PROTOZOA CULTIVATING METHOD FOR ASIAN SEABASS LARVAE USING VEGETABLES AND DRY FISH POWDER

BY

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ABSTRACT

Seed production of marine finfish in Malaysia is still facing problem particularly live feed for starter diet. Aquaculturists are currently using rotifers of the S or SS type, which have sizes from 100 to 200µm. Early stage larvae of some marine finfish species select feeds less than 100µm. In nature, protozoa are consumed by the early stage of marine finfish larvae. Protozoa is a common term for aquatic microorganisms that has been identified to be suitable live feed, as their body sizes are less than 100µm. However, there are very limited studies regarding protozoa culture methods.

Thus, the objective of this study is to examine the density changes of Different Protozoa Species (DPS) using batch and continuous culture methods. Then, Asian seabass larvae were reared using live feeds; rotifers, DPS, and *Euplotes encysticus*.

In batch culture method, DPS and one protozoa species; *Euplotes encysticus* were cultured for the duration of eight days. The diets for DPS and *E.encysticus* culture were given only at the first day of experiment. DPS and *E.encysticus* were continuously cultured for the duration of 11 days in the continuous culture method. Every day, 10%, 20%, and 30% of cultured water was harvested and replaced with new water and diets for DPS and *E.encysticus*. In Asian seabass larval rearing experiment, four types of live feed; rotifer, DPS, *E. encysticus*, and mixed (rotifer, DPS, and *E. encysticus*) were given to larvae from 2 to 10 day-after-hatching. Number of seabass larvae in each group was counted every day and their survival rates were determined.

The result of batch culture method showed that three and two days were optimum to produce the highest density (2463 and 1037 ind/mL) for DPS and *E.encysticus.* Next, the result of continuous culture method showed that 10% and 20% were optimum harvesting amount to produce the highest density of DPS and *E.encysticus.* The discussion of comparison between batch and continuous culture methods showed that the batch culture was the best method for DPS and continuous culture was the best method for *E.encysticus.* The recommended live feed for the starter diet of Asian seabass was mixed live feeds which consist of rotifer, DPS, and *E.encysticus.*

In conclusion, batch culture method is recommended method to culture DPS, continuous culture method is recommended for *E.encysticus*, and mixed live feed is recommended for the larval rearing of Asian seabass.

خلاصة البحث

إن إنتاج يرقات الأسماك البحرية في ماليزيا ،ولا سيما الأعلاف الحية لنظام غذائي أولي، لا يزال يمثل تحديًا. الروتيفر من النوعS أو SS،بمقاسات تتراوح من 100 إلى 200 متر يستخدمها مربو الأحياء المائية حاليًا. تفضل بعض يرقات الأسماك البحرية الوجبات التي يقل طولها عن 100 متر. في الطبيعة ، تستهلك الأوليات البروتوزوا في المراحل المبكرة من يرقات الأسماك البحرية .نظرًا لأن أحجام أجسامهم أقل من 100 متر، فإن البروتوزوا هي كلمة شائعة للكائنات الحية الدقيقة المائية التي تم اكتشافها كعلف حي مقبول. ومع ذلك ،لا يوجد سوى عدد قليل من التحقيقات حول طرق زراعة الأوالي. الهدف من هذه الدراسة هو استخدام طرق الاستزراع الدفعي والمستمر لتقييم تغيرات الكثافة لأنواع مختلفة من البروتوزواDPS . تم بعد ذلك إطعام يرقات القاروص الآسيوي الروتيفر الحيDPS ، ، و *"Euplotes encysticus"*. تمت زراعة DPS ونوع واحد من البروتوزوا , *Euplotes encysticus*،لمدة ثمانية أيام في نهج الاستزراع الدفعي. تم إعطاء النظام الغذائي المزروعDPS و *E.encysticus* فقط في اليوم الأول من التجربة. في نهج الاستزراع المستمر تمت تربية DPS و *E.encysticus* بشكل مستمر لمدة 11 يوم، كل يوم يتم أخذ 10٪ و 20٪ و 30٪ من المياه المستزرعة وتزويدها بالمياه العذبة والأعلاف لـ DPS و *E.encysticus* على التوالي. تم استخدام أربعة أنواع من الأعلاف الحية في تجربة تربية يرقات القاروص الآسيوية: الروتيفيرDPS ، , E. من 2 إلى 10 أيام بعد الفقس ،تم إعطاء اليرقات الروتيفير وDPS و *E. encysticus* بالإضافة إلى خليط (الروتيفير ، DPSو *E. encysticus*). تم حساب عدد يرقات القاروص في كل مجموعة ، وتم حساب معدلات بقائها. أنتج أسلوب الاستزراع الدفعي أقصى كثافة (2463 و 1037 إندون / مل) لـ DPS و *E.encysticus* بعد ثلاثة أيام ويومين على التوالي. أظهرت نتائج طريقة الاستزراع المستمر أن كميات الحصاد 10٪ و 20٪ كانت الأمثل لإنتاج أعلى كثافة منDPS و *E.encysticus*. تم العثور على الاستزراع الدفعي ليكون أفضل نهج لـ DPS بينما وجد أن الاستزراع المستمر هو أفضل طريقة لـ *E.encysticus* بعد مقارنة طرق الاستزراع الدفعي والمستمر. تم نصح الأعلاف الحية المختلطة المحتوية على الروتيفير و DPS و *E.encysticus* لبدء النظام الغذائي لأسماك القاروص الآسيوي. للتلخيص، تم تحديد طريقة الاستزراع الدفعي لـ DPS ، ويوصى باستخدام طريقة الاستزراع المستمر لبكتريا *E.encysticus* ، ويوصى باستخدام العلف الحي المختلط لتربية يرقات القاروص الآسيوية.

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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This thesis is specially dedicated to:

The loves of my life

Abah
(Mohd Shahidin bin Kitan)

Mak
(Norma binti Ariffin)

Families and Friends

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LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization
SR	Survival Rates
DPS	Different Protozoa Species
8DBC	Eight day batch culture

LIST OF SYMBOLS

%	Percent
°C	Degree Celsius
G	Gram
Kg	Kilogram
L	Litre
Ml	Millilitre
µl	Microlitre
Ppt	Parts per thousand
SD	Standard deviation
SE	Standard error

CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

According to Richards et al. (2001), Richards et al. (2003), Yaowu et al. (2009), Beveridge et al. (2013), and Ibrahim et al. (2014), fish is one of the sources of animal protein for human consumption. The demand for fish has increased due to the increasing of human population year by year. The two sectors that provide fish for human are fisheries and aquaculture.

In 2018, the Food and Agriculture Organization (FAO) reported that fisheries are not capable of providing sufficient supplies of fish for humans. In fisheries, the fish is captured in the wild such as in the sea, rivers, or lakes. More fish are captured from the wild to fulfill the demand of fish which leads to overfishing, thus causing the fish resources to decrease (Beveridge et al., 2013).

Aquaculture is the farming of fish, mollusks, crustaceans and aquatic plants under controlled freshwater and seawater conditions (Food and Agriculture Organisation of the United Nations, 1988). The production of fish can be increased by aquaculture (Adewumi and Olaleya, 2011). FAO (2018) reported that aquaculture provided 48% of the total fish production in 2016. Aquaculture can be done in marine water and freshwater environment. Many fish species can be cultured in the whole of the world. Aquaculture can answer the demand of seafood. It also can generate income for fish farmers. According to Pomerey (2002), Apines-Amar et. al. (2012), Jerry (2013), and Shapawi et. al. (2014), marine finfish such as seabass, groupers, and snapper can generate high income due to their high demand in many countries in

Southeast Asia region. Ibrahim et al. (2014) and Yusoff et al. (2016) showed that there are many species being cultured in Malaysia includes grouper, seabass, red tilapia, African catfish, sutchi catfish and climbing perch.

Malaysia is located in equatorial region and belongs to a tropical rainforest climate. Marine finfish larvae in tropical area have small size bodies and small size mouths. This is because of the theory of fish biology, that fish spawn the small size and large number of eggs in high water temperature area (Bone et al. 1996). Based on the studies done by Doi et al. (1997), Nagano et al. (2000), and Hagiwara et al. (2014), they said that groupers, Napoleon wrasse and snappers have small bodies and small mouth openings in their early larval stage, however, these fish are commercially important in tropical regions.

High mortality occurs during marine finfish early larval stage (Ch'ng and Senoo, 2008). The newly hatched larvae are small in size and their mouth sizes also small at their first feeding in tropical region. Early stage larvae of some marine finfish species, such as grouper and snapper select feeds less than 100 μm. So, suitable live feeds with smaller body size than 100 μm are needed for seed production. Therefore, seed production of marine finfish is more difficult in tropical regions than in temperate regions due to lack of suitable live starter feed in the tropical regions.

As a result, the newly hatched larvae of marine finfish at tropical regions are smaller in size than larvae at temperate region. When the larvae are ready to eat, usually they are given rotifer as their first feeding diet. The rotifer was given to the larvae to avoid starvation, thus increasing the survival rates of the larvae.

However, the small mouth size of the larvae makes the larvae difficult to feed on rotifer. So, protozoa which is single-celled organisms that have smaller size than rotifer, but highly nutritious used by aquaculturist as an alternative of first feeding diet for marine finfish larvae.

First feeding diet is very important to the larvae as it will determine the future of the larvae survival rates. Enough feeding during the early stage of larvae also important to ensure that the larvae are not starves. As a result, the larval survival rates will increase accordingly. Thus, many important marine finfish can be produced in future to fulfill the protein demand of human.

1.2 PROBLEM STATEMENTS

Currently, aquaculturists use S or SS type rotifers, which have size from 100 to 200 µm for marine finfish larvae as their starter diet. However, as the mouths of larvae of some marine finfish (such as grouper and snapper) are smaller than rotifer, the larvae are not able to feed on rotifer. This condition causes the larvae to starve and show low survival rates. Trecee and Davis (2012) stated that some species of larvae with a smaller mouth gap need smaller prey than rotifers. Ohs et al. (2013) reported that some marine fish larvae have small mouth gap, so they can eat only small size feeds.

Protozoa which have various body sizes, approximately around 20 to 100 µm have been identified to be suitable live feeds for larval first feeding. This is due to the smaller sizes of protozoa that can fit into the mouth of some marine finfish larvae such as grouper and snapper. Thus, providing the suitable live feeds for the larvae can increase their survival rates. However, as protozoa studies are still limited, there is no study about types of culture methods and efficient methods for the mass production of protozoa.

Some previous studies showed that the survival rates of marine finfish larvae were improved using protozoa as starter diets. According to Rhodes and Phelps (2004), they got success in rearing red snapper using protozoa as a starter diet. In addition, Nagano et al. (2000) also studied larval rearing about grouper *Epinephelus septemfasciatus* using ciliated protozoans. This is due to the small mouth sized of marine finfish that only accept the small feeds at their first feeding. Ohs et al. (2013) reported that some marine fish larvae that have small mouth gap cannot eat large live food organisms. Sufficient amount of protozoa were needed as they are important live feed for the larvae first feeding.

Thus, it is important to study the culture methods of protozoa and the efficient methods for a mass production of protozoa. There are very limited studies regarding starter diet of marine finfish using protozoa and there are still no studies about types of culture methods for protozoa. Researchers only used batch culture to culture protozoa. So, we still do not know the efficiency of other culture method, for example continuous culture method. So, in the present study, protozoa were cultured by both batch and continuous culture methods. After that, the larval rearing experiment of one type of marine finfish larvae; Asian seabass larvae was conducted using rotifer and protozoa as their first feeding to determine their survival rates.

1.3 SIGNIFICANCE OF THE STUDY

The current experiments are essential to determine the most efficient method to culture different protozoa species (DPS) using batch or continuous culture method. By obtaining the most efficient culture method of DPS, the sufficient amount of live feed can be served for starter diet of marine finfish larvae. The usage of DPS as starter diets for marine finfish larvae can improve the larval survival rates. Then, the survival and also growth rates of these fish species could be increased. Results from these experiments can be used by fish farmers to increase the production of marine finfish.

1.4 RESEARCH HYPOTHESIS

i. Batch culture method will produce higher total amount of different protozoa species (DPS) in 3 days of cultivation period.

ii. Continuous culture method will produce higher total amount of different protozoa species (DPS) with 10% of harvesting amounts per day.

iii. Survival rate of Asian seabass larvae will be higher using rotifer with different protozoa species (DPS) as their first feeds than only rotifer feeding group.

1.5 RESEARCH OBJECTIVES

i. To examine the density changes of different protozoa species (DPS) and *Euplotes encysticus* by the batch culture method for 8 cultivation days.

ii. To examine the density changes of different protozoa species (DPS) and *Euplotes encysticus* by continuous culture method with different harvesting groups of 10%, 20%, and 30% per day.

iii. To determine which method between batch and continuous producing the higheest number of different protozoa species (DPS) and *Euplotes encysticus.*

iv. To determine the survival rates of Asian seabass larvae using different starter diets which are rotifer, different protozoa species (DPS), and *Euplotes encysticus*.

CHAPTER TWO

LITERATURE REVIEW

2.1 LIVE FEED FOR FISH SEED PRODUCTION

Live feed is very important as the foods for fish larvae. Many kinds of fish larvae need the live feed. There are many types of live feeds that are including protozoa, rotifers, *Artemia*, and copepods. The survival rates of marine fish under artificial conditions will increase with the proper nutritional composition and suitable size of live feed (Ohs et al., 2013). The live feeds are much more acceptable compared to artificial feeds (Das et al., 2012). Das mentioned that the artificial feed cannot be given to the larvae of fish due to the bigger size of artificial feed compared to the mouth gap of fish larvae. Moreover, artificial feeds are not moving and cannot be detect by the fish larvae. According to Ohs et al. (2013) live feeds have essential nutrients that important for marine fish larvae. According to Walford et al. (1991), Desvilettes et al. (1997), Zhukova & Kharlamenko (1999) and Decamp et al. (2001) enough live feed with ideal size was needed by fish larvae for growth.

2.2 DIFFERENT TYPES OF LIVE FEED FOR FISH SEED PRODUCTION

As been mentioned earlier, there are many types of live feed that exists in nature and also in artificial conditions (aquaculture).

2.2.1 Copepods

Treece and Davis (2000) stated that the common zooplankton in both freshwater and brackishwater are copepods. Most of larvae and juveniles of many finfish and crustaceans in natural is depend on copepods as their food. According to Stottrup (2000), Molejon and Lajonchere (2003), and Kleppel et al. (2005), marine copepods contain many important nutrients such as protein, lipid, carbohydrates, and enzymes that vital for the larval survival, growth, digestion, and metamorphosis. Nauplii, one of the larval stages of the copepods have been an appropriate diets for many marine finfish larvae (Jepsen et al., 2013). The development stages of the copepods showed in Figure 2.1. Cutts (2003) and Drillet et al. (2006b) reported that harpacticoid copepods are epi-benthic copepods (pelagic nauplii) was proven to has many nutritional qualities compared to Artemia and rotifers. Calanoid copepod, *Acartia sp.* was successfully fed by the red snapper (*Lutjanus argentimacula*) compare to the rotifers that bigger in size (Schipp et al., 1999). Rajkumar & Vasagam, 2006 reported that calanoid copepod, *A.clausi* was useful to be a live-food for L.calcarifer at the age of 14 day-after-hatched.

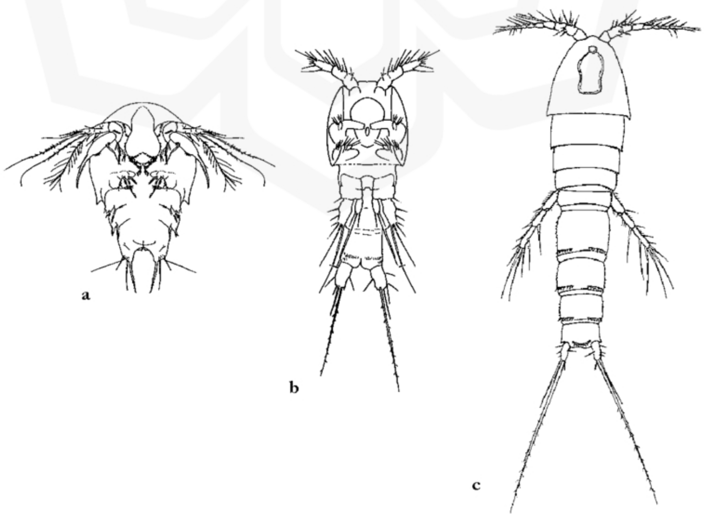

Figure 2.1 Copepods development stages

Gopakumar and Santhosh (2009) reported that most of marine fish larvae consume marine copepods in nature because copepods contain high levels of DHA and PUFA. Some aquaculturists used copepods as live feed in hatcheries. However, Olivotto et al. (2005) reported that the copepods which are fed by the larvae in natural environment not suitable to be used in aquaculture because copepods are difficult to culture. Moreover, copepods are too big to be a first feeding for marine finfish larvae that usually have small mouth gap. The sizes of copepod are around 0.2 to 2.5 mm (Caramujo, 2015; Daniela et al. 2010).

2.2.2 Protozoa (Ciliates)

Ciliates are important as the first-feeding for fish larvae because ciliates are abundant in coastal waters (Kamiyama 1994). Study by Fukami et al. (1999) reported that the gut contents of most fish larvae in Japanese waters contained protozoa, proving that some of the fish larvae consumed protozoa as their starter diet. There are also some previous studies that mentioned about the role of protozoa as a good starter diet for fish larvae in hatcheries. According to Nagano et al. (2000) fish larvae can feed on ciliates at first-feeding in hatcheries. This statement was supported by Rhodes and Phelps (2006), *Fabrea salina* which is one type of ciliates was used in their experiment as the first feeding of Red snapper, *Lutjanus campechanus* which has small mouth gapes. According to Thompson *et al.* (1999) the larvae of Carpas shrimp, *Penaeus paulensis* have better growth rates when fed with flagellates and ciliates. Curds (1975) stated that there are various range of body sizes for protozoa, which are around 25 to 300 µm. Most of the ciliates have the smallest size among live feeds in nature (Taniguchi 1978). The small body sizes of protozoa have an advantage that the