

**Figure 1.** Locomotor activity of a juvenile horseshoe crab maintained first in natural cyclic lighting and then in constant darkness. The data are double-plotted to emphasize periodic events. Black bars indicate periods of activity; large dots indicate no data collection. Stippled regions indicate the solar night (sunset to sunrise). The juvenile was exposed to cyclic lighting from July 10 to July 14, when the porthole was closed to produce constant darkness until July 22.

whether these processes are modulated by one or more circadian oscillators.

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### Stable Isotopic Evidence for Changing Nutritional Sources of Juvenile Horseshoe Crabs

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Horseshoe crabs, *Limulus polyphemus*, are important predators in shallow environments (1). The feeding habits of adult horseshoe crabs have been examined (2), but the natural diet of juveniles has not. Juvenile crabs grow through 16 to 17 instars (3), and the increases in size during development suggest that nutritional sources may change (4). We identified likely components of juvenile diets using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures of juvenile horseshoe crabs and their potential prey species.

To obtain samples for isotope analyses, we collected juvenile crabs from intertidal sand flats at Nauset Beach, Massachusetts, during June and July 2002. Instars of crabs were determined based on data of Carmichael *et al.* (unpublished) on size of juveniles at each instar. To determine the size of juveniles, we measured the widest portion of the crab prosoma to the nearest 0.1 mm using vernier calipers. To assess position of juveniles within food webs, we collected potential prey items by hand and by sieving sediment from 0.3 m  $\times$  0.3 m  $\times$  0.1 m grabs from intertidal areas.

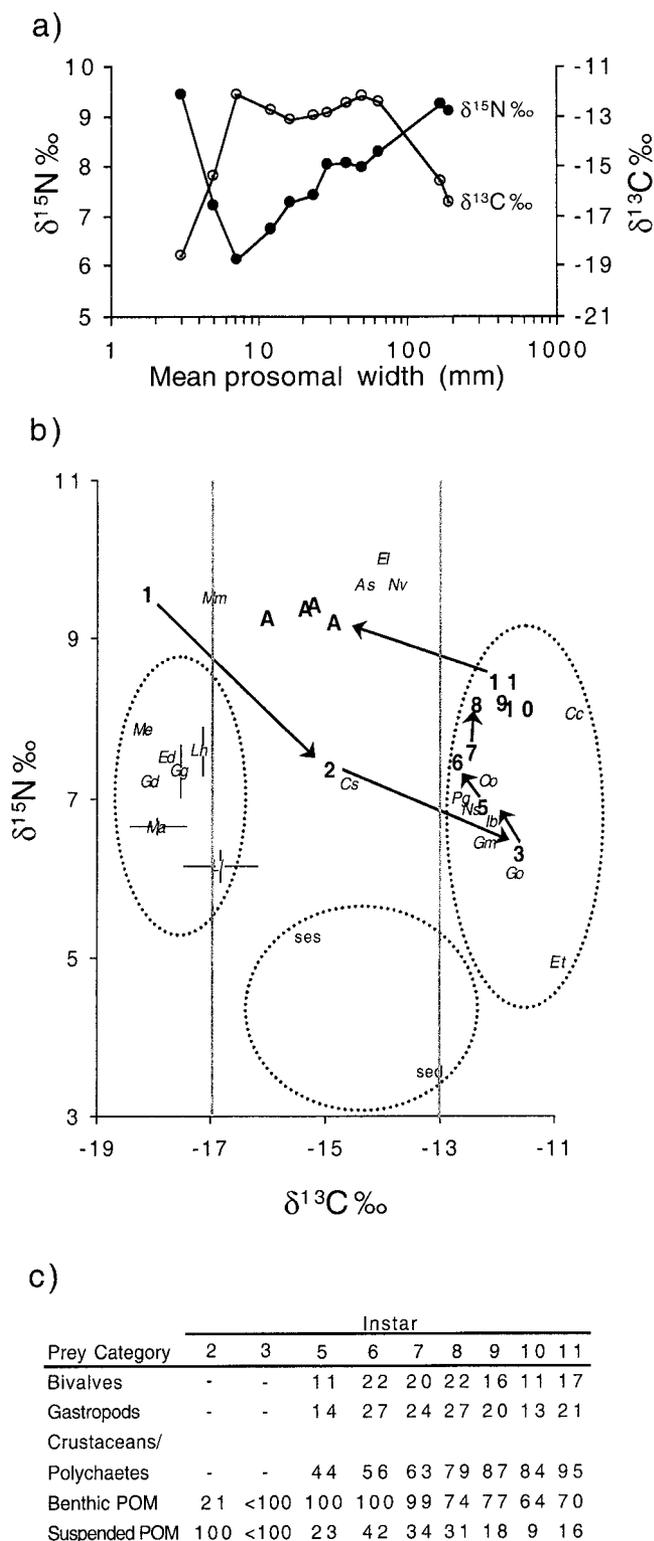
All specimens were dried at 60 °C, ground, and sent to the University of California—Davis Stable Isotope Facility to measure  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of crabs and potential prey species by mass spectrometry. To estimate maximum possible contributions

of different taxa of prey items to the diet, we applied a linear mixing model (5) to the isotope data.

$\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of horseshoe crabs changed as crabs grew (Fig. 1a). Signatures of the first instar juveniles are likely inherited from the parent, since first instars live off yolk made by the parent (6). The signatures of first instars were enriched in  $\delta^{15}\text{N}$  by approximately 0.32‰ and depleted in  $\delta^{13}\text{C}$  by approximately 2.8‰ relative to the average adult signature. These results extend, to an invertebrate, the range of results from studies on birds, which show yolk to be enriched in  $\delta^{15}\text{N}$  and depleted in  $\delta^{13}\text{C}$  relative to the parent signature (7).

Once juvenile crabs reached the second instar, the carbon and nitrogen signatures changed (Fig. 1b). This change in isotope signature coincides with the onset of assimilatory capacity in the digestive tract (3) and with the start of active burrowing behavior (8). Juveniles in instars 2–3 that gained assimilatory capacity may feed on sedimentary organic matter and meiofauna (lower dotted circle, Fig. 1b), and their signature moved steeply to the lower right of Fig. 1b. Eventually, these juveniles became large enough to eat smaller crustaceans and polychaetes on the sediment (right-hand dotted oval, Fig. 1b). As juveniles grew beyond the third instar, their  $\delta^{15}\text{N}$  signatures became heavier, up to instar 11. The shift in  $\delta^{15}\text{N}$  after instar 3 confirms the usual finding that, as

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**Figure 1.** (a) Change in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures of the horseshoe crab with increasing prosomal width. Data points correspond to the sequence of instars; 1–3, 5–11, and two adult samples, respectively. Where there is no visible standard error for the mean prosomal widths, the error is smaller than the symbol. (b)  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  signatures of horseshoe

consumers increase in size, they move up trophic steps because they seek larger prey; and larger prey characteristically show heavier signatures (4). The  $\delta^{15}\text{N}$  signatures of adults remain similar to instar 11, and their offspring then start the isotopic cycle again.

As the horseshoe crab juveniles grew, they evidently made use of mixed diets. To roughly quantify the proportion of different items contributing to the mixed diet, we calculated possible maximal contributions to diets of the different instars (Fig. 1c). For instars 2–3, benthic and suspended matter made up the diet. As crabs went through instars 5–11 (Fig. 1b and 1c), the contribution of polychaetes increased markedly; and molluscs contributed less than a quarter of the diet, considerably less than reported for adults (2).

The change in  $\delta^{15}\text{N}$  values according to life cycle of horseshoe crabs depends on the size of the crab and their prey. The changes in  $\delta^{13}\text{C}$ , in contrast, tell us that as horseshoe crabs grow, they make remarkable shifts in food webs. Judging by the  $\delta^{13}\text{C}$  signature, the instar 2 juveniles probably assimilated food from organisms that derived their nutrition in part from phytoplankton and macroalgal food webs, which have  $\delta^{13}\text{C}$  values of about  $-21\text{‰}$  and  $-17\text{‰}$ , respectively (9), and in part from a food web based on *Spartina alterniflora*, the salt marsh cordgrass, which has a  $\delta^{13}\text{C}$  of  $-13\text{‰}$  (10) (vertical gray lines in Fig. 1b). By instar 3, the juveniles largely shifted to prey that depended primarily on *Spartina* alone. This dependency on the salt marsh-supported food web continued until instar 11. Adult signatures markedly changed from those of juveniles, again turning to the phytoplankton- and macroalgae-based parts of the food web. This pattern likely exists because juveniles largely eat small benthic polychaetes, amphipods, and isopods, whereas adults consume more bivalves (2), which are suspension feeders assimilating phytoplankton.

The isotopic data showed remarkable shifts in the diet and food web position of juvenile horseshoe crabs as they grow. Juvenile diets changed on the basis of prey size, as well as shifting from a food web based on phytoplankton to one supported by salt marsh producers. The crabs then returned to the phytoplankton-based food web as adults. These shifts in position in the food web reflect the changing array of prey consumed by horseshoe crabs of dif-

crabs compared with signatures of potential prey items. Horseshoe crab data points are labeled by instar number until adult. (A) Prey items are labeled by abbreviation: Bivalves—*Mercenaria mercenaria*\* (Mm), *Mytilus edulis* (Me), *Ensis directus* (Ed), *Geukensia demissa* (Gd), *Mya arenaria* (Ma), *Gemma gemma* (Gg); Gastropods—*Lunatia heros* (Lh), *Littorina littorea* (Ll); Amphipods—*Caprella* spp.\* (Cs), *Gammarus oceanicus* (Go), *Gammarus mucronatus* (Gm); Isopods—*Edotea triloba* (Et), *Chiridotia coeca* (Cc), *Idotea baltica* (Ib); Polychaetes—*Orbinia ornata* (Oo), *Neanthes succinia* (Ns), *Pectinaria gouldii* (Pg), *Ampharete* spp.\* (As), *Eteone longa*\* (El), *Nereis virens*\* (Nv); Benthic particulate organic matter (POM)—sediment (sed); Suspended POM—seston (ses). An asterisk (\*) indicates prey items excluded from mixed diet analysis. In cases where multiple samples were pooled, signatures were averaged and error bars are shown. Circles, from left to right, signify dietary groups: molluscs, POM, crustaceans/polychaetes. Grey lines represent expected  $\delta^{13}\text{C}$  values for food webs based on macroalgae ( $-17\text{‰}$ ) and *Spartina* ( $-13\text{‰}$ ). (c) Estimated maximum percentage of potential prey items in the mixed diet of juvenile horseshoe crabs at Nauset Beach, Massachusetts. Percentages were calculated using a linear mixing model (5).

ferent instars and demonstrate that this species depends on widely different food webs. Conservation of horseshoe crab populations, therefore, depends on suitable management that assures both a phytoplankton and macroalgal source, as well as continued accessibility of salt marsh habitats.

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