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FORMATION OF A STRESS-INDUCED CHECK MARK ON THE OTOLITHS OF JUVENILE FISHES: IMPLICATIONS FOR MESOCOSM STUDIES

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ABSTRACT Daily otolith increment widths of spot *Leiostomus xanthurus* and spotted seatrout *Cynoscion nebulosus* were examined experimentally in field mesocosms for 5 to 7 days in various habitat types. Daily otolith increments were used as a surrogate for daily somatic growth so that growth prior to capture and handling could be examined. For both species, possible effects of habitat types were confounded by an overall decrease in daily increment widths during the experimental period when compared to increment widths prior to capture. Several spotted seatrout inadvertently captured during mesocosm deployment provided a means for assessing if there was a significant mesocosm effect or if capture and handling may have caused the decreased increment widths. These "volunteers" were distinguishable from experimental fish by the occurrence of a check mark on the otoliths of the experimental fish. Because experimental increment widths of "volunteers" were not different from pre-experimental widths, handling rather than caging effects appeared responsible for reduced increment widths. While there appeared to be no "mesocosm" effect, handling stress potentially affected growth longer than the 24 h acclimation period we anticipated. Short-term effects of capture and handling of wild fish for mesocosm use should be explored and accounted for in future studies.

INTRODUCTION

Enclosures or experimental mesocosms have been used in aquatic research for a variety of investigations including growth (Sogard 1992, Keller and Klein-MacPhee 2000), survival (Cowan et al. 1992, Stunz and Minello 2001), predation (Elliot and Leggett 1996, Kim and Devries 2001), and ecological risk analysis (Boyle and Fairchild 1997). Mesocosms provide an experimental method for confining test subjects in areas of known environmental characteristics (e.g., substrate type, emergent vegetation) while allowing other abiotic factors (e.g., salinity, temperature) to fluctuate naturally (Cline et al. 1994, Breitburg et al. 1997). As such, mesocosms may be an excellent tool for assessing the relative value of various habitat types to growth and survival of juvenile fishes. However, interpretations of results from mesocosm studies have been criticized on the basis of scale and artificiality (Petersen et al. 1999) and failure to recognize that experimental manipulations or natural phenomena may unequally interact with treatments (Peterson and Black 1994).

To address questions regarding the value of shallow-water habitat types for early growth in juvenile estuarine fishes, we conducted experiments using mesocosms and 2 species of estuarine fish. The main objective of this study was to use growth, as measured by daily otolith increment widths, to assess the relative value of various shallow-water habitat types common in the northern Gulf of Mexico (GOM). For a variety of reasons, we were unable to meet our objectives and post-experimental analyses could not overcome the problems encountered during our investigations. However, unexpected results from these experiments have allowed inferences on the use and effectiveness of mesocosms for in situ experiments such as those performed in this study. The objectives of this paper were to identify the problems encountered as part of this study, suggest means for improving similar designs, and provide evidence on the potential effects capture and handling may have on subsequent short-term growth of juvenile fishes.

MATERIALS AND METHODS

Spot *Leiostomus xanthurus* and spotted seatrout *Cynoscion nebulosus* are common residents in South Atlantic and GOM estuarine systems (Weinstein 1979, Baltz et al. 1993). In northern GOM waters, spot typically recruit to nursery grounds in the early spring (February–March) and seatrout recruit throughout the summer (June–August; Baltz et al. 1993). Their abundance and temporal distribution make them excellent candidates for serial studies examining growth over common habitat types found in the northern GOM. The in situ studies took place in a *Spartina*-dominated saltmarsh, near Fourchon, Louisiana, in March and August, 1992.

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On the day before each experiment began, 20 cylindrical mesocosms were set from a boat to minimize disturbance and to allow any disturbed substrate to settle. Mesocosms were constructed from colorless polycarbonate sheets, measuring 1.25 m in height with a basal area of 0.44 m^2. Three rows of 10 cm diameter openings covered by screens of 5 x 3.5 mm mesh encircled each mesocosm. These mesh openings allowed water and plankton to pass through while effectively retaining experimental fish. A remote water-quality probe, centrally located between mesocosms, monitored temperature (°C), dissolved oxygen (mg/l), salinity (psu), and relative tidal height (cm) during the experimental period.

On the day prior to each experiment, fish were captured by seine in an area adjacent to the experimental site. Individuals were not measured at the time of capture in an attempt to reduce handling stress. Additionally, salinity and temperature were reduced slightly (1–2 psu and 1–2 ºC) in initial holding water to further alleviate stress (Kelsch and Shields 1996). Fish were transferred within the hour to aquaria containing water of ambient temperature and salinity and held for about 12 h to ensure that only healthy fish were used. At the beginning of each experiment, 2 individual fish were randomly selected and placed into each mesocosm (i.e., 4.5 fish/m^2). Typical mean densities for occupied habitats in Louisiana for juvenile spot are 10.8/m^2 (maximum density of 40/m^2) and 2.2/m^2 (maximum density of 10/m^2) for juvenile spotted seatrout (Baltz et al. 1993).

The spot experiment examined 4 habitat types: mud, sand, mud with emergent *Spartina* stems (mud/stem), and sand with emergent *Spartina* stems (sand/stem). The spotted seatrout experiment compared only sand and sand/stem treatments, but did so using 2 different mesh types (the standard mesh of the spot experiment and fine mesh designed to exclude mysid shrimp that were attracted to the chamber structure; see Reinert 1993). The spot experiment lasted the planned 7 days, but the seatrout experiment only lasted 5 days, due to approaching inclement weather. At the end of each experimental period, fish were retrieved by dip net and the enclosed water was pumped through a 333 µm mesh plankton net to ensure that all remaining fish were recovered. Immediately following retrieval, standard length (SL) in mm was recorded and sagittal otoliths were removed from each fish.

In the lab, otoliths were embedded in an epoxy resin and sectioned in the transverse plane to produce a thin section around the core of the otolith (Haake et al. 1982). Otoliths were sanded and polished until daily increments were visible. At this point, if an otolith was not suitable for reading, it was etched with 0.1 N HCl to enhance readability (Secor et al. 1991). All increment measurements were accomplished with a calibrated image processing system on a microcomputer. Increments were measured along the same radius, immediately adjacent to the sulcus groove on each otolith. Pre-experimental increment widths were measured for the 5 daily increments immediately prior to the day of capture. The first experimental increment (i.e., day one of the experiment) and last daily increment (i.e., day 5 or day 7 for seatrout and spot, respectively) were omitted as they represented an acclimation day and an incomplete daily increment. Because otolith increment widths were analyzed in 2 time frames, a split-plot statistical analysis based on time (in days) was used to compare treatments and pre-experimental and experimental increment widths (Maceina et al. 1994).

RESULTS

Spot

Of the 40 spot initially placed into mesocosms, 24 were recovered at the end of the experiment. Mean SL (mm) for experimental fish was 41.1 ± 4.80 *s*. At least one spot was retrieved from each of the 5 mud mesocosms, from 4 of the sand mesocosms, and from 4 of the sand/stem mesocosms. Two mud/stem mesocosms drained completely during the experiment and fish were not retrieved. At least one fish was retrieved from each of the 3 remaining mud/stem mesocosms.

In the split-plot ANOVA, we were unable to detect a significant influence on increment width attributable to substrate type ($P = 0.975$), the presence or absence of emergent *Spartina* stems ($P = 0.379$), or their interaction ($P = 0.288$). Pre-experimental increment widths ($n = 5$ days prior to capture) were pooled to determine a pre-experimental increment growth rate. Comparison of mean pre-experimental increment width (i.e., prior to capture) and experimental increment widths (days 2–6) was highly significantly different ($P \leq 0.0001$). Mean pre-experimental increment width for all spot, was 3.10 ± 0.08 $s_{\bar{x}}$ µm/d (Figure 1). Because no treatment differences were evident, daily increment widths were pooled across treatments for the experimental period. Individual mean daily experimental increment widths ranged from 1.94 to 2.58 µm with an overall group mean of 2.07 ± 0.05 $s_{\bar{x}}$ µm/d (Figure 1). Each otolith displayed a check mark formed on the day of capture, presumably due to the stress of capture and handling.

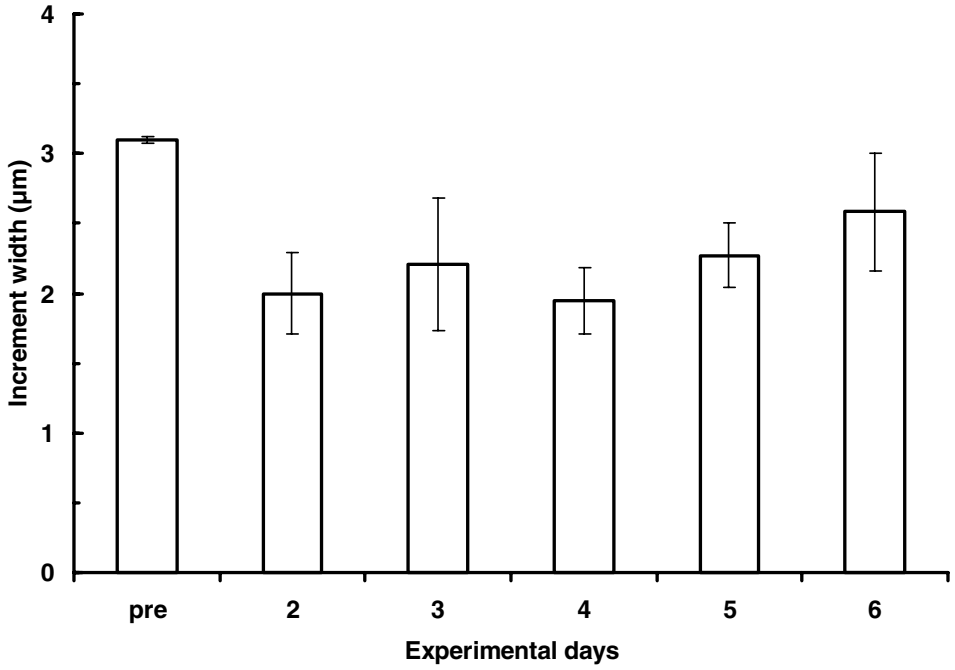

Figure 1. Mean daily increment widths from spot *Leiostomus xanthurus* otoliths for 5 days prior to capture (pooled and labeled as "pre") and during experimental days 2–6, while confined to mesocosms near Fourchon, Louisiana, March 1992. Experimental days 1 and 7 were omitted as acclimation and incomplete days, respectively. Increment widths during experimental days are pooled across treatments. Error bars are 95% confidence intervals.

Spotted Seatrout

At the end of the spotted seatrout experiment, 15 more seatrout were retrieved than were initially placed into the ten mesocosms containing emergent *Spartina* stems. As all of the screened portholes were intact, these extra fish apparently were trapped during initial deployment of the mesocosms. Experimental fish were distinguishable by the presence of a stress-induced check mark similar to those found on the spot otoliths. The "volunteer" seatrout lacked a similar check (Figure 2). Overall, 52 spotted seatrout were retrieved. Three experimental fish were missing (from treatments over bare sand). Mean SL (mm) for experimental fish (n = 37) was 26.6 ± 3.50 s; mean "volunteer" fish (n = 15) SL was 22.2 ± 4.62 s; and overall, mean SL was 25.5 ± 4.30 s.

For experimental fish, the split-plot analysis did not detect any differences in increment width due to the presence or absence of emergent *Spartina* stems (P = 0.629), mesh type (P = 0.834), or their interaction (P = 0.115). Mean daily increment widths were significantly greater during the period prior to capture than during the experiment (P = 0.005, Figure 3). Mean increment width pooled across the 5 days prior to capture was 10.37 µm ± 0.21 $s_{\bar{x}}$, and mean increment widths for the 3 experimental days averaged 9.29 µm ± 0.35 $s_{\bar{x}}$.

We were unable to detect significant differences in overall experimental increment widths (days 2–4) between the 2 groups of seatrout (handled and "volunteer"; P = 0.415). However, increment width comparison of "volunteer" and experimental fish across time was significantly different (P = 0.038), indicating that experimental fish and "volunteer" fish responded differently during the experiment. Increment widths of the experimental fish were lower during the experimental period when compared to the pre-experimental period; however, "volunteer" fish did not show a detectable difference in increment widths between periods (Figure 3).

DISCUSSION

Results from experiments investigating effects of various habitat variables on individual growth of juvenile spot and spotted seatrout were confounded by reduced otolith increment widths during the experimental period. Additionally, we initially relied on the untested assumption that otolith increment width was proportional to daily somatic growth. Although otolith increment widths are usually related to somatic growth and may be used as a measure of recent daily growth (Methot Jr. 1981, Wilson and Larkin 1982, Burke et al. 1993), decoupling of the otolith growth-somatic growth relationship can occur (usu-

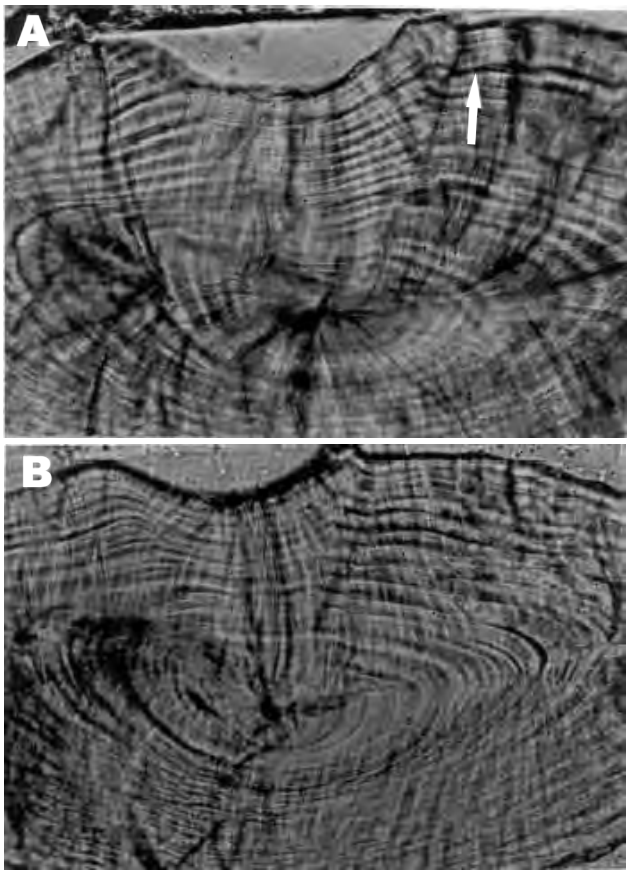

Figure 2. A) Evidence of a stress induced check mark (↗) on the otolith of an experimentally manipulated spotted seatrout. The check mark occurs on the day of capture. B) "Volunteer"seatrout (i.e., those individuals inadvertently captured during deployment of experimental mesocosms and not handled at all) lacked a similar check mark. Experiments were conducted near Fourchon, Louisiana, in August, 1992.

ally under stressful conditions), limiting the reliability of daily increment widths to accurately reflect recent patterns in daily growth (Secor et al. 1989, Mugiya and Tanaka 1992). Because we did not measure fish length prior to experimentation (in an attempt to reduce handling stress), this assumption remains untested and may limit conclusions drawn from the results regarding growth responses to habitat variables. However, the accidental inclusion of "volunteer" seatrout during deployment of the experimental mesocosms may yield inferences to future mesocosm studies examining growth, survival, competition, or other in situ biological investigations of fishes.

Check marks on otoliths are formed when normal calcium deposition has been disrupted, usually in association with periods of stress. The stress may be due to the onset of sexual maturity (Campana and Neilson 1985), degraded environmental quality (Kalish 1992), migration (Kawakami et al. 1998), metamorphosis (Bailey et al. 1977), or handling (Paragamian et al. 1992, Zhang et al. 1995). All fishes that were captured and handled in our study displayed check marks on the day of capture, indicating they had experienced significant physiological stress at that time. Deliberate check-mark induction has been achieved through fluctuations in water temperature (Volk et al. 1994). Although not our intended purpose, we did expose experimental fish to lower water temperatures, which may have contributed to check formation.

Increment widths of experimental fish in both studies decreased immediately following capture and placement in the mesocosms. Short-term periods of stress cause a variety of physiological responses in fishes, one of which is impaired growth (Wedemeyer and McLeay 1981). If increment widths are indicative of (if not always directly related to) somatic growth or stress level, handling stress additionally appeared to manifest itself through reduced daily increment widths. "Volunteer" fish did not have a check mark on their otoliths nor did they experience reduced otolith growth during the experimental period.

Overall, the intended objectives of the study could not be met, primarily because of the design of the experiment (i.e., insufficient duration) and reliance on an untested assumption (i.e., no uncoupling of the linear relationship between somatic and otolith growth under stress). However, the inadvertent inclusion of "volunteer" spotted seatrout in the mesocosms provided a unique opportunity to evaluate the use of wild animals in in situ experimentation. The lack of a stress-induced check mark and no change in increment widths during the experimental period of the "volunteer" seatrout demonstrated that, in this case, there was no detectable mesocosm artifact affecting daily otolith increment widths, and thus, mesocosms may be an effective tools for such studies. However, fish that were handled demonstrated a check mark as well as an immediate reduction in otolith increment widths. Even though we tried to reduce handling stress by not measuring the fish ahead of time and attempting to ameliorate the stress response through reductions in salinity and water temperature, experimental fish were negatively impacted by the experience. A longer acclimation period might have allowed the resumption of normal otolith growth (and presumably somatic growth as well). In salmonids, stabilization of increment widths may take as long as 15–21 d depending on experimental conditions (Neilson and Geen 1984, Molony and Choat 1990). Paperno et al. (1997) examined another sciaenid, juvenile weakfish *Cynoscion regalis* and found that increment widths stabilized within a week of experimental manipulation. Additionally, an independent assessment of increment width formation in free-ranging fishes at the conclusion of in situ experiments

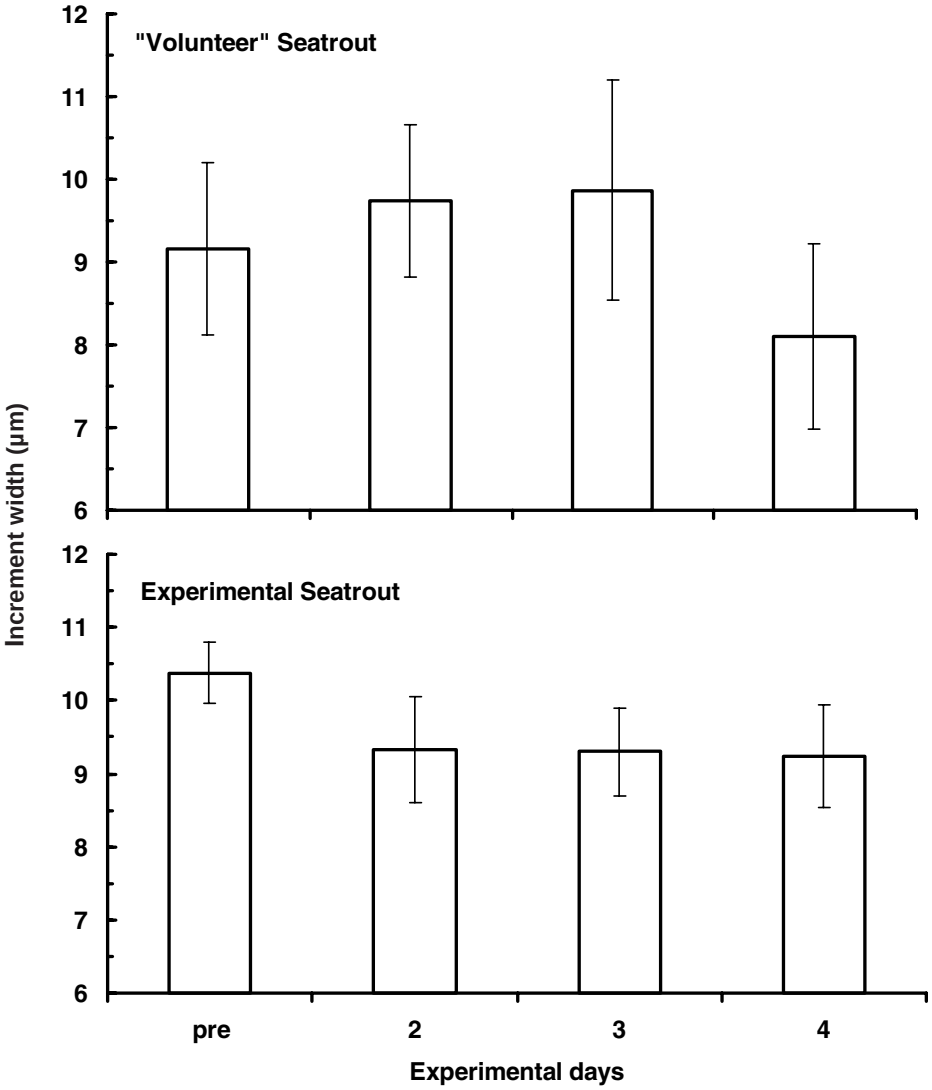

Figure 3. Mean daily increment widths from spotted seatrout *Cynoscion nebulosus* otoliths for 5 days prior to the experimental period (pooled and labeled as "pre") and during experimental days 2–4, while confined to mesocosms near Fourchon, Louisiana August 1992. Experimental days 1 and 5 were omitted as acclimation and incomplete days, respectively. Increment widths during experimental days are pooled across treatments. The top graph shows increment widths of "volunteer" seatrout, and the bottom graph shows increment widths of seatrout that were handled prior to placement into the mesocosms. Error bars are 95% confidence intervals.

(covering the experimental period) would have provided a separate means of verifying growth rates in the wild as compared to those determined during our experiments (Kellison et al. 2003). With these additional procedures and precautions, the use of mesocosms to identify the relative value of estuarine habitat types can be a valuable tool in the study of the early life history of juvenile fishes.

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