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Using $\delta^{13}C$ and $\delta^{15}N$ to determine the migratory history of offshore Louisiana blue crab spawning stocks

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ABSTRACT: During offshore sampling conducted between 2005 and 2007, we discovered large concentrations of the blue crab *Callinectes sapidus* spawning between 4 and 50 km offshore of the Louisiana coast; the greatest concentrations were found on high-relief sandy shoals. We used natural abundance carbon and nitrogen isotopes to evaluate *C. sapidus* migratory source locations within south-central Louisiana estuarine systems as well as residency on the shoals. There was an east-west trend of decreasing $\delta^{13}C$ and increasing $\delta^{15}N$ for offshore crab tissue (muscle and ovary) related to proximity to the Atchafalaya River. This indicates that crabs predominately migrate directly offshore from their home estuary rather than migrating long distances alongshore. Many $\delta^{13}C$ values for offshore crab muscle and ovary were depleted relative to typical salt marsh values, indicating that some female blue crabs migrate directly offshore from low salinity regions higher in the estuary. The convergence of muscle and ovary isotope values towards a proxy for offshore residence (i.e. *Callinectes similis* mean isotope values) indicated that migrating *C. sapidus* utilize offshore prey resources and do not typically re-enter inshore estuaries during the spawning season. A correlation between crab $\delta^{13}C$ values and the body size of shell-associated epibiotic acorn barnacles *Chelonibia patula* indicated that crabs found on shoals acquire offshore isotopic values over time. These findings have important research implications for studies involving animal migrations, as well as for management of this ecologically and economically important species. The findings provide evidence of a direct link between the inshore fishery and female blue crabs captured offshore in unique sandy shoal habitats, which likely support at least 20% of the known Louisiana blue crab spawning stock west of the Mississippi River.

KEY WORDS: *Callinectes sapidus* · Behavior · Feeding ecology · Stable isotopes · Gulf of Mexico · Migration

INTRODUCTION

Blue crabs *Callinectes sapidus* are an ecologically and economically important species with a wide ranging distribution in the Americas from Nova Scotia to Argentina (Williams 2007). Though the timing of blue crab life history events varies with latitude, relative environmental factors that trigger specific events are generally consistent. For example, optimal blue crab zoeal development occurs at salinities between 20 and 32 practical salinity units (psu) and temperatures between 19 and 29°C (Sandoz & Rogers 1944, Costlow & Bookhout 1959). Despite these known and important environmental parameters, much is still unknown about blue crab ecology and how it may differ geographically. This article uses a novel isotopic conceptual model to elucidate a newly discovered aspect of blue crab reproduction — offshore

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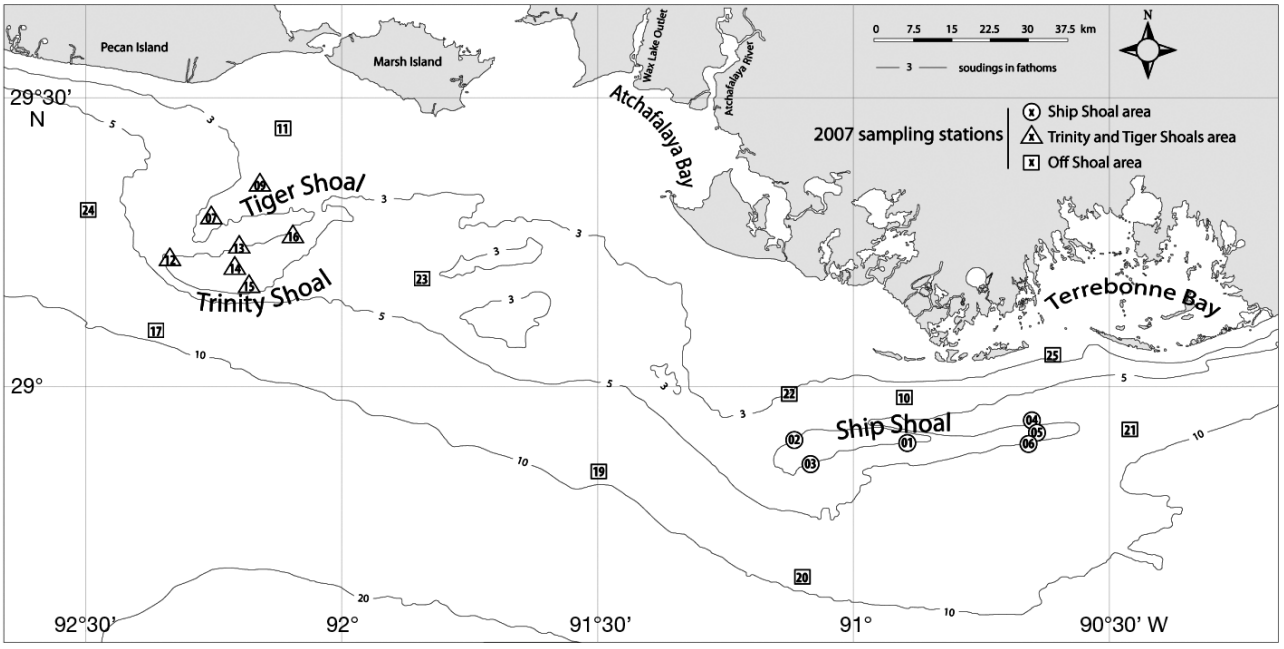

Fig. 1. Station locations within our study area, the Ship, Trinity, Tiger Shoal Complex (STTSC), located off the south-central Louisiana coast

spawning migration — and develops a tracking framework that is applicable for many estuarine systems and species.

Recently, on the Gulf of Mexico continental shelf, large concentrations of spawning female blue crabs were discovered at previously unreported distances offshore, with highest abundances on sandy shoals (Gelpi et al. 2009) located approximately 25 to 50 km from Louisiana's Terrebonne and Atchafalaya Bays (Fig. 1). These shoals are high-relief, subaqueous stands of mostly sandy sediment (water depth 3 to 10 m), and are under-explored areas on the continental shelves that are difficult to sample and are too frequently overlooked by biologists (e.g. Baustian & Rabalais 2009, Baustian et al. 2009). The shoals and the surrounding muddy Mississippi/Atchafalaya River depositional plain constitute the Ship, Trinity, Tiger Shoal Complex (STTSC). The STTSC is heavily influenced by nutrients, freshwater, and sediments associated with the Mississippi and Atchafalaya Rivers (Goolsby et al. 2000), which contribute to large phytoplankton blooms and ultimately an expansive area of seasonal bottom-water hypoxia (Rabalais et al. 2002), though STTSC shoals likely provide a hypoxia refuge due to shallow depths and high concentrations of benthic microalgae (Dubois et al. 2009, Gelpi et al. 2009, Grippo et al. 2009, 2010, DiMarco et al. 2010, Gelpi 2012).

The discovery of abundant spawning populations within the STTSC greatly expanded what was known about blue crab reproductive biology, because spawning grounds located at such distances offshore had not previously been reported (Gelpi et al. 2009). The crabs from the STTSC were 99% female, almost all were carrying eggs or were about to spawn, and in some instances females with late stage eggs also had full ovaries, indicating a constant state of spawning and ovarian replenishment (Gelpi et al. 2009). Internal examination and subsequent analysis suggested that they were in good condition, that their health was not affected by the presence of epibiotic acorn barnacles *Chelonibia patula*, and that they were forming and releasing a new spawn approximately every 21 d (Gelpi et al. 2009). However, the origin and life history of this newly discovered blue crab spawning stock remains unclear.

Under the generally accepted paradigm for female blue crab spawning behavior, females hatch their eggs in nearshore coastal waters with high salinities and then re-enter protected estuarine waters (Daugherty 1952, Tagatz 1968, Adkins 1972, Oesterling 1976, Steele 1987). This behavior was more recently described in greater detail based on observations made from an estuary on the mid-Atlantic coast using a 2 phase migratory pattern where the females take advantage of selective tidal stream transport. First, in Phase I, females travel down-estuary to over-

winter in higher salinity waters and then spawn in the spring. In Phase II, females move further seaward to hatch their eggs and release larvae, with many females returning to the estuary after larval release (Tankersley et al. 1998). Alternatively, recent behavioral experiments using crabs from the Atlantic seaboard have suggested that female blue crabs continue in a seaward migration and do not re-enter inshore estuaries after hatching their eggs (Hench et al. 2004, Forward et al. 2005). In addition, tagging studies from the Florida Gulf coast indicate blue crab migratory patterns there differ from US Atlantic coast estuaries (Oesterling 1976, Steele 1987). These studies revealed that adult female, and to a lesser extent adult male, blue crabs from the Florida Keys to Apalachicola undergo an alongshore migratory pattern with many ultimately concentrating in the vicinity of Apalachee Bay.

The first objective in our study was to determine if STTSC *Callinectes sapidus* engage in a back and forth spawning migration by moving to high salinity water to hatch their eggs and then return inshore to spawn again, or alternatively if most crabs migrate to offshore shoal-based spawning grounds where they engage in multiple spawning/hatching cycles. The second objective was to determine if STTSC blue crabs engage in an alongshore migratory pattern, or alternatively if they migrate directly offshore to spawn on shoals and surrounding off-shoal areas closest to their home estuary.

Stable isotopes have proven to be an invaluable tool to understand trophic linkages and contribution of food sources to an organism's diet (e.g. Fry 2006). They are also increasingly used as a tool to discover migratory routes and understand migratory patterns (Hobson 1999, Rubenstein & Hobson 2004, Newsome et al. 2007, Fry 2011). Developing new isotope methods to track migratory patterns of mobile invertebrate species from ecologically diverse estuaries with differing hydrology, land use, and associated biogeochemical factors is of interest to scientists who seek to understand large scale connectivity within and between systems. Isotope tracking methods can be successfully employed across areas where isotopic compositions of autotrophs (end members) are sufficiently different to distinguish basal food sources, and therefore feeding sites. The STTSC and its adjacent estuaries consist of the interface of a near marine environment with coastal salt, brackish and fresh marshes, with varying amounts of riverine input. If STTSC crabs are migrating from different inshore areas, their carbon and nitrogen isotopic signals should reflect their feeding and migratory histories.

STUDY SITE AND LOCAL ISOTOPIC FRAMEWORK

To address STTSC blue crab migratory dynamics we have developed the following isotopic framework.

(1) Although commonly used to evaluate trophic position (Minagawa & Wada 1984, Post 2002), $\delta^{15}N$ can also be a useful tool in estuarine migratory studies where source areas differ in the amount of freshwater input (Fry 2011). Mississippi/Atchafalaya River waters have elevated nitrate and dissolved inorganic carbon concentrations that are high in $\delta^{15}N$ and low in $\delta^{13}C$ (Fry & Allen 2003). This results in ^{15}N-enriched and ^{13}C-depleted food webs for river-influenced estuaries (Fry & Allen 2003, Fry & Chumchal 2012). Within the STTSC (Fig. 1), isotopic differences between blue crabs that are migrating from areas of high freshwater input (e.g. Mississippi or Atchafalaya River deltas) and areas that no longer have a direct riverine connection and therefore have relatively little freshwater input (e.g. Terrebonne Bay) would be expected. Given the configuration of the Louisiana coast and the dominant westerly direction of the longshore current in our study area, we expect that crabs caught west of 91° 30′ W nearer the Atchafalaya River will have a higher $\delta^{15}N$ signal and lower $\delta^{13}C$ signal than crabs caught east of this longitude (Fig. 1).

(2) In the upper reaches of estuaries in lower salinity waters a relative $\delta^{13}C$ depletion occurs (Deegan & Garritt 1997, Bucci et al. 2007, Fry 2011), and the $\delta^{13}C$ signal should become relatively enriched if crabs moved seaward from inshore estuaries, converging to an offshore range of approximately −14 to −19‰ (Fry 1981, 1983, 1988, 2011, Fry & Sherr 1984, Fry et al. 1984, 2003, Sherwood & Rose 2005), due to high contributions of marine phytoplankton. We therefore expect that an association of the STTSC crabs with the open waters of the Gulf of Mexico will increase their $\delta^{13}C$ values, while their association with Louisiana's inshore estuaries will result in lower $\delta^{13}C$ values.

(3) Benthic microalgae (BMA) have recently been found to be an important component of the offshore autotrophic community of the STTSC, predominately on Ship Shoal (Grippo et al. 2009, 2010, 2011). BMA usually are 3 to 5‰ enriched in $\delta^{13}C$ versus phytoplankton (France 1995, Hecky & Hesslein 1995). To the extent that crabs feed in food webs supported by BMA, we expect the $\delta^{13}C$ signal will be higher on Ship Shoal.

(4) Muscle is typically used as a slow turnover tissue and representative of an animal's long-term diet

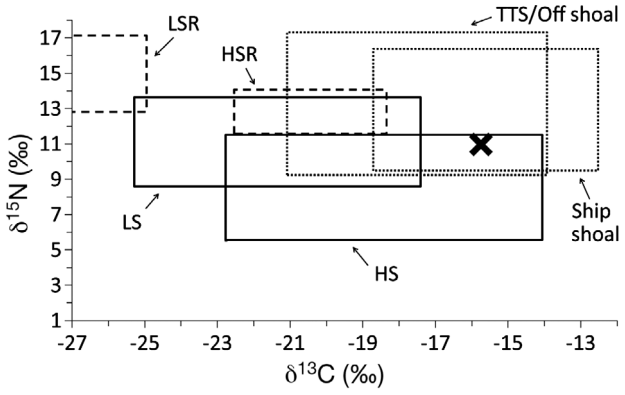

Fig. 2. Inshore boxes (based on Fry 2002, 2011; see 'Materials and methods') that define changing source area isotopes based on riverine and salinity influences: higher salinity without river influence (HS), lower salinity without river influence (LS), higher salinity with river influence (HSR), and lower salinity with river influence (LSR). In addition, 2 offshore residency boxes are defined based on isotopes of potential prey from the Tiger/Trinity/Off shoal areas and Ship Shoal; the mean *Callinectes similis* (✖) is plotted as a proxy for offshore, resident *Callinectes sapidus*

and migratory history (Logan et al. 2006). We therefore assume that turnover of muscle will reflect basal metabolism and that the isotopic composition of the muscle will represent an integration of 'long-term' migratory history. Growth in width does not occur in post-copulation female *Callinectes sapidus* (Churchill 1919), and muscle tissue turnover may be long when growth is slow (Tominaga et al. 2003). In contrast, STTSC crabs replenish their ovaries every 21 d (Gelpi et al. 2009), so we expect that the blue crab ovary will be an indicator of recent diet and migratory history (<21 d). Residency designation for crabs found on the STTSC would thus be indicated if the ovarian and muscle isotopic signals are equilibrated with each other and are within an offshore isotopic range (Fry et al. 2003). Conversely, if the ovarian and muscle isotopic signals differ, and at least one lies outside the range for offshore residents, then migratory connection to the STTSC is indicated. For crabs which are newly recruited to the STTSC from an inshore source, we expect a seasonal convergence in their isotopic carbon signal from an inshore range generally less than −19‰ (Deegan & Garritt 1997, Bucci et al. 2007, Fry 2011) to an offshore (and STTSC) isotopic range of approximately −14 to −19‰ as they become resident. We expect that this convergence will be seen first in the ovary, and then in the muscle.

(5) Because larvae of the epibiotic acorn barnacle *Callinectes patula* requires salinities >25 for survival (Crisp & Costlow 1963), we expect larval settlement to begin shortly after inshore crabs have entered high salinity water, and a convergence of the isotopic composition of the ovary and muscle of STTSC crabs as the body size of their acorn barnacles increases.

(6) The lesser blue crab *Callinectes similis* taken from the STTSC is used here as a proxy for shoal-resident blue crabs. *C. similis* is known to occupy high salinity water on the continental shelf (Williams 1974) and has been found to feed on similar prey types to those consumed by *C. sapidus* (Hsueh et al. 1992). If recently migrated to the STTSC, muscle and ovary convergence of *C. sapidus* isotopic values towards those of *C. similis* is expected.

Using this overall framework, we used a graphic analysis approach to study blue crab migrations by showing expected isotope value ranges in rectangular areas of $\delta^{15}N$ versus $\delta^{13}C$ plots. We defined 4 inshore rectangles and 2 offshore rectangles that characterized the isotope values of locally resident crabs (Fig. 2), and then used the orientations of crab tissue isotopes to these boxes to study migration.

The environmental framework of the 4 inshore boxes used to determine estuarine isotopic ranges are: low salinity with riverine influence (LSR), low salinity without riverine influence (LS), high salinity with riverine influence (HSR), and high salinity without riverine influence (HS) (Fig. 2). Requirements were that the studies involved Louisiana estuarine areas west of the Mississippi River, and contained sufficient data to define at least one of the inshore boxes within a $\delta^{13}C$, $\delta^{15}N$ bi-plot. Two studies with sufficient data were used to construct the 4 inshore boxes (Table 1). We used data on benthic, generalist-feeding finfish from the fresh marsh (salinity <1) environment of the Atchafalaya Basin (Fry 2002) for the LSR box. For the LS box we used data on benthic, generalist-feeding finfish from oligohaline marsh (salinity <3) in the upper Barataria Estuary (Fry 2002, Fry & Chumchal 2012). For the HSR box, we used brown shrimp associated with a meso/polyhaline environment (salinity 20 to 30) near the Mississippi River Bird's Foot Delta (Fry 2011). And for the HS box we used brown shrimp associated with the meso/polyhaline estuaries (salinity 20 to 30) of Barataria and Terrebonne Bays (Fry 2011).

To construct our offshore boxes (Fig. 2) we plotted the carbon and nitrogen isotopes for all benthic macrofauna available from a 2007 study in the STTSC (e.g. Grippo et al. 2011) by area and season (Fig. 3a–c). Examination of STTSC blue crab stomachs showed an STTSC-based macrofauna diet consisting largely of crustaceans, mollusk, and fish (Gelpi 2012). As an initial check on our boxes, we

Table 1. Carbon and nitrogen isotope ranges of animals from source regions of blue crab *Callinectes sapidus* migrations

Area designation	Sampling region	Species sampled	Salinity	$\delta^{13}C$ range	$\delta^{15}N$ range	Source
Low salinity with riverine influence (LSR)	Atchafalaya River (AR)	*Aplodinotus grunniens* *Ictiobus bubalus* *Ictalurus furcatus* *Ictalurus punctatus* *Pylodictis olivaris*	<1	−32. 6 to −25	12.8 to 17	Fry 2002 (appendix)
Low salinity without riverine influence (LS)	Barataria Bay (BB)	*I. furcatus* *I. punctatus*	<3	−25.3 to −17.4	8.5 to 13.6	Fry 2002 (appendix)
High salinity with riverine influence (HSR)	Riverine shrimp from the Bird's Foot Delta	*Farfantepenaeus aztecus*	20 to 33	−22.8 to −18.4	11.6 to 14	Fry 2011 (his Fig. 5 and p 3,11)
High salinity without riverine influence (HS)	Bay shrimp from Barataria & Terre-bonne Bay	*F. aztecus*	20 to 33	−22.8 to −14.1	5.5 to 11.5	Fry 2011 (his Fig. 5 and p 3,11)

plotted the isotopic composition of sediment, phytoplankton, and BMA from Grippo et al. (2011) and examined the pattern for consistency with expected trophic relationships. These bi-plots were examined by area and season. Using the inshore box procedure, we used rectangles to represent the ranges for the bi-plots for Ship Shoal, Trinity and Tiger Shoal, and all off shoal stations (Fig. 3a–c). We collapsed the Trinity/Tiger Shoal and the Off shoal boxes into a single box (TTS/Off) due to their similarity. These groupings of 2 summary boxes: (1) Ship Shoal, and (2) TTS/Off are in agreement with the results of Grippo (2009). We then applied trophic enrichment factors of 1 and 2.5‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively (Fig. 3d), based on Fry & Sherr (1984), Vanderklift & Ponsard (2003), and McCutchan et al. (2003). Finally, as an additional check on the offshore boxes, we plotted mean isotopic values for *Callinectes similis* muscle (Ship Shoal summer mean $\delta^{13}C = -16.6$ and $\delta^{15}N = 12.7$; Ship Shoal fall mean $\delta^{13}C = -17.8$ and $\delta^{15}N = 11.5$; Trinity Shoal summer mean $\delta^{13}C = -15.9$ and $\delta^{15}N = 11.7$). These values also provide estimates for expected isotope values of offshore-resident *C. sapidus*.

MATERIALS AND METHODS

Altogether 229 adult female blue crabs *Callinectes sapidus* were collected from the STTSC (Fig. 1) during 3 cruises in spring, summer and fall of 2007 as outlined in Gelpi et al. (2009), and muscle and ovary tissue were analyzed for isotopic content. A total of 48 lesser blue crabs *C. similis* was taken from Trinity Shoal in summer and Ship Shoal in summer and fall of 2007; their muscle tissue was also analyzed for isotopic content. The gut contents of 31 *C. sapidus* taken from Ship Shoal and off shoal stations immediately north of Ship Shoal were also analyzed for $\delta^{13}C$ and $\delta^{15}N$. Raw isotopic data on potential food web contributors (i.e. sediment, phytoplankton, BMA, and resident macroinfauna) were obtained from recent work in the STTSC by Grippo et al. (2011). Seasonal (April, August, and October) ranges for bottom water salinity, temperature and dissolved oxygen were measured at all stations (Table 2). Station specific values are presented with crab isotope data in Table S1 in the Supplement at www.int-res.com/articles/suppl/m494p205_supp.pdf.

Forceps were used to extract muscle tissue from *Callinectes sapidus* and *C. similis* claws and ovary tissue from the interior of the *C. sapidus* cephalothoraxes. Each tissue sample was washed with fresh deionized water and frozen. All tissues were freeze-dried, ground to a fine powder, and then weighed in tin caps. Isotope analyses were performed by the University of California Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon). Resulting $\delta^{15}N$ and $\delta^{13}C$ values were calculated using the formula:

$$X = [(R_{SAMPLE} / R_{STANDARD}) - 1] \times 1000 \quad (1)$$

where $X = \delta^{15}N$ or $\delta^{13}C$, and R is the ratio of the heavy isotope to the light isotope $^{15}N/^{14}N$ or $^{13}C/^{12}C$, respectively. Vienna Pee Dee Belemnite and atmospheric N_2 were used as standards for carbon and nitrogen, respectively.

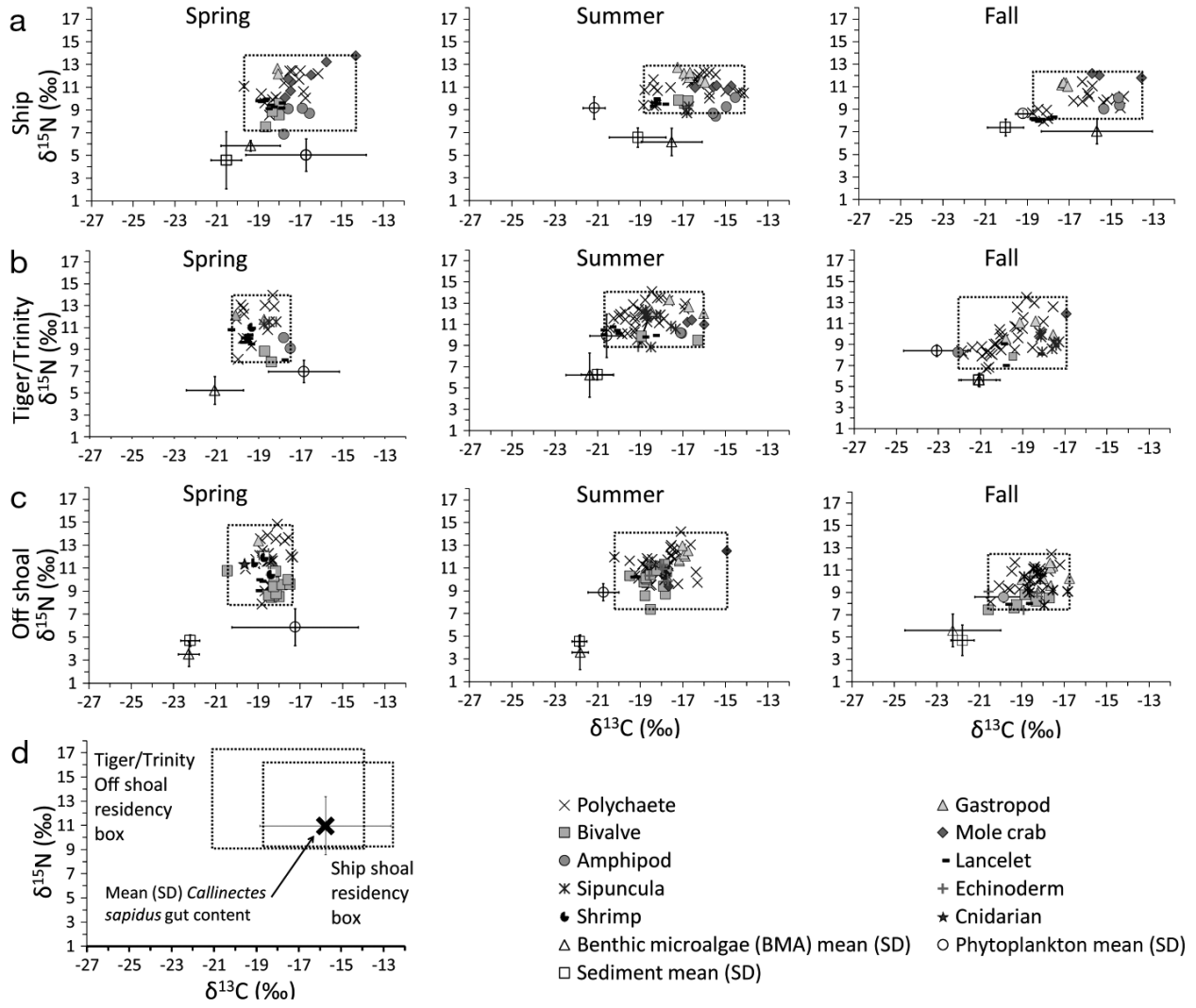

Fig. 3. Carbon and nitrogen isotopes for macrofauna and mean (± SD) of potential contributors to the base of the food web from spring, summer and fall from (a) Ship, (b) Tiger/Trinity, and (c) Off shoal. Offshore composite boxes shown in (d) represent offshore blue crab residency based on macrofauna from: Trinity/Tiger/Off shoal areas, left box (combined from b and c), and the Ship Shoal area, right box (combined from a) illustrating the offshore isotopic framework. Both composite residency boxes and mean *Callinectes sapidus* gut contents (✖) from east area have a +1 and 2.5‰ trophic enrichment factor applied for $\delta^{13}C$ and $\delta^{15}N$, respectively

During analysis, samples were interspersed with several replicates of at least 2 different laboratory standards. These laboratory standards, which are selected to be compositionally similar to the samples being analyzed, had been previously calibrated against NIST Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). A sample's preliminary isotope ratio is measured relative to reference gases analyzed with each sample. These preliminary values are finalized by correcting the values for the entire batch based on the known values of the included laboratory standards. The long term standard deviations of results from this laboratory are 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

Table 2. Ranges for bottom water environmental parameters taken from the Ship, Trinity, Tiger Shoal Complex (STTSC) during 3 cruises in 2007

Season	Salinity	Temperature (°C)	Dissolved oxygen (mg l^{-1})
Spring (Apr 1–5)	24.2–36.3	20.4–23.5	2.5–7.7
Summer (Aug 16–19)	23.9–36.1	27.5–31.3	0.4–5.6
Fall (Oct 5–7)	29.0–33.3	27.5–28.2	5.6–6.5

Because *Callinectes sapidus* ovary tissue exhibited higher lipid content than muscle,

measured C/N ratios were used to provide a lipid-free basis for ovary using the following mass balance equation based on Fry et al. (2003):

$$\delta_p = \delta_o + 6 - (6 \times 3.2)/R_o \quad (2)$$

where δ_p is the $\delta^{13}C$ value of lipid-free tissue (i.e. the ovary value after correction), δ_o is the $\delta^{13}C$ value of the ovary, 6 refers to an assumed 6‰ depletion in lipid C isotopic composition versus muscle (Fry 2002), 3.2 is the average C/N ratio of STTSC blue crab muscle (n = 224) and used here as a proxy for lipid-free ovary, and R_o is the measured C/N ratio of the ovary. On average, ovary $\delta^{13}C$ values were corrected by 1.9‰ due to lipid content of blue crab ovary. Preliminary tests with other correcting models (Kiljunen et al. 2006, Bodin et al. 2007) were not significantly different. Only lipid-corrected ovary values are used in analyses. Corrected and uncorrected values for all tissues are listed in Table S1 in the Supplement.

The contents of blue crab stomachs, taken from the foreguts that were half-full or greater, were kept frozen until freeze dried and ground to a fine powder for carbon and nitrogen isotopic analysis. Because of sand and numerous shell fragments, a portion of each stomach sample was acidified after freeze drying and grinding to remove inorganic carbon for $\delta^{13}C$ analysis.

Muscle to ovary isotopic convergence

The orientation and spacing (i.e. separation) of muscle and ovary isotopes was used to determine convergence patterns for STTSC crabs. Spacing was calculated as the ‰ value (i.e. hypotenuse distance) between muscle and ovary created from tissue differences in $\delta^{13}C$ and $\delta^{15}N$ bi-plots using the Pythagorean Theorem. Based on known salinity-associated changes in $\delta^{13}C$ from fresh to marine systems, we tested the assumption that blue crabs that have recently moved offshore into high salinity water were converging on an offshore/shoal based $\delta^{13}C$ isotopic range, and our proxy value for offshore blue crabs (i.e. *Callinectes similis* muscle). In addition, the acorn barnacle *Chelonibia patula* is a filter feeder with larvae that require high salinity water, between 25 and 30, to develop (Crisp & Costlow 1963). When present, the diameter of the largest adult *C. patula* for each crab was used here as an indication of relative time spent offshore, and plotted against crab $\delta^{13}C$ values for ovary and muscle tissues.

Statistical analysis

Two station groupings within the STTSC were outlined in proximity to the Atchafalaya River as those west and east of 91°30′ W (Fig. 1). Mean values of isotopes were given with standard error of the mean (SEM) unless otherwise stated. Statistical analysis was performed on $\delta^{13}C$ and $\delta^{15}N$ isotope values using linear regression and 2-way analysis of variance (ANOVA), with the main effects of area and season and area × season interactions, using SAS® version 9.1.3 (SAS Institute 2004). Isotope data were transformed when required in order to approximate the assumptions of normality and equal variance.

RESULTS

General isotope pattern

There was a broad range of isotopic values for STTSC blue crab tissues, including both muscle $\delta^{13}C$ (−25.3 to −14.7‰), $\delta^{15}N$ (7.2 to 15.1‰), and ovary $\delta^{13}C$ (−23.6 to −15.1‰), $\delta^{15}N$ (7.2 to 14.7‰) with the widest seasonal range for each tissue occurring in summer. ANOVA revealed substantial geographic and seasonal variation of $\delta^{13}C$ and $\delta^{15}N$ of crab tissues (Table 3). There was a general enrichment of $\delta^{15}N$ for the west station group; $\delta^{15}N$ was significantly greater than the east station group for muscle and ovary for nearly every season. Conversely, $\delta^{13}C$ was more enriched in the east station group with differences significantly greater in the muscle tissue in spring and over all seasons (Table 3), and ovary values for both east and west groups slightly converged towards a narrower offshore range (Fig. 4).

Using published results of previous studies (Fry 2002, his Appendix, Fry 2011, his Fig. 5) involving trophically comparable species from extreme ends of both riverine and salinity influences, we outlined a conceptual isotopic gradient for migratory species' estuarine source locations. The gradient represented by the 4 inshore boxes is oriented from the upper left to lower right on a $\delta^{15}N$, $\delta^{13}C$ bi-plot as follows: LSR to HSR and LS to HS (Fig. 2).

Inshore to offshore convergence

The percentage of crab muscle and ovary $\delta^{13}C$ values that fell within an offshore range of −14 to −19‰ increased seasonally at 30, 35, and 45% for muscle, and 56, 74, and 74% for ovary through

Table 3. *Callinectes sapidus.* Mean ± SEM seasonal and spatial carbon and nitrogen isotope values (‰) for the muscle and ovary tissue of spawning female blue crabs from the Ship, Trinity, and Tiger Shoal Complex (STTSC) in 2007. East and west station groupings are delineated by Stns 19 and 23 (Fig. 1), respectively. Parentheses denote number of observations (n). If Analysis of Variance (ANOVA) interactions were significant then pairwise significance is indicated by lettering

Season	Location	Muscle $\delta^{13}C$	Ovary $\delta^{13}C$	Muscle $\delta^{15}N$	Ovary $\delta^{15}N$
Spring	East	−19.5 ± 0.3 (35) A	−18.5 ± 0.2 (35)	10.1 ± 0.2 (35) D	9.9 ± 0.2 (35) D
	West	−21.8 ± 0.4 (11) B	−20.2 ± 0.6 (10)	13.8 ± 0.3 (11) A	13.4 ± 0.2 (10) A
Summer	East	−19.8 ± 0.3 (79) AB	−18.0 ± 0.2 (79)	11.0 ± 0.2 (79) C	11.3 ± 0.1 (79) B
	West	−19.9 ± 0.3 (73) AB	−18.3 ± 0.2 (70)	12.6 ± 0.2 (73) B	12.8 ± 0.1 (70) A
Fall	East	−18.7 ± 0.4 (23) A	−18.4 ± 0.3 (23)	10.0 ± 0.2 (23) D	10.5 ± 0.2 (23) CD
	West	−19.9 ± 0.5 (8) AB	−18.2 ± 0.4 (8)	12.7 ± 0.3 (8) AB	11.4 ± 0.3 (8) BC
Interaction	Area × Season	$p < 0.05$	ns	$p < 0.01$	$p < 0.01$
Fixed	Area	East > west	ns	West > East	West > East
	Season	ns	Summer > Spring	ns	Spring, Summer > Fall

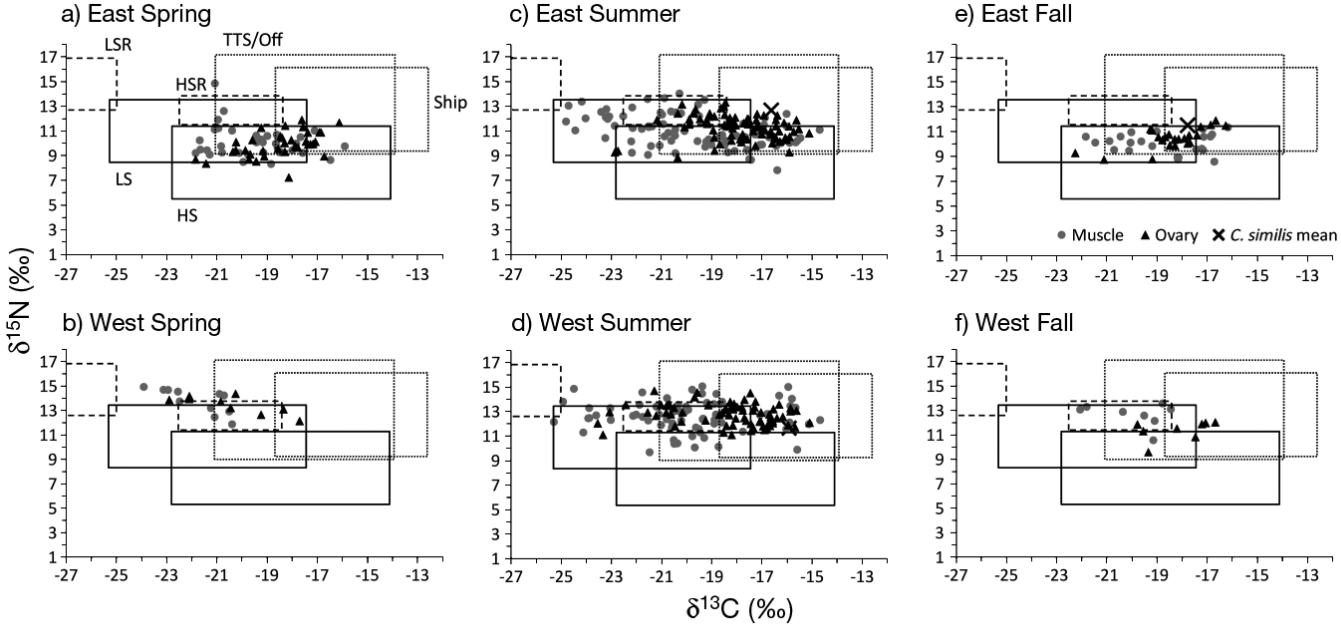

Fig. 4. *Callinectes sapidus.* Seasonal (spring, summer, fall presented from left to right) and spatial plots of carbon and nitrogen isotopes from blue crab muscle (●) and ovary (▲); (a,c,e) east and (b,d,f) west station groupings are delineated by Stns 19 and 23 (Fig. 1), respectively. Boxes are as defined in Fig. 2. Mean *C. similis* (✕) is plotted when available as a proxy for offshore, resident *C. sapidus*

spring, summer, and fall respectively. There was a consistent pattern of greater ovary ^{13}C enrichment relative to muscle when tissues were examined on a per crab basis. Seventy-five percent of crabs taken from the STTSC had ovary $\delta^{13}C$ values greater than those of muscle. There was also an area-based difference in the ovary to muscle spacing for shoal areas (2.4‰ ± 0.1) compared to off shoal (1.7‰ ± 0.2) over all seasons ($F_{1,216}$ =10.5; $p < 0.01$). We also found non-significant trends in seasonal differences in isotope spacing when comparing the mean spring (1.8‰ ± 0.2) summer (2.6‰ ± 0.1) and fall (1.6‰ ± 0.1) values over all areas.

There were generally consistent seasonal patterns in the $\delta^{13}C$, $\delta^{15}N$ bi-plots of east/west groupings in which crab values fell to the lower left for the east station grouping and to the upper left for the west station groupings i.e. $\delta^{15}N$ was higher in the west (Fig. 4). This pattern was especially evident in the spring and fall, with an increase in the spread of points (especially muscle values) in the summer. Fig. 5 provides a more specific illustration of the area based differences in muscle to ovary convergence patterns towards isotopic target values (i.e. mean *Callinectes similis* signature). Some paired muscle-ovary values converged vertically within the residency boxes. The

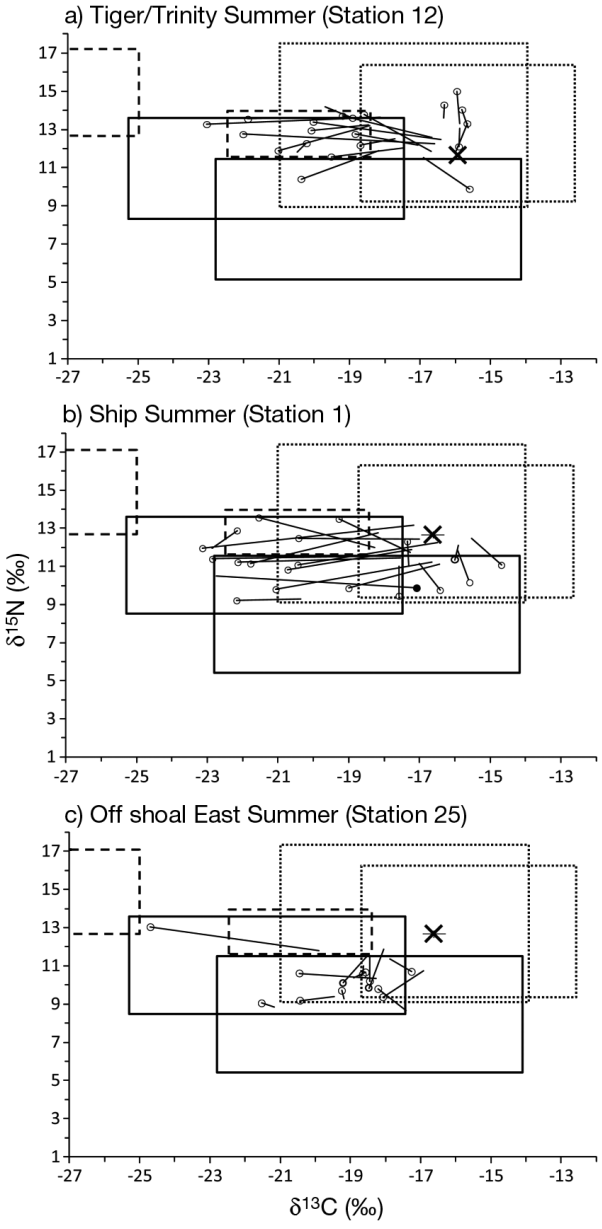

Fig. 5. *Callinectes sapidus.* Plots of muscle (dots) and ovary (tip of line connected to dots) isotopes for representative stations from summer for (a) Tiger/Trinity Shoals, (b) Ship Shoal, and (c) Off shoal east. Mean *C. similis* (✕ ± SEM) is plotted when available. Black dot represents an individual with a deviating migratory pattern (see 'Discussion'). Boxes are as defined in Fig. 2

east station group generally converged from below the $\delta^{15}N$ value of 11.6‰, and the west station group generally converged from above 11.6‰ (Fig. 5a,b).

Of 223 crabs, 130 had no acorn barnacles on their carapace (Table 4). The $\delta^{13}C$ of blue crab ovary tissue was significantly positively related to barnacle diameter (linear regression, $F_{1,222} = 10.02$; $p < 0.01$). A similar analysis with muscle tissue was not significant ($F_{1,224} = 1.7$; $p = 0.19$), though the same general pattern occurred between $\delta^{13}C$ of both tissues and barnacle diameter (Fig. 6a,b). The percentage of *Callinectes sapidus* whose muscle values were within the offshore benthic marine $\delta^{13}C$ range was greatest for crabs with the largest size barnacles, though ovaries were comparatively more enriched for every barnacle size interval. In addition, the percentage of crabs with $\delta^{13}C$ values within the offshore range increased with each barnacle size interval (Table 4). The $\delta^{13}C$ values from all east area crabs with largest barnacle diameter of 1 mm or greater fell within a range of −19.6 to −15.1‰ with a mean of −17.5‰, and values for all west area crabs fell within a range of −20.7 to −15.9‰ with a mean of −17.8‰.

Table 4. *Callinectes sapidus.* Total number of blue crabs whose largest acorn barnacle *Chelonibia patula* was within the respective size intervals, and the percentage of those individuals whose muscle and/or ovary was within the benthic marine offshore $\delta^{13}C$ range of −14 to −19‰

Width (mm) of largest observed acorn barnacle *Chelonibia patula*	*Callinectes sapidus* individuals (n)	Proportion of crabs with tissue within offshore $\delta^{13}C$ range (−14 to −19‰)	
		Muscle	Ovary
0 (no barnacles)	130	0.35	0.58
1–5	45	0.3	0.82
6–10	30	0.37	0.87
11–15	12	0.42	0.92
16–20	6	0.5	1

STTSC blue crab migratory patterns

Over all seasons, 77% of the east station group muscle values fell below the $\delta^{15}N$ value of 11.6‰; in contrast, 87% of western crabs were above 11.6‰ (Fig. 4). Seventy percent of Tiger/Trinity and off shoal individuals' muscle and ovary values were within the TTS/Off box, although there was some overlap with the TTS/Off box and inshore boxes (Fig. 4), likely due to TTS proximity to the Atchafalaya river. Twenty-three percent of Ship Shoal crabs' muscle and ovary tissue isotope values were within the smaller offshore Ship Shoal box, which is probably a better estimator of an offshore residency range (i.e. $\delta^{13}C$ of approximately −19 to −14) than the larger TTS/Off box because

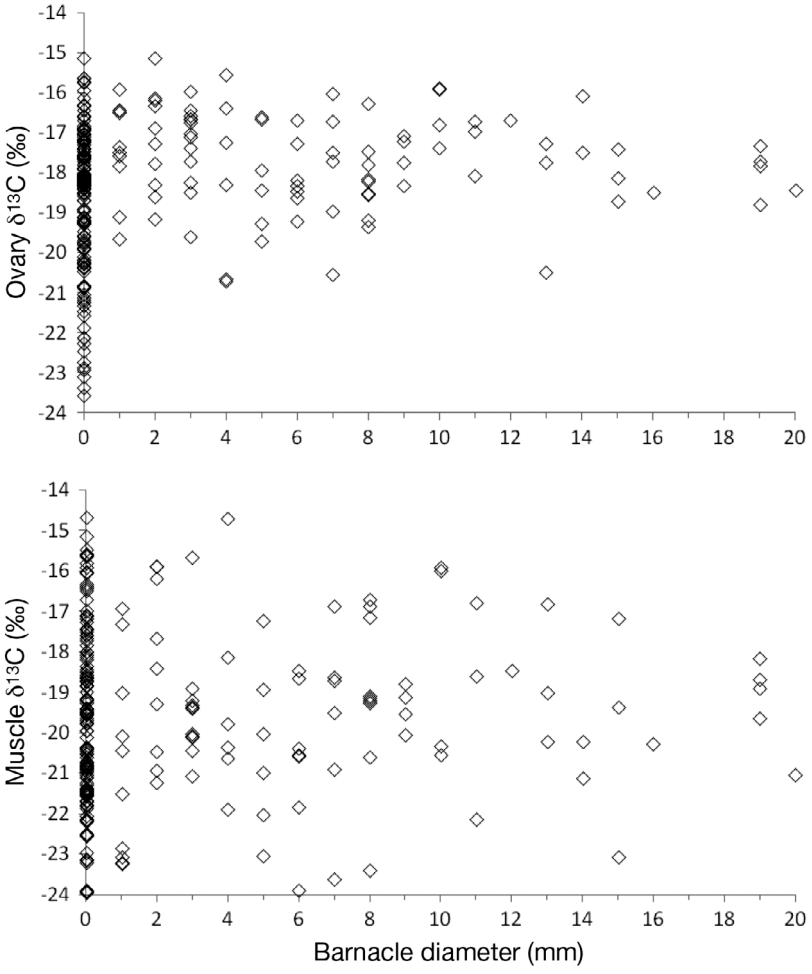

Fig. 6. *Callinectes sapidus.* Relationship of Ship, Tiger, Trinity Shoal Complex (STTSC) blue crab $\delta^{13}C$ (a) ovary and (b) muscle with growth of the epibiont acorn barnacle *Chelonibia patula*

of a reduced riverine isotopic influence at Ship Shoal. Though the largest concentration of crabs in the STTSC was in summer, there was a seasonal increase in the proportion of Ship Shoal crabs that fell within the Ship Shoal box with 13, 25 and 30% of crabs for spring, summer, and fall, respectively, and a seasonal increase of 59, 71, and 79%, respectively, for Tiger/Trinity/Off shoal crabs that fell within the TTS/Off box. Because isotopic gradients occur in relation to changing salinity, we were able to estimate the percentage of crabs from high versus low salinity estuarine areas. The distinction between salinity regimes was especially apparent in the slower turnover muscle tissue where a portion (15%) of summer-caught crabs had muscle $\delta^{13}C$ values indicative of low salinity marsh outside the range of the meso-polyhaline estuaries delineated here by $\delta^{13}C$ values < -22.8‰ (Fig. 4).

DISCUSSION

Migratory dynamics

The results of this study support the prediction that most STTSC female blue crabs migrate to offshore spawning grounds and engage in a continuous spawning/hatching cycle, and do not undertake a back and forth spawning migration between marine and estuarine areas. This conclusion is based on 3 observations: (1) consistent ovary $\delta^{13}C$ enrichment compared to muscle $\delta^{13}C$ that suggests values change with increasing time on the shoals, (2) *Callinectes sapidus* tissue isotope composition converged on that of an offshore resident (*C. similis*) with a similar diet, and (3) correlations between crab isotope composition and body size of an epibiotic barnacle (*Chelonibia patula*) that recruits to the crab carapace only in high salinity water.

Crab isotopes and epibiont analyses suggest that the blue crabs we sampled from the STTSC were not composed of a resident offshore population that had persisted from a previous spawning season, but rather represent a new class of spawning females recently migrated from inshore estuaries. If the crabs taken from our study area were part of a long-term (on the order of many months) resident population, then they would have had very similar ovary and muscle $\delta^{13}C$ — which probably would not have a consistent convergence pattern — and both tissues would be centered near the offshore values of −17 to −18‰. However, the more rapid turnover ovarian tissue of STTSC crabs (~21 d; Gelpi et al. 2009) was typically enriched in ^{13}C compared to the slower turnover muscle tissue. A muscle to ovary convergence pattern is evident (Fig. 4), with the ovary values trending into a narrower range and seemingly pointing towards the isotopic proxy for offshore residence (i.e. *Callinectes similis*) that lies within the offshore residency boxes (e.g. Fig. 5a,b). This suggests a net inshore to offshore movement of female blue crabs based on previously established patterns of isotopic change for other migratory species such as brown shrimp (Fry et al. 2003, Fry 2011).

These results also support the prediction that blue crabs are generally migrating directly offshore from their home estuary and not engaging in an alongshore migration. We base this on the general consistency in crab tissue isotopic composition with that of estuaries closest in proximity to their place of capture (Fig. 4) such that tissues from western grouping crabs had high $\delta^{15}N$ consistent with local Atchafalaya Bay origins and eastern-group crabs had lower $\delta^{15}N$ consistent with Terrebonne Bay origins, reflecting a changing east-west isotopic seascape or 'isoscape'. Despite a west-flowing longshore current in the region, STTSC blue crabs are generally moving in a seaward direction and minimize the east-west migratory distance away from source estuaries. This is in contrast to tagging studies from the Gulf of Mexico east of Apalachee Bay where crabs migrated, long distances in some cases, northwest along the Florida coast (Oesterling 1976, Steele 1987).

In addition to low $\delta^{13}C$ from terrestrial freshwater sources, carbon from primary producers in lower salinity portions of estuaries are also sources of low $\delta^{13}C$, providing a natural isotopic label in comparison to typical marine values. Many blue crabs from the STTSC had low $\delta^{13}C$, particularly in the slower turnover muscle tissue (Table 3, Fig. 4), suggesting that their migration originated from mid-salinity bay environments (Deegan & Garritt 1997) as well as inshore low salinity marsh and/or coastal areas near freshwater input (Fry 2002, 2011, Bucci et al. 2007). This provides evidence that some females undergo a rapid seaward migration occurring on the order of days, from fresher inshore estuaries. This is within their migratory capability, based on an average movement estimate of 5.4 km d^{-1} for females prior to hatching their eggs (Carr et al. 2004). A rapid spawning migration from fresh inshore marsh for Louisiana female *Callinectes sapidus* is in contrast to migratory behavior from higher latitude Atlantic coast estuaries such as Delaware and Chesapeake Bays, where females overwinter in high concentrations in polyhaline zones (Hines 2007 and references therein). Thus, our results call into question whether or not seasonally separated Phase I (i.e. movement from mating locations to the lower estuary before brood production), and Phase II (i.e. movement to the mouth of, or slightly seaward from the estuary; Tankersley et al. 1998) migratory patterns of the US mid-Atlantic coast should be extrapolated to Gulf of Mexico blue crabs.

There were anomalous isotopic patterns for a few STTSC stations and crabs. One example was Stn 25 — the station closest to estuarine shorelines (Fig. 1). Crabs at this station did not consistently display the same isotope convergence towards an offshore $\delta^{13}C$ range, though $\delta^{15}N$ values were consistent with crabs from the eastern station grouping (Fig. 5c). It is possible that re-entry to the estuary is a behavior found in crabs that remain closer inshore, and differs from that of crabs taken from areas such as the STTSC shoals, which lie approximately 25 to 50 km offshore. The variation in muscle to ovary tissue isotopic patterns, such as seen in crabs from Stn 25, could be due to movement in and out of tidal passes, and thus reflects changes in isotopic values that occur over small geographic scales. Another example was the individual from Ship Shoal with a high muscle and low ovary $\delta^{13}C$ (Fig. 5b; Stn 1, black dot). This pattern is consistent with a crab that migrated to the offshore and remained long enough for the slower turnover muscle tissue to equilibrate with offshore isotopic values, then returned to an inshore estuary long enough for the ovary but not muscle to equilibrate, and then migrated to the offshore again where it was caught as a recent immigrant.

It is possible that because STTSC blue crabs were actively spawning, newly acquired energy was allocated more towards ovarian replenishment and less to muscle maintenance. Because female blue crabs do not grow following their terminal molt (Churchill 1919), energy allocation is only to maintain muscle tissue and not growth. If true, the muscle may incorporate the offshore $\delta^{13}C$ signal more slowly, and offshore residence could be masked (isotopically speaking). However, isotopic evidence suggesting the STTSC crabs are relatively new arrivals to the offshore is congruent with epibiont data. There was a correlation between barnacle presence and size with a reduction in the $\delta^{13}C$ range of crab tissue (Fig. 6), providing corroborating evidence that convergence to offshore isotopic values occurs for blue crabs. In addition, only one crab from our spring collections had acorn barnacles attached to the carapace; heavy fouling by epibionts would be expected if crabs had spent much time in a high salinity environment such as the STTSC (Table 2, Table S1).

The summer is evidently a prime migration period to the offshore waters within the STTSC, and the time when crab abundance on shoals was highest. The average muscle to ovary isotopic spacing as well as the spread of isotopic values, especially muscle, is greatest during the summer season (Fig. 4). Lower flow of the Mississippi/Atchafalaya Rivers in summer may allow source areas to diverge more in their isotopic signals. Increased flow of the Mississippi River

in the spring may be responsible for making all areas fresher, while decreased flow after spring likely accounts for a seasonally shifting isoscape. Thus, increased variation in summer crab tissues could reflect changes in isotopic values that occur over small geographic scales — such as those between estuarine ponds, channels, and bays (Fry et al. 2003). Another explanation for greater summertime isotopic heterogeneity is an increase in cross-shelf exchange of crabs from source locations to offshore spawning grounds, possibly because crabs are seeking out high relief shoals as a hypoxia refuge (Table S1, Gelpi 2012).

Importance of the STTSC spawning ground to the Gulf of Mexico blue crab fishery

Our studies within the STTSC have shown Ship, Trinity, and Tiger Shoals peak catch rates are comparable with other well-studied blue crab spawning grounds, such as the lower Chesapeake Bay (Gelpi et al. 2009). Examples in other estuaries have shown that protection of densely populated spawning grounds is extremely important for the viability of the fishery. Beginning around 1991, the Chesapeake Bay blue crab fishery began a period of historically low yields. This decline was highlighted by an 84% decline in mature females (Lipcius & Stockhausen 2002). The recent recovery in the Chesapeake's blue crab fishery was correlated with a decreased fishing effort that targeted migrating females, an end to the winter blue crab dredge fishery targeting females, and greater protection of the Chesapeake Bay blue crab spawning grounds through an expansion of the lower bay spawning sanctuary (Pala 2010). The number of females now residing within the blue crab spawning sanctuary is estimated to be 70% of the total Chesapeake Bay adult female population (Lambert et al. 2006).

Isotopic analysis suggests that there is a direct estuarine-offshore link between STTSC spawning blue crabs and the Louisiana inshore blue crab spawning stock, which supports Louisiana's blue crab fishery valued at approximately 35 million dollars a year (NOAA 2011). Using the known salinity threshold for proper blue crab zoeal development of greater than 20 (Sandoz & Rogers 1944, Costlow & Bookhout 1959) and the areal extent of annual mean salinity greater than 12 from Barrett et al. (1971), we estimate that shoal areas within the STTSC comprise at least 20% of the known blue crab spawning grounds west of the Mississippi River[1].

The recent discovery of large concentrations of spawning blue crabs *Callinectes sapidus* within the STTSC (Gelpi et al. 2009) has not yet resulted in the protection of this largely unexploited population, despite the likelihood that it is a substantial component of the current fishery's spawning biomass. The shoals have been earmarked for sand mining operations, which have recently begun on Ship Shoal (Coastal Protection and Restoration Authority of Louisiana 2012) and could adversely affect the blue crab spawning and foraging habitat. It is also unknown what impacts the Deepwater Horizon oil spill has had, and will continue to have on the habitats and populations within the STTSC. These shoal-based spawning grounds likely benefit the Louisiana blue crab fishery as well as neighboring coastal states along the northern Gulf of Mexico coast. More studies are needed to resolve the extent that females from the STTSC, and shoal areas in particular, are supplying larvae that benefit the inshore fisheries.

CONCLUSIONS

Using nitrogen and carbon natural abundance isotopes, we were able to identify a coastal east-west isoscape based on proximity to the Atchafalaya River, which suggests that female blue crabs are generally migrating in a south-southwesterly direction from source estuaries and concentrating on shoals nearest to those estuaries. Once female blue crabs have migrated to the STTSC, they generally do not continue in a back and forth migratory pattern during the spawning season, but rather remain in the offshore environment in a continuous cycle of spawning and hatching throughout the remainder of that spawning season.

This use of isotopes is a novel approach to assess blue crab population dynamics. Migratory studies of blue crabs have traditionally relied on tagging studies, which are dependent on commercial and recreational fishers finding and accurately reporting the necessary information, often resulting in a low return

[1]Calculated using the proportion of high salinity water zones within 5 different Louisiana coastal areas (5 through 9) delineated in Barrett et al. (1971) versus the combined area of Ship and Trinity Shoals from Gelpi et al. (2009). Area estimates in Barrett et al. (1971, p. 123–124) were interpolated by planimetering water areas within isohalines from upper fresh marsh out to Louisiana barrier islands. We created a grid within the >12 ppt isohaline including marsh, counted the number of grid squares and compared it with the number of grid squares overlain on Ship and Trinity Shoals.

of tagged individuals (Cronin 1949). This new isotope approach has demonstrated a migratory extension of blue crab life history that is important not only for the northern Gulf of Mexico blue crabs, but also potentially useful for gaining knowledge on any estuarine dependent species of ecological/economic interest worldwide.

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