BROODSTOCK MANAGEMENT, REPRODUCTIVE DEVELOPMENT AND LARVAL REARING OF THE MARINE ORNAMENTAL SHRIMP, *Lysmata amboinensis* (de Man, 1888)

By

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Lysmata amboinensis, widely known as "cleaner shrimp," is a favored species in the marine aquarium trade due to its vivid coloration and robust nature. Despite its popularity, attempts to cultivate this species on a large scale have been challenging, primarily due to its extended metamorphosis phase, which can last between 58 and 140 days. This study evaluate the effects of different environmental conditions, specifically salinity and temperature, on major reproductive parameters under controlled laboratory conditions. The study tested the impact of varying salinity levels (30 ppt, 35 ppt, and 40 ppt) on *L. amboinensis* broodstock. The results indicated that while survival rates remained stable across tested salinity, fecundity and egg volume were significantly affected. Broodstock reared at 30 ppt demonstrated the highest values for fecundity, egg volume, and other reproductive parameters ($p < 0.05$). For temperature, the broodstock were divided into four groups (22 ºC, 25 ºC, 28 ºC, and 31 ºC). The shrimp showed a wide temperature tolerance range, surviving from 22 ºC to 28 ºC; however, mortality began at 30 ºC, and no shrimp survived beyond 24 hours at 31 ºC. While the broodstock could endure temperatures as low as 22 ºC, they failed to produce fertile eggs. The optimal temperature for reproduction was 25 ºC, yielding nearly twice the fecundity (1420 ± 193) compared to 28 ºC (851 ± 60) and producing eggs with greater size (0.0613 ± 0.0008 mm^3) and higher biomass (0.2376 ± 0.0395 g). The effects of different feeds on broodstock reproduction were also assessed, using four diets: squid (*Loligo* sp.), mussel (*Perna viridis*), polychaetes (*Marphysa moribidii*), and a combination of these. The squid diet led to substantial egg loss throughout the incubation period (58.67% ± 3.67%) and lower larval production (97 ± 29) ($p < 0.05$). Conversely, the mussel diet produced the highest larval count (568 ± 23).

The study also explored the life cycle stages of *L. amboinensis*, identifying seven phases of complete embryonic development over approximately 13 days. Histological analysis showed that the gastrula stage, where organ formation begins, was crucial for embryonic development. Under laboratory conditions, the shrimp achieved ten zoea stages over 55 days, highlighting the lengthy larval development phase. The study further investigated the effect of different diets on larval by feeding larvae six different treatments, including microalgae (*Chaetoceros* sp., *Tetraselmis* sp.), a commercial diet, and combinations thereof. Starved larvae and those fed solely with microalgae or the commercial diet showed poor survival rates, with the highest mortality recorded in starved larvae. The combination diet of *Tetraselmis* sp. and the commercial feed (TSAD) resulted in a 10% higher survival rate (46.67% ± 5.14%) demonstrating its superior performance in supporting larval growth. However, even the TSAD diet was insufficient to promote complete metamorphosis to post-larvae, suggesting that current feeding regimens still require optimization. Research showed that the productivity under captive condition can be enhanced by improving the *L. amboinensis* broodstock rearing condition and feeding and understanding the biology of the early life of this species.

Keywords: *Lysmata amboinensis*, Larval development, Reproductive performance

SDG: GOAL 13: Climate action, GOAL 14: Life below water

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

PENGURUSAN INDUK, PERKEMBANGAN PEMBIAKAN DAN PENJAGAAN LARVA BAGI UDANG HIASAN MARIN,
***Lysmata amboinensis* (de Man, 1888)**

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Lysmata amboinensis yang dikenali sebagai "udang pencuci" merupakan salah satu krustasea yang paling popular dalam industri akuarium marin kerana warna yang menarik dan lasak. Pelbagai percubaan dilakukan untuk membangunkan teknologi perternakan berskala besar, tetapi kejayaannya masih rendah disebabkan fasa metamorfosis yang sangat lama iaitu sekitar 58 hingga 140 hari. Kajian ini menilai kesan keadaan persekitaran yang berbeza (saliniti dan suhu) pada parameter utama pembiakan *L. amboinensis* di dalam keadaan makmal. Untuk eksperimen saliniti, pasangan induk dibahagikan kepada tiga kumpulan (30 ppt, 35 ppt dan 40 ppt). Keputusan menunjukkan bahawa julat saliniti yang besar ini tidak menjejaskan kelansungan hidup induk. Walau bagaimanapun, saliniti mempengaruhi nilai fekunditi, isipadu telur dan parameter-parameter lain yang diukur di mana induk yang diternak pada saliniti 30 ppt dapat menghasilkan fekunditi dan isipadu telur yang tinggi ($p < 0.05$). Untuk eksperimen suhu, pasangan induk dibahagikan kepada empat kumpulan (22 ºC, 25 ºC, 28 ºC dan 31 ºC). Kelansungan hidup induk bervariasi pada suhu yang berbeza di mana spesies ini mampu bertahan pada suhu serendah 22 ºC dan sehingga suhu 28 ºC. Kematian bermula apabila suhu mencapai 30 ºC. Tiada induk yang hidup selepas 24 jam pada 31ºC. Walaupun spesies ini mampu bertahan pada 22 ºC, induk tersebut tidak dapat menghasilkan telur yang bersenyawa. Induk di bawah rawatan 25 ºC menghasilkan fekunditi (1420 ± 193) iaitu hampir dua kali ganda lebih tinggi berbanding rawatan 28 ºC. Telur yang dihasilkan juga lebih besar isipadu dan tinggi nilai biomasnya. Selain itu, kesan makanan yang berlainan terhadap induk *L. amboinensis* juga dijalankan. Empat jenis diet yang terdiri daripada sotong (*Loligo* sp.), kupang (*Perna viridis*), umpun-umpun (*Marphysa moribidii*) dan makanan kombinasi diberi kepada induk. Hasil kajian mendapati induk yang diberi diet sotong telah kehilangan sebahagian besar daripada telur yang dihasilkan sepanjang melalui tempoh inkubasi (58.67 % ± 3.67 %), dan diikuti dengan pengeluaran larva yang rendah (97 ± 29) ($p < 0.05$). Sebaliknya, diet kupang menghasilkan jumlah larva tertinggi (568 ± 23).

Kajian ini juga meneroka tahap-tahap kitaran hidup *L. amboinensis* dan mengenal pasti tujuh fasa perkembangan embrio yang lengkap dalam tempoh 13 hari. Analisis histologi menunjukkan bahawa fasa gastrula penting untuk perkembangan embrio kerana pembentukan organ bermula. Dalam kondisi makmal, udang ini mencapai sepuluh tahap zoea dalam tempoh 55 hari. Kajian ini juga menyiasat kesan diet yang berbeza pada larva. Larva diberi enam rawatan diet berbeza, termasuk mikroalga (*Chaetoceros* sp., *Tetraselmis* sp.), diet komersial, dan gabungan diet ini. Larva yang tidak diberi sebarang diet serta larva yang makan samaada mikroalga atau diet komersial secara eksklusif menunjukkan kadar kelansungan hidup yang rendah. Gabungan diet *Tetraselmis* sp. dan makanan komersial (TSAD) menghasilkan kadar kelangsungan hidup yang 10% lebih tinggi (46.67% ± 5.14%), menunjukkan prestasi yang lebih baik dalam menyokong pertumbuhan larva. Walau bagaimanapun, diet TSAD masih tidak mencukupi bagi menampung pertumbuhan larva untuk bermetamorfosis hingga ke peringkat post larva. Penyelidikan ini menunjukkan bahawa peningkatan produktiviti bagi *L. amboinensis* di bawah keadaan makmal boleh dipertingkatkan melalui penambahbaikan kondisi penjagaan induk dan pemakanan disamping memahami biologi kehidupan awal spesies ini.

Kata Kunci: *Lysmata amboinensis*, Perkembangan larva, Prestasi pembiakan

SDG: MATLAMAT 13: Tindakan iklim, MATLAMAT 14: Kehidupan di dalam air

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This thesis was submitted to the Senate of the 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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$	US Dollar sign
€	Euro sign
AA	arachidonic acid
ALA	α-linolenic acid
D	dark
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FP	female-phase
g	gram
h	hour
in	inch
L	light
LOA	linoleic acid
MP	male-phase
MUFA	monounsaturated fatty acid
OA	oleic acid
ppt	parts per thousand
PSH	protandric simultaneous hermaphroditic
PUFA	polyunsaturated fatty acid
RM	Ringgit Malaysia
SA	stearic acid
SFA	saturated fatty acid
SH	simultaneous hermaphrodite
US	United States of America
Z1-Z10	zoea I – zoea X

CHAPTER 1

INTRODUCTION

In the 10th century, aquatic ornamental organism have fascinated humans that began in Asia countries such as China and Japan, and later spread to the western cultural in Turkey, Eastern Europe, and the Roman Empire (Rhyne *et al*., 2017a). According to Doar (2007), the book, "*The Aquarium: An Unveiling of the Wonders of the Deep Blue Sea*" published in 1854 have influenced people with new hobby of keeping aquariums at that time. Gosse (1854) in his book stated that keeping aquarium make us attached to the unique sea creatures without the need to diving. Nowadays, keeping aquatic organism in household become among top three in the United States of America (US) petting industry. By the year 2017, there are 2.5 million people in the US keeping saltwater aquaria at their home (American Pet Products Association, 2017). According to Ryne *et al*. (2017b), 4.3 million invertebrates representing 545 species being traded in the US alone in the year 2008.

Cleaner shrimp was among the top demanded invertebrates in the marine ornamental industry and have created special attention due to their dazzling coloration and unique 'personality'. Besides, this high value ornamental shrimp has a major role in maintaining animal health in the reef ecosystem (Fletcher *et al*., 1995). Due to its behaviour, it is commonly known as cleaner shrimp or scientifically referred as *Lysmata amboinensis*.

Most of commercialized marine ornamental organisms including *L. amboinensis* come directly from the wild ecosystems, mainly from coral reefs (Wabnitz *et al*., 2003). However, recent studies have shown a decline in the coral reefs of the world. Based on 27 years of data evaluation on reef condition, De'ath *et al*. (2012) found a major decline in hard coral cover from 28.0 % to 13.8% (2,258 surveys of 214 reefs from the year 1985 to 2012). The aquatic organism collection practices also linked to severe destruction of coral reefs through the use of explosions and poisons like cyanide (Corbin, 2001). Moreover, overharvesting of *L. amboinensis* to meets the demand for ornamental industry might also give an ecological impact in the reef area as this species helps to sustained the health of organisms in this ecosystem.

Efforts have been performed to scale down overexploitation on wild marine ornamental sources and to maintain their sustainability (Corbin, 2001; Calado *et al*., 2003b). Aquaculture or captive culture is recognized as the best alternative to minimize wild collection while sustaining the aquarium industry (Lin, 2004). Aquaculture of ornamental species is a rapid growing sector and often seen as a priority solution in the conservation of reef habitats and relieving collection pressures on wild stocks.

According to Murray *et al*. (2012), the marine ornamental fish that have been successfully bred commercially is still low, approximately 1% to 10%. Presently, the attempts have been made to develop technologies for the mass-scale culture of a few ornamental shrimp including *L. amboinensis*, but with little success (Palmtag and Holt, 2001; Lin *et al*., 2002). Ornamental shrimp that have been successfully reared in captivity is only limited to a few species such as *Lysmata debelius, Lysmata boggessi* and *Lysmata wurdemanni*.

The major bottlenecks for mass production of *L. amboinensis* are connected to its long metamorphosis phases of larvae on the average of five months (Fletcher *et al*., 1995) and most of the larvae died during this period. Besides, the larvae ability to delay the metamorphosis worsen the scenario. Consequently, little development has been made in the culture of *L. amboinensis* in the laboratory compared to other species of cleaner shrimps. Due to increasing demand for captive rearing of *L. amboinensis* in the marine ornamental industry as well as the decline of the world's reefs, research is therefore crucial for culturing this ornamental shrimp.

Thus, the problem statement summary for this study are stated below:

1. Currently, all cleaner shrimp, *L. amboinensis* traded in the marine ornamental industry was collected from the wild, mostly from coral reef ecosystems which cause severe damage through the use of poisons such as cyanide or explosions. Therefore, research need to be done to increase the knowledge regarding this species and minimize overexploitation and the reliability on wild specimens in the future.
2. Captive culture of this highly priced ornamental shrimp is regarded as a sustainable alternative. This species has good market value and the culture is highly promising profitable economic activity.
3. Many attempts have been made to develop technologies for the laboratory culture of a few decapod species including *L. amboinensis*, but with only little success. Longer larvae metamorphosis phases up to five months make this species research development slower compared to other species, especially, research focusing on the larvae. Higher larvae survival will increase the mass production potential. So, the information from this study would be vital for the culture production of this species.

Biological information such as broodstock environment condition and feeding, reproductive and mating behaviour, embryonic development, larvae life cycle and larvae feeding in captivity are essential for the development and the realisation of marine ornamental aquaculture technology and future commercial of *L. amboinensis.*

Therefore, the general objectives of this study are to investigate the broodstock environment culturing condition and feeding in order to optimize better output in captivity. The optimum rearing condition are needed to produce continuously reproductive cycle of berried shrimp. During spawning event, biological data and information on berried shrimp and larvae productions are needed for better captive practices. Going through the literature, very little studies were found on the early life of *L. amboinensis*. The description of embryonic development and larvae life cycle is significant for better output in captivity culture of this species.

The specific objectives of this present study are:

1. To examine the effects of salinity and temperature on the major reproductive parameters of *L. amboinensis*, in terms of broodstock survival and fecundity, egg quality and percentage of relative productivity.
2. To evaluate the effect of widely available fresh frozen diets to *L. amboinensis* broodstock based on biochemical compositions, broodstock conditions, reproductive performances and larval quality.
3. To describe the reproductive cycle, the internal and the external embryonic development, and the larval development stages of this species during cultivation.
4. To determine the effect and the suitability of microalgae and commercial diet to *L. amboinensis* larvae in terms of their survival, zoea stages composition and growth development.

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