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Preliminary observation on breeding three spotted seahorse, *Hippocampus trimaculatus* (Leach, 1814), solely fed with wild caught amphipods under *ex - situ* condition

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ABSTRACT

In this study, we evaluated the reproductive efficiency of the wild caught brooding seahorse population which was compared with captive bred F_1 generation. Aquaculture has been proposed as a solution to address unsustainable trade in seahorse, and for continuous seed production in the aquaculture system information with regard to inter-mating duration and clutch size of the female seahorse are very much needed hence a attempt was made since this information lacks for most of the seahorse studied. The number of juveniles released by wild caught seahorse ranged from 318 to 635, whereas in F_1 generation it ranged from 36 and 238. In captive condition seahorse (F_1) mated two to four times during the experimental period of six months and the time interval between two mating events was between 2 and 63 days. Gestation period ranged from 12 to 14 days at 30ºC. The clutch size of the matured un-paired females ranged from 17 to 106 and the inter clutch release period ranged from 14 to 51 days.

Key words: Captive breeding, Clutch size, Inter-mating duration, Seahorse

The family Syngnathidae differs from other families of bony fishes mainly due to the presence of brood pouch and parental care. Female seahorse transfers the unfertilized telolecithal eggs into the vascularized brood pouch of the males which is located below the trunk region and the fertilization of eggs happens inside the brood pouch (Murugan *et al.* 2008). Monogamous mating system in fish is defined as either time after time spawning with the same partner throughout a breeding season or mutually caring for young ones. The monogamous pair bonding is mainly seen in freshwater fishes and very few in coral reef fishes (Barlow 1986). Limited availability of mates can be an untailored cause promoting monogamy, but monogamy in some fishes is maintained without such limitation (Morley and Balshine 2002). Information on the breeding biology, collectively with courtship behavior of an animal is crucial to know it reproductive strategies. A tryout was made to breed *H. trimaculatus* in captivity to study the breeding behavior of this species which could be useful for the conservation of this species in wild *(in-situ*) as well as for aquaculture industry *(ex-situ*).

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MATERIALS AND METHODS

Brooding males (7) ready to spawn were collected from Mandapam (9°16'39''N; 790°7'27''E), Gulf of Mannar region, Southeast coast of India. The brooding males were individually placed in a rectangular tank (50 cm ×30 cm ×30 cm), whereas the salinity, temperature, pH, dissolved oxygen (DO) and light intensity were maintained at (mean±SD) 31±0.4%, 28±0.5°C, 7.5±0.3, 6.5±0.5 mg/l and 1100±250 Lux, respectively. The brooding males were fed thrice in a day (0945 h, 1300 h and 1830 h) by providing wild caught amphipods *(Eriopisa* spp. and *Gammaraus* spp.) from seaweed and oyster beds of Vellar estuary, Parangipettai at *ad libitum*. The new born juveniles released by the wild caught seahorses were counted and stocked in juvenile rearing tank for rearing experiments.

The juveniles reared under *ex-situ* condition were used for the observation of inter-mating studies. Among the juvenile seahorse reared, courting pairs (14) of F_1 seahorses, approximately 0.9 years old, ranging from 6.5 to 8.2 g in wet body weight and 12.1 to 13.3 cm in standard body length, were maintained in a rectangular tank (1000 cm×500 cm× 500 cm). Each seahorse pairs were tagged as described by (Lipton and Thangaraj 2005), whereas plastic plants were

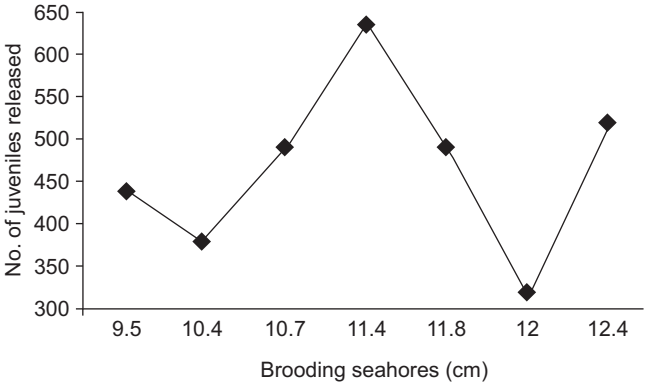

Fig. 1. Reproductive efficiency in terms of juveniles released by the three spotted seahorse, *Hippocampus trimaculatus*

used as holdfasts. The water quality in the breeding tank was maintained as mentioned above, and the photoperiod was maintained at 16 L (0800–2300 hrs) and 8 D (2400–0700 h). The seahorses were fed twice a day (0945 h and 1700 h) with wild caught amphipods *(Eriopisa* spp. and *Gammaraus* spp.) at a daily rate of approximately 15% of the wet body weight. When male and female seahorses completed their copulation, the male seahorses were isolated temporarily and placed in a separate brooding/hatching tank (50 cm × 30 cm × 30 cm) filled with seawater with the same water quality parameters and plastic plants as hold fast until the juveniles were released from the brood pouch. During the gestation period, the male seahorses were fed 3 times (0945 h, 1300 h and 1830 h) a day with wild caught amphipods at *ad lib*. The faeces and uneaten food in the tanks were siphoned out 2 h after each feeding. All statistical analyses were performed using STATISTICA version 6.0. The level for accepted statistical significance was $P<0.001$.

Table 1. Information on breeding pairs of three spotted seahorse, *Hippocampus trimaculatus* (F_1 generation) under *ex-situ* conditions

Breeding data	Mean	SD	Range	n
Adult male length (HT in mm)	12.5	0.8	12.1 – 13.3	14
Adult female length (HT in mm)	12.7	0.91	12.4 – 13.1	14
Adult male wet weight (g)	7.1	0.59	6.5 – 7.4	14
Adult female wet weight (g)	7.8	0.73	6.7 – 8.2	14

RESULTS AND DISCUSSION

Reproductive efficiency of seahorse in terms of brood size from wild population: The number of juveniles released from the seven brooding males ranged from 318 to 635 (466.57±102.53), the highest numbers of juveniles were released by the seahorse with a standard length of 11.4 cm, whereas the seahorse with an size of 12 cm had the smallest brood size (318) among the observed seahorse (Fig. 1). The number of young ones released by *H. trimaculatus* in Vietnam waters varied from 232 to 1783 (Ky and Nga 1995); 400 to 1000 in Chinese waters (Cai *et al*. 1984). Among the observation made on the reproductive efficiency of *H. trimaculatus* from different waters; the present study suggests a low reproductive efficiency this might be due to the result of overexploitation, which might have reduced the size range of the wild population in general.

The present study animal is a medium sized seahorse with a maximum size recorded from Gulf of Mannar region for male seahorse was around 148 mm (Murugan *et al.* 2011). A positive correlation between brood size and body size has been found in most fishes (Breeder and Rosen 1966), whereas this concept is not true in the case of seahorses since maximum brood size has been recorded from the smaller size individual when compared to the recorded maximum

Table 2. Captive breeding of the three spotted seahorse *Hippocampus trimaculatus* during F_1 generation under *ex-situ* Condition

S.No	DOS	Size (cm)		First mating duration	No. of young ones	Second mating duration	No. of young ones	Third mating duration	No. of young ones	4^{th} mating duration	No. of young ones
		Male	Female								
Pair 1	19–11–01	12.5	12.7	17	44	32	57	28	98	-	-
Pair 2	26–11–01	12.2	12.4	24	37	19	69	35	113	-	-
Pair 3	30–11–01	12.9	12.4	33	48	25	77	38	135	53	189
Pair 4	13–12–01	12.5	12.5	13	39	35	42	-	-	-	-
Pair 5	16–12–01	12.5	12.2	16	42	20	59	63	148	36	206
Pair 6	16–12–01	13.2	13.1	22	63	2	60	36	173	41	194
Pair 7	18–12–01	12.7	12.5	28	73	25	97	39	107	28	163
Pair 8	20–12–01	13.3	13.2	16	60	31	83	41	96	37	174
Pair 9	23–12–01	12.5	12.4	23	55	19	71	47	139	-	-
Pair 10	25–12–01	12.7	13.2	19	62	24	89	53	142	55	238
Pair 11	25–12–01	12.8	12.5	31	52	29	61	24	111	42	170
Pair 12	27–12–01	12	12.3	37	63	31	78	28	131	35	179
Pair 13	29–12–01	13.2	13	26	36	37	50	19	105	-	-
Pair 14	29–12–01	12.4	12.6	20	47	27	62	29	129	30	203

•DOS, Day of stocking.

size (Foster and Vincent 2004). The reproductive efficiency in the observed wild male seahorse suggest that comparison between Standard Length (SL) and brood size was significant ($F_{1,5}$= 0.126; r^2=0.025; *P*=0.0042) indicating no correlation between male body size and brood size. There have been no long term data from the same ecosystem assessing the reproductive efficiency of male seahorses and in most of the studies the sampling size is small or not mentioned. So the reproductive efficiency of the wild population can only be determined through long term monitoring protocols, that to an endangered species. At this point the present study provides the baseline information about the reproductive efficiency of seahorse inhabiting Gulf of Mannar region, which in turn would be the yardstick to future research in this aspect for this highly exploited species.

Inter-mating duration of F_1 generation seahorses under ex-situ condition: The pairing of seahorses can be identified through their elaborate courtship behavior which they exhibit before mating, the pair status with regard to its length and wet weight is shown in Table 1. The pairs which showed the first mating behavior were as early as 13 days and as late as 37 days from the day of stocking and the status about the selected pairs is shown in Table 2. Inter mating duration varied from 2 to 63 days (30.36±11.54 days) in the observed 50 mating events during the breeding trial period of six months details shown in Table 2. Among the fourteen pairs one pair mated two times, four pairs mated three times whereas other nine pairs mated four times. There was significant difference between pairs in respect to number of mating ($F_{1,12}$= 48, r^2=0.97, *P*=0.153). Inter-mating period varied significantly among the 14 pairs observed ($F_{1,12}$= 0.003, r^2=0.0003, *P*=0.003). In *H. zosterae* the time from birth of young ones to next copulation was around 17.5±4.4 hours (Masonjones and Lewis 2000). Vincent (1994) observed seahorse mating immediately after the release of young ones from the brood pouch whereas in the present study the seahorses *(H.trimaculatus*) mated within 2days after the release of juveniles, whereas the average duration taken was 30.36±11.54 days. The main lacuna which exists in the breeding study is the inter-mating duration of the pairs, without knowing this parameter initiation of commercial aquaculture practices will not be possible. Continuous breeding is essential for aquaculture operation if that is not achieved, the industry need to depend on wild brooding population which is not accepted/encouraged by the conversationalist and policy makers. Hence the present study was aimed to study the inter-mating duration of *H. trimaculatus*, a widely exploited species. Complex seahorse husbandry requirements mean that they often fare very badly in captivity, although considerable advances have been made in the last decade (Koldewey and Martin-Smith 2010).

Gestation period and Number of young ones released by F_2 generation: Gestation periods varied from 12 to 14 days at 30°C for *H.trimaculatus* under controlled conditions. The number of eggs deposited by the females could not be counted as the eggs were transferred directly into the male's brood pouch. The reproductive efficiency of the female can be directly estimated in the captive bred seahorses by counting the number of young ones released. During the experimental period under *ex-situ* condition the release of new born juveniles varied from 36 to 238 (100.38±54.28, n=50, Table 2). There was a significant difference between the numbers of young ones released between the fourteen pairs during different mating period ($F_{1,12}$= 0.716, r^2=0.056, *P*=0.413). At the end of the gestation period the young ones are released from the brood pouch which happened in the early morning and late evening period. The young ones are released by repeated process of contraction and relaxation similar to that of mammalian pregnancy. In the present study during each contraction 5 to 20 young ones were found to be released from the brood pouch of *H. trimaculatus* and all the young ones were released within a day. Seahorses invest heavily in the development of each of their young ones and the gestation period was relatively constant and it has been reported that environmental parameters and feeding affect embryo growth and development (Foster and Vincent 2004).

Table 3. Clutch size of the unpaired female three spotted seahorse *Hippocampus trimaculatus* in F_1 generation under *ex-situ* conditions

Size in cm (SL)	DTC	NOH	DTC	NOH	DTC	NOH
9.8	34	17	26	65	43	74
10.6	20	23	35	57	-	-
9.9	36	42	19	46	40	89
11.7	26	55	14	49	-	-
10.3	27	43	22	38	-	-
11.8	33	50	26	55	-	-
11.4	24	39	37	49	47	106
10.6	30	32	30	58	-	-
10.9	17	29	24	47	51	97
11.4	39	60	42	40	38	77
11.9	18	52	33	53	42	60
12.2	15	47	28	36	-	-
11.5	32	40	41	60	44	86
10.3	27	85	30	63	47	83

•Duration taken for clutch release (DTC). Number of hydrated eggs in the clutch (NOH).

Under captive condition the number of young ones released by *H.erectus* ranged between 189 and 363 (Lin *et al*. 2008), 400 to 600 in *H. kuda* (Lin *et al*. 2006) and 35 in *H.zostrea* (Masonjones and Lewis 2000). In the present study under *ex-situ* condition the number of young ones released varied from 36 to 238. It has been reported that not all the transferred eggs into the male's brood pouch successfully get fertilized and/or developed properly (Vincent 1994).

Clutch size of un-paired matured female seahorses under ex-situ conditions: Generally during reproduction the cost of investment in egg producing female is always higher than the males. In the case of *H. trimaculatus* the clutch size of

the unpaired females ranged between 17 and 106 (57.3 ±20.2 eggs, Table 3). Significant difference between unpaired females with regard to release in clutch size could be observed in the present observation ($F_{1,12}$= 0.60, r^2=0.004, *P*=0.81). The duration period for the inter clutch releasing period varied from 14 to 51 (31.58±9.73 days, n=36), whereas significant difference between unpaired seahorse pertaining to inter - clutch release could be observed among the 14 pairs ($F_{1,12}$= 0.459, r^2=0.037, *P*=0.51). The mean clutch size of unpaired seahorses was lower (57.3 ± 20.6) than the number of young ones produced by the pairs (100.38 ± 54.28). From this study it is known that the unpaired female doesn't release more hydrated eggs because it accounts about 10–15% of the total body weight (Vincent 1994). This could be a strategy of reducing the cost of energy in the production of un-potential eggs, since an egg clutch is considered as very costly in terms of energy because it contribute 15% of the female body weight (Foster and Vincent 2004).

Vincent (1994) observed entire egg clutches were found dropped during badly aligned mating attempts. The clutch size of the seahorses *H. zostrea* ranged from 5 to 69 (Strawn 1958), up to 1313 eggs for *H. erectus* (Texeira and Musick 2001). In the present study the clutch size of the seahorse *H. trimaculatus*, released during the captive breeding trial varied from 17 to 106. The sizes of the dropped egg clutch for several seahorse species were smaller than the maximum number of young ones released (Foster and Vincent 2004), which coincides with the present observation.

The main bottleneck that scientists and aquaculturists still have to face in seahorse culturing is limited juvenile production (Lin *et al.* 2006), whereas in Indian scenario the research on seahorse is very much limited since this species is placed under Schedule I of the Wild life Protection Act 1972; which means access to collect this animal for research is very much limited. In total 27 countries, primarily from Australia, Brazil, Hong Kong, Ireland, Mexico, New Caledonia, New Zealand, Sri Lanka, UK, USA and Vietnam were recorded as the source of captive-bred seahorses (Koldeway and Martin-Smith 2010) and the seahorse reared under captive conditions are used for marine aquarium trade. At least 13 species are in commercial culture or under research for their culture potential (Koldewey and Martin-Smith 2010).

In the current scenario developing the captive breeding and rearing technique for seahorses is inevitable (Murugan *et al*. 2009), which needs to be promoted as being done for terrestrial animal in Indian context. Keeping the threat to its existence in the wild, the searanching of seahorses has been made as part of the project objective on small scale level (Lipton and Thangaraj 2005); similar effort has been done in Canary Island, Spain, to increase the population of the seahorse *H. hippocampus* (Domßnguez and Ferrer 2009). The attempts taken by various research institutions need to be done on a long term basis to recover the population of this unique fish for which policy makers needs to support the initiative as per the models created for conserving land based animals (for example, project tiger, project bustard and project elephant). The results obtained in the present study reveal the efficiency of this species for aquaculture, which in turn can be used for *in-situ* conservation as well as for marine aquarium trade which is still in hibernation stage with regard to Indian scenario. Further, studies are needed with regard to physiological, ecological, behavioral, and greater precaution should be taken on genetic characteristics of species, since captive breeding techniques can be used for the conservation of seahorses in the coming decades.

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