

**THE EFFECTS OF COD OIL JUICE CONCENTRATION ON THE
SURVIVAL AND GROWTH RATE OF HOI TAI KAI, *Lutjanus rivulatus*
LARVAE**

IVAN KOH CHONG CHU

**PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH**

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Disahkan Oleh

Shigeharu Senoo
(TANDATANGAN PUSTAKAWAN)

PROF. DR. SHIGEHARU SENOO
Nama Penyelia

Ivan Koh Chong Chu
KOH CHONG CHU
IS2003-3519

Ivan Koh Chong Chu
(TANDATANGAN PENULIS)

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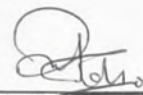


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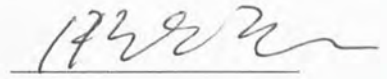
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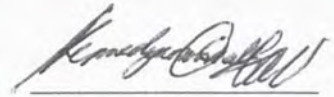
AUTHENTICATION

AUTHENTICATED BY MEMBERS OF DISSERTATION COMMITTEE

1. SUPERVISOR
(Prof. Dr. Shigeharu Senoo)

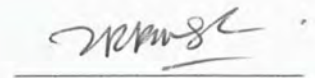


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(Mr. Kennedy Aaron Aguol)

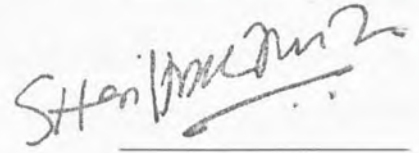


3. EXAMINER 2
(Dr. Sitti Raehanah)

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4. DEAN
(Prof. Madya Shariff A.K Omang)



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UMS, Kota Kinabalu
March 2006



ABSTRACT

Blubberlip snapper, *Lutjanus rivulatus* is a popular marine fish that taste like chicken. Commercial culture of *Lutjanus rivulatus* has been hampered by the mass mortality in early larvae stage. This experiment uses cod oil juice as first feeding. A total of 2 experiments were conducted. Each experiment had 2 replicates. In the first experiment, 100 tails of 1dAH larvae were cultured respectively in 7 liter aquarium at a salinity of 32 ppt and water volume of 6 liters. The larvae were fed cod oil juice daily at concentrations of 0, 0.2, 0.4, 0.6, 0.8ml/l, respectively in triplicates. In the experiment 0ml/l concentration functioned as the control. Results indicated that mean survival rate was the highest in 0.4ml/l. Larvae survived till 6dAH only. In second experiments, all culture conditions were similar except the concentration of cod oil juice fed to larvae. In this experiment, larvae were fed cod oil juice daily at concentrations of 0, 0.3, 0.4, 0.5, 0.6 and 0.7ml/l, respectively in triplicates. Results indicated addition of cod oil juice at any concentrations helped improve survival rate and growth rate. Mean survival rate and mean growth rate was the highest in 0.4ml/l which was not significantly different from 0.3ml/l. The lowest mean survival and growth rate was in control group in which no cod oil juice was added. It was significantly different ($P < 0.05$) than all the other concentrations. However, larvae only survived until 6dAH in all concentrations. This study recommends that the addition of 0.4ml/l of cod oil juice in *Lutjanus rivulatus* larvae culture as supplementary feed for increased survival and growth rate.



ABSTRAK

Blubberlip snapper, *Lutjanus rivulatus*, merupakan sejenis ikan air masin popular yang rasanya seperti ayam. Pengeluaran secara komersil bagi *Lutjanus rivulatus* telah dihalang oleh jumlah kematian yang besar pada peringkat awal larvae. Experimen in menggunakan 'cod oil juice' sebagai makanan pertama bagi larvae. Sejumlah 2 experimen telah dijalankan. Dalam 2 experimen pertama, 100 ekor 1dAH larvae telah dikultur di dalam sebuah akuarium berkapasiti 7l pada saliniti 32ppt and isipadu air sebanyak 6 liter. Larvae diberi cod oil juice pada kepekatan 0, 0.2,0.4,0.6 dan 0.8ml/l, dengan setiap kepekatan cod oil juice mempunyai 3 replikat. Di dalam experimen ini, kepekatan 0 berfungsi sebagai control. Keputusan menunjukkan bahawa penambahan cod oil juice pada sebarang kepekatan membantu meningkatkan kadar kemandirian dan kadar pertumbuhan. Min kadar kemandirian and min kadar pertumbuhan adalah tertinggi pada kepekatan 0.4ml/l. Min kadar kemandirian and min kadar pertumbuhan yang terendah pula adalah di kumpulan control di mana tiada cod oil juice ditambah. Ia mempunyai beza yang signifikan ($P < 0.05$) dengan kepekatan yang lain. Namun demikian, larvae hanya berjaya hidup sehingga 6dAH di dalam semua kepekatan. Eksperimen in mengesyorkan bahawa penambahan 0.4ml/l cod oil juice di dalam pengkulturan larvae sebagai makanan tambahan untuk peningkatan dalam kemandirian dan pertumbuhan larvae.



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LIST OF ABBREVIATIONS

°C	degree centigrade
%	percentage
cm	centimeter
mm	millimeter
kg	kilogram
g	gram
ml/l	millilitre per liter
d AH	day after hatching
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
PUFAs	polyunsaturated fatty acids
HUFAs	highlyunsaturated fatty acids
DO	dissolved oxygen
ppt	part per thousand
IU	International unit
sp.	species
UMS	Universiti Malaysia Sabah
TL	total length
BW	body weight
COJ	cod oil juice



CHAPTER 1

INTRODUCTION

Fish has always been a main source of food for the people in the world. In 2002, up to 93.2 million tonnes of fisheries product was captured globally providing up to 6.2 billion people with fish. However, the world's population has been increasing more quickly than the total food fish supply. The growth rate of the world's population has been increasing exponentially rather than geometrically while capture fisheries production have remained relatively stable with no signs of growth in the last few years. (FAO Fisheries information, 2003)

In Malaysia, the situation is similar, as production of marine capture fisheries has stagnated. Total marine fish landings increased marginally by only 0.01% from 1,272,078 tonnes in 2002 to 1,283,256 tonnes in 2003. In terms of value, there was even a decrease of 4.6% from RM 4.21 billion in 2002 to RM 4.01 billion in 2003. (Annual Fisheries Statistics, 2003). With the current state of fisheries production being unable to answer the growing needs of the population, the onus has turned to aquaculture as the answer.



Aquaculture in Malaysia started in the early twentieth century, with the culture of Chinese carps in the mining pools. In 1984, the Government of Malaysia formulated the national Agriculture Policy for the development of agriculture, which included fisheries and aquaculture. This policy benefited the freshwater culture in the country (Ang, 1990).

In 2003, the aquaculture sector in Malaysia recorded a production of 196,874 tonnes, which constituted about 13.27% of the total fish production, increasing by 6.23% from the production of 2002. Value wise, the aquaculture sector increased by 8.42% from RM 1,081.24 million in 2002 to RM 1,173.31 million in 2003. Out of a total of 21,114 fish farmers/culturist involved in the aquaculture industry in 2003, only 21.0% were involved in the seawater sector. However, the bulk of the aquaculture production was contributed by the marine brackish water and marine sectors with up to 74.63% or 146,926 tonnes of the total aquaculture production in 2003. In marine fish culture, the major species cultured from brackish water pond were snappers and groupers which contributed to 51.54% to this sector. (Annual Fisheries Statistics, 2003).

Among the marine fish, snappers are a popular group of fish that have a high market demand. The snappers are a large and diverse group of robust-bodied, carnivorous fishes. Snappers are fishes that belong to the family of Lutjanidae, order of Perciformes and class of Osteichthyes. There are 21 genera and a total of 125 species of snappers and there are many species that are commercially important. Examples of species of fisheries importance are red snapper (*Lutjanus malabaricus*),



John's snapper (*Lutjanus johni*) and Russel's snapper (*Lutjanus russeli*) (Annual Fisheries Statistics, 2003)

For this experiment, *Lutjanus rivulatus* is the species of fish being used. The common name of *Lutjanus rivulatus* is blubberlip snapper, while it is known as Ketambak in Malaysia (Annual Fisheries Statistics, 2003), "Namifuedai" in Japan (Akazaki, 1984), Gaga in Indonesia and "Maori Seaperch" in Australia (Randall, 1990). *Lutjanus rivulatus* is widely distributed with it being found in the Indo-Pacific Sea from East Africa to Tahiti, north to southern Japan and also in the southern waters of Australia. Its natural habitats are among the coral reef areas (Randall, 1990; Akazaki, 1984).

The popularity of *Lutjanus rivulatus* is mainly due to its good taste. In Asia, especially in countries that have a substantial Chinese population like Taiwan and Hong Kong there is a high demand for this species. It is rumored to taste like chicken thus the name "Hoi Tai Kai" which means sea chicken (Senoo *et. al.*, 2001). In wedding banquets and birthday dinners it is a popular and common. *Lutjanus rivulatus* fetches a high price in the local market in Malaysia, with the wholesale price of RM30-70 (US\$7.89-18.42/kg) (Randall, 1990) and restaurants selling it at around RM80-120 per kilogram. This price is three times more expensive than *L. argentimacultus* of the same genus of *Lutjanus*, which is the most important maricultural fish in Southeast Asia (Randall, 1990).

With a high price and high demand, *Lutjanus rivulatus* is a good potential for culture. However, in Malaysia there is no aquaculture production of *Lutjanus*



rivulatus. (Annual Fisheries Statistics, 2004). This is due to the difficulty in seed production of *Lutjanus rivulatus* which makes the seed supply of *Lutjanus rivulatus* nonexistent. Seed production of *Lutjanus rivulatus* is difficult mainly due to the problem at first feeding.

Conventional seed production of marine fishes in commercial hatcheries is dependent on live on live feed such as rotifer and *Artemia* (Cahu *et al.*, 2001). The rotifer *Brachionus* has been used worldwide as a live feed for the initial stage of larval rearing of marine fishes. There are 3 types of rotifers, L-type, *Brachionus plicatilis*, S-type *Brachionus rotundiformis* and SS-type, *Brachionus* sp. (Su *et al.*, 1997). In Sabah, the species of rotifer that is found and commonly used for larval feeding is *Brachionus plicatilis* which is 130-340 μ m in lorica length. However, the size of a newly hatched *Lutjanus rivulatus* larva is only 1.5mm with a very small mouth size (Senoo. *et al.*, 2002). Therefore, *Lutjanus rivulatus* larvae face a problem in ingesting and consuming the *Brachionus plicatilis* as first feed.

To solve this problem cod oil juice is used to replace *Brachionus plicatilis* as first feed of larvae. Cod oil juice is a liquid solution that was developed by Prof Shigeharu Senoo. Cod oil juice functions to improve the water quality for larvae culture. A mixture of commercial cod liver oil, egg yolk and filtered seawater are blended together in a juice blender for a total of 15 minutes to make a milky white liquid solution called cod oil juice.

As cod liver oil one of the main ingredients, therefore cod oil juice is also rich in EPA and DHA which are omega 3 polyunsaturated oils which are essential for

growth and survival of fish larvae in addition to their energetic role. However, cod oil juice differs from the conventional cod liver oil as cod oil juice includes the addition of a raw egg yolk. Egg yolk is rich in protein which is another essential nutrient for fish larvae. The nutrient composition of a raw egg yolk is shown in table 1.2.

Table 1.1 Nutrient content of 100 grams of raw egg yolk

Nutrient	Value per 100 grams of edible portion
Water	52.31g
Energy	322kcal
Energy	1346kj
Protein	15.86g
Total lipid (fat)	26.54g
Ash	1.71g
Carbohydrate, by difference	3.59g
Fiber, total dietary	0.0g
Sugars, total	0.56g

Source: USDA, (2004)

The molecular structure of cod oil juice is also different from cod liver oil. Cod liver oil does not mix with water but instead forms a layer on the surface. This layer prevents oxygen from mixing with the water. Cod oil juice however, does not form a layer at the water surface but instead segregates rather evenly in the water. The structure is shown in figure 1.1.

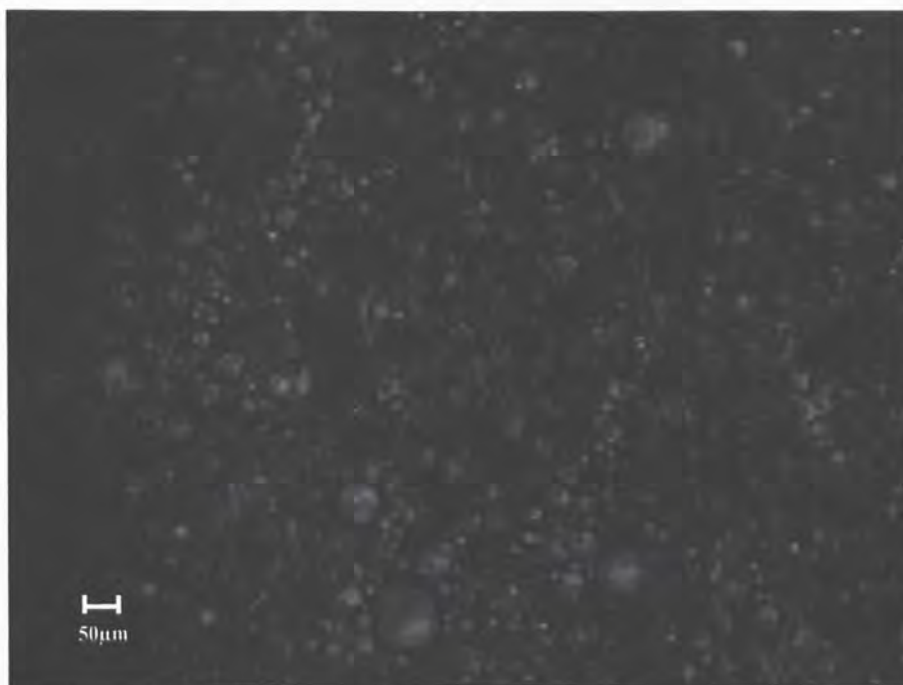


Figure 1.1 Cod oil juice under scanning electron microscope. The cod oil juice structure is unlike other oils it is evenly spread in water

Preliminary experiments were conducted with 1dAH *Lutjanus rivulatus* larvae. 3 types of feed were given to the larvae, cod oil juice, green water and also brown water with 2 replicates for each type of feeding. Green water consisted of *Brachionus plicatilis* zooplankton while brown water consisted of rotifer of a different strain.

For all tanks, culture conditions were identical. Larvae were cultured in 1 ton tanks at a stocking density of 60 individuals l^{-1} . Temperature was kept around 26-27°C, dissolved oxygen above 5.0 $mg\ l^{-1}$ and salinity at 32ppt. In addition, aeration was provided.

Results showed that estimated survival rate of the larvae in tanks being fed cod oil juice were much higher compared with the tanks being fed brown water and green water. In addition, larvae that were fed cod oil juice survived for a longer period; up to



20dAH. Larvae which were fed brown water and green water did not survive more than 10dAH. Observation under microscope showed cod oil juice goblets in the larvae's stomach and gut.

The main purpose of this experiment is to successfully culture *Lutjanus rivulatus* larvae. This experiment places *Lutjanus rivulatus* larvae in different concentrations of cod oil juice which is used to replace *brachiounus plicatilis* as the first feed for larvae. The experiment intends to confirm the positive effects of cod oil juice on newly hatched *Lutjanus rivulatus* larvae. Cod oil juice's ability to function as initial feed will be determined by comparing the growth and survival rate of larvae being fed cod oil juice and with larvae that are not fed. The optimum concentration of cod oil juice for the best survival rate of *Lutjanus rivulatus* larvae will also be determined.

Therefore the main objectives of this experiment are to:

- To determine the positive effects of cod oil juice on *Lutjanus rivulatus* larvae
- To determine the optimum concentration of cod oil juice for the best survival rate of *Lutjanus rivulatus* larvae
- To determine the optimum concentration of cod oil juice for the best growth rate of *Lutjanus rivulatus* larvae



CHAPTER 2

LITERATURE REVIEW

2.1 *Lutjanus rivulatus*

2.1.1 Morphology

Lutjanus rivulatus has a body that is shaped very deep with the dorsal profile of the head deeply sloped. The dorsal fin has a rounded profile while the posterior profile of the anal fin is distinctly pointed. It is also characterized by having a truncate or slightly emarginated caudal fin. *Lutjanus rivulatus* has a brownish body with a reddish tinge. Each of its scales on its sides has a pale brown border and 2-3 small bluish-white spots in the central portion. The head has numerous undulating blue lines while its lips are tan colored and its fins yellowish to dusky grey. In the wild *Lutjanus rivulatus* were found to mature at a size of 37cm in total length and the maximum size recorded was 76cm in total length. (Allen, 1985)

2.1.2 Egg development

Senoo *et al.*, (2002) reported that the newly ovulated eggs of *Lutjanus rivulatus* are shiny white in color. They are transparent and have an unsettled spherical shape. Each egg has an oil globule and a soft covering membrane. Immediately after fertilization, the eggs will absorb water and became fully spherical with a hard covering membrane.



In the same study, Senoo *et al.* (2002) also reported the further morphological changes during the egg development. The blasodisk appears 28 minutes after fertilization and first cleavage occurs at 55 minutes after fertilization. The size of the egg at two celled stage is 0.73mm in diameter. Morula, blastula and gastrula stages occur in the order from 3h AF to 7h AF. Embryo starts to form at 9.12 h AF with the head formation and appearance of Kupffer's vesicle at 11.37h AF. At 13.28 and 14.29 h AF respectively, optic lens and vesicles become visible. The embryo commences movement at 15.38 h AF. The formation of the heart happens at 16.40 h AF followed by active movement of the embryo.

The hatching of *Lutjanus rivulatus* takes about 18 to 21 hours after fertilization before eye pigmentation. The egg development and hatching time is dependent on water temperature during incubation. Egg development of *Lutjanus rivulatus* is observed to be similar to that of other Lutjanids of *L. synagris*, *L. kasmira* and *L. vitta*. (Senoo *et al.*, 2002)

2.2 Effect of eggs size on larvae

Egg size can affect larvae condition. Larvae size, first feeding period and growth rate are examples of the effects that may be linked to egg size.

2.2.1 Relation of egg size with larvae size

Gisbert *et al.* (1999) reported that larvae that were hatched from larger eggs were larger in size were larger than those from smaller eggs in his study on Siberian



sturgeon larvae. The total length, body weight and also yolk sac volume were larger in larvae that were hatched from larger eggs. The same results were reported for some teleost species such as herring (*Clupea harengus*; Blaxter and Hempel, 1963), Atlantic salmon (*Salmo salar*; Thorpe et al., 1984), Arctic charr (*Salvelinus alpinus*; Wallace and Aasjord, 1984), rainbow trout (*Onchorhynchus mykiss*; Springate and Bromage, 1985) and Iceland cod (*Gadus morhua*; Marteinsdottir and Steinarsson, 1998). At the end of the rearing period, the effect of egg size was still evident in larval size based on length and weight but these correlations between egg diameter and fish size disappeared during the juvenile stage

2.2.2 Relation of egg size with initial feeding time

Gisbert *et al.* (1999) also reported that egg size has been found to be positively correlated to initial feeding time in Siberian sturgeon larvae. The onset of exogenous feeding was detected between 9 and 11 days post-hatch, depending on the egg size. Larvae hatched from larger eggs tended to delay the onset of exogenous feeding. This meant that larvae that were larger tended to have a first feeding time that was later than smaller larvae.

2.2.3 Relation of egg size with standard growth rate of larvae

Egg size has no significant influence on the standard growth rate of larvae. Continuing Gisbert *et al.* (1999) study, there was no significant differences in larval SGR between progeny from different females of Siberian sturgeon larvae. Thus, the growth rate of smaller and larger sized larvae were relatively similar.



2.2.4 Relation of egg size on survival of larvae

The study of Gisbert *et al.* (1999) also revealed that egg size of Siberian sturgeon did not provide any advantage as far as survival of young fish was concerned. The same result has been reported in other species that were cultured under favorable conditions. Examples are rainbow trout (Pitman, 1979; Springate and Bromage, 1985), Atlantic salmon (Thorpe *et al.*, 1984), catfish (*Clarias macrocephalus*; Reagan and Conley, 1977), and carp (*Cyprinus carpio*; Zonova, 1973; Tomita *et al.*, 1980). Springate and Bromage (1985) suggested that where size-dependent survival rates were reported, the results might actually reflect a difference between stage of ripeness of eggs, rather than egg size. Therefore, egg size can be concluded to have no direct implications on the survival rate of larvae.

2.3 Larval rearing techniques

2.3.1 Larvae rearing conditions

In natural conditions, fish larvae grow in the most ideal conditions. However, hatchery reared larvae are under controlled conditions. Hatchery reared larvae show a high value of deformations (Divanach *et al.*, 1996) compared to only 4% of wild caught animals that show deformities. (Boglione *et al.*, 2001) Malformations are often associated with growth depression and high mortality rate. This leads to significant loss of money for the hatchery. In addition, fish growing with malformations are sold at a lower price. Therefore it is important to consider the conditions of for the rearing of the larvae.



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