The Ecological Effects of Malathion on Juvenile Walleye

By

Peter D. Delorme

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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.

This project was funded by a World Wildlife Toxicology Fund grant to Drs. Ward and Lockhart, the Department of Fisheries and Oceans and Manitoba CareerStart

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.

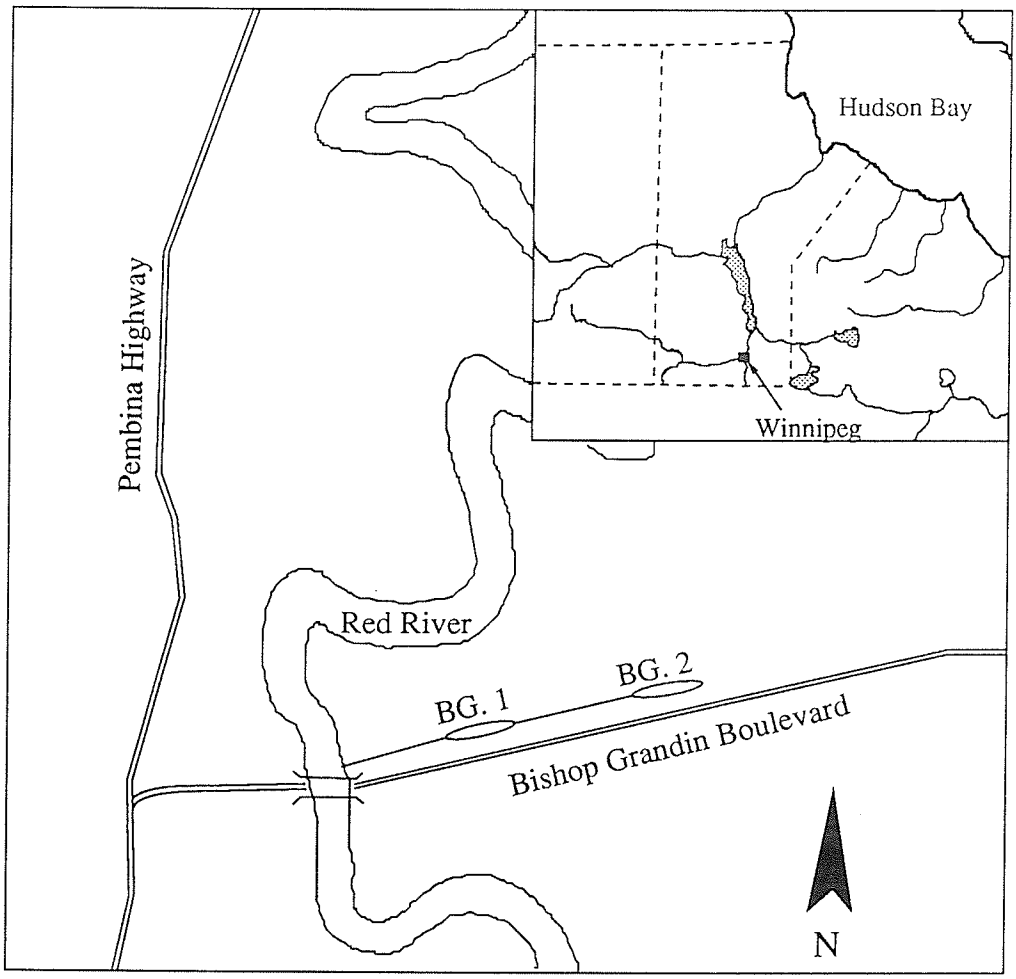
Materials and Methods

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

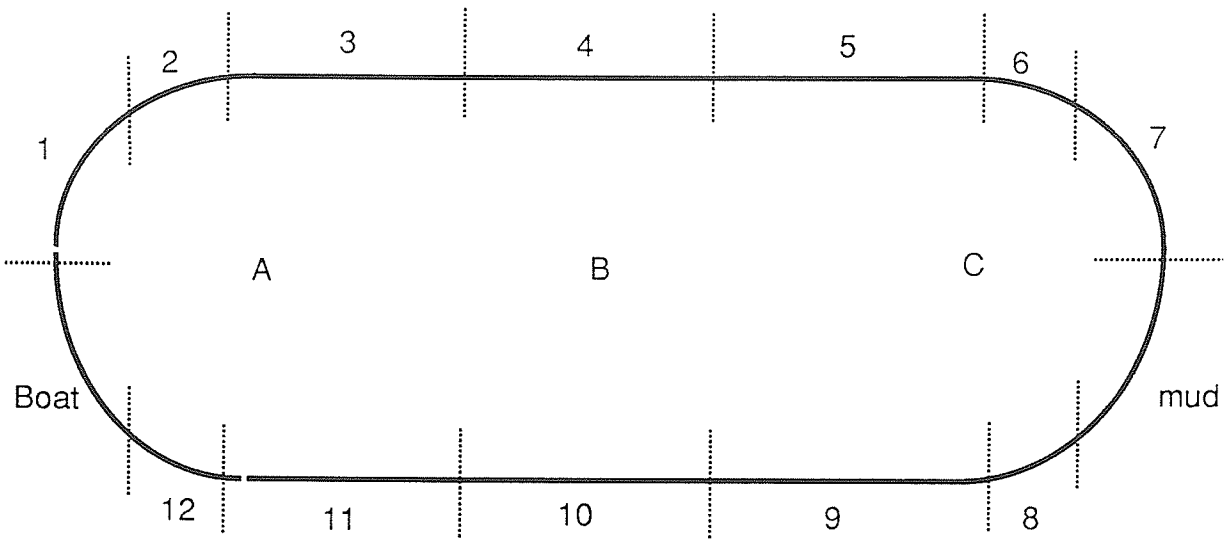
In 1986 fish were sampled from the ponds using an 18.6 x 2 metre net with 6.4 mm mesh, which was hauled over a standard 6.5 x 8.0 m area by wading along the edges of the pond. Each pond was divided into 14 areas of which 12 were seined (Figure 2A). Two areas were not seined, one because the water was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

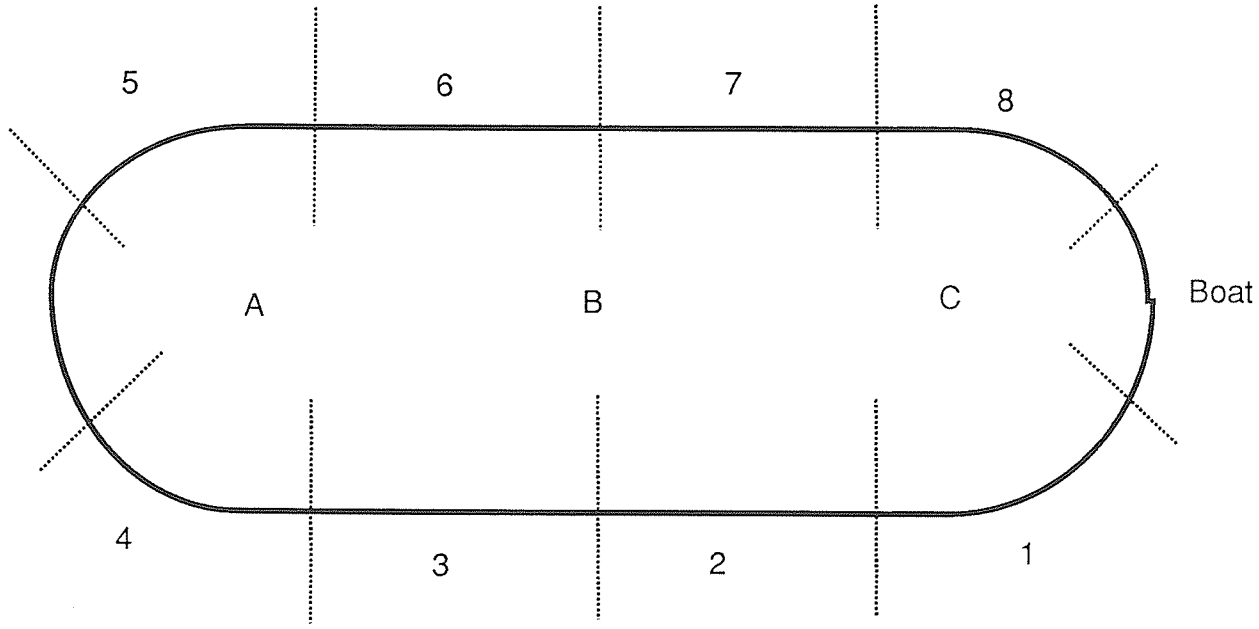
Experiment Number	Date	Exposure		Number of fish returned		Mean Percent Inhibition
		Dose (ppb)	Duration (min)	Treated	Sham Treated	
1	June 25, 1986	1	120	807	820	7
2	July 15, 1986	25	90	420	390	21
3	June 30, 1987	50	90	1084	1195	60
4	July 15, 1987	50	65	525	807	35

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.

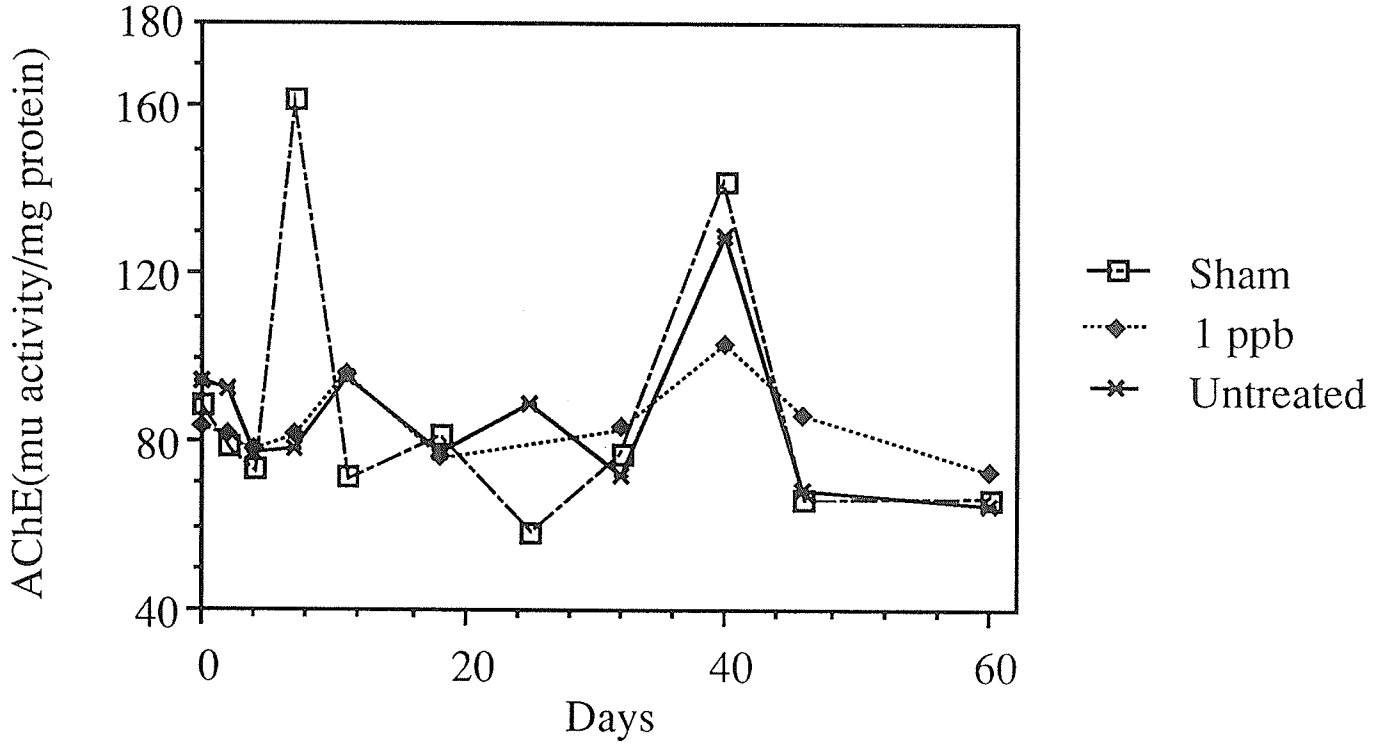

180
160
120
80
40
AChE(mu activity/mg protein)
0
20
40
60
Days
Sham
1 ppb
Untreated

Figure 4. Mean acetylcholinesterase activity levels for 25 ppb malathion treated, sham treated and untreated walleye versus days after exposure.

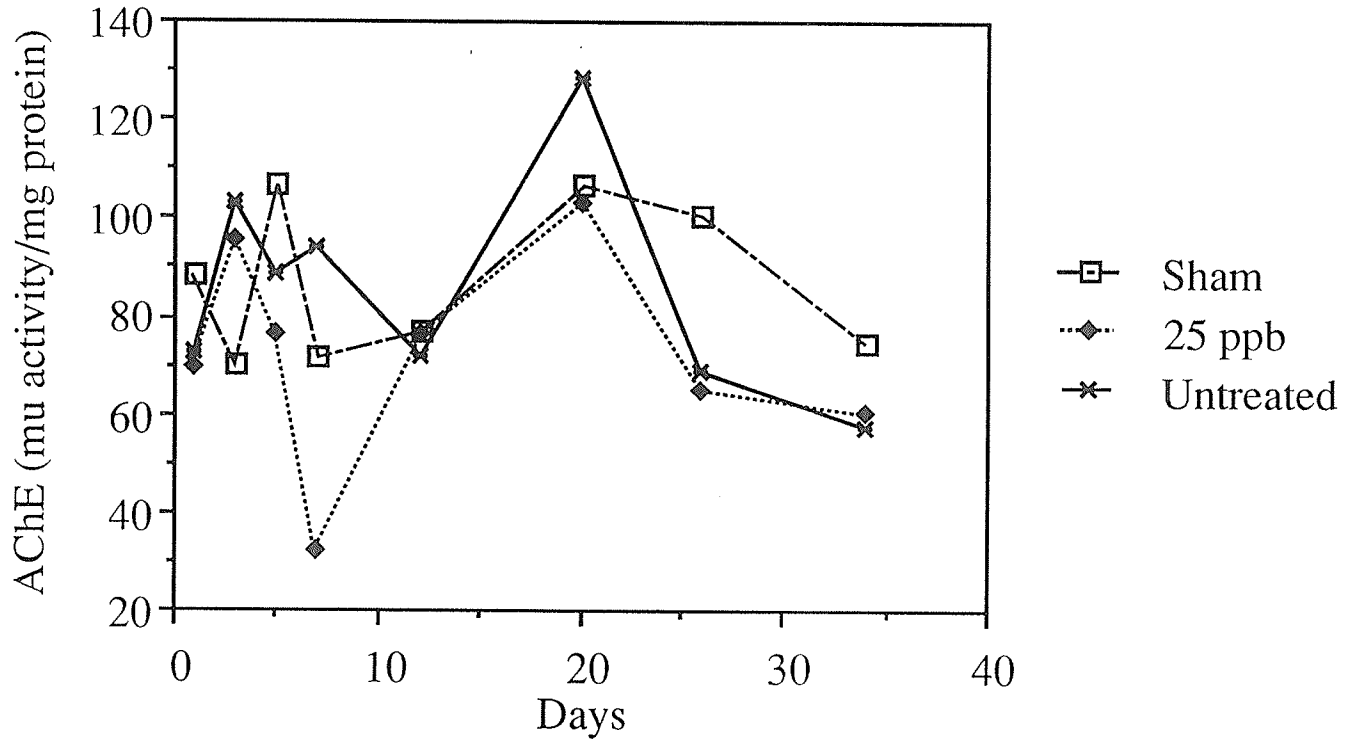
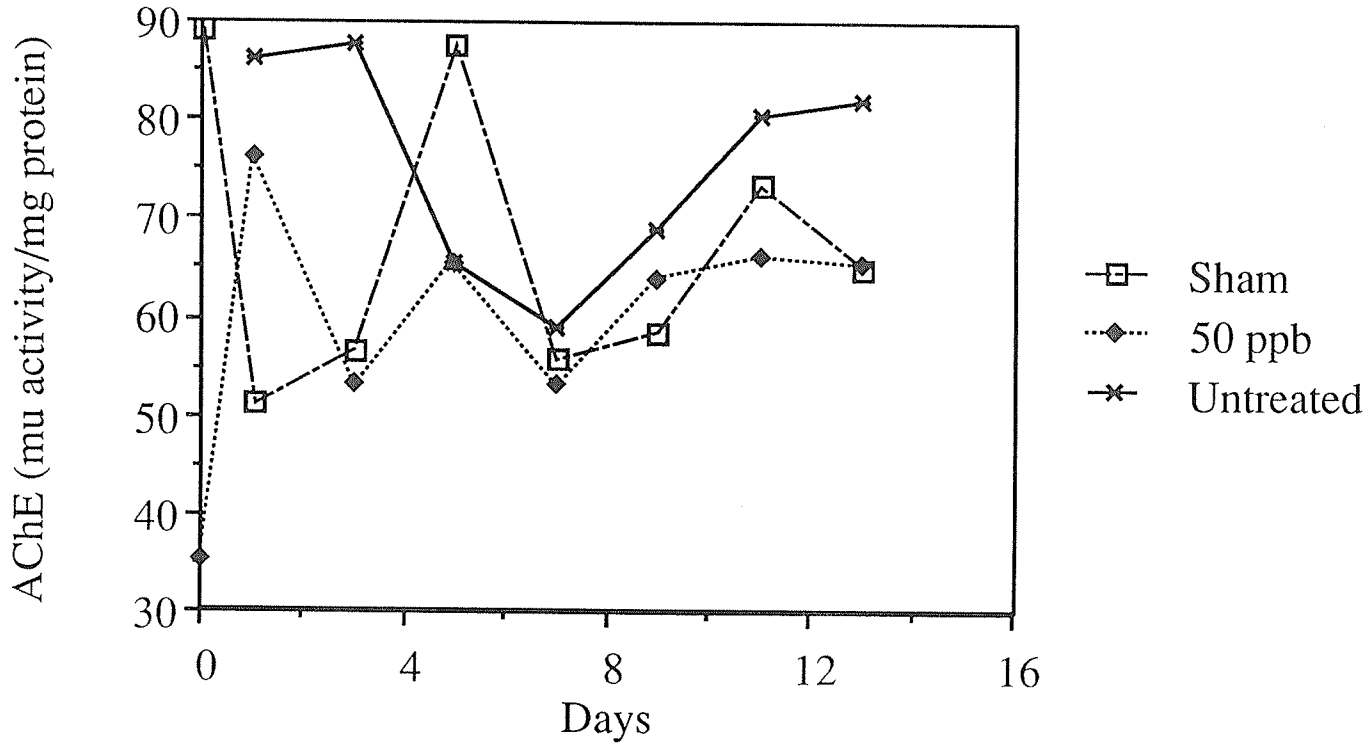

ACh E (mu activity/mg protein)
140
120
100
80
60
40
20
0
10
20
30
40
Days
Sham
25 ppb
Untreated

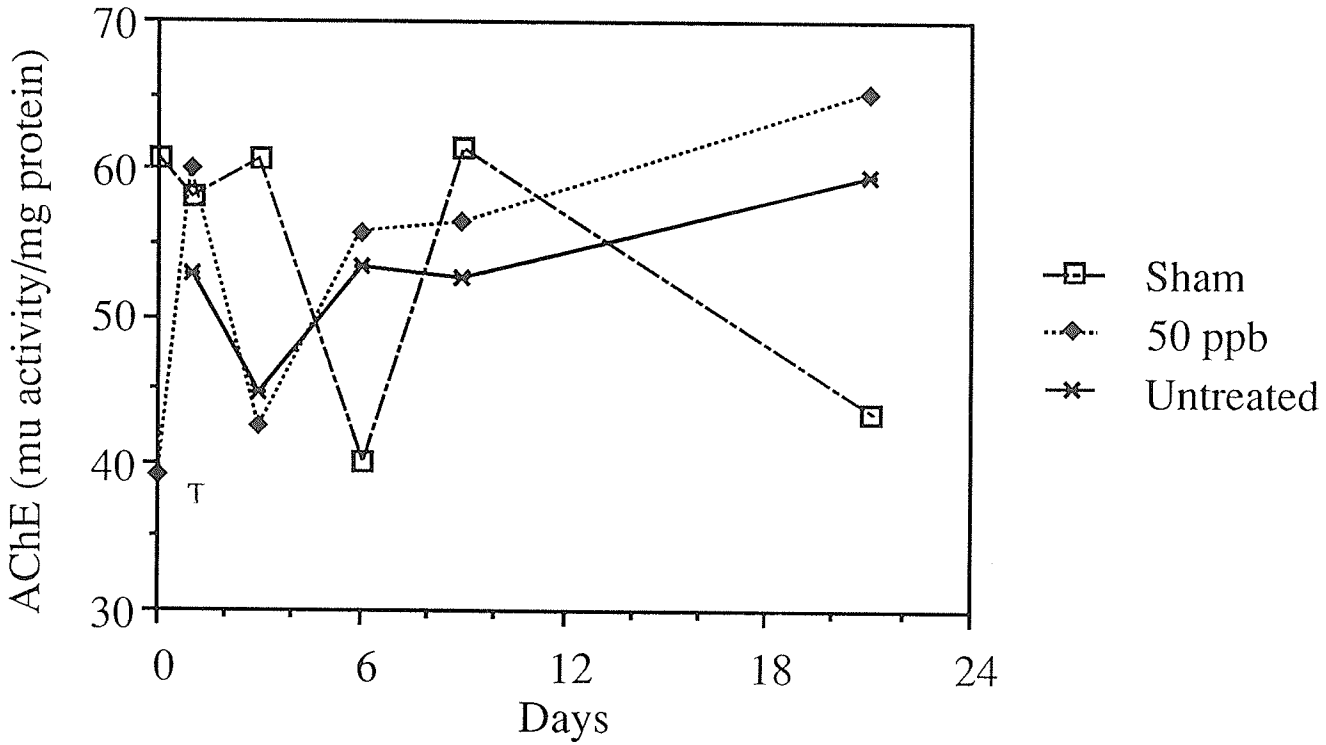
Figure 5. Mean acetylcholinesterase activity levels for 50 ppb malathion treated (experiment 3), sham treated and untreated walleye versus days after exposure.


AChE (mu activity/mg protein)
90
80
70
60
50
40
30
0
4
8
12
16
Days
Sham
50 ppb
Untreated

sham treated and both treated and untreated fish were found on day 5 after exposure (p=0.0065). In this case the difference was caused by anomalously high values for the sham treated fish rather than by low values in the malathion treated group (Figure 4).

The second group of walleye treated with 50 ppb malathion (experiment 4) showed a significant inhibition between the treated fish and the sham treated of 35 % (p=0.0576) on day 0 . As with the first exposure to 50 ppb the inhibition appears to have been short lived. Cholinesterase levels increased to above the level of the sham treated fish on day 1 following exposure (Figure 6). No significant differences were found on any dates between sham treated and untreated walleye.

Figure 6. Mean acetylcholinesterase activity levels for 50 ppb malathion treated (experiment 4), sham treated and untreated walleye versus days after exposure.


70
60
50
40
30
ACh E (mu activity/mg protein)
0
6
12
18
24
Days
T
Sham
50 ppb
Untreated

Growth

In general the 1 ppb exposed fish and the sham treated fish gained weight at a slower rate than the untreated fish for the first 18 days following exposure (Figure 7). Significant differences were found in weight, length and weight/length ratio (Table 2); however these significant differences occurred most often within the first 18 days after exposure.

No significant differences were found in the second experiment (25 $ug \cdot L^{-1}$) in any of the measures used between treated walleye and sham treated walleye on any of the days, nor were there any significant differences found between sham treated and untreated walleye on any of the days (Table 2).

Results of ANOVA's for the first of the 50 ppb experiments indicate that weight and W/L ratio were significantly lower in the sham treated and treated fish on day 1 following exposure (Table 3). Significant differences were also found in lengths between sham treated and untreated walleye on day 5 following exposure and between treated and sham treated on day 9 following exposure. Weights tended to decrease after day 9 for all groups (Figure 8). This may have been caused by an abrupt decline in the pond population of cladocerans (*Daphnia* sp.) which were a major prey item at that time.

In the 50 ppb experiment which involved stocking of minnows as a forage food, the untreated walleye clearly gained more weight than the sham treated walleye and treated walleye (Figure 9). Significant differences in weight and W/L ratio between sham treated and untreated walleye occurred on days 1, 3, 6 and 9 following exposure ($p<0.0020$ in all cases) (Table 3). The W/L ratio was also found to be significantly different between sham

Figure 7. Mean weights for 1 ppb malathion treated, sham treated and untreated walleye versus days after exposure.

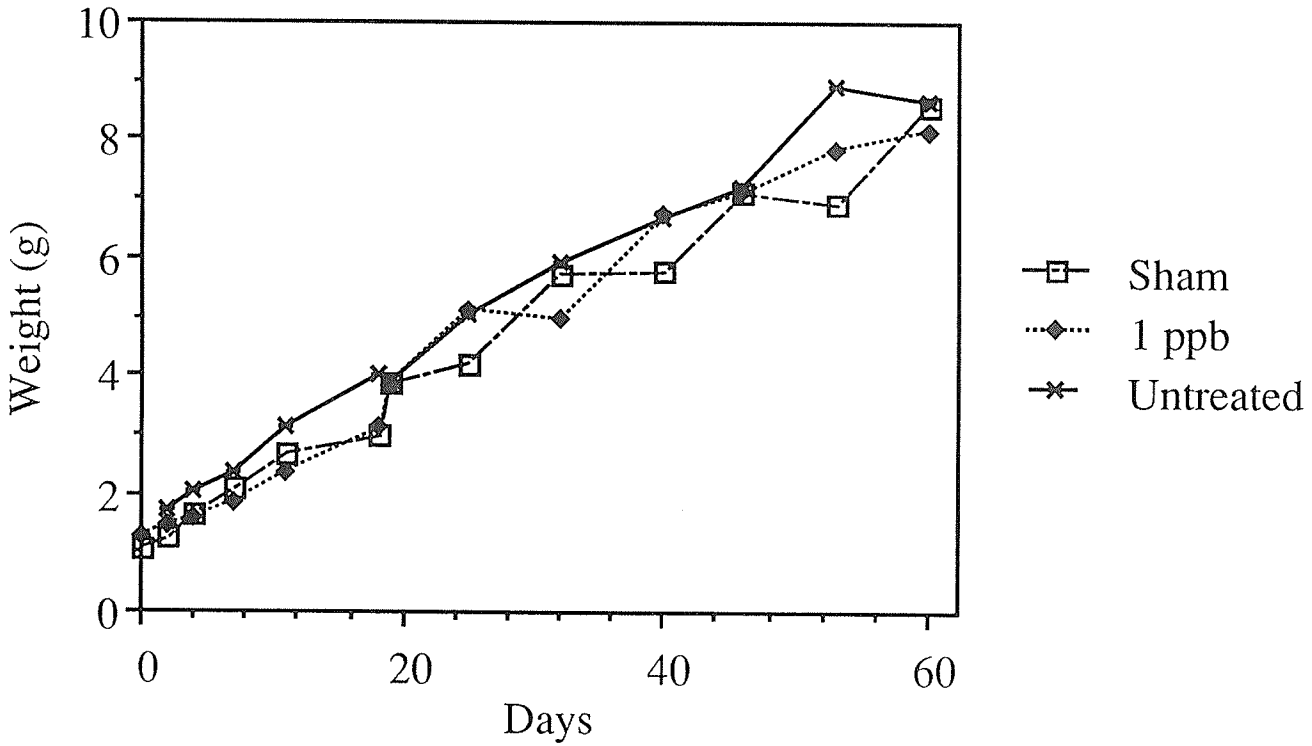

10
8
6
4
2
0
Weight (g)
0
20
40
60
Days
Sham
1 ppb
Untreated

Figure 8. Mean weights for 50 ppb malathion treated (experiment 3), sham treated and untreated walleye versus days after exposure.

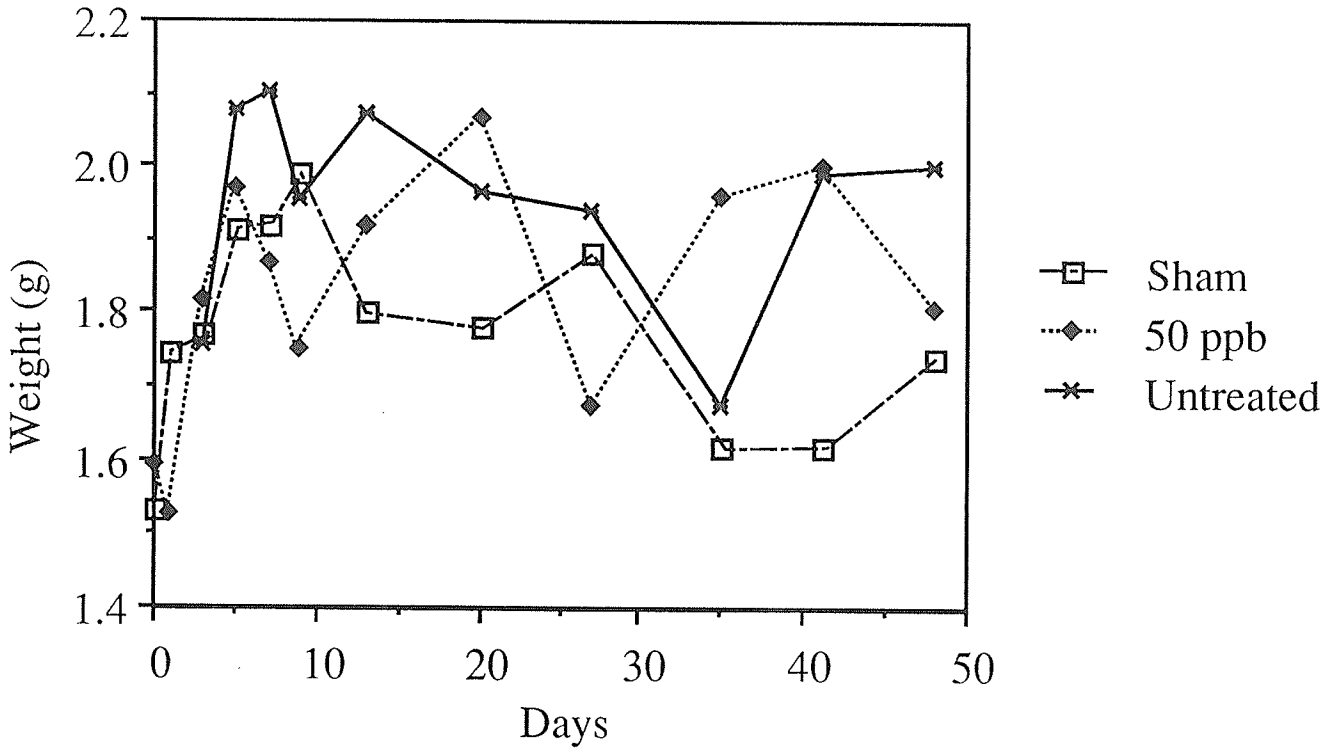

2.2
2.0
1.8
1.6
1.4
Weight (g)
0
10
20
30
40
50
Days
Sham
50 ppb
Untreated

Figure 9. Mean weights for 50 ppb malathion treated (experiment 4), sham treated and untreated walleye versus days after exposure.

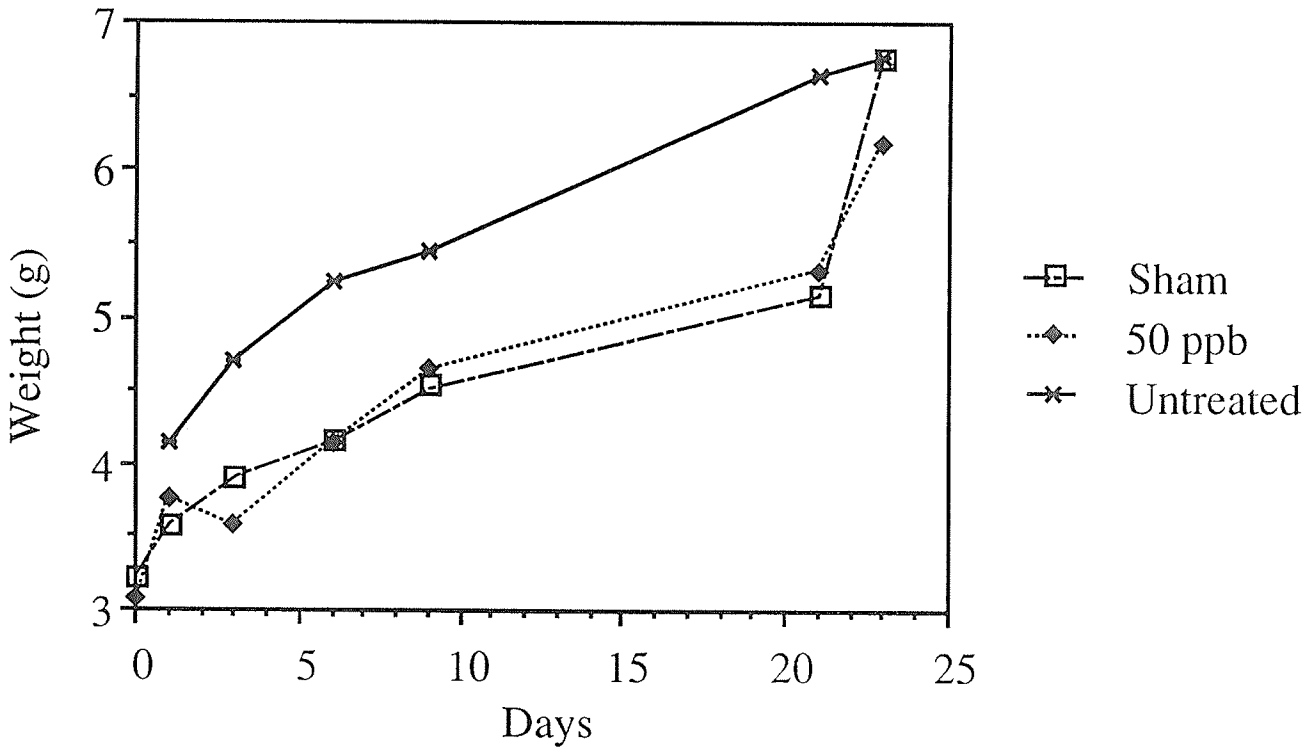

Weight (g)
7
6
5
4
3
0
5
10
15
20
25
Days
Sham
50 ppb
Untreated

Table 2. Geometric means of weight, length and W/L, for malathion treated, sham treated and untreated walleye exposed to 1 and 25 ppb malathion. Probability values from one way analyses of variance (ANOVA) are included.

		Weight (g)				Length (mm)				W/L			
Experiment	Day	Sham	Treated	Untreated	p	Sham	Treated	Untreated	p	Sham	Treated	Untreated	p
1 ppb	0	1.0822	1.2511	-	0.3874	50.0	50.9	-	0.7018	0.0216	0.0246	-	0.2973
	2	1.2755	1.4633	1.7021	0.0219[s]	53.9	55.9	57.1	0.2221	0.0236	0.0262	0.0298	0.0079[s]
	4	1.6613	1.5774	2.0492	0.0112[b]	57.0	56.7	60.2	0.0652	0.0291	0.0278	0.0340	0.0048[b]
	7	2.0869	1.8577	2.3759	0.0181[b]	62.7	60.1	66.2	0.0164[b]	0.0333	0.0309	0.0359	0.0291[b]
	11	2.6785	2.3457	3.1177	0.0001[sb]	68.0	65.0	72.0	0.0001[sb]	0.0394	0.0361	0.0433	0.0001[b]
	18	2.9632	3.1012	4.0020	0.0339	72.1	72.2	78.9	0.0446	0.0411	0.0429	0.0507	0.0384[s]
	19	3.8855	3.8258	3.9140	0.8133	77.5	77.5	77.2	0.9012	0.0501	0.0493	0.0507	0.5874
	25	4.1861	5.0639	5.0362	0.0680	80.2	84.8	84.8	0.1002	0.0523	0.0570	0.0608	0.0576
	32	5.7373	4.9450	5.8925	0.0443	90.3	86.1	90.8	0.0510	0.0635	0.0574	0.0649	0.0500
	40	5.7974	6.7513	6.6894	0.0199[s]	90.3	94.9	94.1	0.0492	0.0642	0.0711	0.0710	0.0140[s]
	46	7.1627	7.1456	7.1829	0.9968	97.5	97.5	97.0	0.9755	0.0735	0.0733	0.0740	0.9730
	53	6.9232	7.8120	8.8931	0.0501[s]	98.0	98.9	107.6	0.0391[s]	0.0762	0.0790	0.0829	0.0608
	60	8.6023	8.1576	8.6938	0.7851	102.6	103.3	103.4	0.9779	0.0838	0.0790	0.0841	0.4209
25 ppb	0	4.0780	4.2721	-	0.6276	76.4	77.7	-	0.5904	0.0534	0.0550	-	0.6542
	1	2.7082	3.0001	3.1653	0.4459	77.6	80.5	84.6	0.1020	0.0349	0.0373	0.0374	0.6952
	3	4.4329	4.1601	4.1550	0.7561	82.9	79.5	78.7	0.3562	0.0535	0.0524	0.0528	0.9627
	5	4.5327	4.9294	5.0362	0.4145	81.4	83.1	84.8	0.6542	0.0557	0.0593	0.0608	0.4529
	7	4.6699	3.7371	5.0422	0.0707	85.9	77.7	85.7	0.0417[b]	0.0544	0.0481	0.0588	0.0881
	12	5.2339	5.4751	5.8925	0.2656	87.1	87.6	90.8	0.2236	0.0601	0.0625	0.0649	0.3120
	20	6.6856	6.8413	6.6894	0.9432	94.1	95.3	94.2	0.8741	0.0711	0.0718	0.0710	0.9713
	26	5.6666	6.4640	7.1829	0.1039	90.4	93.3	97.0	0.1820	0.0627	0.0693	0.0740	0.0805
	33	7.9816	7.5754	8.8931	0.1261	101.1	99.9	107.3	0.0221[b]	0.0790	0.0758	0.0829	0.2809
	40	-	9.7491	8.6938	0.1575	-	109.7	103.4	0.1229	-	0.0889	0.0841	0.2023

[b] - Significant Difference between treated walleye and untreated walleye.

[s] - Significant difference between sham treated walleye and untreated walleye.

Table 3. Geometric means of weight, length and W/L, for malathion treated, sham treated and untreated walleye exposed to 50 ppb malathion. Probability values from one way analyses of variance (ANOVA) are included.

		Weight (g)				Length (mm)				W/L			
Experiment	Day	Sham	Treated	Untreated	p	Sham	Treated	Untreated	p	Sham	Treated	Untreated	p
50 ppb - 1	0	1.532	1.5912	-	0.4750	53.5	53.8	-	0.7672	0.0286	0.0296	-	0.3743
	1	1.7442	1.5268	-	0.0308[t]	55.6	53.5	-	0.0868	0.0314	0.0285	-	0.0206[t]
	3	1.7733	1.8158	1.7567	0.8809	56.0	55.5	56.7	0.5898	0.0317	0.0327	0.0310	0.5059
	5	1.9163	1.9711	2.0772	0.2128	56.9	57.8	59.2	0.0399[s]	0.0337	0.0341	0.0351	0.4561
	7	1.9205	1.8665	2.1053	0.0886	59.4	58.6	61.9	0.0108[b]	0.0324	0.0318	0.0340	0.2123
	9	1.9892	1.7516	1.9535	0.0660	60.9	57.8	60.6	0.0199[bt]	0.0327	0.0303	0.0322	0.1241
	13	1.8048	1.9213	2.0751	0.0820	59.5	60.3	62.1	0.0468	0.0301	0.0319	0.0334	0.1023
	20	1.7817	2.0674	1.9677	0.3817	58.8	61.3	61.0	0.3842	0.0303	0.0337	0.0320	0.3891
	27	1.8836	1.6748	1.9389	0.3638	61.0	58.9	62.1	0.1974	0.0309	0.0284	0.0312	0.4464
	35	1.6210	1.9618	1.6757	0.3730	62.2	63.5	62.1	0.7799	0.0261	0.0309	0.0270	0.2679
	41	1.6206	2.0030	1.9904	0.5255	58.7	62.2	62.9	0.4135	0.0276	0.0322	0.0316	0.5595
	48	1.7422	1.8054	1.9995	0.6025	60.7	60.2	62.5	0.6353	0.0287	0.0300	0.0320	0.5714
50 ppb - 2	0	3.2312	3.0656	-	0.6115	72.6	73.9	-	0.6656	0.0480	0.0465	-	0.5956
	1	3.5875	3.7736	4.1572	0.0132[s]	72.0	73.2	74.7	0.0830	0.0525	0.0541	0.0578	0.0072[s]
	3	3.9257	3.5776	4.7147	0.0001[sb]	73.1	73.2	77.3	0.0001[sb]	0.0570	0.0508	0.0646	0.0001[sbt]
	6	4.1690	4.1483	5.2401	0.0001[sb]	75.0	75.6	79.6	0.0001[sb]	0.0582	0.0566	0.0671	0.0001[sb]
	9	4.5420	4.6562	5.4586	0.0001[sb]	76.8	76.7	81.5	0.0001[sb]	0.0615	0.0642	0.0702	0.0005[sb]
	21	5.1562	5.3107	6.6327	0.3071	81.2	82.1	88.2	0.0542	0.0854	0.0687	0.0821	0.3646
	23	6.7817	6.1802	6.7677	0.0710	88.3	86.4	89.1	0.0276[b]	0.0781	0.0742	0.0778	0.0559

[b] - Significant difference between treated walleye and untreated walleye.
[s] - Significant difference between sham treated walleye and untreated walleye.
[t] - Significant difference between treated walleye and sham treated walleye.

treated and treated walleye on day 3 after exposure (p= 0.0001). Length was significantly different between sham treated and untreated walleye on days 3, 6 and 9 after treatment (p=0.0001 in all cases).

Feeding

Daphnia sp. was the prevalent food item in both the 1 ppb (Table 4) and 25 ppb experiment (Table 5) in 1986 as well as the first 50 ppb experiment in 1987 (Table 6).

All walleye had *Daphnia* sp. present in the stomach during the 1 ppb experiment. Copepods decreased in importance as the fish grew, whereas insects increased in occurrence (Table 4). No difference in food organisms selected were apparent among the three groups of walleye.

Although there were no significant differences among groups in either the mean number of daphnids or in mean total dry weight of stomach contents (Table 7), there was a trend indicating that sham treated and malathion treated walleye consumed less than untreated walleye for the first five days after return to the pond (Figure 10).

As in the data from experiment 1, daphnids were a major component of the diet, present in 100% of the stomachs analyzed in connection with the 25 ppb experiment. Insects were of importance on days 3 and 8 following exposure and amphipods, which were not found in the 1 ppb experiments stomach analyses, were found in several stomachs (Table 5).

Results from the 25 ppb exposure experiment showed a significant difference in the number of daphnids between the untreated and sham-treated fish on day 6 (Table 7). No significant differences were found between the sham-treated and malathion treated walleye or for the total dry stomach

Table 4. Presence/Absence of food items in stomaches of fish sampled during the 1 ppb exposure experiment. Percentages are in parentheses.

Day After Exposure	Group	n	Numbers and percentages of fish with		
			Dapnids	Copepods	Insects
2	Sham	7	7(100)	4(57)	2(29)
	1 ppb	7	7(100)	3(43)	2(29)
	Untreated	7	7(100)	4(57)	1(14)
4	Sham	3	3(100)	0 (0)	0 (0)
	1 ppb	7	7(100)	1(14)	1(14)
	Untreated	7	7(100)	0 (0)	1(14)
7	Sham	7	7(100)	2(29)	4(57)
	1 ppb	7	7(100)	2(29)	1(14)
	Untreated	7	7(100)	1(14)	2(29)
11	Sham	6	6(100)	1(17)	4(67)
	1 ppb	7	7(100)	0 (0)	4(57)
	Untreated	7	7(100)	0 (0)	4(57)
18	Sham	7	7(100)	0 (0)	2(29)
	1 ppb	7	7(100)	0 (0)	0 (0)
	Untreated	7	7(100)	0 (0)	2(29)

Table 5. Presence/Absence of food items in stomaches of fish sampled during the 25 ppb exposure experiment. Percentages are in parentheses.

Day After Exposure	Group	n	Numbers and percentages of fish with			
			Daphnids	Copepods	Insects	Amphipods
1	Sham	7	7 (100)	0 (0)	2 (29)	0 (0)
	25 ppb	7	7 (100)	1 (14)	1 (14)	0 (0)
	Untreated	7	7 (100)	0 (0)	0 (0)	0 (0)
3	Sham	4	4 (100)	0 (0)	3 (75)	0 (0)
	25 ppb	7	7 (100)	0 (0)	6 (86)	0 (0)
	Untreated	7	7 (100)	0 (0)	5 (71)	0 (0)
6	Sham	7	7 (100)	1 (14)	0 (0)	0 (0)
	25 ppb	7	7 (100)	2 (29)	2 (29)	1 (14)
	Untreated	7	7 (100)	0 (0)	1 (14)	1 (14)
8	Sham	4	4 (100)	0 (0)	3 (75)	1 (25)
	25 ppb	5	5 (100)	1 (20)	3 (60)	0 (0)
	Untreated	7	7 (100)	0 (0)	2 (29)	0 (0)
12	Sham	7	7 (100)	0 (0)	2 (29)	0 (0)
	25 ppb	7	7 (100)	1 (14)	1 (14)	0 (0)
	Untreated	7	7 (100)	0 (0)	0 (0)	0 (0)

Table 6. Presence/Absence of food items in stomaches of fish sampled during the 50 ppb exposure experiment. Percentages are in parentheses.

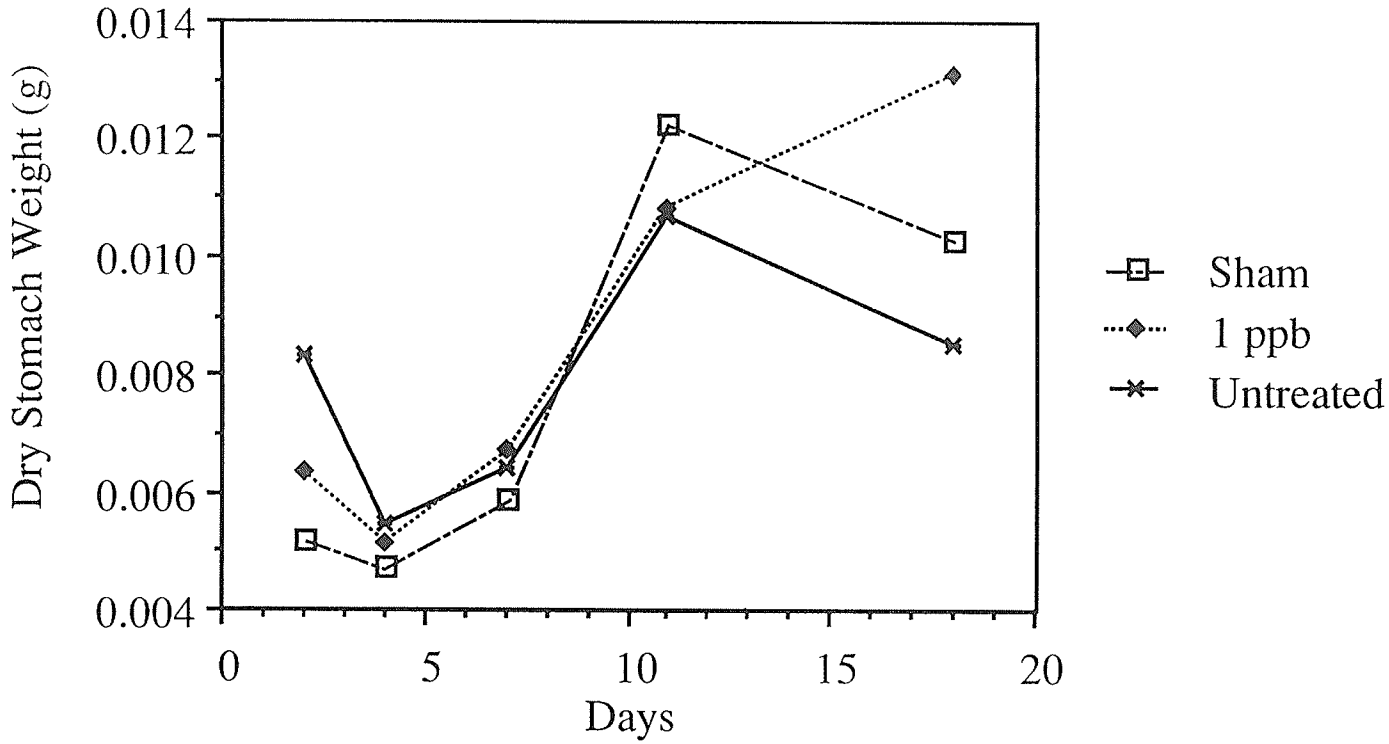
Days After Exposure	Group	n	Numbers and percentages of fish with				
			Daphnids	Copepods	Insects	Amphipods	Crayfish
1	Sham	10	10 (100)	2 (20)	4 (40)	0 (0)	0 (0)
	50 ppb	6	6 (100)	2 (20)	0 (0)	0 (0)	0 (0)
	Untreated	10	10 (100)	1 (10)	1 (10)	0 (0)	0 (0)
3	Sham	15	14 (93)	6 (40)	1 (7)	1 (7)	0 (0)
	50 ppb	15	13 (87)	3 (20)	2 (13)	0 (0)	1 (7)
	Untreated	15	15 (100)	2 (13)	3 (20)	0 (0)	1 (7)
5	Sham	16	16 (100)	10 (73)	1 (6)	0 (0)	0 (0)
	50 ppb	15	15 (100)	10 (67)	1 (7)	0 (0)	1 (7)
	Untreated	15	15 (100)	6 (40)	0 (0)	0 (0)	0 (0)
7	Sham	15	6 (40)	11 (73)	0 (0)	1 (7)	0 (0)
	50 ppb	15	6 (40)	9 (60)	2 (13)	0 (0)	0 (0)
	Untreated	15	5 (33)	5 (33)	4 (27)	0 (0)	1 (7)
9	Sham	15	1 (7)	14 (93)	4 (27)	0 (0)	1 (7)
	50 ppb	15	0 (0)	15 (100)	0 (0)	0 (0)	0 (0)
	Untreated	15	3 (20)	14 (93)	0 (0)	0 (0)	0 (0)
13	Sham	17	2 (12)	14 (82)	3 (18)	2 (12)	0 (0)
	50 ppb	15	0 (0)	13 (87)	3 (20)	2 (13)	0 (0)
	Untreated	15	0 (0)	8 (53)	7 (47)	3 (20)	0 (0)
20	Sham	15	1 (7)	13 (87)	4 (27)	5 (33)	0 (0)
	50 ppb	15	0 (0)	11 (73)	6 (40)	1 (7)	0 (0)
	Untreated	15	1 (7)	12 (80)	6 (40)	2 (13)	0 (0)

Table 7. Mean natural logariths of daphnia numbers and geometric mean total dry stomach weights for malathion treated, sham treated and untreated walleye exposed to 1, 25 and 50 ppb malathion. Probability values from one way analyses of variance (ANOVA)are included.

		Ln number Daphnia				Total Stomach Weight			
Experiment	Day	Sham	Treated	Untreated	p	Sham	Treated	Untreated	p
1 ppb	2	5.39	5.17	5.81	0.2244	0.0052	0.0064	0.0084	0.2335
	4	5.33	5.22	5.45	0.5005	0.0048	0.0051	0.0054	0.8868
	7	5.03	5.66	4.57	0.3185	0.0059	0.0067	0.0064	0.882
	11	4.08	5.51	5.45	0.5415	0.0123	0.0108	0.0107	0.7715
	18	6.45	6.56	5.94	0.2636	0.0103	0.0131	0.0085	0.067
25 ppb	1	2.62	3.10	5.12	0.0948	0.0056	0.0016	0.0044	0.0901
	3	4.22	5.22	6.11	0.2192	0.0070	0.0084	0.0075	0.9097
	6	5.99	6.24	6.44	0.0093[s]	0.0097	0.0081	0.0126	0.1735
	8	4.42	5.61	5.29	0.7058	0.0070	0.0065	0.0510	0.5375
	12	6.55	6.26	6.63	0.2274	0.0107	0.0116	0.0132	0.6189
50 ppb - 1	1	4.19	4.27	4.69	0.4432	0.0053	0.0040	0.0078	0.0051[s]
	3	4.62	4.57	4.83	0.6255	0.0040	0.0061	0.0073	0.0509[s]
	5	5.00	5.39	5.13	0.4436	0.0027	0.0044	0.0074	0.0211[s]
	7	1.76	1.89	1.61	0.8482	0.0020	0.0016	0.0057	0.1608
	9	0.69	0.00	0.69	*	0.0035	0.0026	0.0028	0.4408
	13	3.99	0.00	0.00	*	0.0019	0.0018	0.0027	0.0898
	20	1.10	0.00	0.69	*	0.0018	0.0016	0.0028	0.0128[s]
50 ppb - 2	1	4.06	4.49	3.80	0.6881	0.0077	0.0046	0.0221	0.0072[sb]
	3	3.46	3.19	2.43	0.4193	0.0147	0.0090	0.0334	0.0284[b]
	6	3.56	4.09	1.93	0.0014[sb]	0.0144	0.0103	0.0183	0.1589
	9	3.11	2.57	3.40	0.5829	0.0159	0.0240	0.0265	0.2767

[b] - Significant difference between treated walleye and untreated walleye.
[s] - Significant difference between sham treated walleye and untreated walleye.
* - insufficient data for analysis.

Figure 10. Mean total dry stomach contents weight for the 1 ppb malathion experiment for sham treated, treated and untreated walleye.


Dry Stomach Weight (g)
0.014
0.012
0.010
0.008
0.006
0.004
0
5
10
15
20
Days
Sham
1 ppb
Untreated

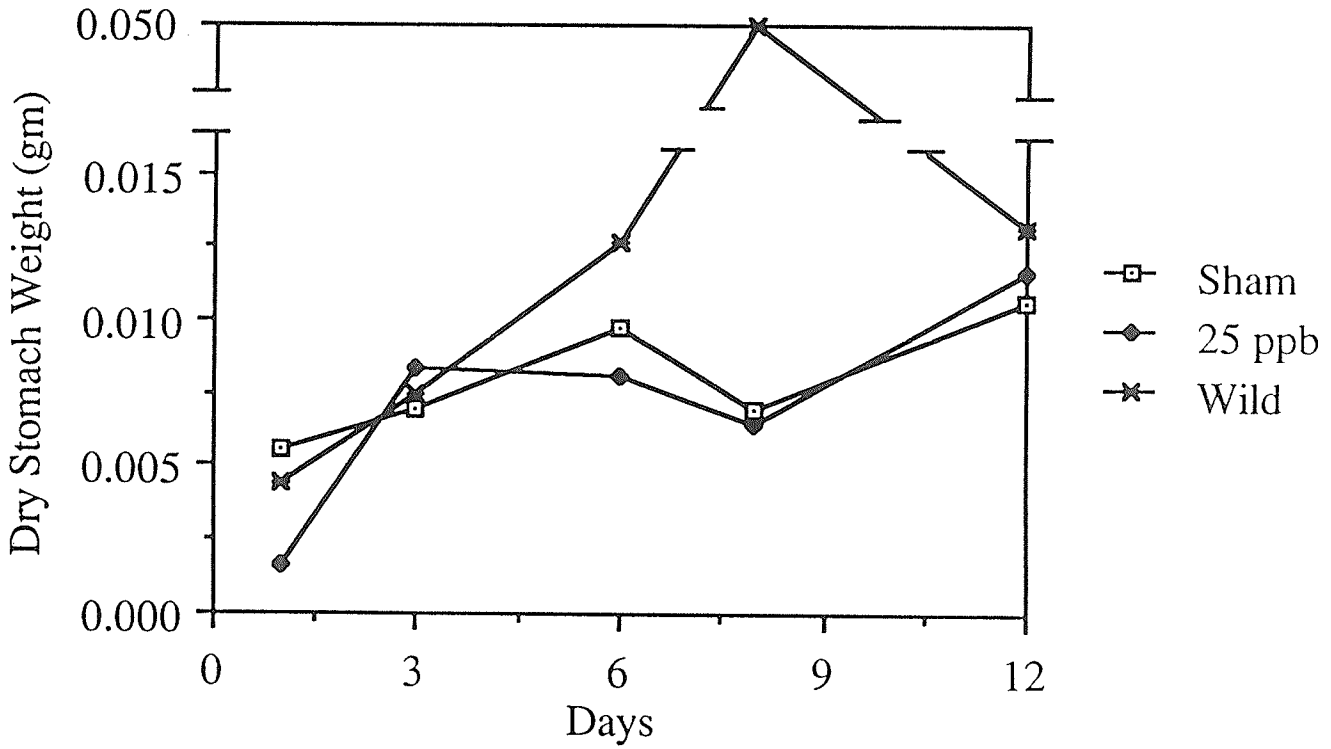
weight on any of the sampling dates (Table 7). Again there was a trend indicating that sham treated and treated walleye consumed less than untreated fish (Figure 11).

Walleye initially fed mostly on daphnids following the first treatment with 50 ppb malathion but later (on day 9) they fed on copepods when the *Daphnia* population declined. Insects and amphipods also became a prey species in later samples (Table 6). Results of analysis of variance indicated that no significant differences occurred on any of the sampling dates in the mean natural logarithm of the number of *Daphnia* between the sham treated fish and either the untreated or the malathion treated fish (Table 7). There were, however, significant differences in the total dry stomach weights between untreated and sham treated on days 1, 3, 5 and 20 (Table 7). This may have been due to an increased number of fish feeding on insects.

As with the 1 ppb and 25 ppb experiments a trend was evident suggesting sham treated and malathion treated walleye consumed less than untreated fish (Figure 12). Data in Figure 12 also indicates that the food supply declined in the BG 2 during the 1987 season because feeding (measured by total dry weight of stomach contents) decreased.

The addition of minnows to the pond as a forage food caused a change in feeding patterns in the fourth experiment. Daphnids became less important as a prey species with the introduction of minnows as did both insects and amphipods (Table 8). Treated fish tended to consume more daphnids than the untreated fish (Figure 13), although the results of the ANOVA show a significant difference in the number of daphnids only on day 6 after treatment ($p=0.0014$) (Table 7). On this date both the treated and sham treated fish consumed significantly more daphnids than the untreated

Figure 11. Mean total dry stomach contents weight for the 25 ppb malathion experiment for sham treated, treated and untreated walleye.


0.050
0.015
0.010
0.005
0.000
Dry Stomach Weight (gm)
0
3
6
9
12
Days
Sham
25 ppb
Wild

fish. Initially the untreated fish had significantly ($p=0.0072$) more in their stomachs than the sham treated fish on day 1 (Figure 14). The reason for the greater dry stomach weight in the untreated fish is that they consumed more minnows than either the treated or sham treated on all dates (Table 8). Malathion treated fish didn't start consuming minnows until 3 days following exposure (1 minnow was found in 13 walleye stomachs analyzed on that day). There was also a trend indicating that sham treated walleye consumed more than treated walleye but less than untreated fish (Figure 15). No significant differences were found in the estimated mean size of minnows consumed by any of the groups of walleye in any of the samples.

Figure 12. Mean total dry stomach contents weight for the 50 ppb malathion exposure (experiment 3) for sham treated, treated and untreated walleye.

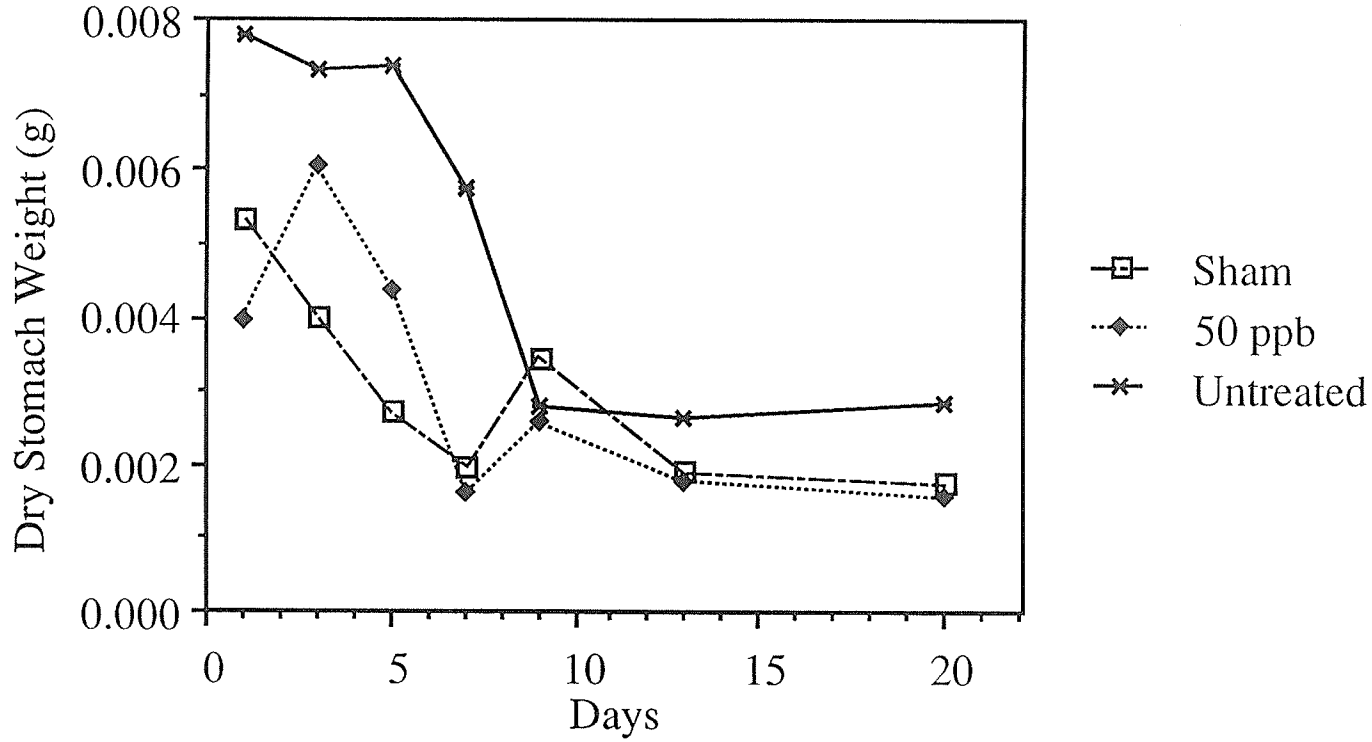

0.008
0.006
0.004
0.002
0.000
Dry Stomach Weight (g)
0
5
10
15
20
Days
Sham
50 ppb
Untreated

Table 8. Presence/Absence of food items in stomaches of fish sampled during the 50 ppb exposure experiment. Percentages are in parentheses.

Day After Exposure	Group	n	Numbers and percentages of fish with					Total Number Minnows
			Daphnids	Insects	Amphipods	Crayfish	Minnows	
1	Sham	15	13(87)	0 (0)	0 (0)	0 (0)	3 (20)	3
	50 ppb	15	13(87)	1 (7)	0 (0)	0 (0)	0 (0)	0
	Wild	14	10(71)	1 (7)	0 (0)	0 (0)	8 (57)	9
3	Sham	15	12(80)	3 (20)	0 (0)	0 (0)	6 (40)	7
	50 ppb	13	9 (69)	0 (0)	1(0.08)	0 (0)	1 (8)	1
	Wild	15	8 (53)	0 (0)	0 (0)	1 (13)	9 (60)	10
6	Sham	15	13(87)	1 (7)	0 (0)	0 (0)	6 (40)	6
	50 ppb	15	15(100)	1 (7)	0 (0)	0 (0)	4 (27)	5
	Wild	15	8 (53)	0 (0)	0 (0)	1 (13)	10(67)	11
9	Sham	15	11(73)	0 (0)	0 (0)	0 (0)	7 (47)	7
	50 ppb	15	9 (60)	1 (7)	0 (0)	2 (13)	10(67)	10
	Wild	15	11(73)	0 (0)	0 (0)	0 (0)	11(73)	17

Figure 13. Mean natural logarithms of daphnid numbers for the second 50 ppb malathion exposure (experiment 4) for sham treated, treated and untreated walleye.

A

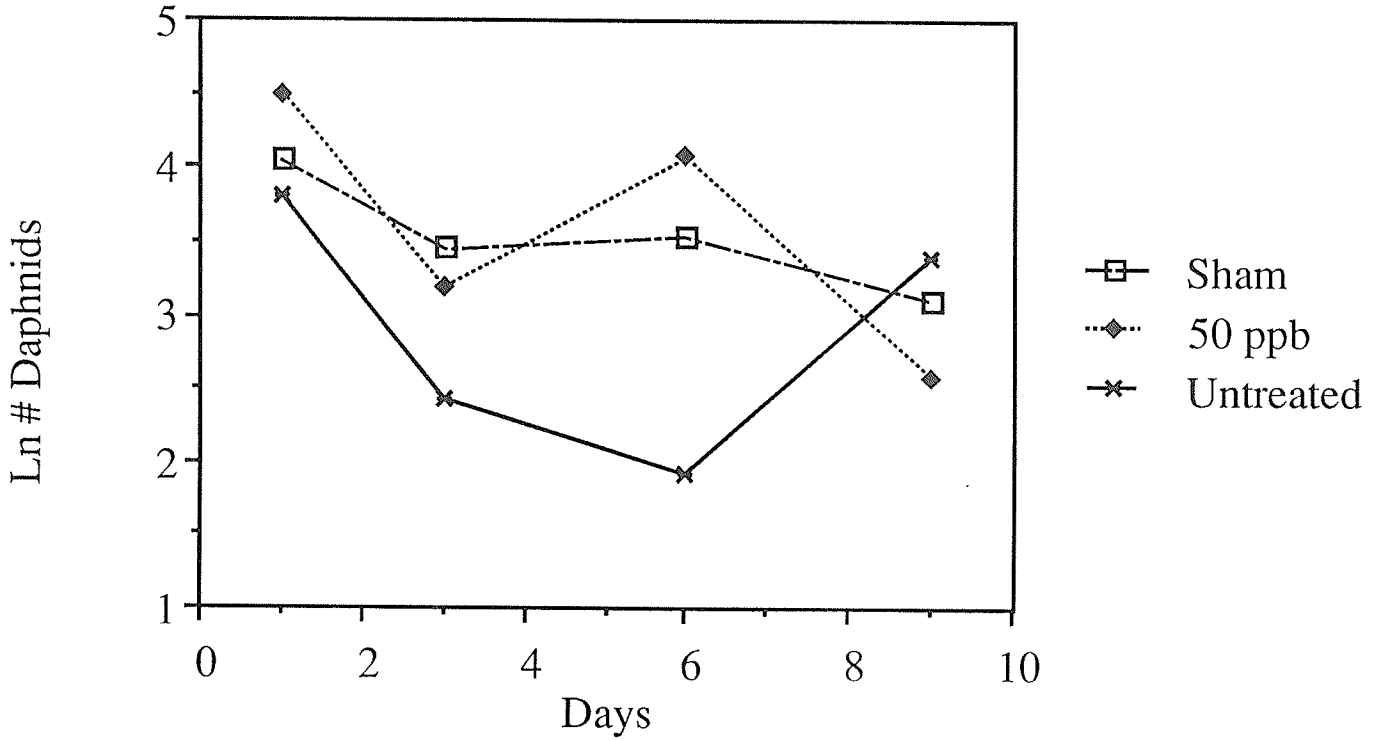


Figure 14. Mean total weight of stomach contents for the second 50 ppb experiment for treated, sham treated and untreated walleye.

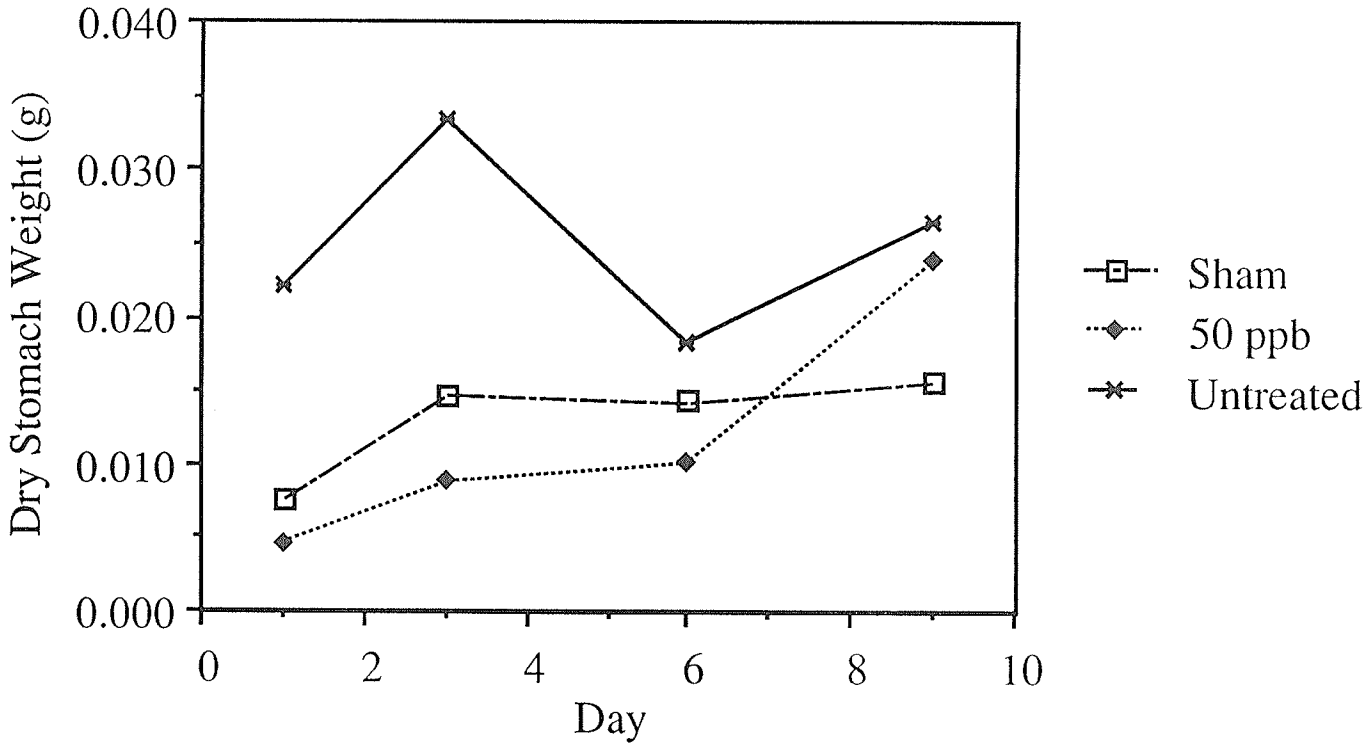
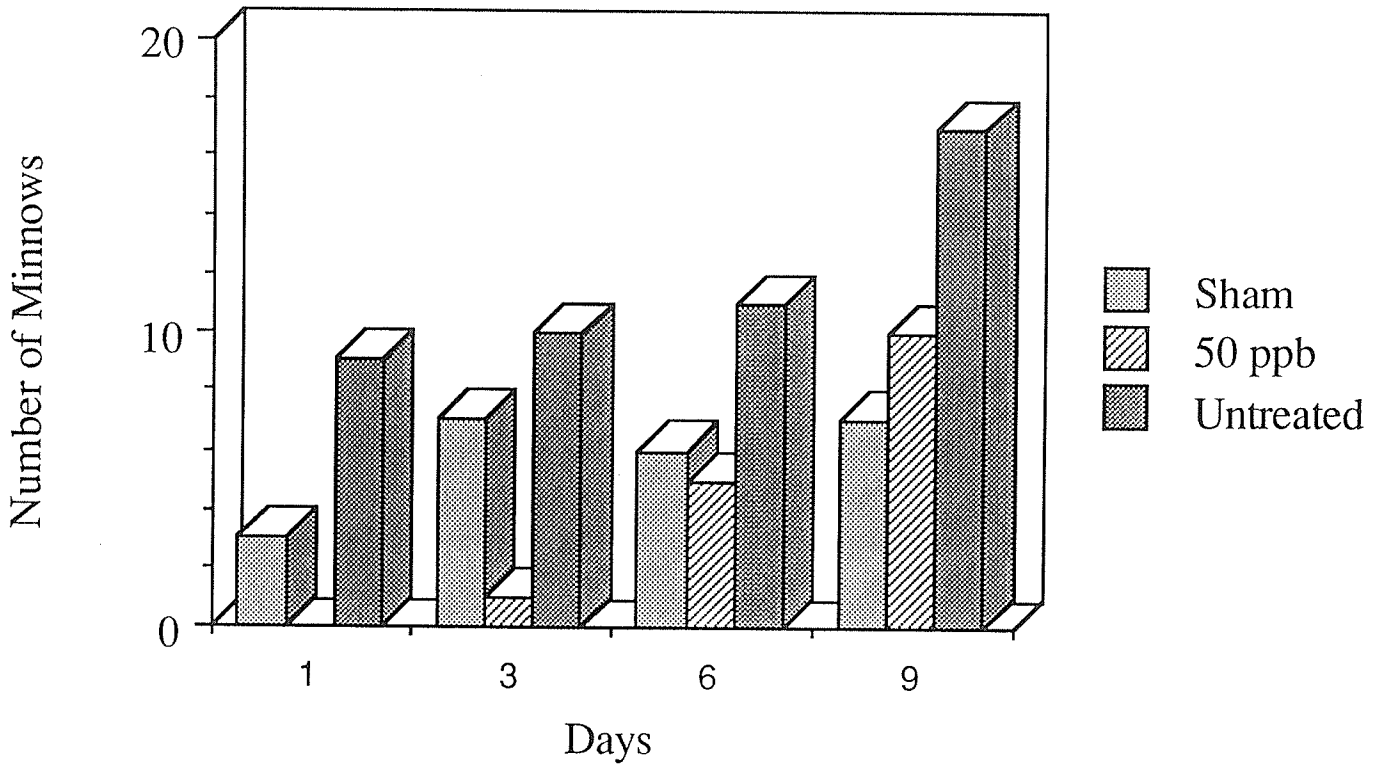

0.040
0.030
0.020
0.010
0.000
Dry Stomach Weight (g)
0
2
4
6
8
10
Day
Sham
50 ppb
Untreated

Figure 15. Total number of minnows found in sham treated, treated and untreated walleye stomachs. Sample size is 15 stomachs for all groups and days except treated fish on day 3 which used 13 stomachs.

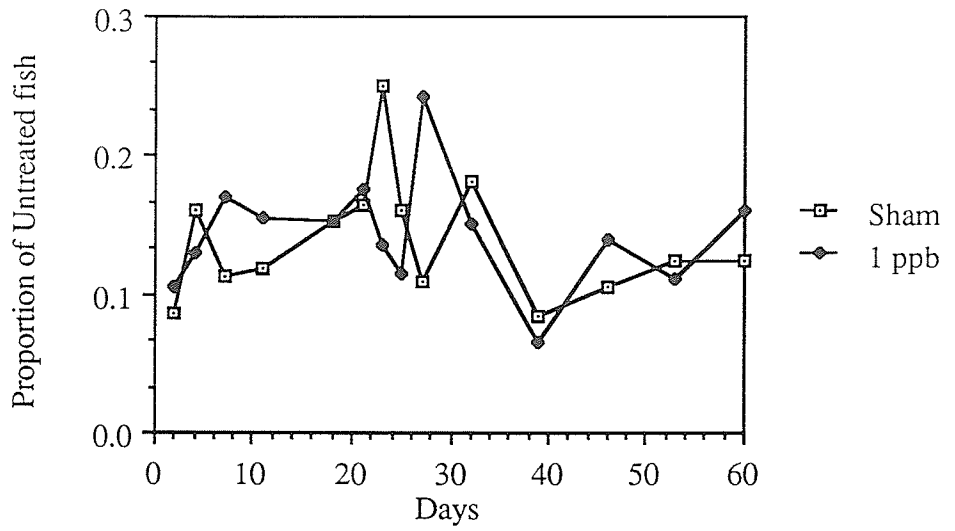

20
10
0
Number of Minnows
1
3
6
9
Days
Sham
50 ppb
Untreated

Survival

Survival was assessed using catch per unit effort (CPUE) as an index of abundance. CPUE was measured as the proportion of sham and treated fish to untreated walleye for a given date in seine hauls. In three experiments and in most samples, the proportions of sham treated and treated fish were similar indicating that there were no survival differences between these two groups. Figures 16A, 16B and Figures 17A and 17B contain one graph for each experiment. The 25 ppb experiment (Figure 16B) shows the treated fish initially at a higher proportion than the sham treated fish. This may have been caused by handling. There is an apparent decline in the proportion of both sham treated and malathion treated walleye in the first 50 ppb experiment indicating a possible mortality in these groups (Figure 17A). In the second 50 ppb treatment, (Figure 17B), the treated fish have a lower CPUE than the sham treated fish. Walleye for the fourth experiment were more stressed from handling when returned to the pond because of a mixing of marked groups in the lab which resulted in an extra sorting just prior to exposure and because of high ambient water temperatures (24^{o} C). This extra handling and temperature stress caused a large mortality in treated walleye. A total of 302 walleye died or were considered close to death following exposure to 50 ppb malathion to be returned to the pond. Furthermore this experiment was shortened because of a large mortality of fish between days 8 and 11. The water temperatures in the pond were at 28^{o}C on days 8 and 11 and were probably higher during the intervening days. Lethal temperature for acclimated walleye is 31.6 ^{o}C (Hokanson 1977, Hokanson and Koenst 1986).

Figure 16. A. Catch per unit effort for experiment 1 (1 ppb), for treated and sham treated walleye. Values are expressed as proportions of untreated fish caught.
B. Catch per unit effort for experiment 2 (25 ppb), for treated and sham treated walleye. Values are expressed as proportions of untreated fish caught.

A


0.3
0.2
0.1
0.0
Proportion of Untreated fish
0
10
20
30
40
50
60
Days
Sham
1 ppb

B

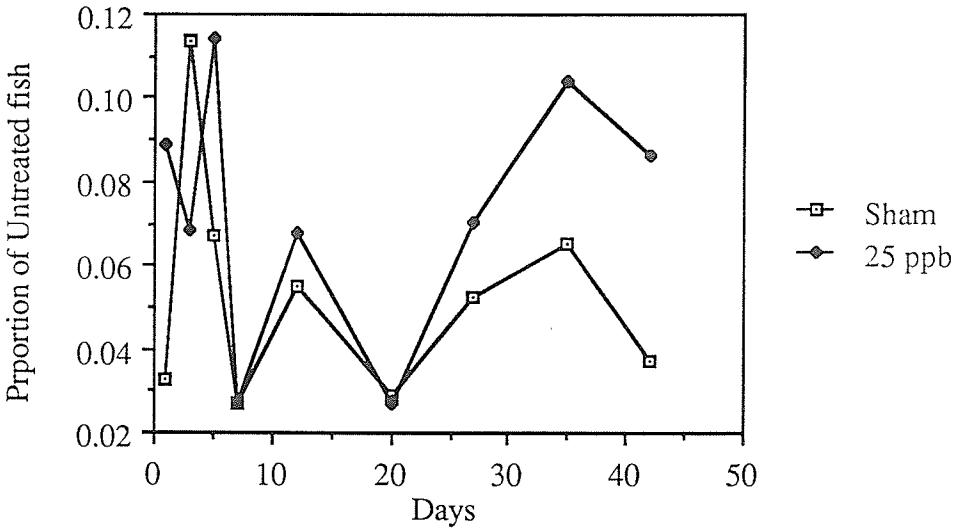
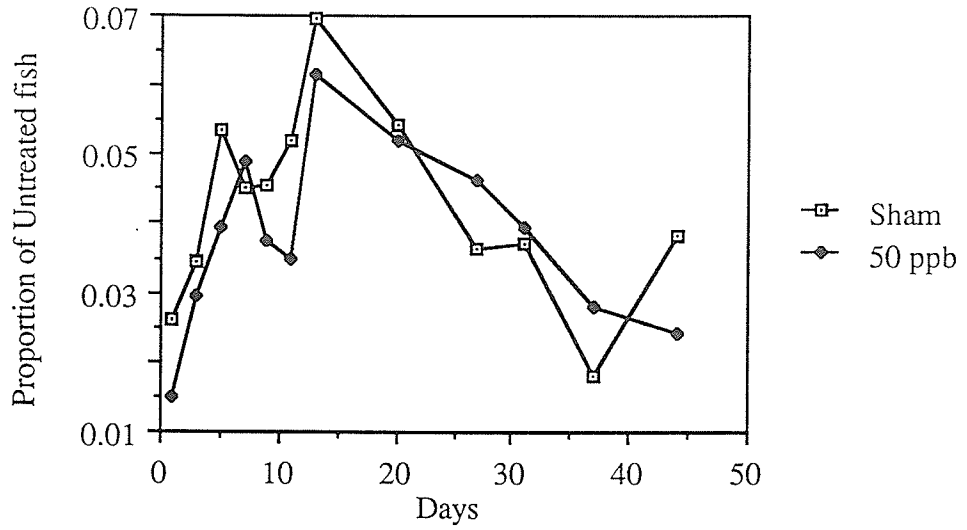
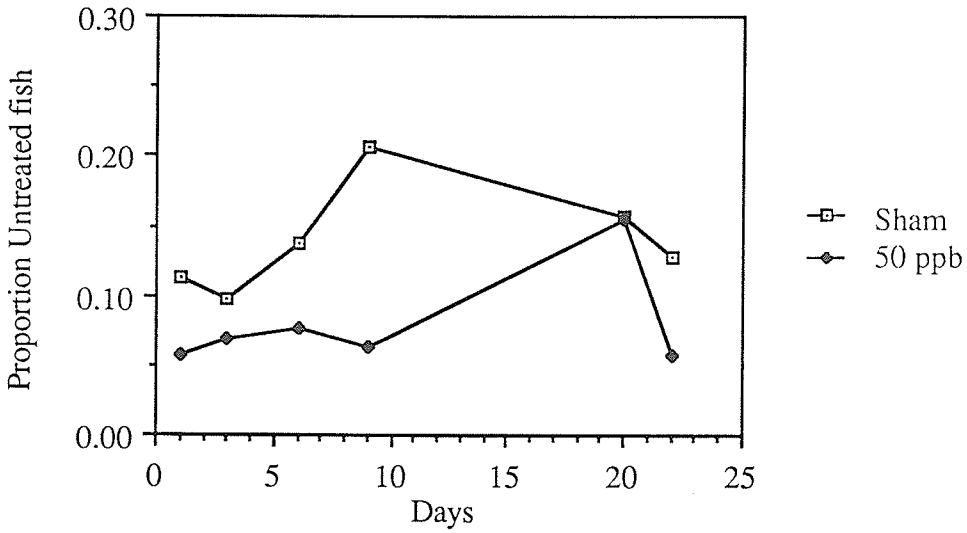

0.12
0.10
0.08
0.06
0.04
0.02
Prportion of Untreated fish
0
10
20
30
40
50
Days
Sham
25 ppb

Figure 17. A. Catch per unit effort for experiment 3 (50 ppb), for treated and sham treated walleye. Values are expressed as proportions of untreated fish caught.
B. Catch per unit effort for experiment 4 (50 ppb), for treated and sham treated walleye. Values are expressed as proportions of untreated fish caught.

A


Proportion of Untreated fish
0.07
0.05
0.03
0.01
0
10
20
30
40
50
Days
Sham
50 ppb

B


Proportion Untreated fish
0.30
0.20
0.10
0.00
0
5
10
15
20
25
Days
Sham
50 ppb

Discussion

The cholinesterase data in general were quite variable for all experiments. This variation may have come from several sources. Unlike Lockhart et al. (1985) whole heads were used for AChE assays instead of just brain tissue. The increased amounts of non-cholinesterase protein may have interfered with the assays causing greater variability. Water temperature has also been shown to influence AChE activity in bluegills (Hogan 1970), higher activities were found with elevated temperatures. It is unlikely that this affected results because water temperatures in the ponds were reasonably constant over the course of the study.

Brain cholinesterase activity has been the most common method used to monitor exposure of fish to Malathion (Lockhart et al. 1985). Inhibition of cholinergic enzymes in fish following exposure to Malathion has been reported by many authors (Weiss 1961, Hogan 1970, Coppage 1972, Lockhart et al. 1985 Ansari and Kumar 1984). Exposure of walleye to 1 and 25 ppb malathion for 2 and 1.5 h, respectively, resulted in non significant inhibitions of 7 and 21% of control (sham treated) values. Inhibitions for the 25 ppb experiment may have been greater, values presented are for one day following exposure because samples for day 0 were lost. Exposure to 50 ppb malathion resulted in significant AChE inhibition of 60% and 35% in experiments 3 and 4 respectively. Although the same malathion dosage was used in experiment 4 it took place 15 days after experiment 3, when fish were larger. The lower level of inhibition agrees with Weiss's (1961) results. He found that AChE activity

activity levels decreased with increasing brain size in several species of fish. Comparison of inhibitions resulting from exposures to 1, 25 and 50 ppb malathion shows inhibition of AChE to be dose dependent.

In all experiments the inhibition was short-lived with AChE activity levels returning to "normal" within 24 h. In both 50 ppb experiments there were large increases in AChE activity in the treated fish on day 1 followed by decreases on day 3. These large increases in AChE activity immediately following inhibition could represent a short term compensatory mechanism to AChE inhibition. Indirect evidence, in the form of increased protein synthesis, for induction of hepatic enzyme systems by malathion was shown by Sahib et al. (1984). If such induction existed, it could account for increases in AChE activity in treated fish shortly after inhibition. The sham treated walleye in the second 50 ppb experiment exhibited a severe drop in AChE activity on days 1 and 3 after exposure. A similar trend was evident in the 25 ppb data but the decrease was not as large. These decreases may be interpreted as a reaction to handling stress, unfortunately no literature could be found to support this interpretation.

In the first three experiments the types of prey items present in stomachs were similar for all groups of fish, indicating that exposure to Malathion and handling did not affect the types of prey consumed. Sham treated and treated walleye tended initially to consume less than untreated walleye in these experiments. In the 1 ppb experiment sham treated and treated fish generally consumed equal or greater amounts than the untreated fish after day 7.

Insects were present in more stomachs after day 7. Possibly, increased daphnid consumption and the additional consumption of insects could account for the approximate equal size of all three groups after day 18. Swenson and Smith (1973) found that growth was related to consumption rate. Further they also found food conversion efficiency in walleye was relatively constant and unaffected by consumption. Feeding patterns of fish in the 25 ppb experiment were similar to those in the 1 ppb experiment with the exception that treated and sham treated walleye did not consume more than untreated fish at any of the times sampled. A single untreated walleye which consumed 9 corixids was responsible for the divergent point in Figure 12. Data from experiment 3 (50 ppb) showed similar feeding trends to the 25 ppb exposure. Sham treated and malathion treated fish consumed less than untreated fish for the first nine days following return to the pond.

The differences in food consumption between sham treated and malathion treated fish in the first three experiments were minor. Consequently, differences between these two groups, taken together, and untreated fish were probably caused by stresses associated with capture, transport and fin clipping. Both sham treated and treated walleye were similarily affected by these sources of stress.

The addition of fathead minnows changed the feeding patterns of all groups of walleye in experiment 4 (50 ppb). Treated and sham treated were found with more daphnids in their stomachs than untreated fish until day 9. In general, treated fish consumed more daphnids than sham treated but had lower stomach content weights. The higher stomach contents weights were caused by the

walleye. Untreated walleye always consumed more minnows than sham treated fish. Sham treated fish, in turn, ate more minnows than treated walleye until day 6. Results of laboratory experiments done by Kenny (pers comm) showed walleye exposed to 25 ppb Malathion consumed significantly fewer fathead minnows than untreated fish and that only 60% of treated fish fed compared with 100 % of controls. Of the 15 treated walleye stomachs sampled on day 1, 2 (7.5%) were empty. Bull and McInerney (1974) found decreased feeding by juvenile coho salmon chronically exposed to Fenitrothion. They also found that this decrease was related to toxicant concentration with fish eating less at the higher concentrations.

Changes in feeding behaviour causing decreased growth have been linked with handling stress in hatchery reared fish (Wedemeyer and McLeay 1981). Pickering et al. (1982) found decreased feeding for two days in brown trout subjected to two minutes of handling, but no significant decreases in growth were found after one month. Decreases in feeding following handling may be species dependent, Wedemeyer (1976) found decreased feeding for 4 to 7 days after handling in juvenile coho salmon, whereas juvenile rainbow trout fed the day after handling. Changes in feeding behaviour may be mediated by conditioning to capture stress. After one week of handling, Schreck (1981), conditioned juvenile coho and chinook salmon to feed almost immediately after having been handled.

In addition to decreased feeding, Pickering et al. (1982) also found significant increases in plasma cortisol, glucose and lactate for

In addition to decreased feeding, Pickering et al. (1982) also found significant increases in plasma cortisol, glucose and lactate for up to three days following handling. Increased plasma glucose is highly correlated with increases in metabolic rate in vertebrates (Umminger 1977). Multiple stress incidents, as were present in this study, have been shown to be cumulative in terms of increased glucose levels in juvenile chinook salmon (Barton et al. 1986). Increases in glucose levels may have been higher in malathion treated fish, Lal et al. (1986) and Mishra and Srivastava (1983) found increased plasma glucose levels in the Indian catfish (*Heteropneustes fossilis*) exposed to Malathion. Lal et al. (1986) determined that increased glucose was utilized to meet stress situations. Increased cortisol levels caused by handling have also been shown to be decreased after conditioning to handling (Schreck 1981), so it is possible that glucose levels may be mediated as well.

Although Malathion treated and sham treated fish did not differ significantly in mean weight of stomach contents, indicating that feeding was not affected by exposure to Malathion, data from both experiments 1 and 3 showed a general trend for treated fish to initially consume more than the sham treated walleye. Kenny (pers comm) in laboratory experiments found juvenile walleye exposed to Malathion oriented and lunged at invertebrate prey more often than untreated walleye and although they were less successful than untreated fish the increased number of attempts more than compensated for the reduced success rate. Others have found hyperactivity in fish exposed to organophosporous insecticides (Matton and LaHam 1969). Bull and McInerney (1974) also found

increased numbers of comfort behaviours involving locomotion at low concentrations (0.001 ppm) of Fenitrothion with coho salmon (*Oncorhynchus kisutch*). Increased swimming activity at the top of the tank was also noted in experiments by Bull and McInerney (1974). Increased activity may affect feeding in the ponds and may also represent an extra energetic cost, affecting growth.

In experiments 1 (1 ppb) and 4 (50 ppb) growth was depressed in the sham treated and treated walleye when compared with untreated fish for a period of approximately 20 days after reintroduction to the ponds. The absence of significant differences in weight, length or weight-length relationship between sham treated and treated fish indicates that exposure to Malathion was not an important factor in causing decreased growth. The trend in these two experiments for growth of both these groups to be less than fish of the untreated group was caused by stresses resulting from handling. In both experiments the trend of sham treated and treated fish to weigh less than untreated walleye lasted about 20 days with no subsequent clear trends. This was contrary to Lockhart et al. (1985) who attributed growth decreases to Malathion exposure after ultra low volume (ULV) application of Malathion to the same ponds. Results here do not support Malathion as being the direct cause for decreased growth.

Experiments 2 and 3 (25 and 50 ppb exposures) showed similar decreases in growth. However, these decreases were not as pronounced as in experments 1 and 4, perhaps because stresses resulting from handling were less severe. Excess handling of walleye utilized for experiment 4 and a longer time spent away from

Length and weight data from experiment 3 were highly variable after day 9 because of the presence of cannibalistic walleye in all groups (sham treated, treated and untreated) in the pond. Cannibalism resulted in a bimodal size distribution for the population, which increased the variability of sample means. McIntyre et al. (1987) found canibalism to be greatest in aquaria which contained only walleye, intermediate in aquaria with walleye and zooplankton and absent in aquaria with fathead minnows. A decline in food availability was probably responsible for the presence of cannibalism in walleye at this time. The general trend after day nine is for a decrease in weight of non cannibalistic walleye. This decrease in growth was probably caused by a decrease in the abundance of *Daphnia*, the primary invertebrate prey. Stomach analyses for experiment 3 showed a decline in mean weight of stomach contents and in the number of *Daphnia* in stomach contents for all groups after day 7. The abrupt change from feeding on *Daphnia* to feeding on copepods, insects and amphipods may have been related to a decline in *Daphnia* abundance. *Daphnia* were always the preferred invertebrate prey item in the other experiments, although copepods and other invertebrate species were always present in the ponds. Swenson (1977) found walleye stomach contents reflected variability in prey availability.

Many studies have reported that there were no differences in growth of fin clipped fish and unclipped fish after periods of a year or more, Maloney (1959) and Churchill (1963) reported that fin clipped walleye fingerlings found no significant differences in weight after a one year period. Brynildson and Brynildson (1967) showed

clipped walleye fingerlings found no significant differences in weight after a one year period. Brynildson and Brynildson (1967) showed no growth differences in brown trout (*Salmo trutta*) fingerlings which were fin clipped, Shetter (1967) had similar results with rainbow trout fingerlings. All these studies were done over a longer period of time than the present one and do not include data on the immediate effects of fin clipping on growth.

One factor which may be partially responsible for the initial differences in growth in all four experiments may be the time taken to treat and mark fish. The current study removed walleye from the ponds for a minimum of 24 h (experiments 2, 3 and 4) and a maximum of 48 h (experiment 1) during which time they were not fed. Fin removal in field studies is most often carried out at the study site with and the fish are returned immediately; however the exposure to Malathion in this experiment precluded clipping fins at the ponds. Time away from the natural environment may explain differences within the first 1 to 2 days after reintroduction but it does not explain continued differences to day 20. Continued differences in growth may be caused by the previously discussed changes to feeding and glucose metabolism caused by handling stress. Effects of handling on feeding lasted 10 to 12 days in all experiments, similar to the amount of time necessary for the fish to adapt to repeated handling (Schreck 1981). During this time decreased feeding might have caused decreased growth for a period of approximately twenty days, the last ten days being the time taken for handled walleye to reach a similar size as untreated fish.

summer indicating exposure to Malathion did not cause a significant mortality. A mortality rate of 5% is low for any natural unstressed population over a period of three months. Survival of walleye exposed to 25 ppb also appeared relatively constant up to day 20 but thereafter the proportions of sham treated and treated fish diverged. Difficulty was encountered in distinguishing marks for this experiment in the field after day 20 due to regrowth of the caudal spines which were removed as a means for identifying the two groups. This regrowth of spines is consistent with findings by Rinne (1976) who found complete regeneration of caudal and dorsal spines in *Tilapia* less than 12 cm TL within one month. Failure to recognize a mark would cause a decrease in apparent proportions of fish with that mark, because we were conservative in identifying marked fish it is likely that the proportions of sham fish are lower than they actually were. The general fluctuations in proportions of sham treated and malathion treated walleye in seine catches might have been caused by sampling procedures but the most probable cause for variations in catches was the contagious distribution of fish. For example the daily range in catches was from 0 to more than 1000 walleye.

The proportions of sham treated and malathion treated fish in experiment 3 increased until day 12, after which they decreased. Because proportions of sham treated and treated fish are similar throughout the sampling periods it can be concluded that exposure to Malathion did not affect survival. The initial increase in proportions after reintroduction to the ponds may have been caused by several factors. Handling stress may have resulted in changed behaviour in

the walleye initially reducing their availability for recapture. Incomplete mixing of marked individuals into the population may have resulted in treated fish and sham treated fish being isolated. Proportions of sham treated and treated fish reached their highest proportions on day 13 after which they to declined. This decline in proportions indicated differential mortality occurred in both groups of walleye. Mortality from starvation may have resulted from the previously discussed decreased food supply in the pond.

Survival of treated fish was lower than sham treated fish in experiment 4 (50 ppb exposure), even after the differential return rates were accounted for. Although only fish that appeared healthy were returned to the pond, it is possible that mortality may have occurred in the treated group after reintroduction. The apparent differences in survival may have been caused by exposure to Malathion or handling stress or a combination of factors. Based on results from experiment 3, which used the same dose but did not have the extra handling, mortality caused by undue stress seems the most likely cause.

Comparison of this study with that of Lockhart et al. (1985) indicates that cholinesterase was inhibited to a greater degree at a lower level of Malathion application in the earlier study. The concentration of Malathion was measured at 8.9 ppb 1 hour after spraying and resulted in 75% inhibition of pre-spray values, whereas in this study a 50 ppb exposure produced 60 and 35% after 1.5 h. Inhibition following aerial spraying took two weeks to return to 80% of pre-spray values whereas exposures to 25 and 50 ppb produced inhibition for less than 1 day. The reason for these major

differences was the length and level of exposure. Experimental design in this study closely resembled a pulse dose at high levels whereas walleye exposed following the aerial spray were exposed to a low dose over the extended period of time required for the Malathion to break down in the pond. The half life of Malathion in the ponds was probably several days (Spiller 1961, Mulla and Mian 1981). Thus a similar level of AChE inhibition can be achieved by short term exposures at high concentrations or longer exposures at lower levels which agrees with Ansari and Kumar (1984) who found inhibition to be both dose dependent and time dependent in zebra fish (*Brachydanio rerio*). Comparison of this study with Lockhart et al. (1985) showed the rate of recovery in malathion exposed fish seemed to be quicker in fish exposed to high concentrations of Malathion for a short time.

Decreased growth of juvenile walleye reported by Lockhart et al. (1985) may have resulted from indirect effects of Malathion on walleye. Although decreased growth was evident to some extent in all four experiments, it was always found in both sham treated and malathion treated fish indicating a handling effect. Since the fish were not handled in the previous study, some other factor must have been responsible for the decreased growth. Data from experiment 3 indicated that a decreased food availabilty can also affect growth of juvenile walleye. Hurlbert et al. (1972) found the cladoceran *Moina affinis* decreased in abundance by 99% following application of the organophosphorous insecticide Dursban (0.028 kg/ha), as did the copepods *Cyclops sp.* and *Diaptomus sp.* Malathion has been shown to be toxic (48 hr EC50 values) to the cladocerans *Simocephalus*

serratus and *Daphnia pulex* at very low levels of 3.5 and 1.8 ppb respectively (Sanders and Cope 1966). These levels are lower than the 8.9 ppb concentration of Malathion reported in the pond by Lockhart et al. (1985). Thus the decreased growth of juvenile walleye in Lockhart et al. 1985 may have been caused indirectly by a decreased food resources (see experiment 3) resulting from the toxic effects of Malathion on invertebrate prey species.

This study has shown that the degree of AChE inhibition was dependent on Malathion dosage; 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. Degree of inhibition may also be related to the length of exposure with high 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 that 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 by 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 groups consumed fewer minnows than untreated fish. Stress caused by handling is the most probable cause for decreased feeding in the sham treated and malthion treated walleye. Decreases in growth of sham treated

and malathion treated fish were probably caused by decreased feeding.

Field experiments such as this one and that of Lockhart et al. (1985) are useful as indicators of ecosystem responses to various stresses. These responses can then be correlated with existing knowledge and further investigated in laboratory studies where various aspects can be more tightly controlled. This study has utilized this type of approach and it has resulted in the confirmation of laboratory derived data and identified areas which may require more study. The importance of using a control group (sham treated fish) to avoid making incorrect interpretations when studying effects of stresses has been demonstrated.

Literature Cited

Ansari, B. A. and K. Kumar. 1984. Malathion toxicity: In vivo inhibition of acetylcholinesterase in the fish *Brachydanio rerio* (Cyprinidae). Toxicol. Lett. 20:283-287.

Barton, B. A., C. B. Schreck amd L. A. Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenlie chinook salmon. Trans. Am. Fish. Soc. 115:245-251.

Brynildson, O. M. and C. L. Brynildson. 1967. the effect of pectoral and ventral fin removal on survival and growth of wild brown trout in a Wisconsin stream. Trans. Am. Fish. Soc. 96:353-355.

Bull, C. J. and J. E. McInerney. 1974. Dehaviout of juvenile coho salmon (*Oncorynchus kisutch*) exposed to Sumithion (fenitrothion), an organophospate insecticide. J. Fish. Res. Board Can. 31:1867-1872.

Churchill, W. S. 1963. The effect of fin removal on surviva, growth and vulnerability of stocked walleye fingerlings. Trans. Am. Fish. Soc. 92:298-300.

Coppage, D. L. 1972. Organophosphate pesticides: Specific level of brain AChE inhibitrion related to death in sheepshead minnows. Trans. Am. Fish. Soc. 101:534-536.

Ellman, G. L., K. D. Courtney, V. Andres Jr., and R. M. Featherstone. 1961. A new and rapid colorimetric determination of acetycholinesterase activity. Biochem. Pharm. 7:88-95.

Hamilton, S. J. and P. M. Merle. 1986. Metallothionien in fish: Review of its importance in assessing stress from metal contaminants. Trans. Am. Fish. Soc. 115:596-609.

Hogan, J. W. 1970. Water temperature as a source of variation in specific activity of brain acetylcholinesterase of bluegills. Bull. Environ. Contam.Toxicol. 5:347-353.

Hokanson, K. E. F. 1977. temperature requirements of some percids and adaptations to the seasonal temperature cycle. J. Fish. Res. Board Can. 34:1524-1550.

Hokanson, K. E. F. and W. M. Koenst. 1986. Revised estimates of growth requirements and lethal temperature limits of juvenile walleyes. Prog. Fish. Cult. 48:95-100.

Hurlbert, S. H., M. S. Mulla and H. R. Willson. 1972. Effects of an organophosphorous insecticide on the phytoplankton, zooplankton and insect populations of fresh-water ponds. Ecol. Mono. 42:269-353.

Klaverkamp, J. F., W. A. Macdonald, D. A. Duncan and R. Wageman. 1984. Metallothionein and acclimation to heavy metals in fish: a review. P. 99-133. *In* Contaminant effects on fisheries. V. W. Cairns, P. V. Hodson and J. O. Nriagu [ed.]. John Wiley & Sons, New York, NY.

Klaverkamp, J.F., M. Duangsawasdi, W. A. Macdonald and H. S. Majewski. 1977. An evaluation of fenitrothion toxicity in four life stages of rainbow trout, Salmo gairdneri. p. 231-240. In Aquatic toxicology and hazard evaluation. F. L. Mayer and J. L. Hamelink [eds]. American Society for Testing and Materials ASTM STP 634.

Lal, B., A. Singh, A. Kumari and N. Sinha. 1986. Biochemical and haematological changes following Malathion treatment in the fresh water catfish *Heteropneustes fossilis* (Bloch). Environ. Pollut. Series (A). 42:151-156.

Lockhart, W. L., D. A. Mettner, F.J. Ward and G. M. Swanson. 1985. Population and cholinesterase responses in fish exposed to malathion sprays. Pest. Bioch. and Physiol. 24:12-18.

Lowry, O. H., N. J. Rosenberg, A. L. Farr and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265-270.

Maloney, J. E. 1953. Survival growth and food of fin-clipped walleye pike fingerlings in Maloney pond Cass County, Minnesota. Minnesota Dept. of Conservation, investigational Report No. 137. 4p.

Matsumura, F. 1975. Toxicology of insecticides. Plenum Press. New York, NY.

Matton, P. and Q. N. LaHam. 1969. Effect of the organophosphate Dylox on rainbow trout larvae. J. Fish. Res. Board Can. 26:2193-2200.

McIntyre, D. B. 1987. Changes in feeding behaviour and the occurrence of cannibalism in the early life history of walleye (*Stizostedion vitreum vitreum*). MSc Thesis, University of Manitoba, Winnipeg, Man.

McIntyre, D. B. and F. J. Ward. 1986. Estimating fork lengths of fathead minnows, *Pimephales promelas* , from measurement of pharyngeal arches. Can. J. Fish. Aquat. Sci. 43:1294-1297.

McIntyre, D. B., F. J. Ward and G. M. Swanson. 1987. Factors affecting cannibalism by pond-reared juvenile walleyes. Prog. Fish. Cult. 49:264-269.

Mishra, J. and A. K. Srivastava. 1983. Malathion induced hematological and biochemical changes in the indian catfish *Heteropneustes fossilis* . Environ. Res. 30:393-398.

Mulla, M. S. and L. S. Mian. 1981. Biological and environmental impacts of the insecticides malathion and parathion on nontarget biota in aquatic ecosystems. Res. Rev. 78: 101-136.

Mulla. M. S., G. Majori and A. A. Arata. 1979. Impact of biological and chemical mosquito control agents on non-target biota in aquatic ecosystems. Res. Rev. 71:121-173.

Pennak, R. W. 1978. Fresh-water invertebrates of the United States. The Ronal Press Company, New York, NY.

Pickering, A. D., T. G. Pottinger and P. Christie. 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. Fish Biol. 20:229-244.

Post, G. and R. A. Leasure. 1974. Sublethal effect of Malathion to three salmonid species. Bull. Environ Contam. and Toxicol. 12:312-319.

Rand, G. M. and S. R. Petrocelli. 1985. Fundamentals of aquatic toxicology. Hemisphere Publishing Company, Washington. 666 p.

Rinne, J. N. 1976. Coded spine clipping to identify individuals of the spiney-rayed fish *Tilapia* . J. Fish. Res. Board Can. 33:2626-2629.

Sahib, I. K., K. R. S. Sambasiva Rao amd K. V. Ramana Rao. 1984. Effect of Malathion on protein synthetic potentiality of the tissues of the teleost, *Tilapia mossambica* (Peters), as measured through incorporation of [^{14}C] amino acids. Toxicol. Lett. 20:63-67.

Sanders, H. O. and O. B. Cope. 1966. Toxicities of several pesticides to two species of cladocerans. Trans. Am. Fish. Soc. 95:165-169.

Schreck, C. B. 1981. Stress and compensation in teleostean fishes: responses to social and physical factors. p. 295-321. *In* Stress and Fish. A. D. Pickering [ed]. Academic Press, New York, NY.

Shetter, D. S. 1967. Effects of jaw tags and fin excision upon growth, survival and exploitation of hatchery rainbow trout fingerlings in Michigan. Trans. Am. Fish. Soc. 96:394-399.

Spiller, D. 1961. A digest of available information on the insecticide Malathion. Adv. Pest Cont. Res. 4:249-335.

Swenson, W. A. 1977. Food consumption of walleye (*Stizostedion vitreum vitreum*) and sauger (*S. canadense*) in relation to food availability and physical conditions in Lake of the Woods, Minnesota, Shagawa Lake and Western Lake Superior. J. Fish. Res. Board Can. 34:1643-1654.

Swenson, W. A. and L. L. Smith. 1973. Gastric digestion, food consumption, feeding periodicity and food conversion efficiency in walleye (*Stizostedio vitreum vitreum*). J. Fish. Res. Board Can. 30:1327-1336.

Umminger, B. L. 1977. Relation of whole blood sugar concentration in vertebrates to standard metabolic rate. Comp. Biochem. Physiol. A. Comp. Physiol. 56:457-460.

Wedemeyer, G. A. 1976. Physiological response of juvenile coho salmon (*Oncorynchus kisutch*) and rainbow trout (*Salmo gairdneri*) to handling and crowding stress in intensive fish culture. J. Fish. Res. Board Can. 33:2699-2702.

Wedemeyer, G. A. and D. J. McLeay. 1981. Methods for determinig the tolerance of fishes to environmental stressors, p. 247-273. *In* Stress and Fish. A. D. Pickering [ed]. Academic Press, New York, NY.

Weiss, C. M. 1961. Physiological effect of organic phosphorous insecticides on several species of fish. Trans. Am. Fish. Soc. 90:143-152.