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Use of Egg Surrogates to Estimate Sampling Efficiency of Striped Bass Eggs in the Savannah River

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Abstract.—Surface push nets have been employed in the Savannah River estuary (SRE) to monitor striped bass *Morone saxatilis* spawning activity since the 1960s, but most intensively since the mid-1980s. These methods provide relative catch-per-unit-effort data for comparing station-to-station and year-to-year changes in egg abundance but do not provide total annual production estimates. To quantify the sampling efficiency of our gear, we investigated the use of gellan beads as striped bass egg surrogates. Gellan beads were comparable to striped bass eggs in size and specific gravity, and are appropriate surrogates for efficiency trials. In 1999 and 2000, we released known quantities of gellan beads to evaluate the efficiency of our egg sampling gear. Efficiency ranged from 1 bead per 11,300 at large (0.009%) to about 1 bead per 300,000 at large (0.0003%), depending on release and recapture locations. The use of egg surrogates was an effective and informative technique for evaluating our standardized egg sampling gear and may be similarly useful for other systems and other species with semibuoyant pelagic eggs. Additionally, the use of egg surrogates uncovered previously unsuspected biases in sampling efficiency because of differences in channel morphology and hydrology within the SRE.

Sampling for pelagic eggs has been employed as a measure of spawning activity and reproduction for a variety of species in various systems (e.g., Polgar et al. 1976; Johnson and Funicelli 1991; Kawaguchi and Moser 1993). These studies produced relative estimates of egg abundance as catch per unit effort (CPUE). Total egg production has been estimated by assuming an even distribution of eggs in the water column and extrapolating based on the cross-sectional area sampled (e.g., Bulak et al. 1993; Rulifson and Tull 1999). Although this method may be appropriate in some cases (particularly single-channeled systems with regulated flow), it is probably less effective in multichanneled systems with diverse hydrology and bathymetry. Additionally, this method is dependant upon the assumption that eggs are distributed evenly throughout the cross-sectional area, a common but often untested assumption.

An empirical estimation of gear-specific sampling efficiency has rarely been attempted. Such an investigation would require an extensive mark–recapture experiment, which is logistically difficult when examining early life history stages such as eggs and larvae. Estimates of sampling efficiency have been attempted mostly by comparing relative efficiency among gear types, towing methods, and mesh sizes (Colton et al. 1980; Hamley et al. 1983; Jessop 1985; Somerton and Kobayashi 1989; Pepin and Shears 1997). Few studies, if any, have attempted to quantify sampling efficiency for a single method by the release and recovery of known quantities of marked individuals or objects. The difficulty in obtaining large quantities of eggs or larvae, then marking and releasing them for later recapture has prohibited such investigations (but see Cobb 1989). Additionally, using actual eggs of imperiled or locally depleted populations may not be possible or feasible. To our knowledge, the use of egg surrogates for sampling efficiency trials has not been investigated until now.

Striped bass *Morone saxatilis* are broadcast spawners that spawn in fresh or nearly fresh waters when water temperatures are between 12°C and 24°C. Depending on the water temperature, fertil-

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ized eggs may float for up to 80 h before hatching and require flows of about 30 cm/s to remain suspended (Albrecht 1964; Talbot 1966). In water temperatures common to the Savannah River estuary (SRE) during the striped bass spawning season, striped bass eggs typically drift 44–48 h before hatching (Bayless 1972). Because striped bass eggs are semibuoyant, they are susceptible to standard ichthyoplankton sampling gear such as bongo nets, push nets, and trawls.

Bow-mounted push nets have been employed in the SRE to determine the location and relative amount of striped bass spawning since the 1960s but most intensively since the mid-1980s (Dudley and Black 1979; Larson 1985; Van Den Avyle et al. 1990; Georgia Cooperative Fish and Wildlife Research Unit, unpublished data, 1990–2000). Methods employed by the Georgia Cooperative Fish and Wildlife Research Unit were standardized in 1990 and have since remained virtually unchanged. These methods provide CPUE data for comparing station-to-station and year-to-year changes in estimates of egg abundance and have been used to assess the recovery of the striped bass population that crashed in the 1980s (Van Den Avyle and Maynard 1994).

In the springs of 1999 and 2000, we evaluated gellan beads as striped bass egg surrogates and used the beads to assess the sampling efficiency of our standardized egg sampling gear. The gellan beads approximated the size and specific gravity of striped bass eggs and were available in known quantities. The objectives of this study were to evaluate gellan beads as striped bass egg surrogates based on physical characteristics and to establish the sampling efficiency (number captured/number released) of our egg sampling gear in the SRE.

Methods

Egg Surrogate Characteristics

Gellan-based compounds are extracellular polysaccharides approved by the U.S. Food and Drug Administration for use in food-based products from frostings to beverages (Food Technology 1990). Coincidentally, gellan beads used in a brand of novelty beverage appeared to be of similar size and shape as striped bass eggs. Technology Flavors and Fragrances, Inc. (TFF, Inc., Amityville, New York) produces the gellan beads used in these products, and the beads are available in a variety of colors and flavors.

Although gellan beads are fabricated to be slightly heavier than water (with a specific gravity of 1.006–1.007; R. Dinteman, TFF, Inc., personal communication), measurements were made to determine the initial specific gravity of beads and the specific gravity of beads following soaking in freshwater for 24 and 48 h. Specific gravity was determined using a sucrose-gradient method (Turchi and Weiss 1988; Centelles and Franco 1989). Twenty-five beads of each color were tested directly out of the packing solution. A range of sucrose gradients (5–13%) were used initially depending on bead color (some colors were denser and required a denser gradient). Additional beads ($n = 25$ for each color by treatment) were soaked in tap water for 24 and 48 h, then tested using a 1% sucrose gradient. Individual gellan beads were placed in 50-mL centrifuge tubes containing the sucrose gradient and allowed to stabilize for 30 s. A syringe fitted with a 15.2-cm, 14-gauge needle was used to draw a 0.5-mL sample of sucrose solution from the layer where the bead settled. The solution was tested for percent sucrose with a Lieca Mark II refractometer. Each sample was independently measured by two readers and the values averaged. The percent sucrose values were temperature corrected to 20°C and then converted to density based on the International Critical Tables of Numerical Data (Anonymous 1927). Specific gravity was calculated by dividing bead density by the density of water at 20°C (0.9982 g/mL).

Because the gellan beads were not perfectly spherical, the mean diameter of the beads was determined by measuring the maximum length and width ($n = 75$) with a dissection scope fitted with an ocular micrometer. Bead diameter was calculated as the average of the two values.

To check for any potential adverse effects of salt water on gellan beads, 15 beads of each color were soaked in each of three concentrations (1, 2, and 3%) of sodium chloride solution for 48 h. An equal number of each color were simultaneously soaked in distilled water for comparison purposes. The general condition and diameter of the beads were checked at 0, 24, and 48 h.

Egg Surrogate Studies

Since 1990, striped bass egg sampling methods in the SRE have been standardized to facilitate year-to-year comparisons. During the ebb tide, a bow-mounted plankton net (0.5 m diameter, 505 μm mesh) is deployed 1 m below the surface and pushed obliquely against the current, from bank to bank, until about 100 m^3 of water is sampled. A General Oceanics flowmeter in the mouth of the

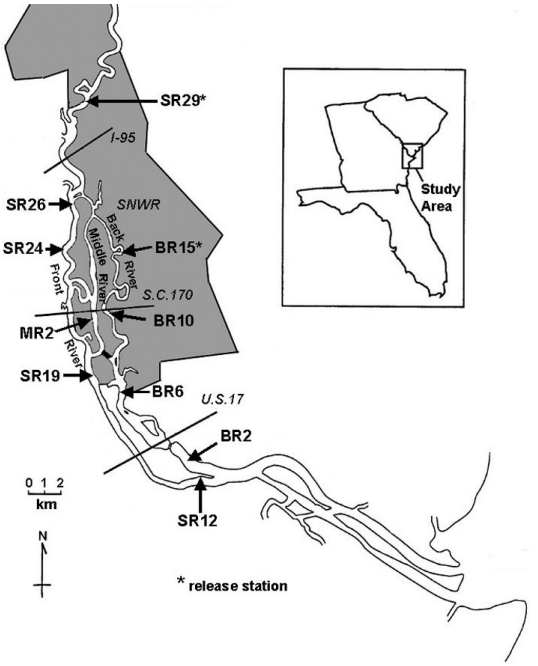

FIGURE 1.—Map of the Savannah River estuary, including major highways, standardized sampling stations, gellan bead release locations (noted by asterisks), and the Savannah National Wildlife Refuge (shaded area).

net measures the volume of water sampled. Five stations are sampled on one day and five different stations the next; thus, stations are sampled every other day during the spawning season (Figure 1). Samples are sorted and striped bass eggs and larvae enumerated.

We obtained 145-kg barrels of gellan beads in a variety of colors from TFF, Inc. We estimated the total number of beads contained in each barrel by determining the number of beads in three 100-mL samples taken from each barrel, weighing them, and extrapolating (mean number beads/g) to the total weight (kg) of beads in each barrel. We soaked beads in freshwater for at least 24 h prior to releasing them.

On multiple occasions during the striped bass spawning season (March–May 1999 and 2000), known quantities of beads were released at current and historic spawning areas. Bead release times generally coincided with times and conditions when striped bass were known to be spawning. All releases occurred at the start of the flood tide. Beads were released in varying quantities to help decipher density-dependent relationships between number of beads at large and sampling efficiency. Bead collection procedures followed our standardized striped bass egg collections, except an additional egg sampling boat was included to simultaneously sample Front and Back river sites (as opposed to five stations one day and another five stations the following day). Sampling for beads occurred for 2 d following release (the average time striped bass eggs would be present in the system following a spawning event). Daily discharge measurements were obtained from the U.S. Geological Survey Water Resources Division station near Clyo, Georgia. Discharge was lagged by 4 d, reflecting the average time required for a water mass to move from Clyo to the estuary (B. Ellis, Applied Technology and Management, personal communication).

Results and Discussion

Egg Surrogate Characteristics

The gellan beads used as egg surrogates varied in number and size. The three types of beads (yellow, red, and purple) used in these studies came in separate barrels, which contained about 2.1, 1.8, and 1.2 million beads per barrel, respectively. The standard error of total beads per barrel ranged from 6,485–57,251. We found that purple (or grape-flavored) beads were packed in a higher density sucrose solution (being the sweetest of the flavors), which yielded fewer per unit volume than the other beads. Conversely, yellow (or lemon-flavored) beads were packed in a solution with less sucrose (being the least sweet of the flavors), which yielded more beads per kilogram. Individual beads were variable in size: purple beads were the largest (mean diameter = $4.9 \pm 0.42_{SD}$ mm), followed by red (mean diameter = $4.6 \pm 0.46_{SD}$ mm) and yellow beads (mean diameter = $4.5 \pm 0.15_{SD}$ mm). Bead diameter did not change following soaking in freshwater (Table 1).

The specific gravity of beads sampled directly from shipping barrels ranged from 1.0067 ± 0.0004 to 1.0324 ± 0.0029 (Table 1). After 24 h in freshwater, yellow beads had the largest decrease in specific gravity—dropping from 1.0324 to 1.0010—while the purple beads only decreased 0.0049 during the first 24-h period, without a decrease during the final 24 h period. The specific gravity of striped bass eggs ranges from 1.0003 to 1.0007 (Hardy 1978), and Savannah River striped bass eggs have been found to have a specific gravity of 1.001 (Bergey et al. 2003). Following at least 24 h of freshwater soaking, gellan beads approximated the specific gravity of striped bass eggs fairly closely; yellow beads, in particular, were the most similar to Savannah River striped bass eggs.

TABLE 1.—Physical properties of gellan beads used in sampling efficiency trials in the Savannah River estuary, Georgia, 1999–2000. Mean specific gravity (±SD) and mean diameter (mm ± SD) were measured directly from shipping barrels (0 h) and after 24 and 48 h soaking in freshwater.

	0 h		24 h		48 h	
Color	Specific gravity	Diameter (mm)	Specific gravity	Diameter (mm)	Specific gravity	Diameter (mm)
Purple	1.0067 ± 0.0004	4.9 ± 0.42	1.0018 ± 0.0003	4.8 ± 0.23	1.0018 ± 0.0003	4.8 ± 0.44
Red	1.0246 ± 0.0051	4.6 ± 0.46	1.0018 ± 0.0002	4.6 ± 0.47	1.0015 ± 0.0003	4.7 ± 0.50
Yellow	1.0324 ± 0.0029	4.5 ± 0.15	1.0010 ± 0.0002	4.4 ± 0.18	1.0007 ± 0.0003	4.4 ± 0.18

In the field, semi-intact beads began appearing during the second day of sampling. We hypothesize that gellan bead breakdown primarily is due to abrasion with the substrate during settlement (which may occur during the slack tidal period, or every 6 h). Additional breakdown may be enhanced during capture and handling. Gellan beads maintained in the laboratory in various salinities did not exhibit a change in physical condition for up to 3 d, and beads stored in their original shipping liquid have maintained condition for several years. Because striped bass eggs normally hatch within 44–48 h under the typical temperature regimes encountered in the SRE during the striped bass spawning season (Bayless 1972), the disintegration of beads within 48 h is fortuitous.

We believe gellan beads to be an adequate surrogate for striped bass eggs. The beads varied in size but were similar to the upper range reported for striped bass (Albrecht 1964; Hardy 1978), although they were larger than Savannah River striped bass eggs (2.77 ± 0.19 mm; Bergey et al. 2003). Soaking beads in freshwater lowered specific gravity close to the range reported for striped bass eggs (Hardy 1978) and to that reported for Savannah River eggs in particular (Bergey et al. 2003). Bergey et al. (2003) reported that striped bass eggs from the Savannah River had a specific gravity of 1.001, similar to the values reported by Bulak et al. (1993) for eggs from the Congaree and Wateree rivers in South Carolina. Overall, the yellow beads were most similar to striped bass eggs in physical characteristics. Because they yield more beads per kilogram, yellow beads are the most economical of the types examined. Additionally, their bright yellow color makes them readily identifiable amid detritus and other debris common in push-net or trawl samples. However, despite slight differences in physical characteristics, using different-colored beads provided the opportunity to track simultaneous releases from multiple locations within the river system.

Egg Surrogate Studies

In 1999, we performed three separate egg surrogate releases (Table 2): two near the head of the estuary (station SR29) and one in the Back River (station BR15). Mean daily discharge during the period was 232 (±22 SD) $m^3 \cdot sec^{-1}$. Forty-nine of the approximately 5.1 million gellan beads released into the upper SRE were recaptured (four purple, 13 yellow, and 32 red; Table 2). Beads released at BR15 were recaptured only in the Back River, but beads released at SR29 were captured

TABLE 2.—Releases and recaptures of gellan beads in the Savannah River estuary, spring 1999 and 2000. Savannah River releases were at SR29 and Back River releases were at BR15 (see Figure 1). Recaptures occurred over a 2-d period following release. Discharge measured at a U.S. Geological Service gauging station near Clyo, Georgia, was lagged by 4 d (the time for a water mass to move downstream to the estuary).

Date	Discharge (m^3/sec)	Bead releases color (*N*)	Recaptures (*n*)	Sampling efficiency ($100 \times n/N$)
		Savannah River releases		
May 18, 1999	215	Purple (1.2×10^6)	4	0.0003%
May 19, 1999	217	Yellow (2.1×10^6)	13	0.0006%
Mar 27, 2000	299	Yellow (3.5×10^6)	25	0.0007%
Mar 31, 2000	223	Yellow (7.0×10^6)	39	0.0006%
		Back River releases		
May 19, 1999	217	Red (1.8×10^6)	32	0.0018%
Mar 29, 2000	320	Red (0.9×10^6)	63	0.0070%
Mar 31, 2000	223	Red (2.8×10^6)	247	0.0088%

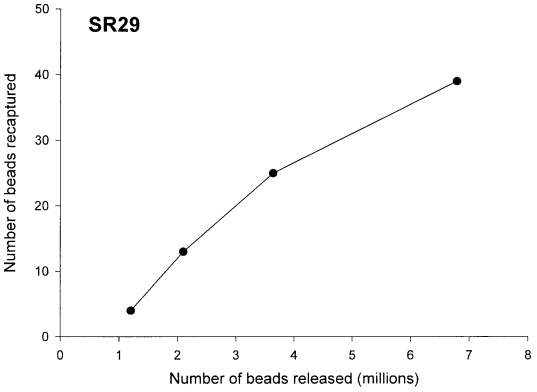

FIGURE 2.—Total number of gellan beads released versus total number recaptured in the upper Savannah River estuary (station SR29), Spring 1999 and 2000.

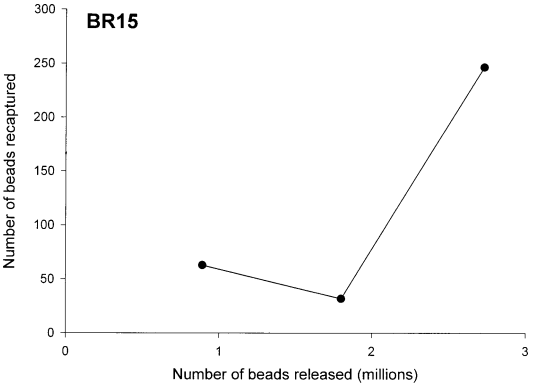

FIGURE 3.—Total number of gellan beads released versus total number recaptured in the Back River, Savannah River estuary (station BR15), Spring 1999 and 2000.

in all three river channels (i.e., Front, Middle, and Back rivers). Mean sampling efficiency for SR29 releases was 0.00045%, and the release at BR15 resulted in a sampling efficiency of 0.0018% (Table 2).

In 2000, we made four separate releases to decipher sampling efficiency: two at SR29 and two at BR15 (Table 2). Mean daily discharge during the March trials was 281 ($\pm$51 SD) $m^3 \cdot sec^{-1}$. As in 1999, beads released at SR29 were recaptured in all three channels, with three being collected as far down the estuary as BR2. Beads released at BR15 were mostly recaptured in the Back River, although three were recaptured at the Middle River station (MR2). Mean sampling efficiency for SR29 releases was 0.00065%, and the Back River releases had a mean efficiency of 0.0079% (Table 2).

The recovery of gellan beads allowed us to investigate the sensitivity of our standardized sampling gear in the SRE. Sampling efficiency ranged from 0.0088% to 0.0003%, or from 1 in 11,360 to 1 in 333,333. Since striped bass females are relatively fecund (163,000 to over 5 million eggs per female; Olsen and Rulifson 1992), our egg sampling protocol would be able to detect almost any amount of spawning effort. Recovery also seemed to be density-dependent. The more beads we released, the more we recovered (particularly in upper estuary releases), although this relationship may not be linear at higher release densities (Figure 2). Additional releases are required to fully investigate the nature of this relationship. Back River recapture efficiency did not show a distinct relationship with release number (Figure 3), but the number of beads released there was relatively low compared with the releases in the upper estuary.

Conclusions

Gellan beads proved to be an effective and informative technique for evaluating our egg sampling gear and may be similarly useful for other species with semibuoyant eggs. Further refinement of sampling efficiency through additional studies over a variety of discharge levels may allow for more quantitative evaluations of egg abundance in the SRE. Understanding sampling efficiency for the various reaches of the SRE under different flows will allow for the estimation of total egg production. Additionally, once these relationships are known for a wider range of river flows, total egg production may be back-calculated for previous years. This estimation of total production will not be constrained by the assumption that eggs distribute evenly throughout the cross-sectional area of the channel and may provide more precise estimates of total production. The methods investigated here may be useful to researchers in other systems wishing to evaluate their sampling methods.

In the SRE, the use of egg surrogates uncovered previously unsuspected biases in sampling efficiency caused by differences in channel morphology and hydrology. Our sampling efficiency seemed to be greater in the reaches of the Back River, which is narrower, shallower, and unchannelized compared with the Front River. We effectively sample a greater proportion of the water column in the Back River and thus sampling efficiency appears to be an order of magnitude greater there than in the Front River. However, addi-

tional sampling is needed to statistically evaluate this relationship. This difference in sampling efficiency among the various channels of the SRE may have led to biased conclusions about the abundance of striped bass eggs in the SRE. Our results suggest that previous assumptions about striped bass spawning activity in the SRE must now be revisited.

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