

Larval Culture of *Tachypleus gigas* and Its Molting Behavior Under Laboratory Conditions

J.K. Mishra

Abstract Horseshoe crab populations along the northeast coast of India are under threat due to degradation of the breeding beaches. To augment the trend, attempts were made to culture the larvae of *Tachypleus gigas* and study its growth rate by enhancing the molting pattern in the laboratory condition. Trilobites of *T. gigas* were cultured on a controlled diet of brine shrimp (*Artemia*) and diatom (*Chaetoceros gracilis*) at 26–28°C and 32–34⁰/00.

Trilobites could molt up to the fourth posthatched juvenile stage within a period of 180 days from the day of hatching of trilobite from the egg membrane as free swimming larval stage. The molting behavior was faster from the first to the third posthatched juvenile stage, i.e., within a period of 90 days. The average growth rate in terms of total body length from the first to second posthatched juvenile was about 63%, and from the second to third posthatched larva was about 38%. The growth rate was found to be about 25% from the third to fourth posthatched juvenile stage, and molting took place 180 days after the day of hatching of trilobite. All the posthatched juveniles had similar morphological features to the adults. The fourth posthatched juveniles exhibited more prominent morphological features with fully grown legs, spines, and segmentation, with a total body length of 45 mm.

Further studies on food-dependent molting patterns of juvenile instars may help to establish a standardized aquaculture method to grow horseshoe crabs in captivity. Sea ranching of these reared animals can be carried out regularly in the holding areas to increase horseshoe crab populations and conserve these precious organisms from the brink of extinction.

J.K. Mishra (✉)

Department of Ocean Studies and Marine Biology, Pondicherry University,
Brookshabad Campus, Post Bag No. 26, Junglighat, Port Blair,
Andaman - 744 103 India
e-mail: jayant64@yahoo.com

1 Introduction

Horseshoe crab populations over the world are now under threat as never before due to several known and unknown factors. Attempts are also being made to restore their populations (Rudloe 1982, Berkson and Shuster 1999, Widener and Barlow 1999, Botton 2001, Tanacredi 2001, Carmichael et al. 2004). Two of the four extant species, *Tachypleus gigas* and *Carcinoscropsius rotundicauda*, are found along the northeast coast of India (Sekiguchi et al. 1976, 1978, Sekiguchi 1988, Chatterji et al. 1988). Their distribution extends from Sunderbans in the extreme northern coast of Bay of Bengal, associated with Gangetic estuarine system (Itow et al. 2004), to the coast of Andhra Pradesh through the coast of Orissa, where they are found in high numbers (Fig. 1). *T. gigas* uses the intertidal zones of Orissa coast, associated with estuaries and creeks for breeding. This is due to favorable conditions such as beach characteristics, estuarine and creek environments, and suitable sand grain size at the breeding beaches (Mishra 1991). The highest number of breeding pairs are found at Balaramgari (Lat. $19^{\circ} 16' N$; Long. $84^{\circ} 53' E$) in the vicinity of Budhabalanga estuary (Mishra 1991, 1994, Mishra et al. 1992).

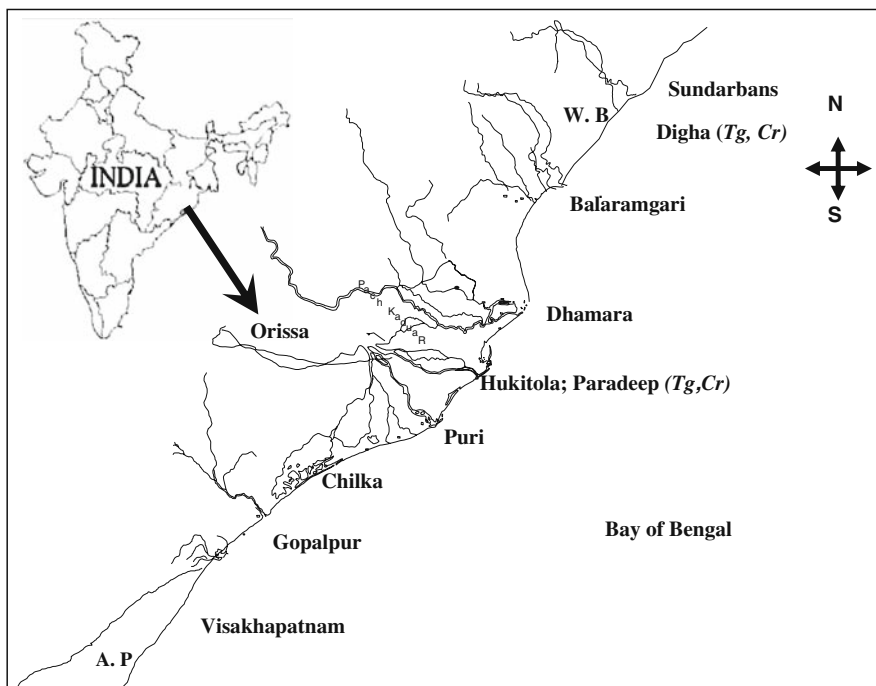


Fig. 1 Map showing the distribution of horseshoe crabs along the northeast coast of India

In recent years, the number of horseshoe crab pairs at Balaramgari has declined, which may be mainly attributed to the destruction of breeding beaches due to various anthropogenic activities as in the case of *Limulus polyphemus* (Botton et al. 2006), fisheries-related activities, and disturbance of the beach sand due to natural calamities. These factors affect the breeding activity and successful recruitment of *T. gigas* along the coasts of India. Though these environmental threats are encountered mainly at a local level, it may become a potential threat in the future and inhibit shoreward migration of the horseshoe crabs for breeding.

Several studies on the developmental biology and culture of juvenile horseshoe crab species have already been carried out at different environmental conditions (Sekiguchi 1973, Brown and Clapper 1981, Jegla 1982, Laughlin 1983, Sekiguchi et al. 1988, Mishra 1991, Chatterjee et al. 2004, Lee and Morton 2005). However, there are no reports on the juvenile molting behavior of *T. gigas* from the trilobite (first instar) stage to different posthatched juvenile instars in response to food and culture conditions under laboratory conditions.

The present study was carried out to culture the larvae of *T. gigas* on a supplemented mixed diet of brine shrimp (*Artemia*) and the diatom (*Chaetoceros gracilis*) and study their molting behavior under laboratory conditions. Attempts were also made to observe the growth rate of the juveniles starting from trilobite to posthatched juvenile instars up to 180 days. The objective was to standardize the technique of rearing of *T. gigas* juveniles in the laboratory. Eventually, sea ranching of horseshoe crab juveniles in the holding areas is planned as a means of restoring natural populations. Therefore, it is important to develop laboratory culture methods that can grow fertilized eggs through the early juvenile stages in an environment that protects them from scavengers and excess human activities in the breeding zones.

2 Materials and Methods

Two hundred freshly deposited fertilized eggs of *T. gigas* were collected from the nests made by breeding pairs at Balaramgari beach immediately at the low tide on 24th-Feb-2002. Eggs were brought to the laboratory and incubated in the laboratory at room temperature of 26–28°C by keeping them in a plastic tray with sand, moistened with seawater. Trilobites hatched out 38–40 days after fertilization. These freshly hatched trilobites were transferred to fiberglass tanks with cartridge-filtered natural seawater of salinity 32–34%. Larvae were reared at a density of two larvae per liter of seawater at room temperature, and the culture water was aerated continuously. A thin sand bed was created in the tank for providing shelter for the larvae and to minimize epiphytic growth (French 1979). Larvae were fed with a substitute diet of *Artemia* and diatom, *C. gracilis*, during the culture period. The larvae were checked daily, and 40% of the culture water was changed. The growth rate was studied by measuring the

total length of the molted individual at each stage by random sampling; total length (TL) of the larvae (starting from the anterior end of the prosoma to the tip of the telson at the posterior end) was measured by using vernier calipers. Simultaneously, prosoma width of the juvenile instars after each molting was also measured.

3 Results

The average total length of the freshly hatched trilobite larvae, which do not possess any tail, was 8 mm (Table 1). These larvae were very active and swam upside down in the culture tank. Trilobites could molt up to the fourth post-hatched juvenile stage (fifth instar) within a total period of 180 days from the day of hatching as free swimming trilobites (Table 1). The rate of molting was faster from trilobite (without tail) to the third posthatched juvenile stage, i.e., within a total period of 90 days from the day of hatching of trilobite. In case of the first juvenile molt, the average growth rate (TL) from trilobite to first posthatched juvenile (first-tailed stage) with the appearance of fully grown tail was found to be 100% within a period of 30 days. The average growth rate was 63% from the first to second posthatched stage, and 38% from the second to third posthatched stage. However, there was a slower growth rate of about 25% from the third to fourth posthatched juvenile stage, which took 180 days after the day of hatching of trilobite. All the posthatched juveniles exhibited similar morphological features to that of the adults. The fourth posthatched juveniles exhibited more prominent morphological features with fully grown legs, spines, and appendages with a total body length of 45 mm. Prosoma width increased

Table 1 Growth increment of larval stages of *T. gigas*

Stages	Days for development	Total body length (mm)	Prosoma width (mm)	Percentage of growth increment (total body length)
Trilobite	38 days after fertilization	8	7	–
First posthatched juvenile	30 days after reached trilobite	16	11	100
Second posthatched juvenile	45	26	16	63
Third posthatched juvenile	90	36	20	38
Fourth posthatched juvenile	180	45	24	25

from 7 mm at the trilobite stage to 24 mm at fourth posthatched juvenile (Table 1). However, the mortality rate during this study period was observed to be very high with an average survival rate of about 30% at the end of the culture. Although there was 100% survival between trilobites and first posthatched juvenile stage, it was about 67% from the first to second posthatched stage. It further declined to about 62% from the second to third posthatched stage, and 30% from the third to fourth posthatched juvenile stage.

4 Discussion

In the present investigation, it was observed that the trilobites exhibited rapid molting under laboratory condition in comparison to the juvenile molting of *Limulus*, which took about 7 months to reach the fourth posthatched juvenile (Brown and Clapper 1981). In all the stages, there was a positive correlation between the total length and the prosoma width (Fig. 2), indicating that the growth takes place in terms of increasing both length and width. It has been reported that salinity influences larval growth in *T. gigas* (Chatterji et al. 2004). In this experiment, natural seawater with a salinity range of 32–34‰ was found to be suitable for larvae culture. In this region of India, trilobites migrate to the sea immediately after their hatching with the receding tide and grow as juveniles in the seawater with little variation in salinity regime. Under experimental culturing conditions, the food given to larvae may have influenced the molting behavior in juvenile horseshoe crabs. However, further studies on food-dependent molting pattern of juvenile instars can help to establish a standardized culture method of this species.

The intermolt period increased with stage, from 30 days between trilobite and first juvenile to about 90 days between the third and fourth juvenile stage (Table 1). Though the reason for this is unknown, the role of ecdysones as molt-promoting hormones (Jegla 1982; Sekiguchi et al. 1988; Shuster and Sekiguchi 2003) deserves further study. But the involvement of a molting protein in

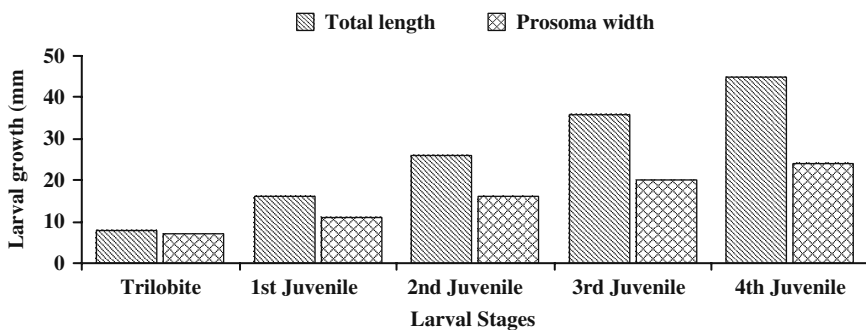


Fig. 2 Total length and prosoma width of different larval stages of *T. gigas*

T. gigas, as in the case of other crustaceans, particularly in barnacles (Satuito et al. 1996) should also be investigated. It may be termed as horseshoe crab molting protein (HCMP). As molting takes place, I hypothesize that the HCMP in the larva may be used, causing depletion in its concentration. Again it may take some time to generate the protein within the body to the required level, which is capable of inducing the molting process. However, it requires further study to clarify the involvement of any such protein in horseshoe crabs.

Sea ranching of laboratory-reared *T. gigas* was carried out by the author on the World Environment Day in the year 2002 at Visakhapatnam (northeast coast of India). The sea ranching of laboratory-reared horseshoe crab juveniles was done in the holding areas as a step to compensate the loss of horseshoe crab population, due to the loss of breeding grounds, and to restore natural populations. Simultaneously, an awareness campaign among the coastal population was conducted to highlight the threats encountered by horseshoe crabs in their natural habitat due to environmental degradation with a view to conserve these precious organisms from the brink of extinction at national and international levels.

Acknowledgments The author acknowledges the Ocean Science and Technology Cell, Andhra University, Visakhapatnam, India for providing laboratory facilities to carry out this research work. Thanks are due to two referees for their critical comments in improving the manuscript.

References

- Berkson J, Shuster C (1999) The horseshoe crab: the battle for a true multiple-use resource. *Fisheries* 24:6–10
- Botton ML (2001) The conservation of horseshoe crabs: what can we learn from the Japanese experience? In: Tanacredi J (ed) *Limulus* in the Limelight. Kluwer Academic/Plenum, New York, pp 47–51
- Botton ML, Loveland, RE, Tanacredi JT, Itow T (2006) Horseshoe crabs (*Limulus polyphemus*) in an urban estuary (Jamaica Bay, New York) and the potential for ecological restoration. *Estuar Coasts* 29: 820–830
- Brown GG, Clapper DL (1981) Procedures for maintaining adults, collecting gametes, and culturing embryos and juveniles of the horseshoe crab, *Limulus polyphemus* L. In: Hinegardner R (ed) *Laboratory Animal Management of Marine Invertebrates*. National Academy Press, Washington, DC, pp 268–290
- Carmichael RH, Rutecki D, Annett B, Gaines E, Valiela I (2004) Position of horseshoe crabs in estuarine food webs: N and C stable isotopic study of foraging ranges and diet composition. *J Exp Mar Biol Ecol* 299:231–253
- Chatterji A, Vijaykumar, R, Parulekar AH (1988) Growth and morphometric characteristic in the horseshoe crab, *Carcinoscorpius rotundicauda* (Latreille) from Canning (West Bengal), India. *Pakistan J Sci Ind Res* 33:352–355
- Chatterji A, Kotnala S, Mathew R (2004) Effect of salinity on larval growth of horseshoe crab, *Tachypleus gigas* (Muller). *Curr Sci* 87:248–250
- French KA (1979) Laboratory culture of embryonic and juvenile *Limulus*. In: Cohen E (ed), *Biomedical Applications of the Horseshoe crab (Limulidae)*. Alan R Liss, New York, pp 61–71

- Itow T, Mishra JK, Ahmed ATA (2004) Horseshoe crabs (King crabs) in the Bay of Bengal, South Asia. *Bull Fac Edu Shizuoka Univ Nat Sci* 54:13–30
- Jegla TC (1982) A review of the molting physiology of the trilobite larva of *Limulus*. In: Bonaventura J, Bonaventura C, Tesh S (eds), *Physiology and Biology of Horseshoe Crabs*. Alan 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*. *Biol Bull* 164:93–103
- Lee CN, Morton B (2005) Experimentally derived estimates of growth by juvenile, *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* (Xiphosura) from nursery beaches in Hong Kong. *J Exp Mar Biol Ecol* 318:39–49
- Mishra JK (1991) Embryonic development and variations in the haemolymph of Indian horseshoe crab, *Tachypleus gigas* (Muller). Ph. D. Thesis, Berhampur University, Orissa, India
- Mishra JK, Chatterji A, Parulekar AH (1992) A freak twin trilobite lava of the Indian horseshoe crab, *Tachypleus gigas* (Muller). *J Bombay Nat Hist Soc* 90:115–117
- Mishra JK (1994) Horseshoe crab, an Amazing Creature of Orissa Coast. Project Swarajya Publ, Orissa, India
- Rudloe A (1982) Man's influence as an environmental threat to *Limulus*. In: Bonaventura J, Bonaventura C, Tesh S (eds), *Physiology and Biology of Horseshoe Crabs*. Alan R Liss, New York, pp 297–300
- Satuito CG, Shimizu K, Natoyama K, Yamazaki M, Fusetani N (1996) Age related settlement success by cyprids of the barnacle *Balanus amphitrite*, with special reference to consumption of cyprid storage protein. *Mar Biol* 127:125–130
- Sekiguchi K (1973) A normal plate of the development of the Japanese horseshoe crab, *Tachypleus tridentatus*. *Sci Rep Tokyo Kyoiku Daigaku Sect B* 14:121–128
- Sekiguchi K, Nakamura K, Sen TK, Sugita H (1976) Morphological variation and distribution of a horseshoe crab, *Tachypleus gigas*, from the Bay of Bengal and the Gulf of Siam. *Proc Jap Soc Syst Zool* 15:13–20
- Sekiguchi K, Nakamura K, Seshimo H (1978) Morphological variation of horseshoe crab from the Bay of Bengal and gulf of Siam. *Proc Jap Soc Syst Zool* 15:24–30
- Sekiguchi K (1988) *Biology of Horseshoe Crabs*. Science House, Tokyo
- Sekiguchi K, Itow T, Yamamich Y (1988) Experimental embryology. In: Sekiguchi K (ed). *Biology of Horseshoe Crabs*. Science House, Tokyo, pp 225–287
- Sekiguchi K, Seshimo H, Sugita H (1988) Post-embryonic development of the horseshoe crab. *Biol Bull* 174:337–345
- Shuster CN Jr, Sekiguchi K (2003) Growing up takes about ten years and eighteen stages. In: Shuster CN Jr, Barlow RB, Brockmann HJ (eds) *The American Horseshoe Crab*. Harvard Press, Cambridge, pp 103–132
- Tanacredi JT (2001) Horseshoe crabs imperiled? The fate of a species 350 million years in the making. In: Tanacredi J (ed) *Limulus in the Limelight*. Kluwer Academic/Plenum, New York, pp 7–14
- Widener JW, Barlow RB (1999) Decline of a horseshoe crab population on Cape Cod. *Biol Bull* 197:300–302