ELSEVIER

Aquaculture 214 (2002) 333–341

Aquaculture

www.elsevier.com/locate/aqua-online

Culturing the oceanic seahorse, *Hippocampus kuda*

S.D. Job[a,*], H.H. Do[b], J.J. Meeuwig[c], H.J. Hall[a]

[a] *Zoological Society of London, Regent's Park, London, NW1 4RY UK*
[b] *Institute of Oceanography, Nha Trang, Viet Nam*
[c] *McGill University, Department of Biology, Montreal, Canada H3A 1B1*

Received 16 October 2001; received in revised form 20 February 2002; accepted 20 February 2002

Abstract

Seahorse aquaculture has received widespread attention due to concerns over declines in wild seahorse populations, and in recognition of their high economic value and marketability. Seahorse aquaculture has great potential to integrate both conservation and sustainable development goals by providing an alternative livelihood option for fishers in source countries who, in the absence of alternatives to fishing, continue to exploit declining seahorse populations. To date, few culturing protocols have been established for tropical species exploited in Indo–Pacific source countries. We here present data on culturing *Hippocampus kuda*, one of the more heavily exploited species in both traditional medicines and marine aquarium trades. We also present results of an experiment testing the effects of three locally available *Artemia* enrichments on *H. kuda* survivorship, sex ratios, and growth to market size (14 weeks of age). Our results indicate that *H. kuda* grows rapidly from birth to 14 weeks, at a rate of 0.9–1.53 mm day^{-1}. Survivorship to market size varied among *Artemia* enrichments and was highest (73%) when seahorses were fed *Artemia* enriched with an emulsion derived from *Acetes* sp., a locally available planktonic crustacean, and lowest (40%) when seahorses were fed *Artemia* enriched with an emulsion derived from local fish. Growth, however, did not vary among enrichments. Sex ratios were not significantly different from the expected ratio of 1:1 nor did enrichment affect sex ratio. The seahorses were only marginally sexually dimorphic at market size (males 3 mm>females in standard length). The rapid growth rate and high survival of *H. kuda* on readily available local *Artemia* enrichments is promising for the development of seahorse aquaculture in source countries.

Keywords: Seahorse aquaculture; *Hippocampus kuda*; Survival; Growth; *Artemia* enrichment

* Corresponding author. Culturing for Conservation, 59B Corinthian Road West, Shelley, WA 6148, Australia.
E-mail address: sureshjob@optusnet.com.au (S.D. Job).

0044-8486/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0044-8486(02)00063-7

1. Introduction

Seahorse aquaculture has been the subject of recent attention both by aquaculturists (Payne and Rippingale, 2000; Woods, 2000a,b) and in the policy domain (CITES, 2001). Interest in seahorse aquaculture reflects concern over overexploitation in the wild with consequent declines in populations, and the recognition that seahorses command high prices and thus may be highly marketable (Vincent, 1996; Lourie et al., 1999). Demand for seahorses appears to be greater than supply, with at least 20 million seahorses traded globally in 1995 (Vincent, 1996). Currently, at least 51 nations and territories around the world are involved in buying and selling seahorses. The bulk of seahorses traded is used in traditional medicine, marine aquarium fish and curios trades. As the overexploitation of wild seahorse populations is driven in part by the high demand, seahorse aquaculture could help reduce overexploitation by supplying the trade with captive-bred fishes. However, it is not clear whether seahorses produced in non-source countries will decrease seahorse exploitation by fishers in source countries.

Seahorse aquaculture has great potential to integrate both conservation and sustainable development goals if it is developed as an alternative livelihood for fishers. Reduction of fishing pressure on overexploited fish species such as seahorses is central to their sustainable management. Reduced fishing pressure is more likely to occur if there are alternatives to fishing as a primary livelihood. Alternative livelihoods, however, cannot be considered in isolation if conservation goals are to be met. The imbalance between supply and demand for seahorses means that as supply diminishes, prices may rise. If prices rise, fishery-derived income may eventually become sufficiently high to entice fishers engaged in alternative livelihoods to reenter the fishery even if the resources are substantially depleted. Thus, alternatives to fishing that actively reduce the demand for wild-caught marine resources are more likely to meet conservation goals than alternatives that only generate income. Seahorse aquaculture, unlike many other alternative livelihoods, can both provide sustainable income and address ongoing market demand by creating a high value alternative to wild-caught animals.

The scarcity of culturing protocols for tropical Indo–Pacific seahorse species constrains the adoption of seahorse aquaculture by seahorse fishers in source countries. The largest known exporters of seahorses are Indo–Pacific developing nations such as Thailand, Vietnam, India, and the Philippines, with the bulk of seahorse fishing occurring in tropical areas. However, information on suitable culturing protocols for most tropical Indo–Pacific species is limited (Truong, 1998). Information on the technical feasibility of culturing heavily exploited tropical seahorse species is essential for the development of seahorse aquaculture that is targeted at reducing overexploitation of wild populations. The present study assesses the feasibility of culturing a highly valued tropical Indo–Pacific seahorse species, *Hippocampus kuda*. Specifically, it aims to measure the growth and survival of *H. kuda* fed with three different *Artemia* enrichments.

The Oceanic seahorse, *H. kuda*, is widely distributed throughout the tropical Indo–Pacific region, from the Indian subcontinent in the west to the Pacific islands in the east (Lourie et al., 1999). It is generally found in a broad range of shallow inshore habitats including mangroves, seagrass beds and estuaries. *H. kuda* is a highly valued seahorse species that is popular in both the traditional medicines and marine aquarium trades

(Lourie et al., 1999). It is one of the most heavily traded seahorse species in many Southeast Asian countries such as Vietnam (for the traditional medicines trade) and Indonesia (for the aquarium trade). Its conservation status is currently listed by the International Union for the Conservation of Nature (IUCN) as vulnerable. Wild populations of this species are likely to face increasing threat unless alternative sources become available.

2. Materials and methods

2.1. Nursery stage

Hippocampus kuda were cultured at the Institute of Oceanography (Nha Trang, Vietnam) in 2000 as part of a joint seahorse conservation project between Project Seahorse (http://www.projectseahorse.org) and the Institute of Oceanography. Three hundred newly hatched *H. kuda* were reared in a 3.5 m^3 circular nursery tank for a period of 42 days. The nursery tank was located outdoors under a translucent roof that transmitted approximately 30% of the overhead sunlight. The young seahorses were fed wild-caught zooplankton (approximately 80% copepods; the remaining 20% included cladocerans and amphipods among others) for the first 10 days. The zooplankton were collected from the mouth of a nearby estuary using a 210 μm plankton net and then sieved through a 500 μm net to remove excessively large planktonic organisms. From the age of 7 days post-hatch, the seahorses were fed 4-day-old unenriched *Artemia* (approximately 1–2 mm in length). From the 12th day post-hatch, 4–6 mm long, wild-caught mysids were added to the rearing tank in addition to the *Artemia*. Prey density in the nursery tank was maintained at between one and four individuals per liter throughout the nursery period. The tank was cleaned twice a day, once in the morning and again in the evening. Nylon netting was added to the tank from the 14th day post-hatch to provide holdfasts for the seahorses.

The nursery tank was linked to a 60,000 l recirculating seawater system. Temperature, salinity, pH, ammonia, nitrite and nitrate levels were measured biweekly in the seawater system. Temperature, salinity and pH values were 30 ± 0.22 °C, 31.74 ± 0.31 ‰, and 8.33 ± 0.05, respectively, throughout the culturing period. Ammonia, nitrite and nitrate concentrations were all less than 0.1 mg/l. The nursery tank was run as a flow-through system with a flushing rate of 1200 l/min. A strainer over the overflow ensured that no seahorses or food items were flushed out of the tank.

Ten seahorses were randomly sampled from the nursery tank each week from the age of 14 days post-hatch and their standard length and height measured. They were then placed in a separate tank from the other seahorses so as not to recount them in the following weeks. Standard length was measured as the sum of the length from the tip of the tail to the mid-point of the cleithral ring and the length from the tip of the snout to the mid-point of the cleithral ring (Lourie et al., 1999). Height was measured as the distance from the tip of the coronet to the tip of the tail (Lourie et al., 1999). All the seahorses in the nursery tank were counted at the age of 42 days post-hatch to assess survival during the nursery stage.

2.2. Grow-out stage

Ninety seahorses were randomly sampled from the nursery tank at the age of 42 days post-hatch. These seahorses were then measured and transferred into 18,147 l rectangular glass experimental tanks (75 cm long by 45 cm wide by 43.5 cm water depth). The experimental tanks were located outdoors under a translucent roof that transmitted approximately 30% of the overhead sunlight, and no additional lighting was provided. The experimental design consisted of three treatments with six replicate tanks for each treatment. Each experimental tank contained five seahorses. Seahorses were randomly assigned to experimental tanks. The experimental tanks were linked to the same 60,000 l recirculating seawater system as described for the nursery tank, and were run as flow-through tanks, with a flushing rate of 150 l/h. The tanks were kept bare except for the provision of short lengths of PVC pipe for the seahorses to use as holdfasts. All tanks were cleaned twice a day.

The three treatments consisted of feeding the seahorses with live adult *Artemia* that were enriched with three different locally available enrichment products. In the first treatment, seahorses were fed with adult *Artemia* that had been enriched with blended whole fish. Fish used were from a range of species sold primarily as animal feeds. Fish used to enrich *Artemia* were bulk purchased in advance, diced into small pieces, mixed thoroughly and then stored in a freezer. The enrichment product was obtained by blending 5 g of the fish pieces prior to *Artemia* enrichment, and filtering it through a 53 μm sieve. Only the filtrate was used to enrich the *Artemia*. Seahorses in the second treatment were fed with *Artemia* enriched with blended *Acetes* sp. (a planktonic crustacean abundant throughout S.E. Asia).The *Acetes*-based enrichment product was obtained using the same protocol as followed for the fish-based product. The third treatment consisted of seahorses fed on *Artemia* enriched with a 1:1 mixture of blended *Acetes* sp. and blended whole fish. In this treatment, 2.5 g each of fish and *Acetes* were blended together and filtered through a 53 μm sieve to produce the enrichment product. In each of the three treatments, the *Artemia* were enriched for 1 h, and then fed to the seahorses. Assessment of *Artemia* gut fullness suggested that enrichment for 1 h was adequate. The seahorses were fed twice a day, once at 10:30 h and again at 16:00 h. The amount of *Artemia* added to the tank for each feed was 2.7 g $\pm$ 0.04 s.e.m. for seahorses aged between 42 and 70 days, and 4.8 g $\pm$ 0.04 for seahorses aged between 71 and 98 days. Uneaten *Artemia* were removed after 2 h.

The standard length (SL) and weight of each seahorse were measured fortnightly before the day's feeding commenced. Standard length was measured as described above. Weight measured was the wet weight, i.e., the seahorses were blotted briefly on filter paper and then weighed. The number of male and female seahorses in each tank was also assessed fortnightly from the age of 70 days post-hatch. The experiment was conducted from the time the seahorses were 42 days of age until they reached saleable (market) size at 98 days of age.

2.3. Data analysis

The effect of *Artemia* enrichment and age on SL, weight, survival, sex ratio and the ratio of head length to body length (trunk length plus tail length *sensu* Lourie et al., 1999) were analyzed separately using a two-factor analysis of variance (ANOVA). Sex ratio was measured as the number of males in a tank divided by the sum of males and females in each tank (juveniles were excluded). The assumptions of ANOVA (Zar, 1998) were assessed prior to evaluating the ANOVA results. The mean values for the seahorses in each tank were used in the analyses to avoid risking a violation of the assumption of independence (pseudoreplication *sensu* Hulbert, 1984). Weight was log transformed to meet the assumptions of ANOVA. The effect of *Artemia* enrichment and age on survival was only statistically tested from the age of 70 days post-hatch onwards as survival was uniformly 100% before that age.

The difference in the SL and weight of male versus female seahorses at 98 days post-hatch was analyzed separately using ANOVA. The ANOVA was first run with sex (male or female) and tank (Tank 1 to Tank 18) as the factors in order to assess the independence of tank effects on the length and weight of males and females. As there was no significant effect of tank on standard length or weight, and no significant interaction between tank and sex, tank was dropped from the analysis. The effect of sex on SL and weight was then analyzed separately using one-way ANOVA, after confirming that the assumptions of ANOVA were met.

3. Results

3.1. Nursery stage (hatching until 42 days of age)

Survival in the nursery tank from hatching until the age of 42 days post-hatch was 97%, with 291 of the original 300 seahorses surviving. Seahorses grew in SL from 30.4 mm $\pm$ 0.37 s.e.m. at 14 days of age (post-hatch) to 72.1 mm $\pm$ 0.70 s.e.m. at 42 days of age (Fig. 1). The relationship between SL (in mm) and age (in days post-hatch) was described by the equation:

$$\text{Standard length} = 10.20 + 1.53^{*}\text{Age},$$

with an adjusted r^2 of 0.99 ($n = 5$, s.e. of estimate = 2.07). The mean growth rate of the seahorses was therefore 1.53 mm/day.

3.2. Grow-out stage (42–98 days of age)

Survival differed significantly among treatments ($F = 10.849$, $df = 2$, $P < 0.001$), but did not vary significantly across ages ($F = 0.744$, $df = 2$, $P = 0.481$), nor was there a significant interaction between age and *Artemia* enrichment ($F = 0.137$, $df = 4$, $P = 0.968$). Survival over the 8-week experimental period ranged from a low of 40.00 $\pm$ 10.33% in seahorses fed fish-enriched *Artemia* to a high of 73.33 $\pm$ 11.16% in seahorses fed on *Acetes*-enriched *Artemia* (Fig. 2).

The mean SL of the seahorses did not vary significantly among the three enrichment types ($F = 1.496$, $df = 2$, $P = 0.231$), nor was there a significant interaction between age and

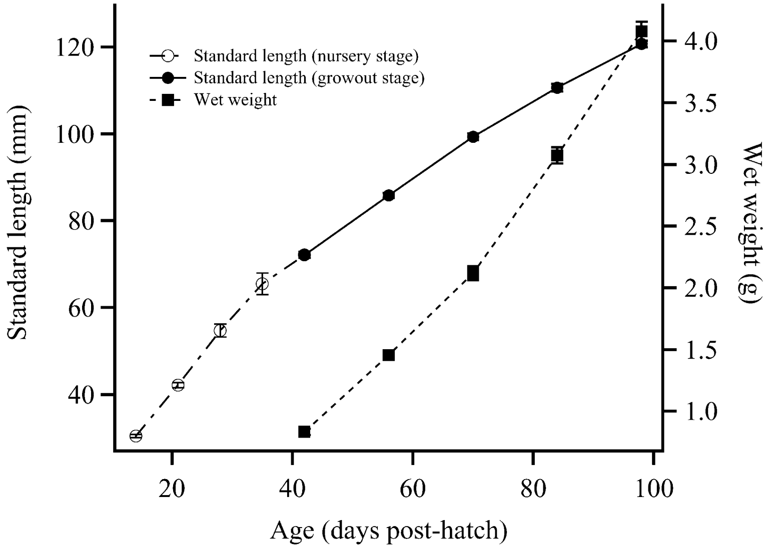

Fig. 1. Change in standard length (left axis) and weight (right axis) with age in *Hippocampus kuda*. All values are means ± s.e.m.

enrichment type ($F = 0.346$, $df = 8$, $P = 0.945$). There was, however, a significant increase in SL with age ($F = 657.421$, $df = 4$, $P < 0.001$). When combined across treatments, the seahorses grew from 72.1 ± 0.70 mm at 42 days of age (post-hatch) to 120.7 ± 0.79 mm at

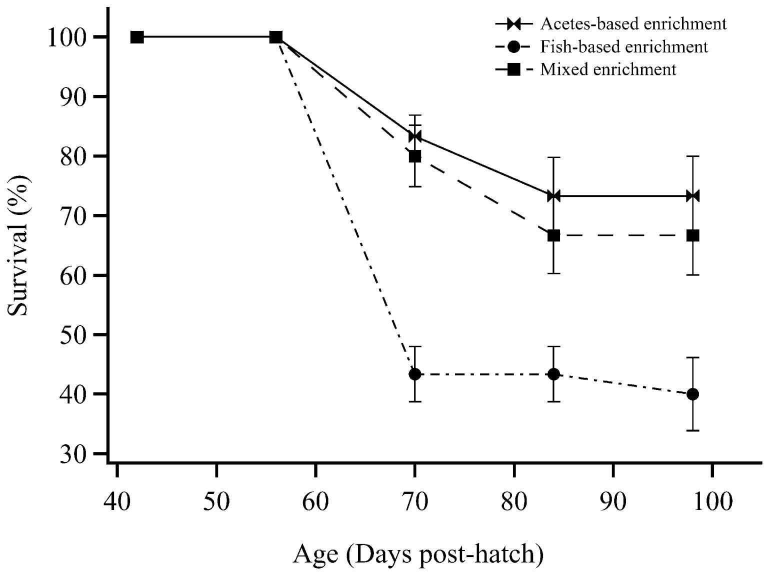

Fig. 2. Survival of *Hippocampus kuda* with three different enrichments. All values are means ± s.e.m.

98 days of age (Fig. 1). The relationship between SL (in mm) and age (in days post-hatch) was described by the equation:

$$\text{Standard length} = 34.85 + 0.90 * \text{Age},$$

with an adjusted r^2 of 0.97 ($n = 89$, s.e. of estimate = 3.24). The mean growth rate of the seahorses (combined across treatments) was therefore 0.9 mm/day.

The mean weight of the seahorses did not vary significantly among enrichment types ($F = 0.913$, $df = 2$, $P = 0.406$), nor was there a significant interaction between age and enrichment type ($F = 0.610$, $df = 8$, $P = 0.767$). The seahorses grew in weight from 0.83 ± 0.025 g at 42 days of age to 4.08 ± 0.077 g at 98 days of age (Fig. 1). The relationship between weight (in g) and age (in days) was described by the equation:

$$\text{Weight} = -1.736 + 0.058 * \text{Age},$$

with an adjusted r^2 of 0.95 ($n = 89$, s.e. of estimate = 0.26). The mean growth rate of the seahorses (combined across treatments) was therefore 0.058 g/day.

The ratio of head length to body length did not differ significantly among treatments ($F = 0.753$, $df = 2$, $P = 0.475$), nor was there an interaction between age and enrichment type ($F = 0.887$, $df = 8$, $P = 0.532$). It did, however, vary significantly with age ($F = 40.821$, $df = 4$, $P < 0.001$). The ratio of head length to body length decreased from 0.231 ± 0.001 in the 42-day-old seahorses to 0.209 ± 0.001 in the 98-day-old seahorses. Older seahorses, therefore, have proportionately shorter heads and snouts relative to the length of their body compared to younger seahorses.

The sex ratio of the seahorses did not vary significantly across treatments ($F = 1.261$, $df = 2$, $P = 0.293$) or ages ($F = 0.004$, $df = 2$, $P = 0.996$), nor was there a significant interaction between age and enrichment type ($F = 0.079$, $df = 4$, $P = 0.988$). The mean sex ratio was 0.556 ± 0.053, and did not differ significantly from a sex ratio of 0.5 ($t = 1.056$, $df = 52$, $P = 0.296$). This suggests that the sex ratio did not differ significantly from an expected value of 1 male:1 female (i.e., 0.5), regardless of enrichment type.

Male seahorses were both significantly longer than female seahorses at 98 days of age ($F = 6.223$, $df = 1$, $P = 0.016$), and significantly heavier ($F = 19.028$, $df = 1$, $P < 0.001$). Male seahorses were 123.10 ± 0.93 mm in SL and 4.43 ± 0.10 g in weight, whereas female seahorses were 119.72 ± 0.93 mm in SL and 3.80 ± 0.09 g in weight.

4. Discussion

Hippocampus kuda appear to display relatively rapid growth compared to that of most other seahorse species that have been cultured using broadly similar techniques. A temperate seahorse species, *H. abdominalis*, for example, achieves a standard length of 43 mm at 56 days of age (Woods, 2000a) and takes a year to reach 110.7 (Woods, 2000b). In comparison, *H. kuda* in the present study reached 85.8 mm at 56 days of age and took only 98 days to reach 120.7 mm. The difference in growth rate may be partially due to differences in the temperatures at which the two species were reared. *H. abdominalis*, being a temperate species, was reared at temperatures of approximately 16 °C compared to

30 °C for *H. kuda* in the present study. However, *H. abdominalis* also grows to a much larger adult size than *H. kuda* (32 cm vs. 17 cm, respectively; Lourie et al., 1999) and thus would be expected to grow more slowly. The growth rate of *H. kuda* remains high even when compared to species of similar size reared at similar temperatures. For example, an Atlantic seahorse species, *H. erectus* (18.5 cm maximum adult size; Lourie et al., 1999), reared at approximately 25 °C, reached a total length of 33.29 mm (assumed to be from the tip of the tail to the tip of the coronet [i.e., height]) at 35 days of age (Correa et al., 1989) in comparison to *H. kuda* which has a height of 57 mm (measured from tip of tail to tip of coronet) at 35 days of age.

While growth in standard length and weight of *H. kuda* did not differ with type of enrichment, the survival of the seahorses was markedly different between the two *Artemia* enrichments that included a crustacean-based enrichment product and the one that did not. Survival to market size was almost twice as high in seahorses fed *Artemia* enriched with *Acetes* sp. compared to seahorses fed *Artemia* enriched with fish. The diet of *H. kuda* in the field appears to be largely crustacean-based: young seahorses feed primarily on copepods, while adults feed on small benthic crustaceans such as amphipods (Do et al., 1998). Therefore, the addition of crustacean-based materials to their diet during culturing may provide essential nutrients that are missing in a solely fish-based diet.

Artemia enriched solely with cultured algae appear to be an unsuitable diet for many marine fish larvae (Sargent et al., 1997), resulting in a search for alternatives to *Artemia* such as copepod nauplii (Payne and Rippingale, 2000). The results from the present study suggest that *Artemia* enriched with *Acetes* sp. may be an adequate diet for culturing some seahorse species. The similarity in growth rates among treatments and the similarity in survival on the enrichment that consisted solely of *Acetes* sp. versus that which consisted of a mix of fish and *Acetes* sp. suggest that relatively small amounts of *Acetes*-based enrichment may be sufficient to obtain reasonably high growth and survival rates in some seahorse species.

Assuming a natural sex ratio of 1:1, the sex ratio of 1:1 displayed in this study suggests that the different diets did not exert a differential effect on the survival of either sex. Other studies have obtained sex ratios ranging from 1.8 females:1 male (Woods, 2000b) to 4 males:1 female (Correa et al., 1989). The reasons for this deviation in sex ratio from the theoretical expected value of 1:1 are uncertain. The difference in the standard length and weight of males versus females at marketable age, while statistically significant, is relatively small in absolute values. In practical terms, the difference in time to market for males versus females is probably negligible, and would not seem to warrant future work on methods to bias the sex ratio in order to increase the proportion of the faster growing sex. The significant change in the ratio of head length to body length during growth in *H. kuda* is consistent with available information on most other marine fish species (Leis and Trnski, 1989). Young fish tend to have relatively large heads, perhaps as a reflection of the importance of feeding at this stage relative to other functions such as reproduction that tend to become progressively more important with development.

The rapid growth and high survival of *H. kuda* obtained in this study bode well for the development of seahorse aquaculture in Indo–Pacific seahorse exporting countries. *H. kuda* is a species that is both heavily exploited in the main source countries, and is highly valued in the traditional medicines and aquarium trades (Lourie et al., 1999). The demonstration that this species can be cultured with relatively rapid growth and high survival, using foods that are readily and cheaply available (fish and *Acetes* for enrichments cost approximately US$1.40/kg as opposed to commercial enrichments costing approximately US$95/kg), suggests that further research is warranted to explore the economic feasibility of large-scale commercial culturing of *H. kuda* in main source countries. The high prices commanded by seahorses in both the traditional medicines and aquarium trades make it likely that seahorse aquaculture could indeed prove commercially viable. Seahorse aquaculture development targeted at seahorse fishers in source countries could provide a sustainable alternative livelihood for fishers while addressing ongoing market demand for seahorses, thus achieving both conservation and development goals.

Acknowledgements

We thank D. Buu, T.H. Ho, P.T.D. Nguyen, S.K. Truong and A. Vincent for their invaluable help. This research was supported by a grant from the Community Fund (UK), and in previous years, grants from the Canadian International Development Agency's Canada Fund for Local Initiatives (Vietnam), The International Development Research Council (Canada), and WildInvest (UK). Ethics approval for this research was obtained from the Zoological Society of London.

References

CITES, 2001. Notification no. 2001/034. Notification to the Parties concerning: Seahorses and other members of the family Syngnathidae. http://www.cites.org/eng/notifs/2001/034.shtml.

Correa, M., Chung, K.S., Manrique, R., 1989. Cultivo experimental del caballito de mariculture, *Hippocampus erectus*. Bol. Inst. Oceanogr., Venez. Univ. Oriente 28 (1 and 2), 191–196.

Do, H.H., Truong, S.K., Ho, T.H., 1998. Feeding behaviour and food of seahorses in Vietnam. In: Morton, B. (Ed.), The Marine Biology of the South China Sea III. Hong Kong University Press, Hong Kong, pp. 307–319.

Hurlbert, S.T., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54, 187–211.

Leis, J.M., Trnski, T., 1989. The Larvae of Indo–Pacific Shore Fishes. New South Wales University Press, Sydney, 328 pp.

Lourie, S.A., Vincent, A.C.J., Hall, H.J., 1999. Seahorses: An Identification Guide to the World's Species and their Conservation. Project Seahorse, London, 214 pp.

Payne, M.F., Rippingale, R.J., 2000. Rearing West Australian seahorse, *Hippocampus subelongatus*, juveniles on copepod nauplii and enriched *Artemia*. Aquaculture 188, 353–361.

Sargent, J.R., McEvoy, L.A., Bell, J.G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture 155, 117–128.

Truong, S.K., 1998. Prospects for community-based seahorse 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.

Vincent, A.C.J., 1996. The International Trade in Seahorses. TRAFFIC International, Cambridge, 163 pp.

Woods, C.M.C., 2000a. Improving initial survival in cultured seahorses, *Hippocampus abdominalis* leeson, 1827 (Teleostei: Syngnathidae). Aquaculture 190, 377–388.

Woods, C.M.C., 2000b. Preliminary observations on breeding and rearing the seahorse, *Hippocampus abdominalis* (Teleostei: Syngnathidae) in captivity. N. Z. J. Mar. Freshwater Res. 34, 475–485.

Zar, J.H., 1998. Biostatistical Analysis, 4th edn. Prentice Hall, New York, 663 pp.