The Ecological Effects of Malathion on Juvenile Walleye

By

Peter D. Delorme

A Thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
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in
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Abstract

Field studies were conducted in storm water retention ponds to evaluate the ecological responses of populations of juvenile walleye (*Stizostedion vitreum vitreum* (Mitchill)) to short term (2 h) exposures to Malathion. Four experiments were conducted with exposures of walleye to 1, 25 and 50 ppb malathion. Exposure to Malathion as well as marking of walleye took place in the laboratory. Sham treated groups of fish were included in all experiments to assess the effects of handling stress. All groups of fish were monitored during the summer for head acetylcholinesterase (AChE) activity levels, growth and feeding.

The study showed that the degree of AChE inhibition was dependent on the dose of malathion, higher inhibitions were found at higher dosage levels. Inhibition of AChE was also related to the size of fish exposed. Lower inhibitions were found in older (larger) fish exposed to the same dose as younger fish. Levels of inhibition may also be related to the length of exposure with higher inhibitions occurring with exposure to low levels of malathion for long periods of time (days) or at high levels for short lengths of time (hours). Comparison of these data with data of others suggests that the length of inhibitory effects may be directly related to the length of exposure.

Growth, feeding and survival were not significantly affected by exposures of up to 50 ppb malathion. Slight increases in feeding on invertebrates in treated fish may have been caused by changes in

feeding behaviour, but these effects were insignificant when compared to the effects caused by handling stress. Piscivory was also decreased in malathion treated walleye. Both treated and sham treated fish consumed fewer minnows than untreated fish. Stress caused by handling is the most probable cause for decreased feeding in the sham treated and malathion treated walleye. Decreases in growth of sham treated and malathion treated fish were probably caused by decreased feeding.

Acknowledgements

During the course of this study there have been many people who have helped with various aspects, given helpful and useful advice and supported me. Dr F. J. Ward as supervisor and Dr. W. L. Lockhart guided me and gave freely advice and comments.

This project would not have been possible without the enthusiasm and help of the graduate students in Dr. Ward's lab, Lenore Ciszewski, Bernie McIntyre and Sue Kenny. Extra special thanks go to Sue who spent many Saturdays doing the cholinesterase assays for the project. Many other graduate students from the Department of Zoology and the Freshwater Institute assisted with the annual fall ritual of seining out the ponds.

Many summer students worked on this project to bring it to successful completion. I am particularly idebted to Bruce Mcullogh and Laura Heuring for their many hours at the microscope helping me do stomach analyses, and for their assistance in collecting field samples. I also wish to acknowledge Sean Bugden and Ann Kong who also assisted in collecting and processing field samples.

Last but by no means least I wish to acknowledge the support given by my wife Denise over the last 3 years.

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Dedication

This thesis is dedicated to my parents, Denis and Marilou Delorme who have sacrificed much so their children could have a good education. Thanks mom and dad.

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Introduction

Recently there has been a great increase in research concerning the use of biochemical indicators for the early detection of contaminants in aquatic ecosystems. Certain classes of organic and inorganic pollutants may produce specific biochemical reactions in fish and/or mammals. These reactions may occur at contamination levels much lower than would cause overt signs of toxicity and long before sub-lethal doses may produce ecological effects. Examples of biochemical indicators include induction of various enzymes such as metallothionein by trace metals (Klaverkamp et al. 1984, Hamilton and Merle 1986), cytochrome P-450 by benz-(a)-pyrene and the inhibition of acetlycholinesterase by organophosphorous insecticides (Lockhart et al. 1985, Mulla et al. 1984).

Malathion is an organophosphorous insecticide which has been widely utilized for control of insects in households, greenhouses, agriculture and public health (Matsumura 1975). This insecticide acts as a neurotoxin (on the nervous system of the animal), inhibiting the enzyme acetylcholinesterase (AChE) (Mulla & Mian 1981). AChE is normally responsible for hydrolyzing acetylcholine into acetic acid and choline (O'Brien 1967 in Rand and Petrocelli 1985). Without this enzyme proper transmission of nerve impulses across synapses cannot occur, usually resulting in paralysis (Rand and Petrocelli 1985). Cholinesterase enzymes can be easily measured and thus offer a convenient method to detect exposure to organophosphorous insecticides.

Acetylcholinesterase has been shown by various scientists to be necessary in fish for proper neurological functioning of the sensory,

integrative and neuromuscular systems (Rand and Petrocelli 1985); Klaverkamp et al. (1977) have shown inhibition of AChE with Fenitrothion alters respiration in rainbow trout (*Salmo gardinerii*). Post and Leasure (1974) found Malathion affected swimming performance in three species of salmonids. Fenitrothion affected feeding behaviour in juvenile coho salmon (Bull and McInerney 1974).

There have been very few if any studies which seek to link (specifically) a biochemical with an ecological effect. The usual studies, such as those already mentioned, look for either a biochemical result or an ecological effect under laboratory conditions. Biochemical indicators may indicate a possibility of contamination, however, very little is known of possible ecological consequences to the organism in the wild.

Effects observed under controlled laboratory conditions may not be apparent in natural conditions. There may be a greater variation in response under field conditions where many more external factors (light, temperature, food availability etc.) probably play an important role in the response. If biochemical indicators are to be used as an early warning system then they must be calibrated against ecological effects in the field. Base line data must be gathered on the variability of these biochemical measures which may be caused by seasonal changes in environment and growth and development of the organism.

Lockhart et al. (1985), reporting on the effects of aerial spraying of Malathion on storm water retention ponds in Winnipeg containing juvenile walleye (*Stizostedion vitreum vitreum*), found that AChE levels were inhibited to 25% of pre-spray values. The results also showed small temporary decreases in catch per unit effort and weight gains in the population.

The current study was done to assess the potential effects of such exposure at a population level but under more tightly controlled exposure conditions. The exposure to Malathion was done in controlled conditions in the laboratory. A group of sham treated fish was included so that effects of handling could be determined. Natural conditions for growth and survival were retained by utilizing a pond population of walleye. The study also used three levels of exposure to Malathion as opposed to the one level in Lockhart et al. (1985).

The major objectives of this study were to (1) monitor the pond populations (treated, sham treated and untreated) for inhibition of AChE., (2) to evaluate the effect of sub-lethal exposure to Malathion on subsequent survival and growth of juvenile walleye under natural conditions and (3) to compare the feeding habits of sub-lethally poisoned fish with sham treated ones with regard to types, quantities and size of prey items.

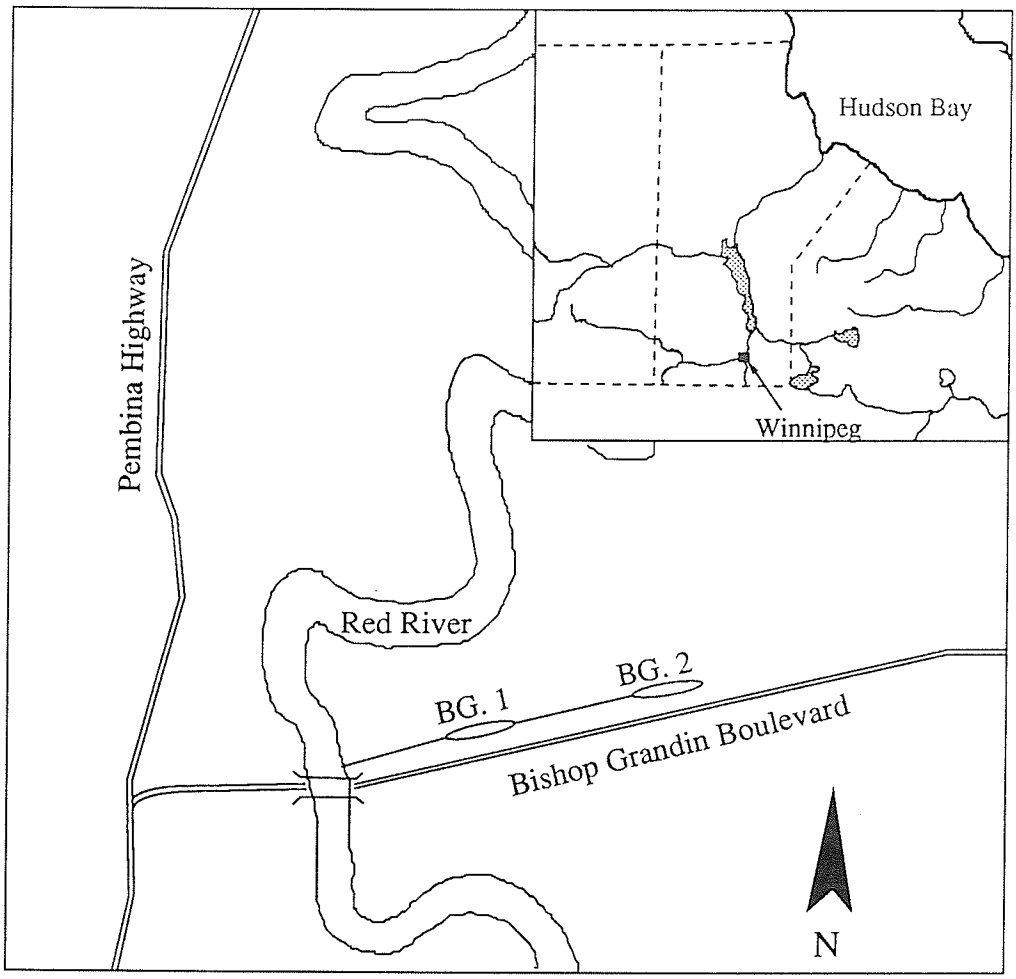
Materials and Methods

Stocking and Experimental Location

On May 22 of 1986 and May 20, 1987, newly hatched walleye fry were obtained from the Manitoba Department of Natural Resources, Fisheries Branch hatchery at West Hawk Lake. These were planted into two storm water retention ponds, Bishop Grandin pond 1 (BG 1) and Bishop Grandin pond 2 (BG 2), located in south Winnipeg on the north side of Bishop Grandin Avenue (Figure 1). The ponds were both approximately 0.7 hectares in surface area, are unstratified and have a maximum depth of 2.0 m, they contained no other fish at the time of stocking. The fry were transported in 45 L bags which were pressurized with 0_2 to facilitate survival. The water temperature in the bags was adjusted slowly to the water temperature in the ponds and then the acclimated fry were released along the margin of the pond in about 1 m of water. Approximately 50,000 fry were placed in each pond in 1986 and approximately 64,000 in each pond in 1987 which were used during the four experiments.

In addition to walleye, fathead minnows (*Pimephales promelas*) were added to BG 1 during the course of the final experiment (4) in 1987 as a forage species. Two thousand (2000) minnows obtained from other storm water retention ponds, located within the city of Winnipeg, were added, at dusk, one day prior to sampling of the pond population for the first four sampling dates in experiment 4.

Figure 1. Location of Bishop Grandin Ponds 1 and 2.


Hudson Bay
Winnipeg
Pembina Highway
Red River
BG. 1
BG. 2
Bishop Grandin Boulevard
N

Marking and Exposure to Malathion

Four experiments were conducted, two in 1986 and two in 1987 (Table 1). For each experiment juvenile walleye were removed from the ponds using an 18.6 x 2 metre net with 6.4 mm mesh. Walleye were taken from the net and placed in 25 L bags of pond water (maximum of 100 fish/bag) and immediately transported to the laboratory. On arrival in the laboratory the fish were placed in 150 L flow through aquaria containing dechlorinated water at ambient pond temperatures. Fish were removed from the aquaria in lots of 10 and placed in MS-222 (tricaine methanosulfonate). Each fish had either the left (sham) or the right (treated) pelvic fin removed and was then placed in one of two 350 L flow through recovery tanks. In 1986 there was no survival in BG 1, thus both experiments were conducted in BG 2. To distinguish the first and second experiments, the second marking in 1986 used the removal of 3 to 4 fin rays from the top (sham) or the bottom (treated) of the caudal fin. In 1987 experiment 3 was conducted in BG 2 and experiment 4 in BG 1.

In all experiments fin clipped fish were allowed to recover for 24 h following marking. Exposure to Malathion in each experiment took place the following day in four 150 L static, aerated glass aquaria. For example fish in experiment 1 were exposed to 1 $ug \cdot L^{-1}$ of Malathion (C.I.L. Domestic, 125 $g \cdot L^{-1}$ A.I.) made by diluting 1 ml of the commercial Malathion to 1 L with dechlorinated water. Then 1.2 ml of the diluted solution was added to each tank to give a final

concentration of 1 ug·L^{-1} and allowed to mix for 5 minutes. Starting times for each aquarium were spaced at 15 minute intervals. Similar procedures were followed for the other three experiments (25, 50 and 50 ug·L^{-1} respectively) except that the dilution of Malathion was adjusted to produce the desired level of exposure. The numbers of fish, exposure durations and exposure concentrations for each of the four experiments are shown in Table 1.

Initial exposures were done for 120 minutes; however at higher concentrations it was not possible to maintain this length of exposure without causing severe mortality. Following exposure the walleye were rinsed with fresh dechlorinated water and returned to the 350 L holding tanks. Fish used in experiment 1 were held for an additional 24 h before being returned to the ponds. In experiments 2 through 4 walleye were returned to the pond 3-4 h following exposure.

Fish (10) were sacrificed from both the sham and treated groups immediately following removal of the treated fish from the Malathion for assesment AChE activity levels.

Field Sampling

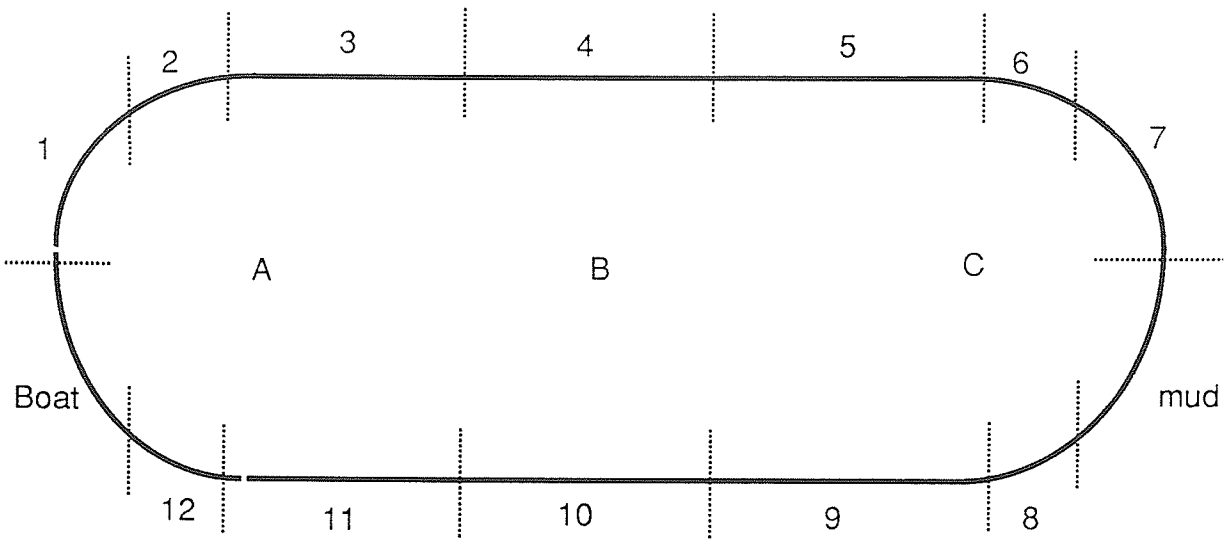
In 1986 fish were sampled from the ponds using an 18.6 x 2 metre net with 6.4 mm mesh, which was hauled over a standard 6.5 x 8.0 m area by wading along the edges of the pond. Each pond was divided into 14 areas of which 12 were seined (Figure 2A). Two areas were not seined, one because the water was too shallow and the other because it was used as a boat landing. The same net was

Table 1. Summary of exposure doses and number of sham treated and malathion treated walleye returned to ponds.

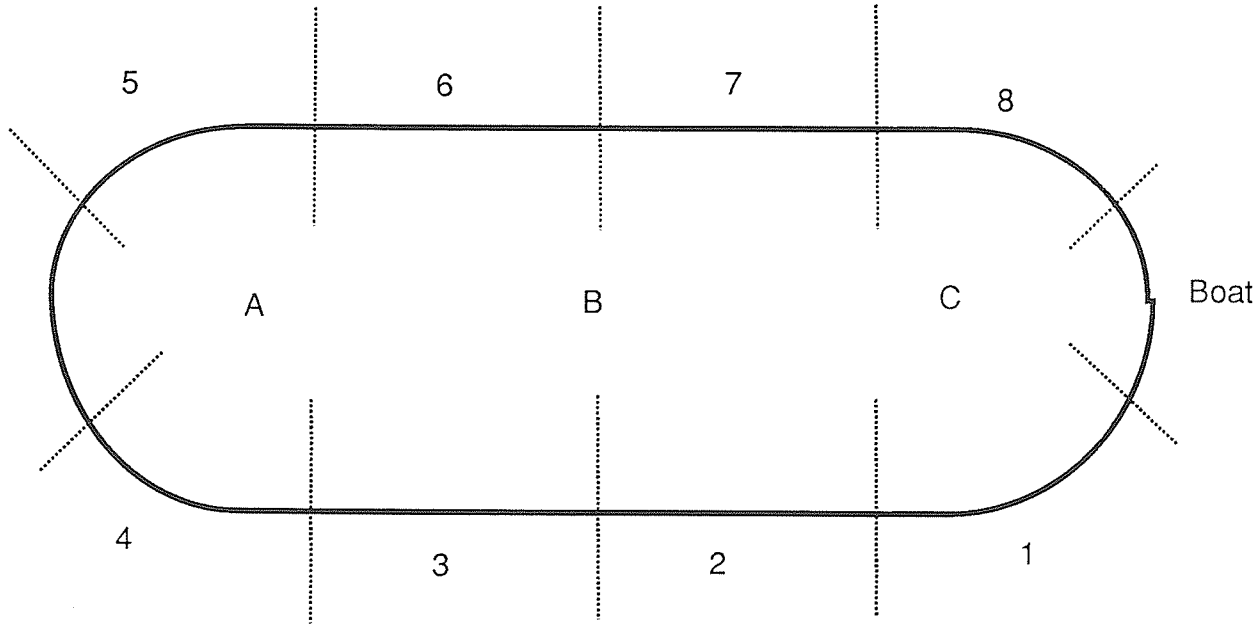
Experiment Number	Date	Exposure		Number of fish returned		Mean Percent Inhibition
		Dose (ppb)	Duration (min)	Treated	Sham Treated	
1	June 25, 1986	1	120	807	820	7
2	July 15, 1986	25	90	420	390	21
3	June 30, 1987	50	90	1084	1195	60
4	July 15, 1987	50	65	525	807	35

Figure 2. Schematic representation of sampling sites on Bishop Grandin Ponds 1 and 2 for A 1986, and B 1987. Numbers indicate seining locations and letters indicate oxygen, pH, depth sampling sites. Modified from McIntyre (1987).

A



B



used in 1987, however a boat was used to tow the net in a semi-circular arc 19 m long, resulting in an increased volume being sampled which included the deeper more central parts of the ponds. Only 10 sites were sampled in each pond in 1987 (Figure 2B). Sampling was done every two to three days for the first two weeks following return to the ponds, after this seining was done on a weekly basis.

Each fish caught was counted according to the type of mark. Sub-samples of marked and unmarked fish (a maximum of 15 from each group in 1986 and 30 in 1987) were taken from each haul. Once sampled, the fish were immediately put into whirl pack bags and the bags placed on ice. Each fish was weighed and the fork and total length measured. Fish used for AChE assays had their heads removed and placed in culture tubes which were stored at -20° C until assayed. Those walleye used for stomach analysis were transferred to a solution of 10% formalin. Samples were sub-divided in the laboratory. In 1986, 7 fish were used for AChE assays; the rest were used for stomach analysis. In 1987, 10 fish were used for AChE assays and 15 for stomach analysis. On dates when not enough fish were obtained for both analyses the bodies of the assayed fish were tagged and numbered and also used for stomach analyses.

Oxygen concentration, pH, water depth and temperature were measured at three stations (Figure 2) in the ponds on each sampling date. Oxygen and temperature were measured using a YSI Oxygen meter (Model 57). A 1 L water sample was taken for pH measurements. The pH was measured in the laboratory using a

Radiometer Model 29 pH meter fitted with a Fisher calomel reference electrode.

Acetylcholinesterase Assays

The assay method for AChE was described by Lockhart et al. (1985). Heads frozen after removal were first homogenized in 2 ml of 0.1 M phosphate buffer (pH 7.2) using a Polytron homogenizer. Heads were homogenized, large ones for 30 seconds, smaller ones for 20 seconds, with tubes immersed in ice. The homogenate was then transferred to centrifuge tubes and centrifuged at 0 to -5 ^{o}C at 14,000 rpm for 20 minutes using a Sorval RC-2B superspeed centrifuge with an SM-24 rotor. The supernatant was then pipetted into clean culture tubes which were kept on ice. The supernatant was then analyzed for AChE activity by the procedure of Ellman et al. (1961) with acetylthiocholine as the substrate and using prepackaged reagents from Boehringer-Mannheim Corporation. Protein in the homogenate was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard. The cholinesterase activities were calculated as milliunits of activity per milligram of protein in the preparation.

Stomach Analyses

Gut contents were studied by removing the entire gut from the esophagus to the anus. The stomach was then separated from the intestines and the contents removed, identified to genus (Pennak

1978) and counted. Individuals in a sub-sample of cladocerans (*Daphnia* sp.) were measured from the anterior margin of the compound eye to the point of inflection on the caudal spine. Metasome lengths of copepods were also measured. A Wild M-5 dissecting scope with an ocular micrometer calibrated to a stage micrometer, was used both for dissecting and measuring organisms.

Lengths of fathead minnows found in walleye stomachs were estimated using the GAP measurement of the left pharyngeal arch and the regression equation found in McIntyre and Ward (1986).

Once removed and sorted, the items were placed in small pre-weighed aluminum dishes and dried for at least 24 h at 150 °C. After drying the containers were re-weighed and weights of items obtained by difference. Total dry weights of stomach contents were obtained by suming the dry weights of the components.

Statistical Analyses

All comparisons of mean values were done using an unbalanced one way analysis of variance (ANOVA). Tukey's multiple comparison test was utilized to determine which groups were significantly different at $p=0.05$. All variables, with the exception of AChE activity, were transformed to their natural logarithms to reduce dependence of the variance on the mean.

Significant differences were sought between sham treated and malathion treated fish and between sham treated and untreated fish. Significant differences between malathion treated fish and untreated

fish were ignored as they include potential effects of both handling and exposure to Malathion. Analyses were done using the general linear models procedures (PROC GLM) in SAS-PC v6.03 (SAS 1985).

Results

Acetylcholinesterase Levels

Walleye treated with 1 ppb malathion (experiment 1) were found to have AChE levels which were inhibited by 7% when compared with sham treated fish on day 0, immediately after exposure. The AChE activity levels for day 0 were not significantly different from those of the sham treated fish according to the results of an ANOVA ($p=0.4909$). Cholinesterase levels were extremely variable over the course of the experiment (Figure 3), especially in the sham treated group. No significant differences in AChE levels were found between sham treated and untreated fish on any of the dates.

Walleye exposed to 25 ppb malathion (experiment 2) exhibited an inhibition of 21% when compared with AChE levels found in sham treated fish on day 1 following exposure. This inhibition was not significant ($p= 0.2271$). No significant differences were found between sham treated and treated or sham treated and untreated on any of the other dates (Figure 4), and as with the 1 ppb experiment, AChE levels were variable in all groups over time.

On day 0 following treatment with 50 ppb malathion in 1987, (experiment 3), a significant ($p=0.0001$) inhibition of 60% of AChE levels found in sham treated fish was found between treated fish and sham treated fish. This inhibition was short lived since by day 1 following exposure, levels in the treated fish were higher than in the sham treated fish (Figure 5). In addition, significant differences in AChE levels between

Figure 3. Mean acetylcholinesterase activity levels for 1 ppb malathion treated, sham treated and untreated walleye versus days after exposure.