# EFFECT OF TEMPERATURE, SALINITY AND DIET ON THE GROWTH OF HARPACTICOID COPEPOD, *Euterpina acutifrons*, IN LABORATORY



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**BORNEO MARINE RESEARCH INSTITUTE**
**UNIVERSITI MALAYSIA SABAH**
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## ABSTRACT

### EFFECT OF TEMPERATURE, SALINITY AND DIET ON THE GROWTH OF HARPACTICOID COPEPOD, *Euterpina acutifrons*, IN LABORATORY

Harpacticoid copepod, *Euterpina acutifrons* is a potential live feed for marine fish larvae due to its high nutritional value and broad range of sizes through their various developmental stages. However, the mass production of this species remains elusive due to culture technique constraints and lack of knowledge of its biology and ecology. Thus, this study was conducted to provide information of the optimum temperature, salinity and suitable diet for culture of *E. acutifrons* in laboratory. Samples used in this study were isolated from Sepanggar Bay, Kota Kinabalu. Pure stock cultures of *E. acutifrons* were maintained in a temperature controlled chamber at 25±2°C with light intensity of 100$\mu$molm$^{-2}$s$^{-1}$ and photoperiod 12:12 light-dark. Observation on the life cycle of *E. acutifrons* shows that they took 9 days-12 days to complete. To determine the optimum parameters for culture establishment, three experiments were conducted. The parameters tested were temperature (25°C, 27°C, 29°C, 31°C), salinities, (5psu, 10psu, 15psu, 20psu, 25psu, 30psu, 35psu, 40psu, 45psu) and the diets; monoalgal species *Isochrysis* (Iso), *Tetraselmis* (Tet), *Chaetoceros* (Cs), *Nannochloropsis* (Nan) and mixed species or binary algal (Iso & Tet, Iso & Cs, Iso & Nan, Tet & Cs, Tet & Nan, Cs & Nan). The results show that optimum temperature was at 27°C with a mean population of 800±100 individuals/150ml and maximum growth of 0.438K, while at 31°C, all copepods were died. Optimum salinity was at 30psu with maximum growth of 0.8755K, similar to water salinity at sampling site. Population growth of *E. acutifrons* was generally higher when fed with binary algal diets compared to mono-algal diets. The best result was obtained from *E. acutifrons* fed with mixture of Cs & Iso with a final population of 1020±75 individuals/150ml and maximum growth of 0.463K. This could be due to content of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in both diets which are essential nutrients for crustaceans growth and development. This work has shown that *E. acutifrons* has the potential to be cultivated in laboratory to meet the demands for live feed in hatcheries.

Keywords: *Euterpina acutifrons*, copepod, live feed, temperature, salinity, diet

# ABSTRAK

*Euterpina acutifrons adalah harpactikoid kopepod yang berpotensi sebagai makanan hidup untuk larva ikan marin kerana nilai nutrisi yang tinggi dan kepelbagaian saiz. Namun demikian, pengeluaran besar-besaran spesies ini masih sukar kerana kekangan teknik kultur dan pengetahuan biologi dan ekologi spesies tersebut. Oleh itu, kajian ini dijalankan untuk menyediakan maklumat tentang suhu, saliniti dan diet yang optimum untuk pengkulturan E. acutifrons dalam makmal. Sampel yang digunakan dalam kajian ini telah diperolehi dari Teluk Sepanggar, Kota Kinabalu. Stok kultur tulen E. acutifrons dikultur dalam ruang suhu terkawal pada 25±2°C dengan intensiti cahaya sebanyak 100μmolm$^{-2}$s$^{-1}$ dan fotokala 12:12 gelap-terang. Pemerhatian terhadap kitar hidup E. acutifrons menunjukkan bahawa E. acutifrons mengambil masa selama 9-12 hari untuk melengkapkan kitaran hidup. Dalam menentukan parameter optimum untuk pengkulturan spesies ini, tiga eksperimen telah dilakukan. Parameter yang diuji adalah suhu (25°C, 27°C, 29°C, 31°C), saliniti (5, 10, 15, 20, 25, 30, 35, 40, 45psu) dan diet; spesies monoalga Isochrysis (Iso), Tetraselmis (Tet), Chaetoceros (Cs), Nannochloropsis (Nan) dan spesis campuran atau alga binari (Iso & Tet, Iso & Cs, Iso & Nan, Tet & Cs, Tet & Nan, Cs & Nan). Hasil eksperimen menunjukkan bahawa pertumbuhan populasi meningkat dengan peningkatan suhu. Suhu optimum adalah 27°C dengan min populasi 800±100 individu/150ml dan pertumbuhan maksimum 0.438K. Manakala, pada suhu 31°C, semua kopepod telah mati. Saliniti optimum pula adalah pada 30psu dengan pertumbuhan maksimum 0.8755K iaitu saliniti yang sama dengan kawasan persampelan. Pertumbuhan populasi E. acutifrons secara amnya tinggi apabila diberi diet alga binari jika dibandingkan dengan diet monoalga. Hasil yang paling baik diperolehi daripada E. acutifrons yang diberi makan campuran Cs & Iso dengan populasi akhir 1020±75 individu/150ml dan pertumbuhan maksimum 0.463K . Ini kemungkinan disebabkan oleh kandungan asid docosahexaeanoic (DHA) dan asid eicosapentaenoic (EPA) pada kedua-dua diet tersebut yang penting untuk pertumbuhan dan perkembangan krustasea. Kajian ini telah menunjukkan bahawa E. acutifrons mempunyai potensi dikultur dalam makmal untuk memenuhi permintaan makanan hidup di hatceri.*

*Kata kunci: Euterpina acutifrons, kopepod, makanan hidup, suhu, saliniti, diet*

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# CHAPTER 1

# INTRODUCTION

## 1.1 Status of Aquaculture and Fisheries

Seafood and fishes in particular are an important source of protein. Seafood sources are widely available worldwide and have high nutritional value (FAO 2004). Fish proteins contain sufficient amounts of all the amino acids used for growth and maintenance of body tissues. Fish protein is also relatively cheaper compared to other types of animal proteins making them an affordable source of protein for the poorer members of society (Tacon, 2000). These advantages have resulted in the rising importance of fishes as the main protein source globally.

According to FAO Fisheries (2010), in 2004, up to 92.4 million tonnes of fisheries product was captured globally to support almost 6.4 billion people. In 2009, the world population increased to 6.8 billion but supply of fisheries product decreased. The demand for fish is not only increasing because of the growing population but also due to a greater awareness of the importance of fish in the diet (Delgado, *et al.*, 2003). Global human consumption increased from around 104.4kg million tonnes in 2004 to more than 117.7kg million tonnes in 2009, resulting in an increase in the global per capita food fish supply from 16.2kg to around 17.2kg during that period. The fish supply from a stagnant capture fisheries will soon be unable to support the growing demand of almost 6.8 billion people in worldwide. Therefore, the aquaculture sector is seen as a solution to the ever growing fish demand (FAO, 2010).

Aquaculture is defined as a farming of aquatic plants and animals useful to human. It is an alternative source of fish that allows fish to reproduce to sustain the population of fishes. Aquaculture in Malaysia began in the early twentieth century with the culture of Chinese carps in the mining pools. The majority of Malaysian prefers to eat marine fish as compared to freshwater fish because marine fish taste better and is abundantly available in the market (Othman, 2008).

The fisheries production in Malaysia, was about 1.4 million tonnes from 2002-2004 with more than 90% of the contributions from captured fisheries sector and the rest from aquaculture. Although the contribution from aquaculture has been small, the production has been increasing from 9.9% of total fish production in 1998 to 13.2% in 2004. Similar to the global trend, aquaculture sectors in Malaysia are potentially increasing in importance as food fish supply (DOF 2004). The aquaculture industry has been targeted to produce about 800,000 tonnes by 2017 (New Straits Times, 2012). This has led to the Malaysian Government undertaking various efforts to improve aquaculture in the country.

## 1.2 Problems in Aquaculture

The major obstacle to produce fishes with commercial value is the utilization of an appropriate live feed during the first feeding phase of the larval cycle (Ohs *et al.*, 2009). First feeding of early larval stage or larviculture is important period for optimal development of marine fish larvae (Agh and Sorgeloos, 2005). During this stage, marine fish larvae require live feed with suitable nutritional value, constituting a suitable size range and stimulating a feeding response for the success of larviculture of many fish species (Ohs *et al.*, 2009). Therefore, it is important to have continuous access to live feed organisms during larviculture.

In Asia, most aquaculture production consists of capturing young or immature wild stocks and culturing them till marketable sizes before selling. This is an unsustainable practice as wild stocks are depleted and sometimes overfished in certain species due to the high demand. A viable alternative would involve the establishment of full cycle culture involving the usage of hatchery produced seeds for aquaculture. However, larviculture of most species remain the bottleneck and main obstacle in establishing full cycle culture. Mortality remains high in most species at larval stages with lack and unavailability of suitable feed at first feeding being the main problem (Sorgeloos and Leger, 1992).

At first feeding, body and mouth or gape size of most larvae are small which limits the size of food a larvae can capture to successfully survive (Chesney, 2005). Formulated feed, though nutritionally sufficient, have been unable to replace live feed with problems pertaining to the digestibility and palatability of the developed feed (Kolkovsky, 2001). The discovery of rotifer and its application in the marine fish culture has revolutionized and helped developed full cycle culture of many species. The brine shrimp, *Artemia*, also is frequently used as food for many marine fish larvae due to its availability commercially.

However, it has been reported that some species of marine fish which have commercial value cannot survive on both rotifers and *Artemia* (McKinnon *et al.*, 2003; Chesney, 2005; O'Bryen and Lee, 2005). The species include groupers *Epinephelus* sp. (Knuckey *et al.*, 2000; McKinnon *et al.*, 2003; Toledo *et al.*, 2005), snappers *Pagrus* sp. (Payne, 2000) and *Lutjanus* sp. (Ogle *et al.*, 2005; Phelps *et al.*, 2005; Su *et al.*, 2005). In contrast, the performance of copepods are excellent compared to the two live preys which are commonly used in aquaculture hatcheries (Stottrup, 2000; Lee, 2003). The advantages of copepods over rotifers and *Artemia* include size flexible as different naupliar, copepodid or adult stages can be chosen according to the mouth size of the larvae (Chen *et al.*, 2006), their nutritional content match the requirements of marine fish larvae (Stottrup, 2000; Evjemo *et al.*, 2003; McKinnon *et al.*, 2003) like DHA and EPA (Bell *et al.*, 2003) and their swimming pattern can stimulate stronger foraging responses in fish larvae (Stottrup, 2000).



## 1.3 Significant of Study

Several species of copepods have shown potential in rearing fish larvae in aquaculture. The preferred species were belong to genera *Acartia*, *Centropages*, *Eurytemora*, *Euterpina*, *Tigriopus*, *Tisbe*, *Oithona* and *Apocyclops* (Stottrup, 2003). The benefits of using copepods as live feed are well known, but reliable supply of copepods remains as a challenge for aquaculture due to technical constraints and is still in progress (Stottrup, 2000, Hagiwara *et al.*, 2001). The major drawback of copepods as live feeds for larviculture compared to *Artemia* and rotifers is their low productivity in mass culture (Milione and Zeng, 2007; Camus and Zeng, 2008).

For aquaculture purposes, temperature and salinity are two parameters used in culture that affect productivity of copepods. Knowing the optimum temperature and salinity will maximize the production of copepod. (Milione and Zeng, 2008). Meanwhile, selecting the most suitable diet for copepods will also increase the production of copepods. According to Ohs *et al.* (2009) copepods have not been successfully cultured using any diet other than live algae. Algae that are used as feed for copepods should be species that are easily available, simple to culture, do not tend to culture crash and support maximum fecundity and development of the copepod (Knuckey *et al.*, 2005; Lee *at al.*, 2006). Therefore, in order to maximize the production of copepods for use as prey, experiments were conducted to find the optimum parameters and suitable diet for the population growth of the copepods. In this study, *Euterpina acutifrons* was chosen due to its wide range of sizes (50µm-580µm), nutritional value and availability.

## 1.4 Objectives of Study

Having a constant supply of live feed such as copepod is one of the key factors for success in aquaculture. Therefore, the factor to be considered at first and foremost in live feed cultivation is how easily it can be propagated. Thus, this study was conducted to establish techniques on cultivation of selected harpacticoid copepod, *Euterpina acutifrons*. To achieve the aim of this study, the following specific objectives were outlined as below:

a) To determine the optimum temperature for the propagation of *E. acutifrons*
b) To determine the optimum salinity for the propagation of *E. acutifrons*
c) To determine the suitable diet for the propagation of *E. acutifrons*

## CHAPTER 2

## LITERATURE REVIEW

### 2.1 Live Feed

Live feed consist of minute zooplankton such as *Artemia* and rotifer and other minute organisms essential in the aquaculture food chain as first food (Faruque *et al.*, 2010). They are fed to altricial larvae, those that remain in undeveloped state when yolk is exhausted (Bengston, 2003). At this stage, they eat live feed because they have small mouth gape and their digestive systems are not fully developed (Govoni *et al.*, 1986). Both Kamler (1992) and Minami (1994) described this stage as the most critical period in the life cycle of fish where heavy mortality occurs during the onset of feeding to the exhaustion of endogenous feeding.

There are many characteristics of live feed which make them suitable as feed during the larval stage. Live feed can stimulate the feeding response of the fish larvae through their movement in the water (Bengston, 2003). This is because during the exogenous feeding, the eyes of the larvae have not fully developed, therefore, the swimming movement of the live feed will attract them (Krebs and Turingan, 2003). Other characteristic is the colour of the live feed that able to attract or stimulate the appetite of the larvae (Olivotto *et al.*, 2010). The bodies of some zooplankton are transparent and become visible to the larvae when they have pigmentation (Zaret and Kerfoot, 1975) and coloured like red as a result of their diets (Thatcher, 1988).

In comparison with formulated feed, Bengston (1993) described live feeds are more palatable due to its soft exoskeleton and contain high water content. These advantages generate good and fast feeding response in larvae of fish (Sorgeloos *et al.*, 2001). Besides that, live feed also must be in an appropriate size of the larvae mouth and gut length (Krebs and Turingan, 2003). Different stages of larvae require different sizes of live feeds. For example, red drum larvae eat small and less mobile rotifer before switching to larger and more elusive *Artemia* as they grow in size (Krebs and Turingan, 2003). Other than that, live feed also contain nutritional compositions essential for the optimal growth and survival of larvae (Payne and Rippingale, 2000; Hernandez Molejon and Alvarez-Lajonchere, 2003; Kanazawa, 2003). They can be enriched using algae before offering them to the larvae for a better result (Olivotto *et al.*, 2006). In aquaculture, various fishes and shellfishes at larval and early post larval stages favored live feed than artificial feed (Das *et al.*, 2007). Those live feed are obtained either collection from natural waters or cultured under controlled conditions.

## 2.2 Copepoda

According to Stottrup (2003), copepods are aquatic crustaceans that get its name from the word Greek *kope* meaning oar and *podos* meaning foots referring to that flat swimming legs that resemble the movement of a paddle. Copepod falls into the second largest Crustacean taxa and has ten orders with approximately 12,000 species that have been described worldwide (Huys and Boxshall, 1991; Humes, 1994). The most cultured copepod in aquaculture falls within three of the ten orders which are Calanoida, Harpacticoida and Cyclopoida (Stottrup, 2006; Stottrup, 2007 ) with their characteristics are summarize in Table 2.1. The Calanoida consists of 2300 species with 25% are freshwater, Cyclopoida with 2450 species and 20% are freshwater while Harpacticoid include almost 3000 species with 10% are freshwater (Bowman and Abele, 1982).

**Table 2.1: Differences between harpacticoid, calanoid and cyclopoid**

| Characteristics | Harpacticoid | Calanoid | Cyclopoid |
|---|---|---|---|
| Habitat | Mostly benthic. All types of waters | Mostly pelagic. Extremely common in marine systems, less in freshwaters | Mostly benthic, many pelagic. More common in freshwater, less in marine systems |
| Size | Smaller than calanoid and cyclopoid; 40µm- | Larger than harpacticoid and cyclopoid; 65µm- | Medium; 65µm-625µm (Hernandez Molejon and |

| | | | |
|---|---|---|---|
| | 620µm (Rhodes and Boyd, 2005) | 850µm (Gopakumar and Santhosi, 2009) | Alvarez-Lajonchere, 2003) |
| Body form | Slightly narrow | Ovoid | Wedge |
| Prosome | Prosome and urosome about the same diameter | Prosome distinctly wider than urosome | Prosome distinctly wider than urosome |
| Urosome | Single long spine often extends from the caudal rami | 4 segments, no abdominal appendages | 5 or 6 segments, sometimes fused |
| Body articulation | No division between body regions | After 6th last thoracic segments | Between 6th and 7th segments |
| 1st antennae (A1) | Shortest (fewer than 10 segments), biramous, rarely as long as cephalothorax | Longest (up to 27 segments), biramous, longer than prosome | Intermediate in length (6-17 segments), uniramous, shorter than prosome |
| Male | Both 1st antennae (A1) geniculate | Right 1st antennae (A1) geniculate | Both 1st antennae (A1) geniculate |



Copepods are widely distributed in marine waters but less in freshwater or estuarine habitats (Stottrup, 2003). Copepods that drift in water are planktonic, those that crawl on the ocean floor are benthic while those living in the groundwater are subterranean species. Different types of copepods have different modes of feeding. They can be herbivorous filter-feeders, detritivorous or omnivorous, feeding on phytoplankton, detritus and bacteria (Rieper, 1978; Huntley *et al.,* 1986). Some copepods species are even cannibalistic as reported by Lazzaretto and Salvato (1992) and Cutts (2003) preying on their own nauplii or copepodites. There are many commensal or parasitic species with both vertebrate hosts such as fish and whales, or invertebrate hosts such as mollusks, sponges and corals (Boxshall and Halsey, 2004).

### 2.2.1 Morphology

Copepod has an elongate segmented body shape based on their type of species or habitats. The body of the planktonic type is narrower while the benthic copepod is broader. The copepod body is divided into three parts consists of a head (cephalosome), a thorax (metasome) and an abdomen (urosome) as shown in Figure 2.1. The head carries a typical median eye and the first and second antennae. The eye is responsible for distinguishing between light and dark. The first pair of antennae is long and conspicuous and differs according to species whereas the second pair of antennae is used for swimming. The maxilliped is used when feeding to not only grasp but also break the food (Stottrup, 2003).

There are 5 pairs of appendages or legs attached to the metasome that are responsible for feeding and swimming. These appendages are useful for identification of species in taxonomical studies as the distribution of the spines and setae on the legs are species specific (Stottrup, 2003). The fifth pair of swimming leg or P5 is differs according to species. In some species of copepods, they could be modified or reduced in females, but enlarged and asymmetrical in males who use them to grasp female during mating (Stottrup, 2003). In other species, P5 may however be reduced or even absent (Dussart and Defaye, 2001).



The urosome is the most posterior end of the copepod, has no appendages and is narrower than the metasome. At the end of the urosome, there is a caudal furca and a pair of short rami. The two symmetrical caudal rami are ornamented with a hair like structure called setae. Male and females are easily identified by their morphological characteristics (Stappen, 1996) that usually develop during the later copepodid stages (Mauchline, 1998). In addition, one or both of the males' antennae is geniculate, meaning it is able to bend at an abrupt angle and is responsible to hold the female during mating (Ianora, 2005).

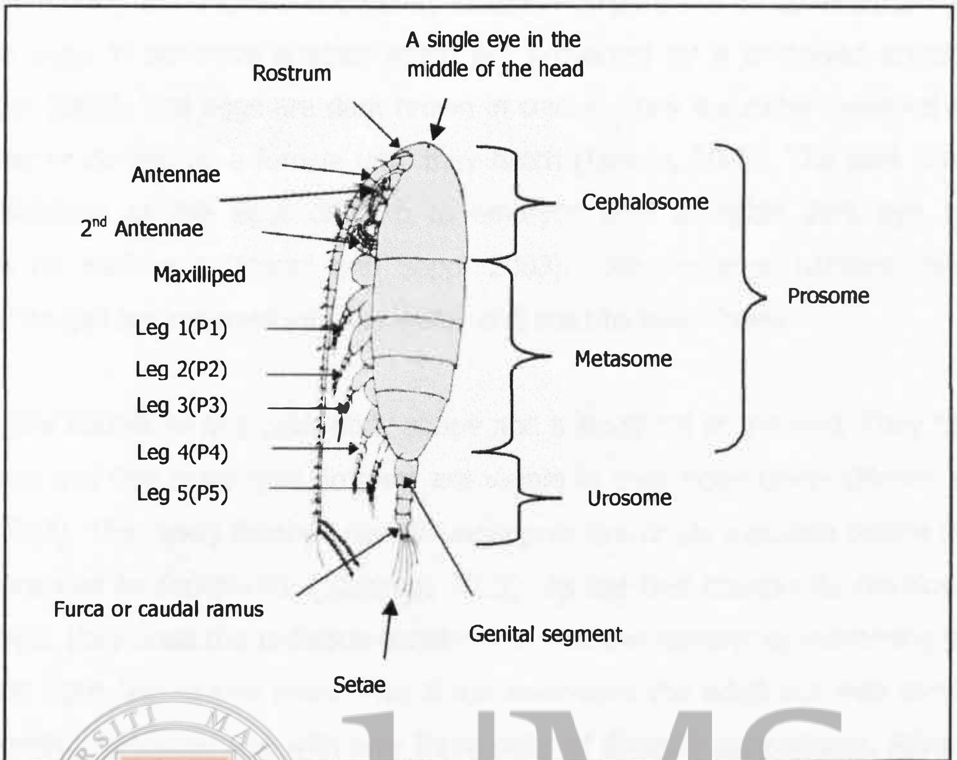


**Figure 2.1: Diagrammatic illustration of the external morphology calanoid copepod**
Source: modified from Mauchline, 1998



### 2.2.2 Life Cycle

Most of the reproduction observed in copepod was sexual reproduction requiring copulation. It occurred when male deposit a spermatophore which is a sac containing sperm near the genital aperture of the female (Stottrup, 2003). Once fertilized, egg was produced in egg sac which was different in the three common orders of copepod used in aquaculture. The females were considered to be matured when they were carrying their first egg sac (Rhyne *et al.*, 2009). Cyclopoids and harpacticoids have their eggs contained within one or two egg sacs (Huys and Boxshall, 1991). Both copepod females carry their eggs by attaching the egg sacs to their genital segments until they hatch. While in calanoid, the eggs are not contained in a membrane but stick on to each other as an egg mass and remain attached to the female (Mauchline, 1998).

Copepods generally have complex life cycles characterized by 13 life stages (egg, 6 naupliar, 5 copepodid and adult) as seen in Figure 2.2. Once fertilized, they produce eggs in spherical shapes which are protected by a chitinised envelope (Stottrup, 2003). The eggs are dark brown in colour. They are either released into the water or carried by a female until they hatch (Ianora, 2005). The dark brown colour lightens as the eggs develop to embryos until a visible dark eye spot appears on each egg (Marini and Sapp, 2003). As the eggs hatches, newly hatched nauplii are released into the water and start to swim freely.

The nauplii have a pear body shape and a small tail at the end. They have no thorax and five small lipid droplets are visible in their body cavity (Marini and Sapp, 2003). The newly hatched nauplii undergoes five or six moulting before they metamorphose to copepodite (Stottrup, 2003). As the first copepodite develop to next stage, they shed the previous exoskeleton and the number of swimming legs increases from one to five pairs. This stage resembles the adult but with simple, unsegmented abdomen and with only three pairs of thoracic appendages. After 13 developmental stages, the copepodite finally takes on the adults form and completes a life cycle (Stottrup, 2003).