UNIVERSITI PUTRA MALAYSIA

CRUCIAL FACTORS AFFECTING REPRODUCTION AND CULTURE OF Hippocampus barbouri (JORDAN AND RICHARDSON 1908)

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

CRUCIAL FACTORS AFFECTING REPRODUCTION AND CULTURE OF *Hippocampus barbouri* (JORDAN AND RICHARDSON 1908)

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Chairman : Annie Christianus, PhD
Institute : Bioscience

Hippocampus barbouri is one of the ten seahorses species found in Malaysia and listed in Appendix II of the Convention on the International Trade of Endangered Species of Wild Fauna and Flora (CITES). Its mere existence is threatened by human, being exploited to fulfil demands from traditional Chinese medicine (TCM) and ornamental trades. It is on demands regardless whether it comes from the wild or cultured. Thus, the establishment of seahorse aquaculture is imminent. Since, there is no documented report on seahorse aquaculture in Malaysia, this study focused on several most important aspects in order to develop suitable technique for the reproduction and culture of *H. barbouri.*

During the acclimatization of wild *H. barbouri*, two common causal factors for mortality were gas bubble disease (GBD) and annelid infestation. Therefore, it was a necessity to overcome this problem to proceed with this study. GBD was taken care by manual removal of gas from the male brood pouch, while annelid infestation was treated with formalin. Subsequent experiment was conducted using different culture systems to determine the most suitable system for the maintenance and breeding of this seahorse. Findings shows that minimum water depth required for the successful spawning of *H. barbouri* is 0.38 m, while the most suitable diet for seahorse broodstock is shrimp post-larvae.

The second objective of the study was to observe and describe the morphological development of *H. barbouri* from juvenile until maturity. Isometric growth pattern was observed during the early developmental stage until it reaches 141 day after birth (DAB), showing the first sign of sex differentiation. Then, from 141 until 168 DAB, male and female *H. barbouri* recorded different growth pattern. The onset of maturity was observed at 164 DAB with the

presence of fully developed brood pouch in male, with standard length and wet weight of 7.16 cm and 1.47 g, respectively.

In the next objective, the effects of physical factor such as culture system, stocking density and salinity on the growth and survival of *H. barbouri* during critical stage (1 to 56 DAB) were investigated. Newborn *H. barbouri* cultured in system with combination of hang-on and under-gravel filter showed the best growth with standard length of 3.74 cm and survival of 75%. Using this culture system, different seahorse stocking densities and water salinities were tested. Results shows that at stocking density of 0.5 seahorse L^{-1} (1 seahorse per 2 L) and salinity of 24 ppt produced the best growth and survival of *H. barbouri.* Scarcity of natural seawater supply has prompted the study on the potential of using artificial sea-salt for the culture of *H. barbouri*. Newborn *H. barbouri* cultured in seawater made from commercial artificial sea-salt known as Red Sea Coral Pro Salt (RSCPS) showed the best growth with standard length increment of 3.13 cm. Element analysis showed that this commercial sea-salt contained similar elements to that of natural seawater.

Feeding experiment was conducted to determine the effect of different *Artemia* enrichment on the growth and survival of newborn *H. barbouri*. Newborn fed with *Artemia* nauplii enriched either using cod liver oil in combination with thyroxine or potassium iodide produced the best growth and survival. A subsequent experiment was carried out to determine the effectiveness of feeding this enriched *Artemia* at different frequencies. Economically, enrichment of *Artemia* using cod liver oil in combination with thyroxine fed twice a week is highly recommended. This study also determine the potential of other feed item such cladoceran *Daphnia* for juvenile *H. barbouri* at the age of two month-old.

In conclusion, seahorse *H. barbouri* can successfully breed in tank system with hang-on filtration unit, at a minimum water depth of 0.38 m. Broodstock of *H. barbouri* can be fed with either post-larvae shrimp or frozen *Mysis* for better reproductive performance. During the development of *H. barbouri* in captivity, critical period with high mortality was observed at 7-11 DAB, with prominent slow growth during the first two months. *Hippocampus barbouri* reared in control condition can reproduce at the age of 168-175 DAB. Tank with hang-on combined with under-gravel filter was the most effective tank system for the culture of *H. barbouri* during early stage at stocking density of 1 seahorse in 2 to 3 L volume. Salinity of 24 ppt was the most suitable salinity for the culture of *H. barbouri*. Red Sea Coral Pro Salt (RSCPS) and Red Sea Salt (RSS) can be used for the preparation of seawater for the culture of *H. barbouri*. At early stage, *H. barbouri* can be fed with *Artemia* nauplii enriched with cod liver oil and thyroxine at frequency of twice weekly. Frozen wild zooplankton or sub-adult *Artemia* were suitable live food for juvenile *H. barbouri* at the advance stage.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

FAKTOR-FAKTOR PENTING YANG MEMPENGARUHI PEMBIAKAN DAN KULTUR *Hippocampus barbouri* (JORDAN AND RICHARDSON 1908)

Oleh

NUR FATIHAH BINTI ABD HALID

Januari 2018

Pengerusi : Annie Christianus, PhD
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Hippocampus barbouri adalah salah satu dari sepuluh spesis kuda laut yang dijumpai di Malaysia dan tersenarai dalam *Appendix II Convention on the International Trade of Endangered Species of Wild Fauna and Flora* (CITES). Kewujudannya diancam oleh manusia, dieksploitasi untuk memenuhi permintaan industri perubatan tradisional Cina (TCM) dan ikan hiasan. Terdapat permintaan yang berterusan terhadap kuda laut sama ada yang ditangkap dari persekitaran semulajadi atau yang dikultur. Oleh itu, penghasilan kuda laut melalui akuakultur adalah sangat diperlukan. Kajian ini memberi fokus kepada beberapa aspek penting untuk membantu penghasilan teknik yang sesuai untuk pembiakan dan pengkulturan *H. barbouri.*

Semasa proses penyesuaian, penyakit gelembung gas (GBD) dan jangkitan annelida menjadi punca utama kematian *H. barbouri* liar. Oleh itu, adalah penting bagi mengatasi masalah ini untuk meneruskan kajian. GBD dapat diatasi dengan mengeluarkan gas dari kantung jantan secara manual, manakala jangkitan annelida boleh dirawat dengan formalin. Eksperimen berikutnya dijalankan dengan menggunakan pelbagai sistem kultur untuk menentukan sistem paling sesuai bagi kultur dan pembiakan kuda laut. Hasil kajian menunjukkan kedalaman air minimum 0.38 m paling sesuai bagi pembiakan *H. barbouri*, manakala pasca-larva udang adalah diet yang terbaik bagi induk.

Objektif kedua adalah untuk memerhati dan menerangkan perkembangan morfologi *H. barbouri* dari juvenil hingga matang. Corak pertumbuhan isometrik diperhatikan semasa peringkat perkembangan awal sehingga perbezaan awal jantina diperhatikan pada umur 141 hari selepas lahir (DAB). Kemudian, dari umur 141 hingga 168 DAB, jantan dan betina *H. barbouri* menunjukkan corak pertumbuhan yang berbeza. Permulaan kematangan diperhatikan pada umur

164 DAB dengan kehadiran kantung induk, apabila panjang piawai dan berat basah kuda laut masing-masing adalah 7.16 cm dan 1.47 g.

Objektif seterusnya mengkaji kesan faktor fizikal seperti sistem kultur, kepadatan dan kemasinan terhadap pertumbuhan dan kemandirian *H. barbouri* semasa peringkat kritikal (1 hingga 56 DAB). *Hippocampus barbouri* yang baru dilahirkan, di kultur dalam sistem yang menggabungkan penapis gantung dan dasar menunjukkan tumbesaran terbaik dengan panjang piawai 3.74 sm dan kemandirian 75%. Dengan menggunakan sistem kultur ini, kesan kepadatan kuda laut dan kemasinan air telah diuji. Keputusan menunjukkan bahawa pada kepadatan 0.5 kuda laut L^{-1} (1 kuda laut setiap 2 L) dan kemasinan 24 ppt, menghasilkan tumbesaran dan kemandirian yang terbaik *H. barbouri*. Kekurangan bekalan air laut semula jadi telah mendorong kajian ke atas potensi menggunakan garam buatan untuk pengkulturan *H. barbouri*. Anak *H. barbouri* yang baru dilahirkan dikultur dalam air garam tiruan komersial dikenali sebagai *Red Sea Coral Pro* (RSCPS) menunjukkan tumbesaran terbaik dengan kenaikan panjang piawai sebanyak 3.13 cm. Analisis unsur menunjukkan bahawa garam komersial ini mengandungi unsur yang serupa dengan air laut semula jadi.

Eksperimen pemakanan telah dijalankan untuk menentukan kesan pengayaan yang berlainan pada *Artemia* terhadap pertumbuhan dan kemandirian *H. barbouri* yang baru lahir. Anak *H. barbouri* yang diberi makan *Artemia* nauplii diperkaya samada dengan minyak hati ikan kod bersama dengan tiroksin atau kalium iodida menghasilkan tumbesaran dan kemandirian yang terbaik. Kajian lanjut dijalankan untuk menentukan keberkesanan pemberian *Artemia* yang diperkaya pada frekuensi yang berbeza. Secara ekonominya, pengayaan *Artemia* menggunakan minyak hati ikan kod dengan tiroksin diberi kepada kuda laut sebanyak dua kali seminggu sangat disyorkan. Kajian ini juga menentukan tahap kesesuaian jenis makanan lain seperti cladoceran *Daphnia* untuk dijadikan makanan kepada juvenil *H. barbouri* yang berusia dua bulan ke atas.

Kesimpulannya, kuda laut *H. barbouri* berjaya membiak dalam sistem tangki dengan penapis gantung, pada kedalaman air minimum 0.38 m. Selain pasca-larva udang, induk *H. barbouri* juga boleh diberi makan *Mysis* yang di sejuk beku bagi peningkatan hasil pembiakan. Semasa proses pembesaran *H. barbouri*, tempoh kritikal dengan kadar kematian yang tinggi diperhatikan pada umur 7-11 hari, manakala pertumbuhan yang perlahan direkodkan pada usia dua bulan pertama. *Hippocampus barbouri* mula membiak pada usia 168-175 hari. Tangki dengan penapis gantung dan dasar adalah sistem tangki yang paling berkesan untuk kultur *H. barbouri* semasa peringkat awal pembesaran pada kepadatan 1 kuda laut dalam 2 atau 3 L. Kemasinan 24 ppt dan garam komersial jenis *Red Sea Coral Pro* (RSCPS) atau *Red Sea* (RSS) adalah sangat sesuai digunakan untuk pengkulturan *H. barbouri*. Pada peringkat ini juga, *H. barbouri* sesuai diberi makan nauplii *Artemia* yang diperkaya dengan minyak hati ikan kod dan tiroksin dengan frekuensi dua kali seminggu. Zooplankton liar yang disejuk beku atau *Artemia* (sebelum dewasa) adalah makanan yang lebih sesuai untuk juvenil *H. barbouri* yang berusia dua bulan ke atas.

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I certify that a Thesis Examination Committee has met on 12 January 2018 to conduct the final examination of Nur Fatihah binti Abd Halid on her thesis entitled "Crucial Factors Affecting Reproduction and Culture of *Hippocampus barbouri* (Jordan and Richardson 1908)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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°C	degrees celsius
%	percent
USD	United States Dollar
µm	micrometer
mm	millimeter
cm	centimeter
m	meter
Ø	diameter
g	gram
µL	microlitre
mL	millilitre
L	litre
ppt	part per thousand
ppm	part per million
N	normality
π	pi (3.14)
S.D.	standard deviation
e.g.	for example
ln	natural logarithm
h	hours
min(s)	minute(s)
sec	seconds

CHAPTER 1

INTRODUCTION

1.1 Background of study

Seahorse is a unique fish under the genus *Hippocampus.* It has horse-like head, tubular mouth, and body covered with rings armoured plate until the tip of their prehensile tail (Hale, 1996; Van Wassenbergh *et al.*, 2011; Porter *et al.*, 2013). In the addition to that, this seahorse is also special since the male develop brood pouch upon maturity (Carcupino *et al.*, 2002) as accessory for nurturing developing embryos.

All 46 seahorse species including the ten species in Malaysia are of great demand in the international trade (Koldewey and Martin-Smith, 2010; O'Donnell *et al.*, 2010). Seahorse is used in traditional Chinese medicine (TCM), since it is believed to enhance energy flows in body, healing effect on health problems, e.g. asthma, high cholesterol, goitre, kidney malfunction and also skin impurities such as acne and nodules (Vincent, 1996; Demunshi and Chugh, 2010). Significant trade of seahorses also involving ornamental industry and curios (Sanders *et al.*, 2008; Perry *et al.*, 2010). Demands for seahorse exceed supply, with about a million seahorses traded yearly, involving 93 nations including Malaysia (Vincent, 1996; Koldewey, 2005a; Vincent *et al.*, 2011).

1.2 Problem of statement

Harvesting seahorses to fulfill the high demands for international trade resulted in the decline of the wild populations (Vincent, 1996; Lourie *et al.*, 1999; Foster and Vincent, 2004). Instead to meet the demand from industry, seahorse populations are also threatened by habitat destruction and loss when exposed to human activities (Hughes *et al.*, 2009). Climate change damages the coral reefs, habitat of many reef-dwelling species including the tropical seahorse (Lourie *et al.*, 2004; Veron *et al.*, 2009). Fishing activity using bottom trawling not only remove large number of seahorse as bycatch, but in the process also ruin the seagrass bed where most seahorse species are found (Scales, 2010). Additionally, anthropogenic activities pollute the coastal area indirectly contributes to the decline of seahorse population (Bell *et al.*, 2003; Rosa *et al.*, 2005; Perry *et al.*, 2010).

Regulatory measures through the International Union for Conservation of Nature (IUCN) and Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) introduced in 2002, assisted in the assessment and monitoring of this overexploited species, however the progress is rather slow

(Rosa *et al.*, 2007). Therefore, production through aquaculture is being proposed as a solution to meet the demand for seahorse reducing the need to depend on the wild population (Olivier, 2003). Various studies have been conducted since early 1990's on the potential establishment of seahorse aquaculture (Giwojna, 1990; Koldewey and Martin-Smith, 2010). To date, very few successes in the seahorse aquaculture, some are even at the pilot stage, and some failed due to problems faced during the culture. Factors like diet, disease and culture technique for seahorse affected the outcome of the seahorse aquaculture (Koldewey and Martin-Smith, 2010; Scales, 2010).

1.3 Significance of study

In Malaysia, there is no successful establishment of seahorse aquaculture (Koldewey and Martin-Smith, 2010). Documented reports were mainly on population and species distribution (Choo and Liew, 2003; Choo and Liew, 2004; Lourie *et al.*, 2004; Choo and Liew, 2006; Lim *et al.*, 2011). Research related to culture of seahorse is very crucial as Malaysia contributes significantly on the international seahorse trade. This led the reduction of their wild population evident by the 70% decrease in numbers of seahorse caught through bycatch (Baum *et al.*, 2003; Giles *et al.*, 2006; Perry *et al.*, 2010). Establishment of seahorse aquaculture requires effort and investment. Particularly on finding solutions for crucial problems like broodstock maintenance, suitable diets, culture technique (newborn, juvenile and adult), water quality and most importantly to counter measure the prime diseases affecting the seahorse. Finding of these studies can be used to develop an economical and practical technique for the reproduction and culture of seahorse.

1.4 Objectives of study

*Hippocampus barbo*uri is one of the local seahorse species inhabit the shallow water of the coastal area, therefore easily exposed to human activities (Lim *et al.*, 2011). As a global trade species, *H. barbouri* is the most common species kept in aquarium due to its uniqueness in appearance and attractive color variation (Kuiter, 2000; Koldewey and Martin-Smith, 2010; Olivotto *et al.*, 2011). This species also contributes in a significant number for traditional Chinese medicine, TCM (Choo and Liew, 2005). *Hippocampus barbouri* has been identified as facing high risk of extinction, thus listed as vulnerable in IUCN Red List and included in Appendix II of CITES. Presently, study on the suitable culture technique for *H. barbouri* is scarce (Payne, 2003; Garcia *et al.*, 2010; Garcia *et al.*, 2012). Successful production of *H. barbouri* is crucial since it has high potential as a candidate for aquaculture due high market price of USD 1200 kg^{-1} in dried form or USD 60-950 per individual as ornamental species (Perry *et al.*, 2010; Vincent *et al.*, 2011). This study was conducted to solve specific problems in relation to the establishment of seahorse aquaculture, particularly for *H. barbouri*. Thus, the specific objectives for this study were:

1) to acclimatize and breed of *H. barbouri* in control conditions
2) to describe and determine the complete life stages of *H. barbouri* in captivity
3) to determine the effects of physical parameters on the growth and survival of newborn *H. barbouri*
4) to determine the effects of enriched diets and feeding preferences on the growth and survival of newborn and juvenile *H. barbouri*

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