Journal of the Marine Biological Association of the United Kingdom, 2012, 92(3), 463–468.
doi:10.1017/S0025315411001196

Sallow-skin horseshoe crabs (late juvenile *Tachypleus tridentatus*) as osmoconformers

YONGYAN LIAO[1], CHANG-PO CHEN[2], HWEY-LIAN HSIEH[2], YUCHENG CAO[1] AND JIEJUN CHEN[1]

[1]Fisheries College of Guangdong Ocean University, Zhanjiang 524025, China, [2]Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan

Sallow-skin horseshoe crabs (late juvenile Tachypleus tridentatus*) from 30‰ salinity seawater were subjected to salinities ranging from 0‰ to 70‰ at 5‰ intervals for 7 days. They were returned to 30‰ salinity for 3 days for recovery. During this period, the survival rate, feeding rate, and body mass of the horseshoe crabs were measured. The sallow-skin horseshoe crabs survived well at 15‰–60‰ salinity, fed well at 30‰–55‰ salinity, gained mass at 0‰–25‰ salinity, lost mass at 45‰–70‰ salinity, and maintained mass at 30‰–40‰ salinity during salinity challenge. During the recovery period, the dehydrated (mass lost) horseshoe crabs rapidly gained mass, whereas the water-retaining (mass gained) horseshoe crabs rapidly lost mass. These results indicate that the sallow-skin* T. tridentatus *horseshoe crabs are osmoconformers and can tolerate a wide range of salinity changes, adapting gradually from inshore low salinity to offshore high salinity.*

Keywords: *Tachypleus tridentatus*, horseshoe crab, osmoregulation, food intake

Submitted 3 December 2010; accepted 12 June 2011; first published online 30 August 2011

INTRODUCTION

The horseshoe crab, *Tachypleus tridentatus* (Leach, 1819), has undergone little morphologic evolution in the last 200 million years (Walls *et al.*, 2002). Although it has escaped many catastrophes that led to mass extinctions (Raup & Sepkoski, 1982), it is now facing extinction (Liao & Li, 2001; Chen *et al.*, 2004; Berkson *et al.*, 2009; Tanacredi *et al.*, 2009). Before the 1980s, fishermen caught 1.5 million *T. tridentatus* every year; however, today, they catch less than 50,000 individuals from the Beibu Gulf of the South China Sea. Thus, in 2004, *T. tridentatus* was classified as an endangered species by the 'Red Book of China' (Wang & Xie, 2004). To manage and protect the rare horseshoe crab resources, a detailed study of its life cycle (Cierpich *et al.*, 2004) and its ability to adapt to environmental changes is necessary.

Horseshoe crabs spawn on sandy beaches (Ehlinger & Tankersley, 2003; Brousseau *et al.*, 2004), and the fertilized eggs hatch into planktonic trilobite larva (Botton & Loveland, 2003), which then settle as benthic larvae in the intertidal zones near the beach (Botton *et al.*, 2003). The early juveniles of the horseshoe crab *T. tridentatus* wander the intertidal mud flats. Their exoskeleton are black, hence, the local fishermen call them black-skin horseshoe crabs. As they moult more and grow gradually, they move into the subtidal shallow water. Between the 11th and the 16th moults, their skins become yellowish, and they are referred to as sallow-skin horseshoe crabs (Liao *et al.*, 2002). The late juveniles live in deeper water (20–40 m) sea areas (Liao *et al.*, 2002). Juveniles moult 17–18 times within 13–14 years to reach sexual maturity (Sekiguchi, 1988). Adults live in the region from the beach and the shallow sea to deeper waters (20–40 m) (Sekiguchi, 1988). Their life history involves the hatching of embryos, the growth of early larvae and late juveniles, and adult life in different sea areas. The habitat changes during the different stages of the horseshoe crab life cycle mean that there are likely corresponding adaptations to different sea environments.

Evidently, a horseshoe crab encounters a number of salinity changes during its life cycle. At present, the effects of salinity change on *Limulus polyphemus* have been revealed clearly on its embryo (Ehlinger & Tankersley, 2004) and larval growth (Jegla & Costlow, 1982; Laughlin, 1983; Ehlinger & Tankersley, 2004), and its osmotic regulation (McManus, 1969; Robertson, 1970; Towle *et al.*, 1982; Towle & Henry, 2003). However, few reports on the effects of salinity change on other horseshoe crabs are available, except for the effects of salinity on larval growth and the moulting of the horseshoe crab *Tachypleus gigas* (Chatterji *et al.*, 2004), on the insemination and hatching of *T. tridentatus* (Li *et al.*, 1999), and on embryo development of *T. tridentatus* (Wang *et al.*, 2001).

Moreover, most of the scanty available research on *T. tridentatus* is restricted to embryos or larvae within one year after hatching. The late juveniles (sallow-skin) are abundantly distributed off Zhanjiang, China (Liao *et al.*, 2001), where the shallow sea area is influenced by rainwater (1588.8 mm/year) and the salinity is lower and more variable (20‰ to 30‰) than in the more stable and comparatively higher salinity of deeper sea areas (32‰ to 36‰). Therefore, experiments on the salinity tolerance and adaptations of sallow-skin *T. tridentatus* were conducted to determine whether they are osmoregulars or osmoconformers (Barrington, 1979).

MATERIALS AND METHODS

Sallow-skin *Tachypleus tridentatus* horseshoe crabs, with average carapace width of 14.5 cm, a body length of

Corresponding author:
C.-P. Chen
Email: zocp@gate.sinica.edu.tw

30.0 cm, and a body mass of about 280 g, were collected from the Dongnan Sea area (20′40″ to 20′21″N and 110′9″ to 110′38″E) of Leizhou Bay, China. The salinity of seawater in Dongnan ranges from 22‰ to 32‰. The depth of the seawater is 20–40 m, at which the horseshoe crabs were collected.

To prepare seawater with varying salinities, natural seawater was taken from the Zhanjiang Sea area, which has a salinity of 28.3‰–30‰ and a pH value of 7.55–8.65. The seawater was filtered through 400-mesh bolting silk after sand filtration, and then adjusted to 30‰ salinity using 'Yuxiabao'$^{\mathrm{TM}}$ instant seawater crystals (97-b type), which were ready for use. Based on the experimental results of the food category and feeding amount (Gao *et al.*, 2003), 0.5–1 cm^2 meat pieces of freshly eviscerated cuttlefish (*Sepia*) were prepared as food. The cuttlefish were bought from the Dongfeng Market of Zhanjiang. During the experiment, the room temperature was 23.5–29.5°C and the water temperature was 22.5–27.5°C. The water in the containers was continuously aerated.

Fifteen salinity levels, from 0‰ to 70‰, at 5‰ intervals were prepared using natural seawater with tap water (pH 7.85) aerated for 24 hours or with seawater crystals, based on the method by Liao & Li (2002), and placed into 55 l plastic buckets (45 l water in each bucket). After being reared at 30‰ salinity for 2 days, the horseshoe crabs were placed into the containers with water with different salinity levels, with 6 horseshoe crabs for each salinity group. The horseshoe crabs were fed 20 g daily at 21:00 every day. At 09:00 the next day, the leftover food was taken out and weighed, and water in each bucket was replaced with freshly prepared water at the same salinity.

After 7 days of the salinity-challenged experiment, the water for all salinity groups was replaced with 30‰ seawater. The other conditions and methods remained the same. The salinity recovery experiment lasted 3 days. The survival rate of the horseshoe crabs at a particular salinity level was calculated with the formula below:

$$S = Ns \div Ni \times 100\%$$

where S is the survival rate, Ns is the total number of the surviving horseshoe crabs at that salinity level after the 7-day experiment, and Ni is the number of horseshoe crabs initially introduced.

The mean relative daily food intake of the horseshoe crabs was calculated using the formula below (Gao *et al.*, 2003):

$$I = F \div M \times 100\%$$

where I is the mean relative daily food intake of the horseshoe crab, F is the total daily food intake, and M is the body mass before the experiment, same as below.

The food intake experiments were all accompanied by the corresponding food solubility tests to correct the amount of food intake.

After the water was emptied with a basket, the *T. tridentatus* were weighed with a Shimadzu electronic scale (Japan), and the precision of the scale was 0.01 g. The body mass experiments were all accompanied by corresponding no-food-intake tests to correct the amount of body mass that resulted from the food intake.

The relative body mass change of the horseshoe crabs on the nth day was calculated by the formula as below:

$$Mc = (Ms - M) \div M \times 100\%$$

where Mc is the relative body mass change of horseshoe crabs on the nth day and Ms is the mean body mass on the nth day.

Significance tests for difference in survival rates were directly carried out based on the expansion of the binomial $(p+q)^n$. SPSS 12.0 statistical software was applied in a two-way analysis of variance to test the significance of the differences in relative food intake and relative body mass. The least significant difference method was used for intergroup comparisons. The differences were considered significant at $P < 0.05$.

RESULTS

Survival

The salinity challenge had a remarkable influence on the survival of the sallow-skin horseshoe crabs (Figure 1). The survival rate was 100% at 15‰–60‰ salinity, which dropped to 83.3% in the 65‰ salinity group, 66.7% in the 10‰ salinity group, and 33.3% in 5‰ salinity. All individuals in the 0‰ and 70‰ salinity groups died. During the salinity recovery period, all horseshoe crabs that did not die during the salinity challenge survived after 3 days.

These results indicate that the sallow-skin horseshoe crabs have a high tolerance for salinity changes. They can live not only in the river estuaries and inshore areas with low salinities, but also in abyssal seas with high salinities.

Food intake

Food intake was higher (with the mean relative food intake being above 2.052%) at salinity 30‰–55‰, and dropped

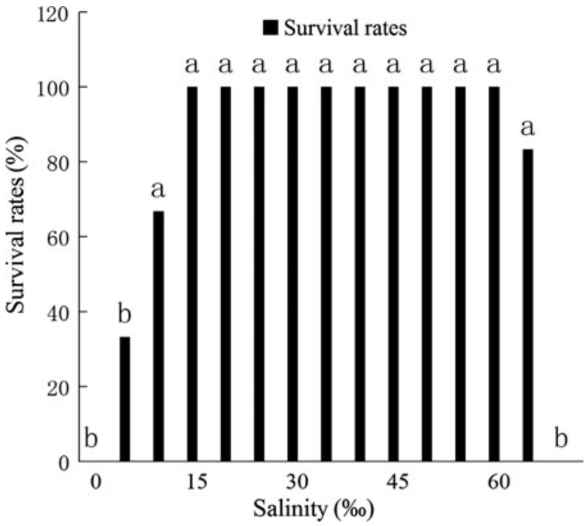

Fig. 1. Effect of salinity challenged on the survivorship of sallow-skin horseshoe crab.

Table 1. Effect of salinity challenge on the mean relative daily food intake of sallow-skin horseshoe crabs with 6 individuals in each salinity group.

Salinity groups (‰)	The relative food intakes of horseshoe crab (%)								Difference significances $P_{0.05}$
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	Mean	
0	0	0	0	0	0	0	/	0	d
5	1.625	1.010	0.789	0.174	0	0.312	0.208	0.588	bcd
10	1.651	1.516	1.245	0.808	0.400	0.441	0.080	0.877	bc
15	1.949	1.901	1.073	1.256	0.786	0.537	0.571	1.153	b
20	1.773	1.431	1.158	0.866	0.772	0.607	0.615	1.032	bc
25	1.865	1.141	1.329	0.911	0.817	0.483	0.454	1.000	bc
30	3.667	2.388	2.013	2.105	1.889	2.059	2.879	2.429	a
35	3.549	3.215	2.160	2.127	2.118	1.819	1.579	2.367	a
40	2.775	2.066	1.829	1.430	2.308	2.130	1.823	2.052	a
45	2.628	1.865	1.823	2.158	2.563	2.739	1.992	2.253	a
50	1.208	0.892	1.371	2.397	2.883	3.607	2.380	2.105	a
55	1.078	0.279	1.181	2.122	3.616	3.581	2.951	2.115	a
60	0.364	0	0.533	0.389	0.394	0.517	0.618	0.402	cd
65	0.706	0	0.243	0.274	0	0	0.082	0.186	cd
70	0.033	0	0	0.106	0	0	/	0.020	d

The '/' denotes the death of the horseshoe crab or no measure; the lower case letters denote the significant difference. There are no significant differences among groups having the same letters, and there are significant differences among groups not having the same letters (similarly in Tables 2, 3 & 4).

significantly at salinities below 25‰ and above 60‰ ($P < 0.05$: Table 1; Figure 2). During the salinity recovery period, the mean relative daily food intake of the sallow-skin horseshoe crabs were similar to that of the salinity-challenged ones (Figure 2), and were over 1.75% at 30‰–55‰ salinity (Table 2).

The optimum feeding salinity for the sallow-skin horseshoe crabs ranges from 30‰ to 50‰. The relatively suitable salinities ranged from 15‰ to 25‰ and 55‰. The most unsuitable salinities are at 0‰ and above 70‰.

The body mass of sallow-skin horseshoe crabs

The changes in body mass of the sallow-skin horseshoe crabs during the salinity challenge revealed a linear relationship between gained mass at low salinity and lost mass at high salinity at salinity levels from 10‰ to 60‰ (Table 3; Figure 2). An inverse linear relationship was observed during the recovery period (Table 3; Figure 2). The water-retaining horseshoe crabs in the low-salinity groups had rapid water loss during the late period of recovery, whereas the dehydrated horseshoe crabs in the high salinity groups had a rapid increase in mass during the early period of salinity recovery (Table 4). These results reveal that the sallow-skin horseshoe crabs are osmoconformers.

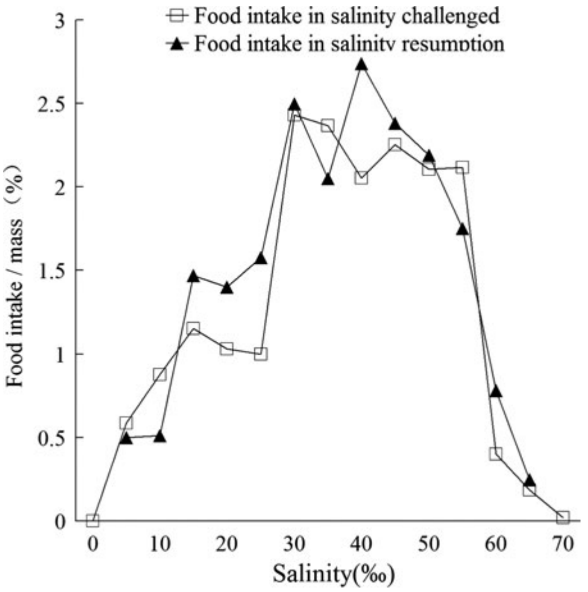

Fig. 2. Effect of salinity challenged on food intake of sallow-skin horseshoe crab.

DISCUSSION

The sallow-skin horseshoe crabs survived well at 15‰–60‰ (Figure 1), fed well at 30‰–55‰ (Table 1), gained mass at 0‰–25‰, lost mass at 45‰–70‰ and maintained mass at 30‰–40‰ during the salinity challenge (Table 3). These results indicate that sallow-skin *Tachypleus tridentatus* can tolerate a wide range of salinity changes, adapting gradually from inshore low salinity to offshore high salinity. The gametes of *T. tridentatus* can be inseminated in a broad salinity range (3‰–48‰), but fertilized eggs can hatch only in a narrow salinity range (16‰–40‰); within this range, the lower the salinity, the higher the hatching rate (Li *et al.*, 1999). Moreover, the hatching rates were 8.67% and 14.50%, respectively, at 10‰ and 15‰ salinity, and the larvae (between the 1st–2nd moults) grew fastest at 20‰ salinity (Wang *et al.*, 2001). Summarizing these data reveals that fertilized *T. tridentatus* eggs can hatch normally from 10‰ to 40‰, and the highest hatching rate and fastest development is at 16‰–26‰ (Wang *et al.*, 2001). Therefore, the embryonic and larval development of *T. tridentatus* is restricted to a narrow range at low salinities, of which 16‰–26‰ was optimal.

The effects of the salinity challenge and recovery on the food intake of the sallow-skin horseshoe crabs coincide with the aforementioned findings. The relative food intake was high at 30‰–55‰ salinity (Figure 2), whereas the relative change in mass of the sallow-skin horseshoe crabs was slight at 30‰–40‰ salinity (Figure 3). These indicate that,

Table 2. Effect of salinity recovery after challenge on the mean relative food intake of sallow-skin horseshoe crabs with 6 individuals in each salinity group.

Items	**The relative food intake of horseshoe crabs after recovery to 30‰ from various salinity levels and the difference significances**												
	5‰	**10‰**	**15‰**	**20‰**	**25‰**	**30‰**	**35‰**	**40‰**	**45‰**	**50‰**	**55‰**	**60‰**	**65‰**
1std (%)	0.751	0.539	2.146	2.264	2.421	2.568	2.560	4.393	3.547	2.789	1.432	0.752	0.077
2ndd (%)	0.317	0.434	1.360	1.244	1.320	2.312	2.325	2.560	2.324	2.217	2.052	0.881	0.106
3rdd (%)	0.433	0.560	0.899	0.690	0.984	2.609	1.258	1.258	1.268	1.558	1.770	0.712	0.559
Means (%)	0.500	0.511	1.468	1.399	1.575	2.496	2.048	2.737	2.380	2.188	1.751	0.782	0.247
$P_{0.05}$	ef	ef	cde	cde	bcd	ab	abc	a	abc	abc	abcd	def	f

compared with the embryos and larvae, the sallow-skin *T. tridentatus* can live at higher salinities. From 16‰–26‰ in the embryo and larval stage, the optimum salinity range of *T. tridentatus* expands to 30‰–55‰ in the sallow-skin stage.

The experimental results, as well as the analysis above, indicate that the sallow-skin horseshoe crabs can live at 15‰–60‰ salinity, feed well at 15‰–55‰, and feed optimally at 30‰–55‰. In hypoosmotic 0‰–25‰ salinity, the horseshoe crab absorbs water and its body mass increases. The lower the salinity, the more the mass increases. In hyperosmotic 45‰–70‰ salinity, the horseshoe crab loses water and its body mass decreases. The higher the salinity, the more the mass decreases. At 25‰–40‰ salinity, the body

Table 3. Effect of salinity challenge on the mean relative body mass of the sallow-skin horseshoe crabs with 6 individuals in each salinity group.

Salinity groups (‰)	**The relative daily body mass changes of horseshoe crabs (%)**							**Difference significances**
	1st day	**2nd day**	**3rd day**	**4th day**	**5th day**	**6th day**	**Means**	$P_{0.05}$
0	9.809	7.495	8.386	8.365	8.471	/	8.505	a
5	8.207	7.926	7.704	6.889	6.704	6.778	7.368	ab
10	5.995	7.331	7.546	7.891	7.676	7.245	7.281	ab
15	4.498	4.798	4.460	4.910	4.760	5.022	4.741	abc
20	3.991	0.045	4.305	4.417	4.709	4.417	3.647	bcd
25	3.544	0.560	2.362	2.735	3.046	2.767	2.502	cde
30	0.568	0.329	0.399	0.681	0.329	0.305	0.435	cdef
35	0.814	0.208	0.972	0.405	0.893	0.366	0.610	cdef
40	−0.694	−1.026	−0.845	−0.814	−0.845	−0.694	−0.820	def
45	−1.561	−1.327	−1.149	−1.289	−1.210	−1.329	−1.311	ef
50	−2.349	−1.572	−2.748	−2.228	−2.357	−2.522	−2.296	f
55	−3.261	−1.886	−3.476	−2.937	−2.937	−2.917	−2.902	f
60	−6.458	−4.321	−6.361	−1.408	−1.360	−1.748	−3.609	fg
65	−8.144	−6.531	−8.119	−8.623	−7.943	−8.144	−7.917	gh
70	−8.441	−8.287	−9.476	−8.915	−9.068	/	−8.876	h

The '-' denotes the body mass decrease.

Table 4. Effect of salinity recovery after challenge on the mean relative body mass of sallow-skin horseshoe crabs with 6 individuals in each salinity group.

Salinity groups (‰)	**The relative body mass changes of horseshoe crabs at different times after recovery (%)**						**Difference significances**
	8 hours	**16 hours**	**24 hours**	**48 hours**	**72 hours**	**Means**	$P_{0.05}$
5	−0.692	−17.486	−2.987	−7.942	−6.375	−7.096	a
10	0.824	−1.691	−3.554	−7.456	−7.759	−3.927	abc
15	0.000	−3.855	−5.091	−6.400	−6.945	−4.458	ab
20	−1.531	−2.049	−2.502	−2.890	−3.300	−2.454	bc
25	1.124	0.874	−3.029	−2.311	−2.530	−1.174	bcd
30	−0.023	−0.164	−0.305	−0.470	−0.986	−0.390	cde
35	1.737	1.292	0.969	−0.969	6.785	1.963	def
40	7.150	3.527	2.002	0.572	0.381	2.726	ef
45	9.268	6.367	5.189	2.821	1.339	4.997	fg
50	12.471	9.290	8.497	5.450	2.198	7.581	gh
55	16.827	12.428	11.356	7.699	2.530	10.168	h
60	12.324	12.961	9.482	5.551	4.489	8.961	h
65	9.576	17.259	11.812	7.913	7.712	10.854	h
Means	5.312	2.981	2.449	0.121	−0.189		
$P_{0.05}$	a	ab	abc	bc	c		

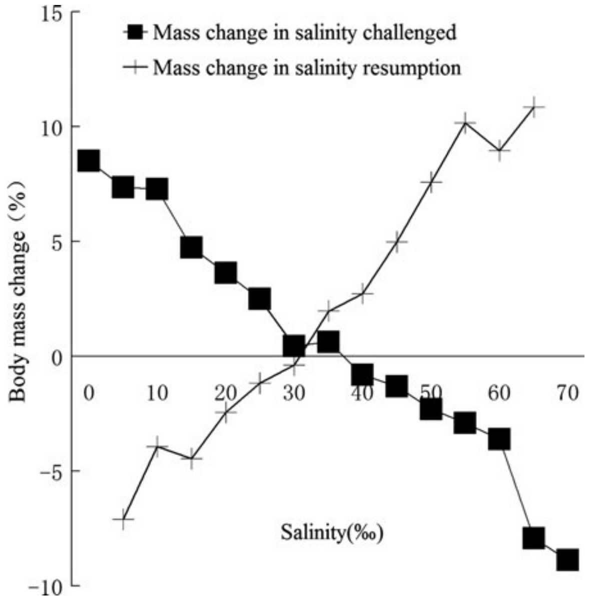

Fig. 3. Effect of salinity challenged on body mass change of sallow-skin horseshoe crab.

mass remained almost stable. At 50‰–55‰ salinity, the food intake of the horseshoe crab was initially low, but it later increased gradually. These prove that the sallow-skin horseshoe crab can tolerate a wide range of salinities (15‰–60‰); however, the comparatively high salinity of 30‰–55‰ is most favourable, with the optimal range at 25‰–40‰.

In the shallow sea of Zhanjiang, the salinity is influenced to a great extent by rainwater. Thus, salinity is comparatively lower. In deeper sea areas, salinity is stable and comparatively higher, ranging from 30‰ to 40‰, which exactly coincides with the narrowest range in which salinity influences body mass changes in our experiment. Therefore, the physiological adaptation to salinity of horseshoe crabs corresponds to its life history and migration route, from its embryonic and larval stage at low inshore salinities to its adult stage at higher salinities in deep sea areas. This reflects the long-term evolutionary adaptation of the living fossil, and indicates that humans should pay more respect and focus on protecting the species (Chen *et al.*, 2009).

REFERENCES

Barrington E.J.W. (1979) *Invertebrate structure and function.* 2nd edition. New York: John Wiley & Sons.

Berkson J., Chen C.P., Mishra J., Shin P., Spear B. and Zaldívar-Rae J. (2009) A discussion of horseshoe crab management in five countries: Taiwan, India, China, United States, and Mexico. In Tanacredi J.T., Botton M.L. and Smith D.R. (eds) *The biology and conservation of horseshoe crabs.* New York: Springer, pp. 465–475.

Botton M.L. and Loveland R.E. (2003) Abundance and dispersal potential of horseshoe crab (*Limulus polyphemus*) larvae in the Delaware Estuary. *Estuaries* 26, 1472–1479.

Botton M.L., Loveland R.E. and Tiwari A. (2003) Distribution, abundance, and survivorship of young-of-the-year in a commercially exploited population of horseshoe crabs *Limulus polyphemus. Marine Ecology Progress Series* 265, 175–184.

Brousseau L.J., Sclafani M., Smith D.R. and Carter D.B. (2004) Acoustic-tracking and radio-tracking of horseshoe crabs to assess spawning behavior and subtidal habitat use in Delaware Bay. *North American Journal of Fisheries Management* 24, 1376–1384.

Chatterji A., Kotnala S. and Mathew R. (2004) Effect of salinity on larval growth of horseshoe crab, *Tachypleus gigas* (Müller). *Current Science* 87, 248–250.

Chen C.P., Yeh H.Y. and Lin P.F. (2004) Conservation of the horseshoe crab at Kinmen, Taiwan: strategies and practices. *Biodiversity and Conservation* 14, 647–665.

Chen C.P., Hsieh H.L., Chen A., Yeh H.Y., Lin P.F. and Wang W. (2009) The conservation network of horseshoe crab *Tachypleus tridentatus* in Taiwan. In Tanacredi J.T., Botton M.L. and Smith D.R. (eds) *The biology and conservation of horseshoe crabs.* New York: Springer, pp. 543–557.

Cierpich S.B., Grady S.P. and Valiela I. (2004) Life history analysis of the juvenile horseshoe crab in Pleasant Bay, Cape Cod. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 207, 175.

Ehlinger G.S. and Tankersley R.A. (2003) Larval hatching in the horseshoe crab, *Limulus polyphemus*: facilitation by environmental cues. *Journal of Experimental Marine Biology and Ecology* 292, 199–212.

Ehlinger G.S. and Tankersley R.A. (2004) Survival and development of horseshoe crab (*Limulus polyphemus*) embryos and larvae in hypersaline conditions. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 206, 87–94.

Gao F.Y., Liao Y.Y. and Ye F.L. (2003) The feed study of the juvenile *Tachpleus tridentatus. Marine Science Bulletin* 22, 92–96.

Jegla T.C. and Costlow J.D. (1982) Temperature and salinity effects on developmental and early posthatch stages of *Limulus.* In Bonaventura J., Bonaventura C. and Tesh S. (eds) *Physiology and biology of horseshoe crabs: studies on normal and environmentally stressed animals.* New York: Alan R. Liss, Inc., pp. 103–113.

Laughlin R. (1983) The effects of temperature and salinity on larval growth of the horseshoe crab *Limulus polyphemus. Biological Bulletin. Marine Biological Laboratory, Woods Hole* 164, 93–103.

Li F., Liao Y.Y. and Dong X.X. (1999) The influence of salinity upon embryogeny of *Tachypleus tridentatus. Journal of Zhanjiang Ocean University* 19, 4–8.

Liao Y.Y., Hong S.G. and Li X.M. (2001) A survey on the horseshoe crabs in the North of South China Sea. *Acta Zoologica Sinica* 47, 108–111.

Liao Y.Y. and Li X.M. (2001) Present situation of horsecrab resources in the sea area of China and tactics of preservation. *Resources Science* 23, 53–57.

Liao Y.Y. and Li X.M. (2002) Researches into the influence of environmental factors upon *Portunus pelagicus. Acta Oceanologica Sinica* 24, 140–145.

Liao Y.Y., Li X.M. and Hong S.G. (2002) Morphology of sallow horseshoe crab (*Tachypleus tridentatus*). *Acta Zoologica Sinica* 48, 93–99.

McManus J.J. (1969) Osmotic relations in the horseshoe crab, *Limulus polyphemus. American Midland Naturalist* 81, 569–573.

Raup D.M. and Sepkoski J.J. Jr (1982) Mass extinctions in the marine fossil record. *Science* 215, 1501–1503.

Robertson J.D. (1970) Osmotic and ionic regulation in the horseshoe crab, *Limulus polyphemus* (Linnaeus). *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 141, 157–183.

Sekiguchi K. (ed.) (1988) *Biology of horseshoe crabs.* Tokyo: Science House Co., Ltd.

Tanacredi J.T., Botton M.L. and Smith D.R. (eds) (2009) *The biology and conservation of horseshoe crabs.* New York: Springer.

Towle D.W. and Henry R.P. (2003) Coping with environmental changes: physiological challenges. In Shuster C.N., Barlow R.B. and Brockmann H.J. (eds) *The American horseshoe crab*. Cambridge, MA: Harvard University Press, pp. 224–244.

Towle D.W., Mangum C.P., Johnson B.A. and Mauro N.A. (1982) The role of coxal gland in ionic, osmotic, and pH regulation in the horseshoe crab *Limulus polyphemus*. In Bonaventura J., Bonaventura C. and Tesh S. (eds) *Physiology and biology of horseshoe crabs: studies on normal and environmentally stressed animals*. New York: Alan R. Liss, Inc., pp. 147–172.

Walls E.A., Berkson J. and Smith S.A. (2002) The horseshoe crab, *Limulus polyphemus*: 200 million years of existence, 100 years of study. *Reviews in Fisheries Science* 10, 39–73.

Wang D.X., Su Y.Q., Wang J. and Liang J.R. (2001) Influence of environmental factors on development of embryo and larvae in *Tachypleus tridentatus*. *Journal of Fishery Sciences of China* 8, 11–14.

and

Wang S. and Xie Y. (2004) *China species red list: Volume 1 red list*. Beijing: Higher Education Press, pp. 1–724.

Correspondence should be addressed to:
C.-P. Chen
Biodiversity Research Center
Academia Sinica, Taipei 115, Taiwan
email: zocp@gate.sinica.edu.tw