

**South Carolina State Wildlife Grant
Striped Bass Stock Enhancement of the Charleston Harbor Estuary
Grant Award SC-T-F10AF00422 (T-53-R-1)
October 1, 2010 – September 30, 2014
Final Report**

Significant Deviations

All project tasks have been completed one year ahead of schedule; therefore, this report represents our Final Report for the project. All project funds have been expended.

Introduction

Striped bass is a long-lived species that inhabits coastal estuaries and rivers along the East Coast of North America from the St. Lawrence River in Canada to the St. John's River in Florida and extending into the Gulf of Mexico (GOM). Striped bass populations throughout their range have been exhibiting population declines during the past decade, and the prevailing causal hypotheses are associated with increased summer temperatures. Bjorgo et al. (2000) found adult striped bass in one southeast coastal river migrating to cooler riverine habitats during the warm summer months. Often the coolest regions of these river systems occur in upstream habitats with extensive riparian shading. Unlike the northern anadromous populations of striped bass that move into coastal estuarine systems in the summer, southern populations tend to move into freshwater habitat for thermal refugia (Scruggs 1957; Combs and Peltz 1982; Bjorgo et al. 2000). Haeseker et al. (1996) found that in shallow estuaries with little freshwater input, the high temperatures of summer severely impact the health and condition of over-summering striped bass with mortality increasing in larger fish. Because of increased temperature associated with global climate change and extreme temperature fluctuations of shallow estuaries, it is possible that coastal populations of striped bass are crowded into available temperature refugia that make them especially susceptible to overfishing. Additionally, Coutant (1985) suggested that as thermal niche partitioning squeezes fish into limited habitat, detrimental results likely include direct mortality, decreased fecundity, and increased susceptibility to starvation, overfishing, and parasites.

Within the Charleston Harbor estuary in South Carolina (SC), drains the small tidally influenced Ashley River. Historically, this estuary supported a striped bass population, but very few young of year (YOY) and adult fish had been observed in the Ashley River since monthly sampling began in the early 1990s (per. com. John Archambault, SCDNR). Widespread stocking of freshwater systems with striped bass fingerlings from the Santee-Cooper river system has been conducted in SC since the early 1960s (Stevens 1975) and during the 1980s after re-diversion of the Santee River. Today, striped bass remain an important and popular recreational fish species in SC and stocking efforts continue, but with interest focused on freshwater systems and little effort in estuarine areas. However, in recent years, the State of SC has set goals to re-establish

historic biodiversity levels, and restoring a key indicator species like striped bass is an important step toward meeting this conservation goal.

Striped bass stock enhancement was initiated in the Ashley River in 2006 with funding from National Fish and Wildlife Foundation (NFWF) and Saltonstall-Kennedy (SK) grant awards that culminated in 2012. The overlap in funding years with the current project (2010-2014) allowed for the continued genetic identification of striped bass released during the SK grant and the completion of ongoing temperature tolerance studies, while actual striped bass production and stocking were continued under new funding from this South Carolina State Wildlife Grant (SWG) project. Since 2012, all production, stocking, genetic identification, and replicated studies have been accomplished using SWG funding.

The original SK grant focused on development of fingerling production in brackish water hatcheries, genetic identification tools, water quality and habitat assessment, species temperature tolerance experiments, and genetic population modeling. The current project refined these tools, examined movement patterns within and between river systems of the Charleston Harbor, evaluated reproductive potential of stocked striped bass in the system, and conducted replicated testing of the metabolic capacity of striped bass at various temperature and dissolved oxygen concentrations found in the Ashley River. The ultimate goal of the combined projects was to develop a restoration model for striped bass in coastal rivers.

I. **Approach**

Objective 1. Continue stocking phase I and phase II striped bass produced from ONFH and WMC into the Ashley River.

Objective 2. Coordinate tissue collection efforts with ongoing state sampling programs and process genetic samples.

Objective 3. Determine if reproductive population is present and determine if spawning is occurring.

Objective 4. Determine summer temperature tolerance of striped bass undergoing swimming challenges in a flume system.

Objective 1. Continue stocking phase I and phase II striped bass produced from ONFH and WMC into the Ashley River.

Production methods

During natural spawning periods each year (April/May), wild striped bass adults were collected from the Santee-Cooper River system and spawned by South Carolina Department of Natural Resources' (DNR) biologists working at the Bayless Hatchery using standard HCG induction methods. Eggs and sperm from broodstock were combined to form genetically unique "families" (1 female and 3 males) which were incubated separately. At five days post-hatch, fry were transported to the respective rearing facility for grow-out. Each rearing facility received fry from four unique genetic families for this project.

Fry from each family were stocked (density 250-500,000/ha) in separate brackish water nursery ponds at the DNR's Waddell Mariculture Center (WMC) in Bluffton, SC and in separate freshwater ponds at the United States Fish and Wildlife Service's (USFWS) Orangeburg National Fish Hatchery (ONFH) in Orangeburg, SC or DNR's Wildlife and Freshwater Fisheries Division (WFFD) Dennis Wildlife Center (DWC) in Bonneau, SC. Fry experienced a 30-40 day nursery period and grew to 25-50 mm TL (phase I development stage). Upon harvest, genetic families designated for spring stocking at phase I were transported to the Charleston Harbor system while families designated for fall stockings at phase II (100-200 mm TL) were held in recirculating aquaculture systems for one week during which they were weaned onto commercial pelleted diets. Once all fish were feeding on the pelleted diet, each family was restocked in separate ponds (density 80,000/ha) for an additional 150 day grow out.

Stocking Design

Ponds were stocked in anticipation of using a 2x2 factorial design for each of the two development stages. This design included freshwater (FW) or brackish water (BW) hatchery production facilities, as well as freshwater (FW) or brackish water (BW) stocking locations in the Ashley River as factors. Each hatchery production facility was expected to produce two unique genetic families for phase I stocking in the spring and two unique genetic families for phase II stocking in the fall. At each development stage, the two unique families from each hatchery were sent to separate locations on the Ashley River for stocking. One family from each facility was stocked in the oligohaline salinity (8-10 g/L) habitats in the Ashley River, while the remaining family from each facility was stocked in the freshwater portion of the river (0 g/L). Stocking locations were the same for both phase I and phase II stockings.

Over the course of this project, it became apparent that phase I stockings were not being recaptured at the same proportion as phase II stockings; therefore, production and stocking efforts were shifted to producing higher numbers of phase II fish in an attempt to bypass the survival bottleneck and increase the abundance of striped bass in the Ashley River.

Objective 2. Coordinate tissue collection efforts with ongoing state sampling programs and process genetic samples.

Fin clip tissue sample collection

Coordination of three of DNR's field sampling programs for collection of striped bass tissue samples was completed during this project. DNR's Marine Resources Division (MRD) conducts monthly independent electrofishing at random sites within the low salinity and freshwater portions of the Ashley River as well as portions of the freshwater Cooper River. MRD also conducts monthly independent random trammel net sampling at 30 randomly selected sites monthly within the Charleston Harbor and estuarine portion of the Ashley River. DNR's WFFD assisted MRD with additional independent random electrofish sampling in the upper reaches of the Ashley River. In addition, samples were collected from dependent non-random electrofish sampling efforts conducted by MRD. Fin clip tissue samples were taken from all captured

striped bass and stored in a non-toxic sarcosyl-urea preservative with appropriate date, location, weight (g) and size (TL) recorded.

Genetic sample processing

All striped bass broodstock used in all statewide hatchery releases during the project period were genotyped using an optimized suite of 12 multiplexed microsatellite markers developed specifically for striped bass. DNA from all field-collected samples was processed and compared to adults to determine the origin of each fish. Genetic parentage analysis was used to identify stocking treatments by family, identify movement between systems, and identify wild striped.

Objective 3. Determine if a reproductive population is present and if spawning is occurring.

Non-random fisheries independent electrofish sample collection efforts were conducted in the Ashley River during spring spawning migrations in March and April in project years 2012-2014. Fish estimated to be greater than 300 mm TL were collected from the river and held in an oxygenated tank on the electrofishing boat. All fish collected were weighed (g), measured (mm TL), and fin clipped. Captured fish had their abdomens gently compressed to determine if they were spermiating running males. All running males were returned to the river. Large striped bass which were not visibly running males were suspected to be females. Suspected females greater than 400 mm TL or 1.0 kg were judged to be at least 3 years old and returned to the DNR's Marine Resources Research Institute (MRRI) for histological examination. Upon return to MRRI, ovaries were removed and weighed (g), and a small sample (5 mm x 5 mm) of one lobe was removed, placed in a labeled Tissue Tek® cartridge, and stored in 10% buffered formalin for histological processing by DNR's inshore fisheries monitoring program.

Objective 4. Determine summer temperature tolerance of striped bass undergoing swimming challenges in a flume system.

Funds from this grant were used to develop and conduct flume experiments in order to further our understanding of how factors such as temperature, dissolved oxygen, and river flow affect the energetic costs of living in the Ashley River, and therefore how these environmental factors may limit striped bass growth, survival, and recruitment in the Ashley River. Metabolic scope is a measurement which can be used to calculate the energetic costs related to environmental conditions. The metabolic scope of hatchery reared striped bass was calculated by measuring oxygen consumption in a series of swimming trials conducted in a 90 L sealed, dissolved oxygen meter-equipped flume tank located within a temperature controlled experimental chamber. Flume trials conducted in 2013-2014 tested phase III striped bass (446.5 ± 62.9 g) at three temperatures (20°C, 25°C, 32°C) and three oxygen concentrations (2.5 mg/L, 3.0 mg/L, 4.0 mg/L), using 5 replicates per a treatment (N=45). As phase II fish (Age 0, 100-200 mm TL) are stocked in the fall and able to bypass their first summer in the wild, phase III fish (Age 1, 200-300 mm TL) represent the size at which fish stocked as phase II experience their first summer in

the Ashley River. The combination of 32°C temperature and 2.5 mg/L dissolved oxygen represent the most extreme summer environmental conditions measured in the Ashley River.

II. **Results**

Objective 1. Stock phase II striped bass produced from the Waddell Mariculture Center into the Ashley, Cooper and Wando Rivers.

Production and Stocking 2013-2014

The final year of this project (Oct 1, 2013-Sept 30, 2014) supported production and stocking of 19,234 phase II striped bass in the Ashley and Cooper rivers in the fall of 2013, the production of 26,116 advanced phase I striped bass stocked in the Ashley River in summer of 2014, and the tagging of 325 phase III striped bass fitted with external nylon dart tags and stocked in the Wando River in the fall of 2014.

On November 14, 2013, one pond of a unique genetic family (L) at WMC was partially harvested and 4,010 phase II striped bass were transported to the Ashley River and stocked at the brackish water site. On December 5, 2013, the remainder of the pond was harvested and an additional 2,862 phase II striped bass from genetic family L were transported to the brackish water release site and stocked for a total of 6,872 released from family L in 2013 (Table 1). On November 15, 2013, a second pond of phase II striped bass at WMC (genetic family DD) was harvested (6,496) and stocked at the freshwater site in the Ashley River. A total of 5,866 phase II striped bass from a third unique genetic family (K) were stocked in the Cooper River on November 19, 2013. In 2013, 13,368 phase II striped bass were stocked in the Ashley River and a total of 19,234 phase II striped bass were stocked in the Charleston Harbor system overall.

Table 1. 2013 phase II (100-200 mm TL) striped bass juveniles stocked in the Ashley River from the Waddell Mariculture Center. Freshwater (FW), brackish water (BW) and Cooper River (CR) release sites are identified, as is the hatchery, fish release sizes (total length and weight) with standard deviations, and genetic family.

Release Location	Fish (n)	TL (mm)	Weight (g)	Genetic Family
BW	6,872	209.0±13.6	101.8±20.8	L
FW	6,496	202.4±21.2	103.9±31.5	DD
CR	5,866	189.6±18.7	85.0±27.0	K

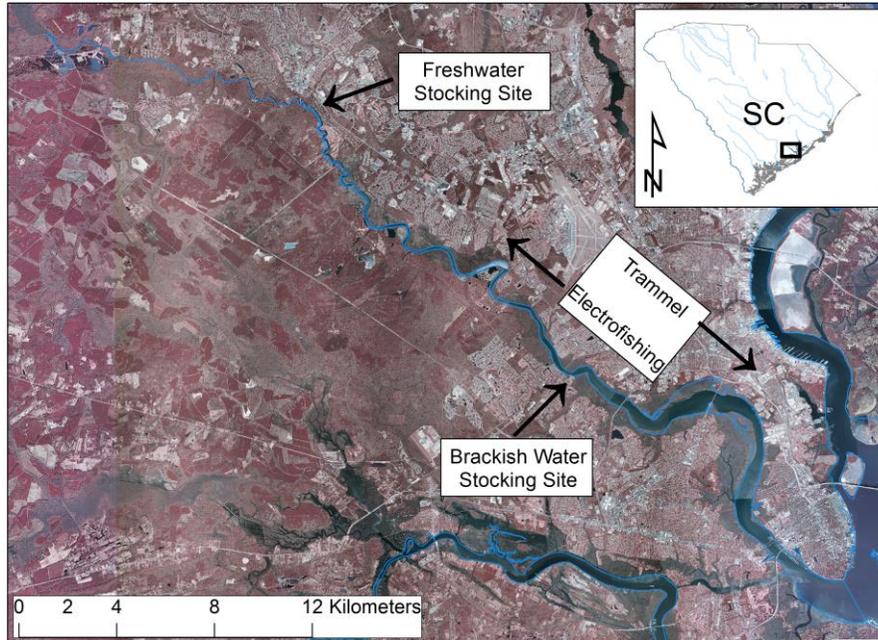


Figure 1. Map of the Ashley River showing locations of freshwater (FW) and brackish water (BW) stocking sites and electrofishing and trammel net sampling strata.

In April of 2014, five ponds at WMC were stocked with approximately 100,000 striped bass fry, each from a unique genetic family, obtained from the WFFD Bayless Fish Hatchery. On July 1, 2014, a total of 12,596 advanced phase I (50-100 mm TL) fish were harvested from ponds at WMC and stocked at the freshwater site on the Ashley River (Table 2). On July 17, 2014, an additional 13,520 advanced phase I fish from the WMC were stocked at the freshwater Ashley River release site. A total of 26,116 advanced phase I striped bass were stocked in the freshwater portion of the Ashley River in 2014.

Table 2. Number of 2014 year class advanced phase I (50-100 mm TL) striped bass juveniles stocked in the Ashley River from the Waddell Mariculture Center. Freshwater (FW) release site is identified as are fish release sizes (weight) with standard deviation, and genetic family.

Release Location	Fish (n)	Weight (g)	Genetic Family
FW	1,658	5.43±0.22	V
FW	2,420	6.82±0.94	R
FW	754	5.77±0.37	J
FW	7,764	3.90±0.42	S
FW	2,421	7.23±0.33	S
FW	1,436	9.16±0.63	E
FW	9,663	14.46±0.31	J

In November of 2014, a total of 325 one year-old striped bass of a unique genetic family (CC) produced at WMC as phase I in 2013 and retained at the Marine Resources Research Institute for use in flume experiments, were large enough to be tagged with external dart tags and stocked in the Wando River (Table 4).

Total Production 2010-2014

Five distinct year classes of striped bass (164,895) were produced with funding from this project (Table 3). A portion of these fish (101,351) were produced in brackish water at WMC, while 63,544 were produced in freshwater at ONFH and DWC. In 2012, production of phase I fish was discontinued and all further production of phase II striped bass was conducted at WMC.

Table 3. Striped bass released in the Charleston Harbor System 2010-2014. Fish were produced at the Waddell Mariculture Center (WMC) in Bluffton SC; Orangeburg National Fish Hatchery (ONFH) in Orangeburg SC; and at the Dennis Wildlife Center (DWC) in Bonneau SC.

Hatchery	2010	2011	2012	2013	2014	Total
WMC	11,230	18,970	25,801	19,234	26,116	101,351
DWC	-	3,442	-	-	-	3,442
ONFH	6,523	53,579	-	-	-	60,102
Total	17,753	75,991	25,801	19,234	26,116	164,895

Of the 164,895 striped bass stocked in the Charleston Harbor system, 76,204 were stocked at the phase II stage (Table 4). A total of 32,097 fish were stocked at the freshwater release site on the Ashley River, while 38,241 fish were stocked at the brackish water release site. Between 2010 and 2013 the mean number of phase II striped bass stocked at the freshwater site was $8,024 \pm 2,962$ and the mean number of phase II striped bass stocked at the brackish water site was $9,560 \pm 5,021$. An additional 5,866 phase II striped bass were stocked in the Cooper River in 2013.

Table 4. Stocking locations in the Charleston Harbor system of genetically unique and identifiable striped bass by development stage and year class. Freshwater stocking site (FW), Brackish water stocking site (BW), Phase I (Age 0, 25-50 mm TL), Phase I+ (Age 0, 50-100 mm TL), Phase II (Age 0, 100-200 mm TL), and Phase III (Age 1, 200-300 mm TL).

Ashley River FW	Phase I	Phase I+	Phase II	Phase III
2010	-	-	11,762	-
2011	35,653	-	4,974	-
2012	-	-	8,865	-
2013	-	-	6,496	-
2014	-	26,116	-	-
Ashley River BW	Phase I	Phase I+	Phase II	Phase III
2010	-	-	5,991	-
2011	26,922	-	8,442	-
2012	-	-	16,936	-
2013	-	-	6,872	-
2014	-	-	-	-
Cooper River	Phase I	Phase I+	Phase II	Phase III
2013	-	-	5,866	-
Wando River	Phase I	Phase I+	Phase II	Phase III
2011	-	-	-	168
2012	-	-	-	200
2014	-	-	-	325
Total	62,575	26,116	76,204	693

During this project, refinements were made to protocols for the acclimation and stocking of striped bass fry to brackish water ponds at WMC. Fry were first held an extra day or two, until day 6-7 post hatch, at the Bayless hatchery and were feeding on *Artemia* nauplii prior to being stocked in the WMC ponds in 2011. Normal pond culture protocols require pond fertilization to bloom natural prey items for larvae and fry; however fertilization created dense phytoplankton blooms in the brackish water ponds and pH was elevated above 9.0 in the afternoons. Ponds filled without adding fertilizer had less dense phytoplankton blooms but more stable pH and *Artemia* nauplii were added to ponds daily to supplement for the loss of natural production.

In addition to eliminating pond fertilization, fry were acclimated to brackish water ponds in several stages. First, freshwater from the Bayless Hatchery was hauled to WMC each time fry were picked up. This freshwater was used to fill 300 gallon fiberglass tanks on the bank of the pond. Fry were introduced into the freshwater from the Bayless Hatchery after sunset and pond water was pumped into the tank and allowed to flow-through overnight to raise the salinity and

temperature to pond conditions. Prior to sunrise, each tank was fed *Artemia nauplii* and tank contents, including the fry, were drained through 10 feet of 2" PVC pipe into the pond. In 2013, survival in three of the five phase I production ponds at WMC was greater than 50%.

Objective 2. Coordinate tissue collection efforts with ongoing state sampling programs and process genetic samples.

2013-2014 Collections

In the final year of this project (July 2013-June 2014) a total of 558 fin clip tissue samples were collected from striped bass sampled in the Ashley and Cooper rivers and analyzed for parentage (Table 5). A total of 267 fin clip samples were provided by the MRD's Inshore monitoring program; 254 from the Ashley River and 13 from the Cooper River. In addition, 260 fin clip tissue samples were provided by monthly randomized fisheries independent electrofish sampling in the upper Ashley River and Shultz Lake conducted with the assistance of WFFD and 31 fin clip tissue samples were provided by MRD through non-random fisheries independent electrofish sampling efforts in the Ashley River in the spring of 2014.

Table 5. Genetic fin clip tissue samples collected by fisheries independent sampling using electrofishing from July 2013-June 2014 and analyzed for parentage. South Carolina Department of Natural Resources Marine Resources Division (MRD) and Wildlife and Freshwater Fisheries Division (WFFD) coordinated sample collection in the upper Ashley River. Non-random sampling occurred only in March and April.

Collector	Sampling	Ashley River	Cooper River	Total
MRD	Random	254	13	267
MRD + WFFD	Random	260	-	260
MRD	Non-Random	31	-	31
Total		545	13	558

With the single exception of a 4.4 kg 2008YC hatchery striped bass stocked into Lake Moultrie as part of the WFFD stocking program, genetic parentage analysis revealed that all of the fish captured in the Ashley River during the final year of the project were originally stocked in the Ashley River as part of our restoration effort (Table 6). No wild fish were identified. Most captures came from stocked phase II stage fish, though both of the 2009YC fish were stocked at the phase I stage from the freshwater hatchery at the freshwater release site (FWIFW). Striped bass from 9 out of 10 phase II treatments stocked during the project were captured in the Ashley River during the final project year. In addition, fish were identified from every year class released from 2009-2013. All five 2013 YC fish recaptured in the Cooper River were identified as coming from the 2013 project stocking in the Cooper River (Table 6).

Table 6. Summary of genetic identification of striped bass collected in the Ashley River (n=539) and Cooper River (n=13) from July 2013 to June 2014 from Phase I (25-50 mm TL) and Phase II (100-200 mm TL) stocking treatments. Unless otherwise noted, all fish were stocked in the Ashley River. Treatments are designated as production facility:stocking location (i.e., FW:FW: freshwater produced, freshwater stocked; BW: brackish water; HW: headwater; CR: Cooper River). Phase I Lakes represents striped bass that were stocked in lakes Marion and Moultrie within the adjacent Santee Cooper River system as phase I fish. Shaded boxes represent treatments that were not stocked. Recaptures of fish are not included in this table.

Capture System	Year Class	Capture (n)	Wild (n)	Stocking Treatment										Phase I Lakes
				Phase I					Phase II					
				FW:FW	FW:BW	BW:FW	BW:BW	BW:HW	FW:FW	FW:BW	BW:FW	BW:BW	BW:CR	
Ashley River	2006	0												
	2007	0												
	2008	1												1
	2009	2		2										
	2010	4							3		1			
	2011	20								8	8	4		
	2012	108									41	67		
	2013	404									194	210		
Cooper River	2013	5											5	

We used a Monte Carlo approach using the program R (R Development Core Team 2009) to test the null hypothesis that the proportion of fish returned from each release site was no different from the proportion of fish that were stocked in the Ashley River. Within the 2013YC, 51.4% of the fish stocked in the Ashley River were stocked at the brackish water site and 52.0% of the fish recaptured were stocked at the brackish water site. The difference was not statistically significant ($p=0.3859$).

Multiple recaptures 2013-2014

Striped bass (18) were recaptured on multiple occasions during the final year of this project (Table 7). The mean number of days between the first and second capture was 287.2 and mean growth was 0.35 mm/d. Sampling sites were ranked in order from most downstream (0) to most upstream (134) and direction movement calculated by subtracting the original capture location from the recapture location. A positive number indicated movement upstream while a negative number indicated movement downstream. A total of six striped bass moved downstream, nine moved upstream, and three were recaptured in the same location both times. Mean directional movement was 16.9 sampling sites upstream (Table 7).

Table 7. A summary of collection data (mean and standard deviation) for striped bass recaptured multiple times within the Ashley River from July 2013 to June 2014 (n=18). Sampling sites were ranked in order from most downstream (0) to most upstream (134).

Recaptures	Days at Large	Growth (mm)	Growth (mm/d)	Directional Movement
Mean±SD	287.2±289.2	104.3±106.7	0.35±0.18	16.9±43.5
Range (min-max)	(6-984)	((-2)-379)	((-0.03)-0.86)	((-33)-108)

Total Collections 2010-2014

Funding for this project supported processing of striped bass samples from July 2010 through June 2014. In the first and second grant years, processing was jointly supported by an SK-funded grant that was completed in the summer of 2012. Collections included multiple year classes from multiple state programs in both the Ashley and Cooper rivers.

A total of 2,142 genetic fin clip samples were collected and analyzed between July 2010 and June 2014 (Table 8). Mean recaptures were highest in the spring and lowest in the fall (Figure 2). Recaptures in the months of November and December were biased by phase II release events during those months; however it’s unclear why the mean number of recaptures in the month of January and February (2-3 months after release) is lower than in the months of March and April (4-6 months after release).

Table 8. Total number of genetic fin clip samples collected from the Ashley River and analyzed for parentage by month and year of collection.

Month	2010	2011	2012	2013	2014	Grand Total
January		1	4	47	22	74
February		5	128	21	104	258
March		69	274	70	110	523
April		53	304	136	121	614
May		33	45	55	65	198
June		15	14	34	34	97
July	9	14	31	25		79
August	17	17	5	11		50
September	5	9	5	7		26
October	6	1	8	9		24
November	7	16	6	29		58
December	63		70	8		141
Grand Total	107	233	894	452	456	2,142

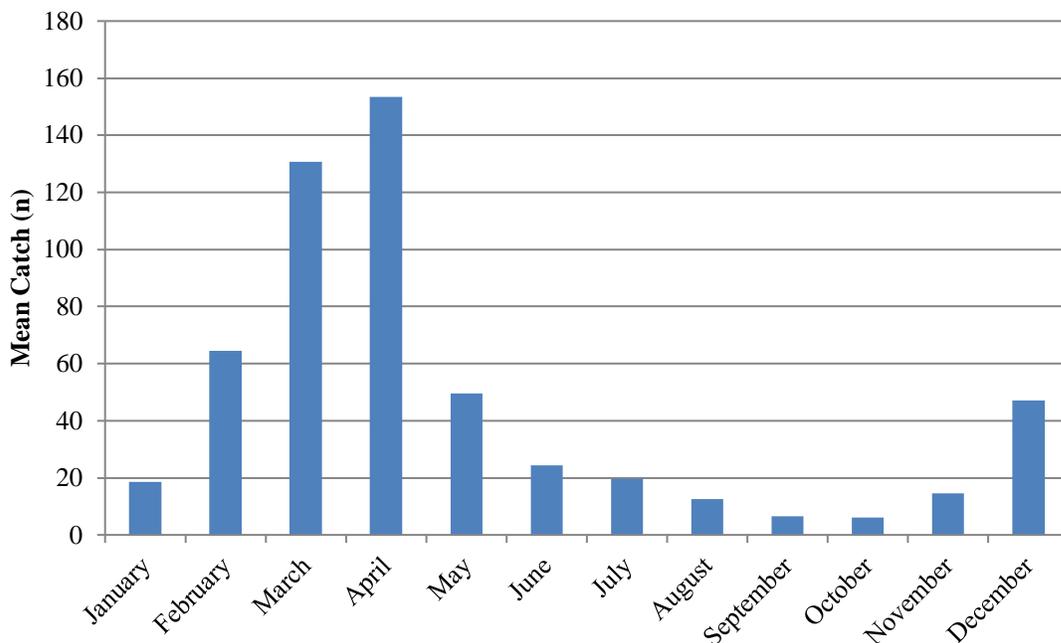


Figure 2. Mean number of striped bass captured in the Ashley River by capture month for collections July 2010 to June 2014.

Using Monte Carlo analysis in the statistical program R, a significant difference ($p < 0.001$) was detected in the proportion of phase I and phase II striped bass recaptured during this project. Phase I striped bass comprised 70.3% of the total number of fish stocked in the Ashley River and only 0.5% of the recaptures (Table 9). Only eleven phase I fish were recaptured in four years. Such a limited recapture rate is insufficient for evaluating production hatchery and release location treatments and therefore all phase I stage fish were removed from further analysis of treatment effects.

Table 9. Total number and percentage of phase I (25-50 mm TL) and phase II (100-200 mm TL) striped bass stocked and recaptured January 2010 to June 2014 for the 2010YC–2013YC. Phase II fish were captured in a significantly higher proportion than stocked (Monte Carlo analysis, R statistical program).

Stage	Stocked (#)	Stocked (%)	Recap (#)	Recap (%)	p value
* I *	166,539	70.3	11	0.5	<0.0001
* II *	70,338	29.7	2032	99.5	

Within the phase II stage of development, Monte Carlo analysis revealed that the proportion of striped bass recaptured from each stocking location was not significantly different than the proportion of striped bass stocked in each location (Table 10, $p = 0.2878$); treatment effects from hatchery were confounded by variability in phase II freshwater production.

Table 10. Total number and percentage of Phase II (100-200 mm TL) striped bass stocked and recaptured from each treatment factor from January 2010 to June 2014 for the 2010YC–2013YC.

Release Location	Stocked (#)	Stocked (%)	Recap (#)	Recap (%)	p value
* FW	32,097	45.6	939	46.2	0.2878
* BW	38,241	54.4	1093	53.8	

Capture Location

Only one striped bass was collected in trammel sampling of the lower Ashley River (AR) over the course of this project and fewer fish were captured in the upper Ashley (UA) section of the river than in the far Ashley (FA) or freshwater Ashley (FWA) sampling sections (Figure 3). While some of this difference is explained by variable sampling effort among sites; creeks and tributaries in the UA section of the Ashley River produced much higher numbers of striped bass than other locations of the river. This pattern could indicate that creeks and tributaries offer more favorable habitat or it may indicate inefficiencies in the electrofishing gear in the upper Ashley collection strata. The river in this section reaches hundreds of meters wide and up to 15 meters deep. If larger striped bass are seeking deep water they may be able to avoid the electrical current of the sampling gear focused along the river bank.

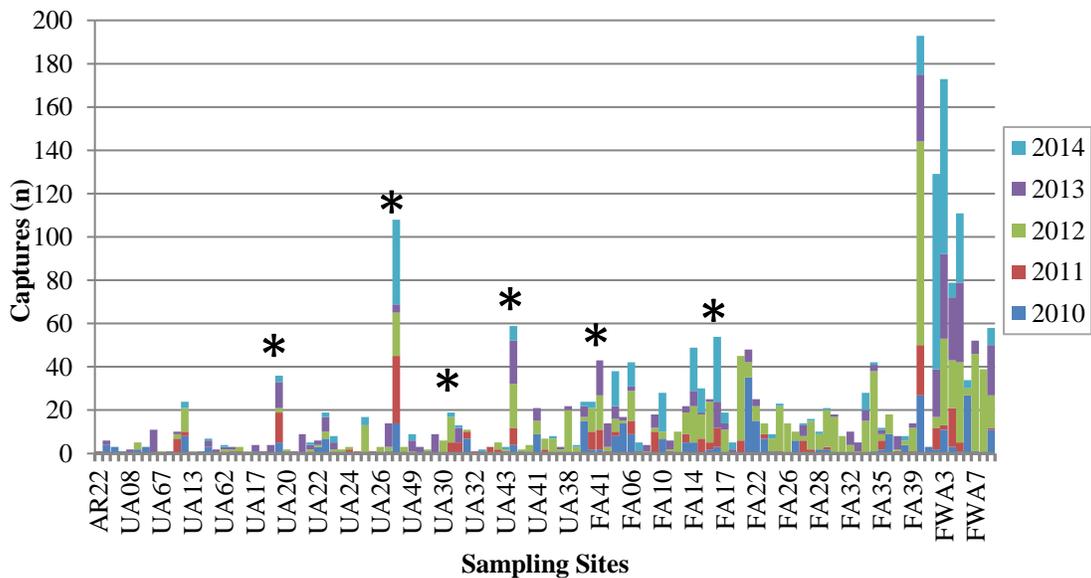


Figure 3. Total number of striped bass captured in the Ashley River by sampling site and collection year. Sampling sites are shown from most downriver site on the left and Shultz Lake at the headwaters of the Ashley River on the right. Trammel net (AR), lower brackish water Ashley (UA), far upper Ashley (FA), and freshwater Ashley (FWA) represent separate sampling strata within the river. (*) Indicates a tributary of the Ashley River.

Capture Timing

Spring migration of striped bass from less effectively sampled sections of the river to more effectively sampled sections upriver may explain the pattern in capture timing observed during this project (Figure 4). Each year class of phase II striped bass released in the Ashley River was captured soon after release during November and December; however, capture numbers increase dramatically during the spawning season in March and April. After May, collections drop until the following spring when capture numbers again increase for two year old fish in the Ashley River. Capture of striped bass aged 3 years and older made up only 1.6 percent of recaptures.

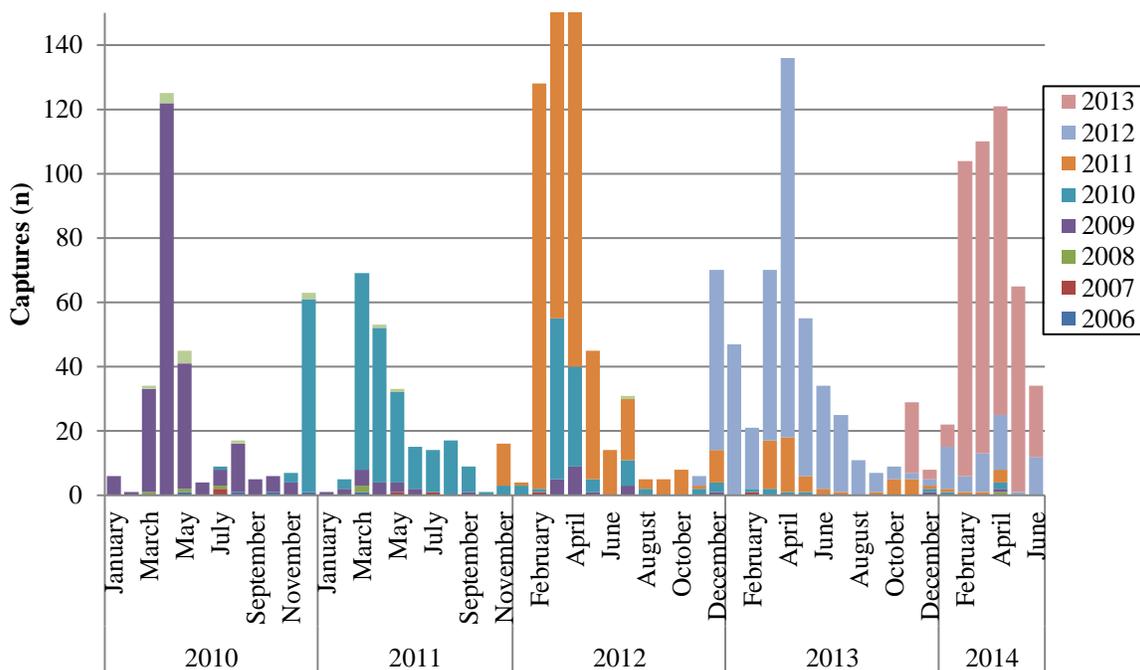


Figure 4. Total number of striped bass captured in the Ashley River between January 2010 and June 2014 by month and year of capture for each year class released in the Ashley River. Capture totals in March (274) and April (304) 2012 exceed charted y-axis with 2011 YC captures.

Multiple Recaptures 2010-2014

Seventy-three striped bass were captured multiple times over the course of the project. The average number of days between the first and second capture was 193.0 and mean growth over that time was 0.24 mm/d (Table 11). No fish were recaptured outside of the Ashley River. A total of 21 striped bass moved downstream, 42 moved upstream, and 10 were recaptured in the same location. Mean directional movement for the second capture was 20.5 sampling sites upriver of the original capture.

Table 11. A summary of collection data for striped bass captured multiple times within the Ashley River from July 2010 to June 2014 (n=73). Sampling sites were ranked in order from downstream (0) to upstream (134).

Recaptures	Days at Large	Growth (mm)	Growth (mm/d)	Directional Movement
Mean±SD	193.0±223.3	66.9±87.0	0.24±0.31	20.5±48.8
Range (low-high)	(6-984)	((-18)-379)	((-1.8)-0.86)	((-73)-132)

External Tagging 2010-2014

Tagging

Over the course of the project, 1,322 striped bass were fitted with external tags (Hallprint) and released in the Charleston Harbor system, with the majority of tags released (n=771) when larger (phase III+, Age 1+, >350 mm TL) hatchery fish were fitted with an external dart tag and stocked directly into the wild at public boat landings (Table 12). While all tagged fish released in the Wando River (n=693) were stocked from the hatchery, tags released in the Ashley River (n=613) represent both hatchery fish tagged at the time of stocking (n=78) as well as striped bass captured from the wild and tagged during MRD fisheries-independent electrofish sampling efforts (n=535) (Table 13). All fish tagged in the Cooper River (n=16) were captured during sampling. From spring of 2012 to the present, nearly all striped bass captured during fisheries-independent sampling with a total length greater than 350 mm have been fitted with an external dart tag and released.

Table 12. Striped bass tagged by source, 2010-2014. Hatchery fish were tagged prior to original stocking while larger striped bass (~ 300 mm TL) captured during both random and non-random independent electrofish sampling efforts were also tagged and released.

Source	Number Tagged and Released	Mean TL (mm)
Hatchery	771	416.2 ± 6.3
Electrofishing Random	123	387.7 ± 20.4
Electrofishing Non-Random	428	289.6 ± 7.2

Table 13. Striped bass tagged by location, 2010-2014.

Location	Number Tagged and Released	Mean TL (mm)
Ashley River	613	331.5 ± 8.6
Wando River	693	403.4 ± 6.4
Cooper River	16	525.6 ± 51.6

Tag Recaptures

Of the 1,322 striped bass externally tagged during the reporting period, 325 were released during November 2014 and have not been at large for long enough to evaluate recaptures. Excluding these fish, a total of 997 fish have been tagged and 62 have been recaptured for a recapture rate of 6.2% (Table 14). Fish captured during electrofish sampling were more likely to be recaptured (41/551, 7.4%) than hatchery fish that were tagged and released at a boat ramp (21/446, 4.7%). This difference in recapture rate is explained by release location. The majority of all externally-tagged hatchery fish were stocked in the Wando River, where independent electrofish sampling is not possible due to a lack of freshwater input. Therefore, all recaptures occurring in the Wando River (18) were fisheries dependent.

Table 14. Recaptures by initial tagging location, 2010-2014. (*) Denotes fisheries dependent sampling effort.

Location	Recapture Rate (%)	Mean Size at Recapture (mm)	Mean Days at Large
Ashley River	7.0	369.9 ± 36.3	115.2 ± 34.4
Cooper River	6.3	549.0 ± 0	72 ± 0
Wando River	4.9*	525.1 ± 49.3*	127.8 ± 85.6*

Movement of Tagged Fish

Recaptured fish were at large for an average of 90 days (range 0-809 days) and were recaptured an average of 7.0 km (range 0-73 km) from the initial tagging site. There was no relationship between time at large and distance traveled ($r^2=0.05$). In the Ashley River, 23.8% (10/42) of tagged fish were recaptured in the same sampling location (within 0.25 km) where tagging occurred, suggesting that striped bass in the Ashley River show some fidelity to certain areas. In the polyhaline Wando River, the majority of striped bass (14/18, 77.8%) were recaptured upriver of the stocking location (~20 mg/L salinity), possibly seeking lower salinity conditions. Movement of tagged striped bass between rivers in the Charleston Harbor system was rare, as only one fish tagged in the Ashley River has been captured outside of the Ashley River, and no fish tagged in the Cooper or Wando rivers have been recaptured outside of their respective rivers. During the reporting period, 21 recreational anglers captured and reported 21 different externally-tagged striped bass and were rewarded with a stock enhancement program t-shirt for their efforts.

Objective 3. Determine if reproductive population is present and determine if spawning is occurring.

Wild recruitment 2010-2014

All striped bass collected in the final project year were identified as stocked hatchery fish. This marks the second collection year in a row no age 0 wild striped bass were identified in the

Ashley River after three consecutive years identifying wild fish 2010-2012 (Table 15). A total of 16 wild striped bass were identified during this project (compared with 11 from phase I stockings). The factors necessary for successful wild recruitment are currently unknown. Striped bass typically require long, moderately fast moving streams for successful natural recruitment; however, the Ashley River is a relatively short tidal river with limited natural freshwater input and reversing flows influenced by the tide. As such, flow reversal may serve to retain wild striped bass eggs and larvae in the Ashley River prior to reaching the settlement stage (5-7 days post-spawn depending on water temperature). Rain events in headwaters of the Ashley River greatly impact flushing rates in the river. It's possible that the timing and amount of freshwater input into the Ashley River watershed may influence natural recruitment success in the Ashley River (Figure 5).

Table 15. Total number of genetic fin clip tissue samples analyzed for each collection year in the Ashley River. Striped bass identified as wild could not be matched to any broodstock used in any stocking programs in the state.

Year	Cultured	Wild	Total
2010	311	11	322
2011	244	2	246
2012	878	3	881
2013	452		452
2014	456		456
Total	2334	16	2357

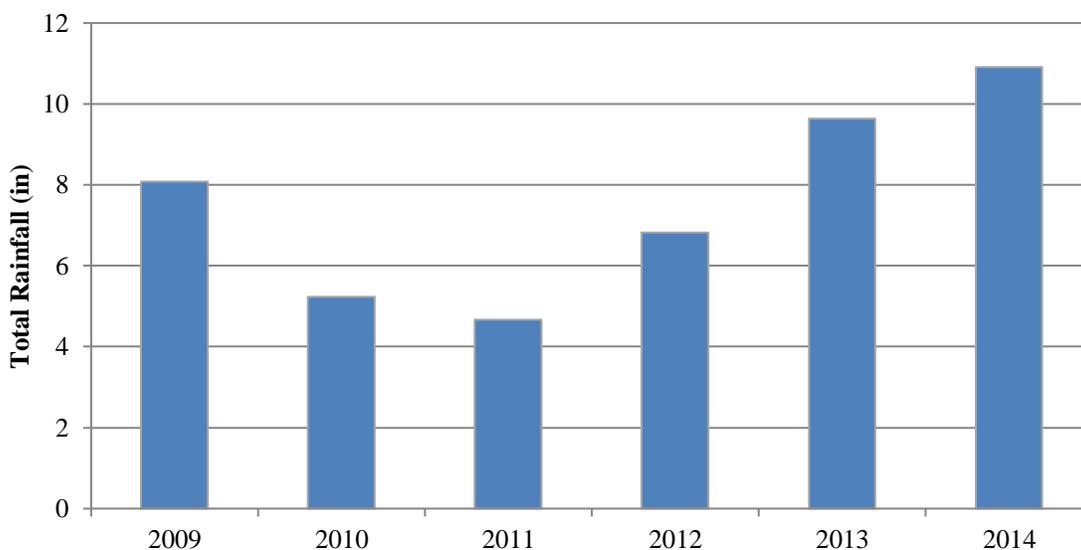


Figure 5. Total rainfall (in) collected at Charleston International Airport for the combined months of March and April in each year.

The source of wild recruitment in the Ashley River is unknown. Wild recruits could have been spawned by wild adults in the Ashley River, or they could have been spawned in an adjacent system and migrated into the river, or they could be offspring of previously stocked fish. Since Ashley River stocking only began in 2006, 2009 would be the first year in which spawning and recruitment could occur from stocked fish. Given that a number of fish identified as wild recruits to the 2009YC and 2010YC were captured at Age 0, it is unlikely that these fish were spawned in a different system and migrated to the Ashley River at such a young age and small size (Table 16). The lack of Age 0 contribution from wild fish to the 2011-2013YC could be an indication of intermittent spawning or recruitment success in the Ashley River due to a number of possible environmental parameters.

Table 16. Striped bass captured (n) in Ashley River, hatchery contribution (%) by year class and age from January 2010 through June 2014. All striped bass were assumed to have an April 1 birthday.

Age	2006 YC		2007 YC		2008 YC		2009 YC		2010 YC		2011 YC		2012 YC		2013 YC		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
0							40	97.5	130	98.5	368	100	178	100	227	100	943	99.7
1					1	100	212	96.2	187	100	371	99.2	278	100	180	100	1229	99.1
2			0	---	4	100	17	94.1	50	100	40	100	30	100			141	99.3
3	0	---	2	100	0	---	13	100	4	100	4	100					23	100
4	5	100	4	75.0	0	---	1	100	2	100							12	91.7
5	0	---	1	100	0	---	1	100									2	100
6	0	---	0	---	1	100											1	100
Total	5	100	7	85.7	6	100	284	96.5	373	99.5	783	99.6	486	100	407	100	2351	99.3

Directed Sampling During Spring Spawning Migrations

Histology 2013-2014

During the 27 non-random sampling days in March and April in the Ashley River between 2012 and 2014, a total of 159 striped bass greater than 300 mm TL were collected and 17 fish were sacrificed for histological examination (Table 17). Of the fish sacrificed, 12 were identified as female and five were identified as male (Table 18). Seven females were collected at Age 3 or Age 4 and all seven showed signs of ovarian development and sexual maturity, including one female captured in 2012 which showed evidence of post-ovulatory follicles indicating that it had recently spawned (Table 18). Gonadosomatic Index (GSI) is the proportion of gonad weight to total body weight for an individual and is often used as a quantitative measure of sexual

maturity. Three of the four largest females captured in the spring had GSIs above 5.0 and all three were Age 3 fish. All females Age 2 years and younger were found to be immature, suggesting female striped bass are reaching sexual maturity in the Ashley River beginning at Age 3. Histological examination of male striped bass captured in the river revealed all males to be either sexually mature or developing by Age 2.

Table 17. Total number of striped bass identified during directed non-random sampling efforts conducted in the Ashley River during spawning migrations in March and April 2012-2014. Striped bass sacrificed for histological evaluation were thought to be females Age 3 or older. Fish < 300 mm TL were counted and not removed from the river beginning in 2013 to maximize river coverage.

Year	Identified (n)	>300 mm (n)	Histology (n)
2012	477	95	11
2013	362	31	2
2014	398	33	3
Total	1237	159	16

Table 18. Sexual maturity of striped bass collected in the Ashley River during the spawning months of March and April 2012-2014. Gonadosomatic Index (GSI) is a measure of the proportion of gonad mass to total body mass. Histological evaluation and staging conducted in MRRI's Inshore fisheries histology lab.

Date	TL (mm)	FL (mm)	Weight (g)	Sex	Gonad Wt. (g)	GSI	Histo Stage	Year Class	Age
4/11/2014	600	565	2270	F	18	0.79	Developing	2010	4
4/3/2014	595	563	2690	F	194	7.21	Developing	2011	3
4/11/2012	548	516	1998	F	167	8.36	Recent Spawn	2009	3
3/13/2012	525	498	1956	F	103	5.27	Developing	2009	3
3/20/2014	519	499	1946	F	28	1.44	Developing	2011	3
3/13/2012	510	483	1656	F	47	2.84	Developing	2009	3
3/5/2013	505	475	1557	M	-	-	Developing	2010	3
3/14/2013	492	466	1668	F	29	1.74	Developing	2010	3
3/8/2012	400	375	804	F	-	-	Immature	2010	2
3/13/2012	392	369	848	M	-	-	Mature	2010	2
3/8/2012	380	360	738	F	-	-	Immature	2010	2
4/5/2012	297	278	247	M	-	-	Developing	2011	1
4/5/2012	276	260	188	M	-	-	Developing	2011	1
4/5/2012	261	245	192	M	-	-	Developing	2011	1
4/5/2012	240	229	126	F	-	-	Immature	2011	1
4/5/2012	240	226	117	F	-	-	Immature	2011	1
4/5/2012	232	217	113	F	-	-	Immature	2011	1

Objective 4. Determine summer temperature tolerance of striped bass undergoing swimming challenges in a flume system

Continuous water quality recordings at two locations in the Ashley River show that, during summer extremes, temperature in the Ashley River can reach up to 33 °C, while levels of dissolved oxygen typically range between 2.5 and 4.0 mg/L. In an effort to identify how these potentially stressful summer condition impact striped bass, previous experiments focused on how temperature, salinity, and food rationing affected growth and survival of phase I striped bass. However, these experiments were unable to identify critical limits of either temperature or salinity for striped bass under static, fully-oxygenated conditions. Funds from this grant were used to develop and conduct flume experiments (Table 19) in order to further our understanding of how factors such as temperature, dissolved oxygen, and river flow affect the energetic costs of living in the Ashley River, and therefore how these factors may limit growth and survival during summer months.

Table 19. Phases of experimental design, troubleshooting, and performance. Trials not used in analysis were used for obtaining preliminary information, or involved equipment malfunction. Phase I (25-50 mm TL), Phase II (100-200 mm TL), and Phase III (200-300 mm TL)

Dates	Size	Total Trials Conducted	Trials Used in Analysis	Purpose
9/19/2013-12/10/2013	Phase I	31	0	Methods development and equipment troubleshooting
12/15/2013-1/16/2014	Phase II	15	12	Preliminary studies to determine appropriate flume flushing time, flow speed, and replicates to use in future trials
5/19/2014-11/14/2014	Phase III	61	45	Determine metabolic scope of fish under summer conditions, using sizes comparable to fish in the wild during their second summer

Metabolic scope is a measurement which can be used to calculate the energetic costs related to environmental conditions, as it represents the potential energy an animal has to fuel all activities (growth, motion, digestion, reproduction, etc.) under a given set of environmental conditions. Metabolic scope is measured as the difference between the standard metabolic rate (SMR, the minimal maintenance of unfed fish) and the active metabolic rate (AMR, the maximum rate of energy expenditure). Metabolic scope was calculated by measuring oxygen consumption in a 90 L sealed, dissolved oxygen meter-equipped flume tank located within a temperature controlled experimental chamber. Fish were acclimated to environmental conditions (temperature and salinity) for at least two weeks prior to testing. One week before use, fish were transferred singly to a holding tank and fasted for 7 days to ensure that digestion did not affect the oxygen consumption. The day before each trial, a single fish was anesthetized, weighed, measured and

transferred to the flume system. Fish were allowed to recover overnight at full oxygen concentration in low flow conditions prior to the onset of the experiment. After an overnight recovery, oxygen in the flume was decreased until the treatment oxygen level was reached, and then maintained for 30 minutes to allow for acclimation. Water flow speed was incrementally increased in the flume by 15 cm/s every 45 minutes. At each speed, oxygen consumption was repeatedly measured over four-minute intervals. Speed was continuously increased until maximum oxygen consumption was reached and fish exhausted in the flume.

Preliminary Flume Experiments Using Phase II

In preliminary studies completed in the winter of 2013-2014, we calculated the metabolic scope of phase II striped bass (206 ± 12 mm TL, 95.6 ± 20.7 g) at three different temperatures (20°C , 25°C , 30°C) and two dissolved oxygen concentrations (2.5 mg/L, 3.5 mg/L, Figure 6) using two replicates per treatment ($N=12$). Since phase II fish are larger than those that would be in the Ashley River during their first summer, these trials were used to determine the best methods to use in future flume trials. Metabolic scope was lower at 2.5 mg/L than at 3.5 mg/L at every temperature. At each oxygen concentration a critical temperature was calculated; this represents the temperature at which metabolic scope is equal to zero, and all energy is used to maintain basal metabolism. The critical temperature is 37.7°C at an oxygen concentration of 3.5 mg/L, and 32.7°C at a 2.5 mg/L. If we compare these requirements with the summer conditions observed in the Ashley River over several years, we see that the temperature rarely rises above 32°C , and oxygen rarely drops to 2.5 mg/L (Figure 7 and 8). When these conditions do occur, it is only on the order of several hours to 2-3 days. Given the rarity of these extreme events, it is unlikely that they would co-occur in the Ashley River. These preliminary data also suggest an optimum temperature of approximately 25°C for this strain of striped bass.

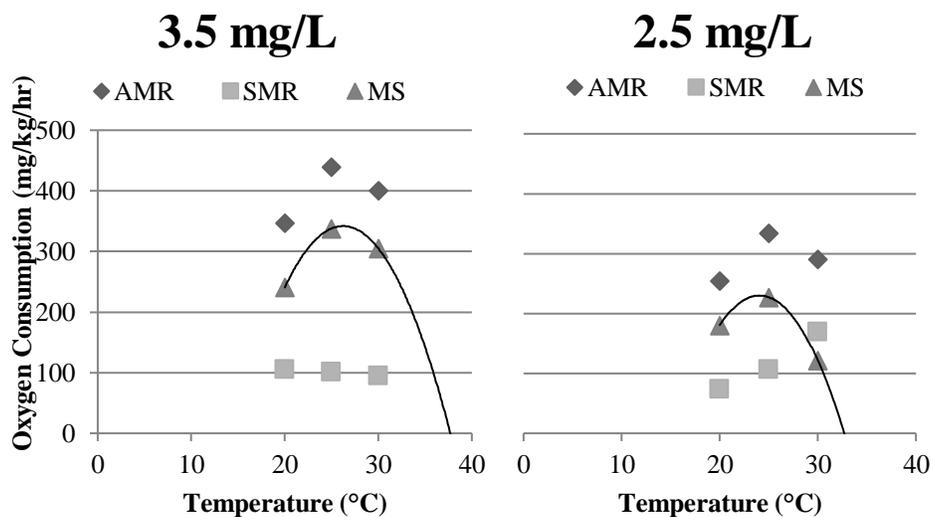


Figure 6. Influence of temperature on active metabolic rate (AMR), standard metabolic rate (SMR) and metabolic scope (MS) at two oxygen concentrations, 3.5 mg/L and 2.5 mg/L, for phase II (100-200 mm TL) striped bass.

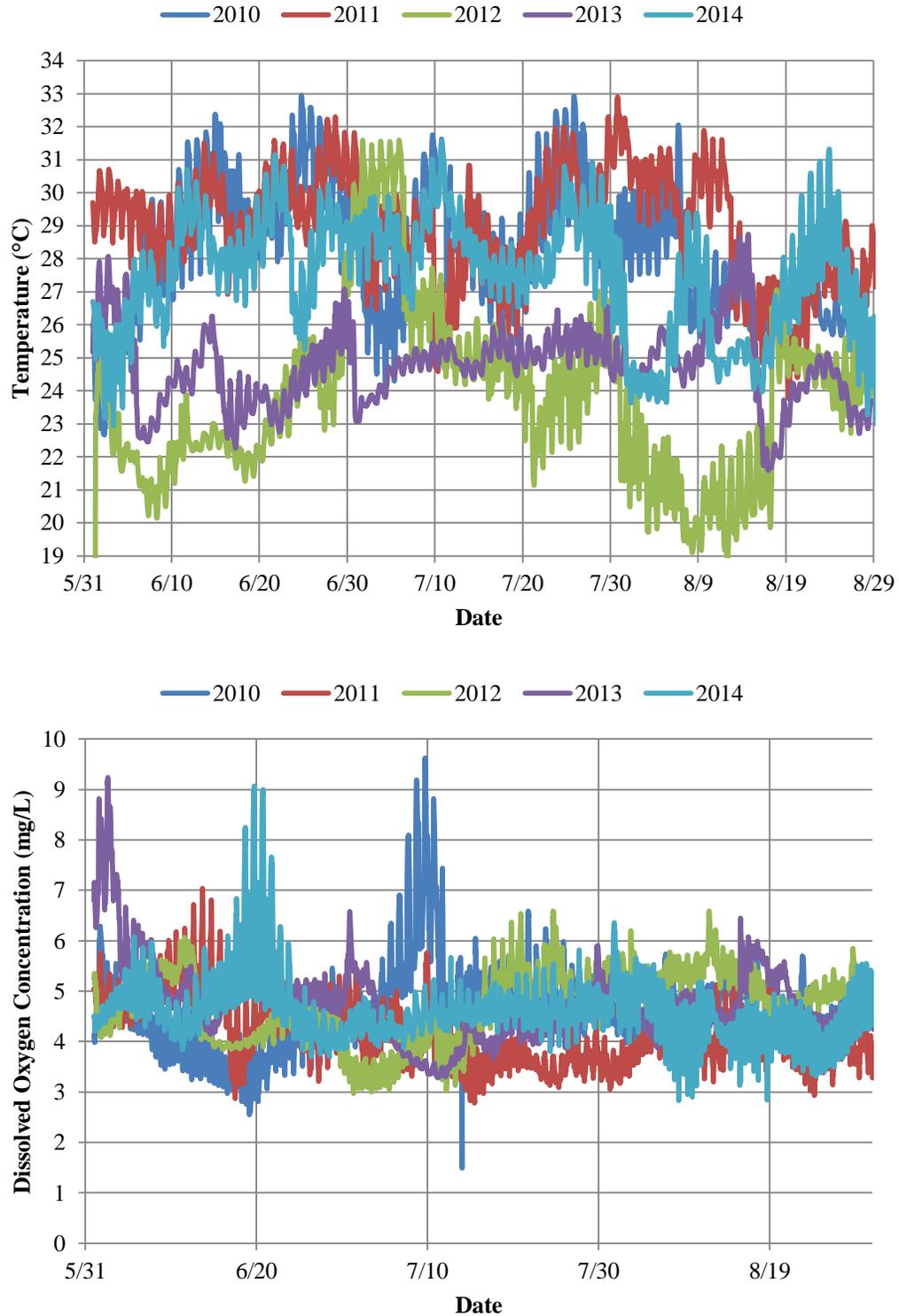


Figure 7. Temperature (°C) and dissolved oxygen (mg/L) levels recorded every 15 minutes in the freshwater Ashley River (FWA) sampling strata from June 1-Aug 30 of 2010-2014.

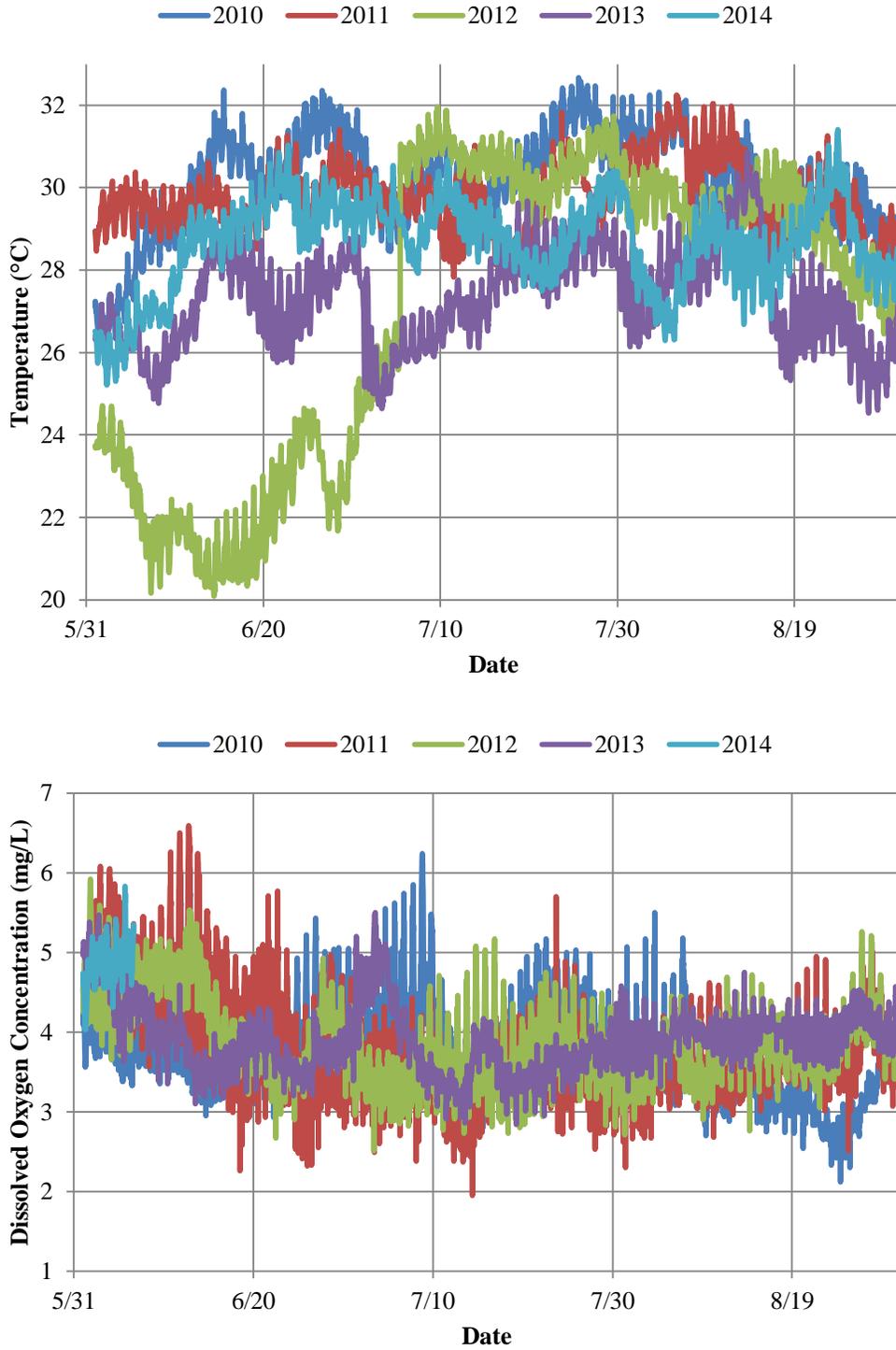


Figure 8. Temperature (°C) and dissolved oxygen (mg/L) levels recorded every 15 minutes in the upper Ashley River (UA) brackish water sampling strata from June 1-Aug 30 of 2010-2014.

Phase III Metabolic Scope Flume Experiment

In 2014, flume studies were expanded to test phase III fish (352±15 mm TL, 446.5± 62.9 g) at three temperatures (20°C, 25°C, 32°C) and three oxygen concentrations (2.5 mg/L, 3.0 mg/L, 4.0 mg/L), using 5 replicates per a treatment (N=45). Unlike our preliminary study, flume trials with phase III striped bass showed that metabolic scope was higher for fish at 32 °C than at 25 or 20 °C (Figure 9, Two-way ANOVA, $p=0.038$, $p=0.072$). While metabolic scope should theoretically decrease at temperatures above 32 °C, these results suggest that striped bass in the Ashley River should be able to manage the energetic costs associated with the high temperatures observed in the Ashley River. Additional trials performed at higher temperatures would be needed to find the critical temperature associated with low oxygen concentrations.

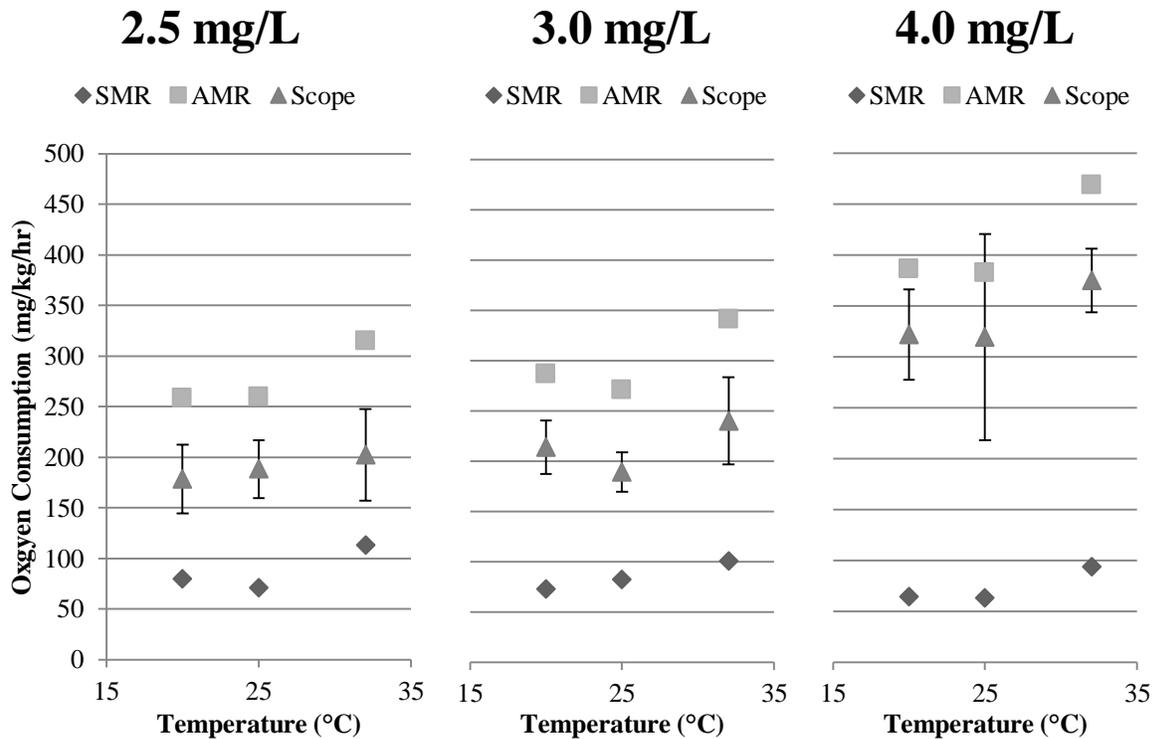


Figure 9. Influence of temperature on active metabolic rate (AMR), standard metabolic rate (SMR) and metabolic scope (MS, ±SD) at three oxygen concentrations, 2.5 mg/L, 3.0 mg/L, and 4.0 mg/L, for phase III (200-300 mm TL) striped bass.

Trials with both phase II and phase III fish showed that metabolic scope decreased with decreasing oxygen concentration. In the phase III trials, scope at 4.0 mg/L was significantly

higher than at 3.0 or 2.5 mg/L (Two-way ANOVA, $p < 0.001$). The measurements of metabolic scope at low oxygen concentrations (3.0 mg/L and below) averaged between 180-240 mg/kg/hr. A portion of this scope is required for motion, engaging in activities such as general swimming, foraging, and escaping predation. We measured water flow in the Ashley River to be 60 cm/s at low tide, representing an approximate maximum flow rate. Assuming that flow is near zero at high tide, over a full tidal cycle the average flow rate would be approximately 40 cm/s. Oxygen consumption from swimming at flows from 15-45 cm/s averaged 40-210 mg/kg/hr at 2.5 and 3.0 mg/L (Table 20), and most fish at these low oxygen concentrations were unable to swim at 60 cm/s. These data suggest that fish may become stressed in moderate-high flow conditions (above 30 cm/s) if oxygen levels stay low over an extended period of time. It is probable that under such conditions, striped bass avoid areas of high flow.

Table 20. Energetic costs incurred by striped bass due to swimming at different flow rates while exposed to low oxygen concentrations.

Dissolved Oxygen	Temperature (°C)	Oxygen Consumption (mg/kg/hr)		
		15 cm/s	30 cm/s	45 cm/s
2.5 mg/L	20	39.53	89.42	178.56
	25	34.55	100.96	186.31
	32	48.42	92.00	202.33
3.0 mg/L	20	41.16	110.69	213.78
	25	34.70	84.88	185.36
	32	47.01	114.41	211.74

These tests do not support the hypothesis that striped bass stocked in the Ashley River cannot survive recorded temperatures of 32 and low oxygen conditions exhibited during the summer months. Hatchery fish exhibited sufficient metabolic scope to endure the energetic costs related to environmental conditions in the Ashley River. This is likely similar for wild recruits which is encouraging as it suggests that the conditions in the system are sufficient for striped bass to survive and eventually repopulate this system.

III. Conclusions

This project supported striped bass stock enhancement in Charleston Harbor from 2010 to 2014. Project funds were used to evaluate the potential for using stock enhancement as a means to re-establish historic levels of biodiversity and restore a key indicator species to coastal rivers in the Southeast with the ultimate goal of developing a conceptual restoration model for striped bass in coastal rivers.

Over the course of the project, striped bass were stocked in the Ashley, Cooper, and Wando rivers of the Charleston Harbor system. Striped bass from each stocked year class and each river were recaptured during this project. While there were multiple immigrants from the Santee-

Cooper lakes captured in the Ashley (n=6), only one striped bass released in the Ashley River was recovered from outside of the Ashley River (Cooper). The persistence of multiple year classes within the Ashley River and the limited number of recaptures outside of the system indicate limited emigration of stocked fish out of the Ashley River.

The capture of wild young-of-the-year striped bass in 2009 and 2010 may indicate spawning activity is occurring in the Ashley River. It seems unlikely that fish collected at age 0 were spawned in a different system and migrated to the Ashley River at such a young age and small size. Histological examination of ovaries from female striped bass collected in the Ashley River revealed that almost 43% of all female striped bass captured above the age of 3 had GSI values higher than 5 and all seven females were categorized as either developing or recent spawners. All females aged two years or younger captured in the Ashley River were found to be immature. Of the 5 male striped bass examined between the ages of 1-3, all were either developing or sexually mature. The lack of substantial contribution of wild fish to the most recent 2012 and 2013 year classes could be an indication that the system is at capacity or environmental conditions were not favorable for natural recruitment of wild fish.

Metabolic scope experiments suggest that striped bass in the Ashley River should be able to manage the energetic costs associated with the extreme high temperatures and low dissolved oxygen concentrations observed in the Ashley River during the summer. These findings confirm the results of temperature tolerance studies conducted on hatchery reared striped bass in previous studies.

The results of this project are encouraging for the future of the striped bass restoration effort in the Ashley River. We have determined the best size and age class of fish to stock (Phase II) to successfully increase abundance to a critical mass that might facilitate wild recruitment and have demonstrated survival of striped bass grown in both conventional freshwater rearing systems and brackish water pond systems. We have also determined that the environmental conditions within the river are sufficient for survival and that fish are becoming sexually mature and returning to the river to spawn. Continued monitoring over the next 5-10 years will be necessary to determine if the stocking effort was enough to restore this key indicator species to the Ashley River. Further, the methodology and results presented here make production and stocking of striped bass in coastal rivers a viable means of reducing the chance of this important species from being listed as a threatened species.