UNIVERSITI PUTRA MALAYSIA

CULTURE OF COPEPOD Oithona simplex (Farran, 1913) AND ITS POTENTIAL AS LIVE FOOD FOR FLOWER CRAB, Portunus pelagicus (Linnaeus, 1758) LARVAE

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FP 2018 61

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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 Master of Science

May 2017

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CULTURE OF COPEPOD *Oithona simplex* (Farran, 1913) AND ITS POTENTIAL AS LIVE FOOD FOR FLOWER CRAB, *Portunus pelagicus* (Linnaeus, 1758) LARVAE

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Research on *Oithona simplex* was carried out to further assess its potential as live food candidate for hatchery crab larval rearing. Although several species copepods from genus *Oithona* was proven to be good for fish larval rearing, no trial had ever been carried out using *O. simplex* during crab larval rearing and its life cycle development of this *O. simplex* has not been studied. All experiments were done at the International Institute of Aquaculture and Aquatic Sciences, Port Dickson and specimens were obtained from nearby coastal waters. The larval development of *O. simplex* was studied under laboratory condition of 30 ºC and with 12 hours light and 12 hours dark photoperiod. The developmental stages were divided into 6 naupliar stages (NI, NII, NIII, NIV, NV, NVI), 5 copepodite stages (CI, CII, CIII, CIV, CV) and an adult (CVI) stage. The different larval stages of this species were described. The life cycle period lasted for 5 to 6 days.

Effect of some environmental parameters on the reproduction and development of *O. simplex* were also studied. This is to gather the optimum environmental condition for the culture of the species. Four different salinity levels viz. 20, 25, 30 and 35 ppt, three different temperatures of 25, 30 and 35 ºC, three different photoperiods representing 12h light (L): 12h dark (D), 1h (L):23h (D), 24h (L):0h (D) and three light intensities of 5, 20, 35 µmol m^{-2} s^{-1} were employed in this study. *Oithona simplex* can tolerate a wide range of salinity of 20-35 ppt. The optimum temperature required for the maximum reproduction of *O. simplex* was 30 ºC. The overall reproductive process was highest under the lowest light intensity (5 µmol m^{-2} s^{-1}) compared to other light intensities. In addition, the overall reproduction and fastest development time were achieved by copepod reared under photoperiod 12h light (L): 12h dark (D) condition. The result of this study showed that *O. simplex* had a high population growth, short generation time, high reproductive potential, and a wide range of tolerance to environmental conditions.

Survival and specific growth rates of flower crab larvae from zoea 1 to megalopa stage were studied by feeding them with three different live food options that comprised of *O. simplex* alone, *Artemia* nauplii in combination with *O. simplex* and only *Artemia* nauplii. The highest survival was achieved by crab larvae fed with the combination of *O. simplex* and *Artemia* nauplii (3.16%), followed by those fed with *O. simplex* (1.31%) and *Artemia* nauplii (0.95%). Meanwhile, the highest specific growth rates were achieved by larvae fed with the combination of both live foods (28.4 %) followed by *Artemia* nauplii only (27.2%) and only *O. simplex* (25.6%). No significant difference was found on the final length of crab larvae in those three different treatments. The result from this study showed that *O. simplex* meets the several requirements needed as live feed for crab larvae which indicated that it can be used to replace partially imported *Artemia* cyst during crab larval rearing.

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

PENTERNAKAN KOPEPOD *Oithona simplex* (Farran, 1913) DAN POTENSINYA SEBAGAI MAKANAN HIDUP KEPADA LARVA KETAM BUNGA, *Portunus pelagicus* (Linnaeus, 1758)

Oleh

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Penyelidikan ke atas *Oithona simplex* dijalankan untuk mengenal pasti potensinya sebagai calon makanan hidup untuk penternakan larva ketam. Walaupun beberapa spesis kopepod dari genus *Oithona* telah terbukti bagus untuk penternakan larva ikan, tiada percubaan yang pernah dijalankan menggunakan *O. simplex* untuk penternakan larva ketam dan perkembangan kitaran hayat spesis *O. simplex* ini belum pernah dikaji. Semua eksperimen telah dilakukan di Institut Antrabangsa Akuakultur dan Sains Akuatik, Port Dickson dan specimen diambil dari perairan pantai yang berdekatan. Perkembangan larva bagi *O. simplex* dikaji di bawah keadaan makmal pada suhu 30 ºC dan 12 jam cahaya:12 jam gelap. Tahap perkembangan telah dibahagikan kepada 6 nauplius (NI, NII, NIII, NIV, NV, NVI), 5 peringkat kopepodit (CI, CII, CIII, CIV, CV) dan dewasa (CIV). Peringkat perbezaan larva bagi spesies ini diterangkan. Tempoh kitaran hidup mengambil masa selama 5 hingga 6 hari.

Kesan beberapa parameter persekitaran ke atas pembiakan dan perkembangan *O. simplex* juga turut dikaji. Ini adalah untuk mendapatkan faktor persekitaran yang optimum bagi penternakan untuk spesies ini. Empat tahap kemasinan berbeza iaitu 20, 25, 30 and 35 ppt, tiga suhu berbeza 25, 30 and 35 ºC, tiga tahap jangka masa cahaya mewakili 12 jam cahaya:12 jam gelap, 1 jam cahaya:23 jam gelap, 24 jam cahaya:0 jam gelap dan tiga tahap keamatan cahaya 5, 20, 35 µmol m^{-2} s^{-1} telah digunakan dalam kajian ini. *Oithona simplex* boleh hidup dalam julat saliniti yang lebar iaitu 20-35 ppt. Suhu optimum yang diperlukan untuk pembiakan maksimum adalah 30 ºC. Pembiakan keseluruhan adalah paling cepat di bawah keamatan cahaya yang rendah (5 µmol m^{-2} s^{-1}) berbanding keamatan cahaya ujikaji yang lain. Sementara itu, masa keseluruhan pembiakan dan perkembangan terpantas telah dicapai oleh kopepod yang diternak dibawah jangka masa cahaya 12 jam cahaya: 12 jam gelap. Hasil kajian ini menunjukkan bahawa *O. simplex* mempunyai tahap pembesaran yang tinggi, selang masa generasi yang singkat, potensi pembiakkan yang tinggi dan ketahanan yang tinggi terhadap faktor persekitaran.

Kelansungan hidup dan kadar pertumbuhan spesifik larva ketam bunga dari zoea 1 ke peringkat megalopa dikaji dengan memberi pilihan makanan hidup yang berbeza: *O. simplex* sahaja, *Artemia* nauplius dengan gabungan *O. simplex* dan hanya *Artemia* nauplius. Kelangsungan hidup yang tinggi diperolehi oleh larva ketam yang diberi makan kombinasi *O. simplex* dan *Artemia* nauplius (3.16%), dikuti yang diberi makan *O. simplex* (1.31%) dan *Artemia* nauplius (0.95%). Sementara itu, kadar pertumbuhan spesifik dari segi berat badan yang paling tinggi telah diperolehi oleh larval ketam yang memakan gabungan kedua-dua makanan hidup (28.4%) diikuti yang memakan hanya *Artemia* nauplius (27.2%) dan hanya *O. simplex* (25.6%). Tiada perbezaan yang signifikasi didapati pada panjang akhir larva ketam dalam ketiga-tiga rawatan tersebut. Hasil daripada kajian ini menunjukkan bahawa *O. simplex* memenuhi beberapa syarat yang diperlukan sebagai makanan hidup untuk larva ketam dimana ia boleh digunakan untuk menggantikan sebahagian pengimportan sista *Artemia* semasa penternakan larva ketam.

ACKNOWLEDGEMENTS

Praise be to Allah, lord of worlds and 'selawat' to the prophet Muhammad S.A.W. Thanks to God that I have finished writing the project report. Special appreciations, dedicated to my supervisor, Prof. Dr. Aziz Arshad for his invaluable guidance, advice, constructive comment, kind suggestions, financially and encouragement throughout my study period. Sincere thanks is extended to my co-supervisor Prof. Dr. Mohd Salleh Kamarudin for providing all the expert advise and suggestions to further improve the research.

I would also like to sincerely thanks Dr. Hazel Monica Matias-Peralta from UTHM, for her assistance in the identification of copepod species used in this study. Further thanks is dedicated to Dr Nur Leena Wong for her guidance and suggestions. Special thanks to Dr. Mahdi Ebrahimi for being the resource person for the fatty acid analysis.

I am deeply thanks to my postgraduate friends especially Ainul, Kak Ika, and Kina for their understanding, support, concern and willingness to help me in order to complete my project. I would like to extend similar appreciation to all my new found friends and staffs at International Institute of Aquaculture and Aquatic Sciences for their kind assistance in ensuring the smooth implementation of the project.

Finally, I would like to dedicate my sincere thanks and appreciation to my great beloved family members for their support, encouragement and understanding throughout the preparation of this project and the whole duration of my study.

I certify that a Thesis Examination Committee has met on (2 May 2017) to conduct the final examination of Nur Syuhada binti Mat Noor on her thesis entitled "Culture of Copepod *Oithona simplex* (Farran,1913) and its Potential as Live Food for Flower Crab, *Portunus pelagicus* (Linnaeus, 1758) Larvae" 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 Master of Science.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

A1	Antennule
ARA	arachidonic acid
an	Antenna
CI - CV	copepodid I – V
CVI	adult
D	Dark
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FAME	fatty acid methyl esters
h	Hour
L	Light
ind	Individual
M	Megalopa
mnd	Mandible
min	Minute
MUFA	monounsaturated fatty acid
mx	Maxilla
NI - NVI	nauplius I – VI
ppm	parts per million
ppt	parts per thousand
PUFA	polyunsaturated fatty acid
SFA	saturated fatty acid
Z1 – Z4	zoea 1 – 4

CHAPTER 1

INTRODUCTION

Portunus pelagius known as a flower crab is one of the commercially important species for local consumption in Malaysia. However almost all landings are from the wild sources and there has not been much crab pond grow outs activities taking place in the country. In comparison to mud crab, flower crab aquaculture is almost nil at present. As the availability of the wild stocks is fluctuating and the practice of wild catching in the long run is unsustainable, the aquaculture development of flower crab must seriously be considered. Apart from that, flower crab culture operations also totally relying on seed collected from the wild, but now it is becoming more and dependent on hatcheries production which is a more dependable source for the future (Azra and Ikhwanuddin, 2015). Nowadays, the commercial development of crab culture, both mud crab and flower crab face some common problems mainly on live food cultures due to the difficulties in maintaining its culture, disease vector and high price of *Artemia* cyst (Allan and Fielder, 2004; Quinitio *et al.*, 2001). According to Ikhwanuddin *et al.* (2012a), investigation and modification of feeding during crab larvae rearing might be useful to improve survival rate of crab larvae. Therefore, research on live foods resources considerably important in establishing a better live food candidate for crab larval rearing. This is because; initial feeding at the early stages of development is one of the main components for successful larval rearing (Le Ruyet *et al.*, 1993). In addition, most larvae would prefer feed that can be easily captured and digested, and also provides good nutrients for growth and survival (Giri *et al*., 2002). Thus, a great comprehension of larval morphology, behaviour, live food and artificial diet requirements, and environmental conditions are important to improve the techniques of larval rearing (Liao *et al.*, 2001).

Most marine hatcheries are dependent on rotifers (*Brachionus* sp.) and brine shrimp (*Artemia* sp.) during the larval rearing period of fish and crustacean (Evjemo and Olsen, 1997; Hagiwara et *al.*, 2001; Takeuchi, 2001). *Artemia* and rotifer are also commonly used as a live food for portunid crab larvae (Suprayudi *et al.*, 2002; Soundarapandian *et al.*, 2007; Baylon, 2009). Rotifers are comparatively easy to culture and it high density culture can also be reproduced rapidly (Lubzens *et al.*, 2001). Despite the advantage, the culture of rotifer may sometime crash easily (Ananthi *et al.*, 2011). As for brine shrimp, it is commercially available in the form of dry cysts. Recently, there has been some form of crisis in *Artemia* cyst availability due to insufficient supply to meet big demands by the aquaculture operators. The decreasing of harvested *Artemi*a from the traditional productive Great Salt Lake, Utah USA, along with implementing a stricter rules of harvesting from those waters were also one of the cause of *Artemia* cyst availability crisis (Conceicão *et al.*, 2010). Other than that, even though *Artemia* is still the most preferred live food during larvicuture of fish and crustacean but this brine shrimp is not cost-effective due to its high market price and the cost tends to increase from year to year following market trend (Akbary *et al.*, 2010).

Marine copepods are the most numerous plankton that exist throughout the worlds's ocean and becoming the natural food sources for many marine fish and crustacean.

Copepods are common zooplankton of seawater, freshwater and brackishwater, that can represent up to 80% of the zooplankton biomass in the water column (Mauchline, 1998). The most commonly used species of free-living copepods in aquaculture belong to the three main orders which are Calanoida, Harpacticoida, and Cyclopoida (Støttrup, 2003). Copepods are abundant in the coastal waters of Malaysia and study by Rezai *et al.* (2004) stated that 177 copepod species belongs to 37 genera and 25 families are found in the Straits of Malacca. However, there is still lack of information and knowledge on these indigenous copepods especially on their potential role as a live food for fish and crustacean larvae. There are several advantages of using copepod as a live food such as improving larval survival (Shields *et al.,* 1999), increasing growth rate (Støttrup and Norsker, 1997), high contents of HUFA and has broad range of body size (Anathi *et al.*, 2011). Despite the advantage, there are still not many works regarding the application of copepod as live food for crustacean larvae. Thus, more research is needed to discover and assess the potential of copepod culture which can enhance the better growth and survivals of crustacean especially crab larvae.

Thus, the summary of the research problem in this study were stated below:

1) At present, the mariculture hatchery operation is completely dependent on imported and expensive *Artemia* cyst which is on the increase from year to year following market trend. Therefore, copepod has been chosen as an alternative live food to replace *Artemia* due to their unique characteristics, good nutritional content and readily available zooplankton in Malaysian coastal waters. By utilizing the naturally available live foods such as copepod to rear the crab larvae, feed costs can be significantly reduced.

2) *Oithona simplex* have been chosen in this study due to overall lack of biological information regarding this species especially on it's larval stages, life cycle reproduction and life development under environmental laboratory conditions. Lacking in information especially on their culture conditions could be one of the reason why mass culture technique is yet to be successful for hatchery copepod production. Thus, the information from this study would be vital for the mass culture production of this species.

3) The use of copepod as live food on the growth and survival of *P. pelagicus* larvae has not been well documented particularly for *Oithona simplex*. Thus, the finding of this study would contribute positively toward rearing of *P. pelagicus* larvae with locally abundant copepod. Higher survival rate of the larvae will pave the way for *P. pelagicus* aquaculture and this will indirectly reduce the dependent on wild catches from the sea and will also support the high growing demands of flower crab production in the country.

The overall aims of this study is to investigate in details about larval stages, culture conditions of *O. simplex* and to examine the use of *O. simplex* as alternative live food with an attempt to replace fully or partially the use of *Artemia* nauplii in crab larvae rearing. Thus, this study was established to address the following objectives:

- To investigate the different larval stages and life cycle of cyclopoid copepod *O. simplex* in the laboratory conditions.
- To examine the reproduction and development of *O. simplex* exposed to different sets of environmental laboratory conditions.
- To determine the growth and percentage survival rate of crab larvae fed with copepod *O. simplex* and in combination with other live foods.

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