ashpit drain and flowing through net 4. Preference for the warmer water currents may lead fish close to the ashpit drain.

In addition to being warmer, the ashpit drain had a markedly higher conductivity in 1977 and 1978 than the other stations. This condition was due to the use of sodium bicarbonate in the coal to increase the efficiency of the electrostatic precipitators. For example, February-April 1978 averages for conductivity at 25°C were 468 μmhos/cm at net 1, 658 at net 2, 805 at net 3, and 1,218 at net 4. The conductivity decreases downstream, but is still elevated at the mouth of the Rocky Run slough (net 2).

Effect of Spring Water Levels on Spawning--

Spring water levels also appear to influence the distribution of spawning fish. In 1976 and 1978 spring flood periods were normal, but in 1977 flooding was greatly reduced (Figure 5). Thus, although large numbers of spawning northern pike enter the slough area each year, they proceed to spawning sites near the cooling lake (net 3) or in the ashpit drain (net 4) only if water levels are high enough to provide suitably flooded vegetation. In 1977 such vegetation was unavailable, and pike remained in marshy areas just above the entrance to the Rocky Run slough area (net 2) or moved further upstream in Rocky Run. This situation is indicated by a reduced catch per unit effort (= total number of pike caught per number of days net was set) for nets in the sedge meadow and ashpit in 1977 compared to other years, but an increased catch per unit effort for the net in upstream Rocky Run (net 1) (Figure 6).

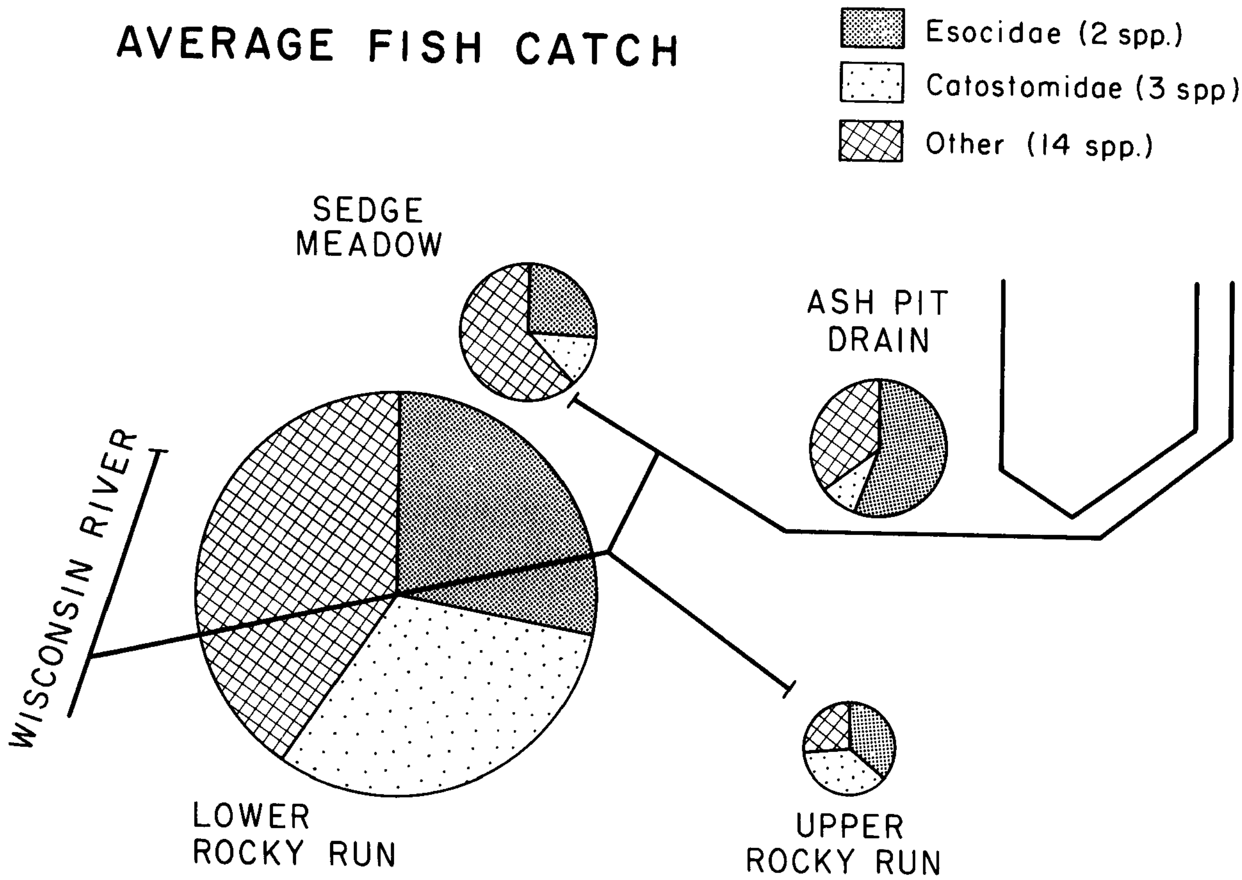


Figure 4. Fyke-net catches averaged over the 3-yr period, 1976-78. Upper Rocky Run corresponds to net 1 in Figure 3, lower Rocky Run to net 2, sedge meadow to net 3, and ashpit drain to net 4. Circles are scaled to indicate relative abundance at each net site.

TABLE 2. WATER TEMPERATURE (°C) AT VARIOUS SITES IN THE SPAWNING MARSH

	Upper Rocky Run (Net 1)	Lower Rocky Run (Net 2)	Access to sedge meadow adjacent to cooling lake (Net 3)	Lower ashpit drain (Net 4)	Mint drain (Net 5)
1976					
March 5	2.8				
13		2.5			
15	3.4	3.6	1.9	8.0	
16	1.7	1.3	1.0	3.7	
17	2.0	2.2	1.8	4.2	
18	4.7	5.0	3.2	7.5	
19	4.5	6.6	3.6	8.6	
20	4.8	5.4	7.7	10.4	
21	3.4	3.6	3.4	3.6	
22		3.6	5.0	7.5	
23	3.1	3.5	3.1	4.5	
24	6.0	7.0	5.8	7.2	
25	7.0	7.2	6.1	6.7	
26	9.2	8.5	6.5	10.6	
27	6.3	6.3	4.5	6.5	
28		6.8	5.1	7.7	
April 8	10.4	11.5	10.5	15.5	
10	10.2	11.8	9.9	11.0	
13	8.8	14.2	10.6	11.4	
15	12.5		15.1	13.4	
20			11.6	12.8	
22		10.2	10.4	10.1	
23	11.5	12.4	12.5	12.7	
28	9.4	11.0	10.5	10.5	
30	10.1	13.2	12.1	10.4	
May 3	5.6	6.5	5.8	4.9	
5	12.7	13.5	13.7	14.2	
7	7.7	10.3	8.4	10.2	
12			13.5		
1977					
March 2	0.5	0.4			
4		2.0			
7	4.0	4.0			
9		6.1			
11		9.4			
14	6.0	6.5		8.0	

(continued)

TABLE 2 (continued)

	Upper Rocky Run (Net 1)	Lower Rocky Run (Net 2)	Access to sedge meadow adjacent to cooling lake (Net 3)	Lower ashpit drain (Net 4)	Mint drain (Net 5)
16	6.5	7.0	5.7	10.0	
18	4.0	4.0	2.5	7.5	
21	5.0	6.0	4.0	7.0	
23	6.0	6.5			
24	5.0	7.7			
28	8.2	9.2	9.0	10.0	
30	8.0	9.7			
April 4	3.0	2.2	0.5	3.5	
5	4.5	3.9	3.8	6.5	
7	10.0	9.0	16.0	14.0	
1978					
March 2		2.8			
3		1.0			
6		0.2			
8		1.0			
10		0.9			
13		1.8			
15		1.0			
17		1.8			
20		3.0		8.0	
22	5.3	5.0		9.0	
24	1.5	2.0		3.5	
27	8.0	5.8			
29	8.2	10.1	9.5	11.0	
30		6.9			
31	13.7	12.5	7.9	16.1	14.1
April 3	7.9	9.9	9.8	11.5	8.2
6	6.8	7.5	9.0	8.5	6.5
8	7.7	8.1		8.0	6.9
10	8.8	9.7	8.9	9.0	8.1
12	8.9	8.3		8.3	
14	11.0	11.1		12.5	
16	11.0	12.0		13.0	

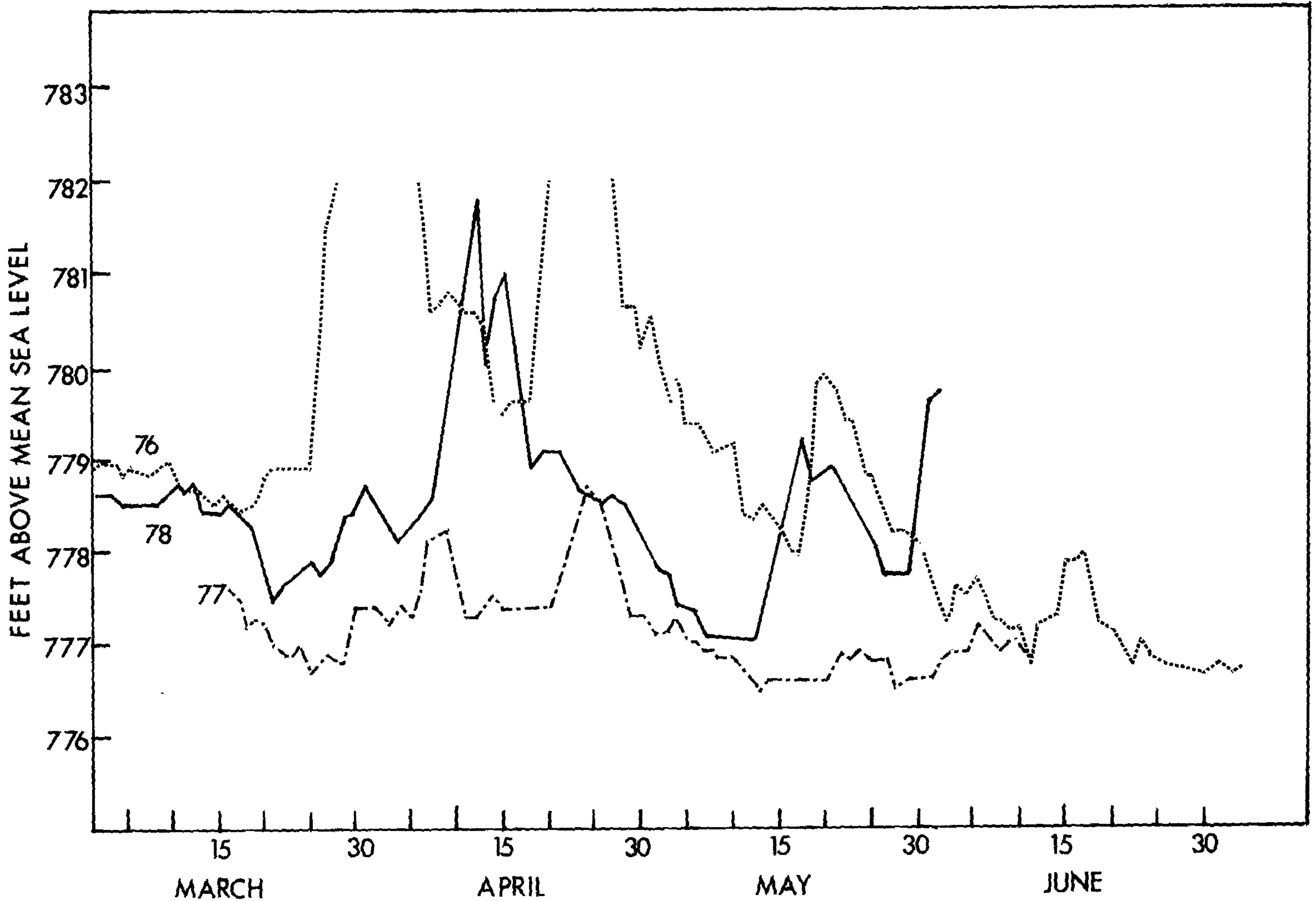


Figure 5. Spring water levels in the spawning grounds at the Columbia site during 1976-78.

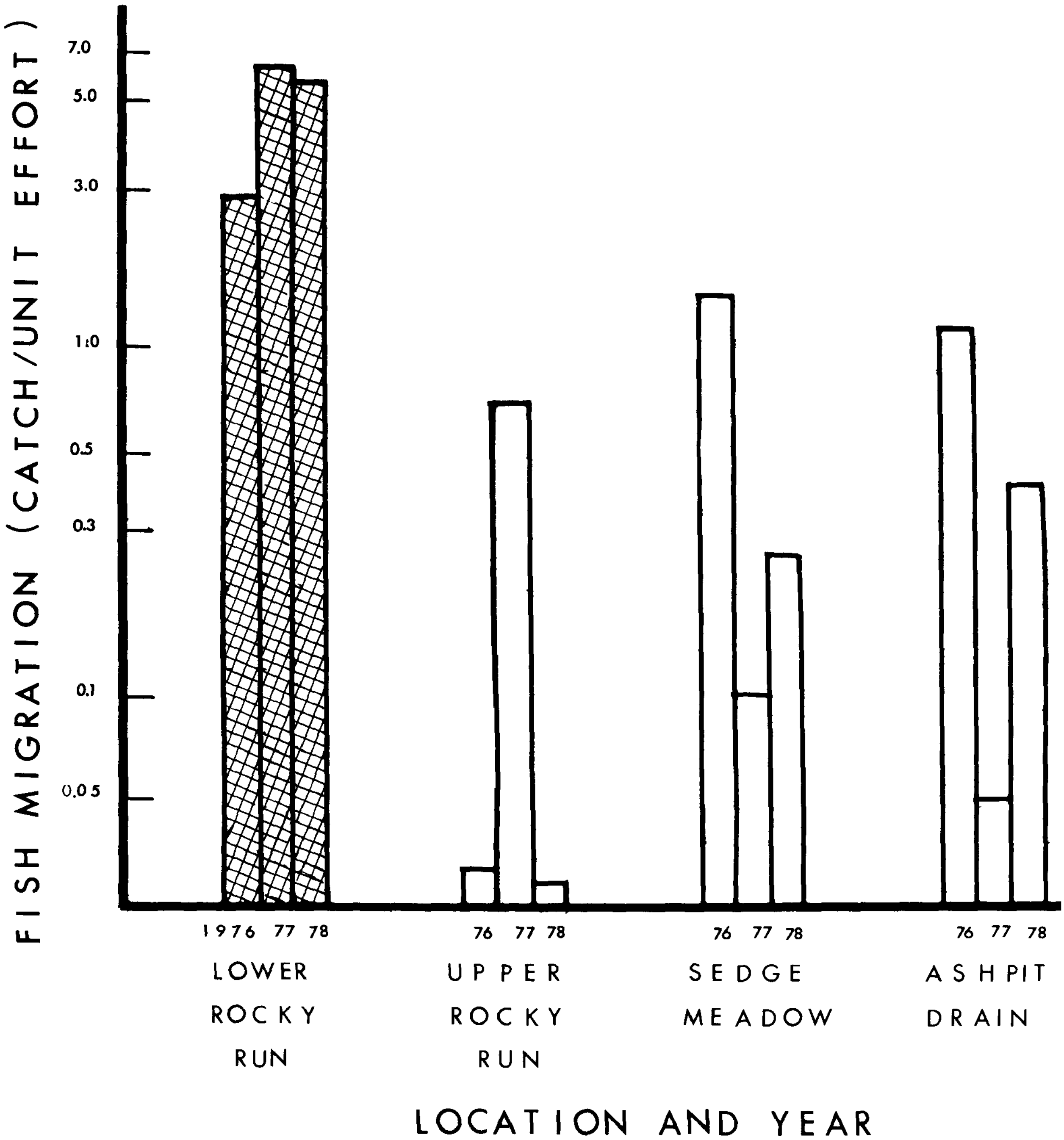


Figure 6. Catch of northern pike per unit effort on the Columbia site during 1976-78. Upper Rocky Run corresponds to net 1 on Figure 3, lower Rocky Run to net 2, sedge meadow to net 3, and ashpit drain to net 4. Catch per unit effort was calculated by dividing the total number of pike caught by the total number of days a given net was worked.

Capture of Pike Fry--

A few northern pike fry were captured in the Rocky Run slough area in 1976. This indicates that eggs were hatching and larvae were surviving in these wetlands (Table 3). About one-third of the fry were caught in the ashpit drain where waters are most affected by ashpit effluent. Small sample sizes precluded any comparisons of growth rates or estimates of fry abundance between sampled areas.

TABLE 3. SIZE (IN MILLIMETERS) OF INDIVIDUAL NORTHERN PIKE FRY CAPTURED IN ROCKY RUN SLOUGH, SPRING 1976

Date captured	Location captured[a]		
	Net 2	Net 3	Net 4
5 May	32,30		
7 May			27
10 May			42
12 May			30,47
14 May	34	25,27,28	30,33,35,35,44,46,47
17 May	48	35,35	
19 May	50,60,70,72	35	
21 May	60,70		
24 May	31	43,43	38
Total	11	8	12

[a]Net 2 was located near the mouth of Rocky Run slough; net 4 was located in the ashpit drain; net 3 was located in the main current channel draining the sedge meadow adjacent to the cooling lake (see Figure 3).

No fry were captured in 1977, however. Following the spawning period that year, 12 fry traps were placed in the marshes and were fished from 1 May to 10 June 1977. Seven traps duplicated the 1976 effort, and five more were set at other locations in the marsh. No fry of northern pike, walleye, or muskellunge were caught. Light trapping on two nights in June 1978 also failed to produce fry.

Because we caught no pike fry in 1977, we suspected a poor year-class. However, the 1977 year-class was represented in our 1978 fyke-netting efforts (refer to Figure 11). Comparisons between year-classes based on the low numbers of fry caught.

Tag Returns--

During the 3 years of fyke netting, 208 northern pike, 9 muskellunge, and 39 walleye were tagged on the station site. To date we have received three tag returns. Two northern pike tagged in early Spring 1978 were caught in May 1978 approximately 17 km downstream by fishermen in Lake Wisconsin near Okee, Wis. A third northern pike also tagged in spring 1978 was caught in October 1978, 5 km downstream in the Wisconsin River.

Effects of Ashpit Effluent on Reproductive Success

Egg mortalities for the field incubation tests were high at all net locations (Figure 7). Eggs incubated in the ashpit drain, however, had a higher survival rate (4.6%) after 10 days than eggs incubated in upstream Rocky Run (3.5%) or downstream Rocky Run (0.2%). Water quality data in the ashpit drain showed higher conductivity and warmer temperatures, while total alkalinity and hardness were reduced (Table 4). Although more material precipitated in sediment traps in the ashpit drain qualitatively it was very different from that collected in sediment traps set in Rocky Run. The ashpit drain material was almost entirely the white flocculent material from the ashpit, whereas the Rocky Run material consisted of heavier, brown, organic sediments that settled on the eggs.

Newly hatched larvae were held at the same sites as the egg incubation tests (Figure 8). Again, survival was better in the ashpit drain (54.8%) compared to downstream Rocky Run (39.7%) or upstream Rocky Run (6.6%). Although yolk-sac fry survival was better than egg survival, we still felt siltation confounded the results and hence turned to controlled laboratory bioassays to assess the effect of the ashpit effluent on fish reproduction.

Trace-Element Analysis of Fry--

Northern pike fry hatched at various locations in the marsh during the above field incubation tests were analyzed for 20 trace elements (Table 5). The 10 days that these fish spent in the marsh covered the embryonically active eyed-egg period through the early larval period. For all incubation sites a general increase in trace-element levels was found in fry as compared to levels in eggs. This observation reflects both embryonic development and the greater ionic constitution of the water compared to waters where the parents were caught (Lake Butte des Morts, Winnebago Co., Wis.). Fry hatched in the ashpit drain contained elevated levels of only one element, sodium, compared to fry hatched at marsh locations not influenced by the ashpit effluent. High iron levels were found in fry hatched in upper Rocky Run but the reason for the elevated iron levels is not known.

Egg Survival in Laboratory Tests--

In the laboratory, pike egg survival was significantly lower in water from the ashpit drain than in water from either a control area in upper Rocky Run or in a natural mixture of ashpit and Rocky Run water (Figure 9). Pike eggs were most susceptible to the toxicity of ashpit drain water during the developmental period following gastrulation (day 3 in Figure 9). Daily mortality rates were significantly greater for eggs hatched in ashpit water only on days 2 through 6 ($p < 0.05$, analysis of variance, Snedecor and Cochran 1967).

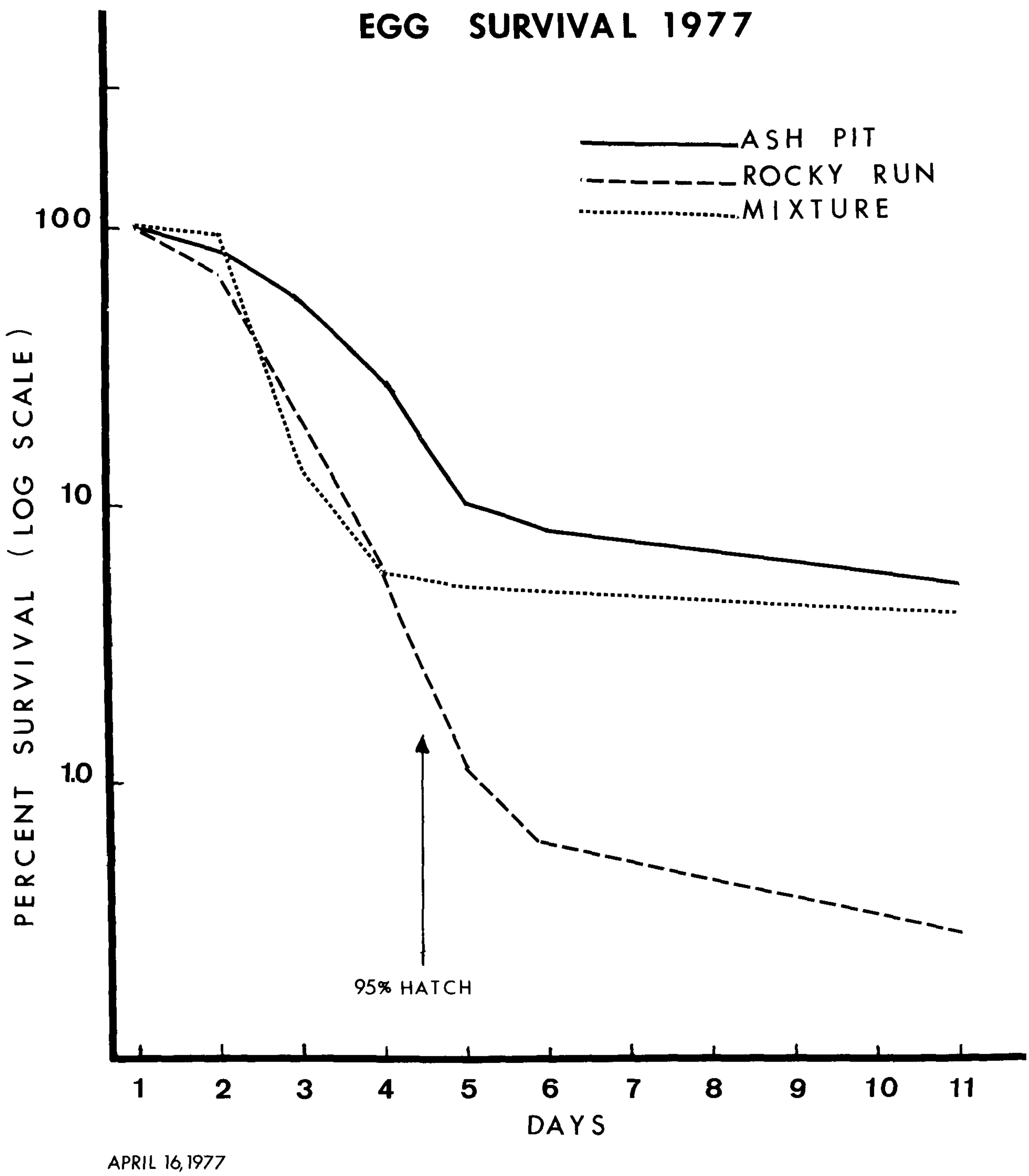


Figure 7. Survival of northern pike eggs hatched *in situ* in the wetlands at the Columbia site during April 1977. The ashpit site corresponds to net 4 and the Rocky Run site to net 1 in Figure 3. The mixture site corresponds to a natural mixture of Rocky Run and ashpit water at net 2 of Figure 3. Ten bottles with approximately 50 eggs in each were used at each site.

TABLE 4. WATER QUALITY DATA FOR VARIOUS STATIONS IN THE MARSH--MARCH 21-MAY 12, 1977

	Upper Rocky Run	Lower Rocky Run	Entrance to sedge meadow	Ashpit drain
Temperature (°C)	12.1[a] ±5.8 (4.5-20.5) 9	13.5 ±6.8 (3.9-23.0) 9	7.95 ±3.8 (3.8-13.0) 4	14.2 ±5.6 (6.2-21.8) 9
Current Speed (cm/s)	25.6 ±5.5 (20.6-36.8) 7	19.9 ±7.3 (10.1-30.3) 6	11.2 ±8.0 (2.6-18.3) 3	19.6 ±10.5 (7.4-44.9) 9
Dissolved oxygen (mg/liter)	0.6 ±6.0 (9.6-12.2) 8	9.8 ±1.1 (8.2-11.6) 8	7.2 ±1.6 (5.8-8.9) 3	9.7 ±0.7 (8.3-10.9) 9
Conductivity (μmhos/cm) @ 25°C	485 135 (381-831) 9	870 460 (448-2,028) 9	670 720 (282-1,747) 4	1,425 490 (443-1,940) 9
Total alkalinity (ppm)	220 35 (155.1-253.0) 8	203 33 149.6-239.8) 8	132 9 (126.5-143) 3	173 33 (133.1-226.6) 8
Hardness (ppm)	301 ±57 (234-368) 8	247 ±61 (150-240) 8	167 ±16 (150-182) 3	187 ±78 (130-352) 8
pH	7.9 ±0.1 (7.7-8.0) 9	8.0 ±0.5 (7.7-9.4) 9	7.8 ±0.9 (7.2-9.1) 4	7.9 ±0.3 (7.4-8.4) 9
Sediment (ml/day)	1.0 ±0.6 (0.5-1.8) 4	0.8 ±1.1 (0.1-2.0) 3	0.8 --- --- 1	2.9 ±2.5 (0.5-6.3) 4
Turbidity (JTU)	36.4 ±12.8 (13.7-54) 8	31.4 ±15.9 (15-57.7) 7	13.9 ±12.1 (0.7-30) 4	19.3 ±6.9 (11-34.7) 8

[a]The first entry in each cell is the mean; the second is the standard deviation; the third is the range; and the fourth is the number of measurements.

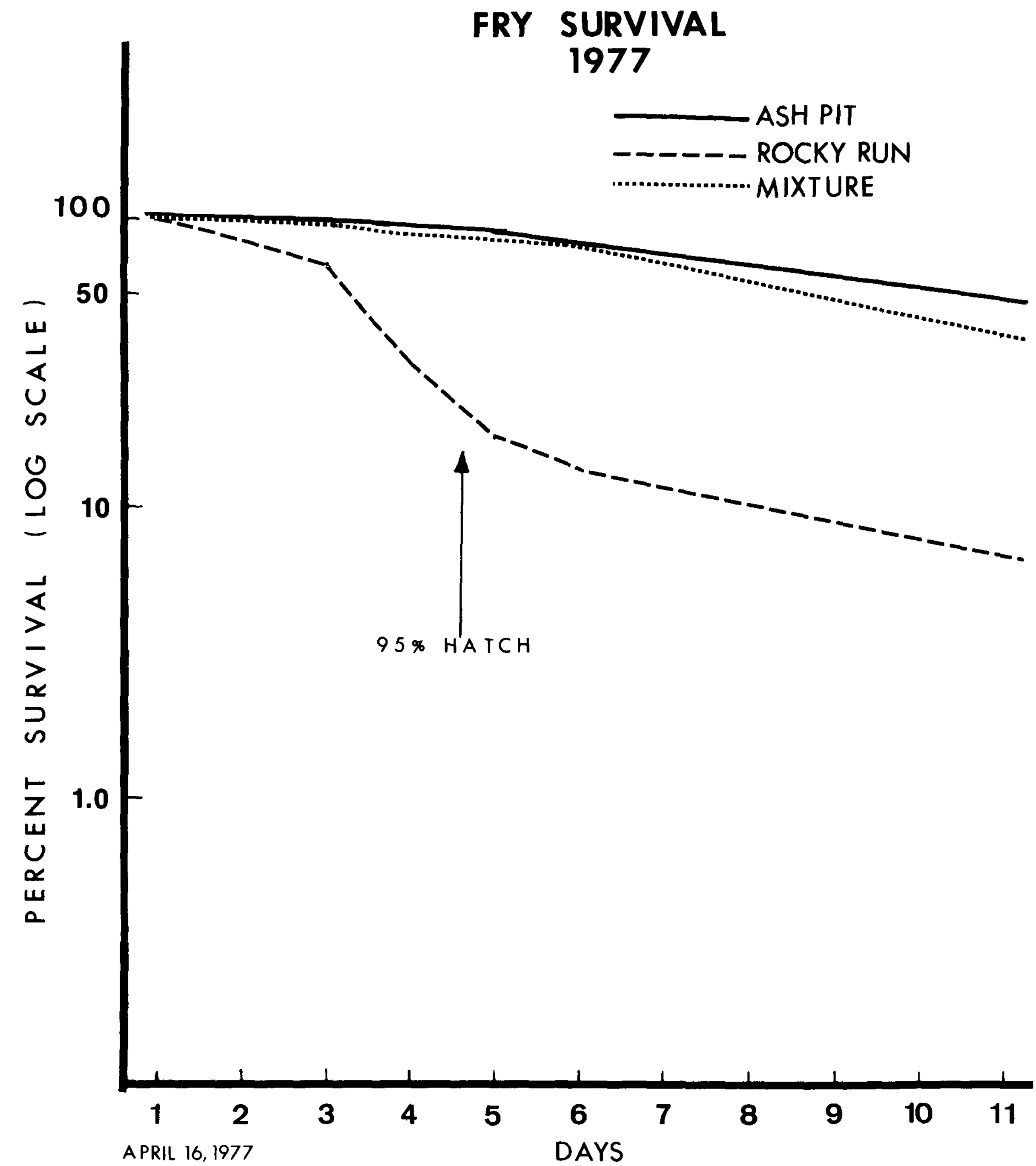


Figure 8. Survival of northern pike larvae placed in wetland at the Columbia site for 11 days in April 1977. The ashpit site corresponds to net 4 and the Rocky Run site to net 1 in Figure 3. The mixture site corresponds to a natural mixture of Rocky Run and ashpit water at net 2 in Figure 3. Ten bottles with 50 larvae each were used at each site.

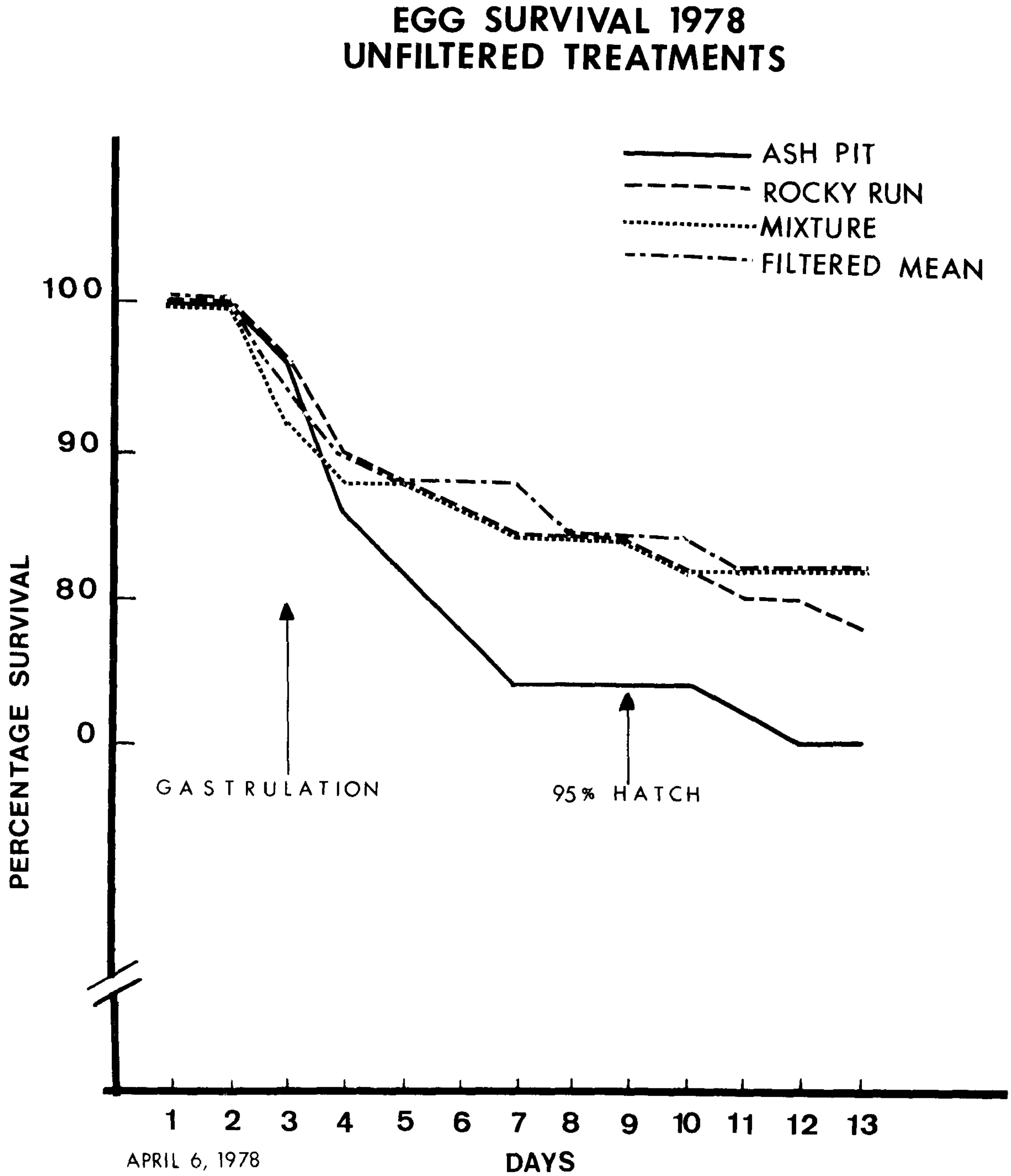


Figure 9. Survival of northern in pike eggs hatched in the laboratory during April 1978 in unfiltered water from the ashpit drain, Rocky Run Creek, and a downstream natural mixture. For comparison, the survival of eggs in filtered water is presented as the mean of the three filtered treatments. Ten lots of approximately 100 eggs each were used for each treatment.

TABLE 5. CONCENTRATIONS OF TRACE ELEMENTS (μg/g=ppm ON FREEZE DRIED WEIGHT BASIS) IN NORTHERN PIKE EGGS AND FRY USED IN THE *IN SITU* BIOASSAY, SPRING 1977[a]

Element	Eggs (before placement in the marsh)	Pike fry hatched at various locations in the marsh: Ashpit drain (n=11)	Lower Rocky Run (n=8)	Upper Rocky Run (n=4)
Br	13±1	25±2	44±2	36±9
Sm	0.04±0.01	0.13±0.01	0.13±0.02	b.d.
La	0.32±0.04	0.58±0.06	b.d.	b.d.
As	0.49±0.08	b.d.	b.d.	b.d.
Sb	0.09±0.01	0.36±0.06	b.d.	b.d.
Na	2,750±27	4,230±50	3,820±70	1,760±120
K	7,133±277	5,100±1,000	6,300±1,200	b.d.
Cr	1.3±0.1	20.0±1.4	12.8±1.9	27.0±5.0
Se	1.6±0.2	9.3±1.1	5.8±1.4	7.0±3.0
Hf	0.05±0.01	0.34±0.10	b.d.	b.d.
Ba	6.0±1.8	69±20	105±34	170±50
Sc	0.07±0.001	0.19±0.01	0.12±0.01	0.91±0.04
Rb	6.8±0.7	b.d.	b.d.	b.d.
Eu	0.01±0.001	0.15±0.03	b.d.	b.d.
Fe	366±16	1,910±100	1,530±160	5,100±400
Zn	65±2.0	222±11	239±12	142±15
Co	0.15±0.01	0.34±0.07	0.51±0.10	1.5±0.3
Ca	2,533±500	58,000±8,000	b	66,000±3,600
Cs	0.04±0.01	b.d.	b.d.	b.d.

[a]Mean = 1 S.D.; b.d. = below detection limits: b = analysis not done.

None of the filtered-water treatments differed in mortality (Figure 10); the percentage survival at the end of the experiment was highest in the Rocky Run filtered water, but this was not statistically significant. Whatever factor caused the increased mortality in the ashpit drain was removed by filtering. Water quality characteristics differed little in filtered and unfiltered samples except that turbidity was reduced in filtered water (Table 6). Ashpit drain water contained a suspended floc

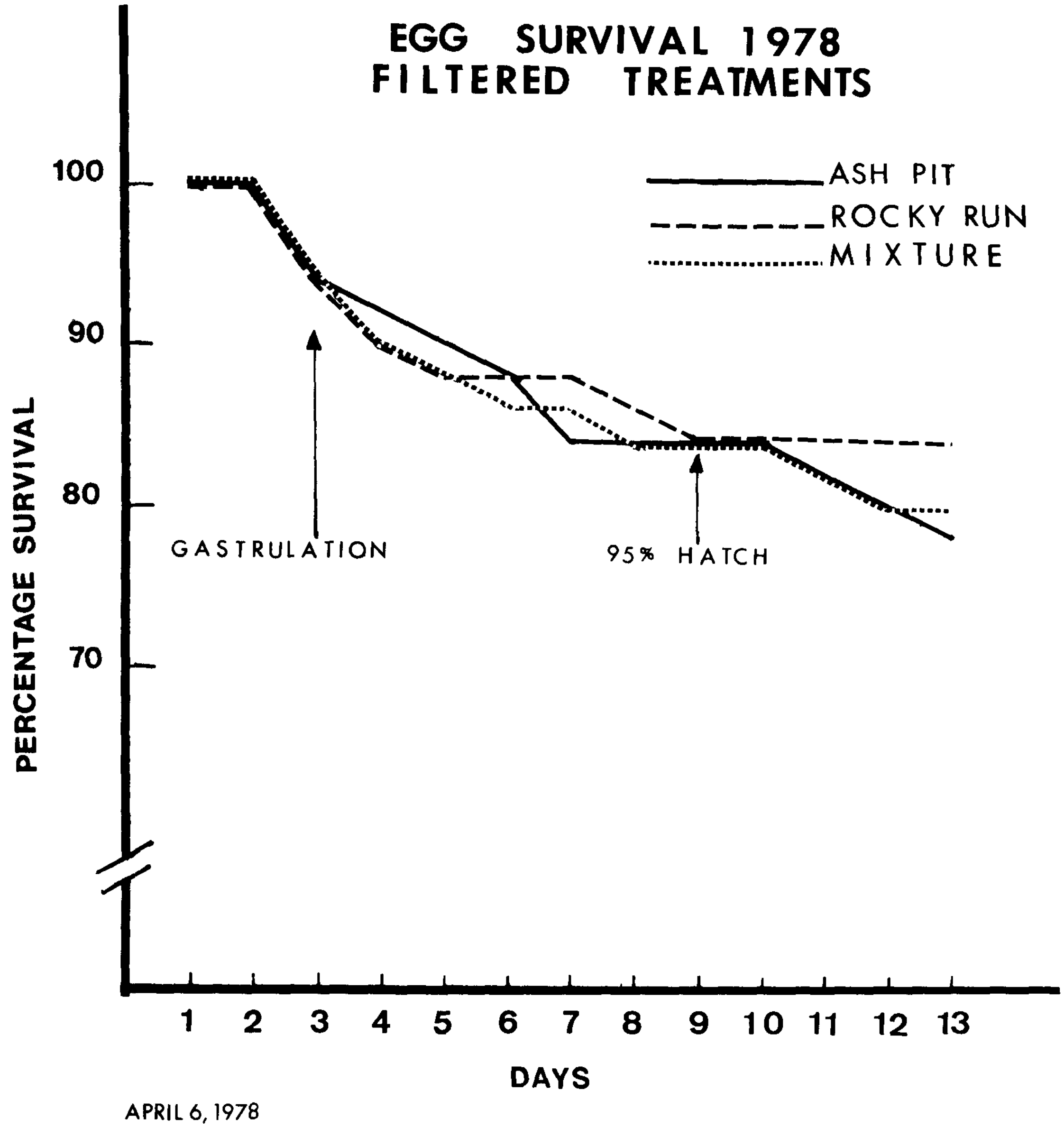


Figure 10. Survival of northern pike eggs hatched in the laboratory in April 1978 using filtered water from the ashpit drain, Rocky Run Creek, and a downstream natural mixture. Ten lots of approximately 100 eggs each were used for each treatment.

TABLE 6. WATER CHEMISTRY FOR 1978 LABORATORY EXPERIMENT

	Dissolved oxygen (mg/liter)	Conductivity (µmhos/cm at 25°C)	Total alkalinity (mg/liter)	Hardness (mg/liter)	pH	Turbidity (JTU)
			Unfiltered Treatments			
Upstream Rocky Run	8.9[a] (8.3-9.7) 7	390 (360-440) 7	185 (118-230) 7	208 (142-247) 7	8.0 (7.8-8.2) 7	1.0 (0.9-1.8) 6
Ashpit	9.0 (7.6-9.8) 7	1,155 (310-1,700) 7	104 (99-122) 7	158 (141-187) 7	7.6 (7.6-7.7) 7	.9 (0.6-3.5) 6
Downstream Rocky Run	9.1 (8.5-9.9) 7	525 (290-630) 7	157 (89-189) 7	189 (184-218) 7	8.0 (7.6-8.2) 7	1.2 (0.6-2.4) 6
			Filtered Treatments (3 µm Millepore filter)			
Upstream Rocky Run	9.1 (8.6-9.6) 7	435 (375-560) 7	184 (115-229) 7	210 (144-250) 7	8.0 (7.8-8.2) 7	0.9 (0.7-1.5) 6
Ashpit	9.2 (8.1-9.7) 7	1,200 (300-1,540) 7	101 (73-118) 7	162 (94-189) 7	7.7 (7.6-7.8) 7	1.3 (0.6-2.3) 6
Downstream Rocky Run	9.0 (7.5-9.7) 7	490 (275-595) 7	160 (79-186) 7	189 (99-220) 7	8.0 (7.7-8.2) 7	1.1 (0.7-2.0) 6

[a]The first entry in each cell is the mean; the second is the range; and the third is the number of samples.

that was filtered out and we suspect that this material caused the difference in toxicity between filtered and unfiltered ashpit drain water. Concentrations of trace contaminants in solution were not thought to be altered significantly by filtering because pH, temperature, and conductivity were similar in both filtered and unfiltered water. Actual trace-element concentrations were not determined, however. Routine water quality measurements showed ashpit drain water to have a higher conductivity and lower pH than water from the other sources (Table 6).

Year-Class Strengths

Our objective was to determine if harmful effects from construction and operation of a coal-fired power plant on a pike spawning ground could be detected as weak or missing year-classes. Having only one preoperational sampling and no preconstruction sampling proved a limitation in attempting to relate habitat changes caused by the plant to spawning success of northern pike. Our data show typical age-dependent mortality for northern pike (Figure 11) with most spawners between 2 and 4 years old.

The 1971 year-class was the first affected by major construction on the generating station site (Table 7). Fish from that year-class were already 5 years old at the time of our first sampling in 1976. If destroying a significant portion of the spawning marsh resulted in a loss of some fixed percentage of pike fry, fewer fish in all subsequent years would reflect this loss. Hence, the northern pike spawning population may be reduced because of habitat loss, but without historical data on the population before 1971, this loss would be undetectable. Since the 1973 year-class was well represented in our samples (Figure 11), construction of the ashpit dikes in 1973 did not result in a detectable reduction of year class strength.

Because the plant began operation in mid-spring 1975, any effects from the ashpit effluent would not be evident until the 1976 spring spawning season. Our 1978 catch data indicate a reduced 1976 year-class. Two-year-old fish constituted a large portion of the 1976 (17%) and 1977 (37%) catches, but represented only 7% of the 1978 catch. Water levels for the 1976 spawning season were similar to those of 1975 and 1974; therefore, adequate spawning habitat was available for all three year classes.

Only in 1977 were enough walleyes captured to allow assessment of population age structure (Figure 12). The 1976 year-class was only 1 yr old at that time and hence not very susceptible to our sampling techniques. As this would be the first year-class to be affected by the ashpit effluent, we can not determine what effects the effluent might have on walleye reproduction. Furthermore, this species naturally undergoes wide fluctuations in year-class strengths (Kelso and Ward 1977).

Importance of this Site to the Wisconsin River Fishery

Wisconsin River wetlands were grouped into 13 major areas of potential northern pike spawning habitat (Figures 13 and 14, Table 8, Appendix B). Because no field data exist to show if northern pike actually utilized these areas for spawning, the comparison of spawning areas was accomplished solely by whether suitable vegetation types existed. Vegetation types were determined by infrared aerial photography. This comparison shows the wetland area on the plant site (bordered by Duck Creek on the north, Rocky Run on the south, and the Chicago, Milwaukee, St. Paul, and Pacific Railroad tracks on the east) to be only a small percentage of the total remaining wetland between the Wisconsin Dells and Prairie du Sac dams (Table 8). Only 13.1% of the deep-water sedge meadow and 0.5% of the shallow-water sedge meadow areas are on the station site. Other areas with substantial deep and

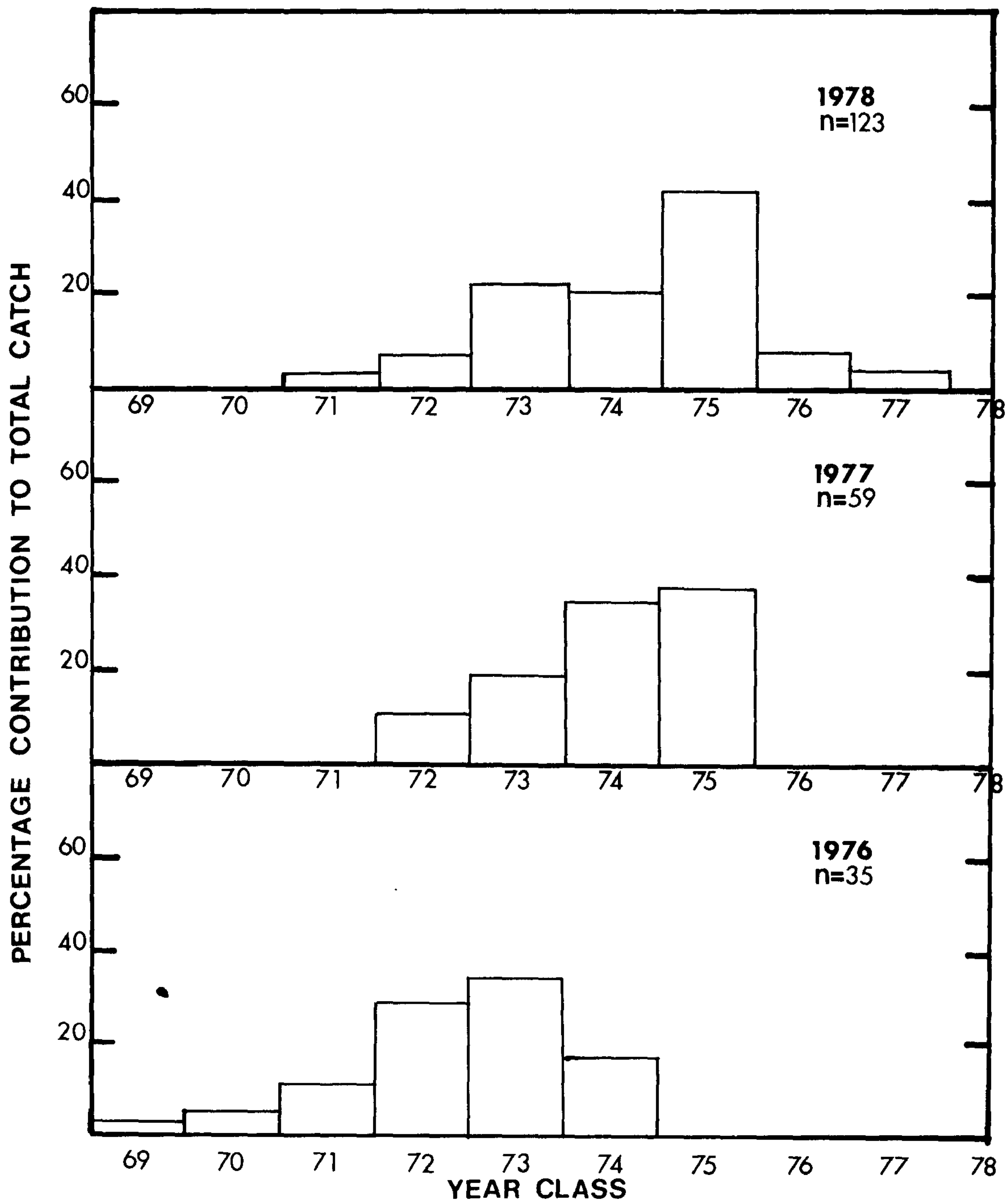


Figure 11. Population-age structure of northern pike caught on the Columbia site in 1976, 1977, and 1978. For each year fish caught in all nets were pooled to form one population. Sample size is given by n; the cooling lake dikes were built in 1971; the ashpit drain was built in 1973, and plant operation began in 1975.

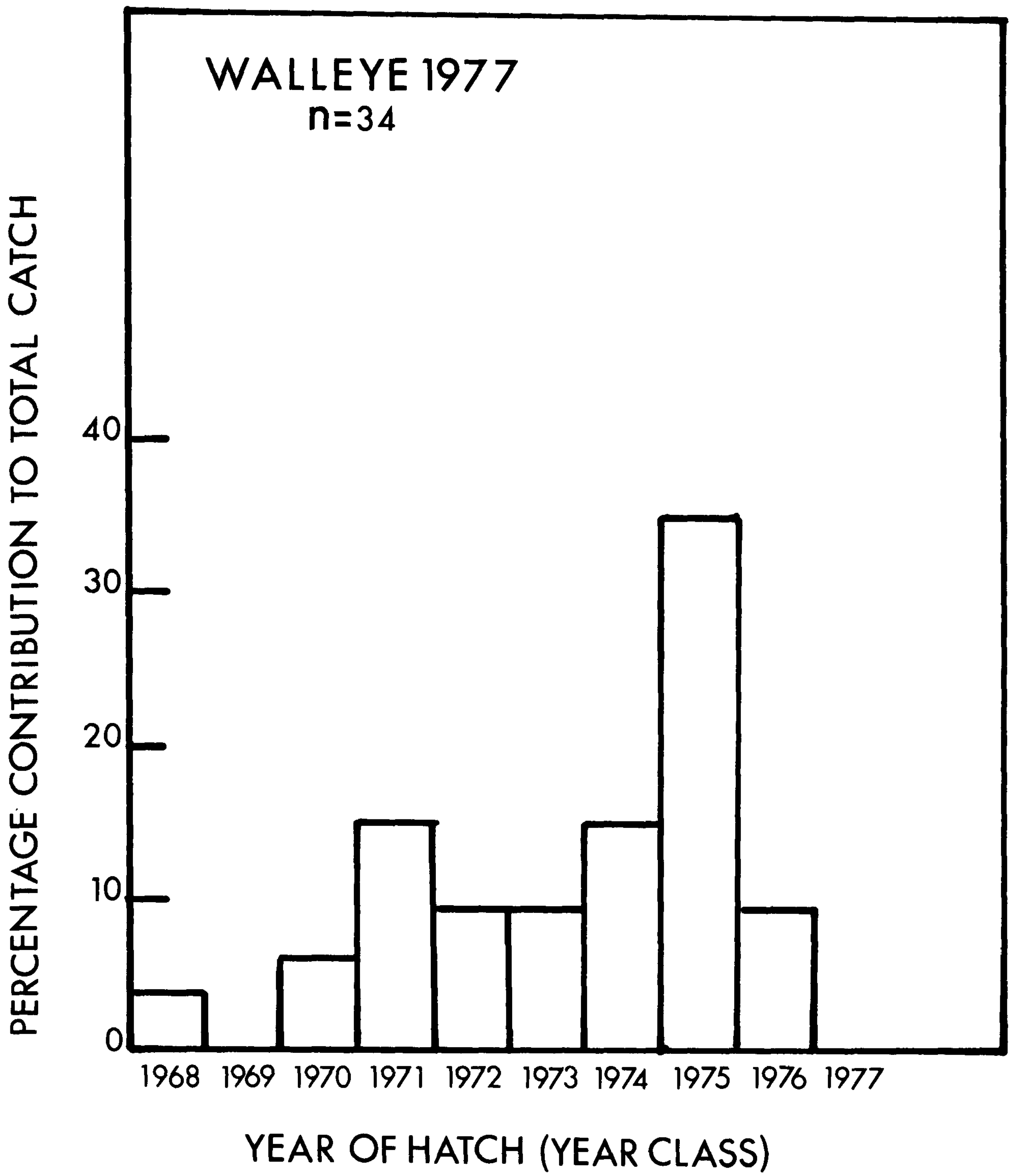


Figure 12. Population-age structure of walleye caught on the Columbia site in spring 1977. Total sample size was 34 fish.

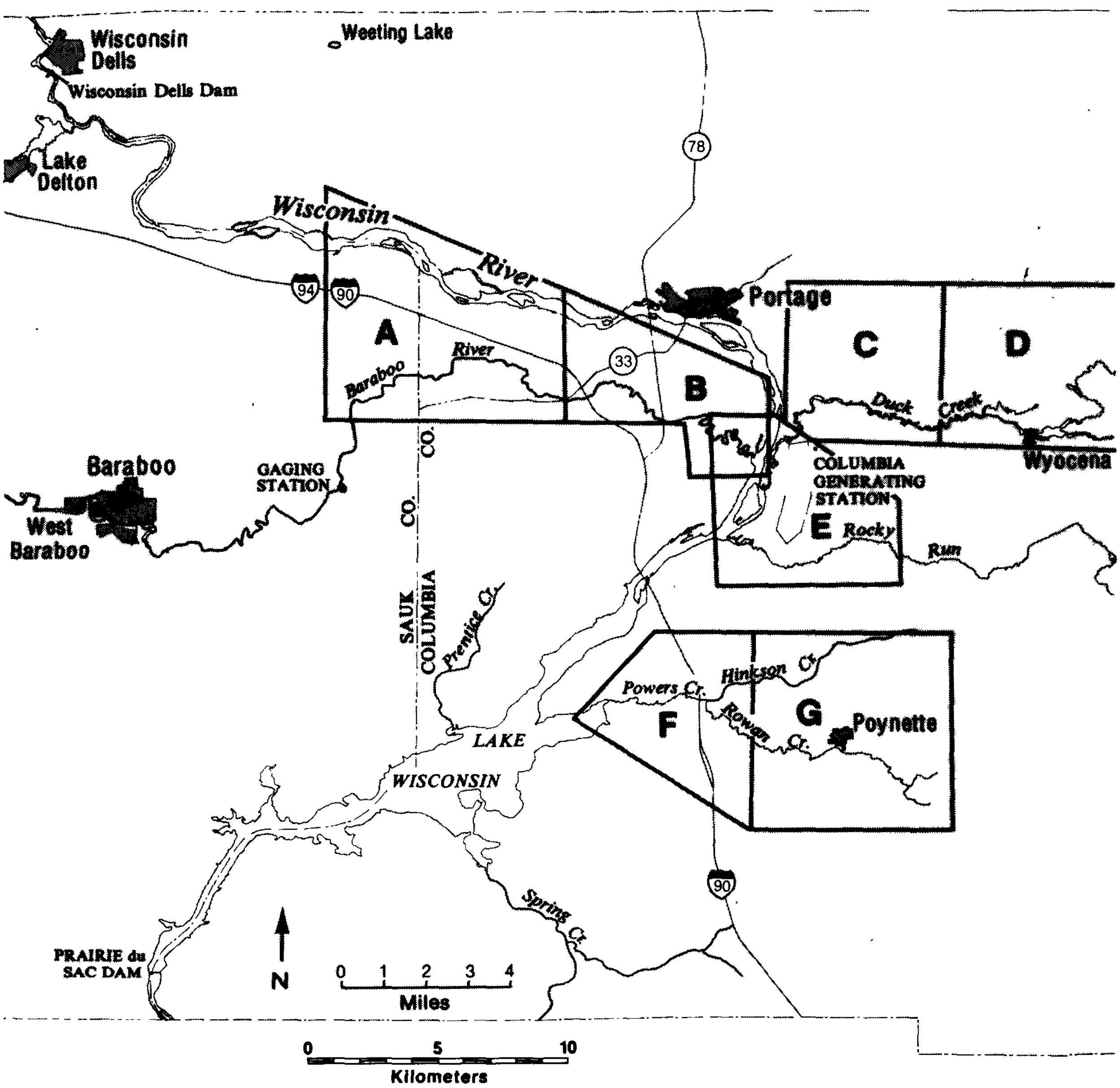


Figure 13. Major areas of potential northern pike spawning habitat in the Wisconsin River and tributaries near the Columbia Generating Station. Figure 14 shows details.

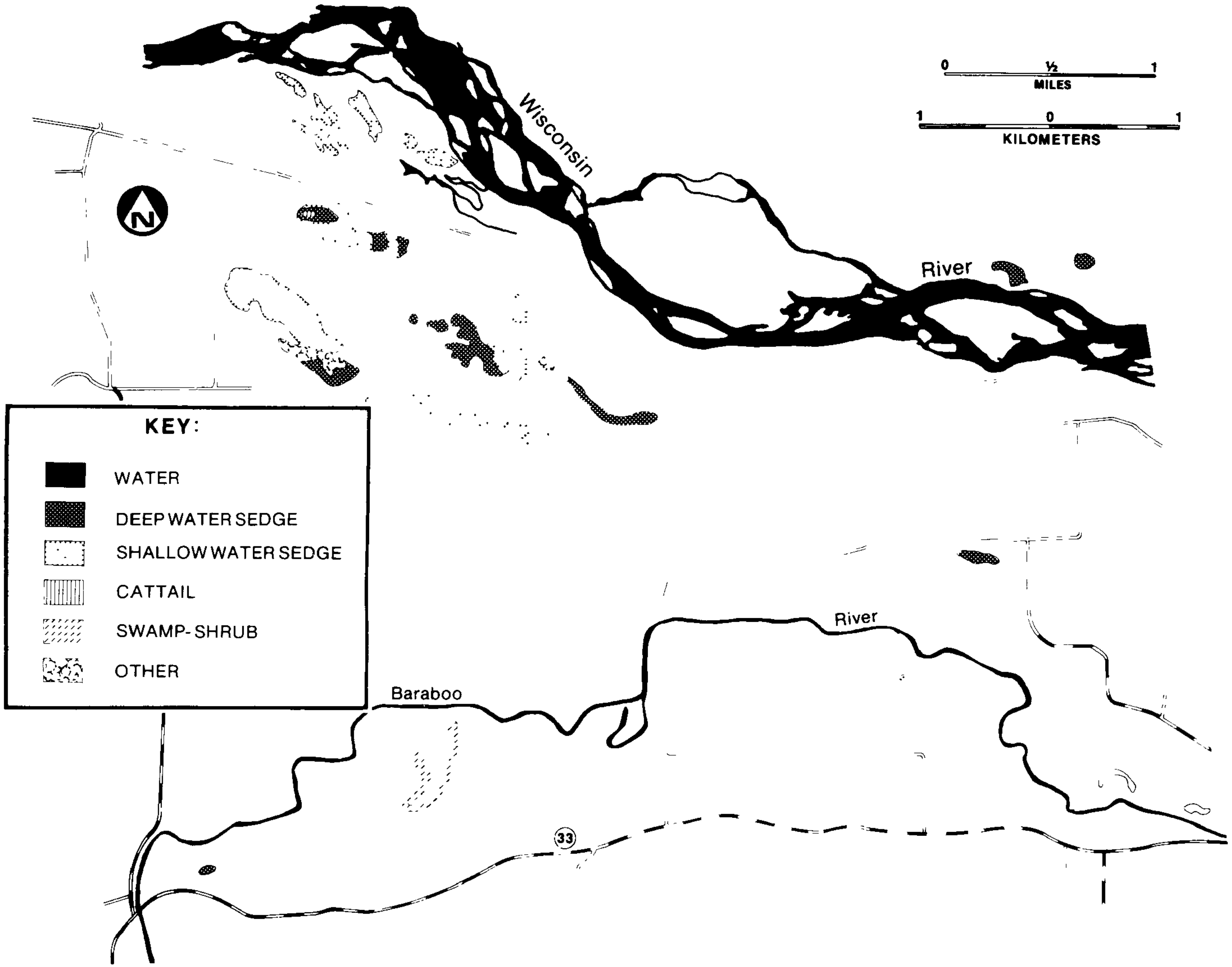


Figure 14a. Detailed map of area A (Figure 13).

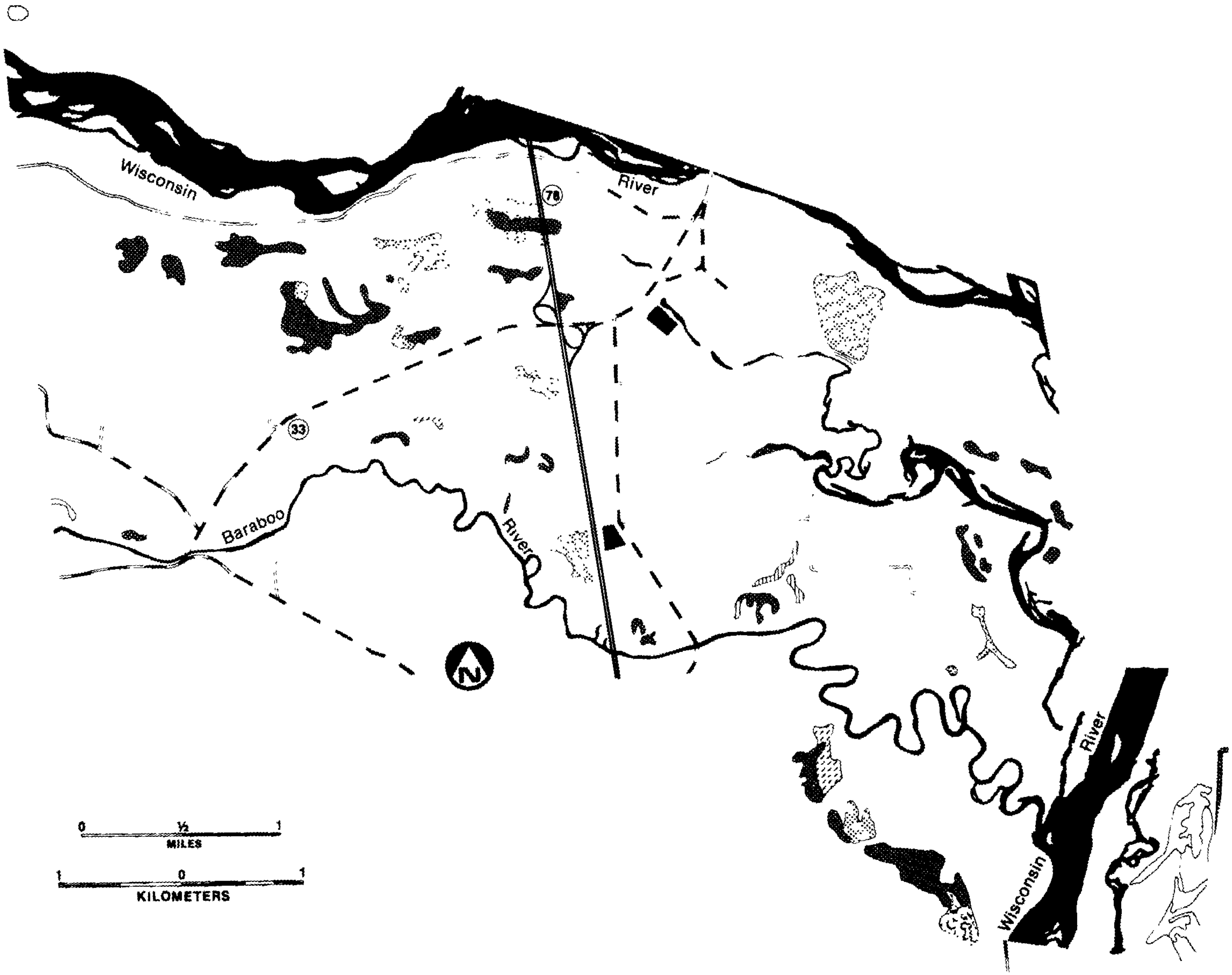


Figure 14b. Detailed map of area B (Figure 13). (See Figure 14a. for key)

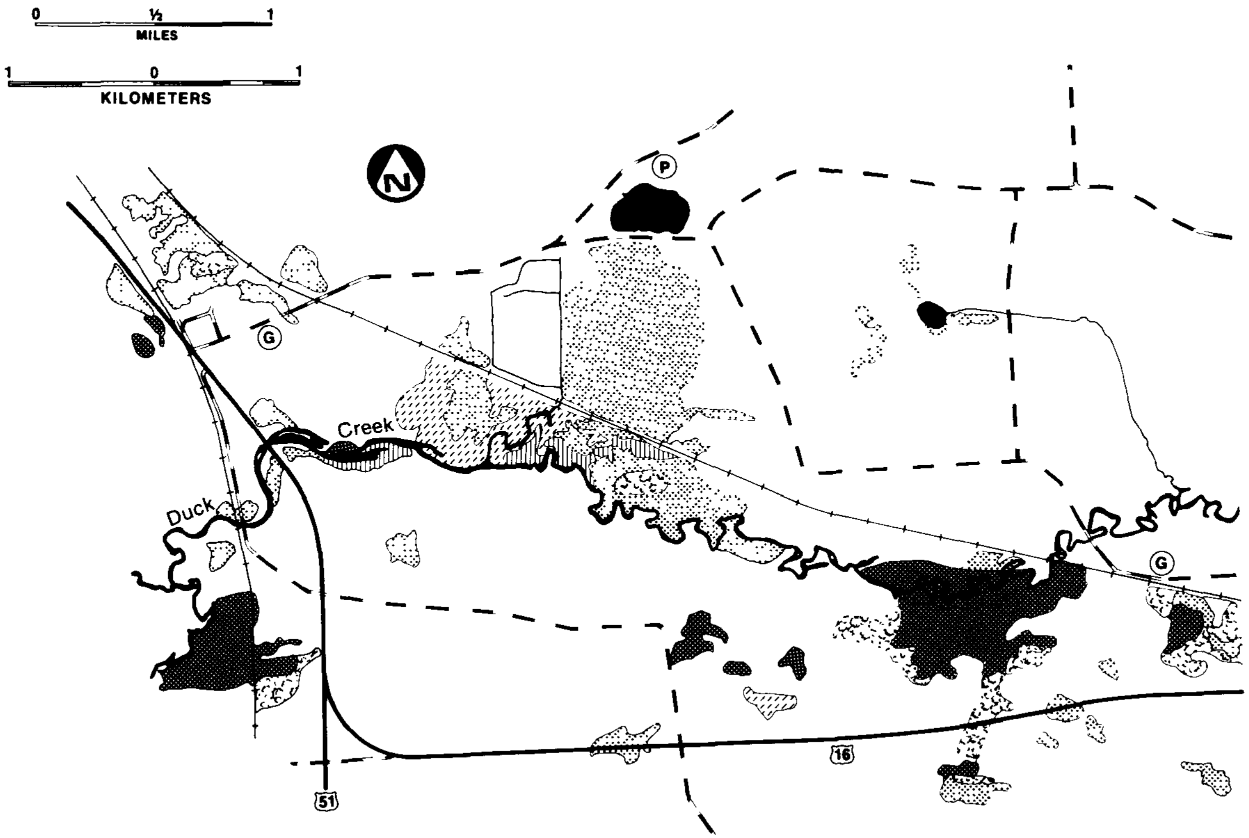


Figure 14c. Detailed map of area C (Figure 13). (See Figure 14a. for key.)

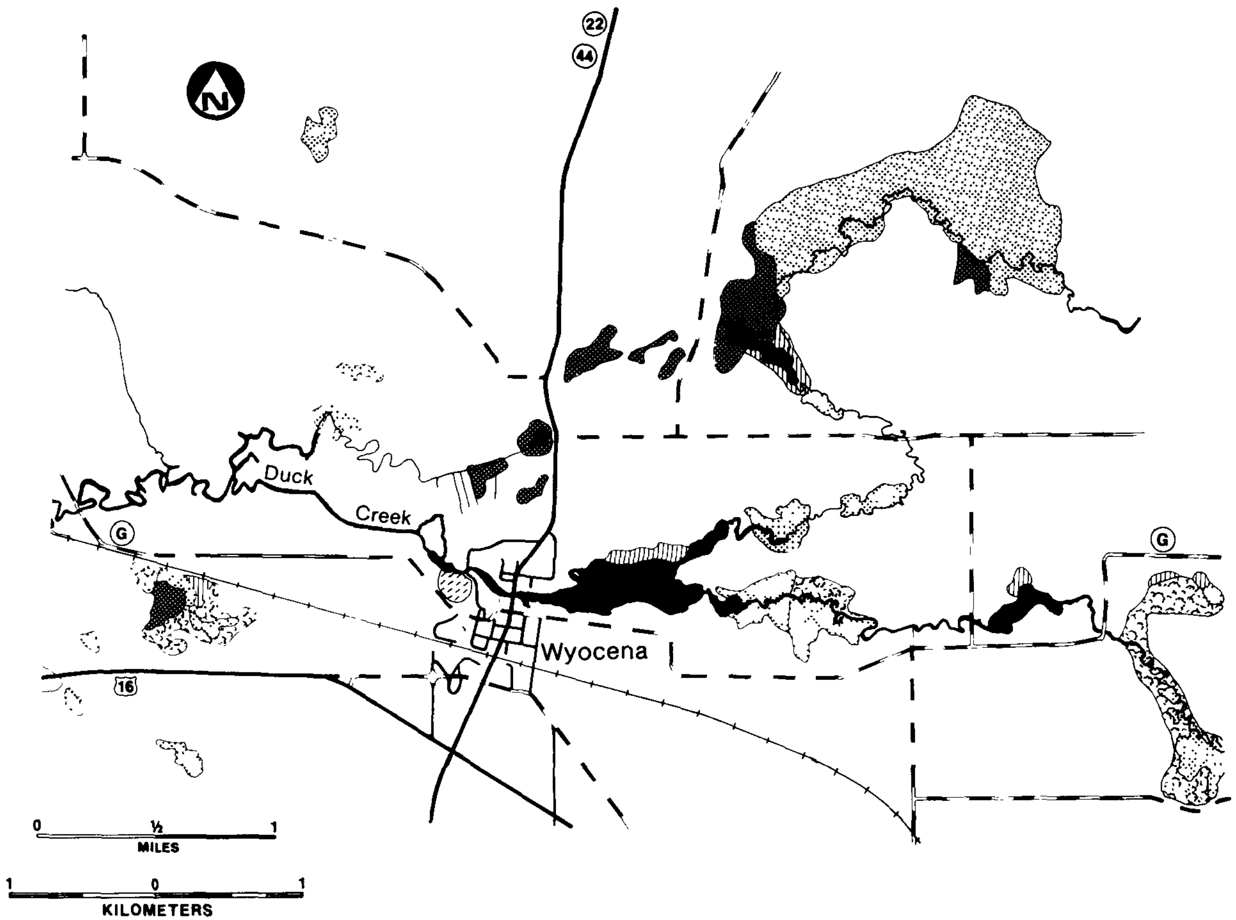


Figure 14d. Detailed map of area D (Figure 13). (See Figure 14a. for key.)

Figure 14e. Detailed map of area E (Figure 13). (See Figure 14a. for key.)

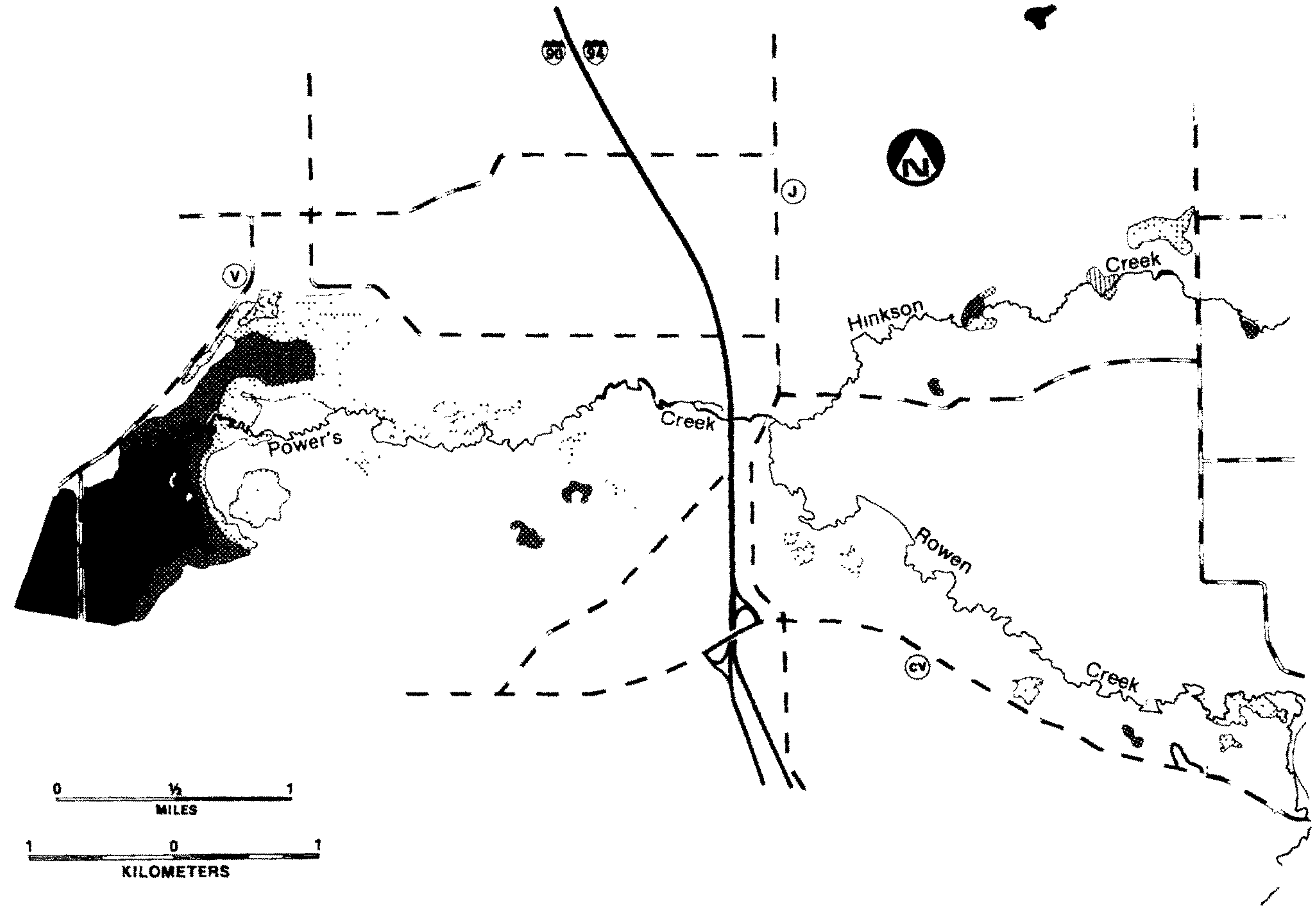


Figure 14f. Detailed map of area F (Figure 13). (See Figure 14a. for key.)

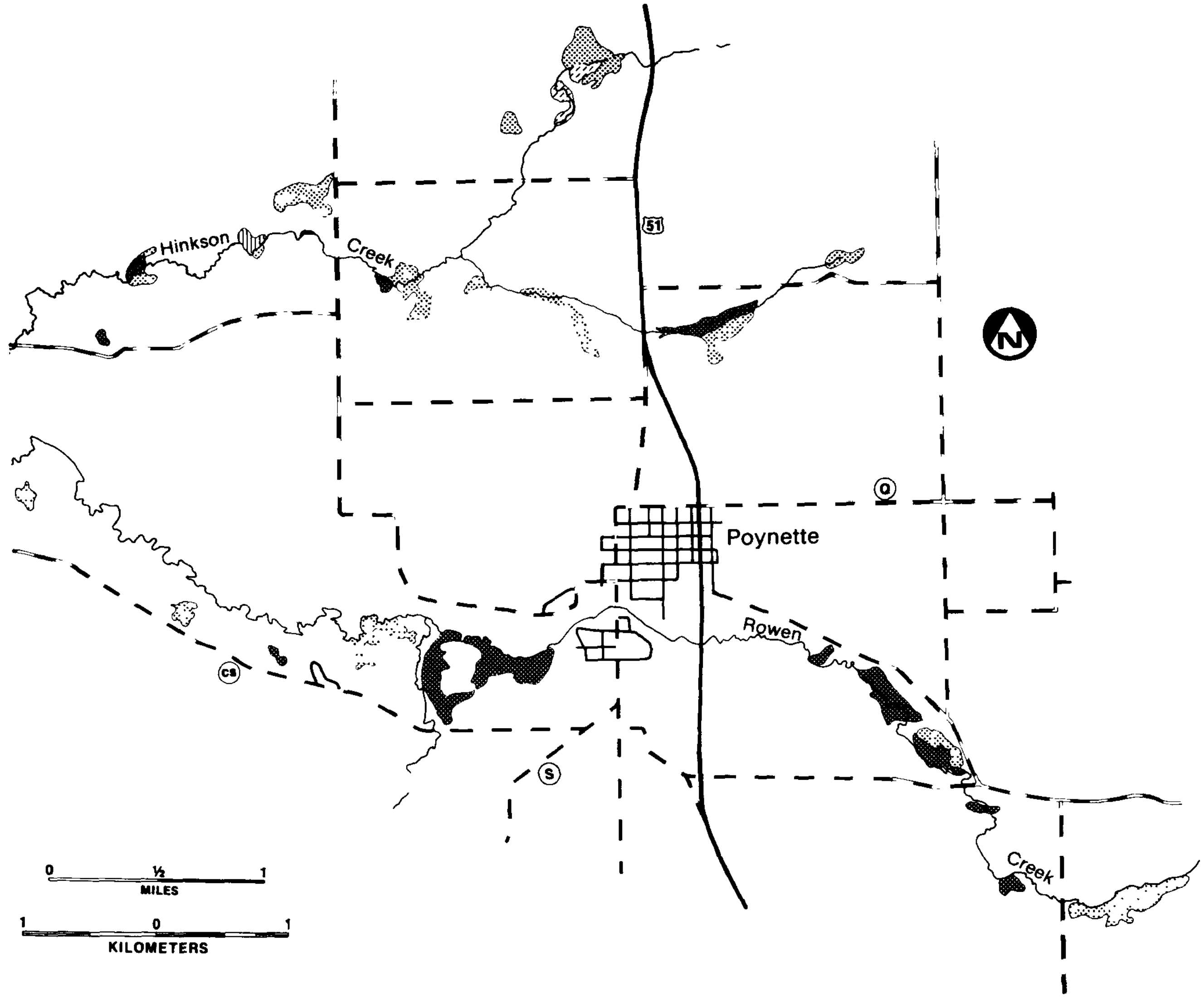


Figure 14g. Detailed map of area G (Figure 13). (See Figure 14a. for key.)

TABLE 7. RELATIONSHIP OF CONSTRUCTION ACTIVITIES TO PIKE YEAR-CLASSES

Date	Event	Effect on year-class
January 1971	First bulldozer, cooling lake, dikes completed by April	Unknown
1973	Ashpit dikes built	None detected
May 1975 to present	Generating station in operation	Possibly reduced year-class for 1976; too early to detect changes in subsequent year-classes

shallow sedge-meadow areas are upper Duck Creek, upper Rocky Run Creek, Powers Creek and its tributaries, and Lodi Marsh.

Although we know from our fyke netting that the Rocky Run A and B areas (described in Appendix B) are not important spawning grounds, they were included in the analysis to avoid biases. On the basis of aerial photography (the criteria used for judging sites where no fish survey data existed) they appeared suitable; therefore, it would have been unfair to exclude them. Information provided by the Wisconsin Department of Natural Resources (J. Chizek, personal communication) indicates that the Lodi marsh is also not used as a pike spawning ground. If these areas are eliminated from the comparison, the station site contains 22.1% of the deep-water sedge meadow and 0.8% of the shallow-water sedge meadow. The relative importance of the generating station wetlands would probably increase even further if fish-survey data for other marshes were available and additional areas were eliminated as spawning grounds.

The largest habitat loss due to construction of the generating station involves replacement of 203 ha of shallow-water sedge meadow with the cooling lake. This represents a loss of 18% of this wetland type formerly occurring in this section of the Wisconsin River. Before destruction of this wetland, the station site contained 28% of the shallow-water sedge meadow likely to be used by spawning northern pike as opposed to the current figure of 0.8%.

DISCUSSION

Our work has documented the use of wetlands on the site of the Columbia Generating Station as spring spawning grounds for several important game fish. The extent of use varies with annual flood levels, but under normal and high water levels fish enter both the sedge meadow adjacent to the

TABLE 8. VEGETATION TYPES FOR VARIOUS WETLANDS LOCATED ALONG THE WISCONSIN RIVER. VALUES GIVEN ARE HECTARES (% OF TOTAL)

Marsh Areas	Deep-water sedge	Shallow-water sedge	Cattail	Swamp-shrub	Canary grass	Grazed sedge	Drained wetlands	Mixed	Floating macrophytes deep water	Bluejoint
Station site (after construction)	100.3 (13.1)	4.4 (0.5)		23.3 (1.6)					17.8 (10.2)	
Duck Creek	124.6 (16.3)	238.2 (26.3)	36.9 (41.1)	55.1 (3.9)			15.1 (9.2)			23.5 (100.0)
Rocky Run A	69.8 (9.1)	41.3 (4.5)	1.3 (1.4)	54.0 (3.8)	1.9 (2.9)	13.2 (100.0)	15.0 (9.1)			
Rocky Run B	179.5 (23.4)	112.1 (12.4)	17.8 (19.8)	144.2 (10.2)	26.2 (38.7)		126.8 (77.2)	26.9 (26.2)	141.5 (81.0)	
Corning-Weeting Lakes	18.6 (2.5)	44.9 (5.0)	31.5 (35.1)	1092.0 (77.1)					11.8 (6.8)	
Powers Creek	54.7 (7.1)	80.9 (8.9)		3.2 (0.2)						
Hinkson-Rowan Creek	41.6 (5.4)	71.2 (7.9)	2.2 (2.5)		4.9 (7.2)					
Lodi Marsh	63.8 (8.3)	222.4 (24.5)		29.5 (2.1)				52.0 (53.6)		
Okee Bay	42.7 (5.6)	11.5 (1.3)								
South Dekorra	21.8 (2.8)	4.9 (0.5)		5.7 (0.4)				22.5 (21.8)	3.5 (2.0)	
Merrimac Inlet	17.5 (2.3)	57.8 (6.4)								
Prentice Creek	7.1 (0.9)	12.6 (1.4)			14.5 (21.4)					
Baraboo River Mouth	24.3 (3.2)	4.1 (0.4)		9.8 (0.7)	20.2 (29.8)		7.4 (4.5)			
Totals	766.3	906.3	89.7	1,417	67.7	13.2	164.3	103.0	174.6	23.5

cooling lake and the ashpit drain. Both of these areas have changed because of the construction and operation of the Columbia Generating Station.

Wetland Water-Level and Vegetation Change

Much of the original sedge meadow at the site was replaced with a cooling lake, and the remaining meadow is changing from shallow to deep-water marsh (Bedford 1977). This transition is the result of a fourfold increase in ground-water discharge rates since the cooling lake was filled in January 1975. Prior to that time, water levels rose during spring floods, decreased frequently in summer to below the soil surface, and rose again in autumn. Water now stands consistently above the soil surface at depths of from 1 to 60 cm. The resulting vegetation change from a community of perennial sedges to one dominated by annual forbs and emergent aquatic species has serious implications for fish reproduction. The fall dieback of sedges leaves much densely matted intact vegetation that provides excellent spawning substrate during spring floods. By contrast, the autumn die-off of annuals and emergent aquatics provides no such vegetation because stalks decompose and are dissipated before spring floods. Because these vegetational changes began in 1975 and accelerated through 1976-77, negative effects on fish reproduction would not have been evident until the 1976 and later year-classes. Since these year-classes are just reaching spawning age, monitoring of adult spawners should be continued.

Effect of Ashpit Effluent on Reproductive Success

Northern pike utilize, and may even be attracted to, the ashpit drain area because of higher water temperatures. This is of interest because the drain contains elevated levels of various trace elements and possibly trace organics. McKim (1977) reviewed 56 life-cycle toxicity tests involving a variety of organic and inorganic chemicals and concluded that the embryo-larval and early juvenile life stages of fish are the most sensitive to toxicants. These are the stages during which the northern pike studied at Columbia have the greatest exposure to the ashpit effluent. The embryo period generally lasts about 2 weeks (Franklin and Smith 1963) and, since juvenile pike do not begin emigrating from nursery areas until 10-24 days after hatching, these young fish are exposed to any chemicals in the ashpit for a minimum of 4-6 weeks. We have observed that some pike may even spend their entire first year in the Rocky Run area. In addition to exposure through the water, young pike may also accumulate toxicants via the food chain. Preferred food items follow a sequence of microcrustacea, insects, and vertebrates (chiefly other pike and tadpoles) with increases in fish size (Hunt and Carbine 1951). These food items accumulate certain trace elements, notably barium and chromuim (Schoenfield 1978) from the ashpit. They may also contain harmful trace organic compounds.

Considering the elevated trace-element levels in ashpit drain water and invertebrates (Magnuson et al. 1980), it is surprising that pike fry hatched there did not show higher element concentrations than pike from unaffected areas. The only elevated element, sodium, is of little toxicological importance. The reasons for the higher iron concentrations in upstream Rocky Run fry are unknown. The small sample biomass and inherent analytical errors cause differences between incubation sites for the other elements to be insignificant.

The lack of apparent biological accumulation of toxic trace elements in pike fry hatched in the ashpit drain does not imply that the ashpit effluent had no negative long-term effects. Because few pike eggs survived to hatching, our sample sizes were small. Also, the 10-day exposure period in the marsh constitutes only a small portion of the 4-6 weeks that the fry minimally spend in nursery areas before emigration. Furthermore, our fry were still in the yolk sac stage when frozen for analysis; hence, they had not yet begun feeding and no uptake via the food had occurred.

Crayfish caged in this same ashpit-drain system accumulated chromium, zinc, selenium, and iron over a 2-month period (Harrell 1978, Magnuson et al. 1980). Trace-element accumulation was also shown for a variety of invertebrates living in the drain (Helmke et al. Unpublished). These studies indicate that long-term exposure probably does result in biological accumulation of trace substances from the ashpit effluent. Yet the survival of organisms and the 1976 catch of pike fry in the ashpit drain indicate that ashpit water is not acutely toxic. Any long-term negative effects from the trace-element contaminants or flocculent precipitate entering the Rocky Run slough will only be evident by monitoring the northern pike population structure during the next several years. Weak or missing year-classes that cannot be attributed to climatic factors will be evidence for such negative effects.

Results for 1976--

Despite the changes in these wetlands, pike continued to use them for reproduction. Successful reproduction in the affected areas was documented in 1976 by the capture of pike fry in both the ashpit drain and the outflow channel from the sedge meadow adjacent to the cooling lake. However, the chemical and physical changes in wetlands were, in many cases, just beginning by 1976, 1 year after the plant began operation. Therefore, successful reproduction in 1976 is no guarantee that reproduction will remain unaffected in future years.

Results for 1977 and 1978--

Despite intensive sampling efforts in 1977, no fry were caught anywhere in the Rocky Run slough area. In 1977, spring water levels were very low and resulted in limited amounts of good spawning habitat. Although some 1977 fry were caught as yearlings in 1978 (Figure 11), evidence of a weak 1977 year-class may appear in future fyke-netting efforts. Sampling with light traps, highly efficient at collecting fish larvae in other areas, failed to catch any pike fry in 1978, despite high spring water levels. Whether these results represent poor hatching success or simply inadequate sampling gear is unknown. Since documentation of the natural hatch proved unfeasible in the extensive Rocky Run slough area, we utilized two other methods for assessing reproductive success: Egg and fry survival bioassays and an analysis of year-class strengths.

Egg and Survival Bioassays

Our attempts to study hatching success by *in situ* bioassays were complicated by factors not directly related to the ashpit effluent. Our hatching rate was less than 5% at all sites; similar work has shown success rates to average 60-70% for Lake Malar, Sweden, and 74% for Lake George, Minn. (Franklin and Smith 1963). We feel that our poor hatch was the result of excessive siltation in the incubation bottles and probably was anomalous. Our results for larval survival were better, but again excessive siltation was a problem. Since accumulation of natural sediments was least in the ashpit drain, the greater survival of eggs and fry there may be attributed to this factor.

Results from the laboratory bioassay indicated that unfiltered ashpit water was toxic to pike eggs, but only during the developmental period following gastrulation. Survivorship was not monitored during the early juvenile period, but based on literature reports, we expect this to also be a sensitive life stage. When ashpit water was filtered, the milky white suspended floc was removed. Since filtering eliminated the toxicity of ashpit water to pike eggs, the implication is that this floc is harmful to egg survival. We aerated the eggs to minimize settling out of any suspended materials. Since such settling out might be greater under some field conditions and since we have observed extensive areas on and near the site where the marsh bottom is covered by the floc, detrimental effects on egg survival are likely.

Analysis of Year-Class Strength

Analysis of year-class strengths revealed no negative effects due to the construction and early operation of the generating station. However, it is too early to assess the eventual effects of the station on pike year-class strengths. Although the station began operating in the spring of 1975, physical and chemical changes in the area's wetlands were just beginning to become obvious in 1976 and 1977. With the operation of an additional power generating unit in 1978, further effects may be expected. Our fyke-netting efforts have provided some background data from which further changes in pike populations might be detected. Since it will be several years at least until the chemical and physical alterations of the marsh result in the establishment of a new equilibrium, monitoring of adult year-class strengths will be an important method of assessing reproductive success of the game fishes.

Success of Tagging

The failure to recapture any fish tagged in previous spawning runs could indicate that fyke nets are inefficient during extreme flood periods or that northern pike do not necessarily return to the same spawning grounds each year. During peak flood periods, water levels were occasionally 1 m over the nets. Hence, fish could move upstream over the net or could use alternate, temporary channels. This hypothesis is supported by the capture of several pike in waters upstream of net 2 (entrance to Rocky Run slough) that had not been captured in that net.

To improve the capture of adult spawners, we suggest that after the upstream spawning run is completed, nets be turned around to catch fish leaving the marsh after spawning. Since water levels are usually lower then, net efficiency should be enhanced. This procedure would also allow an estimate of the adult spawning population by the Petersen tag and recapture method (Priegel and Krohn 1975).

The other explanation for the lack of recapture involves the issue of whether or not northern pike home to certain grounds. Such behavior is well documented in salmon and trout (Hasler et al. 1978) and has been suggested for walleye. Studies on homing in northern pike are scarce, but they do not suggest such a tendency (Franklin and Smith 1963). If homing does occur, however, then loss of spawning habitat on the station site could endanger a genetically distinct pike population and contribute to the genetic impoverishment of the species in this portion of its geographic range. Sampling of alternate spawning marshes for fish previously tagged on the Rocky Run slough area should indicate if pike use alternate spawning areas or return to the same areas yearly.

The three tags returned by fishermen supported our belief that pike spend the majority of the year in the Wisconsin River and particularly in Lake Wisconsin. Therefore, effects from the power plant are likely to be important for only the embryo and early juvenile life stages, which are also the most sensitive to chemical toxicants. This finding demonstrates one of the many roles flood plains play in the functioning of river ecosystems and how far-reaching the effects of flood-plain disturbance can be.

Inventory of Spawning Areas

The inventory of potential spawning areas by infrared aerial photography shows that many alternate sites exist in this portion of the Wisconsin River. Strict inventory by photo-interpretation can be misleading, however, since our data show that fish do not utilize the upper stretches of Rocky Run Creek, even though considerable sedge meadow is available there. Inclusion of such areas in the overall estimate of spawning habitat would underestimate the importance of smaller, but more productive, wetlands. Nevertheless, areas currently affected by the Columbia Generating Station do not constitute the major portion of the wetland areas remaining in this section of the Wisconsin River. Wetlands on the plant site are known to attract large numbers of spawning fish, however, and therefore their importance to the Wisconsin River fishery should not be overlooked. Future degradation of the area wetlands should be minimized or else the loss of further documented spawning marsh is likely.

Final Considerations

We feel that our study has successfully documented the use of areas affected by the power plant as pike spawning grounds. *In situ* egg hatching bioassays were inconclusive, but our laboratory bioassays indicate that ashpit-drain water is toxic to certain developmental stages of pike embryos. One of the major considerations in determining the importance of this area to the Wisconsin River fishery is whether unique homing fish stocks are involved. In addition to northern pike, muskellunge, and walleye, other spring spawning fish, such as catostomids, are affected. If homing is important, then loss of habitat involves loss of these stocks. If fish simply search for the best suitable habitat, then loss of wetlands on Rocky Run Creek might be compensated for by a switch-over to alternate spawning sites, but this could also represent a reduced area for young-of-the-year production.

Finally, the year-classes of 1976 and later should be studied carefully because they will probably reflect the long-term effect of the plant. A series of weak year-classes that cannot be correlated with low water levels or extreme water-temperature changes, and which are not evident in spawning populations at other sites, would be a strong indication of reproductive failure due to operation of the Columbia Generating Station.

SECTION 4

ZINC TOLERANCE IN FOUR GENERATIONS OF FLAGFISH

INTRODUCTION

In Section 2 we observed that, despite the changes in wetland water levels and substrate, northern pike and other game fish continued to use the affected marsh area at the Columbia site for spawning. In this section we will examine a question that may arise at any wetland site affected by the construction and operation of a generating station: Can fish populations adapt genetically to increased trace-element levels in the environment by evolving tolerance? Tolerance is defined as the relative capacity of an organism to grow or thrive when subjected to a normally unfavorable environmental factor. Chronic exposure to toxicants may favor those individuals with a genetic make-up that confers resistance. Through the process of natural selection, the tolerant members of the population survive and transmit the trait of tolerance to their offspring.

LITERATURE REVIEW OF METAL TOLERANCE

Contaminants can cause selective pressures that result in tolerant populations as shown by the many documented cases of pesticide-resistant insects (Crow 1957). The number of insect species and the types of chemicals involved are numerous, but two general observations are noteworthy: (1) the evolution of tolerance is rapid and (2) the mechanisms of resistance are diverse. Among the mechanisms identified are the development of behavior patterns that lessen exposure to the poison, decreased uptake through reduced permeability of the cuticle, and enzymatic detoxification.

The widespread and often indiscriminate use of chemical poisons has subjected many other types of organisms to similar selective pressures. Studies have found pesticide-resistant populations of fish (Vinson et al. 1963), crayfish (Albaugh 1972), frogs (Ferguson and Gilbert 1967), and mice (Webb and Horsthall 1967). In many cases resistance may be an acquired trait through inducible detoxifying enzymes (Webb and Horsthall 1967, Brown 1976), but it can also be inherent in certain organisms regardless of prior pesticide exposure (Crow 1957, Ferguson 1967).

In addition to synthetic organic chemicals, tolerance to trace-element contaminants also has been documented. Studies of both terrestrial plants (Antonovics et al. 1971) and aquatic plants (Stokes et al. 1973, McLean and Jones 1975) show again that the evolution of tolerance is rapid, involves diverse mechanisms, and can occur in response to a variety of trace elements.

Tolerance to metals has been demonstrated in recent studies of invertebrates from contaminated aquatic habitats. Bryan (1976) found the marine polychaete *Nereis diversicolor* to have zinc, copper, silver, and possibly lead-tolerant populations. Tolerance was limited generally to the metals in a particular habitat. Laboratory experiments indicated the mechanism was based on reduced permeability to metal ions. Whether tolerance was an acquired (inducible) or an inherited trait was not determined. B. Brown (1976) found copper and lead-resistant populations of *Asellus meridianus* Rac. in rivers with a history of metal pollution from abandoned mines. Both acute lethal bioassays and chronic growth studies demonstrated tolerance. The persistence of tolerance in second generation organisms from a laboratory culture indicated a genetic basis for this trait.

Despite such demonstrations of the evolution of metal tolerance in aquatic plants and invertebrates, no examples of naturally metal-tolerant fish populations have been reported in the literature. Although waters with high metal contamination frequently contain plants and invertebrates, fish are conspicuously absent (Carpenter 1924, Jones 1958, Weatherly et al. 1967). In waters with slightly elevated metal levels, fish populations exist but reproduction is severely depressed and their long-term survival is uncertain (Van Loon and Beamish 1977, McFarlane and Franzin 1978).

OBJECTIVE OF THIS STUDY

This study tested the hypothesis that if genetic factors partially determine the variation in susceptibility to metal toxicants, the resistance of a fish population can be increased through selection. The ability of fish to develop tolerance is of interest since trace elements are often released by coal-fired power plants at levels that may have severe long-term effects on fish populations even though they are not acutely lethal (Cherry and Guthrie 1977). The approach used was to breed the flagfish (*Jordanella floridae*), a species well suited for extended laboratory studies (Smith 1973), for resistance to the long-term effects of the element zinc.

METHODS

Zinc as an Experimental Toxicant

Zinc was chosen as the toxicant for this experiment because it is a common pollutant whose biological effects are well documented (Skidmore 1964, European Inland Fisheries Advisory Commission 1974). Use of zinc and flagfish provided a convenient laboratory model for the selection processes that would affect other fish species exposed to other trace-element contaminants. Fish exposed to zinc may die from either acute or long-term effects. Acute mortality, the result of extensive gill damage, occurs within the first 4 days of exposure to high zinc concentrations. Death from long-term effects occurs at lower concentrations and involves damage to internal organs. To determine the level of zinc resulting in chronic mortality, groups of flagfish were exposed to various concentrations of the element (Figure 15). A concentration of 0.8 mg/liter was used for the parental generation, but this was increased to 1.0 mg/liter for successive generations to accelerate mortality and increase the selective pressures affecting the population.

To begin the experiment, a stock supply of flagfish was randomly divided into two groups. One group (the selected population) underwent three generations of selection for zinc resistance while the other group (the unselected population) was maintained under the same laboratory conditions but received no zinc exposure (Figure 16). Selection was accomplished by exposing adult fish to a chronically lethal zinc concentration until 50 to 60% of the population had died. To determine if selection was increasing zinc resistance, a subsample of each generation of the unselected population was exposed to zinc along with the selected population and survival times were compared between the two groups.

Biological Procedures

The original stock of flagfish was purchased from a commercial supplier (Ross Socoloff Farms, Bradenton, Fla.) and maintained in tap water in Madison, Wis.

A constant photoperiod of 16:8 h (light:dark) and constant temperature of 25°C were used throughout the experiment. Fish ate frozen brine shrimp supplemented with commercial fish food (Tetra Conditioning Food, Tetra Werke Co.). Because of the hardness of Madison tap water (Table 9), zinc exposures were at a dilution ratio of 1:3 (tap to distilled water). Fish were acclimated to the dilution water for 1 week before exposure to zinc. During the bioassay deaths were recorded at 12-h intervals; death was defined as the failure to respond to a mechanical stimulus.

Breeding proceeded according to methods outlined by Spehar (1976). Breeding tanks were 55-liter aquaria divided into thirds by plexiglass partitions. Eggs, deposited on orlon yarn spawning substrates, were collected daily. Eggs hatched in egg cups made of PVC piping cut to 6-cm lengths and covered with plastic screen at one end. Larvae ate newly hatched brine shrimp nauplii until old enough to take frozen adult brine shrimp. Generation time was approximately 6 months.

Bioassay Procedure

The flow-through bioassays were conducted along guidelines issued by the U.S. Environmental Protection Agency (1975). The characteristics of the tap and dilution water are given in Table 9. Zinc concentrations, conductivity, and temperature were monitored daily; hardness, total alkalinity, pH, and dissolved oxygen were measured weekly. All measurements were taken according to methods outlined by American Public Health Association et al. (1975). Zinc samples, some unfiltered and some filtered through 0.4 μm nucleopore filters, were measured by atomic absorption spectrophotometry. Filtering allows an estimate of how much zinc is actually in solution and hence readily available for uptake by fish (U.S. Environmental Protection Agency 1975). Nominal (added) zinc concentrations,

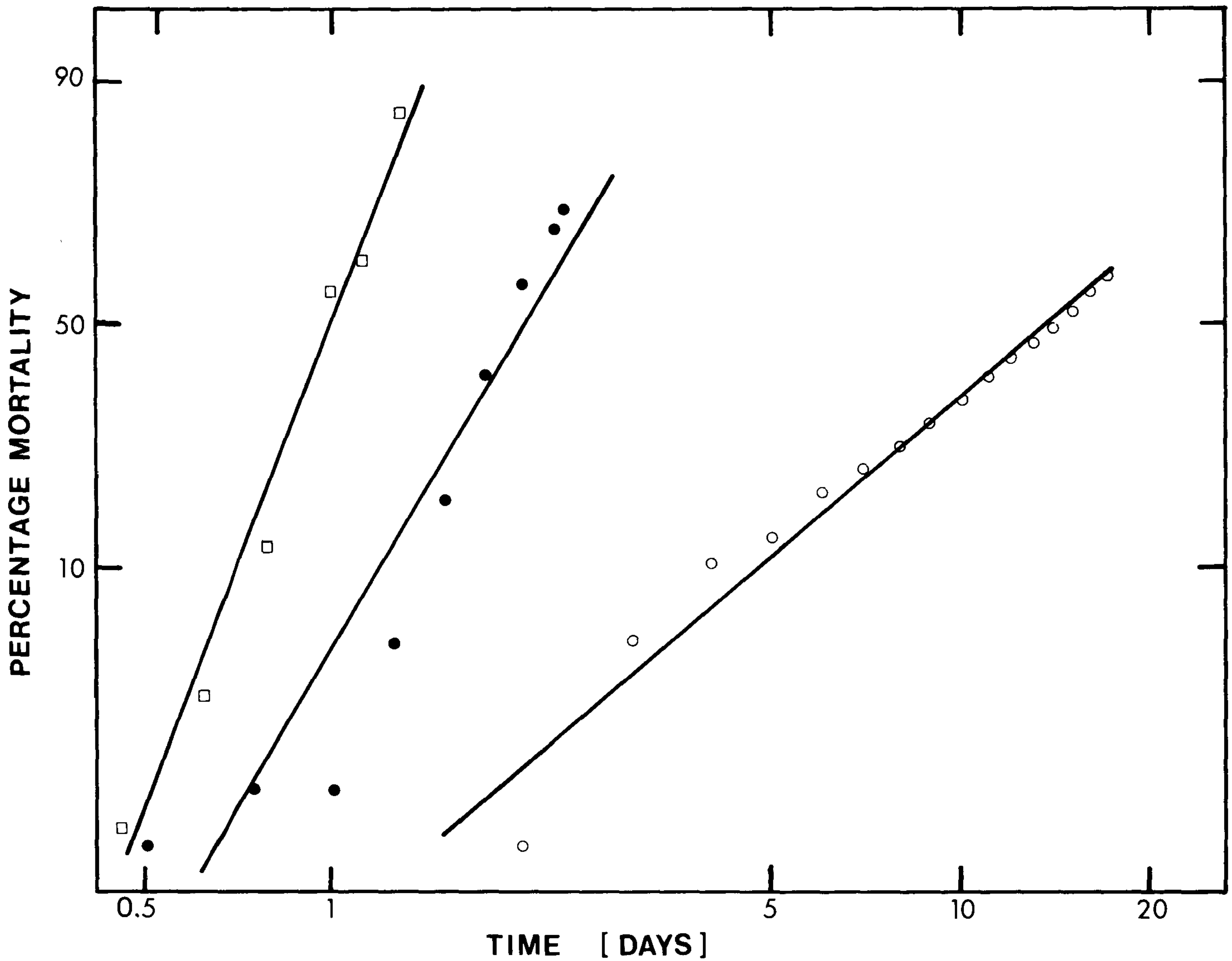


Figure 15. Cumulative mortality (probit scale) as a function of exposure time for flagfish exposed to three zinc concentrations.

□ 5 mg/liter ● 2.5 mg/liter ○ 0.8 mg/liter

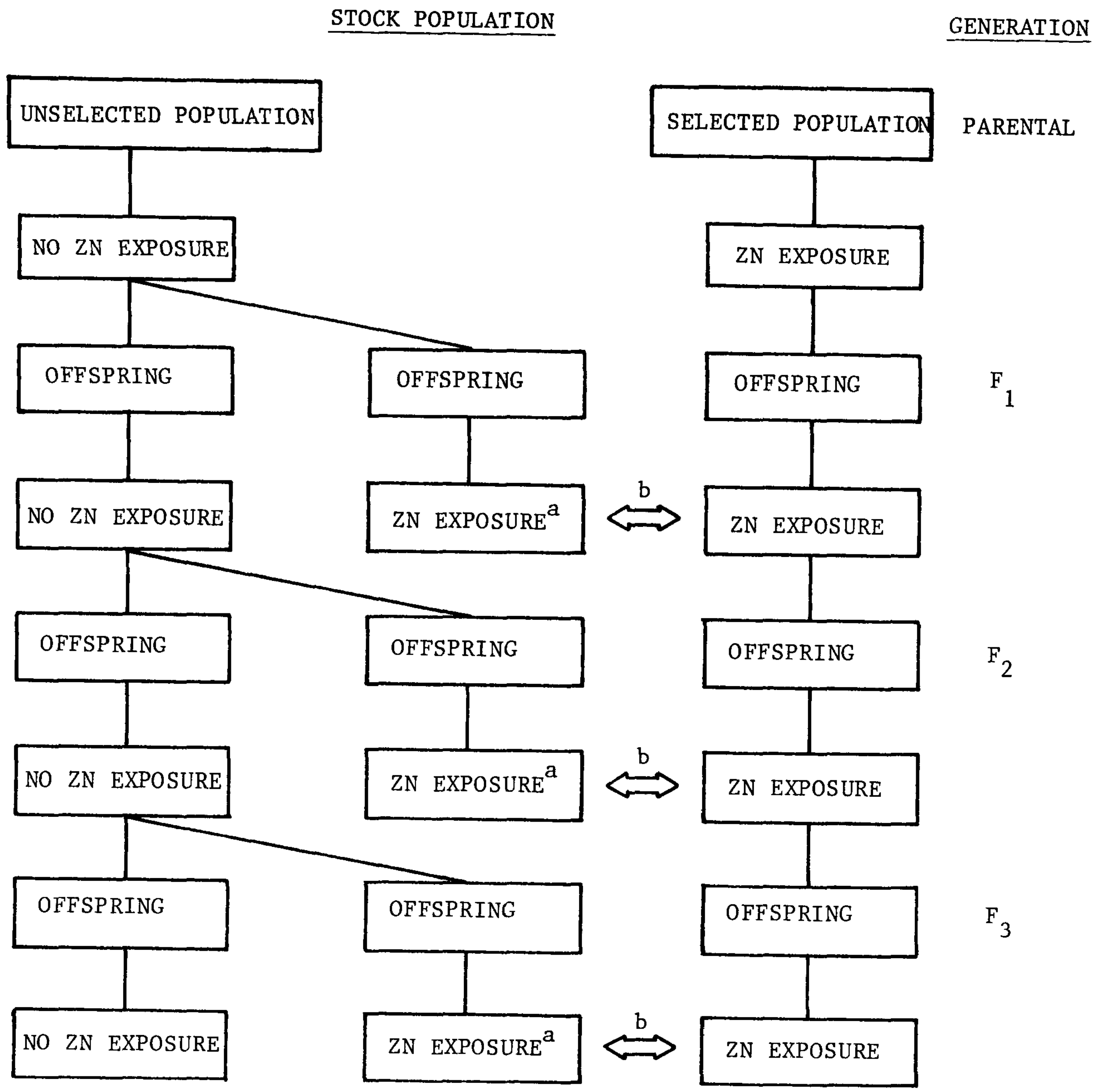


Figure 16. Procedure used in selecting for zinc resistance in laboratory populations of flagfish. An initial stock population was randomly divided into two groups; one underwent three generations of selection for zinc resistance while the other remained as a control population.

[a]Survivors bred to obtain data on spawning success but larvae were then discarded.
[b]Comparisons of survival times between these groups were used to assess effects of selection.

length of exposure, and recovery time before breeding for each generation are shown in Table 10.

TABLE 9. CHEMICAL CHARACTERISTICS OF MADISON, WIS., TAP WATER IN WHICH FLAGFISH WERE RAISED, AND DILUTION WATER IN WHICH ZINC EXPOSURES WERE CONDUCTED

Item	Tap water	Dilution water (weekly samples, all generations)
Hardness (mg/liter $CaCO_3$)	280[a] (235-296)	73 (61-88)
Total alkalinity (mg/liter $CaCO_3$)	b	59 (44-67)
Conductivity (μmhos/cm at 25°C)	590 (510-640)	140 (120-180)
pH	7.4 (7.2-7.8)	7.3 (6.7-7.9)
Dissolved oxygen (mg/liter)	b	7.6 (6.7-8.1)
Zinc (μg/liter)	20 (10-50)	23 (2-45)

[a]First entry in each cell is the mean and the second is the range.
[b]Not measured.

TABLE 10. NOMINAL ZINC CONCENTRATION (ppm), LENGTH OF EXPOSURE (DAYS), AND RECOVERY TIME BEFORE BREEDING (WEEKS), FOR THE ZINC EXPOSURES OF THE PARENTAL AND THREE GENERATIONS OF FLAGFISH

Generation	Nominal zinc concentration (ppm)	Length of exposure (days)	Selection intensity (% dead for selected population)	Recovery time before breeding (weeks)
Parental	0.8	17	60.0	8
First	1.0	17	53.7	3
Second	1.0	6.5	49.3	2
Third	1.0	13	55.4	1

RESULTS--EXPOSURES AND CALCULATIONS

Parental Generation

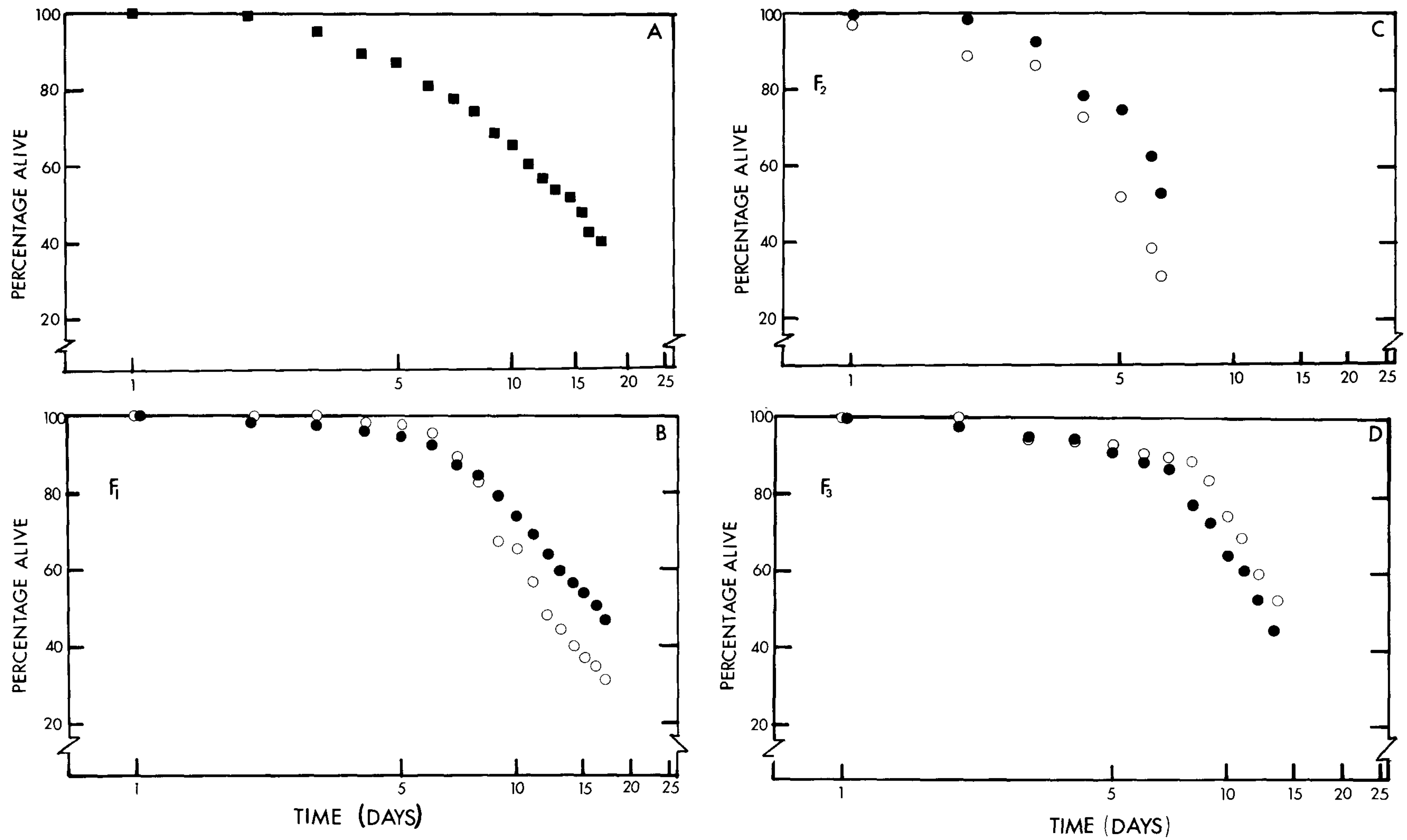
Parental generation flagfish of the selected population were exposed to 0.8 mg/liter zinc for a 17-day period until 60% had died (Figure 17A). Unselected population fish were not exposed to zinc. Approximately 75% of the zinc (Table 11) was in a soluble form considered most toxic to fish (European Inland Fisheries Advisory Commission 1974).

TABLE 11. ZINC CONCENTRATIONS FOR THE PARENTAL, FIRST, SECOND, AND THIRD GENERATION EXPOSURES

Generation	Zn-exposed fish (μg/liter)		Control fish (μg/liter)	
	Filtered	Unfiltered	Filtered	Unfiltered
Parental	575±100[a] (18)	811±48 (7)	25±1 (2)	40±16 (2)
First	750±65 (40)	1,000±140 (11)	22±12 (12)	28±16 (6)
Second	1,122±97 (8)	1,348 (1)	30±20 (2)	40±10 (2)
Third	850±76 (18)	915±9 (2)	< 50 (3)	< 50 (1)

[a]First entry in each cell is the mean ± standard deviation for water samples taken during the course of the zinc exposure; the second is the number of samples.

Survival times of fish exposed to zinc may be correlated positively with fish size (Bengtsson 1974). Therefore, standard lengths of survivors and nonsurvivors of the initial exposure were compared to determine if large size and not an inherent resistance to zinc could explain survival (Table 12). The comparison was based on a two-sample t-test with unequal variances. Males that died and those that survived did not differ in length ($t = 1.12$, d.f. = 85, $p = 0.27$) (Table 12). Females, however, were longer than nonsurviving females ($t = 2.38$, d.f. = 1.34, $p = 0.02$), but the length difference was small. The 95% confidence limits for the length difference shows that surviving females were only 0.3-3.4 mm (0.8-9.9%) longer than nonsurviving females. For fish that died during exposure to zinc, there was no correlation between body length and time to death ($r = -0.06$ for males and $r = -0.11$ for females). Thus, for the size range of fish used,



● Population selected for zinc tolerance.
○ Population not selected for zinc tolerance.

Figure 17. Survival rates of exposed (selected) and control (unselected) flagfish populations over four generations. Survivorship is plotted against elapsed time in days (log scale). A, parental generation; B, first generation of offspring; C, second generation; D, third generation.

selection was not for large size but for some other factor that allowed certain fish to be more resistant to zinc than others.

TABLE 12. STANDARD LENGTHS (MILLIMETERS) OF SURVIVORS AND NONSURVIVORS FOR PARENTAL-GENERATION FLAGFISH EXPOSED TO 0.8 mg/LITER ZINC FOR 17 DAYS

Sex		Nonsurvivors	Survivors
	Mean	36.9	38.1
Male	(S.D.)	(5.3)	(6.7)
	n	106	53
	Mean	34.2	36.0
Female	(S.D.)	(4.2)	(5.4)
	n	83	72

Residual effects on reproduction of a single sustained zinc exposure were determined by comparing spawning data for both experimental and control fish. Since fecundity is affected by fish size, wet weight and standard lengths of fish used for spawning from both experimental and control parental populations were compared with a two-sample t-test with unequal variances (Table 13).

TABLE 13. STANDARD LENGTHS AND WET WEIGHTS OF PARENTAL GENERATION FLAGFISH USED TO PRODUCE THE FIRST GENERATION

Item	Male length(mm)	Female length(mm)	Male wet weight (g)	Female wet weight (g)
Experimental	44[a]	42	3.06	2.43
population	(6)	(4)	(1.13)	(0.58)
(zinc-exposed)	30	30	30	30
Control	47	43	3.48	2.53
population	(6)	(5)	(0.99)	(0.98)
(not zinc-exposed)	27	27	27	27

[a]The first entry of each cell is the mean; the second is standard deviation; and third is number of fish.

No difference in weight or length was found for either males or females ($p > 0.5$). Size differences therefore were not responsible for any fecundity differences. The spawning data, not normally distributed, were analyzed by the Mann-Whitney U test (Siegel 1956). The two populations did not differ in the number of days until the first eggs were laid ($p = 1.00$) or in the percentage hatch of eggs ($p = 0.80$) (Table 14). Experimental fish averaged more eggs per spawn than control fish ($p = 0.03$) with a 95% confidence limit of 0.4 to 13.1 more eggs per spawn. Thus, fish surviving a concentration of zinc lethal to the majority of the population showed no harmful residual effects on reproduction after 8 weeks recovery time.

TABLE 14. SPAWNING DATA FOR PARENTAL GENERATION FLAGFISH[a]

Item	Days until first spawn	Number of eggs/spawn	Percentage hatch
Experimental population (zinc-exposed)	9[b] (5-20) 20	8.2 (0-86.7) 21	74.7 (0-100) 19
Control population (not zinc-exposed)	8 (15-17) 13	7.6 (0-53.6) 15	76.6 (0.4-96.4) 13

[a]Experimental fish survived a 17-day exposure to 0.8 mg/liter zinc. Control fish were not exposed to zinc.
[b]First entry is median; second is range; and third is number of fish pairs.

First Generation

The effect of one generation of selection for zinc resistance was determined by comparing the survival of first-generation fish from both the selected and unselected populations exposed to 1 mg/liter zinc. As in the initial exposure, about 75% of the zinc was in a soluble form (Table 11).

The first deaths from zinc exposure occurred in the first-generation (F_1) selected population (Figure 17B). Once fish began to die in the zinc-exposed unselected population, however, mortality was higher and surpassed the experimental population after 8 days. After 17 days, 68.9% of the unselected population but only 53.7% of the selected population had died. Median survival times were 12 days for the zinc-exposed unselected population and 16.5 days for the selected population. A Mantel-Haenszel test was used to determine the significance of this difference (Snedecor and Cochran 1967). This test computes χ^2 values for the observed deaths during each time period, given the number of fish alive at the start of the period and the null hypothesis that there is no difference in susceptibility to zinc poisoning between the groups. Each time period is treated independently, and the dependency on previous events (as with a parameter such as cumulative mortality) is avoided. Survival times were significantly different ($\chi^2 = 9.9$, $p = 0.001$), indicating that selected population fish were less suceptible to zinc poisoning than unselected population fish.

A comparison of standard lengths of F_1 selected and zinc-exposed control unselected indicated that size differences were not a factor in the increased resistance of the experimental group (t-test, $p = 0.85$).

No significant differences in length or weight for either sex were found that might affect reproduction (Table 15) (t-test, $p > 0.05$). Neither did the groups differ significantly in time to first spawn, average number of eggs per spawn, or the percentage of hatched eggs (Table 16) (Kruskal-Wallis Test, $p > 0.05$, Siegel 1956).

TABLE 15. LENGTHS AND WET WEIGHTS OF FIRST-GENERATION FISH USED FOR SPAWNING

Item	Male length (mm)	Female length (mm)	Male wet weight(g)	Female wet weight(g)
Selected population (zinc-exposed)	34[a] (6) 12	30 (3) 12	1.24 (0.62) 12	0.89 (0.28) 12
Unselected population (zinc-exposed)	37 (6) 8	33 (2) 8	1.53 (0.57) 8	1.12 (0.18) 8
Unselected population (control)	33 (7) 14	32 (4) 14	1.24 (0.81) 14	1.05 (0.37) 14

[a]The first entry is the mean; second is standard deviation; and the third is number of spawning pairs.

Second Generation

The effect of breeding two generations for resistance to zinc was determined by comparing fish survival from both selected and unselected populations when exposed to a lethal zinc level (Figure 17C). Zinc analysis (Table 11) revealed a slightly higher zinc concentration (1.1 mg/liter) than the intended 1.0-mg/liter level.

As in the first generation, second-generation fish from the selected population proved more resistant to zinc poisoning than fish from the unselected population. After zinc exposure of 6.5 days, mortality was 68.5% for the zinc-exposed unselected population, but only 49.3% for the selected population. Median survival times were 5.25 and 6.4 days for the zinc-exposed control and experimental populations, respectively. The difference in survivorship was significant at the $p = 0.004$ level ($x_1^2 = 8.55$, Mantel-Haenszel test).

Selected-population fish were significantly longer ($p = 0.05$) than unselected-population fish with average standard lengths of 23.8 and 25.5 mm, respectively. Considering results from the parental generation, the size difference probably was not a significant source of variability in zinc-tolerance levels between these two populations. The two groups did not

TABLE 16. SUMMARY OF SPAWNING DATA FOR F_1-F_3 GENERATIONS OF FLAGFISH

Generation	Days until first spawn[a]	No. of eggs/spawn[b]	Hatch (%)[a]
F_1			
Unselected pop-control	11 (6-18)[c] 4	0 (0-6.1) 10	89 (2-100) 4
Unselected pop--Zn exposed	7 (5-20) 8	2.2 (0-3.5) 10	90 (50-100) 7
Selected pop--Zn exposed	6 (3-21) 7	3.7 (0-13) 9	81 (4-100) 7
F_2			
Unselected pop-control	4 (4-6) 7	2.4 (0-27.6) 11	84.8 (73.7-100) 7
Selected pop--Zn exposed	7 (4-14) 6	0 (0-6.7) 14	73.7 (0-86.4)
F_3			
Unselected pop-control	33 (23-37) 10	0 (0-12.7) 10	8.7 (83-94) 4
Unselected pop--Zn exposed	25 (18-31) 8	2.0 (0-18.3) 11	99 (89-100) 7
Selected pop--Zn exposed	22 (18-25) 4	0 (0-8.9) 12	92 (71-100) 4

[a]Only spawners producing eggs are included.
[b]Includes all spawners with pairs producing no eggs counted as zero.
[c]The first entry is the median, the second is the range, and the third is number of fish pairs.

differ in the number of days to first spawn ($p = 0.15$), the average number of eggs per spawn ($p = 0.22$), or the percentage of hatched eggs ($p = 0.1$) (Mann-Whitney U Test) (Table 16).

Third Generation

The effect of breeding three generations for resistance to zinc was determined by comparing survival of fish from the experimental and zinc-exposed control populations when exposed to a lethal zinc level (Figure 17D). Zinc water analyses are given in Table 11.

In contrast with the first two generations, mortality was higher in third-generation selected-population fish than in third-generation unselected-population fish. After an exposure period of 13 days, 46.8% of the zinc-exposed unselected population and 55.4% of the selected-population fish had died. Median survival times were 13.5 days for the zinc-exposed unselected population and 12.5 days for the selected population. Despite this decline in tolerance for third-generation experimental fish, the difference in survival between the two groups was not statistically significant ($\chi_1^2 = 1.63$, $p = 0.20$, Mantel-Haenszel test). The two populations did not differ in standard lengths (t-test, $p = 0.59$); hence, size was not a factor in the failure of the experimental population to display superior tolerance.

The only difference in spawning success between selected and unselected population fish exposed to zinc and unselected population not exposed to zinc was that the latter group took longer to produce eggs than the other groups (Kruskal-Wallis Test, $\chi_1^2 = 11.1$, $p = 0.05$) (Table 16).

The results of breeding three generations of flagfish for resistance to zinc are summarized in Figure 18. The absence of a trend toward increased tolerance with continued selection suggests that two confounding factors may be involved, inbreeding depression and carry-over effects. Both phenomena are discussed below.

DISCUSSION

Variability in Zinc Tolerance

These experiments demonstrate considerable variation in the tolerance of fish to zinc. In the parental generation, for instance, some fish died after only several days of exposure to zinc whereas others lived for the entire 17-day period. In a similar study (Bengtsson 1974) some fish survived a 100-day exposure to concentrations of zinc that were lethal to the majority of the population.

As with lethal tests, sublethal studies also reveal considerable variation in the response of organisms to toxicants. The sublethal effects of contaminants are being examined in many bioassays ranging from the subcellular to the community level (McKim et al. 1976, Sprague 1971, G.W. Brown 1976, Maki and Johnson 1976). In a study of the sublethal effects of copper on the cough frequency, locomotor activity, and feeding behavior of brook trout, response variation was evident (Drummond et al. 1973). The cough frequency of some fish increased markedly, whereas that of others did not change.

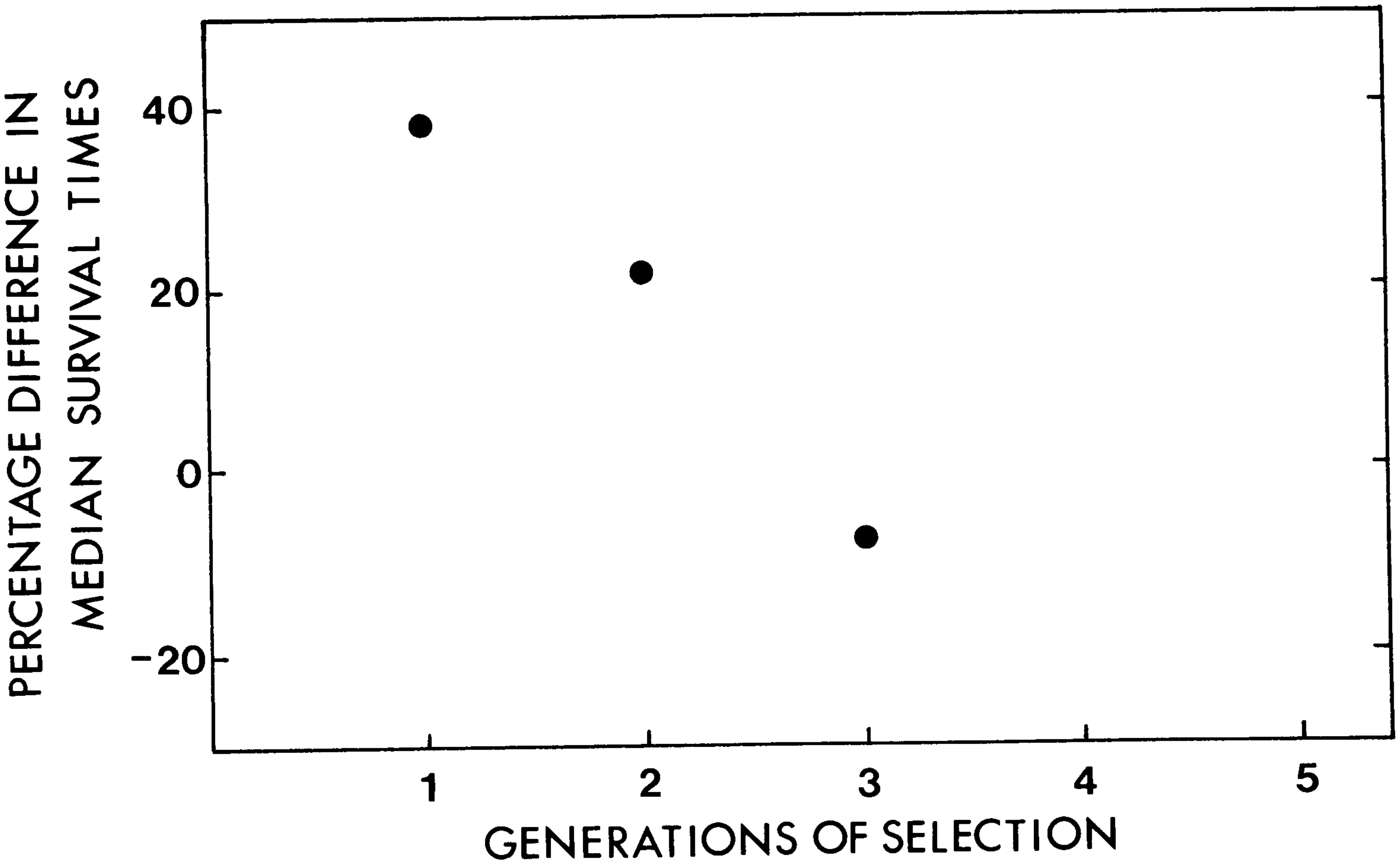


Figure 18. Summary of three generations of selection for zinc resistance in flagfish.

The two sources of this variation, environmental and genetic factors, must be understood if we are to predict the long-term effects of contaminants. Environmental factors alone can have a dramatic effect on an organism's response. In a study by Spehar (1976) flagfish larvae exposed as embryos to zinc and cadmium were more tolerant than unexposed larvae, although both sets were produced by unexposed parents.

Differences in the response of organisms to a pollutant are termed "phenotypic variability" (observable variability due to both an organism's heredity and environment). Even in bioassays with strains of laboratory fish raised under uniform conditions, much phenotypic variability persists. Such variability might be caused by differences in the general health or vigor of individual fish and not by genetic factors conferring resistance to a particular toxicant. A study by Sparks et al. (1972) demonstrates the importance of stress on fish tolerance. When pairs of bluegills were exposed to lethal zinc levels in bare aquaria, dominant fish had a significantly longer survival time. When the experiment was repeated with shelters provided, survival times did not differ. Presumably, subordinate fish were no longer subject to the additional stress of harassment by dominant fish.

If the response variation to toxicants is caused by nongenetic differences in health and vigor of fish, then toxicants should act simply as a general stress on the population, culling out the weak individuals. An increase in the gene combinations conferring resistance should not continually increase the tolerance of the population. Simply stated, selection should not continually increase the resistance of future generations. However, an increase in resistance through successive generations has been demonstrated for many contaminants and many species (see Introduction), indicating that genetic factors must be partly responsible for phenotypic variability.

Differences in the reaction of fish to toxicants also might be caused by factors such as size, sex, age, diet, and degree of acclimation. In this study, as in many others, diet and degree of acclimation were carefully controlled. We found that size had little effect on survival time for the parental generation.

The literature provides conflicting results about the effect of age on resistance to a toxicant. Age was not considered in this study except that only adult fish were exposed to zinc. Jones (1938) found no difference in survival times between juvenile and sexually mature sticklebacks exposed to a range of zinc concentrations. Bengtsson (1974) reported, however, that the resistance of the minnow Phoxinus phoxinus to zinc increased with age. Adelman et al. (1976) found that even for fish of a constant age or size class raised under identical laboratory conditions, variability in response to a toxicant still exists.

The presence of this variation in bioassays, despite uniform laboratory conditions, suggests that genetic differences between individuals may be a significant source of variability. Although genetic studies on zinc and fish have not been done, work on lead and mercury suggests that considerable variability in response to metal pollutants may be genetically based. Burger (1973) studied the inheritance of lead resistance in guppies and obtained a heritability estimate of 0.26 to 0.57 for survival time of fish exposed to acutely lethal levels of lead. [Heritability measures that fraction of the total phenotypic variability due to the additive effects of genes; this part of the phenotypic variability is the most responsive to selection (Crow and Kimura 1970)]. In a study of mercury resistance in steelhead trout, Blanc (1973) estimated the heritability to be 0.5 for survival time in chronically lethal levels of methylmercury.

Despite these demonstrations of genetically based differences in the tolerance of fish to toxic metals, we should be cautious of ascribing the variability found in bioassays to genetic sources. In a study by Rachlin and Perlmutter (1968) guppies still varied in their response to zinc after 31 generations of inbreeding. Since such prolonged inbreeding should have greatly reduced genetic variability, environmental factors appear as the chief source of the variation, although the fish were raised and exposed under uniform laboratory conditions.

Effects of Zinc on Reproduction

This study found that after only 2 to 8 weeks recovery time, flagfish surviving a zinc exposure level lethal to the majority of the population were able to reproduce as successfully as the control population. Similar work with zebrafish indicated that a 9-day period of zinc exposure reduced egg production and egg fertility, but these processes returned to normal levels within several weeks of a return to uncontaminated water (Speranza et al. 1977). Results of these relatively short-term exposures to zinc contrast with results of exposures encompassing the entire life cycle of fish. In the long-term studies, reproduction was reduced significantly although survival was unaffected (Brungs 1969, Bengtsson 1974). Since contaminants in an aquatic environment may be present only intermittently or temporarily (Cairns et al. 1971, Leland et al. 1976), it is important to know if fish have the ability to recover from temporary exposures. Our work suggests that such recovery is possible for fish exposed to zinc.

Whether selection for resistance to lethal effects also causes resistance to sublethal effects could not be determined in this study because the zinc-exposed control and experimental populations for both the first and second generations did not differ in spawning success and no other sublethal aspects were investigated. Such a phenomenon might be expected in fish, however, since it has been demonstrated in metal-tolerant invertebrates (B.E. Brown 1976).

Selection for Resistance

After three generations of selection, the selected line was more tolerant for the first two generations, but showed no difference from the unselected line in the third generation. The absence of any trend toward increased resistance with continued selection (Figure 18) does not necessarily indicate an inability to evolve metal tolerance. The actual decline in the relative tolerance of the experimental population suggests that confounding factors are responsible. Even if none of the variability in metal tolerance was genetic, the experimental population should have remained equal to the control line.

The continued decline in performance of the experimental population suggests the phenomenon of inbreeding depression. Although both lines had a similar number of parents for each generation (Table 17), it has been shown that in populations undergoing selection, selected parents are related more closely than randomly chosen parents (Robertson 1961). Because close relatives are more likely to produce offspring with harmful homozygous recessive gene combinations, the result of such inbreeding is a general depression of survival and vigor in the selected population (Kincaid 1976). In our experiments, parents of the selected population were chosen on the basis of having survived an exposure to zinc, whereas unselected population parents were chosen randomly. Fish surviving the zinc exposure were more likely to be closely related, if survival is genetically based, having inherited favorable genes from a common ancestor. Therefore, the decline in overall health and vigor because of inbreeding would be greater for selected-population fish than for zinc-exposed unselected-population fish. Hence performance of the experimental population would decline with increasing generations of selection.

TABLE 17. NUMBER OF PARENTS CONTRIBUTING LARVAE FOR EACH GENERATION

	Unselected line		Selected line	
Generation	Male	Female	Male	Female
Parental	14	11	20	20
First	16	16	17	17
Second	10	10	11	9
Third	7	7	8	8

Another possible explanation for the decline of the experimental population is the carry-over of harmful effects from mother to offspring through the egg cytoplasm. Exposed fish are expected gradually to lose zinc retained in their body tissue upon returning to clean water. It is possible that females incorporate some of the zinc into the cytoplasm of their eggs and that offspring of females exposed to zinc begin life with elevated zinc levels. According to this hypothesis, exposure of these offspring to zinc later in life results in increased susceptibility (McKim 1977). The magnitude of this carry-over effect would increase with a decrease in the recovery time allotted to females before breeding. Because recovery time was shortened with each generation of selection (Table 10), any carry-over effects work against the direction of selection. By the third generation the parents had had only 2 weeks' recovery time before breeding, and the carry-over effect may have finally negated the effects of selection.

The relative importance of inbreeding depression and carry-over effects in producing the observed decline in zinc tolerance will be tested in future work. Because the selected population showed an initial increase in zinc tolerance and similiar laboratory studies have produced an increase in metal tolerance (Blanc 1973, Burger 1973), it was concluded that fish possess the genetic potential to evolve metal-tolerant populations. Laboratory studies are usually limited to observing a few generations and are often subject to confounding influences (e.g., inbreeding). Hence, ultimate resolution of whether fish can genetically adapt to increased metal levels will come from studies of populations chronically exposed to metal contaminants.

No studies have documented whether natural fish populations in chronically contaminated waters ever realize this genetic potential. Fish are reported to live in a series of Canadian lakes receiving metal inputs from nearby smelters (Van Loon and Beamish 1977), but a more recent study indicates that long-term survival of these fish is uncertain. McFarlane and Franzin (1978) reported that a population of white suckers, *Catostomus commersoni*, suffered severe reproductive impairment in one of these lakes with high zinc levels (141 to 341 mg/liter) although adult survival was relatively unaffected. Similar effects were noted on other fish species from the same lake. The smelter has operated since 1930, and metal concentrations presumably have been increasing since then, although historical data on metal levels in the water are unavailable. The onset of harmful effects on local fish populations indicates that the fish failed to adjust genetically to stressful metal levels during this relatively brief period.

A similar situation exists in the soft-water lakes of the Adirondacks, where aluminum leached from surrounding soils by acid precipitation has killed many fish (Cronan and Schofield 1979). The development of harmful metal concentrations in these lakes is a recent event, and again fish are not adjusting rapidly to this sudden change in their environment.

McIntosh and Bishop (1976) used bluegills from a metal-contaminated and an uncontaminated lake to compare relative survival in an acutely lethal exposure of cadmium. They found no difference in the 96-h LC_{50} value for the populations. In a sublethal exposure of cadmium, they reported a significantly lower cough rate for fish from the contaminated lake than for control fish; no difference was found in breathing rates, however. Whether fish from the contaminated lake actually represented a population exposed to selective pressures for metal tolerance is questionable, however, because metals in the lake were not distributed evenly and it was not known how long fish had been in the contaminated areas.

This study focused on the potential for the rapid evolution of metal tolerance in fish populations. The results suggest that fish possess this potential, but the limited studies of natural fish populations living under chronic metal stress do not support our findings. Further work on fish populations inhabiting waters with high trace-element levels is needed before we can determine if and how fish can adapt ultimately to these conditions. Man's pollution of aquatic systems is extremely rapid on an evolutionary time scale. If this pollution continues, fish populations must adapt at an equally rapid pace to avoid decimation or local extinctions.

SECTION 5

USE OF TEMPERATURE PREFERENCE AND ACTIVITY AS A SUBLETHAL BIOASSAY FOR THE TOXIC EFFECTS OF ZINC TO BLUEGILL

INTRODUCTION

The fly ash emitted from coal-fired generating stations contains microcontaminants, including metal ions, that dissolve and may enter natural water systems. To monitor biological reactions to these toxicants, we need methods that are quick, sensitive, and accurate. Such methods aid in setting standards, monitoring spills, discovering synergistic effects (the cooperative effect of several factors working independently), and monitoring microcontaminant levels in mining runoff and industrial waste waters.

This section documents our effort to test one of these methods: A temperature-preference apparatus. Preferred temperature is a stable behavioral trait for fish (Magnuson and Beitinger 1978). Change in temperature preference indicates a response to some other factor such as stress from starvation (Javaid and Anderson 1967, Stuntz 1975, Stuntz and Magnuson 1976) or pollution (Ogilvie and Anderson 1965, Peterson 1973). In fact, knowledge of the concentration at which contaminants affect fish temperature preference might serve as a useful indicator of sublethal toxicity. Behavioral tests are a more sensitive indicator of these harmful effects than lethality experiments and require less time and space than tests on sublethal chronic effects (Schere 1977, Henry and Atchison 1979).

DESIGN OF THE STUDY

We tested an electronically controlled, temperature-selection apparatus (Neill et al. 1972) for possible use as a tool for the detection of sublethal concentrations of zinc by the bluegill (*Lepomis macrochirus*). This system uses a temporal gradient and provides a record of activity and temperature. Zinc was chosen as the contaminant because its acute and sublethal toxicity are well documented (Cairns and Scheier 1957, Sprague 1968, Brungs 1969, Burton et al. 1972, Waller and Cairns 1972, Cairns et al. 1973). A sublethal zinc concentration (2.5 ppm) was selected on the basis of preliminary experiments and previous studies (Burton et al. 1972, Waller and Cairns 1972, Cairns et al. 1973).

MATERIALS AND METHODS

Selection Apparatus

Nine aquaria, each divided into two compartments with an interconnecting tunnel, were equipped with a system that permits fish to control the temperature of the aquaria. With a constant 2°C temperature difference between the two compartments, the fish controls the direction of temperature change (3°C/h) in the tank. If the fish is in the warmer compartment, the temperature of the whole tank is increasing; if the fish is in the cooler compartment, tank temperature decreases. Temperature is selected on a temporal rather than spatial basis (Neill and Magnuson 1974, Beitinger et al. 1975). A computer continuously monitors and records tank temperatures and movement through the tunnel (activity). Changes due to zinc may occur in the activity rate or selected temperature (e.g., increase, decrease, or diurnal pattern modification).

General Conditions

The water used for the experiments was Madison, Wis., city water diluted at a ratio of 1:7 with distilled water. Madison city water is unusually hard (300 ppm $CaCO_3$), and zinc is less toxic to the bluegill in hard water (Cairns and Scheier 1957). The 1:7 dilution results in hardness of 40 to 50 ppm (Table 18), a level within the range of much previous work with zinc (Cairns et al. 1973). Experiments were conducted during November and December 1976. The 12:12 light-dark cycle included periods of intermediate light levels at dawn and dusk. We analyzed data from eight control fish and 10 zinc-exposed fish. Data were not utilized if a given fish did not pass through the tunnel at least three times during a day or night period. Temperature data from one fish were lost because of thermistor malfunction.

TABLE 18. ROUTINELY DETERMINED CHARACTERISTICS OF WATER USE IN THE TEMPERATURE-PREFERENCE BIOASSAY

Water characteristic	Unit	No. of analyses	Median	Range
pH[a]	--	250	7.6	7.1-8.0
Total hardness[b]	ppm	114	44	24-58
Alkalinity[c]	ppm	114	66	30.90
Dissolved oxygen[d]	% saturation	68	101	96-105
Conductivity	µmhos/cm at 25°C	250	102	73-121

[a]Fisher pH meter Model 150.
[b]EDTA Titrametric method (American Public Health Association et al. 1975).
[c]Methyl Orange indicator method (American Public Health Association et al. 1975).
[d]YSI Model 54A.
[e]YSI Model 33, S-C-T meter.

Fish

Young bluegill (7 to 10 cm) were captured with beach seines and fyke nets in Lakes Wingra and Mendota in Madison, Wis., and placed in holding tanks for at least 1 month before an experiment. The fish were fed trout pellets once daily and were acclimated to experimental light and water conditions. Fish were not fed during the selection experiments.

Procedure

The experiment was started by placing one fish in each aquarium. One side of each tank was set at 21°C, the other at 23°C. After 1 day of fixed temperatures, each fish was allowed to thermoregulate for 2 days before zinc was added to half of the tanks, selected at random. Enough zinc sulfate ($Zn\ SO_4$) dissolved in distilled water was added to both compartments to achieve a concentration of 2.5 ppm zinc in half of the aquaria, selected randomly. Zinc was added only once at the start of the experiment. Distilled water was added to the control aquaria. Water in both compartments of each aquarium was analyzed for zinc daily (Table 19). Fish were allowed to thermoregulate for 4 more days. At the end of the experiment, the bluegill were weighed, measured, and frozen for analysis of zinc concentrations in the gills, liver, and muscle (Table 20).

TABLE 19. ZINC CONCENTRATIONS (ppm) OF WATER IN TREATMENT AND CONTROL AQUARIA[a]

	Zinc-exposed (n=97)		Control (n=86)	
Day	Median	Quartiles	Median	Quartiles
4	1.49	1.33-1.55	0.03	0.02-0.11
5	1.24	1.13-1.31	0.04	0.02-0.06
6	1.13	1.02-1.28	0.04	0.01-0.13
7	1.16	0.99-1.36	0.03	0.06-0.15
8	1.00	0.91-1.21	0.03	0.00-0.11

[a]Analyses were done by atomic absorption photospectrometry.

RESULTS

After the zinc was added, its concentration in the water continuously decreased probably because of absorption on particulate matter and uptake by the fish (Table 19). Neither selected temperatures (Figure 19) nor rates of activity (number of tunnel crossings/h) (Figure 20) of control and zinc-exposed fish differed significantly (Mann-Whitney U Test, $p \leq 0.05$, Siegel 1956). Preferred temperatures on the day before zinc was added and of the day zinc was added were not significantly different in either the control or the zinc-exposed fish (Wilcoxon signed ranks test). There was a trend for

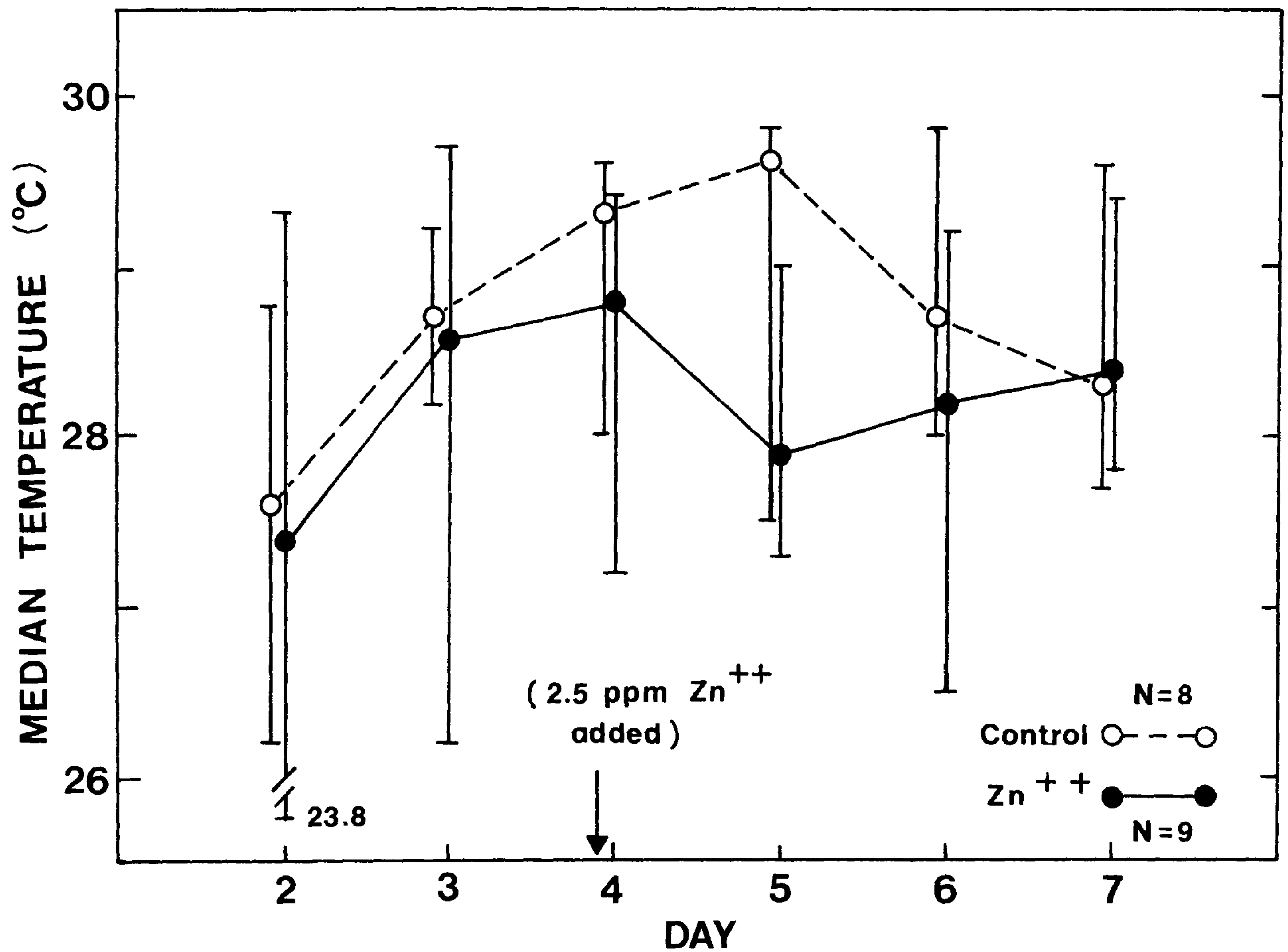


Figure 19. Median selected temperatures of bluegill in control aquaria and in aquaria treated with zinc in a 7-day experiment. Vertical bars represent the 25th to 75th percentiles.

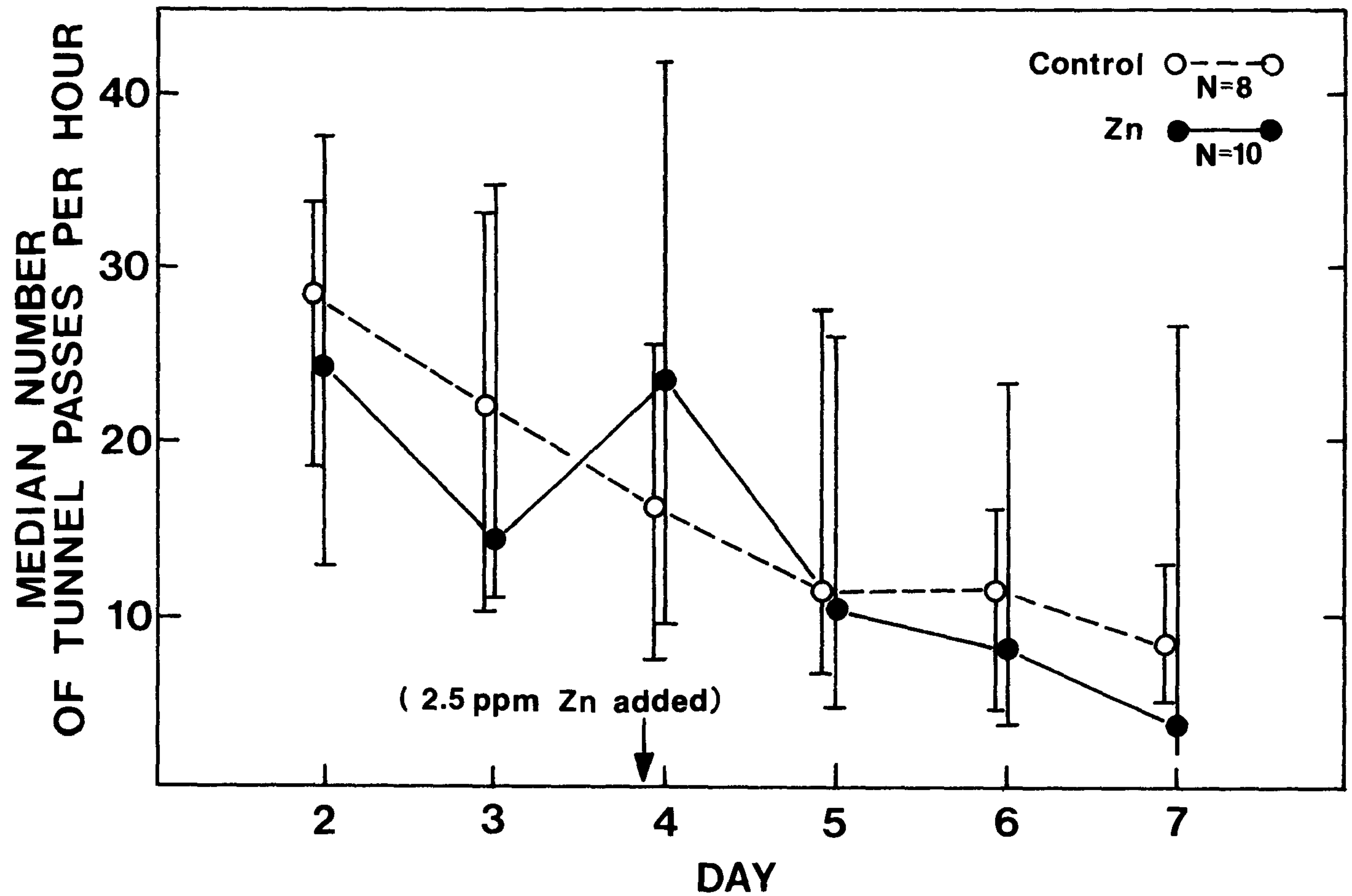


Figure 20. Median number of tunnel passes per hour by bluegill in control aquaria and in aquaria treated with zinc in a 7-day experiment. Vertical bars represent the 25th to 75th percentiles.

TABLE 20. ZINC TISSUE CONCENTRATIONS (ppm) AT THE END OF THE EXPERIMENT FOR RANDOMLY SELECTED FISH FROM TREATMENT AND CONTROL TANKS[a]

	Zinc-treated (n=4)		Control (n=4)	
	Median	Range	Median	Range
Gill	90.4	75.3-107.2	86.8	76.9-89.81
Liver	107.5	90.7-129.8	91.2	83.6-125.2
Muscle	28.6	23.0-33.8	30.8	26.7-34.9

[a]Analyses were done by neutron activation and by each median n=4.

the zinc-exposed fish to prefer a lower temperature, but this lasted for only 1 day. The rate of activity of the control fish, however, was significantly lower ($P \leq 0.5$) on the day distilled water was added than on the previous day. This followed a trend of decreasing activity rates (Figure 20). Activity rates of the zinc-exposed fish were not significantly lower after the addition of zinc; in fact, the median was greater than that of the previous day.

DISCUSSION

Our selection system does not detect sublethal effects of zinc at lower concentrations than other methods tested in water of similar quality. Cairns et al. (1973) detected sublethal zinc concentrations of 2 to 3 ppm by continuously monitoring bluegill movement patterns perceived by light-beam interruptions, and by measuring bluegill ventilation rates. Sprague (1968) found no change in selected temperature of Atlantic salmon (*Salmo salar*) in a horizontal temperature gradient after 24 h of exposure to 0.16 ppm zinc.

The effect of temperature on zinc lethality varies with the type of lethality test and species of fish, but seems greater at higher temperatures. Survival time of rainbow trout exposed to zinc decreases at higher temperature (Lloyd 1960). Temperature stress induced by increasing the temperature at a rate of 1.5°C every 10 min reduced survival time at a concentration of 32 ppm zinc (Burton et al. 1972). Also, at 5.6 ppm zinc deaths occurred in 96 h at 30°C but not at 20°C. Pickering and Henderson (1966) found no significant difference in toxicity to fish at 15° and 25°C, but the trend was for higher toxicity at higher temperatures. Cairns and Scheier (1957) found 100% survival at 18° and at 30°C for overlapping zinc concentrations.

Apparently, a bluegill that behaviorally reduced its temperature while exposed to lethal zinc concentrations would increase its probablity of survival. A nonsignificant trend toward lower temperatures in the presence of zinc was observed in both Peterson's (1976) and the current work, perhaps indicating that further experiments could determine which zinc concentration results in a change in temperature preference.

In conclusion, our method does not appear to be any more suitable as a sensitive indicator of sublethal effects of metal ions than other methods.

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APPENDIX A

NUMBER OF FISH CAUGHT AT EACH SAMPLING STATION, 1976-78

TABLE A-1. NUMBER OF FISH CAUGHT AT EACH SAMPLING STATION, 1976

Species	Station 1	2	3	4
Northern pike (*Esox lucius*)	1	21	8	18
Walleye (*Stizostedion vitreum vitreum*)	0	2	3	1
Muskellunge (*Esox masquinongy*)	0	0	6	3
Largemouth bass (*Micropterus salmoides*)	0	0	1	0
Rainbow trout (*Salmo gairdneri*)	2	1	0	0
Yellow perch (*Perca flavescens*)	0	0	0	1
Spotted sucker (*Minytrema melanops*)	0	2	9	2+[a]
Pirate perch (*Aphredoderus sayanus*)	0	0	3	0
White sucker (*Catostomus commersoni*)	0	12	3	3
Black crappie (*Pomoxis nigromaculatus*)	0	20	0	1
White crappie (*Pomoxis annularis*)	0	1	0	0
Rock bass (*Ambloplites rupestris*)	1	5	0	0
Pumpkinseed (*Lepomis gibbosus*)	0	0	22	2
Bluegill (*Lepomis macrochirus*)	0	0	4	0
Yellow bullhead (*Ictalurus natalis*)	0	2	0	0
Black bullhead (*Ictalurus melas*)	0	3	12	11
Redhorse (*Moxostoma* sp.)	0	1	0	0
Bowfin (*Amia calva*)	0	8	2	0
Carp (*Cyprinus carpio*)	0	many	many	many
Buffalo (*Ictiobus cyprinellus*)	0	many	many	many

[a]+ signifies more than two, but exact number not recorded.

TABLE A-2. NUMBER OF FISH CAUGHT AT EACH SAMPLING STATION, 1977

Species	Station 1	2	3	4
Northern pike (*Esox lucius*)	10	45	2	7
Walleye (*Stizostedion vitreum vitreum*)	0	4	0	0
Muskellunge (*Esox masquinongy*)	0	30	0	0
Largemouth bass (*Micropterus salmoides*)	0	7	0	0
Rainbow trout (*Salmo gairdneri*)	2	7	0	0
Yellow perch (*Perca flavescens*)	0	3	0	0
Spotted sucker (*Minytrema melanops*)	0	54	0	0
Pirate perch (*Aphredoderus sayanus*)	0	0	0	0
White sucker (*Catostomus commersoni*)	11	69	0	0
Black crappie (*Pomoxis nigromaculatus*)	0	25	0	0
White crappie (*Pomoxis annularis*)	0	0	0	0
Rock bass (*Ambloplites rupestris*)	2	20	0	0
Pumpkinseed (*Lepomis gibbosus*)	0	40	0	0
Bluegill (*Lepomis macrochirus*)	0	4	1	0
Yellow bullhead (*Ictalurus natalis*)	0	4	0	0
Black bullhead (*Ictalurus melas*)	0	3	1	0
Redhorse (*Moxostoma* sp.)	1	0	0	1
Bowfin (*Amia calva*)	1	16	0	2
Carp (*Cyprinus carpio*)	0	5	0	0
Buffalo (*Ictiobus cyprinellus*)	0	0	0	0
White bass (*Morone chrysops*)	0	1	0	0
Golden shiner (*Notemigonus crysoleucas*)	0	1	0	0
Chestnut lamprey (*Ichthyomyzon castaneus*)	0	3	0	0
Freshwater drum (*Aplodinotus grunniens*)	0	1	0	0

TABLE A-3. NUMBER OF FISH CAUGHT AT EACH SAMPLING STATION, 1978

Species	Station 1	2	3	4
Northern pike (*Esox lucius*)	0	101	7	12
Walleye (*Stizostedion vitreum vitreum*)	0	1	0	0
Muskellunge (*Esox masquinongy*)	0	0	0	0
Largemouth bass (*Micropterus salmoides*)	0	0	0	0
Rainbow trout (*Salmo gairdneri*)	0	2	0	0
Yellow perch (*Perca flavescens*)	0	0	0	0
Spotted sucker (*Minytrema melanops*)	0	42	0	0
White sucker (*Catostomus commersoni*)	1	40	0	0
Pirate perch (*Aphredoderus sayanus*)	0	0	2	0
Black crappie (*Pomoxis nigromaculatus*)	0	20	0	0
White crappie (*Pomoxis annularis*)	0	0	0	0
Rock bass (*Ambloplites rupestris*)	0	2	0	0
Pumpkinseed (*Lepomis gibbosus*)	0	0	0	0
Bluegill (*Lepomis macrochirus*)	0	3	0	1
Yellow bullhead (*Ictalurus natalis*)	0	11	0	0
Black bullhead (*Ictalurus melas*)	0	2	0	0
Redhorse (*Moxostoma sp.*)	0	0	0	0
Bowfin (*Amia calva*)	1	14	0	0
Carp (*Cyprinus carpio*)	0	21	0	0
Buffalo (*Ictiobus cyprinellus*)	0	0	0	0
Brown trout (*Salmo trutta*)	0	1	0	0
White bass (*Morone chrysops*)	0	1	0	0
Freshwater drum (*Aplodinotus grunniens*)	0	6	0	0

APPENDIX B

MARSHES NEAR THE COLUMBIA GENERATING STATION

TABLE B-1. MARSHES NEAR THE COLUMBIA GENERATING STATION

Marsh area	Description
Station site (after construction)	The area north of County J, west of the Chicago, Milwaukee, St. Paul and Pacific Railroad tracks, south of Duck Creek. Includes the mouths of Rocky Run and Duck Creek.
Duck Creek	Includes all wetlands along Duck Creek east of the Chicago, Milwaukee, St. Paul and Pacific Railroad tracks, up to the dam located along State Hwy 22-24 at Wyocena, Wis.
Rocky Run A	Wetlands south of County Hwy J, east of the Chicago, Milwaukee, St. Paul and Pacific Railroad tracks and west of State Hwy 51. Includes wetlands drained by the mint drain and by Rocky Run Creek. Fyke nets set in both the mint drain and Rocky Run showed few northern pike moved this far upstream to spawn.
Rocky Run B	Wetlands along Rocky Run Creek east of State Hwy 51 including Mud Lake. Unlikely to be pike spawning habitat for reasons given for Rocky Run A.
Corning-Weeting Lakes	Wetlands associated with Corning and Weeting Lakes located north of the Wisconsin River and west of Portage, Wis. Accessible by a small creek flowing 8 km (about 5 miles) south of the Wisconsin River. Ground survey in August 1978 showed no obstructions to fish movement, but very shallow stream flow (4 to 5 cm deep, 1 m across) in upper reaches of the creek.

(continued)

TABLE B-1 (continued)

Marsh area	Description
Powers Creek Whelen Bay	The mouth of Powers (Rowen) Creek east to Interstate 90-94. Includes a portion of Lake Wisconsin known as Whelen Bay.
Hinkson-Rowan Creek	Upstream tributaries of Powers Creek starting at Interstate 90-94 eastward to stream headwaters.
Lodi Marsh	Includes wetlands along Spring Creek from its mouth at the Wisconsin River to marsh upstream of the town of Lodi, Wis. Ground survey in August 1978 indicated two small spillways of about 0.5 m in the town of Lodi which would prevent upstream migration except during spring floods. Fish would have to migrate about 8 km (5 miles) upstream through the town of Lodi to reach suitable spawning habitat.
O'Kee Bay	Wetlands associated with a bay of Lake Wisconsin east of O'Kee, Wis., and a bay east of Pine Bluff at Harmony Grove, Wis.
South Dekorra Inlet	Wetlands associated with a small stream south of Dekorra, Wis., and just south of where Interstate 90-94 crosses the Wisconsin River.
Merrimac Inlet	A bay of Lake Wisconsin and associated wetlands south of Merrimac, Wis. State Hwy 113-78 and the Chicago-Northwestern Railroad tracks cross the bay, but do not prevent access by spawning fish.
Prentice Creek	Wetlands associated with Prentice Creek which joins the Wisconsin River north of Merrimac, Wis.
Baraboo River Mouth	Wetlands located at the mouth of the Baraboo River upstream to where Interstate 90-94 crosses the river.

APPENDIX C

REVIEW OF LITERATURE ON ENTRAINMENT FROM COOLING LAKE INTAKE STRUCTURES

In the appendix, the possible entrainment damage to fish and invertebrate populations at the Columbia site is discussed; the possible damage appears minimal. In addition, a 1978 study of fish entrainment at the site by Swanson Environmental, Inc., has revealed that fish loss due to the present water-intake systems is minor.

The effects of cooling-water intake on aquatic systems have been studied at many power plants over the last 20 years. Although the studies differed in their approach, detail, and conclusions, four general areas of concern have emerged: (1) Removal of animals suspended or swimming in the water column; (2) mechanical injury because of impingement upon intake screens or abrasion in pumps, pipes, and condensers; (3) the toxic effects of biocides used in reducing the fouling of pipe systems by microorganisms; and (4) the various effects of thermal shock during condenser passage.

The removal of animals from the water column, including the impingement of adult and juvenile fish, has become the focus of a federally mandated monitoring program, pursuant to the requirements of Public Law 92-500. Freeman and Sharma (1977) have conducted a survey of these programs, but a summary volume is not complete. The removal aspect of cooling-water intake is relevant to the Columbia site; mechanical, toxic, and thermal aspects of entrainment do not apply. The Columbia station withdraws water from the artificial cooling lake to cool the superheated steam in the turbines. It is essentially a closed system, except that evaporative losses from the lake require a constant input from the Wisconsin River. The "make-up" water is presently pumped from the intake channel to the artificial lake by two 10,000-gal/min pumps. Water is drawn down an intake channel that connects with the river approximately 3,000 ft from the cooling lake. The channel is protected by two bar-grilles and a fish conservation traveling screen.

Studies of mechanical injury and mortality during entrainment have been reported by Marcy (1973, 1976), Carpenter et al. (1974), Ginn et al. (1974), King (1974), Davies and Jensen (1975), and Polgar (1975). Several reviews such as those of Coutant (1970) and Hillegas (1977) have been published. Although survival of damaged organisms is often quite low, it does not appear that the numbers of organisms lost results in serious effects on the aquatic systems.

Biocides such as chlorine are usually used at such low concentrations that they pose no threat to entrained organisms or to the receiving body of water (Marcy 1971, Bass and Heath 1975, Basch and Truchan 1976, Brungs 1976, Seegert and Brooks 1977). However, thermal shock, combined with small amounts of chlorine, has a greater effect than either increased temperatures or chlorine levels alone (Eiler and Delfino 1974, Ginn et al. 1974).

Cooling-systems designers now use predictive tools to minimize impact. Curves and models predict for a given intake design the amount of mechanical damage (Polgar 1975) and the extent of lethal and sublethal thermal effects (Coutant 1970) expected. Models have also been developed by Goodyear (1977), Christenson et al. (1977), and others to forecast effects of removal on given fish populations.

POTENTIAL EFFECTS OF COOLING WATER INTAKE AT THE COLUMBIA SITE

Effects of entrainment of aquatic organisms from the Wisconsin River by the Columbia Generating Station are different from effects seen at most other generating stations. At Columbia there is no direct return of the entrained water to the river. The analogy of the intake acting as a large predator on the river ecosystem (Coutant 1970) is more applicable than in "once through" cooling situations. In assessing potential effects researchers often draw a relationship between the percentage of water in the river used and the resulting effect on the river. However, organisms in riverine communities typically show "patchy" distributions (Whitton 1975), and larger organisms can either avoid the intake channel or electively swim into it.

Zooplankton and Drifting Macroinvertebrates

Zooplankton are too small to be screened out of the intake pumps and are less able to avoid the influence of the pumping current than are larger animals. The percentage of total river flow removed by the intake water at Columbia presently averages 0.3% with a maximum of 1.08%. Assuming that the number of organisms entrained by the Columbia intake is proportional to the volume of river water used, we expect no significant loss of invertebrates from the Wisconsin River. Several other entrainment studies at U.S. power plants (King 1974, Davies and Jensen 1975, Hillegas 1977) did not demonstrate measurable effects in downstream plankton communities even where abundant data were available and generating stations in question diverted up to 30% of the river flow.

Adult and Juvenile Fish

A 1-yr study of fish entrainment at the Columbia site (Swanson Environmental, Inc. 1977) reported the number, species, length, and reproductive condition of fish impinged on the temporary screen box unit and on the traveling screen unit currently in use. Sampling was conducted for a 24-h continuous period once a week. An estimated 14% of the total intake volume was sampled. The catch numbers were extrapolated to estimate total annual impingement as 668 $\pm$ 387 fish/yr (mean $\pm$90% confidence limits). The number of adult and juvenile fish impinged at Columbia is low, and even if all impinged fish die, no effect on the river system should occur.

Fish Eggs and Larvae

The Swanson Environmental, Inc., study (1977) also included sampling for fish eggs and larvae. Submersible pumps were mounted behind the traveling screen unit and pumped the sample water into 423-μm nets. Pump rates were sufficient to prevent fish from avoiding the sampler. Estimated annual entrainment of larval fish was 126,659 ± 93,994 larvae/yr (mean ±90% confidence limits). No northern pike or walleye larvae were caught in the samples. According to a summary of fish-census data for the Columbia site (Wisconsin Department of Natural Resources 1973), northern pike and walleye spawn in the wetland adjacent to Duck Creek. The mouth of Duck Creek is located just upstream from the Columbia intake (Figure 1). Northern pike larvae and fry remain on the spawning marshes until they attain a size of 20 mm at 16-24 days after hatching (Franklin and Smith 1963). Although emigrating larvae of this size would not be able to avoid the intake current, the river currents may be strong enough in early spring to sweep larvae past the intake. Larval walleye are known to migrate from their spawning marshes in intermittent pulses over a 10- to 15-day period (Priegel 1970). By sampling once every 7 days, the period of walleye larval entrainment could have been missed. Walleye larvae may also avoid entrainment by staying in the main currents as they enter the Wisconsin River, therefore bypassing the shoreline by the intake. Newly hatched walleye larvae emerging from similar spawning situations on the Wolf and Fox Rivers in Wisconsin tended to stay in the strongest currents until they reached more lacustrine situations where zooplankton were abundant (Priegel 1970).

In summary, as long as the Columbia intake continues to remove a small percentage of the river flow, we expect no measurable effects of entrainment on the river system. An exception might occur when organism distribution is patchy near the intake, and a significant portion of one year-class (e.g., walleye larvae) is entrained. Aside from acting as a predator by removing organisms from the Wisconsin River, the usual types of entrainment effects (mechanical, toxic, and thermal) do not apply to the Columbia station.

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APPENDIX D

REVIEW OF LITERATURE ON ACID PRECIPITATION

Acid rainfall, the topic of this appendix, is not considered a potential problem for aquatic ecosystems at Columbia because of the high hydrogen-ion buffering capacity resulting from the calcareous nature of the drainage basin.

Recent studies in both North America and Europe have documented the occurrence of acid rains with a pH ranging from 2.1 to 5.0 (Likens and Bormann 1974; Beamish 1974, 1976; Dickson 1975; Schofield 1976). Rainwater is normally slightly acidic, with a pH of 5.7, as a result of the equilibrium reaction between atmospheric carbon dioxide and water forming carbonic acid (H_2CO_4). Both natural and anthropogenic processes, however, can add three strong mineral acids, sulfuric, nitric, and hydrochloric, to atmospheric water with a resulting sharp decrease in pH (Gorham 1976). The most predominant of these acids is sulfuric (H_2SO_4), which can be formed in substantial amounts from the sulfur dioxide (SO_2) produced as sulfur in fossil fuels oxidizes during combustion. Coal normally has between 1 and 3% sulfur, but the percentage can go as high as 6%. Of less importance are nitric acid (HNO_3) and hydrochloric acid (HCl), which are also produced by fossil-fuel combustion through the oxidation of organic nitrogen and chlorine, respectively. These acids may then enter aquatic systems through rainfall or, in northern latitudes, through spring ice and snow runoff.

The work of Cogbill and Likens (1974) illustrates that acid precipitation is likely to remain a problem in certain areas. By graphing isolines of rainfall pH falling over the eastern U.S., they have shown a dramatic increase in the geographic area affected by acid rain, as well as an increase in rainfall acidity for the 10-year period 1956-66.

The initial effects of acid input into lakes and streams depend largely on edaphic characteristics that determine their buffering capacity. All waters so far affected by acid precipitation have been in areas that are geologically highly resistant to chemical weathering and usually have a low concentration of major ions, particularly bicarbonate (HCO_3^-), resulting in a specific conductance less than 50 μmhos/cm (Wright and Gjessing 1976). Acid rainfall into such weakly buffered systems causes a loss of bicarbonate ion and its replacement by sulfate; hence sulfate is the major anion in acidified soft water, whereas bicarbonate predominates in non-acidified soft water. Acidified lakes are frequently found to contain elevated aluminum and manganese concentrations that are attributed to dissolution from surrounding soils. Elevated levels of other heavy metals (Pb, Zn, Cu, Ni) may also exist downwind of major base-metal smelters (Van Loon and Beamish 1977).

Ecological studies concerned with acidification of aquatic ecosystems have focused on fish poulations, since the loss of an exploitable fish population is the most noticeable and economically important consequence of acid precipitation. Fish loss is reported to be a gradual process resulting not from acutely lethal pH changes, but rather from the failure to recruit new year-classes into the population (Beamish 1974). At pH values above the lower lethal level, interference with spawning has been demonstrated in both laboratory and field studies (Mount 1973, Beamish 1976). The presumed mechanism causing reproductive failure is disruption of normal calcium metabolism that prevents females from releasing their ova (Beamish 1976). Long-term effects of acidification on fish populations were summarized by Beamish (1975) as follows: (1) failure to spawn, (2) low serum Ca^{++} levels in mature females, (3) appearance of spinal deformities, (4) decreases in the average size of year-classes, (5) reduction in population size, and (6) disappearance of species from lakes.

Studies have indicated a genetic basis for acid tolerance at the species level (Gjedrem 1976, Robinson et al. 1976, Schofield 1976) and selective breeding of acid-tolerant fish strains has been proposed as a means of stocking waters that have lost their natural populations. The observed rates of population extinction indicate, however, that acidification has been proceeding too rapidly for natural-selection processes to be effective in maintaining fish populations under natural conditions.

Equally as serious as damage to fish are the less conspicuous effects of acid rain on aquatic organisms such as microdecomposers, primary producers, zooplankton, and zoobenthos. Studies in six Swedish lakes, where the pH decreased by 1.4 to 1.7 pH units in the last 40 years, have demonstrated an inhibition of bacterial decomposition with a resultant abnormal accumulation of coarse organic detritus (Hendrey et al. 1976a). Rooted macrophytes, zooplankton, and benthic invertebrates are also stressed by acidification of waters (Hendrey et al. 1976b). Some of the effects of pH on aquatic organisms are summarized in Table D-1.

Although pH measurements of rainfall in the vicinity of the Columbia Generating Station have not been made, it appears unlikely that acid rainfall will noticeably affect nearby aquatic ecosystems for the following reasons: (1) The Wisconsin River, Rocky Run Creek, and nearby waters are well-buffered systems with total alkalinities in the range of 80 to 133 mg/liter $CaCO_3$ and conductivities of 178 to 273 μmhos/cm; (2) winds are predominately from the west and south (Stearns et al. 1977) and, therefore, power plant emissions should miss most of the nearby aquatic systems that are located mainly west and south of the plant; (3) the present pH values of the Wisconsin River (7.6 to 8.2) and Rocky Run Creek (7.6 to 8.2) are well within the recommended safe range of 6.5 to 9.0 for natural waters and have not changed noticeably since the plant began in 1975.

TABLE D-1. SUMMARY OF pH EFFECTS ON AQUATIC ORGANISMS

pH	Effect	Reference
< 3.5	Unlikely that fish can survive for more than a few hours A few invertebrates (midges, mosquito, caddisfly) have been found Few plants (only mosses and algae) have been found	EIFAC 1969 Lackey (1938) Hendrey et al. 1976a
3.5-4.0	Lethal to salmonids and bluegills, limit of tolerance of pumpkinseed, perch, and pike, but reproduction is inhibited Cattail (<u>Typha</u>) is the only higher plant	U.S. EPA 1973
4.0-4.5	Only a few fish species survive, including perch and pike Lethal to fathead minnows Flora is restricted Some caddisflies and dragonfiles are found, and midges are dominant	U.S. EPA 1973
4.5-5.0	Salmonids may survive, but do not reproduce Benthic fauna are restricted; mayflies are reduced Fish populations are severely stressed; a viable fishery is nonexistent	U.S. EPA 1973
	Snails are rare or absent	Hendrey et al.1976a
	The fish community is decimated with virtually no reproduction	Beamish 1974, 1975
	White suckers and brown bullheads fail to spawn, but perch do spawn	Beamish 1975
5.0-6.0	Rarely lethal to fish except some salmonids, but reproduction is reduced Larvae and fry of sensitive species may be killed Bacterial species diversity is decreased, benthic invertebrates are reasonably diverse, but sensitive taxa such as mayflies are absent and molluscs are rare	U.S. EPA 1973
	Fathead minnow egg production and ability to hatch are reduced	Mount 1973
	Smallmouth bass, walleye, and burbot stop reproducing	Beamish 1976
	Roe of roach (<u>Rutelus rutelus</u>) fail to hatch	Milbrink and Johansson 1975

(continued)

TABLE D-1 (continued)

pH	Effect	Reference
6.0-6.5	Unlikely to be harmful to fish unless free CO_2 exceeds 100 ppm Good invertebrate fauna except for reproduction of _Gammarus_ and _Daphnia_ Aquatic plants and microorganisms relatively normal	U.S. EPA 1973
6.5-9.0	Harmless to fish and most invertebrates although 7.0 is near the lower limit for _Gammarus_ reproduction Microorganisms and plants are normal Toxicity of other substances may be affected by pH shifts within this range.	U.S. EPA 1973

Future considerations should be given to the effect of added sulfur emissions when Columbia II begins operation and on the contributions, if any, of the Columbia plant emissions to acid rainfall over distant waters such as northern Wisconsin lakes, some of which are poorly buffered and more subject to acidification.

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APPENDIX E

REVIEW OF LITERATURE ON ALTERNATIVE DISPOSAL OF FLY ASH

Increased national emphasis on the use of coal to meet energy requirements may result in a doubling of coal-ash production from 1975 levels by the year 1995 (PEDCO-Environmental, Inc. 1976). Annual coal-ash production is currently estimated to be 61.9 x 10^6 tons (Davis and Faber 1977) and may be 100 x 10^6 tons by 1985 (Harriger 1977). About 20% of the ash is used for commercial purposes in cement, asphalt and concrete, fertilizer, fire control, road-bed stabilizer, soil aeration, and sanitary landfill cover (PEDCO-Environmental 1976, Theis 1976a, Harriger 1977). Research continues into additional uses for coal ash such as water reclamation, sewage-sludge conditioning, and supplementation of soil sewage micronutrients (Theis 1976a, Furr et al. 1977). Fly ash and lime cause precipitation of phosphorus from natural waters, and the ash seals the nutrients in the sediment; however, the side effects of such treatment may be severe (Theis and DePinto 1976). Fly ash concentrations of 10 to 20 g/liter were toxic to Stone Lake, Mich., fish. High pH, dissolved oxygen depletion, heavy-metal release, and physical clogging and crushing of organisms are other effects that have not been adequately investigated. Fly ash applied to soils can neutralize acid soils and supply calcium and trace elements (PEDCO-Environmental, Inc. 1976); however, the high conductivities of fly-ash-water solutions may result in injuriously high salt concentrations for many sensitive crops (Olsen and Warren 1976). Theis (1976a) suggests the extraction of the following quantities of rare metals from ash: 53.2 kg As/day, 5.2 kg Pb/day, 5.0 kg Cu/day, 49 kg Zn/day, 12.3 kg Cr/day, 730 g Cd/day, and 18.9 g Hg/day.

Despite continuing research the large excess of fly-ash production over demand is likely to continue (Theis 1976a) and, coupled with an average rate of ash production of 0.5 kg/kWh (PEDCO-Environmental, Inc. 1976), will result in large amounts of ash to be disposed of in an environmentally sound manner. The new source performance standards (NSPS) applicable to new power plants prohibit discharges into natural waters from ash-settling ponds (Dvorak and Pentecost 1977). To comply with these regulations, ash from Unit II of the Columbia Generating Station is currently being held in a segregated portion of the ash basin while a site for permanent land disposal is sought and prepared.

Many concerns remain regarding the landfill disposal of coal ash. In addition to the continued threat of surface contamination due to precipitation and overland runoff, ground-water contamination and landfill erosion are significant concerns. Although many of the principles of sanitary landfilling are applicable if consideration is given to the different nature of the contaminants, an expanded study of coal-ash landfills is needed. Information on the leaching and mobility of ash trace constituents is limited (Dvorak and Pentecost 1977), and because of the newness of the disposal method, little is known of the long-term effects of such disposal. Such studies are needed for the creation of standards for land disposal of toxic sutbstances, which is virtually unregulated at the federal level (Fields and Lindsey 1975).

The most widespread concern about coal-ash landfilling is the potential for ground-water contamination by leachate produced when water percolates through the landfill. High salt concentrations in leachate may be a significant problem, especially if it reaches ground-water supplies that are already high in salt. Increased pH due to ash leachate may be a localized problem (Olsen and Warren 1976), but pH is more important because of its effects on metal solubilities and adscription. This potential for metal and other trace-element contamination has received the greatest attention and concern.

The ability of the soil to attenuate contaminants in the leachate is of primary importance in preventing ground-water contamination by any kind of landfill. Waldrip (1975) found that inorganic and organic materials from sanitary landfill leachate are adsorbed by the soil, and many undesirable ions are replaced by desirable ones in an ion-exchange process. He concluded that most ground-water contamination is limited to the immediate vicinity of the landfill because of slow movement of the ground water. The low velocity allows sufficient time for ion exchange, dilution, and dispersion to occur. The landfill contribution to ground-water supply is significantly diminished within a few hundred feet of the landfill.

Griffin et al. (1976) studied the attenuation of metals and other leachate constituents run through laboratory sediment columns. Clay was relatively poor in reducing concentrations of Cl^-, Na^+, and water-soluble organic compounds, but K, NH_4, Mg, Si, and Fe were moderately reduced in concentration, probably by cation exchange with Ca in the soil. Low leachate concentrations were strongly attenuated by small amounts of clay possibly because of precipitation of the metals upon formation of metal hydroxides or carbonates (caused by high pH and high bicarbonate concentration in the leachate). Low leachate concentrations of Al, Cu, Ni, Cr, As, SO_4, and PO_4 precluded interpretation for those substances. Suarez (1974) describes the chemical reactions involving metals leached from sanitary landfills and discusses their relationship with Eh, pH, and dissolved oxygen.

A comparison of fly-ash landfill investigations is necessary to determine the applicability of these sanitary landfill results to the landfill designed expressly for fly ash. Theis (1976b) and Theis and Marley (1976) discuss the potential for ground-water contamination from land disposal of fly ash. They determined the important characteristics of ash to be initial trace-metal concentration, acid-base characteristics, fly-ash concentration in the aquatic system, and the size-fraction distribution of the ash. A combination of field and laboratory studies demonstrated that Cr, Cu, Hg, Pb, and Zn were either released from leachate in insignificant amounts or were rapidly sorbed onto soil particles. The metals As, Ni, and Se, however, occurred in ground water at higher concentrations and appeared able to migrate a greater distance. Sorptive processes could explain the metal leachate behavior in the initial desorption of metals from the ash into water and subsequent adsorption onto the soil phase.

The investigation of a landfill for fly ash from combustion of eastern coal (Harriger 1977, Harriger et al. 1977) is the most comprehensive study to date. The presence of clay-rich soil was determined to be the most important factor affecting water quality. Other factors include composition and quality of the ash, duration of exposure to leaching, pH, oxidation conditions, and surface and ground-water flow patterns. Clay soils were relatively impermeable and found to adsorb or exchange large quantities of ions. Ground-water wells away from the landfill were lower in concentrations of many trace substances, attesting to the benefits of leachate percolation through the soil. Landfill wells often had concentrations of As, Se, Fe, Mn, and SO_4 above the U.S. Public Health Service drinking-water recommendations. Landfill wells also exhibited higher concentrations of Zn, Ca, Cr, Cu, Mg, and K than the off-site wells. The metals Ca, Cr, and Cu were fairly low, however, because of low concentrations in the ash itself, good attenuation by clay, and the prevailing pH conditions.

Analysis of surface waters (streams flowing across the landfill, runoff from the landfill, and ponds formed from precipitation) indicated few effects of the landfill once the water left the site. A stream enclosed by pipe as it crossed the site appeared to receive some ground water and ash-leachate seepage downstream. Concentrations of Fe, Mn, and SO_4 exceeded drinking-water standards, but decreased rapidly downstream. Levels of Ca, Cd, Cu, Fe, Mg, Na, Se, Zn, and SO_4 were higher and pH was lower in ponds on the landfill (especially those with exposed ash deltas) than in control ponds away from the site. Even higher concentrations of metals occurred in the sediments of the landfill ponds, indicating that the contaminants were precipitating out of the water. Metal concentrations were high in runoff water from the landfill, and concentrations higher than in ground water for Cr, Cu, and Zn were evidence of attenuation by clay and restricted metal mobility in ground water. Thus surface runoff must be contained to permit these mechanisms to operate.

The pH and oxidation states of materials in the landfill influence the effectiveness of the attenuation mechanisms. The solubility of most metal ions is increased at lower pH values (Harriger 1977), and thus in acidic leachate metals are not removed as readily by the attenuation processes. Generally, high pH greatly decreases solubility, and only Zn and Cd are considered soluble in the pH range 7 to 8.5 (Theis 1976a). Most Cr is released from ash into the leachate at pH 3, although some is released at pH 6, 9, and 12 (Theis and Wirth 1977). Iron and Mn precipitate at pH greater than 7.5 (Harriger 1977). Fields and Lindsey (1975) conclude that low pH affects ion exchange and adsorption properties of soil. Clays are more effective in adsorbing most metals when the pH is high, although a low pH is best for adsorption of organics. They state that it is best to maintain landfill soils at pH 7.0-8.0. Frost and Griffin (1977) found, however, that

As and Se adsorption by clays is decreased at high pH. Oxidation causes the formation of iron oxides and hydroxides; these precipitate from the leachate and can adsorb other ions (Harriger 1977), thus increasing the purification capacity of the soil.

The relative amounts of lime and amorphous iron oxides in the ash determine the pH of the leachate. Western coals have high amounts of lime (Theis and Wirth 1977), which account for the basic nature of the ash from the Columbia station. The greatest environmental concern with ion pH ashes is the large amount of surface leachable Fe (Theis and Wirth 1977). Theis (1976a) states that a greater amount of metal is likely to be released from ash into ground water than into surface water, because of the lower pH and high CO_2 content of ground water and the consequently greater likelihood of ion exchange from ash into this water.

Research continues into the principles of site selection and design to reduce as much as possible the threat of ground- and surface-water contamination. Little is known about the potential environmental effects of landfills in Wisconsin (Zaporozec 1974) and there have been few long-term studies of solid waste disposal in the United States. Leachate production occurs even in well-designed landfills, especially in humid areas such as Wisconsin (Fields and Lindsey 1975, Zaporozec 1974), but this production can be minimized or controlled with proper site selection and design.

Many investigators suggest the use of liners, either impervious to retain all leachate, or permeable ones to supplement the ability of the soil to attenuate pollutants (Fields and Lindsey 1975, Griffin et al. 1976, PEDCO-Environmental, Inc. 1976, Dvorak and Pentecost 1977). Where clay in native soils is insufficient, a clay liner can satisfactorily mitigate the contamination threat. It has been suggested that ash landfills may have the capacity to seal themselves against leachate loss. As soluble CaO moves into the soil and forms $CaCO_3$, the permeability of the soil may be significantly reduced (Olson and Warren 1976). Fly ash is often deliberately applied to sanitary landfills because of its moisture-adsorbing characteristics (PEDCO-Environmental, Inc. 1976).

Other suggestions to reduce the potential of contamination include vegetating the landfill to reduce erosion by wind or water. Harriger (1977) found that erosion remained a problem when the ash was covered with bare soil. PEDCO-Environmental, Inc. (1976) suggests the use of species tolerant to high pH, boron, and salt. Recommendations for sanitary landfills in southern Indiana include: use of upland sites to avoid runoff from upland areas, sites with soils or intervening materials with high exchange and adsorption capacities, and sites where the water table is much below the bottom of the waste; use of leachate lagoons to prevent surface-water contamination, and avoidance of areas subject to flooding (Waldrip and Ruhe 1974). PEDCO-Environmental, Inc. (1976) presents a detailed discussion of geological, chemical, and engineering aspects of landfill site selection and design. A literature review by Heidman and Brunner (1976) lists references concerning site locations, investigation, monitoring, and management for sanitary landfills. Much of the information in both reports is also applicable to coal-ash landfills.

Several states and agencies have criteria and regulations that should be considered in the construction of coal-ash landfills in Wisconsin. The California State Water Resources Control Board (1975) lists the following: (1) Underlying geological formations with questionable permeability must be permanently sealed, or ground-water conditions must prevent hydrologic continuity; (2) leachate and subsurface flow must be self-contained; (3) sites must not be located over zones of active faulting; (4) limitations are applied if the area is in a 100-yr (or more frequent) flood-frequency class. The U.S. Environmental Protection Agency (1973) recommends the following criteria: (1) Low population density; (2) low alternate land-use value; (3) low ground-water-contamination potential; (4) away from flood plains, excessive slopes, and natural depressions; (5) soil with high clay content; (6) adequate distance from human and livestock water supplies; (7) areas of low rainfall and high evaporation rates, where possible; (8) sufficient elevation over the water table; (9) no hydrologic connection with ground or surface water; (10) use of encapsulation, liners, waste detoxification, or solidification/fixation where necessary; (11) adequate monitoring. Consideration of all these suggestions will significantly reduce, if not avoid entirely, the adverse effects that a fly-ash landfill might have on environmental quality.

It appears that the high pH of the ash expected from Columbia II will substantially reduce the pollution potential from a landfill. The landfill site must be chosen carefully, however, to avoid direct connection with the ground water. A clay or other type of liner will probably be beneficial, if not required, to avoid ground-water contamination. Pipes to collect and recirculate leachate should be used if there is any likelihood of less than complete metal attenuation by the time the leachate reaches the ground water.

SUMMARY

1. Fly ash may be used commercially for a variety of purposes but supply will probably continue to exceed demand (Theis 1976a, PEDCO-Environmental, Inc. 1976, Theis and De Pinto 1976, Harriger 1977).

2. Although recent air and water pollution standards prohibit the discharge of ash or its leachate into surface waters, considerable concern has arisen over the potential adverse effects of the dry disposal of fly ash in landfills.

3. Metal and trace-element contamination of water, particularly ground water, is the most serious concern. Soils vary widely in their abilities to attenuate these pollutants.

4. Clay soils have the greatest capacity for metal adsorption and ion exchange (Griffin et al. 1976, Theis 1976b, Theis and Marley 1976, Harriger 1977).

5. Because of these mechanisms, and the dilution and dispersion in slow-moving ground water, most ground-water contamination is limited to the immediate vicinity of the landfill (Waldrip 1975, Harriger 1977).

6. With proper precautions direct surface-water contamination is usually minimal (Harriger 1977). Appropriate precautions include containment of surface runoff and avoidance of low sites and steep slopes.

7. Better attenuation of metals is usually obtained when the leachate has a high pH. This is caused by the reduced solubility of metals and improved properties of clay under these conditions (Fields and Lindsey 1975, Theis 1976a, Harriger 1977). Fortunately, coal burned at the Columbia Generating Station produces basic conditions in its ash.

8. Where natural soils are not sufficient, clay or impervious liners should be applied to the landfill (PEDCO-Environmental, Inc. 1976, Dvorak and Pentecost 1977). Fly ash appears to have some capacity to form a seal itself (Olson and Warren 1976).

9. Other recommendations to reduce the potential environmental contamination include covering with soil; encouraging vegetation; containing leachate; adequate monitoring; and avoiding sites with high ground water; flooding potential; active faulting; or low elevations.

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16. ABSTRACT

Construction of a coal-fired electric generating station on wetlands adjacent to the Wisconsin River has permanently altered about one-half of the original 1,104-ha site. Change in the remaining wetlands continues as a result of waste heat and ashpit effluent produced by the station. Leakage of warm water from the 203-ha cooling lake is causing a shift in the wetlands from shallow to deep-water marsh. Coal-combustion byproducts enter the wetlands from the station's ashpit drain. Since this area was known to have a diverse fish community and to be a spawning ground for Wisconsin River game fish, we studied the effects of this habitat loss and degradation on fish populations. In laboratory experiments we investigated the use of temperature preference and activity as a sublethal bioassay. In selection experiments we examined the potential of fish to evolve metal-tolerant populations in chronically contaminated environments.

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