REVIEWS IN Aquaculture

Reviews in Aquaculture (2012) **4,** 32–43 doi: 10.1111/j.1753-5131.2012.01059.x

Three decades of horseshoe crab rearing: a review of conditions for captive growth and survival

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Received 27 December 2011; accepted 5 January 2012.

Abstract

Threats to wild horseshoe crab populations and growing interest in their use for research, education and biomedical applications have prompted demand for improved techniques to rear and maintain crabs in captivity. Although numerous laboratory studies have been conducted to determine growth and survival of horseshoe crabs under various conditions, these data have not been compiled and summarized to inform culture practices. We surveyed the literature and analysed the range of available techniques to identify and define a consistent set of conditions for maximum growth and survival of horseshoe crabs in culture. We considered three age classes; embryo, juvenile and adult, and included all extant species (*Limulus polyphemus*, *Carcinoscorpius rotundicauda*, *Tachypleus gigas* and *Tachypleus tridentatus*). We discovered relatively few published studies that clearly related husbandry conditions to growth and survival. Comparison among studies was complicated by inconsistent data collection and reporting techniques. Most published sources reported data for younger age classes, and more studies considered *L. polyphemus* than Asian species. The most commonly reported variables (temperature, salinity, enclosure maintenance and diet composition) showed size dependent and in some cases species-specific effects on growth and survival that will be important in guiding culture efforts. We suggest that future studies give additional consideration to substrate type, water flow, dissolved oxygen concentrations, diet quality and the quantity and frequency of feeding. If laboratory-reared stocks are to be used for propagation and restoration activities, future studies will benefit from closing these data gaps and promoting international data sharing.

Key words: culture, diet, husbandry, salinity, temperature.

Introduction

Over the past century, habitat alteration and anthropogenic use have lead to a decline in horseshoe crabs in many parts of their range (Botton 2001; Mishra 2009a; Shin *et al.* 2009; Zaldívar-Rae *et al.* 2009). Although horseshoe crabs are considered living fossils, having remained largely unchanged for roughly 450 million years, only one American (*Limulus polyphemus*) and three Asian (*Carcinoscorpius rotundicauda*, *Tachypleus gigas* and *Tachypleus tridentatus*) species exist as living relics of that earlier age (Rudkin & Young 2009). Today, horseshoe crabs throughout their range (Fig. 1) are primarily harvested as bait for fishing industries and to produce a bacterial endotoxin indicator and other biomedical products (Berkson & Shuster 1999; Hsieh & Chen 2009; Kreamer & Michels 2009). Horseshoe crabs are also harvested for research in vision, endocrinology and other physiological processes (Berkson & Shuster 1999; Rutecki *et al.* 2004). Horseshoe crabs are recognized as important components of benthic food webs (Botton 2009; Zaldívar-Rae *et al.* 2009), and their eggs supplement the diet of migratory shorebirds along the Atlantic coast of the USA (Gillings *et al.* 2007; Mizrahi & Peters 2009). In some regions horseshoe crabs are also used as a human food source or in traditional rituals (Hsieh & Chen 2009; Shin *et al.* 2009;

Figure 1 Estimated worldwide distribution of American and Asian horseshoe crab species. Owing to the extensive overlap in distribution among the Asian species, data for *Tachypleus gigas* and *Carcinoscorpius rotundicauda* were combined. Data were compiled from Sekiguchi (1988), Itow *et al.* (2004), Zaldívar-Rae *et al.* (2009), Shin *et al.* (2009), UDCEOE (2011) and ERDG (2011).

Zaldívar-Rae *et al.* 2009). There is considerable interest, therefore, in propagating and restoring horseshoe crab populations to support these valuable economic, biomedical, ecological and cultural services (Almendral & Schoppe 2005; Tsuchiya 2007; Mishra 2009a; Hong *et al.* 2009).

Captive rearing has been widely suggested as one option to augment and restore over-harvested native horseshoe crab populations while continuing to support ongoing research and educational activities (Carmichael *et al.* 2009; O'Connell *et al.* 2009; Tsuchiya 2009; Chan 2010; Smith *et al.* 2011). The broadest scale horseshoe crab culture efforts have been reported by Japanese researchers, starting in the late 1980s (Tsuchiya 2009), but the details of this work remain largely unpublished. Although numerous laboratory studies have been conducted over the past three decades to determine growth and survival of horseshoe crabs at different life stages (Fig. 2) and under various conditions, including different enclosure types, sediment types, water conditions and feeding regimes (cf. sources reported in Tables 1–4). These data have not been compiled, organized and analysed to: (i) determine the relative success of the variety of published techniques; or (ii) broadly share this information with the community of invested researchers, managers and educators. As a first step to meet the growing demand for information to efficiently and effectively rear horseshoe crabs, we surveyed available literature to identify and define a consistent set of conditions for rapid growth and high survival of horseshoe crabs in culture.

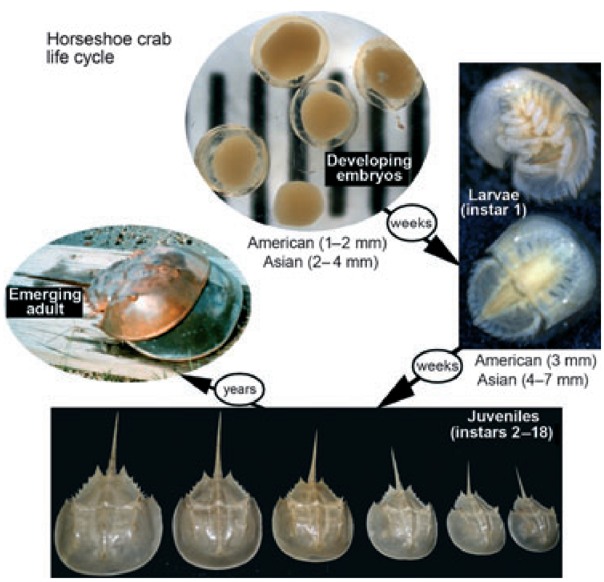

Figure 2 Generalized life cycle for American and Asian horseshoe crabs, showing the approximate size of eggs and larvae and the duration of each life stage, which vary by species and are detailed in Tables 1–4 and associated citations. Images of *L. polyphemus* are shown as an example of each life stage, including preserved developing embryos and larvae, representative juvenile molts, molt to adult stage.

Methods

Literature review

The data for this analysis and review were gathered by searching a broad range of available scientific literature to identify conditions that yielded the maximum values for growth and survival. Sources included books (four), journals (seven), a thesis and a presentation transcript (Tables 1–4). Data pertaining to growth, survival, time in culture, water temperature and salinity, enclosure size, frequency and timing of water changes, diet and substrate type were compiled and separated by reference to embryo, juvenile or adult life stage. Data for the three Asian species (*T. tridentatus*, *T. gigas* and *C. rotundicauda*)

Table 1 Conditions for the maximum growth (in minimum days to hatch) and survival of cultured horseshoe crab embryos reported for each study

Species	Source	Trial length	Growth (days to hatch)	Survival (%)	Temp (°C)	Salinity (g L^{-1})	Enclosure attributes	Maintenance (water change)
L. polyphemus								
	Schreibman and Zarnoch (2009)	1.5 y	Up to 31	>75	–	–	McDonald jar, 40 L min^{-1} (*n* = 300–500)	Flow through
	Ehlinger and Tankersley (2004)	75 d	17	–	30	30–40	1.5 × 1.5 cm (*n* = 1)	Every 2 d
	Sekiguchi *et al.* 1988b	–	14	–	30	34–35	–	–
		–	–	96	30	25	8 × 4.5 cm	Twice per day
	Jegla and Costlow (1982)	–	10	–	30–35	20	3.4 × 3.4 × 3.0 cm (*n* = 1)	Every 2 d
	Brown and Clapper (1981)	–	27	~95	15–32	50–125% RS	9.0 cm petri dish (*n* = 10–50)	Weekly
C. rotundicauda								
	Zadeh *et al.* (2009)	328 d	41–55	99	28	31–35	–	Twice per day
	Sekiguchi *et al.* 1988b	–	34	–	30	34–35	–	–
		–	–	83	30	30	8.0 × 4.5 cm	Twice per day
T. gigas								
	Mishra (2009b)	180 d	38–40	–	26–28	–	Plastic tray	–
	Zadeh *et al.* (2009)	355 d	42–64	98	28	31–35	–	Twice per day
	Chatterji *et al.* (2004)	45 d	42–45	–	27	–	–	–
	Sekiguchi *et al.* 1988b	–	37	–	30	34–35	–	–
		–	–	60	30	30	8.0 × 4.5 cm	Twice per day
T. tridentatus								
	Hong *et al.* (2009)	152 d	–	–	28–30	35	–	Twice per day
	Yan (2008)	2 y	–	15	28	FS	–	–
		90 d	–	1	32	30	9.0 cm petri dish	Once per day
	Sekiguchi *et al.* 1988b	–	43	–	30	34–35	–	
		–	–	98	30	30	8.0 × 4.5 cm	Twice per day

''–'', data not available; RS, raw seawater; FS, filtered seawater; d, day; y, year. Shaded areas indicate the minimum time to hatch and maximum survival among the reported studies.

were grouped because there were too few studies to consider each Asian species separately, and they have similar major life-history characteristics.

Determining growth and survival

We opted to use growth in terms of prosomal width and percentage survival as the objective metrics to evaluate culture success because these were the most commonly measured relevant variables. Whenever possible, growth and survival data were taken directly from original sources without modification. Owing to differences in reporting among authors; however, data were converted to a consistent set of units for comparison of each measurement shown in Tables 1–4. In some cases, we calculated summary values from raw data reported in primary sources to yield comparable data for comparison. For simplicity and clarity, mean growth and survival were reported across the range of instar stages when instars were reared under the same conditions in the same study and had similar growth or survival rates (overlapping standard errors). Some sources tested growth and survival under various environmental conditions; we summarized and reported only the conditions that yielded the maximum values for growth and survival for each age class or species within a study.

Growth

For embryos, we reported growth as the time in culture prior to hatching as it was reported in the original data source (Table 1). For juveniles, data were reported among studies using a variety of different biological metrics (length, weight, molt frequency) and temporal units (days, weeks, intermolt periods). Because prosomal width was the most commonly reported measurement among studies, and most studies reported growth durations that could be converted to weekly periods, we opted to report growth in terms of prosomal width increase in millimetres per week. When growth was reported as a percentage of incremental growth per intermolt time and the prosomal width of at least one instar was given, we calculated the preceding or subsequent molt size, determined the difference in size between molts and divided by the intermolt period to calculate growth rate. In cases where growth was reported as intermolt time or wet weight

Table 2 Reported conditions for the maximum growth in terms of prosomal width (PW) mm wk^{-1} and survival of juvenile American horseshoe crabs, *L. polyphemus,* in culture. If culture conditions were the same, one value was reported for the range of instars

Source	Trial length	Instar	Mean initial PW (mm)	Mean growth (PW mm wk^{-1})	Survival (%)	Temp (°C)	Salinity (g L^{-1})	Enclosure dimensions	Enclosure maintenance (water change)
Carmichael *et al.* (2009)*,†	120 d	1–4	3.1 ± 0.0–8.9 ± 0.1	0.06 ± 0.01 0.03 ± 0.01	87 ± 1 90 ± 1	17–23	34	15 × 24 cm, 20 × 29 cm (*n* = 13)	Every 2 wks
Schreibman and Zarnoch (2009)†	1.5 y	–	Hatchling	0.3 ± 0.2	–	20–25	27–33	320 L downweller, 1.5–2.0 L min^{-1}	3× per week
			6.0	0.6				AS	
Tsuchiya (2007)	3 y	1–16	–	–	–	21	17–34	30 × 20 × 7 cm–45 × 120 × 25 cm	–
Smith and Berkson (2005)	–	–	–	–	–	15–21	~27	–	25–30% every 3–4 wks
Ehlinger and Tankersley (2004)	30 d	–	–	–	100	30	30	1.5 × 1.5 cm plate (*n* = 1)	–
Sekiguchi *et al.* (1988a,b)‡	~7 y	1	3.3 ± 0.3	0.9 ± 0.2	0	30–35	20	6 × 3 cm depth (*n* = 20)	Daily
		2	5.0 ± 0.2	1.4 ± 0.3			25		
		3–5	7.0 ± 0.4	1.0 ± 0.3†			30		
		6–14	14.5 ± 1.2	–			34–35	3.4 × 3.4 × 3 cm–36 × 26 × 10 cm (*n* = 1)	
Laughlin (1983)§	–	1–2	–	20.0 ± 1.0 d	54	30	15	–	5 days per week
		1–2	–	28.0 ± 2.5 d	75	35	25	–	
Jegla and Costlow (1982)§	–	1–3	–	12.3 ± 0.5 d	100	25	30	8.9 cm bowls (*n* = 1)	Every 2 days
		4		24.2 ± 3.9 d	95				
		5–7			30–75				
		1–3	–	15.2 ± 0.5 d	100		40		
		4		24.9 ± 4.8 d	100				
		5–7			65–85				
Brown and Clapper (1981)	1.5 y	2	5.0	0.4	90	19–22	50–125% RS	9 cm petri dish (*n* = 10)	Weekly
		3	6.5	1.7					
		4	9.0	1.3				–	
		5	11.0	3.3					

''–'', data not available; AS, artificial seawater; FS, filtered seawater; d, days (in some cases growth was reported in days to molt rather than PW). Shaded areas indicate maximum growth and survival among the reported studies.

*Two reported growth rates reflect diet differences, which are not shown (algae vs. protein based foods were used in the top and bottom rows, respectively).

†Mean growth was calculated from individual instars among which growth did not significantly differ.

‡Survival of 0% in this case was due to defining the duration of study as the lifetime of the animals, and one animal was reared to instar 14 before death.

§Growth was reported in intermolt days (time between consecutive or non-consecutive molts).

without reference to prosomal width, the values are reported in Tables 1–4, but were not converted and were not included in subsequent comparisons. When possible, the mean ± standard error growth rate (mm $week^{-1}$) was calculated for individual instars, but in some cases, studies reported a single growth measurement across multiple instars. In these cases, we reported a mean growth rate for the corresponding range of instars. When needed, additional notes on the calculations and data conversions are given in footnotes to each table.

Survival

Percentage survival was calculated as the number of individuals alive at the end of a trial, divided by the total number of horseshoe crabs at the start of the trial, multiplied by 100. To convert mortality rates to percentage survival, we multiplied the mortality rate by the total duration of the trial, divided the result by the total number of horseshoe crabs in the trial, multiplied by 100, and subtracted the resulting percentage from 100.

Table 3 Reported conditions for the maximum growth in terms of prosomal width (PW) mm wk^{-1} and survival of juvenile Asian horseshoe crabs in culture. If culture conditions were the same, one value was reported for the range of instars

Species	Source	Trial length	Instar	Mean initial PW (mm)	Mean growth (PW mm wk^{-1})	Survival (%)	Temp (°C)	Salinity (g L^{-1})	Enclosure dimensions	Enclosure maintenance (water change)
C. rotundicauda										
	Zadeh *et al.* (2009)*	328 d	1–7	3.7 ± 0.1	–	60	–	–	17 × 35 cm plastic trays	–
	Lee and Morton (2005)	183 d	–	–	–	67	27–32	26–32	30 × 45 × 30 cm, 47 × 77 × 30 cm	–
T. gigas										
	Mishra (2009b)	180 d	1	7.0	0.9*	100	26–28	32–34 FS	Fiberglass tank (*n* = 2 L^{-1})	40% daily
			2	11.0	2.3	67				
			3	16.0	0.6	62				
			4	20.0	0.3	30				
			5	24.0	–	–				
	Zadeh *et al.* (2009)	355 d	1	7.0 ± 0.3	–	66	–	–	17 × 35 cm plastic trays	–
			2	10.9 ± 0.6						
			3–6	15.4 ± 0.6						
	Chatterji *et al.* (2004)	45 d	–	137–142 mg†	18.5 ± 7.5 mg wk^{-1}	–	27	40	40 L (*n* = 10)	–
T. tridentatus										
	Hong *et al.* (2009)	152 d	1–2	5.8 ± 0.2	0.8 ± 0.2	96	28	35	60 × 50 × 40 cm (*n* = 500)	Daily
	Yan (2008)	2 y	1–4	7.0	–	0.5‡	–	34–35	–	–
	Tsuchiya (2007)	11 y	1–11	6.0	–	–	15–37	–	30 × 90 × 40 cm (*n* = 11); 180 cm^2 (*n* = 1)	Twice per year
	Lee and Morton (2005)	183 d	–	–	–	88	27–32	26–32	30 × 45 × 30 cm, 47 × 77 × 30 cm	–
		61 d	–	21.4–66.1	10% molted	70	18–22	26–32		Weekly
				21.4–41.9	50% molted	75	28–32		46 × 77 × 30 cm	
	Sekiguchi *et al.* (1988a,b)	~8 y	1–10	6.0 ± 0.3	–	0	30	–	8.5 × 4.5 cm (*n* = 20); 3.4 × 3.4 × 3.0 cm–36 × 26 × 10 cm (*n* = 1)	Daily

''–'', data not available; FS, filtered seawater; d, days; y, year (in some cases growth was reported in days to molt rather than PW). Shaded areas indicate maximum growth and survival among reported studies.
*Mean growth was estimated visually from a figure when raw data were not reported.
†Growth was reported in wet weight increase.
‡Survival was calculated based on 20 juveniles that survived from an initial batch of 4000 eggs rather than a previous instar stage.

Table 4 Reported conditions for the maximum survival of adult American horseshoe crabs, *L. polyphemus,* in culture

Source	Trial length	Survival (%)	Temp (°C)	Salinity (g L^{-1})	Enclosure dimensions	Enclosure maintenance (water change)
Smith and Berkson (2005)	–	–	15–21	~27	–	25–30% every 3–4 wk
Ehlinger and Tankersley (2004)	–	–	20–23	30	270 × 170 × 100 cm	Recirculating tank
Brown and Clapper (1981)	1.5 y	82	11–22	–	3600 L (*n* = 57)	Outdoor flow through

''–'', data not available; y, year, wk, week.

Calculating errors and error reporting

When sufficient data were reported in the primary source, the standard error was calculated for each derived mean value. The standard error was calculated from raw data points that did not have associated error. When the error was reported with the raw data in the original source, we reported the error or propagated error for subsequent calculations and data conversions according to Valiela (2000). Errors are not reported in cases where the

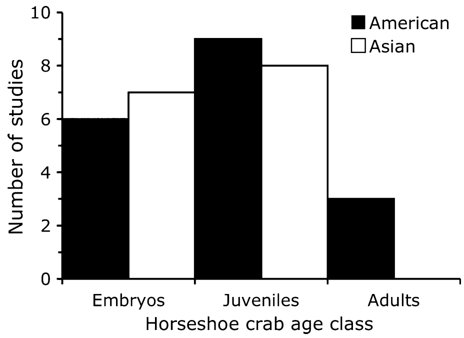

Figure 3 Number of studies reporting data on growth or survival of American and Asian horseshoe crabs in culture, separated by life stage (*n* = 13 total sources, but some sources presented data from multiple studies among different age classes and species, and each study was considered separately).

primary sources did not report the errors and lacked sufficient data for subsequent error calculations (e.g. when a single mean value was reported without error in the original document).

Results

Literature review

Relatively few published sources clearly related husbandry conditions to growth and survival, and comparison among studies was complicated by inconsistent data collection and reporting techniques (note the frequency of blank cells in Tables 1–4). We discovered a total of 13 available sources that reported sufficient detail for comparisons of growth and survival to basic husbandry conditions for embryo, juvenile or adult horseshoe crabs in culture over the past three decades (Tables 1–4; Fig. 3). Many of these sources included studies on multiple species and life stages, which allowed more comparisons than indicated by the number of published sources alone (Fig. 3). In all, we were able to extract data from 13 studies on embryos, 17 studies on juvenile horseshoe crabs (instars 1–16) and three studies on adults held or reared in culture. Most studies reported data for younger age classes and more studies examined *L. polyphemus* than the Asian species (Fig. 3; Tables 1–4) All three studies conducted on adults in captivity focused on *L. polyphemus* (Table 4). Although potentially critical to adequate husbandry conditions in the laboratory, very few studies reported sediment or water-quality attributes (i.e. sediment quantity, composition and texture, dissolved inorganic nitrogen and dissolved oxygen concentrations or pH) or feeding quantities and schedules so formal comparison of these attributes to growth and survival among studies was not possible and they were eliminated from further comparison.

Growth and survival in culture

The duration of the culture studies ranged from 45 days to 2 years for embryos (Table 1), from 30 days to 11 years for juveniles (Tables 2, 3), and up to at least 1.5 years for adults (Table 4). During these culture studies, the reported time to hatching, growth rates and survival showed distinct differences among age classes and, in some cases, among species. For embryos, the hatching time was shorter for the American horseshoe crab, ranging from 10 to 31 days for *L. polyphemus* compared with 34–55 days for Asian species (Fig. 4a). Survival of embryos was high, typically >75% for all species (Fig. 4b). Among the Asian species, minimum hatching time and maximum survival were observed with embryos of *C. rotundicauda*. For juvenile horseshoe crabs of all species, growth rates were most frequently reported for instars 1–5 (Tables 2, 3). Growth rates in terms of prosomal width (PW) were similar among species and instars within this age class (instars 1–5) and ranged from 0.03 to 3.3 mm week^{-1}, with most growth <2 mm week^{-1} (Tables 2, 3; Fig. 4c). Survival of juvenile horseshoe crabs in culture was typically >50% for *L. polyphemus* and 60% for Asian species (Tables 2, 3; Fig 4d). Of the three studies reporting husbandry conditions for adult *L. polyphemus*, only one reported survival, which was >80% (Table 4).

Husbandry conditions

Although we reviewed the entire suite of culture conditions reported among the studies in Tables 1–4, few studies reported sufficient detail to allow comparison between a consistent set of culture conditions and resulting growth and survival. Hence, we opted to analyse the range of values for the two most commonly reported variables, water temperature (°C) and salinity, and highlight the values at which hatching time was low for embryos, growth rates were high for juveniles and survival was high for all age classes (Fig. 5).

Water temperature and salinity

The water temperature and salinity that seemed most conducive to growth and survival of horseshoe crabs in culture varied by species and age class. The most distinct differences were noted between the American and Asian species at the embryo stage when Asian species hatched more quickly and survived better at lower temperatures and higher salinities compared with *L. polyphemus* (Fig. 5, bottom bars). Water temperatures for the most rapid time to hatch and greatest survival were around 29°C for the Asian species and 33°C for *L. polyphemus*, although salinity centred around 33 g L^{-1} for Asian species compared with 22 g L^{-1} for *L. polyphemus*. It is

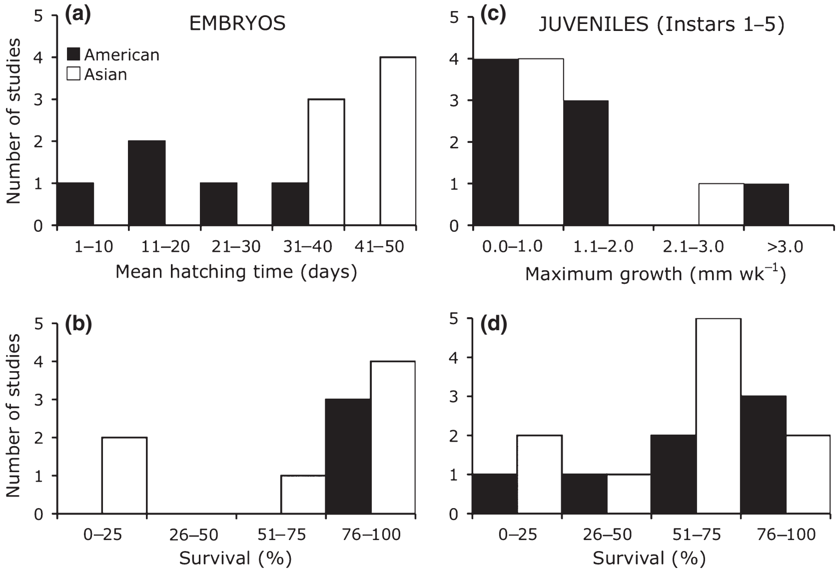

Figure 4 Frequency of (a) reported growth in terms of minimum time to hatch and (b) percentage survival for embryos compared to (c) maximum growth in terms of prosomal width and (d) percentage survival of juvenile horseshoe crabs in culture. Data for the three Asian horseshoe crab species (*Carcinoscorpius rotundicauda*, *Tachypleus tridentatus* and *Tachypleus gigas*) are grouped, but shown separately from the American horseshoe crab (*Limulus polyphemus*).

important to note, however, that the Asian species in these studies were reared (and hence growth and survival responses were tested) over a narrower range of salinities than *L. polyphemus*. Although the range of conditions for maximum growth and survival of juveniles overlapped among species, Asian horseshoe crabs showed a relatively narrower range of preferred conditions compared with *L. polyphemus* (Fig. 5, middle bars). Overall, maximum growth and survival were associated with water temperatures around 26°C for all juveniles and salinity centred around 34 g L^{-1} for Asian species and 31 g L^{-1} for *L. polyphemus*. Of the three studies that reported captive holding conditions for adults, only one reported survival. Hence, it was not possible to distinguish survival responses among husbandry conditions. The range of conditions reported in Table 4 is shown in Fig. 5 for comparison with other age classes. Husbandry conditions for adult *L. polyphemus* overlapped with those for younger age classes, but water temperatures for the holding tanks were generally lower.

Enclosure maintenance

Among the studies we reviewed, the frequency of scheduled enclosure (holding tank) maintenance, including cleaning and water changes, corresponded to the age class. At conditions for the most rapid hatching and maximum survival, the water was changed in the embryo enclosures every other day to daily (Table 1). For juveniles, maximum growth and survival occurred among studies that changed the water weekly to daily, and for adults the frequency of maintenance dropped to monthly, or recirculating and flow-through systems were used.

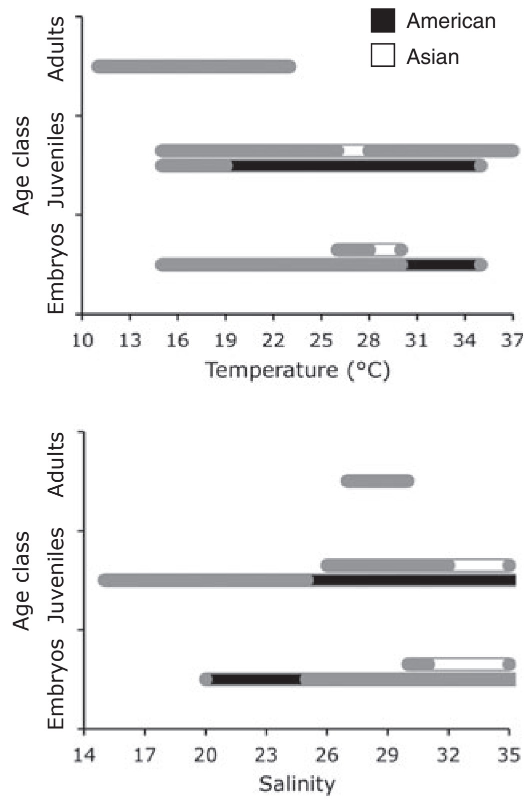

Figure 5 Reported ranges of temperature and salinity values (grey bars) at which embryo, juvenile and adult horseshoe crabs were reared in culture among the studies reported in Tables 1–4. Black and white segments of each bar represent the temperature and salinity ranges at which minimum time to hatch (for embryos), maximum growth (for juveniles) and maximum survival were observed among American and Asian horseshoe crabs, respectively. The single bar for adults reflects data for American horseshoe crabs.

Diet in culture

The most commonly offered food type among the culture studies reported in Tables 1–4 was brine shrimp (*Artemia* spp.), which are abundant and commercially available. Maximum growth and survival were associated with diets of *Artemia* and other mixed invertebrates (e.g. Brown & Clapper 1981; Jegla & Costlow 1982; Hong *et al.* 2009; Mishra 2009b). Horseshoe crabs of both species, however, subsisted in culture on dietary supplements that are not part of their known natural diet, including commercial fish foods, earth worms, rotifers and sea urchin eggs (Jegla & Costlow 1982; Sekiguchi *et al.* 1988a; Hong *et al.* 2009; Schreibman & Zarnoch 2009). In one case, supplementing the diet with algae increased the rate of growth, but reduced survival among juvenile *L. polyphemus* (Carmichael *et al.* 2009). The frequency and quantity of feeding were rarely reported.

Discussion

The relative paucity of data to inform captive rearing of horseshoe crabs may result from two primary factors. First, culture has not been a primary focus of horseshoe crab research. Many researchers, particularly in the USA have considered captive rearing to be difficult, time consuming and impractical (Ruth Carmichael pers. obs., Estuarine Research Federation 2007 biennial conference special session and workshop on horseshoe crab ecology). As a result, many studies on horseshoe crab ecology and biology have been published that have some data relevant to captive rearing, but if culture was not the focus of the study, detailed data on rearing conditions were not necessarily reported. Our results demonstrate that even in cases where culture was a focus, studies often lacked sufficient detail or consistency in data collection and reporting to guide culture practices. Second, many studies conducted in Asian countries, such as Japan, Taiwan and Hong Kong, are only known anecdotally and have either not been published, are not broadly available or are inaccessible owing to language barriers (e.g. Guangyao 1987; Tsuchiya 2007, 2009). This lack of international communication on culture techniques has lead to a potential bias; approximately the same number of studies are accessible on the single American species, *L. polyphemus*, as on all three Asian species combined (Tables 1–4), despite reports of successful rearing of horseshoe crabs from egg to adult in Japan within 3–7 years (Tsuchiya 2009; Chang-Po Chen pers. comm. 2011). It is also important to note that the few published studies on Asian species often included measurements of *L. polyphemus* for comparison, whereas most studies focused on *L. polyphemus* did not address Asian species (Tables 1–4).

Regardless of the reason for the poor data record on horseshoe crab captive rearing, it is clear that these data are now in demand. At the recent International Workshop on Science and Conservation of Asian Horseshoe crabs in Hong Kong (June 2011), seven presentations included data on conditions for captive rearing of horseshoe crabs. The need for data sharing, particularly to encourage and expand horseshoe crab culture, was highlighted as an important component of horseshoe crab conservation and restoration. To this end, it is imperative that researchers publish their data in peer-reviewed journals and include details on all aspects of rearing and holding conditions along with resulting growth and survival data even when husbandry is not the primary focus of a study. Our analyses indicate a need to use multiple metrics for growth and survival or, at a minimum, to apply the most common metrics used in the field (prosomal width and percentage survival) to provide sufficient data for comparison and assessment of rearing conditions for future propagation and research efforts.

Growth and survival in culture

The range and consistency of prosomal width growth rates reported for different instars and species under culture conditions were generally consistent with, but somewhat lower than the rates previously reported for wild stocks in Japan and the USA (Sekiguchi *et al.* 1988a,b; Carmichael *et al.* 2003). Although percentage growth in prosomal width typically decreases with size, particularly among smaller sized horseshoe crabs (instars 1–5), absolute growth in prosomal width for this same size class is relatively constant (Sekiguchi *et al.* 1988a,b; Carmichael *et al.* 2003). These observations suggest that absolute prosomal width, which is a relatively stable growth metric, may best indicate the environmental variables (in this case culture conditions) that most significantly affect growth, providing a functional and comparable metric for growth among all four living species of horseshoe crabs. In contrast to growth rates, survival was more variable within age classes and species (Fig. 4), even under apparently similar culture conditions (Table 1). Although survival was generally high (typically better than 50% for all age classes; Fig. 4) and appeared to improve with increasing age class, chronic mortality after 6 months in captivity has been reported in many studies conducted in the USA (e.g. Brown & Clapper 1981; Smith & Berkson 2005; Schreibman & Zarnoch 2009). Relationships to environmental factors, contamination or disease have not been resolved (Smith *et al.* 2011). It is possible, therefore, that culture studies based on trials shorter than 6 months in duration may report higher survival rates than longer-term studies, regardless of housing conditions, and could confound assessment of suitable husbandry techniques. If unresolved, premature mortality in culture may shorten the time during which individuals can be maintained and grown-out prior to release for restoration or propagation purposes. Longer grow-out times are preferred because they allow release of older age classes, which are larger in size and more likely to survive after release.

Comparison to environmental attributes

In the natural environment, horseshoe crabs are known to be relatively tolerant of a wide range of water temperatures and salinities (Shuster & Sekiguchi 2003; Sekiguchi & Shuster 2009), making these attributes of high interest and among the most commonly reported in culture studies (Tables 1–4). Our results suggest that Asian horseshoe crabs may grow faster and survive better over a narrower range of water temperatures and salinities, and they generally preferred higher salinity water compared with *L. polyphemus* (Fig. 5). Although all horseshoe crab species are distributed in coastal areas and use estuarine habitats (Sekiguchi & Shuster 2009), this finding may reflect an increased adaptation by *L. polyphemus* to the dynamic conditions common to estuarine waters (McManus 1969; Laughlin 1983; Towle & Henry 2003) and may be an important consideration in refining culture methods among the different species. The relatively high tolerance of all horseshoe crabs to temperature and salinity variation, however, makes it difficult to use these attributes to narrow down the specific conditions for maximum growth and survival in culture. Accordingly, although we were able to plot ranges for maximum growth and survival in Figure 5, examination of the full suite of data suggests that growth and survival did not vary consistently or with great magnitude across the range of temperatures and salinities reported among studies.

Defining the most suitable culture conditions is additionally challenging in the absence of data for other variables that might affect growth and survival, but were not consistently reported. For example, sediment grain size, water flow or circulation and dissolved oxygen concentrations have been identified as key defining variables for horseshoe crab habitat in the natural environment (Sekiguchi & Shuster 2009), but were rarely addressed among culture studies (Tables 1–4). Recent studies with embryos and early instar stages of horseshoe crabs found shorter hatching times, more frequent molting and increased growth with increased water circulation or increased dissolved oxygen concentrations (Ruth Carmichael pers. obs.; Chang-Po Chen and Anil Chatterji pers. comm. 2011). The limited available data on culture conditions make it impossible to assess potential interactions among different variables that could further affect growth and survival in culture or susceptibility to infections, which are also poorly understood (Smith *et al.* 2011). These findings highlight the need for more detailed data on conditions for captive rearing in addition to temperature and salinity.

The importance of enclosure maintenance and diet

Along with temperature and salinity, enclosure maintenance and diet composition were two aspects of captive rearing for which most studies reported data. The most important aspect of enclosure maintenance appeared to be higher frequency of water replacement and enclosure cleaning for younger age classes, but movement toward naturalistic conditions (flow through or outdoor holding tanks) for adults. Researchers in Japan also suggest transferring crabs from holding tanks to separate feeding tanks to reduce fouling (Tsuchiya 2007). These age-specific techniques likely reflect the logistical constraints to holding, maintaining, and feeding larger sized crabs, which require larger enclosures, more time to clean and replace holding water, and make frequent maintenance and feeding more difficult.

The diets offered to horseshoe crabs in culture and the growth responses to them were generally consistent with known dietary preferences in nature. The natural diet of horseshoe crabs is typically broad and highly mixed in composition (Zhou & Morton 2004; Botton *et al.* 2003; Carmichael *et al.* 2004). Stable isotope data on *L. polyphemus* confirms that American horseshoe crabs move up food webs as they age and grow (Gaines *et al.* 2002; Carmichael *et al.* 2004). These studies indicate that early instar stages rely largely on detritus and small organic particles adhered to sediments and then forage on polychaetes and small crustaeans as they grow (instar 5+), foraging most broadly as adults and consuming the same small prey along with larger bivalves and gastropods. Although there are no similar isotopic data for Asian species, juvenile *T. tridentatus* and *C. rotundicauda* favour similar small prey, including insect larvae and small polychaetes (Zhou & Morton 2004). Accordingly, the culture studies we analysed (Tables 2–4), which focused on early instar stages, primarily offered and found greatest success with a small crustacean (*Artemia*) and mixed invertebrate diet (Fig. 6). These observations indicate that the size of prey or other food particles is an important dietary consideration along with diet composition.

More research is needed to clearly resolve the importance of diet composition to horseshoe crab growth and survival in culture. Most culture studies offered high protein diets (Fig. 6), which have been suggested to enhance growth rates (Schreibman & Zarnoch 2009). There are few data, however, to determine the relative importance of

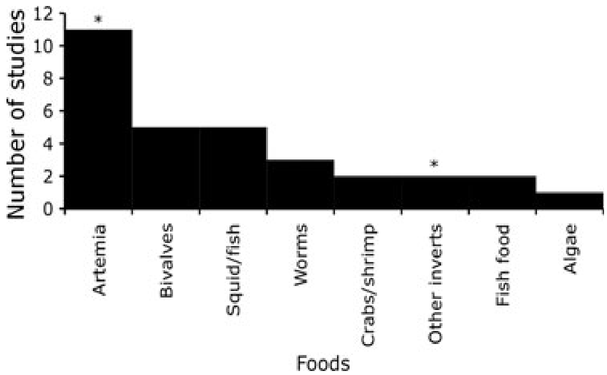

Figure 6 Frequency of different food types offered to juvenile or adult horseshoe crabs from the culture studies shown in Tables 2–4. Asterisks indicate the foods associated with the highest reported growth rates and percentage survival.

protein compared with vegetation-based diets (Carmichael *et al.* 2009; Schreibman & Zarnoch 2009), particularly for young crabs that are known to naturally consume small sediment-derived particles that may not be rich in protein (Gaines *et al.* 2002). One study found lower protein diets resulted in faster growth, but reduced survival among early instars (Carmichael *et al.* 2009). Similarly, recent studies have found that high protein feeds may be poorly assimilated (Schreibman & Zarnoch 2009) and a digestible protein content of ~40% may yield the best growth and assimilation efficiency for *T. tridentatus* and *C. rotundicauda* (Menghong Hu pers. comm. 2011). These data suggest that a mixed diet that includes some vegetation or at least moderates the quantity of assimilable protein may be important. Ongoing studies on protein and energy requirements for American and Asian species will better guide development of formulated diets for future captive rearing (e.g. Schreibman & Zarnoch 2009; Menghong Hu pers. comm. 2011).

Conclusion

If wild populations of horseshoe crabs are to be augmented by laboratory-reared stocks, more consistently collected and detailed data on methods for captive rearing must be reported and made more widely available throughout the international research community. This effort will require more studies to be conducted and placed into the context of previous work as well as an emphasis on international data sharing. Although temperature and salinity are important aspects of the culture environment, more attention should be given to other variables, most notably sediment quality, water circulation and dissolved oxygen concentration. These core conditions along with other aspects of water quality and the quantity and frequency of feeding have been largely unreported in the available published literature. Because horseshoe crabs move up food webs as they age and grow, size appropriate feeding requirements should be considered; a variety of food sources, particle sizes and the relative quantity of assimilable protein may all be important. These findings emphasize data gaps and a need for consistent controlled study of horseshoe crab growth and survival in culture. Improved efficiency and effectiveness of captive rearing will support propagation and restoration efforts in the USA, but also throughout Asia, where crabs stocks have been critically depleted.

Acknowledgements

This work was funded in part by the Dauphin Island Sea Lab through the undergraduate summer research program. We thank Dr Jayant Mishra for providing additional details about his research, attendees of the International Symposium on Science and Conservation of Horseshoe Crabs at Dowling College, NY, who inspired this assimilation, and participants in the International Workshop on Science and Conservation of Asian Horseshoe crabs in Hong Kong, who shared unpublished data. We also thank Ricky Alexander and D. Joe Dalrymple for assistance with background research and imaging, respectively.

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