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Captive Breeding of *Hippocampus reidi* Ginsburg, 1933 (Longsnout Seahorse) in Sri Lanka under Artificial Conditions

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ABSTRACT: *Seahorse trade has become a multi- billion dollar industry across the globe today leaving the wild populations in an endangered situation. Developing a successful captive breeding protocol is thus, necessary for their conservation while creating high income generating opportunities. Hippocampus reidi (Longsnout seahorse) has recently drawn the attention as one of the potential species for non-conventional aquaculture in the world. The aim of the study was to develop a captive breeding protocol for breeding of H. reidi under artificial environmental conditions. Thirty pairs of H. reidi were selected as the broodstock; males and females with average body heights of 16.6 ± 0.4 cm and 15.6 ± 0.2 cm, and average body weights of 20 ± 0.8 g and 18 ± 0.5 g, respectively. This study describes the morphometric identification of the species, broodstock management, rearing of fry and developing different colours for global market. A broodstock feeding regime was developed with a mixed diet of mysid shrimps and estuarine copepods, where the feeding regime at fry stages comprised of enriched brine shrimp nauplii and estuarine copepods. Marketable size was reached at 5-6 months. Survival rate at the marketable size was 65 ± 5% which is comparable to international standards. The results revealed that the successful breeding of H. reidi is possible under artificial conditions in Sri Lanka.*

Keywords: *Captive breeding, feeding regime, Hippocampus reidi, longsnout seahorse, survival rate*

INTRODUCTION

Seahorse is the name given to 54 species of marine fishes in the genus "*Hippocampus*". They are mainly found in shallow marine ecosystems throughout the world. For centuries, people have been enthralled by the equine appearance of these aquatic organisms. Scientists, hobbyists and aquarists are equally fascinated by these mythical creatures. They are truly unique not only because of their unusual shape, but also being the only animals on the earth, where males give birth to young ones (Giwojna, 2002).

Unfortunately, the traditional Chinese medicine trade, the curio trade, the aquarium trade and the global coastal habitat depletion have made them vulnerable to extinct. The global trade in seahorses in the year 2000 was estimated to be around 56 Mt and the present demand for seahorses appears to be greater than the supply (Vincent, 1996; IUCN, 2017).

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As very limited information is available, the whole genus '*Hippocampus*' has been classified as "DD" (Data Deficient) in the IUCN Red List. Further, it is considered as a key organism for a wide range of marine conservation issues. Therefore, many species including the *H. reidi* used for the study are listed as 'threatened' in the IUCN Red List.

At present, seahorse fishing and trading have been restricted all over the world because of their declining population size. All seahorse species are placed in "Appendix II", the list of endangered species by the Convention for the International Trade in Endangered Species (CITES) where it has restricted the legal import and export of seahorses dead or alive. This is a welcome measure for seahorse conservation worldwide and, is essential especially to eliminate wild seahorses from the aquarium trade. This situation has stimulated interest in commercial aquaculture of many seahorse species to supply for the trade (Anil *et al.*, 1999; Fortheath, 2000; Ignatius *et al.*, 2000; Woods, 2000; CITES, 2001; Job *et al.*, 2002).

One of the critical bottlenecks that culturists have to face in seahorse rearing is their low survival of juvenile stages (Scaratt, 1995; Payne and Rippingale, 2000). The species used in the present study, *H. reidi* is recognized as a difficult species to raise in captivity (Giwojna, 2002) and this situation has further aggravated as they have to be reared exclusively on live prey. Thus, the objective of this study was to develop a captive breeding protocol and fry rearing technology of *H. reidi* under artificial conditions in Sri Lanka.

MATERIALS AND METHODS

Location

This research was conducted in a marine aquarium in Chilaw, Western Province of Sri Lanka.

Broodstock

Thirty pairs of adult *H. reidi* were selected from an imported stock from Brazil. Presence of a brood pouch in males and its absence in females was the key feature in sexual dimorphism (Figure 1).

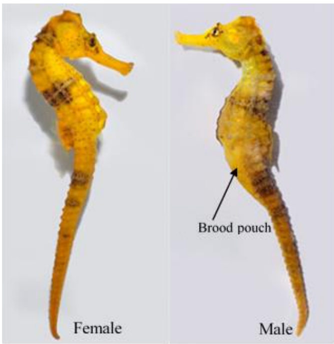

Figure 1. Sexual dimorphism of adult *H. reidi*

Identification of the species

The methods described by Ginsburg, (1933) and Lourie *et al*. (1999) were used in the identification of the species. Thus, morphometric and meristic features were considered for the identification of the species. Measurements and counts were checked with the above standards specified to ensure accuracy (Figure 2).

Body height was taken as the vertical distance from the tip of the coronet to the tip of the outstretched tail when the head was held at right angle to the body (Figure 2). Number of trunk rings, tail rings, pectoral fin rays and dorsal fin rays were counted through visual observations with the aid of a magnifying glass.

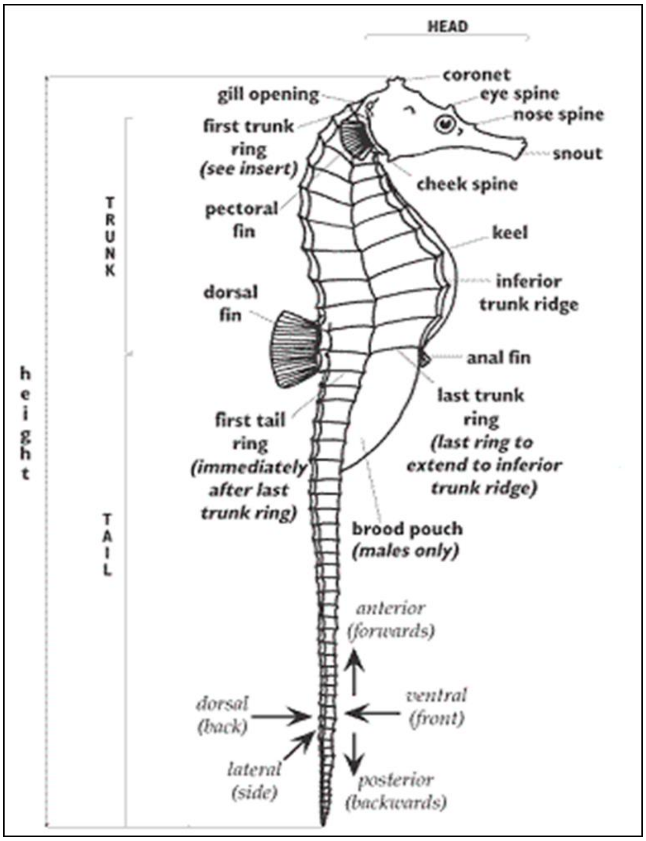

Figure 2. Body parts of a male seahorse.
(Source: Lourie et al., 1999)

Sea water purification process
The method described elsewhere by Hettiarachchi and Edirisinghe (2016) was used for sea water purification. Sea water was directly pumped to a collecting sump and allowed 24-48 hours for sedimentation according to the level of turbidity. The water was then subjected to a series of filtration and sedimentation. Finally, the water was allowed to run through an Ultra -Violet sterilizer before using in the rearing system.

Water quality parameters
Purified sea water in its natural condition was used in the rearing system without regulating water quality parameters by artificial means. However, daily measurement and recording of some water quality parameters were performed routinely as described by Hettiarachchi and Edirisinghe (2016).

A hand-held refractometer was used to record the salinity and specific gravity of water. Water pH was measured using a portable pH meter. A thermometer designed for aquarium purposes was used to measure daily fluctuations of water temperature. Liquid type water quality test kits designed for salt water aquarium use (HANNA Instruments) were used to measure the nitrate and ammonia concentrations.

Broodstock maintenance

Initial body weight and height measurements were taken from adult *H. reidi* selected for the study. Monogamous nature of seahorses was considered in selecting 1:1 male to female ratio in the broodstock.

Thirty pairs of *H. reidi* were initially maintained in two, common broodstock tanks for natural pair formation. The broodstock tanks were made of fiberglass and cylindrical in shape, having a diameter of 120 cm and 95 cm height. Each tank could hold 1000 L when the height of the water column was maintained at 90 cm. Water level was maintained to facilitate their upright swimming behaviour. Each tank was equipped with valves to regulate water inflow and outflow during water exchange.

Pair formation was observed over a period of 30 days, and each identified pair was transferred to separate fiberglass tanks of 500 L in capacity with conical frustum in shape (Figure 3). The system was facilitated to receive natural sunlight as well as natural light/dark regime. Orange, green and red coloured nylon ropes of 10 mm thickness and 45 cm in length were unwound, and tied into a weight and placed at the bottom of the broodstock tanks in a manner to simulate sea grasses as in the natural reef habitat. This setup provided holdfasts to curl the tails around them and stay firmly in the water column. In addition, broodstock tanks were provided with mild aeration.

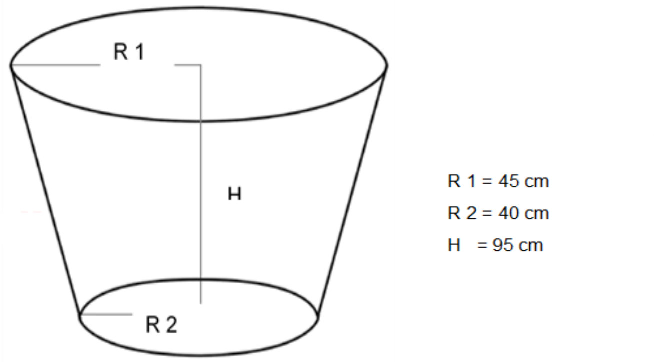

Figure 3. Dimensions of broodstock tanks

Broodstock feeding

Feeding was practiced three times a day (7.00 am, 11.00 am and 4.00 pm). Brine shrimp (*Artemia salina*) nauplii of 24 hours after hatching and a commercial formula 'Selco' (DHA INVE Aquaculture, Belgium) was the morning diet. Wild caught estuarine and coastal mysid shrimps of 5-10 mm in size comprised 90 % of the broodstock diet which was fed twice a day. The habit of feeding where chasing behind a live pray and swallowing the whole live prey with a quick suck, without chewing was closely observed. *Ad libitum* feeding was practiced, and the aeration of tanks facilitated even distribution of live feeds in the water column.

Broodstock tanks were siphoned off twice a day, one hour after feeding in the morning and evening to remove faeces and the dead organisms. Ten minutes before siphoning, aeration was stopped to settle waste matter in the tank bottom. After siphoning, 50% of water exchanged once a day.

Rearing fry stages of *H.reidi*

Newborn *H. reidi* were collected by siphoning off daily from the broodstock tanks early in the morning before commencing feeding.

A 10 mm flexible hose was used to siphon off new-born fry to a 20 L basin. Newborn *H. reidi* were miniature adults of 8-10 mm in size. They were transferred to fry rearing tanks which were of the same type as broodstock tanks illustrated in Figure 3.Water level of the fry rearing tanks was maintained at 90 cm height. Stocking density was 20/ Liter during the first four weeks.

Water flow was regulated, aeration was provided and siphoning was carried out as described for broodstock tanks. Air stones were not used in fry rearing tanks to avoid the problem of ingesting minute air bubbles resulting inflation and mortality. Initial water level of the tanks were maintained at 50 cm and gradually increased to 90 cm at the end of the first week. Water exchange was started 2 days after stocking in the fry rearing tanks.

Feeding regime during fry stages

Feeding regime for different stages of fry, namely 1st week, 2nd week, 2-5 weeks and 6-14 weeks was developed. Daily feeding frequency at all stages of fry was at 7.00 am, 11.00 am and 4.00 pm.

Feeding during the first week after birth

A mixture of live copepodites and brine shrimp nauplii 18 hours after hatching were the starter feeds for newborn *H. reidi*. Harvested estuarine copepods were filtered through 180 μm mesh to obtain copepodites of 250 μm size. Live copepodites comprised of 75% of the diet and the feeding density was three prey organisms per liter. Brine shrimp nauplii were the balance 25% of the diet and fed at a density of 1 nauplii/mL of water.

Feeding and care during the second week

During this stage, fry were capable of swallowing adult copepods. Therefore, filtering was not performed. Diet was composed of 75% copepods and 25% brine shrimp nauplii18 hours after hatching. Feeding density and the frequency were same as in the first week.

Time of feeding, siphoning and water exchange was continued as performed in the first week.

Feeding and care during 2-5 weeks

Fry were fed with diet composed of brine shrimp nauplii (18 hours after hatching) and copepods at equal proportions. Feeding density was four prey/L. Time of feeding was the same as in the previous stage. Orange, green and red coloured nylon ropes of 5 mm in thickness and 45 cm in length were unwound into threads and tied to weights as a bunch and placed on the tank bottom. Due to their low weight, those threads stood in the water column and provided holdfasts to growing fry.

Tanks were siphoned off twice a day and 50% water exchange was performed routinely to maintain water quality.

Care of juveniles from 6 -14 weeks

Six weeks after birth, brine shrimp nauplii (24 hours after hatching) were introduced as a mixed diet with copepods. Other routine practices were as same as performed in the previous stage of fry.

At 14 weeks of age, juveniles were transferred to mass rearing tanks.

Mass rearing of juveniles

Mass rearing tanks were circular shaped cement tanks of 1 m in height and 2 m in diameter. Water level was maintained at 80 cm to have a total volume of 10 000 L. Juveniles were 6-8 cm in size.

During this stage, mysid shrimp was the main component of the diet (90%) with 10 % of brine shrimp nauplii of later nauplii stages. Water exchange (around 80%) was practiced twice a day. Strict hygiene was maintained to keep free from diseases.

Inducement of different body colours

According to the market demand for differently coloured seahorses, juveniles of 6-7 cm height were transferred to glass aquariums for colour inducement. Feeding freshwater mysid shrimps was essential for colour inducement. Juvenile seahorses were individually collected using a concave spoon and were carefully transferred to rectangular glass aquariums of 120 × 60 × 50 cm in size where water level was maintained to a height of 50 cm. Each tank carried 12.5 cm diameter inlet positioned on the top of the tank at the broad side and, an outlet of 25 cm at the opposite side just above the water level. Outlet was covered with a nylon mesh of 5 mm holes to facilitate water outflow without allowing juveniles to escape. Water outlet was located 10 cm below the top level, so that the full water level of a tank was 300 L, where, stocking density was one seahorse per 10 L. Natural sunlight and mild aeration were provided for each tank. Outside walls of the glass tanks were covered with colored polythene. Blue, orange, red and yellow colors were used.

Data collection and analysis

Daily observations and regular data collection were performed throughout the study. Morphometric and meristic data, length of the gestation period, growth rate, age at maturity and survival rates were recorded. In addition, number of fry produced in each spawning cycle by each male seahorse was recorded.

Survival rate was calculated from the number of individuals grown up to marketable stage out of a total number of newborns per spawning. The average survival rate was calculated batch wise. The average of the first spawnings of the 30 spawners were taken as the batch one and the average of the second spawnings of the 30 spawners were taken as the batch two etc. Thus, fives pawnings of each spawner (5 × 30) were considered.

RESULTS AND DISCUSSION

Identification of the species: *H. reidi*

The unusual shape of seahorse created challenges when observing for morphometric and meristic characters of the broodstock. They were flexible when free and curled up to different degrees when handling. All 3 main spines, number of body rings and number of fin rays unique to the species were clearly observable in *H. reidi* (Table 1).

Table 1. Morphometric and meristic features of adult *H. reidi*

Parameter	Description
Eye Spine	clearly visible
Cheek Spine	clearly visible
Nose Spine	clearly visible
Coronet (Group of spines on the top of the head)	clearly visible
Height (from coronet to tip of the tail)	16.3 ± 0.7cm
Number of trunk rings	12
Number of tail rings	33
Number of dorsal fin rays	16
Number of pectoral fin rays	14

Stress during handling

It was important to minimize the stress to the animal when measuring live seahorses. Care was taken to minimize the handling time and to hold them underwater to reduce stress during measuring though it was a difficult task.

Water quality parameters

The water quality parameters during the experimental period was given in Table 2. These values were within the optimum range for most of the marine aquarium species as reported by Hettiarachchi and Edirisinghe (2016).

Table 2. Water quality parameters during the experimental period

Parameter	Value
Salinity	28-37 ppt
Water temperature	28 - 30 °C
Water pH	7.6-8.5
Specific gravity	1.024-1.027
Nitrate concentration	< 40 mg/L
Ammonia concentration	<0.8 mg/L

Reproduction

H. reidi exhibited courtship behaviour 2-3 days before breeding. Courtship behaviour accompanied with change in body colour, swimming side by side, grasping their tails together and drifting upward snout-to-snout while freeing out of the holdfasts. During mating, female seahorse deposited eggs in the male's pouch using its ovipositor. This act takes less than a second and was hardly noticeable. The observed spawning interval was 13-15 days. The male seahorse exhibited a severe pain during spawning. Spawning occurred early in the morning, between 4.00-6.00 am. Usually spawning occurred at the bottom of the tank. After spawning, no parental care was shown by the male, and often they engaged in re-mating within hours.

The average fecundity of *H. reidi* reared under Sri Lankan conditions was 565±45 which was a low value compared to 1000-1500 eggs under different environmental conditions (Vincent, 1990). However, it was not possible to get a clear understanding about how many fry were produced out of them since the fertility of the eggs was not reported by Vincent (1990).

In the present study, the initial body sizes of spawners were different. However, differences in the weight of male seahorses having similar body height may be due to the differences in their ovarian conditions (Table 3).

Table 3. Variation in body size and fecundity of *H. reidi*

Fecundity/ Fertility	Initial Measurements		Number of larvae released (Mean ± SD)	Condition 3
	Height (cm)	Weight (g)		
Fertility of 30 males	16. 3 ± 0.7	20 ± 0.8	565 ± 5	Present study-under aquarium conditions in Sri Lanka
Fertility of 13 males	14.8	13.7	691.4	Silveira and Fontoura, (2010) in Brazil
Fecundity of 3 females	13.6	11.43	839.7	

According to Silveira and Fontoura (2010) an average fecundity rate of 839.66 oocytes per lot and, an average fertility rate of 691.4 offspring per brood pouch were reported. They have reported that, newborns (5–7 mm tall) had no correlation with the height or weight of the male and the number of embryos incubated. Silveira and Fontoura (2010) further reported that, even though many species of *Hippocampus* have characteristics in common (e.g., courtship rituals) they show differences fertility rates, fecundity rates and size of the newborns even within the same species based on the geographical region where they live.

Life during fry stages

Newborn fry (Figure 4) resembled miniature adults. Energetic fry were phototrophic and were 8-10 mm in size. Soon after release, they drifted to water surface. It was quite a passive movement with the aid of mild water currents and then gathered on the water surface in search of planktonic live prey. However, active swimming was not observed until 3-4 days after birth. Upright swimming behaviour observed in adults were not quite obvious in newborn fry.

Fry of *H. reidi* were1.5-2.5 cm at the age of 2-5 weeks. From the second week onwards, they were curling their tails on nylon thread holdfasts. Dias and Rosa (2003) who used random visual census method to observe occurrences of seahorses on each holdfast in natural habitats reported that *H. reidi* has been found using of 18 different holdfasts, of which the green algae *Caulerpa racemosa* and *C. kempfi*, the tunicate *Ascidia nigra*, and the roots of the mangrove plants *Avicennia schaueriana* and *Rhizophora mangle* were reported the most frequent. However under aquarium conditions, *H. reidi* did not pay interest in using green colour holdfasts.

Figure 4. Fry of *H. reidi*

Mass scale rearing

In mass scale rearing, the juveniles reaching sexual maturity could be observed by the colour changes and ritual movements exhibited early in the morning. At sexual maturity they were 160 ± 20 days of age and with the body height of 8 ± 2 cm. Development of brood pouch in males could be observed during this stage. The male to female ratio observed was 1:1.5. They exhibited calm behavior, curling their tails on any object they find and ambushing on prey items.

Inducement of different body colours

Fry of *H. reidi* were pale or dark black in colour at birth and gradualy developed a dark colouration with time. The excellent camouflaging ability of *H. reidi*, was an added advantage for inducing different body colours according to market demand in aquarium trade. In the present study, they gradually changed the body colour when they were transferred to glass aquariums with coloured backgrounds. However, those transferred to blue and orange backgrounds exhibited no colour changes. In the red background, all seahorses turned to a fascinating orange and red colour at the end of 14 days of transfer (Figure 5). Those transferred to yellow background exhibited bright yellow colours (Figure 6).

However, under captive breeding conditions, colour variations of yellow, orange, red, black or mixture of those colours could be observed even in the mass rearing tanks when provided with freshwater mysid shrimps. It was believed that high amount of colour enhancing compounds in freshwater mysid shrimps contribute to colour inducement in growing juveniles.

Growth of juveniles

The growth was not monitored until 14 weeks of age, since handling seemed to be highly stressful for early stages. Initiation of sexual metamorphism could be observed at 14^{th} week. Hora *et al*. (2009) have observed the onset of sexual maturation in about 60 days which was not realistic under Sri Lankan aquarium conditions. In the present study, males and females were separated and cultured separately at 14 weeks of age. From each group, 100 individuals were taken for measuring body height and the weight. First measurement was taken on the 105^{th} day and the 2^{nd} was after another 50 days. After that, it was taken on every 30 days until 11 months of age (335 days). Results showed that the average growth is higher in males than that of females throughout the period (Figure 7).

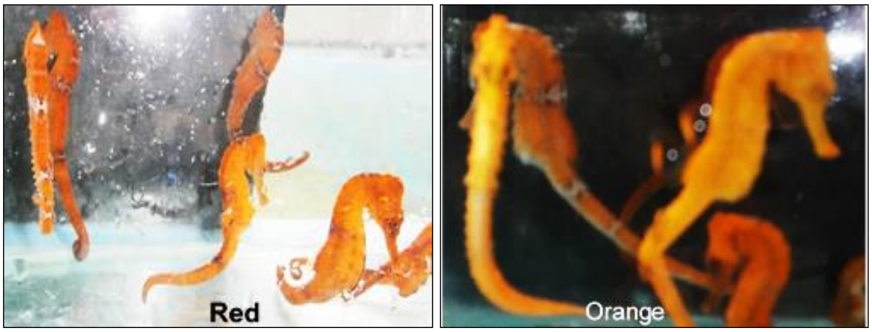

Figure 5. Red and orange *H. reidi*

Hora *et al*., (2009) observed a growth rate of 0.77 ± 0.01 mm/ day from birth to 109 daysin *H. reidi* grown on wild zooplankton, enriched brine shrimp nauplii and mysid shrimps. However, they have observed decreased growth after the onset of sexual maturation in both males (0.31 ± 0.10 mm/day) and females (0.53 ± 0.09 mm/day).

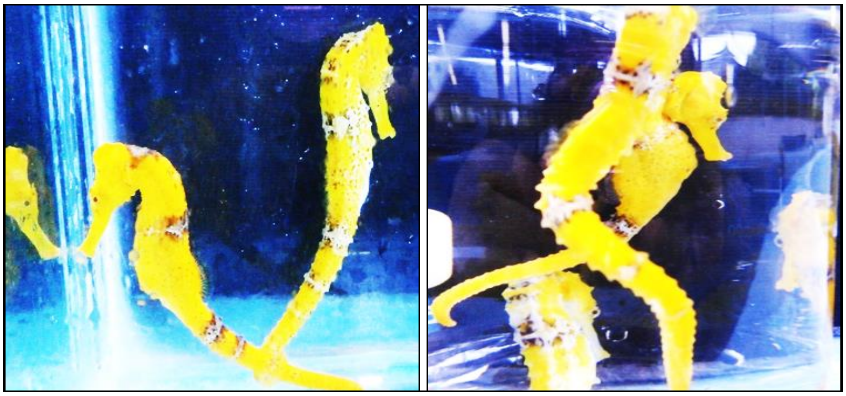

Figure 6 . Yellow *H. reidi*

Attempts to reduce mortality

As reported by Murugan *et al.* (2009) ingestion of air bubbles was found to be the major cause of fry mortality. Fry of *H. reidi* feed at the surface where prey organisms tend to congregate towards sunlight accidently swallow air along with the live feeds. This makes the newborns to lose their equilibrium and to float on the surface of water. When such floating individuals were closely examined, a bubble of trapped air could be observed in the body cavity. Floating made them unable to find food resulting in starvation. This condition was observed during the first two weeks of larval life. This condition could be controlled by having green algae (*Chlorella vulgaris*) at a density of 50,000 cells/mL in the tanks during the first seven days of newborn fry. It was an added advantage even for prey organisms to find their feed. Turbidity in rearing water caused by green algae helped to keep fry away from the surface. From 7-14 days of age, salinity values of fry rearing tanks were lowered to 27 ppt so that fry were less buoyant for surface feeding. As indicated by Kollman (1998) reduced salinity was helpful to reduce parasitic problems in fry.

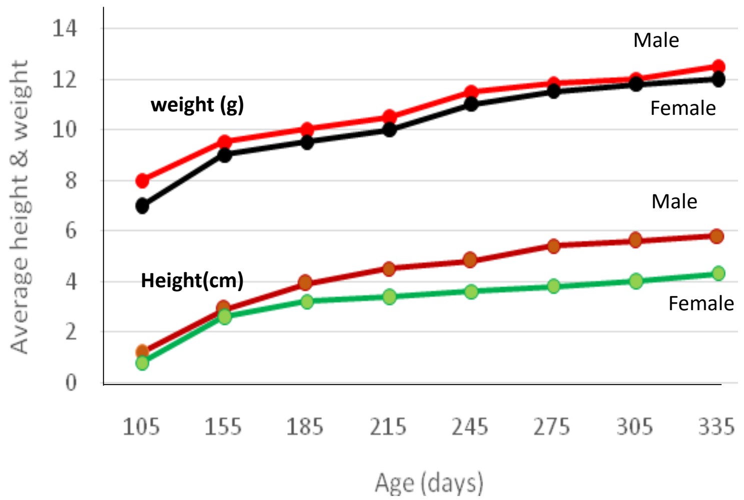

Figure 7. Growth differences in male and female *H. reidi*

Feeding during the first two weeks after birth was a critical period in larval rearing. During this period, proper size and proper type of feed was essential to avoid high rate of mortalities. In the absence of suitable and balanced diets, larval death was observed starting from 5-7 days after birth. As observed by Payne and Rippingale (2001) feeding on calanoid copepods resulted in improved survival and growth. Even though brine shrimp nauplii were important as a feed, it could not be effectively used as the only feed in rearing *H. reidi*. Though juveniles were hardy, excessive handling seemed to be quite stressful, which caused reduced immunity and prone to diseases in subsequent life. Usually, mortality at adult stages was less if the water quality was properly maintained.

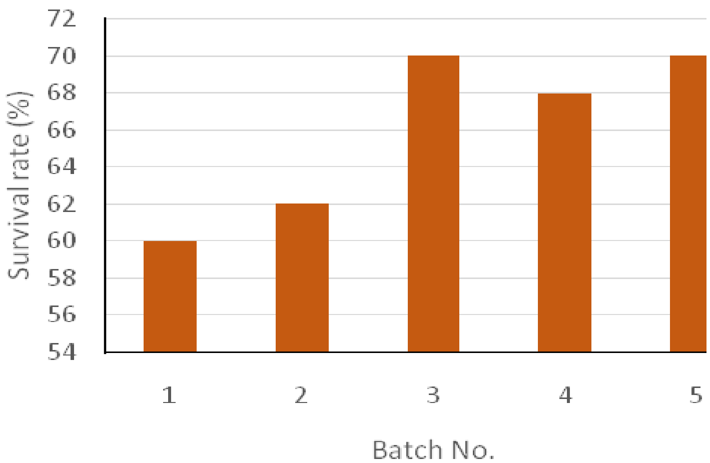

Figure 8. Survival rate of *H. reidi* fry to adult

(One batch represents an average of 30 spawnings)

Survival rate

Survival rate was calculated from the number grew up to marketable stage out of the total number of newborns per spawning. The protocol developed in this study was successful to achieve a survival rate of 65 ± 05% (Figure 8). It was a high survival rate compared to many other studies worldwide as reported by Bull and Mitchel (2002). Providing proper nutrition during the crucial first few weeks of life was one of the main reasons in achieving higher survival rates in this study. Maintenance of proper hygienic conditions and optimum water quality were also contributed to the high survival of growing fry.

CONCLUSIONS

H. reidi can be successfully bred and cultured in captivity under Sri Lankan aquarium conditions with a fast growth and high survival rate of 65 ± 05 %. Results of this study could be used for mass scale captive breeding of *H. reidi* in Sri Lanka with the aim of conservation and export market.

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