Size-based trophic shifts of saltmarsh dwelling blue crabs elucidated by dual stable C and N isotope analyses

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ABSTRACT: Analyses of stable C and N isotopes were used to examine trophic characteristics of blue crabs Callinectes sapidus in relation to body size (15 to 165 mm carapace width, CW) within saltmarsh habitats of the Aransas National Wildlife Refuge, Texas. Blue crab trophic position did not change with increasing body size, and all size classes appear to consume primary consumers and primary producers in relatively equal proportions. However, blue crab δ13C values increased significantly with increasing body size. As body size increased, blue crabs assimilated greater proportions of carbon ultimately derived from Spartina alterniflora. Individuals of approximately 35 mm CW assimilated carbon almost exclusively derived from C3 plants and benthic algae, whereas larger individuals (125 mm CW) assimilated similar amounts of carbon derived from benthic algae and S. alterniflora-derived detritus. Given the complex nature of saltmarsh food webs and the omnivorous diet of blue crabs, the observed size-based trends have important management and conservation applications.

KEY WORDS: Callinectes sapidus · Food webs · Omnivory · Benthic algae · Spartina alterniflora · Whooping crane · Conservation

INTRODUCTION

Blue crabs Callinectes sapidus are important consumers in estuarine food webs from the northern Atlantic coast of the United States as far south as Argentina. As opportunistic foragers, blue crabs consume a diversity of food items including crustaceans, gastropods, fish, bivalves, detritus, algae, vascular plants, zooplankton, and infauna, with the relative importance of each related to its availability in the environment, habitat type, and body size (Laughlin 1982, Alexander 1986, Fitz & Wiegert 1991, Rosas et al. 1994, Meise & Stehlik 2003). In marsh habitats, strong predation pressure by large blue crabs may cause trophic cascades, preventing top-down control of marsh grasses by grazers such as marsh periwinkle Littoraria irrorata (Silliman & Zieman 2001, Silliman & Bertness 2002).

The relative importance of primary production sources ultimately supporting estuarine consumers may depend on factors such as feeding mode, trophic position, habitat type, and location within the estuary (Peterson et al. 1985, Peterson & Howarth 1987, Degan & Garritt 1997, Dittel et al. 2000). Although blue crab diet has been fairly well studied, the relative importance of dietary components supporting growth may differ greatly from the items’ observed frequencies in stomach contents. The relative importance of carbon sources that ultimately support blue crab growth has only been determined for early life stages in few habitat types (Fantle et al. 1999, Dittel et al. 2000). This study complements earlier work on blue crab trophic ecology and fills an important gap by elucidating primary production sources supporting larger, marsh-dwelling crabs. We used dual stable C and N isotope analyses to examine size-based trends in
trophic position and the relative contributions of carbon sources supporting juvenile and adult blue crabs (15 to 165 mm carapace width) within a heterogeneous saltmarsh.

**MATERIALS AND METHODS**

**Field collection and sample processing.** Blue crabs *Callinectes sapidus* and basal carbon sources were collected from 2 saltmarsh locations of the Aransas National Wildlife Refuge (ANWR), Guadalupe estuary system, Texas, during a 2 d period in May 2004. This natural saltmarsh system is characterized by a narrow band of *Spartina alterniflora* in the low intertidal zone, transitioning to a high intertidal marsh composed primarily of succulent halophytes. The high marsh zone also contains numerous ponds that are intermittently flooded with tidal waters from nearby creeks and bays. The mean diurnal tidal range of our study locations was approximately 12 cm (Zeug et al. 2007).

At each location, we sampled 3 closely positioned habitat types: tidal creek, connected pond, and isolated pond. Representative saltmarsh vegetation including both *C*$_3$ succulents and *C*$_4$ grasses was collected at each location. For each sample, several leaves were collected from the same plant, with a minimum of 3 replicates (plants) collected at random positions interspersed among the habitat types at both locations. Samples of benthic algae were collected by carefully removing the thin upper layer of visible mats on submerged surfaces at each habitat type at both locations. Flocculated detritus was collected in a similar manner. Seagrass *Ruppia maritima* was collected from tidal creek and connected pond habitats, but was not present in isolated ponds. All samples were placed in separate sterile bags, labeled, stored on ice, and frozen upon return to the ANWR laboratory.

Blue crabs were collected by seineing and dip netting in each habitat type at both locations. An effort was made to collect individuals of as many different sizes as possible for each habitat. Following collection, crabs were placed immediately on ice for processing at the refuge station later the same day. Because isotopic signatures of crustacean exoskeletons reflect assimilated calcium carbonate derived from the environment, only pure muscle samples were used for stable isotope analysis. Carapace width (CW) of each crab was measured to the nearest 0.1 mm using dial calipers, and a sample of muscle tissue was carefully removed with a scalpel, placed in separate, labeled, sterile bags, and frozen. To obtain pure muscle samples for the smallest size classes, i.e., <30 mm CW, a composite sample was taken by combining muscle tissue from 5 to 15 individuals of similar size collected at the same site.

In the laboratory, samples were thawed, rinsed with distilled water, inspected to remove any contaminants (e.g., shell fragments, periphyton on plant samples), and dried at 60°C to constant weight (minimum of 48 h). Dried samples were ground to a fine powder using a mortar and pestle. Subsamples weighed to 10$^{-4}$ g were pressed into Ultra-Pure tin capsules (Costech Analytical Technologies) and sent to the Analytical Chemistry Laboratory at the University of Georgia, for analysis of carbon and nitrogen isotope ratios. Results of isotopic analysis for each sample are expressed in delta notation (% deviation from a standard material): $\delta^{13}C$ or $\delta^{15}N = \left[(R_{\text{sample}}/R_{\text{standard}}) - 1\right] \times 1000$, where $R = 13C/12C$ or $15N/14N$. The standard material for carbon is Pee Dee Belemnite (PDB) limestone, and the nitrogen standard is atmospheric nitrogen. Standard deviations of $\delta^{13}C$ and $\delta^{15}N$ replicate analyses were 0.28 and 0.12‰, respectively.

**Data analyses.** Blue crab trophic position (Tp) was estimated using the formula: $\text{Tp} = 1 + \left(\delta^{15}N_{\text{blue crab}} - \delta^{15}N_{\text{base}}\right)/F$, where $\delta^{15}N_{\text{blue crab}}$ is the nitrogen signature of the individual being evaluated, $\delta^{15}N_{\text{base}}$ is the nitrogen signature representing the base of the food web (mean $\delta^{15}N$ value of all sources excluding *Borrichia frutescens*, which likely fixes or obtains nitrogen from a different source pool, see ‘Results’), and $F$ is the per trophic level fractionation of nitrogen. We used a $\delta^{15}N$ fractionation of +2.5‰, following the meta-analysis of Vanderklift & Ponsard (2003).

Analysis of variance (ANOVA) was used to compare mean C and N isotopic signatures among sources. When a significant main effect was observed, pairwise comparisons of source means were performed using Tukey’s post hoc procedure. Mean blue crab $\delta^{13}C$ and $\delta^{15}N$ values were compared among habitat types (tidal creek, connected pond, isolated pond) using the same procedure. Linear regression was used to examine relationships between crab size (mm CW) and $\delta^{13}C$ and $\delta^{15}N$ values. The above analyses were performed using SPSS v12.0.1.

The software package IsoSource (Phillips & Gregg 2003) was used to calculate ranges of source proportional contributions to observed blue crab isotopic signatures, because the number of potential sources in our system was too large to permit a unique solution (i.e., $n_{\text{sources}} > n_{\text{isotopes}} + 1$). This procedure uses mean isotope values to calculate all possible contributions from 0 to 100% for each source using small increments, with combinations summing to within a selected mass balance tolerance of the consumer isotope signature retained as feasible solutions. The analysis was performed using mean C and N isotopic compositions, correcting for N fractionation, of blue crab size classes and source groups (*C*$_3$ succulents excluding *Borrichia frutescens*, *C*$_4$ grasses, benthic algae, seagrass, and...
flocculated detritus), with source increments of 1% and mass balance tolerance of 0.03‰. Distributions of feasible contributions, means and the 1 to 99 percentile range are reported for each source (Phillips & Gregg 2003).

RESULTS AND DISCUSSION

Carbon isotopic compositions of sources were well differentiated, with mean δ13C values of –27.3, –20.4, –16.2, –12.8, and –11.7‰ for C3 succulents, benthic algae, seagrass, C4 grasses, and detritus, respectively (Table 1). Flocculated detritus isotope ratios were not significantly different from those of C4 grasses, suggesting that marsh detritus was predominantly composed of material derived from smooth cordgrass Distichlis spicata because it accounts for a much greater proportion of primary producer biomass than the other marsh grass Distichlis spicata. Benthic algae δ13C and δ15N were much more variable than other sources. Sources were not well differentiated by N isotope ratios, except that Borrichia frutescens had a much lower mean δ15N value (0.9‰) than other sources (Table 1), perhaps due to nitrogen fixation or by obtaining nitrogen from a different source pool. The seagrass Ruppia maritima had the highest observed mean δ15N value (8.3‰), which may reflect freshwater inflow to the system.

Among habitat differences in blue crab Callinectes sapidus, mean C and N isotope ratios were not significant (p = 0.093 and p = 0.474, respectively). However, statistical power may have been too low to detect a significant difference in blue crab mean δ13C values among habitats, due to low sample sizes (observed power = 0.469). Blue crabs collected in isolated ponds had slightly depleted δ13C values relative to blue crabs collected in tidal creek and connected pond habitats. The mean carbon isotope ratio of the aquatic community (fishes, shrimps, and crabs combined) in isolated ponds was significantly depleted compared to tidal creek and connected pond habitats (Davis & Hoeinghaus unpubl. manuscript), likely due to differences in dissolved inorganic carbon sources among habitat types associated with decomposition processes (Zieman et al. 1984, Keough et al. 1998).

Mean blue crab trophic position was 2.59 ± 0.38 (between primary and secondary consumers), reflecting the omnivorous diet characterized by earlier studies using stomach contents analysis. Although a direct relationship exists between trophic position and body size for many taxa (Cohen et al. 1993), blue crabs of all sizes occupied approximately the same trophic position in this study (Fig. 1a), feeding on primary producers and primary consumers in relatively equal proportions. Because large blue crabs feed at similar trophic positions as smaller individuals, predatory fishes such as red drum Sciaenops ocellatus that feed heavily on blue crabs (Scharf & Schlicht 2000) may exploit short, ecologically efficient food chains while at the same time consuming optimal prey sizes.

Although trophic position did not change with body size, a significant relationship was observed between carapace width and δ13C (R² = 0.45, p = 0.001; Fig. 1b), indicating a general size-based shift in the relative importance of carbon sources supporting blue crabs. The same shift was observed for each habitat even though blue crab mean δ13C was slightly depleted in isolated ponds compared to the other habitats (Fig. 1). Based on regression estimates, blue crabs of 35, 80, and 125 mm CW have δ13C values of –21, –19, and –17‰, respectively. Increasing δ13C, even when only by 2‰, represents significant changes in the relative contributions of carbon sources that ultimately support blue crabs. In general, as blue crabs increased from 35 to 80 to 125 mm CW, the mean amount of dietary carbon ultimately derived from flocculated detritus increased from 10.1 to 13.7 to 33.1% (Fig. 2). This increase in carbon derived from detritus is concomitant with changes in the contributions of other sources. For example, the estimated contribution of carbon derived

<table>
<thead>
<tr>
<th>Source</th>
<th>n</th>
<th>δ13C</th>
<th>Homog. subsets</th>
<th>δ15N</th>
<th>Homog. subsets</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 marsh succulents</td>
<td>27</td>
<td>–27.3</td>
<td>5.4 ± 1.7</td>
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<td></td>
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<tr>
<td>Borrichia frutescens</td>
<td>7</td>
<td>–28.7</td>
<td>0.9 ± 1.0</td>
<td>a</td>
<td>a</td>
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<tr>
<td>Batis maritima</td>
<td>6</td>
<td>–26.2</td>
<td>7.0 ± 1.4</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Carex sp.</td>
<td>3</td>
<td>–27.9</td>
<td>5.9 ± 0.3</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Salicornia virginica</td>
<td>6</td>
<td>–28.6</td>
<td>5.6 ± 1.3</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Aster tenuifolius</td>
<td>6</td>
<td>–27.4</td>
<td>4.5 ± 0.3</td>
<td>a, b</td>
<td></td>
</tr>
<tr>
<td>Lycium carolinianum</td>
<td>6</td>
<td>–26.8</td>
<td>4.1 ± 2.0</td>
<td>a, b</td>
<td></td>
</tr>
<tr>
<td>Benthic algae</td>
<td>16</td>
<td>–20.4</td>
<td>4.9 ± 4.2</td>
<td>a, b</td>
<td></td>
</tr>
<tr>
<td>Seagrass Ruppia maritima</td>
<td>9</td>
<td>–16.2</td>
<td>8.3 ± 1.7</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>C4 marsh grasses</td>
<td>12</td>
<td>–12.8</td>
<td>6.3 ± 1.2</td>
<td>c, d</td>
<td></td>
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<tr>
<td>Distichlis spicata</td>
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<td>–13.1</td>
<td>5.9 ± 1.0</td>
<td>b</td>
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<tr>
<td>Spartina alterniflora</td>
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<td>–12.6</td>
<td>6.8 ± 1.3</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>Flocculated detritus</td>
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<td>–11.7</td>
<td>6.0 ± 1.7</td>
<td>b</td>
<td></td>
</tr>
</tbody>
</table>
from C₃ succulents decreased dramatically with increasing body size (Fig. 2).

While particulate organic carbon derived from C₃ succulents occurring in the high marsh may be consumed directly or assimilated through consumption of zooplankton or bivalves, the importance of this source suggested for smaller blue crabs by our analysis may also be due to variation in isotope ratios of algae and the use of mean values when calculating source contributions. Because the mean δ¹³C of benthic algae was −20.4‰, contribution of a more depleted carbon source (i.e. C₃ succulents) is necessary to achieve mass balance for 35 mm CW blue crabs (δ¹³C = −21‰). However, even the smallest blue crabs had δ¹³C values within 1 SD of the mean δ¹³C of benthic algae. Several estuarine food web studies have found that algae and C₄ marsh grasses such as Spartina alterniflora are principal carbon sources, whereas C₃ succulents provide little or no contribution to saltmarsh consumers (Currin et al. 1995, Deegan & Garriott 1997, Kwak & Zedler 1997, Moncreiff & Sullivan 2001, Kang et al. 2003). Smaller blue crabs were common in inner-marsh areas dominated by C₃ succulents, supporting the importance of this source illustrated by our mixing model.

Analysis of organic C:N ratios suggests that large blue crabs, ultimately supported primarily by carbon derived from benthic algae, Spartina alterniflora, and detritus (which is predominantly derived from Spartina), may feed either directly or indirectly on benthic algae, while probably only feeding indirectly on Spartina, due to its relatively low nutritional quality (C:N =12.56 for algae vs. C:N = 36.22 for live Spartina). Assimilation of S. alterniflora-derived carbon likely occurs through consumption of grazers such as marsh periwinkle Littorina irrorata (δ¹³C = −17.3‰, C:N = 4.02) and shrimp (δ¹³C = −17.5‰, C:N = 3.23), and of detritus (δ¹³C = −11.7‰, C:N = 11.69). A diet dominated by algae and primary consumers feeding on detritus would result in the observed trophic position and relative carbon source contribution for large blue crabs, while at the same time maximizing the nutritional quality of the diet. Smaller individuals, almost completely supported by algae and C₃-plant-derived carbon, may feed on large amounts of benthic algae, as well as on zooplankton or bivalves to optimize dietary quality.

Fantle et al. (1999) and Dittel et al. (2000) found blue crab megalopae and juveniles in open-water estuarine habitats to assimilate carbon ultimately derived from phytoplankton, shifting to diets incorporating benthic algae or Spartina alterniflora in marsh habitats. Our results illustrate that this general shift continues as blue crab body size increases, and large, marsh-dwelling adults assimilate large amounts of S. alterniflora-derived carbon at our sites.

In addition to important commercial and sport fishes such as red drum Sciaenops ocellatus (Scharf & Schlicht 2000), blue crabs are an important food resource of the endangered whooping crane Grus americana that over-winters in the Aransas National Wildlife Refuge (Chavez-Ramirez 1996). From late October to early April of each year, blue crabs comprise from 62 to 98% of whooping crane energy intake (Chavez-Ramirez 1996), and behavioral studies suggest that cranes preferentially select larger individuals (LaFever 2006). Given the omnivorous nature of blue crabs and the complexity of saltmarsh food webs, this size-based trend in relative importance of energy sources supporting blue crab secondary production has great utility for estuarine ecosystem conservation and resource management.

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Fig. 2. *Callinectes sapidus*. Source contributions resulting in mass balance for consumer $\delta^{13}C = -21$, -19, and -17‰ (representing blue crabs of ~35, 80, and 125 mm CW, respectively). For each source, the distributions of feasible contributions, mean percent contribution, and the 1st to 99th percentile range (in parentheses) are reported.
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