Diet Composition of Juvenile Horseshoe Crabs: Implications for Growth and Survival of Natural and Cultured Stocks

Ruth H. Carmichael, Emily Gaines, Zacharia Sheller, Amanda Tong, Amanda Clapp, and Ivan Valiela

Abstract Horseshoe crabs are valued for economic, ecological, and educational purposes. These values have raised interest in managing natural stocks and culturing crabs for conservation, research, and education. To inform these efforts, we used N and C stable isotopes to define the natural diet of juvenile horseshoe crabs and then assessed effects of different diets on growth and survival of juveniles in culture. In the natural environment, N and C isotope ratios in juvenile horseshoe crabs changed as crabs grew, with larger crabs consuming larger prey. Linear mixing analyses suggested young crabs were supported by high quantities of benthic and suspended particulate organic matter (POM), shifting between marine algae and salt marsh-based food webs, depending on size. In culture, we tested the relative importance of algae, as a proxy for POM, in horseshoe crab diet by feeding juvenile crabs different percentages of algae and prey animals. Initially, juvenile crabs showed a significant increase in size when fed diets >70% protein, but showed a decrease in survival compared to algae-rich diets. Overall, growth rates and survival declined through time during the 128-day study, regardless of diet composition. These data suggest horseshoe crabs require foods from a combination of plant and animal sources. Successful culture or conservation of horseshoe crabs will depend on understanding the relative importance of different food sources at different life stages as well as discerning the balance between factors that increase growth, but reduce survival.

1 Introduction

Interest in culture of American horseshoe crabs, *Limulus polyphemus*, has increased in recent years due to increasing concern over use and conservation of natural stocks. American horseshoe crabs are harvested primarily as bait for the conch and eel fishery, for scientific research, and to produce Limulus

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amoebacyte lysate (LAL), which is used to test medical supplies and equipment for pathogens (Berkson and Shuster 1999, Botton 2002). Horseshoe crabs also are ecologically important as a food source to migratory shorebirds, which fuel a significant ecotourism industry in some regions (Botton 2002). More recently, horseshoe crabs have gained popularity as part of a worldwide movement to integrate science, management, and policy into public education (Botton 2001, Chen et al. 2004, ERDG 2007, O'Connell et al. in press). These interests have prompted curiosity in programs to culture horseshoe crabs for use in classrooms, the aquarium trade, controlled research settings, and emerging aquaculture enterprises to supplement natural stocks (Botton 2002, Carmichael et al. 2003, Chen et al. 2004, Degener 2007, ERDG 2007, O'Connell et al. (2009), Carmichael unpublished data).

Research on horseshoe crab development has provided basic information to establish the physical conditions for culture. Field and laboratory research has defined the basic tolerances of horseshoe crabs to culture conditions, including salinity, temperature, and dissolved oxygen (DO) concentrations (Sekiguchi 1988, Shuster and Sekiguchi 2003). In particular, this work has defined the effects of variation in these parameters on growth and survival of early life stages (Jegla and Costlow 1982, Sekiguchi 1988). There also is a growing body of information on health management of horseshoe crabs in captivity (Smith and Berkson 2005). Despite these advances, however, there are few published reports to guide culture enterprises in the United States, particularly with regard to diet and nutrition.

To successfully rear horseshoe crabs in culture and aid conservation of natural populations, we must understand the diet composition that yields the highest rates of growth and survival. Gut content analyses and limited choice tests suggest that protein sources, particularly bivalves, may be a main component of adult horseshoe crab diet (Shuster 1960, Botton 1984, Botton et al. 2003a). Gut content analyses, however, are limited to detecting the most recent diet and may be biased toward identification of species with hard skeletons or shells that remain in the gut (Hyslop 1980, Alexander et al. 1996). Stable isotope analyses provide an alternative method to discern foods that are nutritionally important by identifying items that were not only consumed but also assimilated (Carmichael et al. 2004). Recent studies among horseshoe crabs suggest a variety of foods, including particulate organic matter (POM) such as algae, may play a significant role in the diet of horseshoe crabs (Gaines et al. 2002, Carmichael et al. 2004, Carmichael and Valiela 2005). Measuring growth and survival of horseshoe crabs fed diets that vary in proportion of animal protein and components of POM, such as algae, may help define artificial diets and improve culture success.

In this study, we sought to inform culture of juvenile horseshoe crabs by better defining their natural diet and using this information to guide development of artificial diets. To do this we took a two-part approach. First, to better define the natural diet of juvenile horseshoe crabs, we analyzed N and C stable isotope ratios in horseshoe crabs and their potential foods in the field. Second,

to test the relative importance of POM to horseshoe crab diet, we used marine algae as a proxy for POM and measured growth and survival of juveniles in culture when fed diets that differed in percentages of algae and prey animals.

2 Methods

2.1 Field Sampling and Stable Isotope Analyses

Methods for the field component of this study are described in detail in Gaines et al. (2002) and Carmichael et al. (2004). Hence, we briefly describe the methods here. Juvenile and adult horseshoe crabs and potential prey items were sampled by hand from Nauset Beach in Massachusetts. Crabs were measured to the nearest 0.1 mm prosomal width and assigned to instar stages according to Carmichael et al. (2003). Benthic and suspended POM were sampled using modified syringe corers and by filtering 1 L of near-bottom water, respectively. All animals, sediments, and water filters were dried to a constant weight at 60°C. Animals and sediments were ground to a powder. All samples were packed in tin capsules and sent to the U.C. Davis stable isotope facility for analysis by mass spectrometry. A linear mixing model was applied to estimate the maximum possible contribution of different components to horseshoe crab diet (Phillips 2001).

2.2 Laboratory Culture

Juvenile horseshoe crabs were cultured at the Downeast Institute of Applied Marine Research (DEI) on Great Wass Island, Maine. Horseshoe crabs were obtained as eggs from Delaware Bay and as juveniles from Pleasant Bay, MA.

2.2.1 Culture Conditions and Apparatus

Crabs were kept in a closed tank under ambient conditions for 2 weeks before the start of the study. Crabs ranging in prosomal width from 2.4 to 9.7 mm were sorted into cohorts based on known instar stages (Sekiguchi 1988, Carmichael et al. 2003) and distributed by prosomal width so that each treatment bin received a total of 13 crabs; one crab (8.90 $\pm$ 0.10 mm), eight crabs (6.72 $\pm$ 0.03 mm), two crabs (4.69 $\pm$ 0.05 mm), and two crabs (3.05 $\pm$ 0.04 mm). We selected these smallest sized horseshoe crabs because they are likely to grow quickly and have similar expected molting rates (typically 2- to 3-week intervals; Sekiguchi 1988, Carmichael et al. 2003). The number of individuals within each size (prosomal width) class depended on availability during field collections.

Horseshoe crabs were reared in 24 plastic bins of two different sizes; 12 (15.4 cm $\times$ 24.2 cm) and 12 (19.8 cm $\times$ 28.6 cm size), without covers (Fig. 1, Bin).

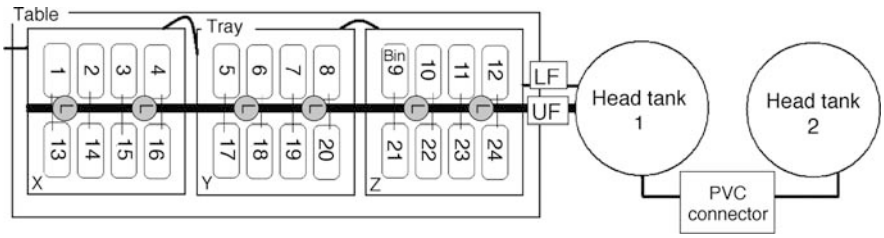

Fig. 1 The horseshoe crab culture apparatus consisted of two head tanks connected by PVC pipe to a table containing three trays (X, Y, Z) of eight bins (1–24). LF = Lower flow from head tanks to trays. UF = Upper flow from head tanks directly to bins. L = location of lights on the upper PVC pipe

The bottom of each bin was removed and replaced with 700 μm mesh, and plastic feet were added to the bottom of each bin to allow water movement into and out of bins from below. The bottom of each bin was covered with 1 l of pre-washed natural sand (700 μm–2 mm), collected from Roque Bluffs Beach, Maine.

Bins were randomly placed into three large, flat trays in which they remained throughout the study (Fig. 1, Tray X, Y, Z). Two large head tanks were elevated above the level of the trays to provide a gravity fed system of continuous water flow. Head tanks were filled daily with ambient seawater at salinity of 34%. Water was serially pre-filtered to 10 μm and heated to 25°C to ensure lower trays maintained temperatures ranging from 17 to 23°C. DO concentration was maintained at 6.0–7.6 mg l^{-1} by using two air stones in each tray, with one additional air stone in the head tank. Trays were supplied by a continuous flow of water at approximately 1 cm s^{-1}, which kept sand moist but held water levels just below the surface of the sand in each bin (Fig. 1, LF). In addition to this continuous flow, each bin was supplied with water from an overhead hose (Fig. 1, UF). This overhead supply of water was regulated by a timer and raised the water level in the bins to ~ 6–10 cm by flowing from 11 pm to 1 am, 3 am to 5 am, and again from 11 am to 1 pm, 3 pm to 5 pm to simulate two 6-h periods of high tide. To reduce temperature variation and likelihood of hypoxia in the bins, we opted to use these two shorter periods of continuously flowing "high tide", spanning 6 h, rather than one longer period of slack high water. Water did not drain completely during the 1-h break, representing peak high tide in our system. To simulate ambient light conditions, we mounted six 60-W natural-sun lights (GE) above the bins (Fig. 1, L). Lights were set on a timer to maintain 12-h light and dark cycles.

To account for potential differences in culture conditions among experimental bins and trays, we regularly measured DO, temperature, and salinity in each head tank and tray, and in two randomly selected bins in each tray. DO and salinity were measured every 2 weeks using a model EDO (LaMotte #7414) DO kit and using an optical refractometer, respectively. Water temperature was measured daily with a thermometer.

To reduce fouling by silt, algae, and *Pseudomonas* sp. (fouling bacteria common to culture facilities), the culture apparatus was cleaned on a regular basis. Head tanks were cleaned with mild soap every week. Every 2 weeks, bins and large trays were scrubbed with a mixture of diluted bleach and detergent, rinsed with fresh water, and re-rinsed with copious quantities of salt water. Sediments from each bin were also washed over a 3 mm sieve with fresh ambient salt water.

2.2.2 Experimental Diets

To determine the relative importance of algae to horseshoe crab diet, we tested four diets differing in ratio of algae to animal matter (referred to as "protein" for simplicity); 90%:10%, 60%:40%, 30%:70%, and 100% protein. Test algae included *Ulva* var., *Enteromorpha* spp. (macroalgae), and *Tetraselmis* sp. (microalgae). Animal protein sources included soft-shelled clams (*Mya arenaria*), blue mussels (*Mytilus edulis*), and polychaetes (*Neanthes virens* and *Glycera* spp.). These species were chosen because they represent a combination known to be part of horseshoe crab diet and are locally available in Downeast Maine and elsewhere along the Atlantic coast to supply natural and cultured stocks of crabs (Gaines et al. 2002, Carmichael et al. 2004, Carmichael pers. obs.). All species were obtained by hand from local beaches in Downeast Maine except *Mya* and polychaetes, which were obtained from local commercial fishermen.

To create diet mixtures, each species was washed thoroughly with clean seawater, patted dry with paper towels, and for bivalves, soft tissues were separated from shell. To ensure equal proportions of each test species contributed to the final algae and protein mixtures used to create the test diets, we pureed each species separately (polychaetes were combined) in a commercial grade blender. Purees were then combined by volume, using batches of 50 ml of each species, to create final "algae" and "protein" stock mixtures, which were subsequently mixed at the test ratios. Food stocks (pastes) were prepared at once for the entire sampling period and frozen at –20°C so that small batches of food could be thawed for feeding as needed. Horseshoe crabs were fed 2.0 g (wet weight) of the appropriate test mixture per bin. We determined this quantity of test diet during week 1, by offering crabs 5.0 g of food mixture per bin and weighing the amount uneaten after 24 h. Food paste was mixed into the sediment every 2 weeks after cleaning but before horseshoe crabs were reintroduced to their bins. Any visible pieces of uneaten food were removed after 24 h to minimize fouling.

2.2.3 Measuring Growth and Survival

To measure growth and survival, juvenile horseshoe crabs were recovered from bins by hand sieving over a 2 mm mesh screen every 2 weeks. Crabs were counted, along with shed molts, and prosomal width was measured (by one

person throughout the experiment) to the nearest 0.1 mm with vernier calipers. We used change in prosomal width as the unit for determining growth because this unit has been widely used (allowing comparison to other studies) and shows relatively little variation within instars among populations (Riska 1981, Sekiguchi 1988). Growth and survival were measured as the change in prosomal width and number of crabs, respectively, through time for the first 56 days of the study and through day 120. After day 56 growth and survival were measured every 4 weeks.

2.3 Statistics

Analyses of variance (ANOVA) were performed to test for treatment effects, including differences in horseshoe crab size and survival between trays, treatments, bin size, and bin location for each date. Regression analyses were used to determine significant differences in growth, survival, or culture conditions (DO, salinity, temperature) through time. Where significant relationships were found, we further performed analyses of covariance (ANCOVA), preceded by a test for homogeneity of slopes (Sokal and Rohlf 1987). This approach was used to determine differences among trays (for changes in temperature through time), treatments (for growth and survival rates), and to compare the initial days of study (through day 56) to the entire study (day 120).

3 Results

3.1 Field Study

3.1.1 Available Foods and Adult Horseshoe Crabs

To provide a context for analysis of stable isotope ratios in juveniles, we first measured ratios in available foods and adult horseshoe crabs. We identified three isotopically distinct dietary groups available to horseshoe crabs at our field sites. These groups included (1) bivalves and gastropods, which clustered near δ^{13}C values for marine algae (Fig. 2; Bi, Ga circle and dark gray line), (2) crustaceans and polychaetes, which clustered near δ^{13}C values for salt marsh grass (Fig. 2; Cr, Po circle and light gray line), and (3) POM, which showed depleted δ^{15}N% compared to other dietary groups and had δ^{13}C% that reflected a mixture of marine algae and salt marsh sources. N and C stable isotope ratios in tissues of adult horseshoe crabs were roughly 9 and –15%, respectively, showing the appropriate 2–4% enrichment compared to the two animal-based dietary groups and reflecting a mixture of marine algae and salt marsh-supported food webs (Fig. 2, A). Refer to Gaines et al. (2002) for the species-specific stable isotope ratios within each dietary group presented here (Fig. 2).

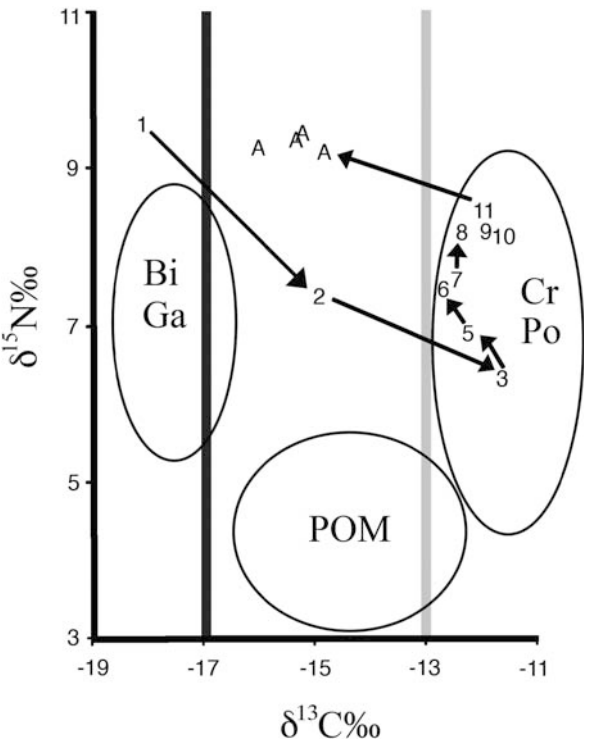

Fig. 2 δ^{15}N compared to δ^{13}C values in juvenile (indicated as instars 1–11) and adult (A) horseshoe crabs and dietary groups (defined in Table 1). Ovals indicate the general region in which isotope values clustered for each dietary group. The gray lines show expected δ^{13}C values for food webs supported by marine algae (–17%) and salt marsh grass (–13%). Arrows depict the isotopic shift in horseshoe crab tissues with growth. Modified from Gaines et al. (2002)

3.1.2 Juvenile Horseshoe Crabs

N and C stable isotope ratios in juvenile horseshoe crabs changed as crabs grew, with young crabs depending primarily on small particles of organic matter (Fig. 2 and Table 1). First instars had stable isotope ratios similar to adult horseshoe crabs (Fig. 2; 1 and A). Second and third instars showed relatively depleted δ^{15}N values compared to other instars (Fig. 2; 2 and 3), and their δ^{13}C values reflected benthic and suspended POM (Fig. 2; POM circle). δ^{15}N% in tissues of instars 5 through 11 increased from ~6% to nearly 9% and maintained δ^{13}C % similar to salt marsh sources (Fig. 2; gray line). Accordingly, stable isotope values of these instars clustered near values for crustaceans and polychaetes (Fig. 2; Cr, Po circle). Linear mixing analyses further confirmed that young crabs fed on high quantities of benthic and suspended particulate organic matter, shifting from POM to animal-based food sources as they grew (Table 1). In fact, instars 2–7 appeared to feed almost exclusively on POM. Among instars 8–11, diets shifted to a combination of primarily benthic POM, crustaceans and polychaetes (Table 1; shaded area).

Table 1 Estimated percent contribution of dietary components to diet for juvenile horseshoe crabs (instar stages 2–11). Data were not available for instar 4. Bi = bivalves, Ga = gastropods, Cr = crustaceans, Po = polychaetes, POM = particulate organic matter. Shaded area shows the components estimated to be most important at each instar (modified from Gaines et al. 2002)

	Instar								
Diet component	2	3	5	6	7	8	9	10	11
Bi	–	–	11	22	20	22	16	11	17
Ga	–	–	14	27	24	27	20	13	21
Cr/Po	–	–	44	56	63	79	87	84	95
Benthic POM	21	<100	100	100	99	74	77	64	70
Suspended POM	100	<100	23	42	34	31	18	9	16

3.2 Laboratory Culture

3.2.1 Growth

In culture, we tested the relative importance of marine algae, a major component of benthic and suspended POM, to horseshoe crab diet by feeding juvenile horseshoe crabs different mixtures of algae and potential prey animals. Mean prosomal width of horseshoe crabs in all treatments increased during the study, showing distinctive jumps in growth, characteristic of molting (Fig. 3; top, between days 14 and 28). Despite this stepped pattern of growth, linear regression best fit these data and explained a majority of variation in horseshoe crab size through time (Fig. 3). Hence, the slopes of the regression lines in Fig. 3 were used to calculate mean growth rates (Table 2).

We also observed significant differences in growth between the first half and second half of the study (Fig. 3). First, during the initial days in culture (through day 56), mean prosomal width increased significantly through time among horseshoe crabs fed diets >70% protein, but not among crabs fed diets higher in algae content (Fig. 3 and Table 2). By day 120, however, all treatments showed significant increases in mean prosomal width through time except crabs fed 40% protein (Fig. 3, bottom). Second, growth rates slowed significantly during the final weeks in culture (test for homogeneity of slopes, d56 vs. d120: $F_{1,27} = 5.15$, $P = 0.03$; Fig. 3, comparison of slopes between top and bottom panels), decreasing from a mean value of 0.10 mm week^{-1} through day 56–0.06 mm week^{-1} through day 120 (Table 2). Overall, mean growth rates also were generally slower among crabs fed primarily algae diets (Table 2), but they did not significantly differ among treatments (test for homogeneity of slopes, d56: $F_{1,6} = 0.01$, $P = 0.91$; and d120: $F_{1,15} = 0.46$; $P = 0.81$).

3.2.2 Survival

The number of juvenile horseshoe crabs in all treatments decreased significantly through time during the study (Fig. 4), with rates of decline slower during initial weeks in culture (test for homogeneity of slopes, d56 vs. d120:

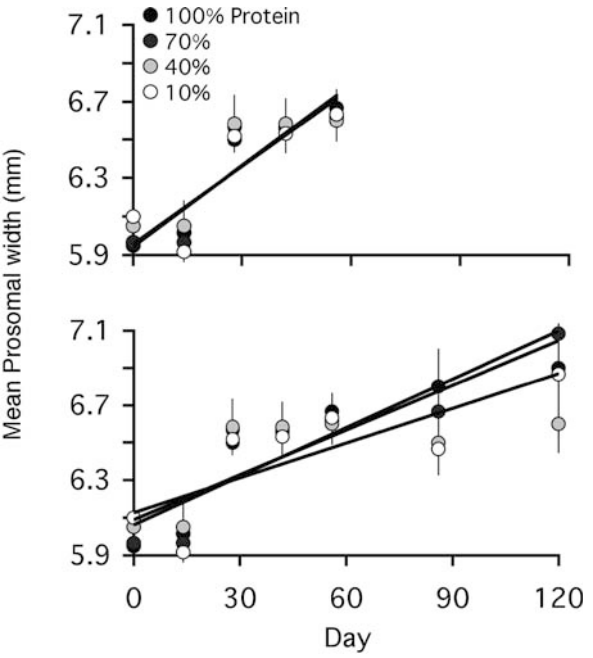

Fig. 3 Mean prosomal width (mm) of juvenile horseshoe crabs in each diet treatment during the first 56 days of the study (*top*) and throughout the 120-day experiment (*bottom*). Percent (%) protein refers to the % of diet mixture comprised of prey animals relative to algae. Error bars show standard error. Where no bars are visible, error was smaller than the symbol. (*Top*, days 0–56: 100%: $y = 0.01x + 5.94$, $R^2 = 0.89$, $F_{\text{reg } 1,3} = 25.93$, $P = 0.01$; 70%: $y = 0.01x + 5.96$, $R^2 = 0.79$, $F_{\text{reg } 1,3} = 11.17$, $P = 0.04$; *bottom*, days 0–120: 100%: $y = 0.01x + 6.09$, $R^2 = 0.82$, $F_{\text{reg } 1,5} = 22.94$, $P < 0.01$; 70%: $y = 0.01x + 6.07$, $R^2 = 0.82$, $F_{\text{reg } 1,5} = 23.03$, $P < 0.01$; 10%: $y = 0.01x + 6.13$, $R^2 = 0.65$, $F_{\text{reg } 1,5} = 9.40$, $P = 0.03$)

Table 2 Growth and survival rates (±se) of juvenile horseshoe crabs in each dietary treatment, as of days 56 and 120. Percent (%) protein refers to the % of diet mixture comprised of prey animals relative to algae. Growth and survival rates were calculated from the slopes of the regression lines plotted in Figs. 3 and 4. *indicate values from regression lines in which $P_{reg} > 0.05$ and not included in comparisons (tests for homogeneity of slopes) among treatments or between days 56 and 120

	Growth rate (mm week^{-1})		Survival rate (no. week^{-1})	
	Day			
Treatment (% protein)	56	120	56	120
100	0.10±0.02	0.06±0.01	–0.45±0.11	–0.53±0.05
70	0.10±0.02	0.06±0.01	–0.43±0.08	–0.61±0.06
40	0.08±0.03*	0.03±0.01*	–0.39±0.07	–0.47±0.04
10	0.08±0.03*	0.04±0.01	–0.27±0.05	–0.55±0.07

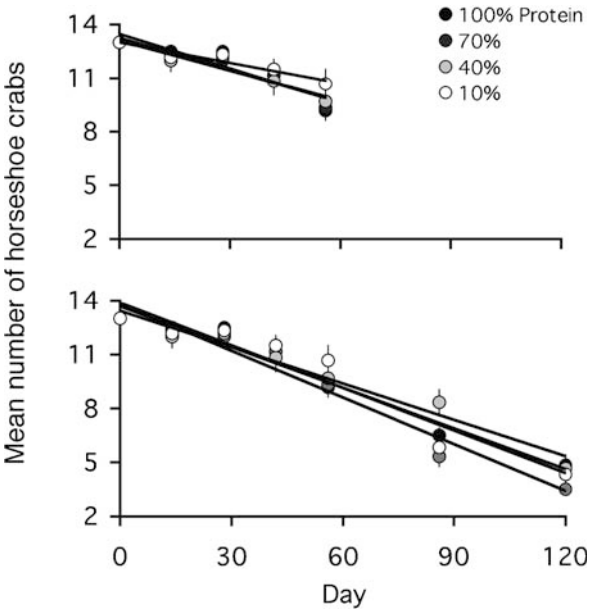

Fig. 4 The mean number of juvenile horseshoe crabs surviving in each diet treatment during the first 56 days of the study (*top*) and throughout the 120-day experiment (*bottom*). Percent (%) protein refers to the % of diet mixture comprised of prey animals relative to algae. Error bars show standard error. Where no bars are visible, error was smaller than the symbol. (*Top*, days 0–56: 100%: $y = -0.06x + 13.47$, $R^2 = 0.84$, $F_{\text{reg }1,3} = 15.50$, $P = 0.03$; 70%: $y = -0.06x + 13.27$, $R^2 = 0.91$, $F_{\text{reg }1,3} = 28.52$, $P = 0.01$; 40%: $y = -0.06x + 13.10$, $R^2 = 0.91$, $F_{\text{reg }1,5} = 30.15$, $P = 0.01$; 10%: $y = -0.04x + 13.00$, $R^2 = 0.91$, $F_{\text{reg }1,5} = 28.99$, $P = 0.01$; *bottom*, days 0–120: 100%: $y = -0.08x + 13.68$, $R^2 = 0.96$, $F_{\text{reg }1,5} = 126.23$, $P < 0.001$; 70%: $y = -0.09x + 13.81$, $R^2 = 0.96$, $F_{\text{reg }1,5} = 119.98$, $P < 0.001$; 40%: $y = -0.07x + 13.42$, $R^2 = 0.96$, $F_{\text{reg }1,5} = 132.54$, $P < 0.001$; 10%: $y = -0.08x + 13.88$, $R^2 = 0.92$, $F_{\text{reg }1,5} = 59.72$, $P < 0.001$)

$F_{1,44} = 6.94$, $P = 0.01$; Fig. 4, comparison of slopes between top and bottom panels). On average, survival rates were –0.39 ± 0.07 through day 56 and dropped to –0.54 ± 0.02 through day 120 (Table 2). Survival rates did not differ among treatments (test for homogeneity of slopes, d56: $F_{3,12} = 0.98$, $P = 0.43$; and d120: $F_{3,20} = 1.05$, $P = 0.39$). Smallest size classes (mean prosomal width 3.1–6.7 mm) showed greatest loss during initial weeks in culture, while largest sized crabs (mean prosomal width 8.9 mm) experienced 100% survival until week 6. In the initial weeks in culture, we also found a pattern of lower survival rates when growth rates were highest (Table 2, d56). Because growth did not change significantly through day 56 for the primarily algae treatments (Fig. 3; top, 10 and 40% protein), however, we could not establish a significant correlation between growth and survival rates during this time (Table 2, d56).

3.2.3 Culture Environment

Despite minor variation in water temperature and DO among trays, we found no relationships between these variables and growth or survival of juvenile horseshoe crabs during our study. Hence, we briefly discuss this variation here, but for simplicity, we excluded these data. Water temperature in each tray (Fig. 1; X, Y, and Z) decreased from an average of 23 to 17°C during the time of the study, as ambient air and water temperature declined. This decrease was significant for trays X and Y. DO concentration in each tray increased during the study, as water temperature declined, but also showed no effect on growth. Mean salinity did not differ among trays during the study. We also found no treatment effects in relationships between growth or survival and tray location or bin size and location within the culture apparatus.

4 Discussion

Stable isotope data provided insight to help identify the components of available diet, which were nutritionally important in the natural environment. These data indicated that juvenile horseshoe crabs used a diet of mixed composition, which changed with horseshoe crab size, and showed reliance on potentially high concentrations of POM. Similarity between δ^{15}N values in first instars and adult horseshoe crabs is consistent with the fact that this life stage does not have a gut and was living off nutrients provided to the egg by adult crabs. Reliance of youngest crabs (instars 2–7) on a diet supported largely by POM is consistent with their small size (roughly 3–22 mm), at which most available prey species were too large to eat (Gaines et al. 2002). The apparent greater use of suspended POM by second instars (~5 mm) suggests this life stage may be resuspended in near shore waters, while instars 3+ (>7 mm) remain settled in the benthos (Shuster 1982, Botton et al. 2003b). It also is possible that smallest instars select a component of pelagic POM such as phytoplankton, either because it is a suitable size or more readily available compared to benthic sources in some estuaries. Instars 8–11 (roughly 30–60 mm) were large enough to begin eating many of the smaller amphipod, isopod, and polychaete species available in the study area (Gaines et al. 2002, Carmichael et al. 2004). Accordingly, the N stable isotope ratios in these larger instars increased as much as 3%, indicating their shift to a higher trophic level and reflecting a mixed diet including prey animals as well as POM (Fig. 2). These data point out that different-sized crabs depend on different food sources, and POM may have a significant influence on horseshoe crabs during their earliest life stages.

Use of marine algae as a proxy for POM, however, did not significantly enhance growth and survival of juvenile horseshoe crabs in culture. The significant increase in size observed first among horseshoe crabs fed higher protein diets suggests that an animal-based diet promoted more rapid molting, at least among our test diets. It is important to consider that POM includes detritus and

living material from a variety of sources including bacteria, salt marsh grasses (which stable isotope analyses also identified as potentially important), terrestrial plants, and aggregates of mixed composition, as well as marine macro- and microalgae (Lee et al. 2004). We tested only two of the many groups of marine macroalgae and microalgae, which may contribute to POM in the natural environment. Although the species we tested are commonly available in areas where horseshoe crabs live and feed, they may not represent the groups most abundant within POM or most nutritionally important. It also is conceivable that POM provides a unique combination of molecules that are not available in any single component. These molecules may include amino sugars, which are building blocks for chitin and common in marine sediments, and astaxanthin, an antioxidant in marine algae that enhances growth and survival in crustaceans (Carmichael et al. 2004, Chien and Shiau 2005). Additional research is needed to better define components of POM consumed by horseshoe crabs and test these as supplements to artificial diets for culture.

It is difficult to evaluate the overall effectiveness of our culture approach because there are few published reports on growth and survival rates of horseshoe crabs in culture with which to make comparisons. The growth increments, time between molts, and survival rates we found were generally consistent with previous lab studies (Sekiguchi 1988, K. Tsuchiya pers. comm.). Our culture growth rates were slower and survival was higher, however, compared to crabs studied in the natural environment (Carmichael et al. 2003, Botton et al. 2003, Chen et al. 2004, Carmichael pers. obs.). This latter finding is not surprising since culture conditions eliminate predation, which is a primary cause of mortality among early instars in nature (Carmichael et al. 2003; Botton et al. 2003). Additional study on survival of juveniles in the natural environment, in the absence of predation, would provide a better context to assess survival in culture. These findings also suggest that our culture conditions lacked some component necessary to support maximum growth, and this component may be available (but not yet identified) among natural POM in the benthos and near-bottom waters.

Despite these challenges, our data provide guidance for refinement of culture conditions for juvenile horseshoe crabs. For example, although not conclusive, our data relating increased growth rates to reduced survival raise concern regarding a possible link between increased molting and mortality during initial days in culture (Table 2). Molting is dangerous business in the natural environment and may be more so in culture. Handling practices may increase physiological stress, and limited space or insufficient water flow may somehow encumber molting or increase exposure to and infection by bacteria or other parasites (Smith and Berkson 2005). We observed ongoing colonization of cages and occasionally horseshoe crabs by *Pseudomonas* sp. among our culture tanks and bins. This group of bacteria has been associated with external lesions on the surface of adult horseshoe crabs (Smith and Berkson 2005). We did not find a clear relationship between *Pseudomonas* and growth or survival of juvenile horseshoe crabs, but exposure of newly molted crabs to these bacteria

may have contributed to reduced survival. Furthermore, the significant decrease in survival rates in the second half of the study suggests that stressors in culture could have a greater effect through time (Figs 3 and 4, bottom).

Considered together, our stable isotope and culture data indicate that a diet comprised of a combination of foods from plant and animal sources is needed to best support growth and survival of horseshoe crabs. Successful culture or conservation of horseshoe crabs will depend on understanding the relative importance of different food sources at different life stages as well as discerning the balance between factors that increase growth, but reduce survival. Discerning the appropriate composition of artificial diet, including particle size and the appropriate mixture of particles derived from animal and vegetation sources, may be particularly important among early life stages.

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