

## **Abstract**

GODBOUT, JASON DOUGLAS. Investigating Interactions between Largemouth and Spotted Bass, Lake Norman, North Carolina. (Under the direction of James A. Rice and D. Derek Aday.)

Spotted bass *Micropterus punctulatus* were recently introduced into Lake Norman, which already supported a healthy largemouth bass *M. salmoides* population. After only ten years, spotted bass now make up about half of the black bass fishery. Because the two species are ecologically similar, and numbers and biomass of largemouth bass have been declining, biologists were concerned that spotted bass were negatively affecting largemouth bass. Additionally, morphological observations suggested the two species were hybridizing. To better understand these issues, hybridization, diet overlap, and habitat use by black bass in Lake Norman were quantified. Genetic analyses confirmed largemouth and spotted bass were hybridizing. Genetic information on individuals was paired with morphological characteristics at juvenile (50 – 100 mm total length, TL;  $n = 60$ ) and adult (300 – 500 mm TL;  $n = 78$ ) life stages, and reliable patterns for field identification of spotted bass, largemouth bass, and hybrids were developed (78-88% correct). To understand potential competition between the taxa, juvenile ( $n = 132$ ) and adult ( $n = 120$ ) black bass were collected for diet and habitat comparisons. Diet information was collected from juveniles from 31 July – 08 Aug 2007, 29 April 2008, and 10 June 2008 at different spatial scales. Zooplankton and benthic invertebrates were collected to estimate availability. Diet information was collected from adult fish from 29 April – 01 May 2008

and from 12 May – 16 May 2008. Habitat information on substrate and cover use was collected from locations of where fish were captured, and habitat availability of substrate and cover was estimated from 300-m transects ( $n = 12$ ) throughout the reservoir. Proportion similarity index and Morisita's index were used to quantify diet overlap by percent by number and percent by occurrence, and 10,000 bootstrap values were generated so that 95% confidence intervals could be estimated. Estimates of habitat selection were calculated with Jacob's improved Ivlev's index of electivity and the Strauss index of selectivity. Estimates of diet overlap were high, and 95% confidence intervals were typically in the upper half of the range of the indices. Based on prey availability samples, selection of invertebrate prey was largely opportunistic and similar between species. Habitat selection of both substrate and cover was similar with few exceptions. Because largemouth and spotted bass are hybridizing in Lake Norman, and overall, they show high overlap in use of prey items and habitat at both juvenile and adult life stages, they are likely competitors in Lake Norman. These findings should guide future research and educate managers and anglers about the potential effects of introducing spotted bass or largemouth bass into lakes already containing a healthy black bass fishery.

Investigating Interactions between Largemouth and Spotted Bass,  
Lake Norman, North Carolina

by  
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## **Biography**

I was born in Augusta, Maine, and I am the oldest of Robert and Charlene Godbout. I was raised in the small town of West Gardiner, Maine with my little sister, Michelle, and spent a lot time with my aunt Cheryl. My father often took me hunting and fishing outdoors. My parents also were very active and supporting of my involvement in the Boy Scouts. After completing high school, I attended the University of Maine at Orono where I narrowed my interest to fisheries management. During the summers while I was completing my undergraduate degree, I did internships in Illinois and Utah where I gained hands-on experience and learned about fisheries.

After completing my Bachelor's degree in 2004, I moved to southern Illinois to work as a Fisheries Technician at the Illinois Natural History Survey. In 2006, I moved to Raleigh to attend North Carolina State University and learn about management of freshwater fisheries under the guidance of Drs. Jim Rice and D. Derek Aday. NC State has provided me with a diversity of experiences, and I have learned much from the faculty, staff, and students. Although I have memories here, I hope to pursue my career in aquatic sciences closer to home where I can spend more time with friends and family.

## **Acknowledgments**

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## Table of Contents

<b>List of Tables .....</b>	v
<b>List of Figures .....</b>	vi
<b>Chapter 1: Morphological models for identifying largemouth, spotted, and hybrid largemouth-spotted bass .....</b> 1	
<b>Abstract .....</b>	2
<b>Introduction.....</b>	3
<b>Methods .....</b>	6
<b>Results .....</b>	14
<b>Discussion .....</b>	17
<b>Acknowledgments .....</b>	24
<b>Tables .....</b>	30
<b>Figures .....</b>	35
<b>Chapter 2: Diet and habitat overlap of largemouth bass and recently introduced spotted bass in a low productivity reservoir..... 43</b>	
<b>Abstract .....</b>	44
<b>Introduction.....</b>	45
<b>Methods .....</b>	48
<b>Results .....</b>	58
<b>Discussion .....</b>	61
<b>Acknowledgments .....</b>	67
<b>Tables .....</b>	73
<b>Figures .....</b>	78
<b>Appendix.....</b>	84

## List of Tables

Table 1.1. Description of expected morphological characteristics of largemouth bass and spotted bass.....	30
Table 1.2. The number (% in parentheses) of largemouth, spotted, and hybrid bass correctly classified by the classification trees .....	32
Table 1.3. Number (% in parentheses) of adult (300 - 500 mm) and juvenile (50 – 100 mm) largemouth, hybrid, and spotted bass.....	33
Table 2.1. Habitat variables and descriptions observed for estimating habitat availability in the littoral zone.....	73
Table 2.2. Prey groups used for estimates of diet overlap between juvenile largemouth and spotted bass.....	74
Table 2.3. Prey groups used for estimates of diet overlap between adult largemouth and spotted bass.....	75
Table 2.4. Number and percent of empty stomachs of largemouth and spotted bass.....	76
Table 2.5. Calculated diet overlap estimates, mean bootstrap, and 95% CIs of 10,000 bootstrap values .....	77
Table A.1. Number of prey items (organized by prey group) eaten by largemouth and spotted bass .....	85

## List of Figures

Figure 1.1. Known morphological characteristics for identifying largemouth (top) and spotted bass (bottom).....	35
Figure 1.2. Morphological characteristics in addition to characteristics displayed in Figure 1 (because some characteristics were only observed on juveniles and some only on adults) used for identifying juvenile largemouth (top) and spotted bass (bottom).....	37
Figure 1.3. Classification trees for differentiating adult (300-500mm) largemouth, spotted, and hybrid bass in the lab and in the field.....	39
Figure 1.4. Classification trees for differentiating juvenile (50-100mm) largemouth, spotted and hybrid bass in the field.....	42
Figure 2.1. Mean relative gut fullness (total wet stomach contents weight/total wet fish weight) of juvenile and adult largemouth and spotted bass.....	78
Figure 2.2. Proportions by number of available planktonic and benthic invertebrate prey groups.....	79
Figure 2.3. Chesson's alpha values ( $\pm$ 95% CI) for juvenile largemouth and spotted bass selection of zooplankton and benthic invertebrate prey groups.....	80
Figure 2.4. Boxplots of mean percent availability of substrates (silt-clay, sand, gravel, pebble, cobble, boulder-bedrock, and other, based on modified Wentworth scale; left axis) from 10 x 2-m plots ( $n \sim 30$ per transect), and percent availability of cover.....	81
Figure 2.5. Values of (A) Jacob's improved Ivlev's electivity index and (B) the Strauss index of selectivity for adult.....	82
Figure 2.6. Jacob's improved Ivlev's electivity index (A) and the Strauss index of selectivity (B) values for juvenile.....	83

**Chapter 1:**

**Morphological models for identifying largemouth, spotted,  
and hybrid largemouth-spotted bass**

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## **Abstract**

Hybridization is common among many closely related fishes, including largemouth bass *Micropterus salmoides* and spotted bass *M. punctulatus*. Although these species are common members of the sportfish community in Midwest and Southeast U.S. reservoirs, fairly little is known about their ecological interactions or the potential for introduction of one species to influence the other. To address these ecological questions and develop appropriate management strategies, reliable field and laboratory identification of each parental species and their hybrid is required. To that end, we collected juvenile ( $n = 60$ ) and adult ( $n = 78$ ) suspected largemouth, spotted, and hybrid bass from Lake Norman, North Carolina, a system with a historically strong largemouth bass fishery that recently experienced a spotted bass introduction. We recorded a suite of morphological data on each individual and correlated those observations with DNA sequences from one mitochondrial and three nuclear DNA markers in an attempt to develop morphological field and lab methods for identifying both parental species and their hybrid. Following confirmation that largemouth and spotted bass were hybridizing in Lake Norman, we used classification tree analyses to form dichotomous keys for field and laboratory identification of each parental species and their hybrid at juvenile (50-100 mm) and adult (300-500 mm) life stages. These keys should provide fishery biologists and managers with a tool to identify these commonly interacting species that closely resemble one another. In addition, these keys should be useful in providing evidence that largemouth and spotted bass are hybridizing before more expensive techniques like DNA sequencing are pursued.

## **Introduction**

Many freshwater fishes hybridize in nature, and some do so commonly (Hubbs 1955; Scribner et al. 2001). Largemouth bass *Micropterus salmoides* and spotted bass *M. punctulatus* are ecologically similar species and co-occur in many reservoirs in the Midwest and Southeast United States. Both naturally hybridize with other black basses (Whitmore and Hellier 1988; Morizot et al. 1991; Koppelman 1994; Avise et al. 1997; Pierce and Van den Avyle 1997; Barwick et al. 2006), yet, based on a review of the primary literature, have not been recorded naturally hybridizing with each other. Hybridization is possible and seems likely, as spawning behaviors and locations are similar in both species and occur at about the same time of year, although spotted bass may nest slightly deeper than largemouth bass (Robbins and MacCrimmon 1974; Vogele and Rainwater 1975; Sammons et al. 1999). Successful management of these species in systems where they co-exist requires methods for reliably identifying each parental species and, if they are mixing, their hybrid.

Several methods exist to quantify hybridization. Early work examined hybridization in laboratory tanks, creating an environment to encourage mixed spawning (Hubbs 1955). Now, naturally spawned fish can be identified as hybrid or pure with a suite of cellular analyses (see review by Ward and Grewe 1994). Black bass species and subspecies can be identified using allozyme markers (e.g., Kassler et al. 2002), which are particularly useful in systems involving only two taxonomic groups. In systems containing three or four groups (e.g., subspecies) that may be hybridizing, confident identification of

individuals becomes more difficult given the allozyme markers currently available (see Kassler et al. 2002). Conversely, nuclear methods that can distinguish largemouth bass (i.e., Florida *M. s. floridanus* and northern *M. s. salmoides* subspecies), spotted bass (i.e., Alabama *M. p. henshalli* and northern *M. p. punctulatus* subspecies), and largemouth-spotted bass hybrids are currently available and offer the opportunity to reliably sort species and hybrids (J. Quattro, University of South Carolina, unpublished data).

Quantifying morphological characteristics in the field and correlating those measurements with genetic data may allow the development of field methods to differentiate hybrid from pure individuals. However, past studies examining hybridization and attempting to correlate morphological field data with genetic data sometimes have produced equivocal results, often as a result of hybrids backcrossing with pure individuals (Avise et al. 1997). Results of studies attempting to use phenotypic characteristics to categorize pure and hybrid individuals range from general failure (e.g., Smith et al. 1995; Avise et al. 1997) to identification with reasonable confidence (e.g., Weigel et al. 2002; Baumsteiger et al. 2005). Even with the potential for failure, the cost savings generated by reliably differentiating hybrids from parental species in the field may make the initial investment in DNA analyses worthwhile.

Understanding hybridization also has important ecological implications. Introgression occurs when hybrid individuals backcross with individuals from at least one parental population (Rhymer and Simberloff 1996). When introgression is occurring, hybrids become particularly difficult to identify. Avise et al. (1997) provide a good

example of introgression between two black bass species in a study that documented a drastic decrease in a smallmouth bass *M. dolomieu* population 10-15 years after spotted bass were introduced, with eventual replacement by spotted bass. Using restriction fragment length polymorphism (RFLP) digests from mitochondrial DNA (mtDNA) and allozyme data, the authors found that >99% of individuals in the sample population were either spotted bass or hybrid spotted-smallmouth bass of F<sub>1</sub> or later generation. More generally, Huxel (1999) investigated introgression through modeling and estimated that native species may be completely replaced by exotics in fewer than five generations. The observation of hybridizing species alone, however, does not explain why species are so quickly replaced. Avise et al. (1997) proposed behavioral, environmental, and ecological changes as additional causes of the shift in dominant species.

Duke Energy biologists working on Lake Norman, North Carolina first collected spotted bass unexpectedly in 2000 (D. Coughlan, Duke Energy, unpublished data). Soon after, biologists assumed spotted and largemouth bass were hybridizing because some individuals were exhibiting intermediate and mixed morphological characteristics. Morphological variation does not necessarily indicate hybridization; for example, bluegill *Lepomis macrochirus* are known to vary morphologically based on habitat preference (Ehlinger and Wilson 1988). Nevertheless, the observation of intermediate and mixed phenotypic traits coupled with many other hybrid black basses observed by investigators suggested the possibility that largemouth bass and spotted bass could hybridize and were likely doing so in Lake Norman. This, coupled with a decline in estimates of largemouth

bass numbers and biomass at early life stages (Abney et al. 2007), caused concern for North Carolina Wildlife Resources Commission (NCWRC) biologists that manage the lake. However, with no genetic data available and no reliable published methods for identification of each species and their hybrid in the field, questions about the extent of hybridization and its effect on the Lake Norman fishery remain unanswered.

The objectives of this study were (1) to determine if largemouth and spotted bass are hybridizing in Lake Norman, and (2) if largemouth and spotted bass are hybridizing, to develop reliable field and laboratory methods to identify each parental species and their hybrids at both juvenile and adult life stages. To determine whether spotted bass and largemouth bass were hybridizing and to validate field identifications, genetic analyses were correlated with detailed morphological observations. Classification and regression tree analyses were used to provide straightforward output that can be used in field or lab settings to identify each group by simply observing reliable morphological traits.

## **Methods**

### *Study Site*

Lake Norman is a large (12,634-hectare), relatively unproductive reservoir on the Catawba River in west-central North Carolina. A nuclear power station and a coal-fired steam plant influence nearby water temperatures due to hot water discharges, and hydroelectric power is generated at the dam. Much of the lake's shoreline is developed, lined with riprap, and has numerous piers and docks.

## *Field*

Boat electrofishing (Smith-Root®, model GPP 7.5) was used to collect two size classes of black bass (50-100 and 300-500 mm total length, TL) for genetic analyses during spring and summer of 2007 and spring of 2008. These size classes were chosen in an effort to develop identification rubrics for both juveniles and adults, as managers are often faced with the need to reliably identify species at all life stages. The ‘juvenile’ size range of 50-100 mm TL was chosen because both parental species retain juvenile coloration from roughly 50 - 100 mm TL, though there is some individual and species-specific variation (Ramsey and Smitherman 1972). For the ‘adult’ size class, only fish >300 mm TL were collected to ensure that each individual was well into adulthood and displaying adult characteristics, which is consistent with previous investigations (Ramsey and Smitherman 1972; Ludsin and DeVries 1997). For the 50-100 mm size class, 26 suspected largemouth bass, 21 suspected spotted bass, and 13 suspected hybrids ( $n = 60$ ) were collected for genetic analyses, and for the 300-500 mm size class, 32 suspected largemouth bass, 32 suspected spotted bass, and 14 suspected hybrids ( $n = 78$ ) were collected. Because suspected hybrids were seemingly uncommon, individuals were targeted for collection (versus random) to allow for sufficient sample size.

After a bass was netted, it was placed in a flow-through livewell until sampling was completed. Total length (mm) and mass (g) of each fish was recorded, and a side-profile digital photograph was taken at a distance of 60 cm for 300-500 mm fish and 25 cm for 50-100 mm fish. Forceps were used to raise the spiny dorsal fin for each photograph. Each

fish was scored categorically using known differences in morphological characteristics (Table 1.1, Figures 1.1, 1.2) compiled from fish field identification books, scientific articles (Ramsey and Smitherman 1972; Pflieger 1975; Page and Burr 1991; Jenkins and Burkhead 1994; Rohde et al. 1996.), and anecdotal information from field biologists. We also described potential intermediate morphological characteristics because some characteristics of hybrids may be co-dominant and appear morphologically intermediate between largemouth and spotted bass, which has been observed in other hybrid Centrarchidae (Whitt et al. 1973). Because hybrid largemouth-spotted bass had not yet been formally described, additional descriptions of characteristics were developed during collection. Each collected individual was assigned a number to ensure that genetic analyses were blind. After being euthanized with MS-222, each bass was placed in an individual bag or wrapped in aluminum foil with its assigned number. Fish were immediately placed in a cooler on wet or dry ice and stored at -20°C upon returning from the field.

#### *Laboratory*

Pyloric caeca were removed from the body cavity and counted. Pyloric caeca counts can be a useful method for differentiating largemouth from spotted bass (Applegate 1966). Spotted bass have 10-13 pyloric caeca (11 and 12 are most frequent), and largemouth bass have 20-33 (24 is most frequent). Because pyloric caeca of largemouth bass are typically branched and those of spotted bass are typically not, the number of tips on pyloric caeca was recorded for fish and used in analysis of characteristics useful for identifying largemouth, spotted, and hybrid largemouth-spotted bass.

### *Genetic Analyses*

Tissues (pelvic fin clips in most instances) were collected from individual fishes and placed immediately in 95% ethyl alcohol until shipped to the University of South Carolina for analysis. Total genomic DNA was extracted from tissues using the DNeasy Blood & Tissue Kit (QIAGEN Inc, Valencia, CA) following the manufacturer's protocols. The presence and quality of total genomic DNA was estimated by visualization on agarose gels stained with ethidium bromide.

The polymerase chain reaction (PCR) was used to amplify one mitochondrial (mtDNA) and three single copy nuclear (scnDNA) loci using aliquots of purified total genomic DNA template. The mtDNA NADH dehydrogenase subunit-2 (ND-2) locus was amplified using primers described by Breden et al. (1999). Nuclear-encoded loci include the second intron of the intertransgenic spacer (ITS-2) locus, the first intron of the beta-actin gene (B-Act), and the fourth intron of the calmodulin gene (Calmod). Primer sequences are found in Presa et al. (2002), McDowell and Graves (2002), and Near (2007) for ITS-2, B-Act and Calmod, respectively. PCR amplifications were performed as separate reactions (i.e., singly for each locus) using cycling conditions and temperatures described in Oswald (2007) with the exception of the Calmod PCR assays that used an annealing temperature of 52°C.

Some individuals were recalcitrant to amplification using primers and cycling conditions described above. Two loci, the mtDNA ND-2 locus and the nuclear-encoded Calmod locus, did not yield sufficient product for direct DNA sequencing in a small

number of individuals. Therefore, we redesigned the initial primer sets based on sequences we had collected for largemouth bass and spotted bass. For ND-2, four new oligonucleotides were designed, two forward and two reverse, that were nested within the original Breden et al. (1999) primer set:

BassND2NewF1: 5' - GGG GAC CAC AAT TAC ATT TGC – 3'

BassND2NewF2: 5' - CAA GCT CCC ACT GAC TCC TTG – 3'

BassND2NewR1: 5' - TGT TGC AGY AAT GGC TGG GGT – 3'

BassND2NewR2: 5' - AGC GAG AGG AAG CGT GAG TTG – 3'

Both forward primers were paired with the Breden et al. (1999) reverse primer, and the two reverse primers were paired with the original forward primer. Contigs were constructed that covered the entire sequence amplified with the original Breden et al. (1999) oligonucleotides.

Similarly, four new oligonucleotides were designed for the Calmod locus, two forward and two reverse, that were nested within the original Near (2007) primer set:

CalmodF1: 5' - ACA GCG AGG AGG AGA TCC GCG – 3'

CalmodF2: 5' - TTT CCG GGT ATT TGA CAA GGT – 3'

CalmodR1: 5' - TTT GAA ACA TGA AAA ATC CCA CAG – 3'

CalmodR2: 5' - TCC GTC CTG GAA ACA TGC GGC ACG – 3'

Both forward primers were paired with the Near (2007) reverse primer, and the two reverse primers were paired with the original forward primer. Contigs were constructed that covered the entire sequence amplified with the original Near (2007) oligonucleotides.

Reaction conditions were as described above, but a 48°C annealing temperature was used in all four reactions.

Sequences from all four loci were compared to a large database of black bass sequences collected from throughout parts of the southeastern United States (SC, NC, GA, FL, AL) and some 30 specimens of northern largemouth bass collected from Minnesota and Wisconsin (Oswald 2007; Quattro unpublished data). Variant nucleotide positions that differentiate commonly encountered species in the southeastern United States were examined in comparisons between ‘unknown’ Lake Norman samples and ‘voucher’ specimens in the black bass sequence database. For the mtDNA sequences, no ambiguous sequences were observed; species assignment at that locus was unequivocal. For the nuclear-encoded loci (ITS-2, B-Act, Calmod), ambiguous nucleotide positions were often observed. These ambiguous positions have two likely sources: heterozygosity due to intraspecific polymorphism and heterozygosity attributable to hybridization between distinct black bass species. Our sequence data from Lake Norman, and elsewhere within the Southeast, suggest that intraspecific polymorphisms are very rare at these nuclear-encoded loci. However, hybridization among species produces heterozygous individuals at individual loci with ambiguous positions defined by fixed nucleotide differences observed between species; this source of ambiguity accounted for all of the observed ambiguities at nuclear-encoded loci in Lake Norman specimens. Because the number of diagnostic nucleotide differences between species is small, labeling animals as ‘hybrid’ was easily diagnosed by visual inspection of the chromatograms. We kept a running count of mtDNA

haplotype (and diagnosed species) and bi-allelic genotypes at all three nuclear loci (by species if homozygous or by species pair if heterozygous).

### *Data Analyses*

The primary objective of our study was to produce straightforward models that could be easily applied by biologists attempting to differentiate between largemouth, spotted, and hybrid largemouth-spotted bass in a field setting. To that end, classification and regression trees are useful because they provide straightforward output in a series of binary splits forming a decision tree that can be easily used and interpreted. A classification tree is grown by binary recursive partitioning until it overfits the data and is then pruned to an appropriately sized tree. We used standard methodology (Breiman et al. 1998) in the creation of our classification trees. Breiman et al. (1998) recommend 10-fold cross-validation (a process in which 10% of the data are removed to create a series of subtrees against which to test the primary tree) to prune the trees. However, small sample size of even one group makes the effective sample size small, which causes concern about inflated variances associated with cross-validation (Breiman et al. 1998). The cross-validation approach resulted in nice separation of largemouth and spotted bass in our study, but did not allow prediction of hybrids, presumably because of the small sample size of hybrids. Because predicting hybrids was a primary goal of this investigation, we created trees with multiple nodes (i.e., nodes for identification of largemouth bass, spotted bass, and hybrid largemouth-spotted bass) by manually pruning to levels that allowed hybrid identification, which was somewhat subjective but necessary for useful, predictive trees.

This approach is consistent with other studies that have used non-standard methods for tree selection, such as subjective judgment for pruning trees (e.g., Stoneman and Jones 2000), removing variables (e.g., Steen et al. 2008), or using stop-splitting criteria (e.g., Iverson et al. 1997). The resulting trees have the advantage of showing structure in the data and providing output that can identify hybrids that appear mostly to have largemouth bass characteristics versus those that appear mostly to have spotted bass characteristics.

The statistical program R® 2.4.0 (R Development Core Team 2006) with rpart package (Venables and Ripley 1999; Therneau and Atkinson 2007) was used to produce the classification trees for identifying largemouth, spotted, and hybrid bass in Lake Norman. Trees were grown until all individuals were classified correctly, which overfit the data. Initially, trees were grown including pyloric caeca to determine whether they were useful for identification. However, because we also wanted to produce a reliable field rubric that could be used on fish to be released alive, pyloric caeca were excluded from a second analysis, resulting in two classification trees. These data are often noisy, and the variable chosen for the best split anywhere in the tree may be only marginally better than others, but can have a strong influence on the evolution of the tree, especially in early nodes (Breiman et al. 1998). The best split is determined by a measure of each variable's 'improvement'; how the variables change relative to one another, rather than the size of the improvement value, indicates if the other splits are also useful. The best splits are chosen with the Gini index, which measures the reduction in impurity at each node, then that value is multiplied by  $n$  to get a measure of each variable's improvement (Therneau and Atkinson 1997).

When the improvement of primary node variables was only marginally different, additional trees were grown excluding the best primary split to explore alternative possibilities, and trees that produced the most parsimonious results (i.e., result that provided a reliable assessment of parentals and hybrids without over-fitting the data) were reported.

## Results

DNA analyses confirmed that largemouth and spotted bass were hybridizing in Lake Norman. Genetic analyses detected alleles from four taxa of black bass: northern largemouth bass, Florida largemouth bass, Alabama spotted bass, and redeye bass *M. coosae*. Only one redeye bass allele was detected, however, and redeye bass are not known to exist in Lake Norman. This allele probably entered the population via a hybrid spotted-redeye bass in the initial stocking of spotted bass. The mtDNA revealed that of the 20 adult largemouth-spotted bass hybrids, 14 had largemouth bass mtDNA, and 6 had spotted bass mtDNA. One juvenile black bass was excluded from data analyses because its morphological characteristics all indicated spotted bass, but its genotype indicated pure largemouth bass, suggesting a mistake in sampling, labeling, genetic analyses, or data recording. Overall, based on the decision trees, our confidence in identifying parental species was higher than or equal to identifying hybrids in all models (Table 1.2). Juvenile models generally predicted more individuals correctly than adult models; however, the adult trees had several more hybrids, which likely compromised some of their overall

predictive ability. In any case, the overall predictive capability of trees was generally high (>85% for all juveniles, and >75% for all adults; Figures 1.3, 1.4).

Our objectives were to classify parental species and hybrids with a reasonable level of confidence; in the case of adults that required creation of three separate trees. The most useful morphological characteristic as the first split (non-terminal node) in the classification tree for adult fish was pyloric caeca, the second most useful was ventrolateral stripes, and the third was jaw morphology. The importance value of the first split was 23.96 for pyloric caeca, 21.26 for ventrolateral stripes, and 18.15 for jaw morphology, which are not substantially different. Their similarity is further indicated by the fact that the percentage of fish correctly identified by each model changes very little (78-81%; Figure 1.3). In fact, many variables were good for separating largemouth from spotted bass (Table 1.3). Therefore, a second tree was constructed by eliminating pyloric caeca to produce a tree that would be valuable for identifying live fish in the field (Figure 1.3B). Finally, in producing the third tree, we used an extra split to further resolve the separation between largemouth bass and hybrids. The final product was a tree with five terminal nodes rather than four, which results in a slight inflation of the classification success (81% versus 78% overall, Figure 1.3C). In sum, the models performed similarly in their overall ability to classify individuals, but differences among the models in their relative success rate for each species remain.

For juvenile fish, pyloric caeca were not important in classification. The most useful morphological characteristic as the primary node in the classification tree was the

shape of the spiny dorsal fin, and the second was the caudal submarginal band (Figure 1.4). The improvement value of the best primary split (shape of spiny dorsal fin) was 19.23, and the second best primary split (caudal submarginal band) was 18.11, which was only marginally different. Therefore, we created a second tree, using the caudal submarginal band as the primary node (Figure 1.4B), to offer an alternative and to demonstrate the similarity in prediction success (90% vs. 88%) when importance values of primary variables are similar. Like adults, other variables appear potentially useful for separating juvenile largemouth from spotted bass (Table 1.3).

The morphological variable selected for the primary node of the models was not always the best choice for an overall model (i.e., for predicting parentals and hybrids). For adults, the first node of a classification tree was not a good indication of how well the tree would predict hybrids. The largest percentage of adult hybrids (48; Table 1.2) was predicted by the tree that excluded pyloric caeca and ventrolateral stripes (Figure 1.3C) and included jaw morphology as the primary node (Table 1.3). The model that excluded pyloric caeca and included ventrolateral stripes (Figure 1.3B) predicted only 19% of hybrids correctly (Table 1.2). This model is clearly not desirable for predicting hybrids, but it does well at predicting parental species. For the juvenile models, the percentage of hybrids and spotted bass correctly predicted did not change in the two models explored, and the percentage of largemouth bass correctly predicted only changed marginally (from 100 to 97%) with the third model (Table 1.2).

## **Discussion**

Our study resolves a number of characteristics useful for reliably distinguishing largemouth bass, spotted bass, and their hybrid at both juvenile and adult life stages. In addition, our decision trees provide a practical tool for field and laboratory identification of these common sportfish. Although not without error, these trees also provide managers and biologists with a field tool that can provide evidence of hybridization. If biologists require definitive answers via genetic analyses, fewer fish would need to be analyzed using our approach because the trees could be used for initial screening. As such, we believe these data will be useful to fishery biologists interested in understanding ecological interactions between these common freshwater species and to managers charged with managing them in systems where they co-occur.

Many of the morphological characteristics we quantified were useful, particularly for differentiating largemouth from spotted bass. Interestingly, the shortest dorsal spine, which had previously been identified as a differentiating characteristic, was not useful in our study. Hybrids appear to be more phenotypically variable, exhibiting a range of characteristics; some closely resemble one or the other parental species, whereas others (more often) exhibited mixed or intermediate morphology. Nevertheless, our trees provide a quick method for biologists to process fish in the field. For example, based on our data, the model for adult bass that uses jaw morphology as the primary node (Figure 1.3C) would correctly predict largemouth bass 9 of 10 times for fish with jaws that end slightly or well past the posterior margin of the eye and with no or few scales on the base of the soft

dorsal fin. Because we quantified a number of characteristics that are also useful in distinguishing species, these additional morphological traits (detailed in Table 1.3) can be used to confirm predictions made by the decision tree.

Other studies have taken similar approaches for identifying closely related organisms. We used an approach similar to Weigel et al. (2002), who used a classification tree to differentiate hybrid westslope cutthroat-rainbow trout *Oncorhynchus clarki lewisi*-*O. mykiss* from pure rainbow trout *O. mykiss* based on combined phenotypic and genotypic data, which differentiated the two groups with reasonable confidence. In another study, Oswald et al. (2003) used twelve characteristics measured from whistles of nine species of oceanic dolphins (Delphinidae) paired with sightings to predict species. To standardize methods in paleoecological studies, Lindbladh et al. (2002) developed a classification tree for identifying pollen grains from three species of spruce *Picea* by measuring characteristics of grains from known, extant tree species. In all of these studies, the goal was to develop a standard, objective method for identifying species with a reasonable level of confidence.

Based on our analyses, we recommend two models for adult bass. Because pyloric caeca can be particularly useful for classifying adult individuals, we recommend the model including pyloric caeca (Figure 1.3A) for fish that will be killed for laboratory analyses. Pure adult largemouth and spotted bass did not overlap in number of pyloric caeca. Although hybrids exhibited a wide range in number of pyloric caeca, using subsequent nodes in the tree (i.e., scales on the soft dorsal fin and presence or absence of a tooth patch)

provided reliable identification of parental species and an initial means for screening for hybrids. For field studies with live-released fish, the two models that exclude pyloric caeca can be used to reliably determine parentals and provide evidence of hybridization. Trees that have small differences in improvement values for alternate variable splits, as observed in our models, indicate the variables are most likely correlated, which causes instability in the tree; however, the alternative trees that use competing splits often result in similar classifications and have similar success rates (Breiman et al. 1998), providing additional confidence in our approach. Essentially, the best split results from random chance associated with the competing variables. For our data, this makes sense because we selected known characteristics of largemouth and spotted bass expected to differentiate the two species. Because of the similarities in improvement values, we investigated and compared alternative trees. Ultimately, for field data, we recommend the model that uses jaw morphology as the primary node because it provides the most parsimonious result, as it reliably predicts both parentals and can be useful for screening for hybrids, versus the model that uses ventrolateral stripes as the primary node, which does a poor job with hybrids.

For juveniles, we recommend the model with shape of dorsal fin as the primary node. The second tree is illustrated to explore alternative splits of the primary node, which caused little change in overall success of the tree (90% to 88%). However, it includes lateral line blotches, a more difficult characteristic to resolve, especially on dead fish, because it involves coloration. Because pyloric caeca were not useful for juveniles based

on the data we collected, the first model including all variables is recommended for both the lab and field. Pyloric caeca are still a useful characteristic for differentiating largemouth and spotted bass. However, pyloric caeca on juvenile fish are easily damaged and removed from small fish when other nearby organs (e.g., liver) are removed, which can make counts inaccurate. This is a particular problem in largemouth bass identification because loss of pyloric caeca may incorrectly result in classification of the individual as a hybrid or spotted bass.

These data are the first we are aware of that document and quantify the morphological characteristics associated with these commonly interacting sportfish. Although we believe these data will be useful to fishery biologists and managers, we recognize several limitations to the interpretation of our data. First, identifying individuals with categorical variables is subjective. We attempted to reduce observer bias by including descriptions of each variable along with photos and labels to illustrate how we scored morphological characteristics of largemouth and spotted bass. Some variables used in this study were more subjective and difficult to score than others. Tooth patch, for example, is an easily observed characteristic, whereas lateral line blotches are more difficult because they involve coloration of the fish, which can be affected by stress (Conte 2004) and the ambient environment (Sugimoto 2002). The spiny dorsal fin used in the model for juveniles is only slightly different between largemouth and spotted bass; however, it proved to be the best first split. We address this concern in two ways. First, because the trees performed similarly overall, we considered alternative trees and considered how easy the

morphological variables included in the trees were to observe. With juveniles, alternative trees included coloration variables, which are less consistent than physical characteristics such as shape of the spiny dorsal fin, so we believe the model including spiny dorsal fin as the primary split is the best choice. Second, we provide illustrations (Figure 1.3) that we believe will be useful for biologists developing their own identification rubric in other systems.

Further, it is important to note that classification trees of categorical variables do provide consistent results. Weigel et al. (2002), for example, found that multiple observers were more consistent with their species designations when using categorical classification trees than when observer designation did not include a systematic approach. Additionally, Lindbladh et al. (2002) point out that experts in identification of species that resemble one another often disagree and that standard methods are more objective. The descriptions and photos included here should allow biologists using the classification trees to more consistently apply the models and provide the opportunity to test the trees in the future with additional genetic analyses paired with the same morphological observations.

Our results suggest many variables are good for differentiating largemouth from spotted bass, but hybrids are more difficult to identify. Because backcrossing has likely occurred, hybrids exhibit a range of phenotypes and are not easily placed into categories. Weigel et al. (2002) also found higher error rates when trying to place introgressed hybrids into categories compared with parental species. Because of the high error rate our models have identifying hybrids, detection of hybrids with the models is likely conservative.

Nevertheless, we wanted to develop methods to identify largemouth, spotted, and hybrid bass with a reasonable level of confidence. Additionally, because there are currently no published methods for identifying hybrid largemouth-spotted bass, we wanted to ensure that we would produce classification trees that would address this limitation.

Body size played an important role in the development of our metrics. We limited our juvenile sample to fish < 100 mm to ensure that juvenile coloration patterns would be retained. We limited our adult sample to fish in the 300-500 mm TL range, to ensure that all sampled individuals would have adult characteristics. We believe that the adult trees would apply to fish greater than 500 mm TL, and anecdotal assessment of these large individuals provides some assurance of that. We chose not to collect fish >500 mm TL because we wanted to avoid removing the largest fish from the population. Fish of intermediate size (100-300 mm TL) can be difficult to characterize because fish in this size range are undergoing ontogenetic changes in color and morphology. Based on our experience, fish >225 mm TL generally display adult characteristics. For fish that retain juvenile characteristics (e.g., caudal spot, caudal submarginal band) beyond 100 mm TL, it may be appropriate to use the juvenile classification tree, however, this hypothesis remains untested.

The ecological effects of hybridizing largemouth and spotted bass in Lake Norman, as in other systems, are unknown. Generally speaking, hybridizing populations can have important ecological effects on populations. Philipp and Whitt (1991) observed that F<sub>1</sub> hybrid Florida-northern largemouth bass had intermediate survival and growth rates in

Illinois ponds when compared with parental species. Illinois is in the native range of northern largemouth bass, and northern largemouth bass had the highest survival rates; Florida largemouth bass, the transplants, had the lowest survival rates. Case studies like this suggest hybrid individuals could have low survival rates. Goldberg et al. (2005) examined survival and fitness of F<sub>1</sub> and F<sub>2</sub> hybrids of genetically distinct populations of largemouth bass from Illinois and Wisconsin after being exposed to largemouth bass virus, and they observed reduced fitness in F<sub>1</sub> hybrids and reduced survival in F<sub>2</sub> hybrids, suggesting outbreeding depression. Finally, Rhymer and Simberloff (1996) conclude that it is best to avoid introductions to prevent the possibility of introgression between similar species and the potential consequences of outbreeding depression. The effects of hybridization on largemouth and spotted bass in Lake Norman are presently unknown, but a concurrent study is considering the implications of ecological interactions between the two parental species (Chapter 2).

The first step in investigating the influence of largemouth-spotted bass hybridization on population and community dynamics is reliable identification of parental species and their hybrid. Our models provide biologists with standard methods to use in field settings that result in reliable assessment of parental species and at least an initial means of screening for hybrids. This information can be used to develop management initiatives aimed at minimizing the potential negative effects of species introductions and to educate the public about the potential unintended consequences, some known and some not, of introducing new species into aquatic systems.

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## Tables

Table 1.1. Description of expected morphological characteristics of largemouth bass and spotted bass, and possible intermediate morphological characteristics of hybrid largemouth-spotted bass used during field identification and later combined with genetic analyses.

Morphological characteristic	Species		
	Largemouth bass	Hybrid bass	Spotted bass
Posterior margin of jaw <sup>a</sup>	Extends well past eye	Ends slightly past eye	Ends before eye's posterior margin
Posterior half of lateral stripe	Solid stripe	Somewhat broken stripe	Broken stripe
Lateral stripe blotching <sup>b</sup>	No blotching; solid stripe	Some blotching, but joined stripe	Clear blotching
Caudal spot <sup>b</sup>	Elongate into caudal rays; joined with lateral stripe	Elongate and separated from lateral stripe	Triangular; does not elongate into caudal rays; separated from lateral stripe
Caudal fin <sup>b</sup>	2-colored: no white submarginal band	3-colored: orange, black, and white, but only faint, white band or white tips	3-colored: orange, black, and white; strong white submarginal band

Table 1.1 (continued)

Shortest dorsal spine	Posterior-most spine <1/2 longest spine	Posterior-most spine ~1/2 longest spine	Posterior-most spine >1/2 longest spine
Base of soft dorsal fin <sup>a</sup>	Scales absent	Few scales present	Many scales present
Tooth patch on tongue	Absent	Small patch or slightly rough area on tongue	Large and clearly present patch on tongue
Ventrolateral stripes	No stripes	Faint stripes	Clear stripes
Soft and spiny dorsal fins	Deep notch between fins; soft dorsal does not raise when spiny dorsal is raised	Intermediate notch between fins; soft dorsal raises slightly when spiny dorsal is raised	Shallow notch between fins; soft dorsal raises when spiny dorsal is raised
Shape of spiny dorsal fin	Strongly convex	Intermediate between strongly convex and gently rounded	Gently rounded

<sup>a</sup>characteristic was only observed on adult (300 - 500 mm TL) fish

<sup>b</sup>characteristic was only observed on juvenile (50 - 100 mm TL) fish

Table 1.2. The number (%) in parentheses of largemouth, spotted, and hybrid bass correctly classified by the classification trees

Model (classification tree)	Adults		
	Largemouth bass <i>n</i> =33	Hybrid bass <i>n</i> =21	Spotted bass <i>n</i> =24
All variables	33 (100)	8 (38)	22 (92)
Excluding pyloric caeca	33 (100)	4 (19)	24 (100)
Excluding pyloric caeca and ventrolateral stripes	33 (100)	10 (48)	20 (83)

Model (classification tree)	Juveniles		
	Largemouth bass <i>n</i> =35	Hybrid bass <i>n</i> =8	Spotted bass <i>n</i> =16
All variables	35 (100)	6 (75)	12 (75)
Excluding pyloric caeca <sup>a</sup>	35 (100)	6 (75)	12 (75)
Excluding pyloric caeca and spiny dorsal fin shape	34 (97)	6 (75)	12 (75)

<sup>a</sup>Same as model with all variables; pyloric caeca did not provide a useful split

Table 1.3. Number (%) in parentheses) of adult (300 - 500 mm TL) and juvenile (50 – 100 mm TL) largemouth, hybrid, and spotted bass determined by genetic analyses displaying morphological characteristics that were systematically observed and used for growing classification trees (see Table 1.1 for descriptions; LMB = largemouth characteristic, HBB = intermediate characteristic, and SPB = spotted bass characteristic).

Morphological characteristic	Adults								
	Largemouth bass n=33			Hybrid bass n=21			Spotted bass n=24		
	LMB	HBB	SPB	LMB	HBB	SPB	LMB	HBB	SPB
Posterior margin of jaw	28 (85)	5 (15)	0 (0)	6 (29)	3 (14)	12 (57)	0 (0)	2 (8)	22 (92)
Base of soft dorsal fin	21 (64)	7 (21)	4 (12)	1 (5)	3 (14)	17 (81)	0 (0)	2 (8)	22 (92)
Posterior half of lateral stripe	28 (85)	3 (9)	2 (6)	6 (29)	4 (19)	11 (52)	2 (8)	1 (4)	21 (88)
Shortest dorsal spine	30 (91)	2 (6)	1 (3)	10 (48)	7 (33)	3 (14)	15 (63)	7 (29)	2 (8)
Tooth patch on tongue	27 (82)	4 (12)	2 (6)	8 (38)	3 (14)	10 (48)	1 (4)	1 (4)	22 (92)
Ventrolateral stripes	18 (55)	15 (45)	0 (0)	3 (14)	5 (24)	13 (62)	0 (0)	0 (0)	24 (100)
Soft and spiny dorsal fins	18 (55)	14 (42)	2 (3)	3 (14)	4 (19)	13 (62)	0 (0)	3 (13)	21 (88)
Shape of spiny dorsal fin	27 (82)	6 (18)	0 (0)	7 (33)	6 (29)	8 (38)	0 (0)	6 (25)	18 (75)
Pyloric caeca	31 (94)	2 (6)	0 (0)	3 (14)	3 (14)	14 (71)	0 (0)	1 (4)	23 (96)

Table 1.3 (continued)

Morphological characteristic	Juveniles								
	Largemouth bass <i>n</i> =35			Hybrid bass <i>n</i> =8			Spotted bass <i>n</i> =16		
	LMB	HBB	SPB	LMB	HBB	SPB	LMB	HBB	SPB
Lateral stripe blotching	0 (0)	35 (100)	0 (0)	2 (25)	3 (38)	3 (38)	0 (0)	1 (6)	15 (94)
Caudal spot	19 (54)	8 (23)	6 (17)	3 (38)	4 (50)	0 (0)	0 (0)	4 (25)	11 (69)
Caudal fin	5 (14)	29 (83)	1 (3)	0 (0)	3 (38)	5 (63)	0 (0)	0 (0)	16 (100)
Posterior half of lateral stripe	29 (83)	5 (14)	1 (3)	2 (25)	3 (38)	3 (38)	0 (0)	2 (13)	14 (88)
Shortest dorsal spine	35 (100)	0 (0)	0 (0)	7 (88)	1 (13)	0 (0)	15 (94)	1 (6)	0 (0)
Tooth patch on tongue	25 (71)	10 (29)	0 (0)	2 (25)	0 (0)	6 (75)	0 (0)	2 (13)	14 (88)
Ventrolateral stripes	33 (94)	2 (6)	0 (0)	2 (25)	6 (75)	0 (0)	1 (6)	15 (94)	0 (0)
Soft and spiny dorsal fins	18 (51)	15 (43)	2 (6)	1 (13)	4 (50)	3 (38)	0 (0)	8 (50)	8 (50)
Shape of spiny dorsal fin	35 (100)	0 (0)	0 (0)	4 (50)	4 (50)	0 (0)	0 (0)	12 (75)	4 (25)
Pyloric caeca	24 (69)	6 (17)	5 (14)	4 (50)	0 (0)	2 (25)	0 (0)	1 (6)	14 (88)

## **Figures**

Figure 1.1. Known morphological characteristics for identifying largemouth (top) and spotted bass (bottom) observed on individuals taken for genetic analysis in an attempt to develop reliable methods for identification. In addition to characteristics illustrated here, intermediate descriptions were also developed for each trait depicted (Table 1.1). Photos were taken and characteristics were observed on black bass in Lake Norman, North Carolina, 2007-2008. Note: scale is cm.

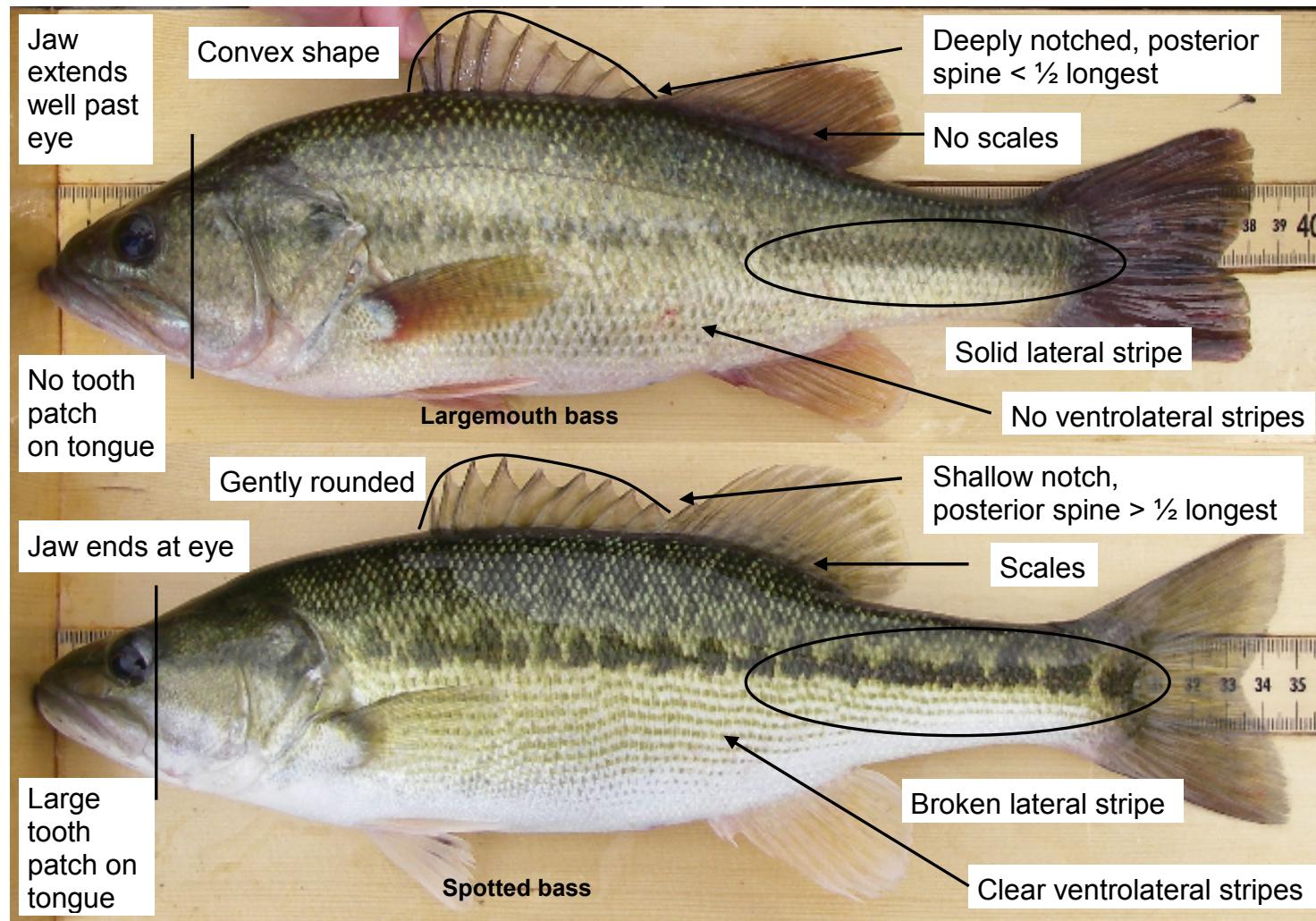


Figure 1.2. Morphological characteristics in addition to characteristics displayed in Figure 1.1 (because some characteristics were only observed on juveniles and some only on adults) used for identifying juvenile largemouth (top) and spotted bass (bottom) observed on bass taken for genetic analysis in an attempt to develop reliable methods for identification. Spiny dorsal was also included because it was useful in the recommended model for juveniles. Photos were taken and characteristics were observed on black bass in Lake Norman, NC, 2007-2008.

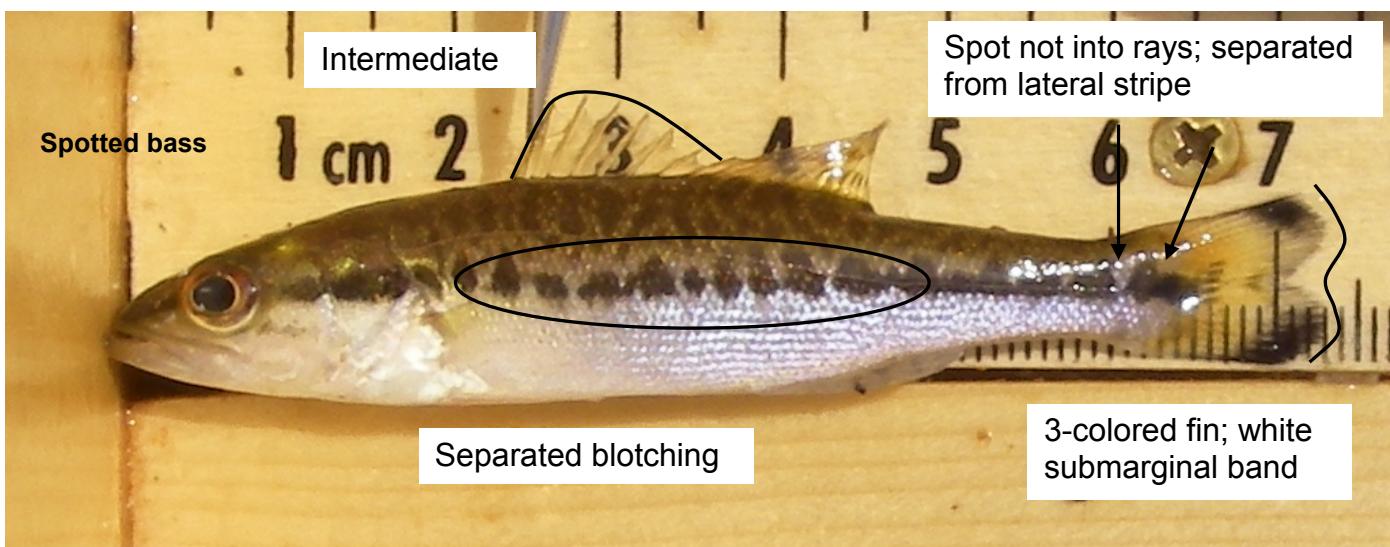
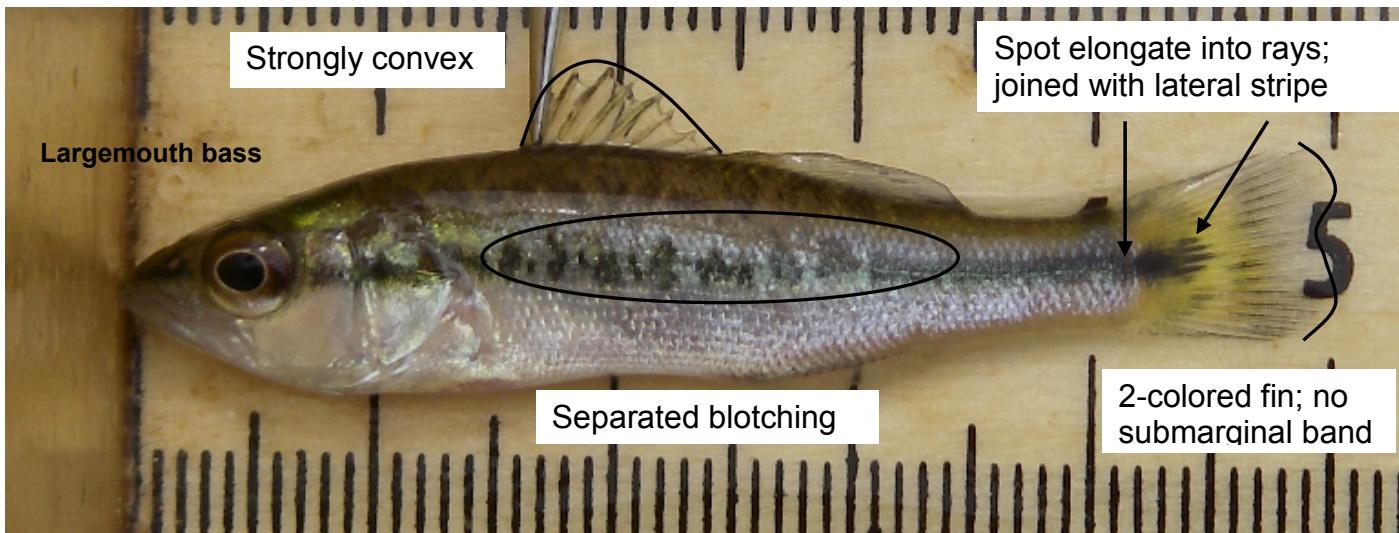
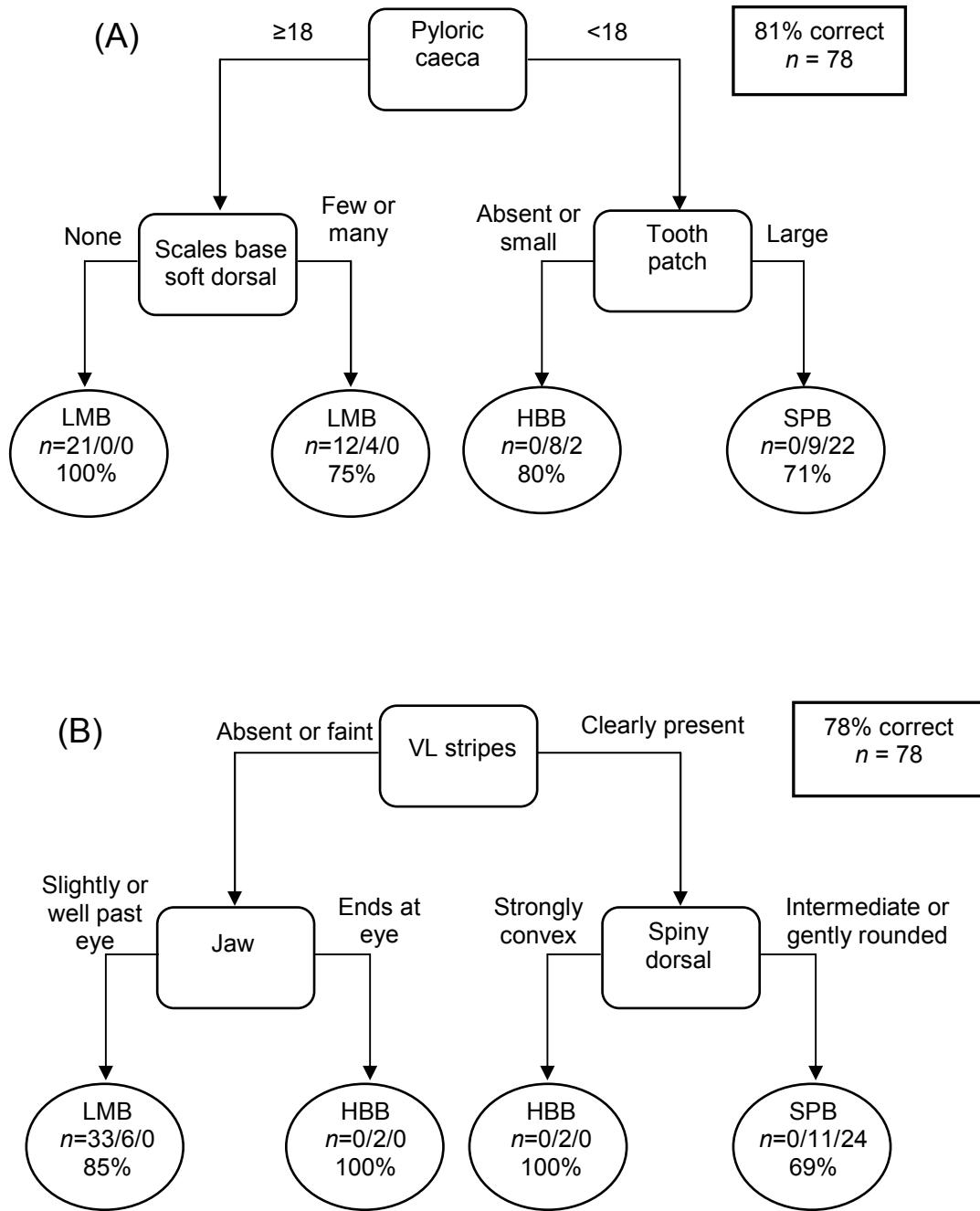
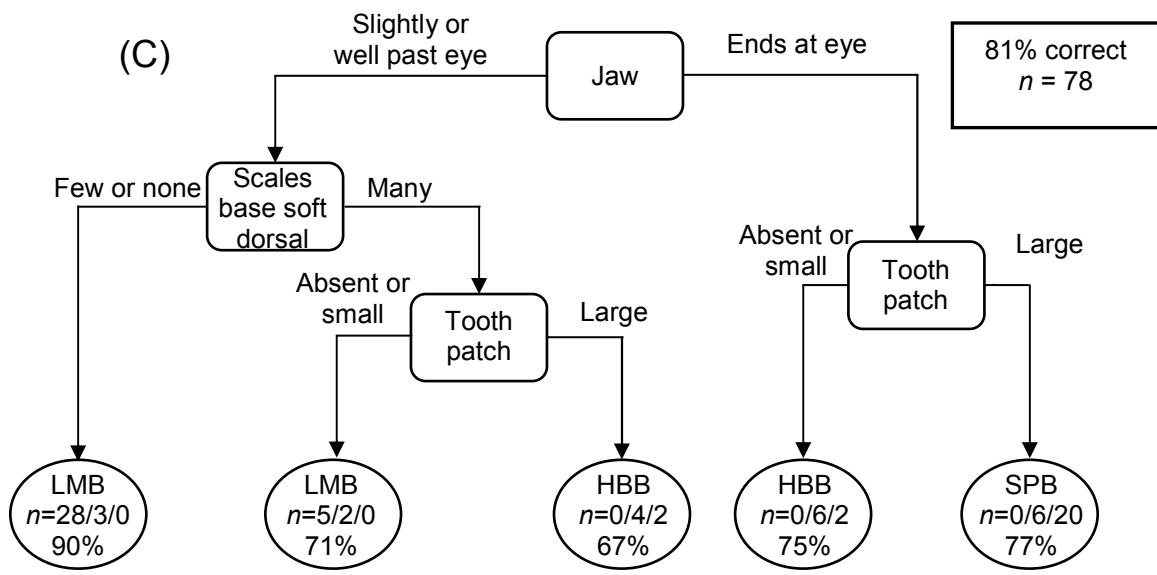


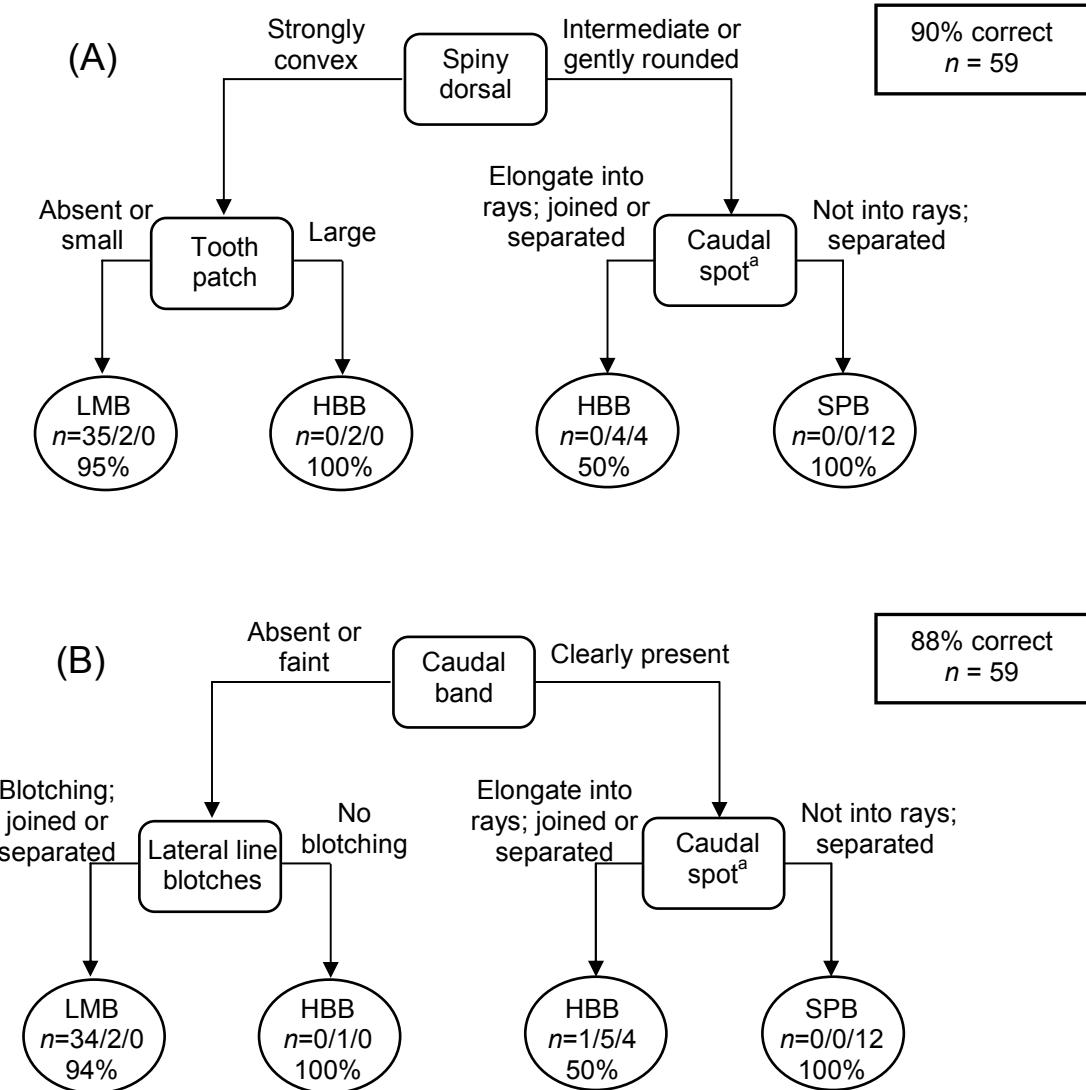
Figure 1.3. Classification trees for differentiating adult (300-500 mm TL) largemouth, spotted, and hybrid bass in the lab and in the field. (A) includes all variables measured, (B) includes all variables except pyloric caeca, and (C) includes all variables except pyloric caeca and ventrolateral stripes. Note that the percentages listed indicate percent correct for that particular node, rather than for the species (see Table 1.2 for percentage correct for each species).

### Classification Trees for Adult Black Bass





## Classification Trees for Juvenile Black Bass



<sup>a</sup>Largemouth characteristic not observed on any individuals

Figure 1.4. Classification trees for differentiating juvenile (50-100 mm TL) largemouth, spotted and hybrid bass in the field using (A) all variables (pyloric caeca were included but were not useful in the model), and (B) all variables except pyloric caeca and shape of the spiny dorsal fin. Note that the percentages listed indicate percent correct for that particular node, rather than the species (see Table 1.2 for percentage correct for each species).

**Chapter 2:**

**Diet and habitat overlap of largemouth bass and recently introduced  
spotted bass in a low productivity reservoir**

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## **Abstract**

The establishment of non-native spotted bass *Micropterus punctulatus* in Lake Norman, North Carolina raised concerns about its potential to affect native largemouth bass *M. salmoides*, a closely related and ecologically similar species. To address this issue, we assessed diet and habitat overlap of juvenile and adult largemouth bass and spotted bass in Lake Norman during 2007-2008. Stomach contents were collected from adult bass 29 April – 01 May 2008 and 12 May – 16 May 2008, and from juveniles 31 July – 08 August 2007, 29 April 2008, and 10 June 2008. Zooplankton and benthic invertebrates were collected to estimate availability. Proportion similarity index and Morisita's index were used to estimate diet overlap of largemouth and spotted bass, and 10,000 bootstrap values were generated to calculate means and 95% confidence intervals. Chesson's alpha was used to estimate and compare diet selectivity of juveniles. Habitat variables were measured at the capture locations of largemouth and spotted bass and were compared to habitat availability estimates, which were quantified along 12, 300-m transects throughout the lake. Habitat selection was estimated using the improved Ivlev's index of electivity and the Strauss index of selectivity. Overall, largemouth and spotted bass overlapped highly in diets and selected for and against similar substrate and cover variables, suggesting that largemouth and spotted bass are potential competitors in Lake Norman throughout their ontogeny.

## **Introduction**

Species introductions by humans are widely cited as a major threat to global biodiversity (e.g., Lovei 1997; Balmford and Bond 2005). In aquatic systems, species introductions are often intended to enhance fisheries, either by adding desirable species or increasing the prey base for desirable fishes, but these additions have often produced unintended consequences (Li and Moyle 1981). For example, Zaret and Paine (1973) observed drastic food web changes in a tropical lake after introduction of a large, piscivorous fish, and Johnson and Goettl (1999) observed significant food web changes in a Colorado reservoir after the introduction of a forage fish. In these examples, although the intent was to enhance local fisheries, the benefits of introduction were short-lived, whereas the costs were long-term.

Among many ecological effects, introductions may lead to competition between ecologically similar native and introduced species. Competitive interactions between two species occur when each species negatively affects the other's population sizes and growth rates (Gotelli 1998). Such effects can occur indirectly via exploitative competition when both species are using the same resource, such as consuming the same forage, or directly via interference competition when one species excludes the other from a resource, such as quality spawning habitat. Because ecologically similar species often have behavioral similarities, they are likely to use the same resources and overlap in space, increasing the potential for competitive interactions.

Lake Norman, located in west-central North Carolina, has traditionally supported a strong largemouth bass *Micropterus salmoides* fishery. Recruitment of largemouth bass in Lake Norman, however, was recently perceived to be declining following the establishment of spotted bass *M. punctulatus* (Abney et al. 2007). Duke Energy biologists unexpectedly collected spotted bass for the first time in 2000 (D. Coughlan, Duke Energy, unpublished data). Because the lake is sampled frequently, spotted bass are thought to have been rare or absent before then. Now, fewer than 10 years since spotted bass were first collected, they make up about half of the black bass fishery in Lake Norman, raising concern that the establishment of spotted bass may be negatively affecting the largemouth bass population.

Largemouth and spotted bass are ecologically similar and presumably competitors in systems where they coexist, yet relatively few investigations have examined interactions between the two species (e.g., Vogele and Rainwater 1975; Matthews et al. 1992; Sammons et al. 1999; Maceina and Bayne 2001), and have often focused on a single life stage or interaction (e.g., diet, habitat). One apparent trend is that spotted bass seem to have a competitive advantage over largemouth bass in systems with low productivity and clear water. Greene and Maceina (2000) found that when both species were present, low productivity reservoirs tended to have higher spotted bass recruitment, whereas eutrophic reservoirs tended to have higher largemouth bass recruitment. Also, Maceina and Bayne (2001) found spotted bass gaining a competitive advantage over largemouth bass after oligotrophication of a reservoir in Georgia. This may be a function of water clarity; in controlled laboratory experiments, Crowl (1989) found that largemouth bass responded to

potential prey faster in turbid water than in clear water. It should be noted, however, that lower productivity systems are typically associated with reduced adult body condition of both species, likely attributable to fewer forage fish (Maceina and Bayne 2001).

Because of the link between low productivity and fewer forage fish (Jeppesen et al. 2000), competition among piscivores may be particularly significant in low productivity lakes and reservoirs, especially in systems with many apex predators. Lake Norman is a mesotrophic reservoir that contains numerous piscivores, including (in addition to largemouth and spotted bass) striped bass *Morone saxatilis* and flathead catfish *Pylodictis olivaris*, which prey on fish as adults. Competition between largemouth and spotted bass, however, may begin much earlier than adulthood. Matthews et al. (1992) estimated high diet overlap between juvenile largemouth and spotted bass, although their sample size of spotted bass was small. Juveniles of both species commonly eat zooplankton and other small invertebrates (Robbins and MacCrimmon 1974), similar to the diets of many other young-of-year and juvenile fish. Because changes in adult populations may be driven by interactions earlier in ontogeny, it is important to quantify the potential for competition at multiple life stages.

Competition for food can influence growth, survival, and recruitment (e.g., Connell 1983). Information on diet and spatial-temporal overlap of different species can be used to gain insight into the potential for competition in an observational field study (Wissinger 1992). Competition for forage is important to understand because it can influence growth (Mittelbach 1988), and body size can ultimately influence an individual's fitness

(Wiegmann et al. 1992). If two species are separated spatially, potential for competition is reduced because they use different habitats and are less likely to be consuming the same prey. Overlap in habitat use does not necessarily indicate competition, but it increases the possibility of competition, particularly during times of limited resources. Although spatial-temporal overlap and diet overlap do not explicitly confirm competition, both are important factors to quantify and consider when evaluating potential competition between or among species. Such information may be useful in elucidating potential impacts of a recent spotted bass introduction on an established largemouth bass population.

The perceived decline in largemouth bass coincident with the recent establishment of spotted bass in Lake Norman was the impetus to examine ecological interactions between these potential competitors. We assessed potential competition between largemouth and spotted bass in Lake Norman by investigating overlap of diets and habitat use between these species as both juveniles and adults. Our objectives were to (1) measure the extent and variability of diet overlap between largemouth and spotted bass, (2) compare feeding intensity of largemouth and spotted bass, and (3) quantify habitat use of largemouth and spotted bass in relation to each other and to its availability.

## **Methods**

### *Study Site*

Lake Norman is a large (12,634-hectare), relatively unproductive reservoir on the Catawba River in North Carolina. Two power plants use the lake for cooling water and

have hot water discharges that influence nearby water temperatures. Much of the lake's shoreline is developed, has numerous docks and piers, and is lined with riprap. Transects used in this study have been in use by Duke Energy biologists for monitoring fish populations since 1993 and are divided into four zones from upstream to downstream as "1" through "4".

#### *Field – Identification of Black Bass*

Largemouth and spotted bass were found to be hybridizing in Lake Norman, so methods for identifying parental species and hybrids were developed (Chapter 1). Classification trees were developed for identifying juvenile and adult largemouth, spotted, and largemouth-spotted bass hybrids by pairing morphological observations with genetic data that differentiated parental species from hybrids with a reasonable level of confidence (81-88% overall success rate). Genetic analyses were used to identify many of the fish collected for diet and habitat comparisons in this study ( $n = 62$  juveniles, 52% and  $n = 46$  adults, 43%); the remaining individuals were identified using the classification trees developed specifically for identifying black bass in Lake Norman based on morphological characteristics (Chapter 1).

#### *Field - Diets*

Pulsed-DC boat electrofishing (Smith-Root®, model 7.5 GPP) was used to collect juvenile (50-100 mm TL) and adult (300-500 mm TL) black bass along 300-m transects in four zones of the lake. During electrofishing, collected fish were placed in a livewell until a transect was completed. Upon completion, total length (TL, mm) and mass (g) of fish were

recorded. Stomach contents of fish  $\geq$ 150 mm TL were removed with a gastric lavage device similar to Light et al. (1983), but modified to use a 12-volt, 2.5-amp, 1363-L-per-hour bilge pump to deliver water, as used by Hakala and Johnson (2004). Gastric lavage is a proven, non-lethal technique to extract stomach contents of largemouth bass (Hakala and Johnson 2004). Briefly, an appropriately sized tube is inserted into the fish's stomach through the mouth and water is pumped into the stomach, which causes the fish to regurgitate its stomach contents. Stomach contents were flushed into a bucket and then rinsed through a fine-mesh net before being stored in 70% ethyl alcohol for laboratory identification. Fish not needed for genetic analysis were released immediately following the gastric lavage procedure. Fish <150 mm TL were euthanized with a lethal dose of MS-222, placed on wet or dry ice in the field, and stored in the lab at -20°C.

Benthic invertebrates and zooplankton were collected to assess availability of prey for juveniles. A 23 x 23-cm Ponar grab sampler was used to collect a benthic substrate sample on each transect, at a depth ranging from 0.5 - 1.5 m. The sample was filtered through a 500- $\mu$ m mesh sieve until it fit into a 1-L jar. Benthic invertebrates were immediately preserved by adding a 70% ethyl alcohol solution containing Rose Bengal, a biological stain. A 1.0-m diameter plankton net (64- $\mu$ m mesh) was used to collect zooplankton with one vertical tow through the entire water column at depths ranging from 3.0 – 5.0 m slightly offshore from each electrofishing transect. Zooplankton were immediately preserved in 70% ethyl alcohol until laboratory processing. Availability of fish prey could not be reliably estimated with the sampling techniques used in this study.

### *Field - Habitat*

After a black bass was immobilized with boat electrofishing, a small, numbered buoy attached by string to a 227-g weight was dropped at the location of fish capture, and a wire tag corresponding to the buoy number was attached to the fish's jaw. After tagging, each fish was immediately placed in a flow-through livewell. After the transect was completed, microhabitat variables (Table 2.1) within a 2-m radius of the location of capture for each individual fish were observed and recorded (Matheney and Rabeni 1995). The habitat variables measured were depth (m), distance from shore (m), substrate type using a modified Wentworth scale similar to deGraaf and Bain (1986), and any associated cover (i.e., pier or piling, woody material, and vegetation).

Habitat availability was estimated at three randomly chosen transects in each of the four zones (totaling 12 transects) of the lake. Each transect was waded approximately 2 m from shore in 10 x 2-m plots (~30 plots per transect), and the microhabitat variables previously described (Table 2.1) were estimated for habitat availability, with the exception of distance from shore. Depth was measured 2 m from the shoreline to estimate slope. Substrate types in each plot were visually estimated in each 10 x 2-m plot and recorded on a percent basis. Vegetation cover was also estimated as a percentage of the 10 x 2-m plot. Total number of piers or pilings (i.e., vertical support of pier or bridge) and total number of coarse woody materials (CWM) were recorded for each transect. Coarse woody material was further classified into simple (largest end >0.1 m diameter, >1.0 m length, approximately horizontal, and 0 to few branches; e.g., logs), complex (largest end >0.1 m

diameter including branching, >1.0 m length, horizontal to vertical, and complex branching; e.g., Christmas trees and logs with complex branching), and vertical (>0.1 m diameter, >0.1 m height, and approximately vertical; e.g., stumps). To standardize enumerated availability variables (e.g., number of piers) to percent availability, the 2-m radius rule used for fish microhabitat association was also used for availability. For example, the availability of one simple CWM (e.g., log) can be considered 4 m wide because within 2 m of each side, a netted fish would be considered associated with the log. Therefore, a log is 4 m of the 300 m transect, or 1.3%. Docks and piers were assumed 1 m wide and therefore a dock's percent availability was 5 m of the 300-m transect, or 1.7%.

#### *Laboratory*

Stomachs of whole, frozen fish were removed in the laboratory and stored in 70% ethyl alcohol until processing. During processing, invertebrates were counted and identified to the lowest feasible taxonomic group, and consumed fish were counted, weighed, and similarly identified; vertebral counts and vertebra shape were used for identification when fish prey were highly digested. For stomachs of juvenile bass, the stomach was weighed ( $\pm 0.01$  g) before and after the contents were removed, and total weight of stomach contents was calculated by subtraction. For stomachs of adult bass, each diet item was weighed individually. Stomach contents from the gastric lavage procedure were processed similarly.

Availability of benthic invertebrates and crustacean zooplankton (copepods and cladocerans) was quantified by enumerating samples in the laboratory. Benthic invertebrate

samples were rinsed through several sieves decreasing in mesh size to 149 µm, picked from remaining substrate materials, and identified to the lowest feasible taxonomic group (most often order or family) using a dissecting microscope at ~12X total magnification. Because zooplankton samples contained large numbers of individuals, subsampling, similar to Welker et al. (1994), was used. Briefly, field samples were filtered through 64-µm mesh and washed into a graduated beaker, and the beaker was filled to a known volume (either 40 or 100 mL, depending on zooplankton density). To randomize samples for subsampling, zooplankton were swirled clockwise, counterclockwise, and back and forth in the beaker at least twice and were subsampled with a 1-mL Hensen-Stemple pipette. Zooplankton in entire 1-mL subsamples were enumerated until at least one family of cladocerans and at least one group of copepods (i.e., cyclopoids, calanoids, or harpacticoids) reached 200 individuals, or 10% of the sample had been subsampled and enumerated. Zooplankton were identified and enumerated using ~25X total magnification.

#### *Analyses - Diets*

To analyze diet overlap, a sufficient sample size of stomachs containing prey items was needed from a short time period because of temporal changes in prey availability. This was particularly important for juvenile fish because of rapid changes in invertebrate communities. Numbers of fish collected dictated the temporal and spatial scales for analyses of diet overlap. Often, it was necessary to pool samples from all transects throughout the lake to obtain sufficient sample size for analyses. Feeding intensity was compared between species via relative fullness of stomachs, which was calculated by

dividing total wet weight of all prey items by total wet weight of the fish and multiplying by 100 (i.e., percent of body weight, Hyslop 1980). Because temperature and fish size influence maximum satiation level ( $C_{max}$ ) of largemouth bass (Niimi and Beamish 1974; Rice et al. 1983), relative gut fullness was compared at similar times to control for seasonal temperature changes, and weight was used as a covariate to control for body size. Analysis of covariance (ANCOVA; JMP® 7.0) was used to compare the mean relative fullness of guts between species using weight as a covariate. Both fish weight and relative gut fullness were log-transformed to approximate a normal distribution. When interactions between species and fish weight were insignificant, slopes were assumed to be equal, and data were analyzed again excluding the species-weight interaction term.

Diet overlap was assessed by comparing percent by occurrence and percent by number of prey groups in the diets of each species using two overlap indices: proportion similarity index (PSI, Schoener 1970) and Morisita's index (M, Morisita 1959):

$$\text{PSI} = \left( 1.0 - 0.5 \sum_{j=1}^n |P_{lj} - P_{sj}| \right) ,$$

$$M = \frac{2 \sum_{j=1}^n P_{lj} P_{sj}}{\sum_{j=1}^n P_{lj} [(n_{lj} - 1)/(N_l - 1)] + \sum_{j=1}^n P_{sj} [(n_{sj} - 1)/(N_s - 1)]} ,$$

where  $j$  = prey group,  $l$  = largemouth bass,  $s$  = spotted bass,  $P_{lj} = n_{lj}/N_l$  and  $P_{sj} = n_{sj}/N_s$ . For prey occurrence data,  $n_{lj}$  = number of stomachs of largemouth bass containing prey group  $j$ ,

$N_l$  = total number of individuals of largemouth bass,  $n_{sj}$  = number of stomachs of spotted bass containing prey group  $j$ , and  $N_s$  = total number of individuals of spotted bass. For prey count data,  $n_{lj}$  = number of diet items of prey group  $j$  in stomachs of largemouth bass,  $N_l$  = total number of prey items consumed by largemouth bass,  $n_{sj}$  = number of diet items of prey group  $j$  in stomachs of spotted bass, and  $N_s$  = total number of prey items consumed by spotted bass.

PSI is a commonly used overlap index (e.g., Bacheler et al. 2004; Kahl and Radke 2006), and M is recommended because of its low bias regarding small sample sizes and number of prey groups (Wolda 1981; Smith and Zaret 1982). M is also recommended for count data (Chipp and Garvey 2007). PSI ranges from 0 to 1, and M ranges from 0 to slightly  $>1$  (see Wolda 1981 for a detailed explanation of the range of M). With PSI, diets  $>0.60$  overlap have been considered similar (e.g., Wallace 1981), and with M, diets  $>0.50$  overlap have been considered similar (e.g., Tallman and Gee 1982); we follow these conventions in our analyses.

Smith (1985) argued that index values do not provide estimates of error and recommended using jackknife or bootstrap procedures to understand the variability associated with index estimates. Therefore, we used the statistical analysis program R® 2.4.0 (R\_Development\_Core\_Team 2006) and boot package (Canty and Ripley 2007) to create 10,000 overlap index values for both PSI and M by bootstrap sampling individual fish stomachs. The distributions of bootstrap index values were used to estimate means and 95% confidence intervals for bootstrapped index values. Because many distributions were

left-skewed, they were transformed with the cubic function (^3) to approximate normality.

Then, means and 95% confidence intervals were calculated, and values were transformed back for reporting.

Categorization of prey groups for these analyses was done separately for juvenile and adult bass. Because of the large number of invertebrate prey taxa in juvenile bass diets, prey items were grouped by habitat association and physical characteristics (i.e., small benthic invertebrates, large benthic invertebrates, planktonic invertebrates, surface and swimming macroinvertebrates, and fish; Table 2.2). These groups were chosen to help identify whether each species was foraging in different habitats or on different sizes of prey. Adult bass, conversely, primarily consume fish and only occasionally consume macroinvertebrates, so prey groupings for adult fish analyses included only fish genera and macroinvertebrates (Table 2.3).

Proportions of prey in diets were compared with proportions available. Chesson's alpha index of selectivity (Chesson 1978) was calculated for individuals of each species:

$$\alpha_i = \frac{r_i / p_i}{\sum_{i=1}^m (r_i / p_i)} ,$$

where  $\alpha_i$  = Chesson's alpha for prey group  $i$ ,  $r_i$  = proportion of prey group  $i$  in diet,  $p_i$  = proportion of prey group  $i$  in environment, and  $m$  = total number of prey groups. If  $\alpha_i$  is  $> 1/m$ , prey group  $i$  is selected for, and if  $\alpha_i$  is  $< 1/m$ , prey group  $i$  is avoided when compared with random foraging. Planktonic invertebrates collected as part of the availability

sampling were further divided into cladocerans, copepods, and *Chaoborus* spp. (Table 2.2) so planktonic availability could be compared with prey of largemouth and spotted bass. Benthic organisms (Table 2.2) in the diet were also compared with those available estimated from benthic invertebrate sampling. Because the availability of prey fish could not be estimated reliably from the sampling techniques used in this study, prey fish were excluded from the selectivity analyses.

#### *Analyses - Habitat*

Summer habitat use by largemouth and spotted bass was examined by comparing the proportions of each habitat used by individuals of each species with the proportion available using Jacobs' (1974) improved Ivlev's electivity index (D) and the Strauss index of selectivity (L, Strauss 1979)

$$D = \frac{r_i - p_i}{r_i + p_i - 2r_i p_i} ,$$

$$L = r_i - p_i ,$$

where  $r_i$  = estimated proportion of habitat  $i$  used by each species and  $p_i$  = estimated proportion of habitat  $i$  available in the reservoir. The calculated index values can range from -1 to 1, with -1 indicating complete avoidance, 0 indicating use in proportion to availability, and 1 indicating always selected for.

## **Results**

### *Diet*

In sum, 132 adult black bass and 120 juvenile black bass were collected for diet and habitat analyses, although 13 adults and 20 juveniles were hybrid largemouth-spotted bass and were excluded from analyses. Because of sample size constraints and some empty stomachs, diets of adult black bass were quantified lake-wide (i.e., by combining collections from all transects) from 29 April to 01 May 2008 ( $n = 25$  LMB and  $n = 9$  SPB) and from 12 May to 16 May 2008 ( $n = 19$  LMB and  $n = 20$  SPB). Sufficient numbers of juvenile black bass were available for analysis lake-wide from 31 July to 08 August 2007 ( $n = 31$  LMB and  $n = 20$  SPB), from a single transect on 29 April 2008 (Zone 2,  $n = 17$  LMB and  $n = 11$  SPB), and from two transects on 10 June 2008 (Zone 2,  $n = 15$  LMB and  $n = 6$  SPB). This resulted in three spatial scales of diet overlap estimates: lake, zone, and transect.

No differences in feeding intensity between adult or juvenile largemouth and spotted bass were detected ( $p > 0.05$  during all five sampling periods; Figure 2.1). Overall, the percent of empty stomachs was similar for adult largemouth and spotted bass (Table 2.4), except for 12 – 16 May 2008, when largemouth bass had 41% empty stomachs and spotted bass had 23% empty stomachs. Most juveniles of both species contained prey, but bass collected in August tended to have more empty stomachs (15-19%) than those collected in April and June (0-9%).

Diet overlap of largemouth and spotted bass was high in all instances. Both the calculated and mean bootstrap values of PSI and M always exceeded 0.60 for juvenile bass (usually  $>0.80$ ), whether based on prey proportion by occurrence or number. The lower 95% confidence limits estimated by bootstrapping exceeded 0.60 for all estimates of M and for PSI estimates in one of three sample periods (Table 2.5). Diet overlap of adults based on both prey number and occurrence also was  $>0.60$  for calculated and mean bootstrap values of PSI and M. However, the lower limits of bootstrapped 95% confidence intervals included 0.60, with one exception (Table 2.5). High diet overlap occurred regardless of spatial scale (i.e., comparisons made at the transect, zone, and lake-wide levels all indicated high diet overlap)

Juvenile largemouth and spotted bass both generally selected for or against similar prey. In samples collected to estimate prey availability, planktonic invertebrates were dominated by either cladocerans or copepods, and benthic prey were dominated by large invertebrates associated with the substrate (Figure 2.2). Juvenile largemouth and spotted bass generally selected similar zooplankton and benthic invertebrate groups, and in most estimates, 95% confidence intervals overlapped neutral selectivity of prey items (Figure 2.3). On 29 April and 10 June 2008, cladocerans were selected by both species (Figures 2.3B and 2.3C), and both species seemed to avoid copepods during all sampling periods, although confidence intervals often overlapped neutral selectivity. Small benthic prey were avoided by both species on 29 April 2008 and by largemouth bass on 10 June 2008 (Figures

2.3B and 2.3C). *Chaoborus* spp. were only detected in fish stomachs and in zooplankton samples during 31 July – 08 August (Figure 2.3A).

Although we could not quantify availability of fish prey adequately to calculate selectivity for fish prey groups, the frequencies of prey items in diets are still informative. The most common fish prey consumed by adults of both species was threadfin shad (Table A.1)

#### *Habitat*

Silt-clay was the most common substrate, followed by cobble, and piers were the main form of cover (Figure 2.4). Riprap was common throughout the reservoir, and it typically fell within the size range of cobble, although some was in the boulder-bedrock size range. Gravel and pebble substrates were least common, as well as the group including other substrates, such as concrete ramps and bricks, occasionally found in the littoral zone. No vegetation was observed, so that cover variable was excluded from analyses.

Habitat use was estimated at capture locations of adult largemouth bass ( $n = 34$ ) and adult spotted bass ( $n = 17$ ) collected lake-wide during spring of 2008. Habitat use (i.e., substrate and cover use) was similar overall for adult largemouth and spotted bass (Figures 2.5A and 2.5B), with few exceptions. Although adults of both species selected specific cover types (vs. random use) in the littoral zone, adult largemouth bass strongly selected complex woody material with weaker positive selection of piers or pilings, whereas spotted bass exclusively selected piers or pilings (Figure 2.5A and 2.5B).

Juvenile largemouth bass ( $n = 24$ ) and juvenile spotted bass ( $n = 28$ ) were collected lake-wide during summer of 2007. Use of substrate and cover was also generally similar for juvenile largemouth and spotted bass (Figures 2.6A and 2.6B), with few exceptions. Largemouth bass avoided silt-clay, whereas spotted bass had a slightly positive selection for silt-clay, and largemouth bass showed a weak selection for pebble, whereas spotted bass were neutral toward it (Figures 2.6A and 2.6B). Both species selected for piers or pilings, complex woody material, and vertical woody material cover, and strongly avoided simple woody material (Figures 2.6A and 2.6B).

## **Discussion**

We assessed interactions between largemouth bass and spotted bass at juvenile and adult life stages by comparing their diets and habitat use. The combined analyses show strong overlap between largemouth and spotted bass; with few exceptions, habitat use and diet of both species were remarkably similar at both juvenile and adult life stages. When ecologically similar species such as largemouth bass and spotted bass use the same resources, both populations may be negatively affected, potentially causing decreases in recruitment levels and changes in the aquatic food web (Mittelbach 1988).

In this study, juveniles and adults of both species preyed upon similar food resources. This suggests that low levels of food resources may lead to competition and poor body condition in both species. Brown and Murphy (2004) observed that body condition of adult largemouth bass increased as forage fish, most notably shad, increased in

late spring and early summer. In our study, threadfin shad were consumed more frequently than any other prey species by both adult largemouth and spotted bass, presumably because they were the most abundant prey item. Because prey fish could not be reliably sampled, selectivity for forage fish could not be assessed for adult largemouth and spotted bass. Nevertheless, the degree of diet overlap shows the two are consuming similar forage as adults. Largemouth and spotted bass may be limited by competition for these important prey items, particularly during times of low availability.

The significant overlap in diet of juvenile largemouth and spotted bass in our investigation is consistent with observations of Matthews et al. (1992) and Clady and Luker (1982). Although we noted some indication of positive selection for cladocerans and avoidance of copepods by both species, overall, the selection of prey items by juvenile largemouth and spotted bass suggested largely opportunistic foraging, much like the observations made on adult largemouth bass by Hodgson and Kitchell (1987). As with adult fish, this suggests the potential importance of competitive interactions in shaping the recruitment and life history of both species. In addition, we found that juveniles of both species consumed smaller young-of-year black bass, which has also been documented in other studies (e.g., Cooper 1937; Post et al. 1998).

Adult largemouth and spotted bass were markedly similar in their use of habitat. The biggest difference we noted between the species was in the use of woody cover; spotted bass avoided it entirely, whereas largemouth bass favored complex woody cover. Vogeles and Rainwater (1975) studied use of brush shelters (a form of complex woody

cover) by different black bass species and found that although largemouth bass preferentially used brush shelters early in the spawning season, spotted bass used brush shelters more than largemouth over the total seven-week time period studied. Our observations also occurred during the spawning season for black bass, and on a similar temporal scale. Although we observed a similar association of largemouth bass with complex woody materials, our findings differed for spotted bass. One interpretation of this result is that largemouth bass are excluding spotted bass from complex woody cover, such as old conifer trees and brush shelters, which are not abundant in Lake Norman. If so, this could also explain the strong selection by spotted bass for piers or pilings, the most abundant cover type in Lake Norman. For substrate use, we found that adult largemouth and spotted bass selected for and against the same substrates. Conversely, Sammons and Bettoli (1999) found largemouth and spotted bass to differ in substrate use; largemouth bass heavily used rip rap and sheltered cove habitats (usually clay bottom and some woody structure), whereas spotted bass were found over rubble (natural rock and usually steep banks) and gravel areas. Other than use of complex woody structure, largemouth and spotted bass seemed to use the same habitat in Lake Norman.

Juvenile largemouth and spotted bass also strongly overlapped in their use and selectivity of habitat. Silt-clay was the only habitat characteristic for which the two species showed opposite selectivity; largemouth bass avoided silt-clay, whereas spotted bass exhibited positive selection for it. Both species selected most strongly for boulder-bedrock substrate, though this selection was not as strong for spotted bass as for largemouth bass.

Conversely, Sammons and Bettoli (1999) found the smallest size range of black bass (mean = 100-200 mm TL) over gravel substrates. Gravel in Lake Norman, however, was estimated as the least abundant substrate and both species showed negative selection for it. Selectivity of cover variables by juvenile largemouth and spotted bass was similar for all types. These results are consistent with the high overlap of habitat use by juvenile largemouth and spotted bass observed by Matthews et al. (1992).

Strong inferences regarding competition between largemouth and spotted bass can be drawn from our study, despite several potential limitations to the interpretation of our results. Because food resources can change substantially over time and space, we had to limit our analyses to times when we could collect enough black bass over a short enough period to reasonably compare diet and habitat use between the two species. As a result, the data we used for juvenile overlap and selectivity calculations spanned a range of spatial and temporal scales: lake-wide over ~1 week, two transects (from one zone) over ~1 hour, and one transect over ~30 minutes. Our data suggest, however, that when prey items are grouped into ecologically meaningful categories, strong similarities between the species emerge regardless of the spatial-temporal scale. Adults were more difficult to collect over a small spatial scale and were only accessible with electrofishing equipment during springtime when fish were spawning. Therefore, data were pooled from all transects for comparisons between the two species. Even when pooling data spatially, however, adults of the two species showed the same degree of high overlap as juvenile fish. These

consistencies in overlap estimates at different spatial and temporal scales may have resulted from largemouth and spotted bass being generalist, opportunistic predators.

Our sample sizes were also relatively small, but our estimates of diet overlap and their associated variability seemed robust to small sample size. Although larger sample size would presumably strengthen our analyses, all of our measures of diet overlap using both the proportion similarity index and Morisita's index were consistently high, and their bootstrap confidence intervals, though wide, were in the upper half of the range of each index, indicating consistent overlap between the two species. Diet overlap estimates of PSI  $>0.60$  and M  $>0.50$ , as seen in this study, have traditionally been considered significant (Wallace 1981; Tallman and Gee 1982). Even our analyses for juveniles on 10 June 2008, which had the smallest sample sizes of each species (15 largemouth bass and 6 spotted bass), resulted in calculated and mean bootstrap values of PSI and M, exceeding the thresholds for significant diet overlap. Given that small sample size should generally bias results in the direction of low overlap (due to individual variability), we believe our approach is conservative in this case. In sum, though our analyses were variable in space and time, and were based on limited sample sizes, we consistently observed similar diet and habitat overlap, suggesting that these patterns are robust to those limitations and that the two species are largely opportunistic foragers and likely competitors.

Like other recent diet studies (e.g., Bozek et al. 1994; Hilderbrand and Kershner 2004; Papastamatiou et al. 2006), we measured diet overlap of largemouth and spotted bass with percent by occurrence and percent by number of ecologically similar prey groups.

More detailed analyses using gravimetric or volumetric measures, when combined with numerical measures in diet studies, are useful for measuring “prey importance” in terms of energetic overlap, which can add valuable information to diet studies (Hyslop 1980).

However, because energetic overlap is not necessary to show potential competition, considerable time and cost associated with a diet study can be saved by using count and occurrence data while still providing sound, effective indicators of diet overlap.

Though largemouth and spotted bass frequently co-occur in reservoir systems (Sammons et al. 1999), few studies on their ecological interactions exist, particularly in systems experiencing recent introductions of one or the other species. We provide evidence for potential competitive interactions between largemouth and spotted bass in both juvenile and adult life stages. Evidence of hybridization (Chapter 1) further illustrates that the two species are interacting and may be affecting population dynamics and community structure in Lake Norman. Evidence that spotted bass populations do better than largemouth bass in systems of lower productivity (Greene and Maceina 2000; Maceina and Bayne 2001) suggests that spotted bass may have an advantage over largemouth bass in Lake Norman, which is a relatively unproductive reservoir. The rapid increase of the spotted bass population to about half of the black bass fishery in Lake Norman just ten years after initial detection also suggests some advantage by spotted bass. However, because of the short ecological history of spotted bass in Lake Norman, it is unlikely that the populations of largemouth and spotted bass have reached equilibrium. Where both species coexist, monitoring of relative proportions of each species through time, especially shortly after an

introduction of one or the other species, should better indicate the potential (or lack thereof) for stable co-existence between the species. Our study provides needed insight into ecological interactions between these common reservoir species, serving as both an initial indication of the effects of the spotted bass introduction in Lake Norman and possible effects of introductions of either largemouth or spotted bass into systems where the other species already exists.

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## Tables

Table 2.1. Habitat variables and descriptions observed for estimating habitat availability in the littoral zone and for estimating microhabitat within a 2-m radius of capture locations of black bass. Substrate type is a simple Modified Wentworth Scale similar to that used by deGraff and Bain (1986).

Habitat variable	Description
Depth (m)	Availability: measured 2 m from shoreline edge Microhabitat: measured at location of fish capture
Distance from shore (m)	Availability: not applicable Microhabitat: measured at location of fish capture
Substrate type (% each)	Availability: estimated within each 10 x 2-m littoral plot (shoreline to 2 m offshore) Microhabitat: estimated within 2-m radius of fish capture location
Silt-clay	<0.0625 mm
Sand	0.0625 mm – 2.0 mm
Gravel	2.0 mm – 16 mm
Pebble	16 mm – 64 mm
Cobble	64 mm – 256 mm
Boulder-bedrock	>256 mm
Associated cover	Availability: total number in 300-m transect Microhabitat: number within 2-m radius of fish capture location
Pier or piling ( <i>n</i> )	Vertical support of dock, pier, or bridge
Woody material ( <i>n</i> )	
Simple	>10 cm diameter, >1 m length, 0 to few branches, and horizontal
Complex	>10 cm diameter, complex branching, and horizontal to vertical
Vertical	>10 cm diameter, >10 cm height, and vertical
Vegetation (%)	Submergent or emergent aquatic vegetation

Table 2.2. Prey groups used for estimates of diet overlap between juvenile largemouth and spotted bass, based on prey items occurring in juvenile black bass diets.

		Invertebrate prey			Fish prey <sup>a</sup>
Benthic		Planktonic	Surface		
Small	Large				
Hydrachnidia	Ceratopogonid larvae	<i>Chaoborus</i> spp.	Collembola	<i>Dorosoma petenense</i>	
Nematoda	Chironimid larvae	Cladocerans	Diptera pupae	<i>Etheostoma olmstedi</i>	
Ostracoda	Coleoptera nymphs and adults	Copepods	Hemiptera	<i>Micropterus punctulatus</i>	
	Ephemeroptera nymphs		Terrestrial invertebrates <sup>b</sup>	<i>Micropterus salmoides</i>	
	Odonate nymphs			<i>Micropterus punctulatus-salmoides</i>	
	Trichoptera			Unknown fish	

<sup>a</sup> Possible species consumed. Prey could rarely be identified to species.

<sup>b</sup> Includes arachnida, hymenoptera, and adult diptera

Table 2.3. Prey groups used for estimates of diet overlap between adult largemouth and spotted bass, based on prey items occurring in adult black bass diets.

	Fish prey				Invertebrate prey
<i>Alosa</i>	<i>Dorosoma</i>	<i>Lepomis</i> <sup>a</sup>	<i>Micropterus</i> <sup>a</sup>	<i>Morone</i> <sup>a</sup>	
<i>Alosa pseudoharengus</i>	<i>Dorosoma cepedianum</i>	<i>Lepomis auritus</i>	<i>Micropterus punctulatus</i>	<i>Morone americana</i>	Diptera pupae
	<i>Dorosoma petenense</i>	<i>Lepomis cyanellus</i>	<i>Micropterus salmoides</i>	<i>Morone saxatilis</i>	Ephemeroptera
		<i>Lepomis gulosus</i>	<i>Micropterus punctulatus-salmoides</i>		Odonata
		<i>Lepomis macrochirus</i>			
		<i>Lepomis microlophus</i>			

<sup>a</sup> Possible species consumed. Prey could rarely be identified to species.

Table 2.4. Number and percent of empty stomachs of largemouth and spotted bass.

Life stage	Date	Largemouth bass	Spotted bass
Adult	29 Apr – 01 May 2008	20/45 (44%)	7/16 (44%)
	12 May – 16 May 2008	13/32 (41%)	6/26 (23%)
Juvenile	31 Jul – 08 Aug 2008	6/31 (19%)	3/20 (15%)
	29 Apr 2008	1/17 (6%)	1/11 (9%)
	10 Jun 2008	0/15 (0%)	0/6 (0%)

Table 2.5. Calculated diet overlap estimates, mean bootstrap, and 95% CIs of 10,000 bootstrap values, based on proportion by number and proportion by occurrence of prey groups, estimated by proportion similarity index (PSI) and Morisita's index (M) for juvenile (50-100 mm TL) largemouth and spotted bass lake-wide in 2007, from one transect Apr 2008, and from one zone Jun 2008, and adult (300-500 mm TL) largemouth and spotted bass lake-wide on both sampling dates. Values of PSI > 0.6 and M > 0.5 were considered significant overlap.

Size class	Date	Proportion by number					
		Calculated index value		Mean bootstrap index value		Bootstrap 95% CI	
		PSI	M	PSI	M	PSI	M
Juvenile	31 Jul – 08 Aug 2007	0.81	0.97	0.77	0.92 <sup>a</sup>	0.50 – 0.93 <sup>a</sup>	0.72 – 1.06 <sup>a</sup>
	29 Apr 2008	0.93	0.99	0.84 <sup>a</sup>	0.95 <sup>a</sup>	0.62 – 0.98 <sup>a</sup>	0.83 – 1.04 <sup>a</sup>
	10 Jun 2008	0.61	0.84	0.62	0.83	0.42 - .088	0.60 – 0.99
Adult	29 Apr – 01 May 2008	0.90	1.01	0.78 <sup>a</sup>	0.97 <sup>a</sup>	0.56 – 0.93 <sup>a</sup>	0.85 – 1.07 <sup>a</sup>
	12 May – 16 May 2008	0.85	0.98	0.75 <sup>a</sup>	0.93 <sup>a</sup>	0.45 – 0.91 <sup>a</sup>	0.73 – 1.07 <sup>a</sup>

Proportion by occurrence							
Size class	Date	Calculated index value	Mean bootstrap index value	Bootstrap 95% CI			
				PSI	M	PSI	M
Juvenile	31 Jul – 08 Aug 2007	0.73	0.98	0.68	0.91 <sup>a</sup>	0.53 - 0.82	0.71 – 1.05 <sup>a</sup>
	29 Apr 2008	0.88	1.08	0.84	1.03 <sup>a</sup>	0.71 – 0.94	0.94 – 1.12 <sup>a</sup>
	10 Jun 2008	0.80	1.03	0.72	0.98 <sup>a</sup>	0.53 – 0.89	0.74 – 1.14 <sup>a</sup>
Adult	29 Apr – 01 May 2008	0.80	1.06	0.66	0.95 <sup>a</sup>	0.43 – 0.86	0.64 – 1.13 <sup>a</sup>
	12 May – 16 May 2008	0.88	1.08	0.71	0.98 <sup>a</sup>	0.52 – 0.88	0.79 – 1.11 <sup>a</sup>

<sup>a</sup>Data were cube-transformed (^3) to approximately normal. Bootstrap mean and 95% confidence intervals were calculated using transformed data and converted back for reporting.

## Figures

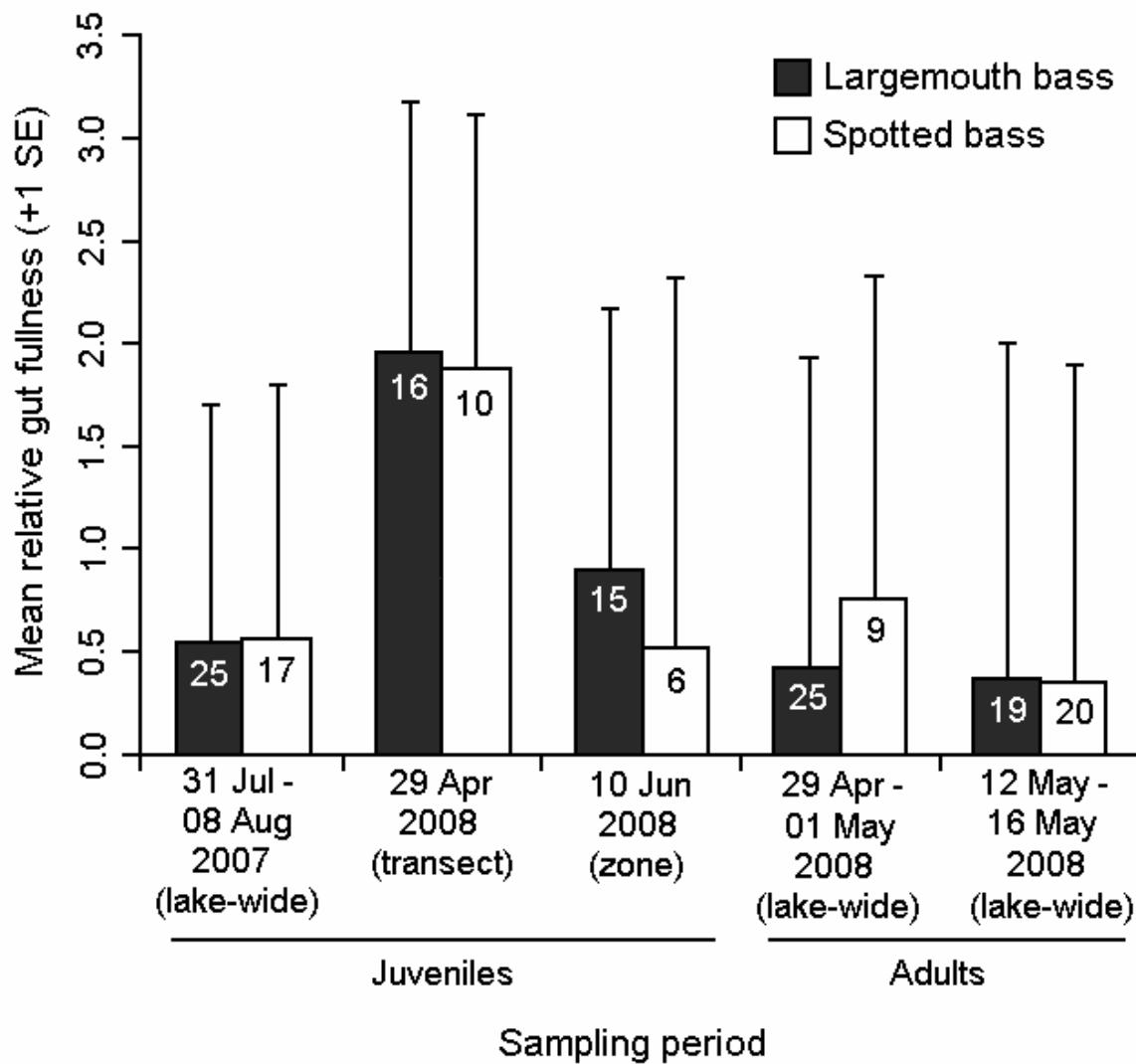


Figure 2.1. Mean relative gut fullness (total wet stomach contents weight/total wet fish weight x 100) of juvenile and adult largemouth and spotted bass ( $\pm 1$  SE). Data were log-transformed to approximate normality, mean and Standard Errors were calculated, and values were transformed back for reporting. X-axis indicates collection period and spatial scale (lake-wide, zone, transect) of each sample. Numbers in bars indicate sample size.

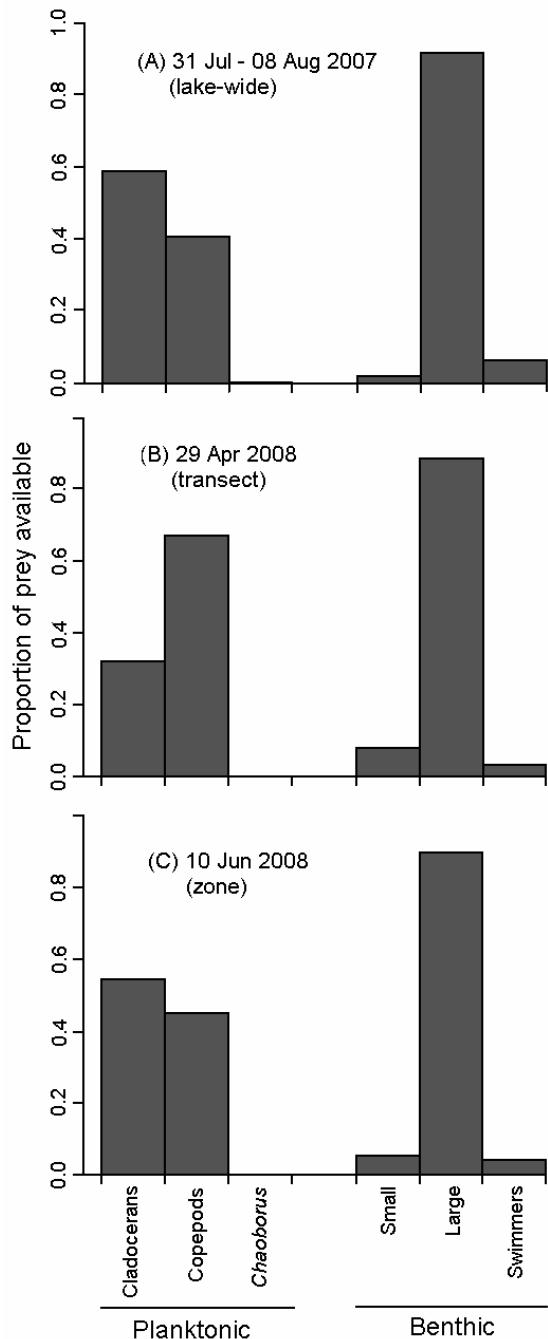


Figure 2.2. Proportions by number of available planktonic and benthic invertebrate prey groups used for calculating electivity index values for largemouth and spotted bass.

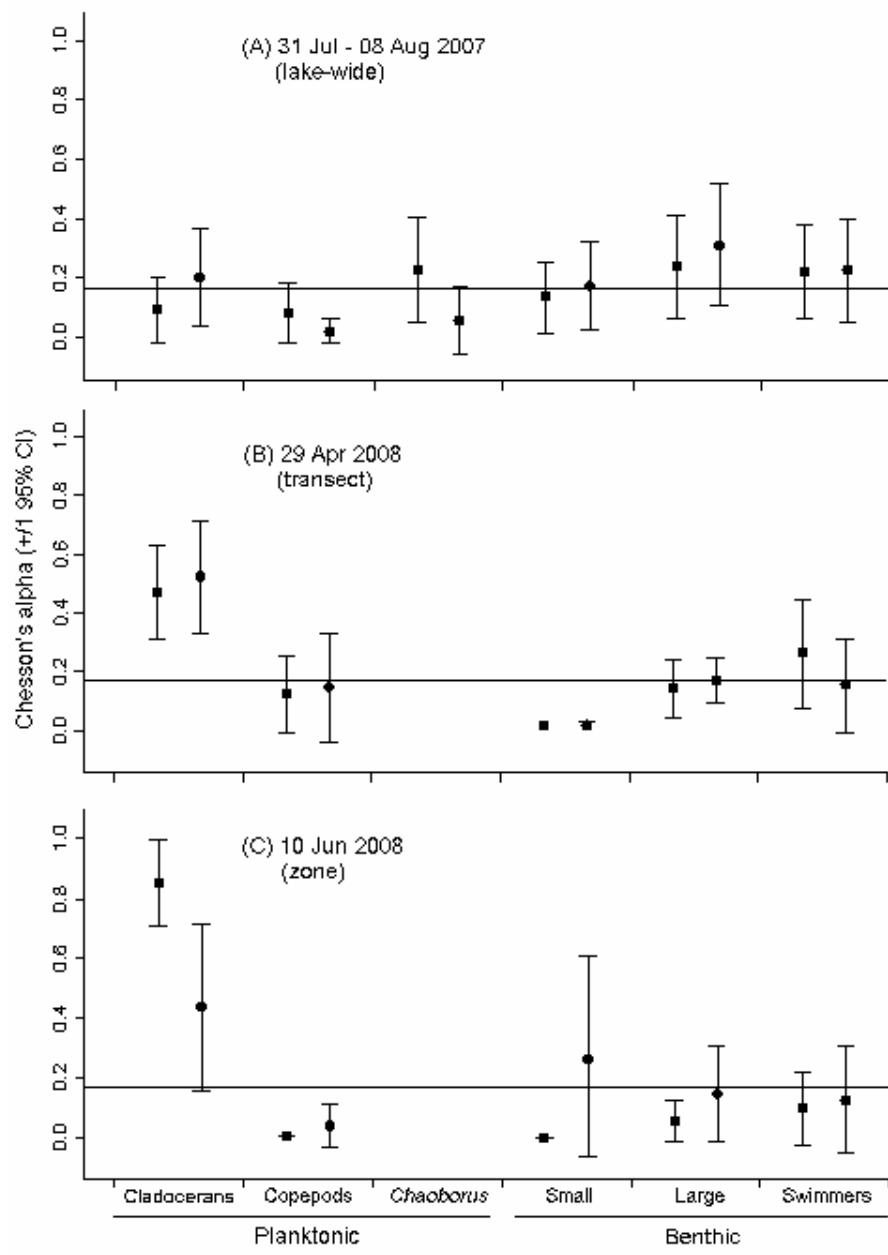


Figure 2.3. Chesson's alpha values ( $\pm$  95% CI) for juvenile largemouth and spotted bass selection of zooplankton and benthic invertebrate prey groups. Estimates are from (A) 31 July – 08 August 2007, (B) 29 April 2008, and (C) 10 June 2008. Squares ( $\blacksquare$ ) indicate largemouth bass, circles ( $\bullet$ ) indicate spotted bass, and the horizontal line indicates  $1/m$  (neutral selectivity).

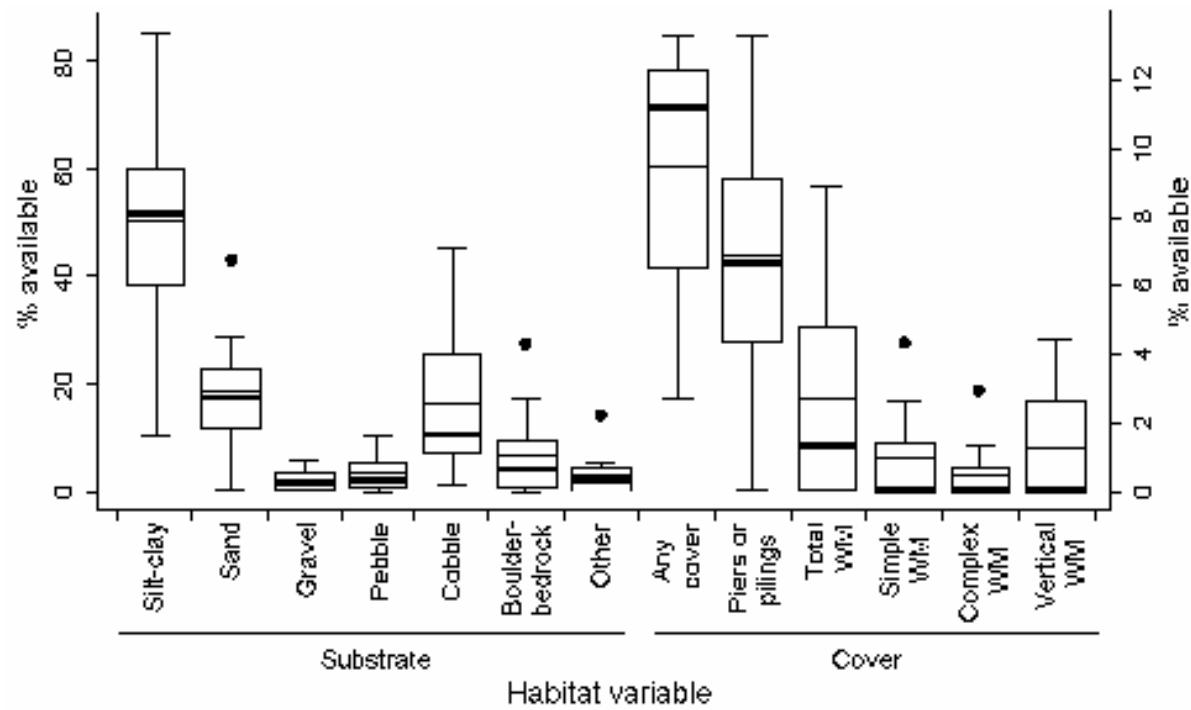


Figure 2.4. Boxplots of mean percent availability of substrates (silt-clay, sand, gravel, pebble, cobble, boulder-bedrock, and other, based on modified Wentworth scale; left axis) from 10 x 2-m plots ( $n \sim 30$  per transect), and percent availability of cover (any cover, piers or pilings, total woody material (WM), simple WM, complex WM, and vertical WM; right axis) from 12 transects sampled during summer of 2007. Vegetation was omitted because none was available. Any cover combines both piers and total WM. Total WM is the percent available based on the sum of simple, complex, and vertical WM. Thick lines indicate median values, thin lines indicate mean values, boxes represent lower and upper quartiles, whiskers represent minimum and maximum values, and dots indicate outliers.

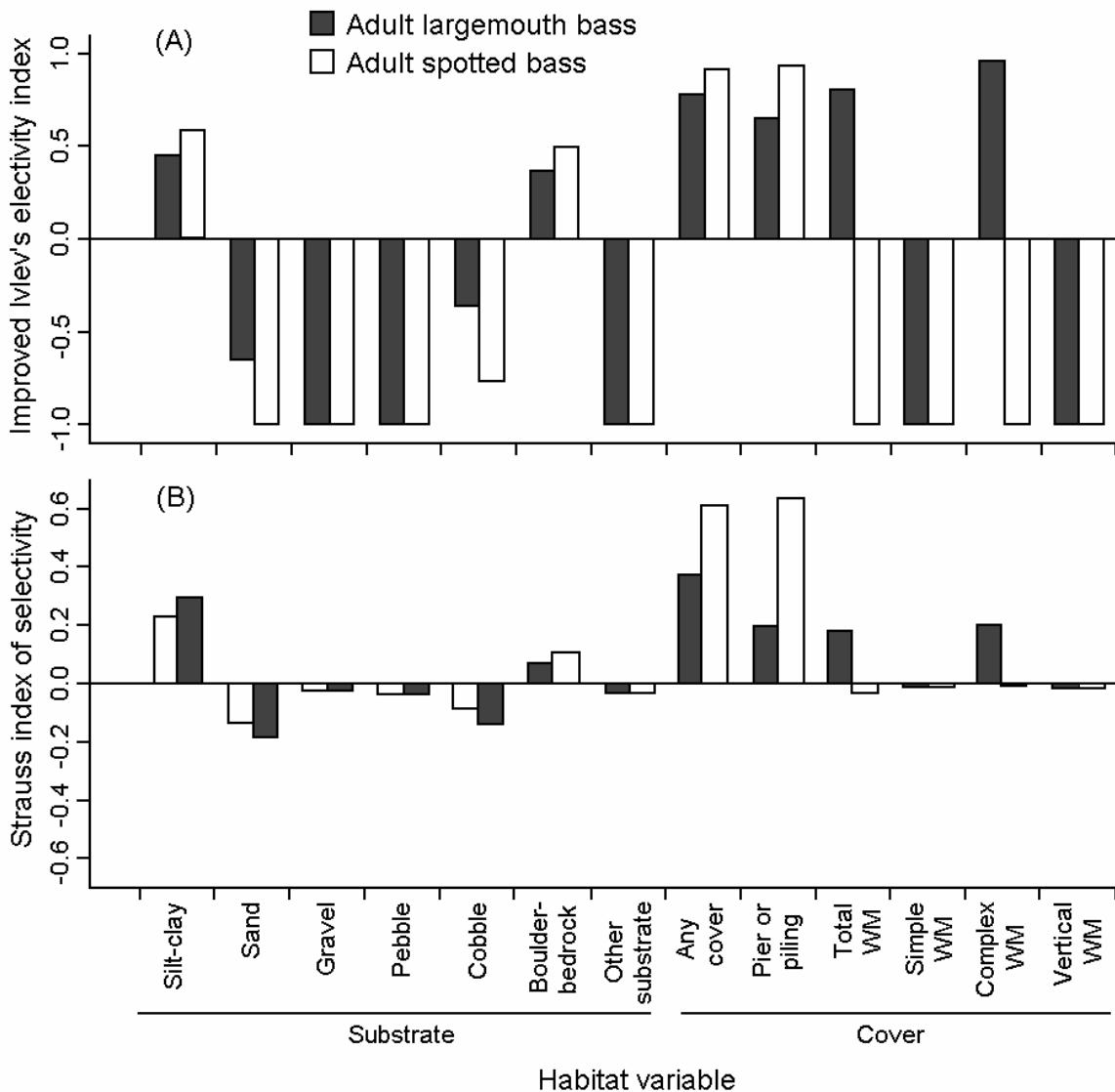


Figure 2.5. Values of (A) Jacob's improved Ivlev's electivity index and (B) the Strauss index of selectivity for adult largemouth and spotted bass for substrate and cover microhabitat selection. Substrate types were estimated using the modified Wentworth scale at the location of capture of each fish. If fish were captured within a 2-m radius of a cover type, they were assumed to be associating with it. Any cover (cumulative total of all cover available) includes piers or pilings, and total woody material (WM); total WM includes simple, complex, and vertical WM. Black bars indicate largemouth bass, and white bars indicate spotted bass.

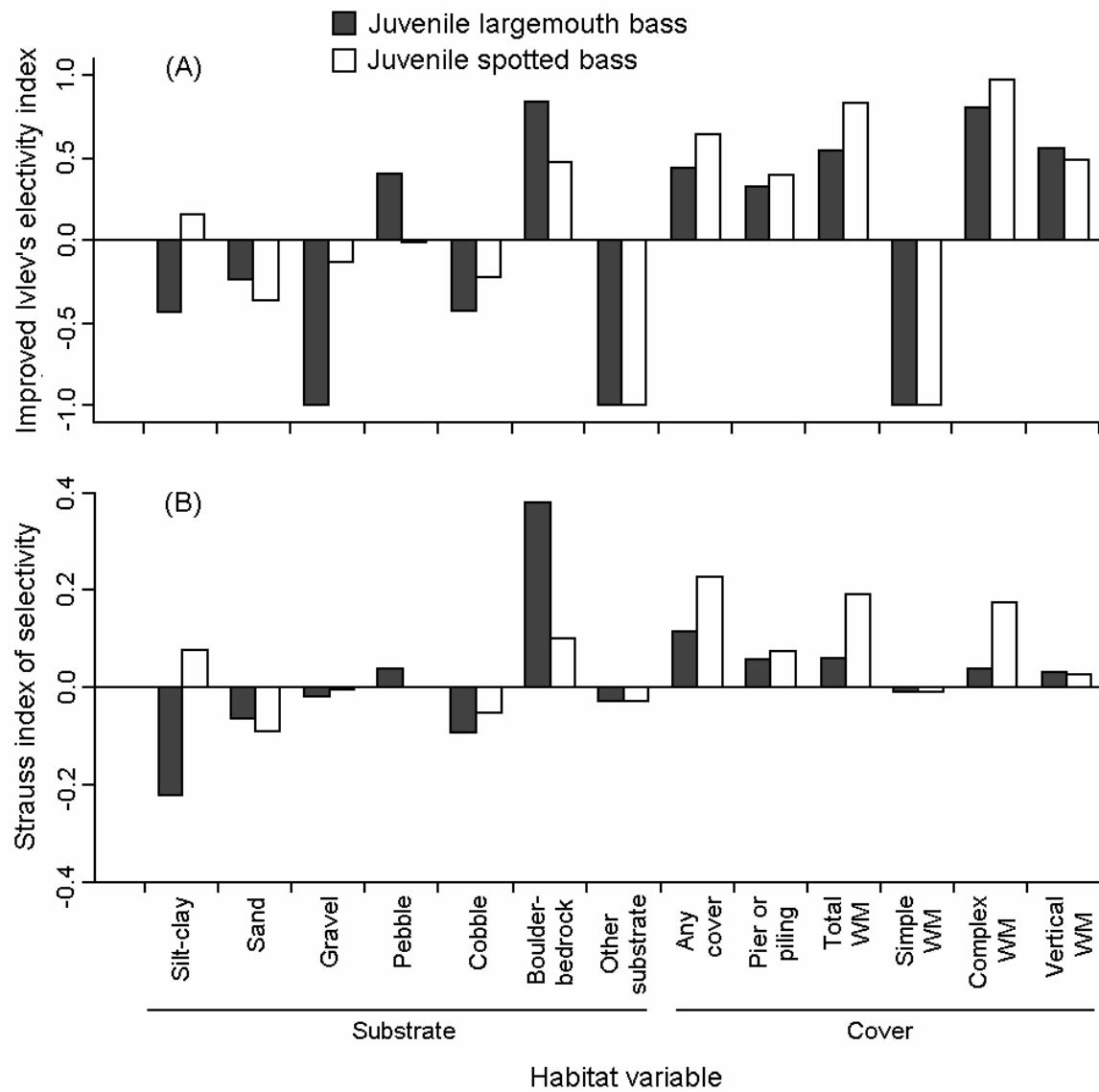


Figure 2.6. Jacob's improved Ivlev's electivity index (A) and the Strauss index of selectivity (B) values for juvenile largemouth and spotted bass for substrate and cover microhabitat. Substrate types were estimated using the modified Wentworth scale at the location of capture of each fish. If fish were captured within a 2-m radius of a cover type, they were assumed to be associating with it. Any cover (cumulative total of all cover available) includes piers or pilings, and total woody material (WM); total WM includes simple, complex, and vertical WM. Black bars indicate largemouth bass, and white bars indicate spotted bass.

## **Appendix**

Table A.1. Number of prey items (organized by prey group) eaten by largemouth and spotted bass at juvenile (50 – 100 mm TL) and adult (300 – 500 mm TL) life stages.

Prey	Juvenile bass						Adult bass			
	31 Jul – 08 Aug 2007		29 Apr 2008		10 Jun 2008		29 Apr – 01 May 2008		12 May – 16 May 2008	
	LMB <i>n</i> =31	SPB <i>n</i> =20	LMB <i>n</i> =17	SPB <i>n</i> =11	LMB <i>n</i> =15	SPB <i>n</i> =6	LMB <i>n</i> =45	SPB <i>n</i> =16	LMB <i>n</i> =32	SPB <i>n</i> =26
<b>Small benthic invertebrates</b>										
Hydrachnidia	3	2	1	0	0	0	0	0	0	0
Nematoda	1	0	0	0	0	1	0	0	0	0
Ostracoda	12	11	1	3	0	5	0	0	0	0
<b>Large benthic invertebrates</b>										
Odonata	1	0	0	0	0	0	0	0	0	0
Ephemeroptera	9	30	0	0	0	7	0	0	0	0
Trichoptera	0	2	0	0	0	2	0	0	0	0
Coleoptera	4	2	0	1	0	0	0	0	0	0
Ceratopogonidae	0	0	0	1	0	0	0	0	0	0
Chironimidae	35	73	167	383	24	7	0	0	0	0
<b>Planktonic invertebrates</b>										
Cladocera	32	475	185	527	768	32	0	0	0	0
Copepoda	95	8	167	366	1	1	0	0	0	0
<i>Chaoborus</i> spp.	133	2	0	0	0	0	0	0	0	0
<b>Swimmer or surface invertebrates</b>										
Hemiptera	0	0	0	0	0	0	0	0	0	0
Diptera Pupae	51	15	5	4	2	0	0	0	0	0
Collembola	0	0	3	1	0	0	0	0	0	0
Terrestrial	24	3	25	1	5	2	0	0	0	0

Table A.1 (continued)

Fish										
<i>Alosa</i>										
<i>pseudoharengus</i>	0	0	0	0	0	0	6	1	0	1
<i>Dorosoma</i>										
<i>cepedianum</i>	0	0	0	0	0	0	1	0	0	0
<i>D. petenense</i>	0	0	2	0	0	0	37	14	32	32
<i>Etheostoma</i>										
<i>olmstedi</i>	0	1	0	0	0	0	0	0	0	0
<i>Lepomis</i> spp.	0	0	0	0	0	0	5	1	6	3
<i>Micropterus</i> spp.	0	0	6	2	0	0	2	0	1	4
<i>Morone</i> spp.	0	0	0	0	0	0	0	0	1	1
Unknown fish	3	2	0	1	1	1	1	0	0	1
Stomachs with Contents	25	17	16	10	15	6	25	9	19	20
Empty Stomachs	6	3	1	1	0	0	20	7	13	6