Growth of four generations of Zebra-snout Seahorse, *Hippocampus barbouri* (Jordan & Richardson, 1908) in captivity

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Abstract: This study was conducted to determine the effect of different generations affecting the size of *Hippocampus barbouri* in captivity. Seahorse in-house breeding was carried out in Fisheries Research Institute, Penang. Adults *H. barbouri* were conditioned prior to breeding. All newborn *H. barbouri* juveniles were transferred to rearing tank once they were born. Growth of *H. barbouri* juveniles was measured at 10 days interval, up to 60 days. Results showed that different F2 *H. barbouri* juveniles recorded the smallest size when compared to other generations at day 10 after birth. However, starting from day 50 after birth to day 60 after birth, F2 *H. barbouri* juveniles recorded the best growth when compared to other generations. Although F3 *H. barbouri* juveniles had better growth from day 10 of birth until day 40 of birth, the growth was limited after day 50 of birth. F4 and F5 *H. barbouri* juveniles had similar finding as F3. One of the possible reasons was due to feeding. At initial stage of life, *H. barbouri* juveniles were fed with newly hatch *Artemia* nauplii. Starting from day 40, *H. barbouri* juveniles were weaned over to live Mysis and adult *Artemia*. Inconsistency supply of live mysids due to monsoon season might affect growth of *H. barbouri*. Moreover, nutritional content of adult *Artemia* was another concern. To conclude, culture of *H. barbouri* in captivity is feasible, where growth of *H. barbouri* can reach maximum height of 72 mm at day 60 of birth, with the survival rate of more than 43%.

Keywords: Seahorse, *Hippocampus barbouri*, inbreeding, growth, captivity.

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I. Introduction

The wild populations of seahorse wild population is now being threatened by incidental by-catch and the loss of habitats in addition to direct fishing pressure either for the purposes of souvenirs, traditional medicines or aquarium trading activities [1-2]. All seahorse species were listed in the Convention on the International Trade in Endangered Species (CITES) [2]. Due to overexploitation or degradation of their natural habitat, seahorse culture has been proposed as one of the solutions to reduce stress on wild seahorse population [3].

Hippocampus barbouri, commonly known as zebra-snout seahorse, is one of the ten seahorse species found in the Malaysia waters and so far, is restricted to Sabah waters [4]. It has been the focus of several research projects, investigating its feeding, breeding, hormones and phylogeography [2]. The International Union for Conservation of Nature (IUCN) has classified this species as "vulnerable" [5]. Culture of seahorses becomes more difficult than expected due to lack of information on the culture techniques and methods [6]. Most studies in Malaysia were on conservation and distribution [4,7] while studies on aquaculture topics such as culture techniques, growth hormones and physical conditions were limited [6,8,9]. The objectives of this study were to determine the effect of different generations affecting the size of *H. barbouri* in captivity and to evaluate the effect of inbreeding in the seahorse.

II. Results and Discussion

For all the generations, broodstock were domesticated and inbred. All the generations produced more than 90 *H. barbouri* juveniles except F4 (Table 1). Number of newborns for each generation was 94.00±55.75, 117.33±67.28, 54.00±13.45 and 99.00±25.51, respectively. At 60 DAB, each of the generations recorded survival of 53.90±20.34, 69.03±7.34, 43.21±3.40 and 71.71±38.79 %.

Table 1: Number of newborn *H. barbouri* and survival at 60 DAB

Generation	Number of new born	Survival (%)
F2	94.00±55.75 [a]	53.90±20.34 [a]
F3	117.33±67.28 [a]	69.03±7.34 [a]
F4	54.00±13.45 [a]	43.21±3.40 [a]
F5	99.00±25.51 [a]	71.71±38.79 [a]

Height of *H. barbouri* juveniles were not sampled after born to reduce stress on the juveniles. Results showed significant differences (P<0.05) between generations at different Day After Birth (DAB) (Table 2). At 10 DAB, height of *H. barbouri* juveniles of generation F3, F4 and F5 were higher with height of 23.53±2.11 mm, 23.77±2.65 mm and 23.13±2.32 mm respectively, as compared to F2 with height of 21.37±2.34 mm (Figure 1).

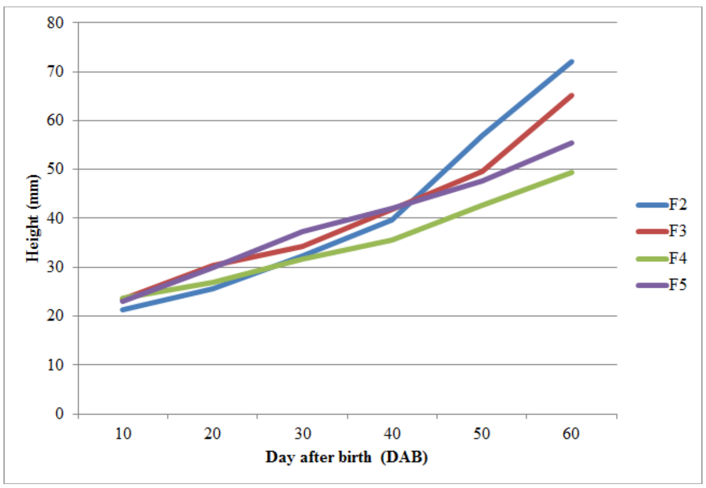

Figure 1: Growth of *H. barbouri* juveniles between generations from 10 to 60 DAB.

For *H. barbouri* juveniles at 20 DAB, generation of F3 and F5 showed significant differences (P<0.05) with height of 30.30±3.14 mm and 29.97±2.68 mm when compared to F2 and F4 with height of 25.57±2.80 mm and 26.90±3.20 mm. When *H. barbouri* juveniles reached 30 DAB, generation F5 was significantly different (P<0.05) when compared to F3 and F4 with height of 37.27±4.13 mm while F2 had no significant difference (P>0.05) when compared to F3 and F4. *Hippocampus barbouri* juveniles of generation F2, F3 and F5 grew better at 40 DAB with height of 39.63±7.62 mm, 41.73±5.34 mm and 42.07±4.27 mm when compared to F4 with height of 35.63±5.14 mm. For *H. barbouri* juveniles at 50 DAB and 60 DAB, generation F2 resulted in best growth when compared to other generations. Generation F3 *H. barbouri* juveniles had better growth from 10 DAB until 40 DAB, the growth was limited from 50 DAB while generations F4 and F5 *H. barbouri* juveniles had similar finding as F3.

Inbreeding is the mating of organisms between relatives, which usually decrease heterozygosity in the gene pool. In current study, all *H. barbouri* was inbred. Inbreeding is normally avoided in aquaculture as it has been frequent reported that traits shows in inbreeding depression in fish species, including reduced survival, fry abnormalities, lowered reproductive success and reduced growth rate [10]. As there is only limited information with regards of effects of inbreeding in aquaculture species has been published, there is no report related to inbreeding in seahorse. Rainbow trout appeared as the most studied subject in this field [11-14]. Hence, knowledge of inbreeding effects in rainbow trout and other species can indicate the traits types that may be vulnerable to inbreeding depression.

Moav and Wohlfarth [15] reported a 15% decrease in relative to growth rate in the inbred carp (*Cyprinus carpio*) produced from full-sib parents. Also, there was a lower recapture frequency from inbred families of Atlantic salmon (*Salmo salar*), in which the lower survival rates were related to inbreeding [16]. In 1971, Aulstad and Kittelsen [11] reported that rainbow trout (*Salmo gairdneri*) fry has deformities with a breeding coefficient of F=0.25. For current study, all newborn *H. barbouri* did not show any deformities and number of newborns of *H. barbouri* did not give any significant differences between generations. The fourth generation (F4), however, produced the least *H. barbouri* when compare to all other generations.

Mrakovcic and Haley [17] reported that inbreeding at levels of half or full sib mating triggered a decrease in fertility, fry survival to 30 days and fry length at 30 days in zebra fish. However, the survival of *H. barbouri* in present study showed no significant differences between generations. The survival rates showed an increasing pattern from 54% in F2 to 72% in F5, with an exception of F4. A study by Su et.al [14] presented that there was a significant inbreeding depression in egg number but not egg size at a relative low level of inbreeding. *Poecilia reticulata* was found to have a less effective immune system towards disease and significantly higher mean parasite intensity due to inbreeding [18]. On the other hand, a study by Aulstad et.al [12] found no depression due to inbreeding for growth of fry. Horstgen–schwark [13] also reported that there is no difference in growth performance between inbred lines and outbred control (F=0.375) in rainbow trout. As mentioned by Thunken et.al [19], theory predicts that the advantages of mating with close kin can

Table 2: Growth of *H. barbouri* juveniles between generations from 10 to 60 DAB.

Generation	Height (mm)					
	Day after birth (DAB)					
	10	20	30	40	50	60
F2	21.37±2.34[b]	25.57±2.80[b]	32.27±4.83[bc]	39.63±7.62[a]	56.97±10.62[a]	72.07±13.00[a]
F3	23.53±2.11[a]	30.30±3.14[a]	34.27±3.72[b]	41.73±5.34[a]	49.57±7.70[b]	65.10±6.21[b]
F4	23.77±2.65[a]	26.90±3.20[b]	31.67±4.41[c]	35.63±5.14[b]	42.60±5.61[c]	49.30±7.16[c]
F5	23.13±2.32[a]	29.97±2.68[a]	37.27±4.13[a]	42.07±4.27[a]	47.67±5.64[bc]	55.40±9.60[c]

Mean ± S.D. with the same superscripts within the same column are not significantly different ($p>0.05$).

override the effects of inbreeding depression, but it is scarce in the animal kingdom. No evidence for inbreeding depression has been found and it is suggested that in *Pelvicachromis taeniatus* inbreeding is an advantage. Therefore, inbreeding caused no adverse effect on cichlid fish. These results were aligned with present study, where no negative effect was recorded.

The use of live food resulted in gonad development and better growth of adult seahorses [20]. Quantity and quality of food marked significance on brood size, and this affected sperm quality and gonad development [21-23]. In this study, only live food was used for both the broodstock and seahorse juveniles. However, Melo et.al [24] mentioned that the nutritional value of feed was the main factor affecting growth of seahorse. Adult *Artemia* were rich in protein but low in carbohydrate and lipids [25]. Craig [26] stated that lipid produced higher energy when compared to carbohydrate and protein. N-3 HUFA is particularly vital for the growth of marine fish. Therefore, the nutritional content of adult *Artemia* might not be enough for the good growth of seahorse.

Nur et.al [9] mentioned that post larvae of white shrimp (PLS) were most preferred feed and showed best reproductive performance with high numbers of spawning occurrences. Broodstocks of *H. barbouri* were fed with live Mysis shrimp and adult *Artemia*. Inconsistency supply of live mysids during monsoon season might affect growth of *H. barbouri*. At monsoon season, *H. barbouri* were only fed with adult *Artemia*, which may affect the reproductive performance of broodstock. *Hippocampus barbouri* broodstock produced lowest brood size when fed with adult *Artemia* [9]. Hence, the growth of *H. barbouri* juveniles between generations was inconsistent.

III. Conclusion

This study focuses on the growth of domesticated and inbred Zebra-snout seahorses, *Hippocampus barbouri*. The outcome is expected to aid in the development of seahorse culture in Malaysia. Breeding and rearing of *H. barbouri* in captivity is feasible, where growth of this threatened marine species can reach maximum height of more than 70 mm by two months old with the survival rate of nearly 50%. Although the growth of *H. barbouri* juveniles between generations was inconsistent, possibly due to feeding, all newborn *H. barbouri* did not show any deformities and number of newborns did not give any significant differences between generations. To conclude, inbreeding caused no negative effect on these seahorses. These findings will generate a base for future research on other local seahorse species and help achieving conservation and commercial goals.

IV. Materials & methods

Experiment was carried out in Fisheries Research Institute (FRI), Batu Maung, Penang from April 2016 to January 2018. Source of seawater was from Batu Maung, Penang, Malaysia. Serial filtration was done for seawater prior to usage. Seahorses were maintained in glass tank of size 90 cm x 45 cm x 50 cm with moderate aeration. Plastic chain was tied with weight to serve as holdfast for the seahorses. Adults *H. barbouri* were conditioned prior to breeding. Seahorses were feed on live Mysis shrimp caught from the wild or live adult *Artemia*. Seahorses were fed twice daily at 0900H and 1600H to *ad libitum*.

Captive bred newborns of *H. barbouri* from second generation (F2) to fifth generation (F5) were used in this study. All newborns were transferred to larval rearing tank of size 90 cm x 45 cm x 50 cm once the juveniles were released from the broodpouch of the pregnant male broodstock. Seahorse juveniles were fed with newly hatch *Artemia* nauplii for first 10 days after birth. Then, *H. barbouri* juveniles were weaned over to enriched *Artemia*. Starting from 20 Day After Birth (DAB), *H. barbouri* juveniles were fed with live adult *Artemia* fed with *Rotifer* or rice flour. From day 40 onwards, live mysis shrimps were introduced to the seahorses as their diet. All *H. barbouri* juveniles were fed to satiation twice daily at 0900H and 1600H.

The growth of *H. barbouri* juveniles was measured at 10 days interval, up to 60 days. Height was taken based on Lourie et.al [27], which is from the top of the coronet to the tip of the straightened tail. After 60 days, the experiment was stopped and all survivors were counted. Five batches of each generation were used for data analysis. The data analysis was performed by software SPSS 21.0, using one way of Analysis of Variance (ANOVA). Tukey test was used to determine the significant difference between treatments. Results of the growth were given as mean ± standard deviation.

Author's contribution: MSI designed the study. MSI and VWCR executed the work and analyzed the data. MSI, VWCR and CKY wrote the manuscript. All authors have read and approved the final manuscript.

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