The Influence of Larval Lampricide (TFM: 3-Trifluormethyl-4-Nitrophenol on Growth and Production of Two Species of Aquatic Macrophytes, *Elodea canadensis* (Michx.) Planchon and *Myriophyllum spicatum* L.[1]

Alan W. Maki[2] and Howard E. Johnson
Department of Fisheries and Wildlife
Michigan State University
East Lansing, Mich. 48823

Introduction

The ecological significance of aquatic plants lies in their ability to oxygenate their environment through photosynthesis (RUTTNER 1966) while providing a unique substrate for periphyton and macroinvertebrate communities (FOERSTER and SCHLICHTING 1965). Their ability to extract and concentrate nutrients from water has only recently been investigated (GERLOFF 1967) and their application for nutrient removal from waste waters while providing a harvestable crop appears promising (McNABB et al. 1972). Implied in the development of such technology is the assessment of possible toxic effects of environmental contaminants and waste water effluents on the growth and production of macrophytes. The limited literature evaluating the effects of toxicants on aquatic macrophytes is reviewed by STANLEY (1974).

This paper outlines a method for the evaluation of toxicant effects on the productive capacity of macrophytes grown in recirculating laboratory stream channels. The toxicant employed, TFM (3-trifluormethyl-4-nitrophenol) is currently used for lamprey control in streams of the Great Lakes drainage. TFM is selectively toxic to sea lamprey larvae, Petromyzon marinus, but has also been employed for the control of rooted aquatic plants (JOSEPHS 1961). When used for aquatic plant control, the exposure time is approximately 2 hours with concentrations up to 100 mg/l required for flowing waters and approximately 15 mg/l concentrations required for standing waters (SCHNICK 1972). This study was designed to define the toxicity of lampricidal concentrations of TFM to two vascular hydrophytes, Elodea canadensis and Myriophyllum spicatum. Replicated toxicity tests were conducted to define both concentration-effect and time of exposure data.

---

[1] Michigan Agricultural Experiment Journal Article Number 7652.

[2] Present Address: Environmental Water Quality Research Dept.
The Procter & Gamble Company
Ivorydale Technical Center
5299 Spring Grove Avenue
Cincinnati, Ohio 45217

Bulletin of Environmental Contamination & Toxicology,
Vol. 17, No. 1 © 1977 by Springer-Verlag New York Inc.

Methods and Materials

The plants were obtained from naturally occurring stocks growing in several outdoor research ponds on the campus of Michigan State University. The plants were collected a few hours prior to the initiation of the toxicity test, brought to the laboratory and individual shoots were cut to a uniform length of 10 or 12 cm. Individual experimental shoots were selected for uniformity by using apical shoots with similar leaf structure, presence of an actively growing vegetative tip and lack of lateral branches or nodes. Six individual shoots were tied with cotton twine in 2 groups of 3 and attached to a 7.62 cm solid glass rod which served to anchor the plants. Twelve individual shoots were set aside for determination of initial biomass at day zero prior to the toxicant exposure.

Exposure to the toxicant was conducted in totally recirculating model streams. Each stream consisted of two parallel wooden channels (243 cm x 29 cm x 19 cm deep) painted white and joined at each end to allow for continual recirculation of 130 liters of nutrient medium; the volume used for all toxicity tests. Current was provided by the action of a variable-speed paddlewheel set to maintain a constant current velocity of about 15 cm/sec. The initial tests were conducted with about 100 lbs of sterile silica sand serving as a substrate but this was discarded as time consuming and unnecessary in later experiments. Uniform light intensity of 400 $\pm$ 50 foot candles was obtained by supporting cool white fluorescent lamps directly above the streams. All experiments were conducted within a controlled environmental chamber maintaining a constant temperature of 17.5-18.0°C.

In order to obtain satisfactory growth of experimental plants within the 2-week period, a nutrient medium was employed in all toxicity tests. The medium used was a modification of Hoagland's culture solution (HOAGLAND and SNYDER 1933). The modified medium, described by GERLOFF (1967), contained one-fifth the concentration of the major elements present in the original medium and full strength concentrations of the trace elements (Table 1). The pH of the final nutrient solution was 7.0 $\pm$ 0.2. No attempt was made to maintain aseptic or algal-free conditions; however, the growth of algal populations was minimized by weekly transfer of growing plants into an adjacent channel circulating newly-synthesized growth medium. Concentrations of from 5 to 35 mg/l of 95% pure analytical grade TFM (Aldrich Chemical Co., Lot No. 060217) were used in the toxicity tests. The appropriate amount of TFM was weighed and dissolved in 15 ml of acetone and added to the experimental channel. Actual TFM concentrations were monitored colorimetrically by comparison with a standard curve (HOWELL and MARQUETTE 1962). Exposures were terminated at about 1, 2, 4, 8, 16, and 24 hours by transferring the glass rod and the six attached shoots into the adjacent channel of TFM-free Hoagland's culture solution for a period of 2 weeks. Six unexposed shoots were used as controls and treated similarly during each test.

Following the two week growth period, individual shoots were separated, total length measured, and each was placed in an individual 5 cm diameter aluminum pan. The plants were then dried at

TABLE 1

Chemical composition of modified Hoagland's nutrient medium for culture of aquatic macrophytes (GERLOFF 1967).

Salt Used	Elements	Final Concentration in Culture Medium (mg/l)
KNO_3	N	42.0
	K	47.0
$Ca(NO_3)_2 \cdot 4\ HOH$	Ca	40.0
	P	6.2
$MgSO_4 \cdot 7\ HOH$	S	12.8
	Mg	9.6
Trace Elements		
KCl	Cl	1.77
H_3BO_3	B	0.27
$MnSO4 \cdot HOH$	Mn	0.27
$ZnSO_4 \cdot 7\ HOH$	Zn	0.13
$CuSO_4 \cdot 5\ HOH$	Cu	0.03
$(NH_4)_6Mo_7O_{24} \cdot 4\ HOH$	Ml	0.01
Fe · EDTA	Fe	0.40

50°C in a forced air oven for 48 hours, weighed to seven place accuracy on a Mettler balance and ashed at 550°C for 1 hour. Pans were allowed to cool in a dessicator and reweighed. The ashed weight was subtracted from dry weight for determination of ash-free dry weight. Each shoot was statistically treated as a single experimental unit and all treatment concentrations were replicated once.

Results and Discussions

Analytical grade TFM inhibited the growth and production of vegetative shoots of Elodea canadensis in all concentrations greater than 5.0 mg/l (Table 2). Exposure periods in excess of 1 hour produced significant reductions in production of plants exposed to increasingly high concentrations of 10.0, 15.0, 20.0 and 35 mg/l TFM. All treatments in excess of 5 mg/l for a period of 24 hours resulted in a net loss in biomass from pre-exposure weights. Reductions in weight also occurred in the highest two concentrations for all exposure periods in excess of 3 hours. Although not actually dead, these affected plants were limp and cyanotic. They had been exposed to the toxicant concentration for a sufficient length of time to bring about a static effect on growth. Given sufficient time and adequate growth conditions they potentially could recover growth ability. However, those individuals exposed to 35 mg/l for periods in excess of one hour, subsequently deteriorated and fragmented to the extent that eventual recovery was not possible.

TABLE 2

The growth (mean length) and production (mean ash-free dry weight) of Elodea canadensis during a two week period following exposure to TFM for different time periods. Values are the mean (1 standard deviation in parentheses) of six individual samples.

TFM Concentration	Pre-exposure	Exposure Period					Control
		1 hour	3 hour	7 hour	15 hour	24 hour	No Exposure
5.0 mg/l							
weight (mg)	22.07(6.81)	25.28(5.18)	29.37(6.53)	29.21(7.87)	28.98(3.59)	25.93(3.60)	30.38(5.11)
length (cm)	10.00	12.65(0.94)	12.73(0.99)	12.83(3.60)	13.42(0.69)	12.58(1.14)	13.13(0.65)
10.0 mg/l							
weight (mg)	17.47(3.47)	46.10(11.71)	36.56(7.56)	33.97(7.66)	21.51(4.57)	16.07(4.20)	38.74(7.27)
length (cm)	10.00	14.75(0.93)	13.34(0.98)	13.20(0.81)	12.13(0.68)	10.08(1.41)	13.62(0.75)
15.0 mg/l							
weight (mg)	21.94(3.62)	40.67(8.54)	26.63(8.15)	24.73(5.07)	17.87(4.20)	11.95(6.50)	36.13(5.60)
length (cm)	10.00	13.84(1.56)	12.52(1.29	11.03(4.28)	6.92(1.37)	5.49(2.01)	13.06(0.76)
20.0 mg/l							
weight (mg)	27.12(8.93)	31.66(11.00)	23.04(13.17)	26.08(4.32)	8.09(4.69)	5.39(8.38)	34.09(2.76)
length (cm)	10.00	11.87(1.73)	9.30(5.32)	11.88(1.49)	2.95(4.61)	2.37(4.02)	12.13(1.16)
35.0 mg/l							
weight (mg)	17.15(4.15)	26.30(4.19)	5.28[1]	1.66[1]	3.83[1]	4.16[1]	35.51(6.76)
length (cm)	10.00	12.61(0.97)	----[2]	----[2]	----[2]	----[2]	13.71(0.80)

[1] Individual plants combined for a single combustion due to deterioration.

[2] Length not measured due to deterioration and fragmentation.

The effects of the toxicant on the primary production measured as mg carbon · 2 week^{-1}, also demonstrate this progressive severity with the length of time plants were exposed (Figure I). The production figures were calculated by subtracting the pre-exposure weight for each treatment concentration from the final weight of the plants following the two week growing period. The figures enable a direct comparison of production following the exposure to five toxicant concentrations. Since exposure to individual toxicant concentrations were done separately with a new group of freshly harvested vegetative tips for each treatment, subsequent effects on production are compared to the production of the control group for that treatment concentration. Negative production figures indicate the progressively toxic effects due to deterioration and fragmentation. The greatest percentage reduction in production occurred after one and three hours exposure for concentrations in excess of 5.0 mg/l.

Myriophyllum spicatum was more susceptible to the toxicant with a slight reduction in growth present at the lowest test concentration of 5.0 mg/l and reductions from 60% to 85% of control biomass in concentrations of 10 to 25 mg/l (Table 3). The relatively high standard deviations, observed in several cases to approximate 40% to 50% of the mean growth and production figures, indicate the variability in growth patterns recorded for this species. Frequently one or more of the six replicates per treatment would initiate lateral branching thereby adding significantly to the final biomass when compared with non-branching replicates. The greatest percentage reduction in biomass occurred after 1 and 4 hours of exposure at all concentrations greater than 5.0 mg/l.

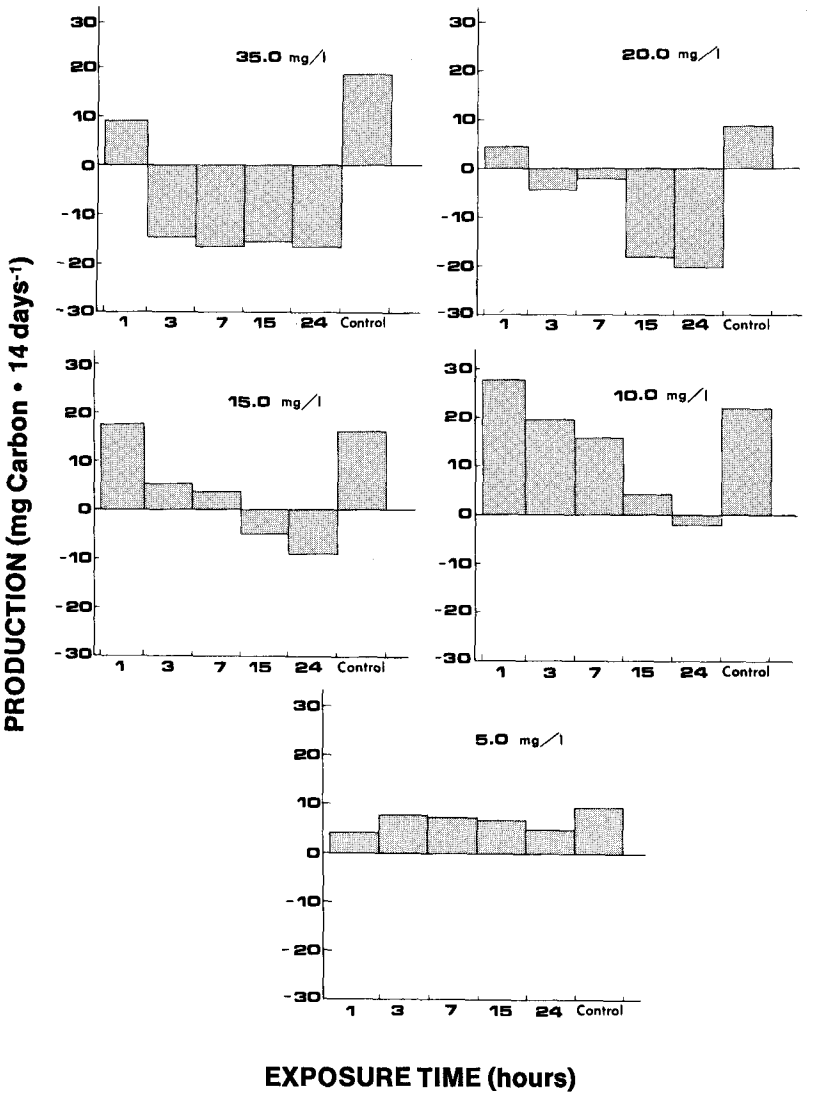

Figure I. The production of Elodea canadensis exposed for 5 time periods to 5 concentrations of the larval lampricide, TFM.

TABLE 3

Growth (mean length) and production (mean, ash-free dry weight) of Myriophyllum spicatum during a two week period following exposure to TFM for different time periods. Values are expressed as the mean (1 standard deviation in parentheses) of six individual samples.

TFM Concentration	Pre-exposure	Exposure Period						Control No Exposure
		1 hour	2 hour	4 hour	8 hour	16 hour	24 hour	
5.0 mg/l								
weight	114.43(41.32)	165.4(33.64)	154.57(37.10)	164.27(26.63)	165.68(31.18)	131.69(41.97)	104.77(50.18)	147.48(20.10)
length	12.00	20.2(1.94)	19.9(2.32)	20.5(1.92)	20.2(2.20)	19.2(2.49)	16.2(2.59)	20.0(1.76)
10 mg/l								
weight	69.02(25.01)	112.74(34.09)	102.65(36.36)	56.35(35.75)	29.69(4.28)	24.78(7.23)	38.51(22.70)	104.29(34.27)
length	12.00	17.8(1.01)	18.0(1.90)	16.0(0.88)	14.8(1.99)	13.7(1.26)	12.5(1.68)	17.7(1.56)
15 mg/l								
weight	73.08(16.48)	91.99(27.12)	65.93(28.68)	39.94(22.60)	25.29(11.59)	34.23[1/]	37.48[1/]	90.96(28.44)
length	12.00	17.3(1.22)	16.3(1.71)	15.0(1.31)	14.6(1.72)	---- [2/]	---- [2/]	17.4(1.26)
20 mg/l								
weight	68.19(26.04)	121.67(42.89)	84.13(47.01)	40.57(20.37)	31.36(12.69)	24.27(12.68)	15.88[1/]	114.09(32.41)
length	12.00	18.8(1.29)	16.2(1.97)	14.7(1.40)	16.5(1.27)	15.3(1.70)	---- [2/]	17.1(2.34)
25 mg/l								
weight	66.21(9.03)	74.35(18.62)	49.87(14.19)	33.27(7.60)	22.17[1/]	12.99[1/]	17.15[1/]	96.88(18.76)
length	12.00	16.0(0.97)	15.0(1.11)	14.4(1.05)	---- [2/]	---- [2/]	---- [2/]	16.4(1.17)

[1/] Plants deteriorated - individual shoots combined for single combustion.

[2/] Length not measured due to deterioration and fragmentation.

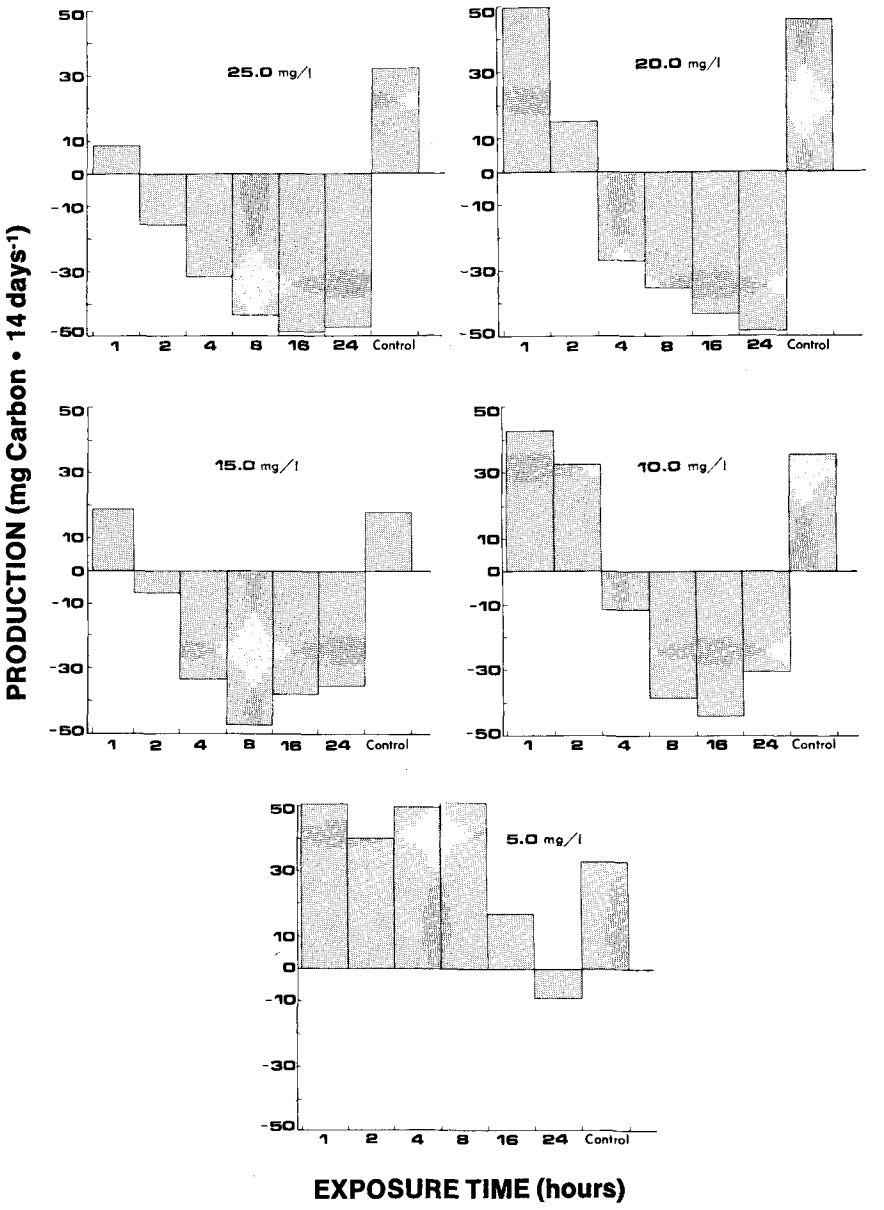

Figure II. The production of Myriophyllum spicatum exposed for 6 time periods to 5 concentrations of the larval lampricide, TFM.

The initial biomass of Myriophyllum shoots used to start all tests was approximately three to four times the weight of the Elodea shoots used in comparable exposures. Subsequently, production of controls and shoots at low exposure levels measured as mg carbon · 2 weeks^{-1} was considerably higher than those values reported for Elodea (Figure II). The greatest percentage reduction in biomass occurred after 1 and 4 hours of exposure at all concentrations greater than 5.0 mg/1. At the 5.0 mg/1 treatment concentration growth reductions were only observed in plants exposed for 8 hours or more.

The toxicant appeared to affect both species similarly. When the individual shoots were placed in the TFM-free channel upon termination of exposure, no anatomical effects such as loss of color or crispness were detected visually. However, within 48 to 72 hours following termination of exposure, the plants from the higher TFM concentrations became cyanotic and lost turgor. Leaves of the affected plants began to slough off and the entire plant rapidly deteriorated during the second week of the growth period. Progressively more severe effects were observed with increasing TFM concentration and length of exposure period.

Field crews utilize toxicity tests and water chemistry nomographs to determine the TFM concentration that will control 100% of exposed larval lamprey (HOWELL and MARQUETTE 1962). Using their nomographs we calculated that our test water would require TFM concentrations of 6 to 7 mg/1 for about 10 hours in an actual lamprey control treatment. Associated stream flora and fauna would also be exposed at this level for the same time period. Interpolation of the present toxicity test data points at 5 and 10.0 mg/1 demonstrates that this concentration could be expected to have a slight negative effect on growth and production of Elodea canadensis, perhaps bringing about an approximate 5-10% reduction in growth rates of exposed plants after a 2 week period. Considering the greater susceptibility of Myriophyllum spicatum this concentration would be expected to bring about a reduction in growth rates and production of exposed plants of the order of 20% within two weeks following exposure.

Acknowledgments

This research was supported by Research Contract No. 14-16-0008-651 from the Bureau of Sport Fisheries and Wildlife and by the Michigan State University Agricultural Experiment Station.

The authors wish to thank Mr. Lester Geissel of the Pesticide Research Center for his technical assistance.

References

FOERSTER, J.W. and H.E. SCHLICHTING: Trans. Am. Micros. Soc. 84, 485 (1965)

GERLOFF, G.C.: Eutrophication: causes, consequences, and correctives. Madison, WI: U of Wisconsin Symposium Proceedings 1967.

HOAGLAND, D.R. and W.C. SNYDER: Amer. Soc. Horti. Sci., Proc. 30,288 (1933).

HOWELL, J.H. and W.M. MARQUETTE: U.S. Fish and Wildlife Serv., Spec. Sci. Rept. -Fish. 418,1 (1962).

JOSEPHS, M.J.: U.S. Patent 3,004,843. Filed Aug. 10, 1959, patented Oct. 17, 1961. 3 p. (1961)

MCNABB, C.D., D.P. TIERNEY and S.R. KOSEK: Mich. State Univ., Inst. Water Research Tech. Rept. No. 24. 23 p. (1972).

RUTTNER, F.: Fundamentals of Limnology. Toronto: University of Toronto Press 1966.

SCHNICK, R.A.: U.S. Fish and Wildlife Serv., Investigations in Fish Control 44,1 (1972).

STANLEY, R.A.: Arch. Env. Contam. Toxicol. 2(4),331 (1974).