OPTICAL DEVELOPMENT, FEEDING DEPENDENCY ON LIGHT INTENSITY AND FOOD COLOUR PREFERENCES IN SEAHORSE, *Hippocampus barbouri*

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CERTIFICATION

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بِسْــــــمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

“Glory be to You; we have no knowledge except that which You have taught us. Verily, it is You, the All-Knowing, the All-Wise” (Al-Baqarah, verse 32).

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ABSTRACT

Seahorse *Hippocampus barbouri* is a species frequently involved in international trade due to its significant economic value and demand in traditional Chinese medicine (TCM), as curios, and in the aquarium trade. In the world of TCM, dried seahorses can cost as much as USD 1,200 per kilogram. Whereas, in the marine aquarium business, captive-bred live seahorses are sold for anywhere between USD 60-950 each, which is more expensive than wild-caught seahorses, which typically sell for USD 1-80 each. Aquaculture addresses overexploitation and global demand, but the main challenge is the low survival rate of pelagic-stage seahorses due to a lack of suitable food and improper feeding and culture techniques. Despite a decade of research, there's still limited knowledge, especially about early development and eye development crucial for their feeding. Seahorses rely on visual cues, such as light intensity, tank colour, and prey contrast. This study aims to fill the knowledge gap on early seahorse eye development and explore physical factors that help seahorses adjust and enhance visual abilities to improve feeding activity. In experiment 1, the optical development of seahorse *H. barbouri* at the early life stage in captivity was examined histologically. Aborted yolk-sac larvae 324 hours after spawning, were still alive and newly released juveniles (0 day after birth, 0 DAB) were sampled until juveniles reached benthic stages at 15 DAB. At yolk-sac larvae, the eyes are fully pigmented with retina formed in a distinguish layer and supplied with optic nerve. Whereas the pigment epithelium in the newborn juveniles shone under surface lighting, indicating the presence of reflective retinal tapetum (eyeshine). Interestingly, the presence of retinal tapetum in seahorse species was first documented in this study. The retina grew progressively in size until 15 DAB without changes in the retina structure. Experiment 2 investigated how different light intensity levels (2100 lux and 1400 lux) from fluorescent and light emitting diode (LED) sources influenced the feed intake, growth, and survival of *H. barbouri* seahorses in pelagic stage. A total of 16 seahorse/tank was used in triplicate for each treatment. At the end of the 15-day culture period, treatment under fluorescent 1400 lux was the most favourable for juvenile growth, survival and feed intake. Experiment 3 was conducted to determine the synergetic effects of tank background and food colouration on the feeding preferences of sub-adult seahorse *H. barbouri* (standard length, SL: 7.56±0.35 cm; wet weight, WW: 0.58±0.10 g). Nine seahorses were reared in triplicate tanks with red, blue and green backgrounds for two weeks with a stocking density of one seahorse/tank. Sergestid shrimp, *Acetes sibogae* (TL:1.00±0.05 cm), were used as food test, dyed with blue, green, red and yellow, whereas natural or non-dyed *Acetes* served as control. Sub-adult *H. barbouri* seahorses were presented with two different colours of *Acetes* simultaneously, with each combination lasting five seconds. Testing continued until the seahorses lost interest in the food colours combination. After about a two-hour break, testing of different colour combinations resumed randomly. Against all backgrounds, the first response was significantly biased toward natural white colour shrimp. This study demonstrated that sub-adult *H. barbouri* has colour preferences. Conclusively, this study fills the gap in our understanding of *H. barbouri*'s eye development during their pelagic stages. A light intensity of 1400 lux from fluorescent light proved optimal for *H. barbouri*'s feed intake, growth, and survival at this stage. When weaning sub-adult *H. barbouri*, offering frozen *Acetes* with natural colouring in tanks with red, blue, or green backgrounds can expedite the process due to the contrast created with the use of light colour against dark colour.

ABSTRAK

PERKEMBANGAN OPTIK, KEBERGANTUNGAN PILIHAN MAKANAN TERHADAP KEAMATAN CAHAYA DAN PEMILIHAN WARNA MAKANAN BAGI KUDA LAUT, Hippocampus barbouri

Kuda laut *Hippocampus barbouri* adalah salah satu spesis yang kerap dijual di peringkat antarabangsa disebabkan nilai ekonomi yang tinggi dan permintaan untuk perubatan tradisional Cina (TCM), sebagai kurio, dan perdagangan akuarium. Harga kuda laut yang telah dikeringkan boleh mencecah USD 1,200 per kilogram untuk kegunaan TCM dunia. Manakala, harga kuda laut hidup yang dibiak dalam kurungan dijual di kedai akuarium sebanyak USD 60-950 seekor, lebih mahal daripada kuda laut liar yang ditangkap dan dijual pada harga USD 1-80 seekor. Akuakultur dapat menangani isu eksploitasi yang tinggi dan permintaan global, namun cabaran utama adalah kadar hidup yang rendah bagi kuda laut tahap pelagik disebabkan oleh kekurangan makanan, cara pemakanan dan teknik kultur yang sesuai. Walaupun telah sedekad penyelidikan dilakukan, masih terdapat pengetahuan yang terhad terutamanya melibatkan perkembangan di peringkat awal dan perkembangan mata amat penting untuk mencari makan. Kuda laut bergantung pada isyarat visual, seperti keamatan cahaya, warna tangki dan kontras mangsa. Kajian ini bertujuan untuk mengisi jurang pengetahuan tentang perkembangan awal mata kuda laut dan faktor fizikal yang membantu kuda laut untuk mengubah dan meningkatkan keupayaan visual bagi memperbaiki aktiviti makan mereka. Dalam eksperimen 1, perkembangan optik kuda laut *H. barbouri* dalam kurungan pada peringkat awal hidup diperiksa secara histologi. Larva yang masih hidup mempunyi kantung yolka yang gugur pada peringkat 324 jam selepas telur disenyawakan dan juvenil yang baru dilahirkan (0 hari selepas lahir, 0 DAB) telah disampel hingga mencapai tahap bentik pada 15 HSL. Pada tahap larva yang berkantung yolka, mata berpigmen sepenuhnya dengan retina yang terbentuk dalam lapisan yang berbeza dan dibekalkan dengan saraf optik. Manakala, epitelium pigmen pada juvenil yang baru dilahirkan bersinar di bawah permukaan cahaya, menunjukkan kehadiran tapetum retina reflektif (sinar mata). Menariknya, tapetum retina (sinar mata) pada spesis kuda laut pertama kali didokumentasikan dalam kajian ini. Saiz retina membesar secara progresif sehingga 15 DAB tanpa perubahan dalam struktur retina. Eksperimen 2 mengkaji bagaimana keamatan cahaya yang berbeza (2100 lux dan 1400 lux) dari sumber lampu pendarfluor dan diod pemancar cahaya (LED) mempengaruhi pertumbuhan, kelangsungan hidup dan pengambilan makanan kuda laut *H. barbouri* semasa peringkat pelagik. Sebanyak 16 ekor kuda laut/tangki digunakan dengan tiga replikat. Pada akhir tempoh 15 hari eksperimen, kajian di bawah rawatan lampu pendafluor 1400 lux adalah yang paling sesuai bagi pertumbuhan, kelangsungan hidup dan pengambilan makanan. Eksperimen 3 dijalankan untuk menentukan kesan sinergi latar belakang tangki dan warna makanan terhadap pemilihan makanan pada kuda laut sub-dewasa *H. barbouri* (panjang piawai, SL: 7.56±0.35 cm; berat basah, WW: 0.58±0.10 g). Sebanyak sembilan ekor kuda laut sub-dewasa dipelihara dalam tangki triplikat mempunyai latar belakang berwarna merah, biru dan hijau selama dua minggu dengan kadar kepadatan satu kuda laut/tangki. Udang sergestid, *Acetes sibogae* (panjang penuh, TL: 1.00 ± 0.05 cm) digunakan sebagai makanan ujian; diwarna dengan warna biru, hijau, merah dan kuning, manakala udang dengan warna semula jadi tanpa sebarang pewarna digunakan sebagai rawatan kawalan. Dua warna *Acetes* yang berbeza diberi secara serentak kepada *H. barbouri* sub-dewasa, dengan setiap kombinasi warna ditawarkan setiap lima saat. Ujian diteruskan sehingga kuda laut tidak menunjukkan respons terhadap kombinasi warna makanan tersebut. Selepas dua jam, ujian kombinasi warna yang berbeza disambung semula secara rawak. Pada semua warna latar belakang, tindak balas pertama signifikan terhadap udang dengan warna semula jadi. Kajian ini menunjukkan bahawa *H. barbouri* sub-dewasa mempunyai pilihan warna dan membantu memendekkan masa penukaran makanan kuda laut kepada makanan sejuk beku. Kesimpulannya, kajian ini mengisi jurang pemahaman tentang perkembangan mata *H. barbouri* pada peringkat pelagik. Keamatan cahaya 1400 lux daripada lampu pendarfluor terbukti memberi kesan optimum terhadap pertumbuhan, kelangsungan hidup dan pengambilan makanan *H. barbouri* pada peringkat ini. Proses penukaran makanan *H. barbouri* sub-dewasa di dalam tangki berwarna merah, biru atau hijau kepada makanan sejuk beku boleh dipercepatkan dengan menggunakan *Acetes* sejuk beku dengan warna semula jadi.

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°C	-	celcius
%	-	percent
>	-	more than
<	-	less than
~	-	approximately

LIST OF ABBREVIATIONS

DAB	-	days after birth
cm	-	centimetre
m	-	metre
m^2	-	meter square
m^2/s	-	per square meter per second
nm	-	nanometre
g	-	gram
TL	-	total length
SL	-	standard length
WW	-	wet weight
GBD	-	gas bubble disease
TCM	-	traditional Chinese medicine
L/h	-	litre per hour
ppm	-	parts per million
ppt	-	parts per thousand
µm	-	micrometre
LED	-	light emitting diode
lx	-	lux
et al.,	-	and others

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CHAPTER 1

INTRODUCTION

1.1 Background of the Research

Zebra-snout seahorse, *Hippocampus barbouri* (Jordan and Richardson, 1908) is a member of Syngnathidae family. The species has been widely reported in international trade because of its high economic value and marketability for traditional medicine, curios and aquarium trade (CITES, 2002; Evanson *et al.,* 2011; UNEP-WCMC 2012; Koning and Hoeksema, 2015). It is one of the most common species kept in aquariums due to its unique appearance and attractive colour variation (Koldewey and Martin-Smith, 2010; Olivotto *et al.,* 2011; Williams *et al.,* 2014). In Malaysia, seahorse *H. barbouri* was found often associated with either the hard coral reef, sponges, seagrasses or seaweeds in Langkawi, Mersing Islands, Banggi Island, Sakar Island, Kudat and jetty in Universiti Malaysia Sabah (Choo and Liew, 2004; Shapawi *et al.,* 2015).

The eye is the primary visual system and is an important sensory organ for seahorses to locate and capture prey (Sheng *et al.,* 2006; Novelli *et al.,* 2015). However, *H. barbouri, H. comes, H. reidi,* and *H. erectus* were observed feeding during the crepuscular hour, suggesting some exciting research questions regarding eye sensitivities (James and Heck, 1994; Perante *et al.,* 2002; Felício *et al.,* 2006; Garcia *et al.,* 2012). However, the previous study mainly focused on the eyes' morphological structure (Sommer *et al.,* 2012; Novelli *et al.,* 2015; Mongkolchaichana *et al.,* 2022), without further discussing the differences in the acquisition of retinal structures. Hence, this might influence the vision-based survival skills of seahorses under low light conditions.

Light receptivity by juvenile fish changes during development, affecting feeding success, behaviour and survival (Owen *et al.,* 2010). As visually guided feeders, seahorses attract and prey upon small moving crustaceans (Lee and O'Brien, 2011; Yip *et al.,* 2015). Nevertheless, live foods and their availability are considered one of the challenging factors for seahorse aquaculture (Martinez-Cardenas and Purser, 2012; Woods, 2007; Murugan *et al.,* 2009). Brine shrimp (*Artemia*), copepods, rotifers and mysid shrimp are commonly used to fed seahorses in captivity (Souza-Santos *et al.,* 2013; de Souza *et al.,* 2020). The live food culture is costly, time and labour-consuming,–whereas collection of live food from the wild is not sustainable with the high potential of disease outbreaks (Lim *et al.,* 2003; Hill *et al.,* 2020; Akbary *et al.,* 2010; Moorhead, 2017). The preferable size of prey increases as seahorses grow due to the relationship between seahorse mouth biometrics and prey selectivity (Teixeira and Musick, 2001; Woods, 2002; Blanco and Planas, 2015). Therefore, the inappropriate size and insufficient nutritional content of live foods might cause low energy intake, resulting in slow growth and poor survival in rearing sub-adult seahorses (Olivotto *et al.,* 2008). Developing economically viable and sustainable feed that provides sufficient nutrients for seahorses is still challenging due to the development and changes in dietary needs (Foster and Vincent, 2004; Murugan *et al.,* 2009).

Captive-produced fish that are well-adapted to eat frozen food are more valuable to consumers, particularly hobbyists because there is less reliance on live food, which simplifies rearing requirements (Lin *et al.,* 2009a; Woods and Valentino, 2003; Vargas-Abúndez *et al.,* 2018). Prey colour, size, shape, brightness, colour contrast between prey and background influences the recognition of prey items, resulting in higher feeding success (Maass, 2007; Kawamura *et al.,* 2017; Ninwichian *et al.,* 2018; Kasumyan, 2019). Many fish species rely on colour discrimination for ecological tasks, including prey detection (Kelber *et al.,* 2003). Previously, food colour affected growth and feed efficiency in Nile tilapias and thinlip mullet (El-Sayed, 2004; Jegede and Olusola, 2010; El-Sayed and El-Ghobashy, 2011; El Sayed *et al.,* 2013). Fatollahi and Kasumyan (2006) study found that African catfish preferred blue food to black background. However, preferred food colour changes with different background colours or brightness used when feeding the fish (Browman and Marcotte, 1987; Luchiari and Pirhonen, 2008; Kawamura *et al.,* 2016a). This may happen due to the contrast between the prey and the environmental factors. Thus, manipulating the background and food colour can potentially increase feeding success. There is no study on the background and food colour preference combinations of seahorses in captivity.

1.2 Problem Statement

Seahorses are well-known as visual feeders, and the optical structure is generally described at a specific stage only (5, 20 and 35 days after birth, DAB) in *H. barbouri* (Mosk, 2004; Mongkolchaichana *et al.,* 2022). Previously, the retinal development of the *H. barbouri* only showed a clear classification of the ten layers at 5th DAB and increased retinal thickness dramatically from the 5th to 35th DAB (Mongkolchaichana *et al.,* 2022). However, the optical development was less detailed, especially at the larvae and early critical pelagic stage (1-15 DAB). Only a few studies have looked at the optical development of seahorses and their visual ability to feed during the crepuscular hour (James and Heck, 1994; Perante *et al.,* 2002; Felício *et al.*, 2006; Novelli *et al.,* 2015). The feeding activity of seahorses might not be limited to the environment with high light intensities and might be species-depending. For successful rearing, it is imperative to provide suitable environments for an animal's visual system to develop optimally and enable prey capture at different stages of maturation. Seahorses are precocial species, as the newborn has already metamorphosed during gestation (Álvarez-Hernán *et al.,* 2019). Differences in the acquisition of retinal structures might influence the vision-based survival skills of seahorses at the early life stage. Many fish species rely on colour discrimination for ecological tasks, including predation, and easy recognition of prey items resulted in higher feeding success (Ma and Qin, 2014; Escobar-Camacho *et al.,* 2017; McLean, 2021). Most previous studies were conducted to determine the factors separately (Utne-Palm 1999; Martinez-Cardenas and Purser 2007). However, determining the synergistic effect of the factors that influence feeding efficiency is crucial for feed development, particularly in understanding the colour preference of prey on the specific background colour. Therefore, the present study was conducted.

1.3 Significance of the Research

This study aims to bridge a knowledge gap by enhancing our understanding of optical development in seahorse species *H. barbouri* during their early life stages, specifically focusing on the importance of species-specific light intensity. Tailoring the light intensity to the specific needs of *H. barbouri* during their early life stages could prove invaluable for their successful propagation. Additionally, the use of color contrast may enhance the visual capabilities of sub-adult *H. barbouri* thus expedite the weaning process and reduce their reliance on live food, particularly when transitioning to frozen feed.

1.4 Objective of the Research

1) To examine the optical development histologically as the sensory organ of seahorse *H. barbouri* in captivity during early development,
2) To determine the effects of light intensity on the growth, survival & feed intake of seahorse *H. barbouri* in the pelagic stage, and
3) To determine the food colour preference of seahorse *H. barbouri* in captivity against different background colour

1.5 Hypothesis of the Research

The optical development of seahorse *H. barbouri* during their early development stages will exhibit distinct histological changes, suggesting adaptation to their visual feeding behaviours. While varying light intensities will significantly impact their growth, survival rate, and feed intake in the pelagic stage, with optimal light conditions leading to improved growth and survival rates. Additionally, seahorse *H. barbouri* will display distinct food colour preferences when presented with various colour backgrounds, favouring food items that contrast with their immediate environment, thereby reflecting their visual foraging strategy.