ASSSESSING ESTUARINE-SCALE POPULATION CONNECTIVITY AND DYNAMICS FOR THE MANAGEMENT OF MARINE FISHERIES

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ABSTRACT

Ian Robert Kroll: Assessing Estuarine-Scale Population Connectivity and Dynamics for the Management of Marine Fisheries
(Under the direction of F. Joel Fodrie)

In response to the degradation of coastal environments and their associated habitats, managers and policy makers have looked to utilize population dynamics and, more specifically, connectivity (i.e., import and export of larvae, juveniles, and adults) in order to rebuild stocks. As the majority of population bottlenecks are thought to result from critical periods experienced in earlier life stages, it is particularly important to discern movement patters during larval and juvenile stages. This study used two estuarine-associated model organisms, the Eastern oyster and the black sea bass, to examine connectivity at the larval and juvenile stages, respectively. A requisite to tracking larval dispersal of the Eastern oyster was to explore the utility of geochemical tagging methods within our study system, the Pamlico Sound. Strong environmental (e.g., temperature and salinity) gradients were present over regional (~ 35 x 15 km quadrants) scales and both larval and settler shells were able to generate distinct, multi-elemental signatures between putative natal and settlement sites. These methods were then applied, with a combination of larval outplanting techniques (i.e., stationary moorings and floating surface drifters), to show that larval dispersal is single-source driven, generally follows wind-driven currents, and leads to high amounts of self-recruitment. However, dispersal pathways are not uniform across the Sound and seasonal and annual dispersal patterns can be highly variable. Geochemical tagging of black sea bass showed that estuarine nurseries, such as oyster reefs, contribute over 89% of the juvenile

black sea bass to the adult stock; however, there is significant annual variation in contribution. The role of estuarine habitats becomes even more complex for the protogynous black sea bass, as fish exhibited carry-over effects (COEs) related to nursery habitats: juveniles that utilized estuarine nurseries transitioned from female to male six months earlier than juveniles that utilized offshore nurseries. This dissertation provides substantial support for the implementation of habitat-based management plans rather than single-species management practices, which cannot account for seasonal and annual variation inherent in dispersal pathways or variable reproductive (and dispersal) potential among subpopulations.

To Jamie Nisse Greenberg,
Your curiosity, passion, and scholarship inspire me daily.

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INTRODUCTION

Global declines in coastal habitats, such as saltmarshes, seagrass beds, mangrove forests and shellfish reefs, have decreased the availability of nursery, foraging, and refuge habitats used by several marine fish and invertebrate (Aarts and Van Den 2003). In response to this, managers and policy makers have looked to utilize population dynamics and, more specifically, connectivity (i.e., import and export of larvae, juveniles, and adults) among subpoputions in order to rebuild stocks. However, most estimates of connectivity are based on theoretical models which are informed by a relatively small number of empirical studies (Cowen and Sponaugle 2009). Therefore, to create effective management plans that protect the distribution and abundance of ecologically and economically valuable species, further research is needed to not only identify dispersal pathways, but also quantify the contribution of specific subpopulations and habitats.

Marine environments present a unique set of obstacles to studying species' movement and distribution. First, nearly all marine organisms move within a three-dimensional space during some, if not all, of their lives. Furthermore, these movements are difficult to see in a relatively opaque sea. Additionally, many early-life histories include a larval stage, in which organisms are microscopic yet rely on large-scale physical processes (e.g., tidal and wave action) to disperse. Traditional terrestrial tagging equipment is also ineffective in marine systems, as sea water is highly corrosive. Additionally, only a small fraction of the ocean has been mapped and nearshore habitats are constantly undergoing changes due to anthropogenic disturbance as well as natural change.

As the majority of population bottlenecks are thought to result from critical periods experienced in earlier life stages (Limburg 2001), it is particularly important for managers to discern movement patterns during larval and juvenile stages. Recent studies have demonstrated higher levels of self-recruitment and longer dispersal pathways than previously assumed (Almany et al. 2007; Puckett and Eggleston 2016); however, larval dispersal is highly dependent on physical factors (e.g., current patterns and tidal forcing), which fluctuate annually, seasonally, and even daily, causing dispersal distances to be highly variable among years or even among neighboring habitats within the same temporal scales (O'Connor et al. 2007, Puckett et al. 2014, Qian et al. 2014). Juvenile connectivity, both between nursery habitats and from nursery to adult habitats, is also of particular interest, as resource availability can vary extensively among putative nursery habitats, exposing organisms to varied environmental conditions during an already vulnerable life stage (Anders et al. 1998). It is also necessary to attribute the role and contribution of juvenile habitats to the adult population in order to preserve essential nursery habitats and adult stock dynamics.

Estuaries comprise a unique domain for connectivity studies, as they are characterized by high environmental spatiotemporal variation (e.g., multiple freshwater input sources) and encompass nursery and adult habitats for several commercially and ecologically important fisheries. The Eastern oyster provides an important model organism for the study of estuarine-scale larval connectivity because of their ecological role as a reef-building, foundation species, commercial fishery status, and metapopulation dynamics. Biophysical models have simulated the dispersal of oyster larvae (over their 2-3 week planktonic veliger phase) and found dispersal distances ranging from 5-40 km, which may limit both connectivity among and local retention within subpopulations (Puckett et al. 2014). However,

there are no empirical data on Eastern oyster larval connectivity and demographic rates across whole-estuarine scales, severely limiting our ability to managing historically overfished stocks.

Although estuarine habitats, such as oyster reefs, were once thought to provide the only nursery ground to the majority of estuarine-associated organisms, recent syntheses have found that estuarine nursery utilization can be facultative, rather than obligate (Able et al. 2005; Nagelkerken et al. 2015). As a result, there is the impetus to understand how organisms like the black sea bass, a socially, economically, and ecologically important member of the snapper-grouper complex, utilize estuarine nurseries and quantify the contribution of estuarine habitats to the adult. Additionally, usage of specific nursery habitats may confer life history benefits beyond the juvenile stage via carry-over effects (Norris 2005). As estuarine habitats are especially vulnerable to climate change and anthropogenic exploitation (Grabowksi et al. 2012), it is important to discern whether the utilization of offshore habitats will increase as estuarine habitat availability decrease and how this may impact population fitness, biomass, and structure (van de Wolfshaar et al. 2015).

The use of the Eastern oyster and black sea bass as model organisms provides an ideal platform by which to examine connectivity at two critically important life stages within ecologically-prized estuarine systems. Examining oyster larval connectivity among estuarine subpopulations not only demystifies the metapopulation dynamics of invertebrate populations but also offers insight into a larval stage experienced by nearly all marine organism. Furthermore, understanding oyster larval connectivity is vital to the successful management of its own commercially-important fishery as well as the success of other shellfish and finfish species that rely on oyster reefs for nursery habitat (Grabowski et al.

2012). Assessing juvenile black sea bass connectivity is necessary to substantiate the contribution of estuarine nursery habitats to adult stocks while also evaluating their role in shaping population dynamics. As managers begin to move toward more habitat-based conservation approaches, there is an emerging need to understand the role of connectivity in sustaining marine populations at various life stages and across entire estuarine scales.

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CHAPTER 1: ENVIRONMENTAL EFFECTS ON ELEMENTAL SIGNATURES IN EASTERN OYSTER, *CRASSOSTREA VIRGINICA*, SHELLS: IMPLICATIONS FOR THE USE OF GEOCHEMICAL TAGGING TO ASSESS POPULATION CONNECTIVITY

Introduction

Researchers have long been interested in the complex larval dispersal patterns that govern early life history and distribution patterns of marine organisms (Young 1990). Current management strategies have only bolstered the impetus to discern population distribution patterns that are driven by larval dispersal, as successful marine reserve design is contingent upon the levels of larval input (e.g., immigration and self-recruitment) and export (e.g., spillover; Gerber et al. 2003, Gaines et al. 2010). Recently, there has been substantial progress in deciphering ranges of dispersal and the degree of self-recruitment within marine populations and ecosystems (Cowen et al. 2009, Puckett et al. 2014), which has allowed researchers to question traditional concepts of connectivity, i.e. to what degree marine populations are demographically open or closed. One of the key challenges in determining the role of larval connectivity in population dynamics and applying this knowledge to management is the ability to test predictions of larval connectivity, especially under variable environmental conditions. For example, larval dispersal is highly dependent on physical factors (e.g., current patterns and tidal forcing), which fluctuate annually, seasonally, and even daily, causing dispersal distances to be highly variable among years or even among neighboring habitats within the same temporal scales (O'Connor et al. 2007, Puckett et al. 2014, Qian et al. 2014).

Estuaries comprise an important domain for connectivity studies, as they are characterized by high environmental spatiotemporal variation (e.g., multiple freshwater input sources) and encompass varying geomorphological components (e.g., creeks, salt water inlets, and marshland). Estuaries also function as important nursery, juvenile, and even adult habitat for many marine organisms (Beck et al. 2001), resulting in the development of distinct subpopulations with varying amounts of larval exchange and connectivity. Finfish connectivity has been examined over many spatial scales, including intra- and inter-estuarine dynamics on both the east and west coasts of the United States (e.g., Able 2005, Fodrie & Levin 2008**)**. However, invertebrate dispersal across estuarine scales has not been as intensively examined, with only a few studies exploring connectivity across estuarine environmental gradients (Becker et al. 2007, Cathey et al. 2012; Puckett et al. 2014 and references therein).

Bivalves, such as the Eastern oyster, *Crassostrea virginica*, provide an important model organism for the study of estuarine-scale larval connectivity because of their early life history characteristics and ecological role as a reef-building, foundation species. *C. virginica* also persists throughout a range of temperatures and salinities commonly found in estuarine systems (Davis 1958). Following successful fertilization, oyster larvae progress through an approximately 2-to-3 week planktonic veliger phase (Medcoff 1939) in which they begin to develop an aragonite-rich prodissoconch shell that is retained after an individual settles on suitable benthic habitat (most typically, gregariously on other adult oyster shells; Stenzel 1964). Recently, biophysical models that simulated the dispersal of oyster larvae among a network of 10 reef units in Pamlico Sound, North Carolina (NC), USA reported that dispersal distance varied around 5-40 km which limited both inter-reserve connectivity and local

retention (Puckett et al. 2014). However, there are no empirical data on oyster larval connectivity and demographic rates within this reserve system. The present study, which evaluates the efficacy of geochemical signatures in shells of larval stage oysters and recent settlers in the waters of Pamlico Sound and beyond, is a key step in testing model predictions of larval connectivity for this and similar bivalve metapopulation.

Because of *C. virginica*'s calcium carbonate shells, geochemical tagging methods can be used to empirically assess larval dispersal and connectivity (Carson 2010, Fodrie et al. 2011). Geochemical tags are based on unique physical and chemical environments experienced by organisms during their larval and post-larval life-history stages. As the organism grows, elements present in natal environments are accreted and stored in calcium carbonate structures (e.g., otoliths in fishes, shells in bivalves), usually through the substitution of anions^{2+} for Ca^{2+} or the entrapment of other contaminants (Bath et al. 2000). Environmental (e.g., temperature and salinity) variations have been shown to further affect the incorporation of trace elements into calcium carbonate structures (e.g., Bath Martin & Thorrold 2005, Becker et al. 2005, Strasser 2008b). These signatures can then be analyzed through the use of specialized mass spectrometry techniques to discriminate natal origin in both fishes and bivalves (Carson et al. 2013). Larvae are exposed to various environmental conditions, while also retained within an estuary, and can therefore be used to further understand the mechanisms of geochemical tagging, via the relationship between salinity, temperature and elemental concentrations, as well as the application of elemental signatures to assess connectivity. Applications of this approach may be used to assess connectivity, thereby improving management and restoration efforts for the species.

The ultimate goal of this study was to develop geochemical tagging as an empirical tool to assess oyster larval connectivity. A requisite for achieving this goal was to first ground-truth tagging methods for oysters via spatially-implicit laboratory experiments with larval oysters, coupled with spatially-explicit field collections of recent settlers. In laboratory mesocosms, we conducted a fully crossed, 3-way, experiment to investigate the effects of temperature, salinity, and seawater concentrations of Mn and Pb on larval (prodissoconch) shell signatures (i.e., elemental ratio, X:Ca). Second, we collected recently settled oysters (hereafter "spat") from sites within the Bogue-Back-Core-Pamlico Sound estuarine system in North Carolina (NC), USA, and examined signatures present in larval shells and outermost portions of settler shells. These geochemical signatures were used to examine natural elemental variability in shells, with respect to salinity and temperature, and to explore discriminatory ability and resolution between sample sites or regions.

Methods

Temperature, salinity, and trace metal manipulations

To investigate environmental effects on larval (prodissoconch) shell signatures, we manipulated temperature, salinity, and elemental concentration of the water surrounding developing oyster larvae. Individual tanks were set up with the following treatments: low (21°C) or high (26.5°C) temperature; low (12.5 ppt) or high (20 ppt) salinity; and ambient (no addition), mid spike (+16 ppb Mn/0.16 ppb Pb addition), or elevated spike (+32 ppb Mn/0.32 ppb Pb) in concentrations of aqueous Mn and Pb. These elements were chosen because of their previous use and importance in elemental tagging studies (e.g., Zacherl et al. 2003, Strasser et al. 2008b). Temperature and salinity treatments were selected based on representative high and low observations in Pamlico Sound at the time of the experiment

(mid-September). Trace metal spikes were calculated to increase the ambient levels of Mn and Pb in seawater, as measured by Statham and Burton (1986) for Mn and Wu and Boyle (1997) for Pb, by 400% and 800% for mid and elevated spike levels, respectively**.**

Three-day old *C. virginica* larvae were obtained from the University of Maryland's Horn Point Laboratory in Cambridge, Maryland, USA. These larvae were mass spawned from a total of 21 males and 28 females and reared in a hatchery system until shipment to the Institute of Marine Sciences (IMS) in Morehead City, NC. Upon arrival, larvae were divided equally into 2, 1.2 L aerated holding tanks filled with a 12.5 ppt seawater mix (ultrapure H_2O added to filtered seawater from Bogue Sound, NC). Over the next 4 days, larvae were acclimatized, with one tank receiving a salinity increase of approximately 2 ppt per day, resulting in a final salinity of 20 ppt, while the other tank remained at 12.5 ppt.

After the acclimatization process was complete, larvae from both holding tanks, now 7 days old, were divided equally into 72 "larval homes", with approximately 1.6×10^4 larvae per home (21.2 larvae cm^{-3}). Larval homes were constructed from hollow PVC tubing capped on each side with nitex cloth, with a 30 μm mesh opening, to allow for the flow of water and food into the home, but prevent larvae from escaping. Homes were then placed into 24 aerated aquarium tanks (35 L), with 3 homes per tank. All tubing, PVC, air stones, and nitex were soaked in a HNO_3 solution and then rinsed thoroughly with ultrapure H_2O prior to its use in the experiment.

Temperatures were maintained at either high or low level by 150 W Aquatop aquarium heaters and salinity levels were established by mixing filtered seawater with ultrapure H_2O until desired salinity was reached. Mn and Pb concentrations were spiked by the addition of 545 μl of Mn + 5.45 μl of Pb or 1090 μl of Mn + 10.90 μl of Pb from1000 ppt

Fisher Scientific reference standard solutions, for mid and elevated spike treatments, respectively. Individual treatments (temperature, salinity, and Mn/Pb spiking) were crossed, to produce a full factorial design with 12 total treatment combinations. Water changes were conducted every other day by removing one-third (~12 L) of water from the tank and replacing it with a freshly made mix. To account for trace element dilution when un-spiked water was added during water changes, tanks with mid or elevated spike treatments were re-spiked with one-third of the original spike (182 µl of Mn + 1.82 µl of Pb or 363 µl of Mn + 3.63 µl of Pb). Immediately following water changes, larvae were fed by depositing dilute Instant Algae Shellfish Diet 1800 (Reed Mariculture; Campbell, California, USA) into larval homes via syringe. The experiment ran for 7 days, until the larvae were 14 days old.

Dissolved oxygen, temperature and salinity were monitored daily with a HACH HQ40d dual input, multi-parameter portable water quality meter. Dissolved oxygen, pH, salinity and temperature measures remained consistent among the treatments throughout our laboratory experiments. Mean dissolved oxygen and pH were 8.68 ± 0.025 mg L^{-1} and 7.72 ± 0.032, respectively. Mean salinity for high and low salinity treatments were 20.7 ±0.091 ppt and 12.8± 0.120 ppt, respectively. Mean temperature for high temperature treatments was 25.7 ± 0.157 °C and 21.3 ±0.104 °C for low temperature treatments.

Although Pb and Mn were manipulated throughout the duration of the experiment, water chemistry was not analyzed. Previous mesocosm work has shown that salinity often affects the relative amounts of specific tracemetals in seawater (e.g., Mn and Sr), whereas temperature is a less consistent factor (Bath Martin & Thorrold 2005). Salinity fluctuations are often a result of freshwater inputs, which dilute seawater trace metal concentrations, and can therefore be corrected for with our replicable spiking procedure (Bath Martin &

Wuenschel 2006). While measurements of specific elements are possible (e.g., Pb), determining the bioavailability of these elements within specific environments can be more challenging (Eggleton & Thomas 2004). Furthermore, the addition of larval oyster food into our mesocosms may complicate traditional elemental detection methods (Bath Martin & Wuenschel 2006). However, larval diet was distributed uniformly to all tanks and thus, while the chemistry of the actual treatments was not verified, we do have reason to assume consistency among treatments. The larval diet used, Shellfish Diet 1800, was cultured in artificial seawater (with a deionized water base), which precludes any suspicions that it may contain above average levels of trace elements.

At the conclusion of these mesocosm incubations, larvae from each home were filtered using nitex cloth (30 μm) and then resuspended in 15 mL of water from their respective tank. A 0.5-1 mL subsample of each larval resuspension was removed and the number of whole larvae were counted. The remaining larval solution was then frozen at -23°C until sample preparation for geochemical analysis.

Spat settlement sampling and site prediction

We collected recently settled spat (see below) across the Bogue-Back-Core-Pamlico Sound (BBCPS) estuarine system of NC sites to assess whether unique elemental signatures existed among estuarine regions that could be used to accurately predict collection sites of individual spat.

Study sites

Spat settlement collectors were constructed by affixing 2-3 wire strings, each containing 12 adult oyster shells, to private and public docks or stand-alone wooden pilings, throughout the BBCPS study system. Settlement collectors were deployed on June 7th and

21st and again on August 1st and 16th of 2012 and retrieved approximately 2 weeks after each deployment as part of an ongoing settlement sampling program (Eggleston and Puckett, *unpubl.data*). Recovered settlement collectors were frozen until individual spat could be counted and removed from adult oyster shells with a tungsten probe.

Sample preparation and LA ICP-MS

Frozen larvae from the laboratory experiments were thawed and approximately 1000 larvae were obtained representing each replicate home. The larvae were then rinsed with ultrapure H_2O and shells were inspected for any remaining tissue. The process of freezing, thawing, and rinsing larvae appeared to remove most soft tissue, and therefore acid and peroxide-which could degrade shells-were not needed nor employed. If larvae were highly translucent (i.e., no tissue present), they were mounted as a concentrated mass on a labeled glass microscope slide covered in double-sided tape. This process continued until larvae from each home were mounted on a slide in haphazard order (i.e., each home was represented by 1 mound of shells; total N=72). The slides then stored in a laminar flow hood until analysis.

Spat from the field settlement collections were thawed and placed individually in 2 mL centrifuge tubes filled with 100 mL of 15% H_2O_2 solution buffered in 0.05 N ultrapure NaOH. Samples were sonicated for 10 min to remove organic material. The H_2O_2 solution was then removed and replaced with a 100 mL solution of 1% ultrapure HNO_3 (OPTIMA grade; Fisher Scientific; Hampton, NJ). Samples were then sonicated for 5 additional min to dissolve any remaining tissue and surface contaminants. Spat were then rinsed three times with ultrapure H_2O and dried overnight in a laminar flow hood. After drying, spat were mounted in haphazard order onto a glass microscope slide with double-sided tape and stored until analysis.

Both larval and spat samples were analyzed using a Thermo-Fisher Element2 inductively coupled plasma mass spectrometer with a Teledyne ATLex 300si-x 193nm Excimer laser ablation unit (LA ICP-MS). To correct for mass bias and instrument drift, National Institute of Technology Standards-certified standards (Reference Material 612, 614, and 616) were run at the beginning and end of every 4 slide sequence (~140 burns). Concentrations of the following elements were quantified from laboratory larval samples: ^{48}Ca, ^{55}Mn, ^{88}Sr, ^{138}Ba, and ^{208}Pb; and from field-collected spat: ^{26}Mg, ^{48}Ca, ^{55}Mn, ^{63}Cu, ^{88}Sr, ^{118}Sn, ^{138}Ba, and ^{208}Pb. These elements were all analyzed in low-resolution mode, and were chosen because of their previous use in uptake and tagging studies of fish otoliths and bivalve shells (Bath Martin & Thorrold 2005; Strasser et al. 2008a,b; Fodrie et al. 2011).

Larval slide-mounts from the laboratory experiment were ablated three times in bulk, using side-by-side line transects of 150 μm with 40 μm spot size and 80% laser intensity. Line transects covered ~2-3 shell lengths, following Becker et al. (2005), and were used instead of burning several individual larvae to reduce the likelihood of pseudoreplication. determine elemental signatures of the spat collection sites, the outermost (most recently formed) section of the settler shell was also ablated twice with 150 μm end-to-end transects with 40 μm spot size and 80% intensity. The larval portion of settler shells was also analyzed to examine potential elemental variation in larval source signatures. Larval shell of each spat sample was identified and sampled in duplicate with side-by-side line transects of 110 μm with 40 μm spot size and 80% intensity. Isotope intensities for replicate burns were averaged and then converted into elemental ratios (X:Ca) for each home or spat/larval shell following Becker et al. (2007). For ease of comparison between laboratory and field experiments, and because X:Ca ratios can yield the same statistical results and significance as partition

coefficients in bivalves (Strasser et al. 2008b), we opted to only utilize and report X:Ca ratios in our analyses.

Data analyses

Temperature, salinity, and trace metal manipulations

A 2-way ANOVA was used to test the effects of salinity and temperature on elemental ratios for the elements that were not spiked during the laboratory experiment (Sr and Ba). Due to the large amount of zero values in certain cases (e.g., undetectable amounts of Ba), Sr ratios and Ba ratios were transformed using a Box-Cox transformation to meet assumptions of normality and homogenous variances. After ensuring no interactive effects of Mn and Pb spikes with Sr or Ba signatures, or nesting effects for homes within individual tanks (using intraclass correlation), all tanks were included in this analysis with individual larval homes treated as replicates (N=6) and temperature and salinity treated as fixed factors.

For spiked elements (Mn and Pb), a three-way ANOVA was used to test the effects of salinity, temperature, and spike level on elemental ratios. Mn ratios were transformed with a Box-Cox transformation, while Pb ratios were transformed logarithmically to meet assumptions of normality. After ensuring no nesting effects of individual tanks, homes were treated as replicates (N=6) temperature, salinity and spike level were treated as fixed factors. For all 4 elements, Tukey's HSD tests were used post-hoc to explore differences among treatment groups

Spat settlement sampling and site classification

Means and standard errors for field-collected larval and settlement shell Sr:Ca and Ba:Ca ratios were calculated and plotted by site to assess spatial variation in geochemical signatures among collection sites. Signatures from larval shells were used to examine

possible temperature and salinity gradients present among natal sites. Additionally, contour plots were used to explore how settler shell elemental concentrations of Mn, Sr, Ba, and Pb varied with temperature and salinity. Contour plots were created using the *graphics* package in R (version 3.0.3). Multiple regression models were then used to quantitatively assess the relationship between salinity, temperature and shell signatures in a natural environment. Because some collection sites did not produce any spat over a given collection period, spat were grouped only by site to increase the sample size and statistical power of our results. A logarithmic transformation of elemental ratio was used as the response variable.

Linear Discriminate Function Analysis (DFA) was performed on Box-Cox transformed ratios to examine spatial variability in settler shell geochemistry and to determine the viability of using geochemical fingerprints to assess connectivity in oyster populations. All 23 sites were used in preliminary DFAs, however the classification success was low, directing us toward independent examination of PS sites from the BBCS sites. Because of spatial autocorrelation in temperature/salinity, PS sites were then grouped by geographic quadrant within PS: Northwest (NW; WC, EH, StP), Northeast (NE; RD, HT), Southeast (SE; OK, CI, WB), and Southwest (SW; OR, SoP, SQ). Each quadrant contained a diagonal of approximately 35 km to the centroid of PS. BBCS sites were similarly broken up into 5 groups based on geomorphology and site location: Bay (JB, WM), Creek (WH, TC), Newport (NeU, NeM, NeL), North (NoU, NoM, NoL), and Sound (BoS, BaS). Jack-knifed classification matrices, without sample replacement, were compared to expected classification matrices, based on random chance, to assess classification success. Sites were additionally grouped based on similar temperature and salinity profiles, however classification success did not improve significantly over geomorphological quadrants so

analysis did not continue with these groupings. Because natal origins are unknown and modeled dispersal pathways for the area (e.g. Haase et al. 2012) have not been empirically validated, no DFA was performed on larval signatures.

Results

Temperature, salinity and trace metal manipulations

Of the initial 1.60 x 10^4 larvae per home, a mean of 8390 ± 920 larvae were recovered, with an average of 128 ± 22.5 actively moving larvae per home. While estimated larval survival was low (based on presence of moving larvae), 0.80 ± 0.14%, survival did not vary significantly by treatment (p=0.524) and was consistent with published values of *C. virginica* larval survival (Davis 1964).

We found a significant interactive effect of temperature and salinity on Sr concentrations in larval shells (F=4.23, df=3, p=0.041; Fig. 1.2). Highest larval Sr concentrations, 5.51 ± 0.752 mmol mol^{-1}, were present in the low salinity (12 ppt), low temperature (21°C) treatment, representing an average increase of 35.1% over the mean concentrations of the other treatments. A similar pattern was observed in Ba concentration with a 572% increase in the low salinity, low temperature treatment as opposed to mean Ba concentrations of the other treatments combined. However, this trend was not statistically significant due to high variance within the treatment, c_v=0.991 (F=1.02, df=3, p=0.383; Fig 1.2b).

Larval shell Mn concentrations increased significantly with spike level. Mean concentration increased from 0.111 ± 0.015 mmol mol^{-1} to 0.568 ± 0.079 mmol mol^{-1} between ambient [0] and mid [+] spike levels, and to 0.802 ± 0.236 mmol mol^{-1} at elevated [++] spike levels, with a 621% mean increase in concentration from ambient to elevated

treatments (F=59.6, df=11, p<0.001; Fig. 1.2). Temperature and salinity did not influence overall Mn concentration (F=1.46, df=3, p=0.228).

Larval shell concentration of Pb was highly variable, with no change in overall concentration with spike level and an overall mean of 0.034 ± 0.014 mmol mol^{-1} (F=1.02, df=2, p=0.361; Fig. 1.2). There was a significant interactive effect of temperature, salinity and spike level (F=3.369; df=11, p=0.0374) seen in the ambient and elevated Pb treatments. Specific comparisons for all examined elements and treatments are provided in Table 1.2.

Settler signatures and site prediction

Both settler and larval shells from field-collected spat showed robust spatial variability in Sr signatures (Fig. 1.3a,b), while elemental concentrations of Sr were typically higher in larval shell than in settler shell (e.g., 73.9% increase in intensity from settler to larval shell at SQ). Strong Sr:Ca gradients were present, with increasing Sr settler shell concentrations when moving northward (e.g., from SQ to EH, a 30.2% increase) and eastward toward inlet openings (e.g., RD and HT, 31.1% increase from SQ). High larval Sr:Ca concentrations were present in the southern Pamlico Sound (e.g., SoP, OR, WB), mean 4.72 ± 0.654 mmol mol^{-1} when compared to concentrations in the northern Pamlico Sound (e.g., EH, StP, WC), mean 3.28 ± .0292 mmol mol^{-1}.

Generally, settler shells displayed less explicit spatial variation with respect to Ba:Ca ratios, although there was a trend of higher intensities at sites closer to freshwater inputs (OR, NeU), with a combined mean of 0.044 ± 0.014 mmol mol^{-1} at these sites, when compared to the overall mean of 0.037 ± 0.016 mmol mol^{-1}. Larval shell Ba:Ca was fairly homogenous along the North-South axis of the PS, however eastern sites near inlets (RD, HT

and even TC) exhibited higher Ba concentrations (e.g., a 127% increase when moving from SQ to RD).

Settler shell elemental concentrations varied greatly along natural temperature and salinity gradients (Fig. 1.4a-d). For Mn:Ca, greater concentrations (> 3.5 mmol mol^{-1}) were found in settler shell collected from mid-salinity (26 ppt), mid-temperature (26 °C) sites, with concentrations declining at lower temperatures and higher salinities (<2 mmol mol^{-1}; Fig. 1.4a). A multiple-regression model verified this, as Mn concentrations were negatively correlated with salinity ($p<0.001$) and positively correlated with temperature ($p<0.001$), with an R^2 value of 0.101. Sr concentrations were greatest (> 3.8 mmol mol^{-1}) at low temperature (<22 °C) and low salinity (< 21 ppt) waters, with concentrations decreasing with increasing salinity and temperature (Fig. 1.4b). Multiple-regression analysis validated this, showing strong, negative correlations between Sr signatures and temperature ($p<0.001$) and salinity ($p=0.007$), with an R^2 value of 0.091. Conversely, observed Ba concentrations were greatest (> 0.06 mmol mol^{-1}) at either end of the temperature range (<18 °C or >28 °C) and at high salinity (<30 ppt; Fig. 1.4c). Pb concentrations were the greatest in higher salinity water (>24 ppt), however concentrations varied across a wide range of temperatures (>16 °C and <29 °C), with highest levels in mid temperature water (Fig. 1.4d). We found no significant correlations between Ba:Ca or Pb:Ca ratios and salinity and temperature.

Differences in settler shell geochemistry were not sufficient to discriminate among locations when including all sites in DFA (classification success of 18.3%). However, when considering only Pamlico Sound sites, jack-knifed classification success rose to 36.5% over a null expected classification success of 22.5%. When sites were divided into quadrants based on location within PS, we achieved an average classification success of 61.0%, a significant

increase over the null expected of 34.1% (Fig. 1.5a,b). Classification success for spat collection location varied greatly between sites and quadrants, ranging from 0-68% correct assignments. The strongest discriminating elemental ratios for quadrant divisions were Sr:Ca, followed by Mn:Ca and Mg:Ca. For BBCS sites, discriminatory ability did not increase substantially when examining them without Pamlico Sound sites (classification success of 20.25%). When dividing southern sites into geomorphological regions (e.g., Bay, Creek), there was a marginal increase in average classification success to 34.9% (Fig. 1.5c). Discrimination was driven, in order of predictive ability, by Mn:Ca, Mg:Ca, and Sr:Ca ratios, based on forward stepwise variable analysis. When consolidating all the BBCS sites into a single "SS" grouping and including Pamlico Sound quadrants, jackknifed classification success rose to 76.5% over a null expected of 23.8% (Fig. 1.5d), however classification success was still highly variable among sites, ranging from 96% (SS) to 0% (NW). The strongest discriminating elemental ratios for these groupings were, again, Sr:Ca, followed by Mn:Ca and Mg:Ca, based on forward stepwise variable analysis (Fig. 1.6). For all grouping combinations, Pb:Ca was the least discriminatory element.

Discussion

Geochemical tags, reflective of spatial gradients in environmental conditions, have been successfully used to identify natal origins, nursery use, and population-level connectivity patterns within a variety of teleost fishes (e.g., Patterson et al. 2005, Bradbury et al. 2011) and bivalves (Becker et al. 2007, Carson 2010, Cathey et al. 2012). The results of our study expand the use of elemental tags to the Eastern oyster by providing the foundation from which to empirically assess population connectivity among estuarine sub-populations. Our study shows that environmental conditions necessary to impart distinct signatures within

oyster shells are reliable over regional (35 km) spatial scales within a large estuarine complex. However, conditions of an individual site (i.e., temperature and salinity) can vary greatly across time and space. Consequently, this approach may be better suited to predicting environmental conditions within a site at a given time, rather than discriminating between specific collection sites. Here, we consider the utility of geochemical signatures in discerning environmental condition over various scales within an estuarine system.

Environmental influence of trace metal signatures

It has been suggested that biological regulation of Sr ions has more influence on shell elemental concentration than salinity or kinetic effects of temperature (Gillikin et al. 2005, Strasser et al. 2008b). However, we observed significantly higher levels of Sr at low salinity and low temperatures in experimental larval oysters and field-collected settlers, supporting the utility of Sr as a marker of abiotic conditions experienced by an individual in tagging studies. If Sr incorporation into oyster shell is biologically regulated (as suggested by Strasser et al. 2008b), it follows that factors affecting metabolism (e.g., temperature) will likely impact Sr signatures. For example, cold water can lead to proportionally heavier calcium carbonate structures (Worthington et al. 1995) as well as altered precipitation rates and elemental incorporation (Bath Martin & Thorrold 2005). For oysters in our study, lower temperatures may have slowed larval growth, resulting in increased proportional accumulation of Sr within the settler shells (sensu Bath Martin & Wuenschel 2006) and thereby allowing the possibility of duel biotic and abiotic regulation of Sr signatures.

Positive correlations between temperature and Ba, and no correlation between salinity and Ba, have been seen in Olympic oysters along the Pacific coast of the United States (Carson 2010). Our laboratory experiments exhibited no significant correlations between

ambient Ba concentration and temperature and/or salinity. In the field however, higher levels of Ba were detected at lower temperatures, a trend also found in clams (Strasser et al. 2008b) and neogastropod shells (Zacherl et al. 2003). There was an anomalous spike in Ba at higher temperatures (>26°C) within the HT site, however this site also experienced the greatest variance in Ba concentrations (Fig. 1.3b). While the specific mechanisms remain unclear, we believe Ba signature can be used dependably to effectively discriminate between temporal environments in geochemical tagging studies.

Previous literature on the geochemistry of bivalve shells has been unable to define a specific relationship between Mn concentrations and temperature and salinity (Siegele et al. 2001, Strasser et al. 2008b). Similarly, Mn elemental ratios in our laboratory experiment did not show temperature or salinity effects, however Mn elemental ratios did scale with increasing spike level. Mn can enter the marine environment via terrestrial runoff, particle re-suspension, and as a product of redox reactions occurring in low-oxygen environments (Limburg et al. 2015). Therefore, we can expect that riverine inputs and localized phytoplankton blooms created hypoxic/anoxic zones that resulted in the strong discriminatory ability of Mn among our study regions. This also explains why higher concentrations of Mn were found within warmer, less oxygen-rich waters (e.g., OR).

While Strasser et al. (2008b) found results similar to ours with respect to Pb concentration in larval clams-no effects of temperature or salinity-they also assert that Pb signatures are more strongly influenced by seawater Pb concentration than temperature or salinity (as in Pitts & Wallace 1994). However, we did not find a relationship between seawater Pb concentration and shell signature in the laboratory and settler shell patterns of Pb were similarly ambiguous. As a result, Pb was not an effective discriminator between

collection sites, or quadrants, and the addition of Pb to our final DFA model did not significantly enhance prediction ability. Pb enters the marine environment via anthropogenic pollutants, but as there are no explicit point sources for Pb within the BBCPS system, it was improbable Pb would have as much discriminatory power as other trace metals. Furthermore, Pb in the water column is often adsorbed to sinking particles and scavenged very quickly by sediments; therefore, it is unlikely that much of it is bioavailable (Bruland and Lohan 2003).

Our initial larval cleaning methodology included rinsing larvae with a mild acid solution, however, significant degradation of shell material was observed and remaining larvae were rinsed only with ultrapure H_2O. While shells were examined visually for signs of remaining tissue, it is possible that residual organic matter or surface contaminants influenced observed elemental patterns. As individual tank environments were monitored and held constant (with the exception of treatment factors), it is unlikely that specific tanks, larval homes, or larvae would have higher contamination risks than others. Nevertheless, differences in cleaning methodology may limit some comparisons of shell chemistry of mesocosm larvae with the larval shell of field-collected spat, which were cleaned with nitric acid. To avoid possible contamination and/or standardization issues, future larval mesocosm studies might consider developing and employing a methodology that utilizes mild acid-washing to clean larval shells.

Application of elemental tagging to assess oyster larval population connectivity

Among the established Pamlico Sound quadrants, elemental tags showed high discriminatory ability and accurately assigned juvenile oysters to their region of collection with a resolution of ~35 km. Comparatively, oysters failed to provide the same discriminatory ability as other bivalves studied in an overlapping area of NC, i.e., ~12 km

resolution found by Cathey et al. (2012) for the hard clam, *Mercenaria mercenaria*, but did deliver close to the 20-30 km resolution found for mussel species *Mytilus californianus* and *Mytilus galloprovincialis* and the 25-75 km resolution found for the Olympia oyster *Ostrea lurida* in San Diego, CA, USA (Becker et al. 2007, Carson 2010). Several factors may be responsible for this dissimilarity in scales between hard clams and oysters, including differing ICP MS methods (dissolving shell in acid as in Cathey et al. 2012 is more integrative and incorporates longer time periods whereas laser ablation in the present study targets specific points in time), potential variations in uptake at the organismal level, and sample site selection and variability. Predicted dispersal distances for *C. virginica* larvae range from 0.1 km to up to 110km (North et al. 2008, Puckett et al. 2014), so our results indicate that elemental tagging can be valuable for refining our understanding of estuarine-scale larval connectivity for these species in the PS as well as in similar estuarine environments (e.g., Chesapeake Bay).

To create our Pamlico Sound quadrants (NW, NE, SE, SW), the area was divided into distinct regions with varying exposure to salt/fresh water influxes and temperature gradients which, based on our larval experiments, could directly affect individual elemental signatures. For example, SQ and OR sites within the "SW" quadrant both receive low salinity inflows from the Tar and Neuse Rivers, which likely elevated levels of Sr in settler shells collected from those sites. Laboratory results indicated elemental signatures of Mn were more dependent on seawater concentration than temperature or salinity. As terrestrial runoff, particle re-suspension, and redox cycling are major inputs of Mn in estuarine environments (Morris et al. 1982), many river-adjacent sites may have uniformly high Mn inputs that degrade signature uniqueness and discriminatory ability. As Sr and Mn offered consistently

high discriminatory ability, similar levels among sites within regional groupings provided greater uniqueness to the overall signature, however, at the scale of individual sites, site proximity and environmental similarity resulted in ambiguous elemental signatures.

Proximity to freshwater sources may also explain the low level of prediction accuracy within and among the BBCS and Pamlico Sound sites. When analyzed individually, the BBCS sites had very low prediction accuracy, driven by a large overlap in predicted site matching between the Newport and North Rivers. As the rivers are adjacent (~6 km apart) and experience comparable surrounding land usage, similar geochemical environments and signatures are to be expected. While including the Pamlico Sound sites (as quadrants) and the singular SS site into the geomorphological DFA (Fig. 1.5d) significantly improved classification accuracy, high between-site variability was likely an additional result of the connection between Pamlico Sound and the Newport and North Rivers.

In general, comparisons made between signatures in larval and settler shells should be interpreted with caution, as the composition of aragonitic larval shells and calcitic settler shells may favor the uptake of specific elements differently (Finch & Allison 2007, Strasser et al. 2008a,b). For instance, we found higher Ba and lower Sr concentrations in a majority of larval shells when compared to their corresponding settler shells (Fig. 1.3 b). Larval shell patterns also indicate a potential departure from traditional models of connectivity within this system. Recent work in the Pamlico Sound suggests that inter-reef connectivity is very low (~2%) and that local retention sustains the sub-populations (Pucket et al. 2014). However, the presence of north-south and east-west gradients in larval shell Sr and Ba, respectively (Fig. 1.5), may indicate multiple larval sources among our study sites. Furthermore, high

variability within sites (e.g., OK) may indicate multiple natal sources exist even within a single area or site where spat have settled.

Given the differences we recorded in larval and settler shell from individual spat, our findings support previous work demonstrating the importance of a larval shell atlas for exploring larval connectivity, such as that utilized by Becker et al. (2007). This is necessary to expand our understanding of larval connectivity and identify potential dispersal corridors within the BBCPS system. Larval outplant experiments would also allow for exposure to other environmental factors not examined in our laboratory experiments, such as ultraviolet radiation, localized primary production, and oxygen concentration, which may affect element uptake (e.g., Eldson & Gillanders 2005). Finally, we recommend the coupling of geochemical tagging data (e.g., based on larval drifter studies) with expanded biological (e.g., surveys of adult oyster density and distribution), and physical (e.g., current and wind patterns) datasets to produce rigorous biophysical models, which can be used to predict dispersal and inform managers.

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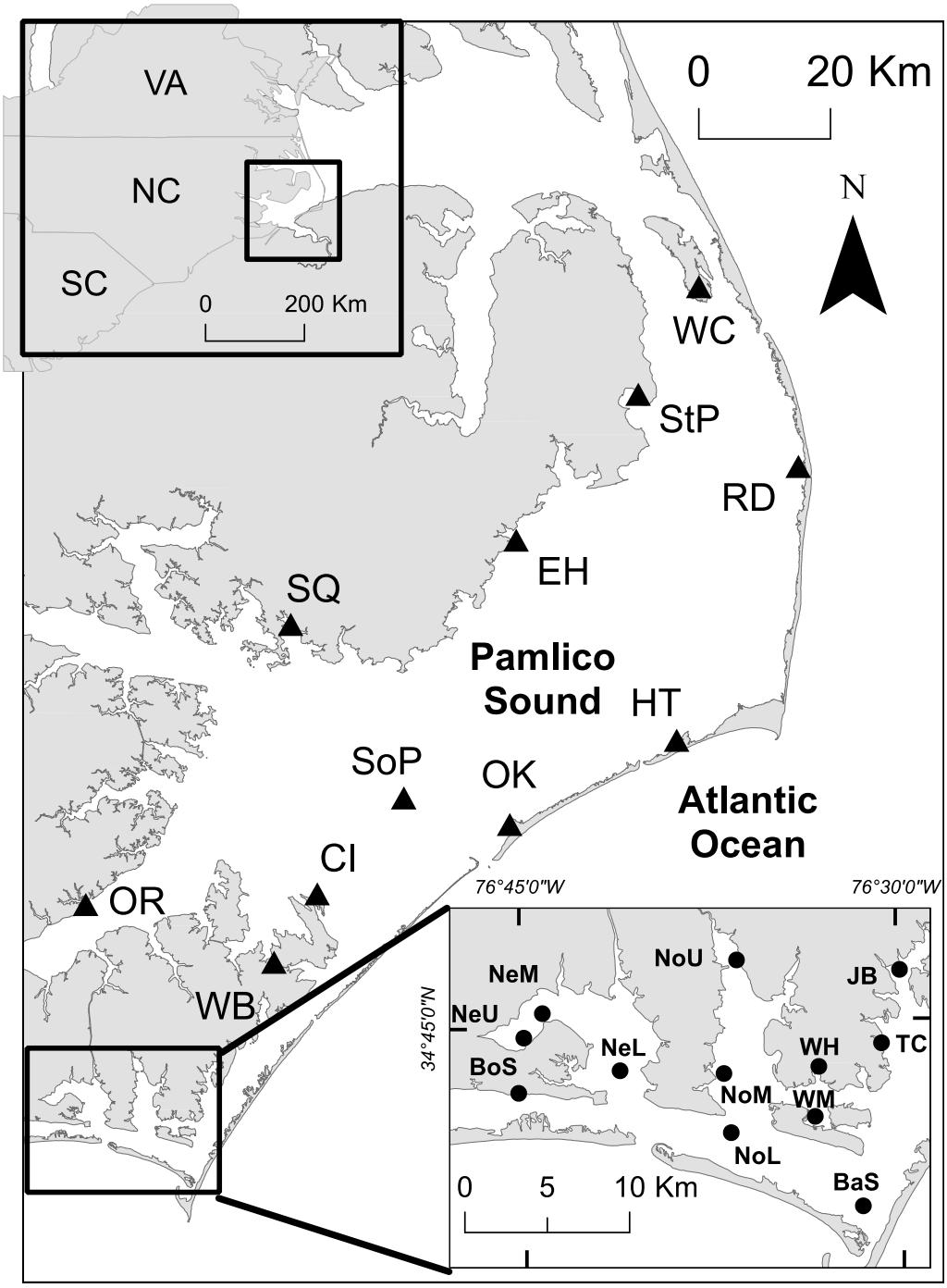

Figure 1.1. Map of spat settlement collection sites within Pamlico Sound, NC (triangles) and Bogue-Back-Core Sound (circles) study system.

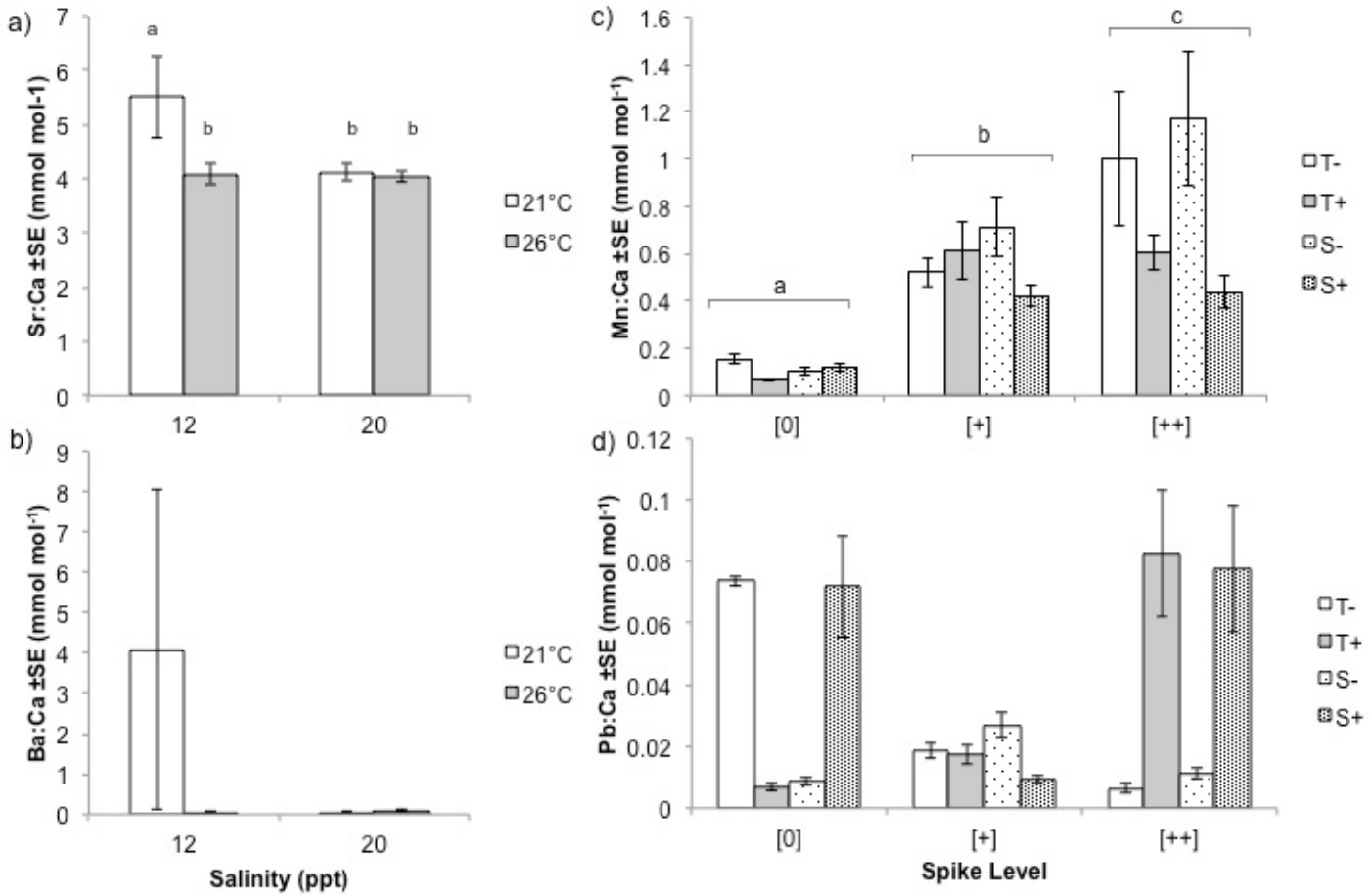

Figure 1.2. Average X:Ca elemental ratios, determined by LA ICP-MS, for larvae exposed to high and low temperature and salinity and ambient [0] , mid [+], and elevated [++] concentrations of: a) Sr, b) Ba, c) Mn, and d) Pb.

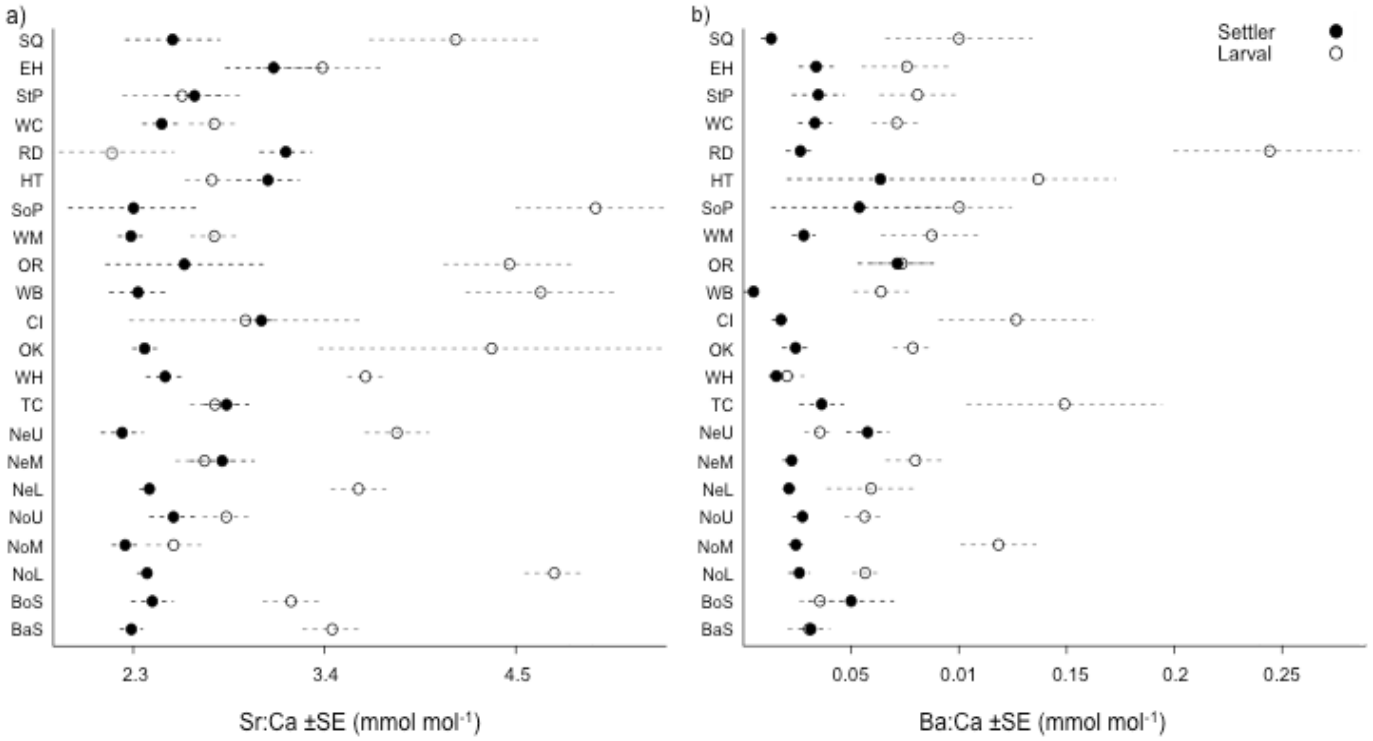

Figure 1.3. Elemental ratios (±SE), X:Ca, of settler and larval components of collected spat shell, by site, for a) Sr and b) Ba. Sites are arranged moving east to west within the northern and southern Pamlico Sound and grouped by geomorphological features in the Bogue-Back-Core Sound

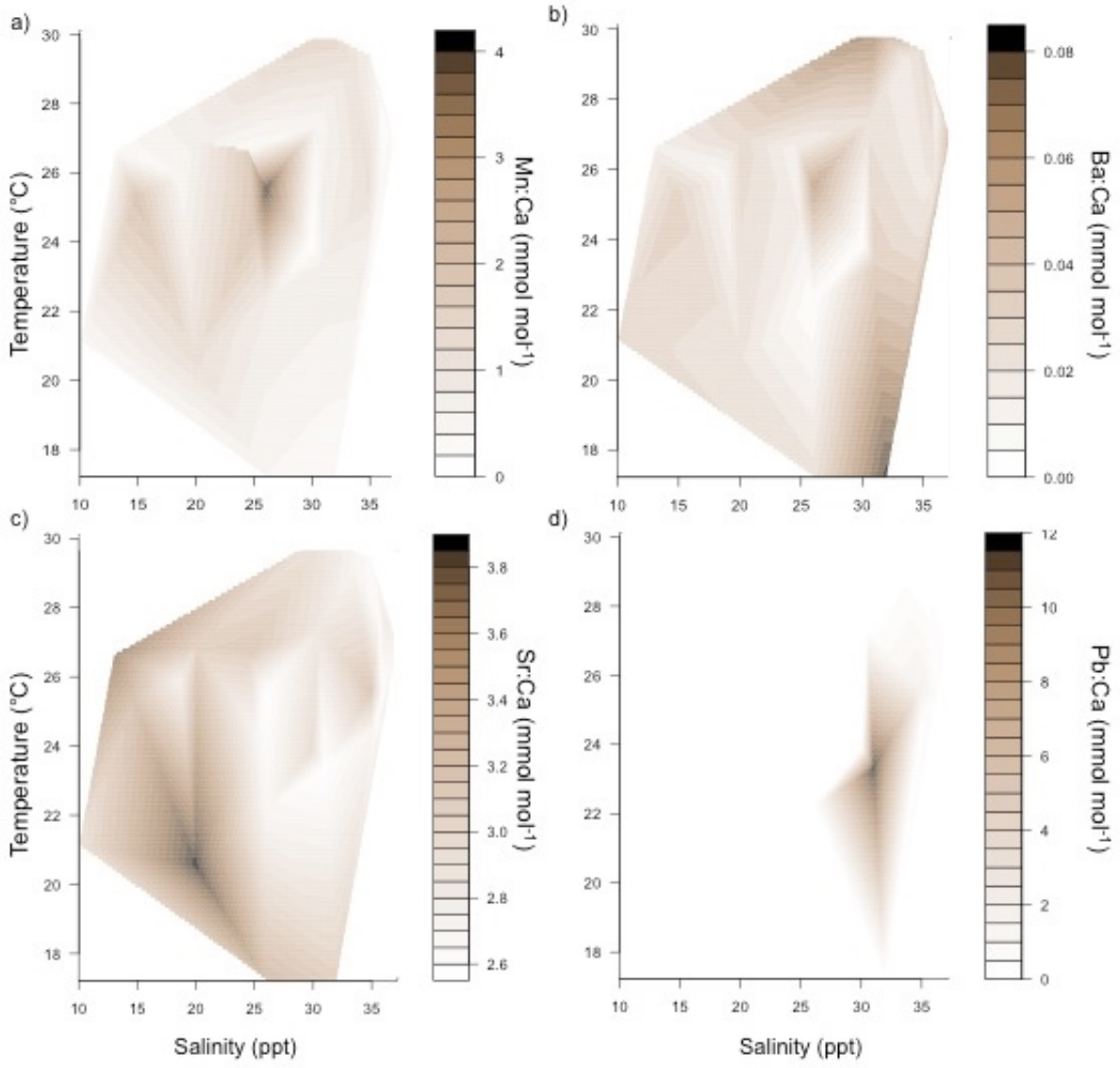

Figure 1.4. Contour plots showing the distribution of elemental ratios for a) Mn, b) Sr, c) Ba, and d) Pb over observed temperature and salinity gradients for all collected individuals throughout the Bogue-Back-Core-Pamlico Sound system.

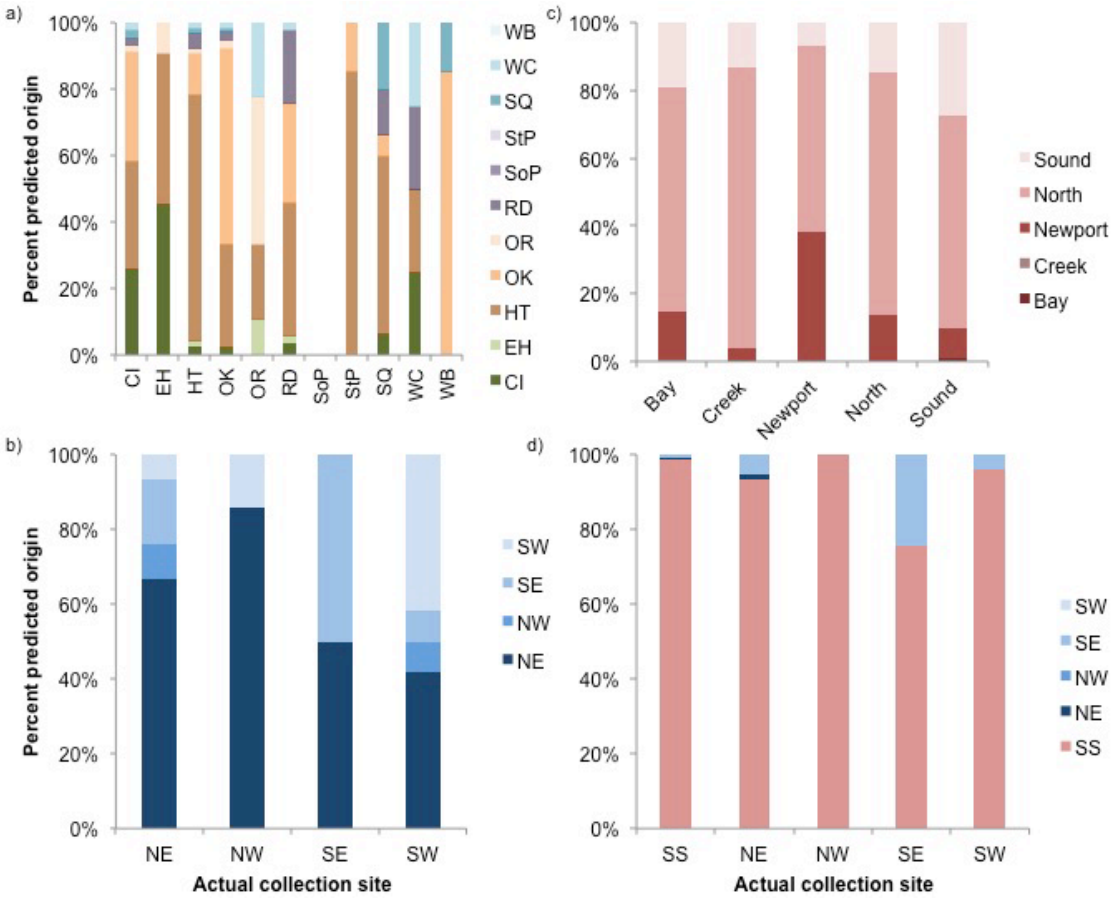

Figure 1.5. Classification success determined by linear discriminant function analysis for a) individual sites within Pamlico Sound, b) regions within Pamlico Sound, c) geographic regions among southern sites (SS), d) and all groupings of sites where spat were collected. The colors represent the predicted collection sites and cumulative percentage correctly identified is displayed on the *y* axis.

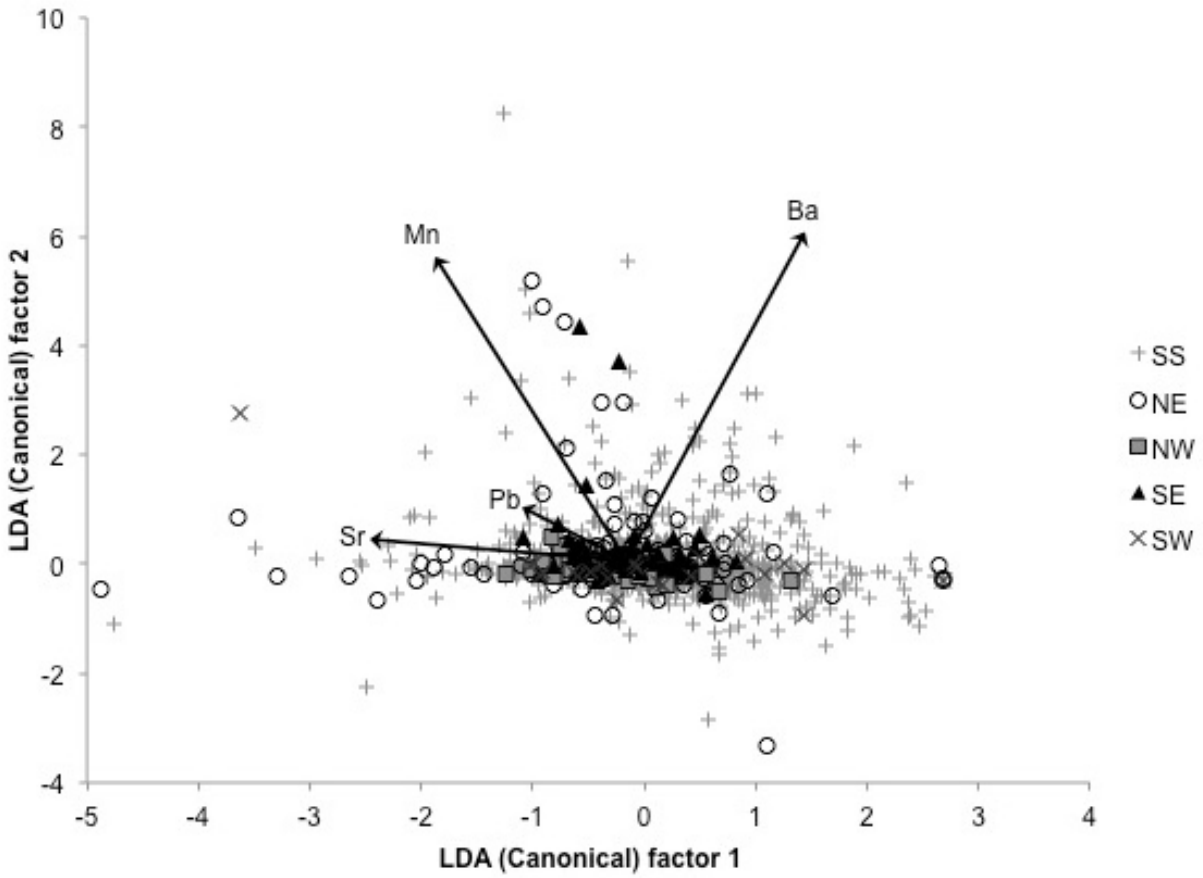

Figure 1.6. Canonical score plots of the linear discriminant function analysis for *C. virginica* settler shells grouped into Pamlico Sound quadrants and adjacent southern sites (SS).

Table 1.1. Mean temperature (± standard error) and salinity (± standard error) measurements for spat collection sites over the collection periods in summer 2012.

Site	Temperature (°C)	Salinity (ppt)
BaS	26.7 ± 2.00	36.9 ± 1.27
BoS	24.4 ± 1.33	35.0 ± 0.98
CI	26.7 ± 1.67	24.5 ± 2.50
EH	26.9 ± 0.44	20.1 ± 0.47
HT	20.6 ± 3.34	31.0 ± 1.00
JB	27.8 ± 0.62	35.5 ± 0.51
NeL	25.6 ± 2.32	35.0 ± 1.04
NeM	25.6 ± 0.87	26.0 ± 1.65
NeU	24.5 ± 1.03	15.3 ± 2.75
NoL	25.6 ± 1.76	36.0 ± 1.00
NoM	27.4 ± 0.73	30.0 ± 1.32
NoU	28.9 ± 0.95	33.7 ± 1.21
OK	24.5 ± 0.41	25.0 ± 1.52
OR	30.1 ± 0.36	20.7 ± 0.95
RD	17.2 ± 1.52	26.0 ± 1.24
SoP	27.6 ± 1.04	25.1 ± 1.92
StP	24.8 ± 1.85	24.8 ± 2.52
SQ	27.4 ± 1.21	18.6 ± 0.78
TC	27.8 ± 0.62	36.0 ± 0.25
WC	26.1 ± 0.56	15.7 ± 1.45
WB	22.2 ± 1.11	25.0 ± 1.00
WM	29.4 ± 0.72	35.0 ± 0.94
WH	29.4 ± 0.05	35.0 ± 1.15

Table 1.2. Analysis of variance (ANOVA) table summarizing the effects of temperature (T), salinity (S), and Mn/Pb spike ([ ]) on *C. virginica* larvae in laboratory experiments (Note: N=number of larval homes).

Element (X:Ca)	Factor	N	df	*F*	p
Sr	T	36	1	2.18	0.142
	S	36	1	2.60	0.108
	T × S	18	3	4.23	0.041*
Ba	T	36	1	1.01	0.316
	S	26	1	1.00	0.317
	T × S	18	3	1.05	0.306
Mn	T	36	1	0.417	0.519
	S	36	1	0.710	0.401
	[ ]	24	2	56.9	<0.001*
	T × S	18	3	1.46	0.228
	T × [ ]	12	4	2.19	0.115
	S × [ ]	12	4	2.62	0.0753
	T × S × [ ]	6	11	2.15	0.119
Pb	T	36	1	0.731	0.393
	S	36	1	0.096	0.757
	[ ]	24	2	1.02	0.361
	T × S	18	3	0.676	0.412
	T × [ ]	12	4	0.177	0.838
	S × [ ]	12	4	0.588	0.556
	T × S × [ ]	6	11	3.37	0.0374*

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CHAPTER 2: QUANTIFYING ESTUARINE-SCALE INVERTEBRATE LARVAL CONNECTIVITY: METHODOLOGICAL AND ECOLOGICAL INSIGHTS

Introduction

The ability to discern larval dispersal patterns is fundamental to the understanding and subsequent management of ecologically and commercially valuable marine species (Young 1990**;** Gillanders et al. 2003). For example, idealized marine reserve design depends on sufficient larval import (via immigration and self-recruitment; Jones et al. 1999; Puckett and Eggleston 2016) and export (e.g., spillover; Gerber et al. 2003; Gaines et al. 2010). Additionally, knowledge of system-specific larval import and export allows managers to allocate resources more effectively: in areas where local retention is higher than overall connectivity, efforts should focus on improving subpopulation demographics to bolster recruitment. Conversely, for sub-populations with relatively high connectivity and low local retention, local demographics become less coupled with recruitment and therefore less important (Almany et al. 2009; Figueira 2009; Puckett and Eggleston 2016). A greater understanding of larval connectivity can also aid in the understanding of year-class fluctuations in commercially important species (Hjort 1914). Knowledge of dispersal patterns, as well as the biological and physical parameters which control connectivity, will also become essential to predicting the effects climate change will have on the resiliency and persistence of future populations (Cowen and Sponaugle 2009).

Studies utilizing natural or artificial tagging methods have greatly enhanced our quantitative understanding of larval connectivity among sub-populations which, in turn, can

inform metapopulation dynamics (Thorrold et al. 2002; Levin 2006; Puckett et al. 2014). With the aid of improved technology (i.e., rapid and inexpensive genetic analyses and high-resolution mass spectrometry), the use of natural tag methods has become more accessible and diverse (Durrant and Ward 2005). Recently, tagging studies have reevaluated the extent of geographic connectivity of marine fishes and invertebrates (Lopez-Duarte et al. 2012), and by demonstrating higher levels of self-recruitment than previously assumed, have helped change the paradigm of marine larval dispersal from passive long-distance to behaviorally-mediated shorter-distance dispersal (Almany et al. 2007).

Geochemical signatures stored within calcified structures, such as fish otoliths, gastropod statocysts, and bivalve shells, are particularly useful for examining marine larval connectivity as many marine organisms begin recording geochemical signatures from egg fertilization (Thorrold et al. 2002; Becker et al. 2007; Kroll et al. 2016). Furthermore, geochemical tags are valuable to connectivity studies because they are capable of discerning between environmentally variable locations (e.g, within or among estuaries) with potentially high spatial resolution (~12 kms; Cathey et al. 2012). A key component of these studies involves the generation of reference signatures (i.e., atlases) from known location to inform multivariate algorithms that assign settling larvae of unknown natal origin to their natal source. For larval connectivity studies, generating reference signatures is traditional accomplished through the outplanting of larvae to stationary moorings (Becker et al. 2007). However, as larval dispersal is believed to be highly dependent on current patterns (Haase et al. 2012), there is a need to understand how utilizing more mobile outplanting methods, such as floating surface drifters, may affect signature generation and predicted connectivity. For example, larvae attached to stationary moorings likely contain a concentrated signature of

natal sites, whereas drifter larvae contain a more integrated signature of the environments larvae are exposed to as they disperse.

Estuarine systems provide an ideal setting in which to develop geochemical tagging methods and explore connectivity as they are characterized by high environmental variation through time and space, encompass varying geomorphological components (i.e., oyster reefs, marshes, barrier islands), and function as important nursery, juvenile, and adult habitats for several marine organisms (Beck et al. 2001). Many vertebrate and invertebrate species form distinct subpopulations, with varying amount of larval exchange and connectivity, within estuarine systems (Kämpf et al. 2010; Vasconcelos et al. 2011; Cathey et al. 2012). While finfish connectivity has been examined over numerous spatial scales within estuaries of the US east and west coasts, as well as abroad (e.g., Able 2005; Fodrie and Levin 2008; Vinagre et al. 2011), we know relatively little about the scale of estuarine larval connectivity for invertebrates, and most of what is known is based on biophysical models (e.g., Reyns et al. 2007; North et al. 2008; Narvaez et al. 2012; Puckett et al. 2014). The few studies that have begun to examine invertebrate larval connectivity in estuarine systems have determined that geochemical tagging methods are a viable tool for connectivity studies (Cathey et al. 2012; Kroll et al. 2016).

The goal of this study was to gain insight into the larval connectivity and dispersal of a commercially and ecologically important invertebrate, the Eastern oyster (*Crassostrea virginica*), within a large, wind-driven estuarine system. A requisite for achieving this goal was to develop an atlas of geochemical signatures (i.e., elemental ratio, X:Ca) from putative natal sites associated with an existing oyster reserve system within Pamlico Sound (PS), North Carolina (NC), USA. The secondary goal of this project was to evaluate the utility of

two different methodological approaches for developing the atlas by outplanting recently spawned oyster larvae to stationary moorings and surface drifters. We compared outplanting methods in their ability to represent distinct natal signatures or potential larval dispersal corridors. Geochemical signatures from outplanted larvae were used to predict the region of origin (NW, NE, SE, SW), within PS, of recently settled oysters (hereafter "spat") collected from several sites across the Sound. Classification successes from discriminant function analysis predicted patterns of dispersal, and diversity indices were used to evaluate connectivity and outplanting techniques.

Methods

Study system and previous application of geochemical signatures

Pamlico Sound, which extends 129 km north-south and 24-48 km east-west, is the largest lagoonal estuary along the eastern North American coastline and is protected from the Atlantic Ocean by a string of barrier islands. Circulation patterns in PS are primarily wind-driven due to its broad and relatively shallow basin (mean depth ~4.5 m). Wind forcing varies greatly over short intervals, however, there are some regular seasonal patterns of southwesterly winds in the spring/early summer and northeasterly winds in late summer/fall (Eggleston et al. 2010). Pamlico Sound contains several important and highly productive nursery and adult habitats (e.g., oyster reef, seagrass) for many estuarine-dependent species. Historically, PS was one of the largest sources of commercially harvestable oysters along the U.S. east coast, however overfishing, poor water quality, disease, and habitat degradation has reduced their abundance by nearly two orders of magnitude within the last century (Lenihan et al. 1999).

Previous connectivity studies within PS have been used to not only investigate dispersal patterns but also highlight the relationship between prevailing wind magnitude and direction and current magnitude and direction. Over a two-year timespan, Reyns et al. (2007) found that 70-96% of the variance in non-tidal current velocities occurred within the direction of primary wind flow, whereas tidal velocities increased with decreasing distance toward inlets. Floating surface drifters have also been used to empirically validate wind-based dispersal models with high levels of success within the PS, indicating that net transport is primarily wind-driven (Haase et al. 2012). More recently, biophysical models have shown that location and date of spawning in combination with frequency of wind reversals and magnitude of wind direction significantly influenced larval dispersal patterns within PS (Puckett et al. 2014).

To increase larval supply and connectivity within PS, the North Carolina Division of Marine Fisheries has established and maintained several no-take subtidal oyster spawning sanctuaries (reserves) over the last 20 years. Within these reserves, ~2-m-tall, cone-shaped mounds were constructed from limestone riprap and oyster shell. In 2006-2008, oyster densities within and among reserve reefs fluctuated over both seasonal and annual times scales, indicating various levels of recruitment success and survival (Puckett and Eggleston 2012). Hydrodynamic models have been used to predict dispersal from these reserves, suggesting that mean dispersal distances vary among reserves from 5-40 km (max distance c. 100km), which can hinder both inter-reserve connectivity and local retention, as reserve areas range from 0.03-0.2 km^2 and inter-reserve distances ranges from 10-120 km (Haase et al. 2012; Puckett et al. 2014). Furthermore, natal location was the primary driving force for

nearly all aspects of dispersal, indicating the need to develop an atlas that incorporates site and region specific geochemical signatures.

A recent study conducted by the authors found that there was substantial spatiotemporal variation throughout PS to successfully apply geochemical tagging methods to *C. virginica* shells and that geochemical signatures in shells could be utilized to discriminate between collection regions within PS (~ 35 x 15 km; Kroll et al. 2016). For this study, we used larval outplanting methods to develop an atlas of geochemical signatures at the following sites in PS (Fig. 2.1): Cedar Island (CI), Crab Hole (CH), Gibbs Shoal (GS), Middle Bay (MB), Ocracoke (OK), West Bay (WBa), and West Bluff (WBl). Sites were chosen because of their proximity to oyster reefs of high density, or broodstock reserves. To capture a wide range of dispersal pathways, we also sampled the following sites within the Sound for newly settled spat: Englehard (EH), Hatteras (HT), North Central (NC), Oriental (OR), Point Peter (PP), Rodanthe (RD), South Central (SC), and Wanchese (WC) (Fig. 2.1a). Following the regions used in Kroll et al. (2016), we bisected PS twice to create four, ~ 35 x 15 km geographic regions (quadrants): Northwest PS (NW): CH, GS, EH, WC, PP; Northeast PS (NE): HT, NC, RD; Southeast PS (SE): CI, OK, WB; and Southwest PS (SW): MB, OR, WBl.

Study species

The Eastern oyster is an important model organism for the study of estuarine-scale larval connectivity because of its early life history characteristics and ecological role as a reef-building foundation species. Following successful fertilization, oyster larvae progress through an approximately 2-to-3 week planktonic veliger phase (Medcoff 1939), in which they begin to develop an aragonite-rich prodissoconch shell that is retained after an

individual settles on suitable benthic habitat (most typically, gregariously on other adult oyster shells; Stenzel 1964). Previous modeling studies have reported mean oyster larval dispersal range of ~5-40 km (Puckett et al. 2014). Recently settled spat are sessile for the remainder of their juvenile and adult life.

Larval outplanting

To (i) examine natal origins of settling *C. virginica*, (ii) identify larval connectivity, and (iii) compare larval outplanting methodology, we deployed larval "homes," attached to stationary moorings and surface drifters during June 2013, June 2014, and August 2014. Outplanting times corresponded to known reproductive peaks of *C. virginica* within PS (Eggleston et al. 2011; Mroch et al. 2012). Larval homes were constructed from hollow PVC tubing capped on each side with 30 μm mesh, nitex cloth to allow for the flow of water, nutrients, and small phytoplankton into the home, yet prevent larvae from escaping. Three-day old *C. virginica* larvae were obtained from the University of Maryland's Horn Point Laboratory in Cambridge, Maryland, USA, acclimatized to local salinity, and then divided into homes, with approximately 1.6×10^4 larvae per home (21.2 larvae cm^{-3}). For further detail on larval home construction and the acclimatization process, see Kroll et al. (2016).

Stationary moorings were constructed with a cement base and marine rope attached to a surface float following Becker et al. (2007). Four larval homes were attached to PVC piping that rested ~ 1 m below the water's surface. Sets of four larval homes were also attached ~ 1 m below the sea surface to Microstar Lagrangian Surface Drifters (hereafter "drifters;" Pacific Gyre; Oceanside, CA). Drifters were also equipped with uBlox GPS receivers and Globalstar Simplex telemetry software for remote tracking. Both drifters and moorings were equipped with a HOBO Water Temp Pro v2 data logger (Onsett; Bourne,

MA). Drifters and moorings were deployed at reserve sites for approximately one week (7 d) to incorporate the chemical signature of the (i) associated site (moorings) and (ii) dispersal pathway (drifters) within the prodissoconch shell. For certain replicates, drifter trials were terminated early in cases of inclement weather, drifter removal by fisherman, or if a drifter ran aground.

In June 2013, stationary moorings were deployed at CH, CI, GS, MB, WB, and WBl. Drifters were deployed from CH, CI, GS, MB, WB. An additional drifter was deployed at WBl, however, the GPS signal was lost and the drifter was never recovered. Drifters and moorings were deployed from CH, CI, GS, OK, MB, WB, and WBl in June 2014, with all drifters successfully retrieved. In August 2014, drifters and moorings were deployed from CH, CI, GS, OK, MB, and WB. Additionally, in August 2014, a mooring, but no drifter, was deployed at WBl. All drifters, except MB, were successfully retrieved during this final trial. To focus resources on the regions with the highest concentrations and densities of oyster reefs (Puckett and Eggleston 2012; Peters 2014), no drifters or moorings were deployed within the NE region of PS during this study. During the drifter sampling periods, wind speed and direction was collected from PS monitoring station HCGN7 (35.2101 N, 75.6997 W) maintained by the National Centers for Environmental Information (ncdc.noaa.gov).

Upon recovery, larval homes were removed from drifters/moorings, resubmerged in water from each collection site, and transported back to UNC's Institute of Marine Sciences (IMS) in Morehead City, NC. Larvae from each home were then filtered using nitex cloth and survival was measured, following Kroll et al. (2016), to compare differences in outplanting methods. Larvae were then frozen until sample preparation for geochemical analyses following Becker et al. (2007).

Spat settlement sampling

Spat settlement collectors were constructed by affixing 2-3 wire strings, each containing 12 adult oyster shells, to the aforementioned stationary moorings at reserve sites, as well as private and public docks or stand-alone wooden pilings at EH, HT, NC, OR, PP, RD, and WC (consistent across all three trials). Although no outplanting was done in the NE region, we did collect spat from that region (e.g., HT, NC, and RD sites) because reefs do persist there and may function as larval "sinks" (Puckett et al. 2016). To ensure we collected spat that were larvae during our drifter/mooring deployment periods, settlement collectors were deployed during each larval home recovery (June 2013, June 2014, August 2014) and retrieved approximately 2 weeks later. Recovered shell-string oyster shells with recently settled spat were frozen until individual spat could be counted and removed from adult oyster shells with a dissecting microscope and tungsten probe, respectively.

Sample preparation and LA ICP-MS

Frozen larvae from homes and spat from field collections were thawed, cleaned (see Kroll et al. 2016 for respective cleaning methods), and mounted on a glass microscope slide in haphazard order. Larvae were mounted as a concentrated mass on a labeled glass microscope slide covered in double-sided tape (i.e., each home was represented by 1 mound of shells), whereas each spat was mounted individually. The slides were then stored in a laminar flow hood until analysis.

Both larval and spat samples were analyzed using a Thermo-Fisher Element2 inductively coupled plasma mass spectrometer with a Teledyne ATLex 300si-x 193nm Excimer laser ablation unit (LA ICP-MS). To correct for mass bias and instrument drift, National Institute of Technology Standards-certified standards (Reference Material 612, 614,

and 616) were run at the beginning and end of every 4 slide sequence (~140 burns). Concentrations of the following elements were quantified from larval and spat samples: ^{26}Mg, ^{48}Ca, ^{55}Mn, ^{59}Co, ^{63}Cu, ^{88}Sr, ^{112}Cd, ^{118}Sn, ^{138}Ba, and ^{208}Pb. These elements were all analyzed in low-resolution mode, and were chosen because of their previous use in uptake and tagging studies of bivalve shells (Strasser et al. 2008a,b; Fodrie et al. 2011; Kroll et al. 2016).

Mounted larvae from drifter and mooring homes were ablated three times in bulk, using side-by-side line transects of 150 μm with 40 μm spot size and 80% laser intensity. Line transects covered ~2-3 shell lengths, following Becker et al. (2007), and were used instead of burning several individual larvae to reduce the likelihood of pseudoreplication. To determine elemental signatures of the spat collection sites, the larval shell (prodissoconch) section of the shell was ablated twice with side-by-side line transects of 110 μm with 40 μm spot size and 80% intensity. Larval shell transects extended from the umbo toward the outward edge of the larval shell following Strasser et al. (2008). Isotope intensities for replicate burns were averaged and then converted into elemental ratios (X:Ca) for each home or spat/larval shell following Becker et al. (2005).

Data analysis

Linear Discriminate Function Analysis (DFA) was performed on Box-Cox transformed larval ratios to determine whether distinct spatial signatures could be identified in larval shell geochemistry between sites, as well as between mooring and drifter approaches. Jack-knifed classification matrices, without sample replacement, were compared to expected classification matrices based on random chance to assess classification success. DFAs were conducted stepwise, with the least significant elemental ratio dropped, as

determined by the *F*-to-remove statistic, until all F-to-remove values >2. As an additional test of outplanted larval predictive ability, signatures from mooring-attached larvae were used as a reference to predict region of origin and termination for each drifter and then compared to actual region of origin and termination. Wind speed and direction was used to create wind rose diagrams and qualitatively compared to observed drifter movement by overlaying a vector field representing magnitude and direction of individual drifter movement (Fig. 2.1).

Geochemical signatures of oyster larvae attached to drifters or attached to stationary moorings were each used to predict larval origin (at both the individual site and regional scale) for spat that settled on shell-string collectors. Separate connectivity matrices were generated for each sampling season, where matrix elements represented the proportion of larvae spawned from each row-referenced site that settled in a column-referenced site. Self-recruitment was determined by calculating the proportion of sampled spat that had settled within their natal region, while connectivity was determined by the proportion of larvae settling within a region that was distinct from their natal region. Predicted connectivity using outplanting attached to surface drifters was quantitatively compared to predictions using outplantings attached stationary moorings with Chi-squared goodness of fit tests. Lastly, Shannon-Wiener diversity indices (H') and evenness were calculated for each region to examine the diversity of natal sources among settlers within individual sites and regions.

Results

Mooring and drifter deployments

In June 2013, all drifters moved northward with the prevailing southerly directed winds. Drifters traveled a net distance of 2.6-21.6 km from their release location, with a mean drift distance of 14.2 ± 1.5 km during a 7 day deployment period (Fig. 2.1b).

Southwest winds, with an average speed of 18.3 ± 0.2 km h^{-1} and gusts of up to 56.4 km h^{-1}, prevailed during this time although strong northeast winds were also present (Fig. 2.1b). Mean daily survival for larvae attached to drifters was estimated at 28.4 ± 7.0% and 34.1± 4.02% for mooring larvae.

During June 2014, drifters in the northern half of PS (CH, GS, and OK) moved southward with the prevailing northeast winds, whereas drifters in the southern half of PS (CI, MB and WBl) moved northward with prevailing winds out of the southwest (Fig. 2.1c). The WB drifter did not adhere to this pattern, moving southward and deeper into the relatively enclosed West Bay area (Fig. 2.1c). Drifters traveled a net distance of 13.1± 2.2 km with a range from 4-24.1 km. North winds of 19.4± 0.5 km h^{-1}, with gusts of up to 46.7 km h^{-1}, prevailed during this sampling period (Fig. 2.1c). Mean daily larval survival was estimated at 24.4 ± 8.2% and 40± 6.2% for drifter and mooring larvae, respectively.

In August 2014, all drifters moved southward from their deployment locations (Fig. 2.1d), despite a prevalent southwest wind with an average speed of 14.0 ± 0.1 km h^{-1} and gusts of up to 37.1 km h^{-1}. Drifter traveled net distances ranging from 5.21-47.8 km with an average of 16.5 ± 6.6 km. Mean daily survival for larvae attached to drifters was 32.3 ± 10.3% and 44.1± 7.0% for mooring larvae.

Self-recruitment and connectivity

Geochemical signatures within settler shells were effective in discriminating geographic regions in PS (i.e., NE, NW, SW, SE) based on atlases developed for both drifting and stationary larvae. Our final DFA models included the following trace elements (as ratios to Ca): Mn, Mg, Cd, and Sn for larvae attached to drifters and Mn, Sr, Ba, and Sn for larvae attached to moorings (Fig. 2.2a-c). Geochemical signatures of regions varied

between years (June 2013 versus June 2014) and seasons (June 2014 versus August 2014). For example, both drifter and mooring larvae presented a trend of increasing average Mn concentration from NW to SE to SW in June 2013 and June 2014, though only significant in June 2013 drifters ($p=0.042$, $F=4.53$, $df=2$ from SE to SW in June 2013; Fig. 2.3a). However, in August 2014, Mn concentrations decreased from NW to SE to SW for both drifter and mooring larvae ($p=0.031$, $F=4.96$ $df=2$ for drifters and $p=0.012$, $F=7.12$, $df=2$ for moorings from NW to SE; Fig. 2.3a,b). Classification success for DFA models based on geochemical signatures from drifter larvae was 93%, 50%, and 88% (compared with the null expected of 62%, 33%, and 50%) for June 2013, June 2014, and August 2014, respectively. Mooring larval models had a classification success was 92%, 77%, and 58% (over the null expected of 56%, 64%, and 44%) for June 2013, June 2014, and August 2014, respectively. Larval signatures from stationary moorings did not effectively predict drifter origin or termination, with predicted origin accuracies of 43.9%, 30%, 46.2% and predicted termination accuracies of 57.1%, 20%, and 30.8% for June 2013, June 2014, and August 2014, respectively.

When comparing mooring and drifter tracking methods, we noted regional consistencies between larval sources and degree of self-recruitment. For example, in June 2013 and June 2014, the southern half of PS supplied the majority of larvae to the rest of PS. Accordingly, self-recruitment was highest in the southern regions because very few immigrants, via connections from the north, settled in southern regions (Fig. 2.4a,b,d,e). In August 2014, signatures of both drifting and stationary larvae suggested that the NW region of PS, may at times, be a more significant natal source than previously inferred from biophysical mod simulations (Puckett and Eggleston 2016) (Fig. 2.4c,f). Despite these broad

similarities, there were also notable differences in the results of mooring and drifter approaches with respect to self-recruitment and connectivity (Table 2.1).

In June 2014, mooring signatures predicted connectivity in line with that of June 2014 drifters: there were high levels of connectivity between the SE and other sites, with the SE as the primary source for NE larvae (82%; Fig. 2.4e). Larval signatures from moorings during June 2014 also predicted some level of self-recruitment within the SW PS (47%) and a SW larval source for NW (33%) and SE (57%) sites in PS. However, June 2013 mooring signatures predicted minimal self-recruitment in the SE (3% of spat; Fig. 2.4d) and high levels of connectivity between the SW and other sites in the SE, NE, and NW (i.e., 92%, 90%, and 100% of spat showed SW natal signatures, respectively).

In August 2014, drifters showed a shift in dominant larval flow from south-north to north-south, with high connectivity between the NW and all other sites. For example, NW origins were predicted for 64%, 99%, 99%, and 100% of spat within SW, SE, NW, and NE regions, respectively (Fig. 2.4c). We also noted elevated self-recruitment within the SW region (32% of spat). Although models based on mooring larvae predicted an increase in supply from the NW during this period, the SE was the primary source for all regions, with 80%, 86%, 71%, and 88% of SW, SE, NE, and NW spat, respectively, being linked to SE origins (Fig. 2.4f). As opposed to previous mooring sampling periods, larval export from the SW was minimal (0% of spat in NE and NW and 3% of spat in SE).

Shannon diversity indices in June 2013 depicted no diversity in larval source for all regions based on drifters (as there was only one, uniform source) and no diversity in larval sources for the NW region based on mooring sampling (Table 2.2). However, 2013 mooring methods did predict higher larval source diversity in other regions, with the highest source

diversity in the SW (H=0.40, 0.33, and 0.86 for NE, SE, and SW, respectively). In June 2014, we saw much higher diversity in larval sources based on both tracking methods than in June 2013 (Table 2.2). Stationary mooring models, again, predicted higher connectivity than drifter models for all regions, except the NE (H=0.76 for drifter versus 0.64 for mooring models). In August 2014, drifter signatures predicted a single larval source for the NW region, with relatively low larval source diversity for NE and SE regions (H=0.09 and 0.07 for NE and SE, respectively) and high diversity within the SW (H=0.76; Table 2.2). Conversely, August 2014 mooring signatures predicted high larval source diversity within the NE (H=0.60), low diversity in the NW (H=0.38), and moderate source diversity in the southern region (H=0.47 and 0.54 for SE and SW, respectively).

Discussion

By combining oyster larval outplanting with geochemical tagging methods, reflective of spatial gradients in environmental conditions, we found that: (1) oyster larval dispersal pathways in PS generally follow the dominant wind flow present during the larval period; and (2) connectivity in PS is generally dominated by single-region larval sources over 2-3 week periods, although the identity of that larval source varies over longer timescales (i.e., seasonally and annually). We also evaluated a novel methodological approach to assessing connectivity using geochemical tags by outplanting larvae in homes attached to surface drifters. To the best of our knowledge, no study, to date, has outplanted larvae on floating drifters and consequently examined how predicted connectivity may differ between larvae outplanted on stationary moorings (traditional approach) and those on floating drifters. We found several coarse consistencies in predicted connectivity between mooring and drifters, such as a dominant south-north larval flow in both June 2013 and June 2014. There were also

significant differences between outplanting methods across all sampling periods, particularly in August 2014, when connectivity based on larvae outplanted on drifters predicted a north to south flow of larvae while moorings predicted a south to north flow. Therefore, one should consider the utility of both drifters and stationary moorings in geochemical tagging studies to discern larval connectivity over estuarine scales of 50-150 km for wind-driven systems.

Connectivity and ecological insights

Two important goals of larval tracking studies are to determine levels of local retention (self-recruitment) and identify the number of sources that drive metapopulation connectivity (i.e., single source vs. multiple sources). Recently, hydrodynamic and now biophysical models have been used to advance our understanding of dispersal ranges and the degree of self-recruitment within marine populations (Botsford et al. 2009; Cowen and Sponaugle 2009; Puckett and Eggleston 2016). While many of these models have allowed us to question traditional concepts of connectivity (i.e., how demographically open or closed a population is), few studies have been able to validate these predictions with empirical studies (*sensu*, Botsford et al. 2009; Carson et al. 2013). Furthermore, models, alone, may not be able to effectively capture dispersal variability at the spatiotemporal scale over which it can occur in the natural environment (Qian et al. 2014). Previous biophysical models constructed by Puckett et al. (2014) and Puckett and Eggleston (2016) for the eastern oyster within the reserve networks in PS have depicted a network of several larval sources (at small <1 km x 1 km reefs scales), with patterns of (1) self-recruitment that were generally higher for reefs in southern PS than northern PS and (2) connectivity, while generally low between reserve sites, was generally directed south to north. At larger 15 km x 35 km regional scales this study identified similar patterns of a south to north larval flow; however, both larval

outplanting methods used in our study predicted predominantly single-source (single-region) connectivity models.

To contextualize our results with Puckett and Eggleston's (2016) model (hereafter, PE), we decreased the resolution of their connectivity matrix to the regional (NW, NE, SE, SW) scale. Although grand mean self-recruitment rates for both studies was comparable (27.8-39.9% of spat in our study versus 34.2% of spat from Puckett and Eggleston 2016), several differences became apparent between regions and tracking methods. Over the 5 year simulation from 2006-2010, the PE model predicted largest, average self-recruitment occurred in the NW, with 39.6 ± 18.5% of spat settling in their natal region. While average self-recruitment for drifter models within the NW was consistent with the PE model (34.2 ± 20.5% of spat), mooring models predicted self-recruitment in the NW to be considerably lower than the PE model (7.1 ± 4.0% of spat). Moreover, when combining our three sampling periods, the highest rates of self-recruitment for both mooring and drifter models occurred within the SE, with 58.4 ± 30.1% and 39.2 ± 25.6% of spat settling in their natal regions, respectively. In the PE model, SE self-recruitment rates were predicted to be 34.2 ± 28.5%. The SW had the least disparity, as PE found self-recruitment at 38.6 ± 15.3% and drifter and mooring models predicted an average self-recruitment of 27.1 ± 9.0% and 37.0 ± 19.0%, respectively.

The differences between our empirically-derived models and the PE model highlight that elemental tagging methods cannot only be used to validate existing models but may also be necessary to fully discern broader connectivity trends within complex estuarine environments. The PE model examined existing and potential reserve sites, and while we were able to examine its results at the regional level, the model itself may be influenced by

the reproductive capability of the individual reserve sites. Therefore, site selection directly impacts predicted connectivity, whereas our use of elemental signatures across a larger scale allows us to account for all sources within a region as sites within the same quadrant are subject to similar environmental variability (Kroll et al. 2016). For example, previous monitoring has shown reef cover and oyster density is lower in the regions with the largest differences in PE versus drifter/mooring model-predicted local retention (i.e., SW) (Puckett and Eggleston 2012; Peters 2014). The inability of purely wind-driven models to account for biophysical mechanisms that reduce larval mortality within the vicinity of source regions (Cowen et al. 2000) and larval ability to orient and navigate toward chemosensory stimuli released by nearby reefs (Kingsford et al. 2002) may also contribute to the increased self-recruitment seen within some regions of our empirical study. Additionally, the use of drifters and moorings to examine larval connectivity may allow us to identify oyster larval dispersal patterns that are not purely wind driven, while the PE model relies on wind-forcing data to generate current velocities, and ultimately, computer particle (larval) velocity (Puckett et al. 2014). Furthermore, the PE model, as well as other dispersal models (e.g., Cowen and Sponagule 2009), depict unidirectional current flow at each time step, whereas strategically placed drifters can reveal nuances in current patterns throughout the system (e.g., Haase et al. 2012). For example, in June 2014, drifters in the north moved southward while drifters in the south moved northward, which is consistent with hydrodynamic seiching patterns observed in PS when winds switch directions (Luettich et al 2002; Haase et al. 2012).

Tracking methods

The life history of marine bivalves may help us to understand why geochemical signatures of larvae outplanted and attached to moorings versus drifters predicted varying patterns of connectivity. During their larval phase, bivalves grow relatively quickly (~6 μm/d for *C. virginica*; Gallager et al. 1986) by accreting shell either laterally (continually extending to the outermost shell) or vertically (on top of other shell; Sprung 1984; His and Maurer 1988). Throughout this period of growth, oyster larvae may be transported from 0.1-110 km (North et al. 2008; Puckett et al. 2014) and can be exposed to a series of different environmental conditions which affect shell geochemistry (Kroll et al. 2016). When ablating the larval shell of spat during LA ICP-MS, the laser may have only ablated the surface, or newest, layers of larval shell, resulting in signatures that are more similar to those of settlement location (i.e., stationary moorings) than to those of larval pathways (i.e., surface drifters).

Despite the differences in connectivity predicted by geochemical signatures in larval attached to drifters and moorings, it is difficult to conclude that one method is superior to the other. Both methods were used to predict seasonal variability in larval dispersal pathways and sources (as shown by H'; Table 2.2), had comparable modeling classification success, and were able to discern spatiotemporal trends of elemental concentrations within oyster shell (e.g., Mn; Fig.2.3). Stationary moorings (i) consistently predicted higher overall diversity in larval sources than drifter-based models (Table 2.2), (ii) are the current standard for larval connectivity studies, and (ii) can successfully discriminate between sites at resolutions of 20-30 km (Becker et al. 2007). However, they were only able to predict

connectivity patterns that were consistent with wind patterns for two of the three sampling periods.

Connectivity models often utilize floating surface drifters as a proxy for larval trajectories and dispersal pathways (e.g., Eggleston et al. 1998; Lugo-Fernández 2001; Haase et al. 2012). Attaching larvae to floating drifters allowed us to not only look at putative dispersal pathways, but also record the geochemical signatures associated with those potential pathways. There are, however, several caveats inherent in the use of drifters. Wild oyster larvae develop depth-regulation and swimming abilities, with speeds up to 2.5 mm s^{-1}, and therefore may be subjected to forces other than the surface winds that control drifter movement (Kennedy 1996; Metexas 2001), but model simulations in well mixed PS suggest that depth regulating behavior has a relatively small effect on larval dispersal patterns compared to location and timing of spawning (Puckett et al. 2014). We also found significantly higher larval mortality within drifter homes, increasing the possibility of elemental contamination from shell degradation and decreasing the amount of larvae available for analysis. Furthermore, the cost of purchasing, operating, and maintaining drifters are significantly greater than those associated with stationary moorings and there is a risk of drifters being tampered with, lost, or removed during the study period.

Reserve design

Connectivity and larval tracking studies have been used successfully to inform management decisions and create new or evaluate existing marine reserve systems (Burgess et al. 2014). As the scale of our study was larger than just reserve sites, we were able to evaluate larval flow on a system-wide scale, rather than evaluate the efficacy of individual reserves within the PS system. Therefore, the results of our study provide key insights into

the early life history of the eastern oyster within this system, as well as build upon our foundation of knowledge for invertebrate larval connectivity and marine reserve design. We found that while some dispersal pathways traversed the Sound (~130 km), the highest amount of larval exchange would likely occur among reefs within the same (or adjacent) regions (~35 km). Connectivity also varied on both a seasonal and annual timescale: dispersal in June 2013 and August 2014 followed a single-source model, with different source regions, and dispersal in June 2014 seemed to be governed by multiple-sources. Additionally, as different regions may experience varied wind-forcing and current flow, dispersal pathways within PS will not always be uniform across space and time. This supports previous work for oysters, as well as for other marine reef-forming organisms, that promotes the implementation of reserves which account for variability in dispersal pathways without compromising self-recruitment within PS (e.g., Almany et al. 2009; Nicol and Possingham 2010; Puckett and Eggleston 2016). Improved accuracy in predictions of population connectivity from larval dispersal models, which can better incorporate multi-directional wind forcing, are also essential to designing and siting successful reserves networks. While larval connectivity is an important factor for the success of reserves, intra-reserve demographics should be carefully considered to ensure a reserve network design that meets the needs of the metapopulation, the management system, and its stakeholders.

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Table 2.1. Chi-squared (χ^2) statistics, degrees of freedom (df), and associated p-values (p) for connectivity matrix comparisons within and among tracking methods, by sampling period.

Tracking method	Sampling periods	df	χ^2	p
Drifter	All	11	842	<0.001
	June 2013/June 2014	11	136	<0.001
	June/Aug 2014	11	521	<0.001
Mooring	All	11	477	<0.001
	June 2013/June 2014	11	178	<0.001
	June/Aug 2014	11	184	<0.001

Table 2.2. Shannon-Wiener diversity indices (H'), evenness (J'), and richness as measures of larval connectivity within and among regions in Pamlico Sound for each tracking method, and by sampling period. H' values of 0 represent a single larval source whereas a value of 1 would represent several larval sources. J' values of 0 indicate one source contributed all the recently settled spat whereas a value of 1 would indicate several larval sources contributed the same amount of recently settled spat.

Date	Tracking method	Region	H'	J'	Richness
June 2013	Drifter	NE	0	0	1
		NW	0	0	1
		SE	0	0	1
		SW	0	0	1
	Mooring	NE	0.40	0.37	3
		NW	0	0	1
		SE	0.33	0.30	3
		SW	0.86	0.78	3
June 2014	Drifter	NE	0.76	0.69	3
		NW	0.56	0.51	3
		SE	0.72	0.65	3
		SW	0.61	0.55	3
	Mooring	NE	0.64	0.58	3
		NW	0.87	0.79	3
		SE	0.96	0.88	3
		SW	0.98	0.89	3
August 2014	Drifter	NE	0.09	0.13	2
		NW	0	0	1
		SE	0.07	0.10	2
		SW	0.76	0.70	3
	Mooring	NE	0.60	0.87	2
		NW	0.38	0.54	2
		SE	0.47	0.42	3
		SW	0.54	0.49	3

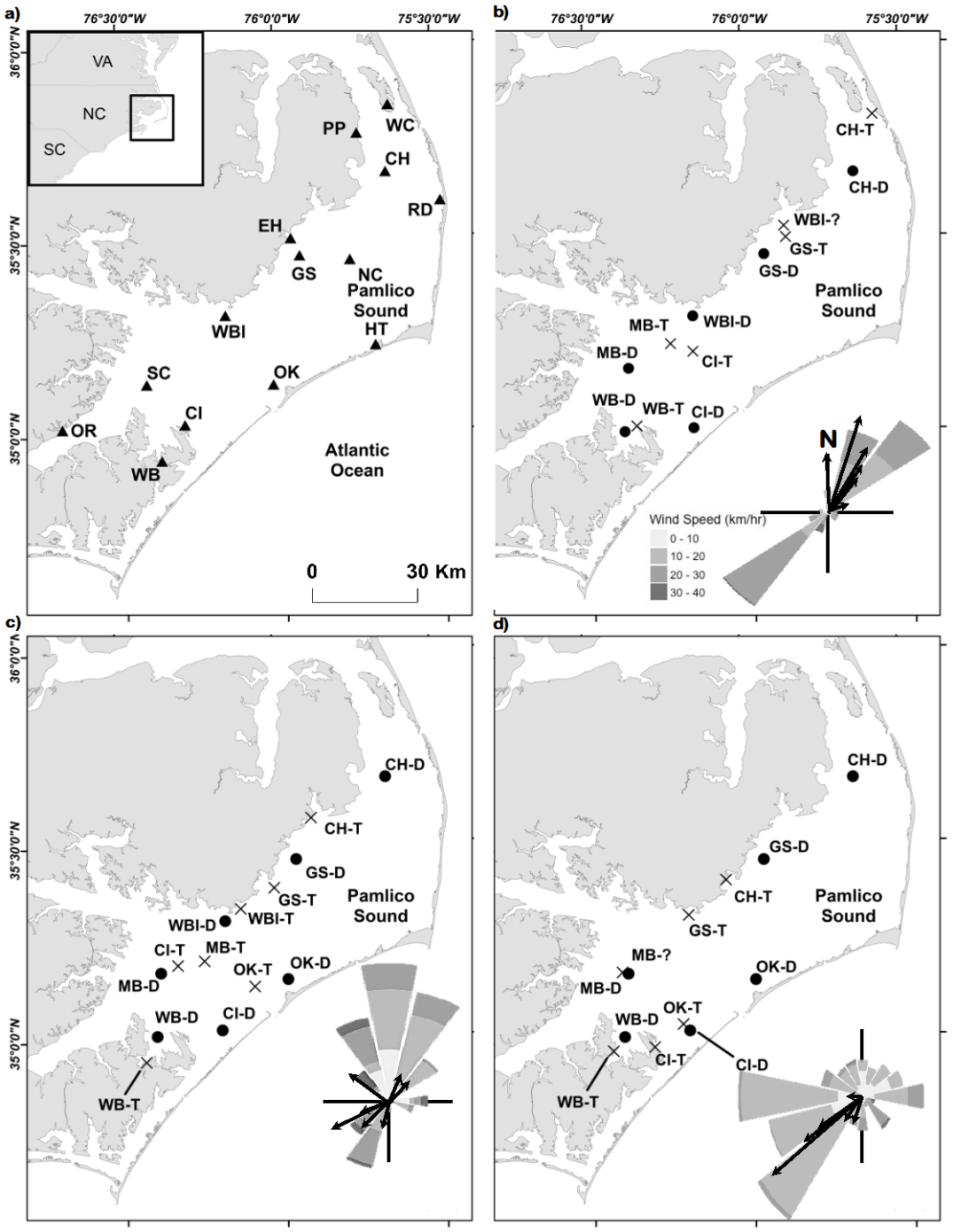

Figure 2.1. Map of a) all spat settlement collection sites within Pamlico Sound, NC and drifter deployment sites (D), termination sites (T), and average wind speed and direction for drifters deployed during b) June 2013, c) June 2014, and d) August 2014. Question marks (?) indicate last known location of lost drifters. Wedge size in wind roses corresponds to measured frequency of wind speed/direction. Arrows on wind rose diagrams correspond to length and direction of drifter path.

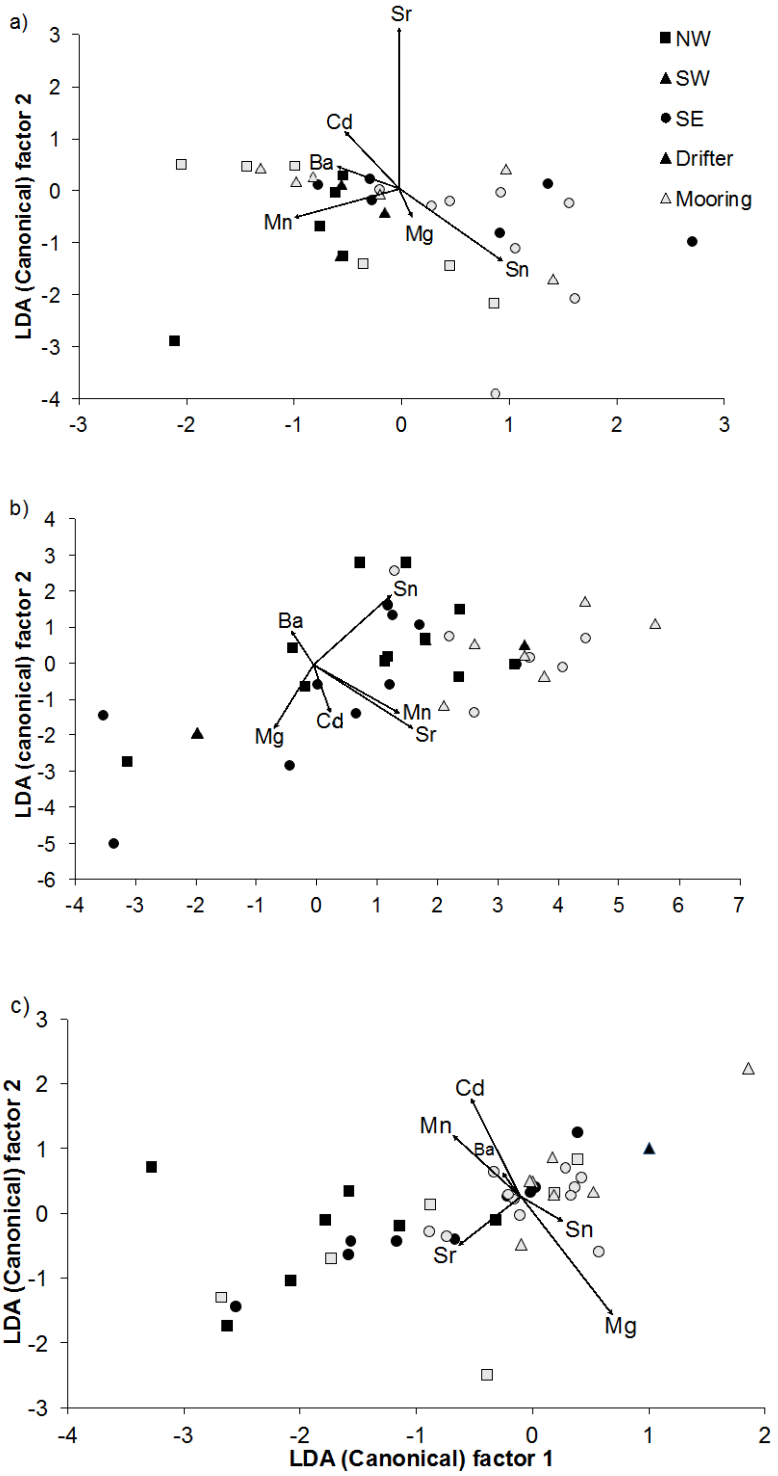

Figure 2.2. Linear discriminant function analysis results for classification of geochemical ratios for oyster larvae housed on surface drifters (black) and moorings (grey), by region (shapes), for a) June 2013, b) June 2014, and c) August 2014.

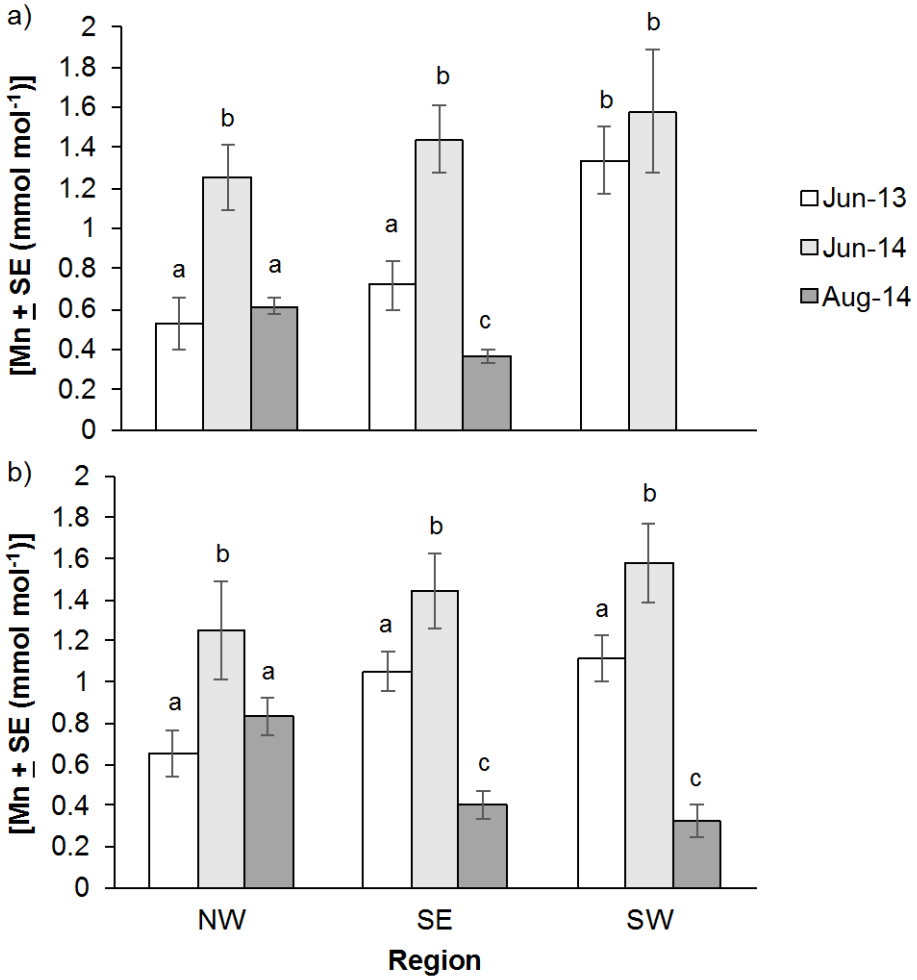

Figure 2.3. Distribution of manganese (Mn) in shells of larvae outplanted to a) surface drifters and b) moorings, by sampling period

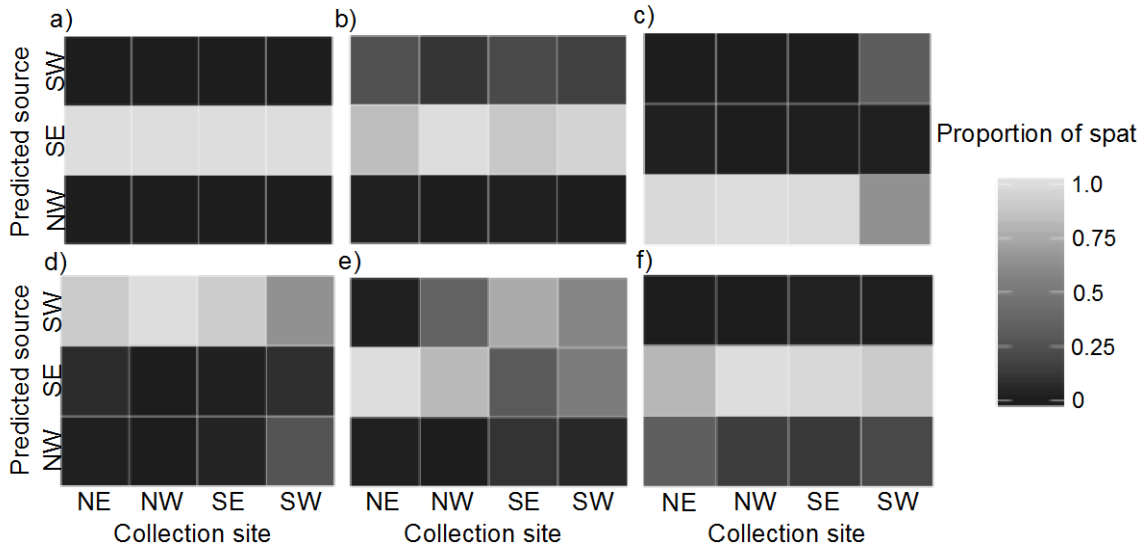

Figure 2.4. Heat map of connectivity matrices showing predicted larval origin for settled oyster spat from each region within PS. Prediction origins are based on a) June 2013 drifters, b) June 2014 drifters, c) August 2014 drifters, d) June 2013 moorings, e) June 2014 moorings, and f) August 2014 moorings. Brighter (lighter) colors indicate higher levels of connectivity between/within regions.

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CHAPTER 3: CARRY-OVER EFFECTS FROM NURSERY HABITATS INFLUENCE REPRODUCTIVE LIFE HISTORY OF A COASTAL MARINE FISH

Introduction

Our understanding of how habitat selection affects the vital rates of organisms and drives population fitness is based primarily on studies that monitor organisms while they remain in a specific habitat (Kittredge 1938; Tupper and Boutillier 1997). However, most mobile species traverse multiple habitats over diel, seasonal, or ontogenetic scales, and therefore may experience several different environments throughout their lifetime (e.g., Polovina et al. 2004; Southwood 2008). Carry-over effects (COEs), in which an event or process that occurs over a given life history stage can affect an individual's future performance, often result from differences in habitat quality and resource availability (Harrison et al. 2011). Therefore, COEs indicate how past habitat utilization may influence lifetime growth, survival, or reproduction of individuals, and ultimately fitness of an individual or species (Searcy and Sponaugle 2001; Norris 2005). For example, copepod-rich diets available only in certain pre-adult habitats have been linked to earlier breeding and larger eggs within Pacific seabird populations (Sorensen et al. 2009). Because COEs are present within individuals and can manifest over protracted timescales (i.e., years), they are difficult to monitor within populations without detailed, paired information on both movements and vital-rate dynamics across many individuals (Harrison et al. 2011).

Nurseries are spatially distinct habitats used by organisms during their juvenile life stages. Given the importance of juvenile habitats in the population ecology of mobile fauna

(e.g., Fodrie and Levin 2008), recent syntheses have attempted to codify robust, generalizable definitions of nurseries. For instance, Beck et al. (2001) defined nurseries as habitats that have a greater, on average, contribution per unit area to the production of individuals that recruit to adult populations than production from other habitats in which juveniles occur. Alternatively, the "effective juvenile habitats" designation highlights the role of expansive habitats that may support lower per-unit-area contribution to adult populations, but may yet be essential for sustaining adult populations (Dahlgren et al. 2006).

Inherently, these contrasting definitions capture multiple reasons why nursery habitats are a model environment to examine COEs and their impacts on population dynamics. First, resource availability can vary extensively among putative nursery habitats, exposing organisms to different environmental conditions during their early life history (Anders et al. 1998). Second, organisms may be particularly sensitive to environmental perturbations during early life-history stages with known sublethal effects on growth and rigor among diverse taxa such as insects (Taylor et al. 1998), amphibians (Pahkala et al. 2001) and fish (Shima and Swearer 2010). If multiple nursery habitats are available, highly productive nurseries may produce individuals characterized by advantageous COEs, such as increased growth rates, which over time may translate into lagged effects on overall fitness resulting from differential mortality or fecundity.

Many marine organisms occupy multiple putative nursery habitats which are spatially distinct from adult habitats (Gillanders et al. 2003). Historically, most fishery species were thought to rely on inshore (e.g., estuarine) habitats for at least some portion of their life history (e.g, juvenile nursery grounds; Günter 1967; Stroud 1971). However, recent syntheses have highlighted that estuarine nursery utilization can be facultative, rather than

obligate, and that some fish may never access inshore habitats and, instead, exclusively utilize offshore (e.g., open coast) habitats (Able et al. 2005; Nagelkerken et al. 2015). As it is likely several species utilize both inshore and offshore nursery habitats, recent research has focused on species-specific studies to quantify nursery contribution to the adult population (Able and Fodrie 2015). The spatial separation and environmental differences between estuarine and offshore nursery habitats provide a valuable lens to assess whether COEs can arise across diverse juvenile habitats followed by ontogenetic migration to a common adult stock, as well as how COEs may influence overall population dynamics.

Here, we use geochemical signatures in fish otoliths to retrospectively determine proportional contributions of two putative nursery habitats, estuaries and offshore, to the adult stock of black sea bass (*Centropristis striata*), which serves as a major fishery along the mid-Atlantic Coast. We also compared juvenile and sub-adult growth rates and percent-male-at-age for subpopulations of fish utilizing either estuarine or open-coast nursery alternatives (determined via otolith-based geochemical tags) to evaluate whether COEs were associated with juvenile habitat use. A requisite for evaluating nursery contributions was building a multi-year library of juvenile otolith geochemical signatures from fish collected in estuarine and open-coast environments.

Materials and methods

Study species

Black sea bass is an economically and ecologically dominant marine fish found along the entire eastern United States coastline as well as into the Gulf of Mexico. Black sea bass are protogynous hermaphrodites, maturing first as female (~2 years) and then as male (~4 years; SEDAR 2011). Adults typically live offshore in waters $\leq$ 100m depth, and spawn

pelagic eggs throughout the spring and summer (Able et al. 1995). It was generally presumed that recently settled juveniles ubiquitously ingress into estuaries in spring and remain in their nurseries until egressing offshore in the fall of that year (September-November; Able et al. 1995; Steimle et al. 1999). However, recent government surveys and anecdotal accounts from fishermen indicate that some juveniles do not move into the estuary, and instead reside in offshore areas throughout the juvenile stage. For instance, 1,890 juveniles (<180mm TL) were captured across 454 offshore trap sets during the 2014 Southeastern Fishery-Independent Survey (SEFIS, C. Schobernd, NMFS, personal communication).

Study area and sample collections

The coastlines of North and South Carolina, from Cape Hatteras, NC to Georgetown, SC, are punctuated by multiple inlets and protected by a network of barrier islands extending from 35.255°N, 72.520°W to 32.204°N, 79.150°W. The continental shelf extends offshore for 45-160 km, reaching a depth of 100 m. To construct a library of juvenile geochemical signatures from putative estuarine and offshore nursery habitats, juvenile black sea bass (<180 mm TL) were collected during summer (May-August) of 2009-2014. Fish were sampled from multiple years to evaluate potential interannual variability (sensu Carson et al. 2013).

To create an atlas of signatures representative of estuarine juvenile habitats along the North and South Carolina coastlines, juveniles were collected from the following estuaries: Bogue Sound (34.724°N, 76.756°W), Radio Island (34.710°N, 76.680°W), Banks Channel (34.208°N, 77.797°W), and Towne Creek (33.336°N 79.186°W). Estuaries were chosen based on prominence, accessibility, and environmental diversity (e.g., temperature, salinity, riverine inputs). While we recognize that not every estuarine environment are represented by

these sites, previous studies have demonstrated that our sampling regime should be sufficient to develop a general estuarine signature that is distinct from that of offshore nurseries (Brown 2006; Fodrie and Herzka 2008). Juveniles from 2009-2012 were collected from Bogue Sound and Radio Island, whereas 2013-2014 juveniles were collected from all four sites. Offshore juveniles from 2009-2012 were collected as part of a previous sampling effort from two cement artificial reefs constructed and managed by the NC Division of Marine Fisheries: AR-315 (34.6722°N, 76.7445°W) and AR-320 (34.6589°N, 76.807°W). Offshore juveniles from 2013-2014 were collected from the continental shelf-break (5-100 km offshore, 20-100 m depth**)** during the NOAA Pisces cruise in July 2014 (Fig. 3.1).

A total of 239 juveniles were collected: 141 from inshore, estuarine habitats and 98 from offshore habitats (Table 3.1). Juveniles from 2009-2012 were sampled as part of NOAA survey programs and collected by hook and line or wire mesh fish traps (1 m x 0.4 m x 0.4 m). Specimens from 2013-2014 were collected by hook and line or chevron traps (1.7 m X 1.5 m X 0.6 m). Upon collection, fish were measured (SL and TL), weighed, and frozen until otolith extraction. The majority of 2009-2012 offshore juveniles were collected only ~3-4 km seaward of estuarine habitats (i.e., AR-315 and AR-320), however, these signatures were consistent with signatures from 2013-2014 when most fish were collected 50-100 km offshore (Fig. 3.2a). Black sea bass that used these offshore juvenile habitats appear to have geochemical signatures that are more distinct from signatures in the otoliths of fish that utilize estuarine habitats than the signatures observed at AR-315 and AR-320. Therefore, we are confident our sampling regime is robust against misclassification based on proximity of estuarine and AR collection sites.

Three-hundred and fifty adult black sea bass were collected offshore during the NOAA Pisces research cruise as part of the SEFIS program in July 2014. Collections were made during daylight hours using chevron traps (1.7 m X 1.5 m X 0.6 m) from 68 sites along the NC and SC coasts (Fig. 3.1). At each site, six traps fished for 90 minutes on soft (e.g. sand) or hard bottom (e.g. rock ledges, gorgonian reefs) habitats (site dependent). Captured adult black sea bass were weighed, measured (SL and TL), and gonads were examined to determine stage of sexual maturity (female, transitioning, or male; following Wuenschel et al. 2011). Fish were then frozen until otolith extraction.

Otolith analysis

Fish otoliths, or ear bones, contain geochemical records that reflect environmental signatures representative of putative nursery habitats (Thorrold et al. 1998). Sagittal otoliths were dissected using sterile scalpels and ceramic forceps and rinsed in ultrapure H_2O (Barnstead Nanopure; Thermo Scientific). Otoliths were wiped with sterile kimwipes (Kimberly-Clark) to remove any organic (tissue) material and placed in plastic microcentrifuge tubes. Samples were sonicated in 15% H_2O_2 solution buffered in 0.05 N ultrapure NaOH for five minutes followed by a five-minute sonication in a 1% ultrapure HNO_3^- (OPTIMA grade; Fisher Scientific). Otoliths were then rinsed three times with ultrapure H_20 and left under a class-100 laminar flow hood overnight to dry.

Entire otoliths were encased in EpoThin® epoxy (Buehler), sectioned along the transverse plane using a Hillquist thin section machine, polished and mounted following Fodrie and Levin (2008). Mounted otolith sections were then cross sectioned using the saw to a width of 250 µm. Cross sections were again polished and rinsed using the aforementioned methods and stored until geochemical analysis. Both juvenile and adult specimens were

analyzed using a Thermo-Fisher Element2 inductively coupled plasma mass spectrometer with a Teledyne ATLex 300si-x 193nm Excimer laser ablation unit (LA ICP-MS). Otoliths were analyzed for the following elements: ^{7}Li, ^{26}Mg, ^{48}Ca, ^{55}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba, and ^{208}Pb. These elements were chosen because of their previous use in uptake and tagging studies of fish otoliths (Bath Martin and Thorrold 2005; Fodrie and Levin 2008).

To attain geochemical signatures representative of estuaries versus offshore, the outermost portions of otoliths from juveniles were ablated. One 150-µm line transect was vertically ablated on each margin (ventral and dorsal) of the otolith. The distance from the core of the otolith to both ablation transects was measured for each juvenile sample. The average distance from core to transect was 1520 µm and 1420 µm for dorsal and ventral sides, respectively. Adult otoliths were then ablated with one 150-µm line transect positioned 1520 µm dorsally from the core and one 150-µm line transect positioned 1420 µm ventrally from the core. All laser transects used a fluence of 4.45 J/cm^2 with a 40-µm spot size, ablated at 10 µ/s. To correct for mass bias and instrument drift, National Institute of Technology Standards-certified standards (Reference Material 612, 614, and 616) were run at the beginning and end of every 24-otolith sequence (~48 burns). Isotope intensities for each burn were converted into elemental ratios (X:Ca) following Fodrie and Levin (2008).

Age and growth

Prior to laser ablations, adult otolith sections were illuminated with reflected light and images were captured with a binocular dissecting scope at 45x magnification with an attached digital microscope camera (DP72; Olympus). A micrometer microscope slide was included in the field view within each image for calibration. Growth bands were measured (µm) using ImageJ software (National Institutes of Health), from the core along the ventral

edge of the sulcus. Age and growth measures were made following the protocol set forth during the most recent ageing workshop for black sea bass (SC Department of Natural Resources 2009). Each otolith was read two times, with overall band size determined by the average of both reads. Total age was calculated by summing the number of growth bands and assuming a January 1 spawn date.

Data analyses

Elemental ratios recorded from juvenile otoliths were Box-Cox transformed to meet assumptions of normality and then analyzed using linear Discriminant Function Analysis (DFA) to create signatures for estuarine versus offshore nurseries. We performed separate DFAs for each year-class. DFAs were conducted stepwise, with the least significant elemental ratio dropped, as determined by the *F*-to-remove statistic, until all F-to-remove values were >2. The final model contained Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca and Pb:Ca, for all years.

Jack-knifed classification success for assigning juveniles to their correct collection location (estuarine versus offshore) was determined for individual collection years before using juvenile signatures to predict nursery habitat for adults. Only 2010 and 2013 had sufficient intra-annual juvenile representation from both habitats for stand-alone DFA. Thus, adults born in 2009, 2011, and 2012 years were pooled and compared to pooled juvenile signatures generated by DFA across those years combined (2009+2011+2012). Notably, 2009, 2011, and 2012 were similar based on precipitation and salinity during the sampling period (summer; ncdc.noaa.gov/cdo-web). Because not every site was sampled every year, classification success was additionally determined for juveniles collected during all years combined. We also used a series of regression trees to compare how orthogonal statistical

approaches predicted the nursery origin of adults that had successfully recruited to the adult stock (Cappo et al. 2005; Mercier et al. 2011).

Juveniles collected from each year were paired with adults born in the same year (e.g., juveniles collected in 2009 and 5-year-old adults collected in 2014) to increase DFA accuracy. The multi-annual atlas (all-years pooled) was additionally used to predict collection location for all sampled adults. Juvenile fish collected in 2014 were used to highlight elemental differences between offshore and estuaries, however, they were excluded from this analysis because no collected adults were born this year (all specimens < 1 year). Because our juvenile samples were only collected 2009-2014 and adult collections occurred in 2014, any adults six years of age and older were excluded from our analysis (i.e., 73 of 350 were excluded in assessment of nursery contribution and COEs).

To explore potential COEs resulting from juvenile habitat use, year one through five growth was compared using serial student's t-tests for each age, between adults from estuarine versus offshore nurseries. Difference in %-male-at-age, between nursery habitat origins, was analyzed using both a likelihood ratio Chi-squared test and binomial logistic regression. After ensuring all assumptions were met regarding normality and homoscedasticity, an Analysis of Variance was used to examine potential effects of age, year, sex, and nursery habitat on log-transformed growth.

Results

Juvenile habitat utilization

Differences in juvenile otolith geochemistry were sufficient to discriminate between estuarine and offshore nursery habitats when including all years in DFA, with a classification success of 75.3% (over a null expected success of 62%). Regression tree analysis provided

very similar results, with a classification success of 75.8%. Because of the comparable results between statistical approaches in this and all subsequent analyses, we only present DFA results hereafter.

Classification success increased when separating juveniles by year. For 2010, 2013, and 2014, classification successes were 93.3%, 90.9%, and 100%, respectively, and 75.5% for 2009+2011+2012 combined (Table 3.1; Fig. 3.2). Mn and Ba were the most influential elements contributing to discrimination among nursery sites for 2010; Sr and Mn for 2013; and Mg and Ba for 2014, as well as for 2009+2011+2012. For all years, combined, otoliths from juveniles collected offshore contained higher concentrations of Ba and Pb than inshore samples (Ba: $t=2.47$, $p=0.014$, $df=140$; Pb: $t=2.93$, $p=0.004$, $df=140$; Fig 2b), while Mg and Li were generally higher in the otoliths of fish collected in estuaries (Li: $t=-0.367$, $p=0.712$, $df=140$; Mg: $t=-0.240$, $p=0.810$, $df=140$; Fig. 3.2b).

When using all sampled juveniles (2014 included), predicted overall nursery habitat contribution to the adult spawning stock was 91% and 9% for estuarine and offshore nurseries, respectively. However, we saw considerable variability in nursery habitat contribution when assignments were based on year-class. For adults born in 2010, 79% (87 of 110) utilized estuarine nurseries, whereas 21% (23 of 110) used offshore nurseries. In 2013, 100% (18 of 18) adults were linked to estuarine nurseries and none (0 of 18) were linked to offshore nurseries (Fig. 3.3). For 2009+2011+2012, combined, habitat utilization was 93% (139 of 149) and 6.7% (10 of 149) for estuarine and offshore nurseries, respectively. Collective nursery habitat contribution for 2009-2013 was 89% and 11% for estuarine and offshore fish, respectively.

Growth and sexual maturity

We found no statistically significant differences in growth based on otolith increment analyses for years 1-5 between adults that had utilized alternate juvenile habitats, even when separating fish by year class ($0.164<p<0.621$). We did note was a non-statistically significant trend of higher growth among individuals that utilized estuarine habitats: estuarine juveniles showed a >5% greater mean growth than their offshore counterparts during the first year of life ($t=-0.994$, $p=0.164$, $df=34$; Fig. 3.4a). Further, there were no statistically significant differences in SL, TL, or weight within each age-class, or overall, for fish associated with either juvenile habitat (Fig. 3.4b).

The ratio of male-to-female adults, aged 2-5, was significantly higher for fish linked to estuarine nurseries ($\chi^2=7.19$, $p=0.027$, $df=2$; Table 3.2; Fig. 3.5). For adults associated with estuarine nursery habitats, adults were 57% female, 31% male, and 12% transitioning. For offshore-nursery associated adults, sampled adults were 74% female, 10% male, and 16% transitioning. Male-to-female ratios between these two subpopulations of black sea bass were not driven by differences in age structure: mean fish age did not differ by the habitat used by juveniles ($t=3.01$, $p=0.102$, $df=53$). The age at 50% transition from female to male for black sea bass utilizing inshore nurseries was also significantly younger, by $\geq$6 months, than that of fish utilizing offshore habitats (based on 95% confidence intervals; Fig. 3.5). Both subpopulations experienced an older age at 50% transition, 4.6 yrs and 5.2 yrs for estuarine- and offshore-associated adults, respectively, than previously reported (3.8 yrs; SEDAR 2011).

Discussion

Many terrestrial and aquatic organisms have complex life histories in which they utilize multiple habitats throughout various life stages (e.g., ontogenetic migrations). While sexual maturation and transition has been linked to habitat-specific resource availability (Bercovitch and Strum 1993; Grether et al. 2001), black sea bass exhibit carry-over effects from nursery habitats that ultimately affect the timing of sexual transition and composition of an adult population. Specifically, black sea bass that utilized estuarine nurseries expressed shorter times to final sexual maturation (female to male). This outcome has several implications for how we conceptualize the role of nursery habitats and the connection between juvenile and adult life history.

Nursery habitat contributions

Productive nursery habitats typically offer a wide range of food resources and refuge (Heck et al. 2003; Stoner 2003) and, as a result, juveniles are thought to primarily utilize these nursery habitats. For example, in marine communities, seagrass beds and oyster reefs within estuaries are frequently described as essential nursery habitats (Beck et al. 2001; Dahlgren et al. 2006). Deegan (1993) further emphasizes their importance, stating that "...fish faunas around the world are dominated in numbers and abundance by species which move into the estuary as larvae, accumulate biomass, and then move offshore." Accordingly, we found that nearly 90% of our sampled adult black sea bass were linked to estuarine nursery habitats, indicating that estuaries encompass the majority, if not all, of the essential nursery habitat (Beck et al. 2001) and effective juvenile habitats (Dahlgren et al. 2006) for black sea bass. However, a 10% contribution from offshore nurseries is ecologically significant and reinforces what Able (2005) and others (e.g., Nagelkeren et al. 2015) have proposed in recent

years regarding the importance and viability of offshore nursery habitats. Similar to Able (2005), we found inter-annual variability in nursery habitat usage, indicating environmental or cohort-specific conditions may influence nursery utilization and production (see below). We encourage future studies to incorporate offshore nursery production when assessing the black sea bass stock and further examine potential sources of variability in nursery habitat utilization (e.g., geographic).

Carry-over effects from nursery habitats

Many studies have noted relationships between nursery habitat availability and juvenile abundance (Meyer et al. 1998; Fodrie and Levin 2008; Rosenfeld et al. 2011; Zobel et al. 2011), yet few are able to provide mechanisms, such as COEs, by which nursery habitats can influence population size and structure. Increased growth and therefore higher survival rates within more productive nursery habitats may result in greater nursery contribution to the adult stock (Beck et al. 2001). While we did not find significant evidence of larger first-year growth in juveniles utilizing estuarine habitats, this is not uncommon: growth differences among fish utilizing more vegetated and/or less disturbed nursery habitats are not routinely observed (Heck et al. 2003; Amara et al. 2007). Therefore, production differences between nursery habitats are more likely related to variability in survivorship than growth.

As we cannot explicitly link differences in growth between black sea bass utilizing estuarine and offshore nursery habitats to differences in timing of female-to-male transition, we instead consider additional drivers with demonstrated potential for influencing sexual transition timing. There is strong evidence that fish experience thermosensitive periods in which 1-2°C temperature shifts during development can bias sex ratios, with warmer

temperatures (e.g., during summer months in estuarine nurseries) consistently resulting in a greater ratio of males (Conover and Kynard 1981; Ospina-Álvarez and Piferer 2008). Though this has never been applied to developmental periods of post-larval organisms (i.e., juveniles), it is possible that warmer temperatures within estuarine nurseries permanently alter levels of sex-hormones within hermaphroditic juvenile fish (Devlin and Nagahama 2002).

Sex determination in fish can also result from the presence of exogenous sex steroids produced by maturing females and adults just prior to a spawning event (Devlin and Nagahama 2002). Specific sex steroids, such as estradiol, have been shown to skew sex ratios from male to female-dominated in adult hermaphroditic fish by increasing the time spent as a female (Ruan et al. 1996) or causing a sex reversal to female after the fish has already transitioned (Chang et al. 1997). Juveniles utilizing offshore nursery habitats typically live in close proximity with maturing female and spawning populations which may increase their exposure to exogenous sex steroids and extend the duration of the female state before transitioning.

Nearly all adult black sea bass live in offshore habitats and therefore estuarine-associated juveniles must undergo an energetically taxing migration into and out of the estuary. In some areas, this distance can measure in the 10s of kilometers (Able et al. 2005) and includes inclement current and circulation conditions. Outward migration occurs in late summer to early fall, and is quickly followed by cooling water temperatures and the less energetically-favorable conditions of winter (Garvey et al. 2004). As adult males typically exert less energy than females in both gamete creation and fertilization (Wootton 1985), it is

possible that energetic requirements associated with migration into and out of the estuary also stimulate earlier transition to reach a more energetically-favorable sex.

Although all larvae were assigned a January 1 spawn date, spawn dates likely range from December to early April (Hood et al. 1994) and may contribute to observed differences in time of sexual transition. Gulf Stream processes primarily control across-shelf transport in the south Atlantic, however, environmental conditions, such as sea surface temperature and seasonal wind-forcing, also affect larval distribution along the coast (Hare et al.1999; Stegmann et al. 1999). Northeast winds, which prevail in early winter, encourage the movement of larvae from offshore spawning grounds to inshore (estuarine) nurseries. However, northwest winds, which are more common in later winter, can cause off-shelf advection of larvae, which may result in increased offshore nursery utilization (Hare et al. 1999). Northeast winds in the first half of the spawning season followed by northwest winds in the later half may result in older fish utilizing estuarine nurseries while younger fish recruit to offshore nurseries. Thus, the observed earlier transition time from female-to-male in estuarine-associated fish may be an artifact of the presumed January 1 spawn date for all fish. Although spawn dates can be more accurately estimated with daily growth rings present in juvenile otoliths (Panella 1971; Campana and Neilson 1985), the readability of daily growth rings significantly decreases in post-juvenile fishes (e.g., adult black sea bass) . Further research is needed to examine whether this pattern in timing of sexual transition will remain after assessing actual spawn dates for fish utilizing estuarine and offshore nursery habitats. Similarly, future studies may also want to consider other potential may arise as a result from the widely used assumption of a universal spawn date.

Conservation considerations of COEs for exploited species

Commercially and recreationally important species typically experience increased harvest pressure with age. Hermaphroditic species are especially vulnerable to overfishing, as high harvest pressure can affect fecundity through sperm and/or egg limitation or by shifting the age of sexual transition (Alonzo and Mangel 2004; Provost and Jenson 2015). Black sea bass have a long history of overfishing, including a disproportionally high extraction rate of males (SEDAR 2011). The fishery was recently declared rebuilt in 2013, however, relics of overfishing and skewed sex ratios likely still exist within the population in the form of uneven adult sex ratios. Current federal regulation prohibits commercial and recreational fishing of black sea bass < 279 mm and < 330 mm (TL), respectively. However, none of our collected fish were male at < 279 mm and only 17% of fish were male at < 330 mm, indicating that anywhere from 83-100% of males within the spawning stock are vulnerable to harvest. Moreover, as the smallest male we collected was 280 mm TL, current regulation practices may place the stock in danger of sperm-limitation.

Estuarine nurseries not only contribute a much greater percentage of black sea bass to the adult stock than offshore nurseries, but also may buffer against severe sperm limitation by increasing the availability of males. As estuaries face numerous conservation threats, such as human development and climate change, the loss of these nursery habitats may have multiple implications for black sea bass: not only will essential nursery/effective juvenile habitat availability decline but fish will also lose access to the associated COEs which may be essential to maintaining population structure. Additionally, population models used for black sea bass, and other estuarine-associate fish species, conventionally only consider one nursery population. Incorporating estuarine and offshore subpopulations, and their associated

reproductive ecology, may not only increase the accuracy and strength of models, but also explore the effects of estuarine habitat degradation and lead to more effective management of critical species.

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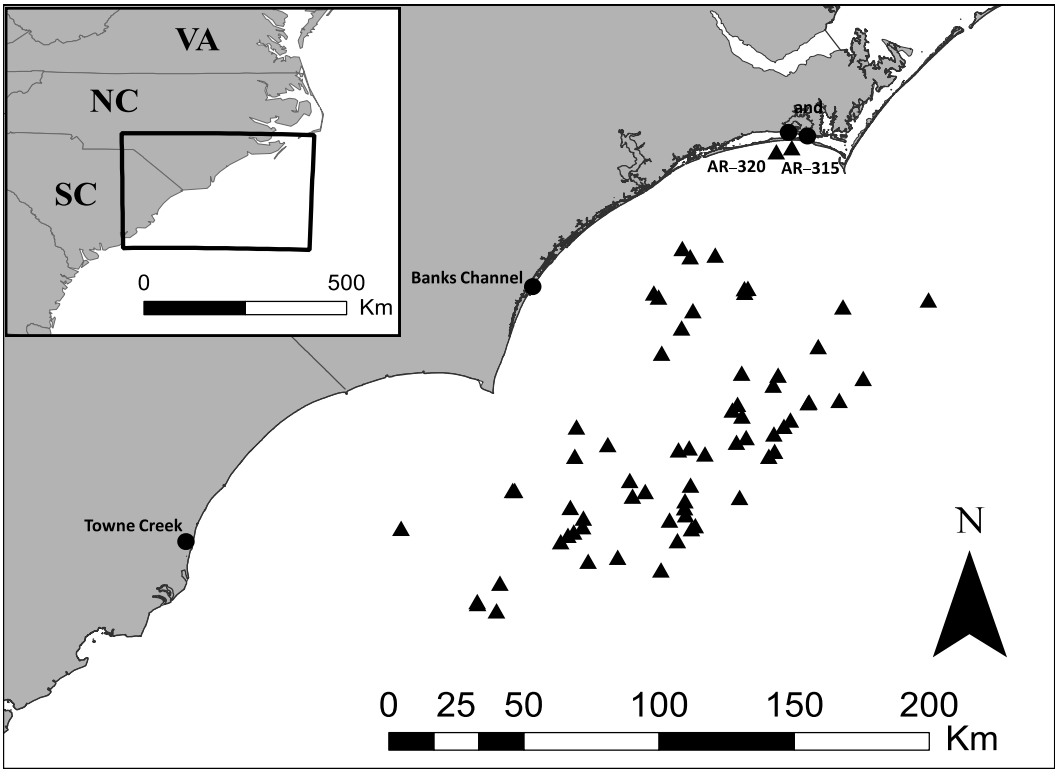

Figure 3.1. Map of southeastern United States displaying collection sites for black sea bass during the July 2014 leg of the South Eastern Fisheries Independent Sampling efforts about the NOAA ship *Pisces*.

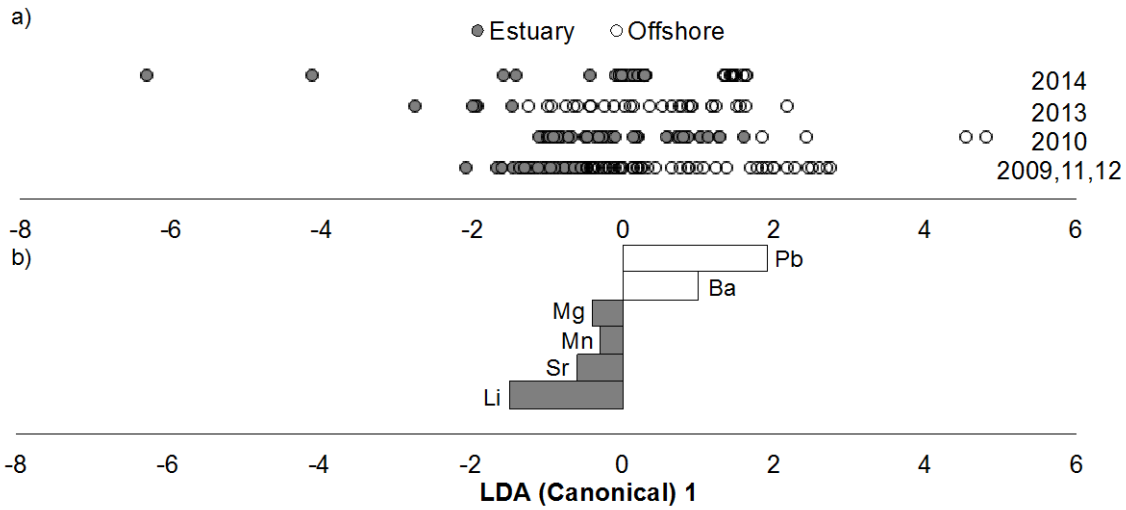

Figure 3.2. Canonical score plot of a) the linear discriminant function analysis for C. striata juveniles grouped by nursery habitat, displayed vertically by year and b) influence of key elements on canonical score.

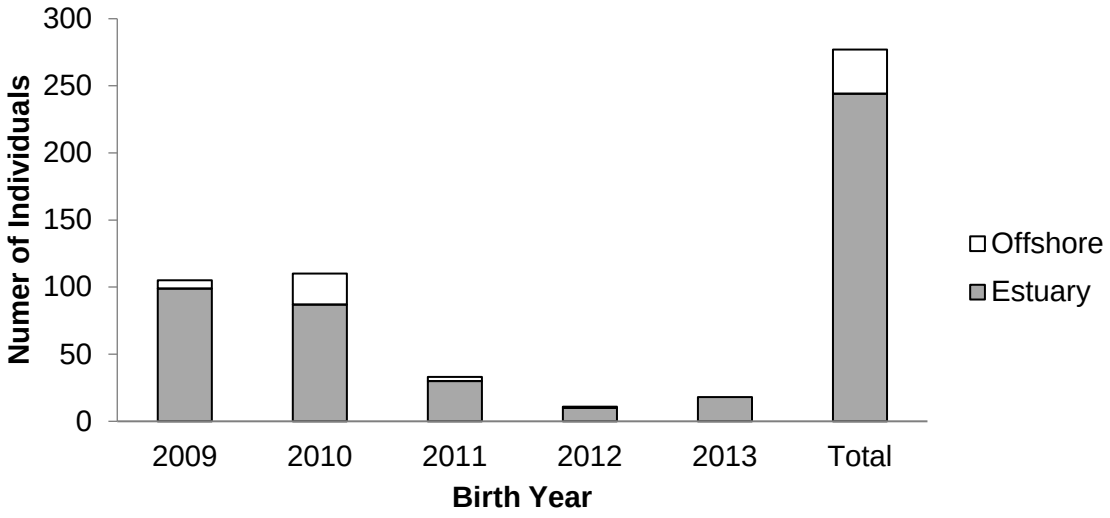

Figure 3.3. Determined contribution of estuarine and offshore nurseries to the adult population of *C. striata*, by year-class.

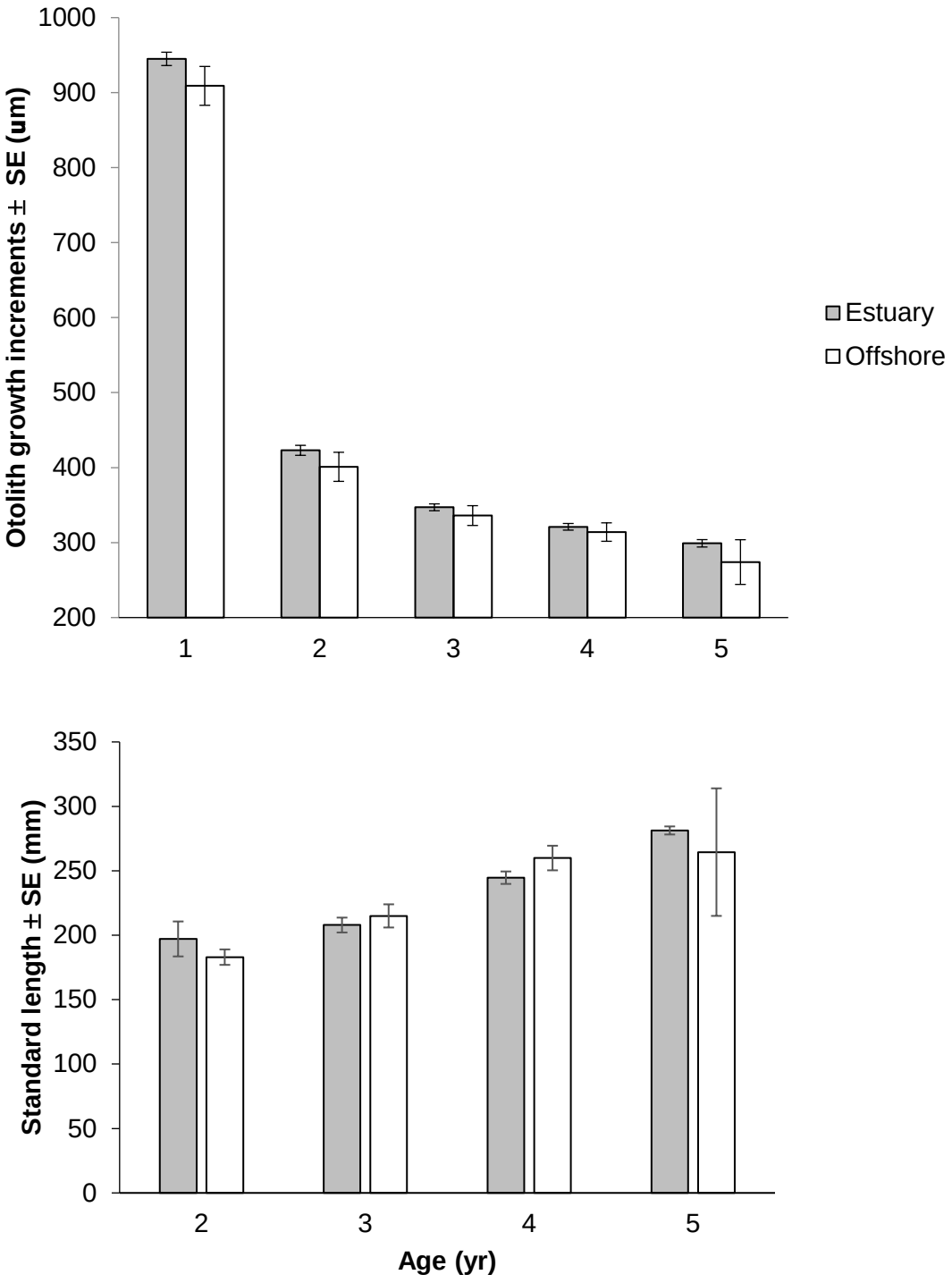

Figure 3.4. (a) Mean growth rates (±SE) of juvenile through adult and (b) mean sizes (SL; ±SE) of adult *C. striata* that utilized estuarine or offshore nursery habitats.

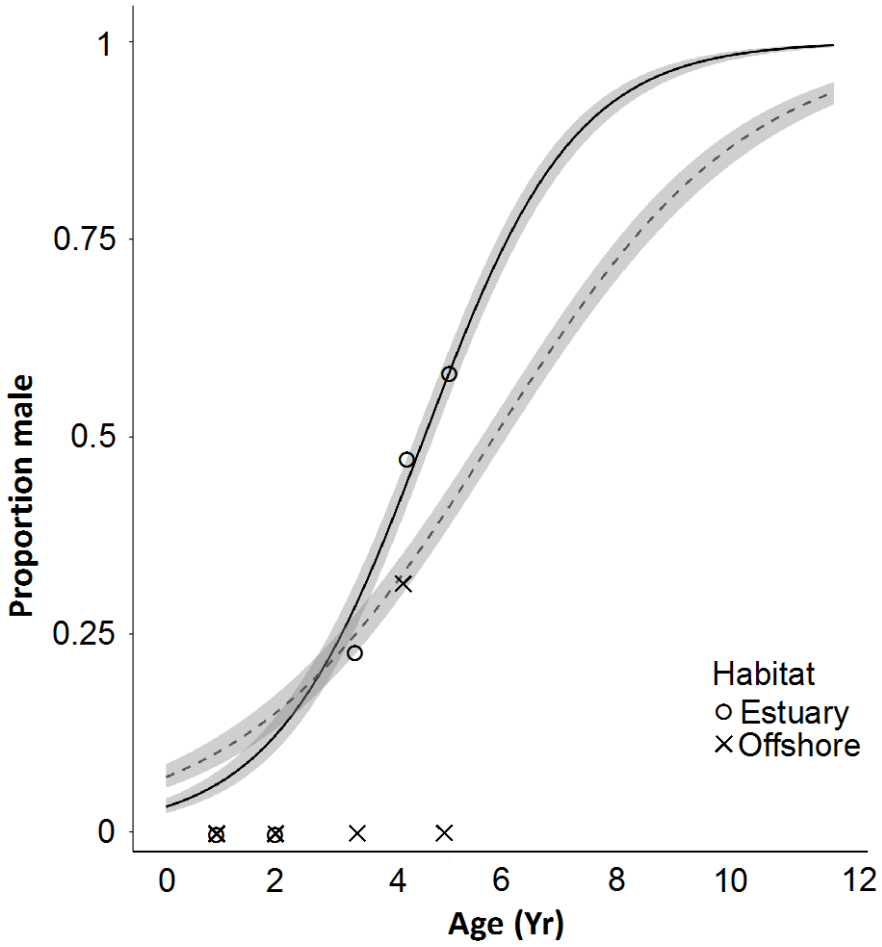

Figure 3.5. Logistic growth projection (±SE) representing predicted percent male for each nursery type fit to observed percent male at age for *C. striata*, by year.

Table 3.1. Number of juveniles collected and classification success, using discriminant function analysis, for *C. striata* from estuarine and offshore nurseries during 2010, 2013, 2014 and 2009+2011+2012 combined.

	2009+11+12	2010	2013	2014
Collected Estuary	31	52	6	52
Percent Correct	74.2%	98.1%	66.7%	100%
Collected Offshore	49	8	27	14
Percent Correct	79.6%	37.5%	96.3%	71.4%

Table 3.2. Number of females, males, and overall percent males for each age class by predicted juvenile habitat.

Age	Habitat	Females	Males	% Male
2	Estuarine	16	0	0
	Offshore	2	0	0
3	Estuarine	26	7	20.5
	Offshore	1	0	0
4	Estuarine	48	35	42.1
	Offshore	19	7	26.9
5	Estuarine	44	68	60.7
	Offshore	2	0	0

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CHAPTER 4: INTEGRATING CARRY-OVER EFFECTS FROM JUVENILE HABITATS INTO POPULATION MATRIX MODELS REVEALS NURSERY CONTROL OF ADULT STOCK STRUCTURE

Introduction

Traditional methods in fishery stock assessment attempt to model commercially important populations with the goal of maximizing long-term harvest yields while sustaining the populations (Shertzer et al. 2008). Models typically incorporate parameters for a population's survivorship, growth, fecundity, and carrying capacity with individuals modeled uniformly: demographic parameters are assumed to apply to all individuals of the same species at the same stage. This practice results in models that are not habitat specific, despite the discovery of several habitat-related bottlenecks that can affect adult stock dynamics (Levin and Stunz 2005; Caddy 2014). In marine environments, most mobile species traverse many habitats over diel, seasonal, or ontogenetic scales and therefore may experience several different environments throughout their lifetime (e.g., Polovina et al. 2004; Southwood 2008). Furthermore, within a population, some individuals utilize different habitats than others during the same life stage, as multiple, alternative habitats are often available (Able et al. 2005). While there is broad qualitative recognition of the importance of specific habitats in supporting fishery production (e.g., nurseries; Beck et al. 2001), the great majority of models currently do not account for the relationship between habitat utilization and fishery population dynamics.

The need to manage marine resources, yet impracticality of conserving all habitats, motivates researchers to provide quantitative links between habitat availability and the vital

rates of organisms (Tupper and Boutillier 1997; Mangel et al. 2006; Fodrie et al. 2009). For example, research has shown that decreasing habitat quality and increasing levels of anthropogenic disturbance can negatively influence growth rates (Amara et al. 2007) while increasing habitat cover, complexity, and configuration can bolster organism survival (Hovel 2003). Differences in predation and survival among individuals utilizing alternative habitats may also increase with the amount of time spent in a specific habitat (Irlandi et al. 1995). As the majority of population bottlenecks are thought to result from critical periods experienced in earlier life stages (Limburg 2001), it is particularly important to understand how habitat utilization during this time can affect vital rates to provide clear, quantitate links between habitat and overall population dynamics and structure.

Nurseries, used by organisms during their juvenile life stages, are spatially distinct from habitats used during other life stages and have a widely-accepted importance in the population ecology of mobile fauna (e.g., Beck et al. 2001; Dahlgren et al. 2006; Fodrie and Levin 2008). Nursery habitats may also provide a unique opportunity to examine how habitat effects can scale to the population level through the presence of carry-over effects (COEs). Carry-over effects, in which an event or process that occurs over a given life history stage can affect an individual's future performance, often result from differences in habitat quality and resource availability found early in life (Harrison et al. 2011). Therefore, COEs indicate how past habitat utilization may influence lifetime growth, survival, or reproduction of individuals, and ultimately fitness of an individual or species (Searcy and Sponaugle 2001; Norris 2005). Black sea bass, a hermaphroditic member of the economically and ecologically important snapper-grouper complex, exhibit COEs related to nursery habitat: fish that utilize estuarine nurseries transition from female to male ~6 months earlier than fish that utilize

offshore nurseries (Kroll et al. 2017). This difference in female stage duration not only represents a tangible link between juvenile habitat and adult population structure but also provides the foundations for a population model that explicitly incorporates habitat into vital rates and life-history.

Here, we use stage-based demographic models (Lefkovitch 1965) to "scale up" previous findings and further explore how juvenile habitat utilization, through the presence of COEs, can affect the population dynamics of black sea bass. More specifically, we used the difference in female-to-male sexual transition times associated with estuarine versus offshore nursery habitats to: (1) construct models with habitat-specific vital rates for fish that utilize alternative nursery habitats (i.e., estuarine or offshore); (2) determine which vital rates (growth, survivorship, or fertility) population stability (λ) is most sensitive to; (3) simulate how shifts in nursery habitat utilization can impact population structure and reproduction; and (4) evaluate how a change in sexual transition time, resulting from juvenile habitat utilization, can affect fertility and ultimately population growth for black sea bass.

Methods

Study species

Black sea bass (*Centropristis striata*) is an economically and ecologically important marine fish found along the entire eastern United States coastline as well as into the Gulf of Mexico. Black sea bass are protogynous hermaphrodites, maturing first as female (~2 yrs) and then as male (~4 yrs; SEDAR 2011). Adults typically live offshore in waters $\leq$ 100m depth, and spawn pelagic eggs throughout the spring and summer (Able and Fahay 1998). It was generally presumed that recently settled juveniles ubiquitously ingress into estuaries until egressing offshore in the fall (September-November; Able and Fahay 1998; Steimle et

al. 1999). However, recent government surveys and scientific studies indicate that a significant portion of juveniles (~10-20%) do not move into the estuary, and instead utilize offshore nurseries throughout the juvenile stage and ultimately recruit to the adult stock (Kroll et al. 2017). Furthermore, Kroll et al. (2017) demonstrated that though there are no apparent demographic differences among fish while they are occupying either estuarine or offshore juvenile habitats, there is evidence that black sea bass utilizing estuarine nursery habitats can transition from female to male roughly six months earlier than fish which utilize offshore nursery habitats (4.6 versus 5.2 yr for estuarine and offshore fish, respectively).

Model construction and parameterization

Stage-based population projection matrices are useful models for populations with spatially-dynamic demographics that may allow us to account for distinct subpopulations based on variability in juvenile habitat utilization (Caswell 2001). Lefkovich matrix models expand upon traditional age-based Leslie models, using discrete ontogenetic stages to examine the population-level consequences of alternative life histories during specific life stages (Fodrie et al. 2009). Matrix population models also lend themselves to sensitivity, elasticity, and life table response experiments, in which potential variations in population growth can be related to nursery-associated vital rates (Caswell 2000).

We developed two classes of stage-based population models (following Fodrie et al. 2009) to explore how the use of estuarine versus offshore nursery habitats effect population growth, sex-ratio, fertility, and overall fitness of black sea bass stocks. We elected for size, rather than age, based models as the progression of the black sea bass life cycle is more closely tied to body size than age (Wenner et al. 1986). We defined four different stages based on the following size classes: larvae (L); juveniles (J); mature females (F); mature

males (M) (Fig. 4.1). Size limits for juveniles (15-170 mm) were calculated using percent-mature-at-age data from SEDAR (2011) and von Bertalanffy (VB) growth curves populated with black sea bass data collected as part of a government-sponsored Southeastern Independent Fisheries Survey program (Kroll et al. 2017). Size limits for stages F (170-300 mm and 170-350 mm for estuarine and offshore-associated models, respectively) and M (300+ mm and 350+ mm for estuarine and offshore-associated models, respectively) were also calculated using VB growth curves paired with percent-male-at-age data from Kroll et al. (2017) (Table 4.1).

Models were analyzed using a one-month time step to match the approximate duration of the larval stage and allow temporal resolution to capture differences in timing of sexual transition between fish utilizing estuarine versus offshore nursery habitats. Using entries specific to each juvenile habitat, the two matrices were constructed to display the change in population structure from time t to $t + 1$:

$$\begin{bmatrix} L_{t+1} \\ J_{t+1} \\ F_{t+1} \\ M_{t+1} \end{bmatrix} = \begin{bmatrix} P_L & 0 & F_F & 0 \\ G_L & P_J & 0 & 0 \\ 0 & G_J & P_F & 0 \\ 0 & 0 & G_F & P_M \end{bmatrix} \times \begin{bmatrix} L_t \\ J_t \\ F_t \\ M_t \end{bmatrix}$$

where G_i (stage growth) is the probability of surviving and advancing form stage I to stage i+1, P_i (stage survivorship) is the probability of surviving and remaining in the same stage, and F_F (fertility) is the reproductive contribution adult females make towards stage L. Both P_i and G_i were calculated from survival (p_i) and growth (γ_i) probabilities (Caswell 2011):

$$P_i = p_i(1-\gamma_i) \quad (1)$$

$$\text{and } G_i = p_i\gamma_I \quad (2)$$

$$\text{where } p_i = e^{(-z_m)} \quad (3)$$

$$\text{and } \gamma_i = [(1-p_i)p_i^{(d_i-1)}]/(1- p_i^{(d_i)}) \quad (4)$$

where z_m is the monthly mortality rate and d_i is the duration of the ith stage (Crouse et al. 1987).

To calculate stage durations (d_i), we again used standard length and otolith band size data from Kroll et al. (2017) to parameterize the following relationship between standard length (SL) and otolith radius:

$$\ln(SL)=0.073+0.0886 \ln(\text{otolith radius}) \quad (5)$$

Otolith growth could then be used to calculate average growth (SL) rate following Hood et al. (1994) and stage-duration for each size class.

Instantaneous natural mortality rates (M) for all stages were derived following Charnov et al. (2012), with the updated $\text{Hoening}_{\text{nls}}$ estimator recommended by Then et al. (2015):

$$M_{est}=4.89 t_{max}^{-0.916} \quad (6)$$

where t_{max} is the maximum age, 12 yrs (SEDAR 2011). Both upper and lower mortality bounds were used in our simulations, with the final models incorporating the values that were best tuned for $\lambda \sim 1$ (a stable population). Fishing mortality (F) was assumed to be zero for stage L and stage J, as federal and state mandates prohibit fishing within those size limits. A fixed F, scaled for our monthly time steps (Z=0.11; SEDAR 2011), was added to M estimates for stages F and M to obtain monthly mortality (z_m).

Average individual fertility (F_F) in the female stage was calculated as:

$$F_F = v[(1+P_F)f] \quad (7)$$

where f is average monthly fecundity of adults, calculated based on the spawning stock biomass (SSB) per individuals reported in SEDAR (2011), P_F is calculated in eqn. 1, and v is egg viability derived by Watanabe et al. (2003).

To simulate the consequences of varying estuarine versus offshore juvenile habitat contribution, we created a combined model, using an integrated female duration (d_F) that reflected the relative degree to which either juvenile habitat was used (i.e., from all juveniles residing in estuaries to all juveniles residing offshore). Additionally, as protogynous fishes are especially vulnerable to sperm limitation (Heppell et al. 2006), we were interested in simulating how the decreased fertility associated with increased offshore nursery habitat utilization and therefore a shorter male, and longer female, phase (due to later female-to-male transition) would additionally stress populations. Because sperm limitation has been shown to reduce fertility by as much as 30% (Alonzo and Mangel), we ran simulations at 1.00, 0.85, and 0.70 F_F for both upper and lower limits of z_F. All simulations were projected over a period of 30 yrs (2.5 generations) using an initial population vector drawn from SEDAR 25 (2011).

Perturbation analyses

Two prominent forms of perturbation analysis are commonly used to examine the effects of individual vital rates on overall population structure: prospective and retrospective analysis (Caswell 2000). Here, we employ prospective analysis (sensitivity and elasticity metrics) to examine how population stability (λ) changes in response to specific changes in one or more vital rate for each matrix model. Sensitivity and elasticity were calculated as follows:

$$S_{ij}=(v_i w_j)/<w,v> \quad (8)$$

$$E_{ij}=(a_{ij}/\lambda)cS_{ij} \quad (9)$$

where w and v are the right and left eigenvectors associated with the dominant eigenvalue, w_j and v_i are the *j*th and *i*th elements of the first right and eigenvector, respectively, <w,v> is the

scalar product of those vectors and a_{ij} are the individual matrix entries (Caswell 2000). Sensitivity and elasticity analysis was run for all models, including experimental matrices with varying z_x, d_F, and F_F.

Retrospective analysis (life table response experiments [LTREs]) was additionally used to examine how observed variation of each vital rate is expressed in the overall variation of λ, or how each vital rate individually contributes to the overall population growth rate. Contributions (C_{ij}) were calculated for each vital rate as follows:

$$C_{ij} = (a_{ij}^{(k)} - a_{ij}^{(.)}) \times S_{ij} | (M^{(K)} + M^{(.)})/2 \quad (10)$$

where $a_{ij}^{(k)}$ is the value of matrix entry a_{ij} in the kth matrix and $a_{ij}^{(.)}$ is the average value of matrix entry a_{ij} from all matrices. S_{ij} is the sensitivity of λ to matrix entry a_{ij} evaluated using the average of the kth and overall average matrices (M) (Caswell 2000). Sensitivity, elasticity, and LTERs were also run for the estuarine and offshore models at reduced (85% and 70%) fertilities.

Results

Simulations, using the upper (z_L=0.99, z_J=0.49, z_F=0.26, and z_M=0.20) and lower (z_L=0.96, z_J=0.47, z_F=0.20, and z_M=0.18) mortality estimates, resulted in population growth (λ mo^{-1}) that ranged from 0.96-1.04, with an average of 0.99 ± 0.02 for both estuarine and offshore nursery associated models. All simulations that resulted in λ<1 involved z_J=0.49, while all simulations where λ>1, used z_J=0.47. Populations with no net growth/decline (λ=1.00) were tuned for both matrix classes with the following vital rates: z_L=0.99, z_J=0.47, z_F=0.26, and z_M=0.18.

Prospective analyses, used to discern how λ responds to changes in individual vital rates, showed similar trends for both estuarine and offshore models and among all mortality

and fecundity values: population growth was most sensitive to changes in juvenile growth rate (G_J) and least sensitive to changes in female fecundity (F_F) (Fig. 4.2a,d), whereas elasticity was highest for female stage survival (P_F) and lowest for larval stage survival (P_L) (Fig 4.2b,e). Retrospective analyses, which used LTERs to determine which vital rates contributed most to variation in λ, was consistent with the prospective analysis in that female stage survival (P_F) contributed highly to overall variation for both estuarine and offshore models (Fig. 4.2c,f). Growth of juveniles (G_J), which our models had the highest sensitivity to, also showed some contribution to variation in λ. However, these analyses diverged as changes in female fecundity (F_F), although not strongly represented by sensitivity and elasticity measures, were also shown to cause significant variation in overall growth for both models. Differences in estuarine versus offshore models were most apparent in our retrospective analysis: the contribution of female fecundity (F_F) to variation in λ was greater for offshore associated populations, while the contribution of female stage survival (P_F) was greater for estuarine association populations. This indicates that changes in female fecundity may be capable of generating larger differences in the population stability of offshore models than in that of estuarine models.

Our primary objective was to examine the effects of female stage duration (d_F), representative of COEs from nursery habitats, on overall population structure of the black sea bass. Using a series of simulated, combined matrices, which integrated the use of both estuarine and offshore nurseries (to varying degrees ranging from all estuarine to all offshore), we found that λ does not fluctuate notably as d_F moves from 100% estuarine to 100% offshore nursery habitat contribution ($1.00<\lambda<1.00$; Table 4.2). However, over a 30-yr time frame, we found a significantly larger (> 400%; t=-26.1, df=8. $p<0.0001$;) number of

mature males that result from a d_F associated with a population where all juveniles utilized estuarine nurseries (260 ± 9.48 males) versus a d_F associated with a population where all juveniles utilized offshore nurseries (62.2 ± 2.28 male) (Table 4.2). Conversely, the mean number of mature females over the same time period did not fluctuate significantly with d_F (t=0.022, df=6, p=0.508).

Changes in the availability of males in a sex-changing species can also affect the population structure and stability through sperm limitation (Alonzo and Mangel 2004). Under high mortality scenarios (z_F= 0.26), both 0.85 and 0.7 F_F caused estuarine and offshore populations to decline (λ<1). At 0.85 F_F, male populations fell to zero by year 25 and year 19 for estuarine and offshore models, respectively. At 0.70 F_F, male populations declined to zero by year 11.8 and year 8.3 for estuarine and offshore models, respectively. Under low mortality scenarios (z_F= 0.20), populations only declined at 0.70 F_F as the male population fell to zero by year 67 and year 58 for estuarine and offshore models, respectively (Fig. 4.3).

Discussion

The concept that habitat selection and utilization of marine fishes can affect individual vital rates and, consequently, adult fishery stocks, is not novel (Thrush et al. 1996; Quinn and Peterson 1998). However, stock assessment and other population models are still largely unable to account for the effects of varied habitat utilization or habitat-associated impacts. Here, we used the existence of newly discovered COEs to quantitatively link juvenile habitat to adult stock dynamics and demonstrate the utility of considering habitat to effectively manage black sea bass populations. Although estuarine and offshore subpopulations showed similar stabilities (λ=1.00), sensitivities, and elasticities, modeling the population as a whole overlooks a key insight as to how habitat can affect stock

dynamics: the number of mature male were 4xs greater in estuarine rather than offshore nursery associated models over the course of 2.5 generations. As nursery habitat contribution fluctuates annually (Kroll et al. 2017), this trend reveals that some cohorts may be more susceptible to sperm limitation and reduced fecundity and subsequently require management strategies more targeted toward male survival. LTERs also highlight differences in the contribution of individual vital rates (C_{ij}) to the regulation of λ: female fecundity (F_F) contributed more in offshore-associated populations whereas female stage survival (P_F) had greater effects in estuarine-associated populations. Modeling the stock as a uniform population neglects these nuances and may overlook the vulnerability of black sea bass populations to unfavorable sex-ratios and sperm limitation.

Model parameterization and uncertainty

Our heuristic approach to vital rate selection minimizes the uncertainty inherent in stage-based matrix population models while also providing an empirical framework that can be generalized to other species with similar life-histories. The monthly mortality rates (z_M) were calculated based on methods used by the National Marine Fisheries Service in stock assessment models and were compared to values empirically derived from field studies within similar habitats (Fodrie et al. 2009). Stage durations were based on a combination of field data collected by both government surveys (SEDAR 2011) and in a previous study performed by the authors (Kroll et al. 2017). Based on the same study, which found no evidence of larger growth within the first year (and beyond) in fish utilizing estuarine versus offshore nurseries, our model assumed that all fish simultaneously transition to the mature female stage, regardless of juvenile habitat utilization. However, growth differences among fish utilizing habitats of varying quality are not routinely observed (Heck et al. 2003; Amara

et al. 2007), indicating that perceived production differences between nursery habitats may be more linked to survivorship than individual growth.

The structure of our matrix models allowed us to directly assess the population-level consequences associated with alternative juvenile habitat utilization by altering female stage durations. We did not vary juvenile mortality by habitat although sensitivity analyses indicate changes in the stage growth of juveniles may have a significant impact on population survival. Therefore, future studies may want to consider additional vital rate differences that may exist between, or result from, either habitat. Additionally, measuring vital rates, such as mortality, among alternative habitats would also be beneficial because habitat-associated COEs, while providing an important pathway through which to link habitat and population dynamics in this study, are an emerging field of study (Harrison et al. 2011) and there is no guarantee that they will be easily identifiable or even present in all habitats.

Although both estuarine and offshore models presented stable (λ=1.00) populations, forward projections consistently produced a low number of adult males. Mature males represented < 1% of our modeled population, which is considerably lower than the equilibrium sex ratio (3:1 female to male) expected for protogynous hermaphrodites (Allsop and West 2004) and the number of mature males recorded during past sampling efforts (~46% of the adult population; SEDAR 2011). To correct for this, we initially employed three strategies: (1) increase male stage duration (d_M) by 2 yrs (24 mo), (2) increase model projection time from 30 to 60 yr, and (3) increase juvenile duration by 4 mo. Both strategies 1 and 2 had little impact on the overall population and require an unrealistic increase in the male stage duration (>15 yr) or an 120 yr model projection time to attain a female to male ratio close to 3:1. Increasing the juvenile stage (strategy 3) caused a net decrease in

population stability from λ=1.00 to λ=0.83. While these manipulations did not substantially bolster our male population estimates, our prospective and retrospective analyses may provide some insight as to why: our model was most sensitive to juvenile and female stages, so altering the male stage duration and overall projection time should not have a large effect on the population. However, altering juvenile stage duration, even by only 4 mo, did have cascading effects on population stability.

An additional strategy used to bolster the number of males was to decrease d_F by 1 yr and extended d_M. This was motivated by data in government surveys, which report ~1 yr shorter female stage durations (d_F) than those recorded by Kroll et al. (2017), and past research, which suggests females transition earlier when male counts are low (Heppell et al. 2006). Reducing d_F resulted in an increased number of males for both estuarine and offshore associated models (1.43×10^4 and 5.19×10^3 males, respectively), however, the populations became less stable than when using initial d_F values (λ= 1.06 and1.05 for estuarine and offshore, respectively). Despite increased λs, the pattern of increased males associated with estuarine nursery utilization (176% greater than the offshore nursery model) was still present, validating our assumptions that estuarine-habitats support greater availability of males.

Developing habitat-based management

An increasing body of literature asserts ecosystem-based fisheries management (EBFM), rather than single-species management, as the premier strategy for ensuring sustainable use of marine resources (e.g., Pikitch et al. 2004; Fulton et al. 2014; Möllmann et al. 2014). An intrinsic part of EBFM is the identification and conservation of critical marine habitats, with the expectation that conserving habitat will translate into increased fish abundance. Despite growing momentum for EBFM, several fisheries management plans still

include catch limits and size restrictions to ensure a sufficient number of juveniles reach sexual maturity (e.g., recruitment; Botsford et al. 1997). However, in protogynous hermaphrodites, size limits often increase harvest pressure on adult male fish, increasing the likelihood of skewed sex-ratios, sperm limitation, and ultimately lower female fertility (Yund 2000; Heppell et al. 2006).

Understanding the link between nursery habitats and adult stock dynamics is essential to the management of black sea bass because the availability and utilization of estuarine versus offshore nurseries encourages population stability for an historically overfished species. Kroll et al. (2017) used elemental fingerprinting to link ~89% of adult black sea bass to estuarine nurseries, however, contribution from offshore nurseries varied from 0-20% annually. Thus, in years where offshore nursery contribution is higher, cohorts will transition later, leaving fewer males available for breeding. In the simulated case where nursery utilization shifts from all-estuarine to all-offshore, our models predicted a 64-76% decrease in male population and rapid population decline ($\lambda<1$). This population decline is exacerbated under higher mortality conditions (e.g., when size-limits displace fishing pressure onto adults) and under conditions of reduced fertility (e.g., sperm-limitation). As estuarine habitats face numerous conservations threats, such as climate change and coastal development (Lotze 2006), the loss of estuarine nursery habitat may not just drive juveniles to offshore nurseries but also distort stock structure, decrease overall population fecundity, and ultimately lead to population collapse. Management strategies that focus on conserving these nursery habitats may not only ensure the success of juvenile cohorts, but also help maintain the population stability of the black sea bass, as well as many other protogynous hermaphroditic members of the snapper-grouper complex.

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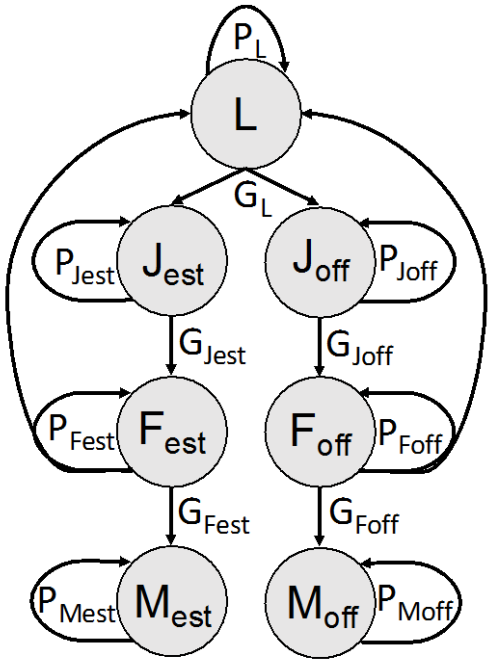

Figure 4.1. Life-cycle diagram used to construct stage-based population matrix models for two black sea bass subpopulations separated by juvenile habitat alternative: estuarine (est) and offshore (off). L: larvae; J: juvenile; F: female; M: Male. P_i is the probability of surviving to remain in the same stage duration and G_i is the probability of surviving and advancing to the next stage during 1 time step. F_F is female fertility (contribution of offspring to the *L* stage).

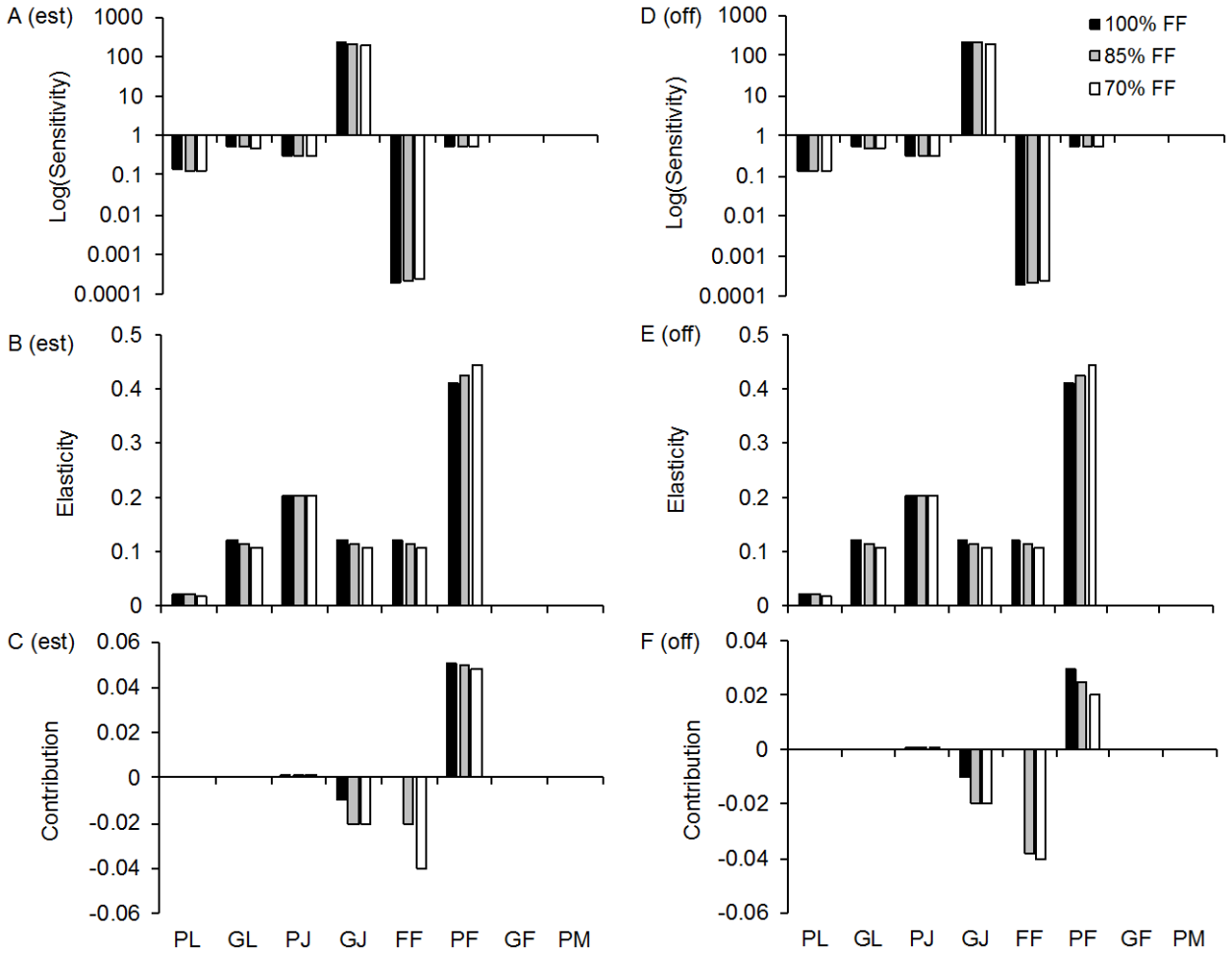

Figure 4.2. Sensitivity (A, D), elasticity (B, E), and contribution (C, F) of each matrix element to changes in λ for fish associated with estuarine(A-C) and offshore (D-F) nursery habitats. Bar colors correspond to models with 0, 15, and 30% reductions in female stage fecundity (F_F)

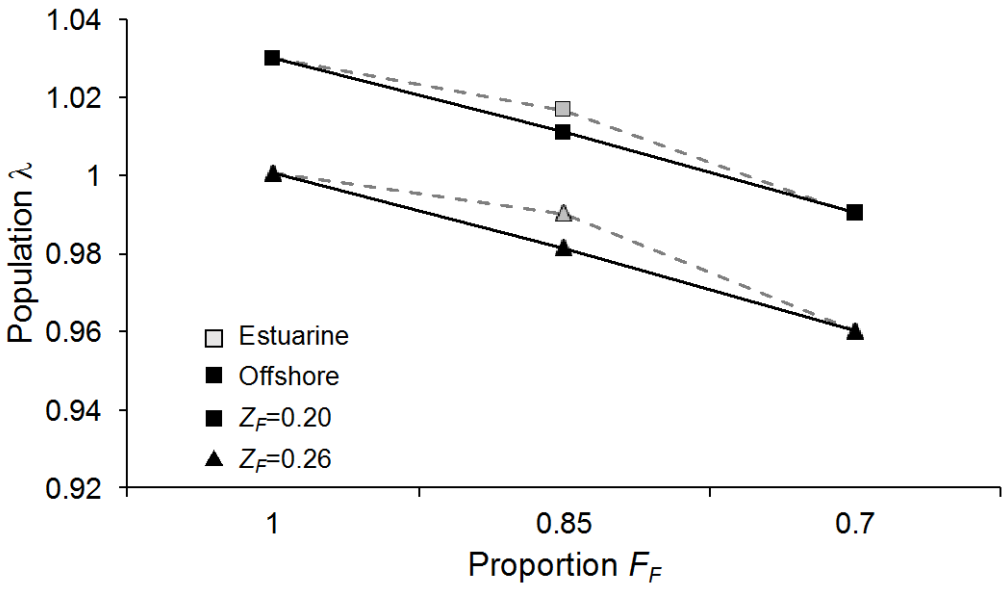

Figure 4.3. Population growth (λ) resulting from 0, 15, and 30% reductions in female stage fecundity (F_F), using two potential female stage mortalities (z_F), for estuarine and offshore-associated populations.

Table 4.1. Name, size classification, source and associated vital rates for each mode stage. *f*: fecundity; z_{high}: upper bound mortality; z_{low}: lower bound mortality; and *d*: stage duration

Stage	Size (mm)	Source	*f*	z_{high}	z_{low}	*d*
L	0-15	Berlinsky et al. (2000)	0	0.96	0.99	1.3
J_{est}	15-170	SEDAR 25	0	0.47	0.49	14
J_{off}	15-170	SEDAR 25	0	0.47	0.49	14
F_{est}	170-300	von Bertalanfy (VB) growth curve based on data from Kroll et al. (2016)	7260	0.20	0.26	39.5
F_{off}	170-350	VB curve based on data from Kroll et al. (2016)	7260	0.20	0.26	45
M_{est}	300+	Kroll et al. (2016)	0	0.18	0.20	89.2
M_{off}	350+	Kroll et al. (2016)	0	0.18	0.20	83.7

Table 4.2. λ; F: number of females (x10^3); and M: number of males resulting from a single model, ran over 30yrs, with varying estuarine and offshore nursery habitat utilization (reflected in female stage duration, d_F).

				Year											
				5		10		15		20		25		30	
Prop. est	Prop. off	d_F	λ	F	M	F	M	F	M	F	M	F	M	F	M
1	0	39.5	1.00	544	56.8	564	58.8	584	60.9	604	62.3	624	64.6	644	67.3
0.9	0.1	40.1	1.00	544	65.5	564	67.9	584	70.3	604	72.7	624	75.1	644	77.6
0.8	0.2	40.6	1.00	544	75.5	564	78.3	584	81.1	604	83.9	624	86.6	644	89.5
0.7	0.3	41.2	1.00	544	87.2	564	90.3	584	93.5	604	96.7	624	99.9	644	103
0.6	0.4	41.7	1.00	544	101	564	104	584	108	604	112	624	112	644	119
0.5	0.5	42.3	1.00	544	116	564	120	584	124	604	129	624	133	644	137
0.4	0.6	42.8	1.00	544	134	564	138	584	143	604	149	624	153	644	159
0.3	0.7	43.4	1.00	544	154	564	160	584	166	604	171	624	177	644	182
0.2	0.8	43.9	1.00	544	178	564	184	584	191	604	198	624	204	644	210
0.1	0.9	44.5	1.00	544	205	564	212	584	220	604	228	623	236	643	243
0	1	45	1.00	544	237	564	246	584	254	604	263	623	272	643	281

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CONCLUSION

Discerning the complex connectivity patterns within marine environments is essential to the management of ecologically and commercially valuable marine species (Young 1990**;** Gillanders et al. 2003). While estuarine habitats are regarded as some of the most productive nursery habitats for several important species (Beck et al. 2001), degradation, due to anthropogenic influences, continues to occur (Lotze 2006). A major barrier to protecting these habitats is the lack of empirical studies which examine the complex, estuarine-scale connectivity patterns during critical early life stages (e.g., larval and juvenile). Understanding the larval connectivity of the reef-forming Easter oyster, for example, is essential to delineating source-sink dynamics within metapopulations and protecting dispersal pathways. Additionally, the ability to ascertain how and to what extent juvenile fishes utilize estuarine habitats is required to predict how declines in habitat availability may impact population structure. To properly manage estuarine habitats, and the fisheries that rely on them, we must understand what drives connectivity within these systems and identify potential links between estuarine habitat utilization and adult stock dynamics.

A requisite to tracking estuarine-scale connectivity among sub-populations of the Eastern oyster was to explore the utility of geochemical tagging methods within our study system, the Pamlico Sound (Chapter 1). Strong environmental (e.g., temperature and salinity) gradients were present over regional (~ 35 x 15 km quadrants) scales and both larval and settler shells were able to generate distinct, multi-elemental signatures between putative natal and settlement sites. These methods were then applied, with a combination of larval

outplanting techniques (i.e., stationary moorings and floating surface drifters), to show that larval dispersal is single-source driven and that pathways generally follow wind-driven currents (Chapter 2). However, dispersal pathways are not uniform across the Sound and seasonal and annual dispersal patterns can be highly variable. Furthermore, self-recruitment occurs at rates up to three times higher than could be predicted by traditional modeling simulations (Puckett and Eggleston 2016). To conserve oyster reefs within this system, reserve networks should be designed to protect primary larval-sources, such as reefs present in the SE quadrant, while also bolster cross-regional dispersal.

The understanding of how organisms utilize juvenile habitats within estuarine systems, and whether these habitats can confer life history advantages, is essential to the conservation of estuarine-associated species. Here, we determined that estuarine nurseries, such as oyster reefs, contribute over 89% of the juvenile black sea bass to the adult stock (Chapter 3), however, there is significant annual variation in contribution. The role of estuarine habitats becomes even more complex for the protogynous black sea bass, as fish exhibited carry-over effects (COEs) related to nursery habitats: juveniles that utilized estuarine nurseries transitioned from female to male six months earlier than juveniles that utilized offshore nurseries. By incorporating this difference in female stage duration into a population model, we were able to explore the population level effects of estuarine nursery utilization and form a novel link between juvenile habitat and adult stock dynamics (Chapter 4). When nursery habitat utilization moves from all-offshore to all-estuarine, we see an over 400% increase in the availability of breeding males, indicating that estuarine nursery habitats may be necessary to buffer against potential sperm limitation that result from current management practices.

Through the quantitative evaluation of oyster reef and black sea bass connectivity, this dissertation provides substantial support for the implementation of ecosystem based fisheries management (EBFM). Single-species management practices, such as catch limits and size restrictions, do not account for seasonal and annual variation inherent in dispersal pathways or the fact that reproductive (and dispersal) potential may vary among subpopulations. For example, as the majority of tracked oyster larval connectivity occurred within adjacent regions, the implementation of marine reserves, spaced ~5-40 km apart, may better protect oyster larval dispersal than existing harvest limitations or farther-spaced reserves. Furthermore, estuarine reserves would also aid in the conservation of mobile fish species by not only protecting juvenile fish while they are utilizing these nursery habitats, but also by increasing their exposure to COEs, which are essential to the maintenance of black sea bass stock structure and fertility.

This work also demonstrates the necessity of an improved modeling framework for the establishment and maintenance of successful reserve networks. Current oyster dispersal models not only underestimate the degree of self-recruitment among subpopulations, when compared with our empirical study, but also do not account for the multi-directional wind forcing which may drive the high levels of spatiotemporal variation (e.g., regional, seasonal, and annual). Similarly, the contribution of estuarine nurseries to the adult sea bass stock, and therefore juvenile exposure to COEs, also vary over seasonal and annual timescales.

Therefore, assuming uniform habitat utilization (as most population models do) can be deleterious in years when higher percentages of fish utilize offshore nurseries and cohorts become more vulnerable to skewed sex-ratios or other habitat-related population effects. As several marine organisms utilize multiple nursery habitats, other COEs likely exist, our model can be expanded to quantify the links between a suite of fisheries and their associated habitats.

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