DEVELOPMENT AND STANDARDISATION OF CULTURE TECHNIQUES FOR CONSERVATION OF YELLOW SEAHORSE, *HIPPOCAMPUS KUDA* (BLEEKER, 1852)

A Thesis submitted to
GOA UNIVERSITY
for the Award of the Degree of
DOCTOR OF PHILOSOPHY
in
MARINE SCIENCES

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Goa University,
Taleigao Goa
March, 2014

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Taleigao Goa
March, 2014

STATEMENT

As required under the University Ordinance 0.19.8 (vi), I state that the present thesis entitled "**Development and standardisation of culture techniques for conservation of yellow seahorse, *Hippocampus kuda* (Bleeker, 1852)**" is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge, the present work is the first comprehensive work of its kind from the area mentioned.

The literature related to the problems analyzed and investigated has been appropriately cited. Due acknowledgements has been made wherever facilities and suggestions has been availed of.

Place: CSIR-NIO, Dona Paula

Date: 28/03/2014

Hrishikesh Babasaheb Pawar

वैऔअप - राष्ट्रीय समुद्र विज्ञान संस्थान
(वैज्ञानिक एवं औद्योगिक अनुसंधान परिषद)

CSIR-national institute of oceanography
(Council of Scientific & Industrial Research)

Certificate

This is to certify that the thesis entitled ***"Development and standardisation of culture techniques for conservation of yellow seahorse, Hippocampus kuda (Bleeker, 1852)"*** *submitted by* ***Mr. Hrishikesh B. Pawar*** *for the award of the degree of* ***Doctor of Philosophy*** *in* ***Marine Sciences, Goa University, Goa*** *is based on original studies carried by him under my supervision. The thesis or any part thereof has not been previously submitted for any degree or diploma in any universities or institutions.*

Place: Dona Paula
Date: 28/03/2014

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"Give a fish to man, and he has food for a day; teach a man to breed fish, and he has food for himself and his family for the rest of their lives."

(Paraphrase of Chinese proverb)

Dedicated to

Late P. R. Kurle

Engine Driver, NIO

For his dedication and willing hand during the entire work

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(Hrishikesh B. Pawar)

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GENERAL INTRODUCTION AND

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CHAPTER 1

GENERAL INTRODUCTION AND REVIEW OF LITERATURE

Seahorses are fascinating and remarkable group fishes, mainly because of their unique body shape (horse-like head, body like a caterpillar, tail like monkey and kangaroo-like brood pouch) (Plate 1.1). Although they are bony fish, they do not have scales; rather a thin skin stretched over a series of bony plates arranged in rings throughout their body (Plate 1.2), each species having a distinct number of rings. They have a horse like head positioned at a right angle to an erect body; eyes that swivel independently much like the chameleon's; a long tubular snout (without teeth) that sucks food; a digestive tract without a differentiated stomach (Rauther, 1925; Stoskopf, 1993). Seahorses swim upright, they swim very poorly by using a dorsal fin, which they rapidly flutter to propel them, and pectoral fins located behind their eyes, which they use for stabilization and steering. Seahorses have no caudal fin, therefore they are poor swimmers. Male and female seahorses are commonly the same size. Males consistently have a relatively longer tail, while females have a relatively longer body trunk. A longer tail may enable a male to support a large caudal pouch while grasping a holdfast, or may give males an advantage in the tail-wrestling exhibited during mating competitions (Vincent, 1990).

1.1 TAXONOMIC CLASSIFICATION:

Seahorses, pipefishes, pipe-horses and sea-dragons are members of the family Syngnathidae, which includes 215 species under 52 genera. The entire family Syngnathidae falls within the order Gasterosteiformes (Plate 1.3). All seahorses have been placed under a single Genus, *Hippocampus*, worldwide total 46 species of seahorses

Plate1.1 Male and female of *Hippocampus kuda*

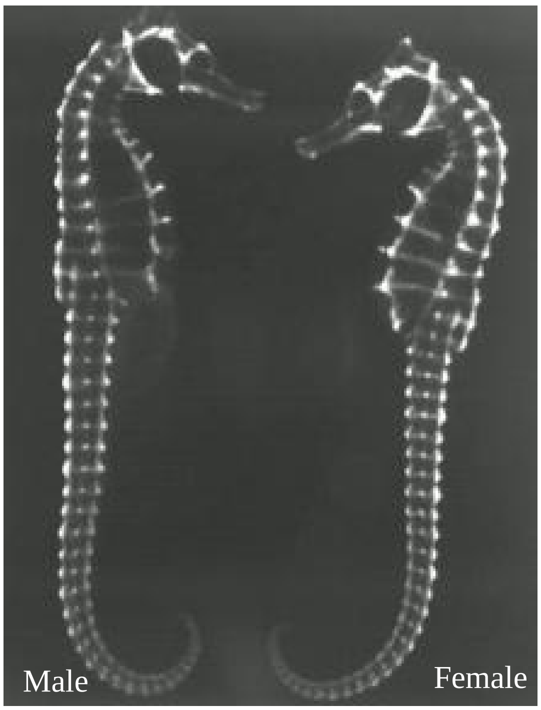


Plate1.2 Skeleton structure of male and female *Hippocampus kuda* (X-ray image)

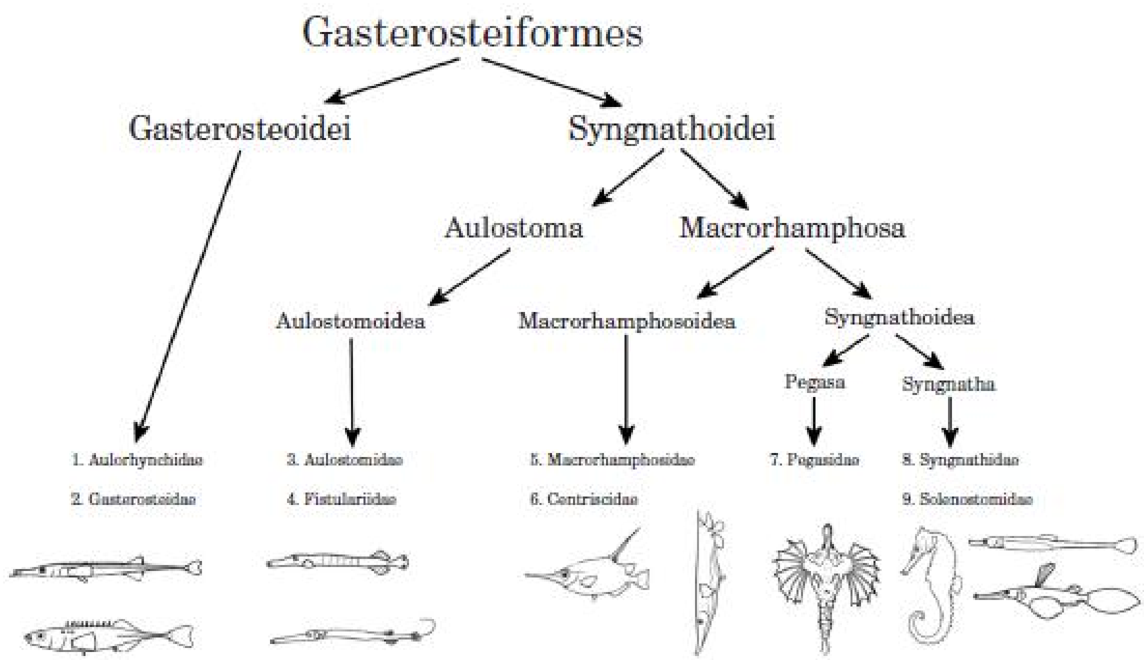


Plate 1.3 Phylogeny of the order Gasterosteiformes (based on Orr, 1995)
(Surce:Foster and Vincent, 2004)

are found (Kuteir, 2009). Seahorses ranged in size from the tiny *Hippocampus denise* (<20mm Ht) to the large *H. abdominalis* (>300mm Ht). Indian waters harbour ten seahorse species *viz. H. kuda, H. trimaculatus, H. fuscus, H. borboniensis,H. histrix, H. kelloggi, H.spinosisssimus, H. mohnikei, H. montebelloensis* and *H. camelopardalis* (Plate 1.4) (Murugan et al., 2008).

1.2 DISTRIBUTION:

Seahorses especially occur in estuary and coastal habitats (6 to 35 ppt salinity), in shallow temperate and tropical waters. They are distributed from 50° N to 50°S, while the highest diversity of species occurs in the Indo-Pacific region (Lourie et al., 1999). While individuals of most seahorse species were found in shallow waters (<30m depth), *H. kelloggi* were reported to depths of 90m in Malaysian waters (Choo & Liew, 2003), and *H. minotaur* were reported from trawls at 100m in Australia (Gomon, 1997).

Along Indian coast seahorses occurs with preference for Palk bay and Gulf of Mannar (Tamil Nadu); whereas sporadic and patchy distributions were observed in Maharashtra, Goa, Karnataka and Kerala as well as reported from Anadaman and Nicobar and Lakshadweep islands (Sreepada et al., 2002; Murugan et al., 2011) (Plate 1.5).

1.3 HABITAT:

Seahorses inhabit many ecologically sensitive aquatic habitats, including coral reefs, seagrasses, mangroves and estuaries (Plate 1.6). They primarily occupy inshore habitats in narrow strips along the coast. Seahorses were regularly reported from the seagrass meadows, whereas very rarely reported from mangroves. Coral reefs are the most commonly preferred habitat for tropical seahorse species whereas, temperate species inhabited seagrasses and algae (Vincent, 1996). Seahorses are also reported from sponges, sea quirts and gorgonian habitats. *H. bargibanti* is a unique kind of seahorse which needs a very specific habitat; it has only been reported from two species of gorgonid corals.

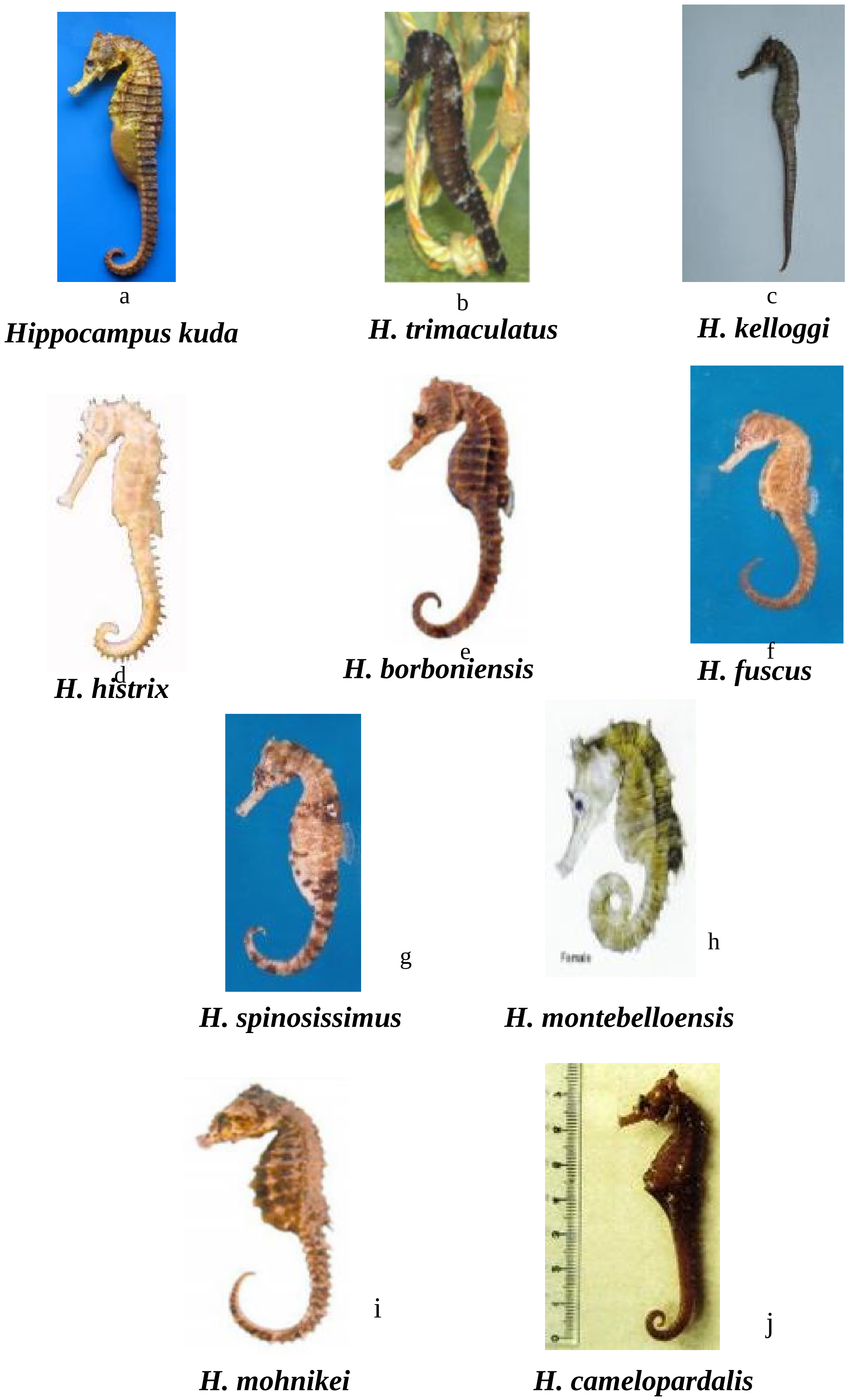
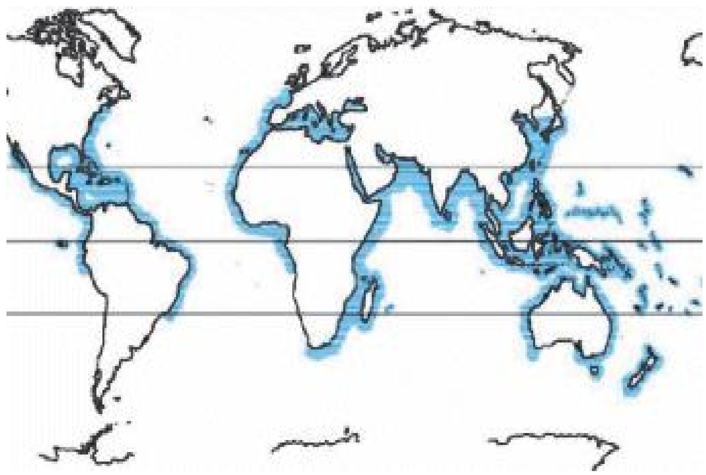


Plate 1.4 Seahorse species reported from India

Source: c,d,e,f,g,h Murugan; j http://www.poseidonsrealm.com/



Source: www.projectseahorse.org

Plate 1. 5 Seahorse distribution around the world

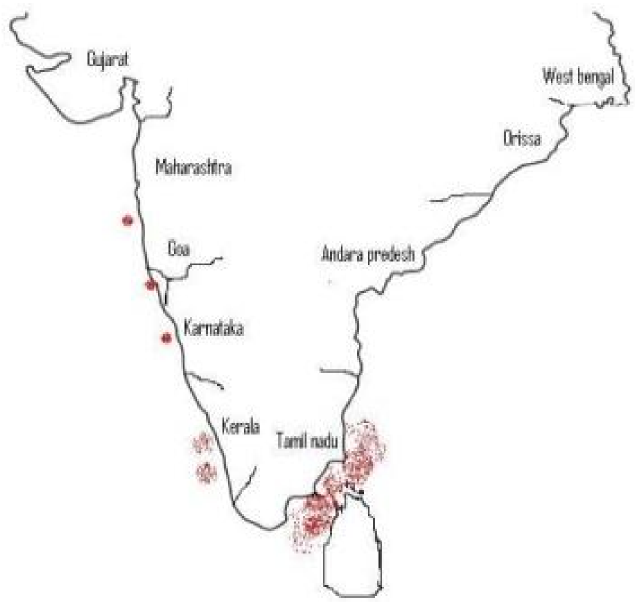


Plate 1. 6 Seahorse distribution around India

a

Coral

b

Seagrass

c

Pneumatophore of Mangrove

d

Sponges

e

Gargonids

Plate 1.7 Seahorse natural habitats

Source: a, b, c and e www.projectseahorse.org

They are most likely to be found resting with their prehensile tails wound around a stationary object (holdfast). Holdfasts could be anything from a sponge to a branching coral, a piece of seagrass or a submerged tree branch. Some species exhibited preferences for particular holdfasts – for example, *H. comes* usually grasped sponges in communities dominated by seagrasses.

1.4 CAMOUFLAGE:

Seahorses bob around in seagrass meadows, mangrove stands, and coral reefs where they are camouflaged by murky brown and grey patterns that blend into the seagrass backgrounds; probably facilitating both prey capture and predator avoidance. They remain virtually immobile for much of the time, can change colour over a few days or weeks to match their background better, and sometimes have long skin filaments to blend better with their habitats. Most seahorses are beige, brown or black, but species specific colour changes include fluorescent orange and deep purple. For example, *H. capensis* individuals were usually mottled brown with darker patches, but were also found in black, green, orange, white or yellow colour forms (Bell et al., 2003). *Hippocampus bargibanti* mimics in colour and form the stalks and polyps of the gorgonian coral to hitch; it clings using its tail (Whitley, 1970). Encrusting organisms sometimes grow on the skin of a seahorse, improving the fish's camouflage (Rauther, 1925). Seahorses often changed colour rapidly when interacting with each other. Both sexes of *H. whitei* brightened in colour from a dark brown or grey to a pale yellow or off-white during pair-wise encounters in the wild (Vincent and Sadler, 1995). During social moments or in unusual surroundings, seahorses turn bright colors.

1.5 HOME RANGE:

Seahorses are poor swimmers, so their home range is very small. Comparatively, the female has a larger home range than the male, a field study on *H. white* showed that the female home range is 6 to 21 m^2 whereas males roam in 4 to 17 m^2 areas (Foster and Vincent, 2004). The small home ranges of *H. breviceps, H. guttulatus, H. hippocampus, H. reidi* and *H. whitei* indicate limited daily movements similar to those in other monogamous syngnathid species (Foster and Vincent, 2004). It is considered that small home ranges have enabled the seahorses to adopt camouflage appropriate for their environment, and to maintain a stable social structure (Foster and Vincent, 2004). Adult dispersal over large distances appeared primarily to occur when adults were cast adrift by storms or carried away while grasping floating debris. Adult *H. fisheri* have been caught at the surface in the open sea (Lourie et al., 1999), while the occurrence of *H. ingens* in the stomachs of tuna suggests they were occasionally found in the pelagic zone (Alverson, 1963), possibly associated with rafts of drifting seaweed. Young seahorses are more likely to disperse than adults. Some species were clearly planktonic immediately after birth, as juveniles were found in plankton samples. Most of the seahorse species are active during the day time, whereas only *H. ingens* were found to be nocturnal and *H. abdominalis* and *H. comeswere* reported active during day and night.

1.6 PREDATORS:

Sub-adult and adult seahorses are presumed to have few natural predators because of their camouflage capabilities, and unpalatable bony plates and spines (Lourie et al., 1999). However, seahorses were reported in the stomachs of large pelagic fishes such as red snapper (Jordan and Gilbert, 1882), dorado, rays, skates, tuna (Herald 1949; Alverson, 1963; Wilson and Beckett, 1970) and dolphin fish. Partial predation by crabs may be a threat to seahorses, as indicated by direct observations of seahorses with shortened tails (Baum et al., 2003). Predation mortality was probably greatest in juveniles that were highly vulnerable to piscivorous fish and planktivorous organisms. The life spans of seahorses, influenced by predation and disease, inferred life spans for seahorse species ranged from about one year in the very small species *H. zosterae* (Strawn, 1953, in situ) to an average of 3 to 5 years for larger species (*H. capensis*, Lockyear et al., 1997).

1.7 REPRODUCTIVE BIOLOGY:

Seahorses are so named for their unique reproductive behaviour, in which the males become pregnant. The male fertilizes and broods the eggs produced and deposited into its pouch by the female during courtship and mating, and in turn delivers the hatchling, after a long period of pregnancy and labour. Most species of seahorses studied so far show unique sexual fidelity and form faithful pair bonds (Vincent and Sadler, 1995), in which case one male and one female mate repeatedly and exclusively giving up opportunities to interact with non-partners. Male pregnancy and monogamy are the extraordinary myths also surrounding them.Smaller seahorse species, such as *H. zosterae*, appeared to mature at 3 months (Strawn, 1953). *H. barbouri, H. fuscus* (Wilson and Vincent, 1998), reached maturity at 4 or 5 months, while many other species were thought to start breeding in the season after birth, at 6 months to 1 year (*H. kuda and H. mohnikei*, Jiaxin, 1990; *H. spinosissimus*, *H. trimaculatus*, Truong andNga, 1995; *H. capensis*, Whitfield, 1995; *H. erectus*, Gardiner, 1998; *H. abdominalis*, Woods, 2000a).

1.8 FOOD AND FEEDING:

Seahorses are ambush predators, and consume primarily live, mobile prey types. While feeding within the water column, they wait until prey comes close to the mouth, whereupon they are drawn up into the long snout with a rapid intake of water. Seahorse eyes move independently of each other, enabling the seahorse to maximize its search area and/or to monitor its environment. They ingest any organism small enough to fit into their snout; mostly small crustaceans such as amphipods, but also fish fry and other invertebrates and they change their diet ontogenetically.

1.9 USAGES:

Ironically, seahorse popularity places them in danger, as they are required in large numbers for use in Traditional Chinese Medicine (TCM), as aquarium fish and curios (Plate 1.7). Sea horses are used as an ingredient in traditional medicine, particularly in China, Japan, Hong Kong, Singapore, Korea, Malaysia and Taiwan. The TCM is used by one fourth of the world population and it is recognized as valid medicine by the World Health Organization (WHO). Seahorses are being used for increasing and balancing vital energy flows within the body, as well as a curative role for such ailments as impotence and infertility, asthma, high cholesterol, goiter, kidney disorders, and skin afflictions such as severe acne and persistent nodules. They are also reported to facilitate parturition, act as a powerful general tonic and as a potent aphrodisiac. Live seahorses are also used as ornamental fish particularly in public aquaria and part of dried seahorses are used as souvenirs. Along Ramnad coast in Tamil Nadu, dried seahorse is used as a medicine to arrest whooping cough in children.

1.10 TRADE:

The world trade of dried seahorses was estimated to be over 20 million individuals (Vincent, 1996). The trade has been expanding globally at a fast pace and the demand exceeds supply. India has a long history of trade on seahorses, which originated along the Southeast coast, particularly in the state (province) of Tamil Nadu. Limited quantities of seahorses were also reported to be collected from the coast of Kerala, and off Maharashtra and Karnataka (Vincent, 1996). A well-established seahorse trade emerged along the South Tamil Nadu coast, especially the Palk Bay and Gulf of Mannar areas, as an alternate fishery to the dwindling resources of sea cucumber (*Holothuria* spp.) along these coasts (Marichamy et al., 1993).

Since the mid-1990s, there has been mounting concern over the decline in some exploited wild stocks of seahorses. In 1995, it was conservatively estimated that more than 56 metric tonnes of seahorses (20 million numbers) were caught for the traditional medicine market. In addition, more than one million live seahorses are caught for aquarium trade, mostly destined for sale in North America, Europe, Japan and Taiwan. The value of seahorses is quite high; the price of dried seahorses in Hong Kong markets is around US$ 150 per kg (Koldway and Martin-Smith, 2010) depending on the species, quality and size. About 80 countries are involved in seahorse exploitation and trade. In response to decline in seahorse supplies in Asian waters, new countries such as Ecuador, Guinea, Jordan, Maldives, Mozambique, Nigeria, Peru and Senegal are now entering the seahorse trade.

In response to a significant increase in international demand, a target fishery for seahorses along the east coast of India in the Gulf of Mannar was started in 1992. Till year 2000 India was one of the largest exporters of dried seahorses globally, exporting at least 3.6 tonnes (~ 1.3 million sea horses) annually, and contributing to about 30% of the global seahorse trade. There is also a significant trade in seahorses as aquarium fishes, as supplements in some specialized cuisine and as curios. Seahorses are exploited both as an incidental catch (by-catch in trawl nets) and target catch, for export. Presently, the exploitation of seahorses is banned. Demand for medicinal purposes has increased 10-fold during the 1980s and continues to grow at an annual rate of about 8% in China alone, predominantly due to China's economic boom which promotes increased consumer-spending on traditional medicines. A similar trend is expected in other countries with large Chinese populations. Such high exploitation rates in India, Indonesia, Philippines, Thailand and Vietnam have resulted in a decline in the population of seahorses by 25-75% over the period of five years. There is also a substantial reduction in the size of the harvested individuals and increased removal of immature males. The indiscriminate exploitation of seahorses coupled with a general degradation of their habitats put their populations under pressure (Plate 1.8).

1.11 GLOBAL SCENARIO OF SEAHORSE AQUACULTURE:

Global scenario of seahorse aquaculture was well explored in a review by Koldewey and Martin-Smith, (2010). According to Koldewey and Martin-Smith (2010) due to increasing demand and trade for seahorses in TCM, aquarium fishes and curios, there has been considerable expansion in the number and size of aquaculture operations and the number of species cultured. According to Moreau et al., 1998, members of the TCM community have indicated a willingness to accept aquaculture animals, although there is a view that these may be less efficacious than those that are wild-caught. In fact, many TCM traders are looking for seahorse aquaculture to meet their requirements. According to Fan (2005), the Shantou Mariculture Test Farm in Guangdong province of South China during the year 1957, was the first facility to produce the first captive-born seahorse, *H. trimaculatus*, which is a widely used seahorse in the TCM industry. However, commercial seahorse breeding was started in the 1970s particularly in China and early 1980s literature from mainland China conveyed the impression that seahorse culturing was well understood (Aquaculture Institute of Shanghai, 1982; Wu and Gu, 1983; Shandong Marine College, 1985; Publicity and Aquaculture Society, 1990). Commercial development and considerable expansion of seahorse aquaculture occurred in the 1990s particularly in Australia, New Zealand and USA. As per a Koldewey and Martin-Smith, (2010) review, 13 species of seahorses were cultured commercially and two species in research. In total 11 countries - Australia, Brazil, Hong Kong, Ireland, Mexico, New Caledonia, New Zealand, Sri Lanka, UK, USA and Viet Nam were recorded as the source of captive-bred seahorses. Seven species, *H. abdominalis, H. barbouri, H. breviceps, H.*

comes, H. ingens, H. kuda and *H. reidi* accounted for more than 99% of the internationally-traded, captive-bred, live animals. Australia and New Zealand were major centers of seahorse aquaculture, defined by the number of operations, with Mexico and China also with multiple operations (Koldewey and Martin-Smith, 2010).

1.12 INDIAN SEAHORSE AQUACULTURE REVIEW:

Breeding of seahorse is simple when compared to other marine ornamental fishes because they are free from the complicated process of sex reversal and sex role reversal (Murugan et al., 2009). Seahorse aquaculture in India has a great potential to integrate both conservation and sustainable development and provide alternative livelihood options to fisher folk as well as in promoting the marine aquarium industry which is still in the hibernation stage even though we have vast resources. After searching Aquatic Science and Fisheries Abstracts (ASFA), Web of Science and popular search engine Google for seahorse research in India, it appears that research on seahorses includes: genetics and population dynamics (Thangaraj and Lipton, 2004; Goswami et al., 2009; Kumaravel et al., 2010a, b; Thangaraj and Lipton, 2010; Thangaraj and Lipton, 2011a; Singh et al. 2012; Thangaraj et al., 2012), Taxonomy and distribution (Salin and Mohanakumaran 2006; Thangaraj and Lipton 2007; Murugan et al., 2008; Murugan et al., 2011; Krishnan et al., 2011; Singh et al., 2012; Yogeshkumar and Geetha, 2012; Rajagopal et al., 2012; Lipton and Thangaraj, 2013), Diseases (Thampiraj 2002; Thampiraj & Lipton 2005; Thampiraj et al., 2010; Sanaye et al., 2013), Ecology and morphometrics (Salin et al., 2005; Lipton and Thanaraj 2007; Bijukumar et al., 2008; Kumaravel et al., 2010c; Thangaraj and Lipton 2011b), Captive rearing, breeding and husbandry (Anil et al., 1999; Ignatius et al., 2000; Naik et al., 2002; Gokulakannan, 2002; Ignatius and Jagadis 2003; Salin et al., 2005b; Lipton et al., 2006; Dhamagaye et al., 2007; Thangaraj and Lipton 2008; Murugan et al., 2009; Pawar et al., 2011, 2012; Tanu et al., 2012; Thangaraj et al., 2012 and Murugan et al., 2013). From all the above mentioned literature, it seems that several attempts were made on eco-biological and captive condition studies on seahorses in India, with limited success.

As per published literature, seahorse research is primarily limited to four institutions from India, *viz*., Central Marine Fisheries Research Institute (ICAR- CMFRI), Cochin, Center for Advanced Study in Marine Biology (CAS), Annamalai University, TamilNadu, Marine Biological Research Station (MBRS), Ratnagiri, Maharashtra and Aquaculture Laboratory, CSIR- National Institute of Oceanography (CSIR-NIO), Goa. As the literature suggests, attempts have been made to breed and rear only three species of seahorses (*H. kuda*, *H. trimaculatus* and *H. kelloggi*) and achieved limited success. Seahorse captive breeding and rearing efforts from various researchers from India is summarized in Table 1.1.

Experimental breeding of seahorse started in the late nineties with efforts from Anil et al., 1999 for yellow seahorse, *H. kuda*. From year 2000 to date various researchers -from different institutions have worked for better understanding of seahorse breeding biology and mass scale rearing development. The main species of research is the yellow seahorse, *H. kuda*, due to its wide distribution and abundance in coastal waters of India (Murugan et al., 2008; Pawar et al., 2011). Till date only four species namely, *H. kellogii* (Balasubramanian, 2002), *H. kuda* (Anil et al., 1999; Ignatius et al., 2000; Naik et al., 2002; Gokulakannan, 2002; Ignatius and Jagadis 2003; Salin et al., 2005; Lipton et al., 2006; Dhamagaye et al., 2007; Thangaraj and Lipton 2008)*; H. spinosissimus* (Unpublished data) and *H. trimaculatus* (Murugan et al., 2009) were studied so far for their biology and captive breeding trial. Breeding trials with the larger sized seahorse species *H. kelloggi* have not succeeded mainly because of the policy hindrances as well as

Table 1.1 Seahorse captive breeding and rearing research published in various journals and Ph. D thesis in India

Author	Species	Year	Institute/University	Source
Anil et al.	*H. kuda*	1999	Karwar Research Station of Central Marine Fisheries Research Institute (CMFRI)	Marine Fisheries Information series no. 162, CMFRI
Ignatius et al	*H. kuda*	2000	Regional Centre of CMFRI, Mandapam Camp,	Marine Fisheries Information series no. 163, CMFRI
Balasubramanian, R	*H. Kelloggi*	2002	Centre of Advanced Study (CAS) in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu	PhD Thesis. 124 pp
Naik et al.	*H. kuda*	2002	Marine Biological Research Station (MBRS), Dr. B.S. Konkan Agriculture Univeristy, Ratnagiri, India	Economica and Environmental Conservation, 69-72
Ignatius and Jagadis	*H. kuda*	2003	Regional Centre of CMFRI, Mandapam Camp, Tamil Nadu	Indian Journal of Fisheries, 50(3):369-372
Salin K. R.	*H. kuda*	2003	Central Institute of Fisheries Education (Deemed University), Mumbai, India	PhD Thesis
Salin et al.	*H. kuda*	2004	Calicut Research Center of CMFRI, Kerala	Ocean Life Food and Medicine Expo 2004 Proceedings, 368-383.
Murugan A.	*H. trimaculatus*	2004	CAS-Marine Biology, Annamalai University, Parangipettai, Tamil Nadu	PhD Thesis. 176 pp
Lipton et al.	*H. kuda*	2006	Vizhinjam Research Centre of CMFRI	Asian fisheries Science, 19: 423-428
Dhamagaye et al.	*H. kuda*	2007	MBRS, Dr. B.S. Konkan Agriculture Univeristy, Ratnagiri, India	Asian fisheries Science, 20: 1-6
Thangaraj and Lipton	*H. kuda*	2008	Vizhinjam Research Centre of CMFRI	The Israeli Journal of Aquaculture Bamidgeh 60(3): 185-189
Murugan et al.	*H. trimaculatus*	2009	CSIR-National Institute of Oceanography, Goa and Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu	Aquaculture 290:87-96
Pawar et al.	*H. kuda*	2011	CSIR-National Institute of Oceanography, Goa	The Israeli Journal of Aquaculture Bamidgeh
Murugan et al.	*H. trimaculatus*	2013	CAS-Marine Biology, Annamalai University, Parangipettai, Tamil Nadu and National Institute of Oceanography, Goa	Indian journal of Animal Science

the constraints to collecting this animal alive since they are caught only in bottom trawl net in deeper sea.

In earlier trials, maintenance of the adult seahorses which were collected from wild was the main challenge in front of researchers (Anil et al., 1999; Ignatius et al., 2000; Naik et al., 2002). Adult seahorses were fed most concisely with adult *Artemia*, amphipods, mysids and various fish larvae collected from wild or cultured inside the hatchery complex. Wild collected pregnant males were often juveniles released 7-8 days after collection. Rearing juveniles which are released by collected pregnant males is a critical task and very low survival and growth was obtained due to lack of proper knowledge about seahorse rearing (Anil et al., 1999; Naik et al., 2002; Ignatius and Jagadis, 2003). At the same time published research from developed countries on seahorse breeding and rearing (Payne and Rippingle, 2000; Woods, 2000a, b; Job et al., 2002) helps to overcome some husbandry related problems. Though survival was low in initial trials (Anil et al., 1999), all environmental conditions like water quality parameters, light-dark cycles and live food organism supply have been developed for Indian seahorse rearing protocols. Use of different live food organisms and culture protocols in seahorse breeding and culture development by Indian researchers is summarized in Table 1.2. Seahorse juveniles are fed most commonly with rotifers, marine copepods and freshly hatched *Artemia* for the initial period of 7-21 days. Recent success in large scale rearing of *H. kuda* (present thesis) and *H. trimaculatus* (Murugan et al., 2009) by investigating different husbandry parameters, including feeding regimes and rearing protocols, will help in development of successful large scale seahorse aquaculture.

Though seahorse captive breeding and aquaculture production is well understood in developed countries, work done in present thesis has great potential as results obtained from seahorse captive breeding and rearing studies are more practical for Indian climatic conditions and resources available.

Table 1.2 Different husbandry parameters and culture protocols for seahorse rearing developed in India, based on published research (A: Adult; J: Juveniles; Jp: pelagic juveniles; Js: Settled juveniles; FRP: Fiber Reinforced Plastic ; L :Light ; D :Dark; d:Days)

Author	Species	Life Stage	Tank size (L)	Tank material	Water and culture parameters					Food items		Survival
					Water temp	Salinity	pH	Photo period	Stocking density/ L	Adult	Juveniles	
Anil et al., 1999	*H. kuda*	A	10000	Plastic pool	26.5-28	32±2	7.9-8.3	-	-	Brine shrimp adult, amphipods, mysids, prawn and fish larvae	Copepods, cladecerons and crab larvae to 2d, freshly hatched *Artemia*nauplii to 2 week, 3-5 d old *Artemia*nauplii grown in mixed algal culture	-
		J	150	Glass	-	-	-	-	10			24% to 70 %
Ignatius et al., 2000	*H. kuda*	A	1000	Glass	28-32	33-35	-	-	-	Mysid, *Artemia*, small crusteceans	Algae with rotifer to 1d, copepod and rotifer to 7d,*Artemia*nauplii to 30d, mysid, *Artemia* and prawn PL	-
		J	1000	FRP tank	-	-	-	-	-			-
Naik et al., 2002	*H. kuda*	A	1200	Glass	25	-	-	-	-	Adult *Artemia*,caridean prawns, guppy fish fry	freshly hatched *Artemia*nauplii, marine cladocera, copepods, caridean larvae to 15d	-
		J							3			-
Ignatius and Jagadis, 2003	*H. kuda*	A	864	Glass	-	-	-	-	-		Copepod and rotifer	
		J	10000	FRP	26-29	30-34	8-8.6	-	-			46.7 at 176d
Salin et al. 2005	*H. kuda*	A	-	-	-	-	-	-	-	-	*Artemia*nauplii and mixed marine copepods*	-
		J	45	Glass	-	30	-	-	2			56% at 60d
Lipton et al., 2006	*H. kuda*	A	1000	FRP	-	-	-	-	-	Adult *Artemia*, mysid and tilapia juveniles	*Artemia franciscana* nauplii to 21d, 4-6 d old *Artemia* to 30d	-
		J	-	-	27.5±0.85	37±1.5	8.03±0.26	-	-			65.22% at 30d
Thangaraj et al., 2006	*H. kuda*	A & J	1000	FRP	26.5-28.0	36.5-37.0	7.8-8.3	12L : 12D	-	-	*Artemia*, mysids	-
Dhamagaye et al., 2007	*H. kuda*	J	27	Glass	27-31	30-32	7-8	-	2	-	Freshly hatched *Artemia*nauplii + rotifer	100% at 15 d
Thangaraj and Lipton, 2008	*H. kuda*	J	25	Plastic	26±0.85	36±0.5	8±0.26	12L : 12D	Four 60d old/10L	-	Wild *Artemia* and mysids	96.7% at 90d
Murugan et al. 2009	*H. trimaculatus*	A	2000	FRP	30.2±0.5	32±1.5	7.6±0.3	16L :8 D	-	Amphipods, Acetes, fish larvae and insect larvae	-	-
		Jp	45	FRP	-	-	-	24L :0 D	2	-	Rotifers, *Artemia*, wildzooplankton	65% at 182d
		Js	188	FRP	30±02	32±1.5	7.4±0.8	16L :8 D	1	-	*Artemia* and copepodites	
Murugan et al., 2013	*H. trimaculatus*	J	250000	-	28±0.5	31±0.4	7.5±0.3	16L :8 D	-	Wild amphipods	-	-

1.13 CONSERVATION MEASURES:

Seahorse biological characteristics such as slow growth rate (they take one year to attain maturity), low natural adult mortality, structured mating patterns (monogamous), low fecundity – with each pair producing about 1000 young per year (very low compared to other fishes), lengthy parental care, sparse distribution, limited mobility, and site fidelity make them vulnerable, particularly to selective fishing pressure (Plate 1.9). The UCN Red List of Threatened Animals includes most of Indo-Pacific sea horse species. The Red List draws the conservation concerns about species, but has no direct legislative or legal implications for trade. In India, fishing of seahorses is banned by the Wild Life Protection Act of 1972.

1.14 SCOPE FOR THE PRESENT STUDY:

Sound knowledge of the life history of species is essential for their conservation and management. Theoretical and empirical analyses show that certain life-history characteristics make species more vulnerable to over-exploitation (Jennings et al., 1999; Sadovy, 2001; Denney et al., 2002). For example, comparative studies have indicated that, all else being equal, species with low rates of natural population increase and small geographic ranges, complex social behaviour and distributions in vulnerable habitats are more likely to be overfished (Sadovy, 2001). In general, populations of species that are common and widely distributed with broad dietary and breeding needs (generalists) are more likely to be resilient to exploitation and disturbance than populations of species with limited distributions and specific needs (specialists).

Decline in wild populations of seahorses have occurred, particularly in western Atlantic and Indo-Pacific waters (Alverson et al., 1994; Vincent, 1996, 1997; Baum et al., 2003; Martin-Smith et al., 2004). Some factors responsible for population decrease are considered to be: fishing pressure for commercial trade, by-catch in fisheries, and degradation and loss of habitat (CITES, 2002). In addition to that, biological features like

Wind trawl **Mechanized trawl**

Gill net **Shore seine**

Castnet **Skin-diving**

Plate 1.8 Fishing methods and its impact on seahorse population

sparse distribution, low mobility, small home ranges, low fecundity, lengthy parental care, mate fidelity and pregnancy of the male rather than the female seahorse. All recognized seahorse species are currently listed on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and are included on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN, 2006). Knowledge of the biological characteristics of affected species would be of major importance in conservation actions, development of breeding programmes and recovery of wild populations. Unfortunately, information available on the distribution, biology and rearing of seahorses is generally scarce and limited to a few seahorse species (Foster and Vincent, 2004).

Seahorses are valued as ornamental fish when alive and dried it forms a major ingredient in the multimillion dollar Traditional Chinese Medicines market. So far, the seahorses are caught only as a by-catch. Because of increasing demand, now a days target fishing is done in many places. Scrutiny of existing literature revealed that less information is available on the global seahorses and very little is available on the taxonomy, reproductive biology, mass scale culture, life cycle of seahorses from Indian waters.

To fill up this lacuna, the present study was undertaken with following objectives.

OBJECTIVES

1. To study the reproductive behaviour of yellow seahorse, *H. kuda* in captivity.
2. To standardise hatchery technique of *H. kuda.*
3. To standardise mass scale culture technique of *H. kuda*.
4. To complete life cycle (F2 generation) of *H. kuda* in captivity.

CHAPTER 2

LENGTH-WEIGHT, LENGTH-LENGTH RELATIONSHIPS AND MERISTIC ANALYSIS

CHAPTER 2

LENGTH-WEIGHT, LENGTH-LENGTH RELATIONSHIPS AND MERISTIC ANALYSIS

2.1 INTRODUCTION:

Seahorses, pipefishes, pipehorses and seadragons are members of the family Syngnathidae, which includes 320 species under 56 genera (Kutier, 2009). The entire family Syngnathidae falls within the order Syngnathiformes. Seahorses, representing the subfamily Hippocampinae, are characterized by a fully enclosed brood pouch with a small opening for the incubation of eggs, vestigial caudal fin, and a prehensile tail (Lourie et al. 1999b, 2004). All seahorses have been placed under one genus, *Hippocampus* and a total of 46 species of seahorses are found around the world (Kutier, 2009). *Hippocampus kuda* is one of the most complicated species of seahorse because the name *H. kuda* was used for all non-spiny seahorses in the Indo-specific. Previous taxonomic work has suggested that at least 15 names for apparent species were merely the synonyms for *H. Kuda*. Lourie et al. (1999) isolated *H. barbouri, H. borboniensis, H. comes, H. fisheri, H. fuscus and H. kelloggi* as species that have been subsumed into the *H. kuda* complex. The escalating trade and worldwide decline of seahorse populations reiterate the need to resolve seahorse taxonomy (Bijukumar et.al, 2008). Identification will make it easier to modify fishing practices appropriately, design protective marine reserves, assess captive breeding potential for seahorses, and develop protective legislation (Anonymous 2008, Bijukumar et.al, 2008).

The length–weight relationship is widely used in fisheries research because it allows the conversion of growth-in-length equations to growth-in-weight and use in stock assessment models (Pauly, 1993; Petrakis and Stergiou, 1995) as well as helps in estimation of biomass from length observations. Length weight relationships are often used to estimate the condition indices, for life history and morphological comparison of populations from different regions (LeCren, 1951; Anderson and Gutreuter, 1983; Petrakis and Stergiou, 1995). Relationships between different types of lengths (length–length relationships), for which little information seems to be available for *H. kuda*, are also very important for comparative growth studies (Froese, 2006).

The present study describes the morphometrics and meristic characters, length-weight and length-length relationships in wet and dry conditions of *H. kuda* from Ratnagiri, North-west coast of India.

2.2 MATERIALS AND METHODS:

Length-length and length-weight relationships for wet and dry *H. kuda* specimens were collected from the Mirya creek (17^{o} 02’ 52’’ N, 73^{o} 16’ 08’’ E) of Ratnagiri, India. Morphometric measurements (Fig. 2.1) were taken on 31 male and 27 female sundried seahorses and 29 male and 30 female wet seahorses. Height (Ht), standard length (SL), trunk length (TrL), hand length (HL) and tail length (TL) measurements were taken by thread. Whereas, snout length (SnL), snout depth (SnD), head depth (HD), spine width (SW), pectoral fin base length (PL) and dorsal fin base length (DL) measurements were taken with the help of caliper (Lourie *et al.*, 1999). Coronet height (CH) was measured as the vertical distance between tip of cornet to first spine of cleithral ring. Trunk depth on 4th trunk ring (TD4) was recorded as narrowest distance between the superior and inferior trunk ridges on

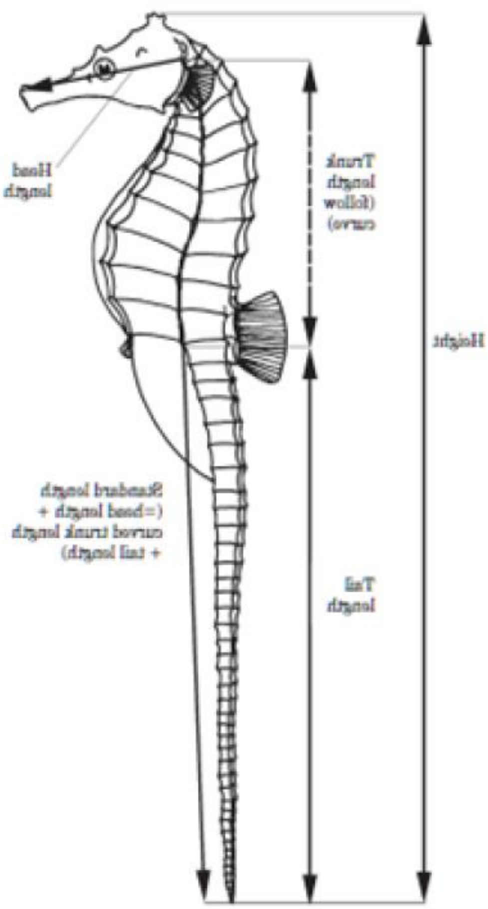


Plate 2.1 Morphometric measurements on seahorse (based on Orr, 1995)
(Lourie, 2003)

the 4th trunk ring whereas trunk depth on 9th trunk ring (TD9) was recorded as narrowest distance between the superior and inferior trunk ridges on the 9th trunk ring. Meristic counts like trunk rings (TaR), tail rings (TaR), dorsal fin rays (DF), pectoral fin rays (PF) and anal fin rays (AF) were taken. Length measurements were taken to 1 mm accuracy whereas weight measurements were recorded to 0.1 mg accuracy.

2.3 RESULTS:

Cornet is round, medium sized and overhanging towards the back with a cup like depression in the top. Live specimens observed, which were collected from sargasssum bed of Mirya creek, were bright yellow, orange with small spots and greenish black in colour. On wet seahorses not real spines, but prominent round bumps with a band like patch on 1, 4, 7 and 11th trunk rings were observed; whereas 5, 9, 13, 17, 20, 23, 26, 29, 32 and 34th or 35th tail rings bumps and patches were observed on dorsal side. Dorsal fin started between 9th and 10th trunk ring and ended between 1st and 2nd tail rings.

2.3.1 MORPHOMETRIC RELATIONSHIPS:

Mean values along with standard deviation, minimum and maximum values of morphometric measurements of male and female *H. kuda* specimens collected from Ratnagiri, North West coast of India, are presented in Table 2.1. The SL, Ht, HL, SnD, HD, TrL, TrD, PFH, AFH, PFBL, DFBL, AFBL, TD4, TD9 and SW of male and female did not show significant difference ($P>0.05$) presented in Table 2.2. Among dry seahorses CH and SL showed significant difference between male and female ($P<0.05$). Female's snout length (1.26±0.02) was significantly bigger than that of the male (1.21±0.02) whereas male's cornet height (0.81±0.02) is more than female's (0.58±0.04). Among wet seahorses HL, SNTL,

Table 2.1 Minimum, maximum, mean and standard deviation (cm) of each length parameter of *Hippocampus kuda*

Morphometric Character		Male			Female			Mean as % of SL
		Min	Max	Mean ± SD	Min	Max	Mean ± SD	
Std. Length	Dry	13.5	20.20	16.70±1.89	13.6	19	16.75±1.42	-
	Wet	12.9	20.50	17.18±1.89	13.3	20.4	17.50±1.55	-
Height	Dry	12.6	18.00	15.06±1.59	12.5	17.2	15.25±1.22	90.63
	Wet	12.1	18.8	15.63±1.64	12.5	18.5	15.85±1.29	90.77
Head Length	Dry	2.52	3.20	2.83±0.20	2.6	3.2	2.87±0.18	17.05
	Wet	2.25	3.30	2.82±0.24*	2.6	3.2	2.97±0.13*	16.71
Snout length	Dry	1.04	1.50	1.21±0.12*	1.05	1.5	1.26±0.11*	7.40
	Wet	0.85	1.40	1.12±0.15*	1.00	1.4	1.24±0.08*	6.80
Snout Depth	Dry	0.30	0.53	0.42±0.05	0.35	0.5	0.41±0.03	2.50
	Wet	0.30	0.50	0.41±0.05*	0.3	0.5	0.44±0.05*	2.44
Head Depth	Dry	1.30	1.90	1.56±0.19	1.28	1.75	1.52±0.12	9.20
	Wet	1.25	1.80	1.46±0.12*	1.35	1.70	1.54±0.10*	8.67
Trunk Length	Dry	3.00	5.50	4.32±0.59	3.5	5.6	4.51±0.51	26.42
	Wet	3.9	5.3	4.54±0.37	4.2	5.4	4.98±0.36	27.44
Tail length	Dry	7.6	11.9	9.58±1.21	7.5	10.8	9.39±0.85	56.65
	Wet	7.6	11.5	9.84±1.03*	7.6	10.9	9.59±0.91*	56.04
Coronet Height	Dry	0.7	0.95	0.81±0.09*	0.35	0.9	0.58±0.20*	4.11
	Wet	0.6	0.95	0.78±0.09*	0.7	1.1	0.85±0.07*	4.69
Pectoral fin height	Dry	0.31	0.6	0.44±0.07	0.35	0.55	0.44±0.05	2.63
	Wet	0.35	0.65	0.53±0.07	0.4	0.5	0.53±0.05	3.04
Dorsal fin height	Dry	0.4	0.7	0.58±0.06	0.45	0.6	0.50±0.03	2.94
	Wet	0.45	0.7	0.56±0.06	0.4	0.65	0.52±0.06	3.11
Anal fin Height	Dry	0.25	0.5	0.37±0.07	0.3	0.5	0.37±0.04	2.23
	Wet	0.5	0.8	0.42±0.05	0.3	0.6	0.42±0.06	2.48
Pectoral fin base length	Dry	0.5	0.72	0.58±0.06	0.5	0.65	0.58±0.07	3.48
	Wet	0.5	0.8	0.63±0.07	0.45	0.80	0.61±0.08	3.58
Dorsal fin base length	Dry	1	1.85	1.39±0.04	1.24	1.65	1.41±0.09	8.37
	Wet	1.15	1.7	1.48±0.15	1.25	1.7	1.48±0.13	8.52
Anal fin base length	Dry	0.15	0.3	0.23±0.3	0.18	0.25	0.20±0.01	1.28
	Wet	0.1	0.35	0.22±0.06	0.2	2	0.46±0.4	1.97
TD4	Dry	0.9	1.4	1.14±1.4	1	1.3	1.17±0.08	6.90
	Wet	0.85	1.35	1.06±0.11	0.8	1.4	1.10±0.13	6.23
TD9	Dry	1.4	2.25	1.77±2.25	1.4	2.1	1.75±0.17	10.53
	Wet	1.5	2.2	1.79±0.17	1.3	2.05	1.79±0.18	1.32
Spine width	Dry	0.5	0.8	0.65±0.8	0.55	0.85	0.66±0.07	3.93
	Wet	0.35	0.8	0.59±0.12	0.45	0.95	0.63±0.10	3.53

Table 2.2 Morphometric relationships of wet and dry *Hippocampus kuda*

aaMorphometric relationships		Male		Female		Pooled	
		r	Regression equation	r	Regression equation	r	Regression equation
Ht on SL	Dry	0.9235	Ht=2.0315 + 0.7801SL	0.9925	Ht=0.9283+0.8549 SL	0.9499	Ht= 1.6032+0.8105 SL
	Wet	0.9643	Ht=1.2737+0.8358SL	0.9816	Ht=1.5869+0.8149SL	0.9708	Ht=1.4516+0.8261SL
TrL on SL	Dry	0.9111	TrL=-0.4185+0.2836SL	0.9339	TrL=-1.1312+0.3365SL	0.9076	TrL=-0.6906 +0.3054SL
	Wet	0.8666	TrL=1.6649+0.1676SL	0.8897	TrL=1.2865+0.2109SL	0.7908	TrL=1.3738+0.1952SL
TL on SL	Dry	0.9901	TL=-1.0740+0.6377SL	0.9743	TL=-1.0740+0.6377SL	0.9777	TL=-0.7965+0.6141SL
	Wet	0.9747	TL=0.7426+0.5293SL	0.9326	TL=0.0909+0.05425SL	0.9316	TL=0.6535+0.5227SL
SnL on HL	Dry	0.8507	SnL=-0.2493+0.5141HL	0.7363	SnL=0.03149+0.4298HL	0.7948	SnL=-0.1498+0.44865HL
	Wet	0.9014	SnL=0.3767+0.5283HL	0.7363	SnL=0.0446+0.4332HL	0.8731	SnL=-0.4183+0.5510HL
HD on HL	Dry	0.7982	HD=-0.4915+ 0.7256HL	0.7523	HD=0.0997+ 0.4948HL	0.7440	HD=-0.1618+0.5966 HL
	Wet	0.8798	HD=0.2250+0.4386HL	0.8290	HD=-0.4039+0.6545HL	0.8626	HD=0.0778+0.4918HL
SW on TD9	Dry	0.7819	SW=0.1467+0.2860TD9	0.8079	SW =-0.0168+0.3866TD9	0.7823	SW =0.0918+0.3214TD9
	Wet	0.8142	SW=-0.4080+0.5600TD9	0.8594	SW=0.0699+0.3875TD9	0.8072	SW=0.2427+0.4754TD9
TD4 on Trl	Dry	0.8886	TD4=0.2135+0.2206 Trl	0.8861	TD4=0.4959+0.0144 Trl	0.8743	TD4=0.3532+0.1815 Trl
	Wet	0.7649	TD4=0.0308+0.2257TrL	0.8187	TD4=-0.3112+0.2845TrL	0.7689	TD4=0.0489+0.2168TrL
TD9 on Trl	Dry	0.8423	TD9=0.3370+0.3324 Trl	0.8333	TD9=0.5421+0.2684 Trl	0.8117	TD9=0.4783+0.2903 Trl
	Wet	0.8467	TD9=-0.0695+0.4083TrL	0.8891	TD9=-0.3512+0.4304TrL	0.7525	TD9=0.3065+0.3115TrL
DFBL on Trl	Dry	0.7899	DFBL=0.2068+0.0868TrL	0.8251	DFBL=0.3054+0.0625TrL	0.7863	DFBL=0.2574+0.0741TrL
	Wet	0.8293	DFBL=0.0212+0.2930TrL	0.7650	DFBL=0.0599+0.3116TrL	0.6869	DFBL=0.4098+0.2245TrL
PFBL on TRL	Dry	0.8651	PFBL=0.0139+0.3194TrL	0.8281	PFBL=0.7562+0.1442TrL	0.8005	PFBL=0.3732+0.2324TrL
	Wet	0.8387	PFBL=-0.3760+0.1974TrL	0.7101	PFBL=-0.0155+0.1428TrL	0.6869	PFBL=0.4098+0.2245TrL

SNTD, HD, TRL and CORH showed significant difference between male and female ($P<0.05$).

2.3.2 MERISTIC COUNTS:

The meristic counts of *H. kuda* recorded on dry and wet seahorses were pooled together (as there could not be any difference in numbers) and presented in Table 2.3. The number of trunk rings in *H. kuda* was 11 in both males and females, whereas in males the median number of tail rings was 35, while in females it was 33. Similarly, median values of pectoral and dorsal fin rays were higher in male fishes by one count. Results of Mann Whitney *U* test showed that the number of tail rings, pectoral fin rays, and dorsal fin rays varied significantly between male and female *H. kuda*.

2.3.3 LENGTH-WEIGHT RELATIONSHIPS:

The estimated regressions for both male and female length weight relationships were significant ($P > 0.05$), with the coefficient of determination (r^2) values being >0.95. The 't' test revealed negative allometric growth in both male and female seahorses. Significant difference between 'b' value of male and female was observed. The estimated values of length-weight parameters are presented in Table 2.4.

In the present study morphometric relationships were established on both wet as well as on dry seahorses separately after considering its major trade for Traditional Chinese medicine in dry form and wet seahorse length-length and length-weight relationships could be used for species identification as well as to study the life history.

Table 2.3 Meristic counts of male and female *Hippocampus kuda*

Meristic character	**Male**				**Female**				***U***
	Min	Max	Mean ± SD	Median	Min	Max	Mean ± SD	Median	
Trunk rings	11	11	11	11	11	11	11.00	11	P<0.05
Tail rings	32	37	35.03	35	33	36	33.74	33	P>0.05
Dorsal fin rays	17	19	17.84	17	17	19	17.33	17	P>0.05
Pectoral fin rays	16	17	16.35	16	15	17	16.14	16	P>0.05
Anal fin rays	4	5	4.06	4	4	4	4	4	P<0.05

Table 2.4.Length-weight relationship of *Hippocampus kuda*

Sex	No.	Standard Length (cm)		Weight (g)		a	b	b 95% C.I.	r^2
		Min	Max	Min	Max				
Male	29	12.9	20.50	5.0129	18.8993	0.0034	2.8533	2.6341-3.0739	0.9605
Female	30	13.3	20.4	5.6778	17.2145	0.0079	2.5256	2.3115-2.7398	0.9542

2.4 DISCUSSION:

Our knowledge on seahorse taxonomy from India is limited to a few reports where morphology has been used as the sole criterion for describing the species. Although it has been reported that nine species of *Hippocampus* occur in our waters (Murugan et al., 2011), no description of species other than *H. trimaculatus* has been made (Bijukumar et al., 2008). The general shape of the sea horse is familiar and easily recognizable, but detailed identification is quite difficult as sea horses often change colour and grow filaments to blend with their surroundings (Lourie et al., 2004). They also lack certain key physical features (e.g. pelvic and caudal fins) as in other fish species, and variation in body proportions and meristic counts used to determine species membership, often overlap among species (Lourie et al., 2004). Such subtleties in species distinctions can lead to 'lumping' of multiple 'cryptic' species under the same name. Over 120 scientific names have been cited for what are now described as 32 different species (Lourie et al., 2004). Lack of knowledge on the identities of seahorses restricts research on geographic ranges, population density and viability, hinders the understanding of trade routes and consumer preferences for particular species. Proper species identification, on the other hand, will help us to modify fishing practices appropriately, design protective marine reserves, and assess captive breeding potential for seahorses. Taxonomic confusion complicates assessment of species' conservation status, forcing unnecessary listing in IUCN Red List of Threatened Species, and greatly increasing the possibility of omitting species at risk.

CHAPTER 3

COURTSHIP BEHAVIOUR AND SPAWNING UNDER LABORATORY CONDITIONS

CHAPTER 3

COURTSHIP BEHAVIOUR AND SPAWNING UNDER LABORATORY CONDITIONS

3.1 INTRODUCTION:

Seahorse aquaculture could help in decreasing the pressure on over-exploitation of wild seahorse populations with captive-bred fishes (Sreepada et al., 2002; Murugan et al., 2009). There has been growing interest in culturing and breeding seahorses in many countries to reduce the pressure on the wild stocks (Woods, 2000a; Job et al., 2002; Wong and Benzie, 2003). In most Asian countries, as well as in Australia, New Zealand, South Africa and USA, technologically developed commercial seahorse aquaculture is currently underway (Koldewey and Martin-Smith, 2010). With increasing interest in developing and standardizing the protocol for seahorse aquaculture and its conservation, information on the reproductive biology of the heavily exploited tropical seahorse species becomes essential. Breeding adult seahorses in captivity has been unsuccessful or unreliable in many cases due to husbandry problems and inadequate nutrition. Many of the seahorse culture programmes have to rely on wild caught pregnant males which then release their young under captive conditions (Vincent, 1996). The behaviour, life history and ecology of some seahorse species have been described in the wild but little is known about their reproductive behaviour and spawning performance under captive conditions (Laksanawimol et al., 2006). For developing aquaculture and improvement in rearing protocols, behavioural studies are useful tools to assess animal response to culture conditions. Furthermore, close observation of animal behaviour under captive conditions help in better understanding of reproductive biology and animal welfare.

The Oceanic yellow seahorse, *H. kuda* (Bleeker, 1852) has a wide distribution throughout the tropical Indo-Pacific region (Lourie et al., 1999) and is found to occupy a broad range of shallow inshore habitats including mangroves, seagrass beds and estuaries. *H. kuda* is one of the most heavily traded seahorse species in many Southeast Asian countries both for traditional medicine (Vietnam) and aquarium fish (Indonesia). And its current conservation status listed by the International Union for the Conservation of Nature (IUCN) is ‘vulnerable’ (IUCN, 2012). Although commercial culture of *H. kuda* and *H. trimaculatus* in China for traditional medicine for several years has been reported (Lin et al., 2006, 2008), seahorse aquaculture is a relatively new industry that has to overcome many husbandry problems, particularly the nutritional requirements (Giwojna and Giwojna, 1999; Foster and Vincent, 2004).

The effects of food enrichment on growth and sex ratio have been reported for *H. kuda* (Job et al., 2002), Dzyuba et al. (2006) which indicated that parental age and associated size of *H. kuda* could affect reproduction and the offspring's growth and survivorship. The effect of temperature and food on gonad, embryonic development and survival rate was examined (Lin et al., 2006, 2008). Laksanawimol et al., 2006 studied alteration in brood pouch morphology during gestation period while Dzyuba et al. (2006) studied fertilization pattern in *H. kuda*. Thangaraj et al., 2006 studied sexual maturity in captive reared *H. kuda* while Ignatius et al., 2000 studied spawning and rearing of *H. kuda* in captive conditions. None of these focused on behavioural studies (mating pattern, courtship behaviour, egg clutch, gestation and spawning) in detail. A greater understanding of seahorse mating pattern and reproduction is important for conservation. In some earlier studies reproductive biology and mating patterns in the sense courtship behaviours, spawning and social monogamy or polygamy was observed in 13 species of seahorses (Foster and Vincent, 2004). Out of which nine are socially monogamous. These include *H. fuscus* (Vincent, 1990), *H. reidi* (Dauwe, 1992; Nijhoff, 1993; Trommelen, 2001), *H. histrix* (Kuiter & Debelius, 1994), *H. capensis* (Grange & Cretchley, 1995), *H. whitei* (Vincent & Sadler, 1995), *H. zosterae* (Masonjones & Lewis, 1996), *H. bargibanti* (Tackett & Tackett, 1997), *H. comes* (Perante et al., 2002), *H. hippocampus* (J. Curtis, unpublished Data). The remaining three *H. abdominalis* (Woods, 2000), *H. breviceps* (Moreau & Vincent, 2004) and *H. guttulatus* (J. Curtis, unpublished Data & Falerio et al., 2008) are reported to be polygamous and one *H. subelongatus* (Jones et al., 1998; Kvarnemo et al., 2000) showed variable mating patterns. Mi, 1993 reported that *H. kuda* did not show monogamous mating pattern in laboratory conditions. The present study describes and discusses the reproductive behaviour and spawning of yellow seahorse, *H. Kuda,* under laboratory conditions. It is expected that the results of this study would help in understanding the reproductive behaviour of *H. kuda* which is important to optimise seahorse reproduction and culture.

3.2 MATERIALS AND METHODS:

3.2.1 STUDY ANIMALS:

Total 38 specimens of *H. kuda* were collected from Mirya and Shirgaon creek, Maharashtra, India (Lat. 17º 1.4' N and Long. 73º 16.4' E to Lat 17º 022' 20.02" N and Long 73º 17' 48.55" E) measuring approximately 13 to 16 cm height (Ht), collected from local fishermen, being incidental catch in their gill nets. Immediately after collection, live specimens were transferred into polythene bags containing ambient seawater and oxygen gas was pumped into the bags which were transported to the Aquaculture Laboratory, National Institute of Oceanography, Goa (India) within few hours after collection. For captive breeding trials, seahorses were reared in a large FRP tank (cap. 1500 l) with rope mesh (thickness, 4 mm) as holdfasts. Optimum water quality conditions were maintained in the brooders tank (Salinity, 26±1 ppt; water temperature, 27± 2ºC Dissolved oxygen, >6 mg l^{-1}; pH (7.6-8.2), NO_2-N (<0.02 mg/l) and NH_3/NH_4 (0 mg/l). Seahorses were fed *ad libitum* twice a day (0700 h and 1700 h.) with different prey organisms [Mysid shrimps (*Mesopodopsis orientalis*), estuarine grass shrimps (*Palaemonetes* spp.) estuarine fish larvae (*Etroplus* spp., *Ambasis* spp. and *Oreochromis* spp.) and mosquito larvae] caught by hand scoop nets (mesh size 0.5 to 1 mm) from a nearby brackish water fish farm. The collected feed were washed thoroughly with 1 ppm Potassium permanganate ($KMnO_4$) and then with fresh water. Photoperiod was maintained with natural day-night rhythms (13 hrs. light: 11 hrs. dark). FRP and glass tanks were cleaned every day and 10-20% water changes made daily before feeding. There was no mortality in adult seahorses in eight months of observation period. Generally considered, seahorses are monogamous in mating pattern and males compete more intensively than females for access to mates (*H. fuscus* Vincnt 1994; *H. zosterae* Masonjones & Lewis, 1996). We used more females than males, sex ratio was 1:1.3 (male: females) in the glass tank whereas 1:1.4 in the FRP tank. Seahorses were observed daily from early morning for daily greetings and courtship. During night behavioural observations were recorded by using dim light. All results obtained are from direct visual observations. The gender was determined by the presence or absence of the brood pouch. Developments of the brood pouch were examined in males. The brood pouch length (PL), pouch depth (PD) and pouch width (PW) were measured accurately to the nearest mm with Vernier calipers following Lourie et al., (2003) recorded in seahorses at the time when brought to laboratory and at fortnightly intervals until they mated and 3-day intervals after mating and before the release of juveniles and after releasing juveniles.

In order to determine the reproductive performance of individual brooders, all (total 16) male seahorses with developed brood pouch were tagged with different coloured rings (diameter 4 mm) tied with nylon twine to their neck. As per their ring colour numbers were assigned to seahorses (Table 3.1). Pouch volume (PV) was calculated as Pouch length x Width x Depth x 0.7 as described by Boisseau (1967 a, b). The relationship between height (Ht) and pouch volume (PV), and Pouch volume (PV) and number of embryos (NE) after release from brood pouch were studied.

3.2.2. COURTSHIP BEHAVIOR AND MATING:

Sexual selection theory described by Trivers, 1985; Williams, 1966; predicted that relative investment made by male and female in their offspring is a primary determinant of sexual selection intensity and patterns of courtship behaviour within species. In species where male investment exceeds that of females, sexual selection theory predicts that traditional courtship patterns should be reversed. Observations indicated that all seahorse courtship occurs in the morning, except on the day of copulation (Vincent, 1994a). Daily behavioural observations for each pair starting on the day of introduction into tanks and continuing until mating occurred were recorded. 14 seahorses (six males, numbered. 2,4,5,7,9,10,11 and eight females) were kept in an all glass tank (0.3 m x 0.15 m x 0.15 m) and remaining 24 seahorses (10 males nos. 1,3,6,8,12,13,14,15,16 and 14 females) were kept in FRP tank (1.5x1x0.9m) to assess the effect of water column depth on courtship and mating. The water level in all glass tanks was 0.42 m and in FRP tank was 0.60 m. As water height plays an important role in copulatory rise, which results into successful mating and egg transfer in seahorses (Murugan 2004; Sobolewski 1997, 1999). Observations were made continuously from the time the pair started courtship until copulation occurred on the day. Courtship behaviour was recorded continuously using the following modified definitions of behavioural patterns as described by Fiedler (1954),

Table 3.1 Number assign to male seahorses and their size

Colour ring	Number assign	Height (cm)	Weight (gm)
Yellow	1	15.3	19.8
Green	2	16.4	25
Red	3	15.8	21.6
Black	4	17.3	25.2
Blue	5	16.2	22.7
Silver	6	17.8	25.6
Golden	7	16.3	23.4
Violet	8	18.2	25.1
White	9	15.3	19.9
Orange	10	15.6	21.4
Grey	11	16.1	21.6
Pink	12	16.2	22.8
Lavender	13	15.6	20.5
Magenta	14	16.2	22.8
Khaki	15	14.9	17.5
Turquoise	16	15.6	20.8

Vincent (1990; 1994a) and Masonjones and Lewis (1996) these are changes in body colouration (Brightening), erection and vibration of body (Quivering), upward head swimming (Pointing), pumping of brood pouch by male (Pumping), males and females swimming side by side (Promenading) and rise up into the water column facing one another (Dry run) and finally copulatory rise and egg transfer. Apart from this, aggressive behaviour such as tucking of males by head (Snapping) and entwining with tails and pushing each other (Wrestling) was also recorded. After mating same colour tag rings were fixed on the respective female to identify the pair and the same numbers were assigned for female seahorses.

3.2.3 EGG CLUTCH SIZE:

Egg clutch refers to all the hydrated eggs produced at a single time by a female. Effective seahorse breeding and culture programmes are likely to be dependent upon the reproductive efficiency of newly matured or matured seahorses. Observations were made on hydrated egg clutch droppings by female seahorses in this study to assess reproductive efficiency. Very few studies, *H. fuscus* Vincent (1994), *H. zosterae* Masonjones and Lewis (2000) recorded egg clutch dropping. Till now the information regarding egg clutch dropping in seahorse reproduction has been overlooked as egg clutch formation takes a substantial amount of energy. It has been reported that a female seahorse drops the hydrated egg clutches into the water if a receptive male is no longer available (Vincent and Sadler, 1995). In the present study egg clutch dropping was observed and these clutches were collected and counted for clutch size and egg size and weight was recorded.

3.2.4 GESTATION PERIOD AND SPAWNING BEHAVIOUR:

Gestation period (or pregnancy) is traditionally defined as the period in which developing embryos were incubating in the body after egg-sperm union to release of developed embryo at parturition (Stolting and Wilson, 2007). If the number of days available for breeding is divided by the average gestation period this will produce an estimate of the maximum possible number of pregnancy events per year or of breeding duration for the species (assuming that the male became pregnant the day after giving birth). When this was multiplied by the maximum possible brood size, this gave maximum annual reproductive output i.e. maximum possible number of young produced by a pair of seahorses (Foster and Vincent, 2004). After egg transfer, colour developmental changes of male brood pouch are visually observed. Brood PW, PV and PD were recorded in mated seahorses at intervals of three days. Pregnant seahorses were weighed before release of juveniles by daily observation of their moving pattern and in view of average gestation period, and after they released them to estimate the relative fecundity (RF). The average individual juvenile weight was estimated dividing the total juvenile weight by the number of juveniles released. All the released juveniles (swimming juvenile + undeveloped juvenile + dead eggs) from each male seahorse were counted, separately. RF was calculated dividing the number of juveniles per body weight (g) before release of the juveniles.

After mating and egg transfer by female seahorse into male brood pouch, gestation period was recorded for each male seahorse. Spawning behaviour of males was observed as they remain attached to the holdfast, cease feeding prior to release of juveniles, swimming at upper level of water in the tank with pumping action (contraction and relaxation) of brood pouch to release juveniles. Gestation period, batch/partial spawning, number of dead/deformed embryos, undeveloped eggs and free swimming juveniles were recorded for each male seahorse.

3.2.5 INTER MATING DURATION AND MATING PATTERN:

After the complete release of juveniles, male seahorses were released back into their respective tanks for estimating the inter-mating duration. All species of seahorses studied in the wild appear to be monogamous within a single breeding cycle, the male accepting eggs from only one female. Many species also form pair bonds that last at least throughout the breeding season (Lourie et al., 2004). The mating pattern of laboratory reared *H. kuda* was observed in this respect too. Paired male and female were identified by their tag colour, daily observations were made for courtship of paired seahorses (may be male or female) with unpaired or individuals from other paired seahorses.

3.2.6 WATER QUALITY:

Water quality parameters such as temperature, salinity, pH and dissolved oxygen were measured weekly by adopting standard methods (APHA, 1998; Boyd, 1981) and presented in table 3.2.

3.2.7 DATA ANALYSIS:

Student *t*-test was used to compare the size of males and females. Regression analysis was used to assess the relation between Height of males, Pouch length, pouch volume and number of embryos. Significance in all statistical tests was judged at a P = 0.05 level. (Snedecor and Cochran, 1967; Zar, 2004).

3.3 RESULTS:

3.3.1 BROOD POUCH DEVELOPMENT:

The relationship between height (Ht) and Pouch volume (PV) of the brood pouch and Ht and pouch length (PL) is presented in Figs.3.1 and 3.2, respectively. The correlation coefficient (r) value between Ht and PV was found to be insignificant ($P>.05$). While, the relationship between Ht and PL was found to be significant (r= 0.6530), ($p<0.05$). This result reveals that PL increases with increase in Ht but there is no relation

Table 3.2 Water quality parameter during rearing and breeding period of *Hippocampus kuda*

Sr. no.	Water parameter	All glass tank	Fiber tank	Breeding tank
1	Temperature (°C)	24 to 26	26 to 28	25 to 27
2	Salinity (ppt)	26 to 28	26 to 28	26 to 28
3	pH	7.3 to 7.6	7.2 to 7.6	7.2 to 7.8
4	Dissolve oxygen (mg^{-l})	5.45 to 6	5.2 to 5.8	5.5 to 5.8

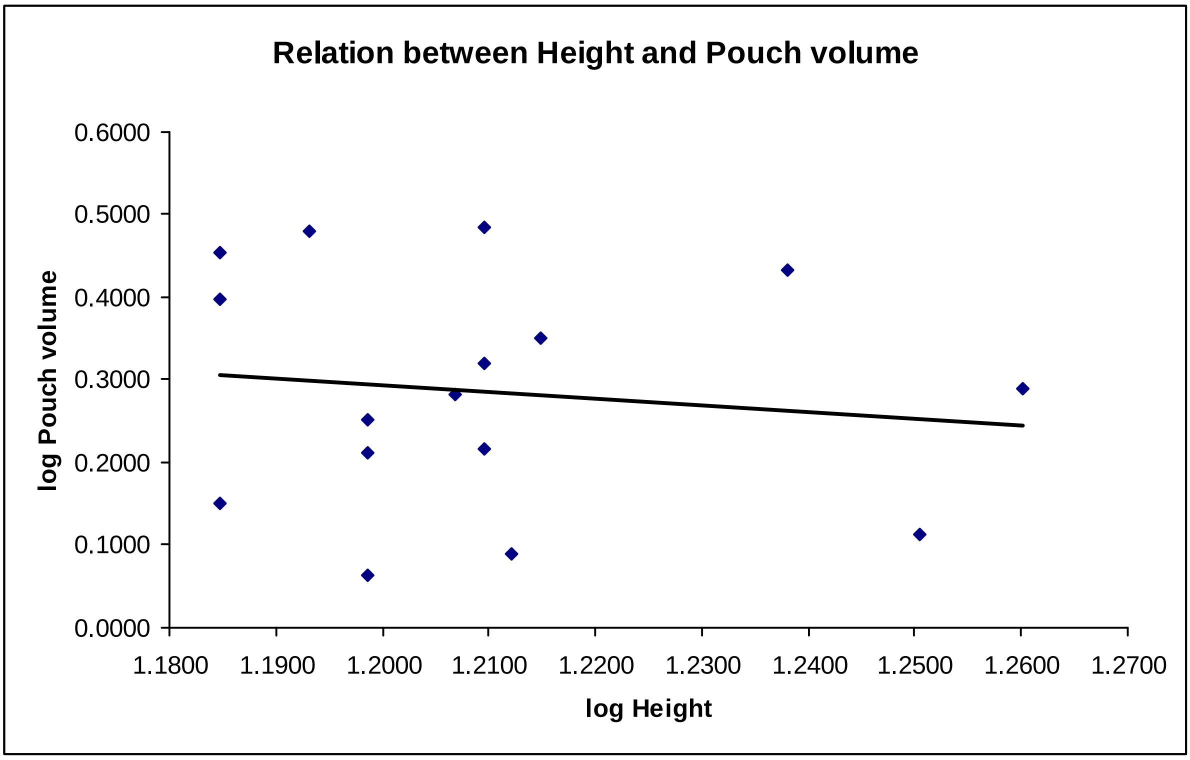


Fig. 3.1. Relationship between height and pouch volume

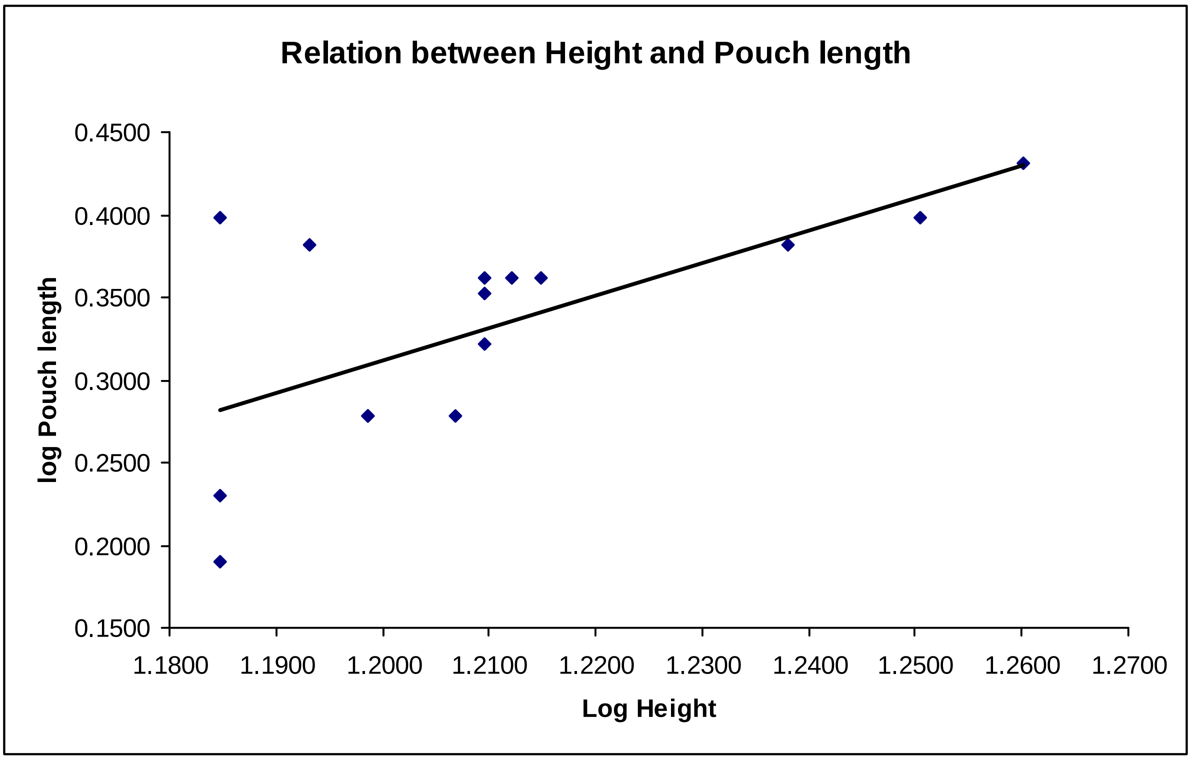


Fig. 2. Relationship between Height and Pouch length

between PV and Ht of seahorses as other two factors such as pouch depth and pouch width are dependent on the number of eggs transferred by the female. The relationship between Pouch volume (PV) and number of embryos (NE) is presented in Fig. 3.3. The correlation coefficient (r) value between pouch volume and number of embryos was found to be significant (r=0.57, ($P<0.05$). In other words, the PV increases significantly with the increase in the number of embryos in the brood pouch.

3.3.2 COURTSHIP BEHAVIOUR AND MATING:

Observations for courtship behaviour such as changes in body colouration, erection and vibration of body, upward head swimming, pumping of brood pouch by male, males and females swimming side by side (promenading) and rise up into the water column facing one another (dry run) and finally copulatory rise were categories under three main phases i.e. 1. Quivering; 2. Pointing and pumping; 3. Rising and copulation.

Initially, courtship occurred every day during the courting period i.e. in the early morning hours. During this phase the male changed its body colour 'Brightening' to create a contrast with the surroundings so as to attract the female. Male seahorses which start showing courtship behaviour become brighter than other males in tank. In this period the male slightly shakes his body and moves around the female, which is called 'Quivering'. Females responded to the courting male by changing their colour and tucking their head 'Pointing' which is a positive response.

In the second phase, the females also changed their body colour and exhibited the pointing posture and the male responded to the female pointing by going near to her and quivering. Then male seahorses inflated and deflated its brood pouch to a balloon shape by pumping the water in and out i.e. 'Pumping'. During this behaviour the males changed the colour of their brood pouch from dark brown to bright yellow to pale yellow. The male held the female with the help of its prehensile tail and swam in the water. Both sexes

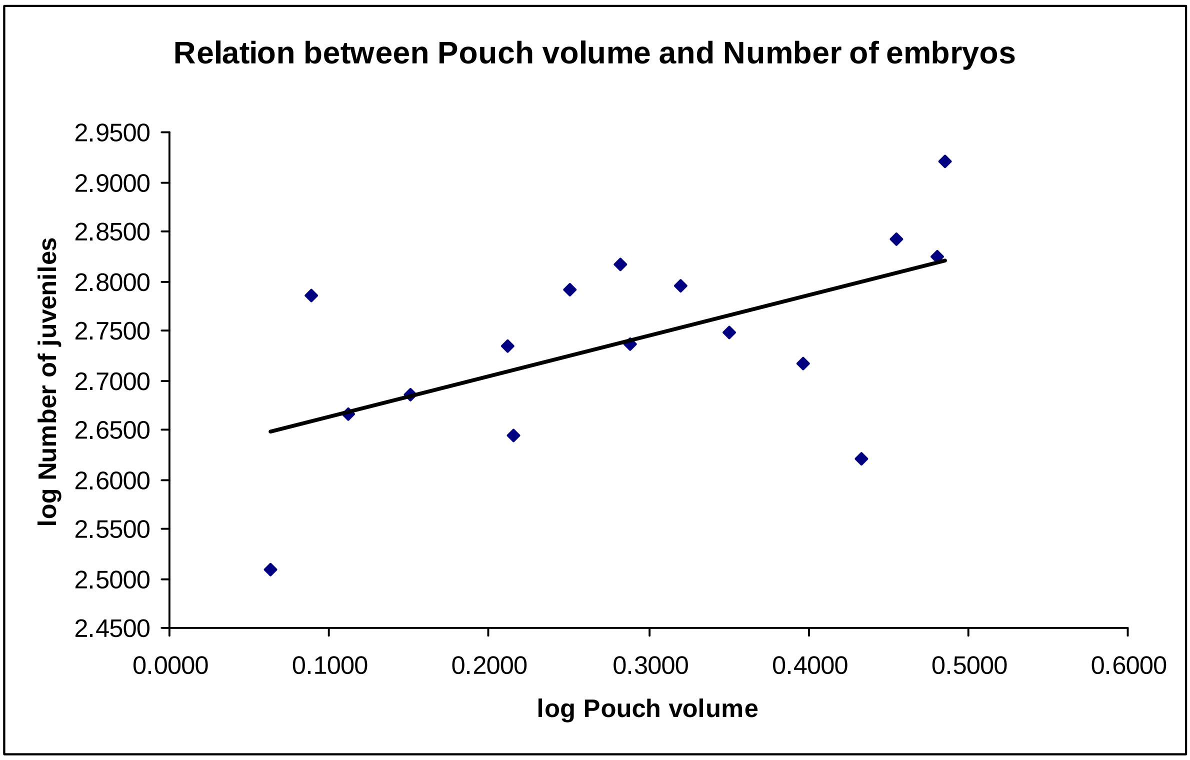


Fig. 3.3 The relation between pouch volume and number of embryos

swam parallel to each other in the tank (i.e. ‘Promenading’) with tails entwined; and the male sometimes touches (physical contact) his body to the female body. Aggression between males was observed at this phase as two males showed wrestling and snapping behaviour by pushing each other with tails entwining and snapping with snout.

In the last phase, that is the rising and copulation phase, the males attempted to get the female away from the holdfast and swam around the tank first and then towards the water surface by pointing the snout upwards. During the copulation phase, both the male and female swam towards the water surface with their head upwards and the tail pointing downwards. Before actual transfer of eggs both male and female rose together vertically several times and act as if depositing eggs (termed ‘Dry run’). After depositing the eggs into the male’s brood pouch with the help of genital papilla, both the fishes snapped their heads indicating the final stage of mating. After mating, the male seahorse rapidly shakes his body with a contracting action of his brood pouch to receive all eggs properly into the brood pouch. The female trunk becomes concave after egg transfer. After mating, the courtship behaviour was not exhibited but the pairs remained attached to the same holdfast. No daily greetings were observed in mated seahorse pairs during the gestation period of the male. In the second mating, courtship was observed to be started on the same day or next day. Males become more aggressive to get eggs from the female and showed courtship behaviour just after releasing young ones by pumping his brood pouch. In such cases, the initial two events i.e. brightening and quivering were less seen and females responded by pointing. Out of the 18 observed courtships, in 38.89 % mating was found on the 1st day, while in 61.11% mating occurred on the 2nd day. Average time duration for courtship was 1.78±0.4469 hrs on the 1st day to 1.64±0.3687 hrs on 2nd day with maximum and minimum courtship period recorded was 2.52 hrs and 1.15 hrs respectively. Courtship duration was longer on the first day (max 2.52 hrs. and min 1.20 hrs.) than on 2^{nd} day (max 2.20 hrs. and min 1.15 hrs.).

In the present study, 12 pairs of laboratory reared *H. kuda* mated successfully. 2 pairs (no. 1 and 7) mated twice and no. 1 pair released young ones twice where as the no. 7 pair released young ones just once and the second mating was observed to have failed. Pair no. 3 and 5 mated 3 times but pair no. 3 released young ones 3 times whereas pair no.5 released young ones two times and the third mating was observed to have failed. Remaining 8 pairs mated and released young ones once during the culture duration of 8 months.

3.3.3 EGG CLUTCH SIZE:

Two females i.e. female seahorse no. 4 in glass tank and no. 14 in FRP tank released their matured hydrated eggs in water. This is particularly due to either no pair formation or unavailability of male partner due to pregnancy. These females dropped their hydrated eggs when their respective partners were under pregnancy. Dropped hydrated eggs were collected and counted for clutch size which was 298 and 472 eggs respectively (avg. length 1.85 ± 0.0029 mm and weight 0.0015 g (Plate 3.1). In the final phase of courtship, paired seahorses rose vertically in the water column to mate and for egg transfer. In case the height of water in aquaria or holding tank was less, the female seahorses were unable to transfer the eggs properly into the brood pouch of the male. We used two types of rearing tanks (glass aquaria (water height 42 cm) and FRP tank (water height 60 cm)) to confirm the role of water column height. It was observed that pairs formed in all glass aquaria (pair no. 5 (with new female) and 7) were unable to transfer eggs into the brood pouch and a clutch of eggs was stuck outside the opening of the brood pouch during copulation and later fell into the tank. These clutch sizes were 395 and 449 (avg. length 1.86 ± 0.003 mm and weight 1.6 mg), respectively. In both cases the copulation duration was less i.e. 5 and 6 Sec. respectively. Female investment in egg clutches represents between 1.7 to 5.7 % of the female's wet body weight.

3.3.4 GESTATION PERIOD AND SPAWNING BEHAVIOR:

Gestation period, batch spawning, no. of dead/deformed embryos and undeveloped eggs and free swimming juveniles for each pair were observed and presented in the Table 3.3. At the end of the gestation period, the male seahorse released young ones by the process of contraction and relaxation of body and flexing of tail. The colour of the brood pouch becomes dark brownish from bright light yellow. The pregnant male seems to become more sluggish, ceases to feed and prefers to rest on the holdfast. At the time of releasing juveniles, the male swam in the upper part of the water column, flexing his tail to create pressure on the brood pouch to spill out juveniles. Gestation period of laboratory reared *H. kuda* were recorded from 9 days to 16 days with an average gestation period of 13.81±1.68 days. Maximum possible pregnancy events in the culture period of 245 days was 17.74 times and maximum possible young ones from one pair of *H. kuda* in the culture period of 245 days was 14793 young ones. It was observed that there was no difference between size and survival of newly born juveniles at different gestation time period. The fecundity of *H. kuda* ranged between 322 and 834 with the size of newly born juveniles ranging from height 7.18 ± 0.076 mm to 8.47 ± 0.0034 mm and weight 0.0023 ± 0.00046 mg to 0.0028 ± 0.0005 mg. The average relative fecundity observed per gram of female and male body weight in the present study was 28.27± 1.7037 and 25.25 ± 1.5721 respectively. Juvenile birth occurred in the morning period. It was observed that juvenile birth was discontinuous and occurred in the duration of 1 to 3 days, with 4 to 140 in number on day one. During the breeding period increase in number of young ones was noticed when the pair repeatedly mated.

Table 3.3: Gestation period and batch spawning in *Hippocampus kuda*

Seahorse number	Mating time	Mated date	Gestation period (days)	1st batch			2nd batch			Mass spawning			Dead/ deform embryos	Un-developed eggs	Total
				Date	Time	Nos.	Date	Time	Nos.	Date	Time	Nos.			
1	11.30	29Aug	9	-	-	-	-	-	-	6 Sep	8.00	526	0	5	526
2	8.30	29 Sep	15	12 Sep	18.30	14	-	-	-	14 Sep	7.30	638	1	3	652
3	8.00	1 Sep	14	13 Sep	6.30	5	14 Sep	11.25	12	15 Sep	0.30	548	0	0	565
4	7.00	3 Sep	16	-	-	-	-	-	-	20 Sep	10.30	435	0	0	435
5	10.45	10 Sep	14	23 Sep	8.45	6	-	-	-	24 Sep	6.00	472	0	0	478
6	13.50	12 Sep	15	25 Sep	7.20	4	25 Sep	17.1	7	26 Sep	8.00	511	0	0	522
7	11.07	17 Sep	14	28 Sep	18.30	24	29 Sep	8.10	79	30 Sep	6.30	506	0	0	609
3	9.45	17 Sep	14	-	-	-	-	-	-	30 Sep	9.25	689	0	4	689
1	7.45	19 Sep	12	-	-	-	-	-	-	30 Sep	8.45	559	1	2	559
8	10.20	8Oct	13	19 Oct	18.15	52	-	-	-	20 Oct	6.45	513	0	4	565
5	9.25	27 Oct	15	-	-	-	-	-	-	10 Oct	10.20	648	0	0	648
3	11.20	03 Oct	14	-	-	-	-	-	-	16 Oct	12.35	391	1	0	391
9	12.45	9 Oct	15	-	-	-	-	-	-	23 Oct	6.10	698	1	0	698
10	9.15	15 Oct	14	-	-	-	-	-	-	29 Oct	7.20	685	0	0	685
11	8.35	24 Jan	15	4 Jan	8.10	27	5 Jan	6.30	39	5 Jan	11.40	584	0	3	650
14	10.15	4 Jan	12	15 Jan	17.10	140	15 Jan	22.20	176	16 Jan	8.35	549	1	0	865

3.3.5 INTER-MATING DURATION AND MATING PATTERN:

It has been documented that the male seahorse is capable of mating again and ready to carry a new batch of embryos immediately after parturition (Foster and Vincent, 2004). Inter-mating duration for the observed pairs of *H kuda* varied from 16 (2 days after the release of first batch of young ones) to 29 days (13 days after the release of first batch of young ones). The observed difference between size of male and female averaged at 1.01± 0.84 cm with a maximum of 2.5 cm. In the 12 observed pairs, males (mean 16.38 ± 0.93) mated with females (mean 15.62 ± 0.67) approximately the same size (paired Students *t-test*, $t_{11}=2.96$, $P<0.05$). Male Seahorses tagged with no. 5 and 14 were observed to court with one unpaired new female seahorse and 12, respectively. In this period male seahorse tagged with no. 12 was under pregnancy. Seahorse no. 5, who already had a pair, courted and mated with a new female on his third mating but in the final phase this mating failed as egg clutch transfer was not completed as copulation time was less (5 s). This clutch stuck outside the male pouch and later dropped into the tank. This observation from the present study revealed that *H. kuda* under laboratory conditions may form groups rather than pairs or switch mates between broods. The time taken for inter-mating, number and length-weight of newly born juveniles by each pair is presented in Table 3.4.

3.4 DISCUSSION:

3.4.1 BROOD POUCH DEVELOPMENT:

The brood pouch of seahorses is the secondary sexual organ progressively developing with sexual maturity in the male. The brood pouch of *H. kuda* is a sealed skin pocket, located on the ventral surface of the tail and with a small opening situated anteriorly (Laksanawimol et al., 2006). The linear relationship of the seahorse size (Ht) and pouch length (PL) indicated that the length of brood pouch increases with the size of

Table 3.4: Intermating duration during culture period of eight months in *Hippocampus kuda*

seahorse no	Height (cm)		1st mating (DOC)	Date	Juveniles			2nd mating (DOC)	Date	Juveniles			3rd mating (DOC)	Date	Juveniles		
	Male	Female			Nos.	length (mm)	weight (gm)			Nos.	Length (mm)	Weight (gm)			Nos.	Length (mm)	Weight (gm)
1	15.3	14.5	86	29Aug	526	7.053 ± 0.083	0.0024 ± 0.0003	-	-	-	-	-	-	-	-	-	-
2	16.4	15.9	86	29Sep	652	7.51 ± 0.38	0.0027 ± 0.00023	-	-	-	-	-	-	-	-	-	-
3	15.8	15.6	89	1Sep	565	7.78 ± 0.29	0.0029 ± 0.00033	-	-	-	-	-	-	-	-	-	-
7	16.3	16.4	89	1Sep	Mating fail	-	-	-	-	-	-	-	-	-	-	-	-
4	17.3	16.8	91	3Sep	435	7.24 ± 0.064	0.0024 ± 0.00043	-	-	-	-	-	-	-	-	-	-
5	16.2	15.2	98	10Sep	478	7.52 ± 0.144	0.0025 ± 0.00032	-	--	-	-	-	-	-	-	-	-
6	17.8	15.4	100	12Sep	522	7.47 ± 0.089	0.0027 ± 0.0003	-	-	-	-	-	-	-	-	-	-
7	16.3	16.4	105	17Sep	609	7.29 ± 0.34	0.0021 ± 0.00019	-	-	-	-	-	-	-	-	-	-
1	15.3	14.5	-	-	-	-	-	108(22)	19Sep	559	7.81 ± 0.074	0.0027 ± 0.0004	-	-	-	-	-
3	15.8	15.6	-	-	-	-	-	118(29)	26Sep	689	8.29 ± 0.25	0.0031 ± 0.0039	-	-	-	-	-
8	18.2	15.7	126	8Oct	565	7.74 ± 0.25	0.0024 ± 0.0034	-	-	-	-	-	-	-	-	-	-
5	16.2	15.2	-	-	-	-	-	116(18)	27 Oct	648	8.47 ± 0.0034	0.0037 ± 0.0003	-	-	-	-	
9	15.3	15.4	127	9 Oct	698	7.21 ± 0.078	0.0028 ± 0.00046	-	-	-	-	-	-	-	-	-	-
10	15.6	14.9	133	15 Oct	685	7.18 ± 0.076	0.0024 ± 0.0003	-	-	-	-	-	-	-	-	-	-
11	16.1	15.2	201	24Dec	650	7.46 ± 0.37	0.0021 ± 0.0004	-	-	-	-	-	-	-	-	-	-
14	16.2	16.4	212	4Jan	865	7.38 ± 0.019	0.0026 ± 0.0006	-	-	-	-	-	-	-	-	-	-
3	15.8	15.6	-	-	-	-	-	-	-	-	-	-	134 (16)	11 Oct	391	8.42 ± 0.007	0.0029 ± 0.0005
5	16.2	15.2	-	-	-	-	-	-	-	-	-	-	135 (19)	14Nov	Mating fail	-	-

male (*P*<0.05). However, it is the PD and PW rather than PL that determines the PV. Pouch volume depends upon the quantity of eggs that are transferred by the female. The size of the male seahorse (Ht) is negatively correlated with the PV thereby indicating that the volume of brood pouch is more dependent on the size of egg clutch that was transferred by the female.

The brood pouch epithelial lining is highly vascularized and may provide developing embryos with gas exchange, osmoregulation and waste removal during their gestation period (Boisseau, 1967). After egg clutch transfer the fertilisation pouch becomes a sealed pocket and all egg pits in the wall of the pouch become compartmentalized and then are enveloped in epithelial tissue until the end of yolk absorption (Boisseau, 1967; Laksanawimol et al, 2006). The embryo becomes embedded within depressions of the interior lining of the brood pouch (Carcupino et al., 1997). As the size of clutch increases it requires a larger brood pouch interface to attach all the eggs individually for respiration, nutrition, etc. which suggests that pouch volume is more dependent on size of egg clutch that are transferred by the female during mating and not on the size of male.

3.4.2 COURTSHIP BEHAVIOR AND MATING:

Courtship displays and coloration have traditionally been viewed as means for the male to convey the information about himself to the female (Andersson, 1994). In case of Syngnathids, the role of daily greetings to establish and maintain pair bond is now well known. Studies on few seahorse species such as *H. fuscus* (Vincent, 1995), *H. whitei* (Vincent & Sadlar, 1995), *H. zosterae* (Masonjones & Lewis, 1996) indicate that both mates maintain their relationship through daily greetings. Similar courtship behaviour was also observed in the present study of *H. kuda* under laboratory conditions. Courtship behaviour lasted for 1-2 days, in some cases courtship started and ended in successful mating on the 1st day itself. In this period three phases were observed. On the first day of courtship we observed phase 1 that is 'Quivering'. Courtship interaction starts daily with change in colour of male seahorse and approach towards the female. Male starts his body quivering and touches the female and swims around the female which also called 'mating dance'. Generally it was observed that the male first starts displaying courtship behaviour. Phase 2 and 3 was observed on the day of copulation which occurred on day 1 or 2 of courtship behaviour. During 2nd phase female responds to the male's brightening and quivering, exhibits her first courtship behaviour by pointing her head. Later on female becomes more active in the initiation of courtship bouts. Both male and female swam in the tank side by side, touching each other's body i.e. 'promenading'. Male seahorse catches the female with his prehensile tail and swims. With quivering and chasing both sexes rise vertically in the water column and try to deposit eggs; such attempts were made 3-4 times before actual transfer of eggs which is termed as 'dry run'. After 3-4 times they rise vertically in the last copulatory rise, the female seahorse places her gentile papilla into male brood pouch opening and transfers her matured and hydrated eggs and at the same time the male releases sperms and fertilizes the eggs. Then after complete transfer both sexes separated and male starts flexing his body and tail to properly set all received eggs in the brood pouch. Then both male and female rest together. The seahorses no. 5 and 14 appeared to court with new female and seahorse female no.12 respectively, and females showed brightening of body. Both male and female swam as the male entwined his tail to female's tail. Both females responded by brightening and showed promenading behaviour, but the courtship of male no. 14 and female no. 12 apparently failed to progress as this female did not respond to repeated brightening and quivering by this male. In another case seahorse male no. 5 courted a new female and both showed all courtship behaviour but this courtship failed at the time of egg clutch transfer due to bad alignment of the pair. This nature of *H. kuda* may suggest that this species may form groups or switch mates between broods than follow a strict monogamous mating pattern in laboratory conditions. It was also observed that after releasing juveniles, the male seahorse is completely ready to accept a new clutch of eggs from the female. In this case the courtship period is one day or less. However, a delay of 4–20 h between birth and subsequent copulation has been reported in a dwarf seahorse, *H. Zosterae*; subsequent reproductive cycles of the same pair was also observed by H. D. Masonjones, (unpublished data). This indicates the aggressive nature of male towards mating and developing their progeny. In this study, active participation of male seahorses in the courtship until the copulation occurs was observed. These findings are consistent with previous studies in *H. fuscus* (Vincent, 1995), *H. zosterae* (Masonjones and Lewis, 1996) where males are more active in courtship and show traditional courtship role in terms of male initiation and active participation and competition to access female seahorse. This result of courtship behaviour studies indicates that *H. kuda* also show traditional courtship roles instead of their high degree of parental care. Sexual selection theory predicts that the relative investment made by males and females in their offspring is a primary determinant of sexual selection intensity and patterns of courtship behaviour within species (Trivers, 1985; Williams, 1966). Courtship behaviour allows individuals to select proper mates to produce healthy generations in order to reduce mortality as cost of both egg clutch and male parental care is very high.

3.4.3 EGG CLUTCH TRANSFER:

During the present study, loose eggs were observed during mating and they ranged from a minimum of 298 to a maximum of 472 hydrated eggs. Female ovaries in *Hippocampus* spp. continuously produce mature follicles of which only a small amount is transferred at each mating. This suggests that females may be physiologically capable of mating immediately after approach of sexual receptive male (Selman et al. 1991). By the previous studies of Masonjones and Lewis (2000), a female seahorse takes at least two days to prepare a clutch of eggs and become sexually receptive and can re-mate before the end of her male gestational cycle. This result indicates that females can prepare a clutch earlier than the normal gestation length, but because of the relatively low density of potential unpaired mates in the field this clutch may drop (Vincent & Sadler 1995). Vincent (1994b) suggested that *H. fuscus* females may need 3 days to mature a clutch of eggs to transfer to her mate. Also, the formation of a new pair may increase the intermating duration of both sexes, due to additional time spent for a new mate and female must hydrate her eggs before spawning (Lourie et al., 1999; Vincent 1994b).

Masonjones and Lewis (2000) and Vincent (1994a) observed that hydrated eggs were dropped by a female seahorse which did not have a pair in the seahorse holding tank. Similar observations were noticed in the present study where *H. kuda* female seahorses tagged with no. 4 and 14 dropped hydrated eggs into the tank. This indicates that a female seahorse can produce matured eggs before completion of her mate’s gestational period and in the unavailability of a partner this clutch of eggs is dropped. Clutch dropping due to badly aligned mating in glass tank of 0.42 m water height was observed. Because of vertical orientation and up-right swimming pattern, a tall aquarium is highly suited for seahorses. At the time of copulatory rise they rose vertically in the water column to transfer eggs from female into male brood pouch. As a general rule seahorses must have two full body lengths of water space above the substrate in order to mate. If the tank is shallow, female seahorses are unable to transfer the eggs into the brood pouch of the male. Vincent (1994a) reported entire egg clutches were found dropped during badly aligned mating attempts. She also reported a drop of 157 eggs for *H. fuscus* (Vincent, 1994a). Therefore, height of the water column plays an important role in the successful transfer of egg clutch into male brood pouch. Sobolewski (1997) reported that *H. abdominalis* never attempted to transfer the eggs in the smallest tank and recommended a tank approximately 1500 mm in height and at least 700 mm wide for breeding. Sobolewski (1999) recommended a tank depth of 5 times the length of the *H. abdominalis* brood stock for the promotion of mating success. Faleiro et al., 2008 suggest that water column depth resulted in mating interruption. Whereas, successful mating of *H. kuda* in the present study were recorded in breeding tanks with a height of 1 m having 0.60 m water height. This suggests that water column depth should be nearly 2 times more than the body size of *H. kuda* for proper mating and egg transfer.

3.4.4 GESTATION PERIOD AND SPAWNING:

At the end of the gestation period the young ones are released from the brood pouch. The young ones are released by repeated process of contraction and relaxation and flexing of tail. In addition, male seahorses may not be active in obtaining food when brooding (Woods, 2000; Woods, 2003; Falerio et al., 2008). In the present study during each contraction 1 to 20 young ones were found to be released in *H. kuda*. Similar observations were noticed for *H. kuda* with 20 to 30 young ones in one contraction by Naik *et al*. (2002), 10 to 15 young ones in *H. kuda* (Gokulakannan, 2002).

Many authors report complete spawning at a single time when all juvenile were released at a time; but we observed partial spawning and it lasted for 2 to 3 days. Release of young ones in batches in the laboratory conditions occurred in an interval of 3 days. Mass release of juveniles was observed on the last (i.e. third day) day of spawning. Similar observation was made by (Mi, *et al*., 1998) in *H. kuda* where the young ones are released in batches at an interval of 2 to 4 days. In the pipefish, *Syngathus scovelli* the hatching of juveniles from the brood pouch of males occurred at an interval of 24 hours (Azzarello, 1991).

Different gestation periods for different seahorse species have been reported. The gestation period for *H. fuscus* ranged between 13-14 days at the laboratory temperature of 28 °C (Vincent, 1990), for *H. reidi* 21 days at 25 °C (Silveira, 2000), for *H. kuda* 20-28 days in coastal waters of Vietnam (Mi, 1993 and Mi *et al*., 1998), 20-21 days in *H. erectus* (Silveira, 2000), 12 days for *H. zosterae* (Masonjones and Lewis, 1996). The gestation period of *H. trimaculatus* was 19 days at 22.5±0.5°C and 16 days at 24±0.5°C and 11 days at 28.5±0.5°C, suggesting that optimal temperature range for its brooding was 26 °C to 30 °C in Chinese waters (Cai *et al*., 1984a). The gestation period for *H. kuda* was previously recorded in the range between 10-24 days (Xu, 1985; Mi, 1993; Mi *et al*., 1998; Truong and Doan, 1994; Truong, 1994). In the present study, the gestation period for *H. kuda* ranged between 9 and 16 days at 26±1°C water temperature in the laboratory conditions.

Ortega-Salas and Reyes-Bustamante, 2006 reported average relative fecundity of 76.43 juveniles per gram of male in *H. ingens* seahorse. While in case of great pipe fish, *Syngnathus acus*, average hydrated oocyte number of females was 29 ± 4.35 and average egg number in pouch of pregnant males was 24 ± 5.16 and embryo number was 34 ± 8.80 (Gurkan et al., 2009). In the present study the average relative fecundity of male and female seahorse was 28.27± 1.7037 and 25.25 ± 1.5721 respectively.

Herald and Rakowicz reported that *H. hudsonius punctulatus* released 253 young ones. Some other workers reported 400 to 1000 in *H. trimaculatus* (Cai *et al*., 1984b), 41.1± 4.02 in *H. fuscus* (Vincent, 1990), 271 to 1405 in *H. kuda* (Troung and Doan, 1994), 1572 to 1753 juveniles in *H. hippocampus* (Vincent 1994), 232-1286 in *H. trimaculatus* (Ky and Nga, 1995), 3 to 16 in *H. zosterae* (Masonjones and Lewis, 1996), 7 to 95 in *H. capensis* (Lockyear *et al*., 1997), 44 to 1751 in *H. kuda* (Hilomen-Garcia, 1999), 502 in *H. reidi* (Silveira, 2000), 721 in *H. abdominalis* (Woods, 2000 b), 150 in

H. fuscus, 50 in *H. hippocampus* (Golani and Fine, 2002), up to 1536 in *H. reidi* (Rosa *et al*., 2002), up to 1552 in *H. erectus* (Dias *et al*., 2002), 1465 in *H. kuda* (Naik *et al*., 2002) 1598 to 1703 in *H. ingens* (Ortega-Salas and Reyes-Bustamante, 2006). In the present study the number of young ones released by *H. kuda* ranged from 321 to 833.

Gestation duration, fecundity rates, maximum brood size records are important to estimate a maximum annual reproductive output. This will help in calculating maximum pregnancy event in the breeding cycle and it gave maximum possible numbers of young produced by a pair of seahorses (Foster and Vincent, 2004). Gestation period of laboratory reared *H. kuda* were recorded from 9 days to 16 days with average gestation period of 13.81±1.68 days. Maximum brood size recorded in the present study was 833 young ones while some workers reported 1405 (Troung and Doan, 1994), 1751 (Hilomen-Garcia, 1999), 1465 (Naik *et al*., 2002) in *H. kuda*. According to Falerio et al. (2008) seahorse mating in captivity resulted in decrease of juvenile number and size. In the present study maximum possible pregnancy events in the culture period of 245 days was 17.74 times and maximum possible young ones from one pair of *H. kuda* in the culture period of 245 days was 14793 young ones. However, Foster and Vincent (2004) reported that maximum annual reproductive output ranged from approximately 500 young ones per pair of *H. capensis,* to over 29000 young ones for *H. kuda*. There is difference in brood size of *H. kuda* in wild and laboratory reared seahorses this suggests that more attention is required for increasing reproductive output. These records suggest the possible reproductive output of pairs in seahorse culture and accordingly may help to manage culture practices in seahorse hatchery.

3.4.5 INTER-MATING DURATION AND MATING PATTERN:

Inter-mating duration refers to the period between two successive broodings of male seahorses. Males of all seahorse species studied till date went through several pregnancies in a single breeding season or in the days available during the breeding period. The number of pregnancies depends upon the length of brooding and length of season. *H. comes* were observed to undergo repeated pregnancies during a year (Perante et al., 2002 while *H. sunelongatus* males had more than one pregnancy per breeding season (Kvarnemo et al., 2000). Vincent (1994 a, b) observed seahorse mating immediately after the release of young ones from the brood pouch. The present study has revealed that the males of *H. kuda* were found to be aggressive to mate immediately after the release of the young ones and the inter-mating duration varied between 16 (2 days after release of young ones) and 29 (13 days after release of young ones) days. In the present study, among the 12 pairs, only 2 pairs (no. 1 and 7) mated twice , pair no. 3 and 5 mated thrice and remaining 8 pairs mated once during the culture duration of 8 months and the inter mating duration ranged between 16 to 29 days. Different period of inter-mating duration has been reported in syngnathids. Franzoi et al. (1993) reported that wild caught pipefish species, *Syngnathus abaster* and *S. taenionotus* can mate 4 times and 2 times, respectively in a single breeding season. *H. kuda* was reported to mate 3 times (Mi, 1992), *H. zosterae* mate twice in a month (Strawn, 1958). Earlier reports revealed *H. whitei* mated 6 times within a period of 141 days (Vincent and Sadler, 1995). Cai *et al*., (1984 a) reported that each individual of *H. trimaculatus* might breed five times , but in practice it breeds once or twice in single breeding period.

Vincent (1994a) reported that male seahorses cannot re-mate while brooding the embryos but female appears to have some potential to mate again before the male gives birth. Woods (2000a) reported that some males had sequential broods with the same female, whereas others had successive broods with different females. However the seahorses and pipefishes derived increased reproductive efficiency from mating with a familiar partner (Gronell, 1984 and Vincent, 1990a). In the case of the two pipefishes *S. typhle* and *N. ophidion* females are the predominant competitors for mates and showed polygamous mating behaviour (Rosenqvist, 1990., Berglund, 2000) and males are more selective than females in accepting mates (Berglund *et al*., 1989).

Mi (1992) found that *H. kuda* showing polygamous behaviour in the laboratory. In the present study, male *H. kuda* seems to court and mate with different females even after having a partner. This competition for mating with more than one female indicates that *H. kuda* may form a larger group or switch mates under captive conditions. However the mating pattern of *H. kuda* in wild is not known. Beside the strict monogamy reported in seahorse species, courtship behaviours in this study indicate that *H. kuda* may tend towards polygamous mating patterns in captivity. Woods (2000a) reported that *H. abdominalis* was polygamous in captivity whereas *H. breviceps* forms mixed larger groups rather than pairs in wild conditions (Moreau and Vincent, 2004). Kvarnemo et al. (2000) reported that in the wild population of *H. subelongatus* mate switching occurred and males of *H. subelongatus* mated with new female in the second of the two broods. They suggest that mate switching is possibly beneficial to males in terms of overall fitness, if the second female was more fecund or showed some other preferred trait that might be naturally or sexually selected.

The polygamous mating pattern observed in this study is due to alteration from natural conditions, where in captive conditions switching mates may be beneficial to both sexes to continue reproduction. For more confirmation on the mating pattern of *H. kuda* information on social behavior and mating patterns in wild is needed and needs to be confirmed through genetic studies.

CHAPTER 4

EFFECT OF TANK BACKGROUND COLOUR ON THE GROWTH AND SURVIVAL OF EARLY JUVENILES

CHAPTER 4

EFFECT OF TANK BACKGROUND COLOUR ON THE GROWTH AND SURVIVAL OF EARLY JUVENILES

4.1 INTRODUCTION:

Within the framework of seahorse culture development, optimization of the environmental conditions and feeding regimes during juvenile rearing are critical for successful captive breeding programmes. Rearing of marine ornamental fishes is often considered problematic because of their bipartite life style (pelagic and settlement behaviour) (Victor, 1991). The initial period of 0-15 DAB (days after birth) when juveniles switch from pelagic to settlement phase is considered very critical in seahorse husbandry (Woods, 2000b). Several attempts to culture seahorses on mass-scale in the recent past have met with limited success as a result of severe problems of high mortality due to inadequate nutrition and disease (Thangaraj and Lipton, 2008; Garcia et al., 2010).

High juvenile mortalities, particularly during the pelagic phase have been attributed to inadequate or inefficient feeding responses. It is possible that the mortalities could be minimised by improved feeding efficiency (Koldewey, 2005; Thangaraj and Lipton, 2008; Garcia et al., 2010) and through manipulation of background (tank) colour. It has been hypothesised that different coloured backgrounds induce a variety of responses in relation to food intake, growth and survival in fishes (Moriya and Miyashita, 1987; Papoutsoglou et al., 2000; Tamazouzt et al., 2000). Several fish species prefer dark tank walls (Naas et al., 1996) as they promote a suitable contrast between the prey and the background colour (Browman and Marcotte, 1987). However, few studies have shown that some fish species prefer light backgrounds (Papoutsoglou et al., 2000; Tamazouzt et al., 2000).

Whereas vast information in assessing the effect of background colour on the performance of many teleost fish larvae is available, comparatively limited research has been undertaken to examine its influence on the growth and survival in seahorses, particularly during the early life stages. Against this background, an experiment was conducted to test the hypothesis that tank colour affects the growth and survival in pelagic phase juveniles of *H. kuda* reared under identical environmental and feeding conditions. The outcome of this study may result in optimising the conditions for juvenile rearing of seahorses and *H. kuda* in particular.

4.2 MATERIALS AND METHODS:

4.2.1 FISH AND FACILITIES:

Newly born juveniles (mean height, 8±0.9 mm; weight 1.9±0.45 mg) from a single brood were used for the experiment. Flat-bottomed transparent circular plastic tubs painted with colours such as yellow, green, blue, red, black and transparent (unpainted), served as rearing tanks. Oil-based enamel paints were applied on external tank surface only to prevent the leaching of paint materials into the rearing seawater. Each experimental coloured tank was filled-up with 5 l of filtered (5 μm) and UV treated seawater. Tanks were illuminated from above with a single 100 W Philips bulb (~700 lx) and a photoperiod of 24 h was maintained throughout the experiment. Gentle aeration with air stones embedded in serrated and muslin cloth (mesh size, 20 μm) wrapped PVC pipes (dia, 60 mm) was provided in order to prevent the formation of aeration-induced currents and excessive air bubbles. Experiments were set up in triplicate and the layout of different coloured tanks was randomized (Plate 4.1). A Juvenile density of 2 fish/l was maintained in each experimental tank.

4.2.2 WATER QUALITY AND FEEDING:

The seawater used for rearing juveniles 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 optimum levels recommended for culture of *H. kuda* throughout the period of experimentation: temperature, (28.5±0.5 ^{0}C), salinity (31±1.5 ppt), dissolved oxygen (6.1±0.6 mg/l), pH (7.6±0.3), NO_2-N (<0.02 mg/l) and NH_3/NH_4 (0 mg/l). Mixed zooplankton collected from the nearby bay-estuarine ecosystem using plankton nets of 60, 120 and 200 μm mesh size, respectively for feeding 0-7 DAB, 8-14 DAB and 15-21 DAB old juveniles. After collection, mixed zooplankton were allowed to settle in a large glass bowl to remove detritus. Mixed zooplankton dominated by copepods (nauplii, copepodite and adults) were fed to juveniles twice a day (10:00 h and 21:00 h) at prey densities of 6-7 cells/ml after carefully siphoning out faeces and uneaten prey organisms (prey mortality due to unsuccessful prey attacks) from the bottom of the tanks. 50% of the rearing water was exchanged twice a day. Our previous feeding experiments confirmed that better growth and survival was achieved when *H. kuda* juveniles were fed with copepods as prey organisms. Before each feeding, prey densities in the rearing tanks were estimated and appropriate adjustments were made to ensure that the densities never fell below 6-7 cells/ml.

4.2.3 JUVENILE GROWTH AND SURVIVAL:

At 7, 14 and 21 DAB, growth measurements in terms of height (Ht) and wet weight (W) were made on three randomly selected juveniles. Height (from tip of coronet to the tip of the uncurled tail) was measured to the nearest 0.5 mm by placing the fish on a submerged

Plate 4.1 Light and dark colour experimental tanks

plastic-covered 1 mm scaled sheet according to the method of Lourie et al. (1999). Wet weight of individual juveniles was measured on an analytical balance to the nearest 0.01 mg (Job et al., 2002).

Juvenile mortality in each experimental tank was recorded daily. Juveniles showing symptoms of horizontal floating and circular movement on water surface at a single place (whirlpool) possibly as a result of air-bubble ingestion (a buoyant air bubble in a juvenile's stomach) were siphoned out immediately. These were recorded as dead in the data as our previous observations confirmed that these juveniles cannot be revived and were found to perturb other healthy juveniles. To prevent potential increased growth rates in juveniles with increased access to feed through the loss of competing tank-mates, dead juveniles were replaced with identifiable tagged juveniles of the same batch and size in order that original stocking densities were maintained. Replaced juvenile seahorses, however were not included in final analyses.

4.2.4 STATISTICAL ANALYSIS:

One way analysis of variance (ANOVA) was used to assess the difference in growth of juveniles reared in different coloured tanks and post-hoc analysis was done by Tukey's multiple comparison test when statistical significance was observed. Kruskal-Wallis test statistic was used to find the differences among the survival rates in different coloured tanks. Significant difference was tested at 5.00% level of significance and represented as $p<0.05$. All results were processed and analysed with the Graph Pad Prism 5 statistical package.

4.3 RESULTS:

4.3.1 JUVENILE GROWTH IN DIFFERENT BACKGROUNDS:

The measured growth (Ht) of *H. kuda* juveniles reared in different backgrounds at 7, 14 and 21 DAB is presented in Table 4.1. Across the six colours tested, juveniles reared in dark backgrounds (red, blue, green and black) grew taller (p<0.05) than those reared in light backgrounds (yellow and transparent). Amongst all treatments and sampled days, consistently higher growth (Ht) was observed in juveniles grown in red backgrounds. The order of juvenile growth in different backgrounds (red>green>blue>black>yellow>transparent) was found to be consistent on all sampled days of 7, 14 and 21 DAB. The height (Ht, mm) attained by juveniles reared in different backgrounds at 21 DAB, was: red (34.58±1.15 mm), green (33.58±1.15 mm), blue (33±0.94 mm), black (31.91±0.58 mm), yellow (29.33±1.12 mm) and transparent (28.75±1.50 mm).

The pattern of juvenile growth in terms of wet weight (W) across the six colours tested (Table 4.2) was similar to that of growth in terms of height (Ht). At 7 DAB, the order of growth (W) in juveniles in tested background colours was found to be red>green>blue>black>yellow>transparent. The pattern of wet weight (W) in juveniles reared in tested background colours at 14 was similar to that of DAB 7, however, post hoc analysis yielded three groups. At 21 DAB, juveniles reared in red (55.43±6.71 mg) and green (52.30±3.63 mg) tanks were significantly heavier (p<0.05) than black (45.7±1.82 mg), yellow (39.53±2.07 mg) and transparent (38.53±2.03 mg) backgrounds but not significantly different (p>0.05) from blue background (49.93±3.05 mg).

Table 4.1 Growth (Ht) in *Hippocampus kuda* juveniles reared in each background colour tank for each of three sampled days of 7, 14 and 21 (mean±sd, n=9) (mm).

Tank background colour	**Days after Birth (DAB)**		
	7 DAB	**14 DAB**	**21 DAB**
Blue	16.75 ± 0.82^{ab}	25.08 ± 1.06^{de}	33 ± 0.94^{hi}
Green	16.83 ± 0.86^{ab}	25.33 ± 1.16^{de}	33.58 ± 1.15^{hi}
Yellow	15.33 ± 0.60^{bc}	22.50 ± 1.00^{f}	29.33 ± 1.12^{j}
Black	16.25 ± 0.52^{abc}	24.16 ± 1.12^{efg}	31.91 ± 0.58^{i}
Red	17.58 ± 1.20^{a}	26.42 ± 1.16^{d}	34.58 ± 1.15^{h}
Transparent	15.17 ± 0.80^{c}	22.08 ± 1.24^{fg}	28.75 ± 1.50^{j}

Values in a column with different superscripts significantly differ (p<0.05).

Table 4.2 Growth (W) in *Hippocampus kuda* juveniles reared in each background colour tank for each of three sampled days of 7, 14 and 21 (mean±sd, n=9) (mg).

Tank background colour	Days of Birth (DAB)		
	7 DAB	**14 DAB**	**21 DAB**
Blue	15.6 ± 1.70^{a}	28.71 ± 1.59^{cd}	49.93 ± 3.05^{gh}
Green	15.87 ± 2.00^{a}	30.38 ± 1.61^{cd}	52.30 ± 3.63^{g}
Yellow	13.37 ± 1.61^{ab}	24.65 ± 2.36^{ed}	39.53 ± 2.07^{ij}
Black	14.13 ± 1.51^{ab}	27.46 ± 2.81^{de}	45.7 ± 1.82^{hi}
Red	16.17 ± 1.73^{a}	32.01 ± 2.01^{c}	55.43 ± 6.71^{g}
Transparent	12.21 ± 1.54^{b}	23.83 ± 3.16^{f}	38.53 ± 2.03^{j}

Values in a column with different superscripts significantly differ ($p<0.05$).

4.3.2 JUVENILE SURVIVAL IN DIFFERENT BACKGROUNDS:

Survival in juveniles (%) reared in different backgrounds on all sampled days is depicted in Fig. 4.1. Kruskal-Wallis statistic indicated a significant variability in juvenile survival across different backgrounds ($p<0.05$). At 7 DAB, the highest and lowest juvenile mortalities were observed in transparent (36.66%) and blue (13.33%) backgrounds, respectively. At 14 DAB, highest juvenile mortality was recorded in transparent background and maximum juvenile survival was noticed in (86.67%) backgrounds. No juvenile mortalities occurred in any tested colours between 15 DAB and 21 DAB. At 21 DAB, the cumulative survival in juveniles was highest in green and blue (70%) followed by red (66.67%) and black (53.33%) backgrounds. Highest cumulative juvenile mortalities were observed in yellow (63.33%) and transparent (56.67%) and backgrounds (Fig. 4.1).

The cause of mortality in juveniles (either due to ingestion of an air bubble and/or starvation) reared in different backgrounds at 7, 14 and 21 DAB is depicted in Fig. 4.1. Ingestion of an air bubble was found to be the major cause and accounted for an average 65% of total mortality in all the tested backgrounds. Juvenile mortality due to air bubble ingestion was more predominant in transparent and yellow backgrounds than in other backgrounds.

4.4 DISCUSSION:

Several attempts to culture seahorses on a mass-scale in the recent past have met with limited success due to inadequate nutrition and disease resulting in heavy juvenile mortalities (Thangaraj and Lipton, 2008; Garcia et al., 2010). Therefore, much effort has been focussed on determining the optimum prey densities, feed type, system design and other conditions necessary for the successful rearing of seahorses (Payne and Rippingale, 2000; Woods, 2000b; Job et al., 2002; Olivotto et al., 2008; Murugan et al., 2009). Typically, much of the

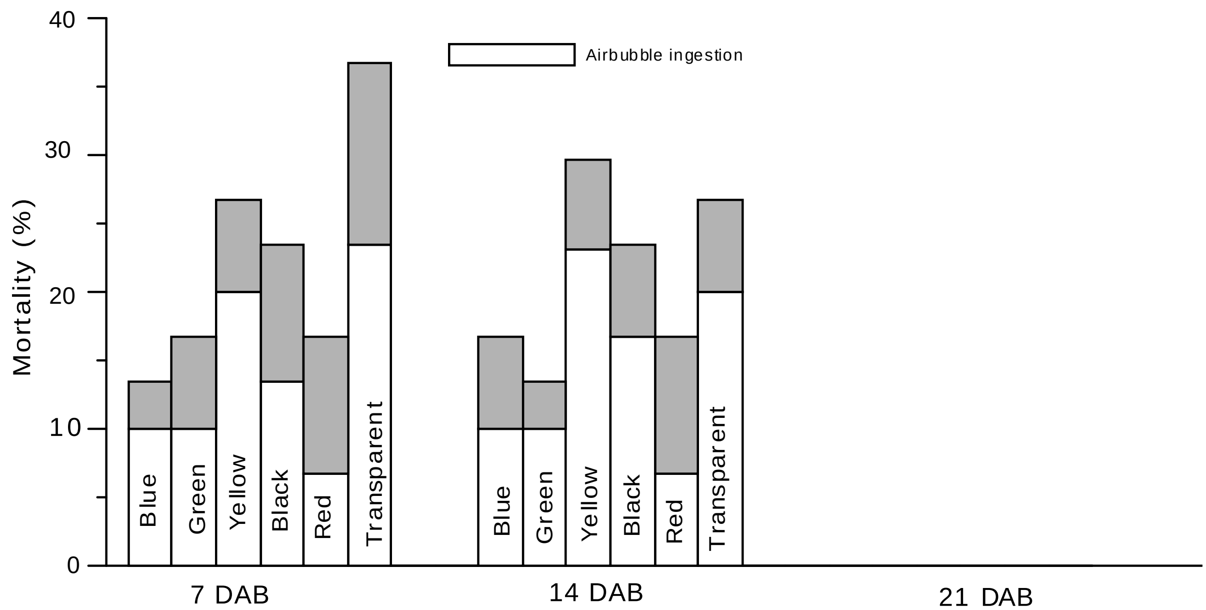


Fig. 4.1 Mortality (%) of *Hippocampus kuda* juveniles reared in different background colours at sampled days of 7, 14 and 21 DAB (days after birth).

research in seahorse husbandry has been conducted predominantly on fish older than two or three months of age, with very limited work being undertaken on early development stages (Hilomen-Garcia et al., 2003; Wong and Benzie, 2003; Woods, 2003). The initial developmental period (0-21 DAB) in seahorse husbandry when juveniles switch from the pelagic to the settlement phase is considered very critical as heavy mortalities are encountered during this period.

Several studies, with a number of fish species, indicate that factors such as prey density; light orientation, intensity and wavelength; tank hydrodynamics as well as the colour and orientation of prey items, affect the ability of larvae to detect, capture and ingest food items (Duray et al., 1996; Martinez-.Cardenas and Purser, 2007; McLean et al., 2008). Seahorse juveniles are no exception to this visual feeding behavior. Therefore, it is important in seahorse husbandry to optimise the prey densities suiting to the needs and consumption rates of juveniles so that food is not wasted, juveniles are not underfed and rearing water is not fouled. Considering the importance of background (tank) colours in influencing the feeding efficiency and survivorship many studies have been carried out in early stages of teleost fish (Blaxter, 1980; Moriya and Miyashita, 1987; Naas et al., 1996; Papoutsoglou et al., 2000; Tamazouzt et al., 2000). Except for studies by Woods (2000b) and Martinez-Cardenas and Purser (2007) in potbellied seahorse, *H. abdominalis*, information on the effect of background colour on growth and survivorship in juveniles of other cultured seahorse species is practically unknown. Woods (2000b) made a comparative assessment of different vessel backgrounds (white, black and clear jars) on attack rate and capture success of *Artemia* by *H. abdominalis* juveniles. Building further on the design of Woods (2000b), Martinez-Cardenas and Purser (2007) evaluated prey ingestion and growth rates in similar aged *H. abdominalis* juveniles under similar environmental conditions but over a greater range of tank colours both for short-term and long-term trials.

Significantly higher juvenile growth (Ht and W) recorded in dark backgrounds might be due to the increased visibility of prey organisms to *H. kuda* juveniles. Prey organisms (copepods) used in this study are translucent in colour which easily merges with light colour backgrounds. Higher incidence of clear copepods in the guts of alevins of Atlantic salmon grown in red aquarium than in blue or green aquariums has been reported by Browman and Marcotte (1987). This might be because of the higher dispersion and scattering of light make prey appear bright in contrast with a dark background (Naas et al., 1996). On the other hand, poor ability to detect and capture the prey by juveniles reared in light colour backgrounds might have affected their feeding efficiency resulting in poor growth.

Few studies assessing the effect of tank colour on performance characteristics (growth and survival) in seahorse species have been reported (Woods, 2000b; Martinez-Cardenas and Purser, 2007). An age-dependent effect of tank colour upon both attack rate and capture success in different stages of *H. abdominalis* juveniles has been reported by Woods (2000b). At 7 DAB, both attack rate and capture success were higher for juveniles contained in clear jars, and capture success was higher in black jars, as compared with white jars. Whereas at 30 DAB, there was no significant difference in attack rate between different jars, although capture success was higher in clear and white jars than in black jars. Martinez-Cardenas and Purser (2007) also tested the effect of tank colour on *Artemia* ingestion, growth and survival in 3, 7 and 42 DAB old juveniles reared in eight backgrounds (clear, white, yellow, orange, red, blue, green, and black) and concluded that tank colour may not be critical in *H. abdominalis* juvenile seahorse culture. Contrasting results reported by Woods

(2000b) and Martinez-Cardenas and Purser (2007) might be attributed to the different *Artemia* enrichments used in the two studies (Woods, 2007). In the present experiment, highest growth (Ht and W) was obtained in juveniles reared under dark background tanks with copepod as prey organisms. Vast differences in results reported by Woods (2000b), Martinez-Cardenas and Purser (2007) and the present study suggest that response to colour may be species–specific. In addition, light intensities which enhance prey detection and capture and manipulation of prey characteristics such as colour can also influence feeding success (Dendrinos et al., 1984).

Observation showed significantly higher growth in *H. kuda* juveniles reared in dark backgrounds is not surprising considering the natural habitat of this species. *H. kuda* typically inhabits shallow coastal waters and estuaries and is often found attached to the thick seaweed vegetation where light penetration is relatively lower. Naas et al. (1996) recommend the use of black background tanks for rearing of marine fish larvae as they provide a light regime that best represents natural conditions. Furthermore, Clarke and Sutterlin (1985) reported that first feeding Atlantic salmon alevins possess innate colour preferences and that preference is flexible; it can be altered by a single associative learning event. Presence of such innate colour preferences in seahorses, however has not been reported. A short-term colour conditioning test determining the strength of *H. abdominalis* juveniles cultured in a specific colour background to show preference for that colour indicated that juveniles failed to display any conditioning to the tank colour in which they were cultured (Martinez-Cardenas and Purser, 2007). Based on this observation, it does appear that preference for particular colour in seahorse juveniles is alterable by a single associative learning event.

Further research studying the stress levels associated with juveniles grown under different backgrounds is warranted.

A good contrast between prey and background may result in improved recognition of food items by seahorse juveniles, and subsequently result in better survival and growth. Significantly higher survival in red>blue=green>black showed that these background colours improve the visual efficiency of seahorse juveniles to detect the prey. Ingestion of air-bubble accounted for 65% of total mortality at 21 DAB in all the tested tank colours. Juveniles reared in light background coloured tanks had significantly higher incidences of air-bubble ingestion compared to the juveniles reared in light background coloured tanks. Ingestion of air bubble during the pelagic phase juvenile rearing of seahorses occurs due to miss-strike. Seahorse juveniles while preying on copepods engulf atmospheric air if they miss the strike and this leads to formation of air bubbles in the digestive tract and early juveniles are unable to take out the ingested air bubbles. Seahorse juveniles with ingested air bubbles lose their balance and are unable to capture the prey, subsequently leading to starvation and ultimately to death. Maximum mortality due to air bubble ingestion occurred in tanks having transparent and yellow colour background. Therefore, it appears that the tank background colour needs to be optimised for different prey organism, light conditions and different species of seahorses. The results of the current study, like those of previous studies were inconclusive from a growth perspective. Nevertheless, the results of the present study help in optimizing the rearing conditions for pelagic phase rearing of the yellow seahorse, *H. kuda*.

CHAPTER 5

MODIFIED METHOD FOR IMPROVING GROWTH AND SURVIVAL OF EARLY JUVENILE

CHAPETR 5

MODIFIED METHOD FOR IMPROVING GROWTH AND SURVIVAL OF EARLY JUVENILE

5.1 INTRODUCTION:

Similar to other marine ornamental fishes, seahorses show a bipartite life style, pelagic phase during the initial two to three weeks and later switch over to settlement phase; this duration is considered very critical in seahorse husbandry (Woods, 2000). High juvenile mortality and poor growth, particularly during the pelagic phase, is considered as bottleneck for mass rearing of seahorses. Optimization of the environmental conditions and feeding regime during juvenile rearing are critical to overcome the poor growth and low juvenile survival rate. Lin et al., (2010) optimised the light intensities, tank colour, stocking densities (Woods 2002). The previous chapter (Chapter 4) documented that the optimization of rearing tank background color results in improved growth and higher survival in pelagic phase juveniles of *H. kuda*.

When rearing juveniles of *H. kuda* in conventional tanks with direct aeration method during the pelagic phase, one is often confronted with the problem of poor juvenile growth and low survival due to air bubble disease. This may be due to the currents induced by aeration which compel juveniles to expend more energy counteracting this; besides, a higher number of unsuccessful preying attempts lead to air bubble ingestion. This limits the mass-scale rearing of juveniles and aquaculture of these seahorse species. Due to the importance of seahorse aquaculture in addressing the global concerns of conservation and livelihood sustenance, even small modifications in the rearing techniques could result in substantial improvement in juvenile growth and survival. Against this background, the conventional juvenile rearing tank has been modified to suit the requirement of new-born juveniles for mass-scale rearing.

5.2 MATERIALS AND METHODS:

Newly born (0 DAB) seahorse juveniles (mean height, 8.56±0.72 mm; weight 2.11±0.63 mg) used for the experiment came from a single captive bred *H. kuda* at the Aquaculture Laboratory of the National Institute of Oceanography, Goa (India). The modified tank (MT) consisted of a set of two identical blue colored FRP (Fiber Reinforced Plastic) tanks (Fig. 5.1 & Plate 5.1). In the first tank, rearing seawater was vigorously aerated (dissolved oxygen >6.5 mg/l) and pumped into the second adjacent tank (main tank) by a submerged aquarium pump (Tullu Pvt. Ltd.). To prevent the formation of water current, a slow water flow (0.5 l/min) was maintained in the main rearing tank. Main rearing tank comprised of three chambers, the first and third chambers were narrow and identical (10X45X30cm) whereas the second (rearing chamber) was wide (40X45X30cm). Main rearing tank was partitioned using transparent plastic frame with a hollow middle portion (20X35cm) covered by muslin cloth (120 µm). Oxygenated water from the aeration tank flows into the first chamber then to the juvenile rearing chamber and lastly to the third chamber and from here, the water returns to the aeration tank by gravity. As both tanks were kept parallel to each other, the water level is always maintained and hence there is no risk of overflow. On the other hand, conventional tanks (CT) (Plate 5.2) are ordinary rectangular tanks (60X45X30cm) where moderate aeration is given during the experiment.

Experiments were set up in triplicate and the layout of different tanks was randomized. Tanks were illuminated from above with a single 100 W Philips bulb (~700 lx at water surface) and an illumination of 24 h was maintained throughout the experiment as previous experience suggested that higher survival was achieved at this

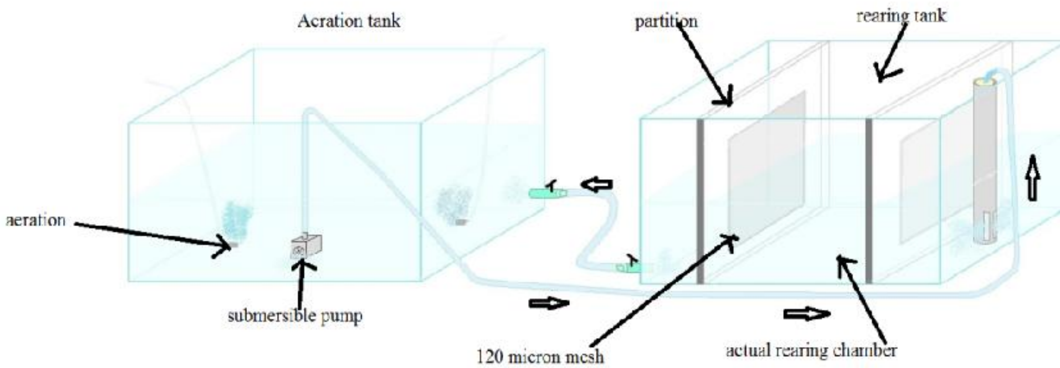


Fig 5.1 Modified Tank (MT) **Line diagram drawn by Felix**

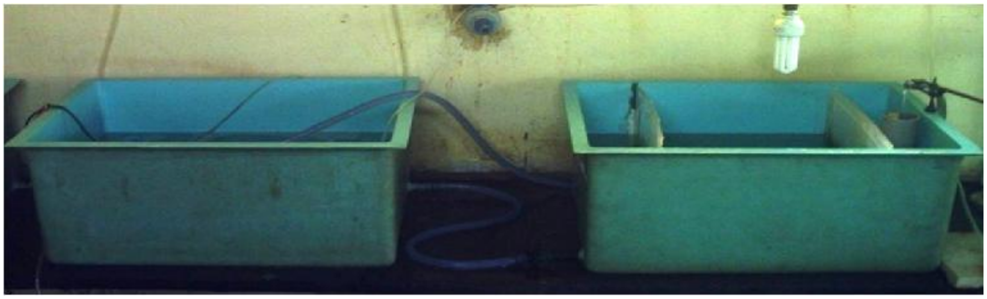


Plate 5.1 Modified Tank (MT)

Plate 5.2 Conventional tank (CT)

photoperiod (Chapter, 4). The seawater used for rearing juveniles was treated by rapid sand filtration, bio-filtration (5 µm) and passed through UV filter. Physico-chemical parameters of seawater in the juvenile rearing tanks fell within the optimum levels recommended for culture of *H. kuda* throughout the period of experimentation: temperature, (27.8±0.6 ^{0}C), salinity (26±1.6 ppt), dissolved oxygen (5.9±0.6 mg/l), pH (7.7±0.2), NO_2-N (<0.02 mg/l) and NH_3/NH_4 (0 mg/l).

During the experiment, juveniles were fed with wild caught mixed zooplankton from the nearby bay-estuarine ecosystem using appropriate plankton nets (120 µm for 0-14 DAB and 200 µm for 15-21 DAB old juveniles). After collection, mixed zooplanktons were allowed to settle in a large glass bowl to remove detritus. Light was provided at one of the top corners of the bowl to attract and gather the zooplankton at one place, to be subsequently separated by siphoning. Juveniles were fed with mixed zooplankton dominated by copepods (>80%) twice a day (1000 h and 2100 h) at prey densities of 6-7 cells/ml after carefully siphoning out feces and uneaten prey organisms (prey mortality due to unsuccessful prey attacks) from the bottom of the tanks. Freshly hatched *Artemia* nauplii (Instar I) were fed to juveniles >14 DAB and their proportion in the total feed offered varied (25% for 15 DAB, 50% to 16-18 DAB and 75% to >18 DAB old juveniles). Before each feeding, prey densities in the rearing tanks were estimated and appropriate adjustments were made to ensure that the densities never fell below 6-7 cells/ml. About 25% and 10% of water was exchanged twice a day in CT and MT, respectively. Prior to each feeding, settled plankton and dust particles attached to the muslin cloth of the second compartment were siphoned off particularly in the second compartment of modified tanks for proper water flow from one compartment to the other. After each feeding, aeration or water circulation was suspended for about 30 min to enable juveniles to prey on prey organisms more efficiently.

Measurement of growth in height ('Ht') and growth in weight ('W') were made on eight randomly selected juveniles at the end of 7, 14 and 21 DAB. The 'Ht' (from tip of coronet to the tip of the uncurled tail) was measured to the nearest 0.5 mm by placing the fish on a submerged plastic-covered 1 mm scaled sheet according to the method of Lourie et al., (1999). The 'W' of individual juveniles was measured on an analytical balance to the nearest 0.01 mg (Job et al., 2002). Juvenile mortality in each experimental tank was recorded daily. Juveniles showing symptoms of horizontal floating and whirlpool behavior due to air-bubble ingestion (a buoyant air bubble in a juvenile's stomach) were siphoned out immediately. These juveniles were recorded as dead in the data as our previous observations confirmed that such juveniles cannot be revived and further were known to perturb other healthy juveniles. Due to differential mortalities observed in CT and MT, a variable density of 2, 1.25 and 0.88 fish/l were maintained in rearing tanks for 0-7 DAB, 8-14 DAB and >15 DAB old juveniles, respectively. Lowering of juvenile density with the progress of rearing period is required to maintain uniform densities in both CT and MT and also to provide relatively more space for older juveniles. The significant difference in survival and growth of *H. kuda* juveniles reared in two different types of tanks was assessed by Mann–Whitney *U* test and Student t-test, respectively (Zar, 2005). All results were processed and analysed with the Graph Pad Prism 5 statistical package.

5.3 RESULTS:

The survival rate and measured growth in 'Ht' and growth in 'W' of *H. kuda* juveniles reared in CT and MT at the end of 7, 14 and 21 DAB is presented in Table 5.1. At the end of 7 DAB, the juvenile survival rate (94.58±2.60%), growth in 'Ht' (17.35±1.30 mm) and growth in 'W' (15.97±2.14 mg) in recorded MT were significantly higher (p<0.05) than those reared in CT (survival, 74.16±8.86%;'Ht', 16.11±0.86mm;

Table 5.1 Comparative assessment of mean (±SD) height and weight of *Hippocampus kuda* juveniles reared in modified and conventional tanks

Days After Birth (DAB)	**Modified tank (MT)**		**Conventional tank (CT)**	
	Height (mm)	**Weight (mg)**	**Height (mm)**	**Weight (mg)**
7 DAB	17.35±1.30	15.97±2.14	16.11±0.86	13.98±1.48
14 DAB	27.06±1.24	32.97±3.26	24.36±1.47	27.46±2.56
21 DAB	34.84±1.38	56.68±5.87	32.26±0.76	47.02±2.74

'W', 13.98±1.48 mg). The juvenile survival (96.66±3.06%), growth in 'Ht' (27.06±1.24 mm) and growth in 'W' (32.97±3.26 mg) recorded at the end of 14 DAB in MT was higher than those reared in CT (survival, 76.66±7.02%; 'Ht', 24.36±1.47; 'W', 27.46±2.56 mg). At the end of experiments (21 DAB), the survival and growth of juveniles were higher in MT when compared to CT but not significant (Table 5.1). Relatively lower rates of juvenile mortality are due to air bubble ingestion recorded in MT (32.56%) compared to CT (68.43%). In MT, final survival (89.35%) at 21 DAB was significantly (p<0.05) higher than in conventional tank (43.22%). Juveniles exhibited settlement behavior (started catching other juveniles with their tail) on 17 DAB in MT while, it was on 18 DAB in case of CT.

5.4 DISCUSSION:

Several attempts to culture seahorses on a mass-scale in the recent past have met with limited success due to inadequate nutrition and disease resulting in heavy juvenile mortality (Lourie et al., 1999; Thangaraj and Lipton, 2008; Garcia et al., 2010; Koldewey and Martin-Smith 2010). Therefore, much effort has been focused on determining the optimum prey densities, feed type, tank background color, system design and other conditions necessary for the successful rearing of seahorses (Woods 2000; Job et al., 2002; Olivotto et al., 2008; Murugan et al., 2009). Typically, much of the research in seahorse husbandry has been conducted predominantly on fish >60-90 DAB with very limited work being carried out on early development stages (Woods, 2003). The initial developmental period (0-21 DAB) in seahorse husbandry when juveniles switch from the pelagic to the settlement phase is considered very critical as heavy mortality is encountered during this period (Chapter, 4). A close observation on the behavior of pelagic phase *H. kuda* juveniles reared in CT led to the conclusion that considerable amount of energy is expended by them by swimming against the current and often juveniles appeared to congregate at one corner of the tank whenever a direct aeration is provided. Furthermore, aeration induced currents pose difficulties for juveniles in targeting prey organisms thereby increasing the number of unsuccessful strikes. This situation causes air-bubble ingestion and ultimately leads to death due to starvation. These observations led to the modifications in the design of CT for achieving higher juvenile survival and growth while maintaining optimum levels of dissolved high oxygen with minimum water movements.

Woods (2007) had conducted a culture experiment on bigbellied seahorse *H. abdominalis* in cylindrical nine liter capacity tanks. All experimental tanks used had circulating water with the vertical movement inlet from the center of the bottom of tank with different background cultured tanks and better growth was found in the first week in the clear background tank. Whereas, at 1 month, there was no significant difference in attack rate between different background tanks, although capture success was higher in clear and white background tanks than in black. Here, none of the researchers studied tank design of any seahorse so results could not be compared. The results of the study highlighted that small improvements in seahorse rearing could be expected to increase the growth and survival of early stage juveniles which is necessary for mass-scale rearing for aquaculture purposes.

CHAPTER 6

MASS SCALE CULTURE

CHAPTER 6

MASS SCALE CULTURE

6.1 INTRODUCTION:

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 (Troung, 1998; Lourie et al., 1999, Murugan et al., 2009). 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, Vietnam and 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). 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).

The problem in culturing seahorses arises as a result of the lack of understanding of their biology. An attempt to culture seahorses on a large scale was unsuccessful and serious attempts to culture seahorses are now underway in most Asian countries. Majority of the projects on the culture of seahorses faced severe problems like mass mortality due to inadequate nutrition and disease. Improved seahorse husbandry and larval rearing will reduce the pressure on the wild population. The reared seahorses can be used as an alternative source for the ones caught in the wild. A technology for the rearing of seahorses will help to develop small scale aquaculture by seahorse fishers. The successful rearing of marine ornamental fishes is perhaps the most rewarding achievement attained by researchers because rearing success is little or less in India. The rearing techniques for mass propagation of young ones of seahorses can be used in stock enhancement programmes to conserve this unique creature. Keeping the above facts in mind, the present study was carried out in the Aquaculture laboratory of CSIR-National Institute of Oceanography, Goa.

This chapter provides basic information about different feedings on growth and survival rate in juvenile rearing and mass-scale culture over a period of 135 days from parturition. 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.

6.2 BROOD STOCK MANAGEMENT AND BREEDING:

Hippocampus kuda brooders were collected from the Ratnagiri region of Maharashtra and were used for the present study. Rectangular blue background FRP tanks (2 X 1.5 X 1 m) ~2500 liter capacity were used for the maintenance of the brood stocks. Before stocking the seahorses, the tanks were disinfected with bleaching powder and fresh water and allowed to sun-dry. Brooder tanks were filled with filtered and UV treaded seawater and brooders were acclimatized to the ambient water conditions before being placed in these tanks. The brooders were fed with amphipods, mysids, fish larvae, mosquito larvae, adult *Artemia* and grass shrimps twice a day at 9:00 am and 5:00 pm. During feeding aeration was stopped for ~30 minutes. The fecal matter was removed periodically and ~20% of water was exchanged daily. The photoperiod was 13 hrs light and 11 hrs dark naturally, water quality parameters such as temperature (25.2-30.4°C), salinity (26.8-34.3 ppt), dissolved oxygen (5.2 to 6.8 mg/liter), pH (7.7 to 8.1) were maintained in the brooder tanks.

As male female pairs formed they were transferred to a cylindrical breeding tank (2 m height X 1 m dia) filled with ~1500 filtered seawater. Ready- to- spawn males (brooding males) were transferred into individual spawning FRP tanks (60 X 45 X 30 cm) filled with ~70 litre filtered seawater. Breeding of seahorse is comparatively easy as the male incubates eggs in his brood pouch for the period of ~2 weeks. During the incubation period the male provides required nutrition and gas exchanges for the development of seahorse babies in the brood pouch.

6.3 STANDARDIZATIONS OF MASS SCALE CULTURE FEEDING PROTOCOL:

Seahorses change feeding antogenically, if suitable feed in sufficient quantity is not provided several times, seahorses stop feeding, which leads to poor growth due to starvation and may go to point of no return. Proper feeding is considered the most important aspect in successful seahorse culture, and was considered a bottle neck problem. Therefore, three different experiments were conducted on different age groups of seahorses to standardize feeding protocol (Plate 6.1).

6.3.1 EXPERIMENT 1

6.3.1.1 INTRODUCTION:

Seahorse juvenile rearing is the toughest part as it has bipertail life stages. For the initial 18 to 21 days they remain in the pelagic stage, which means they swim near the surface of the water. In that situation they face the maximum risk: a major problem being air bubble ingestion. Seahorse babies swim near the surface and feed on the zooplankton which being photactic, generally floats on the surface. While feeding whenever they miss the strike they engulf air from the atmosphere. Several times it is observed that juveniles are unable to

Zooplankton collection

Zooplankton separation

***Artemia* hatching**

Artemia instar stage

***Artemia* growing**

Mysids

Amphipods

Plate 6.1 Various types of live feed used during present study

remove the air bubble once they engulf air and subsequently they lose balance and are unable to catch prey any more. Therefore, proper feeding is very important during pelagic phase.

6.3.1.2 MATERIALS AND METHODS:

Newly born seahorse juveniles (mean height, 8.56±0.72 mm; weight 2.11±0.63 mg) used for the experiment came from a single captive bred *H. kuda*. Modified rearing tanks were used for the present experiment. Experiments were set up in triplicate and the layout of different tanks was randomized. Tanks were illuminated 24 hours a day from the top with a single 100 W Philips bulb. Filtered seawater was used for the experiment and physico-chemical parameters of seawater in the juvenile rearing tanks fell within the optimum levels recommended for culture of *H. kuda* throughout the period of experimentation.

Measurement of growth in height ('Ht') and growth in weight ('W') were made on three randomly selected juveniles at the end of 7, 14 and 21 DAB. The 'Ht' (from tip of coronet to the tip of the uncurled tail) was measured to the nearest 0.5 mm by placing the fish on a submerged plastic-covered 1 mm scaled sheet according to the method of Lourie et al., 1999. The 'W' of individual juveniles was measured on an 'Afcoset' electronic balance to the nearest 0.01 mg (Job et al., 2002).

Newly hatched *Artemia* nauplii and mixed zooplankton dominated by copepods (>85%) were used for the present experiment. The 't' test was used to assess the difference in growth of juveniles reared on *Artemia* nauplii and copepods. Mann–Whitney U test statistic was used to find the differences among the survival rates between treatments. Significant difference was tested at 5.00% level of significance and represented as $p<0.05$. All results were processed and analyzed with the Graph Pad Prism 5 statistical package.

6.3.1.3 RESULTS:

Juvenile growth:

The measured growth (Ht) of *H. kuda* juveniles reared on *Artemia* nauplii and copepods at 7, 14 and 21 DAB is presented in Fig 6.1. Across the two different feeds tested, juveniles reared on copepods grew taller (p<0.05) than those reared on *Artemia* nauplii. Between two different treatments and sampled days, consistently higher growth (Ht) was observed in juveniles grown on copepods. The height (Ht, mm) attained by juveniles reared on copepods at 7 DAB was: (16.72±2.11 mm), 14 DAB (24.72±2.99 mm) and 21 DAB (33.56±4.64mm); whereas seahorse juveniles reared on *Artemia* nauplii at 7 DAB was: (14.61±1.90 mm), 14 DAB (21.23±2.70 mm) and 21 DAB (27.44±1.92 mm).

The seahorse juvenile growth in terms of wet weight (W) (Fig 6.2) fed on *Artemia* nauplii and copepods was similar to that of growth in terms of height (Ht). Across the two different feeds tested, juveniles reared on copepods grew taller (p<0.05) than those reared on *Artemia* nauplii. Amongst two different treatments and sampled days, consistently higher growth (W) was observed in juveniles grown on copepods. The weight (W) attained by juveniles reared on copepods was: at 7 DAB (15.87±2.24mg), 14 DAB (31.81±3.88mg) and 21 DAB (53.76±4.10mg) whereas seahorse juveniles reared on *Artemia* nauplii was: at 7 DAB (11.84±1.52 mg), 14 DAB (22.36±2.51 mg) and 21 DAB (37.38±3.56mg).

Juvenile survival:

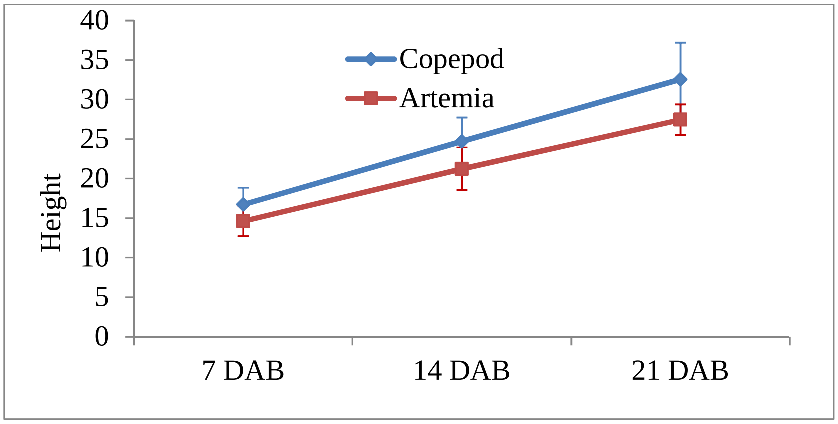


Fig 6.1 Height of pelagic phase juveniles of *Hippocampus kuda* fed with two different feed (copepods and *Artemia* nauplii)

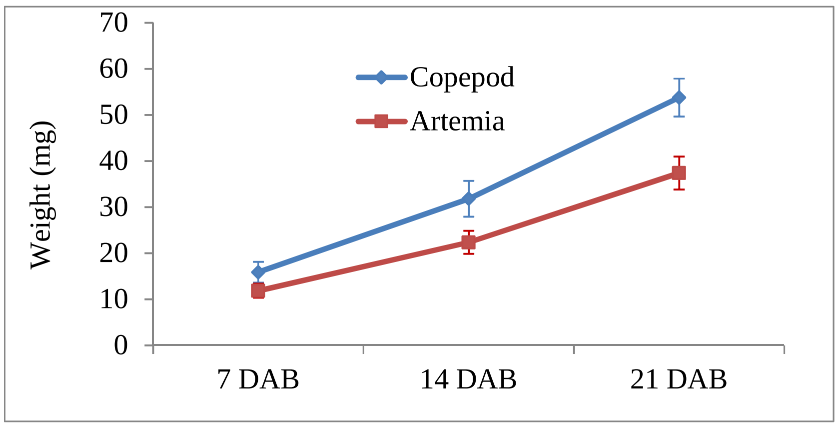


Fig 6.2 Weight of pelagic phase juveniles of *Hippocampus kuda* fed with two different feed (copepods and *Artemia* nauplii)

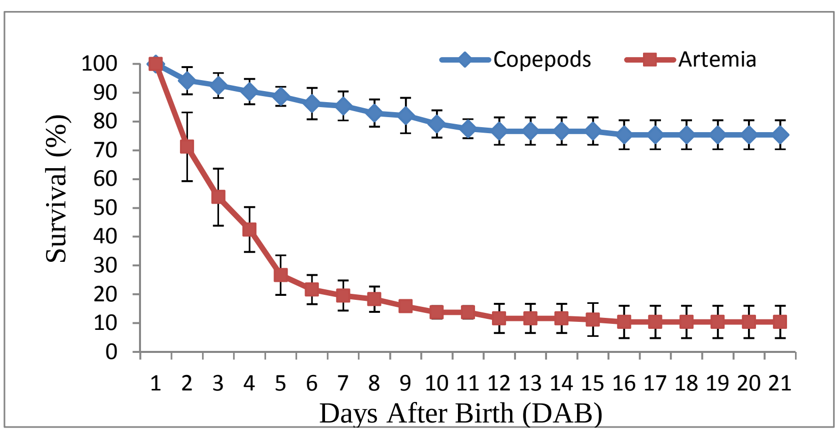


Fig 6.3 Survival of pelagic phase juveniles of *Hippocampus kuda* fed with two different feed (copepods and *Artemia* nauplii)

Survival in juveniles (%) reared on two different feeds is depicted in Fig. 6.3. Mann–Whitney *U* test indicated a significant variability in juvenile survival fed on different feeds (p<0.05). At 7 DAB (85.41±5.05%), 14 DAB (76.66±4.73%) and 21 DAB (75.41±5.05%) seahorse juvenile survival was reported fed on copepods whereas, at 7 DAB (19.58±5.20%), 14 DAB (11.66±5.05%) and 21 DAB (10.41±5.63%) survival was reported in seahorse juveniles fed on *Artemia* nauplii. Results showed significantly higher survival and better growth in pelagic stage juveniles when fed with copepods.

6.3.2. EXPERIMENT 2

6.3.2.1 INTRODUCTION:

Results of the first experiment showed better survival and growth of early seahorse juveniles initially fed on wild caught mixed zooplankton dominated by copepod. But catching wild copepods twice a day is very expensive and laborious. Therefore, a second experiment was conducted to find out whether after 10 DAB seahorse juveniles can be weaned on *Artemia* nauplii/ Instar as *Artemia* cysts are readily available and feed (*Artemia* nauplii) can be produce on demand.

6.3.2.2 MATERIALS AND METHODS:

For the initial 10 days seahorse juveniles were fed on mixed zooplankton dominated by copepods (>85%). On the 11th day seahorse juveniles (mean height, 20.2±2.4mm; weight 18.6±2.8mg) were stocked at the rate of 40 numbers per tank (One/lit) in the modified tank. Two treatments were used *Artemia* nauplii/instar stages and wild caught copepods (>85%). In the first treatment on 11th and 12th DAB newly born *Artemia* nauplii was fed (25% *Artemia* + 75% copepod), on 13th and 14th DAB one day old *Artemia* was fed (50% *Artemia* + 50% copepod), on 15 and 16th DAB 2 days old *Artemia* was given (100% *Artemia*), on 17th

and 20th DAB three days grown *Artemia* was given, on 21th and 24th DAB four day grown *Artemia* was provided, on 25st to 30th five days grown *Artemia* was provided. Whereas, in the second treatment single kind of feed, wild caught mixed zooplankton dominated with copepods, was provided from 11th to 30th DAB. Modified rearing tanks were used for the present experiment. Experiments were set up in triplicate and the layout of different tanks was randomized. Tanks were illuminated from the top for 18 hours with a single 100 W Philips bulb. Filtered seawater was used for the experiment and physico-chemical parameters of seawater in the juvenile rearing tanks fell within the optimum levels recommended for culture of *H. kuda* throughout the period of experimentation.

Measurements of growth in height ('Ht') and growth in weight ('W') were made on three randomly selected juveniles at the end of 15, 20, 25 and 30 DAB. The height and weight were measured as in experiment 1. As in the previous experiment statistical analysis was performed for the data analysis. After 18 days *H. Kuda* juveniles start showing settlement behaviour and started catching each other's tails. Therefore, a hold fast (2 mm nylon fishing net) was provided. Within a week all seahorse juveniles settled on the provided holdfast.

6.3.2.3 RESULTS:

Juvenile growth:

The measured growth (Ht) of *H. kuda* juveniles reared on *Artemia* nauplii and copepods at 15, 20, 25 and 30 DAB is presented in Fig 6.4. Across the two different feeds tested, juveniles reared on copepods and *Artemia* nauplii did not show significant difference (p>0.05) in growth in terms of height. The height attained by juveniles reared on copepods at 15 DAB (27.11±2.57mm), 20 DAB (34.22±4.24mm), 25 DAB (38.89±4.43mm) and 30

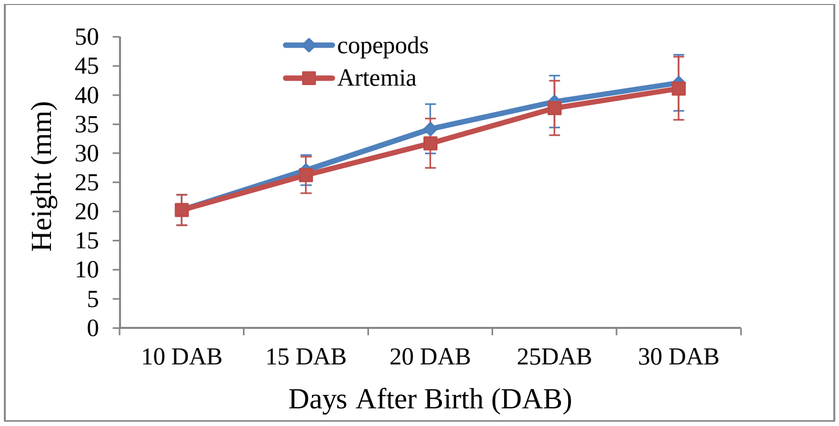


Fig 6.4 Height of 10 to 30 DAB (Days After Birth) juveniles of *Hippocampus kuda* fed with two different feeds (copepods and *Artemia* nauplii/ Instar)

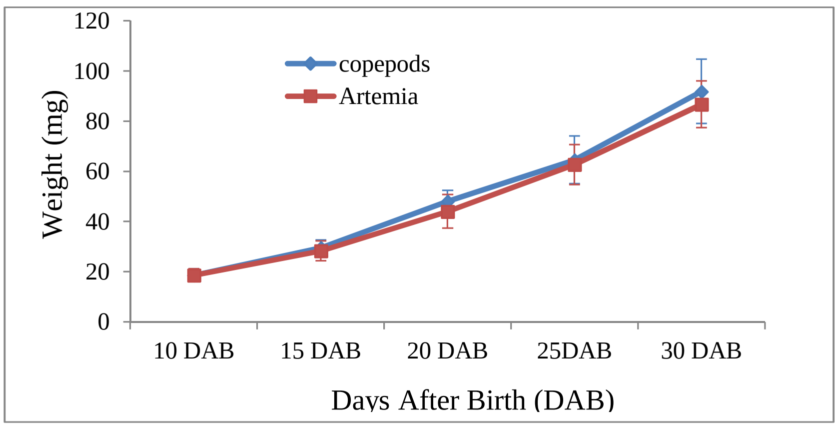


Fig 6.5 Weight of 10 to 30 DAB (Days After Birth) juveniles of *Hippocampus kuda* fed with two different feeds (copepods and *Artemia* nauplii/ Instar)

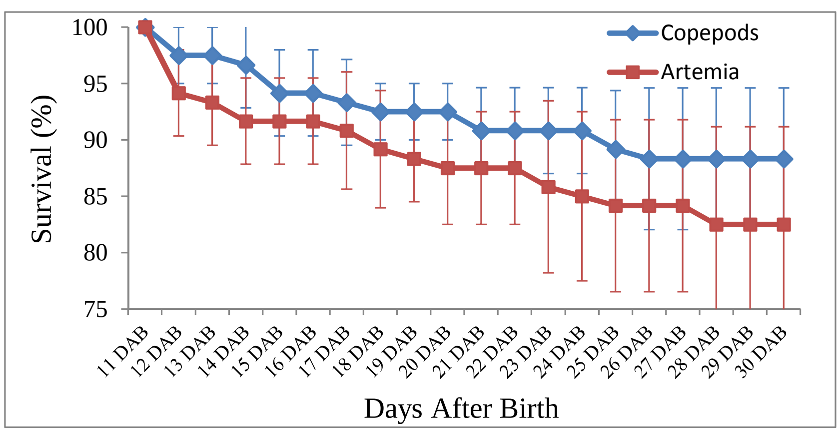


Fig 6.6 Survival of 10 to 30 DAB (Days After Birth) juveniles of *Hippocampus kuda* fed with two different feeds (copepods and *Artemia* nauplii/ Instar)

DAB (42.11±4.81mm) whereas, seahorse juveniles reared on *Artemia* nauplii at 15 DAB (26.28±3.13mm), 20 DAB (31.72±4.23mm), 25 DAB (37.78±4.68mm) and 30 DAB (41.16±5.40mm).

The seahorse juvenile growth in terms of wet weight (Fig 6.5) when fed on *Artemia* nauplii and copepods was similar to that of growth in terms of height. Across the two different feeds tested, juveniles reared on copepods and *Artemia* nauplii did not find significant difference (p>0.05). The weight (W, mg) attained by juveniles reared on copepods at 15 DAB (29.59±3.04mg), 20 DAB (48.11±4.36mg), 25 DAB (65.59±9.51mg) and 30 DAB (91.88±12.77mg) whereas, seahorse juveniles reared on *Artemia* nauplii at 15 DAB (28.33±3.99mg), 20 DAB (44.07±6.65mg) 25 DAB (62.71±8.01mg) and 30 DAB (86.72±9.29mg).

Juvenile survival:

Survival (%) in juveniles reared on the two different feeds is depicted in Fig. 6.6. Mann–Whitney *U* test indicated a significant variability in juvenile survival fed on different feeds (p<0.05). For seahorse juveniles fed on copepods, survival was reported as: at 15 DAB (94.16±3.81%), 20 DAB (92.5±2.5%), 25 DAB (89.16±5.20%) and 30 DAB (88.33±6.29%); whereas, for seahorse juveniles fed on *Artemia* nauplii, survival was reported as: at 15 DAB (91.66±3.81%), 20 DAB (87.5±5%), 25 DAB (84.16±7.63%) and 30 DAB (82.5±8.66%) Results showed there is no significant difference between growth and survival of seahorse juveniles fed with *Artemia* nauplii/instar stages and copepods.

6.3.3 EXPERIMENT 3

6.3.3.1 INTRODUCTION:

After 30 DAB seahorse juveniles start accepting comparatively larger sized prey: they prefer amphipods, mysids, adult *Artemia*, mosquito larvae etc. Therefore, a third experiment was conducted to find suitable feed from 31 DAB to marketable size (135 DAB, 10 cm<).

6.3.3.2 MATERIALS AND METHODS:

On 31st DAB seahorse juveniles (mean height, 31.28.±3.72mm; weight 88.77±9.87mg) were stocked at the rate of 100 numbers per tank in the modified tank. Two treatments were used: amphipods and mysids. Rectangular FRP tanks (2 X 1.5 X 1m) with blue background ~80% filled with filtered and UV treated seawater. About 20% daily water was exchanged through syphoning. Experiments were set up in triplicate and the layout of different tanks was randomized. Natural photoperiod was maintained 12:12 (Light: Dark) Filtered seawater was used for the experiment and physico-chemical parameters of seawater in the juvenile rearing tanks fell within the optimum levels recommended for culture of *H. kuda* throughout the period of experimentation.

Measurements of growth in height ('Ht') and growth in weight ('W') were made on three randomly selected juveniles from each tank at the end of 45, 60, 75, 90, 105, 120 and 135 DAB. Growth parameters height (Ht) and weight (W) were measured as in the previous experiment. The ‘t’ test was used to assess the difference in growth of juveniles reared on mysids and amphipods. Mann–Whitney *U* test statistic was used to find the differences among the survival rates in different treatments.

6.3.3.3 RESULTS:

Juvenile growth:

The measured growth (Ht) of *H. kuda* juveniles reared on mysids and amphipods at 45, 60, 75, 90, 105, 120 and 135 DAB is presented in Fig. 6.7. Across the two different feeds tested, juveniles reared on mysids grew taller (p<0.05) than those reared on amphipods. Amongst all treatments and sampled days, consistently higher growth (Ht) was observed in juveniles grown on mysids. The height (Ht, mm) attained by juveniles reared on mysids at 45 DAB was: (41.67±4.09mm), 60 DAB (54.83±6.32mm), 75 DAB (64.77±10.97mm), 90 DAB (78.89±8.97mm), 105 DAB (89.83±10.98 mm), 120 DAB (101.67±14.45mm) and 135 DAB (117.72±21.97mm) whereas, seahorse juveniles reared on amphipods at 45 DAB was: (39.22±3.24mm), 60 DAB (50.89±5.62mm), 75 DAB (61.72±7.78mm), 90 DAB (71.77±10.18mm), 105 DAB (81.77±13.65mm), 120 DAB (92.61±14.70 mm) and 135 DAB (99.89±16.60 mm).

The seahorse juvenile growth in terms of wet weight (W) (Fig. 6.8) fed on mysids and amphipods was similar to that of growth in terms of height (Ht). The weight (W, mg) attained by juveniles reared on amphipods at 45 DAB was: (224.84±28.51mg), 60 DAB (454.07±52.02mg), 75 DAB (783.09±92.51mg), 90 DAB (1236.82±151.60mg), 105 DAB (1968.17±308.85mg), 120 DAB (2730.41±465.28mg) and 135 DAB (3798.36±706.95mg) whereas, seahorse juveniles reared on amphipods at 45 DAB was: (205.34±21.29mg), 60 DAB (427.58±52.34mg), 75 DAB (742.58±100.57mg), 90 DAB (1199.21±141.27mg), 105 DAB (1725.65±266.23mg), 120 DAB (2475.31±353.17mg) and 135 DAB (3371.81±641.63mg).

Juvenile survival:

Survival in juveniles (%) reared on the two different feeds is depicted in Fig. 6.9. Mann–Whitney *U* test indicated there is no significant variability in juvenile survival fed on

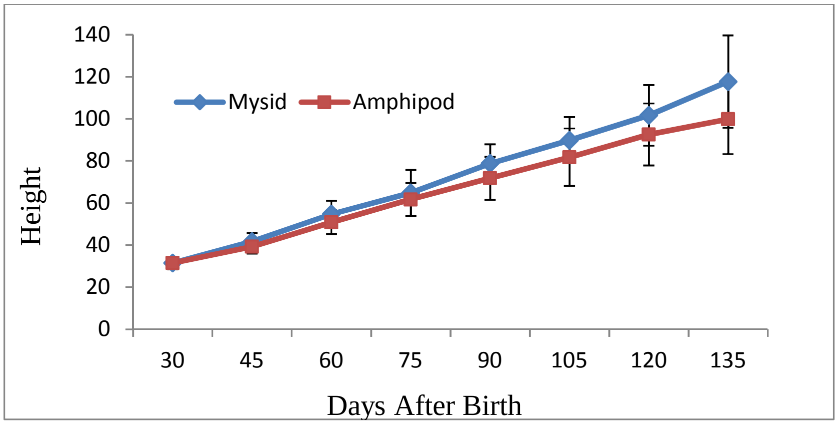


Fig 6.7 Height of 30 to 135 DAB (Days After Birth) *Hippocampus kuda* fed with two different feeds (amphipods and mysids).

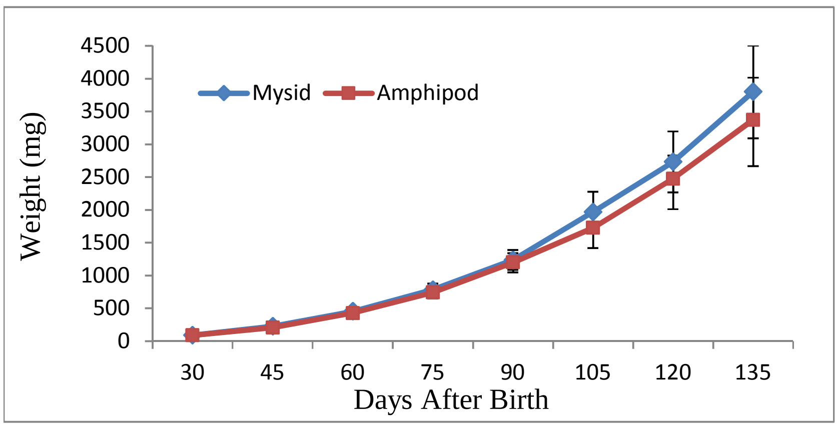


Fig 6.8 Weight of 30 to 135 DAB (Days After Birth) *Hippocampus kuda* fed with two different feeds (amphipods and mysids).

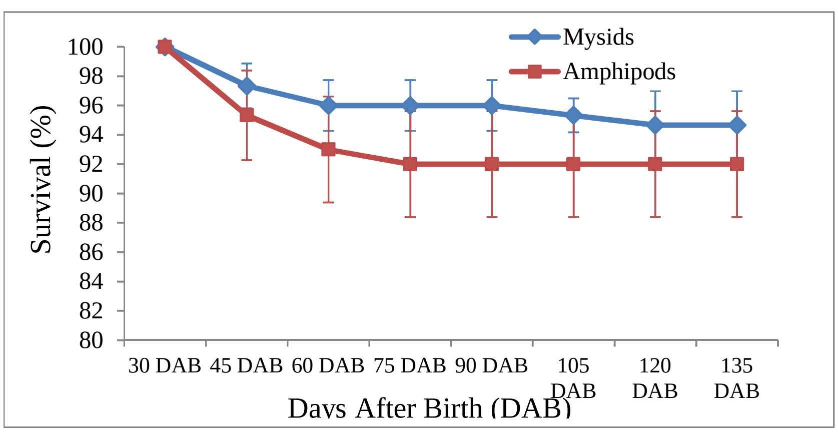


Fig 6.9 Survival of 30 to 135 DAB (Days After Birth) *Hippocampus kuda* fed with two different feeds (amphipods and mysids).

different feeds mysids and amphipods ($p<0.05$). Seahorse juvenile survival when fed on mysids was reported as: at 45 DAB (97.33±1.52%), 60 DAB (96.00±1.73%), 75 DAB (96.00±1.73%), 90 DAB (96.00±1.73%), 105 DAB (95.33±1.15%), 120 DAB (94.66±2.31%) and 135 DAB (94.66±2.31%); whereas, survival of seahorse juveniles fed on amphipods was reported as: at 45 DAB, (95.33±3.05%), 60 DAB (93.00±3.60%), 75 DAB (92.00±3.60%), 90 DAB (92.00±3.60%), 105 DAB (92.00±3.60%), 120 DAB (92.00±3.60%) and 135 DAB (92.00±3.60%).

6.4 DISCUSSION:

After settlement mortality rates are very low; the major problem after settlement is parasitic infection (ciliates). This could be avoided by using VU treated seawater and timely washing of the culture tanks with freshwater and potassium permanganate ($KMnO_4$) followed by sun-drying. Freshwater bath could also be given to treat ciliate infected seahorses. Seahorses of 10 cm and above are considered the ideal size for marine aquaria and Chinese medicine and recommended by conservation scientists for the sustainable use of seahorse resources. In the present investigation 438 no. of juveniles were grown to 10 cm size. *H. kuda* juveniles take 4 to 5 months to attain the size of 10 cm.

Feeding protocol and water parameters:

Feeding protocol which we have been following for the mass scale culture of *Hippocampus kuda* at Aquaculture laboratory, CSIR-National Institute of Oceanography is given in Table 6.1 whereas, recommended water parameters are presented in Table 6.2.

Table 6.1 Standardised feeding protocol for rearing of *Hippocampus kuda* from birth to marketable size

Days After Birth (DAB)	**Feed**
0 to 10 DAB	Copepod nauplii and copepodites
11 to 13 DAB	Copepodites and adult copepods + 25% *Artemia* nauplii in total feed
14 to 16	Copepods + 50% one day old *Artemia*
17 to 19DAB	Two day old *Artemia*
20 to 25 DAB	Three days old *Artemia*
25 to 30 DAB	Five days old *Artemia*
31 to 135 DAB	Mysids / amphipods/ adult *Artemia* / mosquito larvae as per availability

Table 6.2 Recommended water quality parameters for the mass scale culture of *Hippocampus kuda*

Parameter	**Range**	**Optimum**
Temperature	20-30	23-25
pH	7.5 to 8.2	7.8
Salinity	20 to 35	25 ppt
Light	700 to 2000 lux at surface	1000 lux
Photoperiod	1 to 21 DAB 24 hrs 22 to 45 DAB 12 hrs < 46 DAB < Natural day light	24 hrs 18 hrs Natural day light
Dissolved Oxygen (DO)	4 to 8 ppm	6 ppm
DO %	70 to 110	90 to 100

CHAPTER 7

CLOSING LIFE CYCLE IN THE CAPTIVITY

CHAPTER 7

CLOSING LIFE CYCLE IN CAPTIVITY

7.1 INTRODUCTION:

Destruction or degradation of estuarine and reef habitats threatens natural populations everywhere (Lourie et al., 1999) but the commercial heavy extraction of millions of seahorses per year is probably one of the principal factors for the decline of natural populations of these fish in several parts of the world (Vincent, 1996a). Seahorses in dried form are mainly sought for traditional Chinese medicines; in addition to that, live seahorses are used as ornamental fish in aquaria. The aquaculture of ornamental organisms is an alternative to the capture of wild individuals and may reduce the impacts upon natural populations (Vincent, 1996b). It is potentially a boon for the industry of ornamental fishes because various countries, such as Bahamas, Brazil and several states of the USA, have established importation quotas for wild-caught organisms (Tlusty, 2002). An interesting side effect of conducting research for the cultivation of seahorses is the possible furnishing of important life history information that may also be used in conservation and management of exploited species.

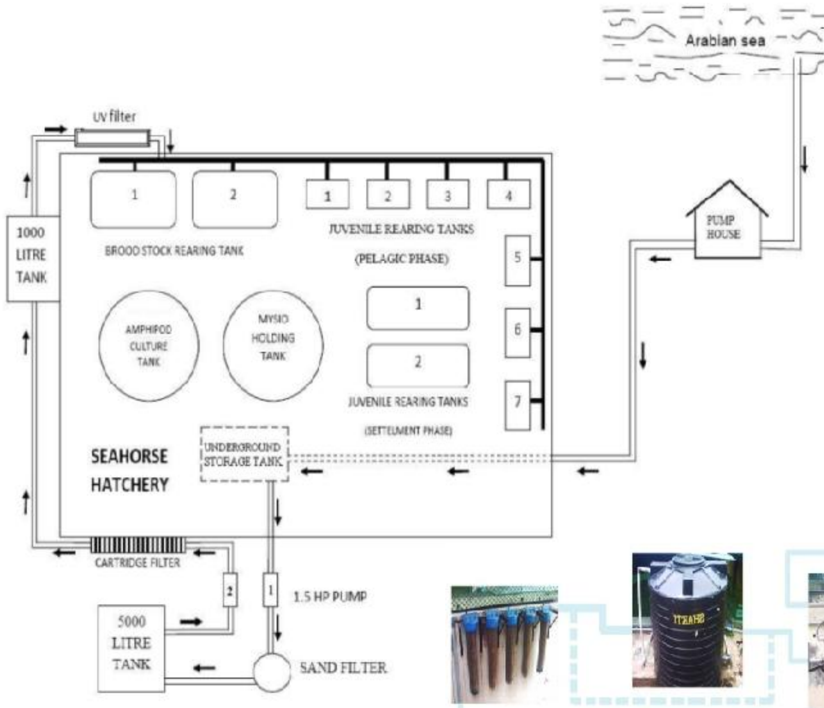
Several attempts to culture seahorses for successive generations under captivity in the recent past have met with limited success as a result of severe problems of mass mortality and low growth rate due to inadequate nutrition and disease. Closing of the life cycle in captivity and production of the next generation of young has not been well documented. Improved seahorse husbandry and rearing could help to reduce pressure on the wild population by ensuring that those animals in captivity live for longer and by creating the possibility of trade.

A successful closing life cycle experiment on breeding the yellow seahorse will help to facilitate the development of small-scale farms by seahorse fishermen, as a viable alternative to wild caught seahorses and conservation initiative.

7.2 MATERIALS AND METHODS:

Adult *H. kuda*, of 122-157 mm (SL, from tip of the snout to tip of the tail, n=33) were collected from Mirya creek, Ratnagiri, India region (17°01’55” N, 73°16’41” E) and were stocked in 1000 litre capacity brooder tanks in 1:1.3 (M: F) ratio. Prior to stocking, the tanks were disinfected with 200 ppm chlorine, washed thoroughly and allowed to sun dry. Brooders were fed twice a day with wild collected mysids and amphipods (0900 hr and 1700 hr). The faecal matter and dead prey were removed twice a day and 10-20% water was exchanged in the morning period (0830 hr) and evening (1630 hrs). Photoperiod of 12L: 12D was maintained naturally. Water quality parameters such as temperature (28±0.43°C), salinity (30±1.25ppt), dissolved oxygen (5.9±0.93mg/l) and pH (7.8±0.51) were maintained in the brooder tank. The courting pairs (n=9) were identified and placed in breeding tank with the capacity of ~1500 litres and the height of the tank was 2 meters with a diameter of 1 m. Ready-to-spawn males (brooding males) were placed in individual FRP tanks (60 X 45 X 30 cm) ~70 lit filled with filtered seawater.

New born juveniles (n=612) released from the male seahorse which was collected from the wild and mated in the *ex-situ* conditions were stocked individually in ~80 lit capacity FRP tanks separately, in three tanks with a water volume of 50 litre @ 4 juveniles/l and 24 hours photoperiod was provided to the triplicate experimental tank using a Phillips



Hatchery layout

Running Seawater facility

Sand filter

Cartridge filter

UV filter

Inside view of seahorse hatchery

Plate 7.1 Seahorse hatchery at NIO, Goa

Line diagram drawn by Felix

bulb (100W). On the 8th day, density was reduced to two juvenile per litter (100 young ones per tank) whereas, on 15DAB and 22DAB juvenile density was adjusted to 75 (1.5 juveniles/l) and 50 (1 juvenile/l) number per tank respectively.

The new born juvenile seahorses were fed with mixed zooplankton dominated with copepods (6-7 nos./ml) in the rearing tank (60 x 45x 30 cm) for the initial period of the ten days. On 11 DAB 25% *Artemia* nauplii was introduced in feed and subsequently the percentage of *Artemia* nauplii was increased (Chapter 6). Density of *Artemia* nauplii was maintained ~7 nos./ml and as the culture progressed they were fed with *Artemia* instar stages. The prey density was counted in the rearing tanks using a plankton counting chamber viewed through a stereo dissecting microscope (Olympus). When the density of copepods (3 nos./ml) fell below those levels, prey were added to the juvenile rearing tanks. As soon as the juveniles showed settlement behaviour holdfasts were provided in the rearing tank so that the juveniles could attach themselves to the holdfasts instead of holding other juveniles thus causing mortality. The photoperiod was altered to 16L:8D once the juveniles exhibited the settlement behaviour (18th day of culture).

After 30 days of culture, juveniles were transferred to ~1000 L FRP blue background rectangular tank (100 juveniles per tank; @ 1 juvenile per 10 L seawater) and natural photoperiod was maintained without providing artificial light. Juvenile height (HT, tip of coronet to tip of tail) and wet weight (mg) were recorded for the entire culture period with a fortnightly sampling to understand the average growth rate. The maturity of seahorses can be recognized by the appearance of brood pouch in the case of males (Plate7.2) and appearance of genital papilla in females (Plate 7.3) and dropping of egg clutch (Plate 7.4). On 180 DAB randomly 80 matured seahorses (F_1 generation 40 males and 40 females) were selected and

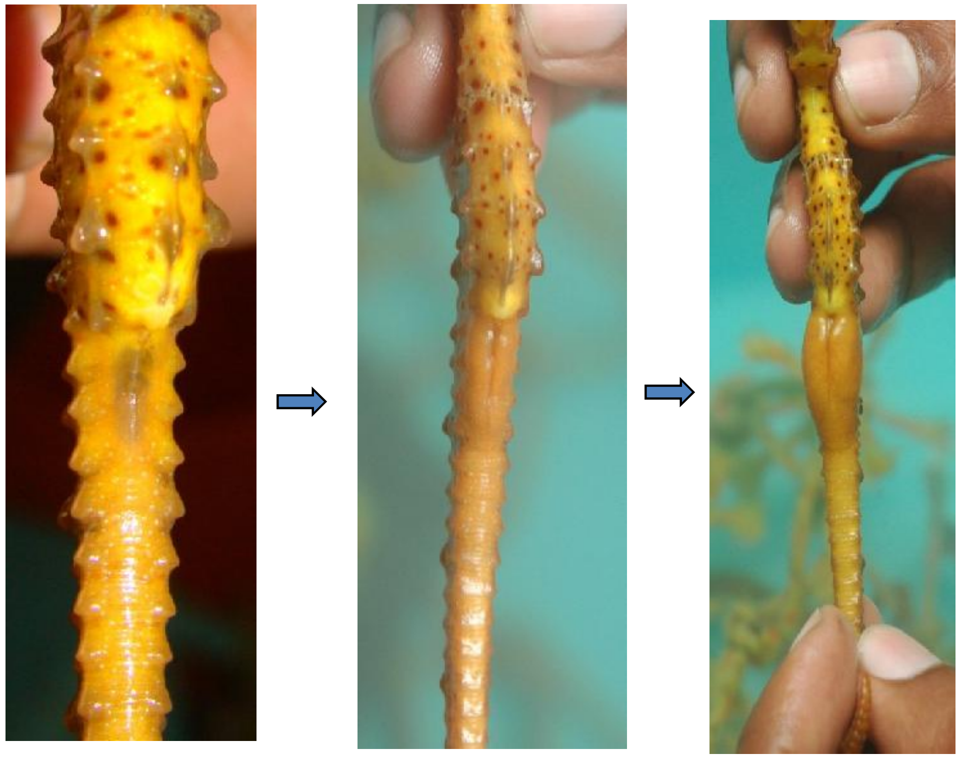


Plate 7.2 Brood pouch development stages

Plate 7.3 Genital papilla in female seahorse

Plate. 7.4 Clutch dropping (eggs)

they were placed in a 2 rectangular FRP tank (male: female 1:1) (200 X 150 X 100 cm) with nylon rope (4mm diameter) as a holdfast and tagged as described in Chapter 3. Multiple choices were provided for seahorses to select their partners mainly because females are very selective in choosing their partners. These brooders were fed *ad libitum* twice a day with amphipods and mysids, whereas natural photoperiod of 12L:12D was maintained. The pair which exhibited courtship behaviour was transferred to a circular tank of 2 m height and 1m diameter; while lengths of the pair were measured (HT) in order to observe any variation in reproductive efficiency with regard to size. The frequency of mating and number of young ones released were recorded for 12 pairs of seahorses as well as egg clutch dropping in matured females (n=9).

7.3 RESULTS:

7.3.1 REPRODUCTIVE EFFICIENCY OF MATURED WILD SEAHORSE MATED IN CAPTIVITY:

Seahorses collected from the wild exhibited courtship and mating behavior in a span of 27 to 54 days of stocking in the brooder tank. Paired seahorses showed courtship behavior 5-6 days before mating, during the courting period the males were more aggressive during early morning and late evening hours. Climax of the courtship was decidedly structured and the female deposited the unfertilized eggs into the male's brood pouch, transfer of eggs was not time specific. Incubation lasted for 12 to 16 days at 28°C water temperature. The brood size ranged from 247 to 766 (Table 7.1) and mean length of the newborn juveniles was 9±0.06 mm (HT). The new born juveniles resembled the adult seahorses in form, lacked a yolk sac and swam freely, exhibiting feeding behavior.

Table 7.1 Breeding of *Hippocampus kuda* collected from wild

Sr. No.	Size (cm)		Mating duration (days)	No. of juveniles released
	Male	Female		
Pair 1	15.4	15.7	27	516
Pair 2	15.4	14.9	33	247
Pair 3	15.8	16	44	766
Pair 4	14.3	13.9	54	519
Pair 5	15.2	14.7	29	634
Pair 6	15.3	15.9	37	471

7.3.2 SURVIVAL AND FEEDING RATE OF PELAGIC JUVENILES:

The survival of juvenile seahorses up to one week was 88%. The ingestion rate of the pelagic juveniles increased, on 6 DOC the ingestion rate on mixed copepod nauplii was 242±39 nos./juvenile/hr. Even though night feeding of week-old pelagic juveniles was observed, the ingestion rate on mixed copepod nauplii was low (86±13 nos./juvenile/hr). Mixed copepodites were provided to pelagic juveniles for the first two weeks thereafter, *Artemia* nauplii were introduced gradually. The highest ingestion rate on mixed copepodites (234±21 nos./juvenile/hr) was observed in pelagic juveniles on the 12 DOC. Ingestion rate of *Artemia* nauplii (198±27 nos./juvenile/hr) was high after the settlement stage and its preference was noticed for a week and then cultured *Artemia* sp. with different instar stages were provided to settled juveniles.

7.3.3 SETTLEMENT DURATION OF PELAGIC JUVENILES:

Almost immediately after birth, juveniles were concentrated on the upper half of the tank and showed pelagic behaviour. At 18 DOC, juveniles started showing settlement behaviour on the nylon mesh. As the juveniles grew, thickness of the holdfast was increased. The nylon mesh was replaced by nylon ropes and bleached coral fragments on the 35 and 67 DOC. Settlement of juveniles on holdfasts was initiated from the 18 DOC, while 100 % juveniles settled within 21 DOC.

7.3.4 FEEDING RATE OF SETTLED JUVENILES:

On the 30 DAB seahorse juveniles started feeding on amphipods, mysids, adult *Artemia*, mosquito larvae and Sergestid shrimp (*Acetes indicus*). As the juveniles grew, there was a shifting of prey items depending on size and it suggested that seahorses preferred larger prey as they grew. During the latter stages of the juvenile were fed as per availability of feed from the wild.

7.3.5 GROWTH RATE AND WEIGHT INCREMENT DURING JUVENILE REARING:

During the laboratory culture for a period of eight months, *H. kuda* attained a maximum height of 151 mm (142.04±13.5mm) and maximum weight of 10943 mg (10067±1115mg). Growth rate was higher during the initial stages and decreased with the attainment of maturity (Fig. 7.1). The average growth rate was 1.25, 1.05, 0.86, 0.75, 0.67, 0.63 and 0.60 and 0.59 mm/day for 30, 60, 90, 120, 150, 180, 210 and 240 days respectively in mass-scale rearing.

7.3.6 SEXUAL DIMORPHISM:

The sexual differentiation in *H. kuda* was possible only after the development of the brood pouch in males. The appearance of a brood pouch in males was first noticed on the 123rd day of culture (94 mm). Pouch development was complete in about 156 days. The female, however, released the first egg clutch in 178 days (105 mm). The number of males and females in the rearing system was 138 and 154 respectively. The sex-ratio was female biased ($P<0.005$) in mass scale condition.

7.3.7 SURVIVAL RATE:

Mortality was high prior to the settlement stage with a survival rate of 87.43% at 18 days of culture. At 30 days, 85.56% of the young ones survived and thereafter the mortality rate was found to reduce. Overall, the survival rate during the eight month rearing period was 76.28% (Fig. 7.2). Mortality was caused mainly due to the ingestion of air bubbles resulting

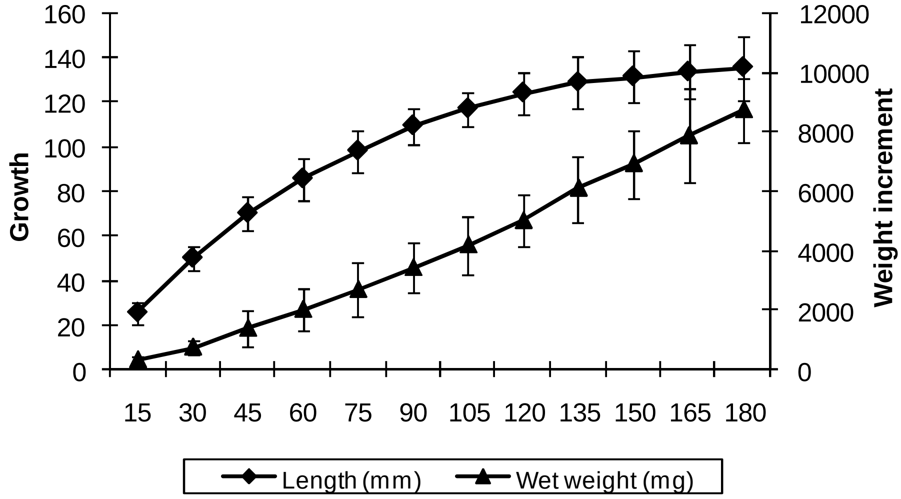


Fig. 7.1 Fortnightly variation in the increment of Growth rate (HT) and wet weight gain (mg) during the rearing of *H. kuda* reared in *ex-situ* conditions.

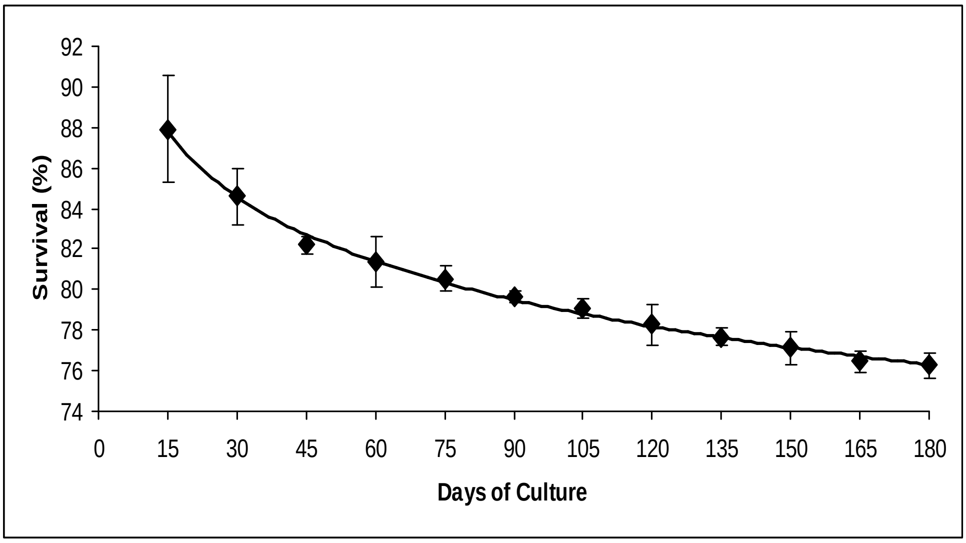


Fig. 7.2 Rate of survival in *H. kuda* reared in the laboratory

in the loss of buoyancy and lack of food intake as well as due to starvation (point-of-no return).

7.3.8 JUVENILE METAMORPHOSIS:

Metamorphosis of larvae to the juvenile stage is completed inside the brood pouch and they are released to the aquatic environment as a juvenile which has the characteristics of an adult seahorse. At birth itself all the fins are well developed and they exhibit free swimming behaviour energetically in search of food. As they grew, body characteristics like the development of tail rings, coronet, eye spine, check spine, trunk keel, appearance of chromatophores, skin filament growth and appearance of brood pouch were observed (Table 7.2).

7.3.9 *EX-SITU* BREEDING OF F_1 GENERATION:

The first mating of F_1 generation seahorses was noticed on the 173 DOC and the number of young ones released was 32 (Table 7. 3) (Plate 7.5). Inter- mating duration for the observed pairs varied from 16 (2 days after the release of young ones) to 43 days (29 days after the release of young ones); however the males gave birth to 32 to 169 young ones. The gestation period varied from 13 to 16 days at 28°C water temperature. During the breeding period increase in number of young ones could be noticed when the pair repeatedly mated, with a mean birth rate of 42.1±9.32 and 85.08±14.92 during the 1st and 2nd mating respectively (Table 7.4). Clutch dropping in female seahorses is considered as an important criterion for understanding sexual maturity. The mean number of hydrated eggs in the egg clutch was 47.4±14 which was on the higher side when compared to the mean number of new born released during the first mating period (Table 7.5).

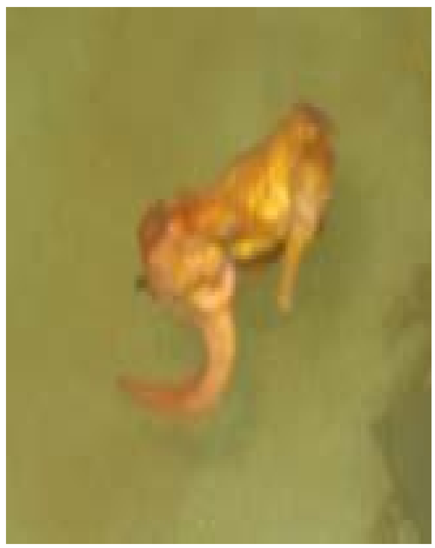
Table 7.2 Morphometric changes of *Hippocampus kuda* during the rearing period.

Days of culture	Morphological changes
1	Anal, pectoral and dorsal fin well developed. 1^{st}, 4^{th}, 7^{th} and 11^{th} trunk ring prominent. Pigmentation was seen in head region
7	Eye spine develop, tail and trunk spines prominent, Cheek spine developed, height increased in coronet. Pigmentation found up to trunk region, gill development
24	Cup shaped coronet with five blunt, check spine develops
35	Coronet and eye spines prominent
43	Star shaped coronet appears, trunk keel appeared
64	Trunk bears large spotted chromatophores pigments, snout became slender
75	Exhibited camouflage behaviour (black to yellow)
88	Appearance of different colour spots on the trunk regions
97	Skin filamentous growth in trunk and snout, keel started appearing in the trunk region
122	Slight brood pouch development
141	Day by which 50% male has pouch development

Table 7.3 Sexual and reproductive development of *Hippocampus kuda* (F_1 generation) reared in captivity

Species	*Hippocampus kuda*
Sex ratio (M:F)	138:154
First sign of pouch development in male	122 DAB
Day by which 50% male had signs of pouch development	141 DAB
First pouch fully developed by any male	163 DAB
Day by which 50% male had fully developed pouch	178 DAB
First free eggs releases in any female	169 DAB
No. of eggs in dropped clutch	74
First mating observed	223 DAB

*DAB = Days After Birth



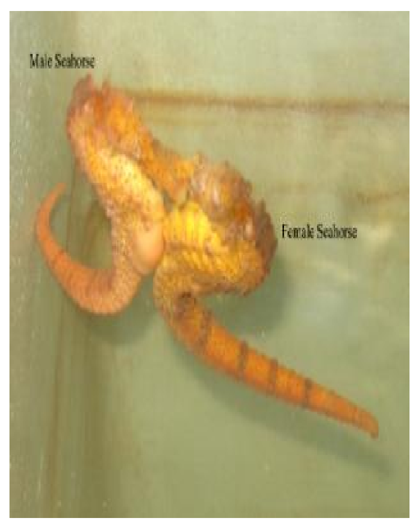


Plate 7.5 Seahorse mating in captivity

Table 7.4 Intermating and duration fecundity *Hippocampus kuda*

S.No	Size (cm)		1st mating (DOC)	No. of young ones	2nd mating (DOC)	No. of young ones
	Male	Female				
Pair 1	13.5	13.7	145	32	174 (29 days)	63
Pair 2	13.1	12.9	162	44	199 (37 days)	75
Pair 3	14.3	14	165	35	191 (26 days)	110
Pair 4	13.5	13.2	170	40	203 (33 days)	93
Pair 5	13.3	13	178	53	202 (24 days)	85
Pair 6	13.7	13.3	182	32	211 (29 days)	97
Pair 7	14	13.8	188	38	222 (34 days)	64
Pair 8	13.2	13.6	195	36	238 (43 days)	77
Pair 9	13.6	13.1	200	42	227 (27 days)	104
Pair 10	14	13.7	203	40	241 (38 days)	88
Pair 11	13.4	14	206	51	235 (29 days)	91
Pair 12	13.7	13.2	210	63	244 (34 days)	74

* DOC = Days of Culture

Table 7.5 Clutch dropping of matured female *Hippocampus kuda* (F_2 generation) reared in captivity

S.No	**Female (cm)**	**No. of hydrated eggs in the clutch**
1	13.4	36
2	13.6	42
3	14	51
4	13.5	39
5	13.2	29
6	13.4	40
7	12.9	66
8	13.3	71
9	12.7	53

7.4 DISCUSSION:

Seahorse populations all over the world experience heavy loss in terms of size and structure due to incidental capture in various fishing gears. Their life history parameters also render them vulnerable to over exploitation (Vincent, 1990). Seahorse farming is an advancement towards species conservation, which should be encouraged despite current legal restrictions concerning wild catch which might be used in the aquaculture trade. Globally at present 11 countries like Australia, Brazil, Hong Kong, Ireland, Mexico, New Caledonia, New Zealand, Sri Lanka, UK, USA and Vietnam are involved in the farming of seahorses (Koldewey and Martin-Smith, 2010). To overcome the concerns of policy makers about wild stocks of seahorses, tagging techniques and genetic tools can be used for sea horse trade to overcome those issues. Successful breeding and rearing programs under captivity can contribute to the utilization of seahorses in the TCM, marine aquarium trade and eventually to their conservation in the wild. Rearing of *H. abdominalis* in farms in New Zealand reduced the demand for wild caught specimens (Forteath, 1996).

7.4.1 REPRODUCTIVE EFFICIENCY OF MATURED WILD SEAHORSE MATED IN CAPTIVITY:

Maintenance of healthy and disease free brood stock is necessary for producing healthy young ones. Live *Acetes* spp. and amphipods have been shown to improve the efficiency of gonad development and decrease the mortality of parent seahorses (Murugan et al., 2009, Lin et al., 2006) whereas in the present study brooders were fed with mysids and amphipods, since these feeds are preferred by them in their natural habitat (Woods, 2002). A temperature range of 26 to 28°C was suggested as the optimal temperature for gonad development and spawning of *H. kuda* (Lin et al., 2006) and 28°C was provided to brooder seahorses during the present investigation. In the present study *H. kuda* which was collected from the wild, mated in the laboratory and released a mean brood size of 525±173.36, with a maximum brood size of 766 juveniles. Higher brood size of 1465 juveniles in pregnant males were recorded from Ratnagiri waters for *H. kuda* (Naik et al., 2002) up to 1751 from Philippine waters, 1025 from Palk Bay region (Gokulakannan, 2002), whereas for other seahorse species like *H. trimaculatus* the brood size was up to 783 from Gulf of Mannar waters (Murugan, 2004), up to 1750 for *H. kelloggi* from Coromandel coast. The present investigation suggests that the reproductive efficiency differs between wild and laboratory conditions and this aspect needs more attention to make advances in this fledgling industry.

7.4.2 SURVIVORSHIP AND FEEDING RATE OF PELAGIC JUVENILES:

Marine finfish rearing is governed by a number of factors like water temperature, stocking density, light intensity and feeding (Teshima 1984, Planas and Cunha, 1999). Water temperature significantly affected the rate of feeding in juvenile *H. trimaculatus* and the optimum was found to be 26°C Sheng et al. (2006) whereas in the present study the water temperature was maintained at 28°C**.** Light is of primary importance since most marine fish larvae including seahorse juveniles are visual feeders (woods, 2000). The positive effect of longer photoperiod on the survival or growth of marine fish larvae have been well documented (Houde and Palko, 1970, Houde, 1974). In the present study, the seahorse juveniles showed high survival rate (87.43% up to settlement period) when continuous photoperiod was provided up to the settlement period. Seahorse juvenile rearing tanks should be located at evenly distributed light to prevent the juveniles from gathering in one area where they compete for space and food (Sheng et al., 2006). By providing the photoperiod for 24 hr the juveniles were found to be evenly distributed in the rearing water column.

The availability of adequate quantities of suitable prey organisms is of crucial importance to the survival of larval fishes (Planas and Cunha, 1999). While the rate of feed intake depends on temperature, feed density and larval weight (Cunha, 1996), the selectivity of prey items is a function of prey detection, visibility and behavior of both predator and prey (Blaxter, 1980). Juveniles of *H. kuda* fed during the day and night up to the settlement stage, when 24h photoperiod was provided. During the pelagic juvenile rearing, a diet of copepod nauplii plays a major role in its growth and survival, where several researchers have preferred this diet when compared to other live feeds (Olivotto et al., 2008; chapter 6). However, the feeding rate of pelagic juveniles on mixed copepod nauplii was 214 and 86 (nos./juveniles/hr) during the day and night time respectively at 6^{th} days of culture showing its peak ingestion during day time. Similar observation has been reported in *H. trimaculatus* (Murugan et al., 2009). The reason behind this difference in ingestion rate may be due to the biological rhythms though clear evidence of the same is still lacking. Being visual feeders with the absence of lateral line, feeding in seahorses is dependent on the availability of light.

Successful and unsuccessful attacks on the prey are expensive and therefore have a direct impact on the growth and survival of larvae (Herbling and Gallager, 2000). The percentage of successful feeding strikes is low at first feeding (Hunter, 1981), but increases rapidly during development. In the present study the number of attacks on the prey varied from 0 to 12 attacks/minute during the day time whereas the attack rate decreased in the night hours (0 to 3/minute) even though 24 hr photoperiod was provided. Early juvenile seahorses showed better growth and survival when reared with dark colour background (Chapter 4) therefore, in our present study blue color was used as a background in the rearing tanks.

7.4.3 SETTLEMENT DURATION OF PELAGIC JUVENILES:

Most of the coral reef fishes exhibit pelagic and settlement behavior, whereas the settlement duration varies within species, groups and modes of spawning (Brothers et al., 1983). In damsel fishes (benthic spawners) *Dascyllus aruanus* and *Abudefduf sexfasciatus* the settlement stage occurred after 17 and 19 days post-hatch, whereas in pelagic spawners *Parupeneus barberinus* and *Acanthurus triostegus* the settlement patterns happened after 35 and 42 days (Juncker et al., 2006). Settlement duration in *H. trimaculatus* was around 15 days (Murugan et al., 2009), for *H. kuda* 19-22 days (Mi et al., 1998), suggesting variation in the time taken for settling to a holdfast. In the present observation *H. kuda* (pouch spawners) juveniles settled after 18 DOC, which coincides with the earlier reports. After the settlement of juveniles in the holdfast, feeding occurred only during the day time, showing the ambush mode of predation. Holdfasts, like nylon mesh, nylon rope, broken coral collected from fish landing sites and plastic plants, were provided as settlement objects. The width of the holdfast was increased as the juveniles grew. During the rearing period of *H. erectus* clumps of plastic eel grass with a blade width of ¼ inch were provided as holdfast (Scarratt, 1996), plastic aquarium plants (twisted *Vallisneria* like) for juvenile seahorse *H. kuda*, *H. fuscus* and *H. barbouri* (Wilson and Vincent, 1998) and 16 mm polyethylene mesh for *H. abdominalis* juveniles (Woods, 2000).

7.4.4 FEEDING OF SETTLED JUVENILES

Rearing of live feeds for reef associated fishes is found to be problematic; rotifers and *Artemia* spp. have been cultured successfully around the world. Limited yields and low productivity have been the result in most attempts at culturing copepods (Olivotto et al., 2008), which play a major role in pelagic stage of seahorse culture. In the present study suitable prey were identified for different stages of their life cycle. The juveniles were fed initially with mixed copepod and subsequently fed on *Artemia* nauplii, amphipods, mysids, sergestid shrimps and fish fry. Feeds like *Artemia* and amphipods were cultured and mysids were collected from the wild which suggest that seahorse culture depends on wild feed collection and similar comments have been reported earlier (Martin-Smith and Vincent, 2006). Feed was provided postpartum because the juveniles lacked yolk sac and were free swimming. Culturing of small numbers of *H. erectus* was carried out with *Artemia* nauplii followed by *Gammarus* spp. and frozen fresh water *Mysis* spp.; however, the success was limited by the number of *Gammarus* spp. available for feeding (Campsen and Paleudis 1995). Mass scale culture of seahorse juveniles in sea cages was done to reduce the cost of feeding since they fed on mobile epifauna such as amphipods and settling crustacean larvae which commonly form part of the early fouling community on the sea cages (Garcia and Hilomen–Garcia 2009).

7.4.5 GROWTH RATE AND WEIGHT INCREMENT DURING JUVENILE REARING:

Juveniles of *H. kuda* were around 9.6 ± 0.11mm SL (mean±S.D); 0.17 ±0.2 mg wet weight (mean±S.D) at birth, whereas the juveniles attained a height of 49.68±5.39 mm, 75.36±6.46 mm, 90.40±10.53 mm,102.88±9.76 mm, 112.76±12.37 mm 120.04±11.26 mm, 127.67±14.83 mm and 134.63±14.44 within a rearing period of 30, 60, 90, 120, 150, 180, 210 and 240 days respectively. Growth rate of 33.29 mm in 35 day old *H. erectus* (Scarratt, 1996), in 4 weeks *H. abdominalis* reached 30 mm in length (Woods, 2000), *H. erectus* and *H. kuda* attained a growth of 75 mm and 30.2 mm in four months and four weeks respectively (Anil et al., 1999, Campsen and Paleudis, 1995). In *H. kuda* a growth of 70 mm in 3 months was observed in pilot studies (Troung, 1998); however a growth rate of 85.8 mm in 56 days, 120.7 mm in 98 days was also observed in *H. kuda* (Job et al., 2002). No significant difference in growth rate for male and female seahorse (*H. kuda*) was observed during the present rearing period. Average growth rate of 0.55 mm/day in the seahorse *H. erectus* (Scarratt, 1996) and 1.53 mm/day in *H. kuda* (Job et al., 2002) have been reported. In the present study the average growth increment of eight month old seahorse *H. kuda* was 0.52 mm/day which was very less when compared to earlier studies.

7.4.6 SEXUAL DIMORPHISM:

Seahorses are dioecious animals where the age of sexual maturation varies among species as well as by seawater temperature, nutrition and environmental conditions (Jiaxin, 1990). In the present study the seahorse *H. kuda* attained sexual maturity at 114 mm and 119 mm in males and females respectively. The quality of food is thought to influence testicular and gonadal development in most fishes and will probably also be true in seahorses with more food leading to faster maturation (Mai et al., 1979). Size to attain maturity in commercial culture practices for *H. kuda* was 12-14 cm (Jiaxin, 1990). Brood pouch development in male *H. kuda* was noticed in 121 days, 85 days in *H. barbouri* and 78 days in *H. fuscus* (Wilson and Vincent, 1998), However, in the present study, pouch development was evident in 156 days. In the present study matured seahorses were fed with mysids and amphipods - further studies on these aspects need priority.

7.4.7 SURVIVAL RATE:

During seahorse rearing the mortality of juveniles was higher during the pelagic phase and reduced on reaching the settlement phase. The pelagic behaviour in seahorses is advantageous in nature by providing access to zooplankton as well as acting as a dispersal mechanism in the wild (Leis, 1991). However, under culture conditions, this phase is problematic leading to ingestion of air bubbles into the gut, hyperinflation of the swim bladder and starvation. Ingestion of air bubbles in the gut was common when preying on rotifer and copepod nauplii and this problem could be noticed up to the settlement stage. The two main constraints in rearing seahorses are the inability to provide sufficiently nutritious first food and the inflation of the gas bladder, a problem afflicting up to 50% of the brood within the first few days (Naik et al., 2002; Woods, 2002; Wilson and Vincent, 1998). After the settlement of juvenile seahorses, fungus, ciliates, bacterial and microsporidian diseases have been reported (Cheung et al., 1980; Vincent and Clifton-Hadley, 1989). However, in the present study, several disease problems were encountered which, are discussed in detail in chapter 9.

The major hold-up in seahorse aquaculture or mass scale rearing is the low survivorship of juveniles during the pelagic stage in cultured species like *H. erectus* (Lin et al., 2008), *H. kuda* (Lu et al., 2001; Job et al., 2002), *H. abdominalis* (Woods, 2002), *H. trimaculatus* (Murugan et al., 2009; Sheng et al., 2006). Despite these problems, high survivorship in seahorses have been achieved which look promising for their consideration as candidate species for aquaculture. In *H. erectus* 71.11% survival was achieved for a rearing period of 9 weeks (Lin et al., 2008), 90% in *H. comes* up to 9 weeks (Job et al., 2006), 65% in *H. trimaculatus* with a rearing period of 26 weeks (Murugan et al., 2009), >80% in *H. abdominalis* up to sexual maturity (Woods, 2000), 73% in *H. kuda* for a rearing period of 14 weeks (Job et al., 2002), whereas in the present study 76.28% survival was achieved for a rearing period of around ~32 weeks.

7.4.8 JUVENILE METAMORPHOSIS:

The new born juveniles had complete body rings and fin rays and similar observations have been reported in *H. trimaculatus* (Murugan, 2004; Cai et al., 1984), *H. kelloggii* (Balasubramanian, 2002), *H. kuda* (Mi et al., 1998, Choo and Liew 2006) from different geographical areas. Variation in morphological development in *H. kuda* could be noticed when compared to previous studies (Lu et al., 2001) and these need validation. During our observation vestigial caudal rays on new born juveniles were not observed despite their occurrence in *H. kuda* (Choo and Liew, 2006) and *H. mohnikei* (Kanou and Kohno, 2001) being reported. Sexual dichromatism was not evident in adults with most seahorses brownish in colour.

7.4.9 *EX-SITU* BREEDING OF F_1 GENERATION:

Availability of research information on captive breeding is very important for any aquaculture species to make advances in this activity. During 1980s, seahorse culture mainly depended on the collection of wild pregnant males (Vincent, 1996) and faced problems due to the lack of breeding techniques (Olivotto et al., 2008, Campsen and Paleudis 1995, Lockyear et al., 1997). Recent research has delivered good results about captive breeding techniques and closing of life cycle in the captivity which might reduce the dependency on brooder from the wild (Plate 7.6). During captive breeding programmes a mean brood size of 269 was reported in *H. abdominalis* (Woods, 2002) 39 in *H. capensis* (Leber and Lee, 1997), whereas in the present study the mean brood size of 78 youngones (F_2 generation) was observed in F_1 generation seahorses in captivity. Observations on the release of hydrated eggs have been reported in seahorses when there is a dearth of mating partners as well as during bad alignment in the act of copulation (Woods, 2002; Leber and Lee, 1997). The

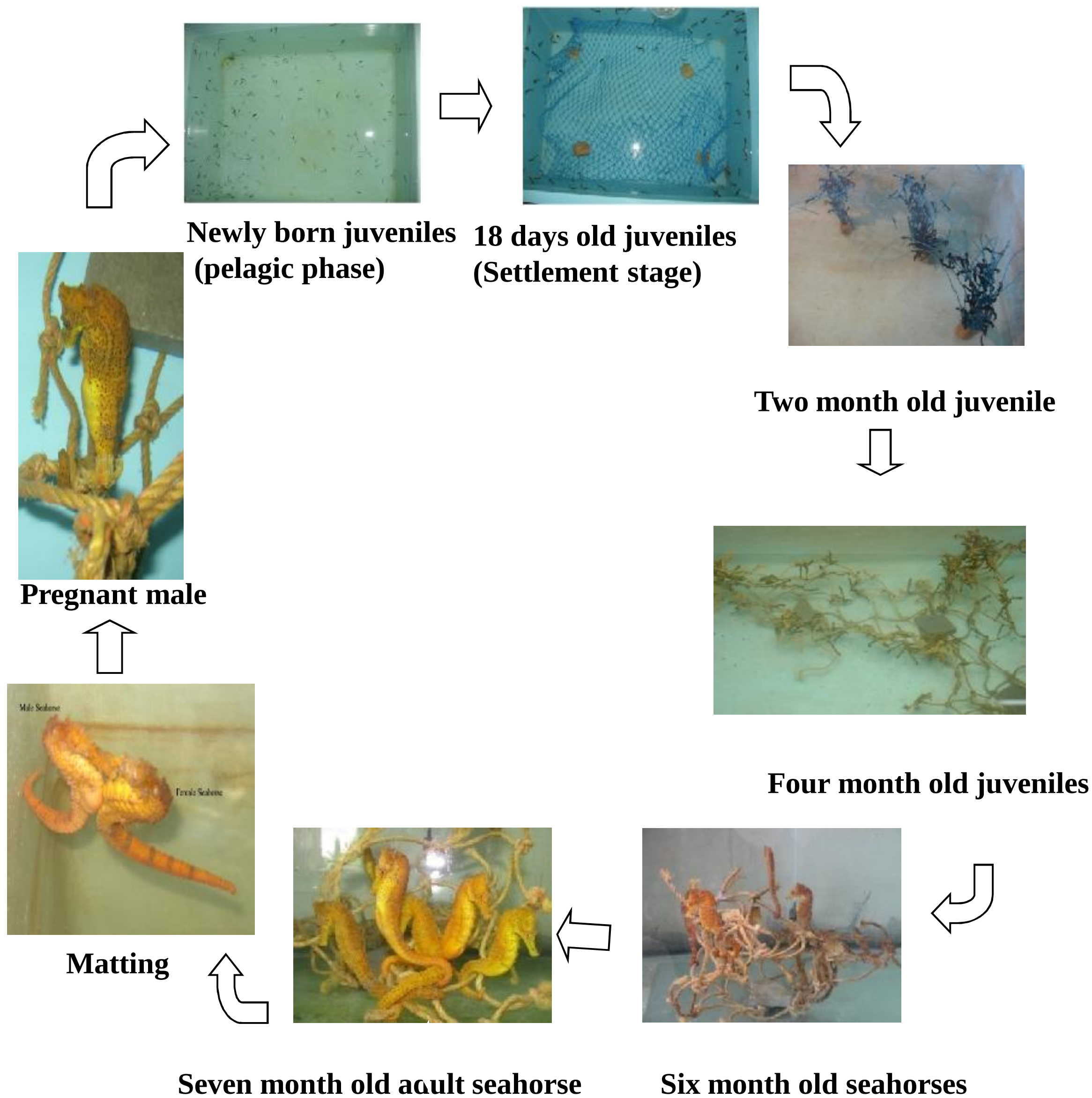


Plate 7.6 Closing seahorse life cycle in captivity

initial observation made in this study about the clutch droppings (47.4 ± 14 eggs) provides information about the reproductive efficiency of newly matured seahorses which is important for breeding programmes. Information regarding clutch dropping in seahorse has been an overlooked topic.

CHAPTER 8

STANDARDIZATION OF VARIOUS ANAESTHETIC DOSAGES ON SEAHORSE

CHAPTER 8

STANDARDIZATION OF VARIOUS ANAESTHETIC DOSAGES ON SEAHORSE

8.1 INTRODUCTION:

The use of anaesthetic agents is a common practice in modern aquaculture and has practical relevance when these agents are required for diverse husbandry manipulations such as the selection of fish, their measurement, sampling, tagging, transportation, artificial reproduction and surgery procedures (Roubach et al., 2005; Weber et al., 2009). The use of anaesthetic agents may reduce these stress-induced damages to the fish, but may also attenuate the physiological response to stress (Weber et al., 2009). A number of chemicals have proved effective in the anaesthetisation of fish, each having its own merits and demerits (Velíšek et al., 2006). The most commonly used anaesthetics in aquaculture are MS-222 and 2-phenoxyethanol (PE). Recent studies have evaluated the anaesthetic efficacy of clove oil, the main active component (70 to 90%) of which is eugenol [2-methoxy-4-(2-propenyl) phenol], in various fish species (Walsh and Pease, 2002; Iversen et al., 2003; King et al., 2005; Mylonas et al., 2005; Roubach et al., 2005; Hajek et al., 2006), as well as benzocaine (ethyl aminobenzoate) (Heo and Shin, 2010; Pramod et al., 2010).

The choice of an appropriate anaesthetic depends mainly on its effectiveness in immobilizing fish, thereby allowing the fish to be manipulated (Gilderhus and Marking, 1987; Burka et al., 1997). An ideal anaesthetic should possess several attributes. They should be non-toxic, inexpensive, simple to administer and result in rapid induction and calm recovery (Treves-Brown, 2000). The efficacy is conditioned by environmental (temperature, pH and salinity) and biological factors (size, weight, lipid content and fish species) (Burka et al., 1997; Ross and Ross, 1999). It is often advisable to identify the lowest effective doses of different anaesthetics in a specified species, as the responses to the same anaesthetic may vary considerably among different species (King et al., 2005).

Seahorses and other syngnathid fishes are in great demand worldwide. They are an important ingredient in traditional Chinese medicine, as well as popular ornamental fish and curios. Different seahorse species are harvested on a large scale, and traded by at least 77 nations in high volumes and various product forms (McPherson and Vincent, 2004). It has been reported that at least 25 million seahorses (>70 metric tonnes, dry weight) are traded globally (Salin and Mohankumran, 2006; Project Seahorse, 2006). Seahorse aquaculture has been expanding considerably in terms of both number and size of aquaculture operations and in the number of species cultured to sustain the ever-increasing trade in traditional medicine, aquarium fishes and curios (Koldewey and Martin-Smith, 2010). Although some researchers have cited the use of anaesthetics in different seahorse species (Woods, 2002; Woods and Martin-Smith, 2004; Koldewey, 2005; Morgan and Bull, 2005; Castro et al., 2008; Mattle and Wilson, 2009; Otero-Ferrer et al., 2010), practical details on their efficacy seldom outlined. Before recommending the use of a particular anaesthetic, a range induction and recovery times must be established to assess its efficacy. Such information would therefore be necessary whenever a culture technology for a potential species is being developed.

Given the growing interest in the culture of seahorses and lack of detailed practical information on the administration of anaesthetics, the objective of the present study was to determine the minimum optimal dosage of four commonly used anaesthetic agents (MS-

222,benzocaine, clove oil and PE) that could be used in the yellow seahorse, *Hippocampus kuda* (Bleeker, 1852). Information on stages of induction and recovery associated with exposure of *H. kuda* to a wide range of concentrations of anaesthetic agents provided here should be of practical interest to seahorse aquaculturists.

8.2 MATERIAL AND METHODS:

8.2.1 FISH AND EXPERIMENTAL FACILITIES:

Juveniles (pelagic phase) released by captive-bred *H. Kuda* were reared in rectangular blue background FRP tanks (capacity, 100 L) at a juvenile density of 2 fish L^{-1} until they attained settlement phase. Thereafter, juveniles were reared in large FRP tanks (capacity, 500 L) secured with different types and sizes of holdfasts depending upon the growth of juveniles. The seawater used was treated by rapid sand filtration, bio-filtration and then passed through ultraviolet radiation. Adequate aeration was provided to the tanks using air blowers and a photoperiod of 12 h l (0700─1900 h):12 h D (1900─0700 h) was maintained using a fluorescent bulb (100 W Philips build) providing a light intensity ~800 lx at the water surface. Juveniles were fed *ad libitum* three times per day (0600, 1400 and 2200 h) with different live prey organisms such as copepodites, *Artemia* nauplii and mysids (*Mesopodopsis orientalis*). The tanks were cleaned daily, and water quality was measured twice a week. The measured physico-chemical parameters of seawater in the rearing tanks fell within the optimum levels recommended for culture of seahorses (Murugan et al., 2009). Temperature (28.5±0.5 °C), salinity (31±1.5 ppt), dissolved oxygen (6.1±0.6 mg L^{-1}), pH (7.9±0.3), NO_2-N (<0.02 mg L^{-1}) and NH_3/NH_4 (0 mg L^{-1}). Healthy adults (mean height: 155.1±9.8 mm; mean wet weight: 11.15±1.68 g) were selected. Seahorses from two different tanks were used to avoid a possible tank effect and were starved 24 h prior to the initiation of anaesthetic experiments.

8.2.2 ANAESTHETIC AGENTS:

The anaesthetic agents MS-222 (tricaine methane sulphonate, HiMedia, India), 2-phenoxyethanol (ethylene glycol monophenyl ether, Sigma Aldrich Chemie, Germany), benzocaine (Ethyl-p-aminobenzoate, HiMedia, India) and clove oil (Sigma Aldrich Co. USA) were used. The doses of the anesthetic agents were prepared a few minutes prior to anaesthetic induction experiments. Since clove oil does not readily dissolve in water (Woody et al., 2002), it was initially diluted in ethanol (ratio of clove oil to ethanol: 1:9) to prepare a stock solution (100 mg ml^{-1}). Similarly, to increase the solubility of benzocaine in water, a stock solution was prepared with ethanol (ratio of benzocaine to ethanol 1:9) to obtain a concentration of 100 mg ml^{-1}. Aliquots of the stock solutions were used to obtain the desired experimental concentrations. MS-222 with its inherent solubility in water was added directly to the tanks to obtain the desired test concentrations. Preliminary trials indicated that the highest concentration of ethanol used in each trial did not have any noticeable analgesic effect on *H. kuda* for at least 20 min. A known volume of 2-phenoxyethanol (PE) was initially mixed with little seawater in a reagent bottle (200 ml) and then stirred to disperse the chemical to form small droplets before adding to anaesthetic induction tanks.

8.2.3 INDUCTION AND RECOVERY STAGES OF ANAESTHESIA:

Since the characteristics that define different stages of anaesthesia induction and recovery in seahorses have not been reported previously, a pilot study was conducted for describing these stages by carefully observing the behavioural responses in *H. kuda*. Clove oil (50 mg L^{-1}) was added to the anaesthetic induction tanks (37.5×22.5×22.5 cm) that were filled with fresh, aerated seawater (capacity, 15 L).Water temperature, dissolved oxygen and pH values in the tanks were 29±0.2 ºC, 6.1±0.5 mg L^{-1} and 7.9±0.1, respectively. Water in each tank was stirred with a glass rod and aerated to facilitate complete mixing. Adult *H. kuda* were netted from the rearing tank and transferred to the anaesthetic induction tank for studying stages of anaesthesia. Changes in the physiological status of the anaesthetized fish were assessed in three consecutive stages as described by Summerfelt and Smith (1990), with slight modifications based on the species-specific behavioural response of *H. kuda*. Different anaesthesia induction stages (IS) and recovery stages (RS) were defined by checking opercular movements, equilibrium and absence of response to tactile stimulus.

For practical purposes, four IS (IS1─sedation, IS2─partial loss of equilibrium, IS3─cessation of swimming and loss of reflex reactivity, IS4─modulary collapse) were considered (Table 8.1). When the seahorses reached IS3, they were immediately netted from the experimental tank, and placed on a wet surface for a period of 90 s. Our previous studies (Pers. obs.) indicated that this time period was sufficient amount of time for taking length and weight measurements or fixing of a tag. Then fish was transferred to a recovery tank (capacity, 100 L) filled with fresh, aerated sea water for recording stages of anaesthesia recovery. Three RS (RS1─Starts operculum and fin movement, RS2─cannot balance head, tapping head on tank bottom, RS3─normal) different parameters were described (Table 8.1). An induction time of 3 min or less with complete recovery in 5 min suggested by Marking and Meyer (1985) was used to establish a new set of anaesthesia induction (IS3) and recovery (RS3) times for different anaesthetics used in the present study.

Table 8.1 Stages of anaesthesia and recovery exhibited by *H. kuda* treated with anesthetics agents (IS- induction stage; RS-recovery stage).

Stage	Descriptor	Behavioural response of the seahorse
0	Normal	Normal swimming and opercular rate; reacts to the external stimuli.
IS1	Sedation	Opercular rate slightly decreased. Slow swimming with slight loss of reaction to external stimuli.
IS2	Partial loss of equilibrium	Striking the water, erratic swimming; increased opercular rate; reaction only to strong tactile and vibration stimuli.
IS3	Cessation of swimming and loss of reflex reactivity	Completely stops swimming and stands on the bottom of the tank, opercular rate decreases slowly, no reaction to stimuli.
IS4	Modulary collapse	Opercular movement ceases due to over dose or longer immersion in anaesthetic solution. Subsequently death occurs.
RS1	Starts operculum and fin movement	Starts operculum movement, starts erratic movement of body.
RS2	Cannot balance head, tapping head on tank bottom	Cannot balance the head, most of the time keep head down trying to get up with tail force. Mouth tapping on the bottom of aquarium tank.
RS3	Normal	Ability to swim normally and regular opercular rate; Reactive to the external stimuli.

8.2.3 ANAESTHETISATION PROCEDURE:

The efficacy of four anaesthetic agents in adult *H. kuda* was assessed by testing several doses of each anaesthetic. The choice of minimum and maximum doses was based on experiments conducted previously on different teleosts (Weber et al., 2009; Gomes et al., 2001). The following doses of each agent were evaluated: MS-222 and clove oil (25, 50, 75, 100, 125 and 150 mg L^{-1}), benzocaine (75, 100, 125, 150, 175 and 200 mg L^{-1}) and PE (250, 500, 750, 1000, 1250 and 1500 µl L^{-1}). Anaesthetic experiments performed were similar to those in the pilot study and three seahorses were exposed to each tested anaesthetic concentration. Fish behaviour at each concentration was monitored individually throughout the induction and recovery stages. The induction and recovery times for four anaesthetic agents were measured under similar experimental conditions using an electronic stopwatch. When a particular seahorse reached stage IS3, it was immediately netted from the anaesthetic induction tank and kept on a wet surface for 90 s. After this, it was transferred to recovery tanks for recording recovery stages.

8.2.4 POST-TREATMENT SURVIVAL:

After anaesthetic treatment, seahorses were transferred to post-treatment tanks (capacity, 60 L) for seven days to assess the behavioural changes and mortality. Seahorses that were exposed to the same concentration of anaesthetic were kept in the same post-treatment tank. During the post-treatment period, seahorses were fed twice a day *ad libitum* with live mysids, and 50% of the tank water was exchanged on a daily basis.

8.2.5 STATISTICAL ANALYSIS:

Kruskal–Wallis test was used to assess the difference in induction and recovery times of different concentrations of the same anaesthetic agent (Zar, 1999). Regression analyses were used to establish relationship between dosage and induction time, as well as dosage and recovery time. All results were processed and analysed with the Graph Pad Prism 5 statistical software (San Diego, California, U.S.A) and graphs were plotted with Grapher 4 windows-based software (Golden Software, Colorado, U.S.A).

8.3 RESULTS:

8.3.1 INDUCTION AND RECOVERY STAGES OF ANAESTHESIA:

Significant differences (P<0.05) in the induction and recovery stages at different concentration levels of the four anaesthetic agents were identified. In general, the induction times decreased significantly with increasing doses for all of the anaesthetic agents evaluated. Induction time (IS3) ranged from 5.59±0.36 min (100 mg L^{-1}) to 1.32±0.09 min (150 mg L^{-1}) for MS-222, from 19.25±0.49 min (75 mg L^{-1}) to 2.30±0.16 min (200 mg L^{-1}) for benzocaine, 7.37±1.29 min (25 mg L^{-1}) to 0.36±0.07 min (150 mg L^{-1}) for clove oil and 19.14±1.09 min (500 µl L^{-1}) to 1.01±0.13 min (1500 µl L^{-1}) for PE (Table 8.2). Recovery times increased with increasing concentrations of anaesthetic agents (P<0.05). Recovery time ranged from 3.00±0.15 (100 mg L^{-1}) to 5.58±0.24 min (150 mg L^{-1}) for MS-222, from 0.41±0.06 (75 mg L^{-1}) to 11.18±0.44 min (200 mg L^{-1}) for benzocaine, 3.58±0.33 (25 mg L^{-1}) to 15.27±50 min (150 mg L^{-1}) for clove oil and 2.50±0.21 (500 µl L^{-1}) to 7.06±0.45 min (1500 µl L^{-1}) for PE (Table 8.2).

The lowest effective doses of different anaesthetic agents evaluated were: 125 mg L^{-1} for MS-222, 175 mg L^{-1} for benzocaine, 50 mg L^{-1} for clove oil and 1,000 µl L^{-1} for PE. MS-222 and PE produced induction and recovery times of less than 3 and 5 min, respectively while benzocaine and clove oil met the induction criterion while exceeding the recovery time limit by about 20 and 30% respectively. The lowest effective concentration of clove oil induced relatively quicker anaesthesia (1.55±0.28 min) and longer recovery (6.25±0.37 min) in *H. kuda*.

8.3.2 COMPARATIVE EFFICACY OF ANAESTHETICS:

A significant correlation was observed between anaesthetic concentration and induction time for all tested anesthetic agents (*P*<0.05), whereas scatter plot yielded inverse exponential relationship (Fig. 8.1). The regression equations of times to reach IS3 and concentrations (C) of four anaesthetic agents were $IS3=45.80e^{-0.023C}$ for MS-222; $IS3=90.31e^{-0.018C}$ for benzocaine; $IS3=7.01e^{-0.018C}$ for clove oil and $IS3=102.7e^{-0.003C}$ for PE. Similarly, a significant correlation (*P*<0.05) was observed between anaesthetic concentration and times to reach RS3 for all anesthetic agents, whereas scatter plot showed direct exponential relationship (Fig. 8.1). The regression equations established for recovery time and concentrations were $RS3=1.130e^{0.010C}$ for MS 222, $RS3=0.102e^{0.023C}$ for benzocaine, $RS3=3.440e^{0.010C}$ for clove oil and $RS3=1.715e^{0.001C}$ for PE.

8.3.4 POST-TREATMENT SURVIVAL:

H. kuda reared in post-treatment tanks did not show any signs of stress and symptoms of disease. No mortality occurred during post-treatment period of 7 days. Seahorses exhibited normal feeding and physiological behaviours.

8.4 DISCUSSION:

Anti-stress agents form an integral component of modern day aquaculture. Strong commercial demand coupled with concerns over the long-term viability of exploiting natural populations have stimulated a renewed interest in breeding and rearing of seahorses. Because stress responses vary widely between species, it is often necessary to screen dosages of different anaesthetic agents for each cultured species (Ross and Ross, 1999). Although the use of anaesthetic agents to reduce the stress associated with handling, transport, confinement, etc., is well established in fisheries andaquaculture research, their administration and efficacy in seahorse husbandry has received scant attention. Some

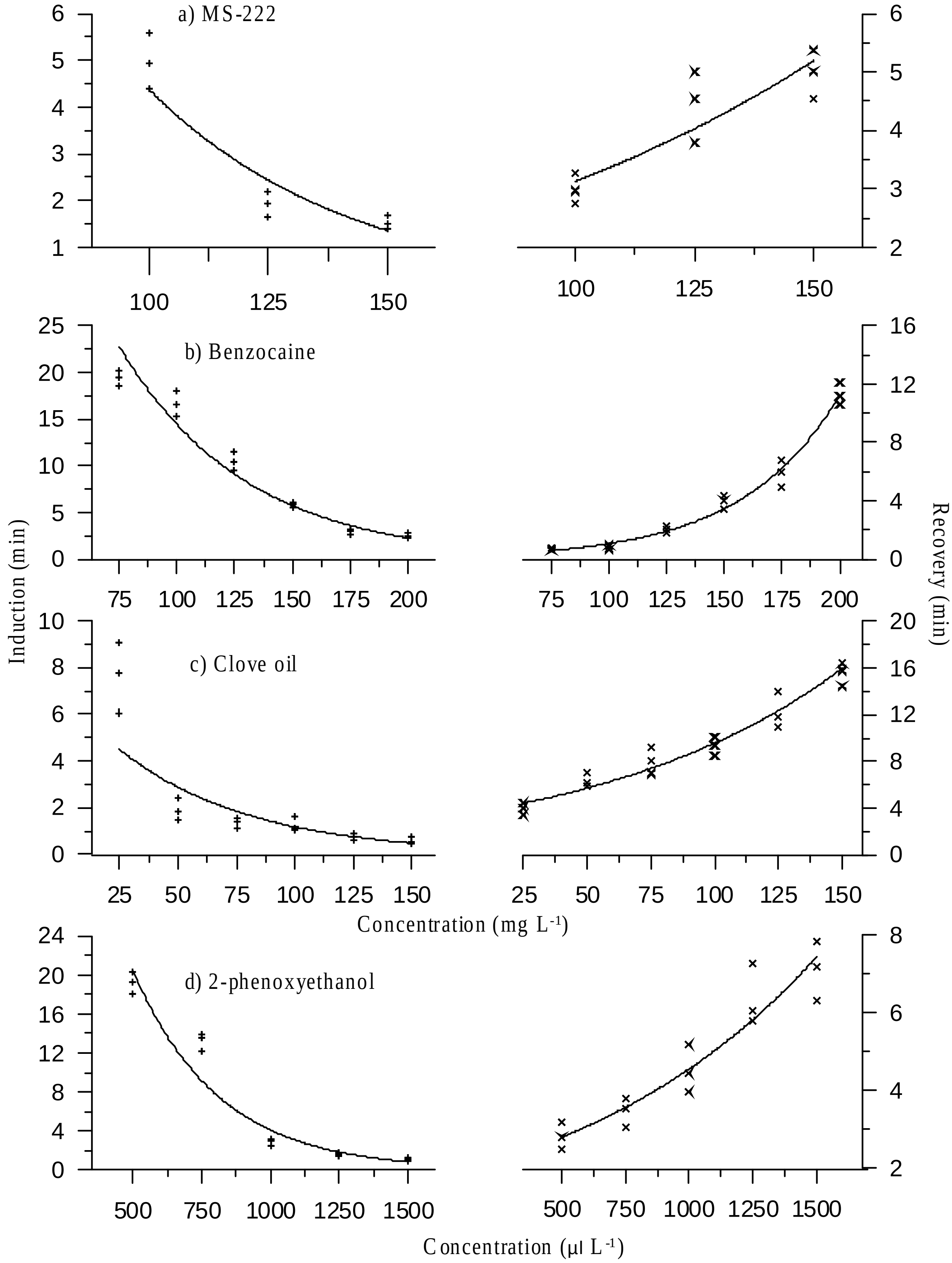


Fig. 8.1 Relation between induction time and anesthetic concentration; recovery time and anesthetic concentration of four different anesthetic agents in yellow seahorse *Hippocampus kuda*

published papers have cited the use of MS-222 (Boisseau, 1967; Bull, 2002; Jones and Lin,2007; Olivotto et al., 2008), AQUI-S (Isoeugenol) (Woods, 2002; Woods and Martin-Smith, 2004; Koldewey, 2005; Morgan and Bull, 2005; Mattle & Wilson, 2009), benzocaine (Wilson et al., 2006; Martins et al., 2010) and clove oil (Castro et al., 2008; Otero-Ferreret al., 2010) in a few seahorse species. For recording length and weight measurements, juveniles of *H. reidi* were lightly anaesthetized at MS-222 concentration of 50 mg L^{-1} (Olivotto et al., 2008). Wilson et al. (2006) used benzocaine at 10 mg L^{-1} for recording weight in *H. abdominalis* juveniles, whereas adults of *H. reidi* were anaesthetized in a benzocaine solution (50 mg L^{-1}) before sacrificing them for histological analysis by Martins et al. (2010). Castro et al. (2008) anaesthetised adult *H. reidi* with clove oil (0.05%) for assessment of diet composition. Also, 25 mg L^{-1}clove oil was found effective andsafe in anaesthetizing *H. hippocampus* juveniles for taking biometric measures (Otero-Ferrer et al., 2010).

Unfortunately, the aforementioned studies do not provide protocols of their administration and detailed information on the anaesthetic induction and recovery times of the various anaesthetic agents. Castro et al. (2008) measured induction and recovery times in anaesthetized adult *H. reidi* with clove oil, but at a single concentration (0.05%). Our study differed from those conducted previously onthe use and efficacy of anaesthetic agents in seahorse species. Firstly, different stages of anaesthetic induction and recovery in seahorse species were defined and secondly, the lowest effective concentrations (LEC) of different anaesthetic agents based on the anaesthetic induction and recovery times using a wide range of doses were determined.

In the present study, the induction times decreased significantly with the increase in concentrations of MS-222, benzocaine, clove oiland 2-phenoxyethanol (PE) ($P<0.05$). The results are in good agreement with previous studies that suggested the induction time decreases inversely according to the concentration of anaesthetic agent in teleost fishes (Mattson and Riple, 1989; Hseu, et al., 1998; Mylonas, et al., 2005; Gullian and Villanueva, 2009; Weber, et al., 2009; Heo and Shin, 2010). On the other hand, recovery times increased progressively with increasing concentration of anaestheticin adult *Hippocampus kuda*. Prolonged recovery with increased anaesthetic dosage has been reported in seven species of tropical reef teleosts (Cunha and Rosa, 2006), sockeye salmon (Woody et al., 2002) and cobia, *Rachycentron canadum* (Gullian and Villanueva, 2009). However, decreasing recovery times with an increase in concentration of clove oil and 2-phenoxyethanol (PE) for European seabass (*Dicentrachus labrax*) and gilthead seabream (*Sparus aurata*) been reported by Mylonas et al. (2005). The dynamics of recovery times in anaesthetized fish seems to be more complex. For example, in Senegalese sole (*Solea senegalensis*), recovery times decreased with increasing doses of PE and metomidate, whereas those for clove oiland MS-222 were dose-independent. The explanation put forward by these authors for such an unusual trend is that with the highest doses, the fish is not in contact with the anaesthetic for long, which would allow faster recovery. Other factors such as differences in the physiological responses of fish to the anaesthetic agents also influence this trend (Weber et al., 2009). This has been exemplified by Keenee al. (1998) who documented lower recovery times with MS-222 compared to clove oil based on different effects on the cardiorespiratory system in rainbow trout. Further studies determining the anaesthetic induction and recovery times in seahorse species exposed to a wide range of anaesthetic concentrations are therefore necessaryfor improved understanding of their interdependency.

The determined LEC of MS-222 (125 mg L^{-1}), benzocaine (175 mg L^{-1}), clove oil (50 mg L^{-1}) and PE (1000 µl L^{-1}) for *H. kuda* were relatively higher than those obtained for teleost fishes. A concentration of 75 mg L−1of MS-222 was effective in anaesthetizing *Gadus morhua* (Mattson and Riple, 1989) and *S. senegalensis* (Weber et al., 2009), respectively. Gomes et al. (2001) documented that application of benzocaine at doses of 100-150 mg L^{-1}resulted in quick induction, total immobilization and fast recovery in juveniles of tambaqui (*Colossoma macropomum*). Cunha and Rosa (2006) recommended 20 mg L^{-1}clove oil dosage for seven tropical reef fishes. Also, Weber et al. (2009) reported 30 mg L^{-1}clove oil was effective in anesthetizing *S. senegalensis*. PE at doses of 600 mg $L^{-1}$and 0.12% produced optimal anaesthetic inductions in *S. senegalensis* (Weber et al., 2009) and in goldfish (*Carassius auratus*) (Yasui et al., 2009), respectively. One of the possible reasons for requirement of higher doses of anaesthetics in seahorses could be related to modified form of general teleost gill pattern (random tufted gills) and narrow opercular opening (small cavity volume) which may entail lower water volumes splashed onto the gills (inadequate irrigation) and existence of slow rate of inhale and exhale water across the gills (Gert Roos, personal communication). It has been reported that entry and excretion of anaesthetics in fish takes place primarily via the gills, whereas internal organs are only slightly involved (Ross and Ross, 1999; Hoskonen and Pirhonen, 2006). Further research on structureof gills influencing the anaesthetic dosage in different teleost species, however is required.

Behavioural response in seahorse to anaesthetic agents is markedly different from those in other teleosts. For example, it is difficult to identify the state of death in seahorses, as many times they do not fall or lie horizontally on the bottom of the tank. Instead, *H. kuda* stand vertically on the bottom of tank and appear to be alive (pers. obs.). This peculiar behaviour of seahorses is often misleading and might lead to overdosing the fish as experienced during our pilot study. This calls for careful observation when anaesthetising seahorses.

The onset of induction (IS3) and recovery (RS3) stages of anaesthesia in *H. kuda* varied significantly with the concentrations of the anaesthetic agent ($P<0.05$). A rapid anaesthetic induction time of less than 180 s and recovery time not exceeding 300 s is considered an ideal for fish (Marking and Meyer, 1985). All the anaesthetics evaluated here met the first criterion, while recovery time's were prolonged in *H. kuda* anaesthetized with clove oil and benzocaine than with MS-222 and PE (Table 8.2). Relatively quicker induction in *H. Kuda* anaesthetized with clove oil may be due to its high lipophilic nature which allows it to adhere easily and penetrate the gill epithelium for adsorption in tissues (Myelomas et al., 2005). On the other hand, delayed recovery might be due to its persistent nature on the gill surface, which effectively increases the recovery time (Sladky et al., 2001; King et al., 2005). Quicker induction (89± 62 s) than recovery (119± 78 s) in adult *H. reidi* anaesthetised with a single concentration of clove oil has been reported by Castro et al. (2008). Although the induction time (115± 28 s) obtained with clove oil inadult *H. kuda* was comparable, the recovery times (375±22 s) however were 3 times longer than those reported for *H. radii* (Castro et al., 2008). Such difference in the respective recovery times might berelated to species, size, physiological status and environmental conditions (Burka et al., 1997; Ross and Ross, 1999).

According to Marking and Meyer (1985), the anaesthetic agent is considered effective if it produces a complete induction within 180 sand recovery within 300 s. In the yellow seahorse *H. kuda*, all four anaesthetic agents evaluated were effective and presented a good margin of safety when compared against the above efficacy criteria. Amongst four anaesthetics; MS-222 and clove oil were found most effective as least concentrations of these are required to achieve the desired induction and recovery in *H. kuda.* However, a number of

Table 8.2 Induction and recovery times for adults of *H. kuda* anaesthetized with various concentrations of four anaesthetic agents. (a) MS-222 (b) benzocaine (c) clove oil and (d) PE. Data are presented as mean ± S.D.

(a)

Stages	Concentrations of MS-222 (mg L^{-1})					
	25	50	75	100	125	150
IS1	4.26±1.13^{a}	1.34±0.16^{b}	0.59±0.12^{b}	0.32±0.08^{b}	0.17±0.05^{b}	0.06±0.02^{c}
IS2	-	3.47±0.26^{d}	3.06±0.12^{d}	2.05±0.19^{e}	0.34±0.06^{ef}	0.21±0.04^{f}
IS3	-	-	-	5.59±0.36^{g}	1.55±0.16^{h}	1.32±0.09^{h}
RS1	-	-	-	0.18±0.03^{i}	0.32±0.11^{ij}	0.44±0.13^{j}
RS2	-	-	-	0.54±0.08^{k}	1.34±0.23^{k}	1.38±0.21^{k}
RS3	-	-	-	3±0.15^{l}	4.26±0.36^{lm}	5.58±0.24^{m}

(b)

Stages	Concentrations of benzocaine (mg L^{-1})					
	75	100	125	150	175	200
IS1	0.41±0.10^{a}	0.42±0.09^{ab}	0.28±0.05^{bc}	0.25±0.06^{bc}	0.19±0.04^{bc}	0.11±0.04^{c}
IS 2	4.13±0.16^{d}	2.58±0.19^{e}	2.22±0.44^{e}	1.09±0.12^{f}	0.46±0.08^{f}	0.35±0.12^{f}
IS 3	19.25±0.49^{g}	16.39±1.22^{h}	10.29±1.04^{i}	5.54±0.15^{j}	2.55±0.19^{k}	2.30±0.16^{k}
RS 1	0.05±0.02^{l}	0.10±0.05^{lm}	0.15±0.05^{lm}	0.45±0.07^{mn}	1.09±0.13^{n}	3.36±0.10^{o}
RS2	0.11±0.03^{p}	0.19±0.06^{p}	0.30±0.08^{p}	1.59±0.12^{q}	3.01±0.13^{r}	3.55±0.26^{s}
RS3	0.41±0.06^{t}	0.50±0.08^{t}	2.02±0.14^{t}	3.56±0.27^{u}	5.54±0.55^{v}	11.18±0.44^{w}

(c)

Stages	Concentrations of clove oil (mg L^{-1})					
	25	50	75	100	125	150
IS1	0.31±0.07^{a}	0.21±0.03^{ab}	0.21±0.04^{ab}	0.12±0.02^{bc}	0.08±0.03^{c}	0.06±0.01^{c}
IS2	0.47±0.16^{d}	0.28±0.03^{de}	0.30±0.07^{de}	0.15±0.2^{e}	0.12±0.03^{e}	0.13±0.02^{e}
IS3	7.37±1.29^{f}	1.55±0.28^{g}	1.21±0.15^{g}	1.16±0.17^{g}	0.45±0.08^{g}	0.36±0.07^{g}
RS1	0.31±0.06^{h}	0.51±0.18^{h}	1.48±0.21^{h}	0.06±0.03^{h}	5.05±0.59^{ij}	6.09±0.39^{j}
RS2	0.52±0.05^{k}	1.10±0.27^{k}	4.11±1.03^{kl}	3.30±0.39^{kl}	7.00±1.06^{lm}	9.05±0.55^{m}
RS3	3.58±0.33^{n}	6.25±0.37^{no}	8.05±1.06^{p}	9.23±0.49^{p}	12.14±1.36^{q}	15.27±50^{r}

(d)

Stages	Concentrations of PE (µl L^{-1})					
	250	500	750	1000	1250	1500
IS1	1.02±0.12^{a}	0.22±0.04^{b}	0.15±0.03^{b}	0.13±0.04^{b}	0.11±0.02^{b}	0.07±0.02^{b}
IS2	5.24±0.52^{c}	1.26±0.06^{d}	0.59±0.05^{d}	0.44±0.07^{d}	0.34±0.05^{d}	0.30±0.06^{d}
IS3	-	19.14±1.09^{e}	13.16±0.55^{f}	2.56±0.22^{g}	1.33±0.11^{gh}	1.01±0.13^{h}
RS1	-	0.05±0.01^{i}	0.14±0.3^{ij}	0.43±0.10^{j}	1.32±0.21^{k}	2.07±0.12^{l}
RS2	-	0.16±0.04^{m}	1.01±0.12^{n}	1.34±0.23^{n}	3.06±0.20^{o}	3.10±0.15^{o}
RS3	-	2.50±.021^{p}	3.28±0.22^{pq}	4.31±0.37^{q}	6.22±0.47^{r}	7.06±0.45^{r}

*Values in the same row indicated with different superscript letters indicate significant differences (P<0.05).

other considerations such as availability, cost-effectiveness, nature ofthe study, legislation and user safety determine the selection of particular anaesthetic for aquaculture.

In many countries, the use of fish anaesthetics is a matter of concern as there are no specific laws regulating their use. The recommendations of the US Food and Drug Administration (FDA) are usually followed. MS-222 is the only FDA-approved anaesthetic for food fish in the USA. The drug is particularly effective on fish because it is highly water and lipid-soluble and readily crosses the gill membrane. It directly acts upon the central nervous system, neuromuscular junctions and ganglion synapses preventing the generation and conduction of nerve impulses (Treves-Brown, 2000). But it is quite expensive and its procurement is also cumbersome as it is not produced locally. On the other hand, clove oil has been extensively used as an anaesthetic agent in aquaculture of fresh water and marine fishes as it is widely available, relatively inexpensive, eco-friendly and relatively safe to fish and humans (Hseu et al., 1998; Keenee et al., 1998; Woody et al., 2002; Iversen et al., 2003). Furthermore, clove oil was found to be effective at much lower concentrations (2.5 times) than with MS-222 in achieving the desired levels of anaesthetic induction and recovery in *H. kuda*. This translates into larger safety margin and increasing cost-effectiveness in favour of clove oil compared to MS-222.

CHAPTER 9

DISEASES IN CULTURE SYSTEM

CHAPTER 9

DISEASES IN CULTURE SYSTEM

9.1 INTRODUCTION:

The complex nature of aquatic ecosystem marked difference between health, suboptimal performance and disease obscure. Disease outbreak in aquaculture is an end result of a series of linked events which involving interaction between host, the environment and the pathogen (Snieszko, 1974). Environment includes not only the water and its components (such as oxygen, pH, temperature, toxins, wastes) but also the kind of management practices (e.g. handling, drug treatments, transport procedures, etc.). Recognition and prevention of disease is a challenge that has to be faced by aquaculture industry and it is important to dealing with available resources effectively.

As most of the cultured fishes, seahorses get stressed when kept at high densities and that stress makes them more vulnerable to infection. With the increasing interest in developing and standardizing the protocols for seahorse aquaculture and its conservation, the information on the disease diagnostics plays significant roles in animal health management and disease control. Moreover, animal health studies in captive conditions helps in understanding the adequate information on solving husbandry problems. The success of closing the life cycle of seahorses under captive conditions will promote marine aquarium trade and export for traditional medicine which may open up new avenues for employment for coastal rural population. In addition, seahorse aquaculture would reduce the fishing pressure on wild population and the developed technique can be used for species recovery programme. In most Asian countries, as well as in Australia, New Zealand, South Africa and in USA, commercial seahorse aquaculture technology development were/are underway (Vincent, 1996) and at present around 24 countries are involved in this venture (Koldewey and Martin-Smith 2010). The seahorse aquaculture is a relatively new industry for India will be addressing many culture related problem. Especially, the technical challenges remain notably in disease, nutrition and the development of species-specific culture techniques.

The observations on different diseases and parasitic infection were recorded at the during the seahorse hatchery. This chapter describes disease related problems observed in culture and rearing of yellow seahorse, *Hippocampus kuda* (F1 and F2 generation) under captive conditions. It is expected that the information on diseases of this species would help in improving and optimize the seahorse aquaculture in India.

9.2 PROBLEMS ENCOUNTERED IN REARING OF JUVENILES:

9.2.1 AIR BUBBLE INGESTION:

Ingestion of air bubble into the gut was found to be the major cause of mortality during pelagic phase juvenile rearing. Seahorse juveniles while preying on live food organisms (copepods nauplii, copepodites and *Artemia* nauplii, etc.) engulf the atmospheric air if they miss the strike and this leads to formation of air bubbles in the digestive tract (Plate 9.1). The early juveniles are unable to release out the ingested air bubbles. Seahorse juveniles with ingested air bubbles exhibit irregular swimming pattern, lose their balance and are unable to capture prey, subsequently leading to starvation and ultimately to death.

SOLUTION:

Appropriate feed density (>6 no. copepods per ml) with dark colour background preferably blue can significantly decrease the false attack and air bubble ingestion. In experiments on effect of background tank colour (Chapter 4) on growth and survival in

Plate 9.1. Air bubble ingestion by seahorse juvenile

pelagic phase juveniles of the yellow seahorse suggested that dark background colour tanks provide better detection of prey and less chance of air bubble ingestion. In addition to that dissolved oxygen levels should be always more than 4 ppm with a minimum water movement with modified tank design (Chapter 5) helps in increasing survival and growth.

9.3 PROBLEMS ENCOUNTERED IN REARING OF ADULTS:

9.3.1 EXTERNAL GAS BUBBLE DISEASE (EGBD):

Gas bubble disease is a non-infectious disease caused by environmental problems in the culture system. Supersaturated gases, not only oxygen but also nitrogen in the water, escape from the water into the body fluids of the rearing fish. Gasses in supersaturated water will try escape out of the water into any medium where the gas saturation level is lower (e.g. into the air, or in the case of gas bubble disease, into the blood and other fluids of the fish). Most commonly they are encountered in the yolk sacs, gills and eyes (and occasionally in fins) as these are the areas of the body which have the most gas permeable membranes. The gas then causes bubbles to form inside capillaries and under the skin, restricting the blood flow and forming hemorrhages and clots. Although the disease often looks disastrous for the affected animal, in many cases the bubbles resolve with few problems for the affected fish. Though, it is non-infectious disease, the most common adverse outcomes are loss of an eye, bacterial infections of the lesions or brain damage.

External gas bubble disease (EGBD) may cause either due to gas super-saturation in the tank water or by bacterial infections where carbon dioxide produced by the bacteria becomes trapped underneath the skin. These subcutaneous (under the skin) air bubbles that may occur anywhere on the head, body, or prehensile tail of seahorse. The situation, wherein gas levels in the water are much higher than normally possible (caused by limited areas for gas escape), causes gas bubbles to form anywhere possible. Because the pressure inside of a seahorse is lower than the pressure of gas super-saturated water, the gas bubbles emerge directly under the skin of the affected seahorse. Since the air bubbles cannot pass through the skin, they remain stranded there until treated or until they can be reabsorbed by the seahorse's own body. The bubbles can cause stress on a seahorse as problems with buoyancy and movement arise.

The gas bubbles appeared on the prehensile tail and snout of seahorses is shown in Plate 9.2. Gasses trapped beneath the skin are believed to be caused by either bacterial infection, by air bubbles in the water column, or by gas super-saturation in the water column, though this remains unresolved.

TREATMENT:

Acetazolamide (trade name 'Diamox®'), is a non-bacteriostatic sulphonamide was used to treat external gas bubble disease in seahorse. Diamox neither kill bacteria as the bacteriocidal potentiated sulfas will, nor inhibit the growth of bacteria as with the bacteriostatic sulfa drug such as sulfathizole. Rather it inhibits production of the zinc containing enzyme carbonic anhydrase which helps to control production and accumulation of carbonic anhydrase which found in most vascular part (tail and brood pouch) of seahorse. The infested seahorses were separated to hospital tank (100 lit capacity) filled with same raring tank water and provided with nylone rope (4mm dia) as holdfast. A 250 mg Diamox® tablet which available at local medical shop was used to treat the infected seahorses. Whole tablet was crushed into fine powder and mixed properly in beaker with some seawater. Stir well to complete dissolve the powder, the dissolved solution added to hospital tank and undisclosed tablet residues were through out. Moderate aeration was provided for proper mixing of solution in all over the tank. After 24 hours 80% water changed and replaced with fresh sea water. Care was taken to

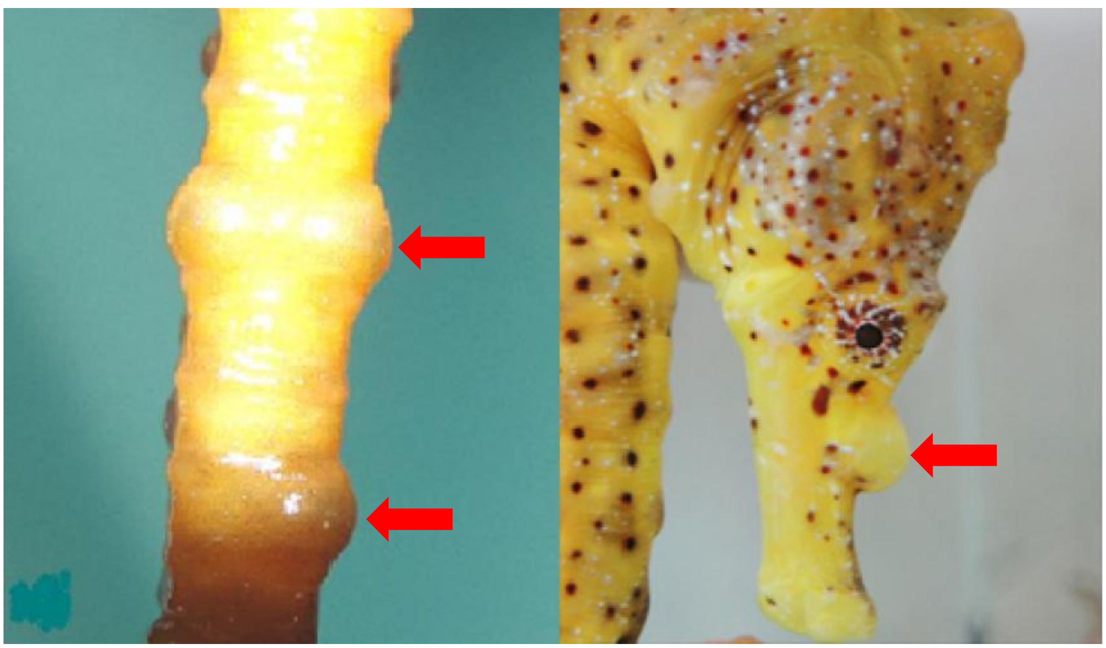


Plate 9.2. External gas bubble disease observed in adult seahorse

avoid difference in water quality. Then fresh solution of Diamox® was prepared and added into the tank as mentioned above and this process was repeated for 3-5 days.

9.3.2 DEEP SKIN ULCER:

A single male specimen of the captive reared spotted yellow seahorse, *H. kuda*, was observed lying dormant at the bottom of the rearing tank. Upon closer examination, it was documented with a necrotic lesion (~ 4mm dia) was present below the dorsal fin and slightly above the brood pouch. Therefore, subsequent studies were carried out to check for the presence of microbial contamination of the lesion, Plate 9.3. The infected seahorse was then transferred into hospital tank (~70 lit) containing fresh filtered UV treated seawater. After microbial analysis, it was observed that out of the 7 isolates, 3 were characterized as to the genus *Vibrio* while another isolate was classified as *Photobacterium*, a member of the Vibrionaceae family. Besides *Pseudomonas* and *Bacillus* were also identified. Since the causative organisms were isolated from the necrotic lesion, for future treatment purposes an antibiotic susceptibility test was performed using motley of antibiotics to check the resistance/susceptibility of the organisms. The resistance/susceptibility of the microbes was then scored with a standard reference. The antibiotic sensitivity test revealed that all isolates tested were sensitive towards Chloramphenicol and uniformly resistant towards Tetracycline, Oxytetracycline and Nalidixic acid.

9.4 PARASITIC INFECTIONS:

9.4.1 PARASITIC INFECTION OF MARINE ICH, *CRYPTOCARYON IRRITANS*:

The outbreak of parasite protozoan ciliates, *Cryptocaryon irritans*, Brown 1951 were noticed during the rearing period which leads to mass mortality of seahorses. *Cryptocaryon irritans* is a holotrichous ciliate that parasitizes marine fishes in temperate and tropical seas. *Cryptocaryon irritans* (Plate 9.4) invades the epithelium of the skin and

Plate 9.3 Deep skin ulcer in Captive *H. kuda*

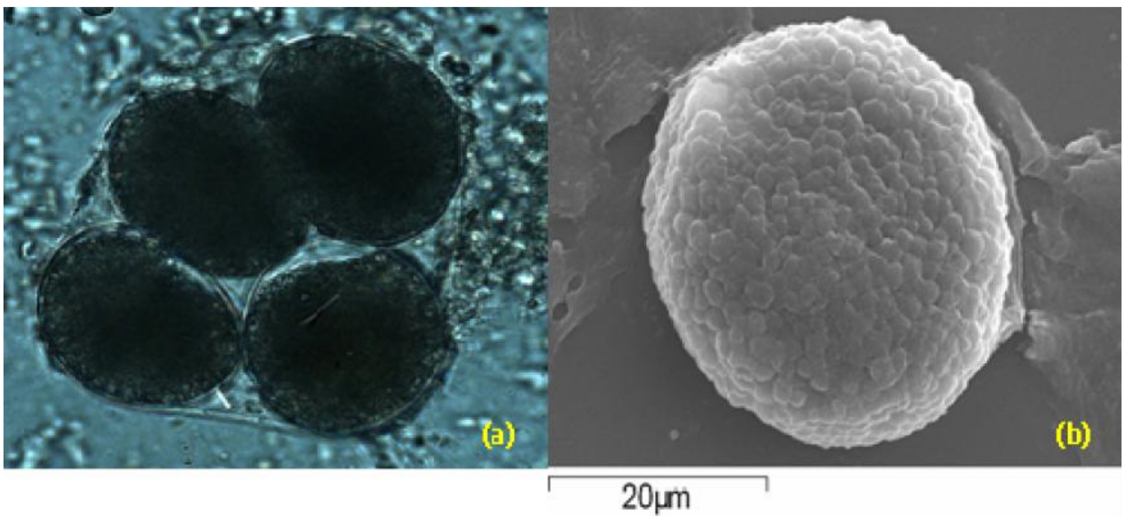


Plate 9.4 The parasite ciliates, *Cryptocaryonirritans* isolated from the seahorse gills
(a) cyst under microscope, division inside the cyst into hundreds of daughter parasites, called tomites, begins shortly thereafter
(b) Scanning Electron micrograph (SEM) of single cyst

gills of fish and affects the physiological function of these organs. Its life cycle includes 3 main stages. The parasitic stage (trophont) lives embedded in the host's skin and feeds on tissue debris and body fluids; the mature trophont leaves the host, adheres to the substrate, encysts and begins to reproduce (tomont); after several divisions, the cyst ruptures, releasing the infective stage (tomites).

The clinical signs of cryptocaryonosis in marine fishes include pinhead-sized whitish nodules, mucus hyper production, skin discoloration, anorexia and respiratory distress. Infected fishes can exhibit behavioural changes, presumably due to pruritus, such as skin tremors, hyperactivity and sudden darting movements. External symptoms observed in infected seahorses includes, fish become lethargic and hover just beneath the water surface. Rapid breathing and expulsion of water in a forceful manner through the opercular opening or the mouth. Repeatedly rubbing head portions by tail with fast up-down movement and loss of appetite. In most cases outbreak come into notice at final heavy infestation. Mortality observed in 2-3 days after visible external symptoms.

TREATMENT:

Cryptocaryon irritans have largely been confined to the examination of infections of captive fish after the parasite has been introduced into aquaria or a manculture facility, often by an unknown source. Indeed, the presence of *C. irritans* is usually detected only after its numbers have built up on confined fish, and the original source of infection in these cases is often a matter of speculation. There is no attack of parasites on other organs of host like intestines, kidneys except gills. Formalin bath treatment (15-20 ppm) found to be very effective in treating this ectoparasite which mainly in the cyst form i.e**.** tomont and after develop into trophonts which attached to gills or body and fed on gill tissues of seahorse. Eradication of *Cryptocaryon initans* was also achieved through switching seahorses between 2 tanks. Weekly switches were needed, the tanks being dried and cleaned between uses. Freshwater bath for 10-15 min. also proved to be helpful before transferring into new tanks.

Seahorse aquaculture face unique challenges for health care due to their anatomy and behaviour. Because of its hard and bony-plated body injections are difficult in addition to that; the semi-closed branchial chambers make gill biopsies prohibitive (Koldewey and Martin-Smith, 2010). The best way to manage disease is through prevention and quarantining of new animals arriving at the culture facility should be carried out as routine. The provision of optimal environmental parameters with a good diet will also reduce health problems. It is clear that the lack of information on the treatment of the common diseases experienced in seahorse culture is a major constraint to the viability of seahorse culture and more research is urgently required (Koldewey and Martin-Smith, 2010).

9.5 DEFORMITIES:

9.5.1 INTRODUCTION:

During the captive rearing period most of the welfare issues concerned with aquaculture activities are not taken under consideration since earlier studies have not reported about the occurrence of two head juveniles, whereas observation on snout and tail deformity in juvenile has been reported due to the role of temperature and nutrient provided to the brooding seahorses (Woods, 2002). Morphological abnormalities in animal kingdom have been reported in vertebrates as well in invertebrates from time to time, however, these abnormalities are not frequent. Captive breeding programmes of food and ornamental fishes have offered several advantages in enhancing the levels of production and conservation of natural resources have produced several changes on cultured fish for human consumption and ornamental purposes. Thus, artificial environmental conditions can affect morphology, physiology and behavior of fishes

(Kihslinger & Nevitt, 2006). Environmental stress can give rise to decreased developmental stability of individuals reared in controlled conditions (Clarke, 1995; Moller et al., 1997) causing morphological abnormalities or deformities. A morphological abnormality due to parasitic infestation has been observed in fishes from *in-situ* populations(Reimchen, 1997; Landsberg, et al., 1998; Schwaiger, 2001). These abnormalities have been attributed to environmental factors in hatcheries such as diet, lighting, population density and concentrations of metabolites (Houde, 1971; Seikai, 1985).

Captive goldfish showed a significant reduced development of sensory (lateral line), structural (number of scales rows above lateral line) and locomotive (fins length) characters under captive conditions. Fin abnormalities including fin anomalies have previously been recorded from several fish species (Wellings, et al., 1976; Cross, 1985; Reash & Berra, 1989). Animals with bicephaly (two head) have been reared successfully in higher vertebrtates and mammals whereas it is reversed in the case of lower vertebrates and invertebrates with rare success. Morphological abnormalities are observed commonly in hatchery-reared flatfishes and are assumed to develop during the period of asymmetric change and tissue differentiation associated with metamorphosis (Seikai et al., 1987). The occurrence of two headed juvenile seahorse have been observed by aquarist whereas no scientific literature has been drafted on this unusual deformity in an unusual fish.

9.5.2 CAPTIVE BREEDING:

Captive breeding trials and mass-scale culture during captive spawning of *H. kuda*, one juvenile released was found to be born with a bicephally. Under normal circumstances, the juvenile inflate the swim bladder and exhibits the pelagic behaviour immediately after the release from the male brood pouch (Woods, 2000). However, the bicephalic juvenile did not exhibit this pelagic behavior and was found to confine on the bottom of the tank which lacked the pelagic behavior. The main reason for that was the independent movement of the head, through this behavior the pelagic movement was restricted while the two head juvenile could able to reach only up to middle of thewater column . According to (Laale, 1984; Holden & Bruton, 1993) polyembryonic fish rarely survive past fry stages. However, one example of unequal sized conjoined twins of rainbow trout, *Onchorynchus mykiss* collected from the wild grew to 1.7 kg (Behnke & Kloopel, 1975). We observed that due to non coordinated movement of the head they could not start the feeding behavior or feeding strike and they could survive for only 62 hrs.

9.5.3 MORPHOMETRIC DETAILS OF BICEPHALIC SEAHORSE JUVENILE:

Laterally conjoined bicephalic twins might have emerged from the unipolar embryonic development with bifurcated heads. Morphometric and meristic measurements were made as per the standard protocols described by (Lourie et al., 1999). Morphometric measurements carried out using ocular micrometer under compound microscope (Olympus BX40) is listed in Table 9.1.

In the observed two headed juvenile, the body was found to be laterally fused from TD4 (between 4^{th} and 5^{th} trunk ring) to TD9 (9^{th} and 10^{th} trunk rings) with a common tail (Fig. 1). At birth, the wet weight of the two headed juvenile was 3.3 mg whereas the mean weight of normal juveniles released by the same parent was 1.7 to 2.3 mg. Similar occurrence of two head spade nose shark has been observed in Karwar without any deformities (Neelakantan et al., 1993), whereas in the similar species collected from Portonova had deformities. In the elasmobranch, Cow nose ray which was carrying the embryo had two head which was landed in Nellore (Leatherland, & Woo, 1998). As far as Indian context is concerned the occurrence of two headed animals has

Table 9.1 Morphometric characters and meristic counts of normal and bicephalic juvenile of yellow seahorse (*Hippocampus kuda*).

Morphometric characters	Bicephalic juvenile		Normal juvenile
	First body	Second body	
Height (Ht) (mm)	8 mm		8 mm
Weight (W) (mg)	3.3mg		1.9 mg
Orbital diameter (OD) (mm)	0.48 mm	0.48 mm	0.49
Snout length (SnL) (mm)	1.1 mm	1.11 mm	1.1 mm
Snout depth (SnD) (mm)	0.6 mm	0.6 mm	0.6 mm
Head length (HL) (mm)	2.4 mm	2.42 mm	2.41 mm
Head depth (HD) (mm)	1.5 mm	1.5 mm	1.5 mm
Trunk length (TrL) (mm)	2.5 mm	2.5 mm	2.7 mm
Trunk width between 4^{th} and 5^{th} trunk ring (TW 4) (mm)	1.6 mm for joined animal		0.7 mm
Trunk width between 9^{th} and 10^{th} trunk ring (TW 9) (mm)	0.9 mm	-	0.8 mm
Trunk depth between 4^{th} and 5^{th} trunk ring (TD 4) (mm)	0.8 mm	0.8 mm	0.8 mm
Trunk depth between 9^{th} and 10^{th} trunk ring (TD 9) (mm)	0.85 mm	0.85 mm	0.8 mm
Tail length (TaL) (mm)	3.5 mm	-	3.6 mm
Dorsal fin length (DL) (mm)	0.7 mm	-	0.7 mm
Pectoral fin length (PL) (mm)	0.5 mm	0.5 mm	0.5 mm
Anal fin length (AL) (mm)	0.3 mm	0.3 mm	0.3 mm
Dorsal fin rays (DF)	18	-	18
Pectoral fin rays (PF)	16	16	17

9.5 The bicephalic seahorse juveniles of yellow seahorse, *H. Kuda*.

been reported from elasmobranchus, whereas the present observation was made in the yellow seahorse, *H. kuda* which released juveniles in captive conditions.

9.5.4 AQUACULTURE:

The incidence of unfavorable environmental conditions during the development of the egg to the embryo stage produces malformed and crippled young, if there is any hatching at all. In aquaculture practices especially in salmon and sea bream, deformity like conjoined larvae and abnormalities in fins and vertebral column development have been noticed (Owusu-Frimpong & Hargreaves, 2000). The reason for those abnormalities might be due to high stocking, lack of nutritious diet, chemicals and temperature of the cultured system, whereas in the present study suggest that the bicepally in seahorse might be due to the physiological activities involved during the gestation period . Double malformations are basically result of egg over ripeness or from immature egg (Bishop, 1998) and thermal shock inductions during egg to embryonic development (Wang & Ryman, 2001). Deformity in marine turtle and birds occurs due to temperature fluctuation as well the contaminants found on the nesting sites (Shikano, et al., 2005; Kocour et al., 2006). The higher incidence of abnormalities under artificial conditions could be partly explained by loss of genetic variability and inbreeding (Anwer & Mehrotra, 1986; Woods, 2000). Abnormalities in aquaculture have negative consequence in selling the products as well as on economic side also. Body deformities in seahorse can be considered as a growing concern since it happens inside the male brood pouch. Deformities like absence of snout and tail has been already reported by researchers Wood, 2002, whereas the occurrence of two head has been not yet scientifically reported world widely.

9.5.5 WELFARE NEEDS:

Knowledge about the effects of captivity and environmental stress on fish populations is basic to culture of any organisms. Environmental stress can give rise to decreased developmental stability of individuals, which may result in reduced performance of fitness components. Animal welfare parameters should be give priority in aquaculture system which might reduce the profit little bit, but provide optimum conditions for the growing species and for sustainable growth of the industry.

CHAPTER 10

GENERAL DISCUSSION AND CONCLUSION

CHAPTER 10

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In recent years, aquaculture of some exotic species has already been initiated in India with permission for culture of Red Tilapia and white leg shrimp, *Litopenaeus vannamei*, through the protocol designed by Coastal Aquaculture Authority (CAA) (FAO, 2004). As seahorses are valuable in marine ornamental fish trade for which there is a high demand , and trade is also increasing for Traditional Chinese Medicine (TCM), supply through aquaculture production has become necessary. With an average individual weight of 10 g, the price of one kilo of dried seahorses in the US market is around US $ 150 (Koldewey and Martin-Smith, 2010) which is very high when compared to other aquaculture shrimps and fishes. By developing proper guidelines, the Ministry of Environment & Forests (MoEF) and The Ministry of Agriculture (MoA), Govt. of India, may constitute a Steering Committee. National level committees may overlook and monitor the seahorse brood stock, seed and grow-out production with help of respective State Fisheries Departments or State Forest Departments for monitoring, controlling and surveillance (MCS) of hatchery/farming facility. In case of violation of prescribed guidelines, a committee constituted by the respective State Government is authorized to call for details from any hatchery/breeding/farm facility registered for seahorse aquaculture. The farmers/entrepreneurs who intend to undertake seahorse hatchery/breeding/grow-out farming shall register with the respective state fisheries department and Marine Product Export Development Authority (MPEDA) using the appropriate format prescribed by the steering committee. Specific recommendations for seahorse aquaculture operations which was considered by 175 parties in a CITES technical workshop concerned with the implementation of the CITES listing for seahorses (Koldway and Martein-smith, 2010) should also follow. Many countries are involved in cultured seahorse trade with license acquired from CITES, monitored through CITES authority of the concerned countries. Since India is a signatory to CITES from year 1976, the management authority of CITES in India is the Additional Director General (Wildlife) cum Director, Wildlife Preservation, MOEF, Govt. of India. A specific recommendation for seahorse aquaculture should be implemented through CITES management authority in India, with registration of captive breeding facility/grow out farm and development of tag and tagging methods to differentiate them from wild-caught animals. The efforts for captive breeding, rearing to large scale rearing of Indian seahorse species has been developing well during these days. Culture technology available for *H. kuda* and *H. trimaculatus* has great potential to provide aquaculture produced seahorse for TCM and marine ornamental fish trade. So, introduction and permission of seahorse aquaculture in India would be advantageous both for conservation of this threatened species and for dependent fisher folk for their employment.

Seahorse aquaculture has been the subject of recent attention both by aquaculturists (Job et al., 2002) and in the policy domain CITES, 2001. Aquaculture is being increasingly cited as a priority solution for reducing the pressures on critical marine habitats from over-exploitation and destructive fishing associated with the trade in wild-caught live reef organisms (Leber and Lee, 1997). Three methods are commonly used to attempt the replenishment of depleted stocks: regulating fishing effort, restoring habitats critical to one or more life stages of the stock and artificially supplementing the reproductive population through restoration or enhancement programs (Botsford et al., 1997). Marine Protected Areas

(MPA's) can act as safe areas for breeding populations inhabiting core regions (Pikitch et al., 2004). However, till date our MPA's in India do not have effective management plans and several implementation issues remain unaddressed. It has been reported that seahorse occurrence is more in sea grass abundant regions of India (Palk Bay) rather than in the coral reef rich region *viz.* Gulf of Mannar (Marichamy et al., 1993; Lourie et al., 2004; Salin and Mohanakumaran, 2006). Since Palk Bay does not have any MPA and illegal fishing occurs in the Gulf of Mannar Marine National Park region, this thesis recommends that seahorse culture is an appropriate approach to conservation since it involves community-based wild stock enhancement programmes which will contribute to the conservation of this unique species through the involvement and empowerment of the local fishing communities.

Wild populations of marine fish stocks are seriously declining worldwide (Jackson, 2001) and these include reef fishes collected for food and aquarium trade (Venkataramani, 2005). As far as India is concerned, the collection of marine ornamental fishes is practiced through target fishing by using methods like, skin diving with scoop net, gill nets and from incidental catches in coral reef fish traps, drag net, bottom trawl net and shore seine operations (Murugan and Durgekar, 2008). In most countries collection of marine ornamental fishes is done through SCUBA diving, this mode of collection is not practiced in India and till now no international trade exists for this marine resource despite domestic demand. India does not figure in the list of exporters of marine ornamental fish even though vast resources are available. The collection of these species from the associated ecosystems has been banned under certain laws and the rationale for such ban is questionable. Till date basic biodiversity studies like fish assemblages in reefs are inadequate although some efforts have begun ((Venkataramani, 2005). Ironically the ban on marine ornamental fish resources makes those resources more vulnerable because these products enjoy good domestic demand. There are several internationally aided projects that promote aquaculture of reef fishes which offer critical support to experimentation in community-based breeding initiatives. Seahorse culture provides an appropriate instance of wild life farming where conservation benefits are certain, and where benefits to local communities are equally attractive. Here, technologies for production have been perfected and the product enjoys a high demand both in domestic and international markets. These are encouraging signals for the marine ornamental fish industry in India to awaken to these innovative conservation options.

Stock restoration or enhancement is gaining increasing popularity and is practiced at various levels worldwide, but it is generally not closely monitored or evaluated. The present study provides information regarding the culture of the seahorse *H. kuda* which can become a conservation measure for the species. The culture of the species can provide the much needed boost to the marine aquarium trade (of cultured fish) in India, currently in a hibernation stage despite financial support from the Marine Products Export Development Authority (MPEDA). Although there are certain technical problems in mass-scale rearing like high mortality during the initial pelagic phase, nutritional disorders and parasites, the development of seahorse culture looks promising. Seahorse culture is an option which can be used to manage and protect seahorse population in the wild, and at the same time can provide a source of livelihood to seahorse fishers and traders. Economic incentives will need to be put into place for the industry to grow, which ultimately reduces the pressure on the wild seahorses. Research institutions and government agencies involved in the conservation of seahorse should give more credence to the importance of carrying out stock-enhancement studies. It is time for decision makers to question the notion that nature will ensure continued stock despite the staggering competition over diminishing coastal resources between the growing fishing sectors and other coastal developmental activities. Till date scant attention has been accorded to studying seahorse distribution along the entire Indian coast (including the islands) and the feasibility of the culture of existing species. In this context the need for a dedicated Seahorse Breeding Center cannot be over-emphasized. .

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List of publications (Peer reviewed journals):

1. **Pawar**, **H.B.**,Sanaye, S.V., Sreepada, R.A., Harish, V., Suryavanshi, U., Tanu, Ansari, Z.A. (2011). Comparative efficacy of four anaesthetic agents in the yellow seahorse, *Hippocampus kuda* (Bleeker, 1852) 31:155-161. ***Aquaculture***
2. **Pawar, H.B.**,Sanaye, S.V., Murugan, A., Sreepada, R.A. andTanu. The effect of tank background colour on growth and survival of juvenile seahorses, (2011). *Hippocampus kuda* (Bleeker, 1852). ***The Israeli Journal of Aquaculture – Bamidgeh*.**
3. **Pawar H.B.,**Ingole B.S. and Sreepada R.A Efficacy of sodium bicarbonate as anaesthetic for yellow seahorse, *Hippocampus kuda*(Bleeker, 1852). 2013, ***International Journal of Research in Fisheries and Aquaculture.***
4. Sanaye, S.V., **Pawar, H.B.,**Murugan, A., Sreepada, R.A., Tanu Singh, Ansari, Z.A. (2013). Diseases and parasites in cultured yellowseahorse, *Hippocampus kuda*(Bleeker, 1852), ***Fishing Chimes,***
5. Tanu; Deobagkar, D.D.; Khandeparker, R.; Sreepada, R.A.; Sanaye, S.V.; **Pawar, H.B.** (2012) A study on bacteria associated with the intestinal tract of farmed yellow seahorse, *Hippocampus kuda* (Bleeker, 1852): characterization and extracellular enzymes ***Aquaculture Research***, 43, 386-394.

Manuscripts communicated to peer reviewed journals:

1. **H.B. Pawar**, S.V. Sanaye, R. A. Sreepada*, B.S. Ingole, Murugan, A.,,Length-weight, length-length relationships and meristic analysis of yellow seahorse *Hippocampus kuda* (Bleeker, 1852) from central North-west Indian coast**.** ***Journal of applied ichthyology*** (IF=0.869)
2. **H.B. Pawar**, S.V. Sanaye, R.A. Sreepada, B.S. Ingole, Félix Haget., A modified method for improving pelagic phase juvenile survival in the yellow seahorse *Hippocampus kuda* (Bleeker, 1852). ***Aquaculture Research***,

National Conferences (Oral presentations):

1. **Pawar H.B.** Sanaye, S. V., B.S.Ingole, Sreepada,Suryavanshi, U., Pawar A.P., Parab P.P., **Simple culture techniques for Indian seahorses** (*National conference on Aquaculture: Prospects & Problems, held at The Institute of Sciences, Mumbai, on 12-13 Oct 2012*).
2. **Pawar H.B.** Sanaye, S. V., B.S.Ingole, Sreepada, R. A., Ansari, Z. A., **Clove oil an alternative anaesthetics for MS-222 for yellow seahorse *Hippocampus kuda***(*National Seminar on Coastal and Island Ecosystems:Conservation and Management, held at NIO, Goa on 16-17th Feb 2012*)
3. **Pawar H. B.**, Sanaye S. V., Sreepada, R.A., Ingole, B.S., Ansari, Z.A., **Closing of lifecycle of vulnerable yellow seahorse *Hippocampus kuda* in captivity: implications for stock replenishment and enhancement** (*National seminar on National Conference on Frontiers in Technologies for conservation of environment held at Dhempe college, Goa on 10th& 11th Feb 2011*.)

Poster Presentations in International and National conferences:

- **Pawar H.B.** Sanaye, S. V., Sreepada,B.S.Ingole ,Standardisations of techniques for captive breeding, rearing and mass culture of yellow seahorse Hippocampus kuda (Bleeker, 1852) ASEAN-India workshop, (19 to 22 March, 2013).
- **Pawar H.B.** Sanaye, S. V., B.S.Ingole, Sreepada,Suryavanshi, U., A.P.Pawar, P.P.Parab, **Seahorse: Flagship species for marine biodiversity conservation**(*National conference on Aquaculture: Prospects & Problems, held at The Institute of Sciences, Mumbai, on 12-13 Oct 2012*).
- **Pawar H.B.,** Sanaye S.V., Sreepada R.A., Ingole B.S., Ansari Z.A. and Sylla S. **Closing life cycle of *Hippocampus trimaculatus* (Leach, 1814) in captivity** (*World Ocean Day presentations on 8th June 2012 at National Institute Oceanography (NIO), Dona Paula, Goa – 403004*).

Popular article (Hindi):

- R. A. Sreepada, A. Murugan, SushantSanaye, UddhavSuryanshi and **H. B. Pawar**, SamudriGhodaEkChamatkariMachili. ***Sagarbodha***. 35-37

Media

- Work has been noted by many local and national news papers (Times of India, Herald, Navhind Times) and fishing chimes journal (Goa Newsletter Section)
- Local Television Channel of Goa noted the work (Link: http://www.goacom.com/news-clip-pro.php?seg=2&cd=2010-03-25).