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Molecular and Behavioral Evidence Suggest Two Distinct Life Histories are Displayed in Smallmouth Bass (*Micropterus dolomieu*) in Lake Erie

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A Thesis Submitted in Partial Fulfillment Of the Requirements for the Degree of Master of Science Department of Biology State University of New York College at Fredonia Fredonia, New York

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Master's Thesis:
Molecular and Behavioral Evidence Suggest Two Distinct Life Histories are Displayed in Smallmouth Bass
(Micropterus dolomieu) in Lake Erie

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Abstract

In Lake Erie Smallmouth bass (Micropterus dolomieu) are an ecologically and economically important species. They are a top littoral predator as well as a popular sport fish. Previous genetic research suggests bass that live and spawn solely in the lake are genetically divergent compared to bass that live and spawn in tributaries to Lake Erie (Borden and Stepien 2006; Borden 2008). In this study we further validate this claim by analyzing 221 individuals from several lake and tributary sites using 7 microsatellite loci. We also provide evidence that suggests there may be two different types of tributary spawning bass based on an isolation by distance statistical test. Our data indicate that there are bass that spawn for multiple years in one stream and there are others that spawn opportunistically in small tributaries throughout the lake. Based on these genetic data it has been hypothesized that these genetic differences are the result of fidelity to different spawning sites (Borden and Stepien 2006; Borden 2008). To test this hypothesis we used radio telemetry to study bass movement patterns during two consecutive spawning seasons. Bass in our study displayed a high degree of fidelity to their spawning location during both spawning seasons with 50 to 85 percent return frequencies at each location. Our results corroborate well with the genetic data published in previous studies and cumulatively these data suggests there are at least two different life histories bass display in Lake Erie.
Background/Significance:

The smallmouth bass, *Micropterus dolomieu*, is a well known sport fish in the United States. Its native range encompasses the Mississippi basin, the St. Lawrence-Great Lake's system, and the Hudson Bay basin (MacCrimmon and Robbins, 1974). Smallmouth bass are part of the Centrarchidae family and inhabit lakes and streams throughout the temperate zone in North America. Bass originally entered the Great Lake system as the last glaciations subsided about 20,000 years ago (MacCrimmon and Robbins, 1974). In the case of Lake Erie’s smallmouth bass population it is important to note that they have never been supplemented by a stocking program and therefore are most likely a genetically pure strain. Thus far, smallmouth bass have proved to be a resilient species in Lake Erie because they have survived ample amounts of anthropogenic abuse via pollution and the introduction of non-native species. As a result of its aggressive predisposition, smallmouth bass are the second most fished sport fish after walleye in New York’s portion of Lake Erie and it is important to make certain that the species is managed properly (Einhouse et al., 2006). In order to properly manage this species it is essential to gain a thorough understanding of the life histories of bass in the lake. A better understanding of bass life histories may also help to ensure the survival of smallmouth bass and to maintain the genetically distinguishable strains that contribute to Lake Erie’s biodiversity.

Environmental Characteristics of Lake Erie

Lake Erie is the shallowest of the Great Lakes and it is divided into three basins: western, central, and eastern (Hartman, 1972). The eastern side of the lake is considered to be oligotrophic where as the western end of the lake is more eutrophic (Jude and Leach, 1999), but in general, the lake is currently in a mesotrophic state. Because the lake is shallow Lake Erie has one of the highest turnover rates and rates of sedimentations of any of the great lakes (Jude and Leach, 1999). These two factors may have a large impact on the fish community structure in the lake. Lake Erie has had a short and intense history with humans. Within the past century it has undergone major changes in its fish community structure due to these interactions. During the time period 1760-1850, Lake Erie became extremely polluted due to all the nutrients being transported into it from the surrounding farmland (Naiman et al., 1995;
Carpenter et al., 1998). It was once known as the Dead Sea of North America because the productivity in the lake was extremely high and therefore created areas of anoxia in the lake. However, its water quality has begun to improve as the result of government action to protect the large quantity of freshwater held in the Great Lakes.

One of the better known federal acts that was implemented to improve the waterways in the United States is Clean Water Act of 1965. Also, the creation of the Environmental Protection Agency has helped to restore and protect the freshwater systems in the United States (Summerfelt, 1999). One well known program that was implemented to rehabilitate Lake Erie along with the other Great Lakes was the Great Lakes Water Quality Agreement of 1972 (Dolan, 1993). This program was used to abate the phosphorus pollution of Lake Erie. Another group that is committed to specifically improving the fisheries in the Great Lakes is the Great Lakes Fishery Commission (GLFC) (Jude and Leach, 1999). This commission was created in 1987 and is mainly involved in the regulatory efforts to control sea lamprey, and support consensus research and management initiatives. Today the GLFC acts in conjunction with fisheries agencies so that all parties with jurisdiction of the Great Lakes are involved in the management process. This program and many others have attributed to a significant improvement in water quality management in the lake. However, the work done by state and federal governments are not the only cause for the improvement of the water quality in the lake.

An unusual factor that has aided in the recent trend in increasing water quality is the accidental introduction of the zebra mussel (*Dreissena polymorpha*) (Dolan, 1993; Nicholls and Hopkins, 1993). These mussels, which are filter feeders, have spread throughout Lake Erie and the other Greats Lakes and research suggests they have transferred much of the nutrients usually found in the pelagic zone to the benthic zone. The oligotrophication of Lake Erie has increased the survival of many bait fish in the lake and allowed a recovery to a declining smallmouth bass species as well (Ludsin et al. 2001). As a whole, many regulatory institutions that have been involved in the regulation of Lake Erie's water quality and the aid of the zebra mussel have helped to clean its water column. Lake Erie's water quality will most likely be a concern well into the future and coupled with these issues managers have also had to address recent concerns about the effects of global climate change on its waters.
Global climate change is transforming ecosystems all across the world and its effects could potentially change the water quality of lakes and streams significantly. Blumberg and Toro (1990) presented evidence that as water temperature increases the dissolved oxygen in the water column decreases due to increased activity within the phototrophic populations. These data suggest that partial anoxia can occur with an increase in water temperature. This may pose problems for the productivity in the lake and can create a form of bottom up control on the fish community in the lake because many of the bait fish such gizzard shad (*Dorosoma cepedianum*) are dependent on the phytoplankton communities in the lake (Ryan et al. 2003).

Along with concerns about productivity in the lake due to global climate change there has also been concerns about its effects on precipitation and ice cover on the lake. It may reduce the amount of precipitation in the area, increase evaporation rates, and reduced ice cover on the lake (Lofgren et al., 2002). These goals are intended to improve the fish community structure in Lake Erie specifically by attempting to create a mesotrophic lake with predators from the *Percid* family (Ryan et al., 2003 and Davies et al., 2005). Constant monitoring of Lake Erie and the implantation of current legislation will aid in the Lake Erie’s recovery from the pollution in the early twentieth century.

**Smallmouth Bass Fishery in Lake Erie**

The smallmouth bass are the second most targeted sportfish by boat anglers from 1988 to 2005 in New York’s portion of Lake Erie (Einhouse et al., 2006). Nearly 6,500 smallmouth bass were harvested in New York alone during the 2007 fishing season and interestingly over 100,000 were caught by boat fishermen over the 2007 season (DEC, 2007). This type of catch and release fishery may help the population flourish in the lake as well as allowing the fishery to increase in popularity. The popularity of smallmouth bass fishing on Lake Erie may also be due to trends of declining *Percids* populations in the lake (Ryan et al, 1999). Throughout the past twenty years the smallmouth bass population has experienced a relatively stable abundance. The success of this fishery is most likely due to good management practices by all of the agencies involved in the supervision of the lakes water quality and fish community.
The fishery is independently managed by five agencies in order to address issues of stakeholders (Ryan et al., 2003). A creel survey given to Lake Erie fishermen found that the total smallmouth bass catch had doubled from 1988 to 2000 (Einhouse et al., 2006). As the managers noticed an increasing interest in the smallmouth bass fishery in Lake Erie their management strategy has changed. Lake Erie is a recreational fishery and recently managers of the fishery have concentrated their efforts on increasing the fishery’s popularity (Einhouse et al., 2006).

Smallmouth bass spawn on reefs 4.6-6.1 meters deep in the lake and some spawn in Lake Erie’s tributaries as well. Those that spawn in the tributaries spawn earlier in the year and this may be related to warmer stream water temperatures in the spring. If bass spawn in tributaries that reach a water temperature ranging between 12-15°C they can potentially provide themselves and their young of the year (YOY) with several advantages (Scott and Crossman 1973; Graham and Orth 1986). Miranda and Hubbard (1994) suggested that larger young of the year have higher survival rates to age-1 due to a decrease in predation. The higher survival rates may also be explained by larger YOY having higher lipid reserves (Thompson et al., 1991). Ludsin and DeVries (1997) also stated that larger YOY become piscivorous early which gives them increased access to prey in the fall. These findings may be related to an increase lipid reserves these YOY experience. In another study YOY largemouth bass differed in size due to consumption rates of invertebrates and this has a dramatic effect on when they become piscivorous (Olsen, 1996). The NYS DEC has also noticed a binomial size distribution in age-0 smallmouth bass through the use of beach seining. The binomial distribution may be related to the YOY that were reared from nesting sites in the tributaries and in the lake itself. However, more research is needed to understand smallmouth bass recruitment in the lake. The DEC is working with SUNY Fredonia in a collaborative effort in an attempt to better understand the life history characteristics in smallmouth bass found in Lake Erie. However, there are other stakeholders in the lake other than the managers and the universities surrounding it.

One important smallmouth bass fishery stakeholder is the recreational fisherman which the NYS DEC and other state agencies work with so the fishery can be managed correctly. The DEC activity quantifies the opinions and successes of the fisherman throughout the year by
using creel surveys. Currently, the fisherman in Lake Erie can fish smallmouth bass throughout the year. There are three seasons which are the first Saturday of May to the third Saturday of June, the first Saturday of June to November 30th, and December 1st to the first Saturday in May. The first season’s minimum length is 50.8 centimeter and a one fish daily limit. This season is limited to finishing in only the tributaries of Lake Erie. These limitations are due to the managers trying to sustain sufficient bass recruitment into the lake (Einhouse et al., 2006). Also, it allows for anglers to fish the smallmouth bass earlier in the season, which may help to increase interest in the sport fishery and increase the opportunity to catch a trophy fish. The second season has a 30.48 centimeter minimum and a 5 fish daily limit. This corresponds with the regulations for all normal smallmouth bass fisheries in the state and with the regulations found in other states (Ryan et al., 2003). The final season takes the anglers through the rest of the year with a catch and release policy. This may help to ensure that the fishery does not have a high mortality rate due to fishing pressure. These three seasons allow the angler a wide array of opportunities to target smallmouth bass throughout the year. They also provide protection for the species so that the anthropogenic effects on the species are not detrimental. Anglers can attempt to fish for smallmouth bass by themselves, through tournament events such as the B.A.S.S. Masters Tournament held in 2007, or through a private charter boats. As a result of the well known fishery, anglers from the states surrounding Lake Erie and elsewhere take advantage of all three venues.

New York State has a unique regulation allowing fisherman to fish smallmouth bass between the first Saturday in May and the third Saturday in June. This is during their spawning season. In most New York lakes bass are not fished for during their spawning season (Quinn, 2002). The DEC Lake Erie unit has recently deviated from the normal state regulations. The new regulation only allows the angler to keep a smallmouth bass that is 50.88 centimeters or larger. Their reasoning behind this new regulation is that it satisfies the enthusiastic fisherman’s desire to catch large fish and prevents illegal angling for the fish in Lake Erie. There has been some concern for the impact on the recruitment for the next year but several studies that suggest this regulation is not detrimental to the fishery. Steinhart et al. (2005) data suggested that there are not any significant impacts on the fry of the nest guarding males as long as the bass
guarding the nest are being released. Most likely the fish being caught during this early season are being released because many do not satisfy the 50.88 centimeters minimum length requirement. Lake Erie's large population of smallmouth bass is most likely not affected by angling pressure during the spawning season. Yet, angling pressure on the smallmouth bass is not the only possible threat to a smallmouth bass' survival in the lake. There are other issues today that do pose a threat to the species.

Lake Erie is also home to many invasive species; the round goby, *Neogobius melanostomus*, is particularly important to smallmouth bass. The round goby is an invasive species in Lake Erie and it is native to the Black and Caspian seas in Eastern Europe. In 1999, the round goby was discovered in eastern Lake Erie (DEC, 2007). This species is of particular important to smallmouth bass because it can affect the survival of the YOY in nests when the guarding male is removed from the nest (Steinhart et al., 2004). However, the effects are usually minimal if the fish is released immediately. The longer the fish are kept from the nest the more eggs the round goby will consumed. If good catch and release practices are implemented then the effect the round goby has on the YOY can be significantly reduced.

Angling can place stress on the fish that are being caught, but as long as appropriate measures are taken while releasing the fish, no lasting damage should be done to the fish.

Adult bass feed on round gobies because they are an abundant and readily available item found throughout the lake. However, the goby can have a negative effect on the survival of smallmouth bass because the goby has the potential of being a carrier of some types of disease. One of the newly identified viruses found in Lake Erie is Viral Hemorrhagic Septicemia Virus (VHSV) (Lumsden et al. 2007). VHSV creates hemorrhaging in the flesh of the bass which can be detrimental to its survival. The introduction to VHSV has only been noted in the past few years because it has been the reason for heavy die offs of gizzard shad in the lake. More research is needed to have a better understanding of its true effects on the smallmouth bass fishery.

Another species that can affect smallmouth bass by interspecific competition are steelhead trout, *Oncorhynchus mykiss*. Smallmouth bass prey on juvenile steelhead trout after the steelhead spawning in the spring and fall. Fayram and Sibley (2000) found that 28% of the
diet of an introduced population of smallmouth bass in Washington consisted of juvenile sockeye salmon. These results may have some meaning to Lake Erie bass and their interactions with steelhead. This is especially a concern because some smallmouth bass spawn in the tributaries in the lake. The overlap of spawning habitat may have a significant impact on steelhead because many of the YOY steelhead can be found in the streams during the spring and some of the summer. More research may be required to understand this interaction between smallmouth bass and steelhead. As a result of the various complex interactions smallmouth bass have within the Lake Erie fishery, managers are always growing in their understanding of the fisheries complexity.

Overall the smallmouth bass fishery in Lake Erie requires a management strategy that involves the managers, universities, and the anglers themselves. Managers consist of state departments from: New York, Pennsylvania, Ohio, Michigan, United States Federal Fish and Wildlife Service, and the Ontario Ministry of Natural Resources. These entities work within each of their jurisdictions to maintain the smallmouth bass fishery. Lake Erie is a very large body of water and managers will always need an in depth information base about the environmental characteristics of the lake, the ecosystem that smallmouth bass live in, and the interactions the species shares with humans.

Smallmouth Bass Life History

Smallmouth bass spawn in between late April and mid-July when the water temperature ranges from 12-23.5°C (Scott and Crossman 1973; Graham and Orth 1986). When the water temperature is in this range males create shallow circular nests in littoral zones of lakes and shallow areas of streams (Breder 1936; Coble 1975; Heidinger 1975). The nests are usually found by some type of structure such as gravel or rocky substrate (Breder and Rosen 1966). Once the nests are created usually one female is courted by the males to the nest. This usually takes place 8-10 m away from the nest (Ridgeway et al., 1989). The male courts to the nest using the female by using contact nips (Ridgeway et al., 1989). Once at the nest the female may test the substrate to ensure it is suitable to lay her eggs on (Ridgeway et al., 1989). Once she determines the nest is sufficient she deposits 2,000 to 30,000 eggs on the nest, depending on
the females' size and age (MacCrimmon and Robbins 1974; Vogele, 1981). The male remains at the nest after spawning is complete and takes role as the sole care giver for the eggs (Breder, 1936; Coble, 1975; Heidinger, 1975). The male protects the fertilized eggs from predation by other fish (Ridgeway et al., 1989). They stay with the nest up to five weeks and care for the eggs and fry by providing aeration using its caudal fin (Breder, 1936; Coble, 1975; Heidinger, 1975). Most male smallmouth bass are semelparous, meaning that they only mate once, and therefore usually monogamous, meaning that males only mate with one female during their life time (Ridgeway et al. 1989; Raffeto et al., 1990; Wiegmann et al., 1992; and Barthel et al., 2008). However, there is a proportion of bass that do return to spawn for multiple years (Barthel et al., 2008). Barthel et al. (2008) noted that little is known about female spawning behavior because they do not partake in spawning activities for a significant portion of time.

Males usually become reproductively active between three to five years old depending on their size (Wiegmann et al., 1997). Males display philopatry, also known as site fidelity, meaning that they will return to within about 100 m of their original nesting site each year (Ridgeway et al., 1991; and Barthel et al., 2008). Whereas females usually become reproductively active around four to five years of age (Latta 1975). There has been some evidence that females display some fidelity to spawning location (Barthel et al., 2008). Barthel et al. (2008) has noted that there can be some variation in these ages depending different life history characteristics. Male parenting plays an important role in YOY survival however this parental role usually last on a few short weeks. After the male leaves the nest the YOY are left to survive on their own. The uniparental male care system is particularly interesting because unlike many other species there is an asynchrony in size between reproductively active male and female. In fish there is a negative allometric relationship between size and metabolic rate as well as positive allometric relationship between body size and energy reserves (Weatherly and Gill, 1987; Shuter and Post, 1990). These relationships are suggested to be related to larger males and females spawning earlier in the spring than smaller males and females.

Young of the year are very vulnerable to many forms of selections and there are many types of pressures that can determine their mortality rates in any given aquatic system. The rate that the YOY grow is critical for successful reproduction (MacCrimmon and Robbins, 1974).
The eggs hatch roughly 60 hours after being laid when the water temperature is at 24°C but it can take up to 10 days for the eggs to hatch when the water temperature is at 13°C (MacCrimmon and Robbins, 1974). Therefore temperature is one of the main factors as to when the egg hatch into fry. Once the eggs hatch, the fry begin feeding on invertebrates and aquatic insects (Easton et al., 1996; Long and Fisher, 2000). After the smallmouth bass reach a size of 125 mm they become piscivorous (Olson and Young, 2003). That time frame is critical for the YOY because their first summer foraging is when they can store energy for their first winter. Winter survival is difficult because fish metabolism slows as a result of the cold waters the fish are in. Smallmouth bass survive through winter because they use their fat reserves for energy. Thus, YOY fat reserves are crucial for their survival because the feeding activity of the YOY is severely restricted during the winter season (Shuter et al., 1980; Shuter and Post, 1990). Also, smaller YOY are less tolerant to extreme winter conditions because they lose their fat reserves faster than larger YOY (Shuter and Post, 1990). Generally temperature can influence the survival of the YOY directly during fertilization and indirectly during the first summer and winter they experience (Christie and Regier, 1973).

Some research has been conducted on bass juvenile stages and most notably, Ridgeway et al. (1991) presented evidence that there is a negative density dependent relationship between the number of nesting adult male bass compared to the total number of males in the population. These data along with other studies have given rise to what is known as the Juvenile Transition Hypothesis which was developed using three lines of evidence (Ridgeway et al. 2002). First, YOY bass only disperse 200 m from the nest they were reared. Second juvenile growth is density dependent. This relationship is particularly strong between the ages of 2-4 and between the ages of 5-6 the strength of the relationship weakens. Finally, adult home ranges are established after mating and these home ranges are traveled extensively. These home ranges are rarely near nest site locations and overlap from year to year. Cumulatively these data suggest that when population size is high there is a rise in competition. Ridgeway et al. (2002) suggest that this competition can be particularly stressful for bass and as a result can slow their maturation. Thus, there are only a small proportion of adult males to mate.
There has been some speculation as to what type of competition drives this negative density dependent relationship. Raffetto et al. (1990) suggested that as competition for mating success increases so does selection for spawning characteristics. This competition may result in many males and females failing to breed during the spawning season (Graham and Orth, 1986). Gross and Kapuscinski (1997) presented evidence that 5.4 percent of the 27 percent of males that acquired eggs for their nests produced 54.7 percent of YOY. Their results may suggest that some males have more advantageous spawning characteristics compared to others. Some studies have found that spawning success may be related to the size of the nesting male because male size increases with brood size (Philipp et al., 1997; Cory and Philipp, 2004). This may be due to increased energy stores in males that are larger in body size or because larger smallmouth bass are usually more reproductively active earlier in the season (Ridgeway et al., 1991). Cumulatively these selective pressures may lead to the evolution of different life histories among smallmouth bass populations.

Barthel et al. (2008) presented evidence that there are two distinct life histories in a river-lake system in Ontario, Canada. They present three lines of evidence using telemetry, mark-recapture, and reproductive surveys that suggest there are two distinct life histories that bass display in their study's location. Using these data, they provide evidence that bass return to either lake or river spawning sites each year and these bass almost exclusively spawn in one location. They also presented corroborative evidence suggesting that male bass display strong site fidelity to their nesting sites in both locations. Females were also restricted to one life history type as well. However, it has not yet been determined if these life history differences exist elsewhere in the natural world. Smallmouth bass life history is a complex and fascinating topic which needs much more research to be conclusively resolved.

Population Genetics Studies

Microsatellites are usually present on non-coding sections of DNA and are known to be highly variable between individuals with in a population. They usually contain tandemly repeated short units of nucleotides where these units are 1-6 base pairs long (Chistiakov et al., 2006). These repetitive sequences can range from 20 to a few hundred base pairs in size.
(Beckman and Weber, 2002). It is thought these hypervariable regions are the result of DNA polymerase slippage during DNA replication. During replication DNA polymerase can dissociated from the DNA strand and during its subsequent reassociation can copy nucleotides sequences it previously synthesized (Schlötterer and Tautz, 1992). As a result multiple copies of the same repetitive sequence can persist in the population over time. Rates of mutations within microsatellite regions of the genome are higher compared to regular non-repetitive sequences of DNA (Li, 1997). As these hypervariable regions are found on non-coding sections of DNA they are commonly used in population genetic studies (Goldstein and Schlotterer, 1999). PCR is commonly used to analyze regions in the genome known as microsatellites.

A well known technique used in population genetic studies is the Polymerase Chain Reaction (PCR) (Saiki et al., 1988). PCR is generally used to amplify a specific location in genome to generate a large quantity of DNA. This occurs by utilizing a thermostable polymerase, primers that are specific the region of the genome of interest, template DNA, and deoxyribonucleotides. These reagents are manipulated in three main phases of PCR; denaturation, annealing, and hybridization. These phases occur at different temperatures such that amplification of the locus of interest is controlled. After amplification has occurred PCR products are generally analyzed by sequencing or mobilization through a gel matrix.

Sequencing is commonly used when analyzing regions of a genome that low amounts of variation because it can detect single nucleotide polymorphisms (Brumfield et al., 2003). On average 3.3 substitutions can be observed in mitochondrial genome over 10,000 years (Billington 2003). Changes within its sequence are likely to accrue slowly over time because mutations are generally are not beneficial and thus natural selection would act against them within the population. Thus, differences between various lineages are likely to be represented by only a few haplotypes which are usually different by only one nucleotide. Sequencing can identify these single nucleotide polymorphisms with ease and thus this technique is used to analyze these types of PCR products. However, sequencing is not always necessary to analyze polymorphisms within a genome. Today highly variable regions in the genome such as microsatellites are used to analyze fine scale genetic differences between individuals with the same species.
If genetic data from two populations within a species are significantly different these data can be used to generate hypotheses about the evolution of these discrete breeding populations. Genetic differentiation can be driven by a variety of forms of reproductive isolation (Palumbi, 1994). One form a reproductive isolation can occur when populations with a species breed in different locations with the same general habitat. The locations that spawning occurs can be analyzed by studying movement patterns of individuals in the species during the breeding season. One way to study movement pattern with a species is acoustic telemetry. This technique is very useful in biology because it can generate fine scale movement differences between individuals with the same species.

*Telemetry as a Means to Study Fish Behavior*

Telemetry is a tool in biology that can be used to monitor movement patterns of individuals in a given species remotely (Cooke et al., 2004). Telemetry has also been used to assess energy expenditure and anthropomorphic impacts (Standen et al., 2002). Movement is monitored using a transmitter that emits a pulse at a very specific radio frequency. A given radio frequency is associated with only one individual. Transmitters can be attached to an individual by a external body harness or can be implanted into the individuals body cavity (McCleave and Stred, 1975).

Internal surgery was initially performed using methods described by Hart and Summerfelt (1975). Common surgical practices include sterilization of equipment, protection of viscera, and suturing the initial incision (Wagner and Cooke, 2005). These initial techniques have generally been accepted by the fisheries community as appropriate despite the lack of little empirical evidence that these techniques are suitable (Wagner et al., 2000). This is of particular concern because if these surgeries affect fish behavior it could severely bias all behavior data collected from these studies. Generally all surgical procedures on nonmammalian vertebrates are overseen by the institutional animal care and use committees. However fish are not included in any IACUC regulations. But, there has been an increasing trend towards standardized guidelines for performing fish surgeries (Jepsen et al., 2002).
Regardless of discrepancies of how surgeries are performed, once the transmitter has been attached to the individuals of interest researchers can begin to track their movements by listening to the radio frequency using radio receivers that usually have some type of antenna attach. These antennas increase the receiver’s ability to detect signals being emitted by the transmitters. Researchers will listen for a pulse signal at the specific frequencies the transmitters were programmed to emit a signal at. Following the completion of data collection researchers simply download the data recorded on the machine. Generally researchers using multiple locations within the study area listen and using these multiple locations researchers can determine the specific location of their test subjects. These data are generally used in conjunction with other environmental data to determine how the species of interest interacts with its environment and others within the same species.

**Population Genetic Studies of Smallmouth Bass**

In recent years, a rudimentary structure of the genetic patterns exhibited in smallmouth bass present in Lake Erie have been published in three papers and a thesis. Borden and Stepien (2006) were the first to analyze the genetic structure of bass in Lake Erie. They used both mitochondrial DNA and microsatellite markers in their analysis. Their results supported the hypothesis that there was some substructure within the lake and they also noted there was strong within site genetic variation. This within site genetic variation was the first hint that there may be a potamodromous, meaning a stream spawning population, present in the lake.

Stepien et al. (2007) analyzed the genetic structuring using genomic DNA samples taken from bass throughout all the Great Lakes and some outgroups and from these data they determine that an isolation-by-geographical-distance prediction was not supported. Therefore genetic variation and the substructure of the bass population within Lake Erie had not evolved by mere geographic distance. Borden (2008) revealed that there are statistically significant differences between bass that spawn in large tributaries of the lake compared those that spawn in the lake itself. Finally, Hahn (2009) analyzed microsatellite data generated from genomic DNA samples taken from bass in small tributary, large tributary, and lake spawning sites. Her data suggested that small tributary spawning bass are genetically divergent as well. Cumulatively these data
support there are three different life histories smallmouth bass display in Lake Erie. All of these authors have shed new light on the genetic structuring displayed in smallmouth bass in the lake and its tributaries.

Recent genetic data suggests that spawning in the small tributaries provide several advantages to the bass (Hahn, 2009; Borden, 2008). Tributary bass are able to spawn earlier in the year because water temperatures in tributaries warm earlier in the spring than the water temperature in the lake itself. These tributaries provide larger reproductively active smallmouth bass a location to spawn earlier in the year because water temperatures in these streams are warmer than the lake itself. Therefore, tributaries offer the YOY more time to grow before their first winter. By allowing bass more time to grow they are able to increase their lipid reserves and size before their first winter, thereby giving them a better chance at over winter survival (Ludsin and DeVries, 1997; Thomson et al., 1991). Also, by spawning earlier males can leave the nest earlier, which allows them more time to increase their fat reserves. Schluter (1996) suggested that these behavioral differences within fish populations can result in greater genetic divergence. Smallmouth bass are an incredibly fascinating fish species because of the versatility in life histories that it displays and thus merits a thorough understanding of its evolutionary past and its fundamental genetic structuring.

**Problem Statement:**

Potamodromy has been noted in other river/lake systems but no one has determined if smallmouth bass exhibit these life history differences in Lake Erie. Hahn (2009) and Borden (2008) provided evidence that there are genetic differences between smallmouth bass that spawn in tributaries compared to the lake itself which suggests that there is a potamodromous strain of smallmouth bass in Lake Erie. It is important to determine if a potamodromous strain of smallmouth bass exists and to gather basic behavioral data pertaining to these potential potamodromous bass because these data can be used when implementing management policies pertaining to this genetically pure smallmouth bass strain.
Objectives:
1) To determine if bass that spawn in streams are genetically divergent using microsatellite loci and population genetic statistics.
2) To determine if smallmouth bass return to the same stream to spawn each year.
3) To determine what types of environmental characteristics are associated with bass movement within their spawning locations.

Approach/Methods:

General Methods:
To determine if the potamodromous bass were genetically divergent to lake spawning bass, genomic DNA was isolated from bass in both tributary and lake spawning locations. A total of 7 microsatellite loci were amplified using the genomic DNA from these spawning locations as template. Genetic data derived from differences in the 7 loci were recorded and analyzed using a variety of different statistical programs to assess if the populations of bass sampled were indeed genetically divergent.

Based on previous genetic studies it has been hypothesized that genetic divergence in smallmouth bass spawning populations is the result of fidelity to spawning location. Acoustic telemetry was used to determine if smallmouth bass do return to the same stream to spawn each year. During the acoustic telemetry data collection environmental characteristics were recorded daily. The raw acoustic data was used to assess if bass returned to their spawning location a consecutive year. These data were also used to determine the relative distance bass moved while they were present in their spawning locations. Both the environmental data and the relative movement data were used in a Linear Mixed Model to determine which environment characteristics were significantly correlated to relative movement.
Genetic Sampling:

This study occurred in New York's portion of Lake Erie. Bass were sampled in four tributaries of Lake Erie: Eighteen Mile Creek (n=21), Cattaraugus Creek (n=32), Canadaway Creek (n=46), and Chautauqua Creek (n=50) (Figure 1). Two spawning locations within the Lake were also sampled: Dunkirk Harbor (n=22), and Van Buren Bay (n=26) (Figure 1). Cassadaga Lake (n=30) was used as an outgroup because it exists in the Mississippi drainage basin. A subset of these genetic samples was taken from bass used in the telemetry portion of this study (see Telemetry Sampling for details). Sampling occurred during the 2007-2010 spawning seasons. When bass tissue was collected the temperature at each location was taken. Bass were collected using a variety sampling techniques including: backpack and boat electrofishing, beach seining, and angling. After sampling was complete all fish were released.

Fig.1. Map of Sampling Sites – There are two lake sites; Van Buren Bay (n=26) and Dunkirk Harbor (n=22). There are six creek sampling sites; 18 Mile Creek (n=21), Cattaraugus Creek (n=32), Canadaway Creek (n=46), and Chautauqua Creek (n=50). Bass sampled in Cassadaga Lakes (n=29) were used as an outgroup because that body of water is in the Mississippi drainage basin.
A fin clip was taken from the caudal fin of each bass and these clips were stored in 95% ethanol until they were taken back to the lab for DNA purification. The fin clips were used to extract total purified DNA using a Qiagen DNeasy kit and all DNA samples were stored at -20°C (Quiagen Inc., Valencia, CA; Borden and Stepiecn, 2006). All DNA samples were used to amplify 7 microsatellite loci; Mdo2, Mdo3, Mdo8, Mdo9, Mdo11, MS19, and RB7 (Table 1) (Malloy et al., 2000; DeWoody et al. 1998). Each forward primer was labeled with an Infrared 700 nm or 800 nm fluorescent tag to optimize multiplexing (Table 1). PCR reactions contained 0.5 mM of dNTPs, 2 μl 10x PCR Buffer which contained 2.5 mM Mg²⁺ (Fisher Scientific Company, LLC), 1 μM Forward Primer, 1 μM Reverse Primer, 0.25 μl (1.25 Units) of Hot start Taq DNA polymerase, and 1 μl of DNA (0.2-1 μg/μl). The exact amount of water was determined by the number of loci being amplified in one reaction. PCR products were amplified using a thermacycler using the following conditions: 2 minutes at 94°C to denature all DNA, followed by 35 cycles of 94 °C for 30 seconds, 52-56 °C for 1 minute (See Table 1), 72 °C for 30. Following the 35 cycles a final synthesis step was completed at 72 °C for 5 minutes. Mdo3, Mdo8, and Mdo11 were multiplexed in one reaction and Mdo5 and Mdo9 were multiplexed in another. All other primer sets were amplified individually. The multiplexed reactions were determined by average band sizes of the PCR products and those that did not overlap were combined into one reaction.
Table 1. Microsatellite loci use genetic analysis – Eight microsatellite loci, orientation (Forward (F) and Reverse (R)), primer sequence, annealing temperature, range of product sizes in base pairs (bp), and fluorescent tag attached to the forward primer.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Orientation</th>
<th>Primer Sequence (5'-3')</th>
<th>Annealing Temperature</th>
<th>Product Size (bp)</th>
<th>Fluorescent Tag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mdo2</td>
<td>F</td>
<td>GCCCTTTCATATTGGGACAA</td>
<td>52 °C</td>
<td>195-203</td>
<td>IR800</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CTGCTCTGGCGTACATTCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mdo3</td>
<td>F</td>
<td>AGGTGCTTTGCGCTACAAGT</td>
<td>54 °C</td>
<td>120-130</td>
<td>IR700</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>CTGCATGGCTTTATGTTGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mdo8</td>
<td>F</td>
<td>GTGAGGACCAGCCAAATGT</td>
<td>52 °C</td>
<td>209-229</td>
<td>IR700</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GGAAGATTGGAGTCCCAAACA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mdo9</td>
<td>F</td>
<td>TTTGATGGGCCTTTGTGTA</td>
<td>54 °C</td>
<td>122-136</td>
<td>IR700</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GACCGGTCTCTGCAATGATT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mdo11</td>
<td>F</td>
<td>TTTGAGAGGGGCGATAAAC</td>
<td>52 °C</td>
<td>170-178</td>
<td>IR700</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GCATCCTCCCAGTTACCCTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS19</td>
<td>F</td>
<td>CAGGATTTCAACTAGGCCAGGC</td>
<td>48 °C</td>
<td>99-127</td>
<td>IR800</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GGAATCATGATTAGGTTGGTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB7</td>
<td>F</td>
<td>GTGCTAATAAGGCTACTGTC</td>
<td>48 °C</td>
<td>111-141</td>
<td>IR800</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TGGTCCCTTAATTTGTGTTGA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCR samples were diluted 1:30 using distilled water and then 1 μl of the diluted sample was added to 2 μl of Li-Cor Blue stop dye. PCR products were combined prior to loading on the gel. The PCR products for Mdo2 Mdo3, Mdo8, Mdo11, and MS19 were combined and diluted together. The PCR products Mdo5, Mdo9, and RB7 were combined and diluted together as well. These combinations of products were determined by size of the PCR samples and the type of fluorescent tag associated with each locus' forward primer.

After diluting the samples, 0.3 μl of the samples were visualized on a 6.5% acrylamide gel using the Li-Cor 4300 Genetic Analyzer. Bands on the gels were scored using SynGene scoring software. Based on the band sizes alleles were designated at all loci for all DNA samples and these data were statistically analyzed.

Genetic Data Analysis:

A similar genetic analysis protocol outlined in Stepien et al. (2007) was used to analyze microsatellite data. All samples were tested for conformance to Hardy-Weinberg equilibrium at each locus. These loci were analyzed using Markov chain Monte Carlo method and 1000
randomizations methods were used to estimate significance (Guo and Thompson, 1992). This statistical test was conducted using GENEPOP (Raymond and Rousset, 1995; Raymond and Rousset, 2004). Using this program linkage disequilibrium and heterozygosity deficiency or excess was tested using arithmetic means of $F_{ST}$ for all loci at each population as well. Significance levels for the Hardy-Weinberg Equilibrium tests were assessed at an $\alpha=0.05$. The $F_{ST}$ was used to assess levels of genetic divergence using FSTAT in GENEPOP (Goudet, 2002; Weir and Cockerham’s, 1984). It is common for sequential Bonferroni corrections to be used to decrease type I errors when multiple tests are conducted using the same data. However sequential Bonferroni corrections decrease the power of testing and as a result type II errors can occur more frequently (Verhoeven et al., 2005; Moran, 2003; Nakagawa, 2004). Thus, when multiple tests were used, p-values were adjusted using false discovery rate method (Benjamini and Hochberg, 1995; Verhoeven et al., 2005). The genetic relationships between different sites was assessed using Nei’s (1972) pairwise genetic distance using the program PopGene. These values were then used in Popgene to create neighbor-joining trees using 1000 bootstrap pseudoreplicates based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method (Saitou and Nei, 1987). A one tailed linear regression was used to assess if the geographic distance between small tributary sites were positively associated with genetic distance, $F_{ST} / (1-F_{ST})$. A one-tailed test was used to determine if the association between geographic distance and genetic distance was directional.

Telemetry Sampling:

In the 2009 and 2010 sampling seasons bass were sampled from one lake site, Dunkirk Harbor (n=10), three tributaries to Lake Erie: Chautauqua Creek (n=25), Canadaway Creek (n=25), and Cattaraugus Creek (n=20) (Figure 2). Of these sampling sites genetic samples were taken from Dunkirk Harbor (n=6), Chautauqua Creek (n=24), Canadaway Creek (n=25), and Cattaraugus Creek (n=6). A tributary of Cattaraugus Creek, Clear Creek (n=10) was also sampled but no DNA samples were taken (Figure 2). Bass were collected using backpack or boat electrofishing. The transmitters were purchased from Advanced Telemetry Systems and they weighed 14 grams; the pulse rate and width were 20ppm and 21 ms respectively. Each transmitter had a battery life of 654 days. Prior to implantation the transmitters were checked
to ensure they worked properly before implanting in a bass. Bass greater than 400 mm in total length were selected for transmitter implantation because this would ensure that transmitter did not weigh more than 2% of the total body mass (Winter, 1983). A tricaine methanesulfonate (200mg/L) solution was used to anesthetize the bass (Knight, 1964). During the sampling process length, weight, type of gonads present (if visible), transmitter frequency, date, fin clip, and GPS location were recorded for each bass. Sex was analyzed during transmitter surgery by visual confirmation of eggs or milt in the peritoneal cavity.

Fig. 2. Map of telemetry sampling sites – There is one lake sites; Dunkirk Harbor (n=10). There are three creek sampling sites; Cattaraugus Creek (n=20), Clear Creek (n=10), Canadaway Creek (n=25), and Chautauqua Creek (n=25).

We placed bass in this solution and when a bass lost equilibrium we conducted the surgery in a V-shaped holding tray. This tray was partially submerged under fresh cold water to keep the gills wet. A 3 cm incision was made on the ventral side of the bass posterior of the pelvic fins. The transmitter was placed into the incision such that the transmitter was facing anteriorly in the body cavity. A small hole was created posterior of the initial incision and the antenna was feed through this hole such that the tail was external to the body. The incision was
closed using 3-4 sutures with (2/0) breaded silk and covered with cyanoacrylate glue (Vet-Bond, 3M Inc.). The surgeries took approximately five minutes to complete. The bass were allowed to recover in a holding net in the stream until equilibrium was regained and all bass were released back into their spawning location with 30 minutes of the surgery.

After surgery transmitter frequencies were tracked at each sampling location using standard operating procedures. Each location had its own designated locations for listening for transmitter frequencies and each frequency was listened to equally. The frequencies were detected ATS R410 or ATS R4500S receivers with a 3 element folding Yagi antenna attached. When a pulse from a frequency was detected the direction of greatest pulse intensity and GPS location where the pulse was heard in decimal degrees were recorded. Each day a location was checked for frequencies, water temperature, and turbidity were recorded. Starting at the beginning of May each location was searched for transmitter frequencies Monday-Friday until no frequencies were observed at the sampling site. All sampling ended by June 30th each sampling year.

Telemetry Data Analysis:

Using GPS coordinates and direction data recorded in the field a single set of GPS coordinates, in decimal degrees, were designated for each bass when it was observed. These data were used to measure relative distance a bass moved between successive observations. Relative distance was calculated by taking the difference between the decimal degree measurement of a given day for a specific bass and the decimal degree measurement from the previous observation for the same bass. These differences were calculated for both latitude and longitude. The differences were then converted from decimal degrees to meters, latitude (1 decimal degree = 111073.2 meters) and longitude (1 decimal degree = 82726.8 meters). All samples sites were located at the latitude of 42°N and thus the longitude conversion was calculated for only that latitude. The meter conversions were used in the Pythagorean theorem equation to solve for the hypotenuse. The hypotenuse was used as a relative measure of bass movement per day. This measure was considered proportional to the actual distance moved per day. Bass transmitter frequency, relative movement, date of observation (using the Julian
calendar), water temperature, turbidity, and sampling location were used to create a linear mix model (LMM) in SPSS. LMM’s were used to elucidate which factors significantly influence relative distance moved during the spawning season because these models take into account repeated measurements per individual. A Binary Logistic regression was also used to assess if turbidity affected relative distance moved per day for bass during the spawning season. For all sampling sites bass were declared dead if their observed transmitter location did not change for two months or more. Canadaway Creek and Chautauqua Creek were sampled in 2010 and will be tracked in the spring of 2011 so that data from these streams can be incorporated into the statistical analysis.

Results:

Genetics Results:

Assessment of Sampling Sites and Microsatellite Loci

Overall 226 bass from seven sampling sites were analyzed using seven microsatellite loci (Table 2). Initially sampling sites were analyzed at the allelic level to asess basic information about the loci used in the population analysis. Canadaway Creek had the highest observed heterozygosity, 0.5776, compared to Cassadaga Lakes which had the lowest observed heterozygosity, 0.4729. Among all sampling sites the average heterozygosity was 0.5258. FIS was used to assess heterozygote deficiency (positive values) or excess (negative values) in each population. Cattaraugus Creek had the highest measure of excess heterozygotes, -0.0619, whereas Dunkirk Harbor had the highest measure of heterozygote deficiency, 0.0711. All sampling sites conformed to Hardy-Weinberg equilibrium at all microsatellite loci (Table 2). Also, the microsatellites used to analyze population structure for bass in Lake Erie showed no evidence for Linkage-Disequilibrium. Conformation to Hardy-Weinberg equilibrium and the lack of evidence for Linkage-Disequilibrium were necessary to use all microsatellite loci in an unbiased fashion during the population analysis.
Table 2. Summary statistics for Sampling Sites- For each location sample size (N), observed heterozygosity (H₀), expected heterozygosity (Hₑ), genetic variation within subsamples (Fₛ), and Hardy-Weinberg Equilibrium probabilities (HWE) are presented.

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>H₀</th>
<th>Hₑ</th>
<th>Fₛ</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canadaway Creek</td>
<td>46</td>
<td>0.5776</td>
<td>0.5703</td>
<td>0.0003</td>
<td>0.2577</td>
</tr>
<tr>
<td>Chautauqua Creek</td>
<td>50</td>
<td>0.5771</td>
<td>0.5643</td>
<td>-0.0296</td>
<td>0.6753</td>
</tr>
<tr>
<td>Eighteen Mile Creek</td>
<td>21</td>
<td>0.4966</td>
<td>0.4963</td>
<td>-0.0012</td>
<td>0.8288</td>
</tr>
<tr>
<td>Cattaraugus Creek</td>
<td>32</td>
<td>0.5179</td>
<td>0.4908</td>
<td>-0.0619</td>
<td>0.7950</td>
</tr>
<tr>
<td>Dunkirk Harbor</td>
<td>22</td>
<td>0.5000</td>
<td>0.4728</td>
<td>0.0711</td>
<td>0.1634</td>
</tr>
<tr>
<td>Van Buren Bay</td>
<td>26</td>
<td>0.5385</td>
<td>0.5725</td>
<td>0.0538</td>
<td>0.4206</td>
</tr>
<tr>
<td>Cassadaga Lakes</td>
<td>29</td>
<td>0.4729</td>
<td>0.4777</td>
<td>0.0122</td>
<td>0.6394</td>
</tr>
<tr>
<td>All locations</td>
<td>226</td>
<td>0.5258</td>
<td>0.5207</td>
<td>0.0064</td>
<td>0.6573</td>
</tr>
</tbody>
</table>

Five of the seven microsatellite loci analyzed in this study showed relatively high levels of polymorphism. MS19 and RB7 had the highest allelic richness among populations with 7 alleles present at each locus (Figure 3). The frequencies for each allele at each locus among all sampling sites were presented in Figure 3. Mdo2 and Mdo11 had the small measures of allelic richness, with two and three alleles observed at each locus respectively. This information could be useful for other studies that that use these microsatellites. Based on these data I would recommend using all loci but Mdo2 and Mdo11 and in their place use more polymorphic loci for a better fine scale analysis.

Among all loci, Mdo11, MS19, and RB7 had negative Fₛ values and thus had excess of heterozygotes (Table 3). Genetic variation was greatest at Mdo9 (Fₜ=0.1324) and lowest at Mdo11 (Fₜ=0.0051) (Table 3). Cumulatively, the average genetic variation observed among all loci was Fₜ=0.0531. The highest measure of genetic divergence was observed at MS19 (Fₛ=0.1.050) and lowest at Mdo3 (Fₛ=0.0405) (Table 4). The average measure of genetic divergence among all loci was (0.0610).
Fig. 3. Allelic Richness and Frequency - The stacked bar chart represents the portion of each allele at each locus. At the top of each bar is the allelic richness for the locus.

Table 3. Summary F-statistics at Each Locus - $F_{IS}$ measures the average departure from Hardy-Weinberg expectations using average deficit of heterozygotes within populations. $F_{IT}$ measures genetic variation in the total sample. $F_{ST}$ measures the reduction in total expected heterozygosity of the entire group of populations due to drift.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Na</th>
<th>Allele Size</th>
<th>$F_{IS}$</th>
<th>$F_{IT}$</th>
<th>$F_{ST}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mdo2</td>
<td>2</td>
<td>197-201</td>
<td>0.0319</td>
<td>0.0879</td>
<td>0.0578</td>
</tr>
<tr>
<td>Mdo3</td>
<td>5</td>
<td>117-139</td>
<td>0.0303</td>
<td>0.0696</td>
<td>0.0405</td>
</tr>
<tr>
<td>Mdo8</td>
<td>6</td>
<td>208-222</td>
<td>0.0113</td>
<td>0.0656</td>
<td>0.055</td>
</tr>
<tr>
<td>Mdo9</td>
<td>5</td>
<td>121-133</td>
<td>0.0764</td>
<td>0.1324</td>
<td>0.0605</td>
</tr>
<tr>
<td>Mdo11</td>
<td>3</td>
<td>169-173</td>
<td>-0.0636</td>
<td>0.0051</td>
<td>0.0646</td>
</tr>
<tr>
<td>MS19</td>
<td>7</td>
<td>102-124</td>
<td>-0.0508</td>
<td>0.0596</td>
<td>0.105</td>
</tr>
<tr>
<td>RB7</td>
<td>6</td>
<td>110-137</td>
<td>-0.0962</td>
<td>-0.0482</td>
<td>0.0438</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>-</td>
<td>-0.0087</td>
<td>0.0531</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Cumulatively, these data also provide information about the utility of these microsatellites in other applications. They also allow for basic genetic composition of each of these loci at each population studied in this research project. Finally, these basic descriptive statistics validate assumptions about each locus made in the statistical models used during the assessment of population.
Assessment of Population Substructure

All sampling sites were tested to determine if there were any significant genetic differences between sampling sites using measures of genetic divergence (\(F_{ST}\)) and genetic differentiation (Fisher's exact G test) (Table 4). Measures of \(F_{ST}\) between two populations are related to variance in allele frequencies (Holsinger and Weir, 2009). Thus if the \(F_{ST}\) was small, the allele frequencies between the populations are very similar and thus little variance (Holsinger and Weir, 2009). However, if the \(F_{ST}\) was high then the allele frequencies between the populations were not very similar, and thus there was a high degree of variance (Holsinger and Weir, 2009). Fischer's exact G test was used as a non-parametric test to compare allelic frequency heterogeneity between populations. This goodness of fit test is comparable to \(F_{ST}\) measurements of population differentiation and does not require the assumption of random mating (Gaudet et al., 1996). It has been suggested that this test is highly sensitive to fine scale differences in populations (Gaudet et al., 1996).

In this study the majority of sampling sites were significantly different from each other for both tests. However, Canadaway Creek and Chautauqua Creek were not significantly different from each other using both statistics (Table 4). Van Buren Bay and Cattaraugus Creek demonstrate consistent genetic divergence compared to Stepien et al. (2007), and Borden (2008). All sampling sites were significantly different from Cassadaga Lakes, which was the outgroup (Table 4). Notably, some measures of genetic divergence and genetic differentiation for Eighteen Mile Creek and Dunkirk Harbor were marginally non-significant using one statistic however were significant using the other.
Table 4. Above the diagonal are measures of genetic differentiation $\chi^2$ and below the diagonal are measures of pairwise $F_{ST}$ based on seven microsatellite loci between all sampling sites. P-values were corrected using FDR and significance was denoted by a *

<table>
<thead>
<tr>
<th></th>
<th>Canadaway Creek</th>
<th>Chautauqua Creek</th>
<th>18 Mile Creek</th>
<th>Cattaraugus Creek</th>
<th>Dunkirk Harbor</th>
<th>Van Buren Bay</th>
<th>Cassadaga Lakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canadaway Creek</td>
<td>..</td>
<td>16.45</td>
<td>24.29</td>
<td>38.54*</td>
<td>33.18*</td>
<td>Infinity*</td>
<td>Infinity*</td>
</tr>
<tr>
<td>Chautauqua Creek</td>
<td>0.001</td>
<td>..</td>
<td>26.12*</td>
<td>29.96*</td>
<td>28.84*</td>
<td>Infinity*</td>
<td>Infinity*</td>
</tr>
<tr>
<td>18 Mile Creek</td>
<td>0.0154*</td>
<td>0.0227</td>
<td>..</td>
<td>14.39</td>
<td>15.16</td>
<td>25.26*</td>
<td>Infinity*</td>
</tr>
<tr>
<td>Cattaraugus Creek</td>
<td>0.0224*</td>
<td>0.0127*</td>
<td>0.002</td>
<td>..</td>
<td>21.49</td>
<td>34.52*</td>
<td>Infinity*</td>
</tr>
<tr>
<td>Dunkirk Harbor</td>
<td>0.016*</td>
<td>0.0099*</td>
<td>0.0094</td>
<td>0.0036</td>
<td>..</td>
<td>19.67</td>
<td>Infinity*</td>
</tr>
<tr>
<td>Van Buren Bay</td>
<td>0.0203*</td>
<td>0.0193*</td>
<td>0.0173*</td>
<td>0.0233*</td>
<td>0.011</td>
<td>..</td>
<td>Infinity*</td>
</tr>
<tr>
<td>Cassadaga Lakes</td>
<td>0.1483*</td>
<td>0.1253*</td>
<td>0.188*</td>
<td>0.1515*</td>
<td>0.1319*</td>
<td>0.142*</td>
<td>..</td>
</tr>
</tbody>
</table>

Nei’s (1978) measure of genetic distance assesses how similar or dissimilar each pair of populations is to each other based on the infinite allele model. Genetic distance between sampling sites was used to generate a neighbor-joining tree which generates a visual representation of the conclusions based on table 4 (Figure 4). Canadaway Creek and Chautauqua Creek were least genetically distance from each other. Cattaraugus Creek and Eighteen Mile Creek group well together. An anomaly in the dendrogram was Dunkirk Harbor clustering with Cattaraugus Creek and Eighteen Mile Creek (Figure 4). However, Eighteen Mile Creek and Cattaraugus Creek are more genetically distant compared to Dunkirk Harbor. Cassadaga Lakes were most genetically distant from all tributary sites as they are in a different drainage basin. Van Buren Bay did not cluster with the rest of the tributary sites and Dunkirk which validates the classification of it being a Lake site.
Two of the three small tributaries clustered very closely with each other and the third was slightly more distinct. If each sampling site was its own discrete population, Canadaway Creek and Chautauqua Creek would have more genetic differences. However, despite being 25 km away from each other these two streams are closely related. This may be evidence of a strain of bass that reproduces in any tributary of Lake Erie and not a distinct tributary. This prediction was tested by plotting $F_{ST}/(1 - F_{ST})$, which is a measure of genetic distance (Rousset, 1997), against geographic distance (km) between the all tributary sampling sites (Figure 6). I excluded Cattaraugus Creek from this tributary analysis because it was significantly different from Chautauqua Creek and Canadaway Creek based on $F_{ST}$ and $\chi^2$ statistics. The $F_{ST}$ and $\chi^2$ statistics for Cattaraugus Creek demonstrate that it is the most genetic divergent stream site. This would have resulted in significant decrease in gene flow between these populations which was most likely the result of fidelity to that specific stream. The small streams all had lower values for both $F_{ST}$ and $\chi^2$ statistics which suggests the opposite is true for these streams. To
have such low values for these statistics it suggests a high degree of gene flow. Also, Stepien et al. (2007) and Borden (2008) both note that Cattaraugus Creek was a distinct genetic strain of bass and my data corroborate well with these statements. In this linear regression there is a significant positive association (p=0.0155) between geographic distance and $F_{ST}/(1-F_{ST})$ among all the small tributary sites (Figure 5). Thus genetic differentiation increases when the distance between small tributaries increases. This association may explain why Eighteen Mile Creek is more genetically distant from Canadaway Creek and Chautauqua Creek.

\[
y = 0.0005x - 0.0093 \\
R^2 = 0.94, \ p=0.0155
\]

Fig.5. Test of Isolation by Distance: A linear regression between genetic distance ($F_{ST}/(1-F_{ST})$) vs. geographic distance.

Cumulatively these genetic data do show some substructure present in smallmouth bass residing in the eastern basin of Lake Erie. They demonstrate that there are significant differences between lake and tributary sites. These tributary sites cluster well to each other in the dendrogram as well. The two lake sites are more distant to the tributary sites, with Dunkirk Harbor more similar to the tributaries than Van Buren Bay. Also there is a significant trend that
the tributary sites become more genetically distant as the distance between the sites increases. These genetic data support the finding present in other smallmouth bass investigations (Stepien et al., 2007; Borden, 2008).

**Telemetry Results:**

**Site Fidelity**

Bass from both tributary and lake sites did display some degree of confirmed site fidelity. In Cattaraugus Creek had 90% of all bass sampled in 2009 returned to the stream in 2010 during the spawning seasons (Table 5). Notably, only 45% of the bass stayed in the stream for more than one day. In Clear Creek 40% of bass returned to spawn in 2010 and 20% of those bass were in either Cattaraugus Creek or Clear Creek for more than one day (Table 5). Bass sampled from Dunkirk Harbor displayed a 50% return frequency and 40% stayed for more than one day (Table 5). Return frequency data are not present for Canadaway Creek and Chautauqua Creek because these bass were sampled in 2010 and I am waiting to conduct sampling during the 2011 spawning season. There were 14 mortalities observed during this study: 3 bass died in Clear Creek, 1 bass died in Dunkirk Harbor, and 10 bass died in Canadaway Creek. No data was observed for 1 bass in Canadaway and 8 bass in Chautauqua Creek. There is no clear explanation as to why so many bass died in Canadaway Creek. Also, no bass have strayed to other sampling locations to spawn the following season.

Table 5. Bass displayed site fidelity to both lake and tributary spawning locations with return frequencies ranging from 40-90%.

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Returned</th>
<th>Returned &gt; 1 day</th>
<th>Deaths</th>
<th>No Data</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattaraugus Creek</td>
<td>20</td>
<td>0.90</td>
<td>0.45</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clear Creek</td>
<td>10</td>
<td>0.40</td>
<td>0.20</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dunkirk Harbor</td>
<td>10</td>
<td>0.50</td>
<td>0.40</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Canadaway Creek</td>
<td>25</td>
<td>N/A</td>
<td>N/A</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chautauqua Creek</td>
<td>25</td>
<td>N/A</td>
<td>N/A</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>
Factors that Influence Bass Spawning Behavior

Data generated during the course of the site fidelity analysis were also used to study bass spawning behavior. Throughout the course of this study bass were observed 667 times and for each observation GPS location of the bass, water temperature, turbidity, and the date were recorded. Linear mixed models (LMM) were used to analyze repeated measures of bass movement during the two consecutive spawning seasons. Using a LMM that had relative bass movement per day as the dependent variable I determined that location (F<sub>4, 324.3</sub> = 7.429, p<0.0005), year (F<sub>1, 320.3</sub> = 4.158, p=0.042), day (F<sub>1, 278.6</sub> = 5.379, p=0.021), and location-day interactions (F<sub>4, 316.1</sub> = 6.895, p<0.0005) significantly influenced relative bass movement during the spawning location. The estimated marginal means for relative bass movement at each location ranged from 168.95 ± 29.24 m in Dunkirk Harbor to 93.43 ± 37.20 m in Clear Creek (Table 6). Year was an important factor in relative movement with a 2009 estimated marginal mean for relative bass movement per day of 165.12 ± 19.45 m. In 2010 relative distance moved per day decreased to 111.05 ± 18.42 m.

Table 6. Estimated marginal means for relative bass movement per day at each location generated from a LMM.

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean (m)</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattaraugus Creek</td>
<td>162.30</td>
<td>18.06</td>
<td>125.53 - 199.06</td>
</tr>
<tr>
<td>Chautauqua Creek</td>
<td>157.91</td>
<td>38.37</td>
<td>82.05 - 233.76</td>
</tr>
<tr>
<td>Canadaway Creek</td>
<td>107.82</td>
<td>32.71</td>
<td>43.20 - 172.45</td>
</tr>
<tr>
<td>Clear Creek</td>
<td>93.43</td>
<td>37.20</td>
<td>19.81 - 167.04</td>
</tr>
<tr>
<td>Dunkirk Harbor</td>
<td>168.95</td>
<td>29.242</td>
<td>110.34 - 227.55</td>
</tr>
</tbody>
</table>

I used a LMM to determine if water temperature, the dependent variable, was significantly different between spawning locations because location and location-day interactions greatly influenced relative bass movement per day. Using this model it was determined that location was significantly important (F<sub>4, 258.7</sub> = 3.226, p=0.013). This model also determined that year (F<sub>1, 441.8</sub> = 21.621, p<0.0005), day (F<sub>1, 285.5</sub> = 137.147, p<0.0005), year-day interactions (F<sub>1, 243.9</sub> = 29.248, p<0.0005), and location*day interactions (F<sub>4, 243.9</sub> = 2.686, p=0.032)
influence water temperature as well. This LMM generated estimated marginal means for water temperature at each location (Table 7). Clear Creek had the highest marginal mean for water temperature, 19.28 ± 0.64°C. Dunkirk Harbor had the lowest marginal mean temperature, 16.10 ± 0.44°C. This is particularly interesting because Dunkirk Harbor has a coal burning plant that discharges warm water into the harbor throughout the year. Estimated marginal means for water temperature for 2009 and 2010 differed by 3°C; 16.30±0.029 and 19.19±0.029 respectively.

Table 7. Estimated marginal means for water temperature at each location generated from a LMM.

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean (°C)</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattaraugus Creek</td>
<td>18.75</td>
<td>0.28</td>
<td>18.18 – 19.31</td>
</tr>
<tr>
<td>Chautauqua Creek</td>
<td>16.11</td>
<td>0.43</td>
<td>15.25 – 16.97</td>
</tr>
<tr>
<td>Clear Creek</td>
<td>19.28</td>
<td>0.64</td>
<td>18.01 – 20.55</td>
</tr>
<tr>
<td>Canadaway Creek</td>
<td>18.24</td>
<td>0.45</td>
<td>17.35 – 19.14</td>
</tr>
<tr>
<td>Dunkirk Harbor</td>
<td>16.10</td>
<td>0.44</td>
<td>15.21 – 17.00</td>
</tr>
</tbody>
</table>

After determining that water temperature was significantly different at each location, a binary logistic regression was used to determine if turbidity was significantly different between spawning locations. The binary logistic regression illustrated that turbidity was significantly different between locations (Waldₐ = 95.86, p<0.0005) and Year (Wald₁ = 20.96, p<0.0005).

The two LMMs and the binary logistic regression suggested that water temperature and turbidity were significantly different between spawning locations. These models also demonstrated that location influenced bass movement. Using this knowledge I used another LMM to determine if turbidity and water temperature influenced relative bass movement per day during the spawning season. This model determined that turbidity (F₂, 319.2 = 8.870, p<0.0005), day (F₁, 134.8 = 7.195, p=0.008), year (F₁, 253.5 = 4.778, p=0.03), and year-turbidity interactions (F₂, 334.3 = 6.604, p=0.002) all significantly influenced relative bass movement per day. The estimated marginal means generated for turbidity demonstrate that bass moved less when turbidity was low (Table 8). The estimated marginal means for relative distance moved...
per day were 164.72 ± 20.41 m in 2009 and 99.36 ± 22.43 m in 2010. What is interesting about this model is that water temperature was not significant despite being significantly different between spawning locations.

Table 8. Estimated marginal means for relative distance moved per day for Low (1), Medium (2), and High (3) measures of turbidity.

<table>
<thead>
<tr>
<th>Turbidity</th>
<th>Mean (m)</th>
<th>Std. Error</th>
<th>95% Confidence Interval Lower Bound</th>
<th>95% Confidence Interval Lower Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60.18</td>
<td>21.28</td>
<td>18.22</td>
<td>102.14</td>
</tr>
<tr>
<td>2</td>
<td>178.27</td>
<td>19.91</td>
<td>138.70</td>
<td>217.83</td>
</tr>
<tr>
<td>3</td>
<td>157.682</td>
<td>33.62</td>
<td>91.44</td>
<td>223.92</td>
</tr>
</tbody>
</table>

As a result of water temperature not being significant in the previous model I used another LMM to determine if turbidity and water temperature were associated with each other using water temperature as the dependent variable. The factors year ($F_{1,451} = 129.621$, $p < 0.0005$), location ($F_{4,541} = 6.124$, $p < 0.0005$), day ($F_{1,451} = 270.174$, $p < 0.0005$), turbidity ($F_{2,541} = 10.251$, $p < 0.0005$), year-day interactions ($F_{1,451} = 143.579$, $p < 0.0005$), year-turbidity interactions ($F_{2,451} = 18.072$, $p < 0.0005$), and location-turbidity interactions ($F_{1,541} = 7.454$, $p < 0.0005$) were all significant. At low turbidity the estimated marginal mean of water temperature was 19.32 ± 0.27. The estimated marginal mean water temperatures for medium turbidity (16.68 ± 0.63) and high turbidity (16.38 ± 0.52) were similar (Table 9). Based on this model water temperature and turbidity are associated with each other because of the high degree of significance between these variables.

Table 9. Estimated marginal means for water temperature for Low (1), Medium (2), and High (3) measures of turbidity.

<table>
<thead>
<tr>
<th>Turbidity</th>
<th>Mean (°C)</th>
<th>Std. Error</th>
<th>95% Confidence Interval Lower Bound</th>
<th>95% Confidence Interval Lower Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.32</td>
<td>0.27</td>
<td>18.80</td>
<td>19.85</td>
</tr>
<tr>
<td>2</td>
<td>16.68</td>
<td>0.63</td>
<td>15.45</td>
<td>17.91</td>
</tr>
<tr>
<td>3</td>
<td>16.38</td>
<td>0.52</td>
<td>15.36</td>
<td>17.4</td>
</tr>
</tbody>
</table>
By utilizing linear mixed models and a binary logistic regression, my data suggest that location, turbidity, and water temperature all influence relative bass movement per day during the spawning season. These data also suggest that water temperature and turbidity are highly associated with each other. Relative distance moved per day for bass varied among the different sampling locations and there was no observable movement trend for lake or tributary locations. Cumulatively, these data elucidate bass behavior during the spawning season by demonstrating how water temperature and turbidity are important at each bass spawning location.

Discussion:

Smallmouth Bass Life History:

My thesis supports a growing body of evidence that bass in Lake Erie display at least two distinct life histories. One life history bass live and spawn in the Lake and is considered a lacustrine strain. This statement is supported by genetic and telemetry data from Dunkirk Harbor bass. These bass were genetically divergent from tributary spawning bass and they demonstrated fidelity to their spawning location for two consecutive spawning seasons (Table 4 and Table 5). The other life history bass display is represented by bass that live in the lake and spawn in tributaries of Lake Erie and this is considered a potamodromous strain. This statement is validated by significant genetic differences presented in my thesis (Table 4). Stepien et al. (2007) and Borden (2008) both present data that suggests that river and lake spawning locations in the Eastern basin of Lake Erie are significantly different from each other as well. Thus my results have been independently confirmed results in other smallmouth bass studies in Lake Erie can collectively they provide strong evidence for my argument. Collectively these data suggest that bass do not represent a homogenous resource in Lake. Thus it is recommended that managers take action at the stock level to protect these various strains of bass.

The outgroup, Cassadaga Lakes, was significantly different to all sampling locations during this study. This is evidence that bass from Lake Erie are a genetically distinct population compared to bass in the Mississippi drainage basin. Stepien et al. (2007) compared the genetic data generated with bass from the Eastern basin of Lake Erie and bass from the Mississippi
drainage basin as well. My data independently confirm findings from Stepien et al. (2007) that suggest these populations of bass are genetically distinct. These data also suggest that the Lake Erie population has never been stocked with bass from the Mississippi basin. A veteran New York State Department of Conservation Aquatic Biologist stationed at the Lake Erie Unit has no knowledge or records that Lake Erie has ever been supplemented by a smallmouth bass stocking program as well (Don Einhouse, pers. comm.).

Based on pairwise $F_{ST}$ and $\chi^2$ statistics Cattaraugus Creek is genetically distinct from other tributary sites with the exception of Eighteen Mile Creek (Table 5). However, note that Eighteen Mile Creek was marginally non-significant using both statistics. Cattaraugus Creek was one of the large river sites that was sampled in Stepien et al. (2007) and Borden (2008) and my findings complement their results. It is interesting to note that Chautauqua Creek and Canadaway Creek are the least genetically divergent ($F_{ST}=0.001$) spawning locations despite being separated by a geographic distance of 25 km. This is in contrast to Stepien et al. (2007) and (Borden, 2008) results, where they noted that smallmouth bass populations that were sampled in rivers only a few kilometers away from each other were genetically distinct. The rivers sampled in both of these papers were large rivers that flow into Lake Erie.

I sampled both large and small tributaries of Lake Erie for my research. These small tributaries do not show strong trends in genetic divergence. Thus I tested if the measures of genetic distance between these small tributaries were associated with geographic distance. Using this test, measures of genetic distance between these small tributaries were significantly associated with geographic distance. Collectively my data suggest that these small tributary bass may be another distinct smallmouth bass population in Lake Erie. My genetic data suggest bass that spawn in these small tributaries may not use a distinct stream to spawn in, but rather they spawn in any small tributary that feeds Lake Erie. However more small tributaries would need to be sampled to confirm this prediction.

What is interesting to note about bass is that most male smallmouth bass are semelparous, meaning that they only spawn once (Raffeto et al., 1990; Barthel et al., 2008). As the literature suggest some bass do display fidelity to their spawning locations however if the bass are semelparous they do not have this opportunity. Perhaps the bass that do display
semelparity are those that spawn in the small tributaries opportunistically, this hypothesis warrants more investigation.

Eighteen Mile Creek and Dunkirk Harbor demonstrated inconsistencies throughout my statistical analysis. Using both measures of genetic divergence and genetic differentiation Dunkirk Harbor was marginally non-significant to Cattaraugus Creek (Table 5). This may have influenced its location in the dendrogram created using Nei’s measure of genetic distance (Figure 5). Based on the dendrogram Dunkirk Harbor was more closely related to Eighteen Mile Creek and Cattaraugus Creek despite being a lake spawning location. Eighteen Mile Creek was significantly different from Canadaway Creek based on the measure $F_{ST}$ (0.0154*) but using Fishers Exact G test it was not significantly different from Canadaway Creek. Compared to the other small tributary site, Chautauqua Creek, Eighteen Mile Creek was not significantly different using Wright’s $F_{ST}$ (0.0227) whereas using Fisher’s Exact G test it was significant. Eighteen Mile Creek and Dunkirk Harbor both had sample sizes less than 30; as a result they may not have represented the complete distribution of allele’s at all seven loci, if true this could explain these discrepancies. Also, Dunkirk Harbor has only been a refuge area for bass for only a period of decades thus it may lack statistical significance as a result.

Both Stepien et al. (2007) and Borden (2008) have suggested that genetic differences observed in their studies were the result of bass displaying strong fidelity to their spawning locations. Some of the smallmouth bass ecology literature presented data that some bass display fidelity to their spawning locations and home ranges (Ridgeway and Shuter, 1996; Barthel et al., 2008). Borden (2008) noted that spawning site fidelity had yet to be confirmed in Lake Erie’s smallmouth bass populations. The work completed in my thesis offer the first evidence of site fidelity in Lake Erie. This investigation suggests that bass from both lake and tributary sites do return to their spawning locations. However, more analysis on lake locations would be beneficial as I only studies two lake sites during this research project. My telemetry data consist of two complete spawning seasons worth of data for one lake site, Dunkirk Harbor, and two tributary sites Cattaraugus Creek and Clear Creek. Using these data my thesis confirmed that some bass display fidelity to their spawning location. The proportions of bass that did return to each of their spawning locations were comparable to those present in Barthel
et al. (2008). Data for Canadaway Creek and Chautauqua Creek bass have not yet been collected. When these data are collected and analyzed I will be able to determine if my prediction about bass spawning in any small tributary will be confirmed.

My genetic data and behavioral data suggest bass in Lake Erie display at least two distinct life histories. Barthel et al. (2008) presented findings from a 7 year study in which they analyzed bass spawning behavior in a River-Lake system in Canada. Their data suggest that bass display a high degree of fidelity to their spawning location and bass that spawn in either the lake or the river have distinct phenotypic characteristics. Barthel et al. (2008) observed that Lake spawning bass were larger than bass that spawned in the tributary. Collectively, data presented in Barthel et al. (2008) suggest that bass do display two distinct life histories and my data corroborate well with these findings.

A unique to this study is the collection of both behavioral and genetic data for the same bass from a variety of lake and tributary sites. Cumulatively these data complement each other well because bass displayed site fidelity to their spawning location and these same spawning locations were genetically distinct from each other.

Smallmouth Bass Spawning Behavior

As a result of tracking bass for two years using radio telemetry techniques, I was able to collect a large data set which contained the location of a bass, water temperature, turbidity, and the date it was observed. Using these data, I constructed a series of LMMs to determine which factors were most important when predicting relative bass movement per day.

The first model created determined that bass movement was significantly different between locations. Water temperature and turbidity were also determined to be significantly different among my sampling locations. Previously I have discussed the potential for tributaries to provide advantages to their YOY. Water temperature is very important for determining when bass spawn (Scott and Crossman 1973; Graham and Orth 1986). Using the models in my thesis water temperature did not significantly influence bass movement during the spawning season. However, turbidity and water temperature were highly associated with each other. Thus, water temperature is likely to play a role in spawning behavior. Todd and Rabeni (1989)
also demonstrated that water temperature was important for bass movement in a stream dwelling population present in Missouri. Based on my estimated marginal means, Dunkirk Harbor had the coldest temperatures (16.10± °C) during the spawning season. However, Chautauqua Creek had the second coldest temperatures (16.11± °C) during the spawning season. Despite this anomaly, the other three tributary sites had water temperatures estimated marginal means ranging from 18.24±0.45°C to 19.28± 0.64. Temperature data from these spawning locations support the tributary advantage hypothesis discussed previously, with the exception of Chautauqua Creek.

Estimated marginal means for relative distance moved per day by bass during the spawning season ranged from 93.43± 37.20 to 168.5± 29.24 meters per day. Todd and Rabeni (1989) analyzed bass movement in a Missouri river and noted that bass movement was positively associated with water temperatures. In contrast, my data suggest that bass move less when water temperatures are high (Table 8 and Table 9). The estimated marginal means for relative bass movement per day was positively associated turbidity as well. Carter et al. (2010) suggested that prey selectivity increases with turbidity in Smallmouth bass. Perhaps the increase in movement observed in this study is the result of the alterations in bass feeding strategies during the spawning season.

Consistently the factors year and day were significant in the LMM constructed. Intuitively the significance of these factors would be expected to vary based on the stochastic nature of environmental variables in these streams and the Lake itself. Tributaries can often be affected by variables associated with run-off. This is true when the different locations sampled during this study are taken into account. I sampled two small tributaries of Lake Erie, one large tributary, a second order stream that feeds into Cattaraugus Creek, Clear Creek, and a lake site. These difference sites will most likely be affected by environmental conditions just because they are present in different locations. This conclusion was validated using the LMMs.

Overall these data have provided some insight on what factors on relative bass movement per day during the spawning season. These models have demonstrated that there are differences in movement among lake and tributary bass. They also suggest that
environment variables from year to year and even day to day influence bass movement. Collectively these data are consistent with known fish behavior.

Conclusions:

Smallmouth bass in Lake Erie are a genetically pure strain based on results from this study and the work of Stepien et al. (2007). This study was conducted in the eastern basin of Lake Erie. Collectively results from this study and others suggest that bass from Eastern basin of Lake Erie are genetically distinct from the central and western basin (Stepien et al., 2007; Borden, 2008; Borden and Krebs, 2009). Thus the importance of maintaining these unique genetic stocks of bass in Lake Erie should be noted by managers.

There is some evidence in my thesis that there may be another genetically distinct strain of smallmouth bass that spawns in only small tributaries of Lake Erie. However, a more extensive genetic analysis for the small tributaries of Lake Erie needs to be conducted for conclusive answer to this question. The shores of Lake Erie are littered with small tributaries that feed the lake and thus provide the opportunity to test this prediction thoroughly.

Lacustrine and potamodromous smallmouth bass strains exist in Lake Erie however it is unknown what proportions are they present in the overall lake population. This question is particularly important because it would allow managers to assess which stocks are more successful at spawning. I have suggested that tributary bass would provide more advantages to themselves and their YOY. In contrast, Barthel et al. (2008) provided evidence that Lake spawning bass are more successful at rearing young. They suggest this is the result of the distinct size differences in smallmouth bass between smallmouth bass and that tributary reared YOY are more susceptible stochastic water flows. A stock identification study would provide some useful insight into this issue.

My thesis research combined behavioral ecology techniques and population genetic techniques together in one study. In a variety of smallmouth bass genetic studies conducted in Lake Erie predictions that spawning site fidelity have resulted in genetic differences among various smallmouth stocks in Lake Erie. Collectively, my field and molecular data provide
evidence that site fidelity is likely to have resulted in the genetic divergence between lake and tributary spawning bass.

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