EFFECTS OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) ON pH, NET OXYGEN PRODUCTION, AND RESPIRATION BY ALGAE

Great Lakes Fishery Commission

TECHNICAL REPORT 63

The Great Lakes Fishery Commission was established by the Convention on Great Lakes Fisheries between Canada and the United States, which was ratified on October 11, 1955. It was organized in April 1956 and assumed its duties as set forth in the Convention on July 1, 1956. The Commission has two major responsibilities: first, develop coordinated programs of research in the Great Lakes, and, on the basis of the findings, recommend measures which will permit the maximum sustained productivity of stocks of fish of common concern; second, formulate and implement a program to eradicate or minimize sea lamprey populations in the Great Lakes.

The Commission is also required to publish or authorize the publication of scientific or other information obtained in the performance of its duties. In fulfillment of this requirement the Commission publishes the Technical Report Series, intended for peer-reviewed scientific literature; Special Publications, designed primarily for dissemination of reports produced by working committees of the Commission; and other (non-serial) publications. Technical Reports are most suitable for either interdisciplinary review and synthesis papers of general interest to Great Lakes fisheries researchers, managers, and administrators, or more narrowly focused material with special relevance to a single but important aspect of the Commission's program. Special Publications, being working documents, may evolve with the findings of and charges to a particular committee. Both publications follow the style of the *Canadian Journal of Fisheries and Aquatic Sciences*. Sponsorship of Technical Reports or Special Publications does not necessarily imply that the findings or conclusions contained therein are endorsed by the Commission.

March 1999

EFFECTS OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) ON pH, NET OXYGEN PRODUCTION, AND RESPIRATION BY ALGAE

Ronald J. Scholefield
U. S. Geological Survey
Biological Resources Division
Great Lakes Science Center
Hammond Bay Biological Station
11188 Ray Road
Millersburg, MI 49759

Kim T. Fredricks
Viterbo College
815 South 9th
La Crosse, WI 54601

Karen S. Slaght
U. S. Geological Survey
Biological Resources Division
Great Lakes Science Center
Hammond Bay Biological Station
11188 Ray Road
Millersburg, MI 49759

James G. Seelye
U.S. Geological Survey
Biological Resources Division
Columbia River Research Laboratory
Cook, WA 98605

TECHNICAL REPORT 63

Great Lakes Fishery Commission
2 100 Commonwealth Blvd., Suite 209
Ann Arbor, MI 48105-l 563

March 1999

TR63/03-99/2500
Printed on recycled paper

TABLE OF CONTENTS

EFFECTS OF THE LAMPRICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL (TFM) ON pH, NET OXYGEN PRODUCTION, AND RESPIRATION BY ALGAE

Ronald J. Scholefield

Kim T. Fredricks

Karen S. Slaght

James G. Seelye

ABSTRACT. The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been used in the United States and Canada for more than 35 years to control larval sea lampreys *(Petromyzon marinus)* in tributaries of the Great Lakes. Occasionally, during stream treatments with TFM, nontarget-fish mortality reaches unacceptable levels. These losses could be due to the presence of sensitive fish species, excess TFM, or a combination of factors that influence the toxicity of TFM, such as delays in daily stream reaeration by algae resulting in extended periods of low pH and low dissolved oxygen (DO). We determined the-effects of a broad range of TFM concentrations on net DO production and respiration by two species of algae, in two culture media (high alkalinity and low alkalinity). The pH and DO in cultures of *Chlorella pyrenoidosa* and *Selenastrum capricornutum* were recorded at time zero and again after a 9-h exposure to TFM under either

lighted or dark conditions. Algal cultures exposed to TFM concentrations typical of those used to control sea lampreys in streams showed only small changes in pH (< 0.1) and small reductions in DO (about 8% in lighted conditions and 11% in dark conditions). Changes in pH and DO of this magnitude probably do not change the efficacy of TFM or cause nontarget fish mortality if algae are the predominant photosynthetic organisms in the stream.

INTRODUCTION

In the United States and Canada, 3-trifluoromethyl-4-nitrophenol (TFM) has been used for more than 35 years to kill larval sea lampreys *(Petromyzon* marinus) in tributaries of the Great Lakes (Applegate et al. 1961). Lieffers (1990) reported that sea lamprey-infested streams usually are treated for 8 to 14 h with TFM at concentrations based on the LC 100 values for larval sea lampreys. Kanayama (1963) correlated the toxicity of TFM with the alkalinity and conductivity of the stream water and developed a guideline for estimating the concentration of TFM needed to treat a sea lamprey-infested stream. Seelye et al. (1988) also developed a TFM-alkalinity regression equation and toxicity charts to estimate the concentration of TFM to be used in stream treatments across a broad range of alkalinities. Klar et al. (1993) reported that the stream treatment application rate of TFM (used by United States Fish and Wildlife Service) is based on water alkalinity, pH, on-site toxicity tests, and past stream-treatment history. A pH-alkalinity prediction chart was developed at the Hammond Bay Biological Station to identify the 9-h minimum lethal concentration of TFM (LC99.9 for larval sea lampreys) and the maximum allowable concentration of TFM (LC25 for nontarget brown trout *(Salmo trutta)).*

Significant, nontarget-fish mortality seldom occurs during TFM stream treatments but can sometimes reach unacceptable levels (Dahl and McDonald 1980). These unacceptable losses could be due to the

presence of sensitive fish species, excess TFM, or a combination of factors that influence the toxicity of TFM, such as delays in daily stream reaeration by algae or extended periods of low pH. For example, TFM treatments of streams that exhibit diurnal variations in pH and dissolved oxygen (DO) could harm fishes and other aquatic organisms if TFM inhibits oxygen production, limits reaeration of the water, or results in abnormally low pH and DO levels. Seelye and Scholefield (1990) reported that low DO levels alone do not increase the toxicity of TFM to larval sea lampreys or rainbow trout *(Oncorhynchus mykiss).* However, lower pH increases the toxicity of TFM to larval sea lampreys and nontarget fish (Dawson et al. 1975; Marking and Olson 1975; Bills et al. 1988; Bills and Johnson 1992). Consequently, exposure to TFM at reduced pH increases the toxicity of TFM to both larval sea lampreys and nontarget aquatic organisms.

Previous studies (Gilderhus et al. 1975; Maki et al. 1975; Maki and Johnson 1976; Dawson et al. 1992) showed that TFM affects growth, oxygen production, and metabolism of aquatic macrophytes and algae, which may lower stream pH and DO. Gilderhus et al. (1975) reported that aquatic vegetation absorbed TFM and that algae absorbed TFM more readily than aquatic macrophytes. Maki and Johnson (1976) reported that TFM alters community metabolism-when TFM was present, algal oxygen production decreased and respiration increased. However, the results of Maki and Johnson (1976) are difficult to extrapolate to conventional TFM stream treatments because they used a longer exposure period (24 h) and a TFM concentration at least two times greater than that routinely used today to control sea lampreys in streams with similar pH and alkalinity.

If a TFM treatment inhibits oxygen production and enhances respiration, the slow recovery of stream pH and DO may extend the normal diurnal decreases in pH and DO, thus increasing the toxicity of TFM. The net result may be unacceptable mortality of nontarget fish. Hence, our objective was to examine, under controlled laboratory conditions, the effects of TFM on algal net oxygen production, reaeration, and respiration during a 9-h TFM exposure at stream-treatment concentrations.

METHODS

Our experimental design consisted of four treatments:

- Two species of algae
- Two alkalinities
- Five concentrations of TFM plus a control
- Two metabolic conditions (photosynthesis and respiration)

Each species of algae was tested under four different conditions. Each test consisted of preparing a fresh high alkalinity (HA) or low alkalinity (LA) algal test solution and running the 9-h TFM-algal exposure tests under lighted or dark conditions. At least four replicates were run for each of the test conditions; however, some tests were repeated five or six times because of unexpected results.

TFM

The lampricide TFM (37.1% active ingredient, isopropanol formulation) was supplied by the American Hoechst Corporation and is the same formulation used in the United States and Canada to control larval sea lampreys in tributaries of the Great Lakes. The TFM concentrations were based on active ingredient (3-trifluoromethyl-4-nitrophenol) and were quantified by the spectrophotometric method described by Smith et al. (1960).

Culture Procedures

We choose *Chlorella pyrenoidosa* and *Selenastrum capricornutum* for our experiments because they are:

- Found in tributaries of the Great Lakes
- Easy to culture
- Easy to enumerate in culture

These latter features assure equal concentrations of algal cells among containers, treatments, and tests. Monocultures of *C. pyrenoidosa* and S. *capricornutum* were obtained from the Carolina Biological Supply Company and cultured under sterile conditions (American Public Health Association 1989) in a water bath-the temperature of the water bath was continuously recorded by a thermograph. The cultures were held at 21°C under continuous fluorescent light (two 200-W bulbs, about $6W/m^2$), aerated continuously, and maintained in logarithmic growth phase by transferring a small volume of culture (3-5 mL containing 1 x 10^5 - 1 x 10^6 cells/mL) to fresh medium each week using sterile procedures. A hemacytometer and compound microscope were used to determine the algal cell densities in the cultures. Two types of culture media were made by adding nutrients to either HA reconstituted water (alkalinity 200 mg/L as $CaCO_3$, hardness 260 mg/L as $CaCO_3$, and pH 8.4) or LA reconstituted water (alkalinity 30 mg/L as $CaCO_3$, hardness 43 mg/L as $CaCO_3$, and pH 7.9) (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975).

Lighted and Dark TFM-Exposure Tests

To determine the effects of TFM on net oxygen production and oxygen uptake of algae, an algal culture was exposed to TFM for 9 h in either lighted or completely dark conditions. Our experimental design required that initial algal-cell concentration be equal among test vessels. Test vessels were 60-mL biological oxygen demand (BOD) bottles. The BOD bottles used for dark conditions were coated with a black rubber material from Plasti Dip, PDI INC. A teflon-coated magnetic stirring bar was placed into each BOD bottle to mix the sample when determining pH and DO values after the 9-h TFM exposure.

Before each exposure, BOD bottles, stirring bars, and culture media were sterilized by autoclaving (National Appliance Company, Steril-Quik, Model 704-9000-D-9). About 3,800 mL of algal test solution was prepared in a magnetically stirred 4-L container. A suitable volume of algae, determined by preliminary 9-h tests, was added to either the HA (177-204 mg/L as $CaCO_3$) or LA (39-75 mg/L as $CaCO_3$) culture medium to achieve cell concentrations that ranged from:

- 1 to 3 x 104 cells/ml for the lighted, oxygen-production tests
- 1 to 3 x 105 cells/ml for the dark, oxygen uptake (respiration) tests

Although greater than under natural stream conditions, these algal cell concentrations were needed to produce measurable changes in DO under the constraints of our laboratory experiment.

For tests in lighted conditions, carbon dioxide and nitrogen were bubbled through the algal test solutions in the 4-L container. The carbon dioxide was used to adjust pH between:

- 8.3 and 8.5 for the HA test solutions
- 7.8 and 8.0 for the LA test solutions

These pH values approximated the pH values found in streams with alkalinities of 200 and 30 mg/L as $CaCO_3$. The nitrogen was used to reduce the DO to about 3 mg/L so that 9-h oxygen production and reaeration could be determined without the samples becoming supersaturated. For tests in dark conditions, carbon dioxide and air were bubbled through the algal test solutions. The carbon dioxide was used to adjust the pH of the test solutions. The air was used to raise the DO level to about 8.8 mg/L (saturation at 21°C) so that the reduction in oxygen could be determined.

While filling the BOD bottles, we:

- Stirred the algal test solution to prevent stratification and stabilize pH and DO readings

- Continuously monitored pH with a Beckman portable Φ F12 pH meter

- Continuously monitored DO with a Wheaton Model 8000 BOD Measurement System

To minimize changes in pH or DO values while filling the bottles, latex tubing was connected to an outlet at the bottom of the 4-L container and used to transfer the algal test solution by gravity flow to the BOD bottles. We followed the DO sampling procedures specified by the American Public Health Association (1989) for sampling from a supply line under pressure. Initial pH and DO values were recorded as each BOD bottle was filled.

Controls

Three control BOD bottles were filled to overflowing with the algal test solution and immediately capped to prevent gas exchange with the atmosphere. Another sample of the solution was removed from the 4-L container and used to determine alkalinity and hardness.

Addition of TFM

An initial volume of TFM was added to the 4-L container holding the remaining stirred algal test solution. Another three BOD bottles were then filled to overflowing and immediately capped. A sample of the TFM-algal test solution was removed from the 4-L container and used to determine the TFM concentration.

A second volume of TFM was added to the TFM-algal test solution to increase the concentration of TFM to the next level. Three more BOD bottles were filled, and another sample of the algal test solution was obtained and used to determine the TFM concentration.

This TFM addition and sampling process was repeated three more times, thereby creating five concentrations of TFM for testing. The five TFM concentrations ranged from:

- 3.0 to 30 mg/L for the HA algal test solution
- 0.3 to 17 mg/L for the LA algal test solution

The 18 BOD bottles (3 controls and 15 TFM-algal test samples) were placed in the lighted water bath and maintained at 21°C for 9 h. The temperature of the water bath was continuously recorded by a thermograph. At the end of the 9-h exposure period, pH, DO, and algal-cell concentration were determined for each BOD bottle. The 9-h exposure period corresponded to the minimum time during a stream treatment that TFM is maintained at or above the LC99.9 for larval sea lampreys (Kanayama 1963).

We used a pH/total alkalinity prediction chart (Klar 1993) to estimate the minimum lethal concentration of TFM (LC99.9 for larval sea lampreys) and the maximum allowable concentration of TFM (LC25 for nontarget brown trout). The LC99.9 values for larval sea lampreys ranged from 5.2 to 6.6 mg/L for the HA tests and from 1.1 to 1.9 mg/L for the LA tests. The LC25 for nontarget brown trout ranged from 12.8 to 17.8 mg/L for the HA tests and from 6.1 to 8.8 mg/L for the LA tests.

Data Analysis

The indirect effect of TFM on pH and DO levels and algal-cell concentrations was analyzed by comparing the 9-h exposure data for the controls with the TFM-algal test samples using both the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney test for multiple comparison (Sigma Stat 1994). Significance was defined asp $\leq$ 0.05.

RESULTS

TFM generally affected *C. pyrenoidosa* less than it affected S. *capricornutum.* Compared to the controls, a 9-h exposure to TFM concentrations equal to the LC99.9 for larval sea lampreys resulted in minor reductions in pH (< 0.1) and DO (< 20%) both in lighted and dark conditions (Figs. l-4). Specifically, with TFM levels at or less than the LC99.9 values, pH changes were not significantly different than those in the control. Even when the TFM concentrations were increased to the LC25 for nontarget brown trout, the change in pH was < 0.2 (Figs. 1, 2). The final algal-cell concentrations did not differ between the controls and the samples exposed to TFM for 9 h.

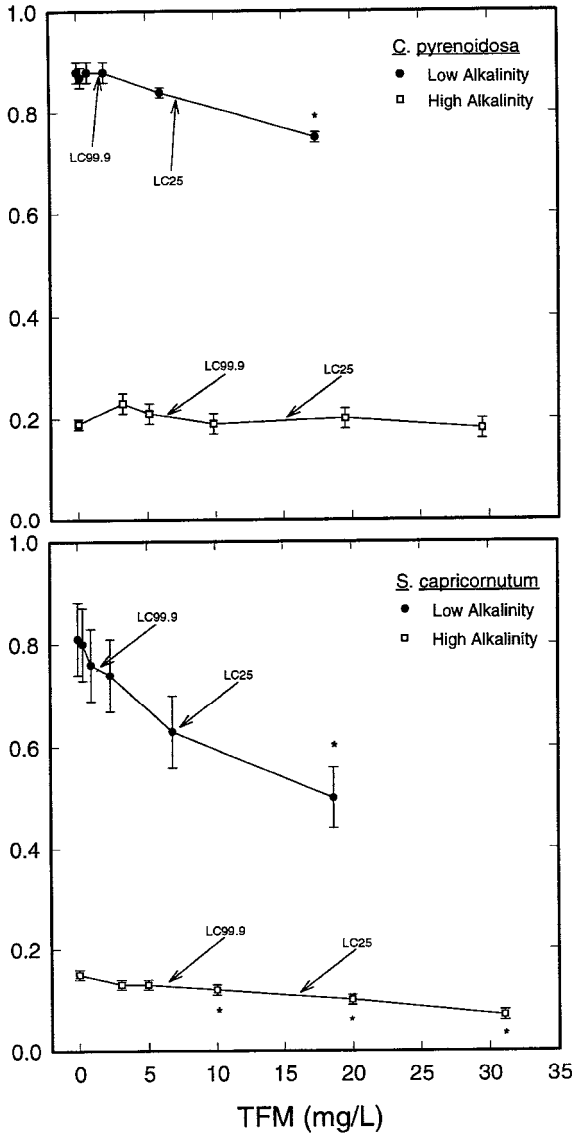

Figure 1. Changes in pH after exposing *Chlorella pyrenoidosa* and *Selenastrum capricornutum* to TFM for 9 h under lighted conditions. The LC99.9 value refers to the concentration of TFM predicted to kill 99.9% of the sea lampreys. The LC25 value refers to brown trout and the maximum permissible concentration of TFM that could be used in a stream treatment. An asterisk (*) indicates significant difference from control.

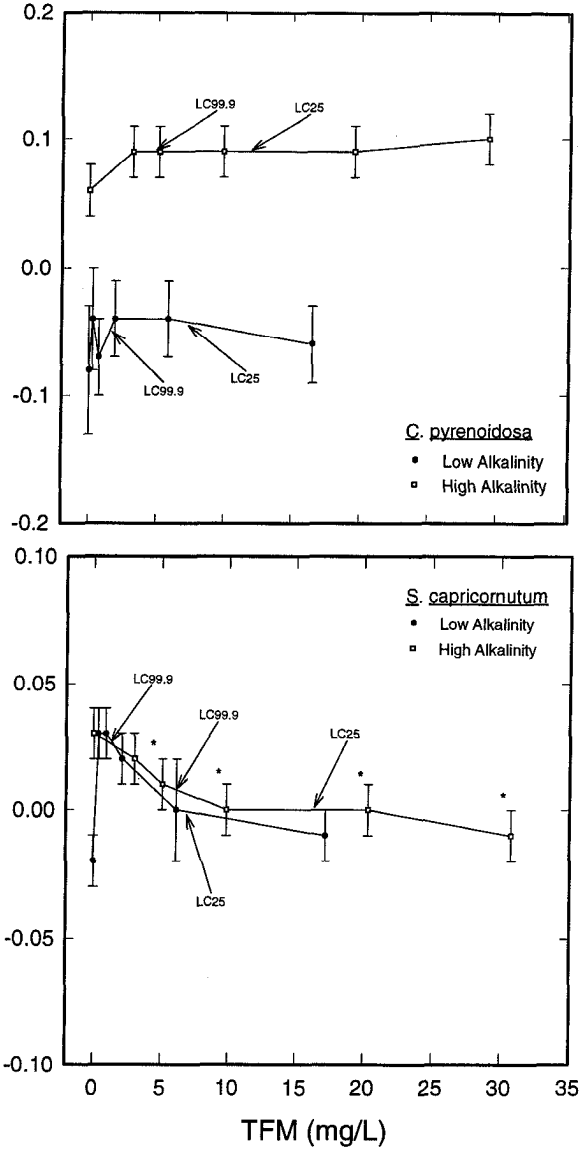

Figure 2. Changes in pH after exposing *Chlorella pyrenoidosa* and *Selenastrum capricornutum* to TFM for 9 h under dark conditions. The LC99.9 value refers to the concentration of TFM predicted to kill 99.9% of the sea lampreys. The LC25 value refers to brown trout and the maximum permissible concentration of TFM that could be used in a stream treatment. An asterisk (*) indicates significant difference from control.

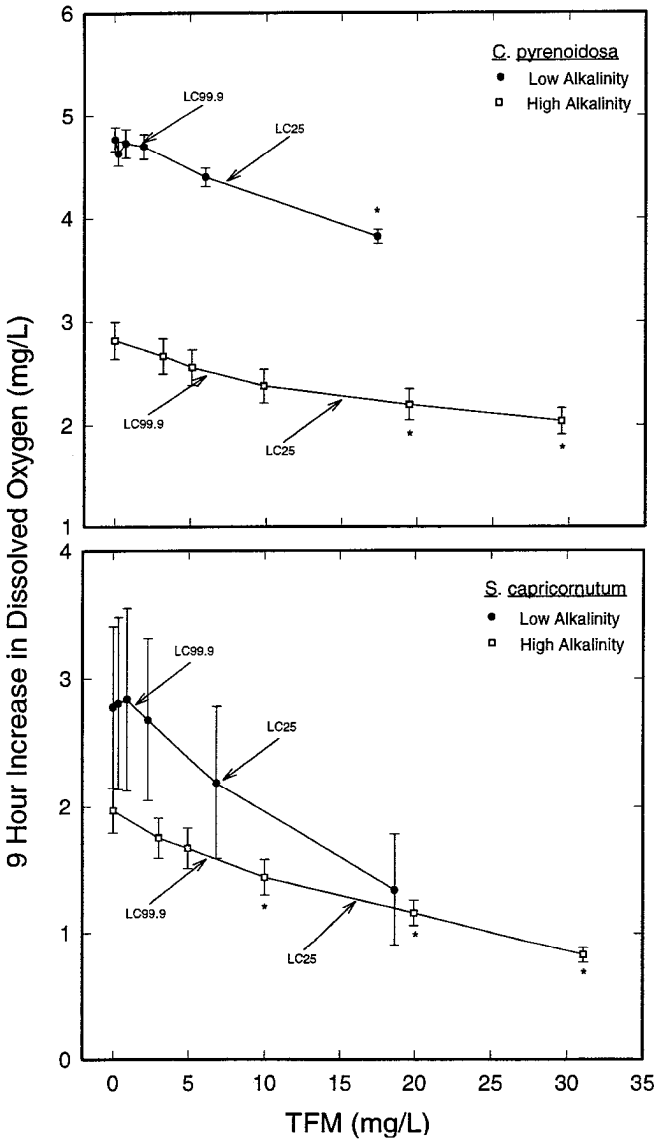

Figure 3. Changes in dissolved oxygen after exposing *Chlorella pyrenoidosa* and *Selenastrum capricornutum* to TFM for 9 h under lighted conditions. The LC99.9 value refers to the concentration of TFM needed to kill 99.9% of the sea lampreys. The LC25 value refers to brown trout and the maximum permissible concentration of TFM that could be used in a stream treatment. An asterisk (*) indicates significant difference from control.

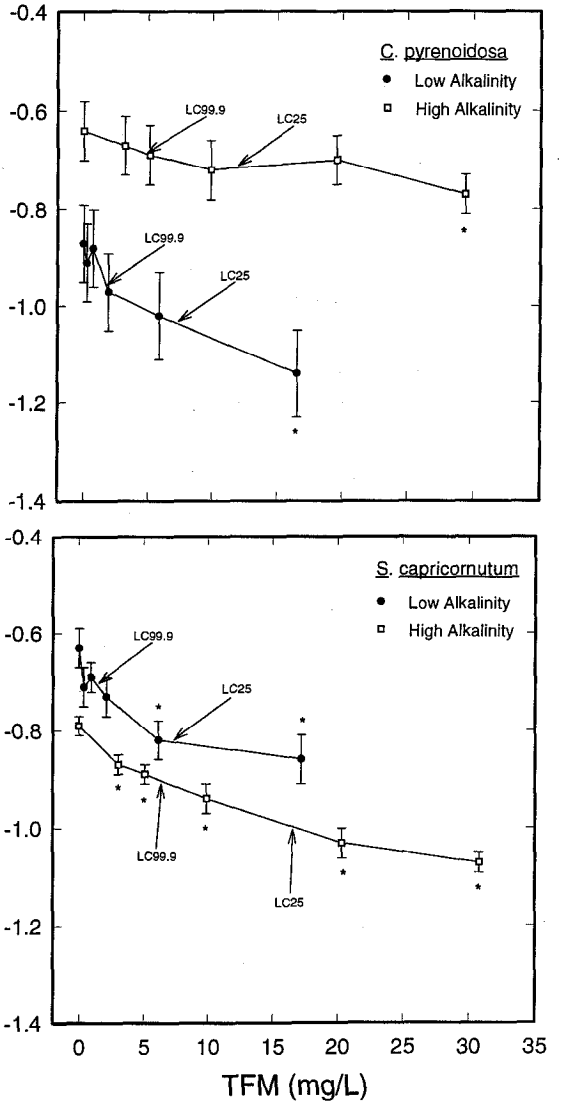

Figure 4. Changes in dissolved oxygen after exposing *Chlorella pyrenoidosa* and *Selenastrum capricornutum* to TFM for 9 h under dark conditions. The LC99.9 value refers to the concentration of TFM needed to kill 99.9% of the sea lampreys. The LC25 value refers to brown trout and the maximum permissible concentration of TFM that could be used in a stream treatment. An asterisk (*) indicates significant difference from control.

Photosynthesis (Lighted) Experiments

Oxygen production in the HA test solution was generally lower than in the LA test solution and was greater for C. *pyrenoidosa* than it was for S. *capricornutum* "(Fig 3)". When the algae were exposed to TFM, oxygen production was slightly inhibited. C. *pyrenoidosa* appeared more resistant to TFM than the S. *capricornutum* (Fig. 3).

In the HA algal test solution with TFM at the LC99.9 concentration for larval sea lampreys, final DO levels in the test vessels were about 11% lower than in the controls for *C. pyrenoidosa (N* = 4) and 19% lower for *S. capricornutum (N* = 5). With TFM at the LC25 concentration for brown trout, final DO levels were about 20% lower than in the controls for C. *pyrenoidosa* and *37%* lower for *S. capricornutum.* For C. *pyrenoidosa,* the final DO levels did not differ significantly between the controls and the samples containing TFM at concentrations lower than the LC25. However, for *S. capricornutum* with TFM levels greater than the LC99.9 for larval sea lampreys, DO levels were significantly lower than those in the controls (Fig. 3).

In the LA test solution with TFM at the LC99.9 concentration for larval sea lampreys, final mean DO levels were about 2% lower than in the controls for C. *pyrenoidosa (N = 4).* For *S. capricornutum (N = 6),* no significant difference was found in the DO levels in the test solution exposed to TFM at the LC99.9 and the controls. With TFM at the LC25 concentration for brown trout, final mean DO levels were about 9% lower than in the controls for *C. pyrenoidosa* and about 21% lower for S. *capricornutum.* These differences were not significant.

Respiration (Dark) Experiments

Exposure of algae to TFM in tests conducted in darkness resulted in a pH change of < 0.1 and a slight increase in oxygen uptake, for both species of algae (Figs. *2, 4).* However, for *C. pyrenoidosa,* neither pH nor DO levels differed significantly between controls and samples for

tests in which TFM was present at concentrations equal to or less than the LC25 concentration.

In the HA algal test solution with TFM at the LC99.9 concentration, oxygen uptake increased by about 8% as compared to the controls for C. *pyrenoidosa (N* = 4) and increased by about 14% for *S. capricornutum* (N = 5). With TFM at the LC25 concentration, oxygen uptake increased by about 13% as compared to the controls for *C. pyrenoidosa* and increased by about 28% for *S. capricornutum* (Fig. 4). For S. *capricornutum* in the HA test solution, oxygen uptake differed significantly between the controls and samples with TFM concentrations at or above the LC99.9 (Fig. 4).

In the LA algal test solution with TFM at the LC99.9 concentration, oxygen uptake increased by about 9% as compared to the controls for C. *pyrenoidosa (N* = 6) and by about *13%* for *S. capricornutum (N = 5).* With TFM at the LC25 concentration, oxygen uptake increased by about 18% compared to the controls for *C. pyrenoidosa* and by about 32% for *S. capricornutum.* The increased oxygen uptake was significant for *S. capricornutum* at the LC25 concentrations (Fig. 4).

DISCUSSION

The results of our study indicate that 9-h TFM stream treatment at the LC99.9 concentration for sea lampreys would change the pH by 0.1 or less in lighted and dark conditions (Figs. 1, 2). These small changes in pH should have little effect on the toxicity of TFM.

In this study, a 9-h exposure to TFM at LC99.9 concentrations for sea lampreys reduced DO production by about 8% in lighted tests and increased respiration by about 11% in dark tests. These results are similar to those of Maki and Johnson (1976). They reported that TFM at a concentration of 9 mg/L decreased community oxygen production by 5% to 10% and increased community respiration by 3% to 50%. Dawson et al. (1992) also reported that TFM inhibited photosynthetic

oxygen production during daylight, but they found no decrease in DO levels in tests conducted in the shade.

The decrease in stream DO is probably related to the uptake of TFM by aquatic plants (Gilderhus et al. 1975), which may inhibit photosynthesis. Hawxby et al. (1977) noted that the most toxic herbicides are those that inhibit photosynthesis rather than some other metabolic process. Huang and Gloyna (1968) reported that the toxic characteristics of phenolic compounds appear to be a function of the substituent groups and their relative substitution position. They also reported that nitrated (para substituted isomers) and halogenated (meta substituted isomers) phenols are the most toxic. The lampricide TFM is a nitrated (para nitro substitute) and halogenated (meta trifluoromethyl substitute) phenolic compound and may impair photosynthesis in aquatic plants.

Maki and Johnson (1976) reported that, in a simulated stream (alkalinity 179 mg/L as $CaCO_3$, hardness 211 mg/L as $CaCO_3$, and pH 7.8) exposure to TFM at a concentration of 9 mg/L did not permanently impair oxygen production by aquatic plants. Oxygen production and respiration rates returned to pre-TFM exposure rates within 24 to 36 h, possibly because TFM residues in aquatic plants rapidly decrease by about 89% to 97% after 24 h when exposed to TFM-free water (Gilderhus et al. 1975). Both studies demonstrated the natural ability of a stream ecosystem to adjust to a temporary exposure to TFM and return to normal conditions within 1 to 4 d. Thus, our data also support the idea that TFM treatments at the LC99.9 concentration for sea lampreys are unlikely to inhibit natural reaeration of a treated stream.

Seelye and Scholefield (1990) reported that low DO alone did not increase the toxicity of TFM to sea lampreys or brown trout. If algae are the predominant source of oxygen in a stream and stream DO levels are not already depressed by other factors, TFM concentrations applied for sea lamprey control should not greatly affect net oxygen production or the available DO in a stream for nontarget fishes.

We conclude that a 9-h TFM stream treatment for sea lamprey control:

- Would change pH levels < 0.1
- Could decrease DO levels from about 2% to 19%
- Would not affect algal concentrations

If algae are the predominant source of oxygen in a stream, TFM concentrations used in most sea lamprey control treatments should not:

- Inhibit algal stream reaeration after a typical diurnal decline in oxygen
- Substantially affect algal net oxygen production or oxygen uptake
- Change DO or pH enough to cause substantial nontarget-fish mortality

Although care must be taken when extrapolating laboratory results to the field, the lack of substantial change in algal-cell concentration, oxygen production, oxygen uptake, or pH indicates that algae should not be greatly affected by sea lamprey control activities.

ACKNOWLEDGMENTS

We thank R. Berryhill and J. Locke for their technical assistance in the laboratory.

REFERENCES

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1989. Standard methods for the examination of water and wastewater, 17th edition. APHA, Washington, D.C.

Applegate, V.C., J.H. Howell, J.W. Moffett, B.G.H. Johnson, and M.A. Smith. 1961. Use of 3-trifluoromethyl-4-nitrophenol as a selective sea lamprey larvicide. Great Lakes Fish. Comm. Tech. Rep. 1: l-35.

Bills, T.D., L.L. Marking, G.E. Howe, and J.J. Rach. 1988. Relation of pH to toxicity of lampricide TFM in the laboratory. Great Lakes Fish. Comm. Tech. Rep. 53: 9-16.

Bills, T.D., and D.A. Johnson. 1992. Effect of pH on the toxicity of TFM to sea lamprey larvae and nontarget species during a stream treatment. Great Lakes Fish. Comm. Tech. Rep. 57: 7-19.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecol. Res. Ser., EPA-660/3-75-009, U.S. Environ. Prot. Agency, Washington, D.C.

Dahl, F.H., and R.B. McDonald. 1980. Effect of control of sea lamprey *(Petromyzon marinus)* on migratory and resident fish populations. Can. J. Fish. Aquat. Sci. 37: 1886-I 894.

Dawson, V.K., K.B. Cummings, and P.A. Gilderhus. 1975. Laboratory efficacy of 3-trifluoromethyl-4-nitrophenol (TFM) as a lampricide. U.S. Fish Wildl. Serv. Invest. Fish Control 63: l-13.

Dawson, V.K., D.A. Johnson, and J.F. Sullivan. 1992. Effect of the lampricide 3-trifluoromethyl-4-nitrophenol on dissolved oxygen in aquatic systems. Great Lakes Fish. Comm. Tech. Rep. 57: 21-33.

Gilderhus, P.H., J.B. Sills, and J.L. Allen. 1975. Residues of 3-trifluoromethyl-4-nitrophenol (TFM) in a stream ecosystem after treatment for control of sea lampreys. U.S. Fish Wildl. Serv. Invest. Fish Control 66: l-7.

Hawxby, K., B. Tubea, J. Ownby, and E. Basler. 1977. Effects of various classes of herbicides on four species of algae. Pestic., Biochem., Physiol. 7: 203-209.

Huang, J., and E.F. Gloyna. 1968. Effect of organic compounds on photosynthetic oxygenation-I. chlorophyll destruction and suppression of photosynthetic oxygen production. Water Res. 2: 347-366.

Kanayama, R.K. 1963. The use of alkalinity and conductivity measurements to estimate concentrations of 3-trifluoromethyl-4-nitrophenol required for treating lamprey streams. Great Lakes Fish. Comm. Tech. Rep. 7: I-10.

Klar, G.T., D.A. Johnson, and T.J. Morse. 1993. pH-alkalinity prediction chart. Manual for the application of lampricides in the U.S. Fish and Wildlife Service sea lamprey *(Petromyzon marinus)* control program including standard operating procedures, Appendix V, p. 278. U.S. Fish Wildl. Serv., Marquette Biological Station.

Lieffers, H.J. 1990. Effects of the lampricide 3-trifluoromethyl-4-nitrophenol on macroinvertebrate populations in a small stream. Great Lakes Fish. Comm. Tech. Rep. 55: l-26.

Maki, A.W., L.D. Geissel, and H.E. Johnson. 1975. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to IO species of algae. U.S. Fish Wildl. Serv. Invest. Fish Control 56: 1-17.

Maki, A.W., and H.E. Johnson. 1976. Evaluation of a toxicant on the metabolism of model stream communities. J. Fish. Res. Board Can. 83: 2740-2746.

Marking, L.L. and L.E. Olson. 1975. Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to non-target fish in static tests. U.S. Fish Wildl. Serv. Invest. Fish Control 60: l-27.

Seelye, J.G., D.A. Johnson, J.G. Weise, and E.L. King, Jr. 1988. Guide for determining application rates of lampricides for control of sea lamprey ammocetes. Great Lakes Fish. Comm. Tech. Rep. 52: l-23.

Seelye, J.G., and R.J. Scholefield. 1990. Effects of changes in dissolved oxygen on the toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to sea lamprey and brown trout. Great Lakes Fish. Comm. Tech. Rep. 56: 6-13.

Sigma Stat. 1994. Statistical Software. Jandel Scientific, San Rafael, CA.

Smith, M.A., V.C. Applegate, and B.G.H. Johnson. 1960. Calorimetric determination of halogenated nitrophenols added to streams as sea lamprey larvicides. Anal. Chem. 32: 1670-I 675.

Technical Report Series

1 Use of 3-trifluormethyl-4-nitrophenol as a selective sea lamprey larvicide. 1961. V. C. Applegate, J. H. Howell, J. W. Moffett, B. G. H. Johnson, and M. A. Smith 36 p.

2 Fishery statistical districts of the Great Lakes. 1961. S. H. Smith, H. J. Buettner, and R. Hile. 24 p.

3 Commercial fish production in the Great Lakes 1867.1977. 1979. N. S. Baldwin, R. W. Saalfeld, M. A. Ross, and H. J. Buettner. 192 p. (Supersedes 1962 edition and 1970 supplement.)

4 Estimation of the brook and sea lamprey ammocoete populations of three streams. 1962. B. R. Smith and A. L. McLain. p. l-1 8. A photoelectric amplifier as a dye detector. 1962. W. J. Ebel. p. 19-28.

5 Collection and analysis of commercial fishery statistics in the Great Lakes. 1962. R. Hile. 34 p.

6 Limnological survey of Lake Erie 1959 and 1960. 1963. A. M. Beeton. 34 p.

7 The use of alkalinity and conductivity measurements to estimate concentrations of 3-trifluormethyl-4-nitro-phenol required for treating lamprey streams, 1963. R. K. Kanayama. 10 p.

8 Synergism of 5, 2'-dichloro-4'-nitro-salicylanilide and 3-trifluormethyl-4-nitrophenol in a selective lamprey larvicide. 1964. J. H. Howell, E. L. King, Jr., A. J. Smith, and L. H. Hanson. 22 p.

9 Detection and measurement of organic lampricide residues. 1965. S. L. Daniels, L. L. Kempe, T. J. Billy, and A. M. Beeton. 18 p.

10 Experimental control of sea lampreys with electricity on the south shore of Lake Superior, 1953-60. 1965. A. L. McLain, B. R. Smith, and H. H. Moore. 48 p.

11 The relation between molecular structure and biological activity among mononitrophenols containing halogens. 1966. V. C. Applegate, B. G. H. Johnson, and M. A. Smith. p. I-20. Substituted nitrosalicylanilides: A new class ofselectively toxic sea lamprey larvicides. 1966. R. J. Starkey and J. H. Howell. p. 21-30.

12 Physical limnology of Saginaw Bay, Lake Huron. 1967. A. M. Beeton, S. H. Smith, and F. F. Hooper. 62 p.

13 Population characteristics and physical condition of alewives, *Alosa pseudoharengus,* in a massive dieoff in Lake Michigan, 1967. 1968. E. H. Brown, Jr. 22 p.

14 Limnological survey of Lake Ontario, 1964 (five papers). 1969. H. F. Allen, J. F. Reinwand, R. E. Ogawa, J. K. Hiltunen, and L. Wells. 60 p.

15 The ecology and management of the walleye in western Lake Erie. 1969. H. A. Regier, V. C. Applegate, and R. A. Ryder, in collaboration with J. V. Manz, R. G. Ferguson, H. D. Van Meter, and D. R. Wolfert. 104 p.

16 Biology of larval sea lampreys *(Petromyzon marinus)* of the 1960 year class, isolated in the Big Garlic River, Michigan, 1960-1965. 1971. P. J. Manion and A. L. McLain. 36 p.

17 New parasite records for Lake Erie fish. 1972. A. 0. Dechtiar. 20 p.

18 Microbial degradation of the lamprey larvicide 3-trifluoromethyl-4-nitrophenol in sediment-water systems. 1973. L. L. Kempe. 20 p.

19 Lake Superior-A case history of the lake and its fisheries. 1973. A. H. Lawrie and J. F. Rahrer. 74 p.

20 Lake Michigan-man's effects on native fish stocks and other biota. 1973. L. Wells and A. L. McLain. 58 p.

21 Lake Huron-the ecology of the fish community and man's effects on it. 1973. A. H. Berst and G. R. Spangler. 42 p.

22 Effects of exploitation, environmental changes, and new species on the fish habitats and resources of Lake Erie. 1973. W. L. Hartman. 44 p.

23 A review of the changes in the fish species composition of Lake Ontario. 1973. W. J. Christie. 66 p.

24 Lake Opeongo - the ecology of the fish community and of man's effects on it. 1973. N. V. Martin and F. E. J. Fry 34 p.

25 Some impacts of man on Kootenay Lake and its salmonoids. -1973. T. G. Northcote. 48 p.

26 Control of the sea lamprey *(Petromyzon marinus)* in Lake Superior, 1953-70. 1974. B. R. Smith, J. J. Tibbles, and B. G. H. Johnson. 60 p.

27 Movement and recapture of parasitic-phase sea lampreys *(Petromyzon marinus)* tagged in the St. Marys River and Lakes Huron and Michigan, 1963-67. 1974. H. H. Moore, F. H. Dahl, and A. K. Lamsa. 20 p.

28 Changes in the lake trout population of southern Lake Superior in relation to the fishery, the sea lamprey, and stocking, 1950-70. 1975. R. L. Pycha and G. R. King. 34 p.

29 Chemosterilization of the sea lamprey *(Petromyzon marinus).* 1978. L. H. Hanson and P. J. Manion. 16 p.

30 Biology of larval and metamorphosing sea lampreys *(Petromyzon marinus)* of the 1960 year class in the Big Garlic River, Michigan, Part II, 1966-72. 1978. P. J. Manion and B. R. Smith. 36 p.

31 Walleye stocks in the Great Lakes, 1800-1975; fluons and possible causes. 1979. J. C. Schneider and J. H. Leach. 54 p.

32 Modeling the western Lake Erie walleye population: a feasibility study. 1979. B. J. Shuter, J. F. Koonce, and H. A. Regier. 42 p.

33 Distribution and ecology of lampreys in the Lower Peninsula of Michigan, 1957-75. 1979. R. H. Morman. 60 p.

34 Effects of granular 2', 5-dichloro-4'-nitrosalicylanilide (Bayer 73) on benthic macroinvertebrates in a lake environment. 1979. P. A. Gilderhus. p. l-5. Efficacy of antimcin for control of larval sea lampreys *(Petromyzon marinus)* in lentic habitats. 1979. P.A. Gilderhus. p. 6-18.

35 Variations in growth, age of transformation, and sex ratio of sea lampreys reestablished in chemically treated tributaries of the upper Great Lakes. 1979. H. A. Purvis. 36 p.

36 Annotated list of the fishes of the Lake Ontario watershed. 1979. E. J. Crossman and H. D. Van Meter. 28 p.

37 Rehabilitating Great Lakes ecosystems. 1979. Edited by G. R. Francis, J. J. Magnuson, H. A. Regier, and D. R. Talheim. 100 p.

38 Green Bay in the future-a rehabilitative prospectus, 1982. Edited by H. J. Harris, D. R. Talhelm, J. J. Magnuson, and A. M. Forbes. 60 p.

39 Minimum size limits for yellow perch *(Perca flavescens)* in western Lake Erie. 1980. W. L. Hartman, S. J. Nepszy, and R. L. Scholl. 32 p.

40 Strategies for rehabilitation of lake trout in the Great Lakes: proceedings of a conference on lake trout research, August 1983. 1984. Edited by R. L. Eshenroder, T. P. Poe, and C. H. Olver. 64 p.

41 Overfishing or pollution? Case history of a controversy on the Great Lakes. 1985. F. N. Egerton. 28 p.

42 Movement and capture of sea lampreys *(Petromyzon marinus)* marked in northern Lake Huron, 198 l-82. 1985. J. W. Heinrich, W. C. Anderson, and S. D. Oja. p. l-14. Response of spawning-phase sea lampreys *(Petromyzon marinus)* to a lighted trap. 1985. H. A. Purvis, C. L. Chudy, E. L. King, Jr., and V. K. Dawson. p. 15-28.

43 A prospectus for the management of the Long Point ecosystem, 1985. G. R. Francis, A. P. L. Grima, H. A. Regier, and T. H. Whillans. 112 p.

44 Population dynamics and interagency management of the bloater *(Coregonus hoyi)* in Lake Michigan, 1967-1982. 1985. E. H. Brown, Jr., R. W. Rybicki, and R. J. Poff. 36 p.

45 Review of fish species introduced into the Great Lakes, 1819-1974. 1985. L. Emery. 32 p.

46 Impact of sea lamprey parasitism on the blood features and hemopoietic tissues of rainbow trout. 1985. R. E. Kinnunen and H. E. Johnson, 20 p.

47 Comparative toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol to ammocetes of three species of lampreys. 1985. E. L. King, Jr. and J. A. Gabel. p. l-5. Solid bars of 3-trifluoromethyl-4-nitrophenol : a simplified method of applying lampricide to small streams. 1985. P. A. Gilderhus. p. 6-12. Toxicity of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2', 5-dichloro-4'-nitrosalicylanilide (Bayer 73) to eggs and nymphs of the mayfly *(Hexagenia* sp.). 1985. T. D. Bills, L. L. Marking, and J. J. Rach. p. 13-24.

48 Pathology of sea lamprey inflicted wounds on rainbow trout. 1986. R. E. Kinnunen and H. E. Johnson. 32 p.

49 Using the lake trout as an indicator of ecosystem health-application of the dichotomous key. 1987. T. R. Marshall, R. A. Ryder, C. J. Edwards, and G. R. Spangler. 38 p.

50 A comparison of two methods for the simultaneous determination of TFM and Bayer 73 concentrations. 1987. Ronald J. Scholefield. p. 1-8. Elimination of ^{14}C-Bisazir residues in adult sea lamprey *(Petromyzon marinus).* 1987. J. L. Allen and V. K. Dawson. p. 9-20.

51 Parasites of fishes in the Canadian waters of the Great Lakes. 1988. Edited by S.J. Nepszy. 106 p.

52 Guide for determining application rates of lampricides for control of sea lamprey ammocetes. 1988. J. G. Seelye, D. A. Johnson, J. G. Weise, and E. L. King, Jr. 24 p.

53 Sterilizing effect of Cesium-137 irradiation on male sea lampreys released in the Big Garlic River, Michigan. 1988. P. J. Manion, L. H. Hanson, and M. F. Fodale. p. l-7. Relation of pH to toxicity of lampricide TFM in the laboratory. 1988. T. D. Bills, L. L. Marking, G. E. Howe, and J. J. Rach. p. 9-20.

54 Economics of Great Lakes Fisheries: A 1985 assessment. 1988. D. R. Talhelm. 54 p.

55 Effects of the lampricide 3-trifluoromethyl-4-nitrophenol on macroinvertebrate populations in a small stream. May 1990. H. J. Lieffers. 28 p.

56 Resistance to 3-trifluoromethyl-4-nitrophenol (TFM) in sea lamprey. July 1990. R. J. Scholefield and J. G. Seelye. p. 1-5. Effects of changes in dissolved oxygen on the toxicity of 3-trifluoromethyl-4-nitrophenol (TFM) to sea lamprey and rainbow trout. July 1990. J. G. Seelye and R. J. Scholefield. p. 6-16.

57 Toxicity of 2', 5-dichloro-4'-nitrosalicylanilide (Bayer 73) to three genera of larval lampreys. October 1992. R. J. Scholefield and James G. Seelye. p. 1-6. Effect of pH on the toxicity of TFM to sea lamprey larvae and nontarget species during a stream treatment. October 1992. Terry D. Mills and David A. Johnson. p. 7-20. Effect of the lampricide 3-trifluoromethyl-4-nitrophenol on dissolved oxygen in aquatic systems. October 1992. Verdel K. Dawson, David A. Johnson, and John F. Sullivan. p. 21-34.

58 Surficial substrates and bathymetry of five historical lake trout spawning reefs in near-shore waters of the Great Lakes. October 1992. T. A. Edsall, C. L. Brown, G. W. Kennedy, and J. R. P. French, III. 54 p.

59 Food ofsalmonine predators in Lake Superior, 1981-87. June 1993. David J. Conner, Charles R. Bronte, James H. Selgeby, and Hollie L. Collins. 20 p.

60 Early changes in the fish community of Lake Ontario. 1995. S. H. Smith. 38 p.

61 Comparison of mark retention and survival of sea lamprey larvae marked by pigment injection and tail clipping. August 1995. William D. Swink, Sidney B. Morkert, and Karen S. Slaght. p. l-8. Comparison of 3-trifluoromethyl-4-nitrophenol (TFM) toxicities to sea lampreys, rainbow trout, and mayfly nymphs in continuouse and interrupted
9-h exposures. August 1995. Ronald J. Scholefield, James G. Seelye, and Karen S. Slaght. p. 9-32.

62 Sensitivity of lake sturgeon (Acipenser fulvescens) to the lampricide trifluoromethyl-4-nitrophenol (TFM) in field and laboratory exposures. February 1999. David A. Johnson, John W. Weisser, and Terry D. Bills. 24 p.