

**CMFRI**

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# ***Course Manual***

*Winter School on  
Recent Advances in Breeding and Larviculture  
of Marine Finfish and Shellfish*

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### Seahorses

Seahorses are fascinating fin fishes in the marine environment. Seahorses have been used in Traditional Chinese Medicine (TCM) for more than 600 years (Vincent, 1995). They are reported in TCM to play a role in increasing and balancing vital energy flows within the body, and as a curative for ailments like asthma, impotency, high cholesterol, goiter, kidney disorders and skin diseases such as severe acne. Although seahorses are considered to be difficult-to-keep fishes in the aquarium, they are among the most popular. The captive aquarium seahorses are wild caught, coming from Indonesia, Philippines and being exported to North America, Europe and Japan (Vincent, 1996). These fishes are also used as curios like jewels, paper weight, key chains and other items owing to their extraordinary shape, the ease with which they dry and their series of external body plates that enable them to retain shape even after death.

Owing to their varied economic uses, the demand for seahorses in the global market is increasing year by year and they are heavily exploited. The trade was expanding globally at a fast pace and the demand always exceeded supply (Lourie *et al.*, 1999a). In India, trade on seahorse, originated in the southern coast, particularly from Tamilnadu along the Palk Bay and the Gulf of Mannar coasts as an alternate fishery in view of the dwindling resources of sea cucumber (Marichamy *et al.*, 1993). India exported more than 3.6 tonnes (1.3 million seahorses) annually constituting about 30.0% of the global seahorse trade (Marichamy *et al.*, 1993, Lipton *et al.*, 2002). In fact in some of the villages, a target fishery existed along the Palk Bay coast; but the landings mostly come as trawl by catch from the Gulf of Mannar region and the Kerala coasts. Most of the catch was dried and exported to Singapore, Hong Kong and Malaysia, apart from their local consumption in limited quantities in folk medicine for treatment of asthma, fits, etc. Most of the Indian exports were destined to Singapore or Hong Kong for TCM (Salin, 2003). Seahorses are often caught incidentally as by catch in trawlers and seines but many are also targeted by some of the fishers, along with chank, other gastropods and sea cucumbers using small nets or by hand collection. Seahorse fisheries tend to damage the wild population severely. Being a subsistence fishery, the reliance on seahorse is increasing in response to the twin pressures of growing demand and declining resources because of overexploitation and destructive fishing techniques. Besides, anthropogenic, industrial, domestic and other disturbances to the habitat cause severe damage to seahorse population.

These threats led to the inclusion of most of the Indo-Pacific seahorse species in the IUCN Red Data Book of Threatened Animals. The entire genus *Hippocampus* has been added to Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Hence, global research on seahorses of late, is oriented towards conservation and management strategies of the fragile population around the world. Aquaculture of seahorse is another important option for conservation through production and propagation in view of the drastic decline in the world seahorse populations. Aquaculture could contribute to restoration effects by producing healthy young of most of the economically important species of seahorses, which would help to ease the fishing pressure on the wild population.

### Reproductive behaviour and breeding strategies

The females deposit pear-shaped eggs in the male's brood pouch where fertilization took place. In syngnathid fishes, Prein (1995) reported a fecundity of 100 to 200 eggs subject to the size variations. In *H. erectus*, the number of eggs varied from 90 to 1,313 (Teixeira and Musick, 2001). Mi (1993) and Mi *et al.* (1998) reported 20 to 1,405 larvae in *H. kuda*. Masanjones and Lewis (1996) recorded that *H. trimaculatus* produced about 400 to 1000 larvae (Cai *et al.*, 1984b) in single spawning.



## Breeding of *H. trimaculatus* in Laboratory conditions

### Maintenance of broodstock

Live adult *H. trimaculatus* collected as by-catch were kept in 1000 liter fiber-reinforced plastic (FRP) rectangular tanks and supplied with filtered seawater and aeration. The tank bottom was distributed with dead corals and sea fans for a near-natural substratum. The bottom of the tanks was cleaned daily and the whole tank drained and cleaned weekly. Adult seahorses were fed with a variety of live feed (*Artemia*, mysids and fish fry) *ad-libitum* along with frozen food at fixed hours. All the seahorses were observed in the culture tank daily for their courtship and feeding behaviours. During egg transfer, some mature eggs spilled out and settled in the tank bottom. These eggs were collected carefully, measured by using a micrometer and photographed at 50X magnification by a camera microscope (Leica MPS 30, Germany).

### Courtship and mating

*H. trimaculatus* displayed distinct patterns of courtship and mating behaviours in captivity. The males underwent rapid changes in their body colouration ranging from normal to lighter shades almost all over the body except the head. During courtship interactions, a male and female came together exhibiting a very characteristic posture consisting of an erect body with head inclining downwards. The males started dilating the opening (mouth) of the brood pouch. They inflated the pouch to balloon-like proportions with water, by swimming forward or by pushing their body forward in a pumping action. When the ripe female is receptive to the courting male, it would reciprocate with its own colour changes and head tucking, typically intensifying the lighter colours such as yellow and white, highlighting the contrast between these colours and their overall darker blotching and banding pattern. A series of short bursts of swimming together in tandem then ensued, sometimes with tails entwined, or with the female tightly rolling its tail up. Copulation normally lasted 6 to 10 seconds and frequently occurred around the midday. After the successful egg transfer, the male repeatedly bent sidewise and contorted his body in an attempt to evenly distribute the eggs within the pouch. All eggs were transferred during the course of copulation, after which the pouch opening was closed to create a water-tight pouch. Pregnancy in male *H. trimaculatus* lasted for 12 to 14 days from the date of egg transfer.

### Embryogenesis

**Egg:** The hydrated eggs were ovoid in shape with one end slightly tapered,  $2.12 \pm 0.019$  mm long,  $1.97 \pm 0.045$  mm wide and  $2.94 \pm 0.3$  mg in weight, with numerous bright range/ red fat globules.

**Yolksac 'larva':** During the yolk sac larval stage, the embryo was somewhat coiled with most of the thoraco-abdominal region in contact with the yolk sac. The anterior and posterior ends of the body were raised from the yolk sac and laterally flattened to form the caudal fin bud. The caudal fin bud was slightly elevated and deviated from the surface of the yolk mass. The caudal bud eventually elongated into the prehensile tail. At this phase, a distinct dorsal fin bud was noted. Paired pectoral fin buds appeared but lepidotrichia within the fins were not yet developed up to this time (*i.e.*, the time of dissection from the brood pouch). No surface amplification was present on these fins. Although the head region was delineated, the rostrum did not emerge. Melanogenesis started on the head region in this stage and some pigment cells were visible along the antero-posterior embryonic axis. A rudimentary heart was seen above the yolk sac and the blood vessels were also visible. Optic vesicles were present on the tip of the head, but the lens placodes were not yet developed.

**Embryo:** As embryogenesis proceeded, the embryo continued to elongate, yet remained coiled around the yolk mass. The anterior end was slightly broadened and was clearly differentiated into the cephalic region. A series of myomeres were aligned along the lateral body region. The eye lens vesicles resided in the centre of the greatly expanded optic cups and the eye was pigmented. As development took place, the yolk reserve gradually got absorbed and the size of the yolk sac reduced. This phase of development was characterized by the morphogenesis of the jaw and the growth of the dorsal and pectoral fins. At this stage, the anal fin first appeared as a swelling posterior to the vent. No surface amplification was seen on the emerging anal fin. The pectoral and dorsal fins were found to be broad and flat with the rays beginning to develop.

The contact of the embryo with the yolk sac was limited to its thoraco abdominal region. The entire prehensile tail and the anterior half of the head were free from the yolk sac. No surface amplifications were found on the emerging anal fin as it was just beginning to develop. The vent, posterior to the anal fin, was as yet not open. Two transverse cartilages developing from the anterior arches represented the initial formation of the upper and lower jaws. The upper jaw was straight, whereas the lower jaw, which was broadly triangular in shape, curved upward to partially cover the median portion of the upper jaw. The branchial arches were covered by an operculum. Chromatophores were fully developed along the lateral body surface, except for the fins.

**Young one:** The internal yolk reserves were completely exhausted and there was no notable protrusion of the extra embryonic yolks at this stage. The elements of the dermal plates were quite visible while the mouth apparatus was elongate, acquiring the typical adult form. The anal fin was completely formed and the vent was open. Microvilli-like rays were present in the anal fin. The dorsal and paired pectoral fins were fully formed with rather sturdy fin rays. The prehensile tail was capable of muscular contraction. The tail musculature became fully functional and adhered to the substrate. Following partruition, the lepidotrichia within the fins became fully calcified and rigid. The dermal scutes of the body armour became further enlarged. The calcified dermal crowns erupted through the epidermis and appeared as a series of spines along the lateral body and down the tail. Coronet and cheek spines were also well developed. Pigmentation increased considerably beyond that of the near-term embryo, except for the region of the abdomen near the vent. The young were able to feed freely upon release from the brood pouch.

**Birth of young ones:** The young became very active and shook loose into the pouch lumen at the end of pregnancy. Before the birth of the young, the oval shaped pouch became nearly spherical in shape. The fish became restless and swam here and there. The breathing frequency increased significantly. Usually the young birth occurred in the early hours of morning. The complete partruition duration extended from 20 to 90 minutes. Initially, the animal bent its trunk and tail towards each other frequently and tried to widen the pouch mouth. As the process of births continued, the bending movement also continued with the trunk bending backward and the tail bending rapidly forward with an ejaculatory movement. During the backward bending, water went inside the pouch while it came out with a force during the rapid forward bend. The young ones got pulled out with the forceful ejection of water from the pouch. Along with the young ones, fine particles and filaments from the broken egg wall also came out. At every ejaculation 3 to 8 young ones came out. Between every two or three ejaculations, the father held the holdfast for rest. All the young ones were not beginning to swim immediately; some were round in shape and settled to the bottom of the tank. After some time later, they stretched their body and started swimming freely.

After the last young one was expelled, the pouch returned to its nearly normal proportion within about 15 minutes, and within half an hour it got completely subsided. During the entire procedure, there was no attempt to rub the pouch on objects as an aid to expelling the young ones. Within hours of partruition, the connective tissue had resumed its former shape. After birth, there was no further parental care by either sex. Newly born seahorses were fully developed with no yolk sac to hinder their swimming or to affect their buoyancy, and possessed hardened fin rays, pigmentation and prehensile tail (Fig. 17). They went straight to the water surface at birth, apparently to fill the swim bladder by gulping air, and were thenceforth able to hunt their prey. The newborn seahorse young measured  $7.0 \pm 0.05$  mm in length and  $0.97 \pm 0.08$  mg in weight.

### Sacred chanks

The sacred chank, *Xancus pyrum* is a gregarious, large, marine gastropod and its dwelling places form distinct chank beds (Nayar and Mahadevan, 1974, Lipton *et al.* 1996a). In addition to the ornamental purposes, the recent demand for chank shells, flesh and operculum led to the increased exploitation. Chank flesh is rich in protein and minerals (Chari, 1966) and the values compared favorably with those of fishes. In the live condition, the shell of the chank is covered by a surface skin, called periostracum, which protects the shell from several environmental factors including corrosive effects. The periostracum in live animals is brown in colour, soft and velvety, which peels off after the animal dies. Upon removal of the periostracum, the shell shows its characteristic milky white appearance.



Although, there are restrictions by the respective state Fisheries Departments, specific exploitation of chanks by long-lines in Kerala (Appukkuttan *et al.* 1980) and by modified trawl nets along Rameswaram coasts in Tamil Nadu (Lipton *et al.* 1996 b) have been reported. Such intense bottom trawl activities also led to depletion of population of chanks in the traditional chank bed areas.

### Chank bed areas

In Gulf of Mannar and Palk Bay, the depth ranging from 5 to 6.5 m with substratum such as dead coral reefs, sand mixed with mud and algae supported the chank settlement and the resulting chank bed area. In addition to the traditional practice of chank diving, chanks were also exploited using a modified trawl net (=chanku madi). The details are presented in a paper (Lipton *et al.* 1996 b). With the operation of such modified trawl nets (which comprises large number of sinkers), the bottom biota is disturbed. Discussions with the traditional chank divers revealed that in Rameswaram area six traditional chank beds ('paars') adjacent to the coral reefs are totally destroyed by the operation of the 'chanku madi'. During the chank diving season, which extends from January to March, they find almost barren seabed, which was earlier flourishing with chanks, holothurians, corals and other mollusks. In addition, the size of chanks obtained from these chank bed areas are also decreased and thus fetch lesser rates. This information is very important in the conservation aspects of chanks.

### Breeding of chanks

The laboratory-reared (maintained) adult chanks exhibited breeding behaviour during the different months. Upon close observation on their breeding behaviour, the males and females can be marked individually and subsequently transferred and reared in the 'brood stock' tanks. The 'brood tanks' are made of FRP with a water holding capacity of 500 lit. Washed sand was provided at the bottom of the tanks up to 20 cm as substratum. Seawater flow rate was adjusted at a rate of 500 ml/ min. They were fed *ad-libitum* with live clams (*Donax cuneatus* and *D. faba*). The sand substratum was changed every month. During the breeding time, the mating behaviour was recorded carefully. After their mating, the females start releasing the characteristic 'ram-horn' shaped egg capsules. The release of egg capsules by the female chank takes a few hours to almost 3 days in some cases. Initially, they secrete a holdfast and paste it to the bottom surface of the tank. Then the female (mother) secretes and makes individual chamber and carefully lays the eggs in to the chamber, which is sealed and this process is repeated till the eggs last. Subsequent to the complete release of the egg capsule the egg capsule stands erect. In general, the mean length of egg capsules of *Xancus pyrum* was about 224 mm, depending on the size of the mother chank. The width of the egg chamber ranged from  $9.64 \pm 0.81$  (minimum) to  $33.0 \pm 4.79$  mm (maximum). Examination of the total number of chambers in each capsule indicates that they vary between 20 and 33 per capsule. From each egg capsule, 99 to 275 (average 222) babies hatch out.

### Release of baby chanks from the egg capsules

Depending on the hydrological conditions of the water and after 30 to 35 days of release of egg capsules, babies hatch out from the egg capsules. Regarding the hatching mechanism, the juveniles of *Xancus pyrum* rasp the wall of egg chamber with their radula and then come out from their respective chamber. The juveniles of *Xancus pyrum* are benthic and very active in creeping movement. At the time of their release, the baby chanks actively move on the surface of the egg capsule and subsequently on the substrata of the rearing tanks.

### Rearing of baby chanks

The babies of the *Xancus pyrum* are carnivores. They feed on very small/young ones of polychaete worms up to 2 months. After two months, according to the size of baby chanks they prey on live earthworm and Neries. After eight months, the baby chanks feed live clams. The growth obtained in experimental studies is given below:

### Growth of baby chanks, *Xancus pyrum*

The baby chanks which hatch out from the egg capsule are of about 09.09 mm in length. After 120 days, they attain an average length of 42.88 mm and after eight months they reach 53.66 mm in length. They attain about 62.0mm after one year of their release from the egg capsule.

**Growth of baby chanks, *X. pyrum* from the day of their release from the egg capsules**

**Growth in terms of**

Days	Length (mm)	MSD (mm)	Weight (g) (mean)
0	09.09 ±0.945	04.07 ±0.469	00.14
120	42.88 ±4.916	20.59 ±2.559	06.36 ±1.625
240	53.66 ±5.256	26.44 ±2.842	17.48 ±5.272
360 *	62.23 ±5.391	31.47 ±2.762	32.35 ±9.021

\* Feed - changed from live earthworm and *Nereis* to live clam (*Donax* sp.)

**Tagging and recapture**

In order to detect the natural growth rate and the migratory behaviour the chanks were tagged using Letro labels with araldite and sea ranched in the Gulf of Mannar and Palk Bay. The results indicated that:

- With the relaxations of chank fishing restrictions, the sacred chank is over-exploited (using modified trawls).
- It is a non-migratory species, which lives in restricted chank beds.
- The sacred chank, *XanCUS pyrum* is a slow growing species with an MSD-wise growth is about 8.0 mm/year.
- Its fecundity is also not very high and it breeds once in a year.

