Biodiversity and Conservation **13:** 1889–1904, 2004.

Conservation of the horseshoe crab at Kinmen, Taiwan: strategies and practices

CHANG-PO CHEN[1,2,*], HSIN-YI YEH[1,2] and PO-FEN LIN[1,2]
[1]*Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan 115, ROC;* [2]*Institute of Fishery Science, National Taiwan University, Taipei, Taiwan 106, ROC; *Author for correspondence (e-mail: zocp@gate.sinica.edu.tw; fax: +886-2-27858059)*

Received 14 August 2002; accepted in revised form 7 July 2003

Key words: Ecotourism, Habitat, Living fossil, Overfishing, Public education, Restoration, *Tachypleus tridentatus*, Threatened species

Abstract. In order to conserve the horseshoe crab, *Tachypleus tridentatus*, a famous 'living fossil' in Kinmen and Taiwan, several investigations related to its basic biology were conducted, and public education/participation programs were implemented. Biological investigations included artificially inducing spawning in the laboratory and field, rearing of juveniles, monitoring of habitat characteristics, and establishing a protected area. The grain size of the sediment of the spawning ground was 0.4–1.8 mm in diameter and that of the juvenile nursing ground was 0.11–0.19 mm. The most suitable water temperature for incubating eggs and rearing juveniles ranged from 28 to 31 °C. A total of 800 ha of the coastal zone on Kinmen was designated a protected area for the horseshoe crab, based on the *Fishery Law*, in December 1999. Fishing is prohibited for the 5-year period from 2000 to 2004. Public education programs were conducted to help local people learn about the basic biology of the horseshoe crab, to become aware of its importance and the necessity for conservation, and to empower them to actively participate in the conservation of this species. In addition, a horseshoe crab conservation program has also been incorporated as an element of the local ecotourism industry. Biotechnology business opportunities that may be created as a result of viable horseshoe crab populations will also be promoted.

Introduction

The horseshoe crab is one of the best-known 'living fossils' (Sekiguchi and Sugita 1980; Rudloe and Rudloe 1981; Shuster 2001) and is a good indicator species for monitoring the health of coastal zones, since it is large enough to be easily observed, and its life cycle is highly dependent on environmental conditions in its coastal zone habitats: adults spawning on the coarse sand grains near the high-tide zone and juveniles nursing on mudflats (Rudloe 1979, 1981; Jegla 1982; Cohen and Brockmann 1983; Botton 1984; Howard et al. 1984; Barlow et al. 1986; Sekiguchi 1988; Sekiguchi et al. 1988; Brockmann 1990; Meury and Gibson 1990; Brockmann and Penn 1992; Penn and Brockmann 1994).

Moreover, as a result of biomedical technology, the horseshoe crab is an even more valuable resource than previously recognized. Limulus amoebocyte lysate is a clotting agent obtained from horseshoe crab blood cells that allows detection of the presence of endotoxins pathogenic to humans in

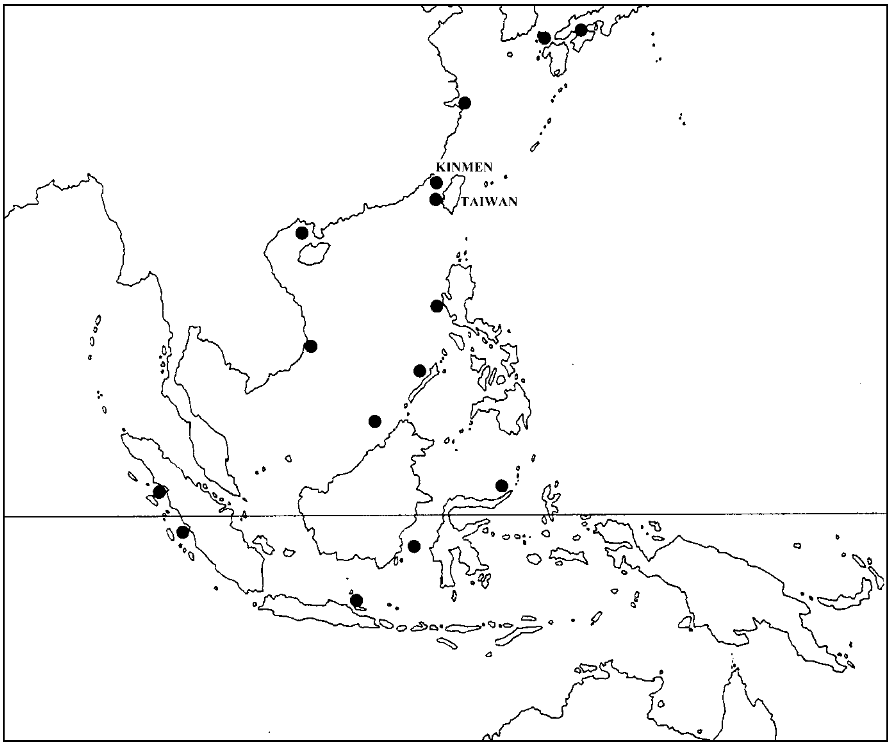

Figure 1. Confirmed distribution of the horseshoe crab, *T. tridentatus*, in southeast Asia (modified from *The Biology of Horseshoe Crabs*).

injectable drugs and implanted medical devices (Mikkelsen 1988; Prior 1990; Swan 2001).

In order to wisely use these natural resources of the horseshoe crab (*Limulus polyphemus*) in Delaware Bay, USA, a multiple-use resource management strategy was developed, in cooperation with birdwatchers, environmentalists, commercial fisheries, biomedical companies, coastal residents, and the eco-tourism industry. Fortunately, horseshoe crabs are sufficiently abundant in that area to maintain healthy and viable populations that can supply the amounts needed by the biomedical industry, to feed migrating shorebirds, but some people doubt whether the large harvest of the population can sustain this fishing pressure (Berkson and Shuster 1999).

However, with the species of *Tachypleus tridentatus*, it is another story (Figure 1). In Japan, it has been protected since 1928, but its population size is still in a poor condition (Botton 2001). The situation in Hong Kong is also bad (Morton 1999). In Taiwan, the status of *T. tridentatus* is even worse than that in Japan. Once it was abundant along the coastal zone of the Taiwan Strait, but due to overfishing, land reclamation, and water pollution, juveniles are no longer found on the eastern side of the Taiwan Strait, that is, in the coastal

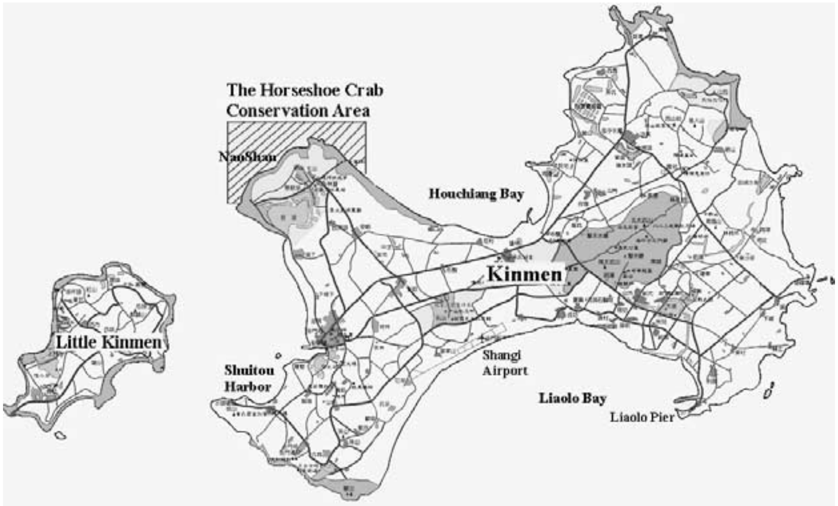

Figure 2. The horseshoe crab, *T. tridentatus*, conservation area at Kinmen.

zones on the west side of the island of Taiwan. Fortunately though, they are still present, but scarce, on the mudflats of Kinmen, an island just off the Fujian Province coast of mainland China (Figure 1).

Moreover, in 1995, there was a proposal to build a harbor in Shuitou Bay in Kinmen (Figure 2). This bay is the only suitable area for constructing a harbor, but it also contains the natural habitats of the horseshoe crab. This means that some of the horseshoe crab's most important remaining natural grounds would be partially destroyed. To help compensate for this loss, we proposed to protect the horseshoe crab off-site at other areas, where suitable adult spawning grounds and juvenile nursing flats can be provided on Kinmen Island.

In 1996 for the first time, we saw these tiny juveniles struggling in the mudflats, and we raised the call to conserve this species by adopting aquaculture principles and techniques such as for inducing spawning and rearing juveniles, in order to increase the population size of the horseshoe crab. Since juveniles presently occur in Kinmen, conservation has initially been focused there. After achieving success in Kinmen, the techniques and skills developed here can be applied to Taiwanese coastal zones.

Materials and methods

Induction of spawning

Laboratory trials

During mating, an adult male grasps a female, and they swim together throughout the day and night to a sandy beach at high tide during the spring

Figure 3. Inducing the horseshoe crab to spawn in the protected area.

tide. In nature, they dig into the sand to spawn; the spawning behavior seems to be triggered by two factors: a flood tide and a sandy substrate. By replicating these biological cues in the lab, adults were moved from a tank without sand to a tank with sand during the flood tide. As a result, we were able to induce them to spawn.

Field trials

To induce spawning in the field, a section of a suitable natural spawning ground was fenced in with net extending to the edge of the beach at high tide. Adults were then moved into this fenced area during the flood tide. Air bubbles emerged when adults were digging into the sand during spawning. Researchers marked these areas and checked whether or not any eggs were present after the tide had receded (Figure 3).

Temperature effects on early life stages

The egg is yellowish, elastic, ball-shaped, and 3 mm in diameter on average. Eggs hatch into the first instars called trilobite larvae, since they look like trilobites without a tail. After molting to second instars, juveniles begin to feed. Newly hatched *Artemia* larvae were used as food (Sekiguchi et al. 1988).

Molting time, size (i.e., carapace length), and the number of book gills were recorded to characterize each instar stage.

In order to predict how long it takes eggs to hatch and the size of the first instars and second instars in subtropical waters, eggs collected from spawning pairs induced to spawn in the lab were incubated at different constant water temperature regimes of 34, 31, 28, and 25 °C in 35‰ seawater, in the absence of sand. First instars were also reared at the above conditions (French 1979; Laughlin 1983).

Temperature effects on hatching time, the size of the first instar, molting time of the first to second instar, and the size of second instar were analyzed using thermal summation (the day–degree rule). This predicts that the product of the developmental period (y) and temperature (T; °C) is constant, $a = yT$ (Pruess 1983; Cossins and Bowler 1987).

Spatial distribution of juveniles in the field

Juveniles live in different tidal zones in the coastal areas of Kinmen. In order to determine the best location for releasing juveniles in the future, juveniles were monitored *in situ*. The monitored method is setting a 125 m length transect line, which is perpendicular to the boundary between sand and mud flat. Five plots were placed at 25 m intervals, and each plot was 5 m in length by 5 m in width. The juveniles were collected and counted at every plot.

Benthic characteristics of spawning grounds and juvenile nursery grounds

Horseshoe crabs can survive only if the habitats where they live are healthy and well protected. Therefore, we characterized the habitats of spawning areas and juvenile nursery grounds on Kinmen. The information can also be used to identify areas for setting aside protected areas where adults can be released to spawn in order to reestablish resident populations in the coastal zones of Taiwan.

Spawning grounds

Older local fishermen said that they used to use the spawning foam to pinpoint the location of a pair of horseshoe crabs during spawning, but in this study we observed no spawning behavior in nature, which may have been due to the low population density of adults. From the above-mentioned experiments of inducing adults to spawn in the field, we found that the spawning site is about 5–15 cm below the beach surface, and so three replicates of sediments were collected 5–15 cm below the beach surface near where we had observed spawning foam.

Sediment samples were wet-sieved through a Wentworth series of screens with mesh openings from 1.0 mm to 63 μm. Silt and clay contents were measured using pipette methods. Median grain sizes and sorting coefficients were also calculated. Detailed procedures were described in Hsieh (1995).

The water content of the sediments was measured as the percent weight loss after oven-drying at 60 °C for 48 h. Water content (% H_2O) = [(wet weight – dry weight)/wet weight] × 100% (Hsieh and Hsu 1999).

Juvenile nursery grounds

In summer, juveniles wander about 3 cm below the surface of the mudflats. Therefore, we collected samples of substrate from 3-cm depths in juvenile microhabitats to determine grain size, total organic carbon (TOC), and total nitrogen (TN). For benthos, 10 cm × 10 cm samples were collected 3 cm below the surface.

The TOC and TN contents of the sediment, expressed as percent dry weight, were determined by cryo-drying the sediment, mixing it thoroughly, and then adding about 8 ml 1 N HCl to remove inorganic carbon. The TOC and TN contents were then measured using an element analyzer (Perkin Elmer EA-2400 Series II) (Hsieh and Hsu 1999).

The benthos, which are potential prey of the horseshoe crab, were sorted through a 0.5-mm sieve after samples were fixed for 48 h with formalin, and then species were identified and counted.

Results

Induction of spawning

Laboratory trials

We first succeeded in inducing a pair of horseshoe crabs to spawn on 4 September 1998, even though the spring tide was not until 9 September. We succeeded again on 18 September, when the spring tide was on the 22nd. This second pair spawned for 5 h and laid about 10,000 eggs. In October, we induced spawning on the day of the spring tide, 22 October. The horseshoe crabs spawned for 2 h, and the female laid 800 eggs. The female dug into the sand with her fifth legs, and moved her book gills rapidly during spawning, then covered the nest with sand. The pair moved to another site and then repeated the same spawning behavior.

Field trials

The fieldwork was carried out as follows at Nanshan, a protected area for horseshoe crabs set up in 1999 on Kinmen (Figure 2). A 14 m × 30 m fence made of netting was erected at the edge of the high tide line on a beach selected as a suitable natural spawning ground on 4 October 2001. Eleven pairs of adults were moved into this protected site 3 h before the high tide. Observers then marked several locations in the water where spawning foam (air bubbles emerging when adults dig into the sand) were present (Figure 3).

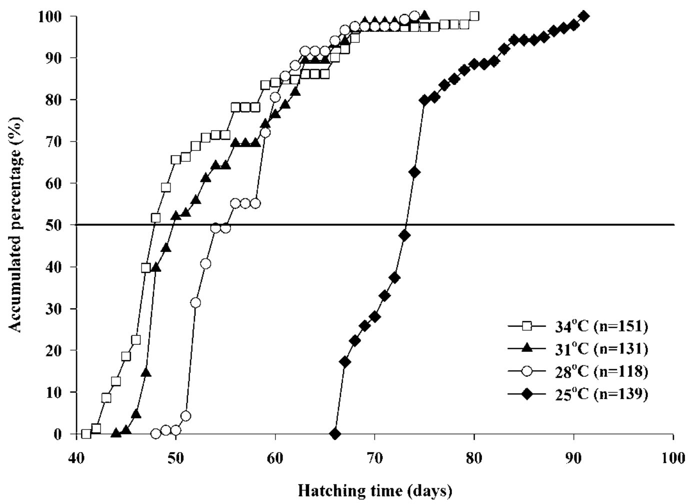

Figure 4. Hatching times and accumulated hatching percentage of first instars at 34, 31, 28, and 25 °C.

One nest was located 4.2 m up from the boundary between the sandy and muddy bottom substrates. It was about 8–10 cm in depth and contained 204 eggs. Another nest was located 27 m from the boundary and was 4–10 cm in depth with 293 eggs. Three other areas with spawning foam were noticed in the upper 6–8 m of the area, but no eggs were found at those sites.

Temperature effect on early life stages

Hatching time

First instars appeared after 42, 45, 49, and 67 days, and lasted 80, 75, 74, and 91 days, respectively, in the 34, 31, 28, and 25 °C temperature regimes (Figure 4). The average time for half of the eggs to hatch significantly differed, being 46 ± 1.8 days (mean ± SD) (n = 151) at 34 °C, 48 ± 1.1 days (n = 131) at 31 °C, 52.8 ± 15.4 days (n = 118) at 28 °C, and 70.6 ± 2.9 days (n = 139) at 25 °C (one-way ANOVA, $p < 0.01$; Table 1, Figure 5).

Values of thermal summation significantly differed among the tested temperatures and were 1564 ± 62.7 day–°C (n = 78) at 34 °C, 1488 ± 35.5 day–°C (n = 68) at 31 °C, 1478.4 ± 40.6 day–°C (n = 65) at 28 °C, and 1765.5 ± 71.8 day–°C (n = 87) at 25 °C (one-way ANOVA, $p < 0.01$; Table 1, Figure 5).

Carapace length of first instars

Average carapace lengths were 5.9 ± 0.1, 5.9 ± 0.1, 6.0 ± 0.1, and 5.9 ± 0.2 mm (n = 20) at 34, 31, 28, and 25 °C, respectively. First instars

Table 1. Average hatching time, thermal summation, and carapace length for first instars at 34, 31, 28, and 25 °C and half molting time, thermal summation, and carapace length for second instars at 34, 31, and 28 °C. Values are expressed as mean ± S.D. *$P < 0.01$; **$0.01 < P < 0.05$.

Age		Temperature (°C)				ANOVA result
		34	31	28	25	
First instar	Average hatching time (days)	46 ± 1.8 (n = 78)	48 ± 1.1 (n = 68)	52.8 ± 15.4 (n = 65)	70.6 ± 2.9 (n = 87)	*
	Thermal summation (day–°C)	1564 ± 67.7	1488 ± 35.5	1478.4 ± 40.6	1765.5 ± 71.8	*
	Carapace length (mm)	5.9 ± 0.1 (n = 20)	5.9 ± 0.1 (n = 20)	6.0 ± 0.1 (n = 20)	5.9 ± 0.2 (n = 20)	**
Second instar	Half molting time (days)	109.0 ± 9.1 (n = 49)	110.9 ± 9.8 (n = 37)	125.6 ± 7.6 (n = 30)	–	*
	Thermal summation (day–°C)	3704.3 ± 307.7 (n = 30)	3439.3 ± 304.3 (n = 37)	3517.7 ± 211.7 (n = 49)	–	*
	Carapace length (mm)	7.6 ± 0.3 (n = 49)	7.9 ± 0.4 (n = 37)	7.8 ± 0.3 (n = 30)	–	**

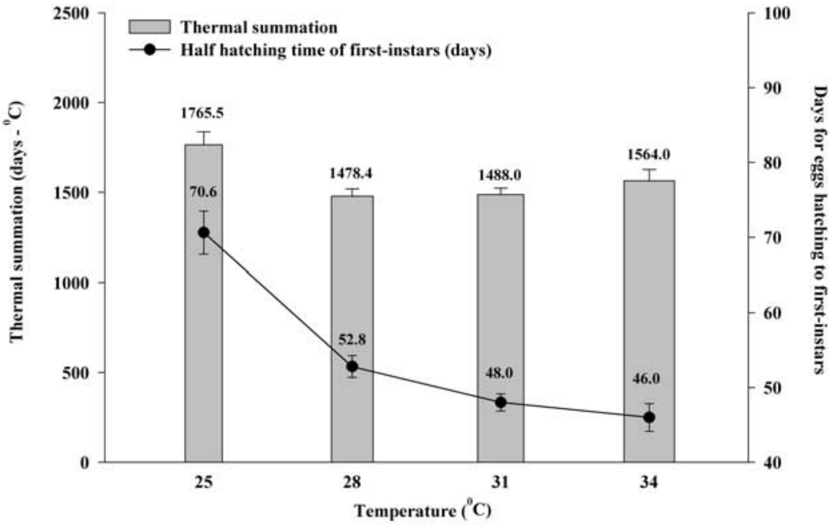

Figure 5. Thermal summation and half hatching times of first instars at 34, 31, 28, and 25 °C.

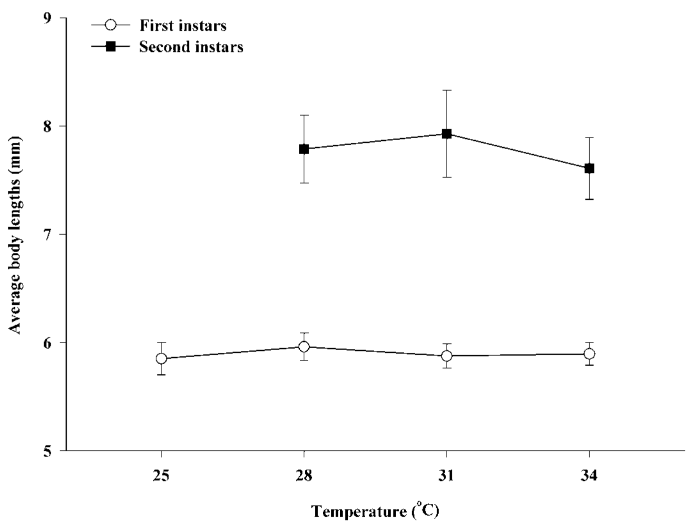

Figure 6. Average body lengths of first instars at 34, 31, 28, and 25 °C and of second instars at 34, 31, and 28 °C.

reared in 28 °C had significantly longer carapace lengths (one-way ANOVA, $p < 0.05$; Table 1, Figure 6), as revealed by Duncan's new multiple range test.

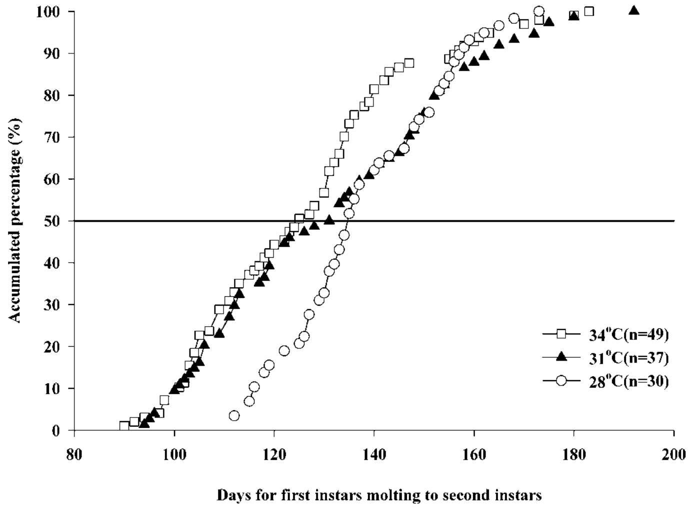

Figure 7. Molting times and accumulated molting percentage of second instars at 34, 31, and 28 °C.

Time required for molting from the first- to the second-instar stage

It took 90, 94, and 112 days for first instars to begin molt at 34, 31, and 28 °C, respectively (Figure 6). The times required for half of the first instars to molt were 109.0 ± 9.1 ($n = 49$), 110.9 ± 9.8 ($n = 37$), and 125.6 ± 7.6 days ($n = 30$), respectively. These half-molt times all significantly differed (one-way ANOVA, $p < 0.01$; Table 1, Figure 7).

Values for thermal summation significantly differed at 3704.3 ± 307.7 days–°C ($n = 49$) at 34 °C, 3439.3 ± 304.3 days–°C ($n = 37$) at 31 °C, and 3517.7 ± 211.7 days–°C ($n = 30$) at 28 °C (one-way ANOVA, $p < 0.01$; Table 1, Figure 8).

Carapace length of second instars

Average carapace lengths significantly differed at 7.6 ± 0.3 mm ($n = 49$) at 34 °C, 7.9 ± 0.4 mm ($n = 37$) at 31 °C, and 7.8 ± 0.3 mm ($n = 30$) at 28 °C (one-way ANOVA, $p < 0.05$; Table 1, Figure 6). And second instars reared at 34 °C had the shortest carapace length, as revealed by Duncan's new multiple range test.

These data indicate that the most suitable water temperature for incubating eggs and rearing juveniles ranged from 28 to 31 °C.

Spatial distribution of juveniles in the field

The carapace size of juveniles increases by about 1.3- to 1.4-fold (Sekiguchi 1988; Meury and Gibson 1990; present data) after each molting in the early

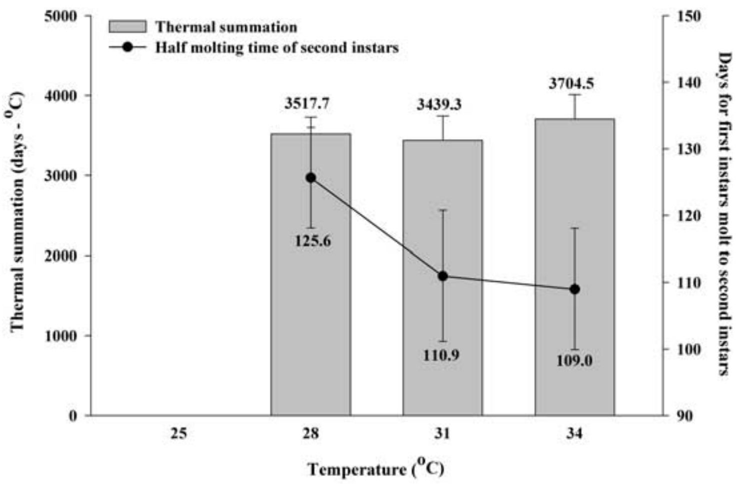

Figure 8. Thermal summation and half molting times of second instars.

stages, so the size distribution curve is typically non-linear. Therefore, each instar can easily be distinguished in the field.

Combining field and laboratory data, we found that first instars had an average carapace length of 5.8 mm with two pairs of book gills. It took about 110 days for first instars to molt into the second-instar stage. The average size of second instars was 8.7 mm in carapace length with three pairs of book gills. It increased to 11.8 mm in third instars with four pairs of book gills. The average carapace length of fourth instars was 15.3 mm with five pairs of book gills. After that, the number of book gills was constant at 5, the sizes of fifth and sixth instars were 21.2 and 30.0 mm, respectively (Figure 9). Because the data on carapace length were insufficient after the sixth instar, juveniles could not be distinguished, so they were combined together as a group.

During the summers of 1997 and 1998, we found many second to sixth instars, but found no first instars. During summer 2001, we monitored juveniles once at the boundary between the sandy and muddy bottom substrates for 125 m across the intertidal zone. We found no first instars at this time either. According to the data, none of the instar stages of juveniles were distributed at a particular tide line (Figure 10), suggesting that juveniles can be released anywhere on the mudflats, thus the best place is probably along the boundary between the sand and mudflat.

Benthic characteristics of the spawning grounds and juvenile nursery grounds

At the spawning ground, sand grain size ranged from 0.4 to 1.8 mm, which corresponds to medium to very coarse sand, with a water content of between

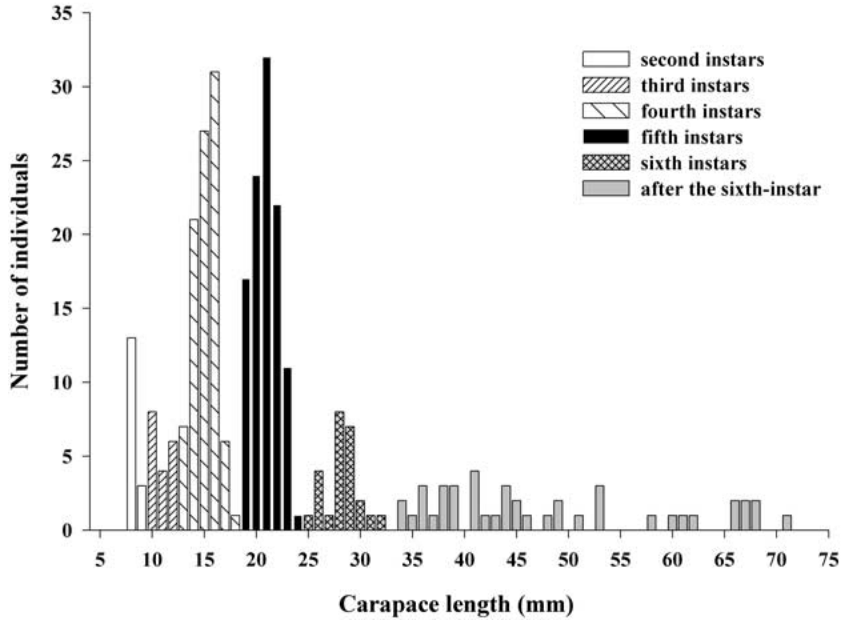

Figure 9. Carapace length for juvenile horseshoe crabs measured during the summers of 1997 and 1998 at Kinmen.

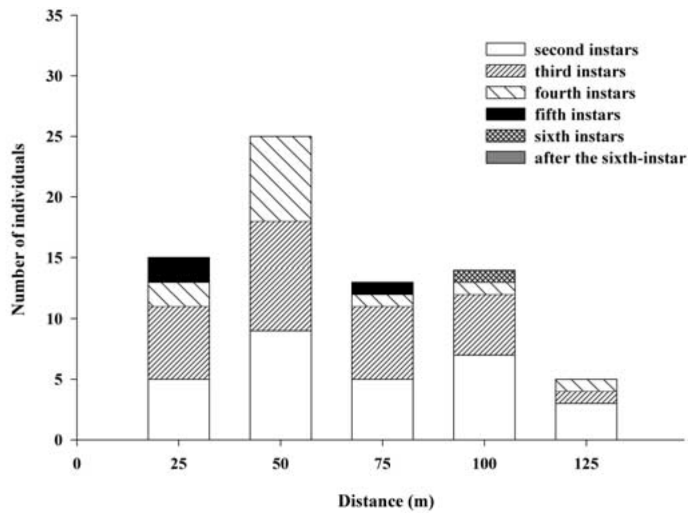

Figure 10. Distribution of juvenile horseshoe crabs along a transect line in the intertidal zone during summer 2001 in Kinmen. Distance (m) indicates the distance off the boundary between sand and mud flat.

9.8 and 13.7%. At microhabitats for juveniles, the sand grain size ranged from 0.11 to 0.19 mm, which corresponds to very fine to fine sand; the silt/clay percentage ranged from 21.8 to 35.0%; the sorting coefficient was 2.08–2.96, indicating that it was very poorly sorted; TOC was 0.35 ± 0.09%; and TN was 0.04 ± 0.01%. The benthos included polychaetes (Glyceridae, Lumbrineridae, Maldanidae, Nephthyidae, Capitellidae, Cirratulidae, Sabellidae, *Polydora* sp., *Scolelepis* sp., *Prionospio membranacea*, and *P. sexoculata*), oligochaetes, molluscs, nematodes, amphipods, decapods, and copepods.

Discussion

Since 1996 when we first saw juvenile horseshoe crabs wandering along the mudflats of Kinmen, we have recognized that the conservation of *T. tridentatus* in Kinmen and Taiwan is not just a biological program, but encompasses social aspects, and that there must be an integrated approach to achieve success. The study of animals and habitats is only a basic part, while building on the consensus of local people at the community level is the most important issue. Scientists as promoters have to contribute basic knowledge and technical skills to local people, cooperate together with them, and even help create an ecotourism industry for the local community.

Fortunately, in former times, the horseshoe crab was already part of the daily life in Kinmen and Taiwan, so it is easy to advocate this commonly remembered event. For example, some places are named after the horseshoe crabs, such as horseshoe crab hill or village, and its shell was commonly used as a utensil to dip water. This utensil was named after the horseshoe crab, and even though the shell itself is no longer in use, the utensil still bears the name of the horseshoe crab. Shells were hung at the tops of doors and walls to protect the house from evil, or they were painted as a tiger, with the faces of Chinese opera characters, etc. The ova and meat were often consumed, and were even used to fertilize crops. So there are intimate connections of this organism with the lives of the people of Kinmen and Taiwan.

Protected areas for sustainability

During the environmental impact assessment for Shuitou Harbor, we proposed to establish a protected area for the horseshoe crab. Finally, a total of 800 ha of the coastal zone on Kinmen (Figure 2) was designated as a protected area for the horseshoe crab, based on the *Fishery Law*, in December 1999. Fishing is prohibited for the 5-year period from 2000 to 2004. The Coast Guard Administration is the agency responsible for enforcing the fishing prohibition.

Management strategies and techniques for monitoring juveniles are currently being developed. Since the setting up of the protected area for the horseshoe crab in 1999, several important events have been conducted. (1) Juveniles occurring at the proposal harbor site were collected and transferred to this protected area by elementary school students during a campaign in 2000. (2) Adults were moved to this protected area for spawning induction in 2001 (as reported in this paper). (3) More than 10,000 individuals of second-instar juveniles produced artificially in the lab were released into this area in 2002. (4) The population size of juveniles has been monitored monthly to the present.

Since we are unable to rear large numbers of juveniles to the third-instar stage, we decided to release second-instar stage juveniles into the protected area. We are currently determining whether or not those released juveniles will survive and increase the population size.

Public education for social action

Public education and ecotourism programs are being conducted to attract the interests of local people. These were designed to help them learn about the basic biology of the horseshoe crab, to become aware of its importance and the necessity for conservation, and to empower them to help conserve this species.

Since the value of horseshoe crabs was not recognized by the local people, a series of public education programs was developed and implemented. These included symposiums, workshops, published material, television programs, and even a popular musician acting as a spokesperson for their conservation. Target audiences for these programs included elementary school students, the administrators at Kinmen National Park, staff from local governments, and the general public.

An exhibition hall especially designed to highlight horseshoe crabs and other species important to the fishing industry in the coastal zones of Kinmen was built and opened in 1999. A pamphlet on the horseshoe crab written in Chinese, entitled *The living fossil*, has been circulated throughout Kinmen and Taiwan since 2000. A Chinese book, entitled *Two billion years of the horseshoe crab*, was published in 2002. Japanese and English versions of the pamphlet will be printed soon.

A slogan in Chinese, 'Love the queen and wife', was created based on the phonetic similarities of the Chinese characters for horseshoe crab and queen and those for habitat and wife. It was adopted to emphasize the necessity of including their natural habitat in any plan to protect the species.

In addition, a group of graduate students was provided support to design a special course on horseshoe crabs for junior high school students in Taiwan. This course was well received by both teachers and students.

Ecotourism

The island of Kinmen has become a tourism hotspot since it was opened to the general public in 1992. The island provides a range of popular features including a national park, military fortifications, ancient Chinese cultural sites, and wild areas. However, the development of the tourism industry has resulted in significant adverse impacts on the natural environments. To help reduce these impacts, the horseshoe crab campaign is also directed at improving the quality of tourism on Kinmen.

Further perspectives

The next steps in this program include establishing a network of stakeholders to further promote horseshoe crab conservation activities and to campaign for listing the horseshoe crab as a species for protection.

Searches will be carried out on the west coast of Taiwan to locate habitats that may be suitable for this species. Once target sites are identified, attempts to restore populations, especially by introducing adults to potential spawning grounds, will follow soon thereafter.

In addition, the values associated with the biodiversity benefits of this precious living fossil and the applicable benefits of the horseshoe crabs to medical care and other industries that can improve our quality of life will be emphasized. Cooperation with scholars in mainland China, Japan, and other countries is also an important element.

We hope that this conservation campaign, as a social movement for cherishing the horseshoe crab, will also provide opportunities to raise public awareness and concern for wetlands and biodiversity in general.

References

Barlow Jr., R.B., Powers M.K., Howard H. and Kass L. 1986. Migration of *Limulus* for mating: relation to lunar phase, tide height, and sunlight. Biological Bulletin 171: 310–329.

Berkson J. and Shuster Jr. C.N. 1999. The horseshoe crab: the battle for a true multiple-use resource. Fisheries 24: 6–10.

Botton M.L. 1984. Diet and food preferences of the adult horseshoe crab *Limulus polyphemus* in Delaware Bay, New Jersey, USA. Marine Biology 81: 199–207.

Botton M.L. 2001. The conversation of horseshoe crab: what can we learn from the Japanese experience. In: Tanacrei J.T. (ed) *Limulus* in the Limelight – A Species 350 Million Years in the Marking and in Peril? Kluwer Academic/Plenum Publishers, New York, pp. 41–51.

Brockmann H.J. 1990. Mating behavior of horseshoe crabs, *Limulus polyphemus*. Behavior 144: 206–220.

Brockmann H.J. and Penn D. 1992. Male mating tactics in the horseshoe crab, *Limulus polyphemus*. Animal Behavior 44: 653–665.

Cohen J.A. and Brockmann H.J. 1983. Breeding activity and mate selection in the horseshoe crab, *Limulus polyphemus*. Bulletin of Marine Science 33: 274–281.

Cossins A.R. and Bowler K. 1987. Effect of temperature on reproduction, development and growth. In: Temperature Biology of Animals. Chapman and Hall, London, pp. 248–293.

French K.A. 1979. Laboratory culture of embryonic and juvenile *Limulus*. Progress Clinical and Biological Research Vol. 29. Biomedical Application of the Horseshoe Crab (Limulidae), pp. 61–71.

Howard H.A., Fiorddalice R.W., Camark M.D., Kass L., Powers M.K. and Barlow Jr. R.B. 1984. Mating behavior of *Limulus*: relation to lunar phase, tide height, and sunlight. Biological Bulletin 167: 527.

Hsieh H.L. 1995. Spatial and temporal patterns of polychaete communities in a subtropical mangrove swamp: influences of sediment and microhabitat. Marine Ecology Progress Series 127: 157–167.

Hsieh H.L. and Hsu C.F. 1999. Differential recruitment of annelids onto tidal elevations in an estuarine mud flat. Marine Ecology Progress Series 177: 93–102.

Jegla T.C. 1982. A review of the molting physiology of the trilobite larva of *Limulus*. In: Bonaventura J., Bonaventura C. and Tesh S. (eds) Physiology and Biology of Horseshoe Crabs: Studies on Normal and Environmentally Stressed Animals. A.R. Liss, New York, pp. 83–101.

Laughlin R. 1983. The effects of temperature and salinity on larval growth of the horseshoe crab *Limulus polyphemus*. Biological Bulletin 164: 93–103.

Meury T.W. and Gibson III. D.G. 1990. Force generation in juvenile *Limulus polyphemus*: effects on mobility in the intertidal environment. Bulletin of Marine Science 47: 536–545.

Mikkelsen T. 1988. The Secret in the Blue Blood. Science Press, Beijing, China.

Morton B. 1999. On turtles, dolphins and, now, Asia's horseshoe crabs. Marine Pollution Bulletin 38: 845–846.

Penn D. and Brockmann H.J. 1994. Nest-site selection in the horseshoe crab, *Limulus polyphemus*. Biological Bulletin 187: 373–384.

Prior R.B. 1990. Clinical Applications of the *Limulus* Amoebocyte Lysate Test. CRC Press, Boca Raton, Florida, pp. 15–25.

Pruess K.P. 1983. Day–degree methods for pest management. Environmental Entomology 12: 613–619.

Rudloe A. 1979. Locomotor and responses of larvae of the horseshoe crab, *Limulus polyphemus* (L.). Biological Bulletin 157: 494–505.

Rudloe A. 1981. Aspects of the biology of juvenile horseshoe crabs, *Limulus polyphemus*. Bulletin of Marine Science 31: 125–133.

Rudloe A. and Rudloe J. 1981. The changeless horseshoe crab. National Geographic 159: 562–572.

Sekiguchi K. 1988. Biology of Horseshoe Crabs. Science House, Tokyo, Japan, p. 428.

Sekiguchi K. and Sugita H. 1980. Systematics and hybridization in the four living species of horseshoe crabs. Evolution 34: 712–718.

Sekiguchi K., Seshimo H. and Hiroaki S. 1988. Post-embryonic development of the horseshoe crab. Biological Bulletin 174: 337–345.

Shuster Jr. C.N. 2001. Two perspectives: horseshoe crabs during 420 million years, worldwide, and the past 150 years in the Delaware Bay area. In: Tanacrei J.T. (ed) *Limulus* in the Limelight – A Species 350 Million Years in the Marking and in Peril? Kluwer Academic/Plenum Publishers, New York, pp. 17–40.

Swan B.L. 2001. A unique medial product (LAL) from the horseshoe crab and monitoring the Delaware Bay horseshoe crab population. In: Tanacrei J.T. (ed) *Limulus* in the Limelight – A Species 350 Million Years in the Marking and in Peril? Kluwer Academic/Plenum Publishers, New York, pp. 53–62.