UNIVERSITI PUTRA MALAYSIA

GROWTH AND SURVIVAL OF Hippocampus barbouri (Jordan & Richardson, 1908) AND Hippocampus kuda (Bleeker, 1852) FED WITH Artemia OF DIFFERENT ENRICHMENTS

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By

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Seahorses from the genus *Hippocampus* are facing tremendous pressure due to over exploitation to satisfy the demand for traditional Chinese medicine and ornamental fish trade. *Hippocampus barbouri* and *Hippocampus kuda* among the seahorse species being heavily traded in Malaysia and Thailand respectively. The establishment of seahorse aquaculture for sustainable supply is imminent in order to prevent the extinction of the species. Seahorse is one of the most sensitive fish species, thus every aspects relate to its growth and survival must be studied thoroughly. Among all, feeding aspect is the most critical.

Artemia sp. is commonly used as live food in the culture of marine fish. However, this zooplankton become less nutritious as it grows, therefore requires enrichment. Suitable enrichment will contribute to growth and survival, specifically during early juvenile stage. This study focused on two main species of seahorses, *H. barbouri* and *H. kuda*. Experiments were carried out at two different locations, Hatchery unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia for *H. barbouri*, while at Training Unit, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand, for *H. kuda*. The objectives of this study were divided into two main parts. First, to estimate the amount of *Artemia* nauplii consumed by seahorses from newborn to juvenile. While adult *Artemia* was used as food for juvenile to adult stage. Second objective was conducted to compare the growth and survival of seahorse juveniles fed with *Artemia* enriched with different enrichments. This research consist of five experimental studies in total. First two feeding experiments utilized *Artemia* nauplii for the feeding of newborn to early juvenile stage for the two seahorse species. The third feeding experiment estimates the amount of adult *Artemia* consumed by adult *H. barbouri* with age of 120 to 300 day after birth

(DAB). Finally, the last two feeding experiments were conducted on *H. barbouri* and *H. kuda* juveniles using *Artemia* metanauplii enriched with different enrichments.

Results of the *Artemia* consumption experiments for *H. barbouri* and *H. kuda,* showed increasing numbers of *Artemia* nauplii consumed for both species. As the age of seahorses increase, the amount of *Artemia* nauplii consumed also increase. Upon reaching the early juvenile stage of 42 DAB, *H. kuda* with the height of 32.32±1.23 mm consumed 28-39 *Artemia* nauplii per feeding. In comparison, *H. barbouri* at 28 DAB with height of 32.51±1.28 mm were able to consume 27-42 *Artemia* nauplii per feeding, similar to the amount consumed by 42 DAB *H. kuda*. As for the average numbers of adult *Artemia* consumed by *H. barbouri*, increasing consumption trend was observed as the age increased. The average numbers of adult *Artemia* consumed at the age of 120, 150, 180, 210, 240, 270, and 300 DAB were 32, 34, 47, 59, 55, 63 and 64 *Artemia*/feeding respectively.
Results from the 60 days feeding experiment showed significantly higher ($P<0.05$) in height of *H. barbouri* juveniles when fed with *Artemia* only, *Artemia* enriched with pellet, and with *Spirulina* sp. as compared to *Artemia* enriched with fresh *Chlorella* sp. and *Chlorella* sp. powder. Survival of *H. barbouri* was significantly higher ($P<0.05$) when fed with *Artemia* enriched with fresh *Chlorella* sp., pellet and *Chlorella* sp. powder. As for the feeding experiment on *H. kuda*, no significant different ($P>0.05$) was observed for the height, weight and survival of juveniles when fed with *Artemia* enriched with pellet and with SELCO emulsion (A1 DHA SELCO).

Based on the findings of this study, it can be concluded that the *Artemia* nauplii can be used as diet to support growth and survival of *H. barbouri* and *H. kuda* from newborn to juvenile stage. Simple feeding enrichment using pellet on *Artemia* may be more cost effective as compared to other enrichments. As for water quality, *H. barbouri* and *H. kuda* juveniles have similar water parameters tolerance. Recommended dissolved oxygen (DO) should be above 4.2 mg/L, with temperature range of 26.5 to 30°C, pH of 7.4 to 8.0, ammonia below 0.25 mg/L and salinity between 26 to 35 psu for the successful culture of *H. barbouri* and *H. kuda* juveniles.

Abstract tesis dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

TUMBESARAN DAN KEMANDIRIAN *Hippocampus barbouri* (Jordan & Richardson, 1908) DAN *Hippocampus kuda* (Bleeker, 1852) DIBERI MAKAN *Artemia* DENGAN PENGAYAAN BERBEZA

Oleh

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Kuda laut dari genus *Hippocampus* mengalami tekanan yang tinggi akibat ekploitasi keterlaluan untuk memenuhi permintaan perdagangan perubatan tradisi Cina dan ikan hiasan. *Hippocampus barbouri* dan *Hippocampus kuda* adalah di antara spesies kuda laut yang giat diperdagangkan di Malaysia dan Thailand, masing-masingnya. Pembangunan akuakultur kuda laut untuk sumber lestari adalah perlu untuk mengelakkan kepupusan spesies tersebut. Kuda laut merupakan spesies ikan yang paling sensitif, justeru itu semua aspek berkaitan tumbesaran dan kemandiriannya memerlukan kajian yang menyeluruh. Di antara kesemuanya, aspek pemakanan adalah yang paling kritikal.

Artemia sp. pada amnya digunakan sebagai makanan hidup dalam pengkulturan ikan marin. Walaubagaimanapun, zooplankton ini menjadi kurang berzat bila ianya membesar, oleh itu ianya memerlukan pengkayaan. Pengkayaan yang sesuai akan menyumbang kepada tumbesaran dan kemandirian, terutamanya pada awal peringkat juvenil. Kajian ini menumpu kepada dua spesies kuda laut, *H. barbouri* dan *H. kuda*. Ekperimen dijalankan di dua lokasi yang berbeza, iaitu di Unit Hatceri, Institut Biosains, Universiti Putra Malaysia, Serdang, Selangor, Malaysia untuk *H. barbouri*, dan di Unit Latihan, Fakulti Perikanan, Kasetsart University, Bangkok, Thailand, untuk *H. kuda*. Objektif kajian ini dibahagi kepada dua bahagian utama. Pertama, untuk menganggar jumlah naupli *Artemia* yang dimakan oleh kuda laut dari peringkat baru lahir ke juvenil. Manakala *Artemia* dewasa digunakan sebagai makanan untuk peringkat juvenil sehingga dewasa. Objektif kedua dijalankan untuk membandingkan tumbesaran dan kemandirian kuda laut juvenil yang diberi makan *Artemia* dengan pengkayaan yang berbeza. Kajian ini keseluruhannya mengandungi lima ekperimen. Dua ekperimen pertama dijalankan dengan menggunakan naupli *Artemia* sebagai makanan

untuk kedua-dua spesies kuda laut, dari peringkat baru lahir ke juvenil. Ekperimen yang ketiga menganggar jumlah *Artemia* dewasa yang dimakan oleh *H. barbouri* dewasa dari umur 120 hingga 300 hari selepas lahir (DAB). Akhir sekali, dua ekperimen dijalankan ke atas juvenil *H. barbouri* dan *H. kuda* menggunakan metanauplii *Artemia* dengan pengkayaan yang berbeza.

Keputusan ekperimen pemakanan *Artemia* oleh *H. barbouri* dan *H. kuda,* menunjukkan peningkatan bilangan naupli yang dimakan oleh kedua-dua spesies kuda laut tersebut. Semakin meningkat umur kuda laut, maka semakin meningkat jumlah naupli *Artemia* yang dimakan. Apabila mencapai peringkat awal juvenil pada 42 DAB, *H. kuda* dengan ketinggian 32.32±1.23 mm boleh memakan 28-39 naupli *Artemia* dalam sekali pemberian makanan. Berbanding dengan *H. barbouri* pada umur 28 DAB dengan ketinggian 32.51±1.28 mm, boleh memakan 27-42 naupli *Artemia* dalam sekali pemberian makanan, yakni agak sama dengan jumlah yang dimakan oleh *H. kuda* pada umur 42 DAB. Manakala purata bilangan *Artemia* dewasa yang dimakan oleh *H. barbouri*, menunjukkan arah aliran yang meningkat dengan peningkatan umur kuda laut. Purata bilangan *Artemia* dewasa yang dimakan oleh kuda laut pada umur 120, 150, 180, 210, 240, 270, dan 300 DAB adalah 32, 34, 47, 59, 55, 63 dan 64 *Artemia*/pemberian makanan masing-masingnya.

Keputusan dari ekperimen pemberian makanan selama 60 hari menunjukkan ketinggian juvenil *H. barbouri* yang ketara ($P<0.05$) bila diberi makan *Artemia* yang diperkaya dengan pelet dan *Spirulina* sp. berbanding dengan *Artemia* yang diperkaya dengan *Chlorella* sp. segar dan serbuk *Chlorella* sp. Kemandirian *H. barbouri* ketara lebih tinggi ($P<0.05$) bila diberi makan *Artemia* yang diperkaya dengan *Chlorella* sp. segar, pelet dan serbuk *Chlorella* sp. Manakala ekperimen ke atas *H. kuda*, tidak menunjukkan perbezaan yang ketara ($P>0.05$) dari segi ketinggian, berat dan kemandirian juvenil yang diberi *Artemia* yang diperkaya dengan pelet dan emulsi SELCO (A1 DHA SELCO).

Berdasarkan hasil kajian, kesimpulan dapat dibuat bahawa naupli *Artemia* boleh digunakan sebagai diet untuk menampung tumbesaran dan kemandirian *H. barbouri* dan *H. kuda* dari peringkat baru lahir hingga juvenil. Pengkayaan *Artemia* yang mudah dengan menggunakan pelet adalah lebih efektif dari segi kos berbanding dengan pengkayaan yang lain. Manakala untuk kualiti air, juvenil *H. barbouri* dan *H. kuda* mempunyai toleransi mutu air yang agak sama. Saranan untuk kandungan oksigen terlarut (DO) harus berada pada paras melebihi 4.2 mg/L, dengan julat suhu 26.5 hingga 30°C, pH 7.4 hingga 8.0, amonia kurang dari 0.25 mg/L dan saliniti di antara 26 hingga 35 psu, untuk kejayaan pengkulturan juvenil *H. barbouri* dan *H. kuda*.

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

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°C	degree celsius
%	percentage
mm	millimeter
cm	centimeter
mL	millilitre
hrs	hours
L	litre
g	gram
mg	miligram
psu	practical salinity unit
ppm	part per million
S.D.	standard deviation
e.g.	for example
L*W*H	length * width * height (Tank measurement)
Wt	wet weight
Ht	height
Svr	survival
Min	minimum
Max	maximum
personal obs.	personal observations

CHAPTER 1

INTRODUCTION

1.1 Background

Seahorse is an unique creature with head positions are at right angles to their body, curved trunks and a prehensile tail. Their skin is made up from a series of bony plates with visible rings around the trunk and tail. In addition, the male seahorse developed a brood pouch upon maturity for carrying eggs transferred from female and nourish them. With this special characteristics it is possible to distinguish between the seahorse and other bony fish. However due to discovery of various species of seahorse there is confusion occurs in the identification of these seahorses. With comprehensive research on their morphological differences and habitat, they can be differentiated with ease. In addition, species from the genus *Hippocampus* have been documented well, particularly on their different morphological characteristics.

Hippocampus barbouri or commonly known as Barbours`s seahorse, is one of the most vulnerable seahorse among the four species found in East Malaysia. As *H. barbouri* existed in different color variation from white, pale yellow to pale brown also pale reddish to orange, with a striped snout, making them the most preferred by fish hobbyist. *Hippocampus kuda* also known as the yellow seahorse, not as attractive as *H. barbouri*, however, due to its larger size, it is most traded for traditional Chinese medicine (Job *et al*., 2002).

1.2 Problem of statement

Seahorses have been receiving the mass attention lately due to the report on the declining in catch rates. The main reason for the decline in the natural seahorse population likely due to overexploitation. Approximately 20 million seahorses from different parts of the world were caught yearly (Vincent, 1996). Environmental pollution is another factor which causes damage to the natural habitat (Scales, 2010), therefore reducing the chances of survival of these seahorses. Seahorses are slow moving predator, thus, the destruction of their habitat made it hard for them to adapt. The demand for *Hippocampus* spp. has multiple as it is being used extensively as one of the ingredient in traditional Chinese medicine, as well as ornamental species and as curio (Chen *et al*., 2015).

Hippocampus kuda and *H. barbouri* are normally found in shallow water, often in seagrass beds, near mangrove with seagrass or clinging to hard corals up to a maximum depth of 10m (Lourie *et al*., 2004). Seahorses with it low density existence, coupled with poor mobility amplified the threat level even more (Foster

and Vincent, 2004). The decreased number of seahorses from Kedah-Langkawi coastal water suggest that the seahorse populations has been greatly affected by human activities, whereby their in-shore habitat making them most vulnerable to land based activities (Short *et al*., 2011). A sudden declined in the numbers of seahorse resulted in the seahorse under the genus *Hippocampus* being red listed as vulnerable species under International Union for Conservation of Nature (IUCN).

Despite having spines on its body, an undesirable trait, *H. barbouri* is still being heavily traded for traditional medicine (Lourie *et al*., 2005). It is a great challenge for the continuous existence of *H. barbouri* and *H. kuda*. Thus it is vital to ensure that the seahorse population can continue thrive. Their sensitivity to handling, transportation, and food preferences are the more reason for low survival of wild capture seahorses.

1.3 Research hypothesis

In the wild, seahorses feed mainly on live food such as zooplankton and small crustaceans. Often wild seahorses upon capture are too weak to feed, only able to survive long enough to be displayed on retail outlet, before they eventually die. In captive condition, they feed through their straw like snout accomplished through suction motion, present a challenging task in providing suitable live feed for them. Often *Artemia* is used as live food for seahorse due to the availability and ease of culture. However, due to the low nutritional value of Artemia, it unable to support the livelihood of seahorse at prolonged period. Thus the hypothesis of this study was:

H_O: Feeding with enriched *Artemia* will support growth and survival of *Hippocampus barbouri* and *H. kuda*.
H_A: Feeding with enriched *Artemia* will not support growth and survival of *Hippocampus barbouri* and *H. kuda*.

1.4 Objectives of study

The development of seahorse aquaculture has managed to produce captive-bred species for the ornamental trades. This captive-bred seahorses have tremendous advantages as compared to wild caught. Cultured species are able to take in frozen food, and its adaptability to tank environment allows proper water parameters management. Establishment of suitable culture method by focusing on water quality and feed will ensure the livelihood of juvenile *H. barbouri* and *H. kuda* in culture condition. Seahorse aquaculture has grown significantly as there is increasing attention and demand for seahorses. However the aquaculture industry is still at developing stage with hurdles to overcome. It

has great potential to provide a sustainable alternative to meet the market demand, and also to reduce pressure on the wild population.

This study was designed to contribute to the establishment suitable culture technique for *H. barbouri* and *H. kuda,* in particularly the feeding aspect. The general aim was to determine the suitability of using enriched *Artemia* as live food for *H. barbouri* and *H. kuda*. Thus, the specific objectives of this study were to:

1. To determine the consumption rate of nauplii and adult *Artemia* consumed by newborn and adult seahorse, *H. barbouri* and *H. kuda*
2. To compare the growth and survival of *H. barbouri* and *H. kuda* juveniles fed with *Artemia* with different enrichments

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