MARINE HARPACTICOID COPEPOD *Amphiascoides neglectus* AS LIVE FEED FOR AQUARIUM CORALS: *Protopalyhtoa* sp. and *Acropora* sp. and FRESHWATER FISH LARVAE *Denio rerio* UNDER LABORATORY CONDITIONS

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master of Science (Biosciences)

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ABSTRACT

Copepods has increasingly becoming a live feed in aquaculture industries. This present study is to validate the potential of harpacticoid, *Amphiascoides neglectus* as a live feed for aquarium corals and freshwater zebrafish larvae (*Denio rerio*) in captivity. A challenging area in the field of coral culture and larval rearing is their diets. In addition, the cultured corals may have different feeding behaviour compared to wild corals. To solve this problem, we carried out the test feeding experiment of two common species of aquarium corals (*Protopalythoa* sp. and *Acropora* sp.) and onto 5 day after hatched (DAH) zebrafish larvae. The *A. neglectus* were cultured under laboratory condition and able to supply nauplii for every 21 days and on day 14 culture, was the maximum yield of nauplii density. The coral samples were fed at two feeding time, morning and evening with two different feeding types, cultured *A.neglectus* and wild copepod. The results showed that there is no significant difference in ingestion rate of the corals at different feeding times (p=0.260). The growth of the corals fed different type of copepod also showed no significant difference (p=0.794). The test feeding experiment on zebrafish larvae were served with *A. neglectus,* Artemia, and mixed diets. On 15 DAH showed that there is significant difference in terms of growth and survival of the larvae fed *A. neglectus* p=0.045 and p=0.033). This study concluded that the *A. neglectus* have a potential to be used as a live feed for aquarium corals and freshwater zebrafish larvae. The *A.neglectus* could be cultured in laboratory under controlled conditions and successfully produced nauplii as a diet for cultured organisms. This result would improve the knowledge about the advantages of using marine copepod as a live feed.

خلاصة البحث

لقد أصبحت مجدافيات الأرجل تستخدم كغذاء للمرجان ويرقات سمك الزرد في المياه العذبة بشكل متزايد. تهدف هذه الدراسة إلى التحقق من إمكانية استخدام الهارباكتيكويدات، وبالتحديد نوع أمفياكويديس نيجليكتوس كغذاء للمرجان ويرقات أسماك الزرد (دانيو ريريو) المحتجزة في المياه العذبة. الوجبات الغذائية في مجال مستنبتات المرجانية وتربية اليرقات هو مجال صعب، وقد يكون للمرجان المستزرع سلوك تغذية مختلف مقارنة بالمرجانية البحري. لحل هذه المشكلة أجرينا اختبار تغذية على نوعين شائعين من المرجان (نوع بروتوباليتوا ونوع أكروبورا) أيام بعد الفقس. تم 5وعلى يرقات سمك الزرد في المياه العذبة البالغة من العمر استزراع أمفياكويديس نيجليكتوس تحت ظروف مختبرية قادرة على توفير يرقات أقصى إنتاج لكثافة 14 يومًا، حيث كان في اليوم 21 لمدة (nauplii)القشريات يرقات القشريات. تم تغذية عينات المرجان في وقتين،صباحًاومساءً مع نوعين مختلفين، وهما الأمفياكويديس نيجليكتوس المستزرع ومجدافيات الأرجل البحرية. أظهرت النتائج أنه لا يوجد فرق كبير في معدل الأكل للمرجان بين أوقات التغذية أظهر نمو المرجان الذي تغذى على نوعين مختلفين من (p=0.260).المختلفة في اختبار التغذية على يرقات سمك (p=0.794).مجدافيات الأرجلاختلافًاكبيرًا الزرد تم استخدام الأمفياكويديس نيجليكتوس والأرتيميا ووجبات غذائية مختلطة. في بعد الفقس أظهرت اليرقات التي غذيت بالأمفياكويديس نيجليكتوس وجود 15اليوم لذلك استنتجت (p=0.033). والعيش (p=0.045)فرق كبير من حيث النمو الدراسة إلى أنه بالإمكان استخدام الأمفياكويديس نيجليكتوس كغذاء حي للمرجان ويرقات سمك الزرد في المياه العذبة. من الممكن استزراع الأمفياكويديس نيجليكتوس في المختبر في ظروف خاضعة للرقابة وإنتاج يرقات القشريات بنجاح كغذاء للكائنات المستزرعة. هذه النتائج من شأنها تحسين المعلومات حول مزايا استخدام مجدافيات الأرجل البحرية كغذاء حي.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biosciences)

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATIONS

ARA	Arachidonic acid
DAH	Day after hatched
DHA	Docosahexaenoic acid
EPA	Ecosapentaenoic acid
PUFA	Polyunsaturated fatty acid
RO	Reverse-osmosis

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

1.1.1 Copepods in Aquaculture

Copepods are among the most important constituents of the zooplankton in all areas and accounted up to 58–71% of the total zooplankton populations of the seas (Rezai et al., 2009). It is a kind of zooplankton that is important in energy transfer from primary producer to high tropic level (Soni & Thomas, 2014). Copepods are used primarily as live feeds for marine and freshwater species. The ability of small organisms, fish larvae, and bigger fish to feed on copepod is due to their small size and swimming behaviour which will encourage the feeding preferences.

Population of human increases and world starts to face less food supply each year (Godfray et al., 2010), and this also due to factors of declining of freshwater and marine food stock sources (Golden et al., 2016; McIntyre et al., 2016). Thus, fish farming or aquaculture activity is a solution to provide cheap and easy animal source food to human (Kobayashi et al., 2015).

However, the major constraint in aquaculture practice is the success in larval fish rearing that is related to the issue of live feed supply. The production of high-quality fish is constrained by the bottleneck of poor fingerling and larvae quality in many commercial marine hatcheries (Zaleha et al., 2012). In larval rearing, the important stage is during the first-feeding in which the larvae need to consume exogenous food after the nutrients in their yolk are used up.

Basically, larvae have an immature digestive system and unable to consume inert diet. The fish larvae detect any information around their environment by using their sensory organs including food which is detected via a wide range of chemical (olfaction and taste buds), visual (eyes) and mechanical (free neuromasts and lateral line) stimuli (Rønnestad et al., 2013). This is where live feeds such as copepods play an important role.

As fish aquaculture, corals aquaculture also being an important sectors nowadays because of their beautiful colors and variations, and at the same time coral reefs are important primarily as a coastal protection, food provider for many marine living organisms and one of the attraction for tourist from all over the world (Halpern et al., 2012). Coral reefs act as food provider because they can be a house for cyanobacteria, fungus and algae (Ainsworth et al., 2010) which are needed by the small plankton which later become food for fish larvae and bigger fishes. In marine ornamental trade (MOT), (Wabnitz et al., (2003) and Rhyne et al., (2012) reported on the important biodiversity of coral reefs to provide many species for exploitation (Delbeek, 2001; Rhyne et al., 2009).

Review by Tlusty et al., (2012), some public disagree with this kind of trade because of their concern about several impacts to over-harvesting, coral reef habitat degradation, and existence of exotic species (Smith et al., 2008; Jones et al., 2009; Rhyne et al., 2009; Schofield et al., 2009). Thus, the effort to develop *ex situ* (aquarium based) and *in situ* (sea based) coral aquaculture has been increased (Pomeroy et al. 2006; Osinga et al. 2012). There are two methods of coral restoration or culture technique to restore reefs either by active and passive methods including seeding production, larval rearing, and fragment transplantation (Edwards & Gomez, 2007; Edwards, 2010). Due to manipulation of basic requirements of the corals, nutrients supply becomes important to get success results in terms of growth, coloration, and shape.

Copepods are numerous (Figure 1.1), but there are three orders commonly cultivated for aquaculture which are cyclopoid, calanoid, and harpacticoid. Calanoid and cyclopoid are planktonic copepods inhabit water column, while harpacticoid is mainly epibenthic.

They are able to adapt to this fluctuation at the even they have the optimum level they preferred. Basically, harpacticoid body range between 0.2 and 2.5 mm (Caramujo, 2015). In aquaculture industry of fishes and shrimp, harpacticoid found to be the most important live feed for newly hatched larvae (Rhodes, 2003 and Drillet et al., 2011). Most species of salmomids, flatfish, drums, and gobies feed on harpacticoid as their diet up to ~35mm length (Drillet et al., 2011; Fleeger, 2005).

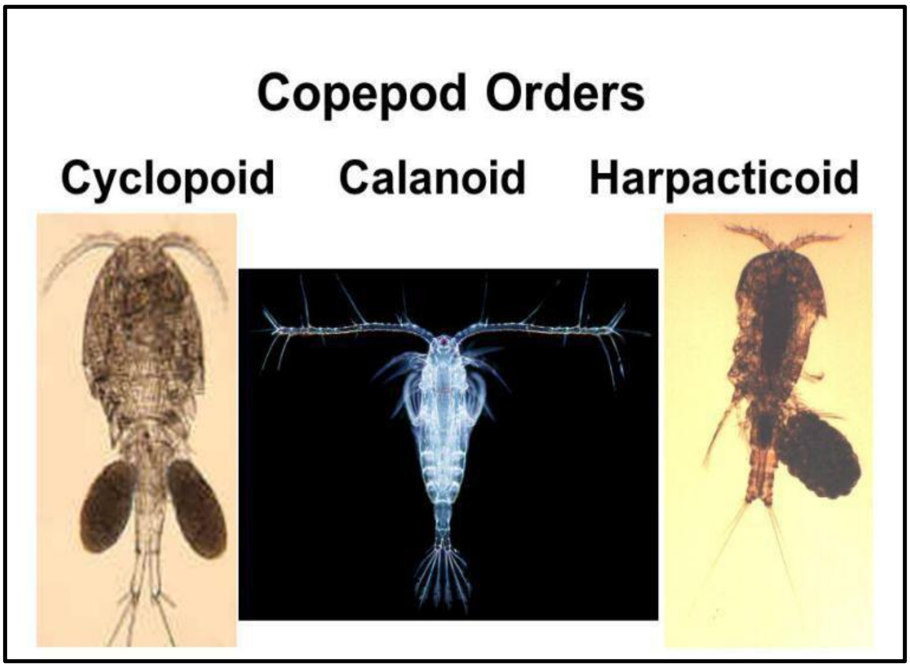


Figure 1.1 Different Order of Copepods
(Source: https://slideplayer.com/slide/1737929/)

1.1.2 Copepods as Live Feed

Nowadays, live feed experiment has become one of the important researches to keep on finding the most suitable live feed for each aquatic species. The usage of formulated diet for larvae is not recommended because the lack of sensory organ and immature digestive system of the larvae which makes them unable to sense the food and to digest it. The aquaculture and aquarium trade really need constant copepod supply in order to support rapid development of their industries (Støttrup, 2000; Payne et al., 2001) as to support the supply from human demand for fish due to declining of captured fisheries (FAO, 2008).

Copepods are also considered as a promising candidate of live feed for culture of marine and freshwater species in hatchery (Qin, 2013). Recently, a new technology has been improved and developed to increase the production of copepod until mass culture to be as live feed (Øie et al., 2017).

1.2 COPEPODS AND CORAL

As stated by Hazel & Fatimah (2015) copepods dominate the zooplankton of most tropical waters including Malaysia. Nakajima et al., (2008) found that copepod showed the highest abundance of the zooplankton communities over a short time intervals even in different size fraction/ stages in coral area of Redang Island, Malaysia. In addition, in coral reef ecosystem, Stella et al., (2011) reported there are at least 869 invertebrate species that have been described as coral-associated, of which 636 are crustaceans, with decapods and copepods being the dominant taxa.

A number of studies have shown that zooplankton is important dietary components of corals and related anthozoans *Stylophora pistillata*, (Ferrier- Pagès et al., 2003), *Stylophora pistillata*, *Galaxea fascicularis*, and *Tubastrea aurea*, (Houlbrèque et al., 2004). Up to now, several studies have explored the relationships between zooplankton feeding and corals growth rate. Due to several natural and anthropogenic threats, coral reefs worldwide reduced (Chavanich et al., 2005, 2009; Wilkinson, 2008; Burke et al., 2011). Culturing coral activity has become an important sector nowadays in order to minimize depending on wild corals as the main source of corals supply. According to Arvedlund et al., (2003), the difficulty to maintain corals in the aquarium and the requirement to replace it regularly will encourage the activity on harvesting from the wild. In addition, aquaculture provides corals for reef restoration projects, an aquarium trade, drug discovery and research (Delbeek, 2001; Leal et al. 2016). However, some techniques of coral restoration still remain at an experimental stage and can be success at scales up to few hectares only (Edwards, 2010). This is because a challenging area in the field of coral aquaculture or coral propagation is the supply of their nutritional needs. The nutritional requirement to maintain corals in a hatchery or in captivity remains the major constraint (Houlbreque and Ferrier-Pages 2009; Leal et al. 2014; Toh et al. 2014).

1.2.1 General Biology of Soft Coral and Hard Coral

Protopalythoa sp. (Phylum: Cnidaria; Order: Anthozoa) is a type of soft polyp coral. Species of the genus *Protopalythoa* consist of colonies of small, thick-walled polyps (Erhardt & Knop 2005). This thick wall has incorporated sand grains and other debris, which provide support and mechanical defense, as species in this genus do not produce a calcareous skeleton (Erhardt & Knop 2005).

This species, since it is in the taxonomic order Zoantharia, it has tentacles surrounding the oral disc in multiples of six (Erhardt & Knop 2005 and Ruppert et al., 2004) on a single polyp (Figure 1.2) total of 66 tentacles counted.

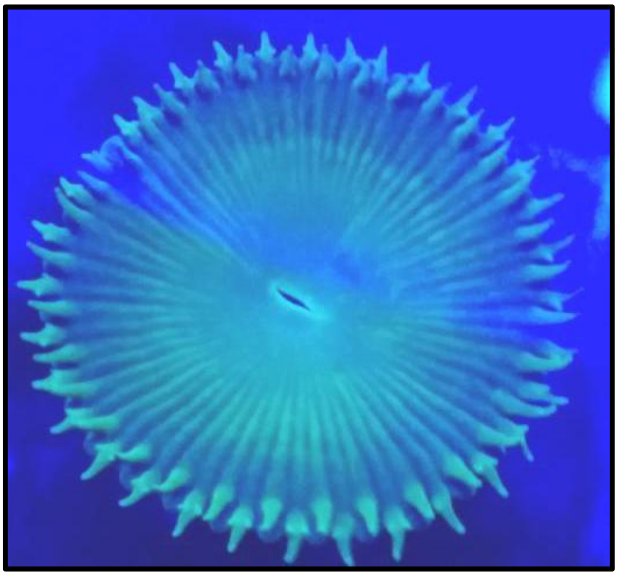


Figure 1.2 Upper View of *Protopalythoa* sp. has 66 Tentacles with the Mouth in the Middle

The *Acropora* sp. (Phylum: Cnidaria, Order: Scleractinian) (Figure 1.3) is a hard or stony coral. The major compositions of coral reef dominant by the sleractinian corals (Shinzato et al., 2011). They are form endosymbiosis relationship with *Symbiodinium* which allow them to fix Carbon Dioxide (CO_2) and form calcium carbonate skeletons (Shinzato et al., 2011).

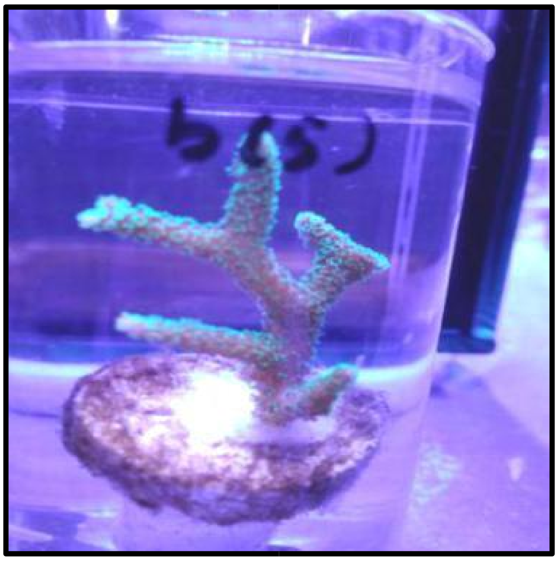


Figure 1.3 *Acropora* sp.

1.3 COPEPODS AND FISH LARVAE

Zebrafish (*Denio rerio*) (Hamilton, 1822) is a popular fish today because, besides being an ornamental species, Zebrafish is known as model organism for many clinical experiments (Lieschke & Currie, 2007), developmental biology and genetics (Grunwald & Eisen, 2002), environmental toxicology (Scholz et al.,2008), drug screening (Barros et al., 2008), evolution (Cañestro et al.,2007) and aquaculture (Ulloa et al., 2011). Due to this, production of Zebrafish under laboratory condition is important. A better understanding of nutritional requirements will help to determine which feed is the best for raising the zebrafish. According to Harper & Lawrence (2010), the zebrafish larvae is able to consume more efficiently when being offered with slow moving and small prey.

Besides, after the development of organs, they generally hunt for larger prey not only in the water column but from bottom surface. The most important character of the prey according to Chesney (2005) is their width. It is important to support the ability of the fish larva to ingest the prey since having limitation of the mouth size (~100µm).

1.3.1 Biology of Zebrafish

Figure 1.4 Zebrafish (Denio rerio)

Adult Zebrafish (Figure 1.4) mostly less than 4 cm total length (TL), the length front snout to the caudal fin. It has laterally compressed body shape and in fusiform, with the terminal mouth facing upwards. The reproductive biology, an approximate complete generation time is 3-4 months, females can spawn every 2-3 days laying several hundred of eggs per mating. Zebrafish able to spawn year round and will released hundreds of synchronous embryos which make the use of them as model animals become cost effective (Clift et al., 2014). The male fish usually will chase the female during mating season (Figure 1.5)

Figure 1.5 Couple of Zebrafish; Female (upper) and Male (bottom)

Zebrafish are euryphagous omnivores possessing a long intestine with a large absorption area and no true stomach (Ulloa et al., 2011). Basically, test feedingresearch begins at 5 day after hatch (DAH) where is the time of gas bladder inflation, the developmental time point that coincides with exogenous feeding in this species (Harper & Lawrence, 2010) in addition, after the fully development of their digestive tract during 4 DAH, they begin to swim freely and hunt for food on the next day (Stärhle et al., 2012).

Laboratory experiments have demonstrated that larval zebrafish prefer to associate with kin, using olfactory (Mann et al., 2003; Gerlach and Lysiak, 2006) and visual (Engeszer and Ryan, 2004). Figure 1.7 showed the embryo of the zebrafish and Figure 1.8 showed the 5 DAH larvae.

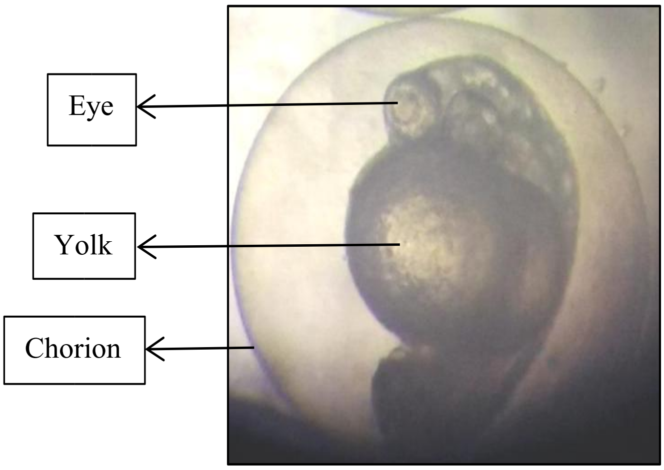
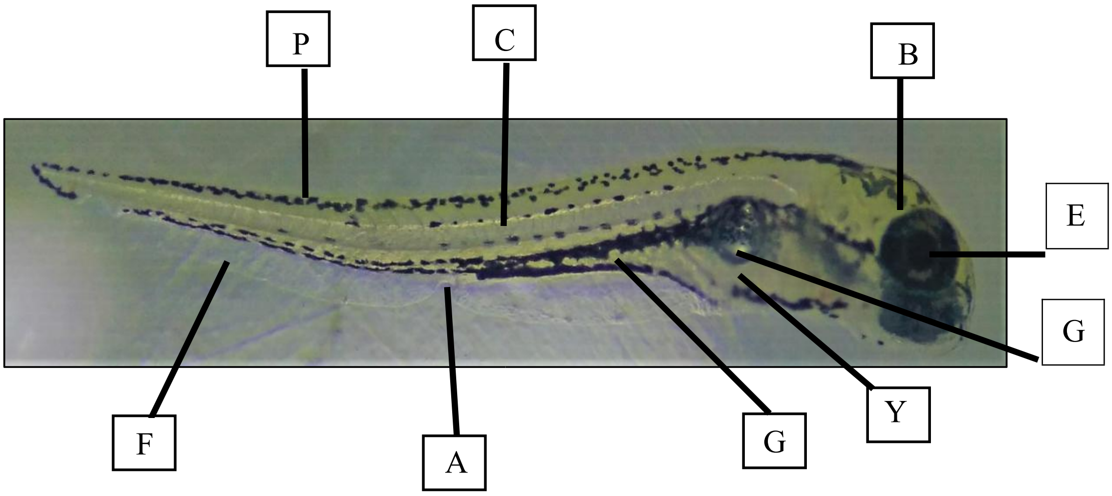


Figure 1.6 Zebrafish Embryo



A-anus; B- brain; C- chord; E- eye; F- fin; G- gas bladder; Gu- gut; P- pigment; Y- yolk

Figure 1.7 Zebrafish Larvae at 5 DAH

1.4 PROBLEM STATEMENT

Several studies proved that marine copepods were high in nutrients and able to be an early diet for cultured organisms. However, the possibility and effectiveness of marine harpacticoid copepod to become a live feed for aquarium corals and freshwater fish larvae was still unclear.

In corals culture industry, this finding is about to help in reducing the operation costs by allowing the culturist to manage the time to feed their corals. Hence, this may avoid food wastes if the coral not ready to be fed yet. Furthermore, to prove the effectiveness of cultured copepod to support the growth of the captive coral.

Besides, the suitability of marine copepod to be a live feed for freshwater fish species also important knowledge to increase the production of healthy larvae. Thus, the growth and survival of zebrafish larvae which are important in bioassay study can be improved.

1.5 SIGNIFICANT OF THE STUDY

The coral feeding still being one of the issues in improving the production of corals (Hii et al., 2009; Wijgerde et al., 2011). Coral feeding is one of the important factors to be examined because in order to make the aquaculture become cost-effective, is the determination of the food demand and the perfect time to feed them (Lin et al., 2002) as well as to maintain the water quality and avoid food wastage (Ali et al., 2010). Previously, aquaculture corals were fed with several diets such as micro dry food, pelagic diatom, and Artemia (Peterson et al., 2008).

Corals listed as multi-million-dollar marine ornamental trade which formerly harvested from the wild source (Thornhill, 2012; Tissot et al., 2010; Tlusty, 2002).