Effects of Salinity on Survival, Growth, and Nutritional Condition of Juvenile Striped Bass: Possible Environmental Factors Effecting Their Distribution in Southeastern Estuaries

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Abstract: Juvenile stages of striped bass (*Morone saxatilis*) depend on estuarine productivity for rapid growth and estuarine habitat diversity for predator protection. The distribution of juvenile striped bass within estuaries may be influenced by salinity. The potential influence of salinity on the suitability of estuaries as nursery areas was investigated in laboratory experiments using four age groups 67- and 91-d post hatch (25 C) and 112- and 133-d (28 C) post hatch of juvenile striped bass reared for 14 days at three different salinities (0, 5, and 10 ppt) representing conditions encountered in different estuarine zones of the Southeastern United States. We examined salinity effects on survival, growth rate, and nutritional condition. Nutritional condition was determined using the liver somatic index (I_L), percent carcass lipid, hepatocyte cell size, and liver glycogen content. Survival exceeded 98% in all treatments. Measured parameters did not vary with salinity in any consistent pattern. Growth rates at 25 C were highest at 10 ppt, but salinity had no affect growth at 28 C. I_L was unaffected by salinity at 25 C but greatest in 0 and 10 ppt at 28 C. Percent lipid was lowest at 10 ppt at 25 C, but salinity had no effect at 28 C. Salinity did not effect on hepatocyte cell size but did influence liver glycogen content at 25 C. The liver glycogen content was lowest at 5 ppt but there were no differences between 0 and 10 ppt. Our results suggest that salinity (0–10 ppt) is not critically important to the growth and condition of juvenile striped bass.

Keywords: Juvenile striped bass, growth, salinity, nutritional condition

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The striped bass (*Morone saxatilis*) is an important sport and commercial fish species in the United States (Huppert 1989). Declining striped bass stocks along the Atlantic Coast in the 1970s and 1980s resulted in numerous studies to determine

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causative factors. Over-fishing, deteriorating water quality and poor survival of early life stages have been implicated in the reduction of adult populations (Chafee 1980, Hassler et al. 1981, Hall et at. 1987). Variability of survival of larvae and juveniles is a major determinant of recruitment in many striped bass populations (Monteleone and Houde 1992), and survival is linked to growth rates during early life stages (Houde 1989). Because predation often is size dependent, slow growth during early life stages may prolong the period of vulnerability for these stages to predation (Rice and Miller 1993).

Most commercially and recreationally important fishes along the U.S. Atlantic and Gulf Coasts inhabit estuaries during one or more life history stages (Weinstein 1979, Bozeman and Dean 1980). Larvae and juveniles of these species depend on the productivity and diversity of estuarine habitats for rapid growth and protection from predators (McDonough and Wenner 2003, Ross 2003). Striped bass spawn upstream and their planktonic eggs are carried downstream to estuaries where eggs hatch and larvae develop. Eggs and larvae are generally distributed in the region above the saltwater interface (Van Den Avyle and Maynard 1994, Secor and Houde 1995).

Many physical and chemical parameters affect fishes in estuaries, but fluctuating salinity is one of the most conspicuous physiological challenges faced during development. Salinity may affect survival, growth, and overall health of larvae and juveniles (Secor et al. 2000) and therefore have an effect on recruitment (Kinne 1964, Otwell and Merriner 1974, Morgan and Rasin 1981). Energetic demands of osmoregulation at extremely high or low salinity may reduce the energy available for growth (Kinne 1964, Brett 1979). Lankford and Targett (1994) showed that growth and condition of juvenile weakfish (*Cynoscion regalis*) in the Delaware Bay differed among spatially disjunct estuarine nursery zones according to salinity.

Wallin and Van Den Avyle (1995) found that stocked and naturally spawned juvenile striped bass were most abundant at locations near the freshwater/saltwater interface in estuaries of the Ogeechee and Savannah rivers in Georgia. In their studies, most of the juveniles occurred in areas where salinity ranged from 0 ppt at low tide to 3.0 ppt and very rarely in salinities approaching 10 ppt at high tide (Sinclair 1996). Juveniles in other systems also depend on the saltwater interface. In Chesapeake Bay juvenile striped bass are found mostly in salinities lower than 3 ppt (Setzler-Hamilton et al. 1981). Fish distribution varied annually in relation to river discharge, and locations of maximum juvenile abundance were just seaward of the saltwater interface. It is unclear if juveniles inhabited these areas to optimize metabolic efficiency, maximize growth, to avoid predators, or some combination.

We compared the effect of salinity on growth and nutritional condition of juvenile striped bass and its interactive effects on age. We hypothesized that growth and nutritional condition of juvenile striped bass would be higher in salinities of 0 ppt than at 5 and 10 ppt. If survival, growth, and nutritional condition are higher in 5 ppt or 10 ppt salinities, this would suggest that salinity is not important in determining the distribution of juvenile striped bass and juvenile striped bass are actively selecting areas of non-optimal salinity.

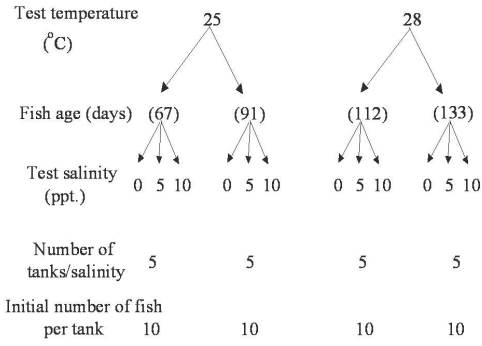

Figure 1. Experimental design of laboratory experiments to test the effects of salinity on survival, growth and condition on four age-classes (67-, 91-, 112-, and 133-d) of juvenile striped bass.

Methods

Experimental Design

We conducted laboratory experiments using four age groups: 67-, 91-, 112, and 133-d striped bass tested at two temperatures [25 C (67- and 91-d) and 28 C (112- and 133-d)]. Within each age-class short-term survival (14-d), growth, and nutritional condition were compared among groups exposed to salinities of 0, 5, or 10 ppt (Fig. 1). Details of the test system and methods for measuring response variables are presented below.

We selected water temperatures that juvenile striped bass would typically encounter in the southern extreme of this species' range during June through August. For example, a juvenile striped bass spawned in March would be approximately 70-d of age in early June when water temperature typically first reaches 25 C in the Savannah River (Wallin and Van Den Avyle, unpublished data). Likewise, temperature usually reaches 28 C in early August, when fish spawned in March would be about 130-d old.

We obtained juvenile striped bass from the Georgia Department of Natural Resources McDuffie Fish Hatchery in Thomson, Georgia, and Richmond Hill Fish Hatchery, Richmond Hill, Georgia. The broods at each hatchery were produced on the same day, and then combined and held in the laboratorty until the prescribed ages. Experimental fish were the progeny from the crossing of four females and eight males at each hatchery. The progeny from the crossings were combined to reduce the influence of genetic variation and the test fish were randomly selected for each temperature and salinity treatment. All brood stock was collected from Lake Lanier, Georgia.

Test System and Protocol

The test system consisted of three source tanks, each supplying five 95-L recirculating test tanks (Fig. 1). Source tanks were held at 0, 5, or 10 ppt salinities. Salinity was maintained by the use of synthetic sea salt brine (Instant Ocean), and each source tank was equipped with a heater to maintain temperature. All tests were

Table 1. Mean (±SE) initial length and weight of juvenile striped bass used in salinity trials.

Temperature (C)	Age (days post hatch)	Length (mm)	Weight (mg)
25	67	39.1 ± (0.3)	688.1 ± (17.5)
	91	51.8 ± (0.4)	1471.4 ± (36.7)
28	112	60.4 ± (0.5)	2144.7 ± (90.1)
	133	75.8 ± (0.6)	4344.4 ± (111.8)

conducted in a temperature-controlled room (20 C) and natural photoperiod (13 h light: 11 h dark).

Ten fish were randomly placed in each test tank and acclimated to test conditions for 14-d. After acclimation, each fish was blotted dry and mass was determined to the nearest 0.01 mg, measured to the nearest 0.01 mm total length (TL), and returned to the test tank for 14-d test period (Table 1). Initial weights did not differ among salinity treatments for any of the groups tested at the start of the tests (ANOVA; $P \geq 0.05$).

During all experiments, fish were fed once daily using Salmon Starter #1 or #2 (Ziegler Brothers; 55.1% crude protein, 15.5% lipid) at 5% of their initial combined body mass. The particle size was adjusted according to the size of the fish. We observed test fish immediately after feeding and no excess food was observed in the tanks so we assumed that all food was consumed. The feeding levels at the end of the experiment trials translated to 1.1% to 4.0% of the final body mass.

After each test run, each fish was blotted dry and the mass was determined. The peritoneal cavities of the fish were opened along a ventral midline incision, and the liver was removed and the mass calculated to estimate the Liver Somatic Index (I_L). A portion of each liver was excised and stored in 10% neutral-buffered formalin solution for histological examination. The remaining section of the liver and the carcass were frozen at –20 C for later lipid analysis.

Fish Responses

Growth and Survival

Survival was calculated as the percentage of fish surviving to the end of the experiment at each salinity treatment. Daily specific growth rate (S_{GR}) was calculated for each test tank as

$$S_{GR} = \{\ln (Wt_f) - \ln (Wt_i) / (14 \times 100\ d^{-1})\},$$

where S_{GR} is the net change in body mass per day, Wt_f = average wet mass of all fish at the end of the experiment, Wt_i = average wet mass of all fish at the beginning of the experiment, and 14 = number of days in the experiment (Ricker 1979). A 2-factor ANOVA was used to determine if S_{GR} varied significantly with salinity or

age within tests conducted at the same temperature. Statistical analyses were done separately for each test temperature. We used contrasts to test the significance of one factor at each level of the other interacting factor. Comparisons among treatment combinations were made with Tukey's post hoc contrasts (Sokal and Rohlf 1981). Differences were considered significant at $P \leq 0.05$. Statistical calculations were made with SAS/STAT (SAS 1988).

Liver Somatic Index

The livers were removed from each fish, weighed, and the liver somatic index (I_L) was calculated as

$$I_L = \{\text{liver mass/fish mass (including the liver)}\} \times 100 \text{ (King 2004).}$$

Results were compared using a fixed-effects nested ANOVA to determine if mean I_L varied significantly with age and salinity. Significance of age and salinity treatment effects and their interaction was tested by using the error mean square associated with variation among tanks. Prior to analysis, data were arcsin transformed to meet the assumptions of the ANOVA.

Lipid Determination

Fish carcasses were thawed and dried to a constant mass at 80 C for 24-h. The bodies were then crushed and placed into extraction thimbles, which were placed in a Soxtec apparatus (2 h immersion, 1 h rinse), where nonpolar lipids were extracted with petroleum ether. After extraction, the mass of lipid was calculated as the difference of the masses before and after extraction. All weighing was done in a sealed glove box with 0% humidity, and all samples were weighed to the nearest 0.01 mg. Differences of mean percent lipids among the salinity treatments were tested statistically using the same methods as for I_L.

Hepatic Histology

Fixed liver samples were routinely processed for histology, embedded in paraffin, cut at 5 µm, and stained by the Periodic Acid-Schiff (PAS) method (Luna 1968). Three 0.015-mm^2 fields (40x) were examined in each liver sample. We used the classification system described by Reimschuessel et al. (1992) to rate the samples for presence of glycogen within the hepatocytes. Five severity codes were used to score the amount of glycogen in each field: (0) absent, (1) minimal, (2) mild, (3) moderate, and (4) severe. Three random cells per field were measured, and the average area of hepatocytes was determined using an image analyzer system with a video camera and calculated using the software program Sigma Scan Pro-3. There were no results for 67-d fish because of improper preparation of the liver samples. Differences among salinity and age were compared using methods described for I_L. Prior to analysis, all data were log-transformed to meet the assumptions of the ANOVA.

Results

Survival and Growth

Survival was greater than 98% throughout the test period. The tanks were checked daily for mortalities and out of the initial 597 fish, 590 survived the salinity tests. Two of the seven mortalities were found in the tanks and cannibalism was assumed to be the cause of mortality for the other five.

S_{GR} ranged from 0.02 to 0.03 g day^{-1} at 25 C and from 0.03 to 0.04 g day^{-1} at 28 C but did not vary with salinity in any consistent pattern. Salinity explained 20% and 57% of the total variation in growth rate (Table 2) and affected growth at 25 C but not at 28 C. At 25 C, growth increased with salinity and was greatest at 10 ppt but the effect differed between ages (Fig 2a). Growth was higher for 91-d fish than 67-d at 5 ppt. At 10 ppt, mean growth rate was faster for 91-d fish than 112-d. There were no significant differences between the fish at different ages 0 ppt (Table 2). At 28 C, growth was fastest at 0 ppt but the effect was only marginal ($P = 0.08$) (Table 2). As with the fish tested at 25 C, the older cohort (133-d) had a mean growth rate higher than the younger (112-d) cohort at all salinities (Fig 2).

Liver-somatic Index

As with growth, I_L varied with salinity, but in no consistent pattern (Figure 2b). Salinity explained 6% and 45% of the total variation in the ANOVA. I_L was affected by salinity at both temperatures. At 25 C, I_L in 0 ppt and 10 ppt was significantly higher than 5 ppt. At 25 C, the I_L values were significantly higher for the older cohort (91-d) than 61-d fish at 10 ppt. At 28 C, I_L was lowest at 5 ppt but the effect differed with age. The I_L of the 112-d cohort decreased with increasing salinity. However, the 133-d cohort had significantly lower I_L (2.83) at 5 ppt than at 0 ppt and 10 ppt.

Lipid Content

Mean lipid content increased with age but the effect of salinity varied between temperature treatments. Salinity explained 1% and 8% of the variation in the lipid content model. Mean lipid content was affected by salinity at 25 C but no significant effects were observed at 28 C (Table 2). Mean percent lipid content at 25 C was lowest in 10 ppt, which was significantly different from 0 ppt but not different from 5 ppt (Figure 2c). Age had a greater effect on lipid content than salinity, explaining 82% and 85% of the variation (Table 2).

Histology Liver Glycogen Score and Histology-Cell Size

Liver glycogen was uniformly distributed throughout all liver samples. One percent of the observed samples received a score of zero. Most of our samples (73.5%) received scores of 3 or 4. Glycogen score did not vary with salinity in any consistent pattern (Figure 2d) and salinity explained <0.01% and 7% of the variation in the ANOVA model. The 91-d cohort mean glycogen scores at 25 C were significantly higher at 0 ppt than 5 ppt but there were no differences between 0 and 10 ppt or 5

Table 2. Results from the two-way analysis of variance for S_{GR}, IL, lipid, glycogen, and cell size for 25 and 28 C of juvenile striped bass tested at 0, 5, 10 ppt salinity. The percent of variation explained is the sums of squares for the source divided by the model sums of squares.

	25 C			28 C		
Source of variation	df	*P*	Percent of explained variation	df	*P*	Percent of explained variation
SGR Model	5	<0.01		5	<0.01	
Age	1	0.72	<0.01	1	<0.01	71
Salinity	2	<0.01	57	2	0.08	20
Age*salinity	2	<0.01	42	2	0.28	9
Error	24			24		
Total	29			29		
LSI Model	17	0.08		17	<0.01	
Age	1	<0.01	39	1	<0.01	85
Salinity	2	<0.01	45	2	<0.01	6
Age*salinity	2	0.95	0	2	<0.01	8
Tank (salinity)	12	0.87	16	12	0.65	1
Error	12			12		
Total	29			29		
Lipid Model	17	<0.01		17	<0.01	
Age	1	<0.01	85	1	<0.01	82
Salinity	2	0.03	8	2	0.58	1
Age*salinity	2	0.89	0	2	0.30	2
Tank (salinity)	12	0.78	6	12	0.10	16
Error	12			12		
Total	29			29		
Glycogen Model	5	0.09		7	0.56	
Age	0	<0.01	28	1	0.43	11
Salinity	2	0.20	0	2	0.14	7
Age*salinity	0	<0.01	19	1	0.71	2
Tank (salinity)	3	0.48	65	3	0.23	80
Error	9			17		
Total	14			24		
Cell size Model	5	0.03		7	<0.01	
Age	0	<0.01	9	1	<0.01	68
Salinity	2	0.45	0	2	<0.01	5
Salinity*age	0	<0.01	45	1	<0.01	12
Tank (salinity)	3	0.09	45	3	0.21	4
Error	9			17		
Total	14			24		

ppt and 10 ppt. Glycogen scores were unaffected by salinity and age at 28 C. Cell size ranged from 3.33 to 5.61 μm^2 in the 25 and 28 C treatments, respectively. Cell size was lowest at 5 ppt at 25 C (Figure 2e); however, this difference was not significant from the other salinities. Cell size increased with salinity at 28 C and the effect of salinity varied with age. Mean cell size for 133-d cohort was three times larger than mean cell size of 112-d fish. Cell size was higher at all salinities for 133-d cohort than 112-d fish.

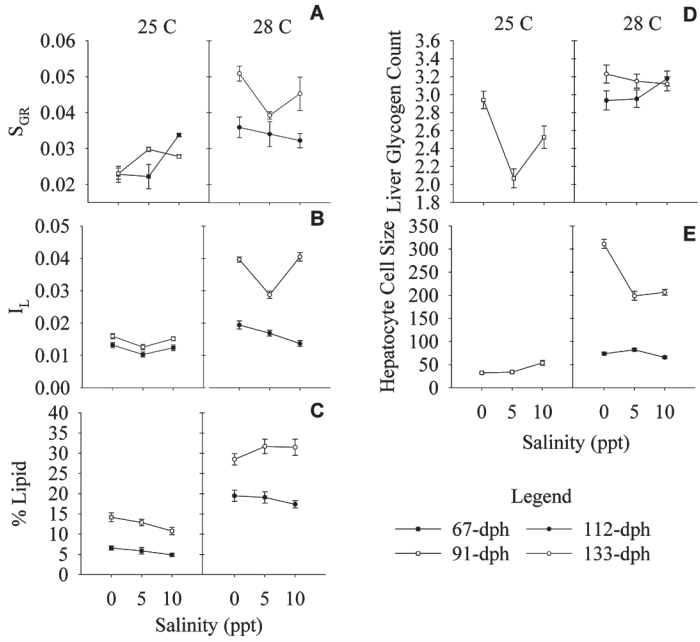

Figure 2. Mean (±SE) specific growth rate (S_{GR}) (A), liver somatic index (I_L)(B), percent lipid (C), liver glycogen count (D), hepatocyte cell size (E), and of juvenile striped bass held at salinities of 0, 5, and 10 ppt. Closed bars represent juvenile striped bass (67- and 91-d post hatch) fish tested at 25 C and open bars represent juvenile striped bass (112- and 133-d post hatch) tested at 28 C.

Discussion

Differences in growth and nutritional condition appeared to result from a complex interaction of several abiotic and biotic factors; they were not adequately explained by any one variable included in this study. From these results, there appeared to be no consistent energetic benefit to juvenile striped bass residing in areas of estuaries differing in salinity from 0 to 10 ppt in temperatures between 25–28 C. Survival rates of juvenile striped bass in our study were inconclusive because most of the mortalities were the result of cannibalism. Almost all other mortalities occurred at 0 ppt. This is consistent with the results of Secor et al. (2000) where 60% of their mortalities occurred in lower salinities (0.5 ppt) for juvenile striped bass in temperatures ranging from 20–28 C. The results from both studies provide evidence to support the juvenile striped bass salinity preference trials by Bogenrider (1997). He concluded that juvenile striped bass tend to avoid freshwater but show no distinct preference for salinities in the range of 5–15 ppt. Other studies have examined the influence of salinity on survival of juvenile striped bass. Krouse (1968) reported greater survival of juvenile striped bass tested at 5 ppt and 15 ppt than at 25 ppt.

We found no consistent relationship between growth and salinity. Lemm et al. (1993) tested the effects of salinity (0, 3, and 10 ppt) on juvenile striped bass and found no differences in growth when fed a salmon diet. Their results did, however, show faster growth rates at 0 ppt when juvenile striped bass were fed a trout feed. Otwell and Merriner (1975) evaluated salinity effects on growth of juvenile striped

bass and found that growth was greater in 12 ppt than in 4 ppt and 20 ppt. Their study was designed to provide evidence for predicting stocking success when age and the temperature-salinity conditions in the stocking areas were known. Experimental differences in acclimation periods and test durations may explain differences among our results. We acclimated fish for 7 days to test conditions and exposed fish to test conditions for 14 days. Otwell and Merriner (1975), however, had no acclimation period and the fish were held for 7 days. Our growth responses at 25 C increased with salinity, which supports the findings of Chesapeake Bay strain striped bass (Secor et al. 2000). However, our remaining growth response to salinity was very similar to those reported by Secor et al. (2000) where they found no response to salinity (0.5–15 ppt) of growth for Santee-Cooper (South Carolina) strain juvenile striped bass. They suggested variation among estuaries might lead to the differences in growth rates.

Nutritional condition of the juvenile striped bass varied among our tests, but as with survival and growth these differences were not pronounced and showed no consistent influences between salinity treatments. Fish store significant amounts of lipids and glycogen in the liver (Hoar and Randall 1971). Measurements from the liver have been used as an indicator of short-term feeding rates (Heidinger and Crawford 1977). We examined the liver-somatic index (I_L) and used it as a measure of nutritional stage, with a low I_L indicating poor condition. Changes in the quantity of fat and glycogen stored in the liver have been shown to cause significant changes in the liver (Phillips et. al. 1960, Knot 1971, Heidinger and Crawford 1977). Large livers and high I_L indicate healthy, well-fed, rapidly growing fish with large lipid and glycogen reserves, but a high I_L may also indicate a fish under stress. The possibility of stress being the cause of the abnormally high I_L in our study was ruled out. We would have expected to observe reductions in growth or other condition indices in response to stress as well. I_L values were higher for 133-d fish compared to the other tested age groups. The high I_L of the 133-d fish is probably because of increased vacuolization of the hepatocytes by lipids. This increase is also supported by the lipid content and cell size data. Lipids are stored throughout many tissues in the body and are used when food intake is not sufficient to support metabolic activities. Lipid content was highest in 133-d fish, and cell size was almost three times that of fish at 112-d fish. The size of the hepatocytes cells is not affected by fish size but depends on the amount of energy stored.

Fish confronted with shifting salinity conditions in estuaries could cope with salinity in two ways: 1) behaviorally, by moving to remain within their preferred salinity range, or 2) physiologically, by tolerating the fluctuating salinity. Moving within a preferred salinity throughout an estuary may indicate a species' low tolerance to fluctuating salinity or an adaptation to maximize growth or optimize nutritional condition within a relatively defined salinity zone. Faster growth and increased condition at a particular preferred salinity will likely reduce the time vulnerable to predation. However, this type of behavior may also have its trade-offs. Increased movement in the estuary may increase the probability of encountering a predator, whereas decreased movement may reduce predator encounter rates thus increasing

the chance of survival. Because of the patchiness often associated with food distribution, fish exhibiting this type of behavior may decrease the frequency of location of food concentrations adequate for survival. Some fishes have shown faster growth rates in fluctuating salinity compared to an optimum steady salinity (Konstantinov and Martynova 1990), which may be true for juvenile striped bass. However, we did not test those effects.

The test fish in our study were the progeny of four females and eight males from each hatchery from the Georgia Department of Natural Resources. It is likely that our test fish lacked the genetic diversity of what is typically expected in the wild. However, restoration of the Savannah River estuarine population of striped bass often depends heavily on hatchery-reared fish from Lake Lanier (T. Will, Georgia Department of Natural Resources, personal communication) of progeny similar to those used in our study. Given the original origins of Lake Lanier fish and the lack of evidence of parental exposure on offspring osmoregulatory ability, we feel that our findings apply to the broader attributes of juvenile striped bass distributions and response to salinity.

Mechanisms and processes that influence the movements and distributions of juvenile striped bass in estuaries are not well understood and need to be further studied. The knowledge could lead to a substantial improvement in understanding the mechanisms and consequences of movements of young fishes. We designed the salinity and temperature combinations that a juvenile striped bass would typically encounter in its southern range. If salinity regimes common to Southeastern U.S. estuaries do not significantly influence survival, growth and nutritional condition of juvenile striped bass, other ecological factors may play a more important role in influencing their distribution. In Savannah River, Georgia, Sinclair (1996) showed that most of the juvenile striped bass were found near or at the saltwater-interface, which were similar to the range of salinities tested in our study. The results of our study suggest that factors other than salinities tested in our study may be contributing to the distribution of juvenile striped bass. Results from this study will hopefully provide insight to the juvenile fish ecology, with a better understanding of spatial distribution of juvenile striped bass to help refine management programs aimed at restoring habitats and maintaining stocks of striped bass.

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