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Stable Isotopic Assessment of Site Loyalty and Relationships Between Size and Trophic Position of the Atlantic Horseshoe Crab, *Limulus polyphemus*, within Cape Cod Estuaries

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Carmichael *et al.* (1) used stable isotope analysis to define the food webs of horseshoe crabs, *Limulus polyphemus*, in Pleasant Bay, Cape Cod, Massachusetts, and also found that these animals forage within relatively small areas of estuaries. In this study, we used field measurements and stable isotope analysis (2) to determine whether the trophic position and site loyalty described by Carmichael *et al.* (1) in Pleasant Bay were also found in two additional Cape estuaries, Stage Harbor and Barnstable Harbor, and to determine how the trophic position of horseshoe crabs might change as adult crabs grow.

Increased population densities and the resulting anthropogenic wastes within watersheds increase nitrogen loads to estuaries (2). Pleasant Bay is part of the Cape Cod National Seashore Reserve and has a low nitrogen load due to the lack of developed land in its watershed (1). Stage Harbor and Barnstable Harbor have relatively greater population densities in their watersheds, and as reported elsewhere in Cape Cod (2), should have greater relative nitrogen loads. The land-derived nitrogen loading rates to the three estuaries are likely to rank in the order of Stage Harbor > Barnstable Harbor > Pleasant Bay (3, 4). Since increased urban development results in heavier nitrogen signatures of estuarine water and biota (2), we opted to study these estuaries with different land use in their watersheds to take advantage of potential resulting differences in δ^{15}N and δ^{13}C signatures (5, 6).

We collected samples during July 2003 at a site in Barnstable Harbor and three sites in Stage Harbor in Cape Cod. We measured the size of horseshoe crabs as the width at the widest region of the prosoma (7). To obtain samples for isotope analysis, we sampled tissue from the last two segments of the second or third walking leg of adult horseshoe crabs (1). To identify some potential foods of horseshoe crabs, we also sampled quahogs (*Mercenaria mercenaria*), polychaetes (*Nereis* sp., *Nephtys* sp., and *Glycera* sp.), seston filtered from 1-l water samples, and sediment from two 10-ml sediment cores taken 3 cm deep, which were pooled into a single sample. All samples were dried at 60°C, ground, and sent to the Stable Isotope Facility, University of California, Davis, for mass spectrometry.

The δ^{13}C signatures of the horseshoe crabs suggest that their diet may have included a mix of polychaetes and quahogs (Fig. 1a). Quahogs, in turn, assimilated carbon from a mixture of seston and sediment, judging from their position relative to values on the δ^{13}C axis. The carbon in seston and sediment was likely initially derived from phytoplankton and macroalgal organic particulates (gray lines in Fig. 1a) (8). Polychaetes seemed to belong to a separate branch of the food web, since they had relatively heavier signatures, and most likely incorporated a mix of carbon from macroalgae and *Spartina* grass (Fig. 1a). These δ^{13}C values are consistent with carbon signatures found in Pleasant Bay (1).

δ^{15}N signatures suggested that the horseshoe crabs consumed a combination of quahogs, polychaetes, and particulate organic matter, given the expected fractionation from food source to consumer of 2‰–4‰ (Fig. 1a) (2). δ^{15}N values for bivalves and polychaetes were 2‰–5‰ heavier than those of particulate matter. In all cases, the δ^{15}N values for each taxon (shown by points within dashed ovals, Fig. 1a) seem to be heavier in Stage Harbor than in Barnstable Harbor, suggesting, as suspected from the different land-use patterns, heavier land-derived nitrogen sources from freshwater inputs into Stage Harbor (5, 6).

Isotopic signatures of individual crabs varied considerably. Part of the variation was associated with different crab size; larger crabs tended to have significantly heavier δ^{15}N signatures ($F = 9.5$, $P = 0.004$) and lighter δ^{13}C values ($F = 13.94$, $P = 0.0005$) (Fig. 1b, c). The increased δ^{15}N signatures may be related to increased prey size among larger crabs (9). The slopes of the regression lines in Figure 1b did not differ, but the intercepts were significantly different (ANOVA: $F = 9.26$, $P = 0.004$). The offset between the regressions shows that Stage Harbor crabs had a heavier δ^{15}N signature than Barnstable Harbor crabs. In Figure 1c, we added a gray reference area that represents the prosomal width and δ^{15}N values for crabs from Pleasant Bay (1), the most pristine of the three estuaries. The Pleasant Bay values were appreciably lighter than those of either Stage Harbor or Barnstable Harbor (Fig. 1b). These isotopic comparisons suggested that the watershed-derived nitrogen loads (and contribution by wastewater) were greater in Stage Harbor than in Barnstable Harbor and that both these estuaries received greater anthropogenic nitrogen loads than Pleasant Bay.

The significant difference in the isotopic signature of horseshoe crabs collected in the three estuaries can also be interpreted to mean that crabs tend to remain foraging in an estuary long enough to acquire δ^{15}N values characteristic of the estuary in which they were found. These results corroborate earlier observations (1) that in spite of the evident mobility of horseshoe crabs, they do tend to remain within relatively circumscribed areas for considerable periods of time.

The δ^{13}C values provided additional information about the foraging and feeding behavior of horseshoe crabs (Fig. 1c). δ^{13}C became lighter as size of crabs increased ($F = 13.93$, $P = 0.0005$) (Fig. 1c). As adult horseshoe crabs grew, signatures shifted from values near the δ^{13}C for the *Spartina*-based side of the food web to values more closely associated with phytoplankton (dashed boxes in Fig. 1c, and Fig. 1a). This transition suggests that as adult crabs grew, a larger percentage of their diet consisted of

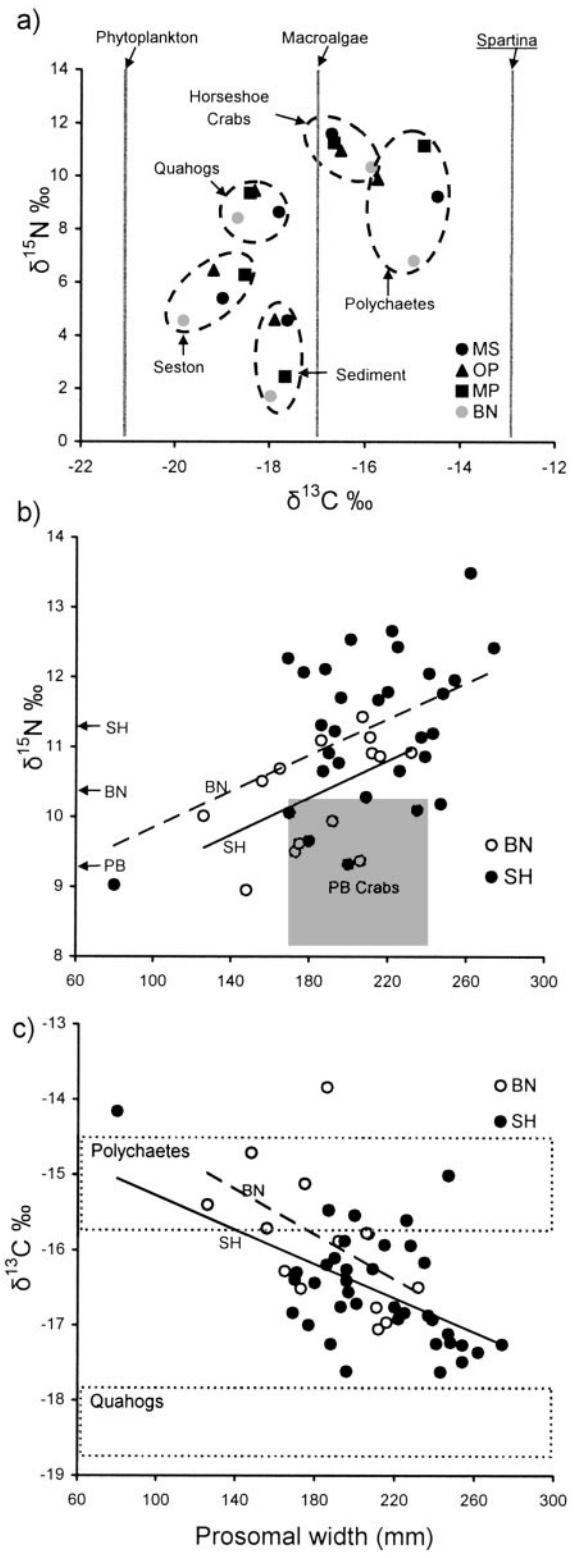

Figure 1. *Isotopic measurements of samples collected in Barnstable Harbor (BN), Stage Harbor (SH), and Pleasant Bay (PB), Cape Cod, Massachusetts. (a) $\delta^{15}N$ and $\delta^{13}C$ signatures of horseshoe crab and other components of the estuary system of BN and SH. The dashed ovals include measurements taken in BN and the three other sites within SH (Main Bay, MS; Oyster Pond, OP; and Mill Pond, MP). The vertical lines show approximate mean value of $\delta^{13}C$ for phytoplankton, macroalgae, and* Spartina *as found in similar Cape Cod estuaries (2, 8). (b) $\delta^{15}N$ value* vs. *prosomal width of horseshoe crabs collected in BN and SH. Regression information for BN:* $y = 0.0137x + 8.9$, $F = 10.81$, $P = 0.002$; *for SH:* $y = 0.014x + 8.41$, $F = 4.48$, $P = 0.05$. *Mean of horseshoe crab $\delta^{15}N$ from SH, BN, and PB [data from (1)] are shown on y-axis. The shaded reference area covers range of PB from (1). (c) $\delta^{13}C$ values* vs. *prosomal width of horsecrabs collected in BN and SH. Symbols as in Fig. 1b. Dashed boxes include range of values for polychaetes and for quahogs for Fig. 1a.*

sources supported by the phytoplankton-based portion of the food web.

The results of this work lend support to earlier findings about the position of horseshoe crabs as generalist predators in the estuarine food webs of Cape Cod; demonstrate that horseshoe crabs are clearly linked to land-derived nitrogen sources; suggest a possible diet shift from *Spartina*-based food sources to more phytoplankton-based sources as adult crabs grow; and show that the crabs exhibit considerable within-estuary loyalty in their foraging habits.

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Literature Cited

1. **Carmichael, R. H., D. Rutecki, B. Annett, E. Gaines, and I. Valiela.** *J. Exp. Mar. Biol. Ecol.* (In press).
2. **Peterson, B. J., and B. Fry. 1987.** *Annu. Rev. Ecol. Syst.* **18:** 293–320.
3. **Eichner, E. M., S. C. Michaud, T. C. Cambareri, B. Smith, G. Prahm, and M. Fenn. 2002.** *Cape Cod Surface Water Nutrient Management Study*. Unpublished report. Water Resource Office, Cape Cod Commission, Barnstable, MA.
4. **Eichner, E. M., K. Livingston, B. Smith, V. Morill, and A. Carbonell. 1998.** *Pleasant Bay Nitrogen Loading Analysis.* Unpublished report. Water Resource Office, Cape Cod Commission, Barnstable, MA.
5. **McClelland, J. W., I. Valiela, and R. H. Michener. 1997.** *Limnol. Oceanogr.* **42:** 930–937.
6. **McClelland, J. W., and I. Valiela. 1998.** *Limnol. Oceanogr.* **43:** 577–585.
7. **Shuster, C. N. 1955.** On morphometric and serological relationships within the Limulidae, with particular reference to *Limulus polyphemus* (L.) Ph.D thesis, New York University.
8. **McClelland, J. W., and I. Valiela. 1998.** *Mar. Ecol. Prog. Ser.* **168:** 259–271.
9. **Valiela, I. 1995.** *Marine Ecological Processes.* Springer-Verlag, New York.