

Author version: Aquaculture: 290(1); 2009; 87-96

Breeding and mass-scale rearing of three spotted seahorse, *Hippocampus trimaculatus* Leach under captive conditions

A. Murugan¹, S. Dhanya², R.A. Sreepada^{1*}, S. Rajagopal³ and T. Balasubramanian³

¹Aquaculture Laboratory, National Institute of Oceanography, Dona Paula-403 004, Goa (India)

²Centre for Marine Living Resources & Ecology, P.B. No. 5415, Kakkanad, Kochi - 682 037, Kerala (India)

³Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu (India)

Abstract

Recent establishment of techniques for captive breeding and closure of the life cycle of some seahorse species is expected to help in achieving the twin objectives of reducing pressure on wild stocks as well as providing alternative subsistence livelihoods to fisher-folk currently involved in seahorse fishing. The relatively higher fishing pressure on the three-spotted seahorse, *Hippocampus trimaculatus* Leach, one of the commercially important species sought in traditional Chinese medicine, necessitated the need to develop techniques for captive breeding and mass-scale rearing for conservation and aquaculture purposes. In this paper, we present data on the reproductive efficiency of captive broodstock and the effect of exogenous factors (light intensity, prey type, ingestion and salinity) on survival and growth of juveniles of *H. trimaculatus*. Significantly higher reproductive efficiency (number of juveniles released) was observed when parent seahorses were fed with amphipods (*Eriopisa* spp.) ($P < 0.05$). The survival of pelagic phase juveniles reared under three different light intensities (1000, 1500 and 2000 lx) differed significantly ($P < 0.05$) with 2000 lx resulting in the highest survival ($77.3 \pm 3.1\%$). An ontogenetic shift in feeding behaviour from rotifer to copepod nauplii was observed in >2 DAB (days after birth) old juveniles. Estimated digestion period (DP) in 6 DAB old juveniles was 3h 20m and the DP reduced as the juveniles grew in size. Salinity tolerance experiments indicated that juveniles and adults of *H. trimaculatus* are able to tolerate salinities not extending below 26 and 17 ppt, respectively. The average growth rates peaked during 15-30 DAB (Ht, 1.64 mm day⁻¹). The first sign of maturity in male (pouch development) and female (dropping of egg clutch) was noticed at 95 DAB and 115 DAB, respectively. The sex ratio of cultured seahorses skewed towards female (1:1.2) and differed significantly from equality ($P < 0.05$). Mean number of newborn juveniles released by first mated males was 65 ± 20 juveniles seahorse⁻¹ (n=9 pairs) and egg clutch droppings by newly matured females was 45 ± 17 eggs seahorse⁻¹ (n=21) in F2 generation. Maximum height (Ht) attained at the end of the culture period of 26 weeks was 125 mm with no significant difference in growth between the sexes ($P > 0.05$). Mean growth attained (Ht, 119.9 \pm 15.3 mm) and survival rate achieved (65%) at the end of 26 weeks of mass-scale rearing are comparable with reports on other seahorse species. It is expected that the results of this study could guide future programmes in hatchery technology and aquaculture of this commercially important fast dwindling seahorse species.

Key words: Captive breeding, Survival, Growth, Mass-scale rearing, Three spotted seahorse, *Hippocampus trimaculatus*

*Corresponding author. Tel: +91-832-2450426; Fax: +91-832-2450606; E-mail: sreepada@nio.org

1. Introduction

The incidental by-catch during commercial fishing operations (Vincent, 1996) as well as targeted fishing practices such as skin diving during high demand periods has pushed seahorses to the edge of extinction, especially in Southeast Asian countries (Marichamy et al., 1993; Pajaro et al., 1997; Job et al., 2002). It has been estimated that over 20 million seahorses are traded annually by more than 45 countries worldwide for traditional Chinese medicine (TCM) (Woods, 2000a). The global trade in seahorses and their Syngnathid relatives as medicine and curios in 2000 was estimated to be at least 56 tonnes of dried animals (Pajaro et al., 1997). India contributes to about 30% of this global trade and most of the catch is taken from Gulf of Mannar and Palk Bay regions (Salin et al., 2005). Further, it has also been reported that small quantities are locally utilized for medicinal and ornamental purposes (Sreepada et al., 2002; Murugan, 2004).

The three-spotted seahorse, *Hippocampus trimaculatus* (Leach, 1814) is a highly valued species in the medicinal and aquarium trades and is one of the heavily traded seahorse species contributing to about 35% of the total seahorse trade for TCM from Southeast Asia (Choo and Liew, 2003; Giles et al., 2006; Salin et al., 2005). Most Indian seahorse exports (~5.3 tonnes dried) to Singapore and Hong Kong comprised this species (Anonymous, 2003). *H. trimaculatus* is widely distributed throughout the tropical Indo-Pacific region, usually inhabiting the shallow regions of inshore waters (Lourie et al., 1999a) and occurs abundantly in the deeper waters (15-25 m) of Gulf of Mannar and Palk Bay regions off the Indian coast (Gokulakannan, 2002; Murugan, 2004). This species is more vulnerable to high fishing pressure due to its smaller size, limited mobility, site fidelity, low fecundity and sparse distribution (Sreepada et al., 2002). High demand in TCM coupled with its vulnerability to intense fishing pressure especially by bottom trawlers and habitat destruction (Salin et al., 2005) could result in drastic reduction in population size. Therefore there is an urgent need to develop and standardise captive breeding and mass-scale rearing of this species for conservation and commercial purposes.

Several attempts to culture seahorses on mass-scale in the recent past have met with limited success as a result of severe problems of mass mortality due to inadequate nutrition and disease (Truong, 1998; Lourie et al., 1999b). Recently, however some of these culture difficulties have been overcome and seahorse aquaculture ventures are currently operating in many countries; Australia (*H. abdominalis*), Sri Lanka (*H. kuda*), Vietnam (*H. kuda*), Indonesia (*H. kuda*), Brazil (*H. reidi*), Mexico (*H. erectus*), Ireland (*H. hippocampus*) and New Zealand (*H. abdominalis*) in order to meet the demand in domestic and international ornamental fish markets (Project Seahorse, 2006).

Compared to the proposition of mass culture of other seahorse species, *H. trimaculatus*, inhabiting different geographical regions, has not been thought of seriously from a commercial standpoint, probably because of it is not favoured by the aquarium trade due to its black to brown colouration.

Except for a few studies, not much information is available on the performance of brooders and optimum conditions for mass-scale rearing of *H. trimaculatus*. The effects of food, temperature and light intensity on feeding behaviour have been documented by Sheng et al. (2006). Furthermore, the effect of starvation on the initiation of feeding, growth and survival rate up to settlement stage has been studied by Sheng et al. (2007). This paper, on the other hand provides basic information about brooder diet, effect of exogenous factors on growth and survival rate in juvenile rearing and mass-scale culture over a period of 26 weeks from parturition. Further, other vital parameters such as duration taken for settlement, maturation and egg clutch releases by F2 generation were also studied. It is expected that the culture methods outlined here will help in developing a sound culture technology for this commercially important and threatened seahorse species.

2. Materials and Methods

2.1. Broodstock maintenance

Juveniles released from captive bred *H. trimaculatus* Leach 1814 (size range, Ht 115-127 mm; n=14 pairs) maintained at Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu (India) served as the material for different experiments (F1 generation). Brooders were maintained in a fluorescent white circular FRP tank (capacity, 2000 l) and bleached fragments of branching coral (*Acropora* spp.) were provided as holdfasts (thickness, 7-11 mm) following the modified protocols suggested by Lockyear et al. (1997). The seawater used in the brooder tank was treated by rapid sand filtration, bio-filtration and passed through ultraviolet radiation. Physico-chemical parameters of seawater in the brooder tanks fell within the recommended optimum levels for culture of seahorses throughout the culture period: temperature, (30.2±0.5 °C), salinity (32±1.5 ppt), dissolved oxygen (6.1±0.6 mg l⁻¹), pH (7.6±0.3), NO₂-N (<0.02 mg l⁻¹) and NH₃/NH₄ (0 mg l⁻¹). The photoperiod of 16L (0700h-2300h):8D (2300-0700h) was maintained using a fluorescent bulb of light intensity ~1500 lx. Different prey organisms (amphipods, *Acetes* spp., larvae of *Chironomous* spp., mosquito and fish) caught from the wild and fed to seahorses.

2.2. Evaluation of brooder diet

Two experimental treatments with live amphipods (*Eriopisa* spp., 8-15 mm) and live sergestid shrimp (*Acetes* spp., 10-20 mm) were selected for evaluating the reproductive efficiency of parent seahorses. These prey organisms were selected as previous studies have indicated their dominance in the gut of adult seahorses (Tipton and Bell, 1988; Gokulakannan, 2002, Murugan, 2004). Each treatment had seven pairs of parent seahorses selected on the basis of courtship behaviour from a group of 70 animals. There were no significant differences between the two treatments in terms of height (Ht), wet weight and condition factors of parent seahorses as measured by Lourie et al. (1999a) and Job et al. (2002) at the beginning of the experiment ($P>0.05$). The size of the brooder tank and the environmental conditions (temperature, salinity, dissolved oxygen and light intensity) were the same between the two treatment tanks.

During the brooder diet evaluation experiment, parent seahorses were fed *ad libitum* twice a day (0800h and 1600h). Aeration was temporarily suspended during feeding to facilitate prey capture by the brooders. Faeces and uneaten prey organisms (prey mortality due to unsuccessful prey attacks) were siphoned out twice a day (1000h and 1730h) and 50% of water exchanged daily (0730h). After completion of copulation, the male seahorses were transferred to the spawning tank (50x30x30 cm). Water quality maintained in the spawning tanks was similar to that of brooder tanks. For comparative assessment of reproductive efficiency of parent seahorses reared with two different feeds, the numbers of new born juveniles under each treatment were counted carefully.

The proximate composition (protein, lipid and carbohydrate) of two prey organisms, amphipod (*Eriopisa* spp.) and sergestid shrimp (*Acetes* spp.) used for evaluating the reproductive efficiency was estimated (dry weight basis) before feeding experiments. Protein, carbohydrate and lipids were determined according to the methods of Lowry et al. (1951), Dubois et al. (1956) and Bligh and Dyer (1959), respectively.

2.3. Rearing of juveniles under different light intensities

Juvenile seahorses initially exhibit pelagic behaviour and hence require 24 h illumination as they are visual feeders. Lack of adequate light could result in wall tapering behaviour around the corners of the experimental tanks and may ultimately lead to starvation and death. Hence, the effect of different light intensities on the survival of new born juveniles (6-7 mm, Ht; n=50) was studied in replicate FRP experimental tanks (50x40x40 cm) having a juvenile density of 1 fish l⁻¹. The experimental animals were fed with copepod nauplii (150-230 µm) at a density of 7 m⁻³. Three

different light intensities of 1000, 1500 and 2000 lx were maintained in each of the triplicate tanks. Survival rate (%) and growth increment (Ht, mm) of juveniles in each set of experimental tanks was recorded daily over an experimental period of 7 days. Further, the cause of mortality (either due to starvation and/or ingestion of an air bubble in juveniles under different light intensities was recorded visually.

2.4. Feed preference of pelagic phase juveniles reared with single food source

The initial period of 0-15 DAB (days after birth) when juveniles switch from the pelagic to the settlement phase is considered very critical in seahorse husbandry. Recognising the importance of optimum size and type of prey organisms in juvenile rearing, an evaluation of feed preferences was therefore made following the method suggested by Payne and Rippingale (2000) with modifications. Two sets of experiments, one with juveniles of 0 DAB and another with 7 DAB juveniles to assess the survival (%) and ingestion rate ($\text{juvenile}^{-1} \text{h}^{-1}$) using a single food source were conducted.

2.4.1. Juveniles of 0-6 DAB

Newborn juveniles (0-6 DAB) were fed with rotifers (*Brachionus* spp., 80-110 μm) and mixed copepod nauplii in the size range of 150-230 μm (*Oithona* spp., *Acartia* spp., *Eucalanus* spp., *Rhincalanus* spp. *Microsetella* spp. and *Euterpina* spp.) at densities of 9 and 7.5 cells m^{-3} , respectively. Experiments were conducted in three replicate tanks (50x30x30 cm) having a juvenile density of 2 fish l^{-1} . A 24 h light intensity of 2000 lx was provided to the experimental tanks as the maximum survival was recorded at this light intensity through a previous experiment (section, 2.3). Prey densities in the experimental tanks were estimated one hour prior to feeding. Adjustments in prey densities were made to maintain the initial densities in the rearing tanks. Survival percentage (%) and ingestion rate (No. preys juvenile^{-1}) were calculated for each feed type on a daily basis.

2.4.2. Juveniles of 7-13 DAB

Prey organisms, copepodites (400-620 μm) and *Artemia* nauplii (Instar-1; 440-480 μm) having densities of 6.7 and 7.5 cells m^{-3} , respectively were supplied to the experimental tanks (50x30x30 cm) having a juvenile density of 2 fish l^{-1} with 3 replicates for each feed type. Survival and ingestion rates for each feed treatment were estimated on a daily basis. The prey density in the experimental tanks was counted at hourly intervals from initial morning feeding (0730h). The prey densities were adjusted to the original densities in the rearing tanks.

2.5. Feed preference of juveniles reared with mixed food source

Seahorse aquaculturists are often challenged by the feed preferences of new born juveniles. For assessing their feed preference, new born juveniles were offered a mixture of rotifers, copepod nauplii, *Artemia* nauplii, copepodites and copepod adults in equal proportion and feed selectivity index (I) and survival (%) was estimated during 0-13 DAB. Enumeration of different prey organisms in the gut and in the experimental tank was done under a zoom stereo dissection microscope (Olympus). ' I ' was calculated using the feed preference formula of Ivlev (1961) $I=(r_i-p_i)/(r_i+p_i)$, where, r_i is the percentage of number food item (i) found in the gut content, P_i is the percentage of food items in the experimental tank. Values for ' I ' thus range from -1 to 1 with '+ve' index values indicating positive food selectivity, '-ve' index values refer to avoidance or inaccessibility of offered food items and '0' index values implies random feeding. Comparative data on survival of juveniles after 13 DAB with single and mixed food sources was recorded.

2.6. Digestion period in juveniles and adults reared with different prey organisms

It is important to standardize the feeding regimes in seahorse aquaculture because they lack masticatory structures and a true stomach and hence only partial digestion takes place in the intestine (Bergert and Wainright, 1997). Digestion period (DP) refers to the time required for a juvenile with full gut to exude the food from the gut (Zhang et al., 2004). The DPs for juvenile and adult seahorses were estimated separately. DPs in 6 DAB and 13 DAB old juveniles were estimated by conducting experiments in two separate FRP tanks (50x30x30 cm) each having a juvenile density of 1 fish l^{-1} ($n=20$). Juveniles were reared with adult copepods ($6\ m^{-3}$) in one tank and with *Artemia* nauplii ($8\ m^{-3}$). Feeding was done in the morning (0730h) for a period of 45 min. The fullness of the gut was determined based on the colour of the trunk region. Those juveniles with full gut were transferred to another tank devoid of prey items for observing the release of faecal matter. For estimating the DP in mature seahorses (~125 DAB), the experimental animals were fed with adult brine shrimp, polychaetes, amphipods (*Eriopisa* spp.), shrimp (*Acetes* spp.) and fish fry.

2.7. Salinity tolerance in settled juveniles and matured seahorses

Juvenile seahorses (Ht, 40-53 mm) that settled on holdfasts and mature seahorses (Ht, 87-102 mm) were used for salinity tolerance studies. Experiments were carried out in 3 replicate rectangular glass aquaria (500 l) filled with seawater having salinity similar to the culture tanks (35 ppt). Each experimental tank was stocked with juvenile and mature seahorses ($n=15$) and plastic plants were provided as holdfasts. During the experiments, fishes were offered wild caught amphipods (*Eriopisa*

spp.) and sergestid shrimps (*Acetes* spp.) *ad libitum* twice daily (0800h and 1600h). Water quality was maintained by daily replacement of approximately two-thirds of the rearing water; siphoning out faecal matter, uneaten food and dead prey items 2 h after feeding; optimal aeration was provided. Dead seahorses, if any, were removed immediately and mortality was recorded. Salinity in the experimental tanks was gradually reduced @ 1ppt for every 48h by adding distilled water (0700h) from their rearing salinity of 35 ppt until 100% mortality occurred. Salinity measurements were made with a calibrated hand-held refractometer (± 1 ppt).

2.8. Mass-scale rearing

For mass-scale rearing experiments, new born juvenile seahorses (n=540) released from a single spawner (Ht, 139 mm) from the F2 generation were transferred to 9 rearing tanks (50x30x30 cm) having a light blue background. The volume of seawater in each rearing tank was 30 l and a juvenile density of 2 fish l⁻¹ was maintained in each rearing tank. Due care was taken not to expose the juveniles to air during their transfer into the rearing tanks as exposure to air could affect their survival. Light-dark cycle of 24h L: 0h D was maintained and a light intensity of 2000 lx was provided initially during the pelagic phase of juvenile rearing (~15 DAB). No aeration was provided during the pelagic phase as the aerator induced water current may impede the predation by juveniles. DO levels were periodically checked at hourly intervals using a portable DO meter (YSI, 85). DO levels in the rearing tanks were always maintained at 5 ± 0.5 mg l⁻¹ by replenishing with oxygenated seawater (average 80% saturation) at 3h intervals. The protocols were standardised by performing repeated trails to yield consistent results. Pelagic phase juveniles were reared on a single diet immediately after birth (0 DAB) as the results in the previous feeding experiments (sections, 2.4 and 2.5) indicated no significant difference in the survival rates between single and mixed diets ($P>0.05$). Rotifers (*Brachionus* spp.) and *Artemia* spp. (nauplii and instar stages) were cultured in the laboratory using microalgae (*Nanochloropsis* sp.). Different prey organisms (copepod nauplii, copepodite and copepod adult) caught from the wild using appropriate plankton nets were fed to juveniles.

Those juveniles which displayed settlement behaviour (holding another juvenile with the prehensile tail) were transferred to 3 settlement tanks (75x50x50 cm) having a juvenile density of 1 fish l⁻¹. Holdfasts of different sizes: nylon mesh, 2 mm; nylon rope, 3 mm; soft plastic plant, 5 mm and coral fragments, 7 mm for juvenile sizes (Ht) of 17 ± 1.8 , 37 ± 3.4 , 70 ± 4.7 , and 85 ± 5.5 mm, respectively were provided. During the settlement phase, the light-dark cycle was reduced to 16L (0700-2300h):8D (2300-0700h) from an initial photoperiod of 24h L. In the settlement tank,

adequate aeration was provided using air blowers and optimal water quality parameters were maintained (DO, 5.4 ± 0.5 mg l⁻¹; salinity, 32 ± 1.5 ppt; temperature, 30 ± 0.2 °C; pH, 7.4 ± 0.8 ; NO₂-N, < 0.02 mg l⁻¹ and NH₃/NH₄ (0 mg l⁻¹). Juveniles were fed with *Artemia* nauplii and copepodites and feeding was done thrice a day (0800, 1200 and 1600h). Siphoning was done 3h after each feeding and 30% of water in the settlement tank was exchanged daily (0700 h).

Data on growth (Ht and wet weight) and survival rate (%) at different stages of mass-scale rearing (from stocking of juveniles to adult stage) were recorded at fortnightly intervals over a period of 26 weeks. Time taken to attain sexual maturity was noted. Signs of maturity in females could easily be identified by the elevated trunk depth, ovary colour (visualised through the abdominal walls), protruded genital papilla and dropping of hydrated eggs (unfertilized eggs) in the water column; while in males, the appearance of the brood pouch was considered a sign of maturity. Further, the data on sex ratio, mating, number of juveniles released from pregnant males (F2 generation) and clutch dropping from matured females (F2) were also recorded.

2.9. Data analyses

All statistical analyses were performed with PC based Minitab Version 8.3 (Minitab Inc., 1991). Measured variables were tested to normality and homogeneity by Cochran's test (Winer et al., 1991). Appropriate data transformations were applied prior to statistical analysis. One-way analysis of variance (ANOVA, Underwood, 1997) was used to assess the differences in survivorship of the juvenile and feed among different treatments. If ANOVA found significant effects, multiple comparisons between different means amongst treatments were then made using the Tukey-Kramer test for highly significant differences (HSD) (Zar, 1996). The Chi-square test was used to analyze the variation in sex-ratio of adult seahorses from a normal sex ratio of 1:1 (Sokal and Rohlf, 1981). Regression analyses were used to assess the growth (height and weight) under different treatments over the entire culture period of 26 weeks. Regression lines were compared by one-way ANOVA. Significance in all statistical tests was judged at a ' P '=0.05 level.

3. Results

3.1. Reproductive efficiency of brooders

Reproductive efficiency (RE) measured in terms of the number of juveniles released by parent *H. trimaculatus* when reared with amphipods and sergestid shrimps was 533 ± 67.1 and 457 ± 52.9 juveniles seahorse⁻¹, respectively. The RE was significantly higher in seahorses fed with amphipods

when compared to that with shrimps ($P<0.05$). Coefficients of regression equations of RE for seahorses reared with two different prey organisms were found to be significantly higher in the treatment with amphipods (ANOVA $P<0.05$). Furthermore, the number of deformed juveniles was found to be lower in brooders fed with amphipods compared to shrimps. However, the relationship between RE and size (Ht) was insignificant ($P>0.05$).

3.2. Effect of different light intensities on survival and growth in juveniles

Survival rate (%) of pelagic-phase juveniles reared under different light intensities of 1000, 1500 and 2000 lx during the 0-6 DAB is depicted in Fig. 1. Mean growth (Ht) and survival rates of juveniles at the end of 6 DAB were 12.4 and 70 ± 2 , 12.7 and 76 ± 2 , 13.1mm and $77\pm 3\%$, respectively at 1000, 1500 and 2000 lx. Survival rate (%) declined progressively ($P<0.05$) over DAB in all the tested light intensities (Fig. 1). Significant differences in the survival rates of juveniles under three tested light intensities was discernible ($P<0.05$). Highest survival and growth rates were achieved in treatment tanks exposed to light intensities of 2000 lx followed by 1500 lx and 1000 lx ($P<0.05$; Tukey-Kramer HSD multiple comparison test).

The cause of mortality of juveniles either due to starvation and/or ingestion of an air bubble in each light treatment was ascertained. Ingestion of an air bubble was found to be the major cause of mortality in all the tested light intensities throughout the 0-6 DAB, while starvation was the primary cause of juvenile mortality during the 0-2 DAB.

3.3. Survival and prey ingestion in juveniles fed with a single diet

Survival rate of newborn juveniles fed with different prey organisms during the 0-6 DAB is depicted in Fig. 2(a). Significantly higher survival ($P<0.05$) was observed in juveniles fed with copepod nauplii ($73\pm 3.1\%$) when compared to rotifers ($29\pm 5\%$). Ingestion of rotifers was highest for 2 DAB old juvenile (186 ± 31 juvenile⁻¹ h⁻¹), whereas lowest was recorded in 6 DAB old juveniles (84 ± 11.5 juvenile⁻¹ h⁻¹). In the case of copepod nauplii, the highest and lowest ingestion rates observed were 223 ± 19 juvenile⁻¹ h⁻¹ in 11 DAB old juveniles and 123 ± 4.5 juvenile⁻¹ h⁻¹ in 7 DAB old juveniles, respectively (Fig. 2b). The survival rate and ingestion rates in 7-13 DAB old juveniles are shown in Fig. 3 (a) and Fig. 3(b), respectively. Higher survival rates were obtained when juveniles were reared with copepodites ($87\pm 3.1\%$) compared to those reared with *Artemia* nauplii ($80\pm 2\%$). Highest rates of ingestion of copepodites (157 ± 3.5 juvenile⁻¹ h⁻¹) and *Artemia* nauplii (159 ± 6 juvenile⁻¹ h⁻¹) was observed, respectively in 9 DAB and 12 DAB old juveniles.

3.4. Feed preference and survival of juveniles fed with mixed diet

Feed preference of juveniles when reared with a mixed diet containing rotifers, copepod nauplii, copepodites, *Artemia* nauplii and adult copepods in equal proportion was evaluated through gut content analysis. Newborn juveniles preferred to feed on rotifers and a shift in feed preference to copepod nauplii was observed in >2 DAB old juveniles (Fig. 4). 'I' for rotifers varied from 0.42 to 0.55 indicating a preference for this prey type among 0-2 DAB old juveniles, whereas for copepod nauplii, the 'I' ranged from 0.33 to 0.53 with a peak in preference occurring in 2-6 DAB old juveniles. *Artemia* nauplii were not preferred by juvenile seahorse, with a peak 'I' of 0.2 by 9-10 DAB old juveniles. However, copepodites were the most preferred by 6-11 DAB old juveniles with corresponding 'I' values ranging from 0.33 to 0.5. Copepod adults were preferred by 10-13 DAB old juveniles with 'I' values ranging between 0.27 and 0.55. A reduction in the ingestion of copepod nauplii and rotifers observed in >6 DAB old juveniles may indicate the onset of an ontogenetic shift in prey preference. The survival of juveniles when fed with a mixed diet during the rearing period is shown in Fig. 5. Survival rate at the end of rearing period of 13 DAB was $77\pm 3\%$.

3.5. Digestion period (DP)

The estimated DP in 6 DAB old juveniles was 3h 20m, while it was 2h 50m in the case of 13 DAB old juveniles. In the case of juvenile seahorses (>75 DAB) reared with polychaetes, sergestid shrimp (*Acetes* spp.) and amphipod (*Eriopisa* spp.), the DP ranged between 1h 45m and 2h 0m. The DP in mature seahorses (>125 DAB) remained within a narrow range of 1 h 20m-1h 30 m.

3.6. Salinity tolerance in settlement-stage juveniles and matured seahorses

Survival rates of settlement-stage juvenile (30 DAB) and adult seahorses (120 DAB) reared under different salinities are depicted in Fig. 6. No juvenile mortality was observed down to a salinity of 26 ppt when the salinity was gradually reduced from a rearing salinity of 35 ppt. The first sign of mortality was recorded in tanks having a salinity of 25 ppt and 100% mortality occurred when the salinity was brought down to 13 ppt. Hence, it appears that juveniles of *H. trimaculatus* can tolerate salinities not extending below 26 ppt. In the case of adult seahorses, the first sign of mortality was observed when the salinity was reduced to 16 ppt and 100% mortality occurred when the salinity was further reduced to 9 ppt. Based on 100% mortality as an index for salinity tolerance, it appeared that adults of *H. trimaculatus* can tolerate salinities not extending below 17 ppt. However, no gender difference with respect to salinity tolerance was observed ($P>0.05$).

3.7. Mass scale culture from pelagic-stage juvenile to adult

3.7.1. Growth, survival, sexual maturity and sex ratio

The average growth rate (Ht) recorded during the mass-scale rearing is shown in Fig.7. Relatively faster growth rates persisted in juveniles up to 60 DAB, with the highest growth rate occurring in the 15-30 DAB (1.64 mm day^{-1}). Mean height (Ht) and weight (wet) attained during the mass-scale rearing of *H. trimaculatus* (from pelagic-stage juveniles to adult stage) were $119.9 \pm 15.3 \text{ mm}$ ($n=230$) and $6.1 \pm 1.1 \text{ g}$ ($n=230$), respectively. Growth rate was higher up to the settlement stage and decreased as the juveniles attained sexual maturity, whereas body biomass increased proportionally to growth (Fig. 8). No variation, however, in growth rate between sexes was discernible ($P > 0.05$).

Survival of *H. trimaculatus* during the mass-scale rearing is depicted in Fig.9. Variations in survival rate were noticeable up to 49 DAB and thereafter no mortality occurred. Higher mortality (24.7%) was observed up to 17 DAB and the overall survival rate obtained at the end of 26 weeks of mass-scale rearing was 65%. Different sexes attained maturity at different times. Females attained maturity within 115-142 DAB with a corresponding size range of 85-104 mm (Ht), whereas males attained maturity earlier than females (95-129 DAB) with corresponding size range of 73-94 mm (Ht). The sex ratio was found to be female biased (1:1.2) and significantly differed from a normal sex ratio of 1:1 (Chi-square test, 2.67; $P < 0.05$).

3.7.2. Breeding of F2 generation

Mating of F2 generation seahorses was observed from 153 DAB onwards. Seahorses exhibited monogamous pair-bonding behaviour although opportunities for polygamous encounters were available for both genders. The egg clutch droppings ($45 \pm 17 \text{ eggs seahorse}^{-1}$; $n=21$) by newly matured females varied between 15 and 75. Mean number of newborn juveniles released from first mating males was $65 \pm 20 \text{ juveniles seahorse}^{-1}$ ($n=9 \text{ pairs}$).

4. Discussion

4.1. Brooder Performance

Recently breeding and culture of seahorses has received considerable attention worldwide for conservation of threatened wild stocks and to sustain the ever increasing trade in medicine and ornamentals (Woods, 2000a; Job et al., 2002; Wong and Benzie, 2003). Brood stock nutrition is one of the most poorly understood and researched areas of finfish nutrition and unpredictable brooder

performance is often considered a bottleneck for successful mass production of seahorse juveniles (Izquierdo et al., 2001). Seahorse aquaculture however, is a relatively new industry with many challenges, particularly low RE and juvenile survival. It has been documented that time to maturity and subsequent RE and survival can be greatly influenced by environmental conditions and food supply (Mai, 1979; Wong and Benzie, 2003; Woods, 2003; Lin et al., 2007).

The nutritional requirements (protein, lipids, carbohydrates, profiles of fatty acids and amino acids), particularly for parent seahorses are largely unknown. Payne and Rippingale (2000) and Payne et al. (1998) suggested that seahorse needs are similar to those of other marine fish. At the beginning of the feeding experiment, we estimated the proximate composition of amphipods and sergestid shrimps. The amounts of crude protein, crude lipid and carbohydrate were 52% and 45%, 11 and 13%, 6 and 8%, respectively in amphipods and sergestid shrimps. The fat content was quite similar in the two tested diets which made any further conclusions about its importance on *H. trimaculatus* metabolism impossible. Similarly, the ~2% difference in the carbohydrate content is unlikely to have had any significant effect upon the results obtained with two tested diets. Unlike fat or carbohydrates, the ~7% difference in protein content may have been an important contributor to the difference in the reproductive efficiency (RE). Faster maturation and higher spawning rates in *H. kuda* have been documented when parent seahorses were fed with live *Acetes* spp. compared to live and frozen mysids (Lin et al., 2007). The differences in crude protein content have been ascribed to the variation in reproductive performance. Recently, Palma et al. (2008) studied the growth parameters of adult *H. guttulatus*, fed with three frozen diets (shrimps, mysids and adult *Artemia*) and concluded that the differences in protein content may be responsible for differential growth rates. Furthermore, it has been documented that the natural food of seahorses contains high crude protein (>75%) and low lipid (3%) contents (unpublished data cited in Lin et al., 2008). Our observation also supports the hypothesis that the protein content of the diet plays an important role in influencing reproductive efficiency. However, these assumptions are based on the proximate composition of diets. The differences between the diets could be fully understood through a detailed analysis of profiles of other nutritional factors such as amino acids, fatty acids, vitamins etc present in diets. The observation of a significantly lower number of deformed juveniles released by parent seahorses fed with amphipods when compared to that with sergestid shrimps may reflect on the nutrient deficiency of the latter which needs further validation. Further, the stress factors induced by nutrient deficient live feed on deformity cannot be ruled out here.

The provision of correctly sized holdfast material is critical in mass culture of seahorses in order to reduce their energy expenditure and maximize growth benefits. During the mass culture of *H. trimaculatus*, seahorse juveniles settled on holdfasts such as nylon mesh (>15 DAB, nylon rope (>35 DAB), plastic plants (>80 DAB) and broken bleached corals (>105 DAB). Different types and sizes of holdfasts have been used during the juvenile rearing of many seahorse species: Clumps of plastic eelgrass in *H. erectus* (Scarratt, 1996), twisted plastic aquarium plants in *H. kuda*, *H. fuscus* and *H. barbouri* (Wilson and Vincent, 1998), polyethylene mesh (16 mm) in *H. abdominalis* (Woods, 2000a) and rope substratum in *H. barbouri* (Payne, 2003). Comparative data on the effectiveness and preference of various holdfasts for seahorse species is lacking.

4.2. Effect of different light intensities

Photoperiod and light intensity are important variables that influence the survival rate in larval rearing of marine fish as they are visual feeders (Blaxter, 1980) and the same holds good with seahorses (James and Heck, 1994). Several fish species have been reared under natural photoperiods (Job et al., 1997; Holt, 2003), while some argue that better growth is achievable if a constant light regime is applied (Barlow et al., 1995; Olivotto et al., 2008) under the assumption that the larvae are able to feed for longer periods. For successful juvenile rearing, it is necessary to optimise the light-dark cycles and the light intensity for each species as it has been reported that seahorses reared with inadequate light will become blind after few days of rearing (Jiaxin, 1990).

Typically, seahorses inhabit shallow coastal areas (Foster and Vincent, 2004) where light intensities may be relatively high, but are capable of feeding effectively at a variety of light intensities. Sheng et al. (2006) examined feeding incidences by *H. trimaculatus* juveniles at different light intensities and found the highest feeding incidence occurred at light intensities of 1834 lx, 1014 lx and 510 lx in 1 DAB, 5 DAB and 10 DAB old juveniles, respectively. In contrast, Wong and Benzie (2003) found no differences in growth or survival in White's seahorse (*H. whitei*), reared at light intensities of 1774 or 10050 lx. In the present study, highest survival rate ($77\pm 3\%$) and growth increment (Ht) of 0.87 mm day^{-1} was obtained in continuous lighting conditions at an intensity of 2000 lx compared to 1500 lx (0.81 mm day^{-1}) and 1000 lx (0.77 mm day^{-1}) (one-way ANOVA, Tukey-Kramer HSD multiple comparison test at $P < 0.05$; $2000 \text{ lx} > 1500 \text{ lx} > 1000 \text{ lx}$). Higher mortalities in 0-6 DAB old juveniles at lower light intensities may be due to engulfing of atmospheric air as a result of unsuccessful prey attacks in the surface (Woods, 2000a, b; Sheng et al., 2007). Starvation related mortality could perhaps be due to the lack of juveniles learning behaviour on how to capture the prey.

Based on our results and that of Sheng et al. (2006), we may suggest that the optimal light intensity for *H. trimaculatus* lies in the range between 510 and 2000 lx under continuous light from the view point of feeding incidence, food intake, survival and growth. Studies concerning light intensities of < 510 lx and >2000 lx would be necessary to further optimise the rearing conditions for juvenile three-spotted seahorses. Our study suggests that survival and average growth rate (0.87 mm day⁻¹) were higher in 2000 lx for up to 6 DAB. On the basis of feeding incidence, Sheng et al. (2006) suggested different light intensities for different period of juvenile rearing (1834 lx for 0-4 DAB, 1054 lx for 5-9 DAB, 510 lx for >10 DAB). However, they did not estimate the survival rate with regard to optimal light intensities recommended by them. Our study differed from Sheng et al. (2006) as we focussed on the survival at different light intensities rather than predation rates as low juvenile survival is often considered a bottleneck for commercialisation of seahorse aquaculture (Woods, 2000a; Job et al., 2002). Research concentrating on both predation and survival rates at different light intensities is therefore necessary.

Illumination is an important factor affecting the distribution of pelagic fish larvae and their prey (Huse, 1994). The positive effect of longer photoperiod on survival or growth of marine fish larvae has also been reported by many researchers (Riley and Thacker, 1963; Houde and Palko, 1970; Yoseda et al., 2008). Seahorse juveniles are no exception to this behaviour. Troung (1998) observed positive phototaxis behaviour in *H. kuda* juveniles and recommended that juvenile rearing tanks should be arranged in such a way that light is distributed evenly in order to prevent juveniles from gathering in one area. A similar behaviour was also observed in the present study and juveniles were seen crowding at the corners of the rearing tank when the illumination was reduced or withdrawn. We may therefore conclude that high light intensities result in high survival rates in pelagic phase juveniles of *H. trimaculatus*. It has been documented that many fish larvae become active with the increase in light intensity as higher light intensity increases prey capture, duration of feeding and rate of prey-searching (Batty, 1987). Studies estimating the light penetration in the culture tanks during different stages of juvenile rearing of seahorse species are however required to confirm this.

4.3. Feed preference of juveniles

The initial period of 0-15 DAB (days after birth) when the juvenile switches from pelagic to settlement phase is considered very critical in seahorse husbandry (Woods, 2000a,b). Determining the most desirable or optimal type of exogenous feed for rearing of fish larvae/juveniles is one of the critical aspects for achieving better growth and survival. Most marine fish larvae are planktonic feeders and appear to predominantly feed on planktonic zooplankton (Werner and Blaxter, 1980). In

the present study, juveniles preferred to feed on copepod nauplii (194 ± 43.2 seahorse⁻¹ h⁻¹) rather than rotifers (84 ± 32.1 seahorse⁻¹ h⁻¹) during the pelagic phase (0-6 DAB). However, rotifers were preferred by up to 2 DAB juveniles (186 ± 31 seahorse⁻¹ h⁻¹). This is in agreement with the studies by Sheng et al. (2006) in *H. trimaculatus* and Job et al. (2002) in *H. kuda*.

A dual phase feeding pattern (24h photoperiod) by fish juveniles has been observed under captive rearing conditions (Sanchez-Vazquez et al., 1996; Sheng et al., 2006). Occurrence of such a feeding pattern in seahorse juveniles is not surprising due to their pelagic behaviour and visual mode of predation. A significant variation in predation rates between day and night was noticed even when 24 h light was supplied ($P < 0.05$). The predation rates of copepod nauplii and copepodites, by 0-6 DAB and 7-13 DAB old juveniles respectively, during the day were significantly higher when compared to night time ($P < 0.05$). Significant variability in predation rates between high and low light intensities of fish juveniles has been reported by James and Heck (1994) in *H. erectus*, Sheng et al. (2006) in *H. trimaculatus*, Jun et al. (2006) in sole, *Cynoglossus semilaevis* and Yoseda et al. (2008) in coral grouper, *Plectropomus leopardus*. Sheng et al. (2006) ascribed this variation to both exogenous and endogenous feeding rhythms in seahorses. However, further research in this direction is required.

4.4. Feed selectivity

Feeding selectivity by fish juveniles is influenced by factors such as encounter rates, prey size, prey availability and ease of capture (Jenkins, 1987; Li, 1999) and the importance of smaller size prey in the diet declines as juveniles grow older. The estimated values of the feeding selectivity index suggested that the juveniles preferred larger size prey as they grew in size. New born juveniles having a mouth aperture of 0.39-0.45 mm preferred rotifers and copepod nauplii. The feed preference shifted to copepodites and *Artemia* nauplii when the mouth size of the juveniles increased to 0.63-0.73 mm. This type of ontogenetic shift in feeding has been documented in syngnathids both in the wild (Teixeira and Musick, 1995; Murugan, 2004) and under laboratory conditions (Job et al., 2002; Sheng et al., 2006).

4.5. Digestion period

Feeding and digestion are two interconnected stages of a single process that determines an animal's net energy and nutrient gain (Penry and Jumars, 1987). Digestive enzymatic activities are notably lower in larvae/juveniles than in adult fishes (Faulk et al., 2007). Feed intake by new born juveniles was quite low when compared to later feeding days and this might be due to poor intestinal development as well as few successful feeding strikes. Digestion period reduced from 3h 20m (5

DAB) to 1h 20m (>125 DAB) with the corresponding increase in feeding intensity as the juveniles grow older. A reduction in digestion time of 30 m has been observed in 10 DAB old juveniles of *H. trimaculatus* compared to 5 DAB old juveniles (Sheng et al., 2006). The gradual reduction in digestion period corresponding to larval growth in finfish has been attributed to the development of the intestine with regard to hormonal secretion (Faulk et al., 2007). Furthermore, quantitative and qualitative changes in gut microflora during different developmental stages of marine fish larvae have been documented (Hansen and Olafsen, 1999). Presence of such gut microflora has not been reported in seahorses as they lack a true stomach and digestion and absorption take place in the intestine.

4.6. Salinity tolerance

Salinity adaptation studies on seahorses are scarce as only few species have been found to inhabit bays and estuaries (Teske et al., 2005). Tolerance of *H. kuda* to lower salinities in seasonally flooded mangroves with fluctuating salinities has been documented by Lourie et al. (1999a). An estuarine seahorse species, *H. capensis* tolerated salinities ranging from 1 to 59 ppt for 7 days (unpublished data cited in Whitfield, 1995). In the present study, juveniles and adults of *H. trimaculatus* tolerated salinities not extending below 26 and 17 ppt, respectively based on the 100% survival. Laboratory reared *H. kuda* juveniles tolerated salinities as low as 15 ppt (Hilomen-Garcia et al., 2003). Reduced adaptability of juveniles of *H. trimaculatus* compared to *H. kuda* might be due to the different ecological niches they occupy. *H. kuda* can be considered as quasi-resident, while *H. trimaculatus* is a resident coastal marine species. Future experiments on salinity tolerance of *H. trimaculatus* in combination with feed intake, growth and reproduction would be helpful in optimising the salinity for aquaculture purposes.

4.7. Mass-scale rearing

Appropriate diet type and quantity of feed are the critical variables for achieving optimal growth and survival in mass culture of seahorses. No consensus exists amongst seahorse researchers on the optimal feed for juveniles rearing due to interspecific and ontogenetic differences (Lunn and Hall, 1998). Seahorse juveniles in the present investigation were reared with a single diet immediately after their birth (0 DAB) as there was no significant difference in survival or growth rate between single and mixed diets ($P>0.05$). A survival rate of 75% from 0 DAB to settlement period (15 DAB) was achieved under mass-rearing conditions. Further, the survival rate did not vary significantly throughout the culture period ($P>0.05$). Muthuwan et al. (2001) while working on the juvenile

rearing of *H. kuda* hypothesised that feeding of juveniles should be started immediately after birth and delaying feeding by 24, 48 and 72 h could result in lowered survival rates of 43.5%, 6% and 0%, respectively from initial survival of 88.1%. Relatively lower survival rates (65%) obtained during the 26 weeks of mass-scale rearing of *H. trimaculatus* compared to *H. kuda* (73%) by Job et al. (2002) might be due to the differences in the standard length (SL) and mouth size of new born juveniles of these two species of seahorses. Length (7-8 mm) and mouth size (0.34 to 0.41 mm) in the former species were comparatively smaller than in the latter species (9-11 mm; mouth size 0.5-0.70 mm) (Gokulakannan, 2002).

As the juveniles grew (0-25 DAB), the shift in feed preference was in this order: rotifer>copepod nauplii>copepodite>*Artemia* nauplii>adult copepod. Juveniles older than 35 DAB are capable of feeding on any prey organisms having size >15 mm in width and 20 mm in length and their only requirement is mobile prey organisms such as amphipods, polychaetes, *Acetes* spp. and fish larvae. In absence of these prey organisms, seahorses were seen preying on mosquito larvae and *Chironomous* larvae. While preying on large organisms, the juveniles made multiple attacks to capture the prey, due to the injury created by those attacks the prey were immobilised (eg. mosquito larvae and sergestid shrimp, visual observation). Perhaps, this behaviour could be exploited when hatchery managers want to wean the seahorses to frozen diets (*Mysis* spp. and *Acetes* spp.). After the settlement period, feeding occurred only during the day time. Night feeding occurred only when seahorses were forced to starve. This type of ontogenetic shift in diet preference has been well documented in seahorse culture (Payne and Rippingale, 2000; Job et al., 2002; Woods, 2005), whereas the feeding behaviour related to this type of ontogenetic shift in controlled conditions requires further validation.

The mean growth (Ht) obtained during the mass rearing of *H. trimaculatus* was found to be 44.5±6, 77.3±10, 94.7±12.8, 106±11.7, 114.2±10.9 and 119.9±15.3 mm at the end of 30-, 60-, 90-, 120-, 150- and 180-DAB, respectively. In contrast, *H. zosterae*, a relatively smaller species grew from 8 to 18 mm in 17 days. The slower growth rate has been attributed to colder water temperature (Strawn, 1958). At the end of 30 days of culture, *H. reidi* attained an average growth of 18 mm (Silveira, 2000) and *H. kuda* reached 31-48 mm in 4-8 weeks of rearing (Troung, 1998). At the end of 4 weeks of culture, the temperate seahorse *H. abdominalis* reached a size of 30 mm (Foreath, 1996). In a comparative growth assessment study on 3 seahorse species, Wilson and Vincent (1998) recorded higher initial growth in *H. fuscus* whereas during the later stages of culture higher growth rates were observed in *H. kuda* and *H. barbouri*. Chang and Southgate (2001) reports that

Hippocampus sp. attained a maximum growth (Ht) of 20.2 ± 0.3 mm in 30 days when juveniles were fed with highly unsaturated fatty acid (HUFA)-enriched live *Artemia* (Selco®; Inve, Belgium). Therefore, it does appear that growth rate of seahorses during initial stages of juvenile rearing is influenced by both exogenous and endogenous factors.

Differential growth rates in a few species under mass culture conditions have been reported. Growth rates of up to 75 mm in 120 days in *H. erectus* (Vari, 1982) and 70 mm in 90 days in *H. kuda* (Truong, 1998) and 110.7 mm in 52 weeks in *H. abdominalis* (Woods, 2000b) have been reported. The relatively higher growth of *H. trimaculatus* (119.9 ± 15.3 mm in 26 weeks) compared with *H. abdominalis* (110.7 mm in 52 weeks) may be due to differences in size, temperature and life-span of the two seahorse species. An average daily growth increment (Ht) of 0.55 mm in *H. erectus* (Scarratt, 1996) and 1.53 mm (Job et al., 2002) and 1.1 mm (Junyi et al., 2002) in *H. kuda* have been recorded. In the present study, the average daily growth increment in one-month-old *H. trimaculatus* was 1.27 mm. No significant effect of gender upon growth rate was found ($P > 0.05$). This is in agreement with study of the potbellied seahorse, *H. abdominalis* by Woods (2000a).

Seahorses are dioecious animals where the age of sexual maturation varies among species and with water temperature, nutrition and other environmental conditions (Jiaxin, 1990). In the present study, *H. trimaculatus* attained sexual maturity at 87-93 mm and the sex ratio was female biased ($P < 0.05$). The sex ratio in wild seahorses has also been found to be female biased (Gokulakannan, 2002; Balasubramanian, 2002; Murugan, 2004). More research aimed at understanding the influence of *in situ* parameters and early juvenile mortality on the sex ratio of cultured seahorses is therefore required.

Rearing of marine ornamental fishes is often considered problematic because of their bipartite life style (pelagic and settlement behaviour) (Victor, 1991). During the juvenile rearing of seahorses, mortality occurs mainly during the pelagic phase and it decreases after settlement (Wilson and Vincent, 1998; Woods 2000a). In the present study, relatively higher mortality was also observed during the pelagic phase (24.7%), the cause of mortality being ingestion of air bubble into the gut and/or starvation. In spite of this mortality, we were able to achieve an average survivorship of 65% from parturition which is comparable to the survival rates of other seahorse species reflecting the good husbandry practices adopted in this study.

The importance of aquaculture in meeting the growing demand for seafood is now well recognised. Captive breeding and mass-scale rearing of marine ornamental fishes as a tool for species

conservation and commercial production also form an important component of the aquaculture industry in several countries (Faleiro et al., 2008; Palma et al., 2008). Non-availability of captive brood stock has been recognised as one of the bottlenecks for large-scale expansion of seahorse aquaculture. Pregnant males are often collected from the wild and such collection could have serious implications on the wild seahorse populations. Therefore, an attempt has been made here to evaluate the reproductive efficiency of captive reared brood stock of an F2 generation. Observations of intermating duration could further optimise the production of juveniles in controlled conditions.

5. Conclusion

As *H. trimaculatus* is vulnerable to high fishing pressure (due to its smaller size) and is a heavily traded seahorse species, there is a great interest in its cultivation. The present study focussed on brooder performance, juvenile rearing and mass-scale culture (26 weeks) in captivity. In spite of the limitations, the husbandry protocol and rearing techniques described here should provide a basis for establishment of sound hatchery and aquaculture technology for this species and help in achieving conservation and commercial goals.

Acknowledgments

The authors are grateful to the Directors of the National Institute of Oceanography (CSIR), Goa (India) and CAS in Marine Biology, Annamalai University, Parangipettai (India) for facilities and encouragement. Funding support came from the Department of Ocean Development (MoES) and the Department of Biotechnology (India). We are also thankful to Dr. Sunil Kadri (UK) for critically going through the manuscript and to three anonymous reviewers for their constructive criticism of the manuscript. Contribution No. 4496 of the National Institute of Oceanography, Goa (India).

References

- Anonymous, 2003. Statistics of Marine Products Exports, MPEDA, Kochi, p. 83.
- Balasubramanian, R., 2002. Studies on seahorses with special references to *Hippocampus kelloggi* (Jordan and Synder, 1902), southeast coast of India. Ph.D Thesis, Annamalai University, India, 124 p.
- Batty, R.S., 1987. Effects of light intensity on activity and food searching of larval herring, *Clupea harengus*: a laboratory study. Mar. Biol. 94, 323-327.
- Barlow, C.G., Pearce, M.G., Rodgers, L.J., Clayton, P., 1995. Effects of photoperiod on growth, survival and feeding periodicity of larvae and juvenile barramundi *Lates calcarifer* (Blotch). Aquaculture 138, 159-168.
- Bergert, B.A., Wainwright, P.C., 1997. Morphology and kinematics of prey capture in the syngnathid fishes *Hippocampus erectus* and *Syngnathus floridae*. Mar. Biol. 127, 563-570.
- Blaxter, J.H.S., 1980. Vision and feeding of fishes. In: J.E. Bardach, J.J. Magnuson, R.C. May and J.M. Reinhart, (eds.). Fish behaviour and its use in the capture and culture of fishes. ICLARM Conference Proceedings, ICLARM, Manila, Philippines, pp 32-56.
- Bligh, E.G., Dyer, W.J., 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.
- Chang, M., Southgate, P.C., 2001. On growth and survival of seahorse *Hippocampus* sp., Juveniles. Aquarium Sci. Conserv. 3, 205-214.
- Choo, C.K., Liew, H.C., 2003. Spatial distribution, substrate assemblages and size composition of seahorses (Family: Syngnathidae) in the coastal waters of peninsular Malaysia. J. Mar. Biol. Assoc. UK. 83, 271-276.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Robers, P.A., Smith, F., 1956. Colorimetric method of determination of sugars and related substances. Anal. Chem. 28, 350-356.
- Faleiro, F., Narciso, L., Vicente, L., 2008. Seahorse behavior and aquaculture: How to improve *Hippocampus guttulatus* husbandry and reproduction? Aquaculture 282, 33-40.
- Faulk, C.K., Benninghoff, A.D., Holt, G.J., 2007. Ontogeny of the gastro intestinal tract and selected digestive enzymes in cobia *Rachycentron canadum* (L.). J. Fish Biol. 70, 567-583.
- Forteath, N., 1996. Seahorse *Hippocampus abdominalis* in culture. Austasia Aquacult. 9, 83-84.
- Foster, S.J., Vincent, A.C.J., 2004. Life history and ecology of seahorses: implications for conservation and management. J. Fish Biol. 65, 1-61.

- Giles, B.G., Troung, S.K., Hoang, D.H., Vincent, A.C.J., 2006. The catch and trade of seahorses in Vietnam. *Biodivers. Conserv.* 15, 2497-2513.
- Gokulakannan, K., 2002. Systematics of seahorses and biology of yellow seahorse *Hippocampus kuda* (Bleeker, 1852) from Palk Bay region, south east coast of India. PhD Thesis. Annamalai University, India. 105pp.
- Hansen, G.H., Olafsen, J.A., 1999. Bacterial interactions in early life stages of marine cold water fish. *Microb. Ecol.* 38, 1-26.
- Hilomen-Garcia, G.V., Reyes, R.D., Garcia, C.M.H., 2003. Tolerance of seahorse *Hippocampus kuda* (Bleeker) Juveniles to various salinities. *J. Appl. Ichthyol.* 19, 94-98.
- Holt, G.J., 2003. Research on culturing the early life history stages of marine ornamental species. In: Cato, J.C., Brown, C.L, (Eds.), *marine Ornamental Species : Collection, Culture and Conservation*. Iowa State Press, Ames, IO, pp. 251-254.
- Houde, E.D., Palko, B.J. 1970. Laboratory rearing of the Clupeid fish *Harengula pensacolae* from fertilized egg. *Mar. Biol.* 5, 354-358.
- Huse, I., 1994. Feeding at different illumination levels in larvae of three marine teleost fish : cod, *Gadus morhua* L., Plaice, *Pleuronectes platessa* L., and turbot , *Scophthalmus maximus* (L.). *Aquacult. Fish. Managt.* 25, 687-695.
- Ivlev, I., 1961. *Experimental Ecology of the Feeding of Fish*. Yale University Press, New Haven. CT, USA. p. 322.
- Izquierdo, M.S., Fernandez-Placios, Tacon, A.G.J., 2001. Effect of brood stock nutrition on reproductive performance of fish. *Aquaculture* 197, 25-42.
- James, P.L., Heck, K.L.Jr., 1994. The effect of habitat complexity and light intensity on ambush predation within a stimulated seagrass habitat. *J. Exp. Mar. Biol. Ecol.* 176, 187-200.
- Jenkins, G.P., 1987. Comparative diets, prey selection and predatory impacts of co-occurring larvae of two flounder species. *J. Exp. Mar. Biol. Ecol.* 110, 147-170.
- Jiixin, C., 1990. Brief introduction to mariculture of five selected species in China (Section 1: Seahorse culture). FAO/UNDP. Report no. RAS/90/002, 31 pp.
- Job, S., Arvedlund, M., Marnane, M., 1997. Captive breeding of coral reef fishes. *Aust. Aquacult.* 11, 333-341.
- Job, S.D., Do, H.H., Meeuwig, J.J., Hall, H.J., 2002. Culturing the oceanic seahorse, *Hippocampus kuda*. *Aquaculture* 214, 333-341.

- Jun, M.A, Zhou, X., Jiang, X.Y., You, L., Zhi-Mang, Z., 2006. Feeding rhythms and growth of the tongue sole, *Cynoglossus semileavis* Gumther, during its early life stages, *Aquacult. Res.* 37, 586-593.
- Junyi, L., Bingji, L., Yanyan, S., Dawei, Y., Huang, K., 2002. The ingestion, growth and ecological conversion efficiency of *Hippocampus kuda* under the intensive rearing conditions. *J. Fish. China.* 26, 61-66.
- Li, J.X., 1999. The technology of seahorse aquaculture. *Forage scan*, 11(11), 36-37 (In Chinese).
- Lin, Q., Gao, Y., Sheng, J., Chen, Q., Zhang, B., Lu, J., 2007. The effects of food and the sum of effective temperature on the embryonic development of the seahorse, *Hippocampus kuda* Bleeker. *Aquaculture* 262, 481-492.
- Lin, Q., Lin, J., Lu, J., Li, B., 2008. Biochemical composition of six seahorse species, *Hippocampus* sp., from the Chinese coast. *J. World. Aqua. Soc.*, 39, 225-234.
- Lockyear, J., Kaiser, H., Hecht, T. 1997. Studies on the captive breeding of the Knysna seahorse, *Hippocampus capensis*. *Aquarium Sci. Conserv.* 1, 129-136.
- Lourie, S.A., Pritchard, J.C., Casey, S.P., Toung, S.K., Hall, H.J., Vincent, A.C.J., 1999a. The taxonomy of Vietnam's exploited seahorses (Family: Syngnathidae). *Biol. J. Linn. Soc.* 66, 231-256.
- Lourie, S.A., Vincent, A.C.J., Hall, H.J., 1999b. Seahorses: an identification guide to the world's species and their conservation. Project seahorse, London, UK, 214 pp.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with Folin-phenol reagent. *J. Biol. Chem.* 193, 265-275.
- Lunn, K.E., Hall, H.J., 1998. Breeding and management of seahorses in aquaria. In: Briefing Documents for the First International Aquarium Workshop on Seahorse Husbandry, Management, and Conservation, Project Seahorse, Chicago, 98 pp.
- Mai, D.Y., Vu, T.T., Bui, L., Tran, M.T., 1979. *Ichthyology*. University and High School Publishers, Hanoi, 360 pp.
- Marichamy, R., Lipton, A.P., Ganapathy, A., Ramalingam, J.R., 1993. Large scale exploitation of seahorse (*Hippocampus kuda*) along Palk Bay coast of Tamil Nadu. *Marine Fisheries Information Service, Technical and Extension Series* 119, 17-20.
- Minitab Inc., 1991. *Minitab reference manual: release 8.2 for PC* State College, PA. 234 pp.
- Murugan, A., 2004. Biology and culture of the seahorse *Hippocampus trimaculatus* (Leach, 1814). Ph.D. Thesis, Annamalai University, India, 176 pp.

- Muthuwan, V., Luang, O., Sawatpeera, S., Chaladkid, S., Noiraksa, T., Teeramaethee, J., 2001. Delay feeding and feeding regimes effect survival of young seahorse *Hippocampus kuda* (Bleeker), Marine Ornamentals 2001: Collection, Culture & Conservation Program and Abstracts. p. 73. Florida Sea Grant College Program, Building 803, McCarty Drive, PO Box 110400 Gainesville FL 32611 USA.
- Olivotto, I., Avella, M.A., Sampaolesi, G., Piccinetti, C.C., Navarro Ruiz, P., Carnevali, O., 2008. Breeding and rearing the longsnout seahorse *Hippocampus reidi*: Rearing and feeding studies. *Aquaculture* 283, 92-96.
- Pajaro, M.G., Vincent, A.C.J., Buhat, D., Pernate, N., 1997. The role of seahorse fishers in conservation and management. Proceedings of the First International Symposium Marine Conservation. Hong Kong Marine Conservation Society, pp. 118-126.
- Payne, M.F., 2003. The seahorse sanctuary. *Austasia Aquacult.* 17, 12-16
- Payne, M.F., Ripplingale, R.J., 2000. Rearing West Australian seahorse, *Hippocampus subelongatus*, juveniles on copepod nauplii and enriched *Artemia*. *Aquaculture* 188, 353–361.
- Payne, M.F., Ripplingale, R.J., Longmore, R.B., 1998. Growth and survival of juvenile pipefish (*Stigmatopora argus*) fed live copepods with high and low HUFA content. *Aquaculture* 167, 237–245.
- Plama, J. Stockdale, J., Correia, M., Andrade, J.P., 2008. Growth and survival of adult long snout seahorse (*Hippocampus guttulatus*) using frozen diets. *Aquaculture* 278, 55–59.
- Penry, D.L., P.A. Jumars., 1987. Modelling animal gut as chemical reactor. *Am. Nat.* 129, 69-86.
- Project Seahorse., 2006. Annual Report, University of British Columbia, p. 20.
- Riley, J.D., Thacker, G.T., 1963. Marine fish culture in Britian. III Plaice (*Pleuronectes platessa* (L.) rearing in closed circulation at Lowestoff, 1961. *J. Cons. Perm. Int. Explor. Mer.* 28, 80-90.
- Salin, K.R., Yohannan, T.M., Mohankumaran, N.C., 2005. Fisheries and trade of seahorses, *Hippocampus* spp., in Southern India. *Fish. Mangt. Ecol.* 12, 269-273.
- Sanchez-Vazquez, F.J, Madrid, J.A., Zamora, S., Iigo, M., Tabata, M., 1996. Demand feeding and locomotor circadian rhythms in the gold fish, *Carassius auratus*: dual and independent phasing, *Physiol. Behav.* 60, 665-674.
- Scarratt, A.M., 1996. Techniques for raising lined seahorse (*Hippocampus erectus*). *Aquarium Frontiers* 3, 24-29.
- Sheng, J., Lin, Q., Chen, Q., Gao, Y., Shen, L., Lu, J., 2006. Effects of food, temperature and light intensity on the feeding of three spot juvenile seahorses, *Hippocampus trimaculatus* Leach. *Aquaculture* 256, 596-607.

- Sheng, J., Lin., Q., Chen, Q., Shen, L., Lu, J., 2007. Effect of starvation on the initiation of feeding, growth and survival rate of juvenile seahorses, *Hippocampus trimaculatus* Leach and *Hippocampus kuda* Bleeker. *Aquaculture* 271, 469-478.
- Silveira, R.B., 2000. Reproductive behaviour and initial growth of seahorse *Hippocampus reidi* Ginsburg (Pisces:Syngnathiformes:Syngnathidae). *Biociencias* 8, 115-122.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry. The Principles and Practice of Statistics in Biological Research*. Freeman, New York, 887 pp.
- Sreepada, R.A., Desai, U.M., Naik, S., 2002. The plight of Indian seahorses: need for conservation and management. *Curr. Sci.* 82, 377-378.
- Strawn, K., 1958. Life history of the pigmy seahorse, *Hippocampus zosterae*, Jordon and Gillbert, at Cedar key, Florida. *Copeia*, 16-22.
- Teixeira, R.L., Musick, J.A., 1995. Trophic ecology of two congeneric pipefish (Syngnathidae) of the lower York River, Virginia. *Env. Biol. Fish.* 43, 295-309.
- Teske, P.R. Hamilton, H., Palsboll, P.J., Choo, C.K., Gabr, H., Lourie, S.A., Santos, M., Sreepada, R.A., Cherry, M.I., Matthee, C.A. 2005. Molecular evidence for long-distance colonization in an Indo-Pacific seahorse lineage. *Mar. Ecol. Prog. Ser.* 286, 249-260.
- Tipton, K., Bell, S.S., 1988. Foraging patterns of two syngnathid fishes: importance of harpacticoid copepods. *Mar. Ecol. Prog. Ser.* 47: 31-43.
- Truong, S.K., 1998. Prospects of community –based aquaculture in Vietnam. In: Morton, B. (Ed.), *The Marine Biology of the South China Sea III*. Hong Kong University press, Hong Kong, pp. 465-474.
- Underwood, A. J., 1997. Experiments in ecology. In: *Their logical and interpretation using analysis of variance*. Cambridge, UK: Cambridge University Press, 504 pp.
- Vari, R., 1982. Fishes of the Western North Atlantic, Subfamily Hippocampinae The seahorse. *Sears Foundation of Marine Research Memoirs, Yale University, USA.* 1, 173-189.
- Victor, B.C., 1991. Settlement strategies and biogeography of reef fishes. In: P.F. Sale (ed.). *The Ecology of Fishes on Coral Reefs*. Academic Press, San Diego, pp. 231-260
- Vincent, A.C.J., 1996. *The international trade in seahorses*, Cambridge, UK: TRAFFIC International, 163 pp.
- Whitfield, A. K., 1995. Threatened fishes of the world: *Hippocampus capensis* Boulenger, 1900 (Syngnathidae). *Env. Biol. Fish.* 44, 362.

- Wilson, M.J., Vincent, A.C.J., 1998. Preliminary success in closing the life cycle of exploited seahorse species, *Hippocampus* spp., in captivity. *Aquarium Sci. Conserv.* 2, 179-196.
- Werner, R.G. and J.H.S. Blaxter, 1980. Growth and survival of larval Herring (*Clupea harengus*) in relation to prey density. *Can. J. Fish. Aquat. Sci.*, 37, 1063-1069.
- Winer, B. J., Brown, D. R., Michels, K. M., 1991. *Statistical principles in experimental design* New York: McGraw-Hill. 3rd edn., 1057 pp.
- Wong, J.M., Benzie, J.A.H., 2003. The effects of temperature, *Artemia* enrichment, stocking density and light on the growth of juvenile seahorses, *Hippocampus whitei* (Bleeker, 1855), from Australia. *Aquaculture* 228, 107-121.
- Woods, C.M.C., 2000a. Preliminary observations on breeding and rearing the seahorse *Hippocampus abdominalis* (Teleostei:Syngnathidae) in captivity. *New Zealand J. Mar. Freshwat. Res.* 34, 475-485.
- Woods, C.M.C., 2000b. Improving initial survival in cultured seahorses *Hippocampus abdominalis* Leeson, 1827 (Teleostei:Syngnathidae). *Aquaculture* 190, 377-388.
- Woods, C.M.C., 2003. Effect of stocking density and gender segregation in the seahorse, *Hippocampus abdominalis*. *Aquaculture* 218, 167-176.
- Woods, C.M.C., 2005. Growth of cultured seahorses (*Hippocampus abdominalis*) in relation to feed ration. *Aquacult. Int.* 13, 305-314.
- Yoseda, K., Yamamoto, K., Asami, K., Chimura, M., Hashimoto, K., 2008. Influence of light intensity on feeding, growth, and early survival of leopard coral grouper (*Plectropomus leopardus*) larvae under mass-scale rearing conditions. *Aquaculture* 279, 55-62.
- Zar, J.H., 1996. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, New Jersey, USA. 481 pp.
- Zhang, H. F, Liu, X.C., Lin, H.R, Liu, F.Y.Z, Wang, Y.X, Huang, G.G, Wang, H.D, Ou, C.H. 2004. Studies on feeding rhythms and daily feeding amount of larval *Epinephelus coioides*. *J. Fish. China.* 28 (6), 669–674.

Legend to Figures

- Fig. 1. Survival of juveniles of *Hippocampus trimaculatus* under different light intensities during of pelagic phase rearing of *Hippocampus trimaculatus*. Vertical bars indicate standard deviation (n=3).
- Fig. 2. Effect of single food source on changes in (a) survival (%) and (b) ingestion rate (juvenile⁻¹ h⁻¹) during pelagic phase juvenile rearing of *Hippocampus trimaculatus* (0-6 DAB). Vertical bars indicate standard deviation (n=3).
- Fig. 3. Effect of single food source on changes in (a) survival (%) and (b) ingestion rate (juvenile⁻¹ h⁻¹) during pelagic phase juvenile rearing of *Hippocampus trimaculatus* (7-13 DAB). Vertical bars indicate standard deviation (n=3).
- Fig. 4. Feed selectivity indices (*I*) of different prey items during the pelagic phase juvenile rearing of *Hippocampus trimaculatus*.
- Fig. 5. Survival of pelagic phase juveniles of *Hippocampus trimaculatus* reared with mixed food source. Vertical bars indicate standard deviation (n=3).
- Fig. 6. Survival of settlement-stage juveniles (30 DAB) and adults (120 DAB) of *Hippocampus trimaculatus* during gradual reduction in salinity from a rearing salinity of 35 ppt.
- Fig. 7. Fortnightly variations in average growth rate (Ht, mm d⁻¹) of *Hippocampus trimaculatus* during mass-scale rearing.
- Fig. 8. Fortnightly variations in weight gain (wet, mg) and growth increment (Ht, mm) during the mass-scale rearing of *Hippocampus trimaculatus*. Vertical bars indicate standard deviation (n=230).
- Fig. 9. Weekly variation in survival rates (%) of *Hippocampus trimaculatus* during mass-scale rearing.

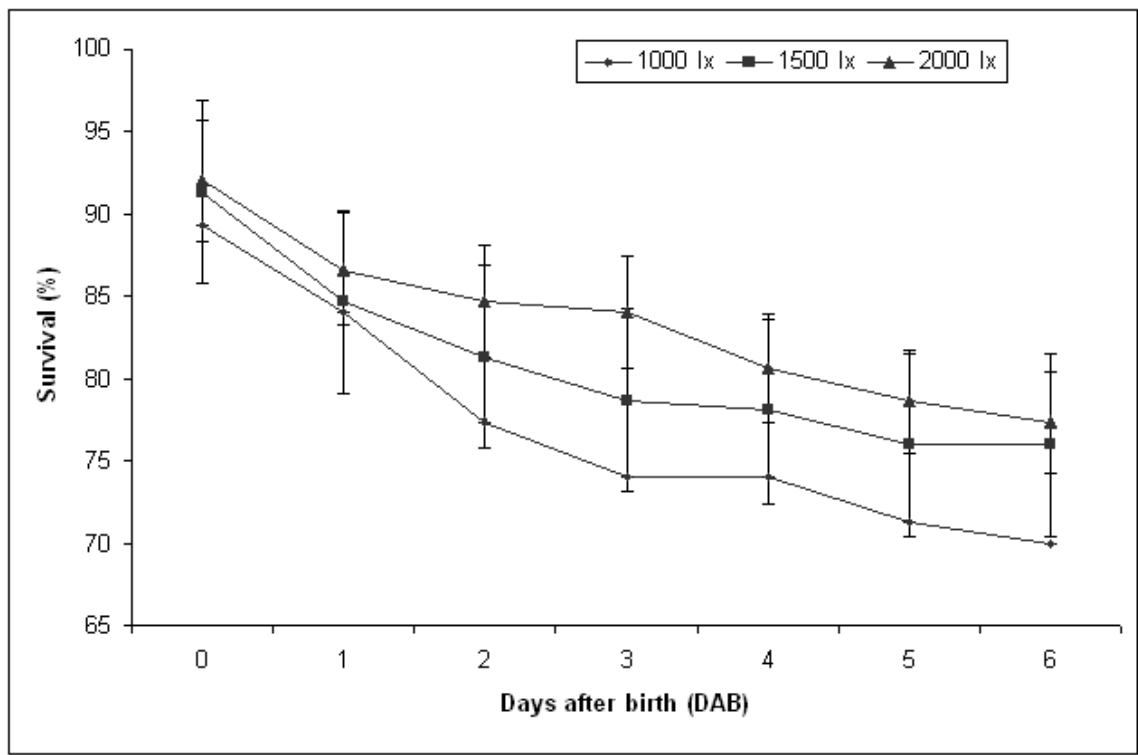


Fig. 1

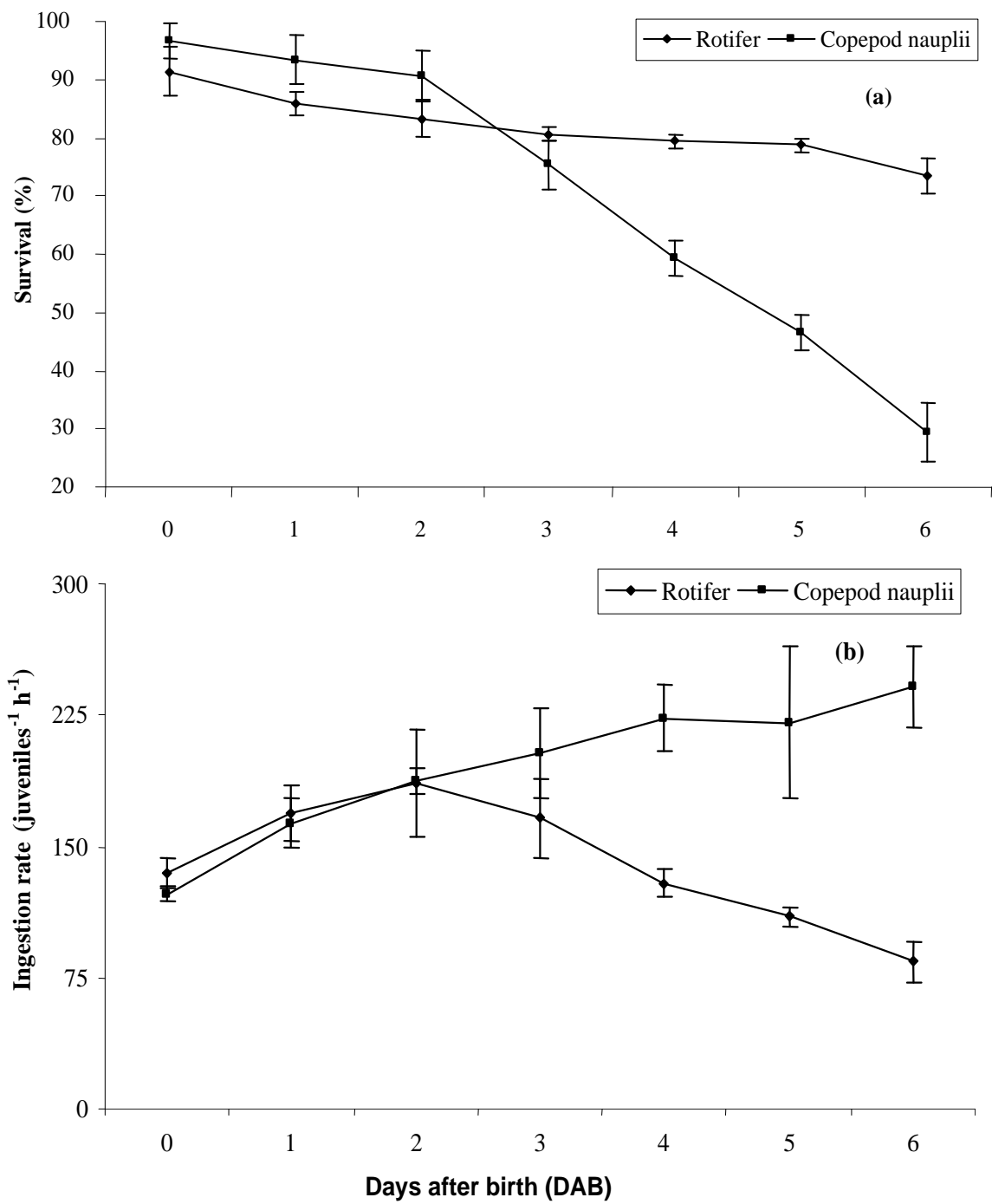


Fig. 2

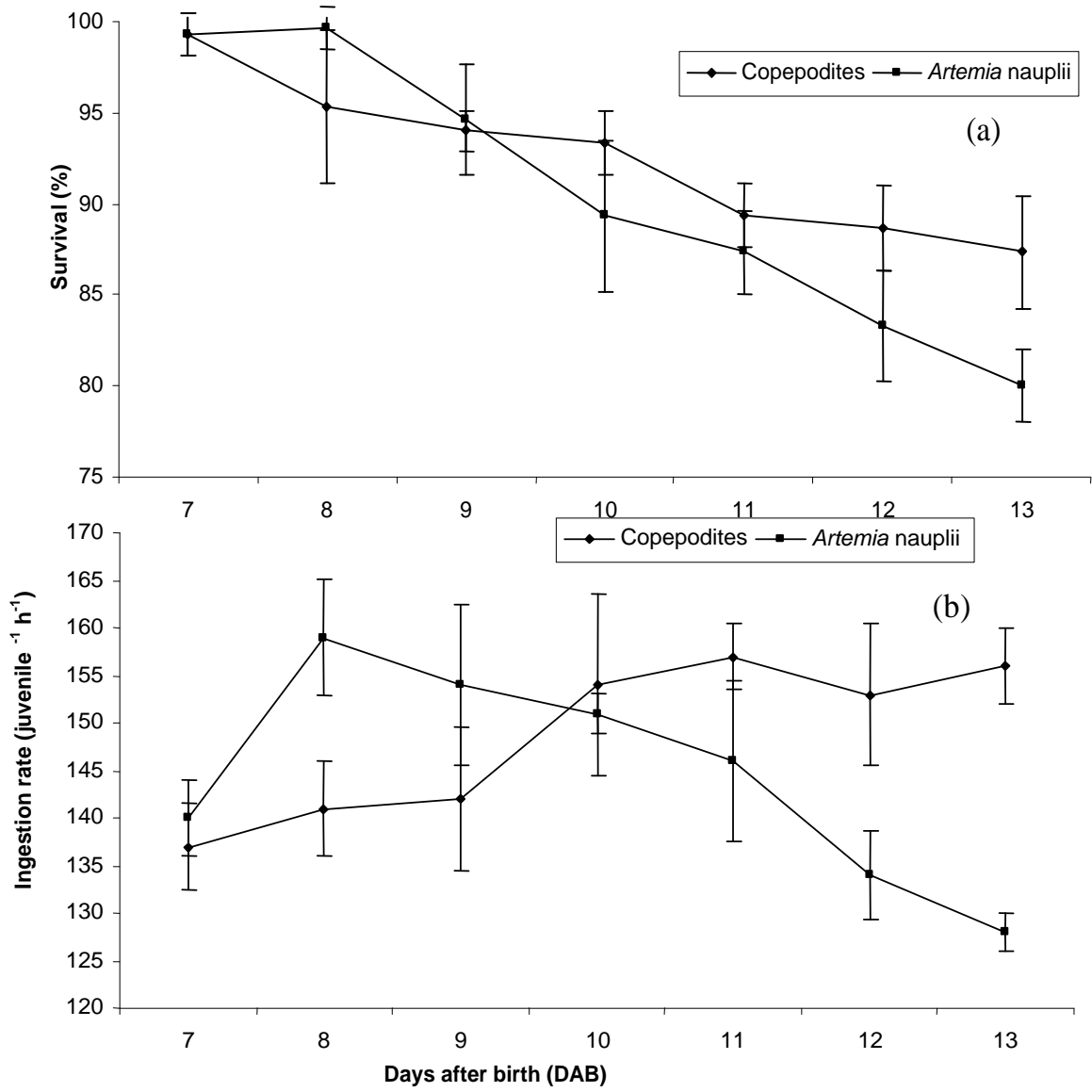


Fig. 3

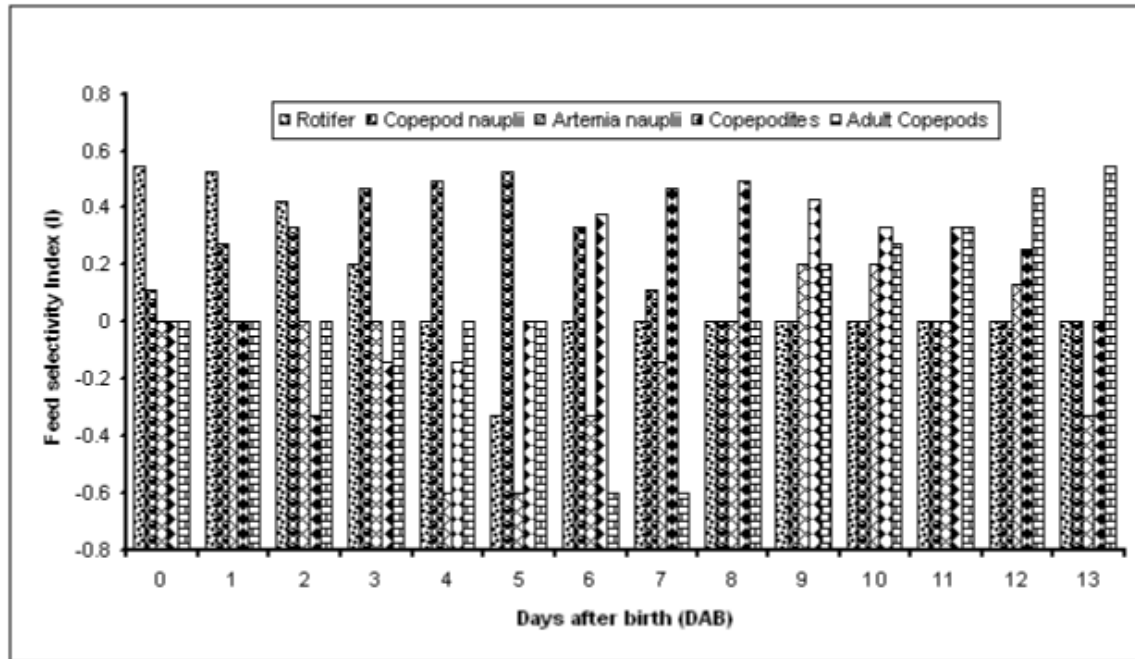


Fig. 4

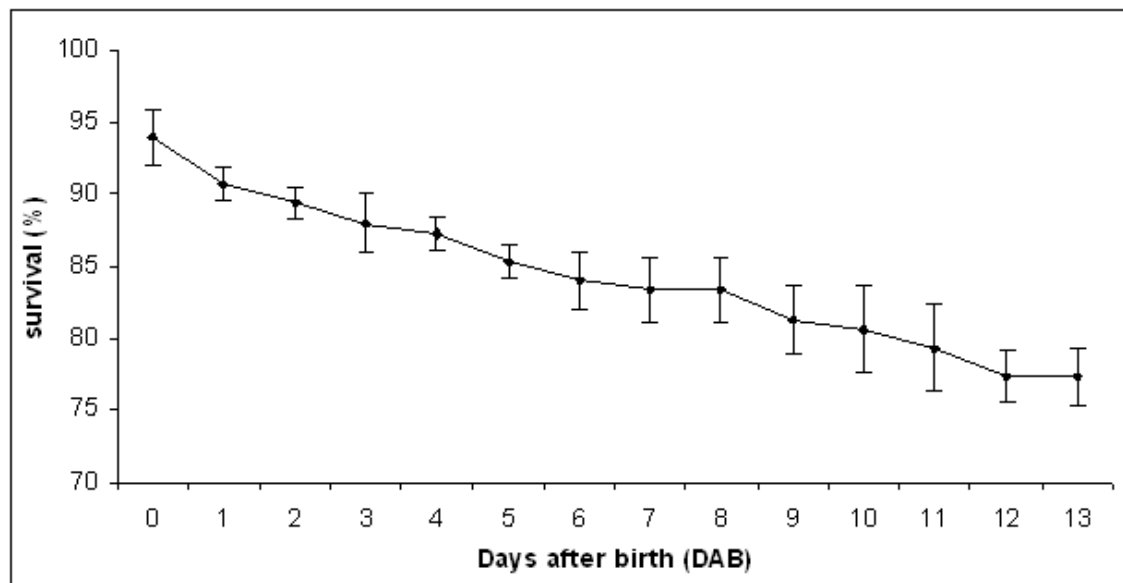


Fig. 5

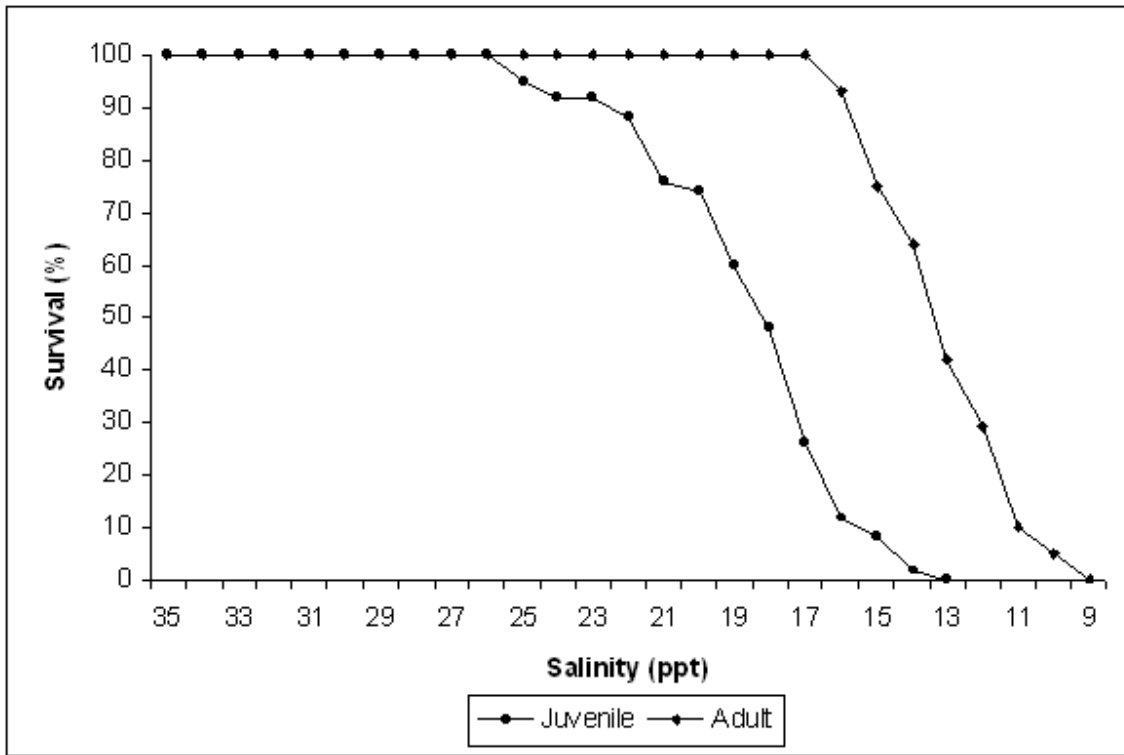


Fig. 6

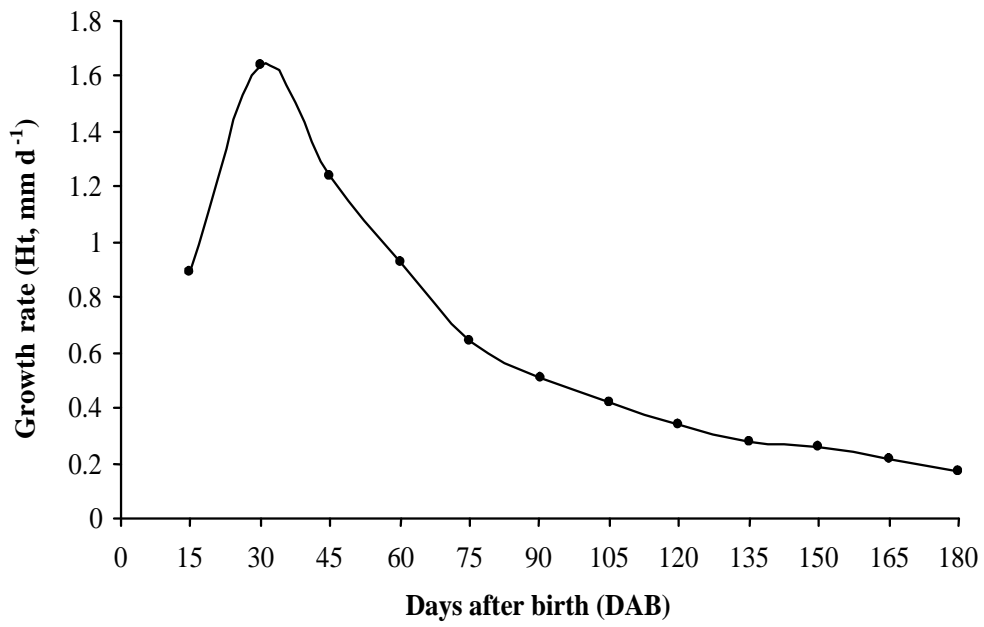


Fig. 7

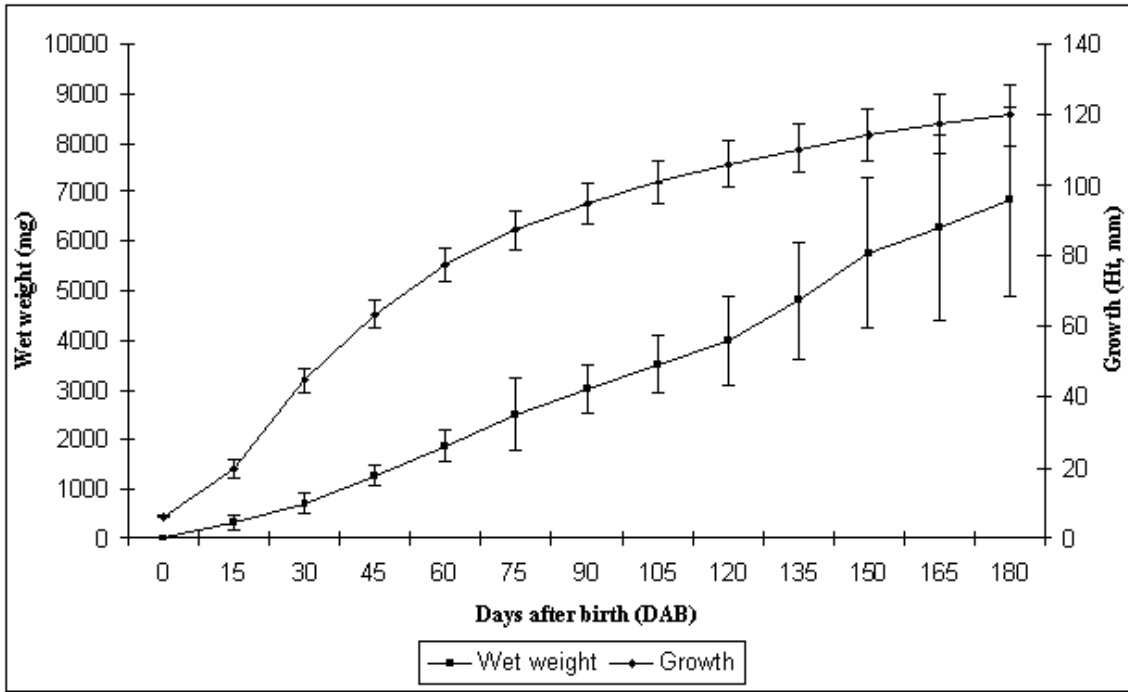


Fig. 8

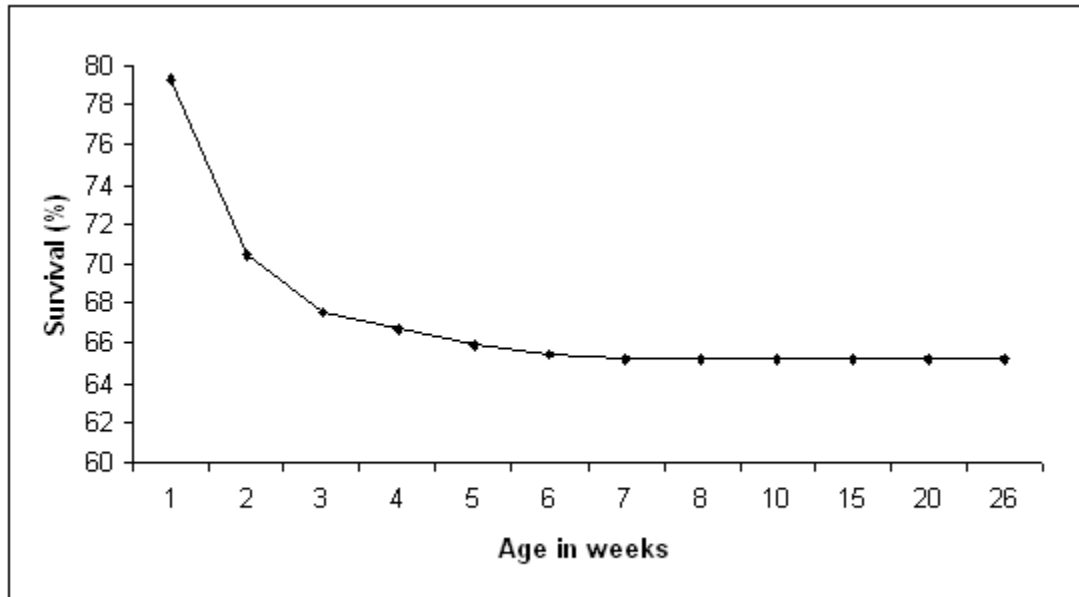


Fig. 9