

QUANTIFYING INDIVIDUAL CONTRIBUTION TO LARGEMOUTH BASS
REPRODUCTION: EXPLORING THE EFFECTS OF SPRING FISHING, HABITAT AND
REPRODUCTIVE BEHAVIOR

By

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ABSTRACT

QUANTIFYING INDIVIDUAL CONTRIBUTION TO LARGEMOUTH BASS REPRODUCTION: EXPLORING THE EFFECTS OF SPRING FISHING, HABITAT, AND REPRODUCTIVE BEHAVIOR

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Largemouth bass (*Micropterus salmoides*) are among the most popular game fish in North America. Bass are also recognized as a keystone species because of their ecological impact on the systems they inhabit. Angling during the spring reproductive period of largemouth bass is controversial because of fears of potential negative population level impacts. However the relative influence of angling versus natural features on male reproductive success and recruitment are not fully understood. Relevant ecological questions about largemouth bass mating behavior such as the number of individuals that contribute to recruitment, and patterns of YOY dispersal also remain, and are important for management because they could mediate angling effects on bass reproduction. We integrated field observations, experimental angling and genetic techniques to assess the relative effects of angling and natural features on male success, document mating behavior and explore YOY dispersal. Analysis revealed that natural features of male nests were more important than angling to a males contribution to recruitment, however nests receiving experimental angling produced roughly 3x fewer YOY than control nests. On average 3.4 females contributed to individual nests in Warner Lake, and 51% of nests had evidence of cuckoldry. Less than 10% of the adult population contributed to recruitment in any one year in either lake. YOY bass dispersed on average 300 m from their nest of origin, and related YOY were no closer together on average than randomly selected YOY bass. Ultimately habitat features may be more important than angling for YOY recruitment and substantial inter-population differences in mating behavior may exist.

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INTRODUCTION

Largemouth bass (*Micropterus salmoides*) are a species of ecological and social importance (Suski and Philipp 2004; Bremigan et al. 2008). Ecologically, largemouth bass impact the water clarity and species composition of waters they inhabit by controlling organisms in lower trophic levels (Carpenter et al. 1985). Carpenter et al. (1987) documented substantial changes in fish and zooplankton communities in systems where bass had either been added or removed. Likewise, Mittelbach et al. (1995) found significant changes in zooplankton composition and documented a decrease in water clarity in a system where bass had been naturally extirpated. Re-introduction of bass resulted in a transition back to the previous state (Mittelbach et al. 2006). The fact that largemouth bass influence the systems in which they reside to such a large degree suggests that largemouth bass are a keystone species (Mittelbach et al. 1995; Estes et al. 2011). Largemouth bass are also among the most popular game species in the United States (Siepker et al. 2007). Bass are a popular target of numerous professional and amateur fishing tournaments, as well as non-competitive recreational angling. Angling during the bass reproductive season is popular among a segment of bass anglers, and bass are particularly vulnerable to angling during their reproductive season. The vulnerability of male bass to angling, coupled with negative implications for the survival of his offspring make the practice and management of spring fishing quite controversial.

During the spring male largemouth bass build highly visible nests in the shallow littoral areas of lakes (Heidigner 1975; Philipp et al. 1997). Females lay eggs in the nest and then leave the male bass, who aggressively guards his brood from nest predators to provide sole parental care (Ridgway 1988; Philipp et al. 1997). This aggressive behavior, coupled with the visibility of nests contributes to the vulnerability of guarding male bass to angling (Kieffer et al. 1995).

Angling has been documented to increase rates of nest abandonment by guarding males (Suski et al. 2003), which results in complete nest failure. Angling is also associated with numerous negative effects on individual bass such as exhaustion and air exposure (e.g. Thompson et al. 2008); however the extent of population level effects of angling is not known.

Part of the uncertainty regarding the effects of angling relative to natural features on bass population recruitment stems from basic questions about largemouth bass reproductive behavior and YOY dynamics that remain unanswered. For example, largemouth bass have been visually observed to be polygamous, yet DeWoody et al. (2000) documented high rates of monogamy in one population using molecular genetic techniques. Knowing the number of mates a male receives is important for generating accurate estimates of effective population size (Nunney 1993). Additionally, very little is known about the dispersal of YOY largemouth bass from the nest of origin to summer habitats. Knowledge about dispersal distance and patterns is needed to determine the relative importance of nest site to post-dispersal survival. Techniques for studying dispersal, however have been limited.

In 2006 the Michigan Department of Natural Resources (MDNR) instituted a spring catch and immediate release (CIR) fishing season for largemouth and smallmouth bass. In the Lower Peninsula the CIR season begins on the last Saturday in April and runs through the Friday immediately preceding Memorial Day. This thesis, in which we present data from two lakes in southern Lower Michigan, is part of the MDNRs effort to evaluate what (if any) effects the new regulation is having on the bass populations of Michigan's Lower Peninsula. Our research also lends insight into other ecological questions about largemouth bass that are relevant to management.

In chapter 1 our first objective was to determine if experimental angling decreased the ability of an individual male to recruit young of year (YOY) bass to the population. We predicted that nests receiving experimental angling would contribute significantly fewer YOY to the population than control nests. Our second objective was to determine the relative influence of experimental fishing and natural features of the guarding male and the habitat of the nest on the number of YOY recruited to the population. We predicted that angling would be the most important factor determining YOY contribution. Our final objective was to evaluate the success of established observational field techniques in locating nests and tracking them through time. We predicted that observational techniques would find the majority of nests in a lake and successfully track nests through time. We achieved these objectives by collecting genetic samples from each observed nest in spring and from fall YOY samples in Warner Lake, MI. At each nest we collected data on the nest habitat, and guarding male characteristics. Finally on a subset of nests we conducted an experimental angling treatment. We used pedigree reconstruction techniques to determine the spring nest of origin of individual summer/fall YOY. Analysis revealed that there was no statistical difference in the number of YOY recruited between nesting bass receiving angling and control bass, but sample size was small and hence limited our statistical power. In fact, the number of YOY recruited from experimentally fished nests, from which the guarding male was caught, was on average three times less than the number recruited from control nests. Natural features of the nest, particularly plant cover and guarding male defense behavior were found to be more influential than experimental angling in predicting the success of males in recruiting YOY to the population. Finally we found that observational techniques did not document the majority of nests located in the lake, and often failed to correctly track the nest through time.

In chapter 2 our objectives were to 1) characterize the mating behavior of largemouth bass and evaluate associations between mating behavior and male characteristics, habitat features of the nest, and the number of females contributing eggs, 2) determine the number of individuals that contributed to YOY samples at the whole lake level, and 3) determine the prevalence of repeat spawning and nest site fidelity.. We achieved these objectives by sampling eggs from spring nests in Warner Lake and summer YOY from Lake Chemung and Warner Lake in 2009 and 2010. We used pedigree reconstruction to determine the number of females and males contributing to eggs from each nest (Warner Lake), and the number of individuals that contributed to the YOY sample from both lakes. Analysis revealed that Warner Lake nests averaged 3.4 females per nest, with 51% of nests identified as having multiple males contributing to the offspring in the nest as well. We found no significant association between number of females and number of eggs. Plant cover was the only significant variable associated with number of females in a nest, with increasing plant cover being associated with fewer females and lower contribution of YOY (Chapter 1).

In chapter 3 our objectives were to measure the distance dispersed from the nest of origin to summer capture site for YOY bass in Warner Lake, and determine if related YOY remained aggregated relative to unrelated YOY in Lake Chemung. We predicted that YOY would remain close to their nest of origin, and that related YOY would remain aggregated relative to unrelated YOY. We collected genetic samples from nests and fall YOY samples in Warner Lake, and YOY samples in Lake Chemung in 2009 and 2010. We used pedigree reconstruction to determine the nest of origin of Warner YOY and measured the straight line distance between their capture site and assigned nest of origin. In Lake Chemung we used pedigree reconstruction to estimate the relationship between YOY, and measured the straight line distance between

capture sites of related YOY bass. For comparison we randomly selected YOY bass from our data set and measured the distance between them to determine the distribution of unrelated YOY. In Warner Lake YOY bass were captured 301 m from their nest of origin on average. In Lake Chemung we found no significant difference in the inter-individual distance between related and random YOY bass. However, related bass varied substantially in the extent of aggregation, with some sibling pairs collected at the same fyke net location and other sibling pairs collected in distant fyke nets.

Ultimately we documented that natural features are important in determining the number of YOY individual male bass contribute to recruitment. Nests located in low cover areas are more likely to contribute to YOY recruitment and attract more females than nests in areas of high cover. Multiple males and multiple females contributing to offspring in a single nest are quite common, at least in our Warner Lake population. We documented substantial dispersal of YOY from their nest of origin over the course of the summer, but found that related YOY bass did not remain aggregated relative to unrelated YOY bass.

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INTRODUCTION

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CHAPTER 1

EXPERIMENTAL EVALUATION OF THE EFFECTS OF ANGLING AND NATURAL FACTORS ON NEST SPECIFIC CONTRIBUTION OF LARGEMOUTH BASS NESTS TO RECRUITMENT

Introduction:

Recruitment is highly variable in fishes (Anderson 1988) and this variability is attributed to both natural and anthropogenic factors. Much attention has focused on the impacts of fisheries on recruitment (Myers and Mertz 1998; Allan et al. 2005; Myers and Worm 2005). Natural conditions (physical environment, food availability) can also cause variation in recruitment (Daskalov 1999; Cushing 1990), and efforts have been made to predict recruitment based on natural environmental variation (Myers 1998). Without understanding the effects and magnitude of natural associations between recruitment and behavioral, demographic and environmental factors researchers cannot fully determine the impacts of fishing and other anthropogenic disturbances on recruitment, resulting in less effective management (Agnew et al. 2002).

Recently recreational fisheries have been recognized to impact local populations similar to commercial fisheries (Post et al. 2002; Cooke and Cowx 2006; Lewin et al. 2006). The impact of recreational fisheries on local fish populations is difficult to document because each population is utilized by relatively few anglers, recruitment in many populations is supplemented by stocking programs, and there is a lack of long term data (Post et al. 2002). Evolutionary effects of recreational fishing have been receiving increased attention as well (Allendorf and Hard 2009). Behavioral traits such as boldness or aggression are often correlated with growth and may be particularly vulnerable to selection because aggressive fish are more likely to be caught (Biro and Stamps 2007). Post et al. (2002) noted that sport fisheries may not be self regulating, as previously believed, [whereby anglers respond to decreasing catch rates by

concentrating efforts elsewhere (Johnson and Carpenter 1994)]. Hard et al. (2008) reviewed evolutionary effects of fishing on salmon populations, and documented a variety of effects including reduced size at age, and changes in spawning timing over time. Estimates of harvest by recreational anglers may contribute substantially to global fish harvest (Cooke and Cowx 2006), highlighting the importance that management agencies address the potential impacts of recreational fisheries to maintain angling opportunities for future generations (Cooke and Cowx 2004).

Catch and release (CR) sport fishing has arisen in part as an effort to mitigate the negative effects of fishing induced mortality by returning captured individuals alive to the water (Arlinghaus et al. 2007). Researchers have documented relatively low mortality rates associated with some CR fisheries (Thompson et al. 2008; Halttunen et al. 2010). However, CR fishing still has the potential to influence fish abundance and genetics through either delayed mortality associated with angling (Cooke and Cowx 2004; Siepker et al. 2007) or disruption of critical life history stages such as reproduction (Cooke and Suski 2005; Siepker et al. 2009). Catch and immediate release (CIR) fishing is a subset of CR fishing in which fish are immediately returned to water in an effort to mitigate negative effects of handling and live well stress (Cooke et al. 2002; Siepker et al. 2007)

Male largemouth bass are particularly vulnerable to angling during the reproductive season because of their visibility and aggressive nature while on the nest (Kiefer et al. 1995). When the guarding male is removed from the nest, either by angling or other means, nest predators may consume offspring in large numbers (Philipp et al. 1997; Steinhart et al. 2004). Parental investment theory predicts that a male guarding fewer offspring is more likely to abandon his nest than a male guarding more offspring (Sargent and Gross 1986; Trivers 1972), and offspring

in nests that have been abandoned have little probability of survival (Heidinger 1975). Therefore if CIR angling results in some removal of offspring by predators the nest may be more likely to fail, even if the male initially returns to the nest. This hypothesis has been supported theoretically (Steinhart et al. 2008) and experimentally (Suski et al. 2003 and Cooke et al. 2008) on closely related smallmouth bass (*M. dolomieu*). Lunn and Steinhart (2010) found that while experimental brood reduction did not increase nest abandonment across all nests, nests that were abandoned were primarily guarded by younger bass and had offspring of an earlier developmental stage, also consistent with parental investment theory. Timing of angling may also be important to the rates of nest abandonment (Ridgway 1988). Male aggression would be expected to increase with offspring age (Ridgway 1988) resulting in bass that are more likely to be caught (Suski and Philipp 2004).

The negative impacts of angling on individual bass are well documented. Thompson et al. (2008) documented sub-lethal physiological changes that fish experience when angled. Bass subjected to angling showed an increase in blood lactate concentrations as a result of air exposure and the anaerobic exercise associated with being caught. Air exposure may also result in the collapse of gill lamellae, which reduces gill surface area and impedes gas exchange (Thompson et al. 2008). Despite the evidence of negative individual effects, population level effects of angling are not as well understood, because very little is known about the total number of fry produced in a lake (but see Ziegenmeyer 2011), the total number of nests that contribute to fall young-of-year (YOY) populations (but see Gross and Kapuscinski 1997) or the extent of compensatory processes that mitigate the loss of some nests through higher survival of offspring from remaining nests during the summer (Ridgway et al. 2002).

Recent studies indicate that vulnerability to angling is heritable in bass (Garret et al. 2002; Philipp et al. 2009). Therefore if angling reduces the fitness of individual bass by increasing nest abandonment, one would expect that fish genetically predisposed to angling vulnerability would systematically contribute less to recruitment, relative to genetically less vulnerable individuals (Philipp et al. 2009). Over time systematically reduced contribution could conceivably result in lower genetic variation in lakes of high fishing pressure, and lower average vulnerability to angling (Philipp et al. 2009). Additionally bass that are not aggressive are generally poor nest guards (Cooke et al. 2007) and would likely contribute less to recruitment under natural conditions. Therefore angling could also be detrimental to overall male quality and average fitness by systematically reducing the number of vulnerable aggressive males that are better nest guards relative to less vulnerable less aggressive males that are poor nest guards.

In many ways knowledge of population level effects of angling has been hindered because of the difficulty of tracking bass offspring throughout and following the breeding season. When compared to other fish, nest-building fishes such as largemouth bass initially provide a somewhat easier context to address questions about individual contributions to recruitment, especially during early developmental stages of the offspring (Raffetto et al. 1990; Philipp et al. 1997; Steinhart et al. 2005; Wagner et al. 2006). However, once offspring disperse from a nest, opportunities to follow individuals based on direct observation are lost. Accordingly, the majority of literature considering male bass reproductive success has been related to observations during early ontogenetic stages [i.e., free swimming up-fry prior to dispersal from nest site (Philipp et al. 1997; Steinhart et al. 2005; Lunn and Steinhart 2010)]. Considering a male successful at the up-fry stage is unsatisfying at several levels, primarily because observing a nest at the up-fry stage is a binary result assessing male nesting success as either a yes or no, and says

very little about the specific contribution of each male to fall recruitment. In order to determine the actual number of individuals contributed by each male, researchers need a means to attribute YOY with their nest of origin. Finally, observational studies provide researchers with little opportunity to evaluate the success of established field methods. For example, observation error may result in nests being missed, or may misclassify nests as successful or failed. If a large number of nests are not located in a lake, the results of an observational study would not extrapolate well to the entire system, particularly if the missed nests are not a random sample of all nests in the lake, with respect to features (e.g., water depth, vegetation density) that may be associated with offspring survival. Therefore a gap exists in the knowledge of the fates of nests between the up-fry stage and fall YOY stage.

Recently genetic approaches have been used to track individuals beyond the ability of observational studies, and thereby gain insight into recruitment in fish populations. Page et al. (2003) used assignment tests to determine the stocked strain of lake trout (*Salvelinus namaycush*) that contributed most to recruitment in the Great Lakes, and found that the spawning adults from hatchery strains that were most abundant contributed proportionally fewer recruits relative to other strains. Gross and Kapuscinski (1997) used restriction fragment length polymorphisms to generate nest-specific genotypes of smallmouth bass nests in order to determine what proportion of nests contributed to fall recruitment. Their study occurred in just one bay of a lake, and genetic techniques have advanced significantly since that time.

Our first objective was to assess if experimental angling reduced the number of males whose nests were observed to reach the up-fry stage and genetically determined to contribute to YOY populations relative to control males. We predicted that males that were experimentally angled would contribute fewer YOY than control males to recruitment. Our second objective was to

assess the relative contribution of fishing, male characteristics and habitat at and around the nests in determining the number of YOY contributed by male bass. We predicted that angling would be relatively more important than natural features of the nest in determining the number of YOY recruited to the population. Our third objective was to evaluate the accuracy of observational techniques in locating nests within a lake and in determining the fate of those nests (in terms of producing up-fry and YOY). Evaluating the success of our observational techniques is important for interpreting the results of our first two objectives and the results of other observational studies. We predicted that observational techniques will accurately track nests through time and are capable of detecting the majority of nests established in a lake.

Methods:

Study Site

We conducted a whole lake study in Warner Lake (N42°28'15"/W85°31'29") in southern Michigan. Warner Lake is an oligotrophic lake with surface area of 24 ha, and a maximum depth of 16 m. We chose this lake because it is private, largely undeveloped (8% of shoreline with residential development), and receives relatively little angling pressure which might have interfered with an angling experiment.

Spring nest surveys

Starting in early May of 2009 and 2010 we conducted surveys for bass nests two to three times per week using three or four person crews. One crew member would drive the boat, one to two members would look for bass nests from the bow of the boat and another crew member was towed behind the boat with a wetsuit and snorkel mask to look for nests. Each time we sampled the lake, crews would proceed in the opposite direction from the previous visit. We surveyed

visible depths (< 3 meters), zig zagging when necessary to cover shallow areas. Upon locating a new nest we recorded the location using a Garmin GPSmap 76 hand held GPS receiver. We noted the substrate (sand, silt, cobble), measured water depth at the nest using a 1.5 m PVC pole with markings every 10 cm and assessed the vegetation cover around the nest. We measured plant habitat using a 1x1 meter quadrat centered over the nest, and visually estimating the total percent cover of plants. We photographed each nest in order to estimate egg abundance in the lab. We estimated the number of eggs in a nest by classifying the nest as either weedy or clean, and sparsely or densely covered (with eggs) and applying class specific (e.g. weedy, dense) estimates of egg density across the area of the perimeter (< 2.5 cm from edge) and interior (> 2.5 cm from edge) of each nest (Bremigan et al. in prep). We sampled roughly 50 offspring per nest across several locations within the nest. Offspring that we sampled at the egg stage were brought back and raised in the lab until hatch. We visually estimated guarding male bass size, and assessed aggression in two ways. First we assessed total anti-predator behavior (TAB) by summing the number of anti predator responses that the bass showed to a model bluegill (9 cm) on a one and a half meter pole over a one minute period (30 seconds at edge of nest and 30 seconds in center) as described by Suski et al. (2003). Those responses could be “yawning”, opening up the mouth and flaring operculum, “rushing”, rapidly swimming up to the bluegill model, or “striking”, hitting or attempting to bite the bluegill model (Suski et al. 2003; Suski and Philipp 2004). Secondly we used a measure of site tenacity to determine how willing a male bass was to remain near his nest during processing. We scored bass that fled their nest as soon as the boat approached, and were not seen again during the processing of the nest as a zero. We assigned bass that were only seen sporadically during the processing of the nest a score of one. Bass that remained in site during the entire time the nest was processed were scored a two.

Finally bass that showed signs of aggression to the snorkeler such as yawning or striking were scored a three. Before leaving the nest site we left a small uniquely numbered Styrofoam float anchored by heavy washers, so that the fate of each nest could be determined through time.

In addition to surveying for new nests we re-visited existing nests each time we surveyed the lake. Upon locating an existing nest, we recorded the presences or absence of the male bass, the presence or absence of offspring, and the stage and condition of the offspring, if present. If we observed neither male nor offspring at an existing nest for three consecutive visits (generally 10 days) we considered the nest failed and did not visit again. We tracked offspring at the up-fry stage for as long as they could still be unambiguously linked to a specific nest to learn how long the male bass continued guarding.

2010 Fishing Experiment

In 2010 we conducted a CIR fishing experiment in Warner Lake to determine if CIR fishing detrimentally affected the number of YOY produced by a nest. We randomly assigned the first new nest of each survey day to either an experimental or control treatments and then assigned every other nest to that treatment to ensure that the treatment and control nests were not clumped in specific areas of the lake. Experienced anglers fished nests in the treatment group once per week until up-fry had dispersed or no offspring or male were observed at nest area. After locating the nest to be fished, we anchored the boat at a distance appropriate for sight fishing (approximately 10 meters from nest but varying depending on conditions). We presented the guarding male with three casts each of four different lures: a Texas rigged worm, a weightless salamander, a standard crank bait, and a top water popper. We selected these lures because they fish the entire water column, and are commonly used by bass anglers. We recorded the outcome

of each cast as miss, strike, or hook. If the bass was hooked a snorkeler entered the water and swam quickly to the nest to ensure that the correct bass had been caught and to observe activity at the nest, such as predation while the bass was away from the nest. We landed hooked bass as quickly as possible. We measured the total length of the fish, and photographed the bass to simulate a realistic angling experience for the fish. We started a stop watch and recorded the duration from the time the bass was hooked until the bass was released, to measure total handling time. In order to measure return time, we used a second stop watch to record the duration from the time the bass was released until the snorkeler indicated that the bass had returned to the nest.

Fall Sampling

We sampled YOY bass using mini fyke nets (6 m lead of 0.6 cm mesh, main cage 1.5 X 0.9 m with 2.5 cm mesh) during two sampling nights (August 6th and September 1st). We set between five and six nets per lake per sampling effort. At each location we recorded depth of the main cage, vegetation abundance, substrate and a GPS location. We noted the abundance of all fish captured, and preserved YOY bass in alcohol. In the lab we measured YOY weight and length and uniquely numbered each YOY bass for genetic analysis.

On September 27th - September 30th 2010 we conducted a bass population survey by boat electrofishing. We divided the lake into four transects of roughly equal shoreline perimeter and captured bass of all sizes. Fish were captured by netting from the front of the boat and marked using an anal fin clip. YOY bass were preserved in alcohol and all other captured fish were released in the middle of the transect in which they were caught at the end of processing. We calculated total population size for each lake and total mature population size (fish over 9") using a Schnabel mark recapture estimate (Richter, 1975) in Microsoft Excel. Captured YOY were

preserved in alcohol. In order to obtain the largest possible sample of YOY bass we used a variety of gear types across two months of sampling recognizing that this limits our ability to make very specific inferences across sample periods because sampling location and gear type differed across sampling event.

Laboratory

We extracted DNA from spring nest samples and fall YOY samples using the QIAGEN DNeasy extraction kit (Qiagen, Inc, Valencia, CA) following manufacturer specifications. We diluted all DNA samples to 20 ng/uL, and then amplified them at 10 microsatellite loci (Table 1): *Mdo 2*, *Mdo 7* (Mallory et al. 2000), *Ms 13* (DeWoody et al. 2000), *Lma 12*, *Lma 21* (Colbourne et al. 1996), *Msf 11*, *Msf 12*, *Msf 38*, *Msf 68*, *Msf 173* (Lutz-Carrillo et al. 2008). We conducted microsatellite polymerase chain reaction (PCR) in 25 uL volumes containing 100 ng of template DNA 2.5 uL of 10X PCR buffer (1 M tris-HCl, 1.5 M MgCl₂, 1 M KCl, 10% gelatin, 10% NP-40, and 10% triton X), and 0.8 mM deoxy-nucleotide-triphosphates (dNTPs), 10 pm fluorescently labeled forward and unlabeled reverse primers, sterile water, and 0.5 U Taq polymerase. Reactions were performed using Robocycler 96 thermocyclers (Stratagene, Inc., La Jolla, California). We visualized PCR products on 6% denatured polyacrylamide gels using a Hitachi FMBIO II sequencer (Hitachi Instruments, Tokyo, Japan). All gels were scored by two experience laboratory personnel and entered into Microsoft Access. To further reduce scoring errors, we analyzed data from all loci with the program Micro Checker (Van Oosterhaut et al. 2005), this program searched for data entry errors, such as data that are not congruent with the microsatellite repeat motif. We calculated summary statistics for each locus including number of alleles, observed heterozygosity and the exclusion probability of sibling identity using the

program CERVUS (Kalinowski et al. 2007) We re-ran 10% of all samples as an error check; error rates averaged 0.81% across all loci and ranged from 0.0% (*Ms 13*), to 1.6% (*Msf 38*).

Analysis

We used multi-locus genotypes to estimate pedigree relationship among YOY and eggs sampled from the nest using programs COLONY (Wang, 2004) and PEDIGREE (Smith et al. 2001; Butler et al. 2004). These programs compare the multi-locus genotypes of each sample to other samples and generate the most likely relationship between the two samples (full sibling, half sibling, or unrelated) using Markov Chain Monte Carlo (MCMC) likelihood methods. However, each program employs different algorithms (Wang 2004, Smith et al. 2001; Butler et al. 2004). Herbinger et al. (2006) tested the congruence of these programs on three salmon data sets and found that COLONY and PEDIGREE agreed on the classification of over 99% of their samples. By using two programs with similar aim but different computation techniques, we can be more confident of our findings when the programs converge on similar answers. To verify the accuracy of pedigree assignments based on our sample populations allele frequencies at all loci surveyed we simulated offspring with known relationships by randomly selecting samples from our data set and “mating” them (randomly selecting one allele from each “parent” at each locus) to generate a new data set of simulated offspring with known parents (Radek unpublished program). We analyzed these offspring with PEDIGREE and COLONY to see how successful the programs were at determining the relationship among these offspring.

To investigate the effects of our fishing treatment, our first objective, we used a single factor analysis of variance (ANOVA) in R (R Development Core Team, <http://www.R-project.org>) to determine if significant differences existed in the number of YOY bass, captured across all

sampling periods, produced by the three treatment groups (control, fished: not caught, fished: caught). This approach allowed us to compare the number of YOY produced among groups but is unable to assess what (if any) effects natural features of the nest may have on the success of individual male bass.

In order to determine the relative influence of natural features such as male size and habitat selection in addition to our fishing treatment, our second objective, we analyzed the number of YOY, sampled across all sample periods, produced by individual nests using a regression tree (CART) analysis (Breiman et al. 1984). This method develops a dichotomous tree which divides the observations into groups that minimizes the within group variation in the response variable (number of YOY) as a function of the predictor variables. We assessed the effects of fishing treatment, habitat features of the nest: depth, substrate, plant cover, twigs, and coarse woody material; guarding male features: size, anti-predator responses, site tenacity and number of eggs; date of nest establishment (see table 1. for variable summaries). CART analysis is useful because CART does not necessitate distributional assumptions about the data and the method is capable of analyzing continuous and categorical variables (De'Ath and Fabricius 2000; De'Ath 2002), and has been used to address similar questions in other black bass species (Rejwan et al. 1999). Additionally the trees generated are easily interpreted. All analysis was conducted in R using the `rpart` function (R Development Core Team, <http://www.R-project.org>), using a 10-fold cross validation and pruning using the 1-standard error rule (Breiman et al. 1984; Venables and Ripley 1999).

In order to contrast between observational and genetic studies, our third objective, we compared the males determined to be successful observationally (offspring observed at up-fry stage), and males determined to be successful using genetic techniques (YOY assigned to males offspring).

We estimated the range of missed nests in the lake based on percent of YOY sample assigned to a nest of origin, and the number of un-sampled families YOY grouped into. Finally, we performed a CART analysis on our observational data with male success to the up-fry stage (binary) as the dependent variable, and habitat features of the nest: depth, substrate, plant cover, twigs, and coarse woody material; guarding male features: size, anti-predator responses, site tenacity and number of eggs; treatment: control, fishing: caught, fishing: not caught, and N/A (see above); date of nest establishment as independent variables, in order to determine if the same independent variables are important to male success to up-fry (observational data) and male success to YOY populations (genetic data).

Results:

Spring sampling and fishing experiment

In 2010 we sampled 33 nests in Warner Lake. Of these 33 nests, 7 were assigned to the control group, and 9 assigned to the treatment group. The remaining nests either failed before they could be fished or were located after hatch at the up-fry stage and were not included. Of the nine nests that were fished, crews caught 55% of the guarding males (5 of 9), handling time averaged 100 s between the time the male was hooked until he was released. On average each nest was fished 1.67 times (once per week of each nests existence), but no guarding male was caught more than once. During the time the male was away from the nest no predation was observed on eggs or larvae within the nest. 4 out of the 5 males captured returned to their nest within five minutes of release, and the fifth bass returned to his nest 30 minutes after release.

Genetic diversity

Per locus estimates of variability differed across the microsatellites surveyed (Table 1) including the number of alleles (2-22), observed heterozygosity (0.171 to 0.883). The combined non exclusion probability of sibling identity (the probability of failing to differentiate between two randomly selected full siblings) across all micro-satellites was 0.0006. PEDIGREE succeeded in assigning an average of 96.6% of simulated data of known pedigree into the correct families, suggesting that we were reliably able to place YOY bass back into their nest of origin.

Assignment of YOY bass to nest of origin

In summer 2010 we sampled 97 YOY bass by fyke netting (August 6th and September 1st) and electrofishing (September 27th - September 30th). 34 YOY bass (35%) were assigned back to a nest of origin by PEDIGREE. Overall PEDIGREE inferred a consistent number of families across 100 runs (56-64 half sib families), and COLONY pedigree consignments were highly concordant. PEDIGREE and COLONY agreed on 92% of full sibling assignments and 99% for half sibling assignments. COLONY tended to split full sibling families assigned by PEDIGREE into multiple half sibling families. However, discrepancies in full and half-sibling assignments did not affect the interpretation that a YOY originated from a specific nest. Therefore, we used the PEDIGREE replicate with the highest likelihood score for subsequent analysis.

Fishing experiment

A single factor ANOVA (Table 2) did not detect significant difference in the average number of YOY produced among the three fishing experiment groups: control (1.28 ± 0.57 YOY/nest; mean \pm SE), fishing: caught (0.4 ± 0.4 YOY/nest; mean \pm SE), fishing: not caught (0.75 ± 0.48 YOY/nest; mean \pm SE) ($F = 0.79$, $p = 0.47$). Despite this nests that were part of the control

group produced on average more than three times as many YOY as nests that were fished and the male caught. A power analysis revealed that sample size may have been too small to detect a significant statistical difference between groups (power = 0.2).

Across natural features of males (male size, aggression), habitat features of the nest and treatment experienced, CART analysis revealed that plant cover and anti-predator responses were the most important factors in determining the number of YOY produced by a nest (Figure 1) explaining roughly 40% of the variation in the sample with plant cover having the most explanatory power. Increased plant cover generally negatively influenced the amount of YOY produced by a nest. On average nests that were located in areas with less than 45% cover produced 1.93 YOY/nest, or roughly 4.5 times more YOY than nests located in areas with more than 45% cover (mean 0.43 YOY/nest). The CART analysis detected a significant interaction between cover, and anti-predator responses such that within the high cover group, males that showed anti-predator responses produced nearly 4 times more offspring (mean 0.7 YOY/nest), than males that did not show anti-predator responses (mean 0.18 YOY/nest).

Evaluation of field methods

Up-fry were observed in 19 of the 33 (57.6%) nests observed in this study. YOY bass were assigned to 15 of the 33 nests (45%). However, up-fry were only observed for 9 of the 15 (60%) nests that had YOY assigned to them. 8 of 19 (42%) nests where up-fry were observed did not have YOY bass assigned to them, and 6 nests that were assigned YOY were never observed to have contained up-fry.

Regression tree analysis (CART) identified nest depth as the most important factor affecting the success of nests to the up-fry stage (Figure 2), explaining 50% of the variation in the data. Nests

in water shallower than 1.025 m successfully reached the up-fry stage roughly 69% of the time (18 out of 26 nests). Nests found in water deeper than 1.025 m failed to reach the up-fry stage roughly 86% of the time (7 out of 8 nests). Nest depth and percent plant cover were not found to be significantly correlated ($r = 0.27$, $p = 0.16$) suggesting that plant cover and depth are not measures of the same thing.

Discussion:

Fishing experiment

The fishing experiment in this study failed to detect an association between fishing and the number of YOY produced by individual male bass nests, but had low power because of small sample size. One explanation is that fishing may not affect the ability of largemouth bass nests to produce YOY bass. Despite the lack of significant differences between treatment groups only 3 of the 9 nests (33%) that were fished produced YOY, and further only 1 of the 5 nests (20%) where the guarding male was successfully caught produced YOY bass. Whereas 5 out of 7 (71%) of control nests successfully produced YOY bass. The fact that only 20% of nests where the guarding male was caught produced YOY, suggests that while the negative effect of fishing was not statistically significant, differences in YOY production among groups may be biologically important. An alternative explanation may be that our fishing methods were not sufficiently detrimental to male bass to cause a significant decline in YOY production. In many respects our fishing treatment was relatively benign compared to what a male bass might normally experience during CIR fishing. Bass were landed as quickly as reasonably possible, and were returned to the water as quickly as possible after capture. Previous studies have indicated that the length of time a bass is played significantly influences return time (Philipp et

al. 1997) Since the boat was anchored to maintain a reasonable casting distance for sight fishing bass were returned to the water relatively close to their nest and were therefore able to return quickly. Importantly no nest predation was observed in any of our treatment nests during fishing trials. Reductions in brood size during angling have been shown to be an extremely important factor determining male abandonment rates (Philipp et al. 1997; Steinhart et al. 2004), and may explain why no significant differences were detected between treatment groups. Finally none of the bass that were captured in our study were captured more than once. In lakes of high fishing pressure a bass could be captured several times (Burkett et al. 1986) during the extended period while guarding a nest.

Overall the amount of plant cover was the most important factor determining the number of YOY produced by individual bass (Figure 1). Lower plant cover (<45%) was associated with higher nest success (1.9 YOY/nest), and higher plant cover (>45%) was associated with lower nest success (0.49 YOY/nest). Within the nests that were located in high plant cover areas nesting male behavior was also an important factor. Males that showed anti-predator responses within the high cover group produced more YOY bass (0.7 YOY/nest) than males that did not show anti-predator responses (0.18 YOY/nest). One explanation for this result is that areas of high cover may have higher densities of nest predators, which may reduce the number of offspring that survive to the YOY stage. Hunt et al. (2002) documented higher rates of nest intrusion by predators in areas of complex structure and corresponding higher rates of male aggression. The interaction between cover and male behavior suggests that habitat features dictate levels of male aggression. Interestingly our findings suggest that natural variation in features such as habitat selection and aggressiveness among males is more important for determining the number of YOY produced per nest than angling in this system, however

sampling size was small (n=9 nests fished), and our angling treatment was relatively benign (see above).

Evaluation of field methods

The combination of genetic and observational approaches that we used in this study provides an opportunity to evaluate the success and assumptions of observational data. An assessment of observational data is important because the inferences made when interpreting observational data may be limited if observational techniques do not sample all male nests or are ineffective at tracking the fate of males nests through time.

Across 33 nests observed standard observational field methods, and genetic techniques detected nest success rates of 57.6%, and 45% respectively. Differences in nest success detected by observational and genetic methods are perhaps not surprising, considering the length of time between the two sample periods (May and June for up-fry and August and September for YOY). However, closer inspection reveals that the nests detected to reach the up-fry stage and those detected to contribute to YOY populations only overlapped 60% of the time (i.e. only 60% of nests found to contribute to YOY populations were detected at up-fry stage). Therefore substantial differences exist between the individual nests that are observationally detected (observed at up-fry) to be successful with those nests that actually contribute to YOY populations. Failure to observe successful nests at the up-fry stage could be important if different factors influence nest success to different stages. For example, analyses revealed that the most important factor influencing survival to the up-fry stage was nest depth (consistent with Wagner et al. 2006). Offspring from shallower nests reached the up-fry stage 69% of the time, while offspring from deeper nests failed to reach the up-fry stage 86% of the time. The most

important factor influencing survival to the YOY stage was a combination of the percent plant cover and level of guarding male aggression (depth was not significantly correlated with plant cover $r = 0.27$, $p\text{-value} = 0.16$). Therefore researchers may reach different conclusions about the relative importance of different habitat features depending on which life stage is considered successful. Additionally it is not clear if the impact of depth on nest success in observational studies is a biological effect or an effect of decreased detection probability of up-fry. For example, it is conceivable that offspring reaching the up-fry stage in deeper water will be more likely to drift away from their nest site as a result of currents induced by wind or boat traffic and may therefore not be associated with their actual nest of origin when located by researchers. Such a mistake would result in the nest the offspring actually came from being considered a failed, and might mistaking lead to another nest being classified as successful, and ultimately lead researchers to conclude that deeper nests fail at a greater rate than shallower nests.

In addition to uncertainty in tracking nests through time, our analysis revealed that we also failed to observe a large proportion of the total nests in the lake. Only 35% of YOY we sampled were assigned to a nest of origin. Assuming that we sampled a random subset of the nests and a random subset of the YOY this indicates that we only detected 35% of the nests in the lake during spring sampling. However, sampling biases likely resulted in a higher probability to detect some nests over others. For example, deep nests, or nests found in very high vegetation are difficult to detect and were likely missed at a higher rate than nests in more visible depths or with less plant cover. Therefore a nest detection rate of 35% likely represents a lower bound on the number of nests missed. An upper bound is provided by the fifteen un-sampled nests that the remaining YOY grouped with (i.e. the YOY not assigned to a nest of origin grouped into 15 unique groups), if no other nests existed in the lake we would have detected 69% of all nests (33

nests detected /48 existing). A nest detection rate of 69% is also unlikely because it would indicate that every nest missed contributed to YOY populations. Therefore, we estimate that the total proportion of nests sampled ranged from 35% to 69%. Gross and Kapuscinski (1997) assigned 62% of YOY they sampled to a nest of origin, and suggested that the majority of unassigned YOY originated from a nest outside their study area (they sampled a single bay within a lake), but acknowledged that some may have originated in nests observed to have failed. The field techniques used here were directly comparable to many studies of nesting black basses (including: Raffetto et al. 1990; Wagner et al. 2006), indicating that there is significant potential for established observational techniques to miss nests. Large numbers of un-sampled nests suggest that attributes of successful males in this study and other observational studies must be carefully interpreted as the characteristics of potentially large numbers of nest guarding males may be unknown (31-65% in this study).

Difficulties in observing nests and associating up-fry with the correct nest of origin likely vary substantially between systems as a function of variables that may influence nest and up-fry observation such as water clarity and depth, and variables that may increase their movement such as current or fetch. Uncertainty in the probability of detecting a nest at the up-fry stage may bias interpretation of observation only studies, by increasing the frequency of nests that are easily found or tracked to the up-fry stage relative to nests that are more difficult to find or correctly associate up-fry with nests.

Conclusions

In our system, percent cover and male aggression were natural features more predictive of nesting success (number of YOY contributed to fall populations) of male largemouth bass than

experimental angling. Researchers should consider the effects and magnitude of natural features when evaluating the impacts of fishing on a system.

We detected substantial differences in the nests that were observed to be successful to the up-fry stage, and nests where YOY were detected using genetic techniques. Therefore, observational data must be carefully interpreted because observational data may fail to detect a substantial number of the total nests, and may be unable to reliably track the fate of a nest through time.

APPENDIX

Chapter 1 Figures and Tables

Appendix

Figure 1. Results of regression tree analysis explaining variation in the number of YOY bass produced per nest. Plant cover was the most important factor influencing production YOY, explaining 35% of the variation within the data. Lower plant cover associated with higher nest success. Within the high plant cover group a significant interaction was detected with guarding male aggression (TAB) explaining an additional 5% of the variation, such that more aggressive males (higher TAB) produced more YOY than less aggressive males (lower TAB).

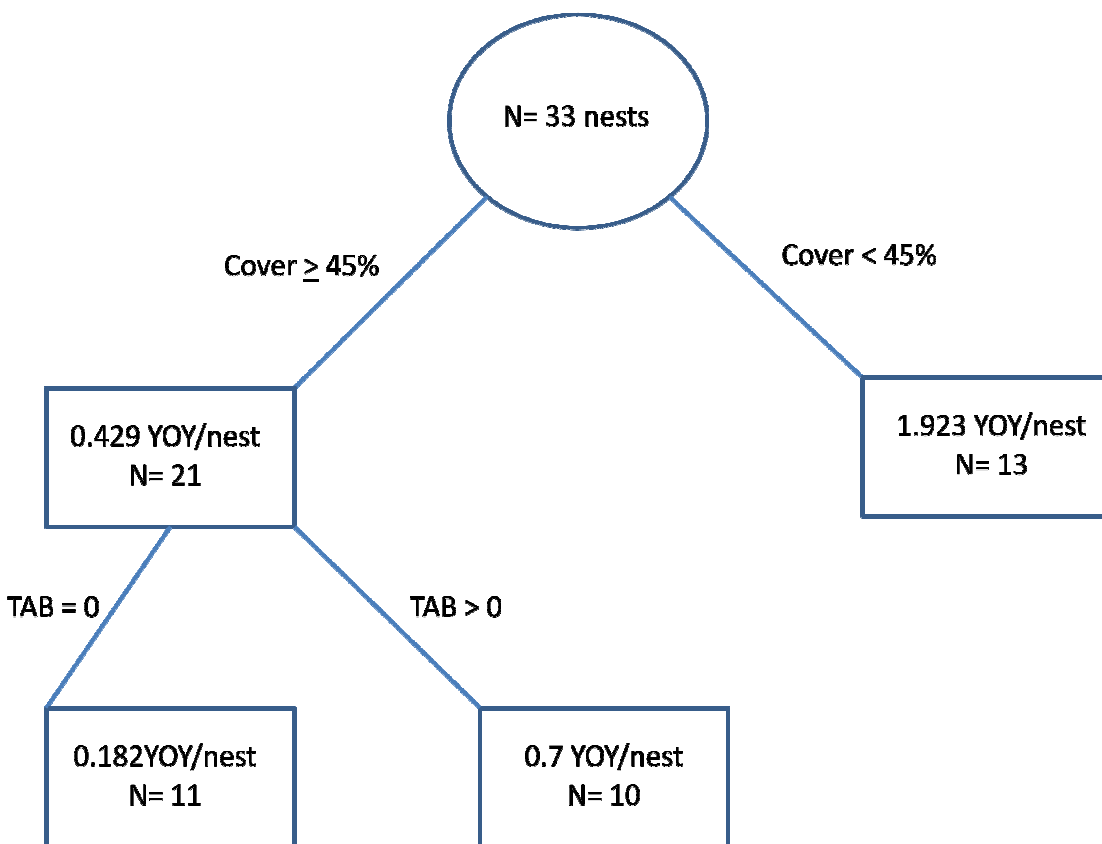


Figure 2. Regression tree analysis for the success of nests to up-fry stage (yes/no). Nest depth is the most important variable determining the success of nests to the up-fry stage, explain 50% of the variation observed in the data.

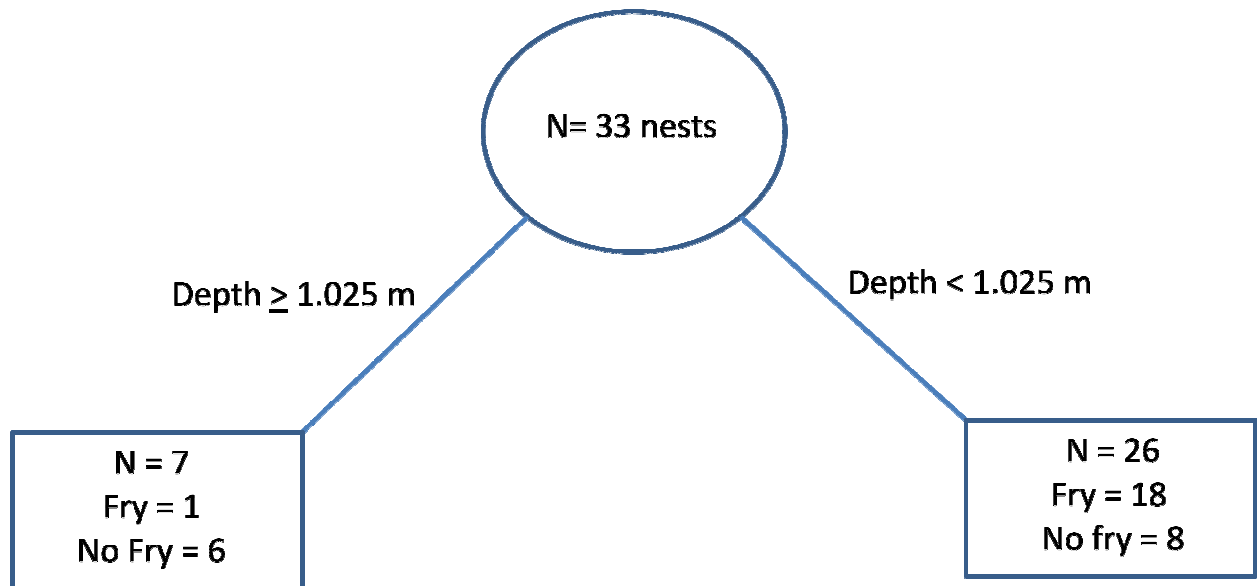


Table 1 Summary measures of genetic diversity for each microsatellite locus [number of alleles (A), observed heterozygosity (Ho), the non-exclusion probability of sibling identity (Ne-SI)] annealing temperature and reference.

| <u>Locus</u> | <u>Annealing (C)</u> | <u>A</u> | <u>Ho</u> | <u>Ne-SI</u> | <u>Reference</u> |
|--------------|----------------------|----------|-----------|--------------|---------------------------|
| Mdo 2 | 57 | 2 | 0.363 | 0.681 | Mallory et al. 2000 |
| Mdo 7 | 55 | 2 | 0.171 | 0.846 | Mallory et al. 2000 |
| Ms 13 | 53 | 3 | 0.484 | 0.577 | DeWoody et al. 2000 |
| Lma 12 | 57 | 2 | 0.289 | 0.727 | Colbourne et al. 1996 |
| Lma 21 | 50 | 7 | 0.699 | 0.444 | Colbourne et al. 1996 |
| Msf 11 | 57 | 8 | 0.724 | 0.416 | Lutz-Carrillo et al. 2008 |
| Msf 12 | 57 | 10 | 0.807 | 0.358 | Lutz-Carrillo et al. 2008 |
| Msf 38 | 57 | 22 | 0.810 | 0.345 | Lutz-Carrillo et al. 2008 |
| Msf 68 | 52 | 14 | 0.798 | 0.372 | Lutz-Carrillo et al. 2008 |
| Msf 173 | 58 | 14 | 0.883 | 0.325 | Lutz-Carrillo et al. 2008 |

Table 2 ANOVA table, comparing the mean (SE) number of YOY produced per nest in each treatment group, and the percentage of treatment nests that were observed to reach the up-fry stage.

| | <u>Control</u> | <u>Fished: Not Caught</u> | <u>Fished: Caught</u> | <u>F</u> | <u>p-value</u> |
|---------------|----------------|---------------------------|-----------------------|----------|----------------|
| N | 7 | 4 | 5 | - | - |
| Number of YOY | 1.28 (0.57) | 0.75 (0.48) | 0.4 (0.4) | 0.79 | 0.47 |
| Fry Yes/No | 71% (0.18) | 75% (0.25) | 60% (0.24) | 0.11 | 0.89 |

Table 3. Mean, range (minimum and maximum) of observations and standard error for quantitative variables assessed at nest site and used in CART analysis. NYOY is number of YOY, date in Julian day, length (in), depth (m), cover (%), twigs (#/nest), CWM coarse woody material (#/nest) , aggress(ion) (site tenacity score 0-3), and TAB (total anti-predator responses) number observed.

| | <u>Mean</u> | <u>Min</u> | <u>max</u> | <u>SE</u> |
|---------|-------------|------------|------------|-----------|
| NYOY | 1 | 0 | 4 | 0.23 |
| Date | 136.4 | 124 | 148 | 1.47 |
| Length | 10.67 | 8 | 14 | 0.29 |
| Depth | 0.89 | 0.7 | 1.7 | 0.06 |
| Cover | 53.7 | 0 | 100 | 6.37 |
| Twigs | 0.67 | 0 | 10 | 0.36 |
| CWM | 0.33 | 0 | 5 | 0.18 |
| aggress | 1.6 | 0 | 3 | 0.13 |
| TAB | 0.61 | 0 | 4 | 0.17 |

LITERATURE CITED

CHAPTER 1

LITERATURE CITED

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CHAPTER 2

GENETIC EVALUATION OF MATING BEHAVIOR IN LARGEMOUTH BASS

Introduction:

Fish display a diversity of mating behaviors (Baylis 1981; Avise et al. 2002 for review), ranging from monogamy to promiscuity. Some species provide no post-ovulatory parental care while others aggressively defend nests and young (Baylis 1981; Gross and Sargent 1985). Knowledge of mating behavior is critical for predicting vulnerability to a disturbance or recovery from disturbance, because mating behaviors may substantially affect population growth and understanding them is therefore important for sound management (Rowe and Hutchings 2003). Mating behaviors are also vital to determining the outcomes of sexual selection because competition between individuals may be strengthened when relatively few individuals mate (Wiegman et al. 1992).

In wild populations visually determining the aspects of mating behavior such as mate number and parentage can be difficult and can lead to erroneous conclusions (Garant et al. 2001; Griffith et al. 2002). For example many bird species were falsely assumed to be monogamous based on visual observation alone. Only when genetic analysis had been conducted was it discovered that extra pair copulations are common (Griffith et al. 2002 for review). In fish, observation of mating behavior is complicated by the need for underwater observation (DeWoody and Avise 2001; Avise et al. 2002).

From a conservation perspective, mating behaviors are particularly important because they can influence the effective size of a population (Nunney 1993), and the number of individuals that contribute to recruitment. Mating systems such as polygamy may increase the reproductive variance among individuals relative to monogamy (Nunney 1993). Therefore if a population is

incorrectly assumed to be monogamous when it is in fact not, effective population size may be estimated incorrectly based on census size alone (Nunney 1993). Incorrect estimates of effective population size could result in a deterioration of genetic diversity faster than was expected under a management regime designed for monogamy.

Genetics techniques provide a means to reliably determine mating systems in wild populations (Avice et al. 2002). For example, parentage analysis allows researchers to determine the parents of an individual by excluding candidate parents from the pool of potential parents using likelihood methods (DeWoody and Avice 2001; Avice et al. 2002; Jones et al. 2010). If parental data are missing, offspring can be grouped into full and half sibling groups to estimate the number of parents contributing to the brood (Smith et al. 2001; Wang 2004). For example Garant et al. (2001) used parentage analysis to reveal aspects of mating behavior in wild salmon (*Salmo salar*). Garant et al. (2001) found that female salmon were not monogamous as previously believed, and that body size was not related to mate number, though mate number and number of young produced were related.

Nest guarding fish provide an interesting study system to evaluate mating behaviors because offspring are concentrated for a period of time, and observations of nest behavior and strategies are possible. The mating systems of bluegill (*Lepomis macrochirus*) have been particularly well studied. Bluegill build nests in large colonies, and have developed three different male strategies: parental males, sneaker males, and satellite males (Gross 1982). Molecular genetic studies have documented that parental males typically sire on average 79% of the offspring found within the nests they guard. The remaining 21% are sired by sneaker or satellite males (Neff 2001).

Largemouth bass (*Micropterus salmoides*) are a nest guarding species for which mating behaviors have not been well studied using genetic techniques. Largemouth bass are generally believed to be polygamous based on observations of offspring at different developmental stages within a nest (Heidinger 1975; Romero and Allen 1975). However these studies do not rule out the possibility of monogamy. DeWoody et al. (2000) used molecular genetic techniques to evaluate this question in largemouth bass, and found that 88% of nests sampled in a single reservoir were composed entirely of full siblings, suggesting that largemouth bass in their study system are highly monogamous. Further genetic evaluation of largemouth bass mating systems is necessary to determine variation in the prevalence of monogamy within and among populations.

Another aspect of mating behavior that is relevant to managers is the number of reproductive events during a lifetime individuals bass have. Semelparous species that only spawn once should invest more energy to ensure spawning success. For example in salmon semelparous species typically have larger eggs relative to iteroparous salmon species (Crespi and Teo 2002).

Breeding failure in semelparous species results in a lifetime fitness of zero and may therefore strengthen the selective forces that disproportionately affect a portion of the population. It is not clear if male largemouth bass spawn in consecutive years. Studies of the closely related smallmouth bass (*M. dolomieu*) have yielded conflicting results with some studies finding that smallmouth bass spawned only once in their lifetime (Raffetto et al. 1990; Baylis et al. 1993), and others documenting smallmouth bass successfully establishing nests in consecutive years and often in the same location (Ridgway et al. 1991; Ridgway et al. 2002). Conflicting results suggest that the mating behaviors expressed in different populations may vary substantially. Waters and Noble (2004) document evidence of repeat spawning and nest site fidelity across

years in a reservoir population of largemouth bass in Puerto Rico, however, whether or not variation exists between populations is not known.

Our objectives were to characterize the mating behavior in closed populations of largemouth bass in two Michigan lakes. Specifically we quantified associations between male characteristics, habitat features, and date of nest establishment to the number of females contributing eggs to a nest and whether mate number resulted in more eggs in a males nest. Factors such as guarding male characteristics, and habitat features around the nest could be important if male or habitat features increase the success of some males relative to others, and thereby increase variance in individual reproductive success. Finally we determined the number and effective number of individuals contributing to young-of-year (YOY) cohorts at the whole lake level in two lakes to determine what proportion of the adult population contributed to recruitment.

Methods:

Study Site

We studied two lakes in southern Michigan. Lake Chemung (N42°34'55"/W83°50'55") is a mesotrophic lake with surface area of 126 ha and has a maximum depth of 21 meters. Because the lake is highly developed (71% of shoreline with residential development) and has a public boat launch, Lake Chemung represents a high fishing pressure lake. The lake hosts several recreational bass tournaments per year. Warner Lake (N42°28'15"/W85°31'29") is an oligotrophic lake with surface area of 26 ha, and a maximum depth of 16 meters. Warner Lake has no public boat launch, is largely undeveloped (only 8% of shoreline developed), and receives relatively little angling pressure.

Spring sampling methods

In May and June 2009 and 2010 we monitored Warner Lake largemouth bass nesting activity. Crews located nests by surveying the shallow water twice each week. Crews consisted of a driver, one to two observers on the bow of the boat and a snorkeler towed behind the boat. When a new nest was found the snorkeler assessed the guarding male's level of aggression in two ways. First we quantified the total number of anti-predator responses (TAB) the male bass exhibited towards a dummy bluegill (9 cm) on a 1.5 m pole, over the course of 30 seconds at the perimeter of the nest and 30 seconds in the center of the nest as described by Suski et al. (2003). Second we measured degree of male site tenacity using a categorical score quantifying the tendency of a bass to stay in the proximity of his nest. The snorkeler scored bass that fled their nests as soon as the boat spotted the nest, and did not return during the processing of the nest as a zero. Bass that initially fled and were occasionally observed during the processing of a nest as a one, bass that moved off the nest initially but were back at the nest every time the diver returned as a two and bass that were openly aggressive to the diver processing the nest, either striking the diver or yawning at the diver, as a three. After assessing the aggression of the bass the diver visually estimated the male bass length (in) and photographed the nest for subsequent egg count estimation in the lab. In the lab nest photographs were classified as weedy or clean, and densely or sparsely covered in eggs. Class specific (e.g. weedy dense) estimates of egg density were applied to the perimeter (<2.5 cm from edge of nest) and interior (>2.5 cm from edge of nest) areas of the nest to determine the total number of eggs in each nest (Bremigan et al. in prep). The diver then measured several habitat features around the nest including water depth using a PVC pipe with markings every 10 cm, substrate, and percentage of total plant cover, using a 1x1 m quadrat centered over the nest. Finally the diver sampled roughly 50 eggs from each nest, to

ensure that sampling did not remove a substantial proportion of the eggs from the nest. Eggs were sampled from several locations throughout the nest, to minimize bias in egg sampling location and enumeration of the number of adults contributing. Eggs were then returned to the lab and hatched in glass containers by nest for genetic analysis.

Using the program PASSaGE v.2 (Rosenberg and Anderson 2011) we calculated the average straight line distance between the nearest neighbors of each nest, and the average distance from each nest to all other nests in the lake (see below). We used nearest neighbor distance and average distance to all other nests as a local and lakewide measure of each nest relative to other nests to determine if proximity to other nests was associated with the number of males and females contributing to the offspring of the nest.

Summer/Fall sampling methods

Once offspring had dispersed from the nest in late June we collected young of year (YOY) bass for genetic analysis in Lake Chemung (2009: Aug. 28th; 2010: Aug. 10th, Sept. 8th) and Warner Lake (2009: Aug. 31st; 2010: Aug. 6th, Sept. 1st). Crews set fyke nets at five locations throughout the perimeter of the lake. Each lake was divided into 10 segments seeking to divide the lakes shoreline evenly among each segment. Crews randomly selected the first segment to set the nets and then put a net in every other segment at the first suitable location. Fyke nets had twenty foot leads with one 0.125 in mesh, and 1 in mesh on the front of the cage. Each net deployed overnight and was emptied the following morning. Crews preserved all YOY bass in 100% alcohol for genetic analysis.

We conducted a mark-recapture survey of each lake over four nights in September in 2009 (Sept 27, Sept 29-30, Oct 8th for both lakes) and 2010 (Sept 27- Sept 30th both lakes) to estimate the population size of adult male bass. Each lake was divided into four segments of roughly equal shoreline distance, all fish were marked and released in the middle of the segment in which they were caught. We calculated total population size for each lake and total mature population size (fish over 9") using a Schnabel mark recapture estimate (Richter, 1975). Total adult population size was needed to calculate the proportion of individuals that contributed to recruitment. All YOY bass captured were preserved in 100% alcohol.

Genetic Analysis

We extracted DNA from all YOY samples from both lakes, and 25 samples per nest (Warner Lake) using the QIAGEN DNeasy extraction kit (Qiagen, Inc, Valencia, CA) following manufacturer specifications. In 5 nests we genotyped 25 samples and then randomly selected 5, 10, 15, and 20 samples and determined how many sibling groups were detected (see pedigree assignment methods below) by each sample size. In 4 out of 5 nests the number of sibling groups stabilized at 15 samples (no further groups detected at 20, or 25 samples respectively), and in all 5 of the groups there was no difference in number of sibling groups between 20 and 25 samples. Therefore to ensure that we had at least 20 samples with a full complement of loci genotyped for as many nests as possible we analyzed 25 samples per nest when available. We diluted all DNA samples to 20 ng/uL, and then amplified them at 10 microsatellite loci (see Table 1): *Mdo 2*, *Mdo 7* (Mallory et al. 2000), *Ms 13* (DeWoody et al. 2000), *Lma 12*, *Lma 21* (Colbourne et al. 1996), *Msf 11*, *Msf 12*, *Msf 38*, *Msf 68*, *Msf 173* (Lutz-Carrillo et al. 2008). We conducted microsatellite polymerase chain reaction (PCR) in 25 uL volumes containing 100 ng

of template DNA 2.5 μ L of 10X PCR buffer (1 M tris-HCl, 1.5 M MgCl₂, 1 M KCl, 10% gelatin, 10% NP-40, and 10% triton X), and 0.8 mM deoxy-nucleotide-triphosphates (dNTPs), 10 pm fluorescently labeled forward and unlabeled reverse primers, sterile water, and 0.5 U Taq polymerase. Reactions were performed using Robocycler 96 thermocyclers (Stratagene, Inc., La Jolla, California). We then visualized PCR products on 6% denatured polyacrylamide gels using a Hitachi FMBIO II sequencer (Hitachi Instruments, Tokyo, Japan). All gels were scored by two experienced laboratory personnel and entered into Microsoft Access. We re-genotyped 10% of all samples as an error check. Error rates averaged 0.81% across all loci and ranged from 0.0% (*Ms 13*), to 1.6% (*Msf 38*). Summary statistics of microsatellite loci (see Table 1) including number of alleles per locus, observed heterozygosity and exclusion probability of sibling identity were calculated using the program CERVUS (Kalinowski et al. 2007). To further reduce scoring errors, we analyzed data from all loci with the program Micro Checker (Van Oosterhaut et al. 2005). This program searched for data entry errors, such as data that are not congruent with the microsatellite repeat motif. We analyzed data by lake and year, different pedigree reconstruction programs PEDIGREE (Smith et al. 2001) and COLONY (Wang, 2004). These programs use different algorithms to group samples into full and half sibling groups based on Markov Chain Monte Carlo (MCMC) estimates of maximum likelihood, determine the number of adults contributing to the sample, and calculate the effective breeding population size of the sample (N_b) (Wang 2004). We visualized the pedigree of each nest generated in COLONY, and interpreted the inferred parent who contributed the majority of offspring to be the guarding male. We grouped pedigrees into three categories: (1) monogamy, (2) polygamy, (3) promiscuity. To establish confidence in pedigree assignments we performed 100 runs of the PEDIGREE program for each data set. Confidence in the results increased when the same groupings of

offspring were generated across replicate runs. Second, by comparing the results of PEDIGREE with the results inferred by COLONY. Confidence increased when the two programs converged on similar pedigree assignments. Herbinger et al. (2006) compared the groupings of COLONY and PEDIGREE using three data sets of salmon and found the programs agreed on 99% of assignments across the three data sets. Thirdly we performed simulations to determine how accurately the programs were able to reconstruct known pedigrees. We simulated multi-locus genotypes of known pedigree using allele frequencies estimated from each lakes bass population (Radek unpublished program). We randomly chose multiple pairs of individuals from our entire sample and then simulated mating by randomly selecting one allele at each locus from each parent to produce groups 52 groups of ten known full siblings. This data set of known full siblings was then analyzed using PEDIGREE and COLONY, and the results of each program compared with the known relationships.

Statistical Analysis

We used single factor analysis of variance to detect differences among pedigree groups (monogamy, polygamy, promiscuity) for variables hypothesized to be associated with the number of females contributing eggs to a nest. Specifically we quantified whether the average number of eggs deposited in the nests of males with only one female (inferred monogamy) differed significantly from the nests of males with multiple females (inferred polygamy) and nests with multiple males and females (inferred promiscuity). We also analyzed whether or not the number of samples processed was significantly associated with the pedigree class inferred for each nest.

To determine if bass spawned during two consecutive years (2009 and 2010) we combined the progeny datasets from both years in Warner Lake and analyzed all data using the PEDIGREE program. Individuals that were sampled at the nests (eggs, larvae or up-fry) from different years and were found to be related at the half sibling level were assumed to have been produced from an adult that spawned in both years. To determine the degree of site fidelity for bass spawning in both 2009 and 2010 we calculated all pair-wise straight line distances between all nests across years using PASSaGE v. 2 (Rosenberg and Anderson 2011). We then used the estimated inter-nest distance matrix to calculate the straight line distance between nests that contained individuals related at the half sibling level across years. We then randomly selected the same number of nests pairs from both years and averaged the distance between them. This simulation was repeated 1,000 times, to generate a distribution of mean distances between randomly selected nests. We compared the distribution of both empirically sampled and simulated data, with a normal distribution using a Shapiro-Wilks test, and compared the distributions using a Kolmogorov-Smirnov test. Analysis was conducted in R (R Development Core Team, <http://www.R-project.org>).

The COLONY program was used to estimate the total number of contributing adults, and the effective number of contributing adults to the YOY samples for each lake (Wang 2004). For this analysis we used samples of YOY bass because they are more representative of the entire breeding population, than samples collected at the nest stage (i.e. includes YOY that originated in nests that were missed or not sampled). We divided the number of contributing adults by the census size estimated from mark recapture data to estimate the N_b/N_c ratio for each year and lake.

Results:

Sampling

In Warner Lake in 2009 and 2010 crews monitored 33 and 35 nests, respectively. We genotyped all nest samples available up to 25 samples per nest in 2009 and 2010. In the fall crews captured 28 YOY in 2009 and 98 in 2010 in Warner Lake and we genotyped all samples. In Lake Chemung crews captured 213 and 298 YOY in 2009 and 2010, respectively, and genotyped all samples.

Genetic analysis

Allelic diversity ranged from 2-22 across loci. Observed heterozygosity ranged from 0.171 to 0.883 across loci, and the combined exclusion probability of sibling identity across all loci was 0.0006. Across 10 runs of simulated data of known relationship the program PEDIGREE assigned 96.6% of samples to the correct full sibling family. Additionally PEDIGREE and COLONY programs concurred on 92% of relationships at the full sibling level and 99% of relationships on the half sibling level suggesting that the microsatellites (see Table 1 for summary stats) used had sufficient power to identify sibling relationships. Because results were similar between programs we used the COLONY results with highest likelihood score because they are easily visualized into nest-specific pedigrees.

Genetic evaluation of individual nests

Across 31 nests sampled in 2009 and 2010 in Warner Lake sampled at either the egg or larvae stage (to ensure that nest of origin is known) we found that 19.4% (n=6) were comprised of a single full sibling family, suggesting that a single male and female (pedigree group 1) had contributed offspring to the nest. 29% (n=9) of nests were comprised of a single half sibling family (pedigree group 2), indicating that all offspring shared one parent in common (assumed to

be the guarding male). We found that 51.6% (n=16) of nests contained multiple half sibling families, indicating that multiple males and females (pedigree group 3) contributed to the offspring contained within the nest. In these nests we assumed that the individual that contributed to the majority of offspring was the guarding male. On average the guarding male contributed to $93 \pm 0.2\%$ (mean \pm SE) of the offspring in the nest, and this value ranged from 66-96%. Across all nests sampled in both years at the egg or larvae stage the average number of females contributing eggs to a nest was 3.4 ± 0.34 (mean \pm SE). In nests where multiple females contributed eggs one female contributed on average $71 \pm 3.6\%$ (mean \pm SE) of the sample to the nest.

Analysis of variance of differences in estimates of nest egg numbers among pedigree classes (see Table 5) did not detect a significant difference in the number of eggs (pedigree group 1 (n=6): 2581 ± 1011 ; pedigree group 2 (n=9): 4110 ± 947 ; pedigree group 3 (n=16): 2500 ± 608 ; mean \pm SE) among nests of different classes ($F = 1.26$ $p = 0.30$), indicating that more females contributing eggs to a nest does not result in more total eggs per nest. However power was low because of small sample size.

None of the variables associated with the guarding male, that might indicate differences in mate quality to female bass such as male size (Hanson and Cooke 2009) differed significantly across the pedigree classes. Male size (pedigree group 1 (n=6): 26.2 ± 2.4 cm; pedigree group 2 (n=9): 27.1 ± 0.9 cm; pedigree group 3 (n=16): 26.0 ± 1.0 cm; mean \pm SE) was similar across all three groups ($F = 0.20$, $p = 0.82$), indicating that male size was not associated with the number of females contributing eggs to a nest. Male site tenacity (pedigree group 1 (n=6): 1.66 ± 0.4 ; pedigree group 2 (n=9): 1.78 ± 0.3 ; pedigree group 3 (n=16): 1.93 ± 0.1 ; mean \pm SE) and anti-predator responses (pedigree group 1 (n=6): 1.16 ± 0.98 ; pedigree group 2 (n=9): 0.11 ± 0.11 ;

pedigree group 3 (n=16): 1 ± 0.31 ; mean \pm SE) also did not differ significantly among pedigree groups (site fidelity $F = 0.29$, $p = 0.75$; anti-predator responses $F = 1.48$, $p = 0.24$).

Percent plant cover (pedigree group 1 (n=6): 83.3 ± 4.9 %; pedigree group 2 (n=9): 40.0 ± 12.2 %; pedigree group 3 (n=16): 59.7 ± 8.5 %; mean \pm SE) was the only significant habitat variable that differed among the pedigree classes ($F = 3.31$, $p = 0.05$). Nests in pedigree group 1 (monogamy) were associated with significantly higher plant cover than the other two groups.

The other nest habitat variables measured water depth (pedigree group 1: 0.95 ± 0.12 m; pedigree group 2: 0.94 ± 0.09 m; pedigree group 3: 0.99 ± 0.08 m; mean \pm SE), and substrate (77% sand, 19% silt, 4% other) were not found to vary significantly among pedigree groups (depth: $F = 0.07$, $p = 0.92$; substrate: $F = 0.64$, $p = 0.53$).

Julian day of nest establishment (pedigree group 1 (n=6): 139 ± 2.2 ; pedigree group 2 (n=9): 132 ± 2.5 ; pedigree group 3 (n=16): 136 ± 2.0 ; mean \pm SE) was not significantly different across pedigree groups ($F = 1.66$, $p = 0.20$).

Neither the local distance to nearest neighbor (pedigree group 1 (n=6): 51.3 ± 25.7 m; pedigree group 2 (n=9): 56.3 ± 20.3 m; pedigree group 3 (n=16): 44.7 ± 10.5 m; mean \pm SE) or lakewide average distance to every other nest (pedigree group 1 (n=6): 297.4 ± 28.2 m; pedigree group 2 (n=9): 296.7 ± 24.0 m; pedigree group 3 (n=16): 325.9 ± 24.0 m; mean \pm SE) differed significantly among pedigree groups (local: $F = 0.14$, $p = 0.87$; lakewide: $F = 0.47$, $p = 0.63$) indicating that differences in location relative to other nests was not associated with the number of females depositing eggs in a nest.

Finally there was not a significant association among the number of eggs analyzed per nest (pedigree group 1 (n=6): 17.5 ± 3.5 eggs; pedigree group 2 (n=9): 21.4 ± 2.6 eggs; pedigree

group 3 (n=16): 18.1 ± 1.9 eggs; mean \pm SE) among the pedigree groups ($F = 0.62$, $p = 0.54$).

The lack of significance in numbers of eggs sampled among pedigree groups indicates that groups were likely not simply artifacts of the sampling regime (i.e. monogamous nests would have been characterized as being polygamous nests if more eggs were sampled).

Repeat spawning and site fidelity

Over both years (2009 and 2010) we identified 8 pairs of nests that had progeny related at the half sibling level in Warner Lake. No groups of full siblings were found. The average distance between nests that shared half siblings across years was 337.01 ± 63.66 m (mean \pm SE). Across 1,000 simulations the average distance between randomly selected nests was 302.31 ± 1.76 m (mean \pm SE). Both distributions were found to be normally distributed using a Shapiro-Wilks test (real: $W = 0.935$, p -value = 0.56; simulated: $W = 0.998$, $p = 0.28$). A Kolmogorov-Smirnov test detected no difference between the distribution of the distance between nests that shared half siblings across years and randomly selected nests ($D = 0.361$, $p = 0.198$).

Effective number of adults contributing to YOY bass

In Lake Chemung during 2009 less than 10% of estimated adult population were parents of the YOY sampled ($N=213$). The number of contributing adults of our YOY sample was estimated to be 81, the effective number of breeders was estimated to be 80 (95% CI: 60-112) and census size of adult bass was estimated to be 966 (95% CI: 640-1973). In 2010 the number of contributing adults was estimated to be 117, the effective number of breeders was estimated to be 118 (95% CI: 90-153) with census size of estimated to be 1966 (95% CI: 1475-2948). In 2009 and 2010 the ratio of contributing adults to census size was estimated to be 0.084 and 0.060 respectively, and the ratio of effective number of breeders was 0.083, and 0.06 respectively.

(Table 4). In Warner Lake during 2009 the number of contributing adults was estimated to be 19, the effective number of breeders was estimated to be 22 (95% CI: 12-43) and census size of adult bass was estimated to be 1132 (95% CI: 728-2074). In 2010 the number of contributing adults was estimated to be 51, the effective number of breeders was estimated to be 55 (95% CI: 39-80) and census size was estimated to be 1392 (95% CI: 841-4022). In 2009 and 2010 the ratio of number of contributing adults to census size was estimated to be 0.017 and 0.037 respectively, and ratio of effective number of breeders to census size was estimated to be 0.02 and 0.04 respectively (Table 4).

Discussion:

Genetic evaluation of individual nests

Overall few of the nests (19.4%) we sampled in 2009 and 2010 were consistent with monogamy. The low rates of monogamy we detected contrasts with the results of the population studied by DeWoody et al. (2000). DeWoody et al. (2000) found that 88% of nests sampled were consistent with monogamy. However differences in analytical techniques (DeWoody et al. 2000 used parentage analysis) and study system (DeWoody et al. 2000 studied a reservoir in South Carolina, USA) may account for these discrepancies. For example variation in abundance of potential nest habitat has been shown to result in different mating strategies in slimy sculpin (*Cottus cognatus*), with abundance of available habitat being associated with monogamy and habitat shortages being associated with polygamy (Mousseau and Collins 1987). The difference between rates of monogamy documented in this study and by DeWoody et al. (2000) adds to the discrepancies regarding bass mating behaviors in the literature, and highlights significant inter-population differences in this species.

The number of females contributing eggs to a nest averaged 3.4 per nest, which is comparable to nests of other Centrarchid fishes. Mackiewicz et al. (2002) found an average of 2.5 females per nest in dollar sunfish (*L. marginatus*), and DeWoody et al. (2000b) found an average of 4.4 females per nest in spotted sunfish (*L. punctatus*). Number of females per nest was not a significant predictor of the number of eggs estimated to be in a nest, suggesting that the number of eggs deposited by individual females varies significantly. It is possible that female largemouth bass exhibit different egg laying strategies, where some choose to lay many eggs in one (or few) nest(s), and other females choosing to scatter relatively few eggs over several nests. Overall, little is known about multiple matings of females in nest making species with male parental care (Coleman and Jones 2011). In fiftenspine stickleback (*Spinachia spinachia*) females have been documented to deposit eggs in multiple nests (Jones et al. 1998). Alternatively an intensive investigation of mottled sculpin (*Cottus bairdi*) found no evidence that females deposited eggs in multiple nests (Fiumera et al. 2002). However, female reproductive success has been shown to increase in Atlantic salmon (*Salmo salar*) that had more mates through increased genetic diversity of offspring (Garant et al. 2005), suggesting a potential benefit to female bass that spawned with more than one male. The majority of nests that contained multiple females generally had one female that contributed the majority of eggs (averaging 71%). The variance in number of eggs laid by individual females in the nests of this study might be explained by the size of the female bass laying the eggs. Larger female bass are more fecund (Heidinger 1975), tend to spawn earlier in the spawning season (Ridgway et al. 1991) and might be laying an equal proportion of their total eggs in each nest as smaller, less fecund females. Although not statistically significant, nests in pedigree group 2 (polygamy) were established earlier on average (day 132 for pedigree group 2 compared with day 139 or 136

for pedigree groups 1 and 3 respectively), and contained more eggs on average (4110 for pedigree group 2 compared with 2581 and 2500 for pedigree groups 1 and 3 respectively).

Unfortunately, we did not have data on female size available in this study to further investigate preliminary trends. Interestingly, this study's findings that the eggs from one female bass made up the majority of eggs deposited in nests with multiple females, is consistent with the findings of DeWoody et al. (2000), for the minority of nests in that study with multiple females.

Plant cover was the only significant habitat predictor of the number of females laying eggs in a nest, with the number of females decreasing as cover increased. One possible explanation is that female bass having difficulty locating nests in densely covered areas. Alternatively nest success was lower in areas of high cover (Chapter 1), and complex habitat associated with plant cover has been shown to result in increased nest intrusion by nest predators (Hunt et al. 2002).

Therefore females may have avoided depositing nests in high cover areas. Habitat features of nests building fishes have been shown to be important in female nest choice, for example in pumpkinseed (*Lepomis gibbosus*), larger females avoid mating with males that build their nests in soft substrates (Danylchuk and Fox 1996). In three-spined stickleback (*Gasterosteus aculeatus*) reproductive success is positively correlated with the plant cover of a nest (Mori 1993).

No nesting male covariates studied were found to be significant predictors of the number of female bass in a nest. Male size was virtually identical across the three pedigree groups indication that females were not preferentially selecting larger males in this population.

However the vast majority of all bass in Warner Lake are relatively small with 99% fish sampled less 356 mm (Utrup, unpublished data), suggesting there may not be sufficient variation for strong female mate choice. However in smallmouth bass size has been shown to be a significant

predictor of reproductive success, with larger males receiving more eggs than smaller males (Hanson and Cooke 2009).

Neither date of nest establishment or local or lakewide clustering of nests was found to be a significant predictor of number of females laying eggs within a nest. The lack of spatial effects documented here is interesting because one might expect isolated nests to have either more females (no other nests in site so lay eggs here), or fewer females (possibly because the nest is located in poor habitat).

We documented substantial evidence of multiple male largemouth bass fertilizing eggs within a nest by observing multiple half sibling families within a nest. Multiple males were detected in 52% of nests found at the egg or larvae stage across two years. Such high levels of cuckoldry are surprising considering its frequency in our study system, and the lack of other documentation of this phenomenon in the largemouth bass specific literature (Avise et al. 2002; DeWoody et al. 2000). On average the male interpreted to be the guarding male contributed to roughly 93% of the offspring detected in a nest, with an average of 7% of eggs being fertilized by other males. Interestingly, 7% of eggs being fertilized by other males is comparable to the rates of cuckoldry reported for other sunfish: dollar sunfish, where approximately 5% of offspring are sired by other males (Mackiewicz et al. 2002), redbreast sunfish (*L. auritus*) where approximately 12% of offspring sired by other males (DeWoody et al. 1998), pumpkinseed sunfish (*L. gibbosus*), where approximately 15% of offspring are sired by other males (Rios-Cardenas and Webster 2008) and bluegill where roughly 21% of offspring sired by other males (Neff 2001).

Repeat spawning and site fidelity

We observed no evidence of site fidelity and relatively low rates of repeat spawning in Warner Lake over the 2009 and 2010 nesting season. The distribution of nests found to contain related offspring across years was not significantly different from the distribution of randomly selected nests, suggesting that nests with related offspring were no closer together than two randomly selected nests. Eight pairs of nest were detected to contain related offspring across years, but it is unclear if these offspring were related through a common mother or common father. In either case substantial evidence does not exist for high rates of repeat spawning between years. A lack of evidence of repeat spawning is important to the management of this system and other systems where this may be the case because, if on average, bass spawn only once in their lifetime, or have long gaps between spawning events nest failure may greatly reduce lifetime fitness. Reducing the number of lifetime spawning opportunities strengthens the selective force of anything that systematically increases or decreases the probability of nest success, and suggests that constant recruitment of cohorts each year is important to maintain a viable population. However, given that we sampled a small proportion of nests in each year (Chapter 1), it is possible that we missed nests that had related individuals from both years. Additionally it is possible that 2009 and 2010 did not represent normal reproductive years in the lake, or that bass spawn every other year.

Effective number of contributing adults

The effective number of contributing adults (N_b) estimated by COLONY varied in both lakes across years. In general a larger proportion of the total adult population contributed to YOY sampled in Lake Chemung with roughly 8% and 6% contributing in 2009 and 2010 respectively (Table 4), relative to Warner Lake in which only 2% and 4% of the population contributed in to YOY sampled in 2009 and 2010 respectively (Table 4). The inferred number of breeders (N_i)

was similar to effective number of breeders in both lakes, and was actually smaller than the effective number of breeders in Warner Lake in both years. This is likely a result of both females and males mating with multiple partners (Nunney 1993). The fact that fewer individuals contributed to recruitment in Warner Lake is surprising considering that Lake Chemung is much more developed and has higher rates of angling (Ziegenmeyer 2011). One explanation may be that nesting habitat is more limiting in Warner Lake relative to Lake Chemung. Warner Lake is much smaller (26 ha relative to 126 ha) but has a similar adult population size (1262 relative to 1466; two year average) suggesting that nesting habitat (no significant difference detected in plant cover between the lakes (Ziegenmeyer unpublished data) may limit the opportunity of some individuals to nest in Warner Lake, thereby increasing individual reproductive variance and decreasing effective number of breeders (Nunney 1993). In Slimy sculpin (*C. cognatus*) populations where nesting sites were abundant, monogamy was highly prevalent, and populations where nesting sites were limiting were primarily polygamous (Mousseau and Collins 1987) which might decrease effective number of breeders within a season relative to monogamy (Nunney 1993). If a similar effect occurred in Warner Lake it might explain why the N_b/N_e ratio was smaller than in Lake Chemung.

Several factors should be considered when interpreting these data. A potential source of error that could influence the findings pertains to the sampling of eggs from each bass nest. Eggs were sampled randomly throughout the nest in an effort to obtain a sample that was representative of the entire egg mass. However, we could not evaluate how effective this method was in achieving this goal. Sampling error has the potential to bias egg sample towards fewer females, or certain females within each nest. Additionally, egg samples were hatched in the lab, and we were unable to determine if the eggs of some females were predisposed to survive lab environments

relative to other females. Differences in the survival of eggs of different females also have the potential to bias the estimates of number of females lower and/or alter the contribution of individual females. Therefore our results are likely conservative in terms of numbers of females contributing to a nest.

Conclusions

Genetic analysis of individual nests documented nests consistent with monogamy and polygamy, and promiscuity. Plant cover was significantly related to the number of females contributing eggs suggesting that male habitat selection may increase or decrease his appeal to females. However, no advantage was detected for males who attracted multiple females to their nest in terms of egg numbers and likely reproductive success unless nest habitats also affect survival to later life stages as documented in Chapter 1. Finally we did not find evidence for substantial spawning in consecutive years or site fidelity across years, though our ability to detect repeat spawning was likely low.

APPENDIX

Chapter 2 Tables

APPENDIX

Table 4. Sample size of YOY (NYOY) bass across lakes and year. Census size (Nc) of adult (> 22.8 cm) bass in the lake, with 95% confidence interval. Number of parents that contributed to sample (Ni), Number of breeders (Nb) as inferred by the program COLONY with 95% confidence intervals. Ratio of contributing adults to adult census size (Nb/Nc), and the ratio of number of contributing adults to number of YOY in sample (Nb/NYOY).

Lake Chemung

| | <u>NYOY</u> | <u>Nc</u> | <u>95% CI</u> | <u>Ni</u> | <u>Nb</u> | <u>95% CI</u> | <u>Ni/Nc</u> | <u>Nb/Nc</u> |
|------|-------------|-----------|---------------|-----------|-----------|---------------|--------------|--------------|
| 2009 | 213 | 966 | 640-1973 | 81 | 80 | 60-112 | 0.084 | 0.083 |
| 2010 | 295 | 1966 | 1475-2948 | 117 | 118 | 90-153 | 0.060 | 0.060 |

Warner Lake

| | <u>NYOY</u> | <u>Nc</u> | <u>95% CI</u> | <u>Ni</u> | <u>Nb</u> | <u>95% CI</u> | <u>Ni/Nc</u> | <u>Nb/Nc</u> |
|------|-------------|-----------|---------------|-----------|-----------|---------------|--------------|--------------|
| 2009 | 28 | 1132 | 728-2074 | 19 | 22 | Dec-43 | 0.016784 | 0.019435 |
| 2010 | 98 | 1392 | 841-4022 | 51 | 55 | 39-80 | 0.036638 | 0.039511 |

Table 5. ANOVA table for all evaluated variables with mean (SE), across the three pedigree groups (1: monogamy, 2: polygamy, 3: promiscuity)

| | Pedigree Group | | | <u>F</u> | <u>p-value</u> |
|----------------------------------|----------------|--------------|--------------|----------|----------------|
| | <u>1</u> | <u>2</u> | <u>3</u> | | |
| N | 6 | 9 | 16 | - | - |
| Samples analyzed | 17.5 (3.5) | 21.4 (2.6) | 18.1 (1.9) | 0.62 | 0.54 |
| Date (Julian) | 139 (2.2) | 132 (2.5) | 136 (2.0) | 1.66 | 0.21 |
| Male Size (cm) | 26.2 (2.4) | 27.1 (0.9) | 26.0 (1.0) | 0.2 | 0.82 |
| Nest Depth (m) | 0.95 (0.12) | 0.94 (0.09) | 0.99 (0.08) | 0.07 | 0.93 |
| % Plant Cover | 83.3 (4.9) | 40 (12.2) | 59.7 (8.5) | 3.31 | 0.05* |
| Coarse Woody Material | 0 (0) | 1 (0.6) | 0.75 (0.5) | 0.58 | 0.57 |
| Aggression Score | 1.66 (0.4) | 1.78 (0.3) | 1.93 (0.1) | 0.29 | 0.75 |
| Total Anti-predator behaviors | 1.16 (0.98) | 0.11 (0.11) | 1 (0.31) | 1.48 | 0.24 |
| Eggs per nest | 2581 (1011) | 4110 (947) | 2500 (608) | 1.26 | 0.3 |
| Avg. dist to nearest neigh. | 51.3 (25.7) | 56.3 (20.3) | 44.7 (10.5) | 0.14 | 0.87 |
| Avg. dist to all nests w/in year | 297.4 (28.2) | 296.7 (24.0) | 325.9 (24.0) | 0.47 | 0.63 |

Table 6. Distributional data for independent variables considered in ANOVA analysis

| | <u>Mean</u> | <u>Min</u> | <u>Max</u> | <u>SE</u> |
|------------|-------------|------------|------------|-----------|
| NYOY | 0.8 | 0 | 4 | 0.21 |
| Date | 135.4 | 124 | 145 | 1.4 |
| Length | 26.36 | 20.32 | 30.48 | 0.28 |
| Depth | 0.96 | 0.5 | 1.2 | 0.05 |
| Cover | 58.80% | 0% | 100% | 6.23 |
| Twigs | 1.35 | 0 | 10 | 0.44 |
| CWM | 0.68 | 0 | 5 | 0.32 |
| TAB | 0.77 | 0 | 6 | 0.25 |
| Moms | 3.4 | 1 | 8 | 0.33 |
| Eggs | 3053 | 269 | 7459 | 475 |
| Local Dist | 50.2 | 0.5 | 181 | 9.6 |
| Lake. Dist | 309.7 | 236.5 | 449 | 14.5 |

LITERATURE CITED

CHAPTER 2

LITERATURE CITED

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CHAPTER 3

A GENETIC EVALUATION OF DISPERSAL IN YOUNG-OF-YEAR LARGEMOUTH BASS

Introduction:

Understanding the dispersal capabilities and patterns of species can provide researchers with valuable information on the distribution and survival of populations and individuals on multiple spatial and temporal scales (Morris 1992; Cadotte and Fukami 2005). For example measures of dispersal distance may be broadly used to infer gene flow between populations over time (Planes et al. 2009). Dispersal distance and patterns may also be used to understand habitat selection (Morris 1987; Morris 1992; Morris 2003). Alternatively estimates habitat permeability provides a framework for predicting dispersal patterns (e.g. Baguette and Van Dyck 2007) and gene flow between populations (Storfer et al. 2007 for review). Dispersal is an important event in the early life history of many fish species because it significantly affects survival probabilities of young (Lomnicki 1980; Hastings 1993). Ultimately dispersal may provide a link necessary to fully understand recruitment processes in fish populations such as density dependent mortality (Derosier et al. 2007).

Despite its ecological importance, dispersal can be difficult to quantify because dispersal often occurs during early ontogenetic stages when individuals are too small to physically tag. One method researchers have used to address this problem is the analysis of trace elements in the otoliths of fish. Researchers have used otolith chemistry to determine the river of origin of American shad (*Alosa sapidissim*) with approximately 90% accuracy (Thorrold et al. 1998). Likewise, Gillander and Kingsford (1996) used similar techniques to quantify the dispersal of blue grouper (*Achoerodus viridis*) from estuary habitats to supplement coral reef populations. These tools are useful for detecting dispersal at broad scales but are dependent on the degree of

environmental heterogeneity and are not likely to have the resolution to detect movement at finer scales, such as within lakes.

Genetics techniques are emerging as a powerful tool to measure dispersal distance and evaluate dispersal patterns at multiple spatial scales (Duong et al. 2011). On broad scales, assignment methods determine the source population of a sample using likelihood methods (see Manel et al. 2005 for review). If the population to which a sample was genetically assigned is not the population within which it was sampled, researchers can infer dispersal has occurred.

Alternatively, on finer scales, parentage (Jones and Ardren 2003 for review) or pedigree reconstruction (Blouin 2003 for review) methods can be used to estimate the relationship between samples, and the distance between related samples can be calculated to infer dispersal distance and patterns. For example Homola et al. (2010) used genetic assignment methods to investigate straying rates and dispersal patterns of stocked lake sturgeon (*Acipenser fulvescens*) into other genetically unique populations of Lake Superior. Kanno et al. (2011) used pedigree reconstruction to detect inter-sibling distance of brook trout (*Salvelinus fontinalis*), and found them to be confined (100-250 m) relative to dispersal potential (4.4 to 7.7 km).

The dispersal of largemouth bass (*Micropterus salmoides*) has not been evaluated using genetic techniques, but bass are ideal for developing such techniques because male bass build nests and guard eggs laid within them (Reighard 1906) until offspring hatch and disperse at roughly 15-20 mm. Therefore, sampling nests provides researchers with a definitive start point (in time and space) for comparisons to samples captured later, relative to other fish that may broadcast eggs over a wide area. Researchers studying a related species smallmouth bass (*M. dolomieu*) used restriction fragment length polymorphisms to “finger print” fry from nests and determined that

average distance between young-of-year (YOY) sampled later in the summer and their nest of origin averaged 88 m (Gross and Kapuscinski 1997).

Dispersal of offspring from their nest site represents a conceptual link that is missing from a more holistic understanding of recruitment in nesting black bass species (and other nesting Centrarchids). Research on largemouth bass has focused primarily either on factors influencing survival during the nesting stage, or growth and survival after dispersal from the nest in the late summer with little spatial linkage. For example, if largemouth bass have limited dispersal abilities then the nest site that a male chooses must be suitable for both nesting success and the survival of YOY as well. Experimental studies conducted by Olson et al. (2003) suggested that plant cover is critical for mitigating predation risk of YOY largemouth bass. Therefore bass nests that are located farther from cover than YOY bass can disperse may be less likely to recruit individuals to YOY populations after the male stops providing parental care. In contrast, nests located close to areas of high quality habitat may contribute disproportionately to YOY populations. However, in order to establish the effect that features of the nest site has on survival to the YOY stage a better understanding of largemouth bass dispersal is necessary.

Although dispersal distance and pathways between nest of origin and young-of-year (YOY) habitat have not previously been reported for largemouth bass, some work has documented the movement of YOY bass through the summer (starting after dispersal from nest site has already occurred). Copeland and Noble (1994) marked YOY bass (average length across years 47.8-49 mm) in June and re-sampled in September. Over both study years 83-90% of YOY bass were found within 58 m of their original capture site. Jackson et al. (2002) studying the same system found that across years YOY bass (length 28-79 mm) movement ranged from an average of 348

m in 1990, to 183 m in 1991. However, these studies did not begin measuring dispersal from the nest of origin and data likely underestimated total dispersal as a result.

Our objectives were to measure the distance that individual largemouth bass traveled from their nest of origin, and determine if related YOY remained spatially aggregated relative to randomly selected YOY. Based on the documented movement of YOY largemouth bass (Copeland and Noble 1994; Jackson et al. 2002), and the dispersal distance from nest of origin to YOY habitat location reported for smallmouth bass (Gross and Kapuscinski 1997), we predict that YOY largemouth bass will remain relatively close to their nest of origin and other related individuals. Because habitat is important to mitigate predation risk of YOY bass (Olson et al. 2003), and other fish (Mittelbach 1981; Werner et al. 1983) we predict that related YOY bass will aggregate in areas of “high quality” habitat and that “low quality” habitat areas will act as dispersal barriers. Therefore related individuals should be found closer together than randomly selected YOY bass.

Methods:

Study site

We studied Warner Lake (N42°28'15"/W85°31'29") and Lake Chemung (N42°34'55"/W83°50'55") in southern Michigan. Warner Lake is an oligotrophic lake with surface area of 26 ha and maximum depth of 16 m. We selected Warner lake because it is largely undeveloped (only 8% of shoreline with residential development) and therefore has been relatively little affected by anthropogenic activity. In this lake we document the dispersal distance of individual YOY bass from their nest of origin to a summer capture site. Lake Chemung is a mesotrophic lake with surface area of 126 ha and has a maximum depth of 21

meters. 71% of the shoreline of Lake Chemung has residential development. Because of Lake Chemung's highly developed shoreline significant heterogeneity in habitat exists providing an opportunity to explore if habitat quality restricts dispersal of related individuals. In this lake we lack information on nests but we address the hypothesis that related YOY bass will aggregated relative to randomly sampled YOY bass.

Nest sampling

In spring 2010 we monitored bass nests in Warner Lake. Crews located nests by surveying the shoreline twice a week in May and June from a boat. Crews typically consisted of three to four members including a driver, one to two members observing from the bow of the boat and a snorkeler towed behind the boat. Upon locating new nests, the snorkeler sampled roughly 50 offspring for genetic analysis, by sampling eggs/larvae from several locations throughout the nest. We transported egg samples to the lab and hatched them before preserving them in alcohol. For nests not located until the up-fry stage, the snorkeler sampled up-fry with a small aquarium net and preserved them in alcohol. At the location of each nest, crews recorded a GPS point using a Garmin GPSmap76 hand held GPS receiver.

YOY sampling

After fry dispersal, crews sampled YOY bass in Warner Lake (2010) and Lake Chemung (2009 and 2010) by fyke netting (6 m leads with 0.6 cm mesh, main cage 0.9 m X 1.5 m with 2.5 cm mesh). We divided the lake's shoreline into twelve segments of approximately equal length. We randomly selected the first segment to receive a fyke net and crews placed a fyke net in every other segment (total of six fyke nets) around the lake at the first suitable habitat for fyke netting located in that segment. We deployed each net in the afternoon and allowed the net to

soak overnight, emptying the net the following morning. Crews set fyke nets In Warner Lake 2010 on August 5th and August 31st 2010. In Lake Chemung we set fyke nets on August 28th (2009), and June 28th (2010). At each site crews recorded a GPS point and preserved YOY bass in alcohol for genetic analysis.

Laboratory methods

We measured the total length and preserved weight of all YOY bass and placed them in individually numbered vials, with recorded date of capture and fyke net of origin, for genetic analysis. We calculated Fulton's condition (Ney 1999) of each YOY bass by multiplying each individual's weight (g) and a constant and dividing by total length (mm) cubed, to test if condition and dispersal distance were related.

We extracted DNA from all YOY samples (both lakes), and up to 25 samples per nest (Warner Lake, see chapter 2) using the QIAGEN DNeasy extraction kit (Qiagen, Inc, Valencia, CA) following manufacturer specifications. We diluted all DNA samples to 20 ng/uL, and then amplified them using 10 microsatellite loci: *Mdo 2*, *Mdo 7* (Mallory et al. 2000), *Ms 13* (DeWoody et al. 2000), *Lma 12*, *Lma 21* (Colbourne et al. 1996), *Msf 11*, *Msf 12*, *Msf 38*, *Msf 68*, *Msf 173* (Lutz-Carrillo et al. 2008). We conducted microsatellite polymerase chain reaction (PCR) in 25 uL volumes containing 100 ng of template DNA 2.5 μ L of 10X PCR buffer (1 M tris-HCl, 1.5 M MgCl₂, 1 M KCl, 10% gelatin, 10% NP-40, and 10% triton X), and 0.8 mM deoxy-nucleotide-triphosphates (dNTPs), 10 pm fluorescently labeled forward and unlabeled reverse primers, sterile water, and 0.5 U Taq polymerase. Reactions were performed using Robocycler 96 thermocyclers (Stratagene, Inc., La Jolla, Ca). We then visualized PCR products on 6% denatured polyacrylamide gels using a Hitachi FMBIO II sequencer (Hitachi Instruments,

Tokyo, Japan). All gels were scored by two experienced laboratory personnel and entered into Microsoft Access. We re-ran 10% of all samples for all loci as an error check. Error rates averaged 0.81% across all loci and ranged from 0.0% (*Ms 13*), to 1.6% (*Msf 38*).

Genetic analysis methods

To minimize scoring and data transcription errors we proofed all genetic data using program Micro-Checker (VanOosterhaut et al. 2004). We calculated summary statistics including number of alleles per locus, observed heterozygosity and exclusion probabilities of sibling identity (see Table 1) using program CERVUS (Kalinowski et al. 2007). We established pedigree relationships (full or half sibling) using the pedigree reconstruction program PEDIGREE (Smith et al 2001; Butler et al. 2004). This program uses a Markov Chain Monte Carlo (MCMC) estimation procedure to estimate relationships between samples based on maximum likelihood using on multi-locus genetics data. We conducted 100 replicate runs of PEDIGREE for each data set to determine the average number of full sibling families; we then selected the individual run that corresponded with the average number of groups and that had the highest likelihood score for analysis. We further assessed the accuracy of the program estimates by simulating known pedigrees based on allele frequency data from our sample and determined how accurately PEDIGREE assigned offspring to known sibling groups. The simulated dataset was created by randomly selecting two sample from our data set and “mating” them (randomly selecting one allele from each parent at each locus) to generate 10 full sibling offspring. We repeated the simulation process 51 times to generate 52 full sibling groups of ten individuals each for 520 total offspring; we then analyzed these 520 samples using PEDIGREE to determine how many PEDIGREE correctly assigned to correct full sibling families.

Estimate of dispersal distance from nest

To measure the dispersal distance of YOY from their nest of origin, our first objective, used the program PASSaGE v.2 (Rosenberg and Anderson 2011) to calculate straight line distances between the nest of origin and the sampling location of YOY bass in Warner Lake. Straight line distance represents a minimum dispersal distance, and often had YOY bass traversing the open water of the lake which may be unlikely due to a lack of cover or predation risk. For comparison we also calculated the shortest perimeter dispersal distance (i.e. the shortest possible path along the perimeter of the lake that maintained roughly the same distance from shore at which the nest was found). While perhaps more biologically plausible, shortest perimeter distance still assumes one-directional movement from nest to capture site along a constrained path.

Dispersal patterns of related YOY bass vs. random

To determine if related YOY bass remained aggregated relative to un-related YOY bass, our second objective, we used the program PASSaGE v. 2 (Rosenberg and Anderson 2011) to generate a matrix of distances between each fyke net in Lake Chemung, and used that matrix to calculate the average distance between all possible pairwise combinations of YOY bass who were full siblings (referred to as “actual data”). For example if two YOY bass were determined to be full siblings, and one was collected in one net, and the other in a second net, the distance between the nets was used as the distance between that YOY pair. Siblings caught in the same net were assigned an inter-individual distance of zero. We averaged inter-sib distance across all full sibling pairs. For comparison, to examine whether inter-sibling distances differed from random (i.e. two random and unrelated individuals in the population) we generated a distribution of average inter-individual distances of randomly selected YOY bass pairs from the sample. To do so, we randomly selected the same number of YOY from our data set, being careful to maintain the family structure observed in the actual data. For example if we observed a full

sibling family with four members in the actual data, we randomly assigned four individuals from the data set into that family when we did our simulation to ensure that the number of comparisons between the actual data set and the random data set remained constant. We repeated the simulation process 1,000 times, and calculated the mean distance between pairs for each iteration, to generate a distribution of mean distances (referred to as “simulated data”) for comparison to observed distribution of distances. We compared the actual dataset with a normal distribution, using a Shapiro-Wilk normality test, to test normality of the data. We calculated the probability of observing the actual average distance between full sibling YOY under the completely random distribution. We performed the simulations and subsequent statistical analysis in R (R Development Core Team, <http://www.R-project.org>)

Results:

Diversity of loci

Across loci alleles ranged from 2 to 22, and observed heterozygosity ranged from 0.171-0.883. Total non-exclusion probabilities of sibling identity across all loci were 0.0006 (see Table 1 for summary).

Accuracy of pedigree assignment

Across 10 runs of the 520 simulated offspring 96.6% of individuals were successfully assigned into known full sibling families. Results indicated that the microsatellites used had sufficient power to accurately determine the pedigree relationships between individuals.

Estimated distances from nest of origin

In 2010 we located and sampled 33 bass nests in Warner Lake and collected 40 YOY bass with six fyke nets across two sampling events (August 5th and August 31st). PEDIGREE assigned 9 of the 40 YOY bass to a nest of origin (i.e. each of the 9 YOY shared at least one parent with multiple members of a nest sampled in the spring) indicating the majority of nests producing YOY were not sampled (see Chapter 1).

We calculated an average straight line dispersal distance of 301.54 ± 41.45 m (mean \pm SE) for 9 YOY where we could identify the nest of origin. The minimum straight line dispersal distance observed was 108 m, and maximum distance observed was 515 m. This measure should be considered a minimum dispersal distance, as distance was calculated as the shortest distance between the two points and did not consider biotic requirements of YOY bass such as plant cover which has been shown to reduced mortality associated with predation in YOY bass (Olson et al. 2003). The shortest perimeter dispersal distances between the nest of origin and capture averaged 577.07 ± 91.41 m (mean \pm SE). The minimum perimeter dispersal distance measured was 165.22 m, and maximum measured perimeter dispersal distance was 965.92 m. YOY bass sampled on August 5th (n= 4) averaged 406.21 ± 38.44 m straight line distance from their nest of origin (mean \pm SE). YOY bass sampled on August 31st (n= 5) averaged 217.81 ± 36.78 m (mean \pm SE) from their nest of origin. YOY sampled August 5th were significantly farther from their nest of origin than bass sampled on August 31st ($t = 3.51$, $p = 0.009$). The condition of YOY bass averaged 0.83 ± 0.03 (mean \pm SE), and differed significantly between fish sampled on August 5th and fish sampled on August 31st (Aug 5th mean = 0.76, Aug 31st mean = 0.89, $t = 2.78$, $p =$

0.027). Distance dispersed from nest of origin was not a significant predictor of YOY condition ($r^2 = 0.11$, $F = 1.99$, $p = 0.20$).

Patterns of YOY dispersal

In 2009 and 2010 we sampled 138 and 140 YOY bass respectively from Lake Chemung each over one night of sampling (Aug 28th 2009, and June 28th 2010) and divided among six fyke nets. The average distance between fyke nets in 2009 and 2010 was 1367 meters, and 1053 meters, respectively, which was not statistically different ($t = 1.37$, $p = 0.18$).

In 2009 PEDIGREE determined that there were between 73 and 81 families of full siblings in Lake Chemung with mean of 77 full sibling families. In 2010 PEDIGREE determined that there were between 102 and 112 families of full siblings in Lake Chemung, with mean of 107 full sibling families.

In 2009 the average distance between YOY related as full sibling was $1,072 \pm 98.19$ m (mean \pm SE). This distribution was not normally distributed ($W = 0.843$, $p < 0.001$), with large ranges of values between full sibling individuals and observed distances between full siblings ranging from 0 m (siblings captured in same net) to nearly 2,500 m (Figure 3). Across 1000 simulations we generated a distribution of mean inter-individual distances with mean 1,202 m and standard deviation of 87.98 m. The means of observed and simulated distributions were not significantly different ($p\text{-value} = 0.08$).

In 2010 results were similar but with a tendency towards shorter distances, likely because sampling occurred earlier in the year. The average distance between full sibling related YOY was 823 ± 74.54 m (mean \pm SE). The distribution of full-sibling inter individual differences was

not normally distributed ($W = 0.783$, $p < 0.001$), and ranged widely between full sibling pairs, with the observed distances between full siblings ranging from 0 meters (siblings captured in same net) to 1,432 meters. The mean of distances between randomly assigned YOY across 1000 runs was 790 meters with standard deviation of 76.09 meters. We did not find a significant difference between the means of full the sibling related YOY and randomly selected YOY (p -value = 0.63).

Discussion:

Dispersal of YOY bass from nest of origin

Dispersal distance of wild YOY largemouth bass from their nest of origin to capture sites in the summer is important because it may provide a spatial link between ontogenetic life stages, that has not been previously documented in largemouth bass. If typical, our results in Warner Lake suggest that YOY bass are capable of dispersing several hundred meters from their nest of origin, over the course of the summer. If the results reported by Gross and Kapuscinski (1997) for smallmouth bass (average 88 m) are typical to their respective species, it seems that largemouth bass are capable of dispersing much farther from their nest of origin than smallmouth bass. If largemouth bass do disperse farther than smallmouth bass, it suggests that nest habitat selection for smallmouth bass may have a stronger impact on YOY survival than for largemouth bass. Additionally limited dispersal may increase the impact of density dependent processes in smallmouth bass (Ridgway et al. 2002) at relevant spatial scales. Alternatively, habitat and prey species differences between our study systems may also explain the difference in dispersal distance between our results and those of Gross and Kapuscinski (1997). Our study was a whole lake study conducted in a relatively small (26 ha) closed system, whereas Gorss and Lapuscinski

(1997) studied Jones Bay (a 6 km stretch of shoreline) in the much larger (5860 ha) Lake Opeongo (Ontario, Canada). It is possible that in a large system YOY bass remain in sheltered areas such as bays.

Studies have documented the importance of habitat to juvenile largemouth bass. For example, both YOY and adult bass achieve highest foraging rates in areas of intermediate plant cover (Savino and Stein 1982; Valley and Bremigan 2002). Additionally Olson et al. (2003) found that bass are less vulnerable to predation in areas of plant cover relative to areas absent of plant cover. YOY bass in this system experienced significant foraging area after dispersing from their nest of origin, which could be important if exposure to larger foraging areas increases growth rate during the first summer, which has been tied to an earlier shift in piscivory and higher probability of surviving the first winter (Olson 1996). Alternatively the dispersal distances observed in this study may represent a strategy to avoid density dependent mortality (Travis et al. 1999). Largemouth bass in our system may be dispersing away from their nest site to avoid competing with nest mates, though further study is necessary to evaluate this hypothesis.

In Warner Lake YOY bass sampled earlier (August 5th) were significantly farther from their nest of origin than bass sample later in the summer (August 31st). YOY sampled August 31st had significantly higher condition indices than bass sampled August 5th, which may indicate improved condition over the summer. However, it is unclear if the significant difference in dispersal distance and condition between sampling periods is biologically relevant. Sample size was very low in both sample periods (n= 4, and n= 5 respectively), and fyke net location was randomly selected for each sample period, so the nets were not set in the same location. This could be important if the relative habitat quality of the fyke net location differed in each

sampling period. Overall, dispersal distance was not a significant predictor of YOY condition, indicating that greater dispersal distance was not negatively influencing YOY condition. Further work with larger sample sized is needed to determine how the distance of YOY from their nest of origin changes throughout the summer.

Inter-individual distance of related and un-related YOY bass

Average dispersal distances of related YOY largemouth bass did not differ significantly from the average distance of randomly selected YOY largemouth bass in Lake Chemung for either study year. These findings were contrary to our predictions and may suggest that YOY largemouth bass disperse randomly from their nest of origin. Interestingly dispersal patterns were more or less identical in 2009 and 2010 despite the fact that samples collected in 2010 were collected roughly two months earlier in the season, suggesting that considerable dispersal occurs early in the season and is essentially random from the beginning. Essentially random dispersal is especially surprising given that Lake Chemung is highly developed (71% of shoreline with residential habitat) and considerably heterogeneity in plant cover exists (personal observation). We predicted that greater habitat heterogeneity would aggregate related YOY bass relative to randomly selected un-related individuals on average. Evidently despite the range in plant cover observed and the potential for higher risk of predation in low plant cover documented by Olson et al. (2003) for largemouth bass in low cover areas, related YOY bass dispersed widely from one another. Had dispersal barriers been present we would have expected to see the majority of observed distances between full sibling pairs to be concentrated at shorter inter-individual distances than the distribution of average random inter-individual distances. However, observed inter-sibling distances were not concentrated at shorter distances than random (Figure 3).

Alternatively recent research indicates a positive relationship between lake shore development

and YOY largemouth bass growth (Gaeta et al. 2011) which may provide the energetic opportunity for increased dispersal distance.

Considerable variability existed in dispersal distances between individual full siblings pairs (Figure 3). One explanation may be that sibling pairs collected close to one another originated from nests located in high quality YOY habitat. If nesting in ideal habitat reduces dispersal distance then bass nesting location may influence the survival of YOY bass beyond the nest stage. However, our results must be somewhat cautiously interpreted because the coarse spatial scale over which we were able to detect movement (i.e. we set six nets per sampling period so there were only 15 possible distances at which YOY could be detected, other than 0). Clearly more work is needed with greater resolution to either support or refute these findings. Interestingly these findings suggest that dispersal barriers may not have existed in Lake Chemung despite its highly developed shoreline, which we expected would concentrate related YOY close to one another.

Future work

This research has laid the groundwork for further investigation of the dispersal of YOY fish in general. By using pedigree reconstruction we were essentially able to “mark” fish as having originated from a specific nest beginning at an earlier (and smaller) stage than would have been possible with a physical tag. Similarly microchemistry approaches would not be feasible at micro-geographic (within-lake) spatial scales. Our approach allowed us to track the dispersal of wild YOY bass in natural systems from an earlier stage than has previously been possible. Determining the dispersal distance and exploring patterns of dispersal among nest mates is an important first step in linking what is known about nesting bass with studies of largemouth bass

during and after their first summer of life. Results from studies such as ours could be used to parameterize models for more holistic studies of largemouth bass recruitment which would take into account nesting, dispersal and first summer survival as predictors of recruitment; such studies can be especially powerful when coupled with detailed habitat data and biological requirements such as prey species. Accurate models of recruitment will likely be of increasing importance in predicting the responses of largemouth bass populations to increases in habitat disturbance, angling or climate change.

APPENDIX

Chapter 3 Figure

APPENDIX

Figure 3. Comparison of inter-individual distances between related YOY bass pairs for observed (black bars) and simulated unrelated (white bars) individuals in Lake Chemung for 2009 (panel a), and 2010 (panel b). Frequency of observed distances is shown for the observed data. For the simulated data, the mean inter-individual distance was calculated for each of the 1000 iterations, and the frequency distribution of those means shown here.

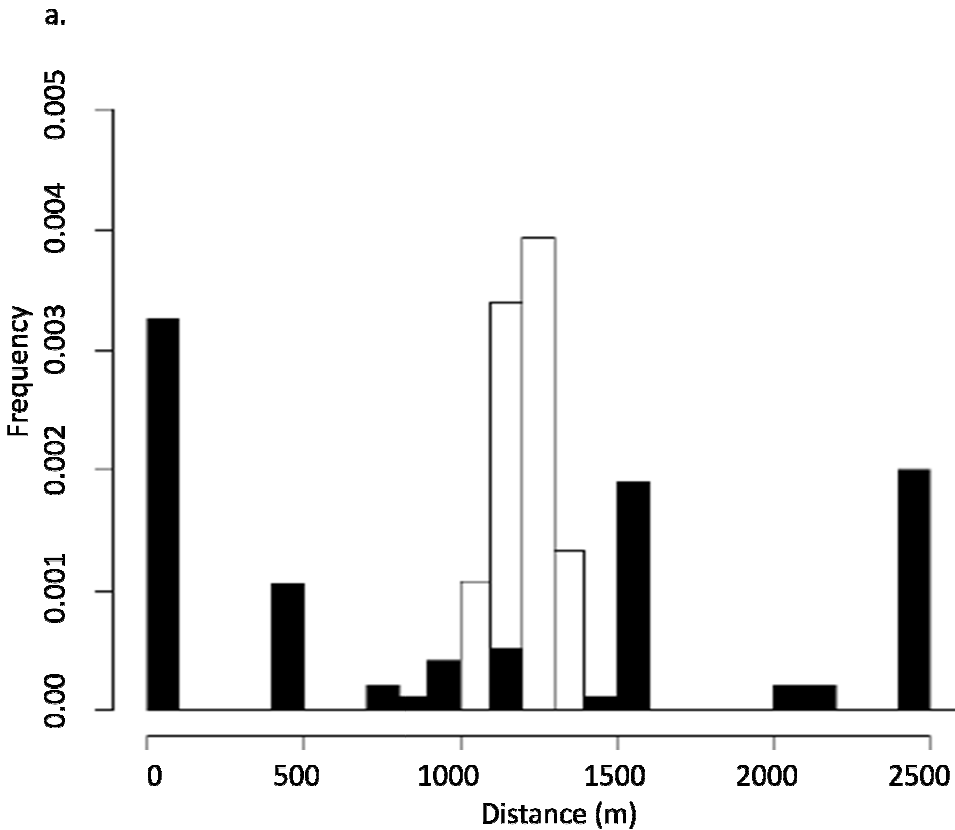
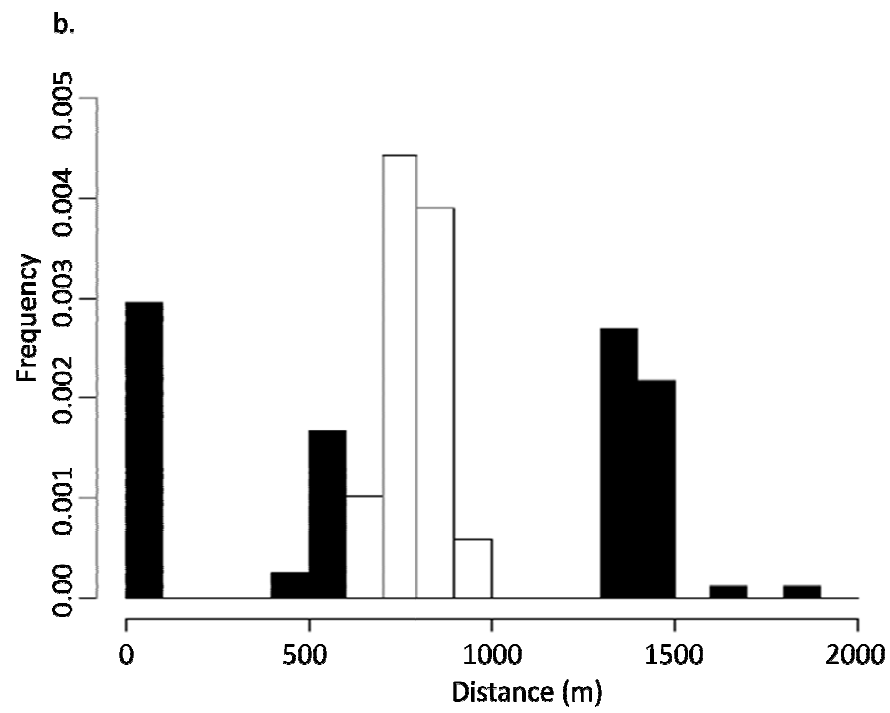


Figure 3 (cont'd)



LITERATURE CITED

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