The effects of Very Low Doses of the Organophosphorous Insecticide (Malathion) on Piscivorous Feeding Performance in Juvenile Walleye, *Stizostedion vitreum vitreum* (Mitchill)

By
Susan A. Kenny

A Thesis
Submitted to
the Faculty of Graduate Studies
University of Manitoba

In Partial Fulfilment
of the Requirements for the degree of
Master of Science
in Zoology

Winnipeg, Manitoba
1990

ISBN 0-315-71788-2

Canada

The Effects of Very Low Doses of the Organophosphorous Insecticide (Malathion) on Piscivorous Feeding Performance in Juvenile Walleye, Stizostedion vitreum vitreum (Mitchill)

BY

SUSAN A. KENNY

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

I hereby declare that I am the sole author of this thesis.

I authorize the University of Manitoba to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Susan A. Kenny

I further authorize the University of Manitoba to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Susan A. Kenny

Abstract

Juvenile walleye (Stizostedion vitreum vitreum, Mitchill) were exposed to short term pulse exposures of malathion in the laboratory. Two experiments were conducted in order to determine the effects of malathion on whole head acetylcholinesterase and piscivory.

The first experiment was conducted on five dates during the 1986 growing season. Whole head acetylcholinesterase and piscivory was measured in groups of fish exposed to malathion. Mortality was observed later in the season when the acetylcholinesterase inhibition reached 25% although inhibitions of this magnitude did not affect the juveniles earlier in the season. Although no linear relationship between the acetylcholinesterase activity and malathion was observed, there was a suggestion of hormesis - stimulatory affects at low concentrations. Piscivory was significantly inhibited on four of the five dates. There was no linear relationship between the inhibition of piscivory and acetylcholinesterase.

The second experiment was conducted in February-March 1987 with juveniles from the 1986 season. Individual walleye, exposed to malathion and allowed no recovery or 24 hours recovery, were videotaped in order to quantify actions involved in the process of piscivory. Higher concentrations of malathion were used resulting in a maximal acetylcholinesterase inhibition of 12% - a significant difference from the control group. Net foraging efficiency was increased in the exposed walleye but they exhibited alterations in their locomotory and sensory components of piscivory. Activity was increased in malathion exposed walleye including upper water activity and increased prey handling losses. Malathion exposed walleye exhibit increased reactive

distances and decreased reactive angles which may limit, in pond environments, the amount of prey seen and decrease consumption.

Short term pulse exposure to malathion results in subtle changes in whole head acetylcholinesterase activity in juvenile walleye. Changes in piscivory are associated with but not related to whole head acetylcholinesterase activity. The changes in piscivory, whether a decline in consumption or alterations in sensory or locomotory components may ultimately affect growth of the individual and the population.

Acknowledgements

This thesis could not have been completed without the assistance of many people. I would like to thank my supervisor, Dr. W.L. (Lyle) Lockhart for his patience, guidance and encouragement throughout this study. I also would like to thank Dr. F.J. Ward who graciously provided laboratory space and who gave me advice and guidance.

Many individuals within the Freshwater Institute provided technical advice and training during my study. I would like to thank D. Metner, N. Grift, Dr. D. Muir, B. Danell, B. Billeck, R. Hunt, J. Gibson, L. Allard and C. Baron.

This study reflects the inspiration and dedication of the group of graduate students associated with Dr. F. J. Ward's Laboratory: Bernie McIntyre, Lenore Ciszewski and Peter Delorme. To the many graduate students in the Department of Zoology, University of Manitoba, I extend my deepest thanks for discussions, assistance and encouragement.

An energetic, gregarious group of summer students managed the tedium of research while never letting their spirits flag. These students are Bruce McCullogh, Laura Heuring and Sean Bugden. Special thanks go to Ann Kong who spent an endless summer of Saturdays doing cholinesterase assays and to Dave Kinnear who courageously broke a toe at the same time that I did.

Finally, I would like to acknowledge the support of my husband, Raymond Kenny. He was always free with advice, support and encouragement.

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

Table of Contents

List of Tables

List of Figures

1. Introduction

1.1 Walleye (Stizostedion vitreum vitreum, Mitchill) Feeding

The walleye (synonyms: pickerel, pike-perch, wall-eyed perch), a member of the perch family (Percidae), is one of the most valuable commercial and sport fish in Canada's inland waters. The walleye is distributed from Great Slave Lake in the northwest and Labrador in the northeast, south to northern Alabama and northern Arkansas and west into Nebraska (Niemuth et al., 1959). Walleye thrive in moderately fertile waters but occur in all lake types which offer suitable spawning sites. Walleye do not have definite home ranges, but rather drift with food sources.

A retinal reflective layer, the tapetum lucidum, and the bundling of rods form macroreceptors to provide walleye with enhanced vision in low light (Braekevelt et al., 1989). Walleye are negatively phototactic in clear water living near or on bottoms of lakes and rivers. They spend the daylight hours in deep, dark waters and then migrate to shallow bars and shoals at night to feed (Ryder, 1977). When waters are turbid or the weather cloudy, they may be active during the day (Ryder, 1977).

Walleye are 'strike feeders' that rely on vision to find and capture food (Mathias and Li, 1981). Once visual contact is made, the walleye orient themselves towards the prey by bringing it into the visual field of both eyes; then they either reject the prey by swimming away, or they strike it until it has been subdued. The food taken by juvenile walleye changes with age; plankton is eaten initially, then insect larvae and finally fish become the predominant diet item after the young walleye reach 75 mm in length (Smith and Pycha, 1960). During the early planktivorous stage, the size of prey is more important than its species composition (Mathias and Li, 1981), indicating

morphology-limited feeding. When the young walleye reach about 60 mm in length they become piscivorous, a shift in habits which depends on the size and availability of suitable prey (Li and Ayles, 1981). During the piscivorous phase, walleye exhibit both species and size selectivity (Parsons, 1971; Davis, 1975; Walker and Applegate, 1976; Li and Ayles, 1981). Walleye take forage fish (including other walleye) of sizes from 30% to 40% of their own body length (Parsons, 1971) in amounts ranging from 1% - 4.7% of body weight daily. Some 32% of juveniles exhibit empty stomachs (Li and Ayles, 1981).

The most important times, in terms of growth and survival, of juvenile walleye are two 'critical periods' of feeding. The first critical period is the shift from yolk feeding to exogenous food when survival depends on encountering suitable and sufficient food. Major changes in morphology and physiology, including tooth and jaw structure, visual acuity, muscular coordination and the ability to swallow, digest and absorb food, are occurring during this phase. Walleye are cannibalistic, especially as larvae. Larval walleye will cannibalize similar size siblings (Cuff, 1980); a phenomenon known as cohort cannibalism (Li and Mathias, 1981). Juvenile walleye will cannibalize those walleye that meet the minimum size difference. Cannibalism in an adult population ranges from a frequency of occurrence of 1% to 4% (Chevalier, 1973) but can be as high as 10% per day in larval culture (Mathias and Li, 1981). Walleye are particularly susceptible to environmental variability and perturbations during this first critical period.

The second critical period of feeding is the switch from planktivorous feeding to piscivorous feeding. This phase is dependent upon the size of the walleye and also upon the size and availability of forage fish.

1.2 Malathion Chemistry

Malathion (O,O-Dimethyl S-(1,2-dicarboxyethoxyethyl) phosphorodithioate) is a broad spectrum organophosphorous insecticide that has been used throughout the world for the control of mosquitoes and agricultural pests. In Manitoba malathion has been applied aerially to control mosquitoes and to limit outbreaks of equine encephalitis. These applications prompted the present study.

Organophosphorous pesticides continue to be widely used because they have a high insecticidal activity, a wide spectrum of action on insect pests and low environmental persistence - that is, they generally break down to form non-toxic products. They are rapidly metabolized in vertebrates with the absence of accumulation and are of relatively low chronic toxicity.

Malathion is a colourless liquid that is highly soluble in most organic solvents, and has a water solubility of 145 mg L^{-1}. In surface waters it breaks down by hydrolysis and photolysis. Hydrolysis of malathion follows different pathways in acid and alkaline media (Wolfe et al., 1977). Malathion is very stable in acid aquatic environments with a half-life between one and two weeks at pH 6 (Cowart et al., 1971) and greater than four years at pH 4 (Wolfe et al., 1977). Malathion has a half-life of 36 hours at pH 8 and 27^{o}C (Wolfe et al., 1977). The half-life of Malathion in distilled water, by photolysis, is 990 hours. However, the actual half-life in a natural water sample where various pathways (physical, chemical, biological) would be active, was only 16 hours (Wolfe et al., 1977). It is marketed in the form of an emulsifiable concentrate containing 30% to 60% active ingredient, an emulsifier and a solvent (eg. xylene).

1.3 Malathion Toxicology

1.3.1 Mode of Action

"The basic mechanism responsible for the toxicity of organophosphorous insecticides is the inhibition of acetylcholinesterase of the nervous system, with the consequent accumulation of excessive levels of acetylcholine" (NRCC, 1975).

Human acute toxicity symptoms range from physical signs such as miosis, ciliary spasm, bronchoconstriction, gastrointestinal cramps, diarrhoea, nausea, vomiting, increased salivation, bradycardia, hypotension, lacrimation, convulsions, coma and death (Murphy, 1985). The long term chronic effects manifest themselves as behaviourial disturbances in man: impairment of concentration, decreased cognitive abilities, mental confusion, hypoactivity, memory impairment, depression, schizophrenic syndrome, anxiety and irritability (Levin and Rodnitzky, 1976).

1.3.2. Acute Toxicity

Table 1 provides a summary of acute lethal toxicity of Malathion to fish. Acutely poisoned fish show signs of muscle paralysis, especially of the fins and respiratory organs, hyperactivity resulting in an over-reaction to stimuli, loss of equilibrium resulting in a swimming pattern with spirals or corkscrews and may have terminal tetany and convulsions (Weiss, 1959).

Table 1. Summary of Acute Lethal Toxicity of Malathion to Fish[1]

Test Organism	Stage or wt (g)	Temp (C)	96-h LC50 95% CI (ug/L)
Coho Salmon	0.9	12	170 (160 - 180)
Cutthroat trout	1.0	12	280 (270 - 310)
Rainbow trout	1.4	12	200 (160 - 240)
Yellow perch	1.4	18	263 (205 - 338)
Walleye	1.3	18	64 (59 - 70)
Largemouth bass	0.9	18	285 (254 - 320)
Fathead minnow	0.9	18	8,650 (6,450 - 11,500)

[1]Johnson and Finley, 1980.

1.3.3. Sublethal Toxicity

1.3.3.1. Acetylcholinesterase

Acetylcholinesterase (E.C. 3.1.1.7) is found at myoneural junctions between somatic motor nerves and skeletal muscles, at synaptic gaps between pre- and post- ganglionic neurons, at parasympathetic nerve-organ junctions of the visceral motor system and within the central nervous system. Acetylcholinesterase is responsible for the breakdown of the neurotransmitter acetylcholine in the neuronal synapse. The inhibition of acetylcholinesterase thus results in an accumulation of acetylcholine in the neuron synapse and this acetylcholine continues to exert its influence on the post-synaptic membrane resulting in over-stimulation of the effector. The mechanism of inhibition of cholinesterase by organophosphorous compounds may be represented by the following scheme; the esterase forms a covalent bond to a serine hydroxyl group to produce a phosphorylated esterase enzyme which is not active catalytically. The phosphorylated esterase may be hydrolysed slowly to regenerate the active enzyme and the esterase activity is restored.

1.3.3.2. Biochemical/Histopathological

In addition to direct effects on the acetylcholinesterase, organophosphorous compounds can affect other components of the cholinergic system indirectly. Thompson and Thomas (1985) reported that soman (an organophosphorous compound used in early gas warfare) can exert a secondary effect by presynaptic alteration of the activity of the synthetic enzyme of acetylcholine, choline O-acetyltransferase (CAT, E.C. 2.3.1.6) in rats. Soman intoxication resulted in a decrease of 50% in CAT activity in all parts of the

brain with up to 85% acetylcholinesterase inhibition. The loss of CAT activity occurs simultaneously with acetylcholinesterase inhibition, thus the decline in CAT activity may be due to the depletion of acetylcholine in the terminal. This loss of functionality and/or death of the terminal may be associated with delayed neuropathy.

Exposure to organophosphorous compounds may effect the integrity of the nerve cell and target tissue. Total lipid and phospholipds of the cord and brainstem declined in rats given malathion (Haque et al., 1987). This lipid breakdown can lead to demylination of the axons.

In addition to the toxicity resulting from cholinesterase inhibition there is the syndrome known as chronic delayed neurotoxicity which is unrelated to the inhibition of cholinesterase. Delayed neurotoxicity, which results in peripheral neuropathy, is characterized clinically and behaviourial by ataxia, loss of reflexes and muscle weakness (Cavanagh, 1964). Soman and sarin have both been found to alter sensory receptor function and conduction velocities which may lead to this syndrome (Goldstein et al., 1987).

1.3.3.3. Behaviour

Behaviourial changes associated with pesticide exposure are well documented in many animal species. Altered behaviourial responses to pesticides documented include locomotor activity (Fingerman and Russell, 1980 ; Davy et al., 1973 ; Timms et al., 1972 ; Rand 1977) , general comfort behaviours - thrusting, flicking (Henry and Atchison, 1979, 1986 ; Bull and McInerney, 1974) , learning/memory (Wolthuis and Van wersch, 1984), feeding (Bull and McInerney, 1974) and growth (Lockhart et al., 1985).

Bull and McInerney (1974) suggested that feeding was the most sensitive indicator of low level contamination by an organophosphorous insecticide, and

a behaviour that had obvious important ecological implications. Predator/prey interactions, which impact on the social organization of a population and on the distribution of food, have been shown to be affected by pesticides and other pollutants (Sullivan et al., 1978, Hatfield and Anderson, 1972 ; Symons, 1973 ; Glickman and Morrison, 1969 ; Wildish and Lister, 1973 ; Tagatz, 1976 ; Brown et al., 1985 ; Schneider et al., 1980 ; Woltering et al., 1978 ; Hedtke and Norris, 1980; Mac, 1981). The typical response in fish is biphasic: at low concentrations, the fish exhibited an irritant response typified by increased comfort behaviours and a decline in social/feeding behaviours; at high concentrations the fish exhibited physiological impairment with a decline in comfort behaviours and a failure to maintain stream position (Bull and McInerney, 1974).

Organophosphorous compounds have induced feeding declines and resulting decrease in weight and growth that may or may not be accompanied by overt behaviourial symptoms. Parathion-treated rat pups exhibited significantly different growth rates compared to a control group (Stamper et al, 1988). A high-dose group of these rats exhibited mild toxicity symptoms for the first few days and a lower dosed group exhibited no symptoms. Lockhart et al. (1985) reported a temporary decline in weight gain by juvenile walleye that had been exposed to malathion following aerial application at 3 oz/acre. It was not clear whether this reduced growth rate in a field population was due to altered cholinergic function or food availability (Delorme, 1988). Glow and Richardson (1965) reported that rats exposed both chronically and acutely to di-isopropylfluorophosphate (DFP) experienced a reduction in cholinesterase and in body weight for 14 days and a 5-7 day decrease in food and water intake. Their results indicated that in acute reductions of acetylcholinesterase, a

central control of feeding was effected whereas in chronic reductions, both peripheral and central cholinergic mechanisms of feeding were involved.

The brain cholinergic system is implicated in spatial, working and reference memory and learning (Ellen et al., 1986, McDonald et al., 1985, Beninger et al., 1985). Memory impairment is one of the recurrent complaints by humans exposed to organophosphorous compounds. Laboratory studies with rats have shown that any alteration in brain cholinergic function via organophosphorous compound exposure results in impaired memory function (Ellen et al., 1986, McDonald et al., 1985, Beninger et al, 1985). Rats trained to press a lever exhibited a suppression in response rate that was correlated to soman induced inhibition of acetylcholinesterase (Brezenoff et al., 1985). Brezenoff et al. (1985) also noted that the rats exhibited a highly variable response to soman, ranging from no overt symptoms through inactivity or tremors to death. Onset of the behaviourial effects ranged from 2 to 30 minutes after treatment with soman but was not related to the dose of soman. Fish exposed to organophosphorous insecticides exhibited altered memory and learning performance. Salmon parr exposed to 1 mg L^{-1} Fenitrothion (96 hour LC50) for 24 hours exhibited a complete inability to learn a simple conditioned response (Hatfield and Johansen, 1972). At lower Fenitrothion concentrations (0.1 mg L^{-1}), the parr still exhibited a high number of wrong responses. This inhibition of learning ability lasted up to seven days. Hatfield and Anderson (1972) reported that Fenitrothion exposed salmon parr were preyed upon more often than unexposed parr (95% as compared to 58%). Clearly, the loss of learning with exposure to Fenitrothion greatly reduced the prey animals' ability to escape capture.

1.3.4. Relationships between Acetylcholinesterase, Exposure and Other Biological Responses

Cholinesterase inhibition has been used as an environmental monitor of organophosphorous insecticide exposure with varying results. Coppage (1975) reported that a brain acetylcholinesterase inhibition of 70% in pinfish resulted in death of the organism. Cook et al. (1976) exposed pinfish to malathion and reported a 10 % mortality rate at only 50% inhibition and 50% mortality rate with 80% inhibition. Eaton (1970) reported that bluegills exhibited 33% inhibition when exposed to 'safe' level of malathion and that the 'harmful' level resulted in an inhibition of 50%. Kennedy and Walsh (1970) exposed pond populations of bluegills to malathion and did not observe any significant difference in acetylcholinesterase levels or growth or survival. Lockhart et al. (1985) reported a slight drop in catch-per-unit-effort and growth in walleye rearing ponds that were sprayed with malathion; this population also showed a loss of brain acetylcholinesterase activity. Weiss (1958, 1959, 1961) reported that only 40% inhibition of acetylcholinesterase activity in freshwater fish brain may be lethal and Nicholson (1961) suggested that even as little as 10% inhibition could be interpreted as evidence of exposure to anti-cholinesterase compounds. In contrast, Gibson et al. (1969) reported that freshwater fish experiencing over 90% brain inhibition of acetylcholinesterase activity after being exposed to organophosphorous compounds failed to develop pronounced symptoms of poisoning and recovered completely when placed in fresh water.

Relationships between the biochemical response (acetylcholinesterase inhibition) and behaviour are quite variable. Rats given a single sublethal injection of malathion exhibited significantly impaired avoidance behaviour

at doses that did not affect blood or brain cholinesterase values - 90% of controls (Kurtz, 1977). Desi et al. (1976) recorded that rats fed 3-5% of the malathion acute oral LD50 for 90 days exhibited significant changes in EEG but normal RBC, plasma and brain cholinesterase. In this instance low doses of malathion disrupted behaviour without significantly reducing cholinesterase activity.

The use of cholinesterase inhibition as a monitoring tool is controversial. Regardless of species and exposure concentration, varying levels of cholinesterase inhibition can be associated with either no overt symptoms, physiological or behaviourial changes, or death. This wide ranging response may be a result of individual differences in the plasticity and 'tolerance' of the cholinergic system.

1.4 Background to the Current Work

In the summer of 1983, the City of Winnipeg declared a health emergency due to the threat of an outbreak of western equine encephalitis. The city and several other communities were sprayed aerially with malathion in order to control the mosquito population. Fish (young walleye) had been planted in city ponds by Dr. F.J. Ward and these were monitored for toxicological effects associated with malathion exposure and for growth and survival. An effort was made to establish a relationship between the sublethal toxicological effects and the whole animal/population response. The study indicated that the walleye's acetylcholinesterase was inhibited by up to 70% - the toxicological response - and that the enzymatic activity did not return to pre-spray levels for up to two weeks. Catch-per-unit-effort (cpue) indicated a slight decrease at the time of spray but the cpue change was probably not significant; growth stopped for a short period after spraying but recovered in a short time. A literature review done in preparation for this project provided little and conflicting information on relationships between sublethal physiological responses and whole-animal responses. Thus this study was undertaken in order to determine whether a relationship could be demonstrated between a sublethal (acetylcholinesterase inhibition) and a whole-animal response using very low exposure levels typical of the actual use of malathion.

2. Materials and Methods

2.1 Source of Fish for Feeding Experiments

Newly hatched walleye fry were obtained from the Manitoba Department of Natural Resources, Fisheries Branch hatchery at West Hawk Lake. These walleye were planted into rearing ponds along Bishop Grandin Boulevard in Winnipeg on May 22, 1986. All juvenile walleye used in these experiments were seined from this source as described by Delorme (1988) and held at the same environmental temperature in the Department of Zoology's Animal Holding Facility.

2.2 Experimental Design Considerations

The whole-animal response chosen was feeding. Walleye feeding on larger organisms more frequently will grow faster, enabling them to attain a size advantage over the average for their year class, sometimes resulting in cannabilism within the population. The feeding response was to be examined in laboratory experiments.

The sublethal response chosen was the inhibition of acetylcholinesterase since malathion is metabolized to its oxygen analogue (malaoxon) which in turn binds to acetylcholinesterase and eliminates catalytic activity.

Exposure protocols were chosen in an effort to mimic environmental conditions. With this rationale, a commercially available malathion (CIL) was chosen over a technical preparation. Commercial preparations of malathion can be more toxic than technical preparations (Haider and Inbaraj, 1986) possibly due to the presence of emulsifiers producing a finer and more uniform dispersion of malathion throughout the exposure water. Malathion obtained from a local retailer (C-I-L brand, 125 mg malathion per litre of formulated

product) was used because it represented the most common source of local exposure.

All exposures were based on theoretical concentration. It was calculated that the 1983 aerial spray, at 3 oz $acre^{-1}$, would produce water concentrations of between 2 ug L^{-1} and 40 ug L^{-1} depending on water depth and mixing processes (Lockhart and Metner, 1983), and an actual concentration of 8.9 ug L^{-1} was reported from ponds in which 75% cholinesterase inhibition was found in the fish. Tagatz et al. (1974) reported that the highest malathion concentrations in a salt marsh after fogging at 6 oz $acre^{-1}$ was 5.2 ug L^{-1} and after ULV spraying at 0.49 ULV only trace amounts (<0.3 ug L^{-1}) persisted in the marsh water for as long as one day. Laboratory exposure concentrations were chosen to include this range for the first experiment. In the Behaviourial Components section of the study preliminary experiments at these concentrations did not produce behaviourial changes and so the maximum concentration was increased to 200 ug L^{-1} for the balance of the experiment.

A single pulse dose was chosen as the most realistic form of environmental exposure. Field exposures change very rapidly often with a single spike input followed by a slow decline in concentration due to degradation processes. Clark et al. (1987) demonstrated that laboratory pulse exposures with rapidly changing concentrations were more predictive than standard toxicity exposures of the non-lethal and lethal effects observed in short-term field exposures.

A two hour pulse exposure was chosen arbitrarily, based mainly on the logistics within the laboratory and also on published literature. This time was probably unrealistically short, but represented a compromise between what was desired and what could be done without excess handling stress. Bull and

McInerney (1974) reported that coho salmon displayed signs of physiological impairment within two hours of exposure to fenthion at 57.8% of the LC50. Secondly, the first experiment was a joint project with a field study (Delorme, 1988) in which juvenile walleye from a common source were brought into the laboratory, exposed to malathion and either returned to the rearing ponds or used in the laboratory feeding study. Thus, a two hour exposure period was chosen to minimize time and stress related to handling. This time period was continued for the Behaviourial Component experiment. The study of those walleye returned to the rearing ponds was the subject of another thesis (Delorme, 1988).

2.3 General Procedures

2.3.1 Biochemical Analysis

Individual walleye heads were placed in pre-weighed, labelled disposable test tubes and held on ice less than one hour. The tubes were weighed to determine head weight. Tubes were then sealed with parafilm and held at -30^{o}C until biochemical analyses were performed. Whole heads were homogenized in 2 ml 0.1M phosphate buffer (pH 7.2) with a Polytron homogenizer for 30 seconds and then centrifuged at 12 000 xg (0 ^{o}C) to prepare a solution/suspension assayed colorimetrically by the method of Ellman et al. (1961) using acetylthiocholine as substrate at 20^{o}C . Reagents, including lyophilized human serum for a quality control measure, were obtained in prepared kit form from Boehringer Mannheim Corp. (BMC Diagnostics, Montreal). The results are expressed as milliunits of enzyme activity per mg of protein (mU/mg).

Protein in the solution/suspension was assayed by the method of Lowry et al. (1951). Lyophilized bovine serum albumin was utilized as a quality control sample and was run with each series.

2.4 Group Feeding Experiments with Field-Captured Fish

2.4.1 Experimental Protocol

The null hypotheses were that malathion had no effect either on acetylcholinesterase activity or feeding activity.

The walleye were planted into the rearing ponds on 22 May 1986. Juvenile walleye were brought into the laboratory for experimentation on five subsequent dates after stocking the young walleye: July 10 (50 days after stocking), July 17 (57), August 9 (75), 20 (90), 27 (97). Ten walleye were randomly distributed into each of four 200 L aquaria illuminated at 30 Lux of Vitalite (12/12 hr). After a 24 hour acclimation period, each group was transferred to an exposure aquarium to receive either 0, 5, 10 or 25 ug L^{-1} (ppb) malathion. On the earliest date (July 10) even lower dosages of 0, 0.1,1 and 5 ug L^{-1} were used. After two hours' exposure, the fish were removed from the treatment aquarium, gently rinsed, and returned to their original holding aquaria. Fathead minnows of known quantity and size distribution, and also a quantity of pond-representative zooplankton were added to each aquarium. All aquaria were isolated from outside disturbances for a two-hour feeding bout. The walleye were then sacrificed for whole-head acetylcholinesterase analysis and the headless bodies were preserved in formalin for a stomach analysis of feeding.

2.4.2 Statistical analyses

The data (acetylcholinesterase and feeding activity) from each date were subjected to a single classification analysis of variance. A Fisher's multiple comparison test was performed to compare data among malathion doses.

2.5 Behaviourial Components Experiment

2.5.1 General Protocol

The feeding observed in the first experiments was repeated with individual fish in order to quantify behaviours associated with feeding. The intent was to determine whether the feeding effect was due to alteration in visual or physical ability factors.

Juvenile walleye were exposed with a two-hour, static pulse dose of malathion at 0, 50, 100, or 200 ug L^{-1}. Ten walleye were exposed at each level; with 5 fish observed immediately after exposure and 5 fish observed 24 hours later. The procedure was repeated five times with order of exposure shifting.

After exposure each fish was transferred to a 60 L (31 cm x 32 cm x 62 cm) observation aquarium with a flow through system at 11^{o}C and 50 Lux of Vitalite. The aquarium had a grid marked at 5-cm intervals placed behind it for distance conversions and a mirror was angled over the aquarium in order to observe the top view in the video tape analysis. An individual walleye was transferred to the observation aquarium and allowed to acclimate for ten minutes. The prey (fathead minnows: 2 from each of the following size groups; 20-25 mm, 26-30 mm, 31-35 mm, 36-40 mm) were introduced into the aquarium via a 10 cm diameter PVC tube at the opposite end of the aquarium from the walleye. Feeding behaviour was videotaped for five minutes. The walleye were then

sacrificed for whole head acetylcholinesterase measurement and the headless bodies labelled and preserved in formalin for stomach analysis.

2.5.2 Behaviourial Analysis

Each sample of 15 minutes (10 minute acclimation, 5 minute feeding) was recorded on videotape. The final five minutes of the videotape were analyzed. Behaviours measured as seconds of activity, seconds of time spent in the upper half of the aquaria, number of orientations, number of lunges, and handling time were observed on a video monitor and recorded with a multi-key recorder. The recorder tape was then decoded (frequency and time of behaviour) for analysis. An orientation was defined as a movement which aligns the predator's body axis towards the prey. A lunge was defined as an attempt by the predator to grasp the prey. The handling time was recorded as the time from capture of the prey until the prey was subdued (usually ingested) or lost. Any unsuccessful handling attempts (i.e. the prey escaped) were recorded as lost handling time.

Swim speed (mean of three observations), reactive angles and reactive distances were measured on a large screen (50 cm) video monitor. The reactive distance was defined as the distance between the snout of the walleye and the prey item when the walleye oriented toward the prey. The reactive angle was the angle between the walleye and the prey at the moment of orientation.

Swimming behaviour was quantified by the time, frequency and location of swimming activity. Any movement was considered a swimming event. Location was defined as either the upper or lower half of the aquarium. For the upper activity data, both times (unequal variances) and frequencies were analyzed by Kruskal-Wallis one-way analysis of variance for each recovery time (SAS/STAT User's Guide, 1988).

Activity levels were assigned to individual fish based on the number of swimming events. The level was a number from 0 to 3. Fish were assigned to group 0 if they did not exhibit any movement. Levels 1 through 3 were based on 0 to 5 movements, 6 to ten movements and greater than 11 movements, respectively. Chi-square and Kruskal-Wallis one-way AOV was used to determine if activity level was affected by the malathion treatment.

Predator efficiency variables include: time to first strike, time to first capture, number of strikes, number of strikes to the first capture, number of captures and net efficiency (Colgan et al., 1986). Net efficiency of predation was defined as the number of successful captures per lunge attempt (Dill, 1983). If the fish did not initiate an attempt the value of 0.00 was given and increasing success was 1.00. One-way AOV and Kruskal-Wallis one-way AOV was used to determine the effect of malathion dose and activity level on net efficiency. The ratio of wet weight of prey consumed to foraging time (handling time and search time) was used as an approximation of the foraging efficiency ratio of E/T. One-way AOV and Kruskal-Wallis one-way AOV was used to determine the effect of malathion dose on foraging efficiency.

Prey consumption (numbers, size, weight) was determined by stomach analysis. Forklengths and dry weights of fathead minnow prey were used.

2.5.3 Statistical Analysis

Unless otherwise specified a split-plot design with dose of malathion as the main plot and recovery time as the subplot was used to analyze the data. A sub-sampling error term was included in the analysis. Analyses of variance (general linear model) and least-significant difference tests were carried out with SAS (SAS/STAT User's Guide, 1988) software. Analysis of variance tested for significance due to malathion dose, recovery time, block and interactions.

Least-significant difference values allowed comparisons of behaviours between malathion dose at each recovery time.

3. Results

3.1 Acetylcholinesterase and Protein Analyses

The acetylcholinesterase test kit is designed for use with human serum; for use here with head extracts it has two limitations; a change in absorbency of 0.400 units per minute can result in non-linear readings, and the lower limit of sensitivity is a change of 0.001 units per 30 seconds. All of the assays performed fell within the acceptable range between these limits.

The amount of protein in the sample was thought to be a potential concern since fish were sampled from all age and size groups available. As a result a protein linearity experiment was performed. Five walleye samples were randomly selected and diluted by 1:2, 1:5, 1:10 and 1:20. The average protein content of these samples was approximately 9 mg mL^{-1}, 4.5 mg mL^{-1}, 1.8 mg mL^{-1}, 0.9 mg mL^{-1} and 0.45 mg mL^{-1}. The assays were linear in all dilutions except at the lowest dilution of approximately 0.45 mg mL^{-1}. Protein content of the Behaviourial components experiment averaged 11.9 mg mL^{-1} and the protein content of the group feeding experiment averaged 23.3 mg mL^{-1}. Although these protein values are higher than in the linearity experiment, there was no indication of non-linearity at the higher concentrations and it was assumed that the assays were linear.

The QC response of the two quality control samples over the two year time period of the study (three technicians, 40 test kits and approximately 4000 samples - note that of the 4000 assays, this thesis presents only 2 experiments with a total of 400 assays) yielded a coefficient of variation of 28.8%. The cholinesterase test kit day-to-day repetition reports a 7.5% coefficient of variation for whole blood. The protein QC response over the period of the

study averaged 0.1023 mg/mL protein (the standard used was 0.101 mg/mL) and the coefficient of variation was 20.6%.

3.2 Group Feeding Experiments with Field-Captured Fish

Juvenile walleye were brought into the laboratory, on five occasions over the growing season, and exposed to malathion, the null hypothesis being that malathion exposure would not result in alterations of acetylcholinesterase activity or feeding activity.

On all experimental dates, the acetylcholinesterase response was variable, with no significant treatment (Malathion dose) effect at the 0.05 level (Table 2), although some individual doses did differ from their respective controls. There was no linear relationship between the acetylcholinesterase activity and the malathion concentration on any date. Days 50, 90 and 97 exhibit typical beta hormetic curves with apparent stimulation at the lower doses and inhibition at the higher doses. Mortality was observed with the highest Malathion treatment in 3 of the 5 dates and on these occasions, the dead fish exhibited lower AchE activities than live fish from the same treatment. Death occurred at acetylcholinesterase inhibitions of greater than 25% on these dates; however, inhibitions of greater than 25% occurred on the two earlier dates with no resulting mortality.

3.2.2 Feeding Activity

On the first experimental date (Day 50) there was a slight increase in numbers of minnows eaten by treated fish over the numbers eaten by controls (Table 3) with a concomitant increase in prey weight recovered from stomachs. Later in the season (Days 75, 90 and 97) the opposite trend was apparent, with significant declines in either the numbers of minnows eaten or in the weight of minnows eaten.

Table 2. Whole head Acetylcholinesterase Activity (mU/mg protein) of juvenile walleye exposed to malathion in the Group Feeding Experiments.

Day	Malathion (ug L^{-1})		AchE Activity mU / mg protein Mean	(n, S.E.)		AchE (% of Control)
50	0		73.03	(6, 5.94)		100 %
	0.1		104.45	(4, 9.22)		+ 43 %
	1.0		45.47	(4, 21.15)	*	- 37 %
	5.0		12.50	(1, .)	*	- 83 %
57	0		64.07	(6, 10.21)		100 %
	5		55.04	(7, 4.71)		- 14 %
	10		46.76	(7, 6.06)		- 27 %
	25		50.78	(6, 5.25)		- 21 %
75	0		80.33	(6, 16.41)		100 %
	5		72.30	(7, 9.87)		- 10 %
	10		86.60	(7, 7.47)		+ 8 %
	25	(Alive)	85.74	(5, 11.05)		+ 7 %
		(Dead)	40.08	(5, 9.96)	#	- 50 %
90	0		79.33	(6, 17.86)		100 %
	5		86.75	(6, 16.76)		+ 9 %
	10		87.09	(7, 7.82)		+ 8 %
	25	(Alive)	69.35	(2, 7.15)		- 13 %
		(Dead)	59.35	(1, .)		- 25 %
97	0		59.01	(7, 6.95)		100 %
	5		64.10	(6, 2.95)		+ 8 %
	10		57.61	(7, 3.90)		- 2 %
	25	(Alive)	68.50	(4, 8.41)		+ 16 %
		(Dead)	42.48	(4, 6.47)	#	- 28 %

* - Significantly different from respective control by alpha = 0.05.
\# - Significantly different from live fish at 25 ug L^{-1} at alpha = 0.05.

Table 3. Feeding Activity of juvenile walleye exposed to malathion in the Group Feeding Experiments.

Day	Malathion (ug L^{-1})	Prey Items / Predator	Dry weight of Stomach content (mg) / wet weight of walleye (g)
50	0	1.1	0.00293
	0.1	1.8 *	0.00829 *
	1.0	1.3	0.00837 *
	5.0	1.9 *	0.00994 *
57	0	n/a	n/a
	5	1.5	0.00605
	10	1.9	0.00847
	25	1.6	0.00721
75	0	2.8	0.00804
	5	1.6 *	0.00586
	10	1.7 *	0.00603
	25	0.8 *	0.00415 *
90	0	1.8	0.02070
	5	1.7	0.00490 *
	10	1.2	0.00459 *
	25	1.8	0.00583 *
97	0	3.0	0.01050
	5	2.7	0.00887
	10	2.6	0.00824
	25	1.6 *	0.00544 *

* - Significantly different from respective control by alpha = 0.05.
n/a - Not available, samples destroyed.

3.2.3. Feeding Selectivity

On all experimental dates only fathead minnows were eaten; zooplankton were not found in any of the stomachs.

The size of fathead minnow prey eaten was normalized in this experiment for predator size by dividing by the walleye length because of the large range in walleye size available on these occasions. Over the experimental time period (Table 4), the mean prey size consumed by the control fish increased from 23.46 mm to 29.40 mm. On days 75 and 90, there was an apparent widening of the prey sizes selected. The Kolomogorov-Smironov test of distributions (sensitive to differences in location, dispersion and skewness) for all dates and exposure concentrations were non-significant (Table 5) indicating that malathion did not affect the size distribution of prey eaten. A one-way analysis of variance on this size ratio was non-significant for all dates.

Table 4. Fathead Minnow size selectivity by juvenile walleye exposed to malathion in the Group Feeding Experiments.

Date	Malathion (ug L^{-1})	Fathead Minnow Forklength (mm) Mean	Variance	Range
Day 50	0	23.46	9.80	15.44 - 27.24
	0.1	24.18	10.01	19.66 - 28.93
	1.0	24.58	7.79	19.66 - 28.08
	5.0	24.48	8.87	21.34 - 28.08
Day 57	0	n/a		
	5	25.10	17.33	17.97 - 35.67
	10	23.55	7.51	20.50 - 25.55
	25	23.97	9.80	18.81 - 28.93
Day 75	0	24.80	17.47	15.44 - 32.30
	5	27.66	27.56	19.66 - 37.35
	10	26.59	31.02	17.97 - 37.35
	25	28.39	46.51	17.13 - 37.35
Day 90	0	27.57	18.15	19.66 - 35.67
	5	22.43	17.06	15.44 - 28.87
	10	24.51	14.21	17.13 - 29.77
	25	25.74	24.11	19.66 - 36.51
Day 97	0	29.40	25.81	23.03 - 40.72
	5	28.42	23.52	19.66 - 39.88
	10	28.79	17.56	23.87 - 39.88
	25	30.78	25.38	23.87 - 39.04

Table 5. The Kolmogorov-Smirnov difference values ($|D|$[1]) between the Malathion exposed juvenile walleye and their respective control on each experimental day in the Group Feeding Experiments.

Day	Malathion (ug L^{-1})	$\|D\|$
50	0.5	0.19
	1	0.21
	5	0.18
57	5	_a
	10	_a
	25	_a
75	5	0.22
	10	0.27
	25	0.23
90	5	0.21
	10	0.42
	25	0.50
97	5	0.08
	10	0.08
	25	0.21

[1] $|D|$ is the maximum difference between ordered distributions.
Missing data is indicated by "a"..

3.2 Behaviourial Components Experiment

Each malathion exposed juvenile walleye was monitored and behaviours related to piscivory were quantified. The null hypothesis was that malathion exposure would not change acetylcholinesterase activity or alter behaviours associated with piscivory.

The walleye tested in this experiment averaged 104.9 mm (+/- 7.265, range 88.0 mm - 130.0 mm) in length and 8.286 g (+/- 2.216, range 4.079 - 15.91 g) in weight.

3.2.2 Acetylcholinesterase Measurement

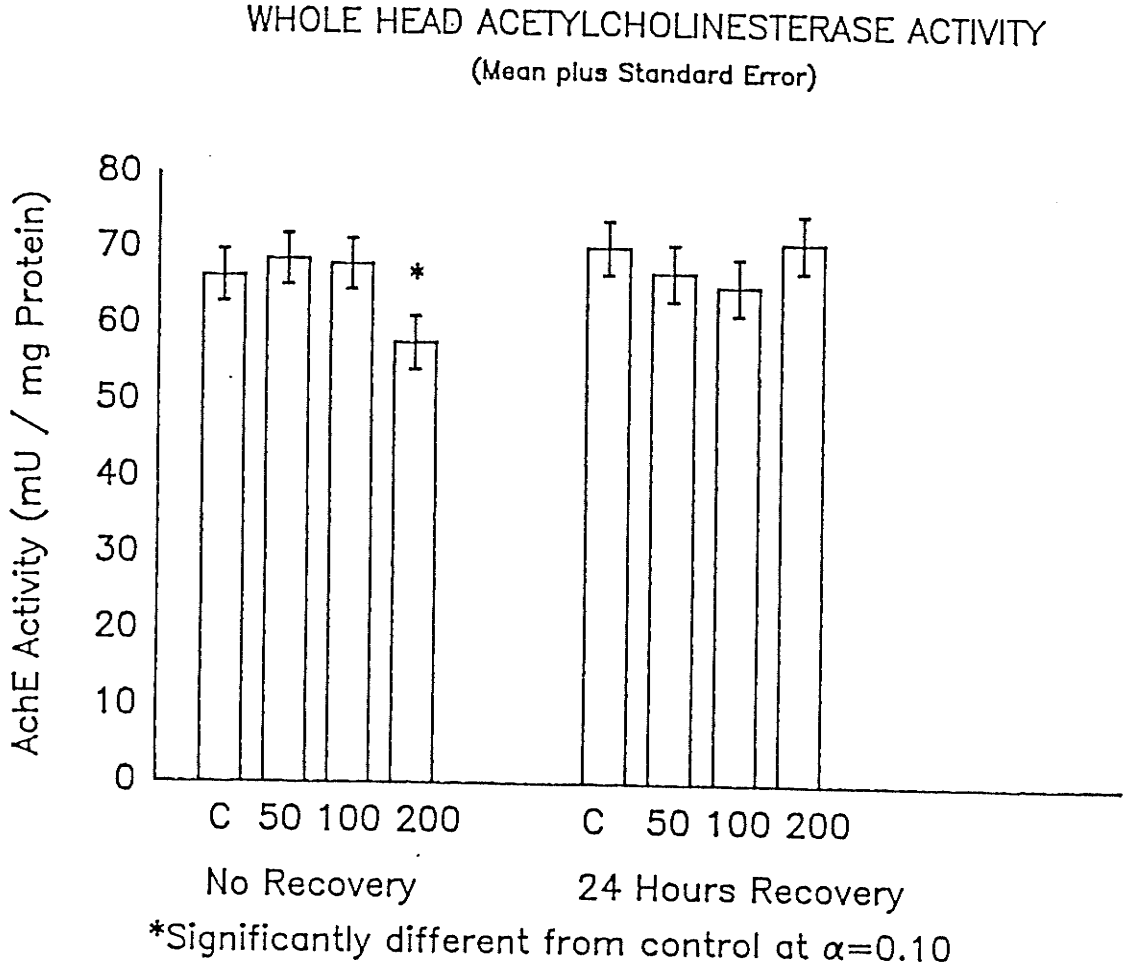
This response is presented in Figure 1. The acetylcholinesterase response was significantly ($F=2.46$, $P<0.0001$) affected by factors within the experimental model. Although malathion dose alone was not a significant variable ($p=0.349$), the recovery time - dose interaction term was significant at $p=0.0283$. There were no significant differences among these recovery time-dose means in a multiple comparison analysis (least significant differences) with alpha = 0.05.

3.2.3 Behaviours

3.2.3.1 Location of Prey

The reactive distance, reactive angle and the number of orientations towards prey were used as measures of the predator's ability to locate and identify suitable prey (Table 6).

Figure 1. Acetycholinesterase Activity (mU/mg protein) in juvenile walleye exposed to malathion in the Behaviourial Components Experiment. Means and Standard Error Bars Presented.


WHOLE HEAD ACETYLCHOLINESTERASE ACTIVITY
(Mean plus Standard Error)
AchE Activity (mU / mg Protein)
80
70
60
50
40
30
20
10
0
*
C 50 100 200
C 50 100 200
No Recovery
24 Hours Recovery
*Significantly different from control at α=0.10

In all malathion doses, in both no-recovery and 24-hours-recovery, there was an increase in the number of orientations although the means were not significantly different from one another (Table 6). The orientation response was significantly affected by the experimental protocol ($F=1.60$, $P>F = 0.0319$) with malathion dose being a significant variable ($P>F= 0.0441$). A comparison of means exhibited no significant differences in orientations between malathion groups except for the 100 ug L^{-1} with 24 hours of recovery group of fish which had twice as many orientations as the controls.

The mean reactive distance was consistently higher than controls in all the treated groups (Table 6). The reactive distance was regressed against the prey size for all treatment groups. In this experiment, there were no linear relationships in any treatment and within the prey size range available. The reactive distance data were analyzed in the standard split-plot ANOVA. The overall experimental protocol did not contribute significantly to the reactive distance response ($F=1.10$, $P>F=0.3593$) although malathion dose ($P>F= 0.0539$) and recovery time ($P>F=0.0696$) did influence the reactive distance. A t-test comparison of means indicated that the 50 ug L^{-1} with no recovery treatment had a significantly higher reactive distance than its control.

In all malathion treatments, the reactive angle is less than that of the control (Table 6). A regression model was run for each treatment with reactive angle dependent on prey size. There was no linear relationship within the prey size ranges within this study. The reactive angle data were analyzed in the split-plot ANOVA design. The experimental protocol significantly affected the reactive angle ($F=1.55$, $P>F= 0.0609$) with malathion dose being a significant variable ($P>F= 0.0212$). Although the angles that the predator reacted to in successful prey encounters declined by approximately half on the no recovery

day, these were not significantly different from their control. After 24 hours of recovery, the reactive angles remained lower in the treated groups than in the control, but again the differences were not significant. The control for the 24 hour recovery was less than the control-no recovery group but this was not a significant difference.

Table 6. Prey localization by juvenile walleye exposed to malathion in the Behaviourial Components Experiment.

Recovery Time (hours)	Malathion Dose (ug L^{-1})	Reactive Distance (cm)	Reactive Angle (degrees)	Orientations (frequency)
0	0	2.44 (8, 0.34)[1]	74.63 (8, 11.21)	1.04 (22, 0.45)
	50	5.85 * (13, 0.50)	31.00 (13, 10.28)	1.36 (25, 0.41)
	100	3.47 (18, 0.51)	34.17 (18, 7.62)	2.00 (14, 0.83)
	200	3.70 (9, 0.93)	35.78 (9, 11.92)	1.71 (14, 0.34)
24	0	4.27 (14, 1.00)	50.43 (14, 9.96)	1.47 (24, 0.42)
	50	7.69 (13, 2.19)	28.15 (13, 9.50)	1.87 (16, 0.43)
	100	5.79 (16, 1.34)	35.81 (16, 8.69)	3.25 * (22, 0.45)
	200	4.57 (16, 0.99)	49.44 (16, 10.02)	1.52 (21, 0.49)

[1] (n, S.E.)
*Significantly different from respective control by alpha = 0.05.

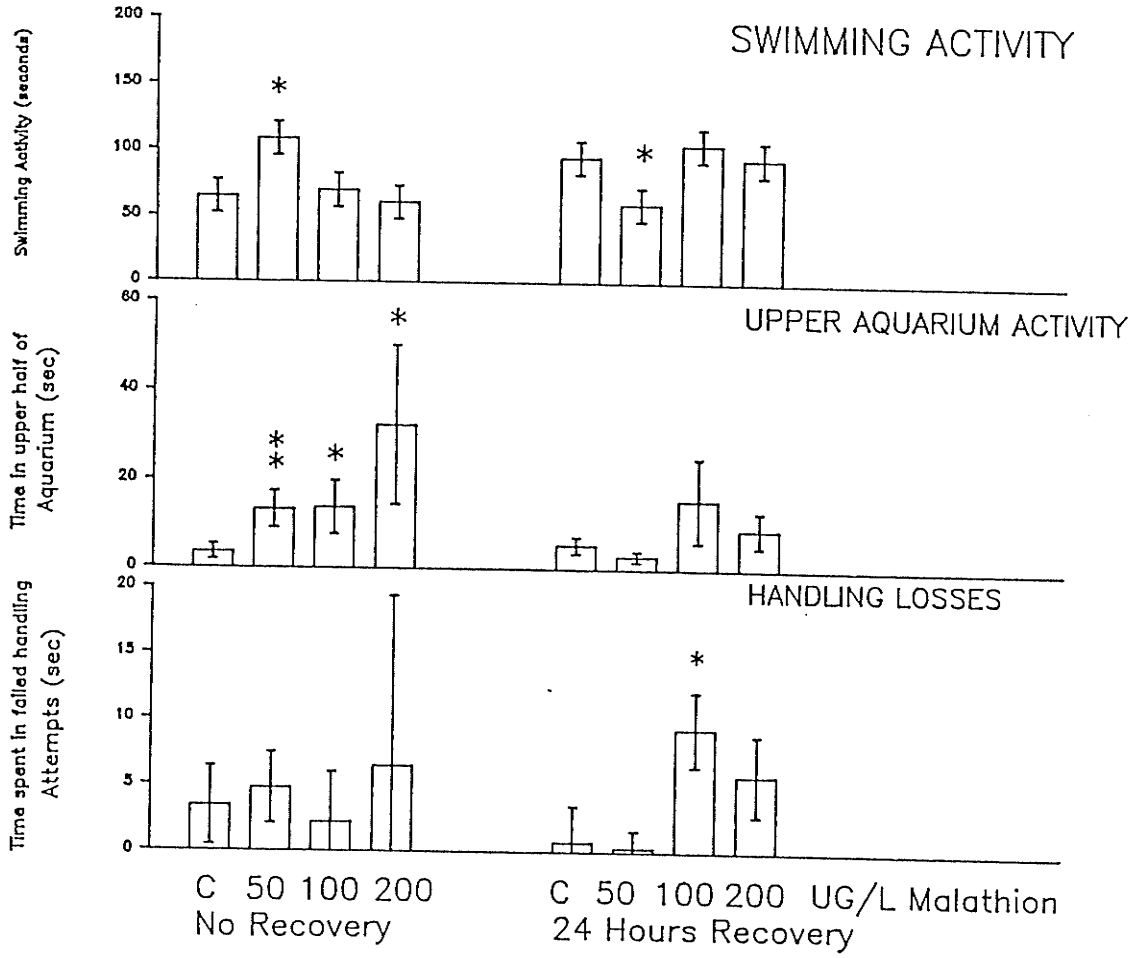
3.2.3.2 Locomotory Activity

Total seconds of swimming activity by the walleye was significantly affected by the controlled factors within the experiment ($F=2.84$, $p < 0.0001$). All interaction terms within the model (day*dose*recovery, dose*recovery and day*dose) were significant. With no recovery, all but the 50 ug L^{-1} group were equally active (Figure 2). The 50 ug L^{-1} group was active for a significantly longer time period. After 24 hours of recovery, this pattern was reversed with the 50 ug L^{-1} group spending less time in swimming activity. There was no linear relationship between the acetylcholinesterase activity and seconds of swimming activity ($r^2 = 0.0036$, $p = 0.5594$).

Malathion affected the amount of time spent in the upper half of the aquaria in the no-recovery group of walleye (K-W=6.46, $p = 0.09$) but did not affect this behaviour in the 24 hours of recovery group (K-W = 0.44, $p = 0.93$) (Figure 2).

The effects of malathion on activity level were analyzed separately for each recovery time. Chi-square analysis indicated that observed activity levels for fish exposed to malathion were significantly different from those activity levels observed in the control group (Table 7). Features of activity levels for no-recovery-time (Table 7) include: all fish at the 50 ug L^{-1} treatment were active, none were inactive; with increasing dose, more fish became completely inactive; and a bimodal distribution emerged at the 100 ug L^{-1} treatment (36% of the fish were completely inactive while 40% of the fish were at activity level 2). After 24 hours recovery, all fish were more active except for the 50 ug L^{-1} treatment. Initially this group had no completely inactive fish and after 24 hours, 40% of the group became inactive.

Figure 2. Locomotory Behaviours of juvenile walleye exposed to malathion in the Behaviourial Components Experiment. Means and Standard Error Bars Presented.



*Significantly different from control at $\alpha=0.10$

**Significantly different from control at $\alpha=0.05$

Table 7. Activity Levels of juvenile walleye exposed to malathion in the Behaviourial Components Experiment.

Recovery Time (hours)	Malathion Dose (ug L^{-1})	Activity Level (frequency) 0	1	2	3
0	0	3	10	6	5
	50 ***	0	5	13	7
	100 ***	9	1	10	5
	200 ***	11	1	5	8
24	0 #	1	6	11	7
	50 ***	10	4	9	2
	100 *	2	3	15	5
	200 ns	3	6	11	5

* Significantly different from respective control at alpha = 0.10.
*** Significantly different from respective control at alpha = 0.001.
ns Not significantly different from respective control.
\# Significantly different from the no-recovery time control at alpha= 0.001.

The controlled factors within the model significantly affected walleye swim speed ($F=1.72$, $p= 0.0198$). On both recovery times, the walleye exposed to the highest level of Malathion (200 ug L^{-1}) exhibited slower swim speeds although these means were not significantly different from their respective controls. There was no linear relationship between acetylcholinesterase activity and swim speed ($r^2= 0.0110$, $p=0.3124$) at either recovery time.

3.2.3.4 Handling Ability

Malathion exposed fish appear to be inefficient in handling (Figure 2). More time was spent in failed predation attempts by malathion exposed fish than control fish ($F=1.44$, $p>F= 0.0739$). The fish exposed to 200 ug L^{-1} of malathion spent at least twice as much time in unsuccessful handling attempts as compared to control fish but this was not a significant difference in a statistical test.

Handling times exhibit a linear relationship with the ratio of prey size to mouth gap diameter of the predator (Bannon and Ringler, 1986):

$$\text{Ln } H_t = a + b*\text{Ln (prey size / mouth gap of predator)}.$$

Handling time data for successful prey encounters was transformed according to this relationship. Mouth gap diameters of the walleye (Ward and McCulloch, 1990) were calculated using the formula:

Mouth Gap diameter = 0.324 + 0.06355*Total Length.

Regression curves are presented in Figure 3. The regression co-efficient and statistics for handling time are found in Table 8. R^2 ranged from 0.29 to 0.76. All of the regressions fit the above model at alpha = 0.05 although the curve for 200 ug L-1 at no recovery had a $r^2=0.29$ with $p=0.0564$ suggesting the model may not be the most appropriate for this data set. R^2 statistics indicated that the model accounted for approximately 60% of the variability in

the data except for the 200 ug L^{-1} treatment where the R^2 drops below 0.35. The general lack of fit at these high doses suggest that another factor (malathion) was affecting the handling time. The intercept and slope of each malathion treatment was compared to its respective control by a two-sample t-test. None of the intercepts or the slopes was significantly different from its control.

Figure 3. Regression Curves of fathead handling times by malathion-exposed juvenile walleye in the Behaviourial Components Experiment.

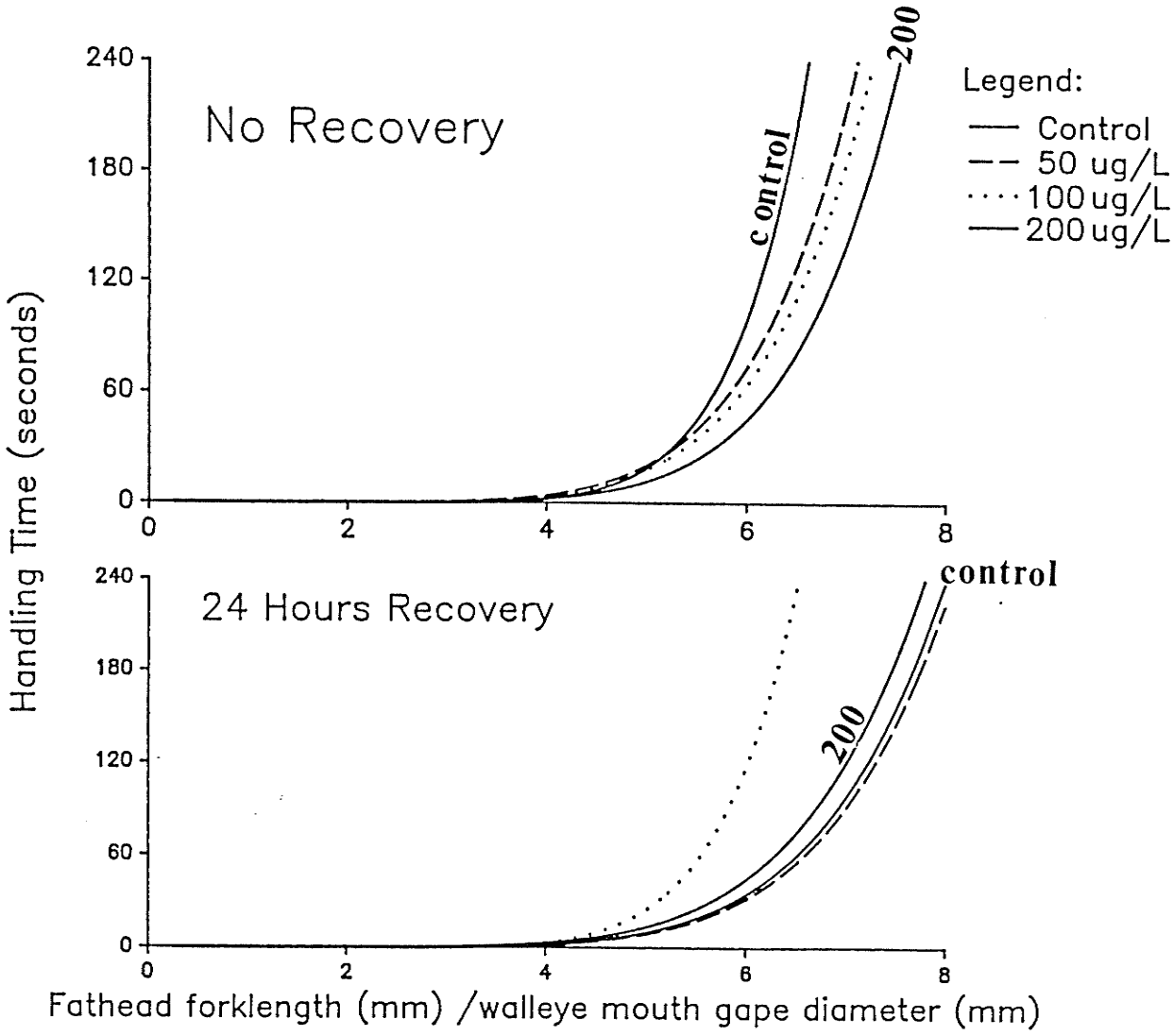

No Recovery
24 Hours Recovery
Handling Time (seconds)
Fathead forklength (mm) /walleye mouth gape diameter (mm)
Legend:
Control
50 ug/L
100 ug/L
200 ug/L
control
200
240
180
120
60
0
0
2
4
6
8

Table 8. Regression co-efficient and statistics of Handling Time of successful prey encounters (Ln H_t = a + b*Ln(prey size/mouth gap)) by juvenile walleye exposed to malathion in the Behaviourial Components Experiment.

Recovery Time (hours)	Malathion Dose (ug L^{-1})	Handling Time Regression: a	b	R^2	Model P
0	0	-11.66	9.07	0.76	0.0023
	50	- 8.25	7.00	0.64	0.0006
	100	- 8.30	6.95	0.56	0.0001
	200	- 9.38	7.36	0.29	0.0564
24	0	- 8.42	6.68	0.66	0.0002
	50	- 8.63	6.75	0.47	0.0047
	100	-10.56	8.55	0.62	0.0001
	200	- 7.71	6.42	0.33	0.0121

3.2.3.3 Predator Efficiency

There was no treatment effect on the time to the first strike (F=0.84, p>F = 0.7171) (Figure 4). All initial predation encounters, if an encounter occurred, took place within the first 90 seconds. The time to the first capture was not affected by the treatments (F=1.14, p>F= 0.3341). First captures also occurred within the first 90 seconds. The number of strikes until the first capture did not differ between malathion doses or recovery time (F=0.85, p>F=0.7063). The experimental protocol significantly affected the number of prey strikes (F=1.69, P>F= 0.0179). The single effect of malathion dose was significant (p>F= 0.0118). There were more strikes by treated fish at both recovery times (Table 9) with significantly higher strike attempts occurring in fish exposed to 100 ug L^{-1} of malathion.

The effects of malathion and activity level on net efficiency was analyzed separately for each recovery time. Net efficiency (the number of captures per attempt) was not affected by malathion dose at either no-recovery-time (F=0.09 DF=3,73) or 24 hours recovery time (F=0.66 DF=3,76) (Table 9). Activity level (Table 10) significantly affected net efficiency for no-recovery-time ($p < 0.00001$) and for 24 hours recovery time ($p < 0.00001$). Fish that were inactive were unsuccessful predators while those fish at activity level 2 exhibited the greatest number of captures per attempt.

Figure 4. Measures of feeding motivation in malation-exposed juvenile walleye in the Behaviourial Components Experiment. Means and Standard Error Bars Presented.

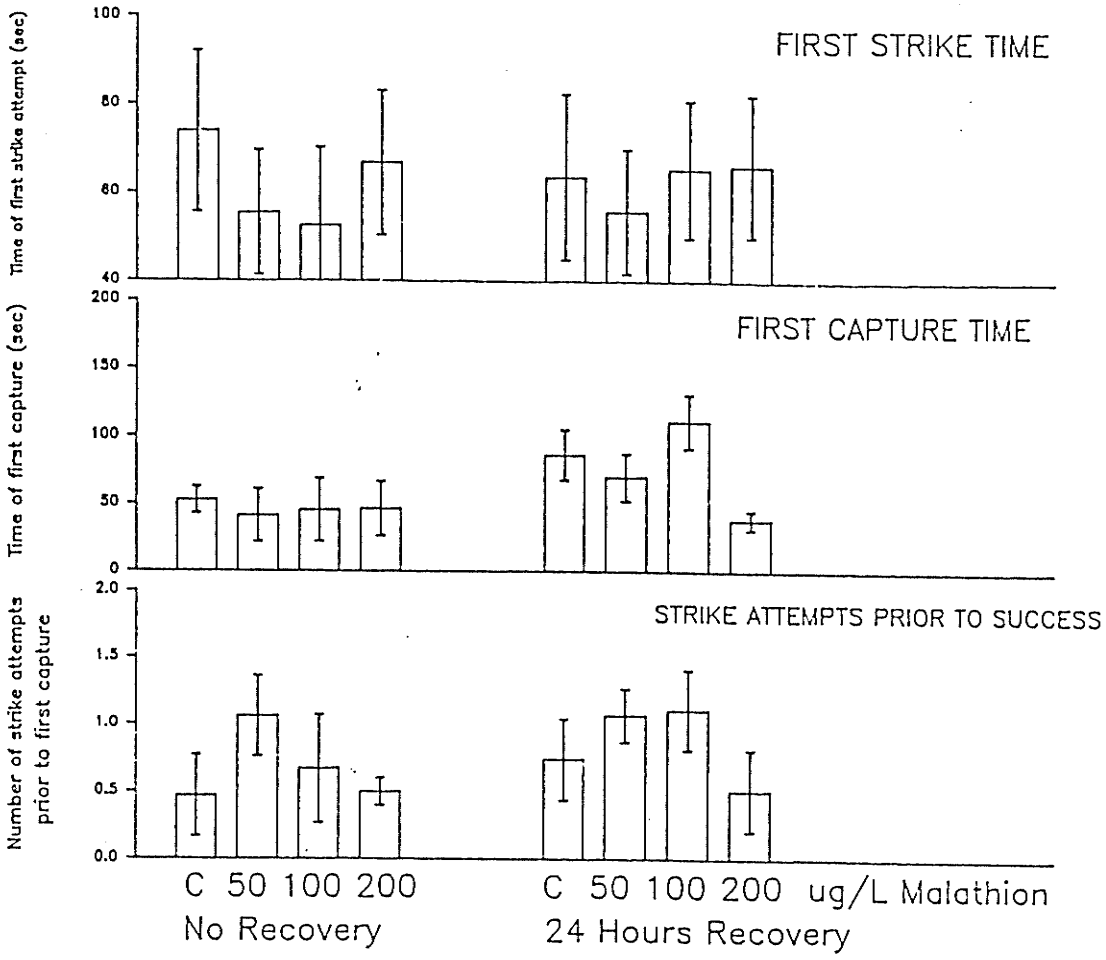

FIRST STRIKE TIME
Time of first strike attempt (sec)
100
80
60
40
FIRST CAPTURE TIME
Time of first capture (sec)
200
150
100
50
0
STRIKE ATTEMPTS PRIOR TO SUCCESS
Number of strike attempts prior to first capture
2.0
1.5
1.0
0.5
0.0
C 50 100 200
No Recovery
C 50 100 200 ug/L Malathion
24 Hours Recovery

Table 9. Predator Efficiency of juvenile walleye exposed to malathion in the Behaviourial Components Experiment.

Recovery Time (hours)	Dose Malathion (ug L^{-1})	Total Strikes	Efficiency	
			Net	Foraging (x 10^{-3} mg sec^{-1})
0	0	0.85 (0.5)[1]	0.21 (0.09)	3.9 (1.9)
	50	1.48 (0.4)	0.20 (0.08)	2.6 (1.1)
	100	2.48 * (0.6)	0.23 (0.08)	35.8 (17.8)
	200	2.00 (0.4)	0.26 (0.10)	8.6 (5.3)
24	0	1.48 (0.4)	0.21 (0.07)	3.6 (1.2)
	50	1.81 (0.4)	0.19 (0.08)	5.9 (3.3)
	100	3.37 * (0.5)	0.34 (0.09)	1.4 (0.4)
	200	1.76 (0.5)	0.29 (0.09)	2.5 (1.2)

[1] (S.E.)
*Significantly different from respective control by alpha = 0.05.

Table 10. Net efficiency (prey captures per attempt) by Activity Level of juvenile walleye exposed to malathion in the Behaviourial Components Experiment.

Activity Level	Recovery Time (hours)	
	0	24
0	0.00 (50, 0)[1]	0.00 (43, 0)
1	0.70 (15, 0.08)	0.60 (18, 0.08)
2	0.76 (7, 0.14)	0.76 (9, 0.08)
3	0.60 (1, 0)	0.63 (4, 0.08)

[1] (n, S.E.)

Foraging efficiency, the wet weight of prey consumed per total foraging time, was affected by dose at no-recovery time (F=2.37 df=3,59 p=0.804) but was not affected by dose after 24 hours of recovery (F=1.41 df=3,63 p=0.2470). With no recovery time period, the 100 ug L^{-1} group exhibited a ten-fold increase in foraging efficiency (Table 9).

3.2.3. 5 Consumption

Prey consumption was measured in terms of the number of successful captures, prey weight and prey size found in the stomach of the walleye (Table 11).

The controlled factors within the experiment significantly affected both the total prey dry weight (mg) consumed (F=1.56, P>F=0.0321) and the number of prey consumed (F=1.52, P>F=0.0408). Consumption, in terms of both dry weight and numbers of prey, did not differ between malathion treatments except at 100 ug L^{-1} which was significantly higher than its control at the no recovery time level. Both weight of prey and numbers of prey items increased between day 0 (no recovery) and 24 hours later.

The frequency of prey sizes found in the stomachs of malathion exposed fish did not differ significantly from their respective control group (Table 12) as determined by Kolmogorov-Smirnov analysis.

Table 11. Feeding Activity in juvenile walleye exposed to malathion in the Behaviourial Components Experiment.

Recovery Time (hours)	Malathion Dose (ug L^{-1})	Prey Consumption wet weight (mg)	Prey Consumption Numbers
0	0	0.15 (21, 0.16)[1]	0.05 (24, 0.19)
	50	0.22 (22, 0.05)	0.07 (25, 0.18)
	100	0.34 * (25, 0.05)	1.20 * (25, 0.17)
	200	0.15 (19, 0.06)	0.52 (25, 0.19)
24	0	0.23 (22, 0.06)	0.81 (25, 0.18)
	50	0.24 (21, 0.06)	0.98 (25, 0.19)
	100	0.19 (23, 0.06)	0.80 (25, 0.18)
	200	0.27 (24, 0.05)	0.92 (25, 0.17)

[1] (n, S.E.)
*Significantly different from respective control by alpha = 0.05.

Table 12. The Kolmogorov-Smirnov difference values (|D|) between the Malathion exposed juvenile walleye and their respective control in the Behaviourial Components Experiment.

Recovery Time (hours)	Malathion Dose (ug L^{-1})	|D|
0	50	0.27
	100	0.14
	200	0.27
24	50	0.24
	100	0.16
	200	0.13

4. Discussion

4.1. Experimental Design

The use of simple systems for behaviourial toxicity testing is documented in the literature (Goodyear, 1972; Woltering et al., 1978; Kania and O`Hara, 1974). Walleye were observed in simple flow through aquaria but this design has been criticized because fish are not challenged to search for, locate, pursue and capture prey (Sandheinrich and Atchison, 1990). Because walleye are visual pelagic feeders (Niemuth et al., 1959), the simple unstructured environment employed in this study was appropriate.

Prey were presented in densities higher than those found in stocking ponds (Li and Ayles, 1981). Sandheinrich and Atchison (1990) criticized the use of high prey densities in behavioral toxicity studies for the lack of challenge presented to the predator. Reported densities of fathead minnows are based on a random dispersal within the aquatic environment but fathead minnows are not randomly dispersed, they school. This study presented the predator with an aggregation of minnows approximating a school found in the environment. With the prey densities provided, feeding behaviours could be elicited without removing the challenge or affecting size selectivity.

A criticism of this study may be that the prey were not exposed to malathion and thus the experiment lacked ecological realism. Fathead minnows are relatively insensitive to malathion with a LD50 (96 hour) of 9000 ug L^{-1} (Johnson and Finley, 1980). The exposures utilized for the walleye may have subtle behaviourial effects on the fatheads but the effect was assumed to be insignificant relative to the potential effects on the malathion sensitive walleye.

4.2 Acetylcholinesterase Measurements

The whole head acetylcholinesterase activity of control fish in the group feeding experiments was 59-80 mU mg^{-1} and the whole head acetylcholinesterase activity of the control fish within the behaviourial components experiment was 57-71 mU mg^{-1}. A wild walleye population (Lockhart et al., 1985) had mean brain cholinesterase activity of 160 mU mg^{-1}. This two-fold difference may be, in part, due to procedural differences. Whole heads were used in this study whereas the Lockhart et al. (1985) used brain tissue. Brain tissue acetylcholinesterase activity is traditionally used to diagnose poisoning. Other tissues including serum or plasma (Lockhart et al., 1973), muscle (Duangsawasdi and Klaverkamp, 1979), gill (Verma et al., 1979), heart (Duangsawasdi and Klaverkamp, 1979) and liver (Verma et al., 1979) experience acetylcholinesterase inhibition upon organophosphorous or carbamate exposure and may be used to diagnose poisoning. The use of whole heads in this study was done for logistical reasons. With fish of a wide size range, fry stage up to 150 mm, it was easy to take a consistent whole head sample, regardless of who dissected the fish. Antwi (1987) used a whole fish heads in order to assess organophosphorous poisoning.

This study employed a modified Lowry protein technique that employs a detergent in the buffer solution to solubilize membranes. This different technique may result in different protein values than those recorded by Lockhart et al. (1985). As nervous tissue cholinesterase tends to be membrane bound - not in solution (Crone, 1971), this modified technique may provide a better estimate of cholinesterase protein.

The higher protein value in whole heads was due to the presence of non-cholinesterase proteins and to the inclusion of membrane bound cholinesterase

and other proteins. This increased protein value would dilute the cholinesterase activity to the lower values found in this study.

Acetylcholinesterase activity did not correlate with any other response variable. There was no simple linear relationship between dose and activity but there was an apparent beta hormetic relationship. Hormesis (Stebbing, 1982) is the term given to the stimulatory effects caused by low doses of potentially toxic agents. Beta hormetic relationships are the most common form of hormesis in which there is a single stimulatory peak at concentrations immediately below those that are inhibitory.

This lack of correlation is consistent with the literature, regardless of species. In a group of acetylcholinesterase inhibited rats, two subgroups presented themselves: one subgroup exhibited no symptoms of intoxication and the other exhibited overt signs of intoxication although their brain AchE values overlapped substantially (Jimmerson et al., 1989). An acetylcholinesterase-independent bimodal distribution of behaviour is evident in this study in the 100 ug L^{-1} with no recovery time group of fish in which half of the fish were completely inactive and the other half were active. Inferences based on a sample population mean of cholinesterase does not reflect values for individuals nor will it predict behaviours for any group of acetylcholinesterased inhibited animals.

4.3 Predation Behaviourial Measures

The only effects of malathion demonstrated in these experiments were the alterations in the sensory and locomotory components of predation.

4.3.1 The Sensory Component

Encountering prey is dependent upon the co-ordination of locomotory activity and the acuity of sensory organs. Recognition and detection is the discrimination of suitable prey which in the case of walleye is determined by vision. The visual acuity of the walleye will contribute significantly to feeding variability. Visual acuity is reflected in the measure of reactive distance (O'Brien, 1979). Reactive distances for visual predators are influenced by environmental factors such as turbidity and light. Reactive distance increases with prey size (O'Brien, 1979).

The distance and angle at which the predator notices prey is the reactive distance and the reactive angle. In this study, malathion exposed walleye exhibited increased reactive distances and decreased reactive angles. The observed increase in reactive distance is inconsistent with the literature in which toxicant exposed fish tend to decrease their reactive distance (Nyman, 1981; Finger et al., 1985; Morgan and Kiceniuk, In Press). Reactive angles of toxicant exposed fish have not been reported in the literature.

Changes in the reactive distance and angle are a function of changes in the environment (light, turbidity) or changes in the acuity of the sensory organ. As no changes in the environment occurred, the change may have occurred in the eye or in the CNS structures involved in vision as a result of malathion exposure. The presence of cholinesterase in the eye and the affect of cholinesterase inhibitors is well documented in the literature. The corneal epithelium has the highest concentration of acetylcholinesterase of any animal tissue and it is assumed that it may have a role in pain perception in the cornea (Fitzgerald and Cooper, 1971). In animals and man, DFP (di-isopropyl phosphofluoridate) a cholinesterase inhibitor, causes prolonged miosis, ciliary

spasm and false myopia (Leopold and Comroe, 1946). Ocular anomolies in humans exposed to organophosphate include visual field stenosis and progressive myopia (Plestina and Piukovic-Plestina, 1978).

Visual stenosis is consistent with the decreased reactive angles observed in this study. The increased reactive distances observed are inconsistent with progressive myopia but are consistent with visual field stenosis. Visual stenosis will affect the ability of a predator to see prey in two ways. Firstly, with a reduced visual field the probability of a walleye seeing any prey is reduced. Secondly, because the immediate search area is narrowed the probability of seeing distant prey is greater than seeing close prey. As walleye lunge at the first prey within its visual field, the first prey item will probably be farther away. This will result in increased reactive distances.

4.3.2 The Locomotory Component

In this study, swimming activity was affected by malathion exposure. Typical beta-hormetic curves (Stebbing, 1982) of low dose stimulation and high dose inhibition were observed with this component. This response is consistent with the work of Bull and McInerney (1974). They described two functional responses of salmon exposed to fenitrothion: at low concentrations, an irritant response marked by increased comfort behaviours (flicking and thrusting) and decreased social and feeding activities. At higher concentrations, physiological impairment was exhibited by decreased comfort behaviours associated with locomotion.

Alterations in swimming behaviour can affect predation. Malathion dose probably affected the activity level of the fish and the activity level

affected the success of predation. Hyperactive fish were more successful predators than hypoactive fish because they had more encounters than hypoactive fish. With the increased number of encounters and because walleye will lunge at any prey they encounter, an increase in predation success is expected.

Capture and ingestion or handling time requires strength, maneuverability and overall co-ordination of jaw and body musculature. Handling efficiency is the ability to subdue and ingest the prey after it is struck. The time spent in lost handling attempts is a measure of the handling inefficiency. Deviations in handling ability can interfere with the capture of prey. Handling time regressions obtained in this study were comparable to published values. Bannon and Ringler (1986) obtained a regression coefficient of $R^2 = 0.6561$ with $P<0.0001$ ($n=674$) on Brown Trout feeding data. Werner (1974) obtained r^2 of 0.774 on bluegills feeding on natural zooplankton prey.

An efficiently foraging fish will gain more energy from its food source than it expended in acquiring that food item. Impairment in swimming behaviour can affect the efficiency of foraging behaviour and decrease the amount of energy available for growth. Although there were deviations in normal locomotory behaviour, this did not impair the efficiency of foraging. The effects of low level acetylcholine stimulation on locomotion increased the search time and the number of encounters and thereby increased the number of captures. This stimulatory effect may only be temporary. The metabolic demand of malathion detoxification, acetylcholine synthesis and release and increased energetic output (overall increase in all physical behaviours and lost predation opportunities) is likely to achieve neither an energetic balance nor provide excess energy for growth.

Hyperactive fish had more entries into the upper half of aquaria and also spent more time in the upper half of the aquaria. Young walleye are subject to predation by fish eating birds and animals but because of they are normally nocturnal and prefer deep water, this type of predation is usually small (Niemuth et al., 1959). The atypical behaviour of increased time in the upper waters displayed by these malathion exposed fish may affect survival by increasing the walleye`s vulnerability to predation. This is consistent with fish that display altered swimming behaviours (impaired schooling) as a result of toxicant exposure. Sublethal cadmium exposure at less than the maximum acceptable toxicant concentration resulted in increased vulnerability of fathead minnows to predation (Sullivan et al., 1978).

4.4 Projection of Laboratory to the field.

Results from the first part of this study suggest that decreases in piscivory occur after exposure to malathion that causes cholinesterase activity to change by 10 %. This value could be appropriately applied to field situations in which fish are the primary food source for the walleye or in situations in which only the walleye are treated.

This predicted decline in piscivory was apparent in a study in which walleye were exposed to malathion in the laboratory and transferred to the field (Delorme, 1988). Walleye exposed to low doses of Malathion, resulting in 35 % acetylcholinesterase inhibition exhibited decreased piscivory. The decrease in piscivory was demonstrated by the lack of fathead minnow prey in stomach samples and the decrease in stomach dry weight content. This cessation of piscivory lasted three days post-exposure and did not return to control levels until 9 days post-exposure.

Malathion exposed, non-piscivorous walleye in the laboratory did not supplement their diet with zooplankton whereas the non-piscivorous field population did have significantly greater numbers of Daphnids in their stomachs (Delorme, 1988). This change from piscivory to planktivory may be due to alterations in the sensory and locomotory components of the feeding behaviour pattern. Decreases in reactive angles would create a smaller visual search area. A feeding walleye would be inclined to eat the first suitable prey within its search field. The most suitable prey in the search field is zooplankton because of its higher density relative to minnow density. Walleyes, in nature, utilize those food items which are most available (Niemuth et al., 1959).

An impairment in piscivory implies a decline in growth. Sandheinrich and Atchison (1990) stated that a statistically significant difference in growth may be seen between controls and toxicant exposed fish that caused a decrease in feeding as low as 10% as compared to controls. The above mentioned field population experienced a 77% decline in food intake, by weight, initially and this decreased intake lasted up to nine days post-exposure. Piscivory was completely inhibited for three days. With this dramatic decline in feeding, decreased growth increments in terms of weight (14.6% lower) and length (6 % lower) was exhibited for up to nine days post-exposure.

4.5 Conclusions

The effect of very low concentrations of malathion can stimulate whole head acetylcholinesterase and stimulate piscivory. Stimulation of all locomotory activity not only results in increased piscivory but also endangers the walleye by making it more vulnerable to predation and may reduce potential growth due to increased energetic costs involved in foraging. At low malathion concentrations, both whole head acetylcholinesterase and piscivory is inhibited. Death of the juvenile walleye (>75 days from planting) occurs when whole head acetylcholinesterase inhibition is greater than 25%.

Very short term exposure to low concentrations of malathion can result in subtle changes in whole head acetylcholinesterase and in piscivory. The affects on feeding could explain the observed decreases in growth following field application of malathion.

5.0 Literature Cited

Antwi L.A.K. 1987. Fish acetylcholinesterase activity after aerial application of temephos in two rivers in Burkina Faso, West Africa. Bull Environ Contam Toxicol. 38(3):461-467.

Bannon E. and Ringler N.H. 1986. Optimal prey size for stream resident brown trout (Salmo trutta): tests of predictive models. Can. J. Zool. 64:704-713.

Beninger R.J., Wirsching B.A., Jhamandas K., Boegman R.J., El-Defrawy S.R. 1985. Effects of altered cholinergic function on working and reference memory in the rat. Can J Physiol Pharmacol 64:376-382

Braekevelt C.R., McIntyre, D.B. and F.J. Ward. 1989. Development of the retinal tapetum lucidum of the walleye (Stizostedion vitreum vitreum). Histol. Histopath. 4:63-70.

Brezenoff H.E., McGee J. and Hymowitz N. 1985. Effect of soman on schedule-controlled behaviour and brain acetylcholinesterase in rats. Life Sciences 37:2421-2430

Brown J.A., Johansen P.H., Colgan P.W. and Mathers R.A. 1985. Changes in the predator-avoidance behaviour of juvenile guppies (Poecilia reticulata) exposued to pentachlorophenol. Can J Zool 63:2001-2005

Bull C.J. and McInerney J.E. 1974. Behavior of juvenile Coho Salmon (Oncorhynchus kisutch) exposed to Sumithion (Fenitrothion), an organophosphate insecticide. J. Fish. Res Board Can. 31:1867-1872.

Cavanagh J.B. 1964. The significance of the dying back process in experimental and human neurological disease. Int Rev Exp Pathol 3:219-267.

Chevalier J.R. 1973. Cannibalism as a factor in first year survival of Walleye in Oneida Lake. Trans Amer Fish Soc 4:739-744.

Clark J.R., Borthwick P.W., Goodman L.R., Patrick J.M. Jr., Lores E.M. and Moore J.C. 1987. Comparison of laboratory toxicity test results with responses of estuarine animals exposed to fenthion in the field. Environmental Toxicology and Chemistry 6:151-160.

Colgan P.W., Brown J.A. and Orsati S.D. 1986. Role of diet and experience in the development of fedding behaviour in largemouth bass, Micropterus salmoides. J. Fish Biol. 28:161-170.

Cook G.H., Moore J.C. and Coppage D.L. 1976. The relationship of malathion and its metabolites to fish poisoning. Bull Environ Contam Toxicol 16:283-290.

Coppage D.L., Matthews E., Cook G.H. and Knight J. 1975. Brain acetylcholinesterase inhibition in fish as a diagnosis of environmental poisoning by malathion, O,O-dimethyl-S-(1,2-dicarbethoxy) phosphorodithioate. Pesticide Biochem Physiol 5:536-542.

Cowart R.P., Boer, F.L. and Epps E.A. Jr. 1971.

Crone H.D. 1971. The dissociation of rat brain membranes bearing acetylcholinesterase by the non-ionic detergent Triton X-100 and an examination of the product. J Neurochem 18:489-497.

Cuff W.R. 1980. Behaviourial aspects of cannibalism in larval walleye Stizostedion vitreum. Can J Zool 58:1504-1507.

Davis R. 1975. Food habits of walleye in a southern Minnesota Lake. Minnesota department of Natural Resources. Division of Fish and Wildlife. Section of Fisheries. Investigational Report No. 333.

Davy F.B., Kleerekoper H. and Matis J.H. 1973. Effects of exposure to sublethal DDT on the exploratory behaviour of Goldfish (Carassius auratus). Water Resour Res 9(4):900-905.

Delorme P.D. 1988. The ecological effects of malathion on juvenile walleye. MSc Thesis. University of Manitoba.

Desi I., Dura G., Gonczi L., Kneffel Z., Strohmayer A. and Szabo Z. 1976 Toxicity of malathion to mammals, aquatic organisms and tissue culture cells. Arch Environ Contam Toxicol 3;410-425

Dill L.M. 1983. Adaptive flexibility in the foraging of fishes. Can J. Fish Aquat Sci 40:398-408.

Duangsawadi M. and Klaverkamp J.F. 1979. Acephate and fenitrothion toxicity in rainbow trout: Effects of temperature stress and investigations on the sites of action. In: Aquatic Toxicology. LL Marking and Ran Kinerli (eds). American Society for testing Materials, Philadephia,PA. pp 35-51.

Eaton J.G. 1970. Chronic malathion toxicity to the bluegill (Lepomis machrochirus Rafinesque). Water Research 4:673-684.

Ellen P., Taylor H.S. and Wages C. 1986. Cholinergic blockade effects on spatial integration versus cue discrimination performance. Behav Neurosci 100(5):720-728

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

Finger S.E., Little E.E., Henry M.G., Fairchild J.F. and Boyle T.P. 1985. Comparison of laboratory and field assessment of fluorene Part 1: Effects

of fluorene on the survival, growth, reproduction and behavior of aquatic organisms in laboratory tests. In T.P. Boyle ED., Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosustems. STP 865. American society for testing and materials, Philadelphia, PA. pp 120-133.

Fingerman S.W. and Russell L.C. 1980. Effects of the polychlorinated biphenyl aroclor 1242 on locomotor activity and on the neurotransmitters dopamine and norepinephrine in the brain of the gulf killifish, Fundulus grandis. Bull Environ Contam Toxicol 25:682-687

Fitzgerald G.G. and J.R. Cooper. 1971. Acetylcholine as a possible sensory mediator in rabbit corneal epithelium. Biochem Pharmacol 20:2741.

Gibson J.R., Ludke J.L. and D.E. Ferguson. 1969. Sources of error in the use of fish brain acetylcholinesterase activity as a monitor of for pollution. Bull Environ Contamination Toxicol 4:17

Glickman S.E. and Morrison B.J. 1969. Some behaviourial and neural correlates of predation susceptibility in mice. Commun Behav Biol 4:261-267

Glow P.B. and Richardson A. 1965. Effect of acute and chronic inhibition of cholinesterase upon body weight, food intake and water intake in the rat. Comp Physiol Psychol 61(2)295-299

Goldstein B.D., Fincher D.R. and Searle J.R. 1987. Electrophysiological changes in the primary sensory neuron following subchronic soman and sarin: Alterations in sensory receptor function. Toxicol Appl Pharmacol 91:55-64.

Goodyear C.P. 1972. A simple technique for detecting effects of toxicants or other stresses on a predator-prey interaction. Trans Am Fish Soc 101:367-370.

Haider S. and Inbaraj R.M. 1986. Relative toxicity of technical material and commercial formulation of malathion and endosulfan to a freshwater fish, Channa punctatus (Bloch). Ecotoxicol Environ Safety 11:347-351.

Haque N., Rizvi S.J. and Khan M.B. 1987 Malathion induced alteration in the lipid profile and the rate of lipid peroxidation in rat brain and spinal cord. Pharmacology and Toxicology 61:12-15

Hatfield C.T. and Anderson J.M. 1972. Effects of two insecticides on the vulnerability of Atlantic Salmon (Salmo salar) parr to Brook Trout (Salvelinus fontinalis) predation. J Fish Res BD Canada 29:27-29

Hatfield C.T. and Johansen P.H. 1972. Effects of four insecticides on the ability of Atlantic Salmon parr (Salmo salar) to learn and retain a simple conditioned response. J Fish Res Bd Canada 29:315-321.

Henry M.G. and Atchison G.J. 1979. Behaviorial changes in Bluegill (Lepomis macrochirus) as indicators of sublethal effects of metals. Env Biol Fish 4(1):37-42.

Henry M.B. and Atchison G.J. 1986. Behaviorial changes in social groups of bluegills exposed to copper. Trans Amer Fish Soc 115:590-595.

Hedtke J.L. and Norris L.A. 1980. Effect of ammonium chloride on predatory consumption rats of Brook Trout (Salvelinus fontinalis) on Juvenile Chinook Salmon (Oncorhynchus tshawytscha) in laboratory streams.

Jimmerson V.R., Shih T-M. and Mailman R.B. 1989 Variability in soman toxicity in the rat: Correlation with biochemical and behaviourial measures. Toxicology 57:241-254

Johnson W.W. and Finley M.T. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory, 1965-78. United States Department of the Interior, Fish and Wildlife Service, Resource Publication 137: 78-115.

Kania H.J. and O`Hara J. 1974. Behaviorial alterations in a simple predator-prey system due to sublethal exposure to mercury. Trans Am Fish Soc 103:134-1136.

Kennedy H.D. and Walsh D.F. 1970. Effects of malathion on two warmwater fishes and aquatic invertebrates in ponds. US Bureau of Sport Fisheries and Wildlife. Technical Paper 55: 13 pg.

Kurtz P.J. 1977. Dissociated behaviourial and cholinesterase decrements following malathion exposure. Toxicol Appl Pharmacol 42:589-594

Leopold I.H. and Comroe J.H. Jr. 1946. Effect of diisfluorophosphate on the normal eye. American Medical Association Archives of Ophthalmology 36:17.

Levin H.S. and Rodnitzky R.L. 1976. Behaviourial effects of organophosphate pesticides in man. Clin Toxicol 9:391-405

Li S. and Ayles G.B. 1981. Preliminary experiments on growth, survival, production and interspecific interactions of Walleye (Stizostedion vitreum vitreum) fingerlings in constructed earthen ponds in the Canadian Prairies. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1041.

Lockhart W.L., Metner D.A., Ward F.J. and Swanson G.M. 1985. Population and cholinesterase responses in fish exposed to malathion sprays. Pesticide Biochem Physiol 23:

Lockhart W.L. and Metner D.A. 1983. Cholinesterase Monitoring following malathion spraying City of Winnipeg, July, 1983. Preliminary Data Report Prepared for Environmental Protection Service.

Lockhart W.L., Metner D.A. and Grift N. 1973. Biochemical and residue studies of rainbow trout (Salmo gairdneri) fololowing field and laboratory exposure to fenitrohion. The Mantioba Entomologiest 7:26-36.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall P.J. 1951. Protein measurement with the Folin Phenol Reagent. J Biol Chem 193:265-275.

Mac M.J. Vulnerability of young lake trout to predation after chronic exposure to PCB's and DDE. Tech Paper US fish and Wildlife Service 105. 1981.

Mathias J.A. and Li S. 1981. Feeding habits of walleye larvae and juvenile: comparative laboratory and field studies. Trans Amer Fish Soc. 111:722-735.

McDonald B.E., Costa L.G. and Murphy S.D. 1988. Spatial memory impairment and central muscarinic receptor loss following prolonged treatment with organophosphates. Tox Lett 40:47-56

Morgan M.J. and Kiceniuk J.W. In Press. The effect of fenitrothion on the foraging behaviour of juvenile Atlantic salmon. Environmental Toxicology and Chemistry.

Morris R.W. 1956. Some aspects of the problem of rearing marine fishes. Bull. Inst. Oceanog. 1082:1-61.

Murphy S.D. Pesticides in:"Casarett and Doull's Toxicology: The Basic Science of Poisons", edited by J. Doull, C Klassen and M. Amdur. New York:MacMillan, 1985, pp.519-581.

National Research Council Canada. 1975. Fenitrothion: The effects of its use on environmental quality and its chemistry. NRCC Publ. No. NRCC 14104. Ottawa.

Niemuth W., Churchill W. and Wirth T. 1959. The Walleye: Life History, Ecology and Management. Wisconsin Conservation Department Publication No. 227.

Nyman H.-G. 1981. Sublethal effects of lead (Pb) on size selective predation by fish Application on the ecosystem level. Int. Ver. Theor. Angew. Limnol. Verh. 21:1126-1130.

O'Brien W.J. 1979. The predator-prey interaction of planktivorous fish and zooplankton. American Scientist 67:572-581.

Parsons J.W. 1971. Selective food proeferences of walleyes of the 1959 year class in Lake Erie. Trans Amer Fish Soc. 3:474-485.

Plestina R. and Piukovi-Plestina M. 1978. Effect of anti-cholinesterase pesticides on the eye and vision. Critical reviews in Toxicology. 6(1):1-24.

Rand G.M. 1977. The effect of exposure to a subacute concentration of parathion on the general locomotor behaviour of the Goldfish. Bull Environ Contam Toxicol 18(2):259-266

Ryder, R.A. 1977. Effects of ambient light variations on behaviour of yearling, subadult and adult walleyes (Stizostedion vitreum vitreum). J. Fish. Res. Bd. Canada 34:1481-1491.

Sandheinrich M.B. and Atchison G.J. 1990. Sublethal toxicant effects on fish foraging behavior: Empirical vs. mechanistic approaches. Environ. Toxicol & Chem. 9:107-119.

SAS/STAT User's Guide. 1988. Release 6.03 Edition. SAS Institute Inc., Cary, NC, USA.

Schneider M.J., Barraclough S.A., Genoway R.G. and Wolford M.L. 1980. Effects of phenol on predation of juvenile rainbow trout, Salmo gairdneri. Environ Pollut 23:121-130.

Smith L.L. Jr and R.L. Pycha. 1960. First-year growth of the walleye, Stizostedion vitreum vitreum (Mitchill), and associated factors in the Red Lakes, Minnesota. Limnology and Oceanography 5:281-290.

Stamper C.R., Balduini W., Murphy S.D. and Costa L.G. 1987. Behaviourial and biochemical effects of postnatal parathion exposure in the rat. Neurotoxicology & Teratology 10:261-266.

Stebbing A.R.D. 1982. Hormesis The stimulation of growth by low levels of inhibitors. The Science of the Total Environment 22:213-234.

Sullivan J.F., Atchison G.J., Kolar D.J. and McIntosh A.W. 1978. Changes in the Predator-prey behaviour of fathead minnows (Pimephales promelas) and largemouth bass (Micropterus salmoides) caused by cadmium. J Fish Res Bd Canada 35:446-451

Symons P.E.K. 1973. Behaviour of young Atlantic salmon (Salmo salar) exposed to or force-fed fenitrothion, an organophosphate insecticide. J Fish Res Board Can 30:651-655

Tagatz M.E. 1976. Effect of mirex on predator-prey interactions in an experimental estuarine ecosystem. Trans Am FIsh Soc 105:546-549.

Tagatz M.E., Borthwick P.W., Cook G.H. and Coppage D.L. 1974. Effects of ground applications of malathion on salt-marsh environments in northwestern Florida. Mosquito News 34(3):309-315.

Thompson T.L. and Thomas W.E. 1985. Organophosphate-mediated inhibition of choline acetyltransferase activity in rat brain tissue. Experentia 41:1437-1438.

Timms A.M., Kleerekoper H. and Matis J. 1972. Locomotor response of goldfish, channel catfish, and largemouth bass to a 'copper-polluted' mass of water in an open field. Water Resource Res 8(6):1574-1580.

Verma S.R., Tyagi A.K., Bhatnagar M.C. and Dalela R.C. 1979. Organophosphate posioning to some fresh water telosts acetylcholinesterase inhibition. Bull Environ Contam Toxicol 21:502-506.

Walker R.E. and Applegate R.L. 1976. Growth, food and possible ecological effects of young-of-the-year walleyes in a South Dakota prairie pothole. Prog. Fish- Cult. 38:217-220.

Ward F.J. and McCulloch B.R. In Press. Relationship between mouth gape of juvenile walleye (Stizostedion vitreum vitreum) and prey size. Verhandlungen Internationale Vereinigun Limnologie.

Weiss C.M. 1958. The determination of cholinesterase in the brain tissue of three species of fresh water fish and its inactivation in vivo. Ecology 39:194.

Weiss C.M. 1959. Response of fish to sub-letahl exposures of organic phophorous insecticides. Sewage and Ind. Wastes 31:580-593

Werner E.E. 1974. The fish size, prey size, handling time relation in several Sunfishes and some implications. J. Fish Res Board Can 31:1531-1536.

Wildish D.J. and Lister N.A. 1973. Biological effects of fenitrothion in the diet of Brook Trout. Bull Environ Contam Toxicol 10(6):333-339

Wolfe N.L., Zepp R.P., Gordon, J.A., Baughman G.L. and Cline D.M. 1977. Kinetics of chemical degradation of Malathion in water. Environmental Science and Technology 11:88-100.

Woltering D.M., Hedtke J.L. and Weber L.J. 1978. Predator-prey interactions of fishes under the influence of ammonia. Trans Amer Fish Soc 107(3):500-504

Wolthuis O.L. and R.A.P. Van wersch. 1984. Fundam Appl Toxicol 4,s195-s208