INVESTIGATING THE SUITABILITY OF NORTHERN MUMMICHOG (*FUNDULUS HETEROCLITUS MACROLEPIDOTUS*) FOR THE ASSESSMENT OF CUMULATIVE AND NON-POINT SOURCE POLLUTION IN PRINCE EDWARD ISLAND ESTUARIES

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ABSTRACT

The overall objective of this study is to evaluate the use of the estuarine species, the northern mummichog (*Fundulus heteroclitus macrolepidotus*) as a monitoring species for the detection of the cumulative impacts of agricultural land use in Prince Edward Island (PEI). The first study investigated the spatial and temporal variability of the somatic indices used to describe fish performance, specifically measures of energy use and storage, as indicated by liver and gonad sizes, and the overall condition of the fish. Three estuaries were sampled once in December 2006 and then on a weekly basis from May through July, with additional sampling periods in August and September 2007 to examine the temporal variability. Spatial variability was assessed by sampling populations at five spots along an estuary at a single time period. Results showed considerable variation both within and between sites over the reproductive season as well as between sites within an estuary. Repeated sampling is required to assess reproductive output in this species and densities of adults and young-of-the-year (YOY) may be the best indicator of environmental stress.

The second study was an effects-based assessment conducted in seven estuaries spanning a range of land-use, agricultural, industrial and/or municipal, and potential nutrient loadings. Over the course of the summer of 2007 somatic measures of fish performance, measures of population density and structure, and biochemical measures of exposure were monitored. The somatic and population measurements focused on the three main processes impacting population integrity: growth, reproduction, and survival; in addition to establishing a potential cause-effect relationship between stressor and response. Mummichog populations appear to be most affected in heavily eutrophic

environments. The most obvious manifestation of this is an increase in recruitment and overall population number. At one site there was a significant increase in fecundity and gonad size; however survival of YOY or eggs appears to be impacted as indicated by lower YOY:adult ratios. Multivariate analysis of environmental variables clearly separates the sites on the basis of both eutrophication indicators such as nitrogen loading, chlorophyll and *Ulva* density in the first principal component and sediment variables in the second principle component. It is thought that the amount of fine sediment at this site may have affected YOY survival. Interestingly, fish found in these higher agriculture areas also experienced significantly lower *in vitro* steroid production. This decline in steroid production does not appear to have a detrimental effect on the reproductive potential of adult mummichog suggesting a possible masking effect of the nutrient enrichment.

Overall, these studies have shown that many of the performance measures typically used in environmental effects monitoring programs, fish condition and reproduction, are not efficient at determining differences among sites. This is likely due to the prolonged reproductive period followed by a short period of winter preparation. However, the abundance of mummichog found at a site combined with lower species richness may be a good indicator of environmental health.

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I leave you with a quote which a friend has reminded me of often as I have struggled through this process.

"Do, or do not. There is no try." - Yoda, Jedi Master

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CHAPTER 1

GENERAL INTRODUCTION

1.1 OVERVIEW

Aquatic ecosystem health has become an increasing concern due to changes in land-use, advances in technology, and an increasing population that has placed considerable strain on aquatic resources (Naiman and Turner 2000). Anthropogenic stressors have altered ecosystem habitats through increasing levels of nutrients, sedimentation and reduced water clarity, and decreased oxygen levels, ultimately leading to changes in aquatic biodiversity (US EPA 2006). In addition, many pollutants are toxic to aquatic biota, affecting reproduction, growth, development, and survival (US EPA 2006, Environment Canada 2006).

There are many questions regarding how best to assess, evaluate, and predict anthropogenic impacts on aquatic ecosystem health. The most common approach used has been derived from classic toxicological sciences and is a bottom-up approach which originates at the biochemical and individual level and works its way up to the community level (Clements 2000). This more mechanistic approach allows contaminant effects to be directly linked to exposures (i.e. cause and effect) and may provide early warnings of population effects (Munkittrick and McCarty 1995). Environmental risk assessment is the predictive discipline that has arisen from the classical bottom-up toxicology approach. However, despite this greater endpoint-response specificity the ecological significance of biochemical responses is still uncertain (Munkittrick and McCarty 1995) because the ability to predict impacts at higher levels of biological organization is at best not validated and at worst not-possible due to emergent properties of more complex systems (Fry 1976; Munkittrick and McCarty 1995). A more ecologically focused approach, termed the top-down approach, looks at the whole ecosystem and then works its way

down to the individual. Although this is a more holistic and integrative approach, and alterations in populations and communities are more definite, it shows no understanding of the mechanisms or causes behind the effects seen (Clements 2000, Munkittrick and McCarty 1995). The field of ecotoxicology emerged from a combination of both of these approaches. By studying responses at several levels of organization simultaneously ecological relevance and mechanistic understanding can both be achieved (Clements 2000).

The observation that fish populations will respond in predictable ways to different stressors was originally proposed by Colby and Nepszy (1981). Later, Colby (1984) classified fish responses to a number of environmental stressor and grouped them into several generalized patterns. This model has served as the basis for a fish monitoring framework for environmental assessment purposes (Gibbons and Munkittrick 1994). Further studies have described new response patterns, many of which are specifically related to contaminant impacts, resulting in a revised monitoring framework (Gibbons and Munkittrick 1994). Based on this framework, Munkittrick and McCarty (1995) proposed an effects-driven mechanistic approach to the study of ecosystem health that will help establish the cause-and-effect relationships between stressors and responses while retaining ecological significance. This approach will enable us to predict the effects of contaminants on populations and communities and thus reduce the environmental impact of specific pollution sources (Gibbons and Munkittrick 1994).

1.2 ENVIRONMENTAL EFFECTS MONITORING – WILD FISH SURVEY

In 1992, amendments were made to the Pulp and Paper Effluent Regulations to include EEM as part of their program to improve the quality of their effluent. Prior to these amendments a number of studies had shown adverse effects to fish populations exposed to pulp and paper mill effluent (McMaster *et al.* 1991; Munkittrick *et al*, 1991). EEM protocols were developed to determine if fishes, their habitat, and the utilization of fisheries resources were being protected by the effluent regulations. The wild fish component of EEM is based upon the effects-based assessment method proposed by Munkittrick and McCarty (1995). EEM is a continuous, site-specific, monitoring program that aims to protect these fisheries resources from detrimental long term impacts through an understanding of the methods by which populations respond to changes in their environment (McMaster *et al.* 2003). Although EEM was designed for point source effluents like pulp and paper, there is hope that it can also be applied to both cumulative or multiple stressor environments and non-point source effluents like agriculture.

Initially, EEM had very low success in estuarine environments. The main problems cited were insufficient numbers of fish caught, and inability to quantify degree of exposure (Courtenay *et al.* 2002). The move towards small-bodied monitoring species, like the mummichog, has improved the success of this program. Smaller bodied fish are presumed to be less mobile, and are generally more common and abundant than their larger counterparts (Courtenay *et al.* 2002). These characteristics should make demonstrating their exposure and responses to contaminants easier.

Over the past 16 years many changes have been made to the EEM program. Specifically, with regards to the fish survey element of the program, there have been

changes in the types of fish used, as well as methods for determining impacts non-lethally, thereby ensuring population continuance.

1.3 FISH POPULATION PERFORMANCE MEASURES

Three major factors which can limit the performance of fish populations are whether there is enough food, whether they use it properly, and whether the population is maintaining an appropriate age structure (Gibbons and Munkittrick 1994). These measures are a reflection of growth, reproduction, and survival that are the essential functions for population continuance.

Fish require energy for metabolic maintenance and survival. Additional energy which is not immediately required can then be stored as lipid or glycogen in tissues, or used for somatic or reproductive growth (Gibbons and Munkittrick 1994). Common measurements of energy storage include condition factor, the ratio of body weight to length, liver size, the ratio of liver weight to body weight, and tissue lipids. Measurements of energy utilization include gonad size, the ratio of gonad weight to body weight, length, weight, size-at-age, fecundity and egg size.

The age structure of a population can be used to measure survival. For example, shifts towards older age classes may suggest recruitment failure, whereas shifts towards younger age classes may suggest selective predation, exploitation of adults or an increase in recruitment due to increased food resources.

1.4 PATTERNS OF RESPONSE

With the idea that the response of a fish species to a particular type of stress is distinct and predictable, some patterns of responses have been categorized (Gibbons and Munkittrick 1994). These generalized response patterns are based on the same three categories of performance measures as discussed above and can be seen in Table 1.1. These patterns can help focus research efforts towards determining the type(s) of stressor(s) which are having the most impact on the fish populations. An example of one such pattern is that of increased food supply, as might be expected in areas of eutrophication. A typical eutrophication response would result in increased energy use and storage. Increased reproduction would result in a larger number of younger fish, thus the age structure of the population would be decreased. These patterns are not definitive, and in some cases only hypothesized for focusing future studies. Given the diversity of fishes, it would not be reasonable to expect similar responses in all cases. Thus patterns of fish population response are still within the realms of ongoing research.

TABLE 1.1. Patterns of population response previously observed in wild fish populations (Gibbons and Munkittrick 1994).

Pattern (age/energy storage/energy expenditure)	Description
-/+/+	Increased food supply
+/0/0	Recruitment failure
+/-/-	Multiple stressors
0/-/-	Food limitation
0/-/0	Niche shift
+/-/+	Metabolic redistribution
+/+/+	Chronic recruitment failure
0/0/0	No response

+ indicates an increase, - a decrease, 0 no change

1.5 CHOICE OF MONITORING SPECIES

The choice of a monitoring species is dependant on several criteria: 1) fish must be present in all sites (both impacted and non-impacted); 2) fish must be found in sufficient numbers to withstand a comprehensive monitoring program; 3) individuals must be relatively resident thereby ensuring exposure to contaminants, and 4) be suitable for the measuring of all the required parameters. Recently there has been a shift towards the use of small-bodied fishes for environmental monitoring. These fishes are usually not commercially or recreationally exploited, have smaller home ranges, and shorter life spans. A shorter life span provides the opportunity for reproductive and growth effects to be expressed sooner and may be more impacted in situations of short term exposure to pollutants.

Estuaries are exceptionally problematic in that many of the fish species are not resident. Estuaries act as pathways and nursery grounds for many migratory species. They are characterized by daily fluctuations in temperature, salinity, and dissolved oxygen. This places considerable physiological demands on fishes that use this environment and influences their overall density, diversity and biomass (Whitfield 1999). In Prince Edward Island, the diversity of true estuarine fish is very low, and of these species, only the mummichog (*Fundulus heteroclitus*) and the fourspine stickleback (*Apeltes quadracus*) were found in sufficient abundance at all of the chosen sampling locations and times examined in this research. Despite meeting the criteria for a monitoring species, the fourspine stickleback was not chosen because of a few characteristics that would make sampling more difficult. These included their relatively small size, and some difficulty with distinguishing adults from young of the year.

Mummichog however, in addition to displaying many of the characteristics of a suitable monitoring species, has been used in the past to monitor the environmental effects of pulp and paper effluent (Leblanc *et al.* 1999) and seafood plant processing effluent (Thériault *et al.* 2007), both point-source contaminants. Their value for monitoring the impacts of cumulative or non-point source contaminants, like agriculture, has yet to be determined.

The mummichog is found along the east coast of North America from the Gulf of St. Lawrence region to Texas (Scott and Scott 1988). They are widely distributed throughout Prince Edward Island's estuaries and are among the most abundant fishes. They have a relatively short life-span (~ 4 years; Fritz and Garside 1975; Kneib and Stiven 1978), high site fidelity (Skinner *et al.* 2005), and are presumed to have a smaller home range than other larger bodied fishes (Griffin and Valiela 2001; Thériault *et al.* 2007). They are sexually dimorphic multiple spawners, and are thought to follow a lunar or semilunar spawning cycle (Taylor *et al.* 1979). Additionally, due to their small size they are not commercially or recreationally exploited.

1.6 BIOCHEMICAL MEASURES OF EXPOSURE

The first interaction between a chemical and a biological system will elicit a biological response (Munkittrick and McCarty 1995; McCarty and Munkittrick 1996). Measurement of this response within an organism allows us to confirm exposure to a specific contaminant or type of contaminant. There are several biochemical measures which have been used extensively within the toxicological field. These measures are known to be sensitive to varying levels of contaminants and are considered to be quite reliable indicators of exposure.

1.6.1 Liver 7-Ethoxyresorufin-*O*-deethylase (EROD) Activity

Cytochrome P450 monooxygenases are a protein family which is involved in the biotransformation of organic chemicals, often leading to the activation of toxic metabolites (Stegeman *et al.* 1992). The reactions catalyzed by these enzymes are referred to as MFO (mixed-function oxidase) reactions. The measurement of EROD (7-ethoxyresorufin-*O*-deethylase) activity is a well-established endpoint for detection of exposure to specific classes of planar compounds, including PAHs, chlorinated dioxins and furans, PCBs, and some natural compounds such as flavones (Whyte *et al.* 2000). EROD induction has been observed in fishes exposed to the insecticide Endosulfan (Coimbra *et al.* 2007), the nematicide Carbofuran (Ghosh *et al.* 2000) and imidazole derivatives commonly found in fungicides (Navas *et al.* 2003).

The CYP1A (cytochrome P450 1A) subfamily is thought to be the main inducible enzyme in fishes, and is most concentrated in the liver (Whyte *et al.* 2000). Measurements of catalytic enzymatic reactions are often the easiest method for detection of CYP1A induction (Whyte *et al.* 2000). EROD induction is a highly sensitive indicator of contaminant exposure in fish, and more recently some connection between EROD induction and detrimental effects in fish has been made (Whyte *et al.* 2000). Thus, there is potential for EROD induction to be used in monitoring programs to identify exposure to particular classes of compounds.

1.6.2 Brain Acetylcholinesterase

Organophosphorus and carbamate pesticides are the most common form of insecticides used in agricultural production. Although they are less persistent than many of the other chemical pesticides (Fulton and Key 2001) they are still among the most

hazardous to nontarget organisms (Büyüksönmez *et al.* 1999). The organophosphate insecticide azinphos-methyl is thought to be the cause of several agricultural related fish kills which occurred in PEI (Mutch *et al.* 2002) and as a result is more restricted in its use.

The mode of action of these pesticides is the inhibition of the acetylcholinesterase enzyme (AChE). The acetylcholinesterase enzyme hydrolyzes acetylcholine thereby terminating nervous stimulation. When this enzyme is inhibited muscle tissue will become overactivated as acetylcholine accumulates at the nerve synapse. Prolonged hyperactivity results in paralysis eventually leading to asphyxia and consequently death (Fulton and Key 2001). The degree of inhibition can thus be used as an indicator and measure of exposure to these classes of pesticides.

1.6.3 Steroid Hormones

Several steroid hormones play an important role in fish reproduction. The sex steroids, primarily 11-ketotestosterone, testosterone, and 17β-estradiol, are responsible for many aspects of gonadal development, including sex determination and differentiation, spermatogenesis/oogenesis, and the development of secondary sex characteristics (Greeley 2002). Some contaminants, commonly referred to as endocrine disrupting compounds (EDCs), can disrupt these sensitive steroid hormone pathways thereby affecting fish reproduction (Arcand-Hoy and Benson 1998). Plasma concentrations of sex steroids have been directly linked with specific stages of the reproductive process. Quantification of these steroids can then be used as an indicator of reproductive impairment (Donaldson 1990).

11-Ketotestosterone is considered to be the main androgen in male teleosts (Kime 1995). It is responsible for the stimulation of spermatogenesis and the induction and maintenance of secondary sex characteristics and reproductive behavior (Greeley 2002).

17β-estradiol is the main estrogen in female teleosts (Greeley 2002). It plays a critical role in oocyte development (Ankley 2004) and is responsible for the induction of vitellogenin production during gonadal growth (McMaster 1995, Ankley 2004). It is also involved in the stimulation of female secondary sex characteristics (Ankley 2004). Plasma 17β-estradiol concentrations are high during the process of gonadal development (McMaster 1995). The completion of steroidogenesis is marked by the lowering of plasma sex steroids, including 17β-estradiol (McMaster 1995).

Testosterone is an androgen which is found in both male and female teleosts, and is the precursor for 17β-estradiol. In females, testosterone increases towards the end of vitellogenesis (Kime 1995) as it is involved in egg yolk production and processes of oocyte maturation (Kime 1995). In males, testosterone is involved in some aspects of spermatogenesis, including spermatocyte formation and spermatogonial multiplication (Greeley 2002). Its presence in both males and females has implicated it in the process of sex differentiation. Additionally, it is likely to be the most important hormone in the negative feedback loop to the brain and pituitary thereby controlling its own production (Bolander 1989).

1.7 LAND USE IN PRINCE EDWARD ISLAND

Prince Edward Island (PEI) has a total land area of 566,560 hectares, of which approximately 261,400 hectares has been cleared for farm use (PEI DAFAF 2008). In

2007, potato production alone used 39,052 hectares (PEI DAFAF 2008), and cattle numbered around 85,000 (Statistics Canada 2006). Intensive agriculture practices have been shown to affect aquatic ecosystems, with erosion, nutrient loading, chemical runoff, and groundwater contamination among the primary stressors.

PEI soils are acidic, have a low organic content, and are comprised mostly of fine sand and silt (Topp *et al.* 1995). This poor soil structure forms very weak aggregates making it highly susceptible to erosion, the process of moving soil from one area to another, which is a major problem associated with agricultural practices. The removal of topsoil decreases the soil's ability to absorb and retain water, especially when exposed to the elements, primarily wind and water. With a substantial part of its land in cultivation, PEI is considered to be at high risk of water erosion (Acton 1995), and the strong ocean winds easily displace the loosely aggregated soils (Topp *et al.* 1995), together resulting in an annual soil erosion rate of approximately 25 tonnes/ha (Smith *et al.* 2002).

Erosion is not only the main source of sedimentation in nearby waterways, but is also a source of excess nutrients and chemicals. With the loss of organic material, and nutrients due to erosion, there is need for additional fertilizers, and thus nutrients, to be used, which in turn can be lost in runoff. Fertilizers can increase crop yield and thus are applied liberally. Phosphate normally binds quite strongly to the soil thus losses are largely due to erosion (Oldham 2008) whereas nitrate does not bind at all and thus is normally lost by leaching into the groundwater (Hatch *et al.* 2002).

Sediment in water often remains suspended thus creating turbid water conditions. Turbid waters degrade fish spawning grounds, stream productivity, and impede respiration and feeding in some animals. An accumulation of sediments in the water can

also alter the streams flow capacity and increase the risk of flooding. PEI has some of the highest amounts of sediment entering surface water from farmland of any other province in Canada (Chambers *et al.* 2000).

Groundwater contamination is another problem associated with agricultural practices. Agrochemicals, fertilizers and pesticides, can leach into the groundwater. This process is increasing in areas of intensive agriculture as the soils are not able to recover in between crops and are becoming more permeable and encourage leaching. The process of livestock production can also generate high levels of soil nutrients due to a heavy application of manure to the land, which can also leach into the groundwater or be lost in runoff (Harker *et al.* 1995). Groundwater contamination results in a major contribution of nitrate to streams and estuaries.

Potato farms are heavily dependant on pesticides, with up to fifteen applications per growing season (Mutch *et al.* 2002). Subsequently at least 22 fish kills between 1994 and 2001 have been either proven or suspected to be related to pesticide run-off, with 16 of these being positively attributed to specific pesticides (Gormley *et al.* 2002). More recently fish kills in the Dunk and Tryon Rivers were detected in 2007. Lethal concentrations of contaminants are rare and usually only occur after a heavy rainfall event. However, sublethal concentrations may occur throughout the year raising concern about their effects on fish reproduction.

Other land uses in PEI include municipal wastewater treatment facilities and industrial food production plants. Both of these produce wastewater that contributes significant loads of nutrients to the aquatic ecosystem. Fish collected downstream of municipal effluents have shown significant nutrient enrichment responses in addition to

some signs of altered endocrine function (McMaster *et al.* 2005). Jarvie *et al.* (2006) found that point sources of phosphorus from municipal effluents were a greater risk for the induction of a eutrophication response in rivers than non-point source agricultural inputs of phosphorus. Food production, specifically potato processing, produces high organic and nutrient rich wastewaters (Smith *et al.* 1975) which may also potentially cause enrichment effects.

1.8 ESTUARIES AND NUTRIENT ENRICHMENT

Estuaries are highly complex ecosystems which are in continual fluctuation. Daily tidal cycles cause changes in water quality parameters and circulation or flushing of pollutants. Estuaries provide important aquatic habitats, acting as nursery grounds for a number of ecologically and commercially important fish, shellfish and invertebrate species, and, until recently, have received little environmental management. In the past, estuaries have served as receiving areas for municipal wastes and have therefore undergone extreme eutrophication (US EPA 2008). Eutrophication results from an increase in the rate of supply of both organic matter and nutrients to an ecosystem (Nixon 1995). Increasing human populations and changes in land cover and use have produced an overabundance of nutrients in aquatic environments, which is especially apparent in areas of intensive agriculture.

In a normal estuarine environment, the supply of key nutrients such as nitrogen and phosphorus are limiting which maintains the balance of species populations within the estuary. Seawater has a naturally low supply of nitrogen. Additionally, in higher salinity environments plants require more nitrogen; thus when salinity increases nitrogen

becomes more limiting (Crain 2007). Eelgrass (*Zostera marina*) is highly effective at removing nitrogen from the sediment, whereas faster-growing algae (phytoplankton, epiphytes, and ephemeral macroalgae like sea lettuce (*Ulva lactuca*)) are better adapted to higher water nutrient concentrations. Eelgrass beds are important habitats for fish and invertebrates because they provide cover, increase habitat complexity, and encourage the settlement of organisms by reducing currents (Vandermeulen 2005). Slight increases in nutrient concentrations will encourage plant growth, both shoots and leaves, as well as increased development of reproductive tissue. Nutrient over-enrichment however, encourages the growth of the faster-growing, opportunistic algae, resulting in large and often harmful blooms (Valiela *et al.* 1997; Hauxwell *et al.* 2003). This elevated growth can reduce or eliminate the growth of submerged aquatic vegetation, like eelgrass, by limiting light penetration, increasing turbidity and increasing epiphytic loads (Duarte 1995; US EPA 2001). Continued nutrient enrichment will result in a changeover from macroalgae to phytoplankton as the dominant producer (Duarte 1995; Fig. 1.1). The relationship between phytoplankton dominance and macroalgal die-off is not clear, however it has been hypothesized that increased phytoplankton growth reduces the light supply to the macroalgae below which reduces their photosynthetic ability (Valiela *et al.* 1997).

A major effect of macroalgal decay is the deposition of large amounts of organic material to the sediment. Changes in sediment chemistry which have already occurred as a result of the eelgrass die-back, low dissolved oxygen, and the production of large amounts of hydrogen sulphide, now have the added influence of elevated carbon content. Together this generates anoxic conditions in the benthic communities of the estuary

which then negatively affect resident fish populations. The destruction of marine habitat, loss of biodiversity, and the smell of decay caused by the proliferation of *Ulva lactuca* has become a common occurrence both in the estuaries of Prince Edward Island and around the globe.

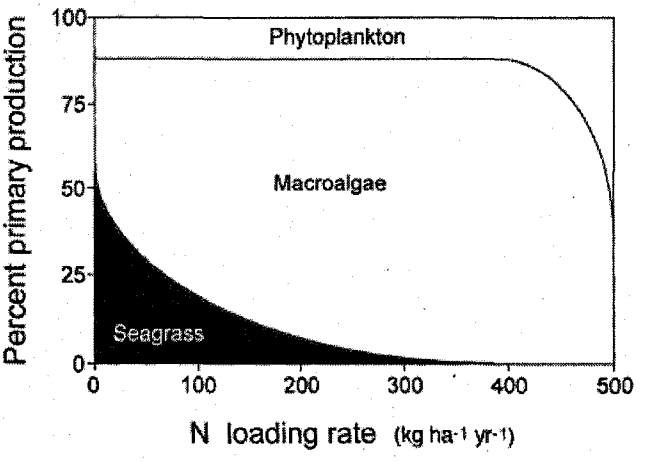

FIGURE 1.1. An example of how increasing nitrate levels in an estuary can alter the proportion of total net production carried out by each type of photosynthetic organism. As the system becomes more nitrate rich the dominant producer switches from seagrass to macroalgae and eventually to phytoplankton. (Figure taken from Valiela *et al.* 1997)

1.9 OBJECTIVES AND HYPOTHESIS OF THESIS

The purpose of this study is foremost to develop and use tools to diagnose the quality of PEI watersheds. By establishing methods for measuring and understanding the responses of wild fish populations to environmental stress, we can then alter our activities to minimize their impacts and measure the progress of those improvements. I hypothesize that the northern mummichog (*Fundulus heteroclitus macrolepidotus*) will respond to pollution related to land use activities in Prince Edward Island estuaries and that these

responses can be measured using population, physiological, and biochemical endpoints. Specifically, I hypothesize that mummichog populations will not differ either spatially or temporally within an estuary. Fish in high agriculturally impacted sites will exhibit either a eutrophication pattern of response (increased reproduction, increased energy storage, increased growth and a decreased age structure), or a metabolic disruption pattern of response (decreased reproduction, increased energy storage and increased age structure). The intensity of this response would correlate with the degree of eutrophication (amount of surrounding land in agricultural production) at a site. Additionally fish at these sites would exhibit a biochemical response (EROD induction, AChE inhibition, changes in *in vitro* steroid production) as a result of pesticide or contaminant exposure.

The main objectives of this thesis are:

- to evaluate the suitability of the mummichog (*Fundulus heteroclitus*) as a monitoring species in PEI estuaries.
    - to gather information on the basic life histories of mummichog in PEI estuaries, by examining the spatial and temporal variability in performance parameters related to reproduction, energy storage, and condition factor within estuaries.
- to assess the effects of multiple stressors as a result of land use activities on PEI estuaries using the mummichog (*Fundulus heteroclitus*) as a monitoring species.
    - to determine efficacy of population, physiological and biochemical measures at detecting effects and prioritizing the severity of both point and non-point sources of contaminants

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CHAPTER 2

ASSESSMENT OF NORTHERN MUMMICHOG (*FUNDULUS HETEROCLITUS MACROLEPIDOTUS*) AS A MONITORING SPECIES FOR NUTRIENT OVER-ENRICHMENT OF PRINCE EDWARD ISLAND ESTUARIES.

2.1 ABSTRACT

Prince Edward Island estuaries are showing dramatic impacts of land-use, particularly due to potato cultivation and the use of chemical fertilizers. In the most severe cases fish kills have resulted from summer anoxic events. Such events have been occurring both more frequently and earlier in recent years. The estuarine resident killifish northern mummichog (*Fundulus heteroclitus macrolepidotus*) has been successfully used as a sentinel for effects of pulp and paper mill effluents in Atlantic Canada and has been proposed as a suitable monitoring species for other anthropogenic impacts. In this study we repeatedly sampled mummichog from estuaries of high (Wilmot), medium (Stanley) and low (North Lake) agricultural activity and measured indices of energy storage and use. Fish at all three sites depleted their energy reserves over winter, as reflected in depressed condition, liver size and gonad size, but then quickly replenished them in May. Condition was consistently lowest at the most agriculturally impacted site. The spawning period was similar at all three sites starting in late May for males and lasting 8 weeks and starting in June for females and lasting 6 weeks. Spawning appeared continuous at all sites without indication of lunar or other periodicity. Reproductive output, inferred from the number of mature eggs in ovaries, was highest at the most agriculturally impacted site, largely due to a peak in mid-June. Despite having the lowest GSI of the three sites, the least agriculturally impacted site showed the highest densities of both adult and young-of-the-year mummichogs. An additional study was conducted in July at five sites along the Stanley estuary to determine spatial variability. Results showed little difference among sites in somatic indices however mummichog density was greatest at the head of the estuary. I conclude that repeated sampling is required to assess reproductive output in

this species and densities of adults and YOY deserve further investigation as a potentially less logistically demanding indicator of habitat quality.

2.2 INTRODUCTION

A federally legislated aquatic environmental effects monitoring (EEM) program was established in Canada in 1992. Initially developed to assess the effectiveness of the pulp and paper effluent regulations in protecting fish, fish habitat, and use of fisheries resources (Munkittrick *et al.* 2002), it is now being used for metal mines (Ribey *et al.* 2002), and has been recommended for use at sewage treatment plants (Kilgour *et al.* 2005). The fish survey portion of EEM, which evaluates changes in indicators of fish growth, reproduction, condition and survival, was developed for use in freshwater environments with point source effluents (Munkittrick *et al.* 2002).

The use of the wild fish component of the EEM program has not been as successful in the more complex marine and coastal environments (Courtenay *et al.* 2002). Many estuarine fishes are highly mobile with both seasonal and/or reproductive migrations making them difficult to monitor and less exposed to point source impacts. Such is the case with the rock gunnel (*Pholis gunnelus*) which was deemed unsuitable as a monitoring species because of its movement offshore prior to gonadal development (Vallis *et al.* 2006). In addition, species diversity in estuarine environments is often quite low and basic biological information sparse, which further complicates the selection of a suitable monitoring species. Mummichog (*Fundulus heteroclitus*), a resident and Atlantic Canada native estuarine killifish, has been frequently proposed (Courtenay *et al.* 1998)

and used (Courtenay *et al.* 2002) as a monitoring species. Mummichog fulfill the most desired characteristics of a monitoring species as they are ubiquitous, have been shown to exhibit high site fidelity (Skinner *et al.* 2002) and respond to point source effluents (Leblanc *et al.* 1997; Thériault *et al.* 2007). Mummichog are asynchronous multiple spawners and are thought to follow a lunar or semilunar spawning cycle (Taylor *et al.* 1979). However, this lunar/semilunar periodicity may be less pronounced in the more northern areas of the mummichog's range (Wallace and Selman 1981). In recent studies it has been acknowledged that there are two distinct subspecies of mummichog. The southern morph (*Fundulus heteroclitus heteroclitus*) can be found in waters from New Jersey to Florida, and the northern morph (*Fundulus heteroclitus macrolepidotus*) are found from Connecticut to Newfoundland, with some mixing in between in the Chesapeake and Delaware Bays (Able and Felley 1986). The majority of studies on mummichog reproductive behaviour has been conducted using the southern mummichog or in the more southern areas. Very few studies have looked at the northern mummichog in the upper reaches of its range and those that have had different results both from the southern mummichog and from each other (Fritz and Garside 1975; Penczak 1985; Leblanc and Couillard 1995; Leblanc *et al.* 1997).

Multiple spawners provide a unique problem for EEM programs. Much of the development of the EEM wild fish endpoints was performed with synchronously spawning fishes that undergo gonadal recrudescence in fall and winter and thus they can generally be sampled with full gonadal development prior to spawning. Accordingly, the existing EEM guidelines for sampling multiple spawners suggest sampling should be done in the spring prior to their first spawn even though with many estuarine fishes the

most substantial gonad growth has not yet taken place at this time. Studies have shown that sampling estuarine species of this nature just once does not provide sufficient information concerning reproductive condition for conclusive results. For example, Leblanc *et al.* (1997) showed a delay in reproductive onset in mummichog exposed to pulp mill effluent, but that once started this population surpassed the reproductive output of the reference site populations thereby suggesting that a single sampling is insufficient for detecting differences.

Prince Edward Island (PEI), Canada provides a challenging environment to validate wild fish population endpoints as most watersheds are dominated by estuaries and most of the environmental stressors are of a non-point nature. The Island is intensively farmed with almost 44% of its total land in agricultural production, a large percentage of which is in potato production (PEI DAFAF 2003). These practices have resulted in severely altered ecosystem habitats and have been the cause of a number of freshwater fish kills over the years (Gormley *et al.* 2005). In addition, elevated nutrient loading to the estuaries has resulted in large macroalgal blooms, specifically sea lettuce (*Ulva lactuca*), which often result in decreases in the native seagrasses (primarily *Zostera marina*) and precipitate anoxic events when the annual algae decays (C. Crane, PEI DOE, pers. comm. 2007). The intensity and locations of these events vary depending on tidal flushing, size of the estuary and magnitude of agricultural nutrient inputs.

The objectives of this study are: 1) to examine the spatial and temporal variability in mummichog populations in estuaries with varying levels of agricultural land use; 2) to determine the onset of spawning, relation to lunar cycle and the number of times a population must be sampled in order to quantify reproductive output; and 3) to gather

information on the basic biology of mummichog in order to better propose how they may be used as an environmental monitoring species.

2.3 METHODS

Study Sites

Three estuaries from watersheds differentially impacted by agriculture were selected for sampling in December 2006 (Fig. 2.1 and Table 2.1). The Wilmot River Estuary, located on the south shore, is in the heaviest potato farming region of PEI and in 2002 the river experienced two large fish kills as a result of pesticide runoff (Gormley *et al.* 2005). North Lake Creek Estuary and Trout River (Stanley Bridge) Estuary are both located on the north shore of PEI, and are thus less tidally influenced (InfoPEI 2008). The mean tidal amplitude for estuaries on the north shore is 0.7 m whereas south shore estuaries have a mean tidal amplitude of 1.8 m (Cairns 2002). North Lake is almost entirely forested and has very little freshwater input. "Stanley", also located in an intensively farmed region, supports a number of commercial mussel (*Mytilus sp.*) farms, and its upper reaches experience yearly anoxic events (C. Crane, PEI DOE, pers. comm. 2007).

Within the Trout River Estuary, five sites were selected to examine the spatial differences in mummichog populations (Fig. 2.1 and Table 2.1.2). The first site, "Gunn's Bridge", was located in the upper reaches of the estuary. Water was densely packed with both healthy and rotting sea lettuce. "Trout", 1.2 km downstream from Gunn's Bridge,

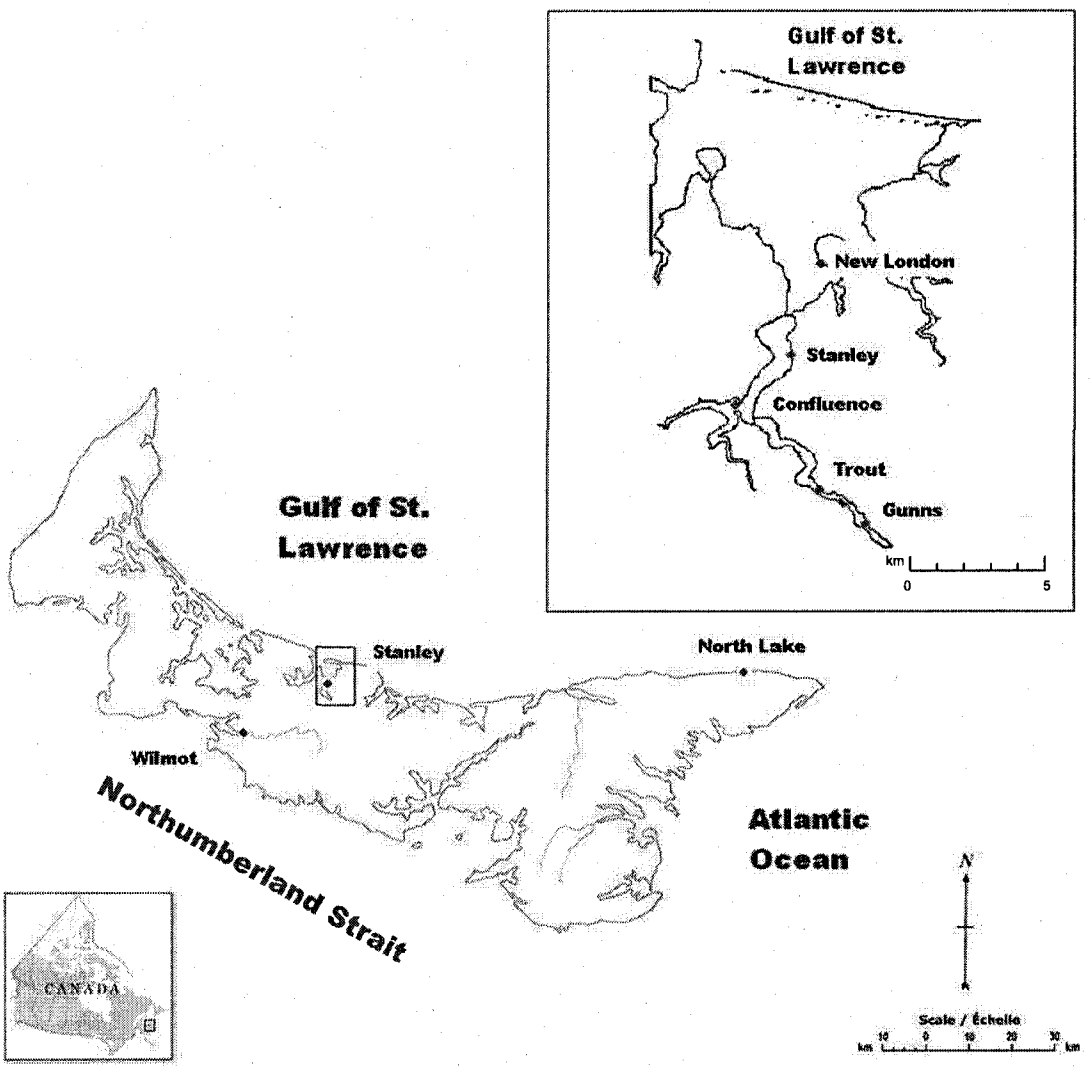

FIGURE 2.1. Map of the three sites used for the temporal study and the five sampling locations used for the spatial study on the Trout River Estuary in Prince Edward Island.

TABLE 2.1.1. Characteristics of sampling sites including location (latitude/longitude), watershed area and percent agricultural land use, maximum tidal amplitude and nitrate loading from freshwater to the estuary.

Site	Lat/long	Watershed		Estuary		Nitrate
		Land Use (% Ag)	Area (km^2)	Area (km^2)	Max. Tidal Amplitude (cm)	Load (kg/ha/y)
North Lake	N46°27.556' W062°05.752'	14.8	47.7	1.1	137	53
Stanley	N46°27.182' W063°27.544'	39.4	53.3	4.1	109	247
Wilmot	N46°23.637' W063°44.560'	74.9	83.4	3.5	216	646

TABLE 2.1.2. Characteristics of sampling sites including location (latitude/longitude), and plant coverage.

Site	Lat/long	Plant Coverage	
		Ulva (%)	Zostera (%)
Gunn's Bridge	N46°25.161' W063°26.296'	100	0
Trout	N46°25.589' W063°26.835'	95	0
Confluence	N46°26.624' W063°28.302'	0	0
Stanley	N46°27.182' W063°27.544'	0.5	23
New London	N46°28.023' W063°27.152'	0	18

was almost as densely packed with sea lettuce and had black anoxic sediment. "Confluence" was located where the three branches of the estuary met. This was a more sandy area of the estuary with very little sea lettuce, and some eelgrass. "Stanley" was the same site used in the temporal part of this study. It was an eelgrass-dominated environment near a freshwater spring and adjacent to a mussel farm. "New London" was located just outside a bridge and in the New London Bay which is partially separated by sand dunes from the Gulf of St. Lawrence. Vegetation in this area was sparse with only a little eelgrass found 12 m from shore.

Environmental Variables

Water temperature, dissolved oxygen, salinity, conductivity, and pH were measured once (YSI 650 handheld meter equipped with a YSI model 600 QS sonde) at all sites during each sampling period indicated below. Measurements were taken at mid-depth from the center of each seine haul area. Aquatic plant habitat was quantified by visual estimation and the percent cover of the major aquatic plants and algae (eelgrass, sea lettuce, green filamentous algae, etc.) was recorded. This estimation was done by running a 15 m transect perpendicular to shore just outside of the seined area. Plant coverage of a 0.5 m^2 area was recorded at 1 m intervals for the length of the transect and averaged to give a single measurement for each site. Assessments were conducted once in May and again in August.

Water samples for nutrient loading were collected in plastic bottles quarterly from 2006 to 2008. At the time of sampling, stream discharge was measured using a Marsh-McBirney model 2000 portable water flow meter. Water samples were filtered using 4

mm, 0.45 μm syringe filters. Nitrate-N and phosphate-P were estimated by suppressed anion chromatography using a Varian model 240 HPLC pump, Varian model 410 autosampler, and an Alltech model 650 conductivity detector. The column was a 150 mm long, 5.5mm ID Transgenomic AN300 anion column. The eluent was 1.7 mM $NaHCO_3$/1.8 mM Na_2CO_3 pumped at 1 mL/min. Estuary area was calculated by contracting a polygon around the estuary using MapInfo Professional version 6.5 and the 2000 Corporate Resource Inventory GIS layer obtained from the PEI Department of Agriculture and Forestry. Nutrient concentrations, stream discharge and estuary area were used to calculate a loading rate in kg/ha/yr.

Sampling Methods

All fish were collected using a 30 m long, 1.5 m deep, 3 mm mesh size beach seine at low tide and sweeps were conducted until a minimum of twenty-five male and twenty-five female adult mummichog (total length > 35 mm; Fritz and Garside 1975; Kneib and Stiven 1978) were captured. Whenever possible, all sites were sampled in the same day and fish were transported back to the laboratory in aerated 20 L plastic pails for immediate processing. Sampling for the examination of temporal patterns was done once in December 2006, repeated weekly from May through July 2007, and then once in both August and September 2007. Fish were killed by spinal severance and total length (1 mm) and total body weight (0.01 g) were recorded. Fish were dissected and liver and gonads weighed (0.001 g). Ovaries were preserved in 10% neutral buffered formalin until further processing. In the August sampling, each site was sampled at four locations thought to be similar and within close proximity to the original sampling sites. Seining

effort was standardized by walking out 15 m, then walking 15 m parallel to shore before returning with the net to shore. All mummichog caught in each seine haul (approximately 225 m^2) were separated into two age classes: young-of-the-year (YOY) and adult, and counted. YOY were determined by size (< 35mm). Sampling to examine spatial variation within an estuary was conducted at five sites along the Trout River Estuary from July 17-19, 2007 using the same methods as previously described except that only adult mummichog (>35 mm) were counted.

Fecundity analysis

Ovaries of twelve randomly selected mummichog per site per sampling period (May through July only) were dissected and all mature eggs were counted. Mature eggs were those in which germinal vesicle breakdown had occurred as indicated by yolk droplets congregated at one pole, and were translucent with yellowish coloration (Kneib 1986, Leblanc and Couillard 1997, Shimizu 1997). From eight of the twelve females sampled, the egg diameter of eight mature eggs were measured with a computer-based image analysis system (PixeLINK PL-A662, PixeLINK Capture SE) attached to a dissecting microscope (Zeiss Stemi 2000-CS).

Data Analysis

Statistical analyses were conducted independently for each sex. Length, weight, and total YOY and adult density were analyzed using analysis of variance (ANOVA). Data were $\log_{10}$ transformed to meet assumptions of normality and homogeneity of variance, and were verified using normal probability plots and the Bartlett's test

respectively prior to analysis. Condition factor, liver size, gonad size and fecundity data were analyzed using analysis of covariance (ANCOVA) on base-10 logarithmically transformed variables, with body size (length or weight) as the covariate. Only sexually mature mummichog were used in the calculation of these results. The assumption of homogeneity of slope was tested by examining interaction between the covariate and the site/station variable. Data are presented as indices for ease of comparison. Indices were calculated from the least square mean generated by the ANCOVA divided by the value of the covariate at which the least square mean was calculated. In this manner, the indices presented are a proportional representation of the least square means tested in the ANCOVA. Carcass weight (total body weight minus liver and gonad weights) was used for all analyses. Condition factor was calculated as corrected least square mean body weight/length^3 x 100, gonado-somatic index (GSI) as least square mean gonad weight/corrected body weight x 100, liver-somatic index (LSI) as least square mean liver weight/corrected body weight x 100. Fecundity was calculated as least square mean number of eggs per gram of corrected wet body weight. Reproductive output was determined by calculating the area under the graph (with 95% confidence intervals) of the average fecundity ($\pm$ SE) of the twelve females as a function of time for the entire reproductive period. All statistical analyses were conducted with SYSTAT 8 software. The critical level of statistical differences for all analyses was assessed at alpha=0.05.

2.4 RESULTS

Environmental Variables

Water temperatures varied among sites with Wilmot averaging higher temperatures in all months but June (Table 2.2; see Appendix A.1 for exact measurements of all variables). Mean salinity ranged from 20-27 ppt at North Lake, 19-25 ppt at Stanley, and 17-24 ppt at Wilmot (Table 2.2). Dissolved oxygen levels were similar at all sites, and dropped significantly in the August sampling, from 12-13 to 0.6-4.6 mg/L (Table 2.2).

Plant and algal coverage (Table 3) differed among the sites. In May, the dominant vegetation at Stanley was eelgrass, while at North Lake and Wilmot the dominant vegetation was sea lettuce. As the summer progressed the sea lettuce became densely packed throughout the water column at both North Lake and Wilmot, however at Stanley the sea lettuce was largely surficial and less decomposed.

Temporal Changes in Somatic Indices

Mummichog collected in May had a reduction in condition from those sampled the previous December (1.2 to 0.9; Fig. 2.2). By the first sampling in May gonad sizes had already doubled in size from that of December. Liver size decreased at the North Lake site but not at Stanley. No fish were collected at Wilmot during the May 2nd sampling period, however at the May 9th sampling Wilmot males had smaller livers than those of the previous December.

Females doubled their liver size and more than tripled their gonad size within one month (May to June, Fig. 2.2). These organ weights remained high until the middle of

TABLE 2.2. Mean monthly (n, range) water quality parameters and plant an algal coverage from May through August at the three sampling sites. Plant coverages are an average of percentages observed at 1 m intervals over a 15 m transect.

Parameter	Month	Site		
		North Lake	**Stanley**	**Wilmot**
Temperature (°C)	May	9.1 (2, 7.3 - 10.9)	11.5 (2, 10.9 – 12.1)	13.8 (2, 12.1 – 15.4)
	June	14.1 (5, 11.6 – 17.3)	17.3 (5, 13.7 – 21.7)	16.6 (5, 12.1 – 21.0)
	July	20.9 (4, 17.3 – 22.7)	22.0 (4, 18.3 – 26.0)	22.5 (4, 17.3 – 23.8)
	August	18.4 (1)	17.4 (1)	19.4 (1)
Salinity (ppt)	May	20.8 (2, 18.1 – 23.4)	19.3 (2, 18.1 – 20.4)	24.0 (2, 23.2 – 24.8)
	June	24.9 (5, 20.5 – 29.4)	19.7 (5, 17.7 – 23.7)	17.4 (5, 13.9 – 22.8)
	July	27.0 (4, 23.7 – 28.4)	24.9 (4, 23.9 – 25.9)	16.6 (13.1 – 19.3)
	August	26.7 (1)	24.4 (1)	21.7 (1)
Dissolved Oxygen (mg/L)	May	11.5 (2, 10.4 – 12.7)	12.0 (2, 11.1 – 12.9)	10.0 (2, 8.0 – 12.0)
	June	9.7 (4, 2.9 – 12.8)	12.0 (4, 4.5 – 19.9)	10.9 (4, 4.6 – 16.5)
	July	12.5 (4, 7.4 – 17.7)	12.6 (4, 10.6 – 14.6)	13.1 (4, 8.6 – 17.2)
	August	0.6 (1)	4.6 (1)	4.0 (1)
Vegetation coverage	May	50% Ulva	40% Zostera, 0.1% Ulva	50% Ulva
	August	76% Ulva	23% Zostera, 0.5% Ulva	93% Ulva

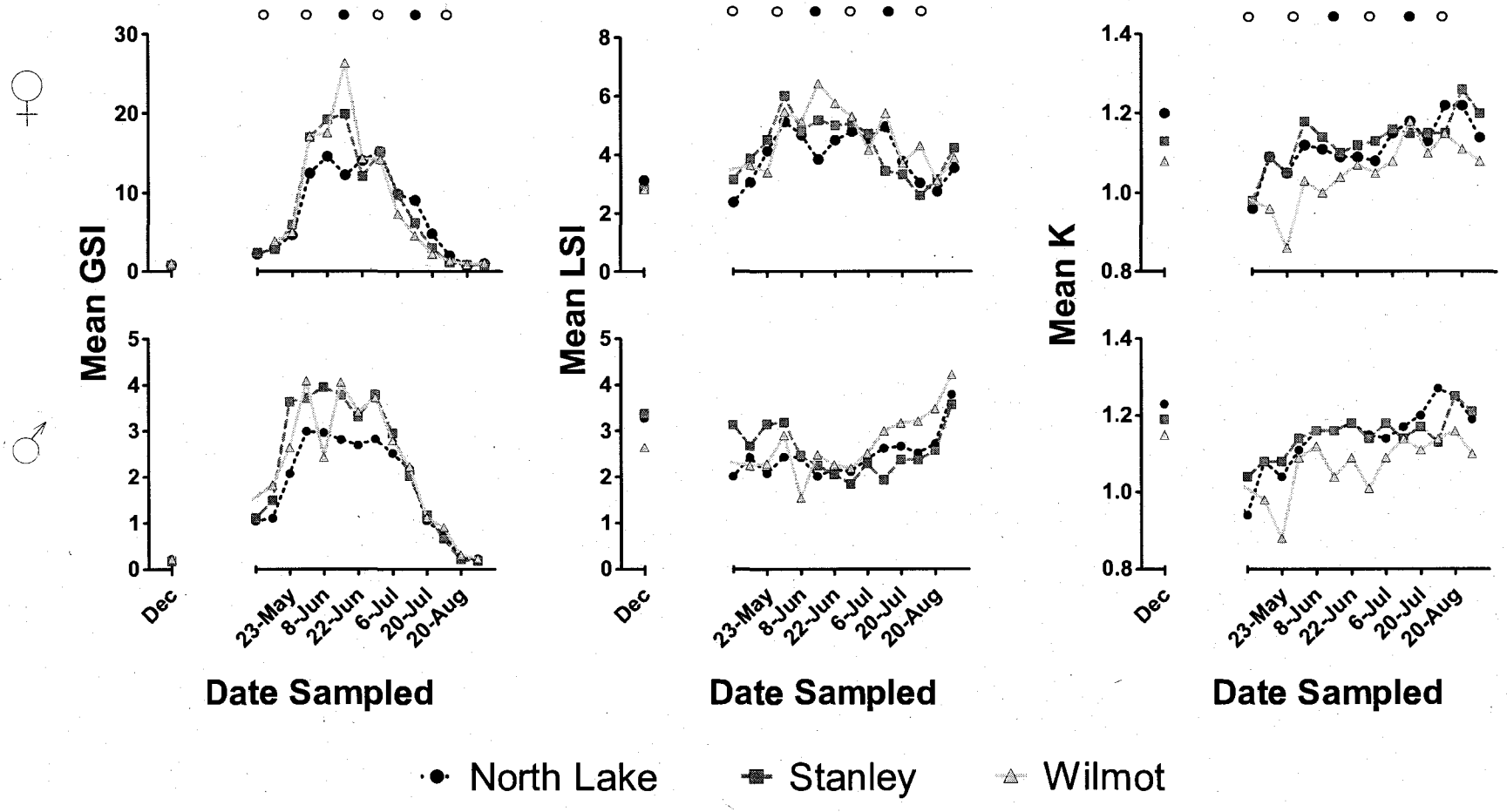

FIGURE 2.2. Mean gonadosomatic index (GSI), liversomatic index (LSI), and condition factor (K) for males and females of all three sites at all sampling times. Open circles indicate full moons and black circles are new moons.

July before starting to decline. Liver weight increased again slightly in the early fall. Condition factor increased throughout May, held steady for June, and then increased from July through August before decreasing slightly in September. The female spawning period for all sites, as indicated by the presence of mature eggs (Table 2.3), began June 1 which corresponded with a full moon and lasted approximately six weeks (Fig. 2.2).

Male gonad size increased rapidly throughout May (1-4%), plateaued in June, and then declined rapidly in July (Fig. 2.2). Male liver size decreased over winter, fluctuated throughout May and June, and increased from July through September. Condition factor increased sharply the 2nd week in May and then through until August when it began to decline. The male spawning period for all sites, chosen based on the first large increase in gonad size until the first major decline, began May 23 and lasted approximately eight weeks.

Between site variability

Wilmot fish had the lowest condition factor in both males and females, while North Lake had the lowest GSI (Fig. 2.2). Significant differences in LSI (Appendix A.2) occurred throughout the sampling with no site being consistently higher or lower at all times. Spawning was continuous and non-cyclical with no lunar periodicity (Fig. 2.2). Carcass weight strongly covaried with both fecundity and egg size. Wilmot and North Lake fish were significantly larger (both length and weight) than the Stanley females; however the relationship was inconsistent in the North Lake fish in which during several time periods they were much smaller than fish at the other sites (Appendix A.2, Table 2.4). There were no significant site differences in the number of mature eggs/gram

TABLE 2.3. The number of mature eggs per gram carcass weight was calculated using the least square means generated from an ANCOVA (with carcass weight as the covariate) from twelve females per site per sampling period. Egg size was determined as an average of eight eggs from each of the eight females, and was only done during the peak spawning period as indicated by the GSI

Date	North Lake			Stanley			Wilmot		
	# eggs/g	Egg size (mm)	Egg size/g	# eggs/g	Egg size (mm)	Egg size/g	# eggs/g	Egg size (mm)	Egg size/g
23-May	0		0	0		0	0		0
1-Jun	15	0.180	0.021	17	0.194	0.022	17	0.191	0.022
8-Jun	15	0.183	0.022	19	0.182	0.022	20	0.187	0.023
14-Jun	10	0.193	0.021	20	0.196	0.022	29	0.190	0.021
22-Jun	17	0.182	0.028	12	0.192	0.030	15	0.196	0.029
29-Jun	12	0.178	0.024	12	0.192	0.026	8	0.181	0.024
6-Jul	12	0.189	0.026	18	0.185	0.027	11	0.178	0.026
12-Jul	13	N/A	N/A	6	N/A	N/A	4	N/A	N/A
20-Jul	9	N/A	N/A	3	N/A	N/A	4	N/A	N/A
27-Jul	2	N/A	N/A	1	N/A	N/A	4	N/A	N/A

carcass weight (Appendix A.3). Stanley and North Lake had significantly different egg sizes/gram carcass weight, with Stanley having larger eggs (Appendix A.3, Tukey $p<0.05$, Table 2.3). Wilmot had the highest total reproductive output of 7435 eggs (95% CI 4711-10468) while Stanley had the lowest with 4790 eggs (3623-5984). North Lake fell in between with a total reproductive output of 5235 eggs (2848-7799; Fig. 2.3). Population density was significantly higher at the North Lake and Wilmot sites ($F_{2,9} = 36.36$, $p<0.001$, Tukey $p<0.05$) than Stanley with North Lake having a larger proportion of YOY than either of the other sites ($F_{2,9} = 20.974$, Tukey $p<0.05$; Fig. 2.4).

The June 14th sampling period provided one exception to all the above trends in female mummichog. The Wilmot fish had an increase in GSI from 17.6 to 26.4% body weight, which corresponded to an increase of 77% in fecundity (Figs. 2.2 and 2.3). The number of mature eggs/gram carcass weight increased from 20.4 to 29.2 (Table 2.3). This peak dramatically increased the total reproductive output of the Wilmot site (Fig. 2.3).

Variance among five sites within the Trout River Estuary

The Gunn's Bridge site in the Trout River Estuary had the longest and heaviest fish and for females had significantly lower LSI (Table 2.5, Appendix A.4). There were no significant differences in any of the other somatic parameters. In comparison to the lower reaches of the estuary, mummichog population density was significantly higher in the upper reaches (Fig. 2.5).

TABLE 2.4. Mean (± SE) length and weight for females and males collected at each of the three sites for all time periods. Within each sampling time, different superscript letters indicate significant differences between sites for each parameter ($p<0.05$).

Sex,	North Lake			Stanley			Wilmot		
Date	N	Length (cm)	Weight (g)	N	Length (cm)	Weight (g)	N	Length (cm)	Weight (g)
Females									
21-Dec	20	7.48 ± 0.34	5.67 ± 0.80	28	7.47 ± 0.20	5.34 ± 0.39	20	6.87 ± 0.27	3.65 ± 0.41
2-May	20	5.93 ± 0.24	2.29 ± 0.33	20	6.76 ± 0.35	4.03 ± 0.82			
9-May	23	8.46 ± 0.19 ^{A}	7.25 ± 0.69 ^{A}	22	8.49 ± 0.25 ^{A}	7.51 ± 0.79 ^{A}	26	9.87 ± 0.21 ^{B}	10.80 ± 0.77 ^{B}
23-May	16	8.44 ± 0.23 ^{B}	7.38 ± 0.61 ^{A}	13	6.78 ± 0.34 ^{A}	3.93 ± 0.61 ^{B}	5	8.40 ± 0.16 ^{B}	5.67 ± 0.29 ^{AB}
1-Jun	20	9.19 ± 0.23 ^{A}	10.85 ± 1.00	20	8.46 ± 0.12 ^{B}	8.87 ± 0.40	20	9.21 ± 0.21 ^{A}	10.22 ± 0.82
8-Jun	20	9.29 ± 0.19 ^{A}	10.94 ± 0.77	20	8.68 ± 0.12 ^{B}	9.27 ± 0.33	20	9.28 ± 0.14 ^{A}	9.97 ± 0.43
14-Jun	20	8.23 ± 0.28 ^{A}	7.59 ± 0.76 ^{A}	20	8.38 ± 0.12 ^{A}	8.15 ± 0.34 ^{A}	20	9.55 ± 0.22 ^{B}	12.29 ± 1.07 ^{B}
22-Jun	20	6.92 ± 0.25 ^{A}	4.64 ± 0.52 ^{A}	20	7.93 ± 0.14 ^{B}	6.71 ± 0.35 ^{B}	20	9.30 ± 0.20 ^{C}	10.61 ± 0.79 ^{C}
29-Jun	20	7.39 ± 0.39 ^{A}	6.23 ± 1.09 ^{A}	20	7.58 ± 0.23 ^{A}	6.25 ± 0.52 ^{A}	20	9.50 ± 0.21 ^{B}	11.04 ± 0.80 ^{B}
6-Jul	20	8.34 ± 0.34 ^{A}	8.97 ± 1.28 ^{A}	20	6.77 ± 0.27 ^{B}	4.64 ± 0.78 ^{B}	20	6.78 ± 0.27 ^{B}	4.19 ± 0.61 ^{B}
12-Jul	20	7.08 ± 0.26 ^{A}	5.21 ± 0.62 ^{A}	20	7.79 ± 0.32 ^{AB}	6.73 ± 0.97 ^{AB}	20	8.50 ± 0.26 ^{B}	8.62 ± 0.73 ^{B}
20-Jul	20	6.21 ± 0.12 ^{A}	3.02 ± 0.18 ^{A}	20	6.84 ± 0.24 ^{A}	4.24 ± 0.51 ^{A}	20	8.97 ± 0.24 ^{B}	8.93 ± 0.71 ^{B}
27-Jul	16	6.47 ± 0.52 ^{A}	5.12 ± 2.00 ^{A}	20	8.00 ± 0.24 ^{B}	6.50 ± 0.61 ^{B}	20	8.24 ± 0.28 ^{B}	7.32 ± 0.73 ^{B}
21-Aug	20	7.97 ± 0.22	6.73 ± 0.57	16	7.43 ± 0.25	5.62 ± 0.56	20	7.94 ± 0.22	6.16 ± 0.54
20-Sep	20	5.70 ± 0.21 ^{A}	2.45 ± 0.32 ^{A}	20	8.14 ± 0.24 ^{B}	7.65 ± 0.91 ^{B}	20	7.01 ± 0.32 ^{C}	4.58 ± 0.69 ^{C}

TABLE 2.4., continued.

Sex, Date	North Lake N	North Lake Length (cm)	North Lake Weight (g)	Stanley N	Stanley Length (cm)	Stanley Weight (g)	Wilmot N	Wilmot Length (cm)	Wilmot Weight (g)
Males									
21-Dec	20	7.59 + 0.22 ^{A}	5.67 + 0.60 ^{A}	13	6.69 + 0.36 ^{AB}	4.09 + 0.67 ^{AB}	20	6.62 + 0.25 ^{B}	3.38 + 0.57 ^{B}
2-May	21	6.07 + 0.21	2.39 + 0.30	20	6.57 + 0.25	3.46 + 0.40			
9-May	22	7.65 + 0.17 ^{A}	5.22 + 0.50 ^{A}	21	8.02 + 0.26 ^{A}	6.19 + 0.71 ^{A}	23	9.02 + 0.23 ^{B}	8.12 + 0.73 ^{B}
23-May	15	8.01 + 0.20 ^{A}	5.96 + 0.66	12	7.28 + 0.18 ^{B}	4.42 + 0.35	4	7.83 + 0.46 ^{AB}	4.71 + 1.03
1-Jun	19	7.30 + 0.25 ^{A}	4.84 + 0.61 ^{A}	20	8.25 + 0.30 ^{B}	7.67 + 0.94 ^{B}	19	7.96 + 0.23 ^{B}	6.09 + 0.25 ^{AB}
8-Jun	20	8.18 + 0.18 ^{A}	7.01 + 0.59	20	8.11 + 0.16 ^{B}	6.89 + 0.54	8	7.81 + 0.30 ^{A}	5.73 + 0.76
14-Jun	20	8.28 + 0.14 ^{A}	7.10 + 0.42 ^{A}	20	7.30 + 0.08 ^{B}	4.80 + 0.18 ^{B}	20	8.49 + 0.21 ^{A}	7.09 + 0.58 ^{A}
22-Jun	20	7.85 + 0.22 ^{AB}	6.34 + 0.68 ^{AB}	20	7.56 + 0.13 ^{A}	5.45 + 0.28 ^{A}	20	8.40 + 0.19 ^{B}	7.08 + 0.52 ^{B}
29-Jun	20	8.24 + 0.31	7.42 + 0.84	20	7.53 + 0.16	5.22 + 0.33	20	8.23 + 0.20	6.40 + 0.60
6-Jul	20	6.64 + 0.24	3.78 + 0.43	20	7.13 + 0.19	4.72 + 0.37	20	6.91 + 0.31	4.27 + 0.57
12-Jul	20	6.40 + 0.24 ^{A}	3.49 + 0.49 ^{A}	20	7.39 + 0.25 ^{B}	5.27 + 0.51 ^{B}	20	7.68 + 0.22 ^{B}	5.61 + 0.44 ^{B}
20-Jul	20	6.26 + 0.23 ^{A}	3.31 + 0.40 ^{A}	20	6.21 + 0.31 ^{A}	3.43 + 0.61 ^{A}	20	7.93 + 0.18 ^{B}	6.08 + 0.41 ^{B}
27-Jul	20	5.67 + 0.17 ^{A}	2.42 + 0.30 ^{A}	20	7.44 + 0.28 ^{B}	5.46 + 0.65 ^{B}	20	8.17 + 0.22 ^{B}	7.14 + 0.57 ^{B}
21-Aug	20	8.05 + 0.26	7.41 + 0.76 ^{A}	20	7.21 + 0.25	5.19 + 0.64 ^{AB}	20	7.20 + 0.27	4.98 + 0.68 ^{B}
20-Sep	20	5.67 + 0.23 ^{A}	2.45 + 0.36 ^{A}	20	6.92 + 0.20 ^{B}	4.57 + 0.40 ^{B}	20	6.97 + 0.09 ^{B}	4.03 + 0.16 ^{B}

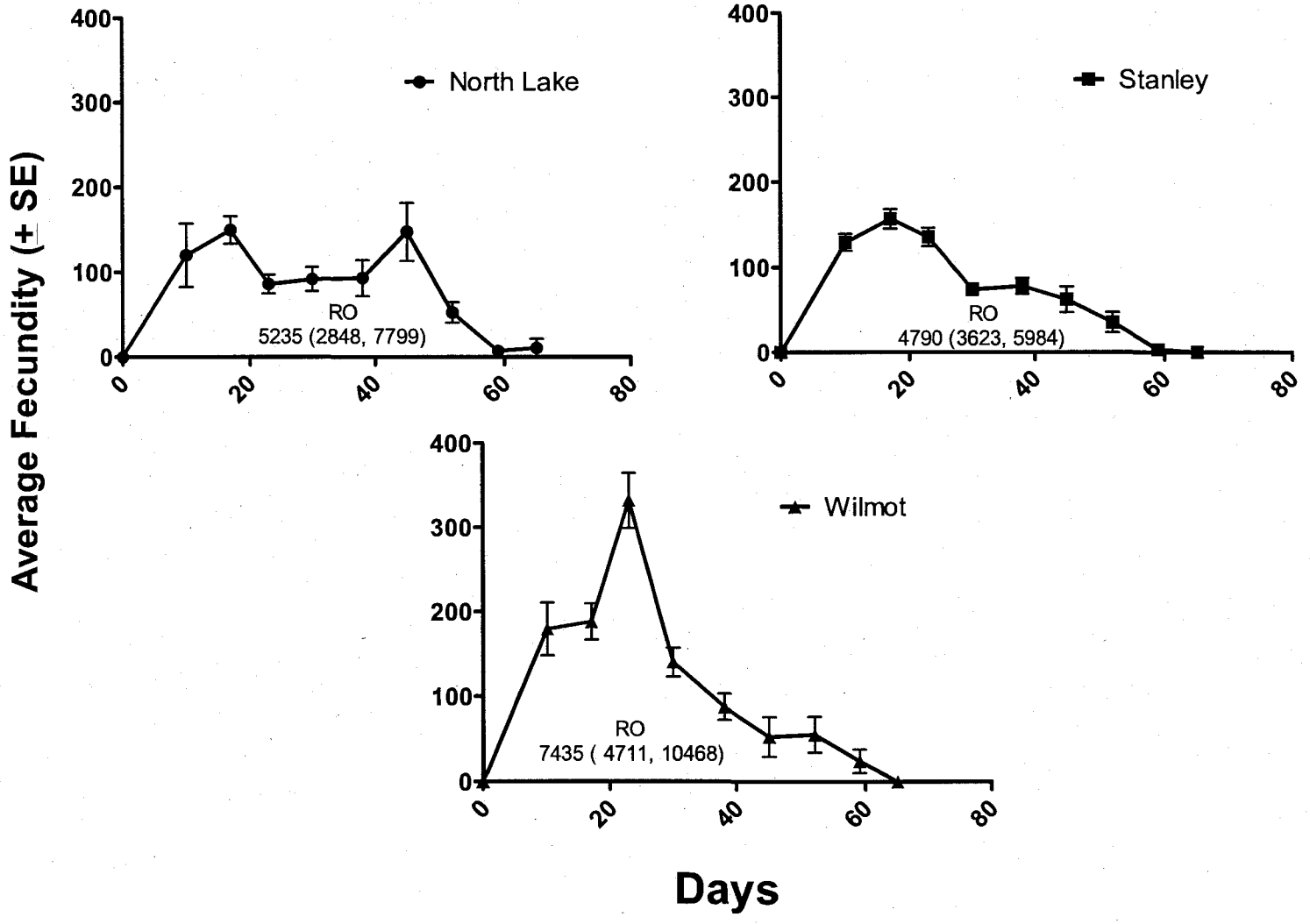

FIGURE 2.3. Average fecundity was determined from twelve females at each site at each sampling time. Average total reproductive output (RO) with confidence intervals was calculated as the area under the curve for the average fecundity (± SE) at each site.

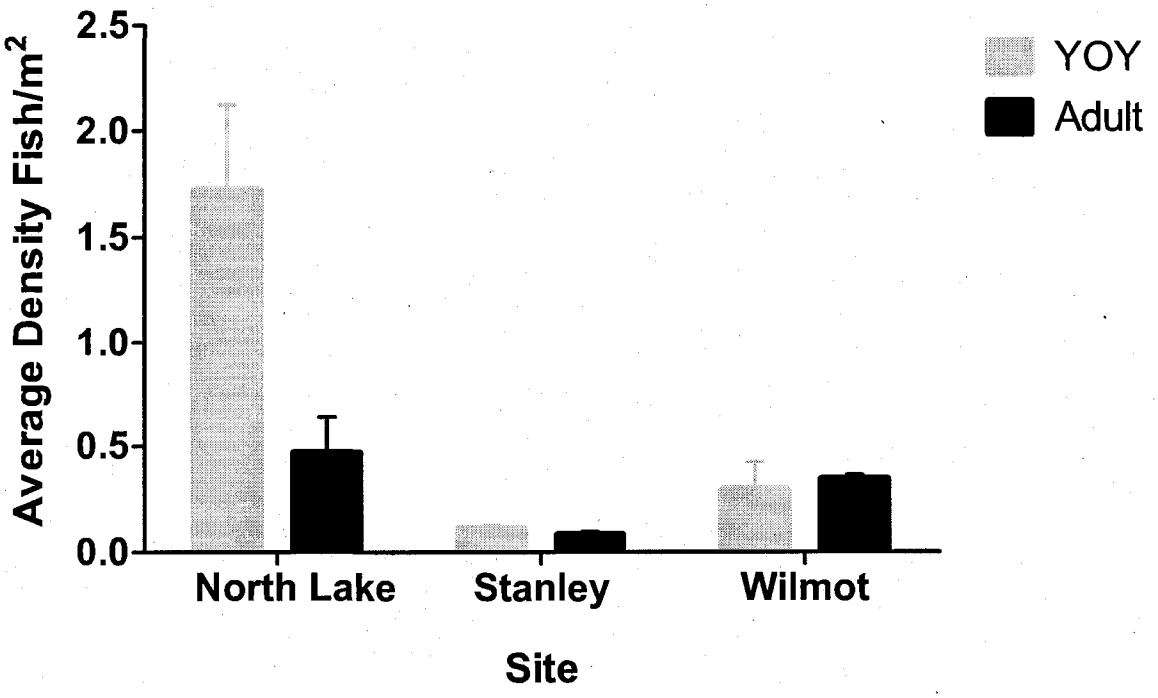

FIGURE 2.4. Average density of mummichog, both adults and young-of-the-year (YOY), per square meter (± SE). Fish were captured by seine net covering approximately 225 m^2 at each of four sites within an estuary and total catch was counted. YOY was determined as any fish ≤ 35 mm.

TABLE 2.5. Mean (± SE) of various parameters of adult male and female mummichog (*Fundulus heteroclitus macrolepidotus*) collected July 17-19, 2007. Sample sizes were twenty males and twenty females per site. Within a row, different superscript letters indicate significant differences between sites for each parameter ($p<0.05$).

Sex	Parameter	Site				
		Gunn's Bridge	**Trout**	**Confluence**	**Stanley**	**New London**
Female	Length (mm)	81.5 (0.23)^{A}	70.8 (0.28)^{B}	80.5 (0.25)^{A}	68.4 (0.24)^{B}	62.3(0.19)^{B}
	Weight (g)	6.72 (0.54)^{A}	4.91 (0.70)^{C}	6.96 (0.69)^{A}	4.24 (0.51)^{CD}	3.02 (0.34)^{D}
	K	1.10	1.16	1.17	1.16	1.12
	LSI	2.51^{A}	3.46^{B}	3.30^{B}	3.29^{B}	3.35^{B}
	GSI	2.70	1.75	2.02	2.78	2.75
Male	Length (mm)	7.79 (0.19)^{A}	6.54 (0.17)^{B}	6.82 (0.28)^{B}	6.21 (0.31)^{B}	5.92 (0.13)^{B}
	Weight (g)	5.98 (0.42)^{A}	3.58 (0.31)^{BC}	4.46 (0.60)^{AB}	3.43 (0.61)^{BC}	2.49 (0.18)^{C}
	K	1.13	1.18	1.19	1.17	1.16
	LSI	1.99^{A}	2.56^{B}	2.72^{B}	2.39^{AB}	2.50^{AB}
	GSI	1.26	0.94	0.99	1.11	1.17

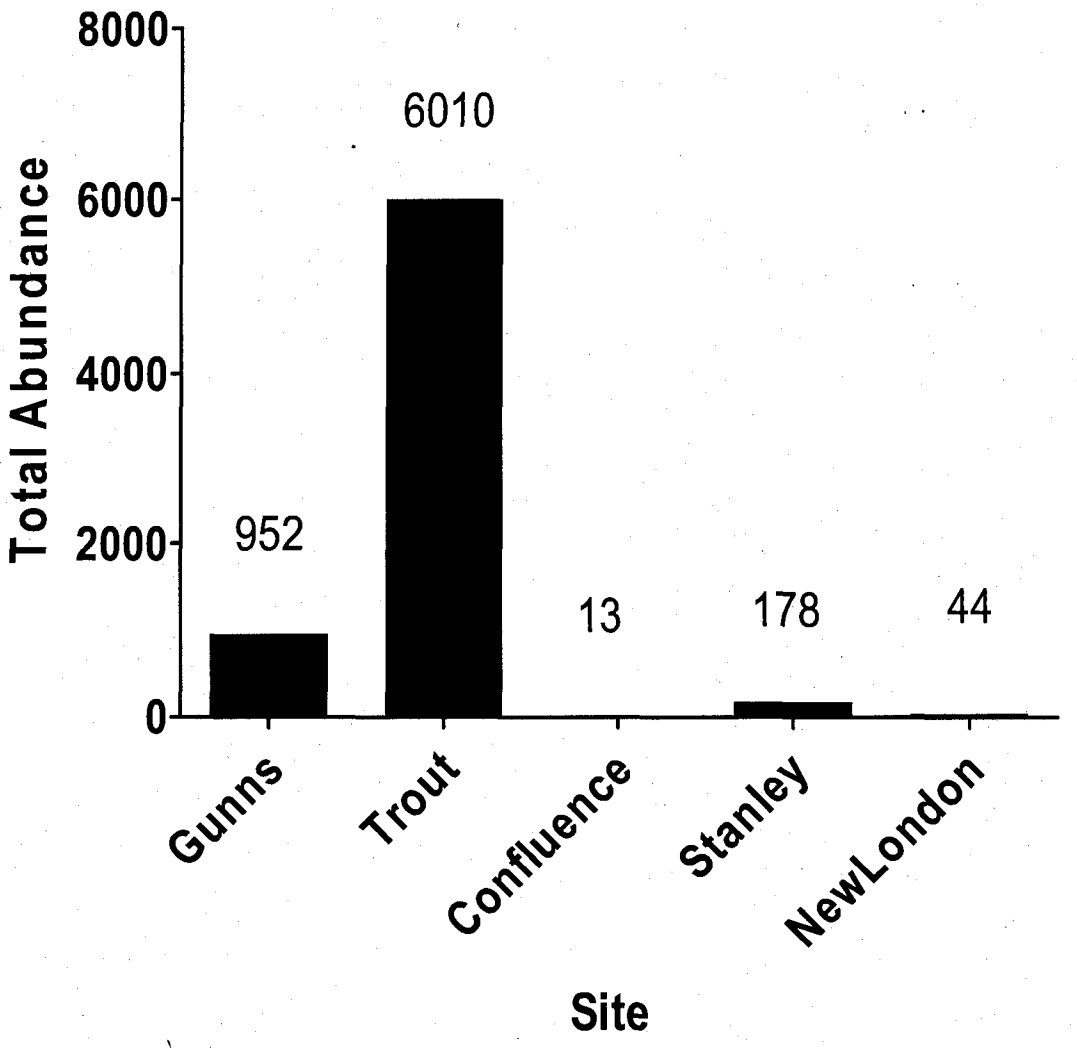

FIGURE 2.5. Total abundance of mummichog (*Fundulus heteroclitus macrolepidotus)*, both adults and young-of-the-year (YOY), captured by seine net covering an area of approximately 225 m^2, at each of the five sampling sites within the Trout/Stanley River estuary between July 17-19, 2007.

2.5 DISCUSSION

This study further describes the life history of the northern mummichog in the Southern Gulf of St. Lawrence and suggests that this subspecies is characterized by one major spawning peak rather than by lunar periodicity as in the southern subspecies. Due to the considerable investment of energy in gonad growth immediately prior to spawning in spring, gonad size measurements before this time were of little value in predicting reproductive output. Thus, our study supports the conclusion that, in order to get a reliable estimate of energy allocation to reproduction and reproductive output, mummichog must be sampled several times throughout their reproductive season as suggested by Thériault *et al.* (2007). Somatic endpoints related to energy storage and utilization were relatively insensitive for the detection of differences in environments both between and within an estuary. Conversely there were dramatic differences found in mummichog population densities which may be a more useful parameter in assessing responses to environmental conditions.

Mummichog have recently been recommended as a premier teleost model for environmental effects monitoring (Burnett *et al.* 2007). However, while they have characteristics that make them suitable as a monitoring species there are clear difficulties that limit the utility of asynchronous multiple spawners to measure reproductive performance. The current Canadian EEM program guidelines recommend sampling fish prior to the initiation of spawning (Munkittrick *et al.* 2002). In single spawning fish this is the time when full gonadal development has been reached and fish are considered to be synchronous in their development. Thus, measurement of gonad size at this time may provide a good approximation of annual reproductive allocation (Fox and Crivelli 1998),

and any alterations in normal energy patterns which may ultimately affect reproduction can then be observed. Obtaining information/developing guidelines for the sampling of multiple spawners is much more difficult due to the rapid onset of vitellogenesis immediately before and during spawning, as well as due to the paucity of knowledge on the various life histories (Munkittrick *et al.* 2002). Without prior knowledge on the spawning pattern of multiple spawners an estimation of reproductive allocation cannot be made, making effective monitoring difficult (Fox and Crivelli 1998). The results presented herein confirm previous suggestions (Thériault *et al.* 2007) that mummichog do not provide enough information to determine site differences with a single sampling event and thus require more frequent samplings in order to be used in environmental monitoring programs.

In the current study, the spawning period of mummichog was continuous and non-cyclical. Gonadal maturity, as determined by the presence of mature eggs in the gonads, was reached at the same time at all sites, and although this coincided with a full moon, it is not clear which environmental factors were most important in the initiation of spawning. Based on this information, determination of an appropriate time for sampling in relation to environmental cues was not possible. Lunar cycle, temperature and photoperiod have all been studied as potential environmental cues, with no consensus being reached on the relative importance of any particular one (Wallace and Selman 1981; Day and Taylor 1984; Taylor 1986; Shimizu 2003). Strong lunar/semilunar periodicity has been observed in several mummichog populations (Taylor *et al.* 1979). However this periodicity has been less pronounced or absent in the more northern reaches of their distribution (Wallace and Selman 1981; Taylor 1986; Shimizu 1997) and

specifically in the subspecies, the northern mummichog (*Fundulus heteroclitus macrolepidus*). The absence of a lunar/semilunar periodicity in northern populations of mummichog may be due to environmental conditions which prohibit longer spawning periods. The spawning period of the mummichog sampled in this study was approximately six weeks. Taylor (1986) found that both timing and duration of spawning seasons differed between populations in various geographic locations, but the water temperature range over which spawning occurred was similar. Sites which reached warmer temperatures sooner had earlier onset and longer duration in the mummichog spawning season. All our sites were in close proximity to one another and varied little in temperature. Furthermore, being in a northern climate, the period of warmer water temperatures and high productivity is compressed. Thus it is possible that to enhance survival of young by providing the maximum scope for growth before winter, the spawning season of mummichog at the northern edge of their range is both shorter and without spawning periodicity.

Mummichog populations sampled in December and again in May showed a large decline in condition factor indicating that they relied heavily on their energy stores over the winter. These stores were replenished during the spring and summer months with the males saving more energy than the females. A similar result was observed by Leblanc and Couillard (1995) in the Miramichi Estuary, New Brunswick, Canada where condition factor increased significantly as the summer progressed, with males doing better than females.

A rapid increase in both gonad and liver weight in females from mid to late May indicated the onset of gonadal recrudescence. The sustained high liver weights

concomitant with the gonad growth in females suggests that the liver is actively participating in reproduction. Rinchard and Kestemont (2003) found LSI to be high throughout the spawning season in the asynchronously spawning bleak (*Alburnus alburnus*). The vitellogenic activity of the hepatocytes also remained constant throughout the bleak's spawning period indicating active vitellogenesis. Other studies on mummichog reproduction found LSI to be increased throughout the spawning period (Selman and Wallace 1983; Cochran *et al.* 1988) and plasma 17β-estradiol (E2) steroid levels to be elevated during this time, further indications that active vitellogenesis is occurring (Shimizu 1997). Similarly, Leblanc and Couillard (1995) found that mummichog sampled from Horton's Creek, Miramichi Estuary, New Brunswick had high LSI right before spawning but it decreased throughout the spawning period, with peaks before each major spawn. These peaks corresponded with increases in plasma E2 which again suggests active vitellogenesis. In comparison, my fish had notably higher overall LSI's than those sampled by Leblanc and Couillard (1995), suggesting my mummichog store more energy in their liver while maintaining reproductive output. This may reflect higher nutrient availability in PEI's estuaries than in the relatively pristine Horton's Creek.

When examined over the entire reproductive season, the reproductive output differed among the sites with mummichog in the higher percent agriculture area having the largest output. However, this increase in the Wilmot population was largely driven by a single mid-June spike in reproductive activity. The Wilmot females were also significantly larger (length and weight) than the Stanley females though interestingly had the lowest condition factors. Fish allocate a base amount of energy for maintenance and

survival with any additional energy being spent on reproduction or somatic growth (Gibbons and Munkittrick 1994). An increase in the food availability, as would be expected in a eutrophic environment, may result in an increased growth rate, earlier age to maturity, and increased reproduction. The difference in the size of fish from the Wilmot site, as well as the increased reproductive output, may be due to such a eutrophication effect.

The density of mummichog was significantly higher in August at the North Lake and Wilmot sites than Stanley site, with North Lake having a much larger proportion of YOY fish than either of the other sites. Sea lettuce was the dominant vegetation at both of these sites and had reached high abundances by the middle of June. Conversely, Stanley was predominantly eelgrass and had very little to no sea lettuce for the majority of the summer. Other studies have shown that enhanced macroalgal production/biomass, sometimes as a result of increased nitrogen loading, has prompted increases in mummichog densities (Kneib 1986; Whitman *et al.* 1997). This apparently rapid and dramatic population growth with increasing resources suggests that demographic population endpoints may prove to be more sensitive endpoints to nutrient enrichment than density-dependent physiological endpoints.

Spatially within the Trout River Estuary, the results showed few significant differences among the sites despite their widely ranging habitat and water quality. The environment in the upper reaches of the Trout River Estuary was much different than that of the other sites. These sites were densely packed with sea lettuce, were subject to late summer hypoxia, had very low tidal flushing, and no eelgrass habitat. The upper reaches of an estuary are known to be very stressful environments for fish populations and are

often characterized by lower species diversity and higher fish abundances (Araujo *et al.*, 1999). In this study, the upper reaches of the estuary, Gunn's Bridge and Trout, had much higher population densities than down river. These data further support the conclusions from the site comparisons that suggest that mummichog populations are strongly resource driven and there are few limitations preventing population density from increasing to the limits dictated by the carrying capacity of the environment. Thus, large differences in population density may be a better indicator of effects of eutrophication than somatic changes for this species.

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CHAPTER 3

EVALUATING CUMULATIVE EFFECTS OF POINT AND NON-POINT SOURCE INPUTS IN PRINCE EDWARD ISLAND ESTUARIES USING THE NORTHERN MUMMICHOG (*FUNDULUS HETEROCLITUS MACROLEPIDOTUS*)

3.1 ABSTRACT

Estuarine eutrophication as a result of land use is increasing worldwide. In Prince Edward Island, where potato production, and consequently the use of chemical fertilizers is intensive, the aquatic ecosystems have become severely degraded. Since 1994, there have been at least 18 pesticide related fish kills, and more recently several anoxia related fish kills, as a result of land use practices. The objective of this study was to examine cumulative impacts on northern mummichog (*Fundulus heteroclitus macrolepidotus*) of nutrient enriched point and non-point source pollution. Fish were sampled on a monthly basis from May through August at seven estuaries spanning a range of land-use and potential nutrient and contaminant loadings. An effects-based assessment and a combination of chemical and biochemical indicators of exposure were used to detect and determine causes of impacts on mummichog populations. Responses of fish were separated by the trophic status of the estuary. Fish at two highly eutrophic north shore estuaries had increased liver size, proportion of young-of-the-year (YOY) and overall fish density. These fish had rapid initial growth rates but lower maximum size. Fish at the third eutrophic site, a south shore estuary, had increased liver and gonad size, increased fecundity, slower growth rate with high maximum size and decreased proportion of YOY with high overall fish density. The three highly eutrophic sites had the lowest *in vitro* steroid production during the prespawning period. Pesticides were not detected in the sediment of any of the estuaries, nor were there any associated responses in enzyme activity. Substrate type may be influencing recruitment and the increased effort being placed on reproduction at the heavily eutrophic south shore site.

3.2 INTRODUCTION

Estuaries represent one of the most diverse and productive aquatic environments. Since industrialization, estuaries have received stress from multiple anthropogenic sources ranging from municipal waste to eutrophication as a result of agricultural land use (Lotze *et al.* 2006). Nutrient loading in areas of intensive agriculture and the deposition of persistent organic pollutants and pesticides are major concerns in estuarine systems. A significant scientific challenge is to tease apart contaminant-mediated impacts from the multitude of other ecological variables.

Agricultural pollution involves multiple stressors which are more complex in their effects due to the widespread and intermittent nature of their delivery to the aquatic ecosystem. These stressors can have a direct effect, such as increased mortality resulting from pesticide exposure, or indirect effect, as in decreased visibility resulting from an increase in turbidity. In complex receiving environments like estuaries that have differing freshwater inputs and tidal flushing, quantifying the amount of exposure and impact of a particular stressor is very difficult. Thus measuring fish responses to cumulative stress will provide an integrated assessment of the system. A fish monitoring framework has only recently been used to study cumulative changes due to multiple environmental disturbances on rivers (Munkittrick *et al.* 2000; West *et al.* 2006). However, there are few studies on cumulative impacts on fish populations in estuarine systems.

Eutrophication has emerged as perhaps the major factor threatening aquatic diversity in estuaries (Cloern 2001; Howarth *et al.* 2002). In northern temperate estuaries, when nutrient enrichment occurs, populations of opportunistic algae, which may include phytoplankton, epiphytes and the macroalgal species *Ulva lactuca* (sea lettuce) explode

(Cloern 2001; Hauxwell *et al.* 2003). These species limit the light which can then reach the benthic eelgrass (*Zostera marina*) resulting in the exclusion of these natural seagrass beds which are important fish habitat (Hauxwell *et al.* 2003). The large algal blooms then decompose creating anoxic conditions in the benthic communities of the estuary which then negatively affect resident fish populations. The destruction of marine habitat, loss of biodiversity, and the smell of decay caused by the proliferation of sea lettuce has become a common occurrence in estuaries.

Many pesticides are highly persistent (Voorspoels *et al.* 2004) and are frequently detected in estuarine sediments (Domagalski and Kuivila 1993; Hack *et al.* 2008; Hong *et al.* 2008). Pesticides can bioaccumulate in the tissues of many marine organisms (Voorspoels *et al.* 2004), and some studies have shown that the level of residues within a fish increase the longer the fish is in the estuary (Butler 1969; Lanfranchi *et al.* 2006). Pesticides which are not acutely toxic to fish may over time become chronically toxic (Domagalski and Kuivila 1993). Indirect effects of pesticide contamination on fish populations include the loss of more vulnerable invertebrate species (Hack *et al.* 2008) and the damage to aquatic vegetation caused by the inhibition of growth (Kennish 1997).

Prince Edward Island (PEI) Canada is intensively farmed, with 44% of its land, approximately 261.4 thousand hectares, in agricultural production (PEI DFAF 2003). Some watersheds have up to 80% agricultural land use. A large percentage of this land is in potato production which has very high agrichemical usage and requires the use of substantial quantities of nitrate-based fertilizers (application rates estimated at 220 kg/ha/yr of fertilizer; Government of PEI 2008). These farming practices have resulted in severely altered freshwater and estuarine habitats and 16 fish kills in streams due to

pesticide run-off have occurred in PEI since 1994 (Gormley *et al.* 2005) with an additional two occurring during this study. Although estuarine fish kills have also been reported as a result of anoxia their exact numbers are not recorded. Lethal concentrations of contaminants usually only occur after a heavy rainfall event; however, sub-lethal concentrations may occur throughout the year. Due to poor soil quality, potato cultivation in PEI is heavily dependant on the use of chemical fertilizers and with strong ocean winds and large areas of exposed soil it is highly susceptible to erosion. Sedimentation due to the loss of soil (estimated at 20-40 tonnes/ha/yr; Smith *et al.* 2002) is also a serious problem in watersheds with substantial proportions of agricultural land. Additional land use activities in PEI include municipal sewage treatment and industrial food production plants. Both of these effluents are responsible for significant point source contributions of nutrients and chemical contaminants to aquatic ecosystems. Altogether, this results in three main stressors in PEI estuaries: nutrient loading, sedimentation and chemical contamination.

The federally mandated Canadian EEM program has established methods for detecting impacts of pollution on fish, fish habitat, and usability of fish resources (Munkittrick *et al.* 2002). The fish survey component of this program is an effects-based assessment which compares indices of growth, reproduction and survival of a monitoring species located in exposed and unexposed areas (Lowell *et al.* 2003). These indices provide information on the health of a population and its prospects for continuance. Any effects observed in the fish population can then be used to identify where and how the system is compromised and thus allow for proper remediation of the watershed.

In estuarine environments the northern mummichog (*Fundulus heteroclitus macrolepidotus*) has been used as a monitoring species. Mummichog are widespread and abundant along the east coast of North America from Connecticut to Newfoundland (Able and Felley 1986), a key criteria for any monitoring species. They have further advantages for environmental monitoring in that they have high site fidelity (Skinner *et al.* 2005), and are presumed to have a smaller home range than other larger bodied fishes (Griffin and Valiela 2000; Thériault *et al.* 2007). Furthermore, they are sexually dimorphic, are not commercially or recreationally exploited, and have been shown to respond to point source effluents such as seafood processing plants (Thériault *et al.* 2007) and pulp mill effluent (Leblanc *et al.* 1997). Mummichog are asynchronous multiple spawners thereby confounding the assessment of indices of energy expenditure and storage for reproductive purposes. Sampling just prior to the first spawn, as recommended by the EEM program for the assessment of multiple spawners, has proven insufficient when mummichog are the monitoring species chosen (Leblanc *et al.* 1997; Thériault *et al.* 2007, Chapter 2).

The purpose of this study was to examine cumulative impacts of estuarine anthropogenic stressors on mummichog using an effects-based population assessment framework in seven estuaries spanning a range of land-use and potential nutrient and contaminant loadings. In order to test the hypothesis that mummichog are being exposed to anthropogenic chemicals associated with either agriculture, or point source effluents, a combination of chemical and biochemical indicators of exposure was examined. Several sampling times were used to aid in the determination of the overall impacts of estuarine stressors. The objective of this study was to combine biological indicators of exposure

and directly measured chemical stressors in order to determine the factors having the most substantial impact on mummichog populations.

3.3 METHODS

Study Sites

Seven estuaries along the coast of Prince Edward Island, Canada with varying degrees of agricultural land-use, nutrient loading and point source effluent sources were selected for sampling in May 2007 (Fig. 3.1 and Table 3.1). The watersheds in PEI are dominated by estuaries, which have highly variable residence times depending on barriers and tidal flushing. However, those located along the north shore have longer residence times than those along the south shore because of lower tidal amplitude (Raymond *et al.* 2002). Sites selected along the north shore included North Lake, Trout River (Stanley Bridge), Indian River, and the Barbara-Weit estuaries. North Lake is almost entirely forested and has very little freshwater input, and has very low nutrient input. "Stanley", located in the peak potato growing region, supports a number of mussel leases, and its upper reaches experience yearly anoxic events, and is considered a medium input site. Indian River and Barbara-Weit are both high nutrient input sites. Indian River is characterized by a sill which alters tidal flushing, and in 2000 the river experienced a fish kill. Barbara-Weit has two additional point source effluents, sewage and potato processing. This estuary has undergone nutrient remediation resulting in demonstrable improvements in estuarine conditions (Raymond *et al.* 2002). However these changes have only resulted in an improvement from hypertrophic to eutrophic status. Sites

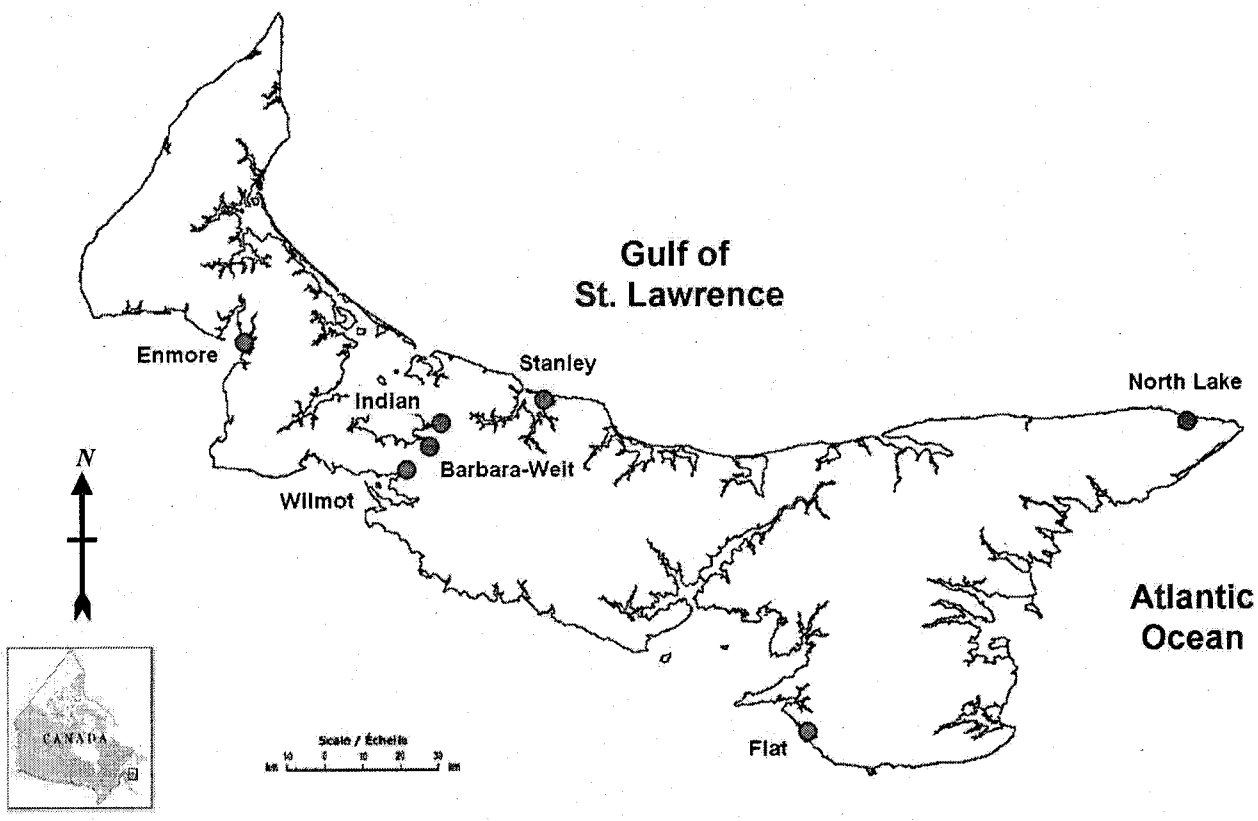

FIGURE 3.1. Map of the seven sampling sites within Prince Edward Island.

TABLE 3.1. Sampling site characteristics including location (latitude/longitude), watershed area and percent agricultural land use, nutrient loading, sediment composition and plant coverage. Maximum tidal amplitude was calculated using the Tides and Currents Pro Software v 2.5B over the months of May through September 2007. Plant coverage of a 0.5 m^2 area was recorded at 1 m intervals over the length of a 15 m transect and averaged to give a single measurement for each site. This assessment was conducted prior to the June sampling.

Site	Lat/long	Watershed		Estuary		Nutrient Load		Sediment			Plant Coverage	
		Land Use (% Ag)	Area (km^2)	Area (km^2)	Max. Tidal Amplitude (cm)	N (kg/ha/y)	P (kg/ha/y)	Organic (%)	Sand (%)	Silt (%)	Ulva (%)	Zostera (%)
Enmore	N46°36.082' W064°02.873'	12.4	42.6	1.6	140	0.15	0.01	1.5	91.9	8.1	0	0
Flat	N46°00.3378' W062°52.790'	15.5	30.1	0.6	274	64	0.45	2.9	100.0	0.0	0	12
North Lake	N46°27.556' W062°05.752'	14.8	47.7	1.1	137	53	0.09	4.9	93.0	7.0	76	0
Stanley	N46°27.182' W063°27.544'	39.4	53.3	4.1	109	247	0.95	1.5	95.5	4.5	0.5	23
Wilmot	N46°23.637' W063°44.560'	74.9	83.4	3.5	216	646	1.06	1.8	92.0	8.0	93	0
Indian	N46°27.667' W063°40.477'	64.9	23.9	1.5	115	421	0.011	1.6	100.0	0.0	87	0
Barbara-Weit	N46°25.938' W063°41.380'	61.9	20.2	1.0	115	334	6.05	4.3	100.0	0.0	93	0

selected along the south shore included Wilmot River Estuary, Enmore River Estuary, and Flat River Estuary. Wilmot is located in the most intensive potato growing area and in 2002 the river experienced two large fish kills as a result of pesticides. Enmore and Flat River are both very low nutrient input sites.

Sampling Methods

Fish were collected using a 30 m long, 1.5 m deep, 3 mm mesh beach seine with a central bag of 1.2 m high by 1.2 m wide and 1.2 m deep, at low tide. Sweeps were conducted until a minimum of twenty-five male and twenty-five female fish of size greater than 35 mm (size cut-off for YOY mummichog; Leblanc *et al.* 1997) were captured. Sites were sampled once per month from May through August (see Table 3.2 for exact dates). During each sampling period, one site was sampled each day and fish were transported back to the laboratory in aerated 20 L plastic pails for immediate processing. Fish were killed by spinal severance and total length (1 mm) and total body weight (0.01 g) were measured. Fish were dissected and liver and gonad weights (0.001 g) were recorded. Organs for biochemical analyses were kept from the first randomly selected twelve males and twelve females, stored in cryovials and snap frozen in liquid nitrogen. These included livers for 7-ethoxyresorufin-*O*-deethylase (EROD) activity and brains for acetylcholinesterase (AChE) activity assays. All samples were transferred to a -80 C freezer immediately post-sampling. Additionally, during the May sampling, a subsample of gonad tissue was taken, weighed, and placed in incubation media for analysis of *in vitro* steroid hormone production. Otoliths and scales (lateral line below the dorsal fin) were taken for ageing purposes.

TABLE 3.2. Sampling dates (day/month) and phase of the moon for mummichog sampled at all seven estuaries from May to August 2007.

Moon phase	Site						
	Enmore	**Flat**	**North Lake**	**Stanley**	**Wilmot**	**Indian**	**Barbara-Weit**
	14/05	13/05	10/05	09/05	12/05	11/05	15/05
New	13/06	12/06	14/06	14/06	14/06	15/06	15/06
	13/07	13/07	12/07	14/07	13/07	14/07	14/07
1st quarter	26/08	19/08	24/08	20/08	23/08	20/08	20/08

In the June and July sampling period eggs were preserved for fecundity analysis. Ovaries were placed in neutral buffered formalin until processing time, at which point they were transferred to 100% ethanol. Mature ova were counted from twelve randomly selected females per site per sampling period. Follicle maturity was determined as those eggs which had oil droplets congregated at one pole, and were translucent with yellowish coloration (Leblanc *et al.* 1997).

Population Structure and Density

In August, each site was sampled at four locations both similar and within close proximity to the original sampling sites. All mummichog caught were separated into two age classes: young-of-the-year (YOY), and adult. YOY were determined by size less than 35 mm. Length measurements were taken on a random sample of fifty YOY and a maximum of two hundred adults whenever possible. At some sites less than two hundred adults were measured due to a limited range of length distribution (Indian), low fish density (Flat), or high temperatures which increased the risk of fish mortality (Barbara-Weit). For length frequency distribution, the length frequency of the YOY and adult subsamples was extrapolated to the total number of those respective age classes captured at each site in order to provide a length frequency curve spanning the entire size range.

Age Determination

Otoliths removed from fish during the June sampling were aged by North Shore Environmental Services (Jon Tost, Thunder Bay Ontario). Otoliths were prepared using the crack-and-burn method (Christiansen 1964) and aged to the nearest year class.

Environmental Variables

Water temperature, dissolved oxygen, salinity, conductivity, and pH were measured once (YSI 650 handheld meter equipped with YSI model 600 QS sonde) at all sites during each sampling period (Appendix B.1). Measurements were taken at mid-depth from the center of each seine haul area. Aquatic plant habitat was quantified once in June by visual estimation and the percent cover of the major aquatic plants and algae (eelgrass, sea lettuce, green filamentous algae, etc.) were recorded. This estimation was done by running a 15 m transect perpendicular to shore just outside of the seined area. Plant coverage of a 0.5 m^2 area was recorded at 1 m intervals over the length of the transect and averaged to give a single measurement for each site. Some changes in plant coverage were noted throughout summer, however they were not quantified. Sediment samples were collected once on August at 10 m depth using a shovel and digging approximately 50 cm deep. Organic and inorganic content, percent moisture, grain size and pesticide content were determined in triplicate for each sediment sample. Moisture was determined gravimetrically after drying for 24h at 70°C, organic content estimated on loss-on-ignition after 4h at 550°C, and inorganic content after 2h at 950°C. Sediment grain size was determined as either coarse (1 mm sieve), sand (75 μm sieve) or silt (< 75 μm).

Sediment Pesticide Quantification

Sediment from each site was freeze dried and soxhlet extractions were performed in duplicate including extraction and recovery blanks. Extraction blanks were made up of sodium sulphate and 500 μL of ^{13}C carbaryl, whereas recovery blanks had sodium

sulphate plus 20 µL of pesticide spike solution and 500 µL of ^{13}C carbaryl. Ten grams of freeze-dried sediment from each site was mixed with anhydrous sodium sulphate, placed in an extraction thimble and soxhlet extracted with 150 mL of 1:1 hexane:acetone spiked with 500 µL of carbaryl recovery standard for 20 h. Extracted samples were then quantitively transferred into glass evaporation tubes and samples were concentrated to 1 mL using a nitrogen TurboVap II Concentration Workstation (Caliper Life Sciences, Ancaster, ON). Samples were reconstituted with 50 mL methanol and evaporated to 1 mL and this procedure was repeated to exchange extraction solvents with methanol.

Aliquots of 1 mL of extract representing an equivalent of 10 g dry weight of sediment were sent to the Aquatic Ecosystem Protection Research Branch at The National Water Research Institute in Burlington, Ontario for pesticide analysis by liquid chromatography/mass spectrometry (LC/MS). Each sample was filtered through a 0.2 µm syringe filter prior to analysis and reduced to 0.5 mL final volume. Samples were spiked with 100 µL of ^{13}C atrazine as an internal standard and then analyzed by a Sciex API2000 (MDS Sciex, Concord, ON) LC-MS-MS system equipped with an atmospheric pressure photoionization (APPI, PhotoSpray) source and an Agilent 1100 HPLC (Agilent, Mississauga, ON). Sample extracts were injected into a reverse phase column (Waters Symmetry C18 3.5 µm, 100 mm x 2.1 mm). Solvent elution profile was 50/50 water/methanol to 0/100 water/methanol in 4 minutes and held for 3 minutes.

In vitro steroids

In vitro production of sex steroids were measured using the protocol described by McMaster *et al.* (1995) and optimized for mummichog by MacLatchy *et al.* (2003).

Briefly, gonads were split into four approximately equal parts for females and 2 for males, weighed (nearest mg) and incubated in either unstimulated (basal, media + 1.0 mM IBMX; phosphodiesterase inhibitor 3-isobutyl 1-mehylxanthine; Sigma, St. Louis, MO) or stimulated (media + 1.0 mM IBMX + 20 IU/ml hCG; human chorionic gonadotropin; Sigma) solution. Incubation of gonadal tissue was conducted for 24 h at 18°C. Media from each well was then removed, placed in individual cryovials, and stored at -80°C until the time of analysis. Concentrations of testosterone (both sexes), 17-β estradiol (females) and 11-ketotestosterone (males) were quantified by radioimmunoassay.

EROD Activity

CYP1A induction was measured using the hepatic mixed-function oxidase catalytic reaction of 7-ethoxyresorufin to the fluorescent molecule resorufin. A modification of the fluorescent plate reader method outlined by van den Heuvel *et al.* (1995) was used, and is described briefly here. Livers were homogenized in 1 ml of an ice-cold cryopreservative buffer (0.1 M phosphate, 1 mM EDTA acetic acid, 1 mM dithiothrietol, and 20% glycerol; pH 7.4) and centrifuged at 10,000 g at 4°C to obtain the post-mitochondrial supernatant (PMS). Samples were then stored at -80°C until the time of analysis. The EROD reaction mixture contained 0.1 M HEPES, pH 7.8 (Sigma), 5.0 mM Mg^{2+}, 0.5 mM NADPH, 1.5 μM 7-ethoxyresorufin (Sigma) and 0.5 mg/ml of PMS protein. EROD activity was determined as a final endpoint on a fluorescence plate reader (Bio-Tek FLx800, Winooski, VT) with 528-nm excitation and 590-nm emission filters.

Protein content was estimated from fluorescamine fluorescence (360-nm excitation, 460-nm emission filters) against bovine serum albumin standards (Sigma).

Acetylcholinesterase Acivity

Brain acetylcholinesterase activity was measured using the method described by Sandahl and Jenkins (2002) and optimized for mummichog (R. Orrego, University of Ontario Institute of Technology, unpublished data). Tissue was weighed and homogenized 1:10 in sodium phosphate buffer (pH 8.0) and 1% Triton X-100 (Sigma), and centrifuged at 1000 rpm for 10 minutes at 4°C to obtain the supernatant. Samples were then stored at -80°C until the time of analysis. AChE activity was determined as changes in absorbance at 12 s intervals for 10 min at 25°C using an absorbance plate reader (Bio-Tek ELx800UV) with 412 nm filters. Tissue and substrate blanks were included for each sample and final well concentrations for all samples were 10 mM AtChI, 10 mM DTNB, and 1 mg/ml brain tissue. To normalize activity, total protein content in the brain was determined by Bradford's method (Bradford 1976) using Coomassie Plus-200 Protein Assay Reagent and bovine serum albumin standard (Bio-Rad, Mississauga, ON).

Statistical Analysis

Statistical analyses were all conducted independently for each sex. Length, weight, total number YOY and adult density, *in vitro* steroid production, EROD, and AChE activity were analyzed using analysis of variance (ANOVA). Condition factor, liver size, gonad size and fecundity data were analyzed using analysis of covariance

(ANCOVA) with body size (length or weight) as the covariate. All somatic variables in both types of analyses were $\log_{10}$ transformed. The assumption of homogeneity of slope was tested by examining interaction between the covariate and the site/station variable. Data are presented as indices for ease of comparison. Indices were calculated from the least square mean generated by the ANCOVA divided by the value of the covariate at which the least square mean was calculated. In this manner, the indices presented are an exact representation of the least square means tested in the ANCOVA. Weight corrected for organ weights (liver and gonad) was used for all analyses. Condition factor was calculated as corrected least square mean body weight/length^3 x 100, gonado-somatic index (GSI) as least square mean gonad weight/corrected body weight x 100, liver-somatic index (LSI) as least square mean liver weight/ corrected body weight x 100. Fecundity was calculated as least square mean number of eggs per gram of corrected wet body weight. All statistical analyses were conducted with SYSTAT 8 software.

Length-at-age relationships were modeled using a modified von Bertalanffy equation of the following form: Length = $L\infty \times (1 - 0.96 \times e(-k \times a^{1.2}))$ where $L\infty$ is the length at infinite time, or maximum length, k is the growth constant, and a is age in years. This equation was empirically derived to minimize residual sums of squares and provide best fit for length-at-age data (van den Heuvel unpublished data). Growth relationships were compared statistically using the residual sums of squares method of Chen *et al.* (1992).

Principal components analysis was performed on environmental parameters using Primer v. 6.1.6 to identify the contributions of those parameters to between-site variability. Environmental parameters were normalized prior to analysis. Somatic

variables were ranked for each site at each time period and their sum added to get a single rank sum value for each site. These sums provided a simplified method for detecting and viewing any consistent trends in the data

3.4 RESULTS

Environmental Parameters

Principal Components Analysis (PCA) revealed that more than 75% of the variability in the environmental variable (58% PC1 and PC2) could be attributed to the first three principal components. The PCA reveals a clear separation of Barbara-Weit, Indian, and Wilmot, the most highly eutrophic sites along the first principal component axis. The loadings to this principal component were strongly influenced by the eutrophication variables such as N loading, chlorophyll a and sea lettuce (*Ulva*) density (Fig. 3.2). The variables indicating eelgrass (*Zostera*) density and tidal flushing worked counter to the eutrophication variable in the first principle component. Sites were further separated on the second principle component on the basis of sediment composition as measured by sand versus silt, and percent organic matter.

Length, Weight, Age and Growth

Length and weight of female mummichog were significantly different among sites for all time periods (Table 3.3.1, Appendix B.2). Length of male mummichog was significantly different among sites for all time periods with the exception of August

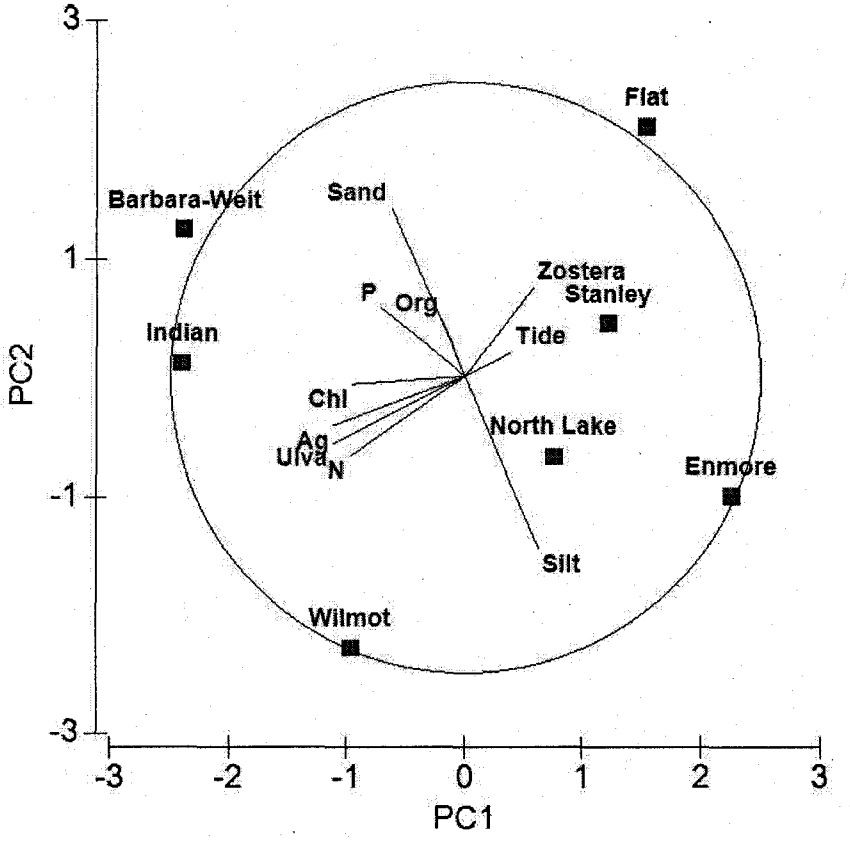

FIGURE 3.2. Principal components analysis of environmental variables related to eutrophication, sediment, and tidal flushing from Barbara-Weit, Enmore, Flat, Indian, North Lake, Stanley and Wilmot for all four sampling periods.

TABLE 3.3.1. Means ± SE of various parameters of adult female mummichog (*Fundulus heteroclitus macrolepidotus*) collected at each of the seven sites at all four time periods. Within each month, different superscript letters indicate significant differences between sites for each parameter ($p<0.05$).

Date	Parameter	Site						
		Enmore	**Flat**	**North Lake**	**Stanley**	**Wilmot**	**Indian**	**Barbara-Weit**
May	N	22	22	23	22	26	20	22
	Length (mm)	75.9 ± 1.2^{ABC}	73.6 ± 2.5^{AB}	84.6 ± 1.9^{C}	84.9 ± 2.5^{C}	98.7 ± 2.1^{D}	67.3 ± 3.9^{A}	81.5 ± 1.8^{BC}
	Weight (g)	4.71 ± 0.27^{ABC}	4.52 ± 0.71^{AB}	7.25 ± 0.69^{BC}	7.51 ± 0.79^{C}	10.80 ± 0.77^{D}	3.98 ± 0.87^{A}	6.39 ± 0.46^{ABC}
	K	1.00^{AB}	0.97^{AB}	1.05^{B}	1.05^{B}	0.95^{A}	1.00^{AB}	1.03^{AB}
	LSI	3.07^{A}	3.33^{A}	2.96^{A}	3.76^{A}	3.50^{A}	3.52^{A}	4.86^{B}
	GSI	3.23^{AB}	4.53^{C}	2.82^{A}	2.73^{A}	3.94^{BC}	3.07^{AB}	4.91^{C}
June	N	20	20	20	20	20	20	20
	Length (mm)	86.5 ± 1.5^{A}	84.1 ± 1.7^{A}	82.3 ± 2.8^{A}	83.8 ± 1.2^{A}	95.5 ± 2.2^{B}	89.4 ± 1.6^{AB}	86.0 ± 2.1^{A}
	Weight (g)	7.83 ± 0.41^{A}	7.77 ± 0.53^{A}	7.59 ± 0.76^{A}	8.15 ± 0.34^{A}	12.74 ± 0.91^{B}	9.82 ± 0.61^{A}	9.17 ± 0.80^{A}
	K	1.00^{AB}	0.99^{A}	1.09^{BC}	1.09^{C}	1.05^{ABC}	1.06^{ABC}	1.10^{C}
	LSI	3.92^{A}	6.56^{D}	3.85^{A}	5.19^{B}	6.41^{CD}	5.27^{B}	5.36^{BC}
	GSI	13.96^{AB}	21.63^{CD}	12.22^{A}	19.91^{CD}	26.36^{D}	19.63^{C}	18.03^{BC}
July	N	20	20	20	20	20	20	20
	Length (mm)	76.4 ± 3.1^{AB}	90.5 ± 2.6^{C}	70.8 ± 2.6^{A}	77.9 ± 3.2^{AB}	85.0 ± 2.6^{BC}	71.4 ± 2.7^{A}	69.0 ± 1.6^{A}
	Weight (g)	6.18 ± 0.75^{AB}	10.39 ± 1.16^{C}	5.21 ± 0.62^{A}	6.73 ± 0.97^{AB}	8.62 ± 0.73^{BC}	5.15 ± 0.73^{A}	4.10 ± 0.38^{A}
	K	1.15	1.01	1.18	1.15	1.19	1.19	1.12
	LSI	3.63^{A}	4.19^{AB}	5.06^{B}	3.41^{A}	4.98^{B}	4.32^{AB}	3.50^{A}

TABLE 3.3.1. Continued

Date	Parameter	Site						
		Enmore	**Flat**	**North Lake**	**Stanley**	**Wilmot**	**Indian**	**Barbara-Weit**
	GSI	2.69	12.25	8.93	6.03	4.30	2.62	3.30
Aug	N	20	20	21	16	20	19	20
	Length (mm)	78.3 ± 1.8^{AB}	75.8 ± 1.8^{A}	79.7 ± 2.2^{AB}	74.3 ± 2.5^{A}	79.4 ± 2.2^{AB}	77.7 ± 3.2^{AB}	85.7 ± 1.7^{B}
	Weight (g)	5.81 ± 0.50^{A}	5.03 ± 0.37^{A}	6.73 ± 0.57^{AB}	5.62 ± 0.56^{A}	6.16 ± 0.54^{AB}	6.32 ± 0.77^{AB}	8.16 ± 0.58^{B}
	K	1.13^{AB}	1.08^{A}	1.22^{CD}	1.26^{D}	1.12^{AB}	1.17^{BC}	1.20^{CD}
	LSI	2.82	2.82	2.78	3.14	3.12	3.17	3.13
	GSI	0.75^{AB}	1.05^{C}	0.72^{A}	0.81^{ABC}	0.95^{BC}	0.69^{A}	0.78^{AB}

TABLE 3.3.2. Means ± SE of various parameters of adult male mummichog (*Fundulus heteroclitus macrolepidotus*) collected at each of the seven sites at all four time periods. Within each month, different superscript letters indicate significant differences between sites for each parameter ($p<0.05$).

Date	Parameter	Site						
		Enmore	**Flat**	**North Lake**	**Stanley**	**Wilmot**	**Indian**	**Barbara-Weit**
May	N	21	23	22	21	23	22	22
	Length (mm)	72.8 ± 1.9^{AB}	71.3 ± 1.7^{A}	76.5 ± 1.7^{AB}	80.2 ± 2.6^{B}	90.2 ± 2.3^{C}	72.2 ± 2.4^{AB}	74.5 ± 0.9^{AB}
	Weight (g)	4.17 ± 0.39^{AB}	3.87 ± 0.34^{A}	5.22 ± 0.50^{AB}	6.19 ± 0.71^{B}	8.12 ± 0.73^{C}	4.21 ± 0.46^{AB}	4.81 ± 0.21^{AB}
	K	1.00^{ABC}	0.99^{AB}	1.06^{BC}	1.05^{BC}	0.96^{A}	0.97^{AB}	1.08^{C}
	LSI	1.84^{A}	2.22^{AB}	2.38^{BC}	2.60^{BC}	2.17^{AB}	2.88^{CD}	3.58^{D}
	GSI	1.95^{BC}	1.81^{BC}	1.12^{A}	1.50^{AB}	1.80^{BC}	1.41^{AB}	2.58^{C}
June	N	20	21	20	20	20	20	20
	Length (mm)	78.1 ± 1.7^{AB}	82.0 ± 1.6^{BC}	82.8 ± 1.4^{BC}	73.0 ± 0.8^{A}	84.9 ± 2.1^{C}	77.9 ± 1.6^{AB}	79.3 ± 1.1^{ABC}
	Weight (g)	5.46 ± 0.39^{A}	6.25 ± 0.40^{AB}	7.10 ± 0.42^{B}	4.80 ± 0.18^{A}	7.09 ± 0.58^{B}	5.55 ± 0.36^{AB}	5.96 ± 0.25^{AB}
	K	1.07^{ABC}	1.02^{A}	1.16^{D}	1.16^{D}	1.03^{AB}	1.08^{BC}	1.10^{CD}
	LSI	1.88^{A}	2.82^{C}	2.01^{AB}	2.25^{ABC}	2.48^{C}	2.36^{BC}	2.68^{C}
	GSI	2.34^{A}	3.94^{B}	2.81^{A}	3.88^{B}	4.08^{B}	3.45^{B}	3.77^{B}
July	N	20	20	20	20	20	20	20
	Length (mm)	73.6 ± 2.7^{BC}	85.2 ± 1.7^{D}	64.0 ± 2.4^{A}	73.9 ± 2.5^{BC}	76.8 ± 2.2^{CD}	68.4 ± 1.8^{ABC}	67.0 ± 2.2^{AB}
	Weight (g)	5.24 ± 0.57^{AB}	7.31 ± 0.54^{C}	3.49 ± 0.49^{A}	5.27 ± 0.51^{AB}	5.61 ± 0.44^{BC}	4.00 ± 0.34^{AB}	3.84 ± 0.45^{AB}
	K	1.17^{B}	1.07^{A}	1.16^{AB}	1.15^{AB}	1.12^{AB}	1.16^{AB}	1.15^{AB}
	LSI	2.24^{AB}	2.19^{AB}	2.63^{BC}	1.92^{A}	2.98^{C}	3.19^{C}	2.26^{AB}

TABLE 3.3.2. Continued

Date	Parameter	Site						
		Enmore	**Flat**	**North Lake**	**Stanley**	**Wilmot**	**Indian**	**Barbara-Weit**
	GSI	1.06	2.69	2.05	2.02	2.28	1.03	1.26
Aug	N	20	20	20	20	20	20	20
	Length (mm)	74.0 ± 1.5	73.2 ± 2.0	80.5 ± 2.6	72.1 ± 2.5	72.0 ± 2.7	79.4 ± 2.7	74.4 ± 1.4
	Weight (g)	5.12 ± 0.32 ^{AB}	4.88 ± 0.48 ^{A}	7.41 ± 0.76 ^{B}	5.19 ± 0.64 ^{AB}	4.98 ± 0.68 ^{AB}	7.07 ± 0.75 ^{AB}	5.36 ± 0.37 ^{AB}
	K	1.19	1.15	1.26	1.26	1.17	1.24	1.23
	LSI	3.37 ^{CD}	2.64 ^{AB}	2.71 ^{ABC}	2.57 ^{A}	3.48 ^{D}	3.70 ^{D}	3.27 ^{BCD}
	GSI	0.17 ^{AB}	0.22 ^{ABC}	0.23 ^{BC}	0.21 ^{AB}	0.32 ^{C}	0.15 ^{A}	0.21 ^{AB}

(Table 3.3.2, Appendix B.2). Weight of male mummichog, however, was significantly different among sites for all of the time periods (Table 3.3.2, Appendix B.2). With the exception of the July sample, Wilmot had the largest (length and weight) fish, compared to the other estuaries. Flat River fish were significantly larger in the July sampling for both males and females.

Ages of fish ranged from one to four years with the majority of fish sampled being in the two-year-old age class, and males having a larger number of older fish (Table 3.4). There were significant differences between sites for both sexes ($♀F_{6,131}$ = 11.226, $p< 0.05$; $♂F_{6,133}$ = 10.852, $p<0.05$). Enmore fish were primarily age 3 with some age 1 fish and were significantly different from all other sites, with the exception of Flat males (Tukey's, $p<0.05$). Males at Flat were significantly different from those at North Lake, Stanley and Indian (Tukey's, $p<0.05$). Wilmot had a greater proportion of age 3 and age 4 fish than other sites and females were significantly different from North Lake (Tukey's, $p<0.05$). Stanley fish were all age 2 fish. Barbara-Weit, North Lake, and Indian all had some age 1 fish.

Growth curves, as assessed by the modified von Bertalanffy function, differed significantly among sites for male and female mummichog caught in the June sampling ($♀F_{18,117}$ = 2.353, $p< 0.05$; $♂F_{18,119}$ = 5.080, $p<0.05$; Fig. 3.3.1 and 3.3.2, Table 3.5). Growth of female mummichog caught at Wilmot was significantly increased from those caught at Enmore, Flat and Stanley (Appendix B.3). Indian and Flat females also differed significantly. Male mummichog caught at Wilmot and North Lake had significantly increased growth from that of males caught at Barbara-Weit, Indian, Stanley and Enmore.

TABLE 3.4. Total number of mummichog aged, mean age, number of fish at each age, maximum length (Lmax), and growth constant (k) calculated using the ages of fish, keeping the sexes separate, and a modified von Bertalanffy equation for fish sampled in June at all of the sites.

	Females					Males				
Site	**N**	**Mean Age**	**# at Age**	**Lmax (cm)**	**k**	**N**	**Mean Age**	**# at Age**	**Lmax (cm)**	**k**
Enmore	20	2.8	1: 0 2: 4 3: 16	9.063	0.936	21	2.9	1: 0 2: 2 3: 17	8.662	0.663
Flat	19	2.1	1: 0 2: 17 3: 2	11.400	0.541	19	2.5	1: 0 2: 11 3: 9 4: 1	8.903	0.899
North Lake	20	1.9	1: 4 2: 14 3: 2	9.670	0.962	20	2.1	1: 0 2: 18 3: 2	10.511	0.629
Stanley	20	2.0	1: 0 2: 20 3: 0	12.047	0.500	20	2.0	1: 0 2: 20 3: 0	9.000	0.708
Wilmot	19	2.3	1: 0 2: 14 3: 4 4: 1	10.672	0.863	20	2.4	1: 0 2: 12 3: 8	10.616	0.572
Indian	20	2.2	1: 0 2: 17 3: 3	9.121	1.613	20	2.0	1: 2 2: 16 3: 2	8.094	1.617
Barbara-Weit	20	2.0	1: 1 2: 18 3: 1	9.241	1.202	20	2.2	1: 1 2: 16 3: 2 4: 1	8.032	2.076

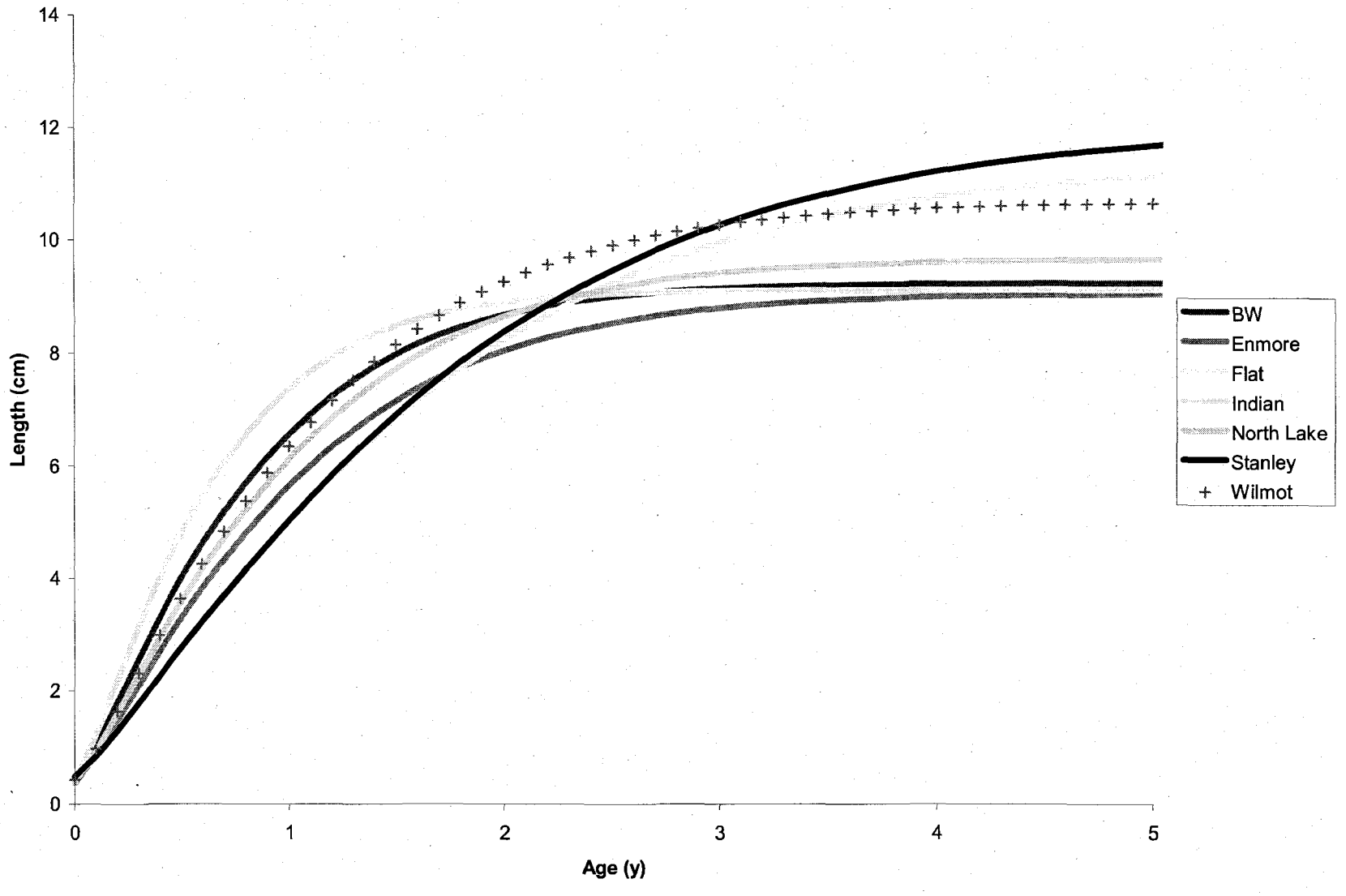

FIGURE 3.3.1. Growth curves for female mummichog, calculated using the ages of fish, and a modified von Bertalanffy equation for fish sampled in June at all of the sites.

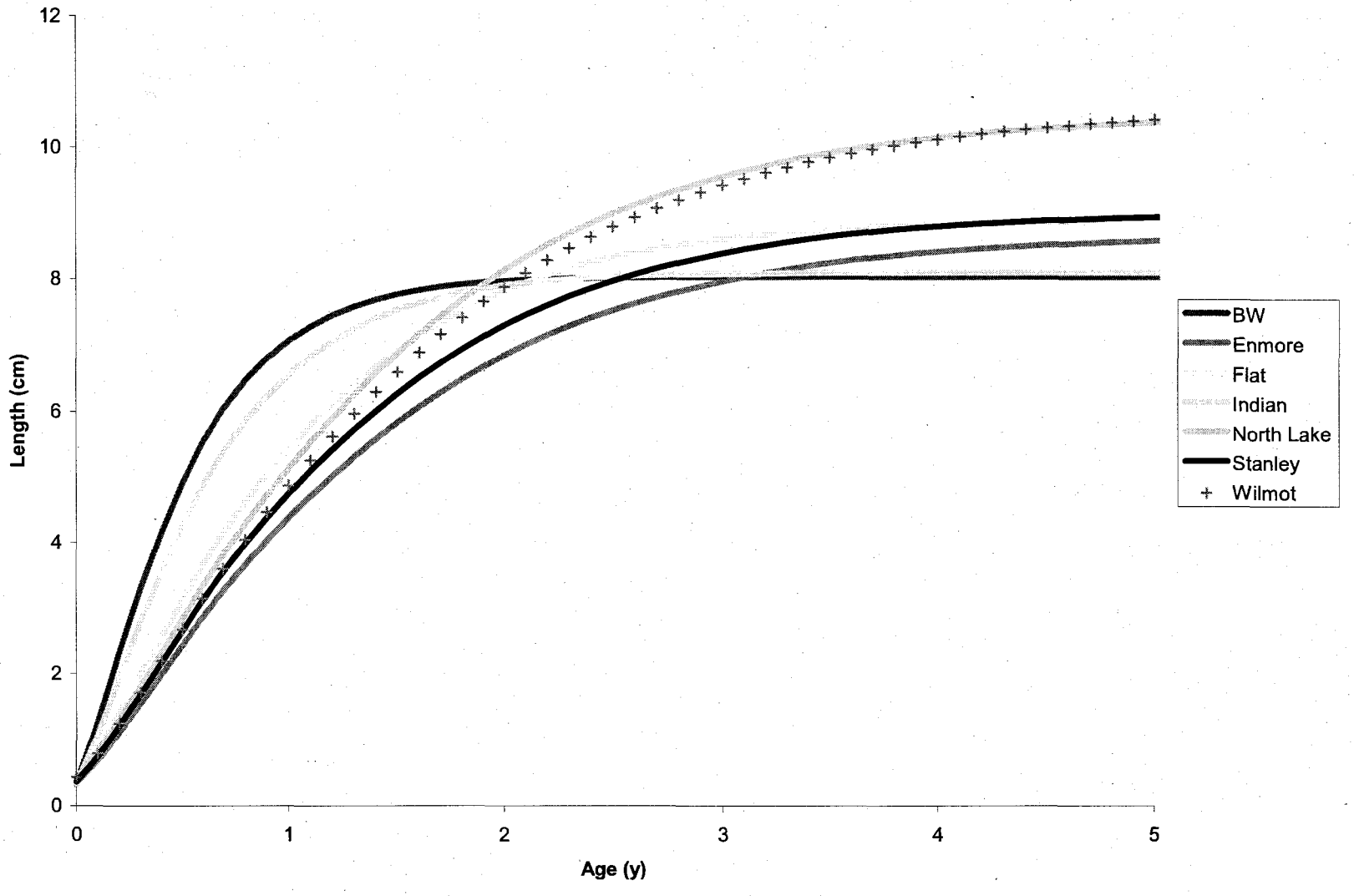

FIGURE 3.3.2. Growth curves for male mummichog, calculated using the ages of fish, and a modified von Bertalanffy equation for fish sampled in June at all of the sites.

TABLE 3.5. Mean (± SE) lengths of age 2 fish sampled during the June sampling period at all of the sites. Difference superscript letters indicate significant differences among sites ($p<0.05$).

	Females		Males	
Site	**N**	**Mean Length (± SE)**	**N**	**Mean Length (± SE)**
Enmore	4	8.05 ± 0.31^{A}	2	6.85 ± 0.35^{AB}
Flat	17	8.24 ± 0.14^{A}	11	7.83 ± 0.19^{ABC}
North Lake	14	8.61 ± 0.21^{AB}	18	8.13 ± 0.11^{C}
Stanley	20	8.38 ± 0.12^{A}	20	7.30 ± 0.08^{A}
Wilmot	14	9.29 ± 0.21^{B}	12	7.88 ± 0.09^{BC}
Indian	17	8.91 ± 0.18^{AB}	16	7.98 ± 0.14^{C}
Barbara-Weit	18	8.73 ± 0.19^{AB}	16	8.04 ± 0.11^{C}

Significant differences also occurred between Enmore and Flat, and between Stanley and both Indian and Barbara-Weit.

Length of fish of the two-year-old age class also showed significant differences between sites for both females and males ($♀F_{6,97} = 4.272$, $p = 0.001$; $♂F_{6,88} = 7.694$, $p<0.001$). For female mummichog the Wilmot fish grew significantly larger than the fish at Enmore, Flat, and Stanley. The mean length of females of age 2 ranged from 8.05 ± 0.31 at Enmore to 9.29 ± 0.21 at Wilmot (Table 3.5). For male fish, Stanley had significantly smaller length-at-age than Indian, North Lake, Wilmot, and Barbara-Weit. Comparisons between male fish at Enmore and other sites were limited by the low number of age 2 fish caught at Enmore (n=2). The mean length of males ranged from 7.30 ± 0.08 at Stanley to 8.31 ± 0.11 at North Lake.

Condition Factor, LSI, and GSI

Significant differences in condition, liver size, and gonad size among the sites and at the different sampling periods were observed (Tables 3.3.1 and 3.3.2, pgs 75-78). Condition factor increased steadily throughout the summer in both sexes with Flat being consistently and significantly lower than the other sites. Livers in both males and females had increased size at the Wilmot, Barbara-Weit, and Indian sites. In females, the liver and gonad sizes increased significantly from May to June (Table 3.3.1). Wilmot females experienced a six-fold increase in GSI during this time. This increase was significantly different from that of both Enmore and North Lake which only experienced four-fold increases. Interactions between site and body weights occurred during the July analyses,

however, with the exception of Flat and North Lake, all of the sites had decreased to gonad sizes resembling those observed in May.

Rank sums (Table 3.6) were used to simplify trends in indices over the four sampling periods (Table 3.7). Trends were similar in both males and females. Consistently observed patterns were that condition factor was highest at North Lake and Stanley, and lowest at Flat. Liver size was highest at Wilmot, Indian and Barbara-Weit. Gonad size was highest at Flat and Wilmot. Wilmot also had the largest mummichog as compared to all other sites.

Fecundity

Fecundity (Table 3.8), the mean number of mature eggs, differed significantly among sites in June ($F_{6,75} = 4.838$, $p < 0.001$) and July ($F_{6,40} = 2.840$, $p=0.021$). Wilmot fish had higher fecundity in June and Flat fish had higher fecundity in July. The number of mature eggs per gram carcass weight (Table 3.8) varied in June from 10.12 at North Lake to 31.99 at Wilmot, and in July from 1.29 at Barbara-Weit to 17.58 at North Lake. In June there was a strong pattern of the more agriculturally impacted sites having higher fecundity.

Length frequency and population density

Fish populations at all sites were dominated by YOY in August (Fig. 3.4). Wilmot had a lower proportion of YOY and a higher proportion of larger fish compared to the other sites. Indian and Barbara-Weit had a significantly greater total abundance of adult ($F_{6,21} = 7.852$, $p<0.001$) and YOY ($F_{6,21} = 11.117$, $p<0.001$) mummichog (Fig. 3.5). The

TABLE 3.6. Rank sums of somatic measurements collected over the four time periods for each sex at each site. Ranks at each time period were calculated from highest to lowest measurement values, thus lower ranks indicate a higher organ or body measurement. Ranks were then summed to get a single overall number for each parameter at each site.

Sex, Site	Parameter				
	Length	**Weight**	**LSI**	**GSI**	**CF**
Female					
Enmore	16	19	22	21	20
Flat	18	20	15	6	27
North Lake	18	17	22	21	9
Stanley	18	15	16	16	9
Wilmot	7	8	12	10	19
Indian	19	18	11	23	15
Barbara-Weit	16	15	13	15	13
Male					
Enmore	18	21	22	21	15
Flat	16	18	18	9	26
North Lake	13	12	18	18	7
Stanley	18	16	22	16	10
Wilmot	11	11	13	8	25
Indian	19	17	8	25	16
Barbara-Weit	17	17	11	15	12

TABLE 3.7. Ranks (from highest to lowest) of fish characteristics grouped according to the summary categories of age, energy allocation and energy storage for all seven estuaries. Where endpoints are separated by sex, female then male rank is indicated, separated by a comma.

	Site	Age Structure		Energy Allocation			Energy Storage		Demographics	
		Mean Age	YOY:Adult Ratio	Growth Lmax	GSI June	Fecundity June	K	LSI	YOY Density	Adult Density
Highly	Wilmot	2, 3	6	3, 1	1, 1	1	5, 6	2, 3	4	5
Impacted	Indian	3, 6	2	6, 6	4, 5	2	4, 5	1, 1	2	2
	Barbara-Weit	6, 4	3	5, 7	5, 4	4	3, 3	3, 2	1	1
Moderately	Stanley	5, 7	4	1, 3	3, 3	3	2, 2	5, 7	7	7
Impacted	Flat	4, 2	5	2, 4	2, 2	5	7, 7	4, 4	6	6
	North Lake	7, 5	1	4, 2	7, 6	6	1, 1	6, 5	3	3
	Enmore	1, 1	7	7, 5	6, 7	7	6, 4	7, 6	5	4

TABLE 3.8. The number of mature eggs per gram carcass weight was calculated using the least square means generated from ANCOVA (with carcass weight as the covariate) from eight females per site per sampling period. The fecundity (mean number of mature eggs) (± SE) was calculated using the same eight females at each site. Different superscript letters indicate significant differences among sites ($p<0.05$).

	June		July	
Site	**# mature eggs/g carcass weight**	**Fecundity**	**# mature eggs/g carcass weight**	**Fecundity**
Enmore	11.25	82.33 ± 12.59^{AB}	5.81	30.42 ± 11.24
Flat	16.41	98.58 ± 15.94^{ABC}	16.41	183.33 ± 53.42
North Lake	10.12	86.58 ± 11.40^{A}	17.58	53.17 ± 12.69
Stanley	19.50	135.83 ± 10.84^{ABC}	7.29	35.83 ± 11.85
Wilmot	31.99	332.17 ± 32.70^{C}	5.53	55.25 ± 20.94
Indian	23.39	183.00 ± 23.90^{BC}	5.61	18.50 ± 12.55
Barbara-Weit	12.35	124.33 ± 24.12^{AB}	1.29	0.92 ± 0.92

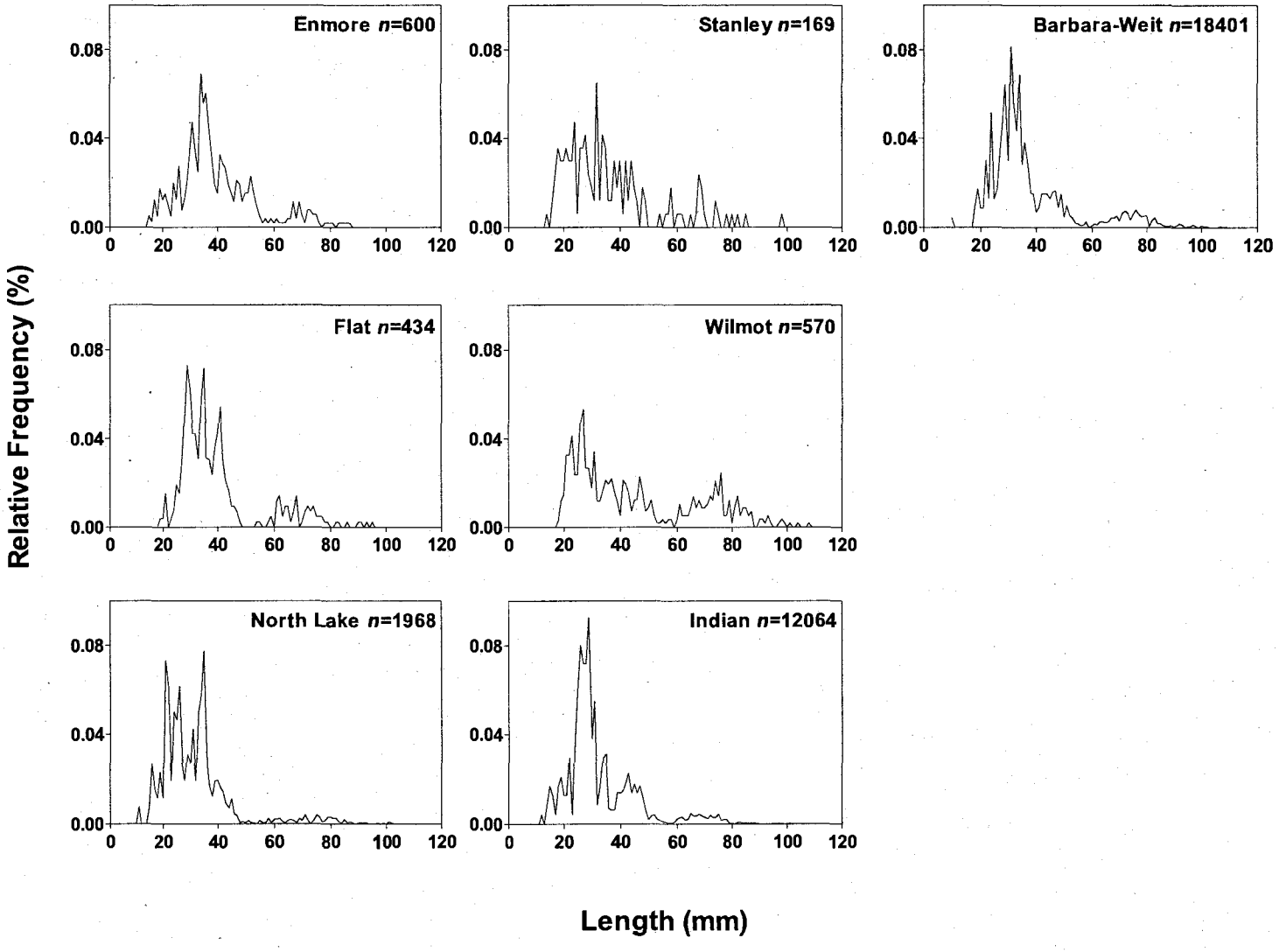

FIGURE 3.4. Length frequency distributions for mummichog collected at all seven estuaries in August 2007. Frequencies are given as % of sample for comparison purposes.

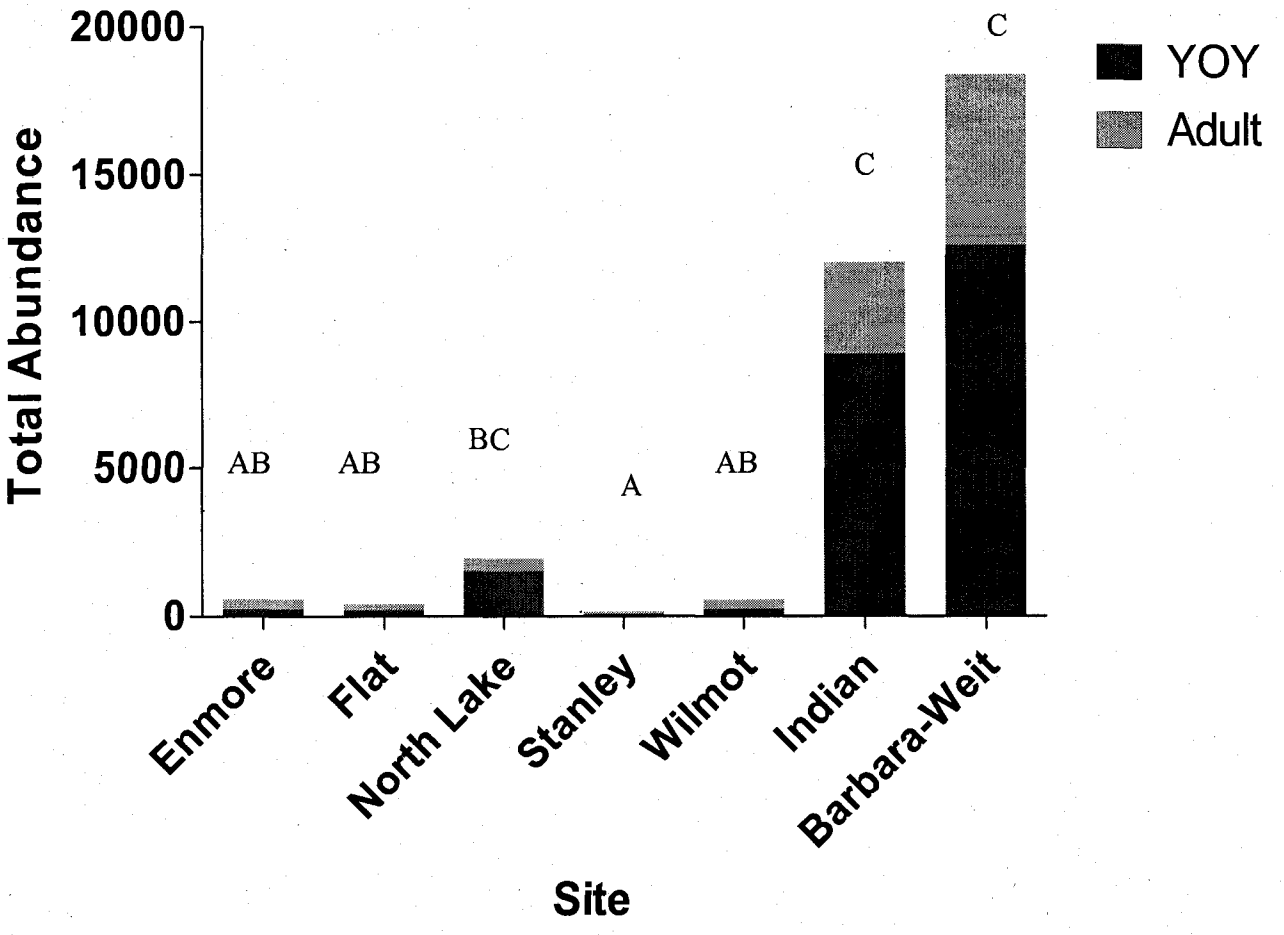

FIGURE 3.5. Total abundance of young-of-the-year (YOY) and adult mummichog caught in four seine hauls (approximately 225 m^2 each) conducted in August 2007 at each of the seven estuaries, PEI. Different letters indicate significant differences among sites, $p < 0.05$

ratio of YOY to adult fish ranged from 0.5 - 3.5, with North Lake, Indian and Barbara-Weit being the highest.

Chemical and biochemical indicators of exposure

Pesticide levels in sediments were all below detection limits (Table 3.9). The EROD levels in males ranged from 5 – 26 pmol/min/mg for all sites over the 4 sampling periods (Table 3.10). There were significant differences between sites at each sampling period with EROD levels increasing as the summer progressed. AChE activity was not depressed at any of the sites during any time period (Table 3.10). Significant differences occurred in the July sampling with Indian mummichog having higher AChE activity ($F_{6,68} = 4.6$, $p < 0.001$). *In vitro* steroid production was significantly different in the female fish, with the higher nutrient input sites, Wilmot, Indian and Barbara-Weit, fish having lower production of both 17β-estradiol ($F_{6,50} = 6.85$, $p < 0.001$) and testosterone ($F_{6,49} = 5.95$, $p < 0.001$; Fig. 3.6). No significant differences were observed in the male fish.

3.5 DISCUSSION

The main goal of this study was to examine the impacts of cumulative, point and non-point source pollution on mummichog populations and determine the stressor(s) having the most substantial influence. An effects-based assessment in combination with biochemical analyses was conducted in seven estuaries spanning a range of agricultural land-use and additional point-source inputs. Eutrophication appeared to be a primary

TABLE 3.9. Detection limits for pesticides analyzed using a Sciex API2000 (MDS Sciex, Concord, ON) LC-MS-MS system equipped with an atmospheric pressure photoionization (APPI, PhotoSpray) source and an Agilent 1100 HPLC (Agilent, Mississauga, ON).

Pesticide	**Detection Limit**
Imidacloprid	61.4 pg/g
Metribuzin	7.3 pg/g
Metobromuron	47.9 ng/g
Metalaxyl	4.2 ng/g
Linuron	9.6 ng/g
Carbaryl	6.4 ng/g
Azinphos methyl	36.6 ng/g

TABLE 3.10. Mean (± SE) 7-ethoxyresorufin-*O*-deethylase (EROD) activity (pmol/min/mg) and mean acetylcholinesterase (AChE) activity (U/mg protein) in male mummichog collected from all seven sites at all time periods. Differences among sites within a time period ($p < 0.05$) are denoted by different superscript letters.

Site	EROD (pmol/min/mg)				AChE (U/mg protein)			
	May	June	July	August	May	June	July	August
Enmore	12.6 ± 2.1^{BC}	12.5 ± 1.7^{B}	18.7 ± 1.8^{B}	18.5 ± 1.0^{AB}	1.48 ± 0.14	1.57 ± 0.05	1.75 ± 0.10^{A}	2.24 ± 0.19
Flat	10.3 ± 1.5^{ABC}	11.6 ± 0.6^{AB}	16.1 ± 1.7^{B}	25.7 ± 2.5^{B}	1.62 ± 0.14	1.62 ± 0.07	1.64 ± 0.17^{A}	2.01 ± 0.23
North Lake	15.8 ± 1.1^{C}	11.3 ± 1.1^{AB}	8.6 ± 1.5^{A}	17.9 ± 1.6^{A}	1.51 ± 0.14	1.53 ± 0.09	1.93 ± 0.08^{AB}	2.19 ± 0.18
Stanley	11.0 ± 1.0^{ABC}	7.4 ± 1.0^{A}	16.2 ± 1.2^{B}	24.8 ± 2.1^{AB}	1.30 ± 0.28	1.55 ± 0.06	1.56 ± 0.07^{A}	2.28 ± 0.11
Wilmot	15.4 ± 0.7^{C}	10.9 ± 0.9^{AB}	16.8 ± 1.2^{B}	21.3 ± 1.5^{AB}	1.53 ± 0.20	1.58 ± 0.10	1.47 ± 0.07^{A}	2.20 ± 0.18
Indian	5.3 ± 2.0^{A}	10.0 ± 0.8^{AB}	15.2 ± 1.6^{AB}	21.2 ± 1.3^{AB}	1.43 ± 0.17	1.78 ± 0.05	2.53 ± 0.45^{B}	2.29 ± 0.16
Barbara-Weit	8.1 ± 0.5^{AB}	8.8 ± 1.3^{AB}	21.0 ± 1.6^{B}	25.9 ± 2.0^{B}	1.69 ± 0.11	1.64 ± 0.07	1.92 ± 0.09^{AB}	2.13 ± 0.21

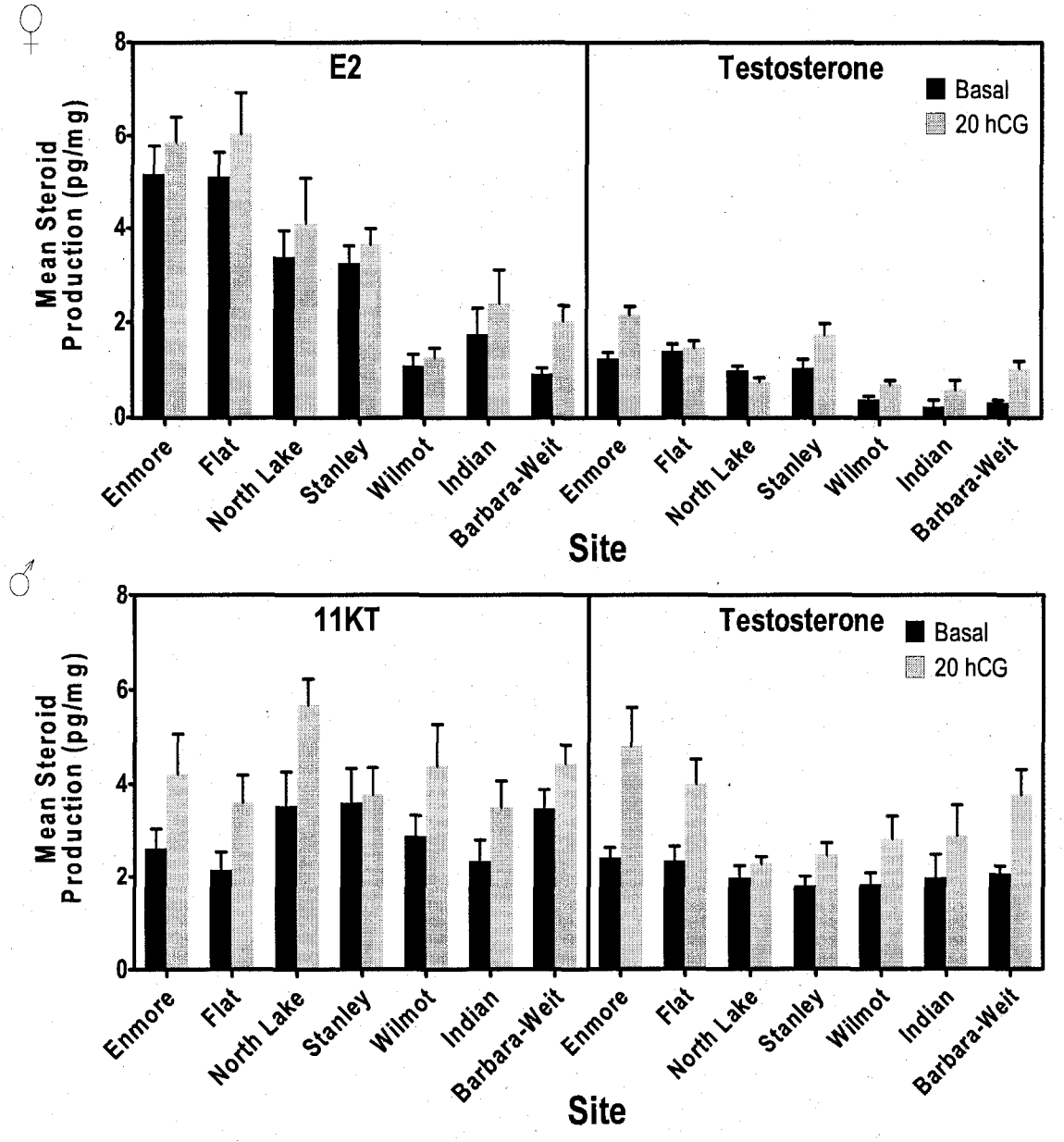

FIGURE 3.6. Mean (± SE) steroid production (pg/mg) for eight females or males per site collected during the May sampling period.

stressor affecting mummichog populations. Their responses to this stressor however did not fit the typical pattern outlined by Gibbons and Munkittrick (1994). The abundance of mummichog in heavily eutrophic estuaries was significantly increased, with a much higher proportion of YOY fish. Liver size, an indicator of energy storage, was also larger at these sites. Other changes in growth, energy storage and use were more variable and not consistently linked with trophic status.

Gray and Munkittrick (2004) studied the impacts of high agricultural activity, primarily potato production, on slimy sculpin (*Cottus cognatus*) populations in freshwater streams. Sculpin exhibited increased growth and condition, decreased liver and gonad size, decreased fecundity, decreased proportion of YOY and overall lower population density. Another study using the creek chub (*Semotilus atromaculatus*) as a monitoring species found reduced growth rate which resulted in decreased size-at-age and increased age-to-maturity (Fitzgerald *et al.* 1999). They also found YOY to be either limited or absent from many of the impacted sites. In comparison, our study showed an increase in liver size, fecundity, YOY, and overall population density in response to agricultural impacts. Growth, condition factor and gonad size could not be consistently associated with the trophic status of the estuary. One major difference between my study and previous studies on the impacts of agricultural activity was that my study was conducted on estuarine populations. Fish in these environments are better adapted to abrupt changes in water quality and increased turbidity. Thus some of the main stressors associated with agricultural activity (increased sediment and pesticides) may have little impact on estuarine fish populations. Nutrient enrichment however leads to important changes in the estuarine environment which may alter energy use and storage in fish populations in

different patterns from those seen in streams which were agriculturally impacted. Estuarine ecosystems undergo continual tidal flushing which often serves to move the nutrients back and forth through the estuary. This could potentially increase the severity of impacts related to nutrient enrichment on the fish populations. Furthermore, the mummichog is a highly tolerant and adaptable species (Nacci *et al.* 2002, Jones *et al.* 2008) that appears to benefit from the environmental conditions created by agricultural inputs. Studies conducted within our lab have indicated that mummichog thrive on the increase in amphipods resulting from increases in sea lettuce density (S. Gill, unpublished data; K.A. Campbell, unpublished data).

Three of the estuaries, Wilmot, Indian, and Barbara-Weit, separated out as being similar in terms of eutrophication. These sites had increased nitrate and phosphate loadings, high chlorophyll a and percent coverage of *Ulva*, and a high percentage of agricultural land surrounding the watershed. Mummichog sampled in these estuaries were thus expected to follow the typical eutrophication pattern outlined by Gibbons and Munkittrick (1994). This pattern is one of increased growth and reproduction resulting in a decreased age structure (higher proportion of YOY). Fish at Indian and Barbara-Weit had increased liver size, population density and proportion of YOY. Growth was very fast and plateaus earlier indicating that fish are reaching a much smaller maximum size. Wilmot fish responded differently with increased liver and gonad size, higher fecundity, but lower population density and proportion of YOY. Growth was slower but maximum size was much larger. Although neither one of these patterns exactly matched the typical eutrophication pattern they are both quite similar. This result also indicates that eutrophication was not the sole stressor on these environments.

Barbara-Weit and Indian are both small north shore estuaries and are thus less tidally influenced than Wilmot. Reduced flushing/increased residence time can allow nutrients to accumulate, macroalgae to proliferate and water quality to deteriorate resulting in increased hypoxic/anoxic events (Cloern 2001). Hypoxic conditions have been related to decreased growth rates in a number of fish species (Stierhoff *et al.* 2003; McNatt and Rice 2004). Initial growth rate at these sites was quite rapid and maximum size was attained sooner. Since typically hypoxia occurs later in the summer when maximum algal growth is reached it is possible that there was no initial effect on growth rate. Food availability may have been greater prior to hypoxia. The lower fecundity and maximum growth at these sites may also be a result of increased population density. Fish do not grow as large because of space requirements, yet food is plentiful so faster growth is possible. Increased fecundity and gonad size is not necessary because there are a greater number of fish reproducing and surviving. The estuaries, being much smaller, are more likely to experience density effects. Some study has been done on density dependant effects. One study showed that population fluctuations in density and growth of common sardine, *Strangomera bentincki*, and anchovy, *Engraulis ringens* have a strong density dependant component (Pedraza-Garcia and Cubillos 2008).

Wilmot fish expend greater energy in reproducing but survival rate of YOY is low compared to the other eutrophic sites. One major difference between these sites is the substrate composition. Wilmot substrate had greater silt content which may explain why survival of young was impacted. Wilmot River is known to incur heavy turbidity during rain events at higher levels than the smaller Indian and Barbara-Weit streams. Furthermore, the Indian estuary is buffered by a freshwater impoundment and thus is less

likely to incur serious siltation than Wilmot. Increased silt may smother eggs, reduce the abundance and variety of macroinvertebrates or clog fish gills. A study by Engstroem-Oest and Mattila (2008) found that larval pike survival and eventual recruitment into the population was negatively impacted by increased algae-induced turbidity. Other studies on freshwater salmonid populations have found decreased survival of eggs and alevin as a result of increased loads of fine sediment (Cunjak *et al.* 2002; Curry and MacNeill 2004). The reduction in YOY at Wilmot does not appear to be a result of adult reproductive dysfunction. Despite the reduction in steroid hormones at the beginning of the reproductive season fecundity at this site was greatly increased in comparison to all other sites.

In vitro steroid production in females clearly illustrated a delay in gonadal maturation for the highly eutrophic sites, Wilmot, Indian and Barbara-Weit. This delay in maturation cannot be attributed to lower water temperatures or differences in photoperiod, suggested cues for reproduction in mummichog (Day and Taylor 1984, Taylor 1986). Water temperatures in May at both Wilmot and Indian were elevated compared to the other study sites. Condition was lower at both Wilmot and Indian at this time suggesting that perhaps the availability or quality of food was impacted, though this does not appear to be the case for fish at Barbara-Weit. Leblanc and Couillard 1997 found a similar delay in the onset of reproduction of mummichog located in estuaries impacted by bleached-kraft mill effluent (BKME; pulp and paper). They also found that these fish invested more intensely in reproduction than fish from other sites despite this initial delay. Their results confirm our own observations, suggesting that perhaps the

nutrient enrichment at these sites allows for increased reproduction and higher overall population densities.

Fecundity in June followed eutrophication status of the estuaries quite closely. However in July this pattern was lost. Other studies in New Brunswick found that the spawning period of mummichog was nearly finished by beginning to mid July (Leblanc *et al.* 1997; McMullin 2008). We also found this to be true for mummichog caught in PEI estuaries (Chapter 2). Thus the fecundity measured in July is less representative of the seasonal reproduction for our sites.

Pesticides were not detected in the sediments of any of the estuaries we studied. This coincided with a lack of EROD induction in our fish. Significant differences among sites and times did exist. This appears to be more related to seasonal effects then to an actual exposure to toxicants. In comparison, mummichog exposed to pulp mill effluent (with known CYP1A agonists) in the Miramichi Estuary, New Brunswick Canada had a 2.5 fold induction from those of reference fish (Couillard and Légaré 1994). Additionally, in a study on the induction of cytochrome P4501A by polychlorinated terphenyl formulation Aroclor 5432 in mummichog, the control fish had baseline levels of ~ 30 pmol/mg/min (Gallagher *et al.* 1995). The EROD levels in the fish sampled in this study were all less than 30 pmol/mg/mm, further indication that induction was not occurring.

Acetylcholinesterase enzyme activity was not inhibited in my fish. Within recent years, due to a number of suspected related fish kills, the organophosphate (OP) insectide azinphos-methyl, has been phased out of use in Canada (Pest Management Regulatory Agency, Health Canada 2004). OPs are generally rapidly degrading (Fulton and Key 2001) and thus their continued presence in our sediment samples would be unlikely.

My study showed that mummichog located in areas of intense agriculture and increased nutrient loads due to point and non-point source inputs had increased abundance and liver size. Survival of YOY was impacted at the Wilmot site, which may be related to substrate type. There was a significant difference in density of fish between impacted and non-impacted sites. Due to the highly adaptable and resilient nature of the mummichog we propose that the abundance of mummichog captured at a site in comparison to other species may be a better indicator of ecosystem health.

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CHAPTER 4

CONCLUSIONS

4.1 CONCLUSION

The first objective of this M.Sc. research was focused on evaluating the suitability and methodology for the use of the northern mummichog (*Fundulus heteroclitus macrolepidotus*) as a monitoring species. A study of the temporal and spatial variability in measures of energy use and storage for mummichog populations in three estuaries was conducted.

The null hypotheses were that mummichog somatic parameters (length, weight, condition factor, gonad and liver weights) would not differ temporally. Thus the combination of sampling times would not provide a detectable difference between sites of varying pollution levels. It was also hypothesized that mummichog would not follow a lunar cycle and populations would not differ spatially within an estuary. This study illustrated tremendous variability in somatic endpoints over the spawning period and thus the null hypothesis of no temporal difference was rejected. The mummichog sampled in this study were characterized by one major spawning peak with no apparent lunar periodicity. The timing of this peak was different at each of the estuaries thus it was difficult to obtain a reliable estimate of reproductive allocation. It was concluded from these data that if mummichog are to be a useful monitoring species, multiple sampling periods around the main spawning peak must be used to gauge reproductive output. Furthermore, as most gonadal development occurs in the two weeks prior to first spawning, gonad size does not provide a useful tool to estimate gonadal energy allocation prior to this time.

The second null hypothesis was that mummichog population density and somatic parameters would not differ between different parts of an estuary. Despite substantial

differences in environment between upper and lower estuaries, somatic parameters were not sensitive enough to detect differences with the exception of liver size, length and weight. Fish located in the more impacted (eutrophic) upper estuary were longer, heavier and had larger livers, Differences in population densities were strongly indicative of habitat quality with higher population densities in the more impacted upper estuary. This suggests that mummichog populations respond to nutrient enrichment conditions through rapid proliferation. Somatic indices are typically density dependent and in the mummichog such responses may be tempered by the ability of this species to rapidly increase populations to utilize existing resources.

The second study examined the cumulative impacts of point and non-point source inputs on mummichog populations over a four month period using a combination of biochemical and somatic parameters. The null hypothesis was that mummichog would not respond to eutrophication with a typical pattern of increased reproduction, increased energy storage, increased growth, and a decreased age structure (more YOY; Gibbons and Munkittrick 1994). The degree of eutrophication would be detectable as a greater intensity of effects in fish caught at the more nutrient-enriched sites. Eutrophication was thought to be directly related to the nutrient loading and percent of surrounding land in agricultural production. It was also hypothesized that fish at the high agricultural sites would not exhibit a metabolic disruption pattern that may be a result of exposure to pesticides. This pattern is one of increased age structure, increased energy storage, and decreased energy utilization (reproduction; Gibbons and Munkittrick 1994).

Environmental variables showed a separation of the seven sites into three that were most affected by eutrophication and four sites that were less affected. Results of this

study showed two patterns of response to these environmental factors. The first was decreased pre-spawning steroid hormones in females, increased liver size (energy storage), population density and proportion of YOY. This response was observed in fish at the Indian and Barbara-Weit sites. The second response was decreased pre-spawning steroid hormones in females, increased liver and gonad size, increased fecundity and population density, decreased proportion of YOY with a skewed population structure towards larger, older fish. This response was observed in the Wilmot site. The major difference between the three sites was sediment type. The first two sites had sediment composed primarily of sand and organic matter whereas the latter site had a larger proportion of fine silt sediment. Neither one of these responses were typical of either a eutrophication or a metabolic disruption pattern according to previously documented fish response patterns. In addition, pesticides were not present in detectable levels in sediment within the estuaries, nor did the biochemical measures of exposure AChE or MFO indicate exposure to organic compounds. This may suggest that they are flushed out of the system fairly rapidly, or are not persistent.

Mummichog have many characteristics which would make them suitable monitoring species. They have high site fidelity, sufficient abundances at all sites, short life spans, and early age to maturation. Estuarine fish are naturally hardier than their freshwater counterparts due to their continually fluctuating environment. Thus they are less likely to fit the patterns of response outlined in the cumulative effects assessment or EEM frameworks (based on Gibbons and Munkittrick 1994). It is not surprising then to find that the mummichog does not respond in typical fashion to the eutrophication in Island estuaries. However, the mummichog does appear to respond to the increased food

availability at the eutrophic sites. The increase in density, particularly YOY, may prove to be a useful indicator of eutrophication. Somatic indices are of less importance in the mummichog because of their prolonged and highly variable spawning period. As well, energetic parameters may be influenced by intraspecific competition. Since many responses to environmental effects are species specific it is important that we understand what our species of choice is telling us. Thus more study on the mummichog's response pattern to a variety of pollution types is needed before we can begin to understand the differences we detected. In the meantime I conclude that the population density of the mummichog in relation to that of other species present may prove to be a useful indicator of environmental effects.

From the data collected in this study, the impact of nutrient enrichment appears to be one of the foremost threats to PEI's estuaries. The increasingly large algal blooms resulting in anoxic events linked to nutrient enrichment have resulted in a degraded environment in which mummichog appear to thrive. With regards to nutrient source, the results from North Lake in particular indicate that other sources aside from agricultural land use can be involved in this eutrophication or that other factors such as tidal flushing can be significant modifiers of system responses to inputs. Determining all the potential sources of nutrients will aid in a better understanding of mummichog responses.

4.2 FUTURE CONSIDERATIONS

Estuaries are complicated environments for conducting EEM assessments. A major research need was the selection of a suitable monitoring species. The results of this

study have led to many additional questions with regards to both the estuarine environment and the mummichog and its particular response patterns. The following list includes some possible suggestions for future research.

- More study on the movements of mummichog. The use of deeper water habitats pre- and post-spawning and the schooling behavior of the different age classes could prove useful in population surveys.
- An assessment of food availability at monitored sites. Environmental factors which may influence the growth and energy availability to the mummichog could be used to explain site differences.
- Determining the impacts of fine sediments on survival of young. Assessing impacts of sedimentation on egg survival and other biota could be used to understand differences in recruitment.
- Laboratory investigations of EROD and AChE enzyme activity in response to controlled amounts of pesticides. Detailed investigations of baseline and seasonal variations in biochemical activity in mummichog need to be conducted. This information can then be used to monitor changes in fish population responses in conjunction with input of particular agricultural stressors.

4.3 REFERENCES

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APPENDIX A:

WATER CHEMISTRY AND STATISTICAL RESULTS FOR CHAPTER 2

TABLE A.1. Water quality parameters at all time periods from May – August 2007 for the three estuaries, North Lake, Stanley and Wilmot, PEI. Missing readings are marked N/A.

Site	Date	Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mg/L)	Dissolved Oxygen (% sat)
North Lake	May 9	10.9	18.1	10.38	N/A
	May 23	7.3	23.4	12.7	107.6
	June 1	12.5	20.5	12.8	N/A
	June 8	11.55	29.43	2.9	32.4
	June 14	14.6	22.1	N/A	N/A
	June 22	14.64	24.75	11.5	129
	June 29	17.29	27.89	11.73	144.3
	July 6	17.3	28.33	10.98	135.8
	July 12	22.33	23.72	17.66	233.3
	July 20	21.23	28.44	13.89	N/A
	July 27	22.73	27.65	7.43	102.4
	Aug	18.4	26.7	0.6	7.3
Stanley	May 9	10.9	18.1	11.12	N/A
	May 23	12.1	20.4	12.87	136
	June 1	13.7	17.7	12.2	N/A
	June 8	16.56	19.06	4.52	52
	June 14	16.7	18.6	N/A	N/A
	June 22	17.88	23.68	11.39	138.7
	June 29	21.68	19.62	19.92	253.6
	July 6	18.3	23.94	10.61	130
	July 12	22.34	25.87	12.94	172.8
	July 20	21.46	25.16	12.16	N/A
	July 27	25.97	24.46	14.56	205.9
	Aug	17.4	24.4	4.6	55.8
Wilmot	May 9	15.4	24.8	8.01	96.3
	May 23	12.1	23.2	12.04	125.8
	June 1	12.1	18.9	12.15	N/A
	June 8	17.77	13.85	4.57	52.3
	June 14	15.2	22.8	N/A	N/A
	June 22	16.87	17.16	10.25	117
	June 29	20.95	14.4	16.46	197.6
	July 6	17.28	19.3	11.74	136.9
	July 12	23.79	15.94	14.77	191.6
	July 20	21.25	17.84	8.62	N/A
	July 27	27.73	13.1	17.24	235.8
	Aug	19.4	21.7	4.0	49.3

TABLE A.2. Analysis of variance (ANOVA) on log transformed total length and body weight, and analysis of covariance (ANCOVA) on log transformed total length, gonad and liver weight by carcass weight on female and male northern mummichog (*Fundulus heteroclitus macrolepidotus*) collected from 3 estuaries, Wilmot, North Lake and Stanley, in PEI from December 2006, May 2-September 2007.

Date	Parameter	Females			Males		
		df	F	P	df	F	p
Dec	Length	2, 65	1.111	0.335	2, 50	4.698	0.014
	Weight	2, 65	2.695	0.075	2, 50	6.255	0.004
	Condition Factor	*	*	*	2, 49	19.164	0.000
	Gonad Size	*	*	*	2, 49	0.583	0.562
	Liver Size	2, 64	4.084	0.021	2, 49	1.710	0.191
May 2	Length	1, 38	3.993	0.053	1, 39	2.229	0.144
	Weight	1, 38	4.329	0.044	1, 39	4.035	0.052
	Condition Factor	1, 37	0.336	0.566	1, 38	9.803	0.003
	Gonad Size	1, 37	0.507	0.481	1, 38	0.129	0.721
	Liver Size	1, 37	11.371	0.002	1, 38	14.537	0.000
May 9	Length	2, 68	14.548	0.000	2, 63	10.968	0.000
	Weight	2, 68	7.924	0.001	2, 63	6.657	0.002
	Condition Factor	2, 67	16.496	0.000	2, 62	5.164	0.008
	Gonad Size	2, 67	11.386	0.000	2, 62	11.533	0.000
	Liver Size	2, 67	3.545	0.034	2, 62	2.080	0.134
May 23	Length	2, 31	10.532	0.001	2, 28	3.640	0.039
	Weight	2, 31	9.461	0.001	2, 28	2.984	0.067
	Condition Factor	2, 30	11.277	0.000	2, 27	12.124	0.000
	Gonad Size	2, 30	1.994	0.154	2, 27	13.014	0.000
	Liver Size	2, 30	3.829	0.033	2, 27	6.379	0.005
June 1	Length	2, 57	5.279	0.008	2, 55	4.883	0.011
	Weight	2, 57	2.255	0.114	2, 55	5.028	0.010
	Condition Factor	2, 56	21.414	0.000	*	*	*
	Gonad Size	2, 56	12.224	0.000	2, 54	15.547	0.000
	Liver Size	2, 56	5.238	0.008	2, 54	9.918	0.000
June 8	Length	2, 57	5.455	0.007	2, 45	5.181	0.009
	Weight	2, 57	2.848	0.066	2, 45	1.062	0.354
	Condition Factor	2, 56	30.364	0.000	2, 44	0.876	0.424
	Gonad Size	2, 53	6.401	0.003	2, 44	5.206	0.009
	Liver Size	2, 56	0.715	0.493	2, 44	14.042	0.000
June 14	Length	2, 57	10.246	0.000	2, 57	18.805	0.000
	Weight	2, 57	7.851	0.001	2, 57	12.973	0.000
	Condition Factor	2, 56	1.249	0.295	2, 56	18.534	0.000
	Gonad Size	2, 56	86.594	0.000	2, 56	21.398	0.000
	Liver Size	2, 56	43.418	0.000	2, 56	5.971	0.004
June 22	Length	2, 57	36.187	0.000	2, 57	5.675	0.006
	Weight	2, 57	33.224	0.000	2, 57	3.071	0.054

TABLE A.2. Continued.

Date	Parameter	Females			Males		
		df	F	p	df	F	p
	Condition Factor	2, 56	1.922	0.156	2, 56	11.510	0.000
	Gonad Size	2, 56	3.329	0.043	2, 56	6.510	0.003
	Liver Size	2, 56	4.014	0.023	2, 56	0.762	0.471
June 29	Length	2, 57	16.740	0.000	2, 57	2.897	0.063
	Weight	2, 57	13.916	0.000	2, 57	2.118	0.130
	Condition Factor	2, 56	1.434	0.247	2, 56	22.265	0.000
	Gonad Size	2, 56	0.152	0.859	2, 56	2.949	0.061
	Liver Size	2, 56	1.254	0.293	*	*	*
July 6	Length	2, 57	9.816	0.000	2, 57	1.083	0.346
	Weight	2, 57	10.543	0.000	2, 57	1.536	0.224
	Condition Factor	2, 56	5.822	0.005	2, 56	9.567	0.000
	Gonad Size	*	*	*	2, 56	2.648	0.080
	Liver Size	2, 56	2.135	0.128	2, 56	1.461	0.241
July 12	Length	2, 57	6.624	0.003	2, 57	8.752	0.000
	Weight	2, 56	5.493	0.007	2, 57	7.351	0.001
	Condition Factor	*	*	*	*	*	*
	Gonad Size	*	*	*	2, 56	0.629	0.537
	Liver Size	2, 55	11.965	0.000	2, 56	11.462	0.000
July 20	Length	2, 57	15.896	0.000	2, 57	15.896	0.000
	Weight	2, 57	12.691	0.000	2, 57	12.691	0.000
	Condition Factor	2, 56	1.547	0.222	*	*	*
	Gonad Size	2, 56	3.458	0.038	2, 56	0.523	0.595
	Liver Size	2, 56	1.915	0.157	2, 56	8.849	0.000
July 27	Length	2, 53	10.603	0.000	2, 57	37.025	0.000
	Weight	2, 53	8.966	0.000	2, 57	29.654	0.000
	Condition Factor	2, 52	5.317	0.008	2, 56	10.150	0.000
	Gonad Size	2, 52	5.284	0.008	2, 56	2.309	0.109
	Liver Size	*	*	*	*	*	*
Aug	Length	2, 53	1.676	0.197	2, 57	3.661	0.032
	Weight	2, 53	0.992	0.378	2, 57	4.529	0.015
	Condition Factor	2, 52	20.118	0.000	2, 56	8.077	0.001
	Gonad Size	2, 52	5.623	0.006	2, 56	3.237	0.047
	Liver Size	2, 52	1.487	0.236	2, 56	8.073	0.001
Sept	Length	2, 57	25.121	0.000	2, 57	17.168	0.000
	Weight	2, 57	25.126	0.000	2, 57	15.453	0.000
	Condition Factor	*	*	*	2, 56	15.930	0.000
	Gonad Size	2, 56	2.164	0.124	2, 56	3.079	0.054
	Liver Size	2, 56	1.573	0.216	2, 56	2.889	0.064

TABLE A.3. Analysis of variance (ANOVA) on egg diameter, and analysis of covariance (ANCOVA) on log transformed fecundity by carcass weight on northern mummichog (*Fundulus heteroclitus macrolepidotus*) collected from 3 estuaries, Wilmot, North Lake and Stanley, in PEI from June 1-July 29, 2007.

Date	Parameter	df	F	p
1-Jun	Egg diameter	2, 20	4.794	0.020
	# mature eggs	2, 28	0.499	0.612
8-Jun	Egg diameter	*	*	*
	# mature eggs	2, 32	2.041	0.147
14-Jun	Egg diameter	2, 20	2.607	0.099
	# mature eggs	2, 32	37.731	0.000
22-Jun	Egg diameter	2, 20	4.033	0.034
	# mature eggs	2, 32	2.887	0.070
29-Jun	Egg diameter	2, 20	15.148	0.000
	# mature eggs	2, 32	1.670	0.204
6-Jul	Egg diameter	2, 18	2.935	0.079
	# mature eggs	2, 27	0.591	0.561
14-Jul	# mature eggs	2, 22	1.502	0.245
20-Jul	# mature eggs	2, 15	0.630	0.546
29-Jul	# mature eggs	*	*	*

TABLE A.4. Analysis of variance (ANOVA) on log transformed total length, body weight, and analysis of covariance (ANCOVA) on log transformed total length, gonad and liver weight by carcass weight on female and male northern mummichog (*Fundulus heteroclitus macrolepidotus*) collected from 5 sites within the Trout estuary in July 2007.

Parameter	Female			Male		
	df	F	P	df	F	p
Length	4, 95	12.708	0.000	4, 95	10.006	0.000
Weight	4, 95	11.994	0.000	4, 95	9.297	0.000
Condition Factor	4, 94	2.711	0.035	4, 94	2.017	0.098
Gonad Size	4, 94	2.775	0.031	4, 94	2.181	0.077
Liver Size	4, 94	5.290	0.001	4, 94	4.321	0.003

APPENDIX B:

WATER CHEMISTRY AND STATISTICAL RESULTS FOR CHAPTER 3

TABLE B.1. Water quality parameters from May through August at the 7 sampling sites. Time of sampling varied based on tides and location of sites. Missing readings are marked N/A.

Site	Month	Time	Temperature (°C)	Salinity (ppt)	Dissolved Oxygen (mg/L)	Dissolved Oxygen (% sat)
Enmore	May	10:20	13.6	19.7	8.8	94.6
	June	09:50	16.0	23.6	N/A	N/A
	July	11:45	23.7	19.0	11.7	155.2
	August	11:00	18.9	24.3	8.3	103.2
Flat	May	15:00	13.8	26.4	10.8	122.3
	June	09:30	11.3	28.8	N/A	N/A
	July	07:00	17.3	28.7	11.1	137.5
	August	12:00	15.5	13.2	5.8	62.5
North Lake	May	11:00	11.5	23.3	10.4	107.6
	June	10:55	14.6	22.1	N/A	N/A
	July	18:00	22.3	23.7	17.7	233.3
	August	06:50	18.4	26.7	0.6	7.3
Stanley	May	10:20	10.9	18.1	11.1	136.0
	June	08:10	16.7	18.6	N/A	N/A
	July	12:25	22.3	25.9	12.9	172.8
	August	10:00	17.4	24.4	4.6	55.8
Wilmot	May	13:30	15.4	24.8	8.0	96.3
	June	07:00	15.2	22.8	N/A	N/A
	July	13:10	23.8	15.9	14.8	191.6
	August	08:15	19.4	21.7	4.0	49.3
Indian	May	12:00	15.6	11.0	14.7	N/A
	June	08:30	17.0	23.9	N/A	N/A
	July	10:55	23.2	9.8	7.9	94.9
	August	13:45	18.1	24.0	6.2	75.2
Barbara-Weit	May	08:00	12.6	19.7	14.5	157.5
	June	12:00	16.3	19.0	N/A	N/A
	July	09:15	23.2	21.1	6.9	90.7
	August	13:05	25.6	21.5	7.7	106.0

TABLE B.2. Analysis of variance (ANOVA) on log transformed total length and body weight, and analysis of covariance (ANCOVA) on log transformed total length, gonad and liver weight by carcass weight on female and male northern mummichog (*Fundulus heteroclitus macrolepidotus*) collected from seven estuaries in PEI on a monthly basis from May 2007 to August 2007. * indicates an interaction (<0.05) between site and body weight.

		Females			Males		
Date	**Parameter**	**df**	**F**	**p**	**df**	**F**	**p**
May	Length	6, 150	19.333	0.000	6, 147	11.35	0.000
	Weight	6, 150	12.669	0.000	6, 147	8.892	0.000
	Condition Factor	6, 149	3.981	0.001	6, 146	4.665	0.000
	Gonad Size	6, 149	19.596	0.000	6, 146	8.790	0.000
	Liver Size	6, 148	7.406	0.000	6, 146	17.139	0.000
June	Length	6, 133	5.335	0.000	6, 134	6.554	0.000
	Weight	6, 133	7.964	0.000	6, 134	4.793	0.000
	Condition Factor	6, 132	4.854	0.000	6, 133	11.578	0.000
	Gonad Size	6, 132	13.035	0.000	6, 133	23.663	0.000
	Liver Size	6, 132	22.135	0.000	6, 133	10.302	0.000
July	Length	6, 133	8.954	0.000	6, 133	10.119	0.000
	Weight	6, 133	7.597	0.000	6, 133	7.591	0.000
	Condition Factor	*	*	*	6, 132	1.984	0.072
	Gonad Size	*	*	*	*	*	*
	Liver Size	6, 132	6.053	0.000	6, 132	8.397	0.000
August	Length	6, 128	2.682	0.017	6, 132	2.377	0.033
	Weight	6, 128	3.214	0.006	6, 132	2.830	0.013
	Condition Factor	6, 127	16.902	0.000	*	*	*
	Gonad Size	6, 127	5.682	0.000	6, 131	6.159	0.000
	Liver Size	6, 127	1.205	0.308	6, 131	7.959	0.000

TABLE B.3. Non-linear regressions on total length and age provided residual sums of squares which were then used to statistically compare differences in growth among sites as described in Chen *et al.* 1992. Sexes were analyzed separately. Female and male northern mummichog (*Fundulus heteroclitus macrolepidotus*) were collected from 7 estuaries in PEI during June 2007 and aged by otoliths using the crack and burn method.

Pairwise Comparisons		**Females**			**Males**		
Site 1	**Site 2**	**df**	**F**	**p**	**df**	**F**	**p**
Enmore	Flat	3, 33	2.269	0.109	3, 34	3.233	0.037
Enmore	North Lake	3, 34	1.603	0.232	3, 35	7.448	0.000
Enmore	Stanley	3, 34	0.331	0.715	3, 35	0.415	0.702
Enmore	Wilmot	3, 33	6.971	0.001	3, 35	11.398	0.000
Enmore	Indian	3, 34	1.815	0.183	3, 35	1.715	0.205
Enmore	Barbara-Weit	3, 34	0.899	0.489	3, 35	2.344	0.100
Flat	North Lake	3, 33	1.962	0.155	3, 33	2.076	0.136
Flat	Stanley	3, 33	0.181	0.672	3, 33	2.118	0.130
Flat	Wilmot	3, 32	4.478	0.010	3, 33	2.535	0.081
Flat	Indian	3, 33	3.494	0.028	3, 33	1.388	0.294
Flat	Barbara-Weit	3, 33	1.955	0.156	3, 33	2.449	0.089
North Lake	Stanley	3, 34	0.584	0.639	3, 34	10.989	0.000
North Lake	Wilmot	3, 33	1.792	0.188	3, 34	0.582	0.640
North Lake	Indian	3, 34	0.653	0.607	3, 34	3.953	0.017
North Lake	Barbara-Weit	3, 34	0.147	0.639	3, 34	5.722	0.003
Stanley	Wilmot	3, 33	3.572	0.025	3, 34	3.264	0.035
Stanley	Indian	3, 34	1.920	0.162	3, 34	4.595	0.008
Stanley	Barbara-Weit	3, 34	0.576	0.643	3, 34	7.765	0.000
Wilmot	Indian	3, 33	1.683	0.212	3, 34	6.437	0.001
Wilmot	Barbara-Weit	3, 33	1.767	0.193	3, 33	9.658	0.000
Indian	Barbara-Weit	3, 34	0.270	0.712	3, 34	0.284	0.714