International Research Journal of Biological Sciences ISSN 2278-3202
Vol. 3(1), 6-10, January (2014) Int. Res. J. Biological Sci.

Courtship Behaviour, Brood Characteristics and Embryo Development in Three Spotted Seahorse, *Hippocampus trimaculatus* (Leach, 1814)

Lipton A.P.[1] and Thangaraj M.[2*]
[1]Marine Biotechnology Laboratory, Vizhinjam Research Centre of Central Marine Fisheries Research Institute, Vizhinjam, Thiruvananthapuram- 695 521, Kerala, INDIA
[2]Centre for Advanced Study in Marine Biology, Annamalai University, Parangipettai- 608 502, Tamilnadu, INDIA

Available online at: www.isca.in, www.isca.me
Received 14th August 2013, revised 17th September 2013, accepted 17th November 2013

Abstract

The three spotted seahorse, Hippocampus trimaculatus is one of the important species being sold in Chinese market. In India, this species is mainly distributed along the south Indian coast, especially in the southeast and west coast. In southwest coast of India, this is the dominant species (79.68%) followed by H. kuda (9.89%), H. kelloggi (8.33%) and H. fuscus (2.08%). Hippocampus trimaculatus has very sharp hook-like cheek and eye spines; quite flat in appearance; narrow head; no nose spine. Colour pattern is golden orange, sandy coloured or totally black; may have large dark spots on the dorso-lateral surface of the first, fourth and seventh trunk ring and it can be easily distinguished from other seahorse species. During this study in captive condition the eggs of H. trimaculatus were ovoid in shape with one end slightly tapered, 2.12 ± 0.019 mm long, 1.97 ± 0.045 mm wide and 2.94 ± 0.3 mg in weight, with numerous bright orange/ red fat globules. The observed brood size was 389 ± 56.11. The newly expelled baby's length was 7.00 ± 0.05 mm and weight was 0.97 ± 0.08 mg. Three distinct embryonic stages such as yolk sac larva, term embryo and youngone were observed during their development.

Keywords: Seahorse, *Hippocampus trimaculatus*, egg size, brood size, embryo development.

Introduction

Seahorses are one of the members of the family Syngnathidae and thirty three species come under the single genera, *Hippocampus*[1,2]. They are found throughout the world in shallow, coastal tropical and temperate waters and are more abundant in the Indo-Pacific region[3]. They are distributed in high density along the China coast among seaweed and in seagrass from Vietnam to Korea[4]. Among all seahorses, *H. trimaculatus* was the most common species on sale in Vietnam markets[5]. *Hippocampus trimaculatus* is one of the predominant species of seahorses distributed along the Southeast and west coasts of India and has been overexploited in an unsustainable manor[6]. The unclear taxonomy of seahorse is due to the limited morphological variation among species, poor type descriptions, independent designation of the same name for different species, and the ability to changing their body colour and growing skin filaments to match their surroundings[4]. As per Lourie *et al.*[3] the standard morphometric and meristic character analysis may clear the taxonomical ambiguity. They are: trunk rings, tail rings, pectoral and dorsal fin rays, trunk length, tail length, coronet height, head length, snout length, snout depth and head depth.

The unique parental care offered by male seahorse is useful in assessing the influence on the size and quality of their progeny. The females produce larger eggs with large males but ultimately the paternal size determines the reproductive output in the paternal mouth brooding fish, *Pterapogon kauderni*[7,8]. However, the males play a major role in determining survival and reproductive success in seahorse as they provide a lengthy protection, aeration, osmoregulation and nourishment of developing embryos, depending on egg size in the pouch[9]. As a female transfers the entire clutch to one male, it indirectly controls the size of brood[9]. The clutch size of *H. kuda* varied from 61 to 325 depending on the maternal body mass. There was no significant correlation noted between paternal body mass and brood size suggesting dominant role of females in determining brood size only. But the long and healthy babies were produced by larger males[10]. Though, many more studies were recorded by several workers in many seahorse species worldwide, the courtship behavior and embryo developmental information is lacking in this *H. trimaculatus* in Indian waters. Breeding behaviour such as courtship role, egg transfer and expulsion of babies and also adult size, in young size and brood size in *H. trimaculatus* in captive condition is reported in this article.

Material and Methods

Animal collection: Adult live seahorses (6 male and 4 female) were collected from Tuticorin area, Gulf of Mannar, South India. The morphometric, meristic, colour, sex were recorded as per standard protocol suggested by Lourie *et al*[4]. Each fish was tagged with collar tags with specific number for easily distinguish with each other. The seahorses were acclimated to laboratory conditions in 1000 L fibre reinforced plastic (FRP) tanks supplied with filtered seawater, mild aeration and water circulation. The brood stock was fed with adult *Artemia* twice every day morning and evening. Besides this, *Mysids* and *Tilapia* juveniles were also occasionally provided *ad-libitum*. Routine husbandry practices such as removal of waste and 25% of water exchange was carried out daily.

Water quality parameters: The water quality was maintained as temperature 27.5 ± 0.85^0C, dissolved oxygen 4.29 ± 0.24 ppm; salinity 35 ± 0.25ppt and pH 7.53 ± 0.26 with photoperiod 10L/14D cycle.

Daily observation: The adults were observed daily for breeding and feeding behavior. Occasionally due to lack of suitable male to the matured females or some times during egg transfer, many eggs were dropped down. All these eggs were collected and counted at 10X magnification in a dissection microscope (Olympus) and twenty eggs from each clutch were measured by micrometer. During egg transfer almost all eggs were deposited directly in to the male's pouch so the best available estimate of female clutch size was male brood size[16]. The occasionally dead male seahorses were dissected out and embryos were observed under a light microscope (Leica) and count the brood size.

Results and Discussion

Courtship behavior: Courtship occurs usually in the early morning. The initiation involves a series of colour changes and postural displays. Dilating the opening of their brood pouch slightly, males inflated the pouch to balloon like proportions with water by swimming forwards or by pushing their body forwards in a pumping action, then closing the pouch opening. At the same time they would lighten their pouch in colour to white or yellow. Males also brightened their body colour, typically intensifying the yellow colour. Males would repeatedly approach their selected female with their head tucked down, and the dorsal and pectoral fins rapidly fluttering. If the female were not receptive, they would ignore the male, who would then approach another potential partner. If no females in the tank were receptive, the males would stop displaying and deflate their pouches by dilating the pouch opening and bending forwards, expelling the water outside.

If a female is receptive to a 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 patterning. 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. After coming to rest, the male would attempt to get the female to swim towards the water surface with it by repeatedly pointing its snout upwards. If the female responded by also pointing its snout upwards then the final stage of courtship followed.

This involved both male and female swimming directly upwards towards the water surface with both their heads pointing upwards and tails pointing down wards. Sometimes if they reach the water surface one or both seahorses, and heard to snap their heads. To transfer its egg to the male, the female would face the male, slightly above it. Pressing the base of its abdomen against the male's pouch it would squirt the eggs through the opening in front of male's pouch. During some egg transfer, many eggs were spilled out and settled in the tank bottom.

After the successful egg transfer, the male would repeatedly bend sidewise and contort its body in an attempt to evenly distribute the eggs with in the pouch and the body coloration begins to dull. Females would also dull their colouration and their abdomens size gets reduced following egg transfer. After 12-14 days from the date of egg transfer fully developed babies comes out.

Egg and brood size: The hydrated eggs were ovoid in shape with one end slightly tapered, 2.12 ± 0.019 mm long, 1.97 ± 0.045 mm wide and 2.94 ± 0.3 mg in weight, with numerous bright orange/ red fat globules (figure-1). Each egg weighed 2.94 ± 0.3 mg and the brood size was 389 ± 56.11.

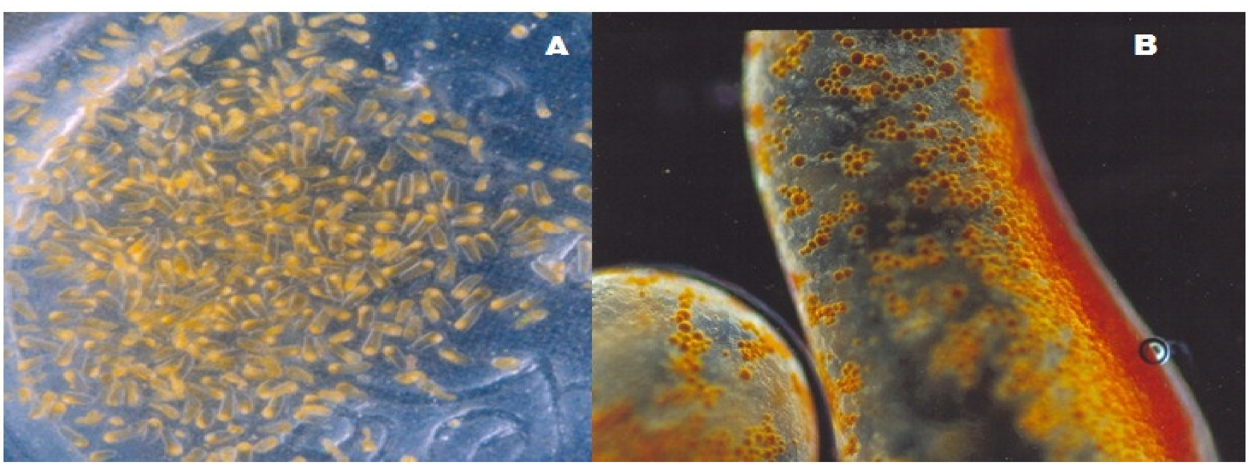

Figure-1
(A) Egg mass, (B) Enlarged portion (50X)

Yolksac 'larva': During the yolk sac larval stage, the embryo was somewhat coiled with most of the thoraco-abdominal region in contact with the yolksac (figure-2). The anterior and posterior ends of the body were raised from the yolksac and laterally flattened to form the caudalfin bud. The caudalfin bud was slightly elevated and deviated from the surface of the yolkmass. The caudal bud eventually elongated into the prehensile tail. At this phase, a distinct dorsalfin bud was noted. Paired pectoralfin buds appeared but lepidotrichia within the fins were not yet developed up to this time (i.e., the time of dissection from the broodpouch). 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 anteroposterior embryonic axis. A rudimentary heart was seen above the yolksac 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.

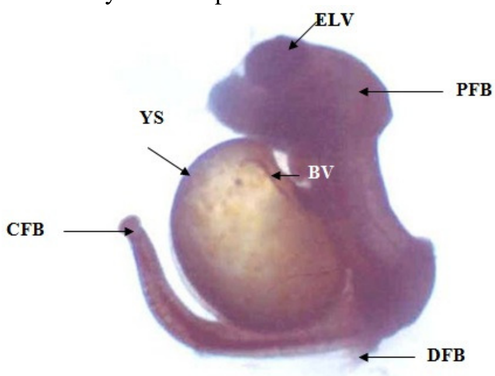

Figure-2
Yolk Sac "Larva" with ELV (Eye Lens Vesicle), PFB (Pectoral Fin Bud), DFB (Dorsal Fin Bud), CFB (Caudal Fin Bud), YS (Yolk Sac), BV (Blood Vessel)

Term embryo: As embryogenesis proceeded, the embryo continued to elongate, yet remained coiled around the yolkmass (figure-3). 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 yolksac reduced. This phase of development was characterized by the morphogenesis of the jaw and the growth of the dorsal and pectoralfins. At this stage, the analfin first appeared as a swelling posterior to the vent. No surface amplification was seen on the emerging analfin. The pectoral and dorsalfins were found to be broad and flat with the rays beginning to develop. The contact of the embryo with the yolksac was limited to its thoracoabdominal region. The entire prehensile tail and the anterior half of the head were free from the yolksac. No surface amplifications were found on the emerging analfin as it was just beginning to develop. The vent, posterior to the analfin, 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.

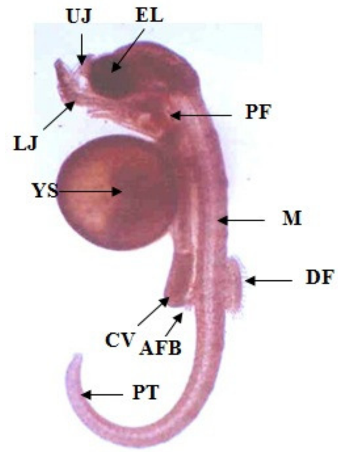

Figure-3
Term Embryo with M (Myomeres) DF (Dorsal Fin), AFB (Annal Fin Bud), PT (Prehensil Tail), CV (Closed Vent), YS (Yolk Sac), LJ (Lower Jaw), PF (Pectoral Fin), UJ (Upper Jaw), EL (Eye Lens)

Youngone : The internal yolk reserves were completely exhausted and there was no notable protrusion of the extraembryonic yolksac at this stage (figure-4). The elements of the dermal plates were quite visible while the mouth apparatus was elongate, acquiring the typical adult form. The analfin was completely formed and the vent was open. Microvilli-like rays were present in the analfin. The dorsal and paired pectoralfins were fully formed with rather sturdy finrays. The prehensile tail was capable of muscular contraction. The tail musculature became fully functional and adhered to the substrate.

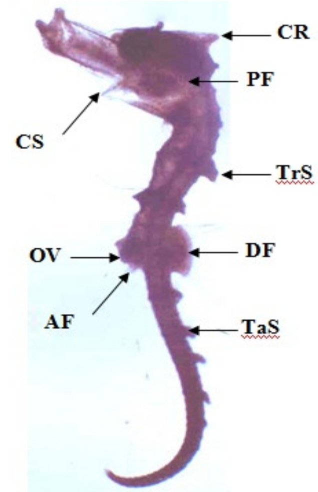

Figure-4
Newborn baby seahorse with TrS (Trunk Spine), DF (Dorsal Fin), TaS (Tail Spine), AF (Anal Fin), OV (Open Vent), PF (Pectoral Fin), CR (Coronet), CS (Cheek Spine)

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 broodpouch.

Young birth: Before the birth of the young the oval shape pouch became nearly spherical in shape. The fish became restless and swim here and there. The breathing frequency became increase. Usually the young one birth occurs in the morning (between 6.30 to 8.30 AM). The complete parturition duration extended from 20 to 90 minutes.

Initially the animal bends the trunk and tail towards each other frequently and tries to widen the pouch mouth. When births occurred, it was doing continuously such a movement; backward bend and rapid forward bending the tail come forward with an ejaculatory movement. During the backward bending the water goes inside the pouch during the rapid forward bend the water come out with a force and along the water young ones are pulled out. Along the young ones fine particles and filaments also come out (it may be the egg wall and waste materials). In every ejaculation 3-8 young ones come out. Between every two to three ejaculations, the animals hold the holdfast and get rest. All the young ones are not swum immediately; some are round in shape and settled in the bottom of the tank. After sometimes later the stretch the body and swim freely. After the last young one was expelled, the pouch returned to nearly normal proportions within about 15 minutes and within an hour it had completely subsided. During the entire procedure, there was no attempt to rub the pouch on objects as an aid to expelling the young ones.

Observations on the breeding behaviour of male and female *H. trimaculatus* in the present study indicated that seahorses in captivity formed monogamous breeding pairs, with daily greetings. Males competed more intense by for access to mates than the females. The males brightened up during courtship and actively sought for females that were brightened and ready to respond. These results conform to the earlier observations of Vincent[11,12] about the courtship behaviour of *H. fuscus* in the laboratory and those of Vincent and Sadler[13] in the field studies on *H. whitei.* Both these species were monogamous with a single male and female mating repeatedly and exclusively over the course of reproduction. Masonjones and Lewis[14] also observed that the dwarf seahorse *H. zosterae* formed monogamous pairs that courted during early morning hours until mating took place.

The eggs of *H. trimaculatus* were spherical with a tapered end, and 2.12 ± 0.019 mm long, 1.97 ± 0.045 mm wide and 2.94 ± 0.3 mg in weight. The egg diameter ranged from 1.3 to 2.45 mm as that of other seahorses like *H. abdominalis, H. breviceps, H. comes, H. erectus, H. fuscus, H. kuda, H. reidi, H. subelongatus, H. whitei,* and *H. zosterae*[15,16,17]. Brood size is the number of youngones per pregnancy released by a male in one birth. The brood size (389 ± 56.11) in *H. trimaculatus* during this investigation varied from that of earlier reports. For example in Vietnam, Nguyen and Do[19] reported brood size of up to 1783 per brood in *H. trimaculatus*. The mean brood size in other male seahorse species ranged from an average of 250 to maximum of 2000 depending on the species at captive conditions[3,9,18,20,21,22]. The smaller seahorse, *H. zosterae* released only about five offspring per cycle[14].

In the present study, three distinct stages of embryonic development in *H. trimaculatus* were observed. Eyes developed much earlier than the other organs. During development, the eye size did not show much variation, unlike the head which showed distinct variation. The snout length showed an increasing trend with the increasing head length. The trunk length, tail length and dorsalfin base also increased in the second stage. Earlier studies reported that in *H. trimaculatus,* the length of the tail gets slightly longer than the body in six days after fertilization in Chinese waters[22]. The present study also confirms that the tail gets elongated in the near-term embryo stage. As the present observations many previous reports on the pipefishes, *Syngathoides biaculeatus*[23] and *Syngnathus abaster*[24], many significant morphological differences in embryo development has been observed. The embryo of *H. trimaculatus* could be distinguished from the embryos of *S. biaculeatus* and *S. abater,* midway through development, i.e., just prior to hatching. The yolksac larva has a well developed head together with the formation of the pectoralfin buds. At this stage, a developing caudalfin was evident in the pipefish embryos, whereas the seahorse embryo lacked any indication of a caudalfin fold as it appeared as a dorsalfin bud. In the term embryo stage, the prehensile tail of *H. trimaculatus* is fully formed and capable of muscle contraction. The orientation of the head of this species embryo at 90° angle normal to the body axis also occurred at near-term embryo stage, whereas the head of pipefishes continued to develop in line with the body axis. In *H. trimaculatus,* their characteristic dermal armour had completely formed prior to their release from the broodpouch as noted by Cai *et al*.[15]. In the case of *H. trimaculatus*, the subsequent sojourn in the broodpouch is not only the time period during which these differences are established, but it also allows the young seahorse to develop to a morphologically more advanced state than the newly hatched pipefishes described elsewhere[23,24]. Calcification of the dermal skeleton while in the pouch confers a subsequent advantage of protection to the young seahorse, because they will be heavily armoured upon their release from the pouch.

Conclusion

The series of developmental stages of the embryo of three spotted seahorse *H. trimaculatus,* were described and characterized based on morphological features identified by examination of dead embryo using Leica light microscope. The brood size in seahorse in nature as well as in captive rearing conditions can be influenced by a number of stock-specific, environmental and behavioural factors including sex-ratio alterations. It is not clear whether the seahorses in the natural conditions release the same number of eggs and size as noted in the captive rearing conditions. It is recommended that a study on the breeding biology of *H. trimaculatus* at natural habitat should be done and documenting each stage of the embryo for their entire life cycle.

References

1. Dawson C.E., Fishes of the Western Atlantic. Family Syngnathidae. The pipefishes. Subfamily Doryrhamphinae and Syngnathinae, Sear Foundation for Marine Research, Memoir, Yale Uiversity. **1(8)**, 1-172 **(1982)**

2. Lourie S.A. and Vincent A.C.J., A marine fish follows Wallace's line: the phylogeography of the three spot seahorse (*Hippocampus trimaculatus*, Syngnathidae, Teleostei) in Southeast Asia, *J. Biogeography*, **31**, 1975-1985 **(2004)**

3. Lourie S.A., Vincent A.C.J. and Hall H.J., Seahorses: an identification guide to the world's species and their conservation. London, Project Seahorse, 214, **(1999a)**

4. Lourie S.A., Prichard J.C., Casey S.P., Truong S.K., Hall H.J. and Vincent A.C.J., The taxonomy of Vietnam's exploited seahorses (family Syngnathidae), *Biol. J. Linn. Soc*., **66**, 231-256 **(1999b)**

5. Vincent A.C.J., The international trade in seahorses. *TRAFFIC International*, Cambridge, U.K., 163, **(1996)**

6. Lipton A.P. and Thangaraj M., Present status of seahorse fishing along the Palk Bay coast of Tamilnadu, *Mar. Fish. Infor. Ser. Tech. Ex. Seri*., **174**, 5-8 **(2002)**

7. Kolm N., Females produce larger eggs for large males in a paternal mouthbrooding fish, *Proc. R. Soc. London B*, **268**, 2229-2234 **(2001)**

8. Kolm N., Male size determines reproductive output in a paternal mouthbrooding fish, *Anim. Behav*., **63**, 727-733 **(2002)**

9. Vincent A.C.J., A seahorse father make a good mother, *Nat. Hist*. 34-43 **(1990a)**

10. Thangaraj M., Lipton A.P. and Ajith Kumar T.T., Interrelationship among reproductive traits in Indian seahorse, *Hippocampus kuda*, *Asian J. Biol. Sci*., **5(5)**, 263-267 **(2012)**

11. Vincent A.C.J., Seahorse exhibit conventional sex roles in mating competition, despite male pregnancy, *Behaviour*, **128**, 135-151 **(1994)**

12. Vincent A.C.J., A role for daily greetings in maintaining seahorse pair bonds, *Ani. Behav*., **49**, 258-260 **(1995)**

13. Vincent A.C.J. and Sadler L.M., Faithful pair bonds in wild seahorses, *Hippocampus whitei*, *Behaviour*, **50**, 1557-1569 **(1995)**

14. Masonjones H.D. and Lewis S.A., Courtship behavior in the dwarf seahorse, *Hippocampus zosterae*, *Copeia*, **33**, 634-640 **(1996)**

15. Cai N., Xu Q., Yu F. and Wu X., Development of the embryo of *Hippocampus trimaculatus*, *Studia Marina Sinica*, **23**, 101-104 **(1984a)**

16. Vincent A.C.J., Reproductive ecology of seahorses, Ph.D. thesis. University of Cambridge, 107, **(1990b)**

17. Mi PT., Kornienko ES. and Drozelov A.L., Embryonic and larval development of the seahorse, *Hippocampus kuda*, *Russian J. Mar. Biol*., **24(5)**, 325-329 **(1998)**

18. Woods C.M.C., Preliminary observations on breeding and rearing the seahorse *Hippocampus abdominalis* (Teleostei: Syngnathidae) in captivity, *New Zealand J. Mar. Fresh. Res*., **4**, 475-485 **(2000)**

19. Whitfield A.K., Threatened fishes of the world: *Hippocampus capensis* Boulenger, 1900 (Syngnathidae), *En. Biol. Fish*., **44**, 360-361 **(1995)**

20. Lockyear J.F., Kaiser H. and Hecht T., Studies on the captive breeding of the Knysna seahorse, *Hippocampus capensis*, *Aquarium Sci. Conserv*., **1(2)**, 129-136 **(1997)**

21. Golani D. and Fine M., On the occurrence of *Hippocampus fuscus* in the eastern Mediterranean, *J. Fish Biol*., **60**, 764-766 **(2002)**

22. Cai N., Xu Q., Yu F. and Wu X., Studies on the reproduction of the seahorse *Hippocampus trimaculatus*, *Studia Marina Sinica*, **23**, 92-100, **(1984b)**

23. Dhanya S., Rajagopal S., Ajmal Khan, S. and Balasubramanian T., Embryonic development in alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785), *Curr. Sci*., **88(1)**, 178-181 (2005)

24. Silva K., Monteiro N.M., Almada V.C. and Viera M.N., Early life history of *Syngnathus abaster*, *J. Fish Biol*., **68**, 80-86 **(2006)**