STATEWIDE RESEARCH - FRESHWATER FISHERIES

JULY 1, 1998 THROUGH JUNE 30, 1999

ANNUAL PROGRESS REPORT
F-63

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STUDY PROGRESS REPORT

STATE: South Carolina PROJECT NUMBER: F-63

PROJECT TITLE: Fisheries Investigations in Lakes and Streams - Statewide

STUDY TITLE: Research

JOB TITLE: Development of Reservoir-Specific Largemouth Bass Management Models

Summary

During the project period July 1, 1998 - June 30, 1999 recent literature dealing with black bass management was reviewed and summarized. Spring electrofishing sampling data provided by the fisheries districts were reviewed and analyzed by reservoir, and preliminary estimates of parameters for recruitment, growth, and mortality of largemouth bass populations were extracted when sufficient data were available. Inconsistencies in otolith aging within and between districts were detected, leading to a workshop to standardize aging procedures and resolve differences in interpretation of otolith microstructure. As part of the new standard, subsamples of otoliths collected by the districts will be sent to Eastover for verification of age assignment. The standardized sampling protocol developed previously was sent to outside experts for review and comment.

Introduction

The importance of largemouth bass (*Micropterus salmoides*) to sport fishing in South Carolina is well known. A survey of freshwater anglers commissioned by the South Carolina Wildlife and Marine Resources Department (SCWMRD), predecessor of the South Carolina Department of Natural Resources (SCDNR), in 1990 found that 28% of all anglers fished for largemouth bass (Logan, 1990). Of anglers who targeted a particular species, 37% fished for

largemouth bass. According to a national survey conducted by the U.S. Department of the Interior et al. (1993), approximately 50% of resident and non-resident anglers in South Carolina fished for black bass, primarily largemouth bass, in 1991. Logan (1990) reported that 48% of survey respondents felt that SCWMRD should pay more attention to the management of largemouth bass, and significant numbers supported harvest restrictions as management options.

Considerable effort is expended annually by district fisheries biologists in South Carolina to monitor the status of largemouth bass populations in reservoirs and streams. Techniques for conducting angler creel surveys, spring electrofishing and summer/fall cove rotenone sampling were standardized to facilitate the analysis and interpretation of data. Kirk (1989) summarized a decision-making process regarding management options that could follow from evaluation of the harvest potential of largemouth bass, based on data generated from standardized surveys and sampling. However, there are no definitive guidelines that management biologists must follow when making management recommendations.

Birth, growth, and death are dynamic processes which operate continuously and interactively on populations of living organisms. Population structure, however it is measured or expressed, is the cumulative result of these processes (each actually a rate function) at any point in time. Structural indices (age structure, length structure, relative condition) provide snap-shots which help to characterize the status of a population, but rate functions (recruitment, growth, and mortality) are needed to assess the dynamics of a population.

Historical spring electrofishing in South Carolina consisted primarily of the collection of largemouth bass length and weight data. Such data was useful for the computation of two structural indices: length structure and relative condition. Inferences were often made about recruitment and mortality from length structure representations and about growth from relative

condition representations. However, rate functions can be estimated meaningfully only if the time step is known. Therefore, accurate and precise aging studies are essential elements of a sampling program.

In 1995 the Freshwater Fisheries Section of SCDNR approved a statewide management plan for black bass, including largemouth bass. Management goals were established to provide continuity and guidance to department personnel and the public, while the need for site-specific management authority was recognized. Having such guidelines would promote uniform, consistent assessments of black bass populations, and could enhance public understanding of and support for the process of managing the fishery. One goal common to all four species of black bass was to develop, maintain, and enhance the biological databases needed to make sound management decisions. Such databases can be used to define reservoir-specific management options, depending on the results of structured and objective assessment of a population.

While this agency still does not have a centralized database management system in place for freshwater fisheries, a step in that direction was taken during the first phase of this study (Bulak et al. 1998). A standardized protocol for collecting spring electrofishing data was approved and implemented, and a standardized data-entry program was distributed to each fisheries district. Data collected annually by the fisheries districts are now sent to the Fisheries Research Lab in Eastover for compilation and analysis using computer programs developed for that purpose. Current and historic data are then used to produce site-specific estimates of largemouth bass population parameters.

Accuracy in aging is extremely important in fisheries science and has critical implications for management. Age provides the time line upon which a number of rate functions, among them growth, mortality, and recruitment are based. In order to have a good understanding of the

dynamics of a population, the underlying age information must be reasonably correct. Otherwise, significant misinterpretations of data can result. This point became clear when we first started looking at largemouth bass data from Lake Thurmond. Fish had been collected for age analysis in 1995 and again in 1997. Age and length data for the two years were used to create age-length keys, which were then used to compute age-frequency distributions for the largemouth bass population of Lake Thurmond. In the absence of any significant change in the population between 1995 and 1997, the two keys should have produced comparable distributions. In fact, the age-frequency distributions were quite different, with mean length at age based on the 1997 key considerably longer than the value produced by the 1995 key. In December, 1998, we undertook an evaluation of the 1997 Lake Thurmond otolith set and discovered that the otoliths were consistently under-aged. That discovery led to an effort to standardize the otolith aging process and to establish a quality control procedure whereby ages determined by the Districts would be reviewed and verified.

The objective of the present study is to develop a quantifiable protocol for identifying and ranking management options within a system through compilation, analysis, and interpretation of existing largemouth bass population data.

Materials and Methods

An aging workshop was held on February 2, 1999. All district biologists and their assistants were invited to participate. Technicians, student interns, and others who assist biologists with otolith aging were also given the opportunity to attend. Dr. Jeff Isely of the South Carolina

Cooperative Fish and Wildlife Research Unit at Clemson University provided an overview of otolith formation and served as an expert in matters of annulus interpretation. Several district biologists brought largemouth bass otolith samples to the workshop to resolve questions about interpretation of annuli. Biologists were subsequently asked to submit otoliths collected during sampling in 1997 and/or 1998 to Eastover for review and analysis.

Spring electrofishing data collected in 1998 in accordance with the South Carolina Largemouth Bass Sampling Plan (SSP) were obtained from the districts and compiled and analyzed using programs developed previously. Metrics for recruitment, growth, and mortality were recalculated for the Lake Thurmond largemouth bass population based on revised age assignments using a combined 1997/1998 age-length key. The key was applied to data collected during spring electrofishing for five years from 1994 through 1998, though the standardized sampling protocol was not applied until 1997. Catch per unit effort (CPUE) of age-1 fish was used as an index of recruitment. CPUE was also computed in terms of length categories, using the five-cell model of Gabelhouse (1984). Stock density indicies (PSD, RSD-15, and RSD-20) were computed for each reservoir using the traditional method of Gabelhouse (1984) as described by Anderson and Neumann (1996). Annual mortality was estimated for fish in age classes 2 to 4. Von Bertalanffy growth parameters were estimated for separately for 1997 and 1998 using revised aging data.

The South Carolina Largemouth Bass Sampling Plan was sent for review and comment to five individuals with expertise designing and conducting electrofishing surveys of fish populations. Two of the reviewers have academic backgrounds in fisheries research and three have backgrounds in fisheries management with state natural resource agencies.

Results and Discussion

Largemouth bass otoliths from 4 reservoirs in 1997 and 9 reservoirs in 1998 were obtained from Districts 1, 2, 3, 5 and 6. In addition, several otoliths from Lake Wateree largemouth bass collected in 1998 were evaluated jointly with District 4 personnel to confirm that District personnel were interpreting annuli and marginal growth correctly and assigning appropriate ages to otoliths. Most Districts (2, 3, and 4) read otoliths whole and stored them dry in vials or coin envelopes. District 2 also sectioned otoliths from Lake Russell fish in 1998 because the whole view was "poor". Districts 1 and 5 sectioned all otoliths before reading them. District 5 stored the second otolith from each pair in alcohol. It was observed that otoliths stored in vials were generally in better condition than those stored in coin envelopes; many of the latter were broken into two or more pieces. Otoliths stored in alcohol were less readable than those stored dry. In fact, none of a subsample of whole otoliths from District 5 could be read reliably after being stored for a year in alcohol. The numbers of otoliths aged by district personnel and by Eastover project personnel are summarized in Table 1 by reservoir and year. Otoliths from Lake Brown largemouth bass collected in 1997 were read and reported separately (Appendix A).

Agreement with ages determined by District personnel varied greatly between Districts and sometimes within Districts between reservoirs. The greatest level of agreement occurred with reads made by District 5 personnel on 100 sectioned largemouth bass otoliths collected from lakes Marion and Moultrie. Though more than half of the otoliths were from fish age 5 or older, we disagreed with the age assignment of only one fish, a 10 year old fish District personnel called 9. In other Districts, initial agreement ranged from a low 14% to a high of 95%. Agreement usually improved when only fish younger than age 5 were included in the comparison, but not always (Table 1).

Usually, low levels of agreement resulted from a systematic difference in the way the margin of the otolith was characterized. Largemouth bass collected in the spring can be problematic to age with certainty because annulus formation occurs during that time of year. The biologist must decide if marginal growth past the last clearly defined annulus is growth that occurred after the annulus was formed earlier in the year in which the fish was collected, or if it represents a full year of growth since the annulus was formed the previous year. Such decisions become particularly difficult the later in the spring the collection is made, because the chances increase that an annulus formed early in the year of collection could be mis-characterized as having been formed the previous year. For the most part, when spring electrofishing collections are made at water temperatures between 15 and 20°C, in accordance with the South Carolina Standardized Sampling Plan, annulus formation for that year can be assumed not to have occurred, and the age of the fish is equal to the number of formed annuli plus one. If the additional annulus for the margin is not included, the fish will be under-aged. Low levels of agreement resulting from systematic under-aging are being resolved in Districts 2 (all reservoirs) and 3 (Lake Murray) now that the nature of the problem is understood.

Table 1. Percent agreement between fisheries districts and Eastover in age assignments of largemouth bass otoliths sent to Eastover for age verification, by reservoir and year of collection. Percent agreement is given for all ages of fish and for fish less than 5 years old. Lake Thurmond otoliths were read once by district personnel, then re-aged using different criteria for interpreting the margin. Notes indicate whether otoliths were read whole or sectioned, and whether whole otoliths were stored dry or in alcohol.

Reservoir	Year	# Aged by Dist.	# Aged by Eastover	% Agreement		Notes
				All Ages	Age<5	
Blalock	1997	93	39	90	96	Sectioned
Greenwood	1998	146	17	94	91	Whole (dry)
Keowee	1998	204	51	100	100	Sectioned
Lyman	1998	82	42	95	100	Sectioned
Marion	1998	156	25	100	100	Sectioned; whole available (in alcohol)
Moultrie	1998	117	25	96	100	Sectioned; whole available (in alcohol)
Murray	1998	124	79	77	76	Whole (dry)
Russell	1998	83	56	27	28	Both whole (dry) & sectioned
Secession	1997	70	18	28	31	Whole (dry)
Secession	1998	55	14	14	25	Whole (dry)
Thurmond	1997	114	102	24[a] 97[b]	24[a] 100[b]	Whole (dry)
Thurmond	1998	121	64	17[a] 89[b]	20[a] 89[b]	Whole (dry)
Wateree	1998	286	-[c]	?	?	Whole (dry)
Wylie	1994	0	28[d]	-	-	Whole (dry)

[a] Percent agreement based on initial aging by District personnel
[b] Percent agreement after re-aging by District personnel
[c] An unknown number of questionable-age otoliths were jointly read by District 4 and Eastover

personnel.
[d] Otoliths collected by Eastover personnel while assisting Duke Power biologists in 1994.

Spring electrofishing data for 1998 were received from Districts 1, 2, 3, 4, 5 and 6, though data from District 1 did not arrive in time to process for this report. District 6 sampled Lake Brown using an older methodology; those data were not processed because the dataset did not include all variables required. Selected population parameters are summarized in Tables 2a-d for the five major reservoirs for which data were available. Comparisons between reservoirs in different districts should be performed with caution. However, the larger mean length at age suggests that largemouth bass grew faster in lakes Marion and Moultrie through age-3, after which the early growth advantage of lower coastal plain reservoirs disappeared (Table 2a). Lake Wateree yielded the highest catch per unit effort overall (Table 2b), and higher catches of stock, quality, and preferred length categories of fish than the other four reservoirs (Table 2c). Stock density indices for lakes Thurmond, Greenwood, and Wateree are within the ranges suggested by Willis et al. (1993) for balanced largemouth bass populations (Table 2d). Stock density indices for lakes Marion and Moultrie are closer to the ranges Willis et al. (1993) suggested as appropriate for a "big bass" fishery, though they may be artificially high due to a recruitment failure of the 1996 year class (Age-2 fish in Table 2b), at least in part.

Population parameters for Lake Thurmond are summarized for five years of data in Table 3a-d. Recruitment of largemouth bass in Lake Thurmond was variable and appeared to decline from 1994 to 1998 (Table 3a). Catch per unit effort (CPUE) of age-1 fish ranged from 16.2 (1998) to 39.6 (1994) (Table 3b), with a mean of 28.9 and a standard deviation of 10.28. Annual mortality was estimated as 65% (R^2=0.94). In 1997, asymptotic length L_∞ was 469.1 with a growth coefficient *k* of 0.46 (fish <175 mm TL assumed to be age-1 and t_0 held constant at -0.024). In 1998, using the same assumptions, L_∞ was 510.2 and *k* was 0.42.

Table 2a-d. Comparisons of largemouth bass population parameters in selected South Carolina reservoirs, 1998. Age-related parameters were computed from age frequency tables based on corrected 1998 age-length keys.

2a. Mean total length (variance) in cm, by age.

Age	Thurmond	Greenwood	Wateree	Marion	Moultrie
1	16.4 (1.82)	15.9 (1.37)	18.4 (1.62)	15.5 (3.35)	17.7 (4.82)
2	29.0 (1.40)	28.6 (1.75)	28.6 (1.61)	32.8 (1.93)	32.5 (2.81)
3	34.5 (1.86)	35.7 (0.89)	36.3 (1.58)	39.5 (1.70)	38.3 (1.07)
4	39.3 (1.19)	41.7 (2.16)	40.0 (0.69)	40.9 (1.88)	40.7 (2.21)

2b. Catch per unit effort (no./hr) by age. Total includes fish older than 5.

Age	Thurmond	Greenwood	Wateree	Marion	Moultrie
1	16.4	9.5	10.6	19.7	10.2
2	19.3	17.1	22.3	8.2	7.5
3	7.3	7.6	18.8	3.0	5.9
4	3.1	2.0	7.4	7.0	6.3
5	3.1	3.6	4.7	6.5	6.3
Total	50.0	43.6	73.4	57.8	50.0

2c. Catch per unit effort (no./hr) by length category. Range of TL (mm) for each category is in parentheses.

Length Category	Thur	Greenwood	Wateree	Marion	Moultrie
Prestock (<200)	14.7	9.1	6.9	15.2	5.4
Stock (200 - 299)	15.3	11.1	20.4	6.5	6.1
Quality (300 - 379)	12.7	13.5	19.8	7.7	10.3
Preferred (380 -	6.9	8.4	25.3	22.7	22.9
Memorable (510 -	0.4	1.6	1.2	5.7	5.1
Trophy (≥630)	0.0	0.0	0.0	0.0	0.2

2d. Stock density indices.

Index	Thurmond	Greenwood	Wateree	Marion	Moultrie
PSD	57	68	69	85	86
RSD-15	21	29	40	67	63
RSD-20	1	5	2	13	12

Table 3a-d. Comparisons of largemouth bass population parameters in Lake Thurmond by year, 1994-1998. Age-related parameters were computed from age frequency tables based on a combined, corrected 1997 and 1998 age-length key. Standardized sampling began in 1997.

3a. Mean total length (variance) in cm, by age.

Age	1994	1995	1996	1997	1998
1	18.0 (1.36)	17.1 (1.77)	15.6 (2.52)	18.0 (1.48)	16.3 (1.79)
2	27.2 (2.14)	27.9 (1.17)	27.1 (1.93)	27.1 (1.78)	28.7 (1.29)
3	35.2 (1.51)	33.8 (1.56)	34.5 (0.90)	34.8 (1.51)	34.5 (1.49)
4	39.2 (1.08)	38.9 (1.05)	38.2 (1.06)	39.0 (1.05)	39.4 (1.32)

3b. Catch per unit effort (no./hr) by age. Total includes fish older than 5.

Age	1994	1995	1996	1997	1998
1	39.6	32.1	36.5	20.0	16.2
2	29.2	52.7	26.1	26.3	18.7
3	12.4	12.0	9.2	10.3	7.6
4	7.1	4.5	3.7	5.1	3.5
5	3.2	2.4	2.4	2.6	3.1
Total	92.8	106.0	78.5	64.8	50.0

3c. Catch per unit effort (no./hr) by length category. Range of TL (mm) for each category is in parentheses.

Length Category	1994	1995	1996	1997	1998
Prestock (<200)	29.4	25.7	30.5	14.6	14.7
Stock (200 - 299)	33.2	50.1	26.8	25.4	15.3
Quality (300 - 379)	20.0	23.1	16.3	16.9	12.7
Preferred (380 - 509)	9.7	6.5	4.4	7.4	6.9
Memorable (510 - 629)	0.5	0.5	0.5	0.5	0.4
Trophy (≥630)	0.0	0.0	0.0	0.0	0.0

3d. Stock density indices (from Gabelhouse, 1984).

Index	1994	1995	1996	1997	1998
PSD	48	38	44	49	57
RSD-15	16	9	10	16	21
RSD-20	1	1	1	1	1

Comments on the South Carolina Largemouth Bass Sampling Plan were received from all five reviewers and were favorable, though most offered suggestions for improving the plan to better meet its stated objectives. All liked the objective of accounting for spatial heterogeneity in reservoirs. While individual reviewers mentioned specific concerns about the plan, none of them had the same concerns. One reviewer thought that several of the instructions in the plan needed to be clarified. Clarifying those instructions could resolve some concerns expressed by other reviewers. The suggestions will be taken into consideration when the sampling plan is finalized.

Recommendations

1. All largemouth bass otoliths collected during spring electrofishing should be stored dry in vials. Otoliths should be read as whole mounts by District personnel. Otoliths aged as >3 years should be sectioned and mounted, then reread.
2. All largemouth bass otoliths should then be sent to Eastover, where 25% will be randomly subsampled for age verification.
3. Agreement of 90% or better between Eastover and District age assignments will be considered satisfactory for fish less than 5 years old. If agreement is less than 90%, an effort will be made to resolve differences. If differences cannot be resolved, those fish will be omitted from analyses involving age.
4. Finalize revision of South Carolina Largemouth Bass Sampling Plan, incorporating recommendations of outside reviewers where applicable.
5. Develop metrics for all reservoirs for which sufficient data are available and statistically assess spatial heterogeneity in population parameters.

6. Once metric compilation is completed, develop statewide management recommendations using site specific modeling.

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Appendix A

Lake Brown Largemouth Bass Otolith Aging Evaluation

Lake Brown Largemouth Bass Otolith Aging Evaluation

Methods

Otoliths from largemouth bass collected by Fisheries District 6 personnel from Lake Brown in Barnwell County, SC, during spring electrofishing in 1997 were examined to determine age of fish. The otoliths were stored dry in coin envelopes. Many were broken into two or more pieces, either during extraction or in storage. Prior to aging, they were cleaned by immersing them in water and teasing off adhered tissue. Broken pieces were aligned in a blackened watch glass and examined under a dissecting microscope to determine how much of each otolith remained and whether the pieces could be used to determine age of the fish. Whole (or pieced-together) otoliths were read independently by two experienced readers and assigned an age when both readers agreed. If the readers disagreed, questionable otoliths were read independently as whole mounts a second time. The right otolith (when available) was then embedded in polymerized resin, sectioned transversely, mounted and polished following procedures in Secor, Dean and Laban (1991) in an effort to assign a correct age to the fish. Three undisputed otoliths were also sectioned to verify ages determined from whole otoliths. Sectioned otoliths were read twice by a single reader.

Once ages were determined, fish were assigned to year classes to facilitate analysis of length data. An age-length key was developed and the age-frequency distribution for the population was extrapolated. Mean total length of each year class was calculated in order to estimate growth rate of the Lake Brown largemouth bass population.

Results and Discussion

Otoliths from 30 fish were prepared and read as whole mounts. Ages were assigned to 21 fish (Table 1). Nine disputed otoliths were read a second time, and the two readers agreed on the

ages of eight fish. However, only four sectioned-otolith ages corresponded with those from whole-mounts. The five otoliths whose ages couldn't be resolved were omitted from further consideration. Sectioned and whole-mount ages were identical for the three undisputed otoliths (Table 1). Year classes were assigned to the 25 fish whose otolith ages were resolved. No age-1 fish were included in the sample. Largemouth bass less than 175 mm TL are not routinely aged under the standardized spring electrofishing protocol because they are assumed to be age-1. However, one fish in the present study measuring 171 mm TL was found to be age-2. All smaller fish were assumed to be age-1, an assumption which may or may not be correct. Otolith-aged fish ranged from two (1995 year class) to six years old (1991 year class)(Table 1).

The age-length key (Table 2) and age-frequency distribution (Table 3) indicate that some length groups were not subsampled according to the standardized sampling protocol, which for small reservoirs required otoliths from 10 fish per 25-mm group between 175 and 474 mm TL, when possible. The age-frequency distribution may therefore be biased due to the small sample sizes used to develop the age-length key. Nevertheless, mean total lengths, with standard deviations, were computed from the age-frequency table for age-1 through 4 fish and are given in Table 4. Length-at-age values obtained in the same manner for largemouth bass populations in five major reservoirs in South Carolina are included for comparison. Largemouth bass in Lake Brown grew more slowly through age-2 than fish in larger reservoirs, but after age-2 they closed the gap. This suggests a possible food bottleneck for younger bass in Lake Brown, which may be overcome as they grow larger.

The results of this relatively small sample suggest that otoliths which are easily aged as

Table 1. Total length, weight, sex, age and year class of fish collected for ageing from Lake Brown during spring electrofishing sampling in 1997. Age was determined independently from whole otoliths by two readers. If they disagreed, otoliths were read whole a second time by the same two readers, then sectioned and read by a single reader. Three undisputed otoliths were also sectioned and read. Year class was assigned

when both readers agreed on whole-otolith age and sectioned-otolith age corresponded.

				Otolith Age						
				Whole				Sectioned		
				Reader 1		Reader 2		Reader 1		
Fish ID	TL (mm)	Wt (g)	Sex	Read 1	Read 2	Read 1	Read 2	Read 1	Read 2	Year Class
1	299	373	M	2	3	3	3	2	2	
2	381	691	M	4		4				1993
3	389	837	M	4	4	3	4	5	5	
4	212	104	I	2		2				1995
5	345	497	M	4	4	3	4	4	4	1993
6	409	979	M	6	6	5	6	5	5	
7	344	528	M	3		3				1994
8	394	867	M	6	5	5	6	6	6	
9	400	882	F	4		4				1993
10	376	753	M	4		4				1993
11	295	283	F	3	3	2	3	3	3	1994
12	335	436	M	4		4				1993
13	323	451	F	3	2	2	2	3	2	1995
14	215	111	I	2		2				1995
15	195	74	I	2		2				1995
16	220	134	I	2		2				1995
17	171	62	I	2		2				1995
18	350	573	M	4		4		4	4	1993
19	292	312	F	2		2				1995
20	260	209	F	2		2				1995
21	347	546	M	3		3				1994
22	363	636	M	4	4	3	4	4	4	1993
23	274	262	M	2		2				1995
24	405	968	M	6	6	5	6	6	6	1991
25	403	898	F	3		3				1994
26	265	264	I	2		2				1995
27	226	139	I	2		2		2	2	1995
28	203	103	I	2		2				1995
29	190	68	I	2		2				1995
30	187	80	?	2		2		2	2	1995

Table 2. Age-length key for largemouth bass collected from Lake Brown during spring electrofishing in 1997. Number of fish aged by length group.

CM Grp (midpoint)	No. Aged	Age 0	1	2	3	4	5	6
16.2	1			1				
18.7	3			3				
21.2	4			4				
23.7	1			1				
26.2	3			3				
28.7	2			1	1			
31.2	1			1				
33.7	4				2	2		
36.2	2					2		
38.7	2					2		
41.2	3				1	1		1
Total	26	0	0	14	4	7	0	1

Table 3. Age-frequency distribution of largemouth bass collected from Lake Brown during spring electrofishing in 1997, based on age-length key.

CM Grp (midpoint)	No. Sampled	Age 0	1	2	3	4	5	6
6.2	1		1					
8.7	3		3					
11.2	7		7					
13.7	3		3					
16.2	2		1	1				
18.7	3			3				
21.2	8			8				
23.7	1			1				
26.2	3			3				
28.7	3			2	2			
31.2	1			1				
33.7	10				5	5		
36.2	7					7		
38.7	12					12		
41.2	13				4	4		4
43.7	10							
46.2	6							
48.7	2							
51.2	4							
53.7	4							
56.2	1							
58.7	1							
61.2	2							
Total	107	0	16	20	11	28	0	4

Table 4. Mean total length (cm) with standard deviation, by age group, of largemouth bass populations sampled in six South Carolina reservoirs during spring 1997. Means were calculated from age-frequency

distributions derived from age-length keys for each population. Fish less than 175 mm TL are normally assumed to be age-1 when computing an age-frequency distribution. However, in Lake Brown, one fish measuring 171 mm was found to be age-2.

Age	Reservoir					
	Brown	Greenwood	Marion	Monticello	Moultrie	Thurmond
1	11.2 (2.50)	17.8 (4.28)	16.3 (4.77)	25.0 (2.89)	20.5 (3.73)	18.0 (3.07)
2	22.8 (4.01)	29.8 (3.20)	32.0 (2.57)	35.5 (3.45)	32.6 (4.64)	27.3 (3.12)
3	35.5 (4.89)	35.5 (3.27)	37.7 (2.47)	40.7 (3.60)	36.5 (2.90)	34.7 (2.82)
4	37.5 (2.40)	40.1 (3.12)	40.6 (2.73)	45.4 (2.46)	40.4 (3.16)	38.8 (2.79)

whole mounts hold up well when sectioned, while those which are difficult to age as whole mounts can be just as troublesome after sectioning. Furthermore, multiple reads on difficult otoliths may provide a false sense of obtaining an accurate age estimate through consensus.

Recommendations

1) Continue collecting otoliths from Lake Brown largemouth bass during spring electrofishing.

2) Follow the standardized sampling protocol for age/growth to collect an adequate, representative sample of otoliths.

3) Rinse and place otoliths in vials rather than in scale envelopes in order to minimize breakage.

4) To characterize growth patterns accurately in populations with known or suspected growth bottlenecks, there should be no lower limit on size of fish subsampled for aging. Extrapolate additional 25-mm size groups downward from those set by the standardized sampling plan.

5) Consider implementing management options aimed at relieving the “stunting” of age-1 and age-2 fish in the reservoir.

Literature Cited

Secor, D. H., J. M. Dean, and E. H. Laban. 1991. Manual for otolith removal and preparation for microstructural examination. Electric Power Research Institute. Technical Publication 1991-01, Belle W. Baruch Institute of Marine Biology and Coastal Research, University of South Carolina, Columbia.

JOB PROGRESS REPORT

STATE: South Carolina PROJECT NUMBER: Sea Grant

PROJECT TITLE: Fisheries Investigations in Lakes and Streams - Statewide

STUDY: Research

JOB TITLE: Inventory of the fish community of tidal freshwater wetlands of Cooper River

Introduction

An overall goal of this sampling effort is to assess fish community structure as a function of major habitat type in Cooper River ricefields. To achieve this goal, three factors have been considered. First, species differences in the fish community will be taken into account. The species composition ranges from resident fishes, which are typically small, such as bluefin killifish, *Lucania goodei*, and least killifish, *Heterandria formosa*, to more mobile fishes, which are typically larger, such as largemouth bass, *Micropterus salmoides*, and striped mullet, *Mugil cephalus*. Second, differences in the types of vegetation that occur in the ricefield will have to be considered, especially for the resident fishes that live in and among that vegetation. The open water (OW) vegetation includes subitidal, submergent plants that are buoyant and whose stems are very flexible. The vegetation type LEP is comprised of subtidal, emergent plants, which have rigid stems and form a dense mat over the water's surface. The intertidal, emergent vegetation, also know as ITEM, contains plants having stems and leaves extending above the surface of the water. Finally, logistics will have to be considered as the boat and gear selected will have to be used in some confined areas where water depth can be ~ 2 meters (m) at high tide and less than 0.2 m at low tide.

Four study sites were selected along the Cooper River (Figure 1), and our sampling efforts focused on the Dean Hall (DH) and Bonneau Ferry (BF) ricefields. The DH ricefield is at river kilometer 45.9 (= river mile 28.5, Williams et al. 1984), which is ~ 1.6 kilometers (km) below The Tee, river kilometer 48.1 (= river mile 29.85, Williams et al. 1984). The Tee is where the mouth of the East Branch (33.04′07″ North latitude, 79.55′28″ West longitude) of the Cooper River intersects the West Branch. Approximately 3 km upriver from Tee on the East Branch is the BF ricefield. For our sampling purposes, the power lines crossing the East Branch arbitrarily mark the most western edge of the BF ricefield, whereas, French Quarter Creek is opposite approximately the most eastern edge.

Our sampling efforts focused on DH and BF because of their geographic proximity to one another and their contrasting vegetative cover (Table 1). Open water vegetation comprises the largest cover class in BF, and this includes plants such as coontail (*Ceratophyllum demersum*), fanwort (*Cabomba caroliniana*), Egeria (*Egeria densa*), and Hydrilla (*Hydrilla verticillata*). Based on personal observation, an increasingly important coverage class in BF is LEP, which includes Primroses (*Ludwigia* spp.), water hyacinth (*Eichhornia crassipes*), and smartweed (*Polygonum* spp.). In contrast, DH is dominated by the ITEM vegetation. This includes pickerel weed (*Pontederia cordata*), arum (*Peltandra virginica*), alligatorweed (*Alternanthera philoxeroides*), and giant cutgrass (*Zizaniopsis miliacea*).

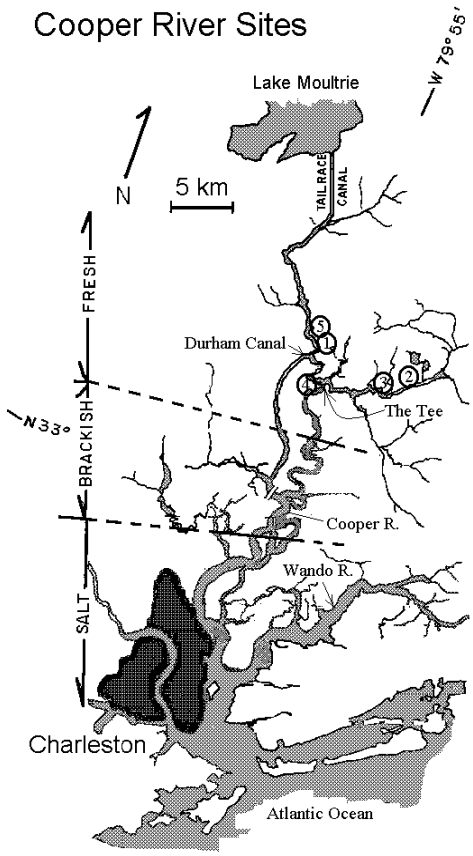

Figure 1. Landmarks

and locations of study sites along the Cooper River. 1=Mulberrry Field; 2=Bossis Field;

3=Bonneau Ferry Field; 4=Dean Hall Field; 5=USGS gauge at Pimlico

Table 1. Cover classifications (% of total area) of study sites from NAPP photos taken near high tide during winter in 1973 and 1994.

Cover Class	Bossis Plantation area= 74 ha		Bonneau Ferry area= 96 ha		Mulberry Field area= 137 ha		Dean Hall area= 63 ha	
	1973	1994	1973	1994	1973	1994	1973	1994
OW	33	2	77	43	88	68	17	2
LEP	6	16	0	9	0	5	0	1
ITEM	42	53	2	20	6	6	39	73
UC	19	29	21	26	3	21	44	24

Cover classes are OW (open water), LEP (*Ludwigia*, *Eichornia*, and *Polygonum*), ITEM (intertidal

emergent mix), and UC (unclassified). The OW habitat contains submerged aquatics (*Cabomba*, *Ceratophyllum* and *Egeria*). The ITEM habitat is dominated by *Pontederia*, *Peltandra*, *Alternanthera*, and *Zizaniopsis*.

Materials and Methods

[Evaluation of Gear for Sampling Resident Fishes]

A literature review was done to evaluate the different types of gear used to sample fishes in vegetated habitats (Kushlan 1981, Freeman et al. 1984, Zimmerman et al. 1984, Kilgore et al. 1989, Miller et al. 1990, Chick et al. 1992, Jordan et al. 1997, Rozas and Minello 1997). Based on that review, we chose to evaluate a 1-meter^2 mesh throw trap and a 1-meter^2 aluminum drop trap built to sample the resident fishes. Field trips were made to collect preliminary data and to determine the accessibility of sampling different sites within a ricefield. Those data and information were used to design the method to be implemented in year 2 of the study.

[Electrofishing]

During this first year of the study a preliminary electrofishing survey was done to determine the effects of tide stage and ricefield on the number of species and number of individual fish collected during the electrofishing of 100 meter transects. Four transects were established in each ricefield. The results from those surveys were used to design the electrofishing survey to be implemented in year 2 of the study. Additionally, we explored the DH and BF ricefields to try and determine what locations and conditions would allow us to block the channels in each ricefield so we could compare the effectiveness of our electrofishing efforts.

[Rotenone Sampling]

Rotenone samples were taken three times, twice in DH and once in BF, to assess if these samples differed substantially in species composition compared to drop trap sampling and electrofishing. On September 14, 1998, rotenone was applied to a section of a small channel in

the downriver area of DH. The dimensions of the section where the rotenone was applied were ~25 m (l) x ~3.7 m (w) x ~0.82 m (d). Seines nets were stretched across the ends of this section of channel, and rotenone was applied to the enclosed area at low, slack tide. Potassium permanganate was applied to the surrounding water to detoxify any rotenone that spread beyond the nets. On October 4, 1998, rotenone was applied in the same manner to a section of a larger channel in the central area of DH. The dimensions of the enclosed section were ~50 m (l) x ~4.0 m (w) x ~1.2 m (d). Also, on that same date, rotenone was applied to an isolated patch of primrose in the upriver area of BF. The patch of primrose was approximately circular and occupied an area of ~215 m^2 with a depth of ~1.35 m. Block nets were placed around the circumference of the patch of primrose and rotenone was applied to the enclosed area. The application was done at a high, slack tide and potassium permanganate was also applied to the surrounding water. Fish were immediately collected after each rotenone application, identified to species and enumerated.

[Purse Seine]

A purse seine was manufactured for this study by Nylon Net Company. The manufacturer did not adhere to the designs sent with the order. Consequently, we had to modify the purse seine. We found a flaw with the purse seine when we used it in an OW ricefield in the Cooper River. While the purse seine can be deployed to enclose an area, the hauling in of the seine to collect fish produces a gap whereby fish could escape. Mark Homer, who deployed such a purse seine in the Homer and Williams 1985 study, said that he was aware that such a gap occurred. We considered additional modifications to the purse seine that might eliminate this problem, but concluded that they would not be worth the money and effort so we opted not to use the purse seine in this study.

Table 2. List of species and method by which they were collected in the Dean Hall and Bonneau Ferry ricefields. The sampling period includes preliminary samples taken from July 1998 to February 1999 and routine drop trap sampling, March and May,1999, and routine electrofishing, April and June 1999.

		Collection Method		
Scientific name	Common Name	Drop Trap Sampling	Electrofishing	
Amiidae				
Amia calva	Bowfin		X	
Anguillidae				
Anguilla rostrata	American eel	X	X	
Aphredoderidae				
Aphredoderus sayanus	Pirate perch	X	X	
Atherinidae				
Labidesthes sicculus	Brook silverside			X
Menidia beryllina	Inland silverside		X	
Belonidae				
Strongylura marina	Atlantic needlefish		X	
Bothidae				
Paralichthys lethostigma	Southern flounder		X	
Centrarchidae				
Lepomis punctatus	Spotted sunfish	X	X	
Lepomis auritus	Redbreast sunfish	X	X	
Lepomis microlophus	Redear sunfish	X	X	
Lepomis macrochirus	Bluegill		X	
Enneacanthus glorisus	Bluespotted sunfish	X	X	
Micropterus salmoides	Largemouth bass	X	X	

Table 2. (Continued)

Scientific name	Common Name	Collection Method: Drop Trap Sampling	Collection Method: Electrofishing
Clupeidae			
Dorosoma cepedianum	Gizzard shad		X
Cyprinidae			
Notemigonus crysoleucas	Golden shiner		X
Notropis spp.*	Shiner		X
Eleotridae			
Dormitator maculatus	Fat sleeper	X	X
*Eleotris pisonis**	Spinycheek sleeper		X
Esocidae			
Esox americanus	Redfin pickerel	X	X
Esox niger	Chain pickerel	X	
Fundulidae			
Lucania goodei	Bluefin killifish	X	X
Lucania parva	Rainwater killifish	X	X
Fundulus heteroclitus	Mummichog	X	
Fundulus confluentus	Marsh killifish	X	
Fundulus chrysotus	Golden topminnow	X	
Gerreidae			
Eucinostomus argenteus	Spotfin mojarra		X
Gobiidae			
Gobionellus shufeldti	Freshwater goby	X	X
Ictaluridae			
Noturus gyrinus	Tadpole madtom	X	

Ameiurus catus	White catfish	X	X

Table 2. (Continued)

Scientific name	Common Name	Collection Method: Drop Trap Sampling	Collection Method: Electrofishing
Ameiurus natalis	Yellow bullhead		X
Ictalurus furcatus	Blue catfish		X
Lepisosteidae			
Lepisosteus osseus	Longnose gar	X	X
Moronidae			
Morone americana	White perch		X
Mugilidae			
Mugil cephalus	Striped mullet		X
Ophichthidae			
Myrophis punctatus[*]	Speckled worm eel	X	
Poeciliidae			
Gambusia holbrooki	Mosquitofish	X	X
Heterandria formosa	Least killifish	X	X
Poecilia latipinna	Sailfin molly	X	
Soleidae			
Trinectes maculatus	Hogchoker	X	

[*] Indicates a tentative species identification, which needs to be verified by Mr. Fred C. Rohde of the North Carolina Division of Marine Fisheries.

Table 3. Absolute and relative abundance of fishes collected in the Dean Hall and Bonneau Ferry ricefields in the fall of 1998 by rotenone application. The volume of water sampled is indicated in parentheses.

Scientific name	Species Code	DH 09/14/98 (~76 m^3) n	%	DH 10/04/98 (~240 m^3) n	%	BF 10/04/98 (~291 m^3) n	%
Anguillidae							
Anguilla rostrata	AEL	12	5.7	24	6.3	8	1.6
Aphredoderidae							
Aphredoderus sayanus	PIP	0	0	2	0.5	0	0
Atherinidae							
Menidia beryllina	ILS	2	1.0	0	0	1	0.2
Bothidae							
Paralichthys lethostigma	SFL	1	0.5	0	0	0	0
Centrarchidae							
Lepomis punctatus	SOS	19	9.1	66	17.2	4	0.8
Lepomis auritus	RBS	27	13	17	4.4	0	0
Lepomis microlophus	RES	1	0.5	9	2.3	0	0
Lepomis macrochirus	BLG	0	0	6	1.6	0	0
Enneacanthus glorisus	BLS	0	0	1	0.3	0	0
Micropterus salmoides	LMB	3	1.4	11	2.9	0	0
Cyprinidae							
Notemigonus crysoleucas	GLS	0	0	7	1.8	0	0
Esocidae							
Esox americanus	RFP	1	0.5	0	0	0	0
Esox niger	CHP	2	1.0	0	0	0	0
Fundulidae							

Lucania goodei	BFK	0	0	20	5.2	13	2.5

Table 3. (Continued)

Scientific name	Species Code	DH 09/14/98 (~76 m^3) n	%	DH 10/04/98 (~240 m^3) n	%	BF 10/04/98 (~291 m^3) n	%
Lucania parva	RWK	0	0	72	18.8	19	3.7
Fundulus heteroclitus	MMC	0	0	4	1.0	0	0
Gobiidae							
Gobionellus shufeldti	FWG	68	32	37	9.7	7	1.4
Ictaluridae							
Noturus gyrinus	TPM	3	1.4	3	0.8	0	0
Ameiurus catus	WCF	0	0	31	8.1	0	0
Poeciliidae							
Gambusia holbrooki	MSQ	69	33	67	17.5	287	56.0
Heterandria formosa	LSK	1	0.5	5	1.3	173	34.0
Poecilia latipinna	SFM	0	0	0	0	1	0.2
Soleidae							
Trinectes maculatus	HCK	0	0	1	0.3	2	0.4
Totals		209		383		515	

Results

[Evaluation of Gear for Sampling Resident Fishes]

We sampled the small marsh fish in the different types of vegetation using a 1 meter^2 mesh throw trap and a 1 meter^2 aluminum drop trap. Only the 1 meter^2 aluminum drop trap was heavy enough to sample fish effectively in all 3 vegetative types. Three modifications were made in deploying the aluminum drop trap to make sampling easier and more effective. First, we modified a john boat so the trap could be released from a boom. Second, we used a pruning saw to cut through the vegetation enclosed within the trap, allowing us to push the trap further into the substrate. Finally, we used a bar seine to obtain cleaner samples, that is they contained less detritus, that we could process more quickly. The bar seine had the dimensions that fit the interior dimensions of the drop trap.

Twenty-four species of fish have been collected using the drop trap (Table 2). During late January and late February 1999, a total of 38 drop samples were collected from BF and DH ricefields to collect preliminary data. Twelve drop traps were taken in OW vegetation, 15 in LEP vegetation, and 11 in ITEM vegetation. Two topminnow species, mosquitofish *Gambusia holbrooki* and least killifish, *Heterandria formosa*, were numerically dominant, which is consistent with the findings from other Southeastern wetland studies. On average, the highest density of fish, number of fish per meter^2, was greatest in the LEP vegetation, 71 fish m^{-2} (standard deviation, s.d. = 146.3). The averages in the OW and ITEM vegetation were 25 (s.d. = 49.5) and 18 fish (s.d. = 43.7) m^{-2}, respectively.

A final survey design was developed from our experience in collecting that preliminary data. The drop trap data will be used to answer two questions: 1. Do the DH and BF ricefields

differ in the abundance and biomass of fish? 2. Do the three different types of vegetation differ in the abundance and biomass of fish? Fishes will be collected using the drop trap and bar seine every other month. The drop trap is a 1 cubic meter aluminum box that has no top or bottom to it. When released from the boom on a john boat it penetrates the dense vegetation in the ricefields and forms a tight seal with the substrate. Water depth is measured within the trap, and the vegetation is removed from the interior of the trap, picked through to remove any fishes, drained, and then weighed. Fishes are then removed with a bar seine. The fishes are then preserved, and in the laboratory the species are identified, enumerated, and dried. The drop trap provides us with a quantitative sample of the abundance and biomass of the fishes in the ricefields.

Because the tides restrict accessibility within a ricefield, each ricefield is divided into 3 blocks, consisting of an upriver block, central block, and down river block. Within a ricefield, the sampling for fishes will be done over a three days period, one block per day. Based on our preliminary data on fish density among the vegetation types, a stratified survey design is warranted. Within a block within a ricefield, 3 drop traps will be taken in OW vegetation, 5 will be taken in LEP vegetation, and 2 will be taken in the ITEM vegetation. On a given day, the drop traps taken among the vegetation types within a block within a ricefield constitute subsamples (Brown and Austen 1996). To avoid pseudoreplication (Hurlbert 1984), we plan to average the response variables, fish density and fish biomass, from the drop trap subsamples taken within the vegetation types. The data from survey will be analyzed by analysis of variance (ANOVA) design that will be a randomized blocks and factorial design. The factors in this design include block (3 levels), rice field (2 levels), and vegetation type (3 levels). We will test for the main effects of block, ricefield and vegetation type and for the interaction of rice field by vegetation type on fish density and fish biomass.

[Electrofishing]

Larger and more mobile species of fish were collected using an electrofishing boat (Table 2). The effects of ricefield and tide stage on the total number of fish species and total number of individuals collected by electrofishing were examined in a preliminary survey that used 4 permanent transects in each rice field. Each transect was 100 m long and was sampled during four tide stages. The first tide stage (TS1) was +2 to +4 hours above low tide, TS2 was +4 hours above low to high tide, TS3 was from high tide to -4 hours before low tide, and TS4 was -4 to -2 hours before low tide. The maximum number of species collected during a survey was 12, and the minimum was 1. Striped mullet, *Mugil cephalus*, and largemouth bass, *Micropterus salmoides*, were two of the species collected. Based on a two-way analysis of variance (ANOVA), the total number of species collected differed significantly ($F_{1,24}$ =6.59, P=0.017) between the BF and DH rice fields. On average, seven species were collected in DH, whereas only four species were collected in BF. The effect of rice field accounted for 15% of the variation in the total number of species. Tide stage was also a significant effect ($F_{3,24}$ =3.40, P=0.034) and accounted for 24% of the variation in this response variable. Significantly fewer species, ~ 4, were collected at TS3 than at any other tide stage, which had averages of 5 to 8 species. The rice field by tide stage interaction was not significant ($F_{3,24}$ =0.76, P=0.524).

The maximum number of individuals collected during an electrofishing survey was 53, and the minimum was 1. The total number of fish collected was not significantly different among the rice fields ($F_{1,24}$ =0.76, P=0.390). The total number of fish differed significantly among the tide stages ($F_{3,24}$ =3.26, P=0.039), however, an outlier was identified. This outlier was the maximum value, which was collected during TS1. After the removal of this outlier, the effect of tide stage

was no longer significant ($F_{3,23}$ =2.32, P=0.102) on the total number of individuals collected. The rice field by tide stage interaction was never significant with the either the total data set or with the outlier removed.

That preliminary data were used to design the electrofishing survey. Electrofishing data will be collected on the alternate months in which no drop trap sampling is scheduled. Those data will be split into two seasons, which will be analyzed separately. In DH four channels will be selected and permanent transects will be established within these channels. In BF eight transects will be established with 4 of these being in channels, to correspond to the DH transects, and the other 4 being randomly selected. Given the vegetative borders that define channels in DH, a criteria for selecting four of the 8 channels in BF will be that a channel must have some sort of vegetative border. Each transect will be 200 meters long and marked with poles every 50 meters.

Based on the initial electrofishing survey, an average of 12 minutes was needed to electrofish a 100 m transect and 18 minutes to process the fish collected. Extrapolating those numbers to a 200 m transect, gives 24 and 36 minutes, respectively. Also based on that initial survey, the greatest number of species are collected during the time from 2 hours past the low tide up until high tide, in other words, the incoming tide. During that 4 hour interval, four 200 m transects can be electrofished. With a total of 12 transects, the electrofish study will require 3 field days each month.

The fishes collected during the electrofishing of a transect will be identified and total length and mass will be measured. Summing the mass of each fish will give the total biomass of fish per transect. The other response variables will include the number of fish collected per transect and the number of species per transect. I will test if these variables differ among the rice fields using an ANOVA.

We also observed that the channels in BF are best defined and most likely to be effectively blocked by nets when a spring, low tide occurs. We can electrofish under those conditions to quantify efficiency using a multiple depletion strategy. The channels in DH are more clearly defined and can also be blocked by nets. Those channels are too shallow to electrofish at a spring low tide, but they can be sampled approximately 2 hours after the low. We will also use the multiple depletion strategy to quantify efficiency in Dean Hall.

[Rotenone Sampling]

The species collected using rotenone were a subset of the species that have been collected through drop trap sampling and electrofishing (Table 3). Mosquitofish were a numerically dominant component of the fish community in both DH and BF, and more species were collected in DH than in BF.

Discussion and Recommendations

Pooling across preliminary and routine drop trap sampling, twenty-four species of fish have been collected in the ricefields. Two of the species, *Poecilia latipinna*, the sailfin molly, and *Fundulus confluentus*, the marsh killifish, had not been previously recorded as being found in the ricefields in the Cooper River. Two other tentatively identified new species for the ricefields are *Eleotris pisonis*, the spinycheek sleeper, and *Myrophis punctatus*, the speckled worm eel. As noted in other drop trap studies of wetland fishes (Chick et al. 1992, Jordan et al. 1997, Jordan et al. 1998), we have found the number of fish collected per drop trap is quite varied, ranging from zero to over 200. To take account of such skewness in the drop trap subsamples, I will take the median number of fish within a vegetation type within a ricefield block as the response variable to be analyzed as opposed to the average. For such skewed data the median better characterizes the central tendency of the data than the average (Sokal and Rohlf 1981). The every other month

drop trap sampling in the BF and DH ricefields began in March 1999 and will be completed in January of 2000. If an association between fish abundance and biomass patterns with vegetation type based on the DH and BF sampling is detected from the analysis, we will test if those patterns generalize by sampling additional ricefields, Bossis Plantation and Mulberry Field (Figure 1), during 2000. We plan to use vegetation and bathymetric maps of these fields prepared by Dr. Joseph Kelley from The Citadel so that we can select our sample sites aprori for this test of vegetation type affecting the fish community.

By using the drop trap, we have also documented that two species of crayfish, *Procmbaus (Ortmannicus) lepidodactylus* and *Procambarus (Scapulicambarus) troglodytes*, occur in the BF and DH ricefields. The proposal, "Crayfish in the tidal freshwater wetlands of the Cooper River, SC: the interaction between an omnivore and successional stages of aquatic macrophytes",was submitted to the South Carolina Sea Grant Consortium to try and obtain funding to study these crayfish.

The routine electrofishing study in BF and DH began in April 1999 and will be completed in February of 2000. After this, the Bossis Plantation and Mulberry Field ricefields will also be sampled by electrofishing, but not as frequently as the BF and DH ricefields.

Currently, our drop trap sampling is restricted to either the edges of the beds of OW and LEP or approximately one boat length into these beds. A fan boat is being built, which should allow us to sample further into these areas of the ricefields. An additional modification that might help the drop trap sampling is to deploy a drop/throw trap that is 0.75 m (l) x 0.75 m (w) x 1.25 m (h) instead of our current 1 m^3 drop trap. This taller and narrower trap may work better given the number of logs and narrow channels that make deploying the 1 m^3 difficult. Also, two people could probably deploy the taller and narrower trap as opposed to three people being used now.

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JOB PROGRESS REPORT

STATE: South Carolina PROJECT NUMBER: F- 63

PROJECT TITLE: Fisheries Investigations in Lakes and Streams - Statewide

STUDY: Survey and Inventory

JOB TITLE: Genetic Survey of South Carolina Bluegill populations

Introduction

The bluegill, *Lepomis machrochirus*, is native to much of the eastern United States as well as Canada and Mexico (Rhode et. al., 1994.). In the southeastern United States two subspecies exist. They are *L. m. machrochirus* and *L. m. purpurescens*, or coppernose bluegill. Pure populations of the coppernose bluegill are restricted to peninsular Florida. The *L. m. machrochirus* subspecies inhabits southern states west of Georgia to Texas. An intergrade zone exists that includes South Carolina and Georgia (Avise and Smith, 1974).

These two subspecies are distinguished genetically by their differences at two allozyme loci, aspartate aminotransferase (*sAAT-1**) where two alleles are possible, and esterase (*sEST-1**) where three alleles are possible. At *sAAT-1**, *L. m. purpurescens* populations are fixed for the *sAAT-1*58* allele while *L. m. machrochirus* populations are fixed for the *sAAT-1*100* allele. At *sEST-1** *L. m. purpurescens* populations are fixed for the *EST-1*100* allele while *L. m. machrochirus* populations possess either or both of the alleles *EST-1*96* and *EST-1*94*.

Avise and Felley (1979) collected bluegill from eight South Carolina reservoirs, four on the Savannah and four on the Santee-Cooper drainages. They found that on each drainage clinal allelic variation existed, with the relative proportion of alleles typical of the coppernose bluegill decreasing as you move North.

The objectives of this study were to 1) re-sample populations included in the 1979 study to

determine if they had changed genetically, and 2) sample several additional populations to provide a more complete baseline of bluegill genetics across the state. Genetic relationships among South Carolina populations were determined, as well as genetic relationships among South Carolina and Georgia populations.

Methods

Bluegill were collected from each of seven populations by electrofishing from 12/96 to 9/97. Lakes Hartwell and Murray were sampled in the 1979 study. Lakes William C. Bowen and Wateree, and the Little Pee Dee, Edisto, and Combahee Rivers were sampled for the first time. Total lengths and weights were recorded for each fish, except for those from Lake Bowen where only total lengths were recorded. Liver, muscle and eye tissues were extracted from each fish and immediately placed on dry ice. Otoliths were extracted from each fish from the five lake populations and stored for future reference.

Tissue samples were shipped to Auburn University for genetic analysis. Samples were analyzed using horizontal starch gel electrophoresis according to the procedures of Steiner and Joslyn (1979), Philipp et al. (1982) and Norgren et al. (1986). Genotypes and allele frequencies were determined for the two loci diagnostic for *L. m. machrochirus* and *L. m. purpurescens*, *sAAT-1** and *sEST-1**. Allele frequencies for Lakes Hartwell and Murray were compared to those from the 1979 study using the G-test (Sokal and Rohlf, 1969). Genetic relationships among all seven South Carolina populations were calculated using Rogers' (1972) genetic similarity. Dendograms of these relationships among South Carolina populations, and among South Carolina and Georgia populations were generated.

Results

Bluegill (N=189) were collected from all populations sampled. Table 1 lists the number

collected and mean lengths and weights by population. Twenty five fish from each population were examined electrophoretically and allele frequencies were computed (Table 2). All

Table 1. Mean lengths and weights for bluegill collected from South Carolina populations in 1996-1997.

			Length (mm)			Weight (g)	
Population	Date	No. Collected	range	mean	sd	mean	sd
Murray	12/3/96	25	153-202	172.4	13.8	97.8	29.7
Wateree	12/5/96	25	148-190	163.7	10.2	74.3	14.8
Hartwell	12/10/96	25	113-174	140.1	16.5	45.8	18.2
W.C. Bowen	3/24/97	25	85-204	132.0	31.2	-	-
Combahee	6/10/97	26	68-230	133.6	47.7	69.4	76.8
Edisto	9/4, 9/17, 9/18/97	34	62-167	111.5	28.7	28.4	24.2

populations were intergrades, possessing a combination of alleles from both subspecies.

Allele frequencies for Lakes Hartwell and Murray varied some from those reported in 1979 (Table 3). Lake Hartwell showed no change at *sAAT-1** but did have a significant (p=.05) increase in the purpurescens allele at *EST-1**. Lake Murray showed a significant decrease in the purpurescens alleles at both loci.

Figure 1 shows the dendogram generated for the seven populations surveyed in South Carolina. Two major groupings are observed. The Combahee and Little Pee Dee Rivers form one group. These two coastal river populations possessed the highest percentages of purpurescens, or southern alleles. The remaining five populations form a second group, with

Lakes Hartwell and Bowen forming a sub-grouping. Lakes Hartwell and Bowen are the two northern most populations surveyed, and possessed the lowest percentages of the purpurescens alleles.

South Carolina and Georgia populations were closely related according to the genetic similarity dendogram for both states combined (Figure 2). Three of the four main groupings observed contain South Carolina populations along with wild and hatchery Georgia populations. For both dendograms, clusters are generally consistent with geographic proximity of populations. An allelic cline is evident where the proportion of alleles typical of the *L. m. purpurescens* subspecies is most common in the Coastal Plain and decreases as you move toward the Piedmont.

Discussion

Differences in allele frequencies on Lakes Hartwell and Murray between the 1979 and 1997 surveys are statistically significant. This could be in part due to sampling error. Sample sizes are small, for the 1997 survey (N=25) compared to the 1979 survey (N=320). In the past we have considered a sample size of 25-30 individuals sufficient for comparisons of population allele frequencies. Recent studies, however, have led us to question this. While samples of that size may be suitable for evaluating general trends in allelic proportions over a large geographic area, they may still be too small for evaluating differences between individual populations, or in the same population over time.

Table 2 Allele frequencies at two diagnostic loci for South Carolina Bluegill populations surveyed. Alleles *sAAT-1*58* and *sEST-1*100* are typical of the southern subspecies of bluegill.

Locus/Allele	L. Murray	L.Wateree	L.Hartwell	L. Bowen	Combahee R.	L. Pee Dee R.	Edisto R.
*sAAT-1**							
(N)	(25)	(24)	(25)	(25)	(24)	(23)	(24)
100	.80	.58	1.00	.96	.22	.43	.69
58	.20	.42	.00	.04	.78	.57	.31
*sEST-1**							
(N)	(22)	(20)	(23)	(18)	(24)	(25)	(25)
100	.23	.27	.24	.06	.37	.36	.30
96	.61	.60	.61	.64	.58	.46	.46
94	.16	.13	.15	.30	.04	.18	.24

Table 3. Allele frequencies at two diagnostic loci for bluegill populations surveyed in 1979 and 1997 with corresponding G test statistics. An * indicates a G value significant at p≤.05.

Locus/Allele	L. Murray			L. Hartwell		
*sAAT-1**	1979	1997	G	1979	1997	G
(N)	(320)	(25)		(320)	(25)	
100	.61	.80	7.8*	.96	1.00	.002
58	.39	.20		.04	.00	
*sEST-1**	1979	1997	G	1979	1997	G
(N)	(320)	(22)		(320)	(25)	
100	.39	.23		.03	.24	
96	.54	.61	7.2*	.77	.61	12.96*
94	.07	.16		.20	.15	

Figure 1. Genetic similarity (Rogers 1972) of seven South Carolina bluegill populations surveyed in 1997.

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.90            .92            .93            .95            .97            .98            1.00
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.90              .92              .93      .95              .97              .98              1.00
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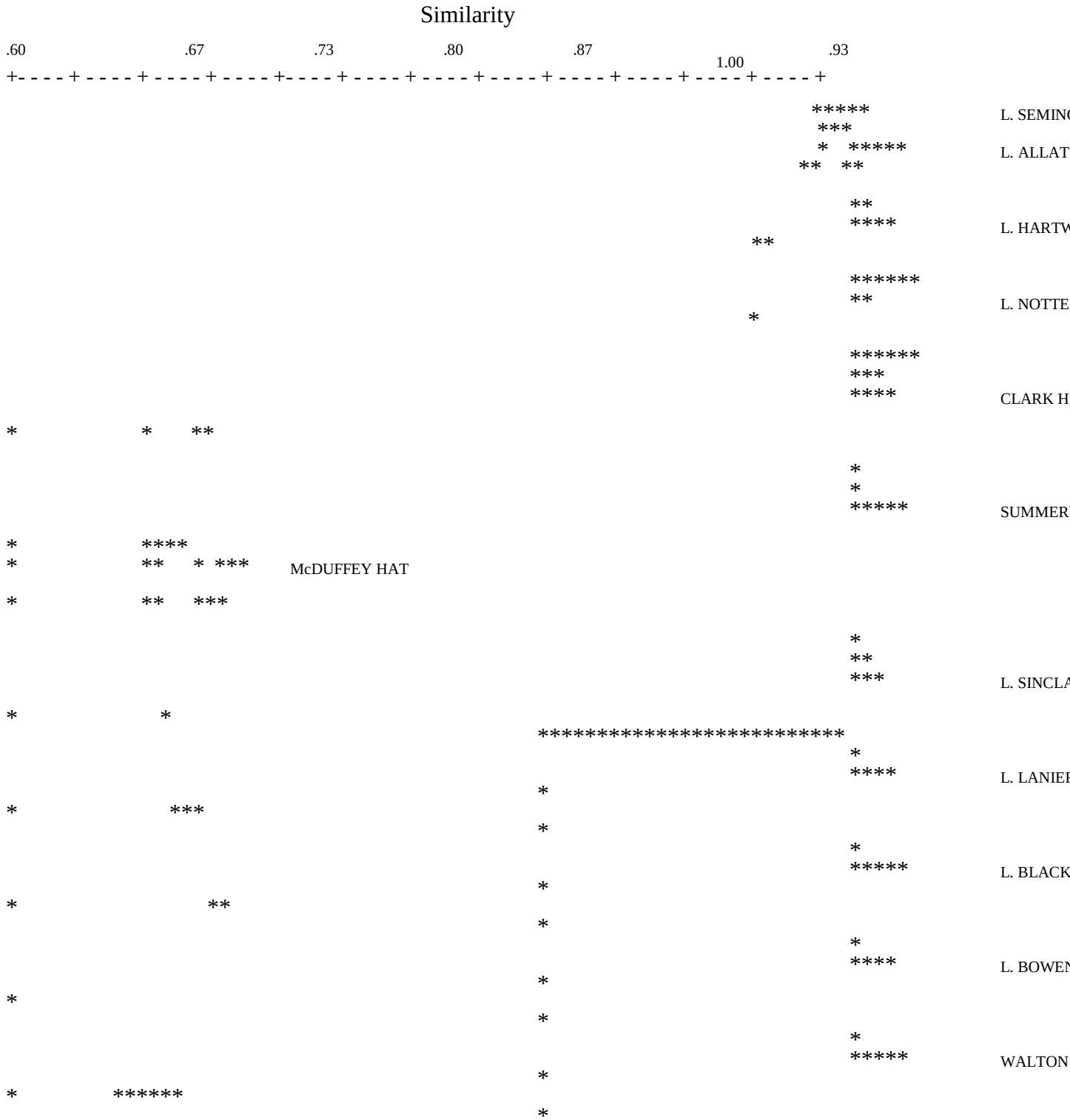
Figure 2. Genetic similarity (Rogers 1972) of wild bluegill populations from South Carolina (N=7), with wild populations and hatcheries from Georgia (N=18).



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South Carolina and Georgia populations are closely related. A geographic cline is evidenced by allele frequencies for the two states, where the proportions of alleles typical of the *L. m. purpurescens* subspecies are most common near the coast and decrease as you move northwest. This mirrors a cline reported in 1995 by Bulak et al. for largemouth bass populations of the Carolinas. In that study it was proposed that natural selection was acting to maintain allelic clines within the broad hybrid zone that South Carolina is a part of. The same may be true for bluegill populations. The southeastern distribution of alleles of the northern and southern subspecies of each of these fish are similar.

This study provides evidence that selective pressures could be acting on bluegill populations to maintain an allelic cline. It does not provide any direct evidence as to what those specific selective pressures are, nor how strong they may be. Care should be taken when supplementing wild bluegill populations to use fish that are genetically similar to wild stocks in that area. Ideally fish would be produced from broodstocks collected from populations receiving supplemental stockings.

Recommendations

Reconsider the number of individuals needed for genetic surveys where population comparisons will be made. Regionalize bluegill hatchery stocks so that each region of the state receives fish most closely related to wild stocks of the area.

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JOB PROGRESS REPORT

STATE: South Carolina PROJECT NUMBER: F- 63

PROJECT TITLE: Fisheries Investigations in Lakes and Streams - Statewide

STUDY: Survey and Inventory

JOB TITLE: Relative performance of two strains of largemouth bass in state lakes

Introduction

Two subspecies of largemouth bass *Micropterus salmoides*, the Florida largemouth bass *M. s. floridanus* and the northern largemouth bass *M. s. salmoides*, exist and readily interbreed in both hatchery and reservoir environments (Isely et al., 1987, Gilliland and Whitaker 1989, Philipp and Witt 1991). The native range of the Florida subspecies (FLMB) is restricted to peninsular Florida. The northern subspecies (NLMB) is native to waters north along the Atlantic coast states from Maryland and west to the Mississippi (Philipp et al., 1983).

South Carolina is located in the broad hybrid zone between the ranges of the two subspecies. A statewide allozyme study of largemouth bass confirmed that South Carolina populations were hybrids (Bulak et al., 1995). This study also showed the existence of a geographic cline within South Carolina where the relative abundance of alleles typical of the Florida subspecies decreased from southeast to northwest. The relative frequency of alleles that are fixed for the Florida subspecies ranged from 98% in Lake Moultrie, a Coastal Plain reservoir, to 36% in Lake Wateree, a Piedmont reservoir. It was suggested that natural selection played a role in maintaining this allelic cline.

Physiological and ecological differences among FLMB, NLMB, and their hybrids have been documented. A number of studies have shown a difference in the response of the FLMB, NLMB, and their hybrids to various temperature regimes (Fields et al., 1987, Charmichael et al.,

1988). Other studies have shown differences in timing of spawning, growth rate, reproductive success and survival of the two subspecies (Philipp and Witt 1991, Maceina et al. 1988, Gilliland and Whitaker 1989, Isely et al. 1987).

The objective of this study was to examine performance differences between Lake Wateree and Lake Moultrie genetic strains of largemouth bass found in South Carolina. Two newly renovated state owned lakes, Wallace and Sunrise, were stocked with largemouth bass fingerlings from each strain. Strains were produced on separate hatcheries from broodfish collected from Lakes Wateree and Moultrie. Each strain received either a single or double oxytetracycline mark prior to stocking. Lakes Wallace and Sunrise were stocked with equal proportions of each strain. The objective will be achieved by measuring growth of stocked bass at age-1 and age-3 and by monitoring the long term temporal change in juvenile genotypes.

Methods

Sunrise Lake, a 20 acre lake in Lancaster County, and Lake Richard B. Wallace, a 280 acre lake in Marlboro County, were renovated during the summer of 1996. Largemouth bass for experimental stockings were produced from adult bass collected from Lakes Moultrie and Wateree. Lake Moultrie broodfish were collected by electrofishing in March of 1993 and were housed separately from other stocks at Cheraw State Fish Hatchery. Lake Wateree broodfish were collected in early Spring of 1997 and transported to Cohen Campbell Fisheries Center where they were stocked directly into a spawning pond separate from other stocks. Each group of broodfish was allowed to spawn. Resulting fry were harvested from as many schools as possible to maximize the number of parents contributing to the gene pool, and were grown out to fingerlings.

Prior to stocking fingerlings from each strain were marked by immersion for 6 hours in a

500 ppm solution of oxytetracycline. Moultrie strain largemouth bass were double marked, first on 4/16/97 as fry, and then on 5/5/97 as fingerlings. Wateree strain largemouth bass were single marked as fingerlings on 4/25/97.

Each lake was stocked with equal numbers of each strain at the rate of 100 fish per acre in April and May of 1997. Lake Wallace was stocked with 28,000 and Sunrise Lake with 2000 largemouth bass. (Lakes were stocked in October 1996 with a combination of bluegill *Lepomis macrochirus* and redear *L. microlophus* fingerlings at the rate of 1000 per acre.) Wateree strain fingerlings were stocked on 4/25/97. Moultrie strain fingerlings were stocked on 5/5/97. Total lengths were recorded for a sample of 100 fingerlings from each strain at time of stocking. One hundred additional fingerlings from each strain were transported to the Berry's Mill Hatchery near Traveler's Rest and held in separate ponds for use in mark evaluation and genetic analysis.

Ponds at Berry's Mill were harvested on 11/6/97 and sagittal otoliths, liver, and muscle tissue were collected from each individual. Known single and double marked otoliths were randomly coded and given to an experienced reader for evaluation. Otoliths were mounted, sectioned and polished to the core. Presence or absence of a mark on the otolith was determined with a flourescent compound microscope.

Liver and muscle tissues were stored at -80°C for genetic analysis. Horizontal starch gel electrophoresis was performed according to Norgren (1986). Gels were stained for four enzymes which are diagnostic for the Florida and northern subspecies of largemouth bass. These are aspartate aminotransferase (*sAAT-2**), isocitrate dehydrogenase (*sIDHP-1**) and superoxide dismutase (*sSOD-1**) from liver tissue, and malate dehydrogenase (*sMDH-B**) from muscle tissue. Alleles typical of the northern subspecies are *sAAT-2*100* and *sAAT-2*110*, *sIDHP-1*100*, *sMDH-B*100*, and *sSOD-*147*. Alleles typical of the Florida subspecies are

*sAAT-2*126* and *sAAT-2*139*, *sIDHP-1*121*, *sMDH-B*114*, and *sSOD-1*100*. A genetic baseline was determined for Lakes Moultrie and Wateree using data from an initial statewide survey (Bulak et al., 1995) and data collected from large and small fish for a related performance study. Allele frequencies of each stock was compared to baseline genetic data for source populations using the G-test (Sokal and Rohlf, 1969).

Lakes were sampled in the Spring and Summer of 1998 for collection of juveniles and age-1 adults, and in Summer of 1999 for collection of juveniles. Adults were collected in 1998 by electrofishing from Lake Wallace on March 31 and April 4, and from Sunrise Lake on May 22. Total length and weight were recorded for each individual. Sagittal otoliths were collected from each largemouth bass and stored in the dark until processed for mark determination. Liver and muscle tissues were collected from each individual and stored at -80°C for genetic analysis. Seining for juveniles was conducted on both lakes in May of 1998 and June of 1999. A variety of areas and habitats were sampled.

Otoliths collected from age-1 largemouth bass were mounted, sectioned and polished to the core for mark determination. Marks were evaluated by two independent readers using a flourescent compound microscope. Otoliths were determined to be single marked, double marked or unmarked by each reader. Those otoliths that were not agreed on after consultation were thrown out. Growth at age-1, in mm/day, was compared for Moultrie strain and Wateree strain fingerlings in each lake using the T-test.

Results

Size at stocking was similar for the Moultrie and Wateree strains. Moultrie strain fingerlings averaged 24.4 mm total length (n = 102, std = 2.6). Wateree strain fingerlings averaged 23.3 mm total length (n = 92, std = 6.2).

Mark evaluations were completed on a set of 68 otoliths. Because of questionable origin made evident by genetic analysis, 8 sets of otoliths were thrown out. Of 27 Wateree strain fish 100% were correctly identified. Of 33 Moultrie strain fish 91% were correctly identified.

Genetic analysis was completed for hatchery fingerlings of each strain, and comparisons made with historic data from wild stocks (Table 1.). Fingerlings of the Wateree strain were similar to the wild Wateree stock at three of four loci. However, at the *sIDHP-1** locus the Wateree strain fingerlings possessed significantly (p=0.05) more of the *sIDHP-1*100* allele which is typical of the northern subspecies. Fingerlings of the Moultrie strain differed markedly from wild lake Moultrie stock at three of the four loci examined. They possessed significantly more of the *sAAT-2*100,110* alleles, the *sIDHP-1*100* allele, and the *sMDH-B*100* allele, all typical of the northern subspecies.. Fingerlings of the Moultrie strain possessed *sMDH-B*100* at a frequency of 20% although broodstock from Lake Moultrie were known to be fixed for *sMDH-B*114*.

Those fish possessing the *sMDH-B*100* allele were also found to be single rather than double marked. This poses a problem, as they are undistinguishable, both genetically and by mark, from the Wateree strain fish. For the purposes of this report, all single marked fish are considered to be of the Wateree strain.

Largemouth bass adults were collected by electrofishing from Lake Wallace on 4/31/98 and 5/22/98. Fish averaged 274.1 mm total length (n = 104, std = 28.2) and weighed an average of 359.3 g (n = 104, std = 123.5) Largemouth bass adults were collected from Sunrise Lake on 5/22/98. These fish averaged 235.7 mm total length (n = 92, std = 17.3) and weighed an average of 171.7 g (n = 92, std = 49.8).

Clear marks were detected on 49 of 104 otoliths sampled from Lake Wallace, and on 44 of

92 otoliths sampled from Sunrise Lake. Twenty-one percent of otoliths from Lake Wallace were determined to be unmarked, and 32% were not readable due to cracks or occlusions. From Sunrise Lake 22% of otoliths were read as unmarked and 29% were not readable.

Marked fish were identified to strain (1 mark = Wateree, 2 marks = Moultrie), and growth rate by strain was computed for each lake (Table 2). Differences in growth rate between the two genetic strains were tested for each lake using the T-test and were not significant.

Despite efforts to sample a variety of areas and habitats, no juvenile largemouth bass were collected from either lake in 1998, nor from Lake Wallace in 1999. Thirty juvenile largemouth bass were collected from Sunrise Lake in 1999 and are stored frozen pending genetic analysis. Juvenile collections on Lake Wallace will be attempted again in the Fall of 1999 by electrofishing.

Discussion

The marked genetic difference between Moultrie strain fingerlings and Lake Moultrie broodfish is a concern, especially at the *sMDH-B** locus. It indicates that not all of the fingerlings stocked as Moultrie strain were produced from Lake Moultrie broodfish.

When they were collected in 1993 all Lake Moultrie broodfish underwent liver and muscle biopsies. Tissues were analyzed so that the alleles expressed at each loci for every fish was known. None of 112 fish biopsied possessed the *sMDH-B*100* allele.

Table 1. Allele frequencies (proportions) for largemouth bass used to stock study lakes, with historic data for reservoirs where stocks originated. A + indicates allele frequencies significantly different from survey data.

	Lake Wateree				
Lake Moultrie					
Locus/Allele	Historic Data	1997 Fing.		Historic Data	1997 Fing.	
sAAT-2*						
100, 110	146 (0.66)	26 (0.69)		47 (0.10)	16 (0.23)	+
126, 139	74 (0.34)	12 (0.31)		443 (0.90)	54 (0.77)	+
sIDHP-1*						
100	116 (0.48)	37 (0.69)	+	11 (0.02)	12 (0.16)	+
121	124 (0.52)	17 (0.31)	+	455 (0.98)	64 (0.84)	+
sMDH-B*						
100	141 (0.61)	39 (0.70)		0 (0.00)	16 (0.20)	+
114	91 (0.39)	17 (0.30)		494 (1.00)	64 (0.80)	+
sSOD-1*						
147	143 (0.57)	29 (0.54)		82 (0.19)	17 (0.24)	
100	107 (0.43)	25 (0.46)		344 (0.81)	55 (0.76)	

Table 2. Mean growth rate at age-1, in mm/day, for Moultrie and Wateree strains of largemouth bass stocked in Lake Wallace and Sunrise Lake with corresponding T-test statistics and probabilities.

	Lake Wallace				Sunrise Lake			
Strain (N)	Rate (mm/d)	(N)	T	Prob>|T|	Rate (mm/d)		T	Prob>|T|
Moultrie	0.75	(13)	1.29	0.2038	0.54	(19)	-0.64	0.5245
Wateree	0.72	(31)			0.55	(30)		

Eight out of 40 Moultrie strain fingerlings were homozygous for *sMDH-B*100* meaning they inherited that allele from both parents. All other fingerlings were homozygous for *sMDH-B*114*. The presence of the northern allele and lack of heterozygotes indicate that the fish possessing the northern allele were spawned in a different pond and from a group of parents other than the Lake Moultrie broodfish.

Fish possessing the *sMDH-B*100* allele also possessed a different oxytetracycline mark from other Moultrie fingerlings. Moultrie fingerlings were marked twice, first as fry when harvested from the spawning pond, and then as fingerlings when taken from the hatchery for stocking. All eight of the fish homozygous for *sMDH-B*100* had only the later mark.

There are a number of possible explanations for the presence of the fish homozygous for *sMDH-B*100*. The first is that the Moultrie strain fingerlings were contaminated on the hatchery. This would have occurred sometime after the marking of fry but prior to the second marking, with the source of contamination either in the grow out pond or the fish house.

A second explanation is that the Moultrie strain fish were contaminated in the holding pond

at Berry's Mill with fish of the single marked Wateree strain. The two strains were housed in adjacent ponds separated by an earthen dike. A third explanation is that the samples collected from Berry's Mill were mishandled and some Wateree strain fish were improperly coded as Moultrie strain. The probability that 8 fish chosen at random from the Wateree strain will all be homozygous for *sMDH-B*100* is P = 0.002.

There is also the possibility that genetic and/or otolith interpretations of the known stocks were incorrect. This will be further investigated by reviewing those otolith samples and genetic records.

If the Moultrie strain fingerlings were in fact contaminated prior to stocking, the effects on the experiment can be assessed. Our experimental design called for the lakes to be stocked with equal proportions of each strain. Performance would be assessed by measuring growth of stocked fish at age-1 and age-3, and by the long term monitoring of allele frequencies of subsequent year classes.

In fact, the lakes were stocked with 50% Wateree strain fingerlings, 40% Moultrie strain fingerlings, and 10% fingerlings of unknown origin. Because the fingerlings of unknown origin are single marked they are indistinguishable from fish of the Wateree strain. Of the marked fish collected from lakes Wallace and Sunrise, 61% and 70% respectively were single marked. Growth assessments of the Wateree strain include those fish of unknown origin. Assessment of reproductive success of the Moultrie and Wateree strains by following changes in allele frequencies of subsequent generations will be difficult because of the unbalanced stocking, and the inability to quantify the contribution of the unknowns.

While these factors negatively impact our ability to draw conclusions regarding the performance of the Moultrie and Wateree strains, valuable information can still be obtained.

Genetically the 8 unknown fish are similar to the Wateree strain. Though as a group they possess more northern alleles, individually they are not distinguishable from a Wateree strain fish. Growth can still be compared between the Moultrie strain and the more northern, single marked fish.

Comparison of growth at age-1 do not show significant differences between the strains for either lake. Larger sample sizes would increase our ability to detect differences. Although about 100 fish were collected from each lake, only about half of these are included in analysis. A number of otoliths examined were either unmarked, or marked but too difficult to read because the core was occluded by cracks. Those samples that were too difficult to read should be reexamined using the other otolith.

Largemouth bass in Sunrise Lake grew much slower in their first year than those in Lake Wallace. While no water quality measurements were taken a visual inspection of the two lakes indicated they were managed quite differently. Lake Wallace appeared to have received more than adequate fertilizer applications; it was deep green with no visibility below the surface in some areas. Sunrise Lake was very clear throughout. If fertilizer applications were made at Sunrise Lake they were not effective. Both of these lakes were stocked at the fertilized rate of 1000 bream/100 bass per acre.

Recommendations

Continue study. Place emphasis on processing otoliths from selected samples and repeat analysis with larger sample size. Collect individuals from 1999 year class from Lake Wallace by electrofishing. Perform genetic analysis on 1999 year class from both lakes and on age-1's collected in 1998 and compare. Pending results, collect age-3 largemouth bass in 2000 and repeat growth comparison. Ensure that all state lakes are managed optimally with regard to liming and

fertilization regimes.

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JOB PROGRESS REPORT

STATE: South Carolina PROJECT NUMBER: F- 63

PROJECT TITLE: Fisheries Investigations in Lakes and Streams - Statewide

STUDY: Survey and Inventory

JOB TITLE: Relative performance of two strains of largemouth bass in farm ponds

Introduction

Two subspecies of largemouth bass *Micropterus salmoides*, the Florida largemouth bass *M. s. floridanus* and the northern largemouth bass *M. s. salmoides*, exist and readily interbreed in both hatchery and reservoir environments (Isely et al., 1987, Gilliland and Whitaker 1989, Philipp and Witt 1991). The native range of the Florida subspecies (FLMB) is restricted to peninsular Florida. The northern subspecies (NLMB) is native to waters north along the Atlantic coast states from Maryland and west to the Mississippi (Philipp et al., 1983).

Genetic differences between the two subspecies are measurable at four diagnostic enzyme coding loci (Philipp et al., 1983). The differences at two loci, aspartate aminotransferase (*sAAT-2**) and isocitrate dehydrogenase (*sIDHP-1**), are fixed meaning one allele or combination of alleles is present only in populations of the Florida subspecies and the other only in populations of the northern subspecies. At a third locus, malate dehydrogenase (*sMDH-B**), Florida populations are fixed for a Florida allele, while northern populations may be fixed for a northern allele or possess a combination of northern and Florida alleles. At the fourth diagnostic loci, superoxide dismutase (*sSOD-1**), northern populations are fixed for the northern allele while Florida populations possess a combination of the northern and Florida alleles. Alleles typical of the northern subspecies are *sAAT-2*100* and *sAAT-2*110*, *IDHP-1*100*, *sMDH-B*100*, and *sSOD-*147*. Alleles typical of the Florida subspecies are *sAAT-2*126* and *sAAT-2*139*,

*sIDHP-1*121*, *sMDH-B*114*, and *sSOD-1*100.*

South Carolina is located in the broad hybrid zone between the ranges of the two pure subspecies. A statewide allozyme study of largemouth bass confirmed that South Carolina populations were hybrids (Bulak et al., 1995). This study also showed the existence of a geographic cline within South Carolina where the relative abundance of Florida alleles decreased from southeast to northwest. The relative frequency of alleles that are fixed for the Florida subspecies ranged from 98% in Lake Moultrie, a Coastal Plain reservoir, to 36% in Lake Wateree, a Piedmont reservoir. Bulak et al. (1995) suggested that natural selection played a role in maintaining this allelic cline.

Physiological and ecological differences among FLMB, NLMB, and their hybrids have been documented. A number of studies have shown a difference in the response of the FLMB, NLMB, and their hybrids to various temperature regimes (Fields et al., 1987, Charmichael et al., 1988). Other studies have shown differences in timing of spawning, growth rate, reproductive success and survival of the two subspecies (Philipp and Witt 1991, Maceina et al. 1988, Gilliland and Whitaker 1989, Isely et al. 1987).

The objective of this study was to examine performance differences between Piedmont and coastal genetic strains of largemouth bass found in South Carolina. Privately-owned ponds were used as study sites. Each pond was stocked with either a coastal or Piedmont strain of largemouth bass. The objective will be achieved by measuring growth of stocked bass at age-1 and age-3, and by monitoring the long term temporal change in juvenile genotypes.

Materials and Methods

[Pond Selection]

Ponds were selected prior to stocking. A list of all pond owners purchasing fish from the

South Carolina Department of Natural Resources was obtained. Through a series of phone interviews and pond visits, study sites were chosen based on the following criteria:

- size 1-3 acres
- either new or properly renovated
- little potential for invasion by wild fish
- agreement with pond owner to allow access for data collection

Ponds had been stocked in October with bluegill *Lepomis macrochirus* and redear *L. microlophus* fingerlings. Pond owners were advised that largemouth bass fingerlings would be delivered to their pond and they should not stock the ponds with bass from any other source.

[Broodfish collection and fingerling production]

Largemouth bass for experimental stockings were produced from adult bass collected from Lakes Moultrie and Wateree. Lake Moultrie broodfish were collected by electrofishing in March of 1993. Lake Wateree broodfish were collected by electrofishing in March of 1994. In 1994 and 1995 each group of broodfish was allowed to spawn. Resulting fry were collected and transferred to grow-out ponds where they were raised to a total length of approximately 25mm. Fry were harvested from as many schools as possible to maximize the number of parents contributing to the gene pool.

Size at stocking and frequencies of alleles characteristic of the NLMB and FLMB were determined for each stock of fingerlings. Forty fingerlings from each stock were weighed (gm), measured (TL mm) and preserved in 100% isopropyl alcohol for future reference. Two sets of 100 fingerlings from each stock were placed on dry ice and stored frozen for allozyme analysis. Horizontal starch gel electrophoresis was performed according to Norgren (1986). Gels were stained for the four allozymes diagnostic for the northern and Florida bass subspecies. Allele

frequencies of fingerlings were compared to source lake populations using the G-test (Sokal and Rohlf, 1969).

Tissue samples were taken from anesthetized L. Wateree broodfish (Leitner and Isely, 1994) prior to spawning in 1995 and allozyme analysis was performed. The purpose of this was to identify and remove from the broodfish pool any individuals possessing a rare allele at the IDHP-1* locus.

[Stocking]

One half of the ponds in each region were stocked with Moultrie fish and the other half with Wateree fish. Prior to the first day of stocking, ponds were chosen at random for stocking with the Lake Moultrie strain. As each pond was chosen, its closest neighbor was assigned the Wateree strain. This ensured a uniform distribution of each strain throughout each region. Only one strain (Moultrie or Wateree) was hauled per day and the truck was flushed and stocked with fresh fingerlings each morning. Largemouth bass were hand counted and stocked at the rate of 50 and 100 fingerlings per acre for unfertilized and fertilized ponds, respectively.

At stocking and during regular pond visits, pond owners were advised of steps they should take to best manage their ponds. Recommendations included stabilization of banks, control of aquatic weeds, liming, and sufficient fish harvest.

[Water quality monitoring]

Selected water quality parameters were analyzed from each pond to define productivity differences among ponds. Parameters measured were hardness and alkalinity, at appropriate intervals, and pH, temperature and chlorophyll-a concentration throughout the growing season. Hardness and alkalinity were measured using a standard Hach kit with a digital titrator. Temperature and pH measurements were made using an Orion field pH meter equipped with a

Ross electrode. Chlorophyll-a was determined with a Turner Filter Fluorometer Model 111. Prior to calculating chlorophyll-a concentrations, the fluorometer was calibrated. A series of known concentrations of chlorophyll-a were read at each of four sensitivity settings. Using the values obtained, calibration factors were derived to convert fluorometric readings of unknowns at each sensitivity setting to chlorophyll-a concentrations, as follows:

$$F_s = \frac{C_a}{R_s}, \quad \text{where}$$

F_s = calibration factor for sensitivity setting S,

R_s = fluorometer reading for sensitivity setting S,

C_a = concentration of chlorophyll-a, µg/L.

In the field water samples for chlorophyll-a determination were taken from 0.3 m below the surface at three sample sites on each pond. Sample sites followed the pond's stream gradient with an upper or inflow site, a middle, and a lower or outflow site. Each sample was inverted to mix any particles that may have settled and 50 ml were measured for filtration. The filter paper, with the filtrate, was carefully rolled, blotted, and placed in a glass vial with 0.7 ml of 10% magnesium carbonate solution. Tubes were capped and stored in the dark on dry ice for transport to the lab, where they were stored frozen for later analysis. When samples had been frozen for at least 24 hours, 6.3 ml of acetone were added, yielding a 90% buffered acetone solution in the tube. Samples were placed in the refrigerator overnight for thawing. The freeze-thaw cycle ruptures the phytoplankton cells, releasing the chlorophyll pigments into solution (H. N. McKellar, pers. comm.). Sample tubes were removed from the refrigerator, shaken and the solution was pipetted off and centrifuged at 3,000 g's for about 15 minutes for clarification. An amount, generally 0.1-4.0 ml, of each sample was carefully measured to the nearest 0.1 ml, removed to a cuvette,

and diluted with 90% buffered acetone. This dilution was placed in the fluorometer for a reading. To account for pheophytin, the solution was then acidified with one normal HCl and allowed to sit for one minute before being read again. The formula used for determining chlorophyll-a concentration (µg/L) was:

$$Chl_a = \frac{F_s (r) (R_b - R_a)}{V}$$

where,

F_s=conversion factor from calibration,

r=2 (R_b/R_a, as determined with pure chlorophyll-a for the instrument),

R_b=Reading before acidification,

R_a=Reading after acidification, and

$V = \frac{\text{Volume of sample}}{\text{Volume of extract}}$.

Mean annual water quality parameters were computed for each pond. Mean pH, hardness, and alkalinity were the simple average of measurements taken throughout the sampling season (n=1-3). Mean annual chlorophyll-a concentration was computed by first taking the mean of the three samples for each sampling event and then taking the average of these means for each pond.

[Fish collections]

Adult largemouth bass were collected by electrofishing from each pond at one and three years post stocking. Ponds stocked in 1994 were sampled for adult largemouth bass from 6/15-7/27/95 and from 6/12-8/21/97. Ponds stocked in 1995 were sampled from 6/11-6/19/96 and from 6/1-6/26/98. At one year post stocking, where possible, we collected 10% of the number stocked with a minimum of 20. All fish were weighed, measured, and returned to the pond. Scales or otoliths were collected from fish that were suspiciously large or small, for age

verification. Fish that were older than age one were noted and not included in further analysis. Growth rate for each fish was computed as:

$$\text{growth rate} = \frac{\text{length at harvest - length at stocking}}{\text{days since stocking.}}$$

Mean growth rate at age-1 of largemouth bass was computed for each study pond.

Bass were collected from all ponds at 3 years post stocking by electrofishing and angling. Electrofishing was used on the initial sampling visit to each pond. All fish collected were weighed, measured and fin clipped to avoid resampling. A length-frequency histogram was constructed in the field so that apparent age classes could be visualized. Scales were taken for age estimation from some fish from each size group, and from all fish that appeared to be older than age-1. For ponds stocked in 1995 the largest fish collected and several from the smaller size classes were sacrificed. Otoliths were taken as well as scales from these fish. An estimate of how many age-3 bass were collected from each pond was derived from length frequency data and an initial look at scale samples. Ponds where it did not appear at least 4, age-3 largemouth bass were collected were sampled again using a combination of angling and electrofishing effort. Age was estimated from scales, and otoliths where available, by two independent readers. Mean growth rate at age-3 was computed.

From 1-3 years post stocking young of the year (yoy) largemouth bass were collected annually from each pond for allozyme analysis. A beach seine was pulled along the edges of the pond until at least 20 yoy were collected. These fish were measured, wrapped in tinfoil and immediately placed on dry ice. They were transported to the lab where they were stored frozen for analysis at the previously discussed four enzyme coding loci.

[Statistical analysis]

Water quality variables pH, hardness, alkalinity and chlorophyll-a concentration were tested for normal distribution using PROC UNIVARIATE (SAS, 1987). Variables that were not normally distributed were log transformed. For 1994 data only, hardness, alkalinity and pH were evaluated as predictors of chlorophyll-a concentration with linear regression analysis.

Because of expected variation among ponds, atypical ponds were identified and not included in analysis of growth. These included ponds where introductions of wild fish or poor water quality had an effect on forage availability.

PROC MIXED (SAS, 1996) was used to identify factors that were significant predictors of largemouth bass growth rate. The effects study site (pond), region, strain, the interaction of region and strain, and each water quality variable (non-log transformed) were tested.

In evaluating growth at age-1, of the four water quality variables tested only pH contributed significantly to the model. All other water quality variables were excluded from the model. The LSMEANS statement (SAS, 1987) was used to compute the mean growth rates for each region and type adjusted by the mean value of the significant covariate pH. The adjusted mean growth rates were tested for differences between region and type. Additional tests were performed with mean pH set at 6 and 8 to insure that the relationship was essentially the same at all pH values.

In evaluating growth at age-3, no water quality variable contributed significantly to the model. All four water quality variables were removed from the model and Proc Mixed was used to test region, strain, and their interaction as predictors of growth rate.

Allele frequencies of juveniles collected were calculated at each of the four diagnostic loci. For 1995 and 1996 samples, allele frequencies for each strain were compared to those of parental stocks using the G-test (Sokal and Rohlf, 1969). For juveniles collected in 1997 allele

frequencies were computed for each region/strain combination. The G-test was used to test differences between regions for each strain stocked. A trend showing an increase or decrease in Florida type alleles for any group would be an indication of selection.

Results

[Pond selection and stocking]

Twenty-four ponds were stocked in 1994. Of 12 Coastal Plain ponds, 7 were stocked with the Wateree and 5 with the Moultrie strain. Of 12 Piedmont ponds, 6 were stocked with the Wateree and 6 with Moultrie strain.

Thirteen ponds were stocked from May 19 - May 23, 1995. Of six Coastal Plain ponds, four were stocked with Moultrie and two with Wateree strain. Of seven Piedmont ponds, four were stocked with Wateree and three with Moultrie strain. A stocking summary is provided in Table 1.

Moultrie and Wateree strains were of similar size at stocking in both 1994 and 1995. In 1994, Moultrie fingerlings (N=41) averaged 26 mm TL (sd=3.3) and 0.2 grams while Wateree fingerlings (N=39) averaged 34 mm TL (sd=1.8) and 0.4 g (sd=0.08). In 1995, Moultrie fingerlings (N=44) averaged 32 mm TL (sd=3.9) and 0.3 g (sd=0.19) while Wateree fingerlings (N=40) averaged 25 mm TL (sd=2.7) and 0.12 g. Standard deviations for weight for 1995 Wateree and 1994 Moultrie stocks could not be calculated because some fingerlings were weighed in batches.

Table 1. Number of ponds and total acres stocked with distinct strains of largemouth bass in 1994 and 1995.

Region	Strain	Number Ponds	Total Acres
Piedmont	Wateree	10	19.0
	Moultrie	9	12.3
Coastal Plain	Wateree	9	16.2
	Moultrie	9	12.5

Allele frequencies of stocked fingerlings were generally consistent with source populations (Tables 2 and 3). Lake Moultrie fingerlings were not significantly different (P=.05) from wild Lake Moultrie stock at any of the four loci examined in either 1994 or 1995. Lake Wateree fingerlings from 1994 were significantly different from Lake Wateree wild stock at *sMDH-B** (P=0.05) and potentially at *sIDHP-2** (P=0.001). At *sMDH-B**, the stocked fingerlings possessed the northern allele in significantly higher numbers than the wild stock. Analysis at *sIDHP-2** indicated that stocked fingerlings possessed a rare allele, *sIDHP-2*142*, in significantly higher numbers than wild Lake Wateree stock. The presence of this rare allele in the 1994 stock has not been confirmed. No juveniles produced from that stock from 1995-1996 have been found to possess it. The survey of Wateree parental stocks also found no individuals that possessed the rare allele. Some parents were removed from the broodfish pool by otters or poachers between time of spawning and the time of survey.

Lake Wateree fingerlings from 1995 were significantly different (P=0.05) from Lake Wateree wild stock at *sAAT-2** and at *sIDHP-2**. At *sAAT-2** the stocked fingerlings possessed

the Florida alleles in significantly higher numbers than the wild stock. At *sIDHP-2** the stocked fingerlings possessed the northern allele in significantly higher numbers than the wild Lake Wateree stock.

[Water quality monitoring]

Ponds were sampled for water quality parameters three times during the 1994 growing season and twice during the 1995-1997 growing seasons. Samples were taken in June, August and September/October in 1994, in June and August in 1995, in June/July and October in 1996, and in July and October in 1997 . A wide range of water quality conditions were encountered from pond to pond (Table 4 and 5). Mean values for pH for 88% of ponds were between 6.5 and 9, the range at which fish grow best (Crochet, 1992). Fifty seven percent of ponds averaged 20 mg/l or higher for both hardness and alkalinity, the minimum concentration considered to provide adequate buffering capacity and support a healthy phytoplankton community (Crochet, 1992). High variance for hardness and alkalinity at certain ponds is due to the liming of those ponds to increase hardness and alkalinity during the course of sampling.

Table 2. Allele frequencies for Wateree strain largemouth bass fingerlings used to stock study ponds in 1994 and 1995, with survey data of allele frequencies for L. Wateree where stocks originated, and subsequent F_1 and F_2 generations. Alleles, or allele pairs, listed first are fixed (sAAT-2*, sIDHP-2*) or dominant in the Northern subspecies. Alleles listed second are fixed or dominant in the Florida subspecies. An * indicates a significant difference from survey data at P=0.05 and a ** at P=0.001. A + indicates a significant difference from fingerlings stocked at P=0.05 (filial generations from 1994 fingerlings are compared to survey data at sIDHP-1*).

Locus/Allele	Survey data		1994 fing.	F_1		F_2
1995 fing.	F_1					
	N=122	N=100	N=240	N=262	N=100	N=56
*sAAT-2**						
100, 110	0.66	0.65	0.71	65	0.44*	0.34
126, 139	0.34	0.35	0.29	0.35	0.54*	0.66
*sIDHP-1**						
100	0.47	0.18**	0.44	0.45	0.66*	0.45+
121	0.52	0.50**	0.56	0.55	0.34*	0.55+
142	0.01	0.32**	0.00	0.00	0.00*	0.00
*sMDH-B**						
100	0.60	0.73*	0.61+	0.64+	0.60	0.48
114	0.40	0.27*	0.39+	0.36+	0.40	0.52

*sSOD-1**						
147	0.57	0.58	0.56	0.59	0.64	0.65
100	0.43	0.42	0.44	0.41	0.36	0.35

Table 3. Allele frequencies for Moultrie strain largemouth bass fingerlings used to stock study ponds in 1994 and 1995, with survey data of allele frequencies for L. Moultrie where stocks originated, and subsequent F_1 and F_2 generations. Alleles, or allele pairs, listed first are fixed (sAAT-2*, sIDHP-2*) or dominant in the Northern subspecies. Alleles listed second are fixed or dominant in the Florida subspecies. An * indicates a significant difference from survey data. A + indicates a significant difference from fingerlings stocked.

Locus/Allele	Survey data	1994 fing.	F_1	F_1	F_2	1995 fing.
	N=116	N=52	N=156	N=181	N=100	N=84
*sAAT-2**						
100, 110	0.10	0.19	0.00+	0.07+	0.14	0.07
126, 139	0.90	0.81	1.00+	0.93+	0.86	0.93
*sIDHP-1**						
100	0.02	0.00	0.00	0.05+	0.02	0.00
121	0.98	1.00	1.00	0.95+	0.98	1.00
142	0.00	0.00	0.00	0.00+	0.00	0.00
*sMDH-B**						
100	0.00	0.00	0.00	0.00	0.00	0.00
114	1.00	1.00	1.00	1.00	1.00	1.00
*sSOD-1**						
147	0.19	0.14	0.29+	0.17	0.13	0.25+
100	0.81	0.86	0.71+	0.83	0.87	0.75+

Table 4. Water quality parameters measured on 1994 stocked study ponds, Summer 1994-Fall 1996. Values are mean values for the three year course of sampling. Standard deviations are in parenthesis. Individual ponds are grouped by strain stocked (M = Moultrie, W = Wateree) and region (C = Coastal Plain, P = Piedmont) .

Pond Name	chl-a(µ g/l)(SD)	pH(SD)	hardness(SD)	alkalinity(SD)
M/C				
Mulberry	2.2 (0.8)	5.3 (3.8)	36.2 (35.4)	16.8 (14.3)
Price	4.6 (1.6)	8.1 (0.82)	34.7 (5.1)	25.2 (7.2)
Gollihugh	7.2 (3.0)	8.9 (0.73)	42.4 (9.1)	40.7 (22.8)
M/P				
Adams	2.7 (0.8)	7.9 (1.2)	13.0 (7.6)	11.2 (9.6)
Kirby	3.8 (1.4)	7.7 (1.0)	9.3 (3.2)	9.7 (1.9)
Cline	3.1 (10.3)	6.6 (2.8)	11.4 (2.8)	7.8 (1.7)
Lockridge	4.9 (1.0)	7.7 (0.9)	15.7 (5.1)	18.2 (5.5)
Beer, G	7.1 (1.3)	7.3 (0.6)	10.2 (2.9)	13.6 (0.4)
W/C				
Gift	2.9 (0.7)	7.9 (0.8)	54.0 (9.8)	45.0 (8.7)
Shelley	3.6 (1.3)	7.1 (0.7)	3.2 (1.4)	3.3 (1.8)
Carrol	4.9 (2.0)	6.4 (4.4)	50.8 (12.1)	35.6 (7.0)
Britton	7.0 (3.2)	7.5 (1.8)	16.5 (7.1)	21.8 (13.6)
New	6.3 (2.1)	6.9 (3.1)	15.1 (4.8)	11.4 (5.9)
Chelsea	8.8 (3.5)	7.7 (1.6)	41.5 (31.8)	27.4 (5.1)

W/P

Childress, C.	3.2 (0.4)	7.2 (0.4)	22.2 (3.3)	24.7 (1.9)
Coble	4.0 (0.7)	8.4 (1.1)	20.9 (1.4)	24.4 (3.0)
Meeks	4.6 (1.1)	8.2 (0.9)	14.9 (2.1)	15.0 (1.7)
Thackston	5.5 (1.4)	6.9 (3.2)	34.6 (6.3)	31.8 (5.9)
Beer, D.	9.5 (4.5)	8.8 (1.1)	35.7 (4.1)	22.5 (10.5)
Benfield	9.4 (4.0)	8.3 (1.0)	38.2 (9.3)	37.3 (8.0)

Table 4 continued

Pond Name	chl-a(µ g/l)(SD)	pH(SD)	hardness(SD)	alkalinity(SD)
Others				
Childress, K.$_{d}$	5.7 (1.5)	7.1 (0.2)	42.9 (0.8)	41.0 (5.3)
Davis$_{d}$	10.4 (3.2)	9.8 (0.2)	33.6 (13.6)	(-) (--)
Harrison$_{d}$	2.9 (--)	7.7 (--)	54.1 (--)	53.0 (--)
Minchey$_{d}$	4.8 (2.1)	8.1 (0.8)	44.6 (17.2)	32.5 (19.1)
Turner$_{d}$	4.2 (1.0)	7.2 (0.2)	35.7 (1.3)	41.6 (1.60)
Bennet$_{+}$	3.9 (1.3)	8.6 (0.8)	21.3 (7.5)	23.7 (7.4)
English$_{+}$	5.7 (2.7)	7.8 (3.4)	82.5 (50.2)	72.5 (30.7)
Helmly$_{+}$	4.4 (2.0)	6.1 (4.2)	56.8 (12.7)	90.2 (43.5)
Sims$_{+}$	5.0 (1.6)	5.8 (2.9)	32.4 (9.1)	19.1 (3.9)

$_{+}$ = ponds not included in growth analysis, but retained for future study
$_{d}$ = Ponds removed from study

Table 5. Water quality parameters measured on 1995 stocked study ponds, Summer 1995-Fall 1997. Values are mean values for the three year course of sampling. Standard deviations are in parenthesis. Ponds are grouped by strain stocked (M = Moultrie, W = Wateree) and region (C = Coastal Plain, P = Piedmont).

Pond Owner	chl-a (mg/L)(Std)	pH(Std)	hardness(Std)	alkalinity(Std)
M/C*				
Hughes	7.3 (3.0)	8.3 (1.1)	24.3 (1.3)	18.3 (3.7)
Shields	5.2 (1.2)	6.8 (3.3)	172.8 (33.4)	137.2 (24.4)
Platts	6.4 (3.2)	6.9 (3.5)	15.5 (4.6)	74.0 (14.6)
McCants	3.6 (0.8)	8.0 (0.9)	84.4 (6.2)	44.1 (7.2)
M/P				
Workman	7.8 (2.8)	8.0 (1.2)	16.9 (2.6)	17.5 (4.8)
Freeland	6.9 (3.6)	7.8 (0.9)	9.8 (1.8)	9.6 (2.5)
Patterson	8.3 (3.2)	7.3 (0.4)	15.3 (2.7)	19.2 (3.1)
W/C				

Ravenel	3.0	(1.1)	7.8	(0.3)		90.9				86.0
					(12.4)			(8.5)		
Branton	3.2	(1.6)	6.8	(3.4)		119.3	(27.0)			74.8
								(15.8)		
W/P										
Hanvey	4.6	(0.9)	7.1	(0.4)		28.1	(7.7)			20.8
								(3.8)		
Harrison	3.6	(1.3)	8.2	(0.9)		30.0	(5.7)			25.4
								(5.6)		
Holland	3.5	(1.2)	7.7	(0.7)		34.3	(5.6)			25.0
								(7.3)		
McGee	6.0	(5.5)	8.6			32.8				30.1
				(1.0)	(10.3)			(11.4)		

[Fish collections]

Age-1 largemouth bass were collected from 26 of 27 ponds, sampled from 6/15 to 7/27/95 and from 12 of 13 ponds sampled from 6/11 to 6/19/96. Mean growth rates were calculated for each pond by region and strain stocked (Table 6 and 7).

Largemouth bass age-1 to age-3 were collected from 23 of 24 ponds sampled in 1997, and from 12 of 12 ponds sampled in 1998. Age estimates were determined for 240 fish using scales for 1997 collections and otoliths for 1998 collections. Fifty-seven age-3 largemouth bass were identified.

Scales were also read for 1998 collections. These age estimates will be compared to those from otoliths for the same fish. Results will be used to verify the reliability of ages estimated from scales for this study.

Juvenile largemouth bass (n=1901) were collected with beach seines from 19 of 27 ponds in 1995, from 32 of 37 ponds in 1996, from 28 of 36 ponds in 1997, and from 10 of 12 ponds in 1998. Number collected per pond ranged from 10 to 33 for all but 6 ponds from which less than 10 fingerlings were collected. Average total length of fingerlings ranged from 29 mm to 134 mm for each pond sampled. Fingerlings were stored frozen until allozyme analysis. Analysis was completed for fingerlings collected from 1995 through 1997. Fingerlings collected in 1998 were lost due to a meltdown in the freezer in which they were stored.

[Data analysis]

Raw data for chlorophyll-a concentrations and alkalinity values were not normally distributed. A $\log_{10}$ transformation resulted in a normal distribution for both and transformed data was used for these two variables in linear regression analysis.

Chlorophyll-a data from 1994 was significantly (p=.05) related to pH, alkalinity, and

hardness. The equations produced by the linear regression analysis were:

a. log10(chlorophyll-a) = 0.17 * pH+ 0.14 ; $R^2 = 0.17$

b. log10(chlorophyll-a) = 0.40 * log10(alk) + 0.86 ; $R^2 = 0.19$

c. log10(chlorophyll-a) = 0.44 * log10(hard) + 0.82 ; $R^2 = 0.10$.

While all equations are significant, relatively low R-squared values indicate other factors are affecting chlorophyll-a concentration in the study ponds.

Data from five atypical ponds were removed from the data set. Largemouth bass from two of the ponds, Helmly and English, were stunted due to limited or zero bream reproduction and therefore minimal forage availability. Three other ponds, Bennet, Minchey, and K. Childress, were removed because introduced fish had severely impacted forage availability to stocked largemouth bass.

Mean growth for all age 1 largemouth bass collected in 1995 and 1996 was 0.57 mm per day (sd=0.09,N=539). Growth was computed for each fish at 386-474 days post stocking. Analysis showed that region and pH were significant predictors of growth rate. The test of least squares means showed no significant difference between growth rates of the two strains or between the interactions of strain and region. The difference between regions was significant at p=.05 with largemouth bass stocked in Coastal Plain ponds growing faster, 0.61 mm per day (sd=0.11,N=215), than those stocked in Piedmont ponds (0.55 mm per day, sd=0.09,N=324).

Mean growth for all age-3 largemouth bass collected in 1997 and 1998 was 0.29 mm/day (sd=0.04, N=57). Growth was computed for each fish at 1107-1197 days post stocking. Analysis showed no significant difference in growth rate due to strain or the interaction between region and strain.

Table 6. Mean growth rate for age-1 largemouth bass collected in 1995. Individual ponds are grouped by strain stocked (M = Moultrie, W = Wateree) and region (C = Coastal Plain, P =

Piedmont).

Pond Name	Growth Rate (mm/d)	Standard deviation	No. Adults
M/C			
Mulberry	0.59	.04	17
Price	0.65	.07	15
Gollihugh	0.66	.03	18
M/P			
Cline	0.45	.09	14
Adams	0.48	.03	9
Beer, G	0.54	.03	14
Kirby	0.57	.07	20
Lockridge	0.58	.09	6
W/C			
Britton	0.46	.08	11
New	0.62	.03	21
Chelsea	0.65	.04	19
Carrol	0.67	.03	20
Gift	0.72	.05	3
Shelley	0.74	.03	3
W/P			
Childress, C	0.44	.03	21
Benfiel	0.52	.03	19
Meeks	0.55	.02	19
Thackston	0.58	.03	29
Beer, D.	0.59	.02	15
Coble	0.64	.05	18
Others			
Helmly$_{+}$	0.30	.02	8
Bennet$_{d}$	0.36	.08	12
English$_{+}$	0.42	.05	6
Sims$_{+}$	0.44	.10	15
Childress, K.$_{d}$	0.49	.16	19
Minchey$_{d}$	0.73	.06	13

$_{d}$ = ponds removed from the study

$_{+}$ = ponds not included in growth analysis but retained for future study

Table 7. Mean growth rate for age-1 largemouth bass collected in 1996. Individual ponds are grouped by strain stocked (M = Moultrie, W = Wateree) and region (C = Coastal, P = Piedmont).

Pond Owner	Growth Rate (mm/day)	Standard Deviation	No. Adults
M/C*			
Hughes	0.70	0.07	19
Shields	0.59	0.02	20
Platts	0.48	0.04	19
McCants	0.66	0.16	7
M/P			
Workman	0.55	0.12	26
Freeland	0.62	0.12	19
Patterson	0.53	0.03	21
W/C			
Ravenel	0.78	0.06	8
Branton	-	-	0
W/P			
Hanvey	0.60	0.04	17
Harrison	0.57	0.07	30
Holland	0.38	0.07	11
McGee	0.59	0.07	19

There was a significant difference (p=0.05) between regions, with fish in the Coastal Plain growing more (0.31 mm per day, sd=0.04, N=29) than fish in the Piedmont (0.27 mm per day, sd=0.04, N=28).

Allozyme analysis was completed and allele frequencies computed for fingerlings collected in 1995 through 1997. Table 2 (Wateree strain) and Table 3 (Moultrie strain) include data collected in 1995 and 1996. This includes allele frequencies for F_1 and F_2 generations from 1994 stocked ponds and F_1 generations from 1995 stocked ponds. G-test comparisons showed significant deviations (P=0.05) from Wateree strain parental stocks at one locus each for both the F_1 and F_2 generations from 1994 stocks, and the F_1 generation from 1995 stocks. (Because of the potential discrepancy in *sIDHP-1** data for the 1994 Wateree strain, comparisons at that locus were made using allele frequencies for the Lake Wateree largemouth bass population.) Significant (P=0.05) deviations from Moultrie strain parental stocks were present at two loci each for both the F_1 and F_2 generations from 1994 stocks, and at one locus for the F_1 generation from 1995 stocks.

The latest allele frequency data from all ponds are those collected in 1997 and is included in Table 8. This includes the pooled data from F_3 and F_2 generation fingerlings from ponds stocked in 1994 and 1995, respectively. Allele frequencies are reported in four groups as defined by the two regions and two strains stocked. G-test comparisons were made between regions for each strain. For ponds stocked with the Wateree strain, significant differences exist at all four loci. At three of these loci, Piedmont ponds possess significantly more alleles typical of the northern subspecies than Coastal Plain ponds. For ponds stocked with the Moultrie strain differences between regions are significant at two loci, where Coastal Plain ponds possess more alleles typical of the Florida subspecies.

Table 8. Allele frequencies for fingerlings collected in 1997 from ponds stocked in 1994 and 1995. G-test statistics are reported for comparisons between regions for each strain stocked. A G value of 3.84 is significant at p=.05 (*), and of 6.63 at p=.01(**). Abbreviations are P=Piedmont, C=Coastal Plain, W=Wateree strain, M=Moultrie strain. N = number of fish sampled.

Locus/Allele	P/W (N)	C/W (N)	G	P/M (N)	C/M (N)	G
*sAAT-2**	(209)	(128)		(192)	(102)	
100/110	0.46	0.55	5.56*	0.12	0.06	6.01*
126/134	0.54	0.45		0.88	0.94	
*sIDHP-1**	(188)	(125)		(173)	(104)	
100	0.47	0.36	7.23**	0.02	0.01	1.47
121	0.53	0.64		0.98	0.99	
*sMDH-B**	(186)	(117)		(189)	(92)	
100	0.73	0.46	43.88**	0.06	0.00	11.14**
114	0.27	0.54		0.94	1.00	
*sSOD-1**	(185)	(126)		(181)	(103)	
100	0.36	0.48	8.04**	0.84	0.81	1.04
147	0.64	0.52		0.16	0.19	

Discussion

Differences in growth for fish stocked in the Coastal Plain vs. those stocked in the Piedmont followed the same trend from age-1 - age-3. Throughout the study fish exhibited significantly greater growth in the Coastal Plain, a milder climate with a longer growing season.

High pond to pond variation, and small sample sizes of age-3 largemouth bass, may affect our ability to detect growth differences between largemouth bass strains in each region. Only

N=12-17 age-3 fish are included in each of four groups defined by region and strain.

A study design where ponds were stocked with equal numbers of both strains would have minimized the effect of pond to pond variation, increasing our power to detect growth differences due to strain. This approach was not chosen because of difficulty in marking the fingerlings. A larger sample size of all age-3 largemouth bass also would have added to the power of our data set. Unanticipated difficulty in collecting 3 year olds could have been avoided by total sampling (i.e. draining and rotenone renovation) of each pond. This was not considered due to the private ownership of each pond site.

Changes in allele frequencies of largemouth bass fingerlings over time will provide direct information on what genotypes are most successful in each region. Results for 1997 collections indicate selection in the piedmont ponds favors alleles typical of the Northern subspecies. As successive generations are added to the database, our power in detecting a shift in allele frequencies due to selection will grow.

Reported genetic data for 1994 fingerlings of the Wateree strain appears to us to be incorrect. We have not been able to resolve the apparent discrepancy and therefore have not used those data in comparing filial generations with original stocks.

Genetic comparison of Wateree fingerlings produced in both 1994 and 1995 illustrates the need to ensure as many parents as possible are contributing to hatchery stocks.

Recommendations

Continue study. Compare age estimates from scales and otoliths for adults collected in 1998. Apply results of that comparison, if approriate, to adults collected and aged in 1997 and repeat analysis of differences in growth at age-3. Complete genetic analysis of 1999 progeny (F_3 and F_4 generations) and analyze differences between regions for each strain.

Implement standard hatchery procedures that are aimed at maximizing genetic diversity and minimizing unnatural selection. These would include maximizing the number of parents contributing to each year class produced, and avoiding inbreeding events by regular collection of wild broodstock and not adding hatchery produced fish to the broodfish pool.

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Prepared by: Jean K. Leitner Title: Biologist

JOB PROGRESS REPORT

STATE: South Carolina PROJECT NUMBER: F- 63

PROJECT TITLE: Fisheries Investigations in Lakes and Streams - Statewide

STUDY: Survey and Inventory

STUDY TITLE: Fishery surveys - Statewide Fisheries Research

JOB TITLE: Genetic survey Brown Trout at Walhalla State Fish Hatchery

Introduction

The brown trout, *Salmo trutta*, is native to Europe, northern Africa and western Asia. Brown trout were first introduced to North America in 1883 in New York and Michigan. They are now commonly propagated and stocked throughout southern Canada and much of the United States (Rhode et al. 1994, Page and Burr 1991). In South Carolina brown trout are produced at Walhalla State Fish Hatchery from a broodstock maintained on site.

Genetic variation, or heterozygosity, is an important component effecting the overall fitness of trout stocks. Over the long term, it provides them with the flexibility to adapt to changing environmental conditions (Krueger er al. 1981, Danzmann et al. 1989). In a hatchery environment there are a number of factors that can result in reduced heterozygosity of stocks. Founder effects occur when a small number of individuals are used to establish a hatchery population (Hansen and Mensberg 1996). Genetic bottlenecks can affect hatchery stocks when some event acts to reduce an established population. Inbreeding is a concern when hatchery produced fish are held over for use as brood, potenially resulting in the pairing of closely related individuals (Kincaid 1976, 1995).

Selection over time can also result in changes in the genetic makeup of hatchery stocks. Natural selection within the hatchery environment may favor particular genotypes, and can differ

from the selective pressures fish face in the wild. Human selection, both purposeful and inadvertent, can also play a role (Kincaid 1995).

It has been shown that reduced genetic variation in hatchery broodstocks of trout can result in reduced fitness and survival of their progeny (Alexander and Hubert 1995, Alendorph and Phelps 1980). The purpose of this study was to compare allele frequencies of the Walhalla brown trout broodfish population to those calculated in a 1988 survey, and to determine present levels of genetic diversity.

Methods

Forty brown trout, 20 males and 20 females, were collected from the broodfish population at Walhalla National Fish Hatchery (now Walhalla State Fish Hatchery) in January of 1998. Liver, muscle and eye tissues were excised from each fish, placed in labeled cryogenic vials, and stored on dry ice for transport to the lab. Samples were stored at -80 degrees Celsius until shipment to Auburn University for genetic analysis.

Genetic analysis was performed at thirty-six biochemical loci (Table 1), consistent with those used in 1988, using horizontal starch gel electrophoresis following the procedures of Steiner and Joslyn (1979), Philipp et al. (1982) and Norgren et al. (1986). Allele frequencies were calculated. The G-test (Sokal and Rohlf, 1969) was used to compare allele frequencies to baselines established in 1988.

F-statistics, also called inbreeding coefficients, were calculated. F_{IS} measures loss of heterozygosity in individuals due to nonrandom mating within their sub-population (1988 or 1998). F_{IT} measures the reduction in heterozygosity of an individual relative to the total population (1988 and 1998 combined). F_{ST} measures the effects of population subdivision, or the reduction in heterozygosity of a sub-population due to random genetic drift (Hartl and Clark,

1989). When F-statistics are close to zero, then random mating has occurred and genetic variation is distributed as if there is one population.

Table 1. Enzymes and loci analyzed in 1998 survey of Walhalla National Fish Hatchery brown trout brood fish.

Enzyme	Number of loci	Loci abbreviations
Aspartate aminotransferase	4	*sAAT-1,2,3,4**
Aconitate hydrase	2	*sAH*, mAH**
Alcohol dehydrogenase	1	*ADH**
Asenylate kinase	1	*AK-1**
Creatine kinase	3	*CK-A,B,C**
Esterase	2	*EST-1,2**
Fructose bi-phosphate aldolase	1	*FBALD**
Fumerate hydratase	1	*FH**
Glutamate dehydrogenase	1	*GLUDH**
Fructose-biphosphatase	1	*FBP**
Glycerol-3-phosphate dehydrogenase	1	*G3PDH**
Glucose-6-phosphate isomerase	3	*GPI-1,2,3**
Isocitrate dehydrogenase	2	*sIDHP-1,2**
Lactate dehydrogenase	4	*LDH**
Malate dehydrogenase	2	*sMDH*, mMDH**
Malic enzyme	2	*sMEP**
Pepdidase	1	*PEP**
Phosphogluconate dehydrogenase	1	*PGDH**
Phosphoglucomutase	1	*PGM**
General protein	1	*PROT*
Superoxide dismutase	1	*sSOD**

Results

Brown trout tissues collected in 1988 and 1998 were analyzed at thirty-six enzyme loci. Six of these loci were polymorphic. Allele frequencies at these six loci were tested for differences between the two surveys using the G-test. Significant differences were detected at two loci, *sAAT-3** and *FBP**. At *sAAT-3** the 1988 survey indicated the presence of the *sAAT-3*78* allele at a frequency of 0.16, while the 1998 survey did not detect this allele. Rather, all fish surveyed in 1998 were fixed for the *sAAT-3*100* allele. At *FBP** the opposite situation exists. All fish surveyed in 1988 were fixed for the *FBP*100* alllele. However, the 1998 survey detected an alternate allele, *FBP*90*, at a frequency of 0.236. Table 2 lists allele frequencies at the six polymorphic loci for each survey, the corresponding G-test statistics, and the number of fish included in analysis.

F-statistics were calculated for each of the six polymorphic loci and are listed in Table 3 along with their means. F_{IS} and F_{IT} values for two loci, *FBP** and *GPI-2**, are considerably higher than for the other loci. Both of these values deal with inbreeding in individuals, with elevated values indicating a lack of heterozygotes.

Discussion

Allele frequency differences between the 1988 and 1998 surveys could be due to a number of factors. The addition of an allele at *FBP** may be due to the introduction of fish to the hatchery population. Annual reports for Walhalla National Fish Hatchery for 1988 state that a strain of brown trout, not previously tested on the station, was brought on site and was intended to be crossed with the Walhalla brown trout. Though there is no mention that they were ever used for this purpose, allele frequency data indicate otherwise. The loss of an allele at *sAAT-3** could

be due to selection in the hatchery against certain genotypes. The introduction of fish could also play a role here, if the introduced fish lacked the allele that was lost. F_{IS} and F_{IT} values are considerably higher for *FBP** and *GPI-2** than for the other loci examined. Elevated F statistics indicate a lack of heterozygotes in a population. This can be due to inbreeding or to selection against heterozygotes.

If inbreeding was in fact a problem with the Walhalla brown trout population, one would expect the F values to be more evenly distributed across all loci. It appears more likely that some selective force within the hatchery is acting on the loci *FBP** and *GPI-2**. Overall F-values do not indicate significant loss of genetic diversity over the ten year span from the 1988 sampling and the 1998 sampling.

Table 2. Allele frequencies and number of fish analyzed at six polymorphic loci for brown trout sampled from Walhalla Fish Hatchery in 1988 and 1998. Corresponding G-test statistics are included for survey comparisons. A G-test statistic of 3.84 or greater (*) is significant at P=0.05.

Locus and alleles	1988 Survey (N)	1998 Survey (N)	G
*sAAT-3**	(50)	(38)	
78	0.160	0.000	13.36*
100	0.840	1.000	
*FBP**	(50)	(36)	
90	0.000	0.236	25.39*
100	1.000	0.764	
*G3PDH**	(50)	(35)	
	0.090	0.171	2.86

72			
	0.910	0.829	
100			
*GPI-2**	(50)	(40)	
	0.951	0.875	3.27
100			
	0.049	0.125	
106			
*sMDH**	(50)	(38)	
	0.020	0.053	1.39
0			
	0.980	0.947	
100			
*mMDH**	(50)	(38)	
	0.740	0.789	0.59
100			
	0.260	0.211	
152			

Table 3. F-statistics calculated at all polymorphic loci for brown trout collected from Walhalla Fish Hatchery in 1988 and 1998.

Locus	F_{IS}	F_{IT}	F_{ST}
*sAAT-3**	-0.042	0.049	0.087
*FBP**	0.461	0.533	0.134
*GPI-2**	0.358	0.370	0.018
*G3PDH**	-0.167	-0.150	0.015
*sMDH**	-0.046	-0.038	0.008
*mMDH**	0.131	0.134	0.003

Mean	0.107	0.148	0.047

Hatchery populations are vulnerable to loss of genetic diversity due to inbreeding. They may also experience genetic change over time due to onsite selective forces, both environmental and human. These changes can result in reduced fitness of fish produced for stocking. Hatchery protocols in place at Walhalla have been successful in protecting stocks from significant inbreeding. There is evidence, however, that selection may be acting on the broodfish population causing a shift in the proportion of heterozygotes over time.

Recommendations

Continue with hatchery protocols aimed at protecting against inbreeding. These should include attempts to maximize the number of parents contributing to each spawning season. Periodic introduction of brown trout from appropriate off-hatchery sources to the brood pool can help minimize effects of selection on progeny ultimately produced for stocking. Introduced fish should be of the same strain. A routine genetic survey of hatchery stocks should be performed periodically, or anytime managers suspect a problem, so that appropriate corrective measures can be taken.

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JOB PROGRESS REPORT

STATE: South Carolina PROJECT NUMBER: F- 63

PROJECT TITLE: Fisheries Investigations in Lakes and Streams - Statewide

STUDY: Survey and Inventory

JOB TITLE: Growth dynamics of larval striped bass in Lake Marion mesocosms

INTRODUCTION

Larval growth dynamics play a critical role in determining recruitment. Relatively slow growth increases time spent in highly vulnerable life stages, increasing mortality potential (Shepherd and Cushing 1980; Houde 1987). Rutherford and Houde (1995) showed that average annual cohort mortality rates of Potomac River striped bass larvae were inversely related to growth rates. Pepin (1989) demonstrated that the mean instantaneous growth rate of a population is largely a function of the available food.

In the Santee-Cooper system, evidence exists that the timing, location, and density of zooplankton influences recruitment. Bulak et al. (1997) and Chick and Van Den Ayvle (1999) found higher densities of zooplankton, especially rotifers in the headwaters of Lake Marion as opposed to the incoming tributaries. Bulak et al. (1997) noted highest recruitment was associated with a spawning cohort that hatched in Lake Marion. Chick and Van Den Ayvle (1999) reported that zooplankton density levels that supported successful larval foraging in laboratory experiments were present in Lake Marion in two of three study years, but were never observed in the inflowing tributaries.

The objective of this study was to quantify the growth and feeding selectivity of striped bass larvae in the headwaters of Lake Marion. Previous efforts to obtain samples of striped bass larvae from Lake Marion were unsuccessful. Thus, field experiments were conducted in large

scale mesocosms, designed to simulate the foraging environment found in Lake Marion.

METHODS

Monitoring

Water quality and zooplankton densities in Lake Marion's headwaters were monitored during the spring of 1996 and 1997. One sampling site, Quetti Cut (33°35'36" x 80°30'36"), was located in the Santee River, just before the river entered Lake Marion. Three other sites were located in Lake Marion. These sites were at Marker 159 (33°34'07" x 80°30'25"), Brown's Lake (33°34'06" x 80°29'04"), and Dergan's Creek (33°35'06" x 80°28'52"). Surface water temperature and conductivity were routinely monitored using a YSI conductivity meter; water transparency was measured with a secchi disk. Temperature and dissolved oxygen profiles at 1, 3, and 5m were occasionally taken during the study.

Ambient zooplankton densities were estimated by two methods. Vertical hauls to a depth of 5 m were taken with a 0.5 m diameter plankton net (mesh = 80 µm). Alternatively, 2.5 L water samples were collected at depths of 1, 2.5, and 4 m with a whole water sampler. Zooplankton samples were preserved in a solution of 3% sugared formalin and Rose Bengal. All sampling took place during daylight hours.

Experiments were conducted in 1.25 m^3 mesocosms (5 m long x 1 m diameter with 20 µm mesh walls) that were suspended in Lake Marion. Three frames, each holding three mesocosms, were constructed of angle iron and styrofoam floats; each frame was securely anchored to the lake bottom. The mesocosms were attached to the frame. The top end of each mesocosm was held approximately 0.5 m above the lake's surface while the bottom was tied shut and a small weight was attached to maintain a vertical position in the water column. In both study years, mesocosms were deployed in Brown's Lake, a submerged oxbow lake of Lake Marion. Average depth in

Brown's Lake was nearly 10 m; the area surrounding Brown's Lake was generally 2 to 5 m deep.

Striped bass larvae that were stocked into the mesocosms were obtained from the Bayless striped bass hatchery, Bonneau, SC. The goal was to obtain 2-3 d old striped bass larvae from one mating for each experiment. At the hatchery, the number of larvae obtained from rearing aquaria with a dip of a 250 ml beaker was measured at least three times. Using this estimate, we aimed to put approximately 400 larvae in each mesocosm. The larvae were transported to the lake in sealed, oxygenated, shipping bags. Transport survival of striped bass in the shipping bags was estimated. Several hundred larvae were preserved at the hatchery to provide an estimate of the length and weight at stocking.

In 1996, the study objective was to maintain total zooplankton densities of 100, 1,000, and 10,000/L in the mesocosms. Each zooplankton treatment was replicated three times. Larval striped bass samples were obtained at the end of the experiment; they were preserved in 5% formalin.

In 1997, 'low' and 'high' zooplankton treatments were replicated in four mesocosms. Striped bass larvae from a high and low density treatment were sampled and preserved every 3-4 days until the last mesocosm was harvested.

Preserved striped bass larvae were weighed and measured in the lab. Measurements of standard and total length of individual larvae were made using an ocular micrometer; larvae that were severely bent or damaged were not measured. Dry weight of individual larvae was also obtained. Aluminum weighing vessels were ashed at 425°C, cooled in a dessicator, and then weighed to the nearest µg on a Cahn electrobalance. Larvae were then placed in the pre-weighed vessel, dried at 60°C for 24 hours, and cooled in a dessicator. The weighing vessel was then re-weighed and larval weight was determined.

Zooplankton to initially stock the mesocosms were obtained by concentrating the catch of

multiple, vertical zooplankton pulls. Total zooplankton per liter of the concentrate was checked periodically in the field using a 1 ml Sedgewick-Rafter cell and inverted compound microscope. Once the concentrate reached the desired density, zooplankton were stocked into the mesocosms.

Zooplankton densities in the mesocosms were monitored during each experiment. A 2.5L whole water sample was obtained at depths of 1, 2.5, and 4 m. Samples were combined and the average density of zooplankton was determined. Depending on results, zooplankton were either added to or taken from - with a plankton net - the mesocosms to maintain the desired densities.

Preserved zooplankton samples from the field and mesocosms were enumerated and identified in the lab. The entire sample was poured into a 10 mm diameter PVC pipe fitted with a 39 µm screen. If visual inspection indicated the sample did not contain many organisms (i.e. < 1,000), the entire sample was rinsed into a 15 ml graduated sedimentation tube. The sample was then allowed to settle for about one hour, allowing the preserved zooplankton to settle to the bottom. After one hour, the top layer was pipetted off, leaving a final volume of 4-5 ml in the sedimentation tube. The sample was then placed in several, 1 ml Sedgewick-Rafter cell for enumeration of the total sample. If the sample did contain many organisms, the sample was diluted to 25, 50, 100, or 200 ml., depending on the concentration of the sample.

In 1997, food contents of striped bass larvae were determined. Larvae were placed in glycerin and the stomach was teased out, intact, using dissecting pins. Under a hood, the stomach was then placed in a drop of CMCP-10 high viscosity mountant (Polysciences, Inc., Warrington, PA) containing Rose Bengal stain. Stomach contents were teased out with dissecting pins. A cover slip was then placed on the sample and sealed to the slide with clear fingernail polish. Stomach contents were then identified under a compound microscope.

RESULTS

Water quality in the study area was intermittently monitored in 1996 and 1997. In 1996, monitoring occurred on April 3, 12, and 18 and May 15 and 22. Surface water temperature ranged from 14.0°C at Marker 159 on April 3 to 28.5°C at Dergan's Creek on May 22. Mean surface water temperature and conductivity were somewhat greater at Dergan's Creek than Marker 159 or Brown's Lake (Table 1). In 1997, monitoring occurred on April 9, 16, 22, and 29 and May 7 and 20. In 1997, surface temperature ranged from 17.1°C at Marker 159 on April 9 to 23.5°C at Dergan's Creek on May 20. Mean surface water temperature, conductivity, and secchi disk transparency were somewhat greater at the Dergan's Creek sampling station while dissolved oxygen was lower (Table 1).

Lake Marion zooplankton densities varied temporally and by sampling gear and were dominated by rotifers (Tables 2 and 3). On days when samples were collected with both types of gear, densitites obtained with 2.5 L whole water samplers were generally greater than those obtained with vertical plankton net hauls (Tables 2 and 3). On 11 of 12 vertical haul sampling dates, total zooplankton density was less than 100/L. On whole water samplng dates, 4 of 12 samples had a total zooplanton density of more than 100/L. A peak density of 791 rotifers/L was recorded at Brown's Lake on 5/26/97 while a peak density of 101 copepod nauplii/L was recorded on 5/29/97 at Brown's Lake; both samples were collected with the whole water sampler.

Zooplankton exhibited spatial variability and patchiness. On 5/1/97, 5 whole water samples were taken from Brown's Lake where rotifer and adult copepod densities ranged from 78 to 982/L and 0 to 12/L, respectively. On 5/29/97, mean total zooplankton densities ranged from 10 to 267/L at the four sampling sites, with highest densities at Dergan's Creek..

A total of 44 types of zooplankton were identified from Lake Marion (Table 4). Of the 27 genera of

collected rotifers, Synchaeta, Polyarthra, Keratella, Conochilus, and Brachionus were the only ones whose percent occurrence exceeded 5% in either 1996 or 1997. Cyclopoids dominated the adult copepods while Bosmina was the dominant cladoceran. Larval nematodes dominated the 'other' types grouping of zooplankton. Mean lenngth and width were measured for 19 types of zooplankton (Table 5).

Table 1. Mean surface temperature (°C), dissolved oxygen (mg/L) at depth of 1m, conductivity (µmhos/cm), and secchi dish transparency (meters) at three sites in the headwaters of Lake Marion in April and May, 1996 and 1997. Sample size in parenthesis.

Location	Year	Temperature	Dissolved Oxygen	Conductivity	Transparency
Marker 159		18.4 (5)	-	78 (5)	0.8 (4)
Brown's Lake	1996	19.0 (5)	-	82 (5)	1.0 (4)
Dergan's Lake		20.0 (5)	-	96 (5)	1.0 (4)
Marker 159		19.7 (5)	6.6 (3)	81 (6)	0.8 (6)
Brown's Lake	1997	19.6 (5)	6.4 (3)	87 (6)	0.8 (6)
Dergan's Lake		20.2 (5)	5.7 (3)	100 (6)	0.9 (6)

Table 2. Mean density (N/L) of zooplankton in upper Lake Marion, 1996-97. Samples were obtained with vertical hauls of a 0.5m diameter net.

				Copepoda			
Year	Date	Sites	Rotifera	Nauplii	Adults	Cladocera	Other
	4/24	1	53.7	0.6	0.5	0.5	1.3
	4/29	3	12.6	1.6	0.2	1.2	0.8

1996	5/07	3	1.1	0.7	0.1	0.1	0.6
	5/15	3	2.0	0.2	0.1	<0.1	0.4
	5/22	3	10.0	0.2	<0.1	0.1	0.5
	4/09	4	3.0	0.8	0.1	0.2	1.8
	4/16	4	2.8	0.6	<0.1	0.1	1.7
	4/22	4	14.0	1.6	0.1	0.1	2.2
1997	4/30	4	2.3	0.8	0.2	0.1	2.0
	5/07	4	2.3	0.7	0.1	0.1	2.0
	5/20	4	8.8	0.8	0.1	0.1	0.9
	5/29	4	104.7	1.7	0.2	0.4	6.6

Table 3. Mean density per liter of zooplankton in upper Lake Marion, 1996-97. Samples were obtained with 2.5L whole water samples taken at 3 depths at several sites.

				Copepoda			
Year	Date	Sites	Rotifera	Nauplii	Adults	Cladocera	Other
	4/03	3	5.4	2.7	0.1	0.6	0.8
	4/04	3	3.0	2.0	0.1	0.3	0.7

	4/12	3	5.7	2.3	0.1	0.3	0.8
1996	4/18	3	3.5	1.9	<0.1	0.4	3.1
	4/22	1	33.9	0.7	0.4	0.7	4.1
	5/01	3	305.3	10.1	2.9	2.3	49.2
	5/07	2	11.9	3.3	0.3	0.9	3.9
	5/15	2	29.3	1.2	0.3	0.4	5.8
	5/20	1	23.5	0.0	0.3	0.0	0.9
1997	5/23	1	649.9	0.1	0.0	0.3	3.3
	5/26	1	790.7	0.0	0.0	0.7	31.3
	5/29	1	160.7	101.1	4.7	1.3	14.3

Table 4. Percent occurrence, by major type (bold), of the zooplankton community of upper Lake Marion and experimental mesocosms. Data was obtained in April/May, 1996 and 1997. A 0.5 m, 80 micron net was hauled vertically to obtain Lake samples; a 2.5 L whole water sampler was used to sample the mesocosms.

Type	Lake Marion		Mesocosms	
	1996	1997	1996	1997
Rotifera				
Synchaeta	75.12	33.88	22.97	67.42
Polyarthra	7.08	22.68	36.22	14.04
Keratella	4.19	38.42	16.61	9.44
Conochilus	7.04	2.62	11.50	5.14
Brachionus	5.01	0.91	2.00	0.06
Filinia	0.18	0.17	0.14	0.02
Monostyla	0.06	0.29	<0.01	0.03
Lecane	0.01	0.06	-	-
Mytilina	0.01	0.01	-	-
Platyias	0.07	-	0.01	-
Notholca	0.01	0.01	-	-
Kellicotia	0.07	0.13	0.01	0.01
Trichocerca	0.02	0.33	0.02	0.11
Hexarthra	<0.01	0.01	0.01	-
Ploesoma	<0.01	0.10	0.10	0.99
Asplanchna	0.84	0.08	10.03	<0.01
Testudinella	0.01	0.03	<0.01	<0.01
Euclanis	0.25	0.14	0.07	0.06
Gastropus	0.01	>0.01	<0.01	0.01
Pompholyx	0.01	>0.01	<0.01	-
Collotheca	0.01	0.02	0.32	2.62
Encentrum	0.01	0.01	-	-
Ascomorpha	<0.01	>0.01	-	-
Cephalodella	-	0.07	-	0.01
Macrochaetus	-	0.01	-	-
Lepadella	-	0.04	-	0.01
Manfredium	-	0.01	-	0.01
Copepoda				
Cyclopoid	85.64	83.66	68.07	57.65
Calanoid	14.36	16.34	31.93	42.35
Cladocera				
Bosmina	74.82	48.71	93.06	98.67
Chydorus	15.90	34.18	0.76	-
Daphnia	2.30	2.83	1.52	-
Ceriodaphnia	3.58	5.35	2.87	-
Diaphanosoma	3.31	8.86	0.78	-
Holopedium	-	0.07	-	0.66
Sida	0.09	0.11	1.00	0.66
Others				
Nematoda	88.93	81.41	98.84	98.85
Corbicula	1.30	13.36	-	-
Chironomidae	5.22	3.21	0.63	0.95
Ostracoda	2.30	0.82	0.15	0.05
Oligochaeta	0.59	0.72	0.03	-
Acarina	1.00	0.25	0.26	0.15
Ephemeroptera	0.22	0.14	-	-
Plecoptera	0.37	0.07	0.07	-
Amphipoda	-	0.01	0.01	-

Diptera	0.07	-	0.01

Table 5. Mean length and width (microns) of selected zooplankton collected from Lake Marion, SC, in spring of 1996. Standard error denoted in parentheses.

Type	N	Mean Length	Mean Width
Rotifera			
Synchaeta	50	136 (4.94)	91 (2.42)
Polyarthra	50	96 (1.82)	66 (1.13)
Keratella	50	125 (2.53)	58 (1.13)
Conochilus	50	84 (1.41)	65 (0.79)
Asplanchna	50	311 (10.84)	209 (6.60)
Brachionus	50	135 (3.39)	106 (3.22)
Ploesoma	13	124 (16.65)	89 (10.83)
Collotheca	19	86 (3.28)	49 (1.07)

Monostyla	10	109 (5.27)	97 (3.17)
Euclanis	22	154 (8.02)	99 (5.55)
Copepoda			
nauplii	50	161 (7.14)	92 (3.42)
adult cyclopoid	50	551 (29.15)	202 (8.11)
adult calanoid	37	807 (42.00)	212 (9.11)
Cladocera			
Bosmina	50	260 (8.27)	193 (8.21)
Daphnia	5	735 (110.20)	318 (62.94)
Ceriodaphnia	24	476 (43.54)	257 (24.26)
Diaphanosoma	10	588 (40.91)	203 (9.72)
Chydorus	33	278 (11.84)	224 (6.55)
Other			
Ostracod	7	291 (40.45)	163 (32.95)

Experiment 1.

The first mesocosm experiment was initiated on 4/27/96 and harvested on 5/8/96. Water temperature averaged 22°C. Due to poor survival during transport, striped bass larvae were stocked into the mesocosms on two dates. Six-day old larvae with a mean standard length of 5.48 mm (Standard error (SE) = 0.02) and a mean weight of 144.88 mg (SE = 1.45) were stocked into one set of three treatments on 4/27. Eight-day old larvae with a mean standard length of 6.20 mm (SE = 0.02) and a mean weight of 144.88 µg (SE = 1.45) were stocked into the other two treatments on 4/27.

Zooplankton densities were monitored on three separate days. Densities increased with time, peaking near the end of the experiment (Table 6). Among treatments, there was not a substantial difference in density, which averaged nearly 200 total zooplankton per liter (Table 7), though, nauplii, adult copepod , and cladocera density were highest in the highest zooplankton treatment. Only 58 larvae were harvested. Negative weight growth occurred in 6 of 9 treatments, though 2 of 3 high zooplankton treatments did have positive growth rates; seven of nine treatments experienced positive increases in length (Table 8).

Table 6. Mean density per liter of zooplankton on three dates in three experimental treatments within Lake Marion mesocosms. For each treatment, there were three replicates. Samples were taken at three depths with a 2.5 L whole water sampler.

				Copepoda			
Exp.	Dates	N	Rotifera	Nauplii	Adults	Cladocera	Other
	4/29	9	59.2	0.9	0.9	3.4	9.7
1	5/2	9	82.0	1.9	0.7	2.2	9.1
	5/6	9	431.2	6.3	1.5	9.2	44.4
	5/16	9	528.0	1.6	0.4	2.0	25.5
2	5/20	9	1482.1	8.5	0.6	11.9	39.2
	5/22	9	2451.1	21.1	1.6	28.3	31.5

Table 7. Mean density per liter of zooplankton in three treatments within Lake Marion mesocosms in 1996. There were three replicates of each treatment. Samples were taken at three depths with a 2.5L whole water sampler.

					Copepoda			
Exp.	Dates	Treatment	N	Rotifera	Nauplii	Adults	Cladocera	Other
		100	9	183.5	1.7	0.7	1.7	12.6
1	4/29-5/6	1,000	9	232.5	2.5	0.6	2.7	26.3

		10,000	9	156.3	4.9	1.9	10.3	24.4
		100	9	1155.5	2.1	0.3	1.6	33.5
2	5/16-/522	1,000	9	1682.0	12.0	0.6	9.0	33.6
		10,000	9	1623.7	17.1	1.7	31.5	29.0

Table 8. Survival and growth of striped bass larvae in Lake Marion mesocosms of varying zooplankton (Zoop) densities. Six and eight day old larvae were stocked on 4/27 and 4/29/96, respectively; 17-day old larvae were harvested.

Treatment (Zoop/L)	Replicate	No. of survivors	Mean SL[1], mm (N)	Growth, SL/day	Mean weight, µg (N)	G[2]
	L	7	6.52 (4)	0.04	140 (4)	-0.08
100	M	2	6.78 (2)	0.12	171 (2)	0.16
	U	3	5.78 (2)	-0.05	103 (2)	-0.39

	L	8	6.21 (8)	0.00	132 (8)	-0.14
1,000	M	2	5.24 (1)	-0.02	65 (1)	-0.80
	U	9	6.35 (4)	0.02	122 (4)	-0.22
10,000	L	9	7.58 (9)	0.15	314 (9)	0.73
	M	10	7.35 (7)	0.17	216 (7)	0.40
	U	8	6.50 (5)	0.03	133 (5)	-0.13

[1] - SL = standard length

[2] - G = instantaneous rate of growth from stocking to harvest

Experiment 2

The second mesocosm experiment was initiated on 5/13/96 and harvested on 5/24/96. Water temperature averaged 23.7°C, generally increasing as the experiment proceeded.. Due to poor survival of striped bass transported to the lake on 5/13/96, larvae were stocked into the mesocosms on two dates. Three-day old larvae with a mean standard length of 5.57 mm (Standard error (SE) = 0.02) and a mean weight of 178.52 mg (SE = 1.95) were stocked on 5/13. Four-day old larvae with a mean standard length of 5.46 mm (SE = 0.02) and a mean weight of 179.69 µg (SE = 1.45) were stocked on 5/14; transport survival of these larvae was good.

Zooplankton densities were monitored on three separate days. Densities increased from approximately 500 to over 2,500 total zooplankton per liter at the end of the experiment (Table 6). Generally, zooplankton density increased among treatments as intended (Table 7), though, there was a relatively small difference between the high and intermediate density treatments.

A total of 353 striped bass larvae were harvested.(Table 9). Positive growth was observed in all treatments with greatest growth occurring in the high zooplankton treatment (Table 9). The instantaneous rate of growth (G) averaged 1.13 and 1.64 in the low and high zooplankton treatment, respectively.

Table 9. Survival and growth of striped bass larvae in Lake Marion mesocosms of varying zooplankton (Zoop) densities. Three and four day old larvae were stocked on 5/13 and 5/14/96, respectively; 14-day old larvae were harvested.

Treatment (Zoop/L)	Replicate	No. of survivors	Mean SL[1], mm (N)	Growth, SL/day	Mean weight, µg (N)	G[2]
	L	17	7.68 (17)	0.22	596 (17)	1.20
100	M	14	7.52 (13)	0.20	501 (14)	1.02
	U	41	7.74 (42)	0.22	580 (42)	1.17
	L	8	8.12 (8)	0.26	719 (8)	1.39
1,000	M	1	7.55 (1)	0.21	305 (1)	0.53
	U	20	7.58 (19)	0.21	642 (19)	1.27
10,000	L	131	8.29 (50)	0.28	878 (50)	1.59
	M	74	8.14 (50)	0.26	859 (50)	1.57

U	47	8.54 (46)	0.30	1058 (46)	1.77

[1] - SL = standard length

[2] - G = instantaneous rate of growth from stocking to harvest

Experiment 3

The third mesocosm experiment was initiated on 5/16/97 and harvested on 5/29/97. Water temperature averaged 21.7°C (N=2). Two-day old larvae with a mean standard length of 5.33 mm (SE = 0.02) and a mean weight of 91.27 µg (SE = 5.12) were stocked on 5/16.

In general, the 'high' zooplankton treatment had greater densities of zooplankton than the 'low' treatment during the experiment (Table 10). Densities were similar in both treatments at the start of the experiment but declined in the 'low' and steadily increased in the 'high' treatment (Table 10). Total zooplankton per liter averaged 390 and 844 total zooplankton per liter in the 'low' and 'high' treatments, respectively.

A total of 2,210 striped bass larvae were harvested from 6 of 8 treatments (Table 11); no striped bass larvae were recovered on 5/20 from either the 'low' or 'high' zooplanton treatment. Growth was similar at in both treatments when larvae were 9 and 12 d old, but, growth at 15 d of age was greater in the 'high' treatment (Table 11). The pattern of growth appeared to follow associated changes in zooplankton density during the experiment.

The stomach contents of 211 striped bass larvae were examined and identified. Bosmina was the numerically dominant food items found in larval stomachs (Table 12). Besides cladocerans, cyclopoids, Keratella, and chironomid larvae appeared numerically important to the larval diet. Only 8 of 132 stomachs from 9 and 12 d old striped bass larvae were classified as 'full'. However, 35 of 81 stomachs from 15 d old larvae were 'full'(Table 13). Relatedly, during weighing and measuring of larvae, a striped bass larvae was found in the stomach of a 8 mm SL larvae.

Table 10. Mean density per liter of zooplankton in two experimental treatments within mesocosms. There were four replicates of each treatment. Samples were taken at three depths with a 2.5L whole water sampler.

				Copepoda			
Date	Treatment	N	Rotifera	Nanplii	Adults	Cladocera	Other
5/20/97	Low	4	13.5	0.2	<0.1	0.5	1.5
5/23/97	Low	3	213.2	0.2	0.0	<0.1	4.2
5/26/97	Low	2	607.0	0.3	0.3	0.0	21.0
5/29/97	Low	1	292.1	1.5	0.3	0.1	12.4
5/20/97	High	4	16.2	0.7	0.9	0.4	2.0
5/23/97	High	3	383.3	3.5	0.2	0.8	22.0
5/26/97	High	2	793.0	5.7	1.3	3.0	28.7
5/29/97	High	1	1169.3	26.7	3.3	8.0	62.7

Table 11. Survival and growth of striped bass larvae in Lake Marion mesocosms of varying zooplankton (zoop) densities. Two day old larvae were stocked on 5/16/97.

Treatment (Zoop/L)	Age (d) at harvest	No. of survivors	Mean SL[1], mm (N)	Growth, SL/day	Mean weight, µg (N)	G[2]
	9	914	5.80 (45)	0.07	143 (45)	0.45
Low	12	285	6.50 (48)	0.12	217 (48)	0.87

	15	250	6.54 (44)	0.09	239 (44)	0.96
	9	48	5.83 (6)	0.07	102 (6)	0.11
High	12	631	6.28 (49)	0.10	157 (49)	0.54
	15	82	7.18 (32)	0.14	412 (32)	1.51

[1] - SL = standard length

[2] - G = instantaneous rate of growth from stocking to harvest

Table 12. Food items of 211 larval striped bass from Lake Marion mesocosms in 1997.

Organism	Number found
Bosmina	210
Copepod eggs	160
Adult cyclopoid copepod	109
Keratella	53
Chironomidae	25

Diaphanosoma	10
Insecta	8
Copepod nauplii	7
Daphnia	7
Brachionus	3
Ceriodaphnia	2
Polyarthra	1
Amphipoda	1
Oligochaeta	1
Acarina	1

Table 13. Stomach contents of larval striped bass in Lake Marion mesocosms in 1997.

		Stomach Content		
Date	Treatment	Empty	Some Food	Full
May 23	Low 2	20	20	4
May 26	Low 3	20	20	4
May 29	Low 4	8	20	20
May 26	High 3	20	22	0
May 29	High 4	3	15	15

DISCUSSION

Water quality monitoring showed differences in water quality from the eastern (Dergan's Creek) to the western shore (Marker 159) of Lake Marion; eastern shore waters were clearer, warmer, and were more conductive. These differences were expected as the eastern shore of the lake is primarily fed by runoff from Santee Swamp while the western and central basins receive water from Santee River. Zooplankton monitoring indicated that densities were generally greater on the eastern shore, as opposed to the western and central basins. If growth rate is a function of zooplankton density and growth rate increases survival potential, this raises the possibility that cross-lake transport to eastern shore waters may improve recruitment potential of striped bass

larvae. In this shallow basin, wind speed and direction may play an important role in transporting larvae to optimal nursery grounds; further examination of data is needed.

A 2.5 L whole water sampler tended to give higher estimates of zooplankton density than vertical hauls with a plankton net. The difference in efficiency should be quantified so historic data, usually obtained with vertical plankton net hauls, can be corrected in the individual-based, striped bass management model for the Santee-Cooper system.

The study demonstrated the usefulness of mesocosms to evaluate specific hypothesis regarding larval striped bass. While efforts to control zooplankton density were relatively successful, experience gained in this effort indicates that nearly daily monitoring and adjustment is needed to keep densities at desired levels. Future efforts could gain insights on other variables, such as predator density and contaminants in the water column.

Obtained data support the hypothesis that transport of eggs and larvae to Lake Marion and relatively high zooplankton abundance increase the recruitment potential of striped bass (Bulak et al. 1997; Chick and Van Den Ayvle,1999). Mesocosm studies generally showed growth rate increased as zooplankton densities increased. Increased growth rate can be associated with increased survival potential by decreasing the duration of the highly vulnerable, early larval stage.

Growth rates (i.e. G) obtained in parts of this study are nearly 10 times greater than the published range in growth rate estimates for larval striped bass as reported by Chick and Van Den Ayvle (1999). This supports past efforts with the individual-based striped bass management model to ‘localize’ bioenergetic functions (Bulak 1997) so that growth observed in Lake Marion could be replicated by the model. These high growth rates also demonstrate the capability of striped bass to grow rapidly - increasing recruitment potential - when optimal conditions are encountered. These high growth rates may question the low growth potential of Santee-Cooper striped bass, as

suggested by Conover et al. (1997). As Chick and Van Den Ayvle (1999) point out, faster growth may result with encounters with 'high quality' prey items. The ability of an 8 mm larvae to ingest a fish larvae suggests they have the ability to take-in 'high quality' food items**.** Further analysis of these data, additional literature search, and peer-review is needed to clearly define the knowledge gained from this study.

RECOMMENDATIONS

1. More rigorously examine these data by a) increasing statistical examination, b) increasing literature review, and c) sending out this and future efforts for review and comment.
1. Use growth data to adjust bioenergetic component of the striped bass management model.
2. As funds and personnel become available, use mesocosms to further evaluate the recruitment process in upper Lake Marion, inspecting other variables such as predation rate, environmental contaminants, and wind-driven transport.

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