UNIVERSITI PUTRA MALAYSIA

EFFECTS OF CRITICAL FACTORS ON GROWTH AND SURVIVAL OF SEAHORSE JUVENILES, *Hippocampus barbouri* (JORDAN & RICHARDSON,1908)

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IB 2019 12

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By

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

May 2019

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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May 2019

Chair : Annie Christianus, PhD
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Seahorse under the genus *Hippocampus*, has a total of 44 species worldwide. Its unique features which include horse-like head, kangaroo-like pouch and monkey-like tail, made it much sought after by the ornamental fish industry. Seahorses contribute ecologically, economically, and culturally to the human kind. However, the overexploitation of seahorses for traditional Chinese medicine (TCM), aquarium trade and curios resulted in the rapid decline of wild seahorse population. Seahorse aquaculture has been proposed as one of the solution to reduce stress on wild stock as well as to support the TCM industry. However, maintaining survival of seahorse juveniles in captive condition proved to be quite a challenge. Production techniques have been developed for very few species but yet to achieve their optimal rearing conditions. Rearing conditions namely critical factors were not well studied. Hence, the objectives of this study were to compare the growth and survival of *H. barbouri* juveniles under different conditions. Factors considered include nursing duration, water surface flow rate, stocking density, temperature, salinity, photoperiod, light intensity and feeding. Different ages of *H. barbouri* juveniles were used to determine the most suitable day to be transferred from nursing tank into rearing tank. *Hippocampus barbouri* juveniles of 3, 5, 7 and 9 day-old were used for this experiment. Upon completion, a follow up study on 9, 12 and 15 day-old *H. barbouri* juveniles were used to determine the best day to transfer juveniles from nursing tank to rearing tank. *Hippocampus barbouri* juveniles that nursed for 9 days before transferred to rearing tank were found to have the best growth and survival compared to other treatments. Results from the follow up experiment showed that *H. barbouri* juveniles nursed for 12 days has the best survival. Hence, all other experiments were conducted using 12 day-old *H. barbouri* juveniles. For experiment on surface water flow rate, three flow rate 0.056, 0.077 and 0.143 m/s were used. At the end of study, the lowest flow rate 0.056 m/s showed the best growth and survival. Stocking density of 0.3, 0.4 and 0.5 juv/L were used to determine the best stocking density for *H. barbouri* juveniles. Results show no significant difference ($p>0.05$) for growth. While stocking density of 0.5

juv/L showed the best survival ($p<0.05$) of *H. barbouri* juveniles. As for experiment on temperature, three temperatures used were 25, 28 and 31 °C. Results showed that *H. barbouri* juveniles cultured at 25 °C showed the best growth and survival. For experiment on salinity, 25, 30 and 35 ppt were tested. Growth and survival of *H. barbouri* juveniles were the best when cultured at 30 ppt. Photoperiod used in this study were 8L:16D, 12L:12D and 16L:8D. Results showed that photoperiod of 12L:12D produced the best height and weight of *H. barbouri* juveniles with 37.71 mm and 0.165g, with survival of 80%. As for experiment on light intensity, natural light with intensity of 700 lx, fluorescent light 1400 lx and LED light 2100 lx were used. At the end of study period, light intensity of 1400 and 2100 lx resulted in best growth and survival of *H. barbouri* juveniles. Feeding experiment was conducted to determine the best *Artemia* enrichment for growth and survival of *H. barbouri* juveniles. Three treatments used were newly hatched *Artemia* nauplii, *Artemia* enriched with marine fish pellet and *Artemia* enriched with cod liver emulsion. Growth and survival of *H. barbouri* juveniles fed with Instar II *Artemia* enriched with cod liver emulsion showed significantly better results ($p<0.05$) when compared to all other two treatments. To conclude, the critical factors determined in the current study may aid aquaculturist, particularly those interested in the culture of this unique species. In addition, the success in seahorse aquaculture, may directly benefits the ornamental fish industry for sustainable supply of seahorses.

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

KESAN FAKTOR KRITIKAL TERHADAP TUMBESARAN DAN KEMANDIRIAN JUVENIL KUDA LAUT, *Hippocampus barbouri* (JORDAN DAN RICHARDSON, 1908)

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Kuda laut di bawah genus *Hippocampus* mempunyai sejumlah 44 spesies di seluruh dunia. Ciri uniknya dengan kepala berbentuk kuda, mempunyai kantung seperti kangaroo, dan mempunyai ekor seperti monyet, menjadikannya pilihan ramai dalam industri ikan ornamental. Kuda laut menyumbang dan mempengaruhi manusia dari segi ekologi, ekonomi dan kebudayaan. Walaubagaimanapun, ianya terlalu dieksploitasi untuk perubatan tradisi Cina (TCM), perdagangan akuarium dan kraftangan, sehingga menyebabkan pengurangan mendadak pada populasi semulajadi. Pengkulturan kuda laut dicadangkan sebagai penyelesaian untuk mengurangkan tekanan terhadap stok semulajadi, pada masa yang sama menyumbang kepada industri TCM. Walaubagaimanapun, mengekalkan kemandirian kuda laut dalam keadaan terkawal menjadi satu cabaran. Teknik penghasilan untuk beberapa spesies telah dibangunkan tetapi masih belum mencapai tahap optima. Keadaan pengkulturan yakni faktor kritikal masih belum dikaji sepenuhnya. Oleh itu, objektif kajian ini adalah untuk membandingkan tumbesaran dan kemandirian juvenil kuda laut, *H. barbouri* dalam beberapa keadaan. Faktor yang dikaji adalah jangka penjagaan anak, kadar kelajuan air permukaan, kepadatan stok, suhu, saliniti, jangkamasa cahaya, intensiti cahaya dan pemakanan. Beberapa peringkat umur kuda laut digunakan untuk menentukan masa yang paling sesuai untuk penjagaan anak sebelum dipindahkan ke dalam tangki ternakan. *Hippocampus barbouri* juvenil berumur 3, 5, 7 dan 9 hari selepas lahir digunakan untuk ekperimen awal. Kemudian, kajian susulan dijalankan ke atas *H. barbouri* juvenil berumur 9, 12 dan 15 hari untuk menentukan masa yang sesuai untuk pemindahan ke tangki ternakan. *Hippocampus barbouri* juvenil kuda laut berumur 9 hari menunjukkan tumbesaran dan kemandirian terbaik berbanding dengan rawatan yang lain. Manakala hasil kajian susulan menjukkan *H. barbouri* juvenil yang dijaga selama 12 hari memberikan kemandirian yang paling tinggi. Oleh itu, semua ekperimen dijalankan dengan menggunakan *H. barbouri* juvenil berumur 12 hari. Kajian kesan kelajuan air pemukaan dijalankan dengan menggunakan kadar kelajuan 0.056, 0.077 dan 0.143 m/s. Pada akhir kajian, didapati bahawa kelajuan air yang

paling rendah 0.056 m/s memberikan tumbesaran and kemandirian yang terbaik. Kepadatan stok pada 0.3, 0.4 dan 0.5 juv/L digunakan untuk kajian ini. Keputusan menunjukkan tiada perbezaan ($p>0.05$) pada tumbeasaran *H. barbouri* juvenil. Manakala stok kepadatan 0.5 juv/L memberikan kemandirian yang terbaik ($p<0.05$) untuk *H. barbouri* juvenil. Untuk ekperimen suhu, tiga suhu yang digunakan adalah 25, 28 dan 31 °C. Keputusan menunjukkan bahawa *H. barbouri* juvenil yang dikultur pada 25 °C memberikan tumbesaran dan kemandirian terbaik. Kajian saliniti adalah menggunakan saliniti 25, 30 dan 35 ppt. Tumbesaran dan kemandirian *H. barbouri* juvenil adalah terbaik pada 30 ppt. Jangkamasa cahaya yang digunakan adalah 8L:16D, 12L:12D dan 16L:8D. Hasil kajian menunjukkan bahawa jangkamasa cahaya 12L:12D memberikan tinggi dan berat terbaik iaitu 37.71 mm dan 0.165g, dengan kemandirian tertinggi sebanyak 80%. Manakala untuk kajian intensiti cahaya menggunakan cahaya semulajadi dengan intensiti 700 lx, cahaya fluoresen light 1400 lx dan cahaya LED 2100 lx. Pada akhir kajian, didapati intensiti cahaya pada1400 and 2100 lx memberikan tumbesaran dan kemandirian terbaik untuk *H. barbouri* juvenil. Ekperimen pemakanan dijalankan untuk menentukan jenis pengkayaan *Artemia* yang sesuai untuk tumbesaran dan kemandirian *H. barbouri* juvenil. Tiga rawatan yang digunakan adalah naupli *Artemia*, *Artemia* diperkaya dengan makanan ikan marin dan *Artemia* diperkaya emulsi hati ikan kod. Tumbesaran dan kemandirian terbaik ($p<0.05$) *H. barbouri* juvenil adalah dengan pemberian *Artemia* diperkaya emulsi hati ikan kod berbanding dengan dua rawatan yang lain. Kesimpulannya, faktor kritikal yang ditentukan dalam kajian ini boleh membantu penternak, khususnya yang berminat dalam kultur spesies unik ini. Di samping itu, kejayaan akuakultur kuda laut boleh memberi manfaat secara langsung kepada industri ikan hiasan untuk bekalan yang mampan.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my family especially my parents for their continuous trust and support in terms of financially, mentally and spiritually along the way.

I sincerely thank my advisor, Dr. Annie Christianus for her supervision, efforts, patience, motivations and advices. Her guidance helped me in all the time of research. I could not have imagined having a better advisor and mentor for my study journey. Besides, my appreciation goes to my supervisory committee: Prof. Dr. Muta Harah Zakaria @ Ya and Dr. Chong Chou Min, for their insightful comments, critiques and encouragement, also for the tough questions which enlightened me to widen my knowledge from different perspectives.

With a special mention to my fellow senior and labmate: Dr. Nur Fatihah binti Abd Halid and Len Yung Wung for knowledge input and help. Not to forget, a special gratitude goes out to lecturers and staffs from Institute of Bioscience, UPM and Department of Aquaculture, UPM for their assistance and help. In particular, I am thankful to Mr Farhan, Mr Dinul, Mr Fareeq, Mrs Wahida, Mrs Shafika and Ms Hafizah for their kindness and great helping hand.

My heartfelt thanks to Mr Saupi Ismail and his team (especially Mr Fadzil and Mr Zukri) from Aquarium Tunku Abdul Rahman, Fisheries Research Institute, Batu Maung, Penang for the opportunity to join the team and give access to the research facilities. The research would not be possible to conduct without their precious support.

I am grateful for all. Thank you.

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

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°C	degree celcius
%	percent
m	meter
cm	centimeter
mg	milligram
ml	millilitre
mm	millimeter
µm	micrometer
mg/L	milligram per litre
m/s	meter per second
g	gram
g/L	gram per litre
I.U.	International unit
L	litre
hrs	hours
juv/tank	juveniles per tank
juv/L	juvenile per litre
ppt	part per thousand
ppm	part per million
L:D	light: dark
lx	luminous flux (lux)
S.D.	standard deviation
D.A.B	day after birth
USD	united states dollar
RM	ringgit malaysia

CHAPTER 1

INTRODUCTION

1.1 Background of study

Seahorse under the genus *Hippocampus*, family Syngnathidae, has a total of 44 species (IUCN, 2018). It is categorized as fish but with horse-like head, chameleon-like eyes, kangaroo-like pouch and monkey-like tail. Size of seahorses ranged between 2 and 30 cm in height (Lourie, 2000) and male seahorse is responsible for parental care instead of female (Vincent, 1990). Although seahorses are not commonly known, it has ecological, economical, medicinal and cultural important (Vincent *et al.*, 2011). There were 93 countries including Malaysia involved in the trade of almost a million seahorses yearly (Vincent, 1996; Koldewey, 2005; Vincent *et al*., 2011). Trade survey showed a reduction in seahorse landings worldwide (Vincent *et al*., 2011). In Vietnam, catch of seahorse decreased to 60 % within 3 years, while Malaysia 70 % (Baum *et al.*, 2003; Giles *et al*., 2006; Perry *et al*., 2010). The demand for dried seahorse was high especially for traditional Chinese medicine (TCM) and trade of live seahorses for aquarium was quite significant (Vincent, 1996; Giles *et al*., 2006).

Hippocampus barbouri is a tropical seahorse, known to inhabit shallow and sheltered bay or lagoons, particularly in *Halimeda* (calcareous seaweed) beds (Choo & Liew, 2004). This species was listed in CITES Appendix II as vulnerable, mainly due to habitat degradation (Short *et al*., 2011), overexploitation (Foster & Vincent, 2004) and by catch (Scales, 2010; Vincent *et al*., 2011). It is popular due to its spiky appearance and various colouration (Kuiter, 2000; Koldewey & Martin Smith, 2010; Olivotto *et al*., 2011; Lourie *et al*., 2004). It was suspected that a minimum of 30 % declined in population occurred over the past 10 years (Pollom, 2017).

1.2 Problem statement

Overexploitation of seahorses from non selective fishing gear for traditional Chinese medicine (TCM) industry, aquarium trade and curios contributed heavily on reduction of wild seahorse population (Vincent, 1996; Parry Jones & Vincent, 1998; May & Tomoda, 2002; Alves & Rosa, 2006; Qian *et al.,* 2008; Vincent *et al.,* 2011). Besides, degradation of seagrass habitat due to destructive trawl fisheries, development and pollution threatened seahorse population (Hughes *et al*., 2009; Short *et al*., 2011). Due to species-specific effect as well as low survival (Scarratt, 1995; Forteath, 1996; Lin *et al*., 2008), culture of seahorse juveniles remained as a bottleneck in the industry. One of the major setbacks is due to early stage mortality during critical period This occurred in species like *H. trimaculatus*, *H. kuda* and *H. abdominalis* (Lin *et al.,* 2006; Sheng *et al*., 2006; Martinez-Cardenas & Purser, 2012). Critical period for each species is different due to species-specific effect. First feeding, change of prey or prey size, feed transition at different life stages, early juvenile inherent mortality or stress due to

negative response to new environment were among the problems faced by aquaculturist (Lin *et al.,* 2006; Sheng *et al*., 2006; Martinez-Cardenas & Purser, 2012). Hence, seahorse aquaculture has been proposed as one of the solution to reduce stress on wild stock as well as to support the TCM industry (Olivier, 2003).

1.3 Justification

Majority studies were mainly on conservation, management and species distribution (Choo & Liew, 2004; Foster & Vincent, 2004; Choo & Liew, 2005; Lourie *et al*., 2005; Perry *et al*., 2010; Vincent *et al*., 2011). There was no reported successful establishment of seahorse aquaculture in Malaysia (Koldewey & Martin-Smith, 2010). Reports on culturing seahorse juveniles particularly early juveniles (1-3 months old) were either limited or study period was short (7-15 days) (Wong & Benzie, 2003; Lin *et al*., 2010; Celino *et al*., 2012; Souza-Santos *et al*., 2013; Martinez-Cardenas & Purser, 2016; Hora *et al*., 2017). Tremendous challenge is in overcoming the low survival of seahorse juveniles in culture condition for most of the species (Lin *et al.*, 2008). Factors like culture techniques, suitable physical parameters, water quality and suitable feed were crucial for growth and survival of adult and juveniles seahorses (Koldewey & Martin-Smith, 2010; Scales, 2010).

1.4 Significance of study

All *Hippocampus* were red listed in IUCN and listed in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Anon, 2003). Most of the species were data deficient or fall under category of vulnerable (IUCN, 2018). Seahorse aquaculture is essential for the successful establishment of seahorse aquaculture as well to reduce stress on wild seahorse population (Olivier, 2003). Seahorse produced through aquaculture will be able to support the ornamental and TCM market by improving the industry through determination of suitable culture condition especially for the juveniles. Thus the finding of this study is crucial to contribute to the improvement of survival and culture of seahorse juveniles.

1.5 Objectives of study

Current study was aimed to solve issue on growth and survival of *H. barbouri*, especially at the juvenile stage to contribute to the development of seahorse aquaculture industry. Hence, the objectives of this study were:

1. To determine the nursing duration, suitable surface water flow and stocking density of captive bred *H. barbouri* juveniles
2. To observe the growth and survival of captive bred *H. barbouri* juveniles cultured under different temperature and salinity

3. To determine the effect of photoperiod and light intensity on growth and survival of captive bred *H. barbouri* juveniles
4. To observe the growth and survival of captive bred *H. barbouri* juveniles fed with *Artemia* with different enrichments

1.6 Research hypothesis

Hypotheses of this study were as stated below:

H_0: Critical factors will not affect the growth and survival of *H. barbouri* juveniles

$H_{1:}$: Critical factors will affect growth and survival of *H. barbouri* juveniles

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LIST OF PUBLICATIONS

Journals

Er, W. C. V., Christianus, A., Muta Harah, Z. and Chong, C. M. (2017). Significance of water flow rate and period of nursing on the growth of juvenile seahorse, *Hippocampus barbouri* (Jordan and Richardson, 1908). *Journal of Survey in Fisheries Sciences*, 4(1): 1-7.

Manuscript Submitted

Er, W. C. V., Christianus, A., Muta Harah, Z., Chong, C. M. and Saupi, I. Foraging dependency of *Hippocampus barbouri* juveniles to photoperiod and light intensity.

Er, W. C. V., Christianus, A., Muta Harah, Z. and Chong, C. M. Influence of stocking density and temperature on the growth and survival of seahorse juveniles, *Hippocampus barbouri* (Jordan and Richardson, 1908).

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