No.

# REPORT OF THAILAND AND JAPAN JOINT COASTAL AQUACULTURE RESEARCH PROJECT

## (APRIL 1984-JANUARY 1986)

## No. 2

APRIL 1986

JAPAN INTERNATIONAL COOPERATION AGENCY

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## PREFACE

The Thailand and Japan Joint Coastal Aquaculture Project was begun in April, 1981, for the purpose of developing coastal aquaculture in Thailand which will contribute to the production of new protein sources in coastal areas and increase employment opportunities for the Thai people.

Five years ago, the National Institute of Coastal Aquaculture was still weak in activities because it was lacking in research personnel and facilities. But by now, every research plant has been equipped with well trained personnel and adequate facilities. I believe this to have been the main achievement of the joint research project.

This report is a compilation of research results from the five year period. Many of the results offer new insight and indicate where further, detailed study is necessary.

I think it is appropriate to say that the Thailand and Japan Joint Coastal Aquaculture Project has been a success, and that this success has been made possible through the unceasing efforts of many biologists and experts both in Thailand and Japan.

Finally, I would like to express my personal gratitude to all NICA staff members for their help and cooperation during this most rewarding five year period.

March 31, 1986

Munekazu MASUO
JICA Team Leader

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# Effect of food density on food consumption for larval seabass, *Lates calcarifer*

Tida Pechmanee, Juadee Pongmaneerat and Masato Iizawa

## ABSTRACT

Larval seabass, *Lates calcarifer*, of 4 different sizes were used to examine the effect of rotifer density on food consumption by using 6 dirrerent food densities. The amount of consumed rotifers increased with augmentation of food density and became constant at densities of more than the following values;

| | |
|---|---|
| $\simeq$ 4 ind./ml | 4–5 day-old larvae |
| $\simeq$ 8 " | 7–8 " |
| $\simeq$ 12 " | 10–11 " |
| $\geq$ 32 " | 13–14 " |

The food density at which the amount of consumed rotifers became constant was nearly identical to that at which the proportion of digested individuals to total rotifers in the digestive tract became constant.

The equation for the number of rotifers in the digestive tract during the 120 minutes following food distribution changed from type: $R = c(1-e^{-bt})$ to type: $R = at\,e^{-bt}$, and the peak at which the consumed amount changed from an increment to a decrement came sooner as the larvae grew.

## INTRODUCTION

The effect of food density on the growth and survival of marine fish larvae has been studied by many authors (e.g. Houde, 1973, 1977 and 1978; Wyat, 1972; Barahona-Fernandes and Girin, 1977; Werner and Blaxter, 1980). The authors state that a high density is generally favorable to obtain fast growth and high survival during the larval stage.

In regard to the relation between food density and amount of consumed food, larval *Plecoglossus altivelis* fed on rotifer at a density of more than 0.3 ind./ml, and the lowest limit of suitable density for food consumption is 0.9 ind./ml (Katsuya et al. 1975). The amount of rotifers consumed by *Pagrus major* larvae aged 7–23 days decreases at a density of less than 2 ind./ml, and the daily amount of consumed individuals at a density of 5 ind./ml is identical to that at a density of 10 ind./ml (Kitajima et al. 1974). Neither does the difference between 5 ind./ml and 10 ind./ml clearly influence the daily amount of rotifers consumed by *Oplegnathus fasciatus* larvae aged 7–15

days (Fukusho, 1979), and there is also no difference in the amount of rotifers consumed by *Acanthopagrus schlegeli* larvae aged 7 and 19 days at a density of more than 3 ind./ml (Yamamoto et al. 1977). The optimum densities of rotifers are estimated at 4 ind./ml and 4–5 ind./ml, respectively for *Fugu niphobles* larvae aged 6 days and *Dicentrarchus labrax* larvae aged 14 days, (Iizawa, 1979 and 1983).

Tongrawd and Suteemeechaikune (1983) examined the effect of food density on the daily amount of rotifers consumed by *L. calcarifer* larvae of 2.59–4.19 mm (total length). According to their results, the daily amounts of rotifers consumed by larvae of 2.59–4.19 mm show no difference at densities of more than about 10 ind./ml and 15–20 ind./ml, respectively.

In the present study, we examined the effect of food density on the amount of rotifers in the digestive tract, and tried to clarify the variation in consumed amounts within 120 minutes after feeding.

## MATERIALS AND METHODS

The experiment was carried out 4 times at various stages of larval development (at the 4–5 days, 7–8 days, 10–11 days and 13–14 days) at the National Institute of Coastal Aquaculture (NICA) from May 1 to May 11 in 1985. The stocking densities were 40 larvae per litre for age 4 to 11 days and 20 larvae per litre for age 13–14 days. The tested fish were collected from a roofed, outdoor rearing tank of 26 $m^3$ stocked in six, 30 ℓ tanks of polycarbonate (water volume 25 ℓ) without food for about 18 hours (From 16:00 to 10:00). Rotifer, *Brachionus plicatilis*, was used as food in this experiment, cultured using *Tetraselmis* sp. as food in a tank of 26 $m^3$. Its lorica length was 149.0 ± 18.0 mm. It was S-strain rotifer, distinguished from L-strain rotifer by the differences in its lorica size and the shape of its occipital spines (Fukusho and Okauchi, 1983). Densities were set at 1, 2, 4, 8, 16 and 32 ind./ml for all trials. Ten larvae were picked up with a pipette every 30 minutes after feeding and anesthetized in a solution of quinaldine (20 ppm), then fixed in 5% formalin. The collected fish samples were dissected under a binocular microscope to count the number of consumed rotifers and classify them as digested (lacking or almost lacking body content) or non-digested individuals (remaining intact or nearly intact). The lengths and weights of the larvae and the water conditions for each trial were as follows;

| Age (days) | Total length (mm) | Wet weight (mg) | W.T. (°C) | Salinity (‰) |
|---|---|---|---|---|
| 4–5 | 2.90 ± 0.124 | 0.3186 | 27.5–27.6 | 33.6 |
| 7–8 | 3.719 ± 0.143 | 0.5203 | 27.5–27.6 | 33.6 |
| 10–11 | 3.972 ± 0.214 | 0.8281 | 28.6–28.8 | 33.6 |
| 13–14 | 5.100 ± 0.259 | 2.0802 | 28.8–28.9 | 30.8 |

Aeration was assured at a rate of 100–267 ml/min for each trial through an airstone in each tank.

## RESULTS

1. Variation in the number of rotifers in the digestive tract (Figure 1).

Larva aged 4–5 days

The number of consumed rotifers increased with the time following food distribution and had a tendency to become stable after 60–90 minutes. The digested individuals already appeared at 30 minutes and its number was always greater than that of non-digested rotifers. The latter, on the other hand, had a tendency to increase with a rise in the food density, while the former showed no striking difference among densities of more than 2 ind./ml. The total number of consumed rotifers consequently tended to increase with density augmentation.

Larva aged 7–8 days

The number of consumed rotifers increased for about 45 minutes and then decreased at densities of 2, and 8 ind./ml, but continued to increase at densities of 16 and 32 ind./ml. The digested individuals were always much greater in number than the non-digested ones. The numbers of digested and total rotifers increased with density augmentation.

Larva aged 10–11 days

The maximum number of consumed rotifers appeared earlier (about 30–45 minutes after feeding) than in the case of previous larvae.

Larva aged 13–14 days

The number of consumed rotifers was much greater than that of the previous larvae at densities of more than 4 ind./ml. The changing pattern from an increment to a decrement in the number of consumed individuals was nearly identical to the larva aged 10–11 days.

2. Comparison of the food density

Figure 2 shows the fluctuation in mean number of rotifers in the digestive tract during the 120 minutes (average of the numbers at 30, 60, 90 and 120 minutes) after feeding at the different food densities. This number increased with density augmentation and became constant at more than a certain density, which is estimated for each larva as follows;

| | |
|---|---|
| 4 ind./ml | 4–5 day-old larvae |
| 8 " | 7–8 " |
| 12 " | 10–11 " |
| 32 " | 13–14 " |

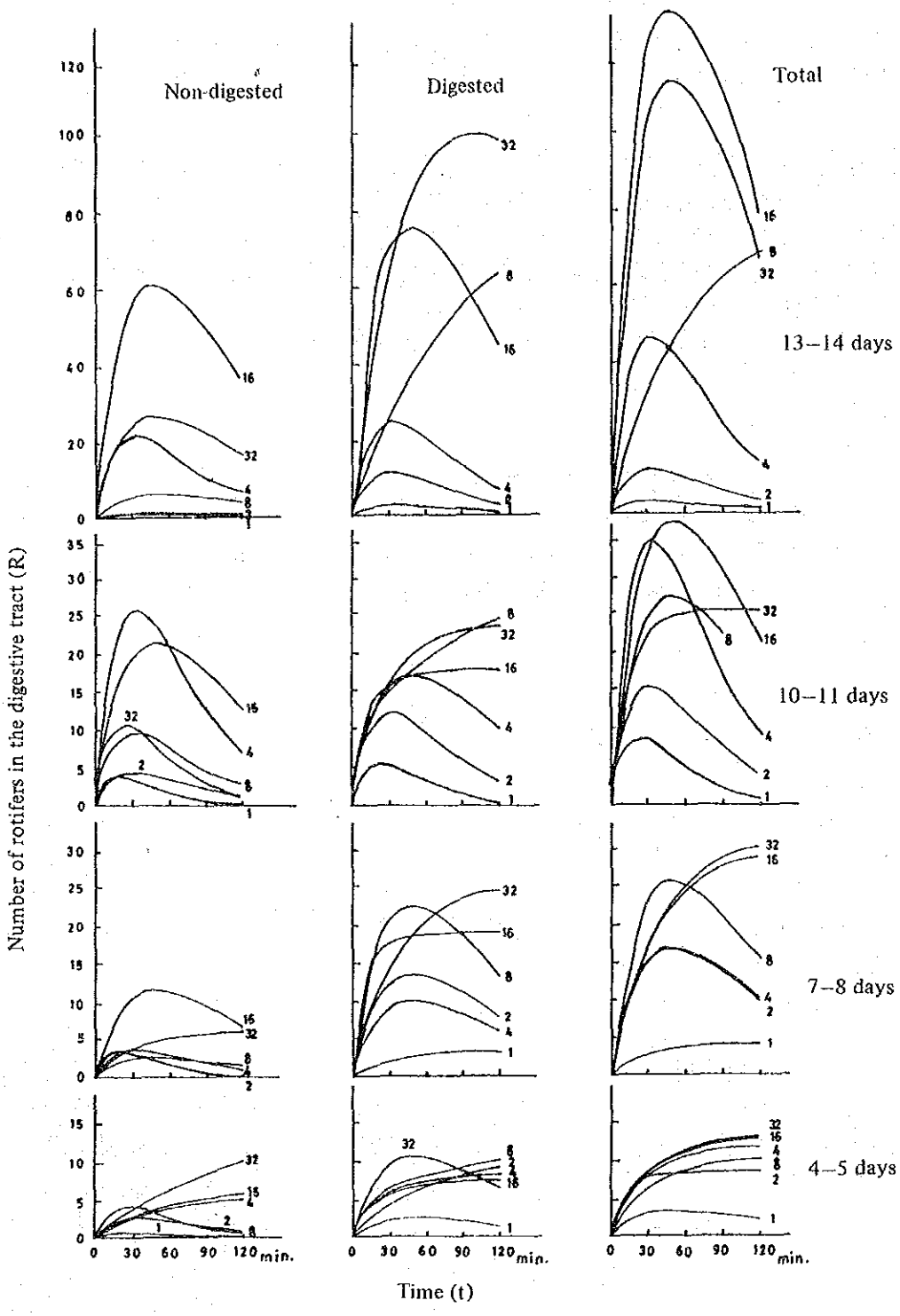



Figure 1. Theoretical curve of the variation in number of rotifers in the digestive tract during the 120 minutes.
1–32: Food densities (ind./ml)

Equations for the curves in Figure 1.

4–5 days

| Food density | Non-digested | Digested | Total |
|---|---|---|---|
| 1 ind./ml | $R=0.08t\ e^{-0.04t}$ | $R=0.12t\ e^{-0.02t}$ | $R=0.17t\ e^{-0.02t}$ |
| 2 " | $R=0.21t\ e^{-0.03t}$ | $R=10.0(1-e^{-0.019t})$ | $R=8.46(1-e^{-0.08t})$ |
| 4 " | $R=5.5(1-e^{-0.02t})$ | $R=8.03(1-e^{-0.039t})$ | $R=11.61(1-e^{-0.039t})$ |
| 8 " | $R=0.46t\ e^{-0.04t}$ | $R=2.23t^{0.31}$ | $R=10.33(1-e^{-0.03t})$ |
| 16 " | $R=0.53t^{0.50}$ | $R=7.09(1-e^{-0.057t})$ | $R=12.76(1-e^{-0.037t})$ |
| 32 " | $R=15.64(1-e^{-0.008t})$ | $R=0.57t\ e^{-0.02t}$ | $R=13.33(1-e^{-0.03t})$ |

7–8 days

| | | | |
|---|---|---|---|
| 1 ind./ml | – | $R=0.09t\ e^{-0.01t}$ | $R=0.11t\ e^{-0.01t}$ |
| 2 " | $R=0.45t\ e^{-0.05t}$ | $R=0.73t\ e^{-0.02t}$ | $R=0.91t\ e^{-0.02t}$ |
| 4 " | $R=0.29t\ e^{-0.03t}$ | $R=0.54t\ e^{-0.02t}$ | $R=0.92t\ e^{-0.02t}$ |
| 8 " | $R=0.13t\ e^{-0.02t}$ | $R=1.23t\ e^{-0.10t}$ | $R=1.40t\ e^{-0.02t}$ |
| 16 " | $R=0.63t\ e^{-0.02t}$ | $R=18.52(1-e^{-0.10t})$ | $R=29.67(1-e^{-0.03t})$ |
| 32 " | $R=5.70(1-e^{-0.037t})$ | $R=25.78(1-e^{-0.026t})$ | $R=31.46(1-e^{-0.027t})$ |

10–11 days

| | | | |
|---|---|---|---|
| 1 ind./ml | $R=0.53t\ e^{-0.05t}$ | $R=0.50t\ e^{-0.04t}$ | $R=0.96t\ e^{-0.04t}$ |
| 2 " | $R=0.35t\ e^{-0.03t}$ | $R=0.97t\ e^{-0.03t}$ | $R=1.27t\ e^{-0.03t}$ |
| 4 " | $R=1.27t\ e^{-0.03t}$ | $R=0.93t\ e^{-0.02t}$ | $R=2.83t\ e^{-0.03t}$ |
| 8 " | $R=0.78t\ e^{-0.03t}$ | $R=4.19t^{0.37}$ | $R=1.97t\ e^{-0.02t}$ |
| 16 " | $R=1.16t\ e^{-0.02t}$ | $R=17.7(1-e^{-0.07t})$ | $R=2.03t\ e^{-0.02t}$ |
| 32 " | $R=1.15t\ e^{-0.04t}$ | $R=23.8(1-e^{-0.035t})$ | $R=25.4(1-e^{-0.07t})$ |

13–14 days

| | | | |
|---|---|---|---|
| 1 ind./ml | $R=0.02t\ e^{-0.01t}$ | $R=0.23t\ e^{-0.02t}$ | $R=0.23t\ e^{-0.02t}$ |
| 2 " | $R=0.07t\ e^{-0.02t}$ | $R=0.93t\ e^{-0.03t}$ | $R=0.95t\ e^{-0.03t}$ |
| 4 " | $R=1.79t\ e^{-0.03t}$ | $R=2.01t\ e^{-0.03t}$ | $R=3.78t\ e^{-0.03t}$ |
| 8 " | $R=0.34t\ e^{-0.02t}$ | $R=83.3(1-e^{-0.012t})$ | $R=78.0(1-e^{0.017t})$ |
| 16 " | $R=8.35t\ e^{-0.02t}$ | $R=4.10t\ e^{-0.02t}$ | $R=7.17t\ e^{0.02t}$ |
| 32 " | $R=1.49t\ e^{-0.02t}$ | $R=2.72t\ e^{-0.01t}$ | $R=6.17t\ e^{-0.02t}$ |

The proportion of digested individuals to total rotifers in the digestive tract decreased with density augmentation and became constant at nearly the same density as above-mentioned for each larva. This constant ratio is estimated as follows;

| | |
|---|---|
| 65.3% | 4–5 day-old larvae |
| 74.1% | 7–8 " |
| 57.7% | 10–11 " |
| 51.8% | 13–14 " |

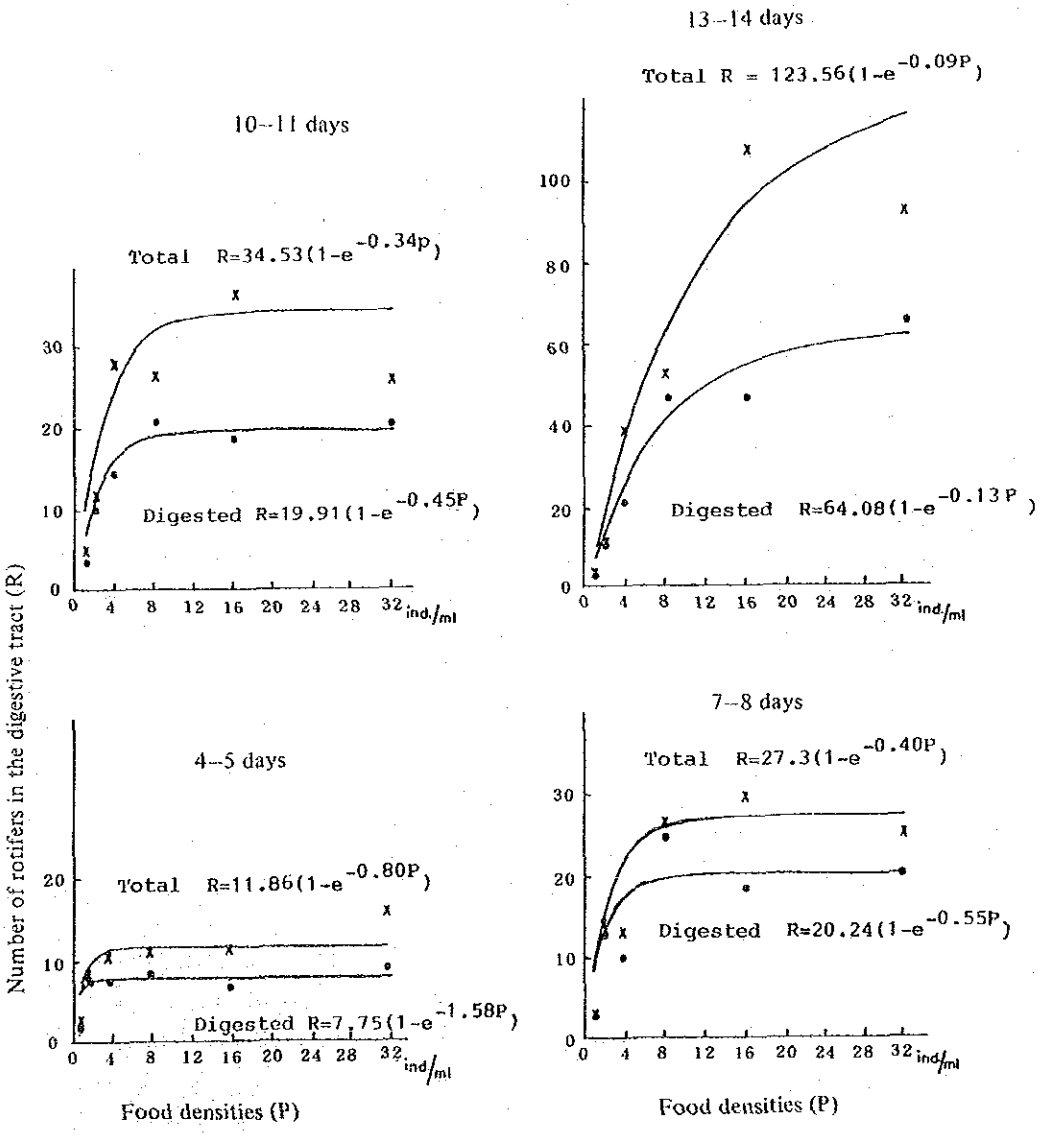



Figure 2. Mean number of rotifers in the digestive tract during 120 minutes (counted every 30 minutes) after feeding at different food densities.

3. Average and maximum amounts of consumed rotifers (Figure 3).

The average amount of rotifers that one larva had in its digestive tract in the presence of a sufficient quantity of food (rotifer density: more than the density above-mentioned for each larva) is obtained from Figure 2 as follows;

| | |
|---|---|
| 12 ind. | 4–5 day-old larvae |
| 27 " | 7–8 " |
| 35 " | 10–11 " |
| 124 " | 13–14 " |

The relation between this amount (R: number of rotifers) and the total length of larva (TL, mm) is shown as the following equation; $R=0.13\ TL^{4.14}$

This amount is about 2.2–3.2% of the daily amount of consumed rotifers per larva of the same species obtained by Tongrawd and Suteemeechaikune.(1983, $R=19.68\ TL^{3.0475}$).

The maximum amount of rotifers that one larva has in its digestive tract in the presence of a sufficient quantity of food is as follows;

| | | |
|---|---|---|
| 30 ind. | for larva aged | 4–5 days |
| 52 " | " | 7–8 " |
| 96 " | " | 10–11 " |
| 345 " | " | 13–14 " |

The relation between this amount and the total length of larva is shown as the following equation; $R=0.23\ TL^{4.40}$

This amount is about 5.1–8.3% of the daily amount of consumed rotifers.

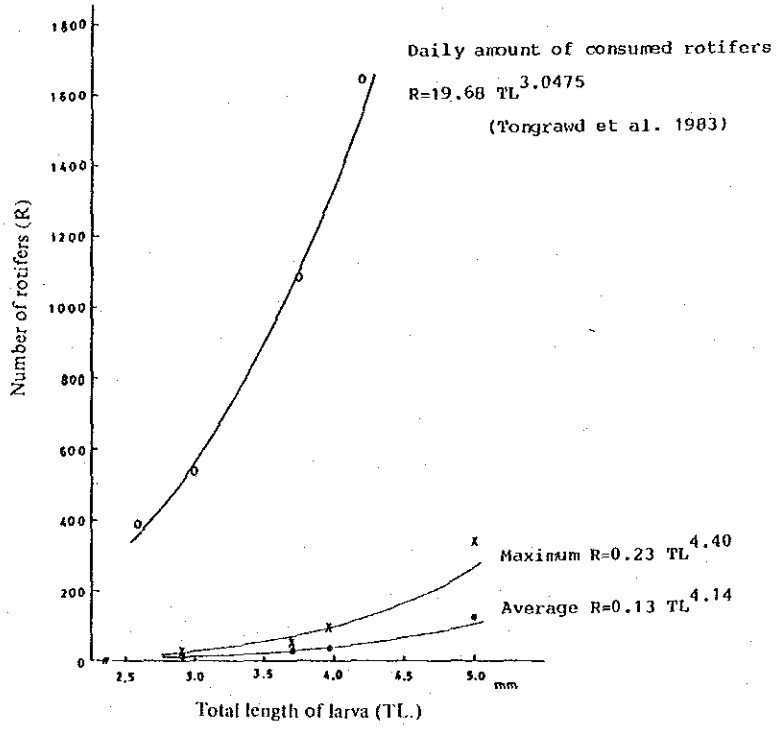
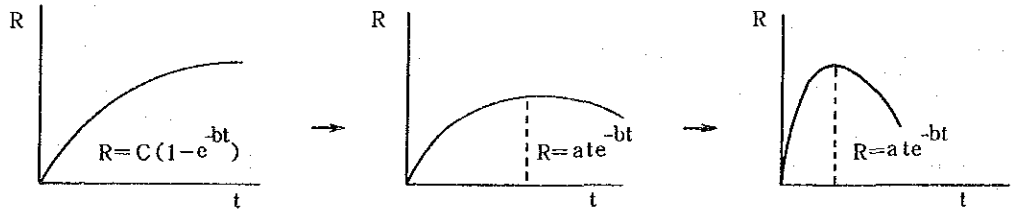



Figure 3. Average and maximum amounts of rotifers in the digestive tract, compairing daily amounts of consumed rotifers

## DISCUSSION AND CONCLUSION

The amount of consumed food increased with the augmentation of food density and became constant at more than a certain density as was also reported by Ivlev (1965) and Iizawa (1979 and 1983). Iizawa (1983) states that the rotifer density at which the amount of consumed rotifers becomes constant is nearly identical with the food density at which the proportion of non-digested individuals to total rotifers in the digestive tract becomes constant for *Dicentrarchus labrax* larvae. He deduces from these results that the rythms of ingestion, digestion and defecation should be stabilized in the presence of a sufficient amount of food. The present study for *L. calcarifer* seems to be quite similar to results obtained with *D. labrax*.

With regard to the variation in the number of rotifers in the digestive tract within the elapsed time, the number increased with the time and had a tendency to become constant after 60–90 minutes for larvae aged 4–5 days. This tendency is also seen for puffer larvae, *Fugu niphobles*, aged 6 days (Iizawa, 1979) and seabass larvae, *D. labrax*, aged 14 days (Iizawa, 1983). The number of consumed rotifers, however, didn't become constant, but decreased after about 45 minutes for larvae aged 7–8 days, and after 30–45 minutes for larvae aged 10–11 days and 13–14 days. These results show that the equation for the number of rotifers in the digestive tract within the elapsed time changes from type: $R=c\,(1-e^{-bt})$ to type: $R=at\,e^{-bt}$, and the peak at which the number of rotifers changes from an increase to a decrease comes sooner as the larvae grow. This phenomenon is figured as follows;




R : Number of rotifers in the digestive tract
t : Time following food distribution
a, b, c: Coefficient

We examined the variation in the number of rotifers in the digestive tract during the first 120 minutes after feeding but no observation was conducted for food density after feeding. The rapid decrease of food density may bring about this phenomenon in the case of older larvae: as the food density might suddenly decrease because of eating after a certain number of minutes, the larvae may be unable to take in food. Or, perhaps the older larvae, which have more developed digestive organs, may stop feeding until the amount of food in the digestive tract decreases in measure, while the smaller larvae continue to eat. Fushimi (1983) examined the daily variation of the number of rotifers consumed by red seabream larvae, *Pagrus major*, aged 4 days. According to his examination, the number of rotifers in the belly does not decrease so clearly, but continues to be maintained during the daytime in the presence of a sufficient amount of food (the food density at the morning feeding: more than 3.5 ind./ml of S-strain rotifer).

The following is a comparison with other fishes of the relation between maximum amount of consumed rotifers and daily amount of consumed individuals;

$$r = \frac{\text{Daily amount of consumed individuals}}{\text{Maximum amount of consumed rotifers}}$$

| | | |
|---|---|---|
| *P. major* (Kitajima et al., 1976) | 5–10 | L-strain |
| *Oplegnathus fasciatus* (Fukusho, 1979) | 2.1–4.2 | " |
| *D. labrax* (Iizawa, 1983) | 1.68–9.78 | " |
| *Lates calcarifer* | 12.0–19.6 | S-strain |

Thus, the seabass larvae, *L. calcarifer*, consumes daily a number of rotifers 12 to 19.6 times as large as the maximum number of individuals in the digestive tract, and this value is much higher than the values for the other fishes. This probably suggests that the metabolism, that is, the digestibility, of this larva may be greater than that of other fishes, or that the S-strain rotifer may be more digestible than the L-strain rotifer. The augmentation of digestibility is strictly related to the development of digestive organs (Tanaka, 1975) and to the increase in digestive enzymes (Kawai, 1975). Iizawa (1983), who examined the time of digestion of *D. labrax* larvae, states that the digestive capacity increases considerably after the differenciation of the gastric gland, when the larval stage of fishes is completed (Tanaka, 1971 and Yasunaga, 1975). The amount of rotifers consumed by a *L. calcarifer* larva increases in great measure at age 13–14 days and it seems that the distribution of sole rotifers is not enough to feed the larva of this age. In fact, the *Artemia* nauplii is used for the larvae from 10 days after hatching in the production of this species (Maneewong et al. 1984). The digestive organs of this species must, therefore, develop greatly at about this age. In order to clarify the implication it would be necessary to examine the histological observation of the digestive organs.

## ACKNOWLEDGEMENTS

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# Effect of food density on food consumption for juvenile seabass, *Lates calcarifer*

Duangrat Dhesprasith, Tida Pechmanee and Masato Iizawa

## ABSTRACT

Juvenile seabass, *Lates calcarifer* of 4 different sizes were used to examine the effect of copepod density on food consumption by using 6 different food densities. The amount of consumed copepods increased with augmentation of food density and became constant at densities of more than the following values;

| | |
|---|---|
| $\leq$ 0.2 ind./ml | 14–15 day-old juvenile |
| $\simeq$ 0.4 " | 17–18 " |
| $\simeq$ 0.8 " | 20–21 " |
| $\simeq$ 0.8 " | 23–24 " |

The food density at which the amount of consumed copepods became constant is nearly identical to that at which the proportion of digested individuals to total copepods in the digestive tract became constant. This constant proportion decreased with the growth of fish.

Average and maximum amounts of copepods in the digestive tract increased in proportion to the growth of fish in body weight.

## INTRODUCTION

Using copepods as food is known to have a good effect on the survival rate and the vitality of larvae and/or juveniles in rearing of marine fish; e.g. *Pagrus major* (Fushimi and Hashimoto, 1969 and Kitajima, 1978), *Oplegnathus fashiatus* (Fukusho et al. 1973) and *Lateolabrax japonicus* (Yamashita et al. 1973).

Some experiments on the amount of consumed copepods have been carried out for juveniles of *P. major* (Kitajima, 1976), *O. fasciatus* (Fukusho, 1977) and *Dicentrarchus labrax* (Iizawa, 1983).

Iizawa (1983) examined, on the other hand, the effect of copepod density on food consumption for larval and juvenile seabass *D. labrax* by using wild copepods collected by Barnabe's methods (Barnabe, 1978) in marshes and oxidation ponds. He stated that the amount of consumed food increased with augmentation of food density and became constant at densities of more than a certain value, and that the fish had a rythm of ingestion and defecation in the presence of sufficient food.

In the previous report (Pechmanee et al. in this volume), we reported this effect for the larval stages of *Lates calcarifer* (4–14 day old larvae) by using rotifer *Brachionus plicatilis* as food, and observed similar phenomenon to the results of Iizawa (1983).

In the present study, we examined this effect for the juveniles of this species (age 14–24 days) by using reared copepod *Tigriopus japonicus*.

## MATERIALS AND METHODS

The experiment was carried out 4 times for the juvenile stages of *Lates calcarifer* (at age 14–15 days, 17–18 days, 20–21 days and 23–24 days) at the National Institute of Coastal Aquaculture (NICA), from May 13 to May 23, 1985. The methods used in this experiment were the same as for the previous report (Pechmanee et al. in this volume). The stocking densities were 10 fish per litre in 30 ℓ tank for all trials.

The food used for this experiment was the copepod *Tigriopus japonicus* (length and width of cephalothorax: 0.766 ± 0.166 mm and 0.287 ± 0.064 mm, respectively, wet weight of an individual: 45.5 μg) which remained in a net of 200 μm mesh after being sieved. It was cultured in 26 $m^3$ concrete tanks (water volume: 25 $m^3$) using fish meal, rice bran and bread yeast as food. The food densities were set at 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 ind./ml.

The lengths and weights of fish and the water conditions for each trial were as follows;

| Age (days) | Total length (mm) | Wet weight (mg) | W.T. (°C) | Salinity (‰) |
|---|---|---|---|---|
| 14–15 | 5.86 ± 0.67 | 3.90 | 28.5–29.5 | 33.3 |
| 17–18 | 7.31 ± 0.82 | 5.27 | 27.8–28.2 | 33.0 |
| 20–21 | 9.41 ± 1.50 | 10.53 | 27.6–27.9 | 33.0 |
| 23–24 | 11.10 ± 1.84 | 20.36 | 26.8–27.0 | 33.9 |

## RESULTS

1. Variation in the number of copepods in the digestive tract (Figure 1).

14–15 day-old

The numbers of consumed copepods increased with the time after feeding and had a tendency to become stable after 90–120 minutes. The numbers of non-digested copepods were always very small compared to those of digested individuals at any density of food. The curves for all the densities were quite similar in the numbers of non-digested, digested and total copepods.

17–18 day-old

The numbers of consumed copepods showed the same tendency to increase as those for the previous fish. The numbers of non-digested individuals was again smaller than that of digested individuals, but this difference was not as large as for the previous fish.

20–21 day-old

A considerable difference appeared in the numbers of non-digested copepods according to the food density; these numbers, at densities of 0.2 and 0.4 ind./ml, were clearly smaller than those at densities of more than 0.8 ind./ml. The numbers of digested individuals dit not, however, show so clear a difference among these densities, and the maximums of those numbers appeared 30 to 60 minutes after feeding.

23–24 day-old

The numbers of non-digested copepods at a density of 0.2 ind./ml were much smaller than those at the other densities. The maximums of these numbers appeared 30 to 60 minutes after feeding at densities of 0.2, 0.4, 0.8 and 6.4 ind./ml, while the numbers continued to increase during the testing period (120 minutes after feeding) at densities of 1.6 and 3.2 ind./ml. The numbers of digested individuals continued to increase at any density of food.

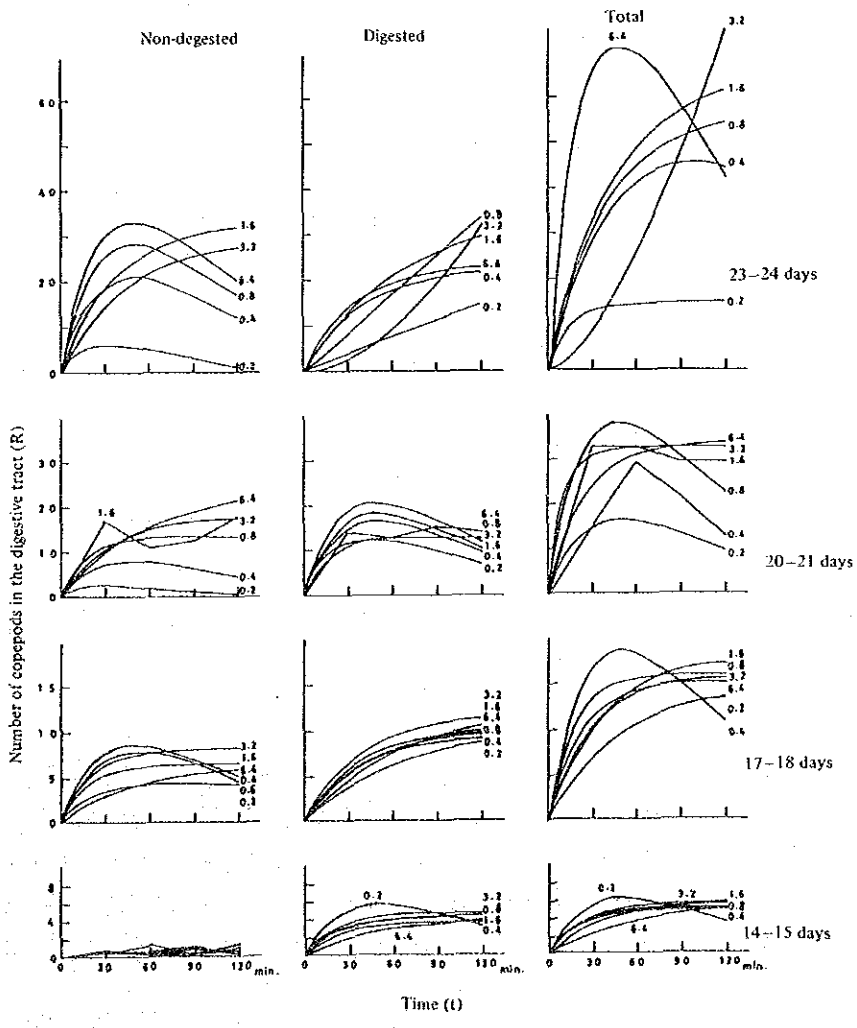


Figure 1. Theoretical curve of the variation in number of copepods in the digestive tract during the 120 minutes after feeding. ,0.2–6.4: Food densities (ind./ml)

Equations for the curves in Figure.

14–15 days

| Food density | Non-digested | Digested | Total |
|---|---|---|---|
| 0.2 ind./ml | – | $R=0.32t\ e^{-0.02t}$ | $R=0.34te^{-0.02t}$ |
| 0.4 " | – | $R=3.85(1-e^{-0.055t})$ | $R=4.84(1-e^{-0.59t})$ |
| 0.8 " | – | $R=4.63(1-e^{-0.057t})$ | $R=5.27(1-e^{-0.052t})$ |
| 1.6 " | – | $R=4.42(1-e^{-0.044t})$ | $R=5.73(1-e^{-0.032t})$ |
| 3.2 " | – | $R=4.95(1-e^{-0.50t})$ | $R=5.84(1-e^{-0.046t})$ |
| 6.4 " | – | $R=4.50(1-e^{-0.020t})$ | $R=5.50(1-e^{-0.020t})$ |

17–18 days

| | | | |
|---|---|---|---|
| 0.2 ind./ml | $R=4.49(1-e^{-0.080t})$ | $R=10.61(1-e^{-0.015t})$ | $R=14.30(1-e^{-0.023t})$ |
| 0.4 " | $R=0.48t\ e^{-0.02t}$ | $R=9.82(1-e^{-0.027t})$ | $R=0.61t\ e^{-0.01t}$ |
| 0.8 " | $R=0.33t\ e^{-0.01t}$ | $R=10.22(1-e^{-0.026t})$ | $R=16.04(1-e^{-0.061t})$ |
| 1.6 " | $R=0.29t\ e^{-0.01t}$ | $R=12.64(1-e^{-0.016t})$ | $R=17.98(1-e^{-0.028t})$ |
| 3.2 " | $R=8.33(1-e^{-0.054t})$ | $R=12.20(1-e^{-0.026t})$ | $R=16.02(1-e^{-0.034t})$ |
| 6.4 " | $R=6.50(1-e^{-0.020t})$ | $R=10.45(1-e^{-0.029t})$ | $R=15.28(1-e^{-0.046t})$ |

20–21 days

| | | | |
|---|---|---|---|
| 0.2 ind./ml | $R=0.23t\ e^{-0.03t}$ | $R=0.68t\ e^{-0.02t}$ | $R=0.91t\ e^{-0.02t}$ |
| 0.4 " | $R=0.45t\ e^{-0.02t}$ | $R=0.64t\ e^{-0.02t}$ | – |
| 0.8 " | $R=0.63t\ e^{-0.02t}$ | $R=0.92t\ e^{-0.02t}$ | $R=2.10t\ e^{-0.02t}$ |
| 1.6 " | – | $R=1.01t\ e^{-0.02t}$ | – |
| 3.2 " | $R=17.92(1-e^{-0.032t})$ | $R=1.15t\ e^{-0.02t}$ | $R=1.49t\ e^{-0.01t}$ |
| 6.4 " | $R=25.13(1-e^{-0.016t})$ | – | $R=33.99(1-e^{-0.042t})$ |

23–24 days

| | | | |
|---|---|---|---|
| 0.2 ind./ml | $R=0.54t\ e^{-0.03t}$ | $R=0.20t^{0.90}$ | $R=15.02(1-e^{-0.068t})$ |
| 0.4 " | $R=1.16t\ e^{-0.02t}$ | $R=23.46(1-e^{-0.024t})$ | $R=1.25t\ e^{-0.01t}$ |
| 0.8 " | $R=0.95t\ e^{-0.01t}$ | $R=0.27t^{1.01}$ | $R=57.75(1-e^{-0.025t})$ |
| 1.6 " | $R=33.38(1-e^{-0.027t})$ | $R=36.56(1-e^{-0.014t})$ | $R=66.29(1-e^{-0.022t})$ |
| 3.2 " | $R=29.96(1-e^{-0.022t})$ | $R=0.007t^{1.77}$ | $R=0.038t^{1.59}$ |
| 6.4 " | $R=1.84t\ e^{-0.02t}$ | $R=24.27(1-e^{-0.027t})$ | $R=2.27t\ e^{-0.01t})$ |

2. Comparison of the food density

Figure 2 shows the fluctuation in mean number of copepods in the digestive tract during the first 120 minutes (average of the number at 30, 60, 90 and 120 minutes) after feeding at different food densities. There was no clear difference in this mean number for the 14–15 day-old juveniles in spite of augmentation of food density. This number increased, however, for the more aged fish

with density augmentation and became constant at densities of more than a certain value, which was estimated for each fry as follows;

| | |
|---|---|
| ≃ 0.4 ind./ml | 17–18 day-old juveniles |
| ≃ 0.8 " | 20–21 " |
| ≃ 0.8 " | 23–24 " |

The proportion of digested individuals to total copepods in the digestive tract did not clearly change for the 14–15 day-old juveniles (80–90%). This proportion for juveniles aged 17–18, 20–21 and 23–24 days decreased, however, with density augmentation and became constant at nearly the same density at which the number of copepods in digestive tract became constant as above-mentioned (constant ratio; 52.7, 50.9 and 44.2%, respectively).

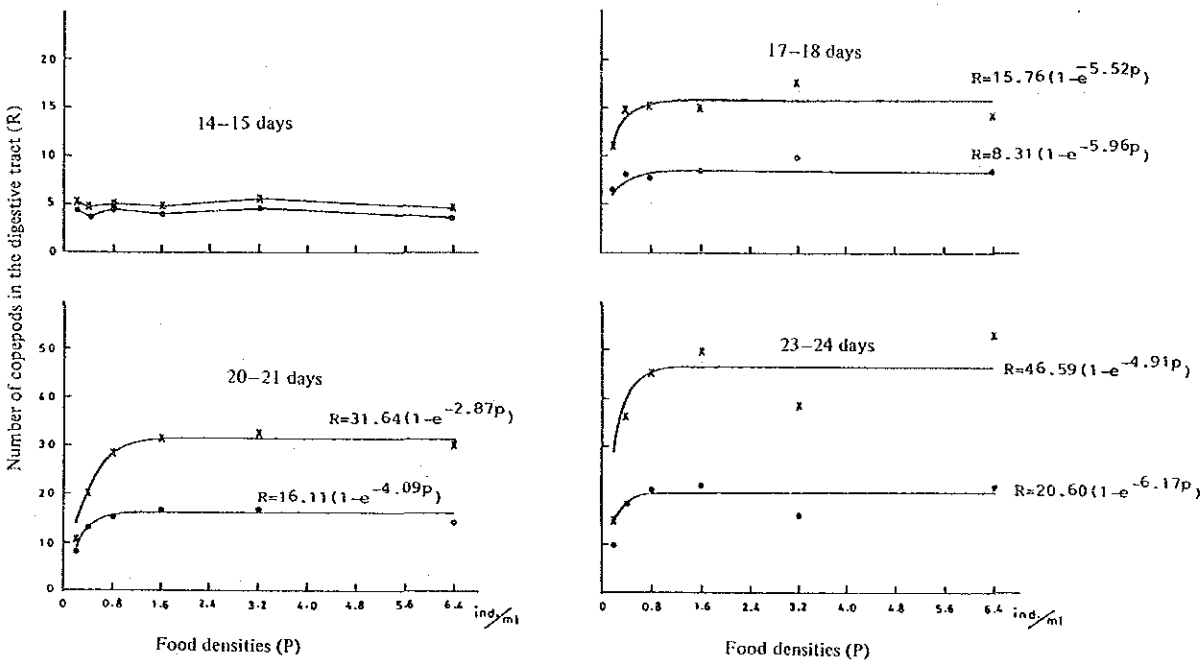


Figure 2. Mean number of copepods in the digestive tract during 120 minutes (counted every 30 minutes) after feeding at different food densities.
x Total number of copepods in the digestive tract
● Number of digested copepods

3. Average and maximum amounts of consumed copepods (Figure 3).

The average amount of copepods that one fish had in its digestive tract in the presence of a sufficient quantity of food (copepod density: more than the density above-mentioned for each juvenile) is obtained from Figure 2 as follows;

| | |
|---|---|
| 5 ind. | 14–15 day-old juvenile |
| 16 " | 17–18 " |
| 32 " | 20–21 " |
| 47 " | 23–24 " |

The relations between this amount (Rn and Rw: number and weight (mg) of copepods) and the total length (TL, mm) and the body weight (BW, mg) of fish are shown as the following equations; $Rn=0.01\ TL^{3.44}$ and $Rw=0.11\ BW+0.06$.

The maximum amount of copepods that one fish had in its digestive tract in the presence of a sufficient quantity of food is as follows;

| | |
|---|---|
| 15 ind. | 14–15 day-old juvenile |
| 37 " | 17–18 " |
| 83 " | 20–21 " |
| 171 " | 23–24 " |

The relations between this amount and the total length and the body weight of fish are shown as the following equations; $Rn=0.02\ TL^{3.73}$ and $Rw=0.42\ BW-0.73$.

Thus, both the average and maximum amounts of copepods in the digestive tract increased in proportion to the growth of juvenile in body weight.

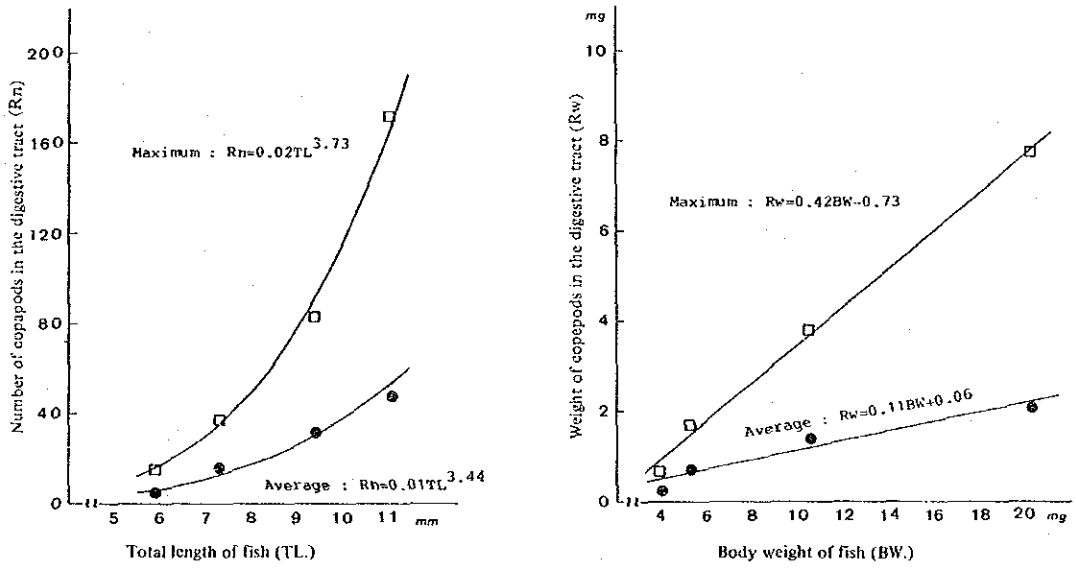


Figure 3. Average and maximum amounts of copepods in the digestive tract according to the growth of fish.

## DISCUSSION AND CONCLUSION

The amount of consumed copepods increased with the augmentation of food density and became constant at densities of more than about 0.4. 0.8 and 0.8 ind./ml, respectively, for juveniles aged 17–18, 20–21 and 23–24 days. This amount did not, however, change clearly at densities with 0.2 to 6.4 ind./ml for the 14–15 day-old juveniles. It suggests that a density of 0.2 ind./ml (the minimum tested density) was sufficient for the feeding of these fry, and some lower densities for them should have been tested to observe the above-mentioned phenomenon, which was also

reported in the feeding of fish fry of several species (Ivlev, 1965), puffer larvae, *Fugu niphobles* and seabass larvae and juveniles, *Dicentrarchus labrax* (Iizawa, 1979 and 1983) and seabass larvae *Lates calcarifer* (previous report; Pechmanee et al. in this volume).

The proportion of digested individuals to total copepods in the digestive tract decreased with the augmentation of food density and became constant at nearly the same densities as above-mentioned. This phenomenon was also observed in the larval stages of this species fed on rotifer (previous report; Pechmanee et al. in this volume) and seabass larvae and juveniles, *D. labrax* (Iizawa, 1983). The constant proportion of digested individuals to total copepods decreased, on the other hand, with the growth of fish as follows; 80–89%, 52.7%, 50.9% and 44.2%, respectively, for juveniles aged 14–15, 17–18, 20–21 and 23–24 days. The activity of the 14–15 day-old juveniles might not be yet strong enough to easily catch the copepod; therefore, it should take time for the fish to take in new food and they hold, consequently, a high percentage of digested copepods in their digestive tracts. But, the fish could catch the copepod easier with the development of catching activity, that is, the percentage of non-digested copepod in their digestive tracts increased as they grew.

As concerns the variation in the number of copepods in the digestive tract within the elapsed time, the results did not show a clear changing of equations for the number of consumed copepods with the growth of fish (from $R=c(1-e^{-bt})$ to $R=at\ e^{-bt}$), as was observed in the larval stages of this species fed on rotifers, but the equations were still generally of the type $R=c(1-e^{-bt})$. This difference may indicate that the copepod is less digestible for juveniles aged 14 to 24 days than the rotifer for larvae aged 4 to 14 days. The equations of $R=at\ e^{-bt}$ appeared, however, frequently in the number of digested copepods for the 20–21 day-old juveniles and of non-digested copepods for the 23–24 day-old juveniles. These facts suggest the development of a digestive tract and the augmentation of digestive power of the fish against the copepod.

It has been observed that a single dose of *Artemia* nauplii frequently caused heavy lethargy of larvae and high moltality of various marine fish after they were fed nauplii for a period of 1 or 2 weeks (Fushimi, 1971: Fujita, 1973; Kitajima, 1978). But the combined feeding of *Artemia* nauplii and copepods (e.g. *T. japonicus*) could improve this situation (Fushimi, 1971). In addition, many researchers have reported that the feeding of copepods has a good effect on the survival rate and the vitality of larvae in rearing of marine fish, as mentioned in the introduction of this report. In regard to the seed production of seabass, *L. calcarifer*, no kind of copepod has ever been used, and a single dose of *Artemia* nauplii (for about one week, Maneewong et al. 1984) has never given as high a mortality of larvae as that was reported by Fushimi et al. (1971). Fujita et al. (1980) assert that the occurrence of this phenomenon varies with the fish species as well as with the place of production of *Artemia*, for the nutritional value of *Artemia* is related to this phenomenon in terms of EFA (essential fatty acid) content. In future studies, it will be necessary to examine this point and to consider the use of copepods for biological and economical interests in the seed production of this species.

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# Experiment on rearing fry of seabass *Lates calcarifer*, from 1 to 12 days old at different densities

Sujin Mancewong, Niwes Ruangpanit, Tanan Tattanon and Prakit Kraisingdecha

## INTRODUCTION

Technical expertise, well designed procedures, and the skillful manipulation of many variables are all necessary for the mass production of fry of seabass, *Lates calcarifer*. Also necessary is a knowledge of the factors which can effect the survival rate, growth rate, and amount of production of seabass fry. One important factor to consider is the suitable stocking density for fry at various ages. This study was designed to observe the survival and growth rates of seabass fry reared from 1 to 12 days old at densities of 50, 80, 110 or 140/ℓ and determine the suitable density for rearing them.

## MATERIALS AND METHODS

500 ℓ tanks were used as rearing vessels for seabass fry. The tanks were filled with 450 ℓ of sea water at a salinity of 25 ‰ and one day old seabass fry were stocked into the tanks at densities of 50, 80, 110 and 140 fry/ℓ, three tanks for each density.

A sufficient amount of live rotifers was given to the fry from 2 days old. From 8 days old, nauplii of brine shrimp were also given to the fry. Dirt and waste deposited on the tank bottom were siphoned off, and 50–100% of the water was replaced by fresh filtered sea water daily. pH, and nitrite-N content of the water were checked daily before the water exchange. The size of the fry was measured every 3 days until the experiment was completed.

## RESULTS AND CONCLUSION

Number of surviving fry at the end of the experiment and the survival rate are shown for each tank in Table 1. The highest survival rate of 85.9% was recorded at a density of 110 fry/ℓ. The survival rate at a density of 140 fry/ℓ was the second lowest at 68.3%.

Table 1. Number of surviving fry and survival rate at the end of the experiment.

| | Tank | Stocking number | Surviving number | Survival rate (%) |
|---|---|---|---|---|
| 50 fry/ℓ | | | | |
| | 1 | 22,500 | 19,000 | 84.4 |
| | 2 | 22,500 | 20,000 | 88.8 |
| | 3 | 22,500 | 19,000 | 84.4 |
| Average | | 22,500 | 19,333 | 85.9 |
| 80 fry/ℓ | | | | |
| | 1 | 36,500 | 85,000 | 68.5 |
| | 2 | 36,500 | 26,000 | 71.2 |
| | 3 | 36,500 | 28,000 | 76.7 |
| Average | | 36,500 | 26,333 | 72.2 |
| 100 fry/ℓ | | | | |
| | 1 | 49,500 | 28,000 | 56.6 |
| | 2 | 49,500 | 26,000 | 52.5 |
| | 3 | 49,500 | 36,000 | 72.7 |
| Average | | 49,500 | 30,000 | 60.6 |
| 140 fry/ℓ | | | | |
| | 1 | 63,000 | 38,000 | 60.3 |
| | 2 | 63,000 | 47,000 | 74.6 |
| | 3 | 63,000 | 44,000 | 69.8 |
| Average | | 63,000 | 43,000 | 68.3 |

Average total lengths of fry of different ages are shown for each initial density in Table 2. The largest average total lengh of 5.2 mm was attained by fry reared at the lowest initial density. Those reared at the highest initial density attained the smallest size of 4.2 mm in the experiment period. The fry in the tanks with initial density at 50/ℓ grew so well that they were fed only with brine shrimp nauplii from 11 days old.

Table 2. Average total length (mm) of fry by age.

| Age (days) | 50 fry/ℓ | 80 fry/ℓ | 110 fry/ℓ | 140 fry/ℓ |
|---|---|---|---|---|
| 1 | 1.24 | 1.24 | 1.24 | 1.24 |
| 5 | 2.99 | 2.91 | 2.71 | 2.93 |
| 8 | 3.31 | 3.24 | 3.16 | 3.29 |
| 11 | 4.58 | 3.93 | 4.10 | 3.91 |
| 13 | 5.16 | 4.51 | 4.27 | 4.19 |

pH, temperature and nitrite-N content of the water for each rearing density are summarized in Table 3. There was no significant difference in pH value between different initial densities, while nitrite-N content seemed to be a little higher at the higher initial densities. This higher nitrite-N content may have some relation to the poor growth of fish in the tanks with higher initial densities.

Table 3. pH and nitrite-N content of rearing water during the experiment.

| | Item | 50fry/ℓ | 80fry/ℓ | 110fry/ℓ | 140fry/ℓ |
|---|---|---|---|---|---|
| pH | av. | 8.0 | 8.0 | 7.8 | 7.9 |
| | max. | 8.2 | 8.1 | 8.1 | 8.1 |
| | min. | 7.8 | 7.8 | 7.8 | 7.7 |
| $NO_2$-N (mg/ℓ) | av. | 0.024 | 0.023 | 0.032 | 0.035 |
| | max. | 0.059 | 0.058 | 0.182 | 0.089 |
| | min. | 0.010 | 0.010 | 0.013 | 0.013 |

As the conclusion of the present experiment, it can be said that the initial stocking density of one day old fry should be 50fry/ℓ at highest. If the stocking density is higher than that, survival and growth rates of fry will drop.

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# Experiment on rearing fry of seabass, *Lates calcarifer*, from 13 to 29 days old at different densities

Sujin Maneewong, Niwes Ruangpanit, Tanan Tattanon and Prakit Kraisingdecha

## INTRODUCTION

In the nursing of fry of seabass, *Lates calcarifer*, one important factor to consider is the suitable density for each age of fry. Maneewong et al. (1981) had successfully nursed seabass fry aged 24–30 days at densities of 2 to 5/ℓ with a survival rate of 85.3%. This experiment was undertaken to determine the highest possible density to rear healthy fry aged 13–29 days.

## MATERIALS AND METHODS

Nine, 500 ℓ tanks were used as rearing vessels which were filled with filtered sea water at a salinity level of 25–30 ‰ . The water was aerated to maintain a disolved oxygen concentration of more than 5ppm. Seabass fry of 13 days old were stocked into the tanks at densities of 10, 20 and 30 fry/ℓ, three tanks for each density.

Throughout the experiment period, brine shrimp were fed to the fry 3 times per day. Water flea, *Moina* sp., was added to the brine shrimp from 20 days old and minced fish meat from 25 days old.

Each day, food remnants were siphoned off, and pH, and nitrite-N content of the water was checked. Then, 80–100% of the water was replaced with fresh, filtered sea water.

## RESULTS AND CONCLUSION

The number of surviving fry at the end of the experiment and the survival rate for each tank are shown in Table 1. The average survival rates for fry reared at densities of 10, 20, and 30 fry/ℓ were 77.7%, 87.7% and 90.0%, respectively. The lowest survival rate occuring at the lowest density could be attributable to cannibalism among fry. Fry reared at higher densities were too small in size and in too poor health to eat each other, which might result in higher survival rates.

Table 1. Number of surviving fry and survival rate at the end of the experiment.

| Tank | | Stocking number | Surviving number | Survival rate (%) |
|---|---|---|---|---|
| 10 fry/ℓ | 1 | 5,000 | 3,250 | 65.0 |
| | 2 | 5,000 | 4,400 | 88.0 |
| | 3 | 5,000 | 4,000 | 80.0 |
| Average | | 5,000 | 3,883 | 77.7 |
| 20 fry/ℓ | 1 | 10,000 | 8,650 | 86.5 |
| | 2 | 10,000 | 8,900 | 89.0 |
| | 3 | 10,000 | 8,750 | 87.5 |
| Average | | 10,000 | 8,766 | 87.7 |
| 30 fry/ℓ | 1 | 15,000 | 13,100 | 87.3 |
| | 2 | 15,000 | 14,070 | 93.8 |
| | 3 | 15,000 | 13,350 | 89.0 |
| Average | | 15,000 | 13,506 | 90.0 |

The growth of the fry reared at each density, measured in total length every 3–4 days, is shown in Table 2. The highest growth was for fry reared at the lowest density of 10 fry/ℓ. The growth for fry reared at higher densities was much slower than this. At the end of the experiment, the surviving fry from each level of density could be divided into 3 groups according to size, in total length; large group 22.75 ± 2.5mm, medium group 15.56 ± 1.5mm and small group 19.29 ± 2.0mm (Table 3). It is shown that a higher percentage of fry fell into the larger size categories at the lower rearing density. At a density of 30 fry/ℓ, only 8.0% of the fry fell into the large and medium groups, whereas 77.2% of the fry fell into those groups at a density of 10 fry/ℓ.

Table 2. Average total body length (mm) of fry by age.

| Age (days) | 10 fry/ℓ | 20 fry/ℓ | 30 fry/ℓ |
|---|---|---|---|
| 14 | 6.16 | 6.36 | 6.10 |
| 17 | 8.07 | 7.53 | 7.27 |
| 20 | 9.43 | 8.53 | 8.17 |
| 25 | 12.43 | 9.87 | 8.47 |
| 29 | 16.23 | 13.07 | 12.93 |

Furthermore, most of fry reared at the low density of 10 fry/ℓ were well able to feed on minced fish, whereas, very few of those fry reared at high densities could eat minced fish, and most of them still fed on brine srimp and water flea. Also, the good health of fry reared at the lowest density was noticeable in their normal shape and in their body color changing to brown. In contrast, the poor health of fry reared at the higher densities was noticeable in their heads being too large and in their body color, which was black.

Table 3. Size distribution of fry at the end of the experiment.

| Tank | | Large size | | Medium size | | Small size | |
|---|---|---|---|---|---|---|---|
| | | Number | % | Number | % | Number | % |
| 10 fry/ℓ | 1 | 750 | 23.1 | 2,200 | 67.7 | 300 | 9.2 |
| | 2 | 700 | 15.9 | 2,200 | 50.0 | 1,500 | 34.1 |
| | 3 | 800 | 20.0 | 2,200 | 55.0 | 1,000 | 25.0 |
| | Total | 2,250 | 19.3 | 6,600 | 56.7 | 2,800 | 24.0 |
| 20 fry/ℓ | 1 | 500 | 5.8 | 2,050 | 23.7 | 6,100 | 70.5 |
| | 2 | 300 | 3.4 | 2,100 | 23.6 | 6,500 | 73.0 |
| | 3 | 250 | 2.9 | 2,000 | 22.9 | 6,500 | 74.3 |
| | Total | 1,050 | 4.0 | 6,150 | 23.4 | 19,100 | 72.6 |
| 30 fry/ℓ | 1 | 300 | 2.3 | 1,000 | 7.6 | 11,800 | 90.1 |
| | 2 | 220 | 1.6 | 850 | 6.0 | 13,000 | 92.4 |
| | 3 | 250 | 1.9 | 600 | 4.5 | 12,500 | 93.6 |
| | Total | 770 | 1.9 | 2,450 | 6.0 | 37,300 | 92.1 |

pH and $NO_2$-N content of the rearing water duirng the experiment are summarized in Table 4 for each rearing density. There was no significant difference in pH, while $NO_2$-N content was highest at the density of 20 fry/ℓ, followed by 30 fry/ℓ, and lowest at 10 fry/ℓ. The lowest $NO_2$-N value occuring may have some relation to the healthier conditions of fry at the lowest density.

As a result of the present experiment, it can be concluded that a stocking density higher than 20 fry/ℓ is too high to rear fly from 13 to 29 days old. The maximum density at which fish can grow normally falls between 10 and 20 fry/ℓ.

Table 4. pH and $NO_2$-N content of rearing water during the experiment.

| Item | | 10 fry/ℓ | 20 fry/ℓ | 30 fry/ℓ |
|---|---|---|---|---|
| pH | av. | 7.8 | 7.8 | 7.8 |
| | max. | 8.0 | 8.0 | 8.0 |
| | min. | 7.5 | 7.4 | 7.5 |
| $NO_2$-N | av. | 0.051 | 0.088 | 0.070 |
| | max. | 0.330 | 0.402 | 0.206 |
| | min. | 0.006 | 0.006 | 0.009 |

## REFERENCE

Maneewong, S. et al. 1981. Propagation of sea bass, *Lates calcarifer* (Bloch). NICA Contribution No. 1. (in Thai with English abstract).

# Experiment on nursing fry of seabass, *Lates calcarifer*, from 1.0cm to 2.5cm at different densities

Sujin Maneewong, Niwes Ruangpanit,
Tanon Tattanon and Prakit Kraisingdecha

## INTRODUCTION

Most hatcheries distribute fry of seabass, *Lates calcarifer* to fish farmers at a total length of 2.5cm or longer.

Due to the cannibalistic nature of the fish, size grading is of prime importance. The first grading should be done at the end of the second week after hatching, since after that the bigger fish can eat the smaller ones. The easiest method of grading is to use buckets with pores through which only fry smaller than a certain size can pass.

Stocking fish at the right density and of the same size would reduce the rate of cannibalism. The growth of the fish would be faster and more homogeneous as well. For this reason, the optimum stocking density must be discovered.

Maneewong *et al.* (1981) had nursed seabass fry aged 24–30 days at densities of 2 to 5 fry/ℓ and produced a survival rate of 85.3%. More detailed information on the optimum rearing density is required for practical seabass seed production. This study was designed to determine the suitable stocking density for rearing seabass fry from 1.0 to 2.5cm.

## MATERIALS AND METHODS

Nine, 500 ℓ tanks were filled with filtered sea water and were aerated. Seabass fry of 1.0cm in total length were placed into the tanks at densities of 2, 3 and 4 fry/ℓ, three tanks for each density. The fry were fed about 7 hours/day (9.00–16.00) by siphoning with 0.6cm pipes from 20 ℓ plastic tanks which contained minced fish meat and water. Food remnants were taken out and 80–100% of the water was changed daily. Size grading for 2.5cm fry was done every 5–8 days using plastic buckets with pores 1/4 inch in diameter. The experiment was set up with a completely randomized design with 3 treatments and 3 replications:

Treatment A: 2 fry/ℓ
Treatment B: 3 fry/ℓ
Treatment C: 4 fry/ℓ

The survival rates of seabass fry at three density levels were statistically compared using the analysis of variance and Duncan's new multiple range test.

## RESULTS AND CONCLUSION

Survival rate of seabass fry till the end of the experiment is shown for each tank in Table 1. Number and percentage of 2.5cm fry at each size grading are shown in Table 2 for each tank.

The results show that the survival rate at a density of 2 fry/ℓ was significantly higher than at the other two densities. There was no significant difference between the survival rates for 3 and 4 fry/ℓ.

The results also show that fry at a density of 2 fry/ℓ grew fastest to 2.5cm, within 47 days, while those at higher densities grew to 2.5cm more slowly, within 52 days.

These results indicate that seabass fry reared at a density of 2 fry/ℓ show the best survival rate and the shortest time for growing from 1.0 to 2.5 cm.

Table 1. Number of surviving fry and survival rate at the end of the experiment.

| Tank | | Stocking number | Surviving number | Survival rate (%) |
|---|---|---|---|---|
| 2 fry/ℓ | 1 | 1,000 | 981 | 98.1 |
| | 2 | 1,000 | 990 | 99.0 |
| | 3 | 1,000 | 938 | 93.8 |
| Average | | 1,000 | 970 | 97.0 |
| 3 fry/ℓ | 1 | 1,500 | 1,353 | 90.2 |
| | 2 | 1,500 | 1,353 | 90.2 |
| | 3 | 1,500 | 1,170 | 78.0 |
| Average | | 1,500 | 1,292 | 86.1 |
| 4 fry/ℓ | 1 | 2,000 | 1,734 | 86.7 |
| | 2 | 2,000 | 1,712 | 85.6 |
| | 3 | 2,000 | 1,765 | 89.1 |
| Average | | 2,000 | 1,737 | 86.9 |

Table 2. Number of 2.5cm fry at each time of size grading. Figures in brackets show percentage to the total number of fry grown to 2.5cm.

| Tank | | 1st grading | 2nd grading | 3rd grading | 4th grading | 5th grading | Total |
|---|---|---|---|---|---|---|---|
| 2 fry/ℓ | 1 | 155 (15.8) | 477 (48.5) | 288 (29.4) | 61 ( 6.2) | – | 981 (100) |
| | 2 | 203 (20.5) | 481 (48.6) | 257 (26.0) | 49 ( 5.0) | – | 990 (100) |
| | 3 | 169 (18.0) | 452 (48.2) | 254 (27.1) | 63 ( 6.7) | – | 938 (100) |
| Average | | 176 (18.1) | 470 (48.5) | 266 (27.4) | 58 ( 6.0) | – | 970 (100) |
| 3 fry/ℓ | 1 | 156 (11.5) | 273 (20.6) | 509 (37.6) | 249 (18.4) | 161 (11.9) | 1,353 (100) |
| | 2 | 156 (11.6) | 599 (44.3) | 448 (33.1) | 120 ( 8.9) | 30 ( 2.2) | 1,353 (100) |
| | 3 | 85 ( 7.3) | 202 (17.3) | 329 (28.1) | 293 (25.0) | 261 (22.3) | 1,170 (100) |
| Average | | 132 (10.2) | 360 (27.8) | 429 (33.2) | 221 (17.1) | 151 (11.7) | 1,292 (100) |
| 4 fry/ℓ | 1 | 73 ( 4.2) | 674 (38.9) | 406 (23.4) | 149 ( 8.6) | 432 (24.9) | 1,734 (100) |
| | 2 | 112 ( 8.5) | 815 (47.6) | 450 (26.3) | 151 ( 8.8) | 184 (10.8) | 1,712 (100) |
| | 3 | 202 (11.5) | 828 (47.0) | 481 (27.3) | 80 ( 4.5) | 172 (10.0) | 1,763 (100) |
| Average | | 129 ( 7.4) | 772 (44.4) | 446 (25.7) | 127 ( 7.3) | 263 (15.1) | 1,737 (100) |

## REFERENCE

Maneewong, S. *et al.* 1981. Propagation of seabass, *Lates Calcarifer* (Bloch). NICA Contribution No. 1. (in Thai with English abstract).

# Experiment on feeding fry of seabass, *Lates calcarifer*, from 3 to 12 days old with different kinds of food

Sujin Maneewong, Niwes Ruangpanit, Tanon Tattanon and Prakit Kraisingdecha

## INTRODUCTION

One of the most important factors which effects the survival rate of fry of seabass, *Lates calcarifer* is the food fed to the fry. Living rotifer, *Brachionus plicatilis*, is commonly used for feeding 2–7 day old fry because it is a good quality food organism of suitable size which enables the larvae to grow fast and healthy.

Normally, live, cultured rotifer is collected by filtration or pumped directly into the fish rearing tank to feed the fry. The mass production of rotifer means feeding them with minute algae, such as *Chlorella* and *Tetraselmis*, yeast or protozoans. The process is not only hard work, but it also takes time and requires facilities. Also, at times, the mass production of the rotifer is not stable, a factor which effects the seed production of seabass. In order to avoid these kinds of problems, various attempts have been made to use other kinds of foods in place of live rotifer.

An experiment was made using 30 ℓ tanks to compare effectiveness of live rotifer, frozen rotifer, and boiled egg yolk as the food for seabass fry (Pechmanee et al. 1984). The result of the experiment showed that frozen rotifer and boiled egg yolk were inferior as food for seabass fry.

In the present experiment, similar materials were used as food for seabass fry reared in the large tanks which are used in practical seabass seed production.

## MATERIALS AND METHODS

Eight 26 $m^3$, rectangular, concrete tanks were used as rearing vessels of seabass fry. Rearing water was filtered seawater with a salinity of 30 ‰, and the aeration rate was set equally for all tanks. One day old seabass fry were stocked into the tanks. The stocking number for each tank is shown in Table 1.

The feeding experiment began when the larvae were 3 days old. The kinds of food used in the experiment were live rotifer, frozen rotifer, boiled chicken egg yolk, and a steamed mixture of instant milk and chicken egg as shown in the same table. The last two items were screened through a 100 μ mesh, and only small particles were used for the feeding.

For tanks of live rotifer, 30–50% of the water was changed each day. Live rotifer was added to the tanks at a density of 10–30 rotifers/ml. In other tanks, water was kept running in daytime to avoid pollution of water in the tanks. The exchange rate of these tanks was 80–100% per day.

## RESULTS AND CONCLUSION

The experiment was terminated when the fry receiving the various kinds of food reached 12 days old. The survival rate of the fry in each tank is shown in Table 1. The average survival rate of fry fed with live rotifer was 36% and the larvae were very healthy and uniform in size. All of these fry were able to feed on nauplius of brine shrimp when the experiment was finished.

The average survival rate of fry fed with frozen rotifer was 1.9% and the fry were not healthy. Some fry could not feed on nauplius of brine shrimp after the experiment. During the experiment, it was observed that the fry ate the frozen rotifer, but they grew slowly. The fry became weak and their mortality was very high after they were 6 days old. Swimming behavior was not normal and the fry could not swim against the current caused by aeration. Only a small number of fry could form schools, while the majority were dispersed by the aeration. Another problem was that, while frozen rotifer float on the water surface, as they thaw they sink and pollute the bottom of the nursing tanks.

The average survival rates for fry fed with boiled egg yolk and mixture of instant milk and egg were 1.7% and 1.4%, respectively. It was observed that the fry could eat the food, but they did not grow. They became weak and their mortality was very high when they were 5 days old. All of the surviving fry in both feeding groups were very thin and smaller than the fry fed with live or frozen rotifer.

It can be concluded that live rotifer is the most suitable food for fry 3—12 days old. However, it may be possible to use frozen rotifer, which showed a higher survival rate than boild egg yolk, and mixture of egg and instant milk, as supplemental food for 2 or 3 days when there is a shortage of live rotifer.

Table 1. Kind of food, and stocking number and survival rate of fry at the end of the experiment.

| Tank | Kind of food | Stocking number | Surviving number | Survival rate (%) |
|---|---|---|---|---|
| 1 | Live rotifer | 1,000,000 | 326,000 | 32.6 |
| 2 | Live rotifer | 500,000 | 204,000 | 40.8 |
| 3 | Frozen rotifer | 546,000 | 10,000 | 1.8 |
| 4 | Frozen rotifer | 510,000 | 10,000 | 2.0 |
| 5 | Egg yolk | 490,000 | 10,000 | 2.0 |
| 6 | Egg yolk | 520,000 | 7,500 | 1.4 |
| 7 | Egg+instant milk | 492,000 | 8,400 | 1.7 |
| 8 | Egg+instant milk | 485,000 | 5,000 | 1.0 |

## REFERENCE

Pechmanee T., P. Kraisingdecha and N. Ruangpanit. 1984. Feeding experiments on rearing the early stage of seabass, *Lates calcarifer*, using rotifer, *Brachionus plicatilis*, frozen rotifer and boiled egg yolk as food. Report of Thailand and Japan Joint Coastal Aquaculture Research Project. No.1.

# Study on nursing seabass larvae, *Lates calcarifer*, in earthen pond

Supot Chungyampin, Boonsong Sirikul, Chaiyuth Chantanachooklin,
Suchart Techanarawong and Vichal Wattanakul

## INTRODUCTION

At present the seabass, *Lates calcarifer* Block, is an important culture food fish in Thailand. A large number of fish farmers, both in Thailand and in other countries of the region, is interested in seabass culture. These countries, including Australia, Hong Kong, Taiwan and Malaysia, have a high demand for this species. There are two parts in seabass culture:

1. Larval rearing to supply fry to fish farmers in country and to sell abroad.
2. Rearing seabass in grow-out cages and ponds to marketable size.

The demand for seabass fry is great in this country and in foreign countries, so the price is rather high. Nursing larvae can be done only in hatchery tanks, which requires investing a lot of money. A series of food types is offered to the fish as they develop, beginning with the rotifer *Brachionus plicatilis* which is fed on green algae, *Chlorella* sp. To feed one tank of seabass larvae requires two, identical tanks of rotifer culture, and these in turn require three tanks of *Chlorella* culture. After 8–10 days, rotifers are replaced successively by *Artemia* nauplii, the freshwater cladocerans *Moina* and *Daphnia*,. and, finally, by minced fish. This experiment was conducted to examine the possibility of rearing seabass larvae from age 4 days in an earthen pond, using only fertilizers, in order to help poor fish farmers who have their own ponds.

## MATERIALS AND METHODS

A half rai earthen pond was prepared by clearing it up and killing all fish that are enemies of seabass larvae, using tea seed cake at a concentration of 10 ppm. Limestone, at a rate of 100kg/rai, was added to the pond to adjust the pH of the water to conditions suitable for fish culture.

Five days before the seabass larvae were transferred, chicken manure, at a rate of 100 kg/rai, and chemical fertilizer N:P:K–16:20:0, at a rate of 40 kg/rai, were put into the pond.

Then, water was added to the pond to a depth of 0.40m.

100,000 seabass larvae were transferred to the pond at a stocking rate of 200,000 larvae/rai. One week after fish larvae were transferred chicken manure was added by putting it on 10 platforms in the pond. 5 kg of manure was put on each platform.

Pond water analysis was done every 2 days by checking pH, salinity, temperature, dissolved oxygen and alkalinity. Zooplankton content and types of dominant species were checked by microscope in laboratory at the same intervals as water analysis.

After seabass were nursed for 27 days to about 1 month old, they were harvested by seining. Size grading was done by using a plastic basket with a hole size of 7/32 inch, in order to calculate price of fry.

## RESULT

After 100,000 seabass larvae were nursed from age 4 days in an earthen pond for 27 days, their harvest brought in 4,657 fry, for a survival rate of 4.7%. The total number of fry was divided into 4 size groups according to total length in cm as follows:

| Group | Total length (cm) | Number of fish | Percentage (%) |
|---|---|---|---|
| 1 | 1.7–2.4 | 1,074 | 23.1 |
| 2 | 2.5–2.9 | 2,675 | 57.4 |
| 3 | 3.0–3.5 | 855 | 18.4 |
| 4 | 3.5 < | 53 | 1.1 |
| | Total | 4,657 | 100.0 |

An estimate of the operational costs of nursing seabass larvae in an earthen pond is as follows:

### Expense

| | | |
|---|---|---|
| 1. 100,000 seabass larvae at age 4 days (0.05 baht/larva) | 5,000 | baht |
| 2. Tea seed cake, 3 kg (35 baht/kg) | 105 | baht |
| 3. Limestone, 40 kg. (1.50 baht/kg) | 60 | baht |
| 4. Chemical fertilizers, 30 kg. (2.40 baht/kg) | 72 | baht |
| 5. Chicken manure, 180 kg. (0.40 baht/kg) | 72 | baht |
| 6. Fuel and other expense | 700 | baht |
| Total | 6,009 | baht |

### Income

| | | |
|---|---|---|
| 1. 1,074 seabass fry, 1.7–2.4 cm in total length (1.50 baht/piece) | 1,611 | baht |
| 2. 2,675 seabass fry, 2.5–2.9 cm in total length (2 baht/piece) | 5,350 | baht |
| 3. 855 seabass fry, 3.0–3.4 cm in total length (3 baht/piece) | 2,565 | baht |
| 4. 58 seabass fry, 3.5–5.9 cm in total length (3.5 baht/piece) | 185.50 | baht |
| Total | 9,711.50 | baht |

The profit was about 3,702.50 baht in a pond area of ½ rai in only one month.That is not so bad for fish farmers who have their own earthen ponds.

Dominant types of zooplankton in the earthen pond include 4 types of zooplankton, mosquito larvae, cladocera, copepod and rotifer (Table 1).

Table 1. Dominant types and amounts of zooplankton in the earthen pond

| Date | Water sample in ml | Amount of zooplankton/ml | | | Remark |
|---|---|---|---|---|---|
| | | cladocera | copepod | rotifer | |
| 26 April 84 | 335 | – | – | – | Mosquito larvae were numerous in the first 9 days. |
| 16 May 84 | 275 | 10 | 1 | – | |
| 18 May 84 | 220 | 5 | 1 | – | |
| 23 May 84 | 120 | 6 | 15 | 9 | |
| 25 May 84 | 135 | 1 | 7 | 2 | |
| 28 May 84 | 120 | 3 | 11 | 16 | |
| 30 May 84 | 150 | 4 | 28 | 49 | |
| 1 June 84 | 140 | – | 18 | 171 | |
| 6 June 84 | 190 | 2 | 20 | 141 | |

Water properties in the earthen pond were suitable for seabass culture during the experiment period (Table 2).

Table 2. Chemical and physical water properties in the earthen pond.

| Date | Water temp. °C | pH | $O_2$ (ppm) | Salinity (ppt) | Alkalinity |
|---|---|---|---|---|---|
| 26 April 84 | 33.1 | 6.9 | 7.4 | 22 | – |
| 16 May 84 | 32.8 | 7.4 | 8.1 | 22 | – |
| 18 May 84 | 33.6 | 7.9 | 9.2 | 21 | – |
| 23 May 84 | 35.2 | 7.1 | 6.7 | 14 | – |
| 25 May 84 | 33.4 | 7.5 | 6.9 | 15 | 93.0 |
| 28 May 84 | 30.4 | 7.1 | 6.3 | 18 | 116.5 |
| 30 May 84 | 29.3 | 7.1 | 6.4 | 21 | 123.5 |
| 1 June 84 | 33.3 | 7.7 | 7.7 | 22 | 122.5 |
| 6 June 84 | 33.2 | 7.0 | 6.5 | 19 | 123.0 |
| Average | 32.7 | 7.3 | 7.2 | 19.3 | 115.7 |

## CONCLUSION

One hundred thousand seabass larvae of 4 days after hatching were grown in an earthen pond for a period of one month. They attained a size of between 1.7–3.5 cm which is suitable for selling and transferring to a nursery net cage. The survival rate was 4.66%, which is rather low. However, when the investment for nursing seabass in a ½ rai earthen pond is calculated, the result is a profit of about 3,702.50 baht in only one month. Due to the use of fertilizers, four dominant species of zooplankton were found; mosquito larvae, cladocera, copepod and rotifer. The water quality in the earthen pond was suitable for growing seabass larvae, and no diseases or parasites occurred during the nursing period.

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# Preliminary study on rearing fry of grouper, *Epinephelus malabaricus*

Seed Production Unit*

## INTRODUCTION

Since 1984, the Seed Production Unit of the National Institute of Coastal Aquaculture has been conducting preliminary studies on the rearing of fry of grouper, *Epinephelus malabaricus*, with the coorportaiton of the Satul Fisheries Station which supplied the fertilized eggs for the work.

The present report deals with the results of the rearing experiments conducted in 1984 and 1985. Before going any further, we would like to thank the Chief of the Satul Fisheries Station and the staff who kindly provided the fertilized eggs of grouper for our experiments.

## MATERIALS AND METHODS

### Rearing in 1984

The fertilized eggs were transported from the Satul Fisheries Station on18 December, 1984. 500 ℓ plastic tanks were used for incubation of the eggs and rearing of hatched fry. Tanks were supplied with filtered sea water.

Rotifer, *Brachionus plicatilis*, at a density of 5–10 inds./ml, and *Tetraselmis* sp. at 2–5 x $10^4$ cells/ml were given to the fry as initial food from the 2nd to the 20th day after hatching, while brine shrimp nauplius was given from the 20th to 35th day. Then, minced fish or shrimp meat was introduced as food from the 35th to the 53rd day. Some mosquito larvae were also given to the fish fry during this period.

During the first 15 days, 50% of total water was changed daily by continuous flowthrough. Later on, 50% of total water was changed also, but without continuous flowthrough.

### Rearing in 1985

Four rearing experiments of the grouper were conducted using the fertilized eggs transported from the Satul Fisheries Station, the first experiment from 21st March, the scond from 25th March, the third from 25th April and the fourth from 18th September, 1985.

The eggs were incubated in 500 ℓ plastic tanks. Hatched larvae were transferred to a roofed, outdoor tank with a capacity of 26$m^3$ , filled with filtered sea water to half capacity.

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*Niwes Ruangpanit, Sujin Maneewong, Tanan Tattanon, Prakit Kraisingdecha, Paitoon Akkayanont and Sunit Rojanapitayagul.

Rotifer at a density of 5–10 inds./ml and *Tetraselmis* sp. at $2 \times 10^4$ cells/ml were given as initial food on the 1st day after hatching. *Tetraselmis* sp. was given every day as food for rotifer in the rearing tank at a density of about $2 \times 10^4$ cells/ml, so that the density of rotifer was maintained at about 15–20 inds./ml from the 3rd to the 15th day after hatching of the grouper fry. Nauplius of brine shrimp was given from the 15th to the 60th day after hatching. The rotifer diet was slowly phased out over 10–15 days as brine shrimp nauplius was given. *Moina* sp. was given by the rubber tube dropping method in the corner of the rearing tank from 30th to the 60th day after hatching. Minced fish meat was introduced from the 40th day by the same method. The daily supply of *Tetraselmis* sp. to the rearing tank was continued until the 55th day after hatching.

The rate of water change was set at 10%, 20% and 30% on the 1st, 2nd and 3rd day after hatching, respectively. From the 4th day, 50% of the water was changed every day throughout each experimental period.

## RESULTS

### Rearing in 1984

Survival rate of fry during the first 15 days of rearing and growth of fry during the whole experiment period are shown in Figs. 1 and 2.

The newly hatched larvae were 1.92 ± 0.07 mm in total length. Three to four days after hatching, the melanophores of the alimentary canal increased in size. At this stage, the larvae became free swimming and began to feed on rotifer. Because their mouth size was 0.150–0.185 mm, they could eat only small size rotifer or newly hatched rotifer which was 80 x 120 $\mu$ in size.

Heavy mortality occurred between 3 and 5 days after hatching, as shown in Fig. 2.

On the 15th day, the fry were 4.18 ± 0.33 mm in total length with long dorsal spines and ventral spines. Around 30–35 days after hatching, at a length of 11.32 ± 1.29 mm, the fry began to eat fish or shrimp meat, and also ate mosquito larvae which were given to them as supplemental food.

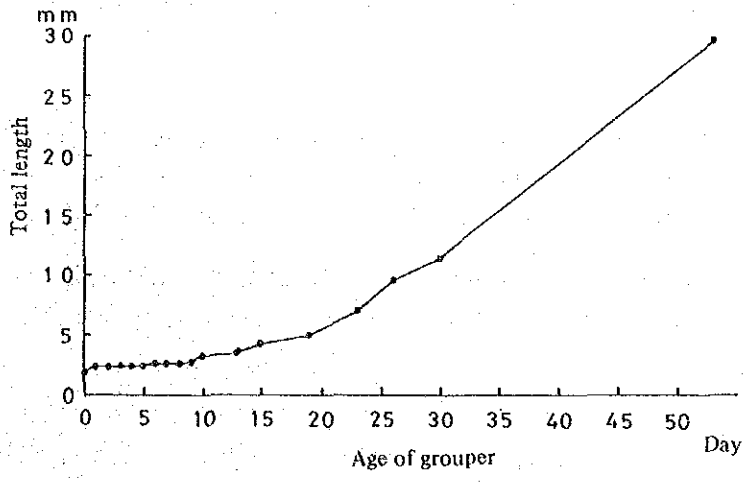


Fig. 1. Growth of larvae and juveniles of grouper in the rearing experiment in 1984.

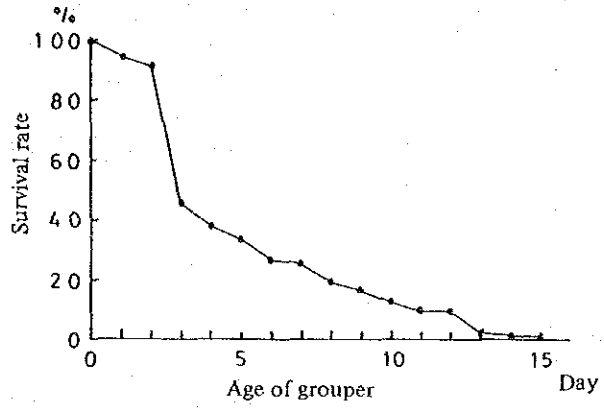


Fig. 2 Survival rate of larvae and juveniles of grouper in the rearing experiment in 1984.

The fry continued to decrease in number almost linearly from the 5th to 15th day after hatching.

Finally, after the 53 days of rearing, 52 juvenile groupers, of 29.9 ± 4.8 mm in total length and 0.52 ± 0.175 g in body weight, survived.

Water temperature was 24–30°C, salinity was 28–30‰, and pH was 8.0–8.2 throughout the experiment period.

## Rearing in 1985

First rearing

560,000 newly hatched larvae were used for the rearing experiment. Heavy mortality occurred on the 11th day after hatching. All remaining larvae died on the 12th day. The salinity was 27–33 ‰ and pH was 8.22–8.7 during the period.

Second rearing

Groups of 563,200 and 448,800 newly hatched larvae were reared in two separate tanks. Heavy mortality occurred between 6 and 7 days after hatching in both tanks, so all remaining larvae in the two tanks were gathered into one tank. All the larvae died by the 19th day after hatching. Water temperature was 27.5-29.5°C, salinity was 27–33 ‰ and pH was 8.24–8.80 throughout the rearing period.

Third rearing

210,290 newly hatched larvae were used for this rearing experiment. Finally, 61 days after hatching, 10,262 juveniles of 1.5–3.0cm in total length survived. The survival rate was 4.9%. The size distribution of surviving fish was as follows:

| | | | |
|---|---|---|---|
| Large size | 2.5–3.0cm | 62 fish | 0.6% |
| Medium size | 2.0–2.5 cm | 1,200 fish | 11.7% |
| Small size | 1.5–2.0 cm | 9,000 fish | 87.7% |

These juveniles were reared separately in different tanks until 78 days after hatching, then transferred to a net cage in open sea. Fish transferred to the next cage were 2,855 in number and 3.6 cm in average total length. The size distribution of these fish was as follows:

| | | | |
|---|---|---|---|
| Large size | 5–6 cm | 55 fish | 1.9% |
| Medium size | 4–5 cm | 800 fish | 28.0% |
| Small size | 3–4 cm | 2,000 fish | 70.1% |

After 3 months of nursing in the net case, about 1,000 fish survived.

Fourth rearing

Groups of 336,000 and 408,000 newly hatched larvae were used for this rearing experiment. All of the fish died by the 41st day after hatching because of white spot disease. Water temperature was 25.3–27.0°C, salinity was 31–34 ‰ and pH was 7.4–8.7 throughout the rearing period.

## DISCUSSION

Heavy mortality of grouper larvae occurred during the 3–11 days after hatching. From observation, the major causes of larval mortality were the weakness of the larvae after hatching and the unavailability of food. The size of rotifer fed to the larvae might have been too big. Pechmanee (1983) reported that the size of rotifer cultured with *Tetraselmis* sp. as food had a lorica length of 154–185 $\mu$. The mouth size of the grouper larvae 3–11 days old was 150–183 $\mu$.

Also, some environmental conditions might have effected the survival rate of the larvae. Further study should be made in this respect.

Other remarks about grouper larval rearing are:

1. The larvae were sensitive to the environment. They died easily from shock at every age of rearing. Death caused by shock disappeared when their total length became more than 2.5 cm or when body pigmentation was nearly completed, as in the adult stage, about 60 days after hatching.

2. The larvae were very different in size, especially 15–20 days after hatching, but grading them by size could not be done until body pigmentation was nearly completed.

3. Cannibalism occurred about 25–30 days after hatching, when the dorsal spine length in proportion to the total length began to decrease.

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Pechmanee, T. 1983. Experiments on the culture of rotifer (*Brachionus plicatilis*) with various kinds of food. Technical Paper No.3. NICA, Dept. Fish., Thailand: 157–180.

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# Larval rearing and development of grouper, *Epinephelus malabaricus* (Bloch and Schneider)

Sujin Maneewong, Paitoon Akkayanont, Juadee Pongmaneerat and Masato Iizawa

## ABSTRACT

Larval rearing of grouper *Epinephelus malabaricus* was conducted with a water-flow system and with cultured copepod *Tigriopus japonicus* as additional food, compared with the ordinary rearing system. No efficient results on the survival and growth of larvae were obtained from these two rearing trials. Some meristic characteristics of the larvae and juveniles were described as well as morphological observation of their development during these rearing trials. Rotifer, *Brachionus plicatilis* was examined to determine whether its size would be suitable as a beginning food for the larvae of this species. The amount of consumed rotifers was also investigated for 2–15 day old larvae. Finally, the difficulty of larval rearing of this species was discussed.

## INTRODUCTION

Fish of the genus *Epinephelus* are very important for commerce and are highly esteemed as food fish in Southeast Asia. Some studies on net-cage culture in these areas have been reported for *E. salmoides* by Teng et al. (1978), Teng and Chua (1978 and 1979), Chua and Teng (1980) and Hu and Lin (1985).

Ukawa et al. (1966) reported the spawning habits and the early life history of *E. akaara*. The development of larval and young stages of this species was described by Mito et al. (1967). Teng and Ho (1979) described also the development of eggs obtained by using hormone injection, as well as the larval rearing. Natural spawning in indoor culture tanks was reported by Miki et al. (1983). In recent years, seed production of this species in large scale has been attempted by JASFA (1983) in Japan.

Hussain et al. (1975) reported the spawning behavior and egg development of *E. tauvina*. The larval rearing and development of this species were also described by Hussain and Higuchi (1980). Chen et al. (1977) conducted the artificial spawning of this species, and described embryonic and larval development.

Murai et al. (1984) described the spawning season in wild and the natural spawning of *E. fasciatus* in captivity.

In Thailand, Phuket Brackish water Fisheries Station (1985) succeeded in obtaining eggs of *E. tauvina* by natural and artificial fertilization in recent years, and reported also studies on grow breeding of *E. tauvina* and *Cromileptes altivelis*.

With regard to *E. malabaricus*, Sutemechaikul et al. (1985) described artificial breeding conducted by using hormone injection in Satul Brackishwater Fisheries Station, and development of eggs and larvae. Recently, natural spawning has occurred in captivity and larval rearing has been tried in this station. Since then, spawned eggs have also been transported to the National Institute of Coastal Aquaculture (NICA) in Songkhla for trying to rear the larvae in large scale. The survival rate of the larvae has been very low and its best was about 5% after 60 days rearing from hatching in a 26 $m^3$ rectangular tank; 10,262 fry measuring 15–30 mm in total length survived from 210,290 newly hatched larvae in a preliminary rearing trial on Apr.–Jun. 1985.

In the present study, we tried to improve the rearing techniques in order to obtain higher survival of the larvae, and described the larval and juvenile development as well as some other biological characters.

## MATERIALS AND METHODS

### Collecting and transporting of eggs

Eggs of *Epinephelus malabaricus* spawned naturally at night by parent fish stocked in two rectangular tanks measuring 10 x 4 x 2 m depth (75 $m^3$ capacity) were collected by a scoop net (1.5 x 4 m; 300 $\mu$m mesh) at the Satul Brackishwater Fisheries Station on the next morning. The fertilized eggs were packed in polyethylene sacks measuring 50 x 75 cm (50,000 – 100,000 eggs per sack) with 6 $\ell$ seawater and 12 $\ell$ oxygen per sack, and then transported by car to the National Institute of Coastal Aquaculture (transport time: 3 hours).

### Rearing trial I

Transported eggs were introduced to two, roofed concrete tanks of 26 $m^3$ (5.8 x 3.8 x 1.2 depth; 25 $m^3$ capacity) on 12 November 1985; $10^6$ eggs to each tank. Hatching rate of the eggs was estimated at approximately 80%. The newly hatched larvae were reared for 61 days, from 13 November 1985 to 13 January 1986. The rearing was conducted from the first day with two aeration systems; using sole 4 airstones (ordinary airstone system) and using 6, half-cleaved PVC pipes (136 cm long and 7.4 cm diameter), one end of which was fixed on a concrete base and each of which possessed an airtube for air-water-lifting, which made the water circulate (water-flow system).

The rearing was carried out in standing water for the whole period. The water exchange rate was 10–20% for the 2nd to 5th day and then 50–80% per day. The newly changed water was previously treated with calcium hypochloride ($CaOCl_2$) 20 ppm for removing turbidity which was very strong during the monsoon seasons in this region (October to March). The water was strongly aerated with this medicine for 24 hours and was reserved without aeration for another 48 hours before being introduced to the rearing tanks.

Larvae were fed on cultured zooplankton; rotifer *Brachionus plicatilis*, nauplius and adult *Artemia salina* and water flea *Moina* sp.. Minced fishmeat was added to the rearing tanks from 40 days after hatching (Fig. 1). The rotifer and *Artemia* were fed in the morning (9:00–10:00). Food density was kept about 10 to 20 rotifers/ml. Green water *Tetraelmis* sp. was introduced 2-5 x $10^4$ cells/ml in density from the first day of feeding to 20 days after hatching, as a food of rotifer and to keep the water condition good for the larvae. No measurements of food density were made for other prey. Every morning the settled, organic waste was removed from the rearing tanks.

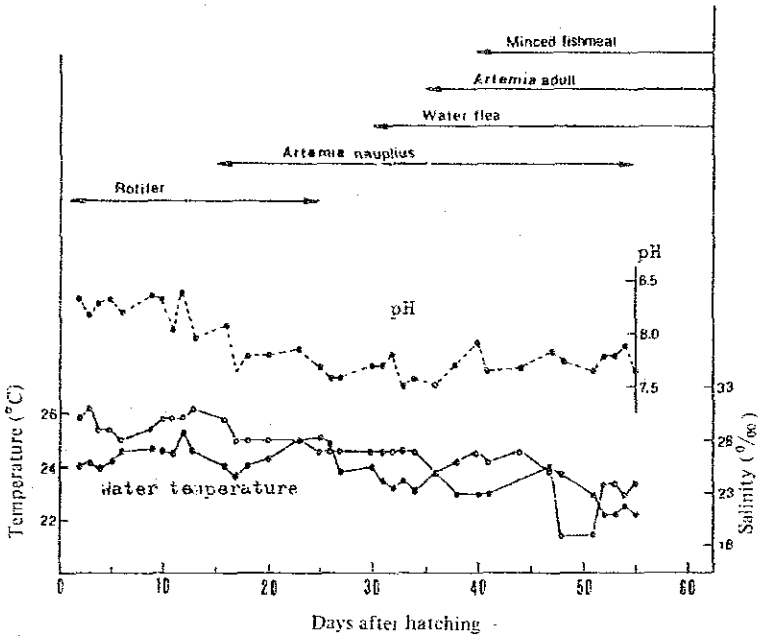


Fig. 1. Rearing conditions for *Epinephelus malabaricus* in a 26 $m^3$ concrete tank. Horizontal bars show the feeding period of the various food organisms.

## Rearing trial II

Transported eggs were hatched out in two, 500 ℓ polycarbonate tanks. The newly hatched larvae were introduced to the rearing tanks of 26 $m^3$ and reared for 30 days, from 18 September to 17 October 1985. The stocking number of the newly hatched larvae was about 330 x $10^3$ in one tank and about 400 x $10^3$ in the other. Copepod, *Tigriopus japonicus*, including nauplius, copepodite and adult was added, 4.08 x $10^6$ individuals per day on average, to the former rearing tank, from 13 days after hatching to the end of rearing trial. The copepod was cultured in 100 $m^3$ outdoor, circular tanks of concrete, using fishmeal and ricebran as food. The water of both the rearing tanks was aerated by the ordinary airsystem (described in rearing trial I). The other rearing techniques and feeding scheme were similar to rearing trial I.

## Determination of meristic and morphometric characters

Total body length of fish were measured every day after anesthetizing 20–30 sampled fish in 20 ppm quinaldine solution for both the rearing trials, under a binocular microscope or a profile projector. After the length measurements, the specimens were preserved in 5% formalin solution.

Two to four months after preservation, 10--20 specimens of each day's sampling were measured in mouth sizes [mouth breath (MB) and mouth height (MH); Fig. 2] and length of dorsal second and ventral spines, as well as total body length. Morphological observations were made to demonstrate the larval and juvenile development.

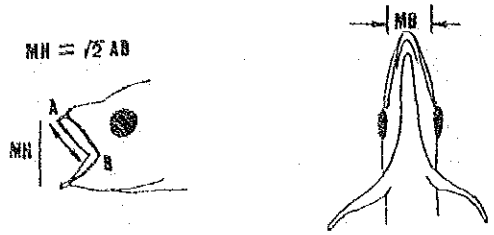


Fig. 2. Measurement of mouth height (MH) and mouth breadth (MB)

Size composition and amount of consumed rotifer

30 larvae, 2 days old, were sampled 3 hours after the first feeding from the rearing tank of the ordinary airstone system in rearing trial I. After the anesthetization in quinaldine, the sampled larvae were dissected under a binocular microscope to measure the lorica length and width of rotifers consumed by the larvae. The sizes of rotifers in the rearing tank were also measured to compare with the former.

The number of rotifers in the digestive tract was counted, following the dissection of larvae after the fixation in formaline, for each 10 larvae, 2 to 15 days old, which were sampled from the rearing tanks in rearing trial II.

## RESULTS AND DISCUSSION

### Rearing trial I

The larvae of *E. malabaricus*, until about 2 weeks after hatching, showed strong mucus on the epidelmis of their bodies in some preliminary trials of larval rearing. And a sudden mortality was sometimes observed at early larval stages, due to suffocation in a group, caused by the phototaxis of the larvae. The larvae were very adhesive to one another in the group because of their strong mucus. In order to avoid this sudden mortality of the larvae, the water was made to circulate in the rearing using the ordinary airstone system. The water conditions of these two rearing tanks were 23.8 ± 0.8°C, 27.0 ± 2.8‰ (salinity) and 7.87 ± 0.27 (pH), and 23.7 ± 0.8°C, 27.1 ± 2.7‰ (salinity) and 7.95 ± 0.25 (pH), respectively, during the experiment.

There was no significant difference in the growth of fish between these two aeration systems (Fig. 3). Fig. 4 shows the relationship between the total length of the fish and the length of the dorsal second spine during these two rearings. The water-flow system did not have any effect on the growth of dorsal second spine, either. The dorsal second spine grew rapidly and its maximum

relative-length came up to 60% of the total body length at about 6 mm. Then, the relative-length of the dorsal second spine decreased slowly and became about 10–15% of the total body length at the end of the rearing.

The numbers of surviving fish at the end of the rearing were 1400 and 2900 for the water-flow system and the ordinary airstone system, respectively. It seems, therefore, that the water-flow system had a negative effect on the survival of the larvae. This may have been due to fatigue of the larvae caused by the continuous water flow through day and night, or caused by a too strong water flow for the larvae. This might be improved by stopping the water flow in the nighttime or by weakening the water flow. Meanwhile, it seems that the water flow could decrease the chance of cannibalism which was observed in the ordinary airstone system rearing from about 20 days after larval hatching. Anyway, further investigation is necessary to determine effectively the best water-flow system for the larval rearing of this fish.

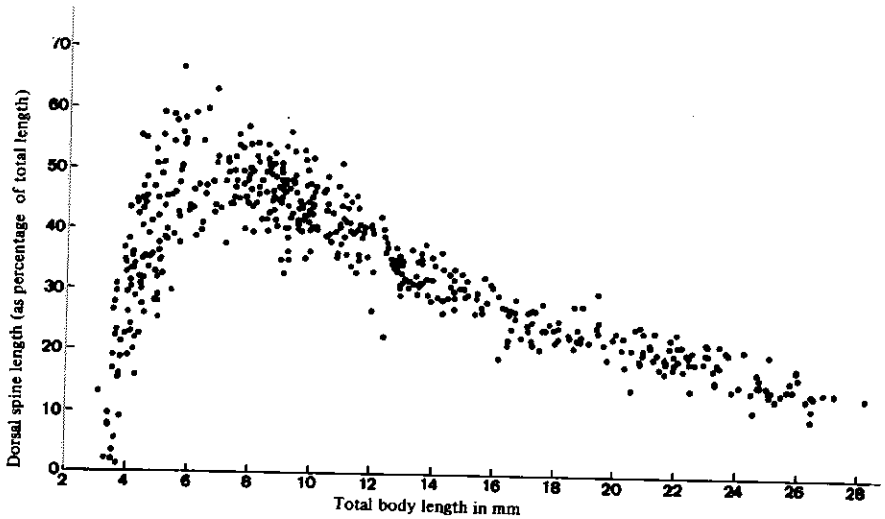


Fig. 4. Growth of the dorsal second spine relative to the total length of *Epinephelus malabaricus*, reared with water-flow system (solid circles) and with ordinary airstone system (open circles).

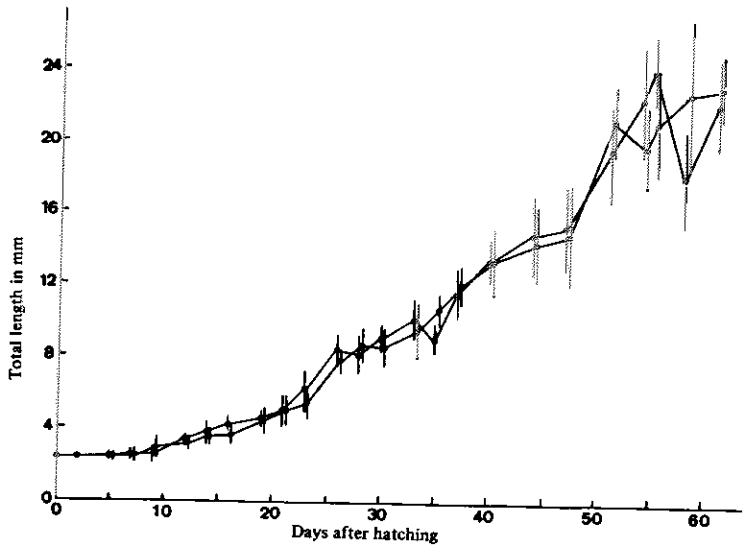


Fig. 3. Growth of *Epinephelus malabaricus*, reared with water-flow system (solid circles) and with ordinary airstone system (open circles). Vertical bars = standard deviation.

## Rearing trial II

The rearing was carried out to compare the difference in the growth and survival of grouper fry *E. malabaricus* between using copepod *T. japonicus* and not using it as additional food. The water conditions of these two rearing tanks were 25.7 ± 0.6°C, 33.8 ± 0.4‰ (salinity) and 8.14 ± 0.21 (pH), and 25.5 ± 0.5°C, 33.8 ± 0.4‰ (salinity) and 8.19 ± 0.18 (pH), respectively, during the experiment.

No significant difference appeared in the growth and survival of the fish during the 30 days rearing (Fig. 5); 10.98 ± 1.90 mm and 10.16 ± 2.12 mm in total length at the end of the rearing for using and not using the copepod, respectively. The number of surviving fish was roughly estimated at 30,000–40,000 in both the rearing tanks. The exact number of surviving fish could not be counted on day 30, because of the strong sensitivity of the fish (die easily from shock).

Many researchers have reported that the use of copepods as additional food showed a good effect on the survival rate and the vitality of larvae and/or juveniles in rearing of marine fish (e.g.: Fushimi and Hashimoto, 1969; Fukusho et al. 1973; Kitajima, 1978). However, no clear effect was obtained in this experiment. In this rearing, we could not feed the sufficient amount of the copepod to the larvae everyday, because the culture of the copepod was very instable (there was sometimes a lack of the copepod for feeding). It will, therefore, be necessary to carry out some experiments in smaller scale, in order to examine in detail the effect of feeding the copepod on the rearing of this fry.

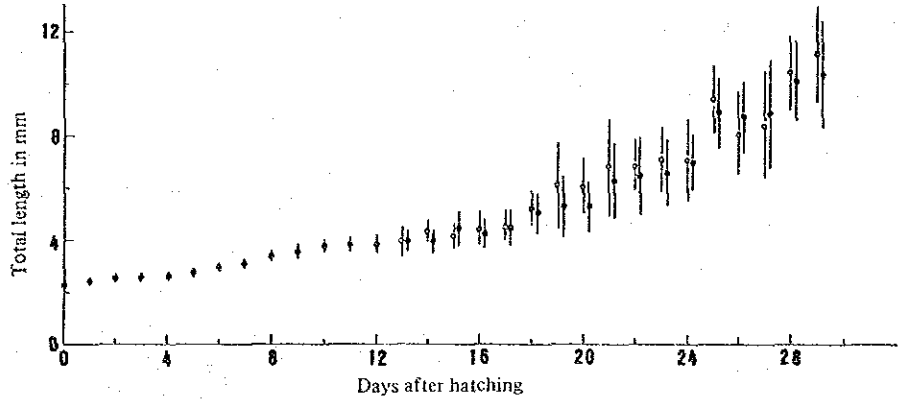


Fig. 5. Growth of *Epinephelus malabaricus*, reared using copepod, *Tigriopus japonicus*, as additional food (open circles) and not using it (solid circles). Vertical bars = standard deviation.

## Embryonic development

The fertilized eggs of *E. malabaricus* were spherical in shape, measuring 0.852 ± 0.010 mm in diameter. The eggs hatched after 19 hours at 29–30°C and 29–30‰ (salinity) (Sutemechaikul et al. 1985).

## Development of larvae and juveniles

Newly-hatched larvae (Fig. 6a).

The newly-hatched larvae measured 2.27 ± 0.09 mm in total length and carried a large yolk sac of 1.55 ± 0.10 mm in length and an oil globule of 0.18 ± 0.01 mm in diameter at the posterior end of the yolk sac. The eyes were unpigmented and the mouth was closed. The narrow, straight gut was visible. The otic vesicle was seen behind the posterior region of the head.

2.37 mm in total length (Fig. 6b).

One day after hatching, the yolk was partly absorbed but the mouth was still closed. Pectral fins and jaw buds appeared, and a urinary bladder was visible. But the anus was still closed.

2.50 mm in total length (Fig. 6c).

Two days after hatching, the yolk was mostly absorbed and the oil globule reduced to a negligible size. The mouth and anus opened. The eyes were pigmented. Melanophores appeared midway along the dorsal and ventral portion of both the body and the alimentary canal. Some melanophores were also observed at the ventral side of the tail.

2.80 mm in total length (Fig. 6d).

The buds of dorsal second and ventral spines appeared on day 5. The melanophores increased and were almost wholly distributed midway of the body and at the alimentary canal.

3.30 mm in total length (Fig. 6e).

The buds of dorsal second and ventral spines grew, and the latter began to project out of the fin-fold on day 7.

3.65 mm in total length (Fig. 6f).

On day 10 the bud of dorsal second spine began to project out of the fin-fold. The ventral spines extended and had some melanophores on their tips.

4.20 mm in total length (Fig. 6g).

The dorsal first spine appeared on day 15. The dorsal second spine extended and had some melanophores on its tip. The gill arches developed. Many spinelets appeared on the anterior and posterior margins of both the dorsal second and the ventral spines.

6.00 mm in total length (Fig. 6h).

The dorsal third spine appeared by day 20. The caudal anlage appeared and some caudal rays were shown at the ventral portion of the tail. A long spine developed at the posterior margin of the preoperculum, and a short spine above the orbits. The nares appeared as a single opening. Around this age, the length of the long dorsal spine became nearly identical to that of the ventral spines.

9.87 mm in total length (Fig. 6i).

At day 24 the fish possessed the adult dorsal (XI, 15), ventral (I, 5) and anal (III, 8) fins. The nares began to constrict at the center. Many melanophores appeared on the surface of caudal peduncle. The dorsal second spine became longer than the ventral spines.

17.0 mm in total length (Fig. 6j).

On day 45 the fish possessed the adult pectral 17 and caudal 21 fins. The spines and rays of each fin became differenciated as in the adult. The length of the dorsal second and ventral spines became shorter in comparison to the total body length. The melanophores were well developed on the dorsal surface on the body, and especially on the top of the head and on the operculum.

28.0 mm in total length (Fig. 6k).

By day 60 the body form completed metamorphosis. The lateral lines were clearly visible. The body pigmentation was accomplished with numerous melanophores distributed all over the body.

The development of larval and juvenile stages of *E. malabaricus* was very similar to that of *E. tauvina* (Hussain and Higuchi, 1980). However, melanophores were distributed midway along the dorsal and ventral portion on both the body and the alimentary canal of *E. malabaricus* at 2 days after hatching (2.50 mm in total length), while the melanophores did not appear on the dorsal portion of the body at early larval stages of *E. tauvina*.

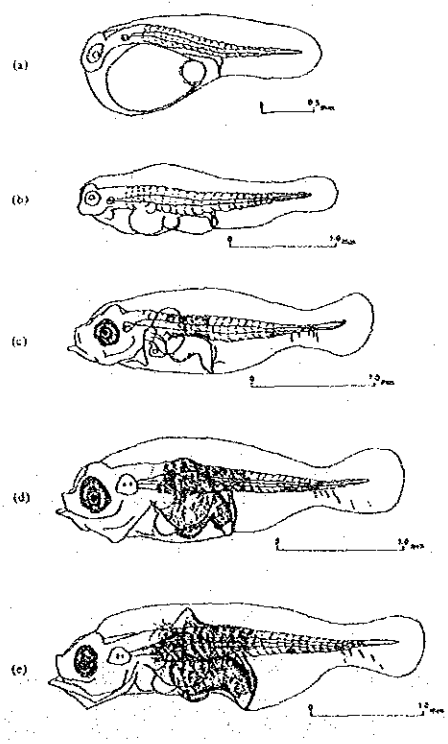

Fig. 6 (a-e).

Development of larvae, *Epinephelus malabaricus*
(a) Newly-hatched larva, 2.27mm.
(b) One day old, 2.37mm. (c) 2 days old, 2.50mm.
(d) 5 days old, 2.80mm. (e) 7 days old, 3.30mm.

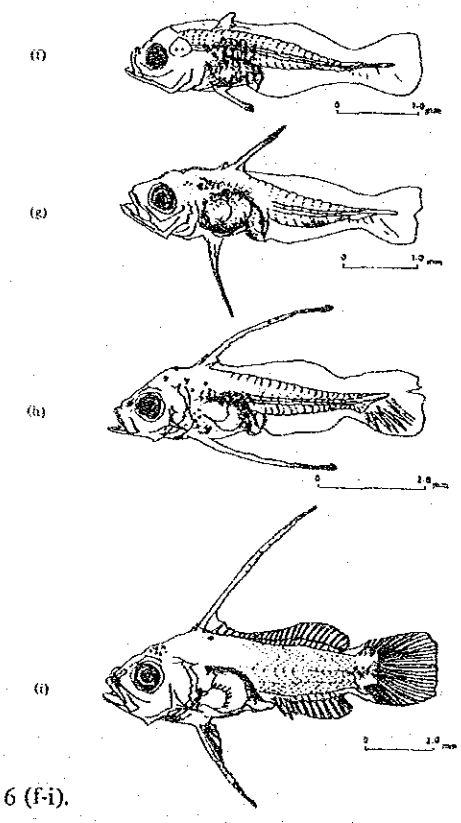

Fig. 6 (f-i).

Development of larvae and juveniles, *Epinephelus malabaricus*.
(f) 10 days old, 3.65mm. (g) 15 days old, 4.20mm.
(h) 19 days old, 6.00mm. (i) 24 days old, 9.87mm.

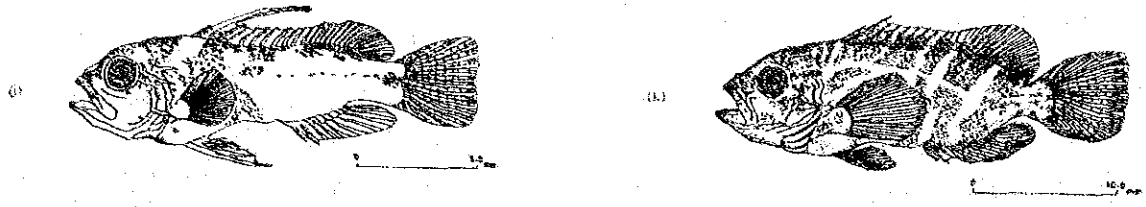

Fig. 6 (j and k). Development of juveniles, *Epinephelus malabaricus*.
(j) 45 days old, 17.0mm. (k) 60 days old, 28.0mm.

## Growth of dorsal second and ventral spines

The long projecting dorsal and ventral spines, which are the most striking feature of grouper larvae (Hussain and Higuchi, 1980), were evident in this species until the fish reached to 24--26 mm in total length at about 2 month after hatching. Fig. 7 shows the relationship between the total length and the lengths of the dorsal second and the ventral spines during the 30 days rearing after hatching. The ventral spines were slightly longer than the dorsal second spine until 4.5 mm in total body length. Then, the dorsal second spine grew much faster than the ventral spines until about 6 mm in total body length, at which fish had the maximum relative-lengths of the dorsal second and ventral spines (about 60% and 50% of the total body length, respectively). The relative-lengths of these spines decreased slowly and the difference between the lengths of these spines also diminished, as the fish grew further.

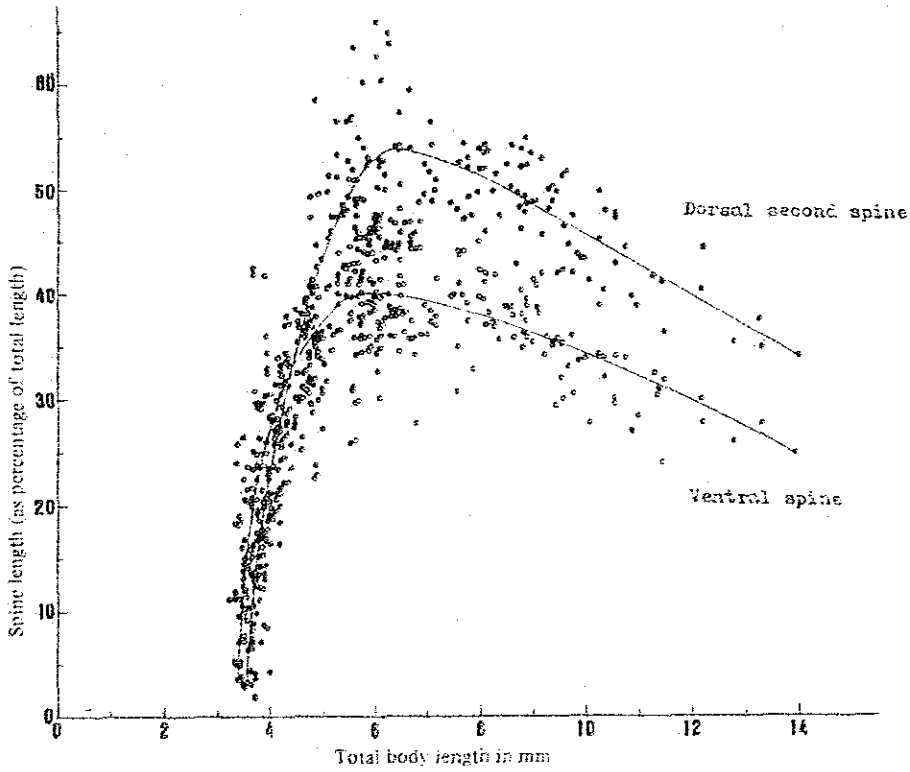


Fig. 7. Changes in the length of the dorsal second (solid circles) and ventral spines (open circles) as a percentage of the total length of *Epinephelus malabaricus*.

Mouth size

Fig. 8 shows the growth of the mouth breadth and mouth height (MB and MH, respectively; shown in Fig. 2). The relationship between these two lengths and the total body length (TL) was represented as the following equations; MB = 0.15 TL – 0.10 and MH = 0.28 TL – 0.53. The mouth height was slightly smaller than the mouth breadth until about 3.3 mm in total body length, at which point these two equations crossed. After this crossing point the mouth height became much greater than the mouth breadth.

Mouth size is a very important factor for feeding in larval rearing of fish. Especially, mouth breadth (MB) or mouth height (MH) of fish larvae can be a factor limiting prey size (Yasuda, 1960; Shirota, 1970; Iizawa, 1983). As the mouth height was smaller than the mouth breadth until about 3.3 mm in total length of *E. malabaricus* larvae the month height is considered as the limiting factor of prey size until this larval stage. After this stage, the factor limiting prey size should change to the mouth breadth. Yasuda (1960) stated that proper size of prey (body width or height) was nearly identical to mouth breadth of predator. On the other hand, Shirota (1970) assumed that body width of prey should have been equal or smaller than an opening rate of mouth (50 to 75% of mouth height). Moreover, Iizawa (1983) reported that body width of prey consumed by seabass larvae, *Dicentrarchus labrax* and its suitable size ranged 33 to 100% and 70 to 90% of their mouth breadth, respectively. Consequently, it is important for larval rearing of fish to feed on prey having its body width slightly smaller than the mouth limiting factor (mouth breadth or height). We investigated only the mouth size in this study, but it would be very interesting to examine the relationship between prey size and mouth size of this species.

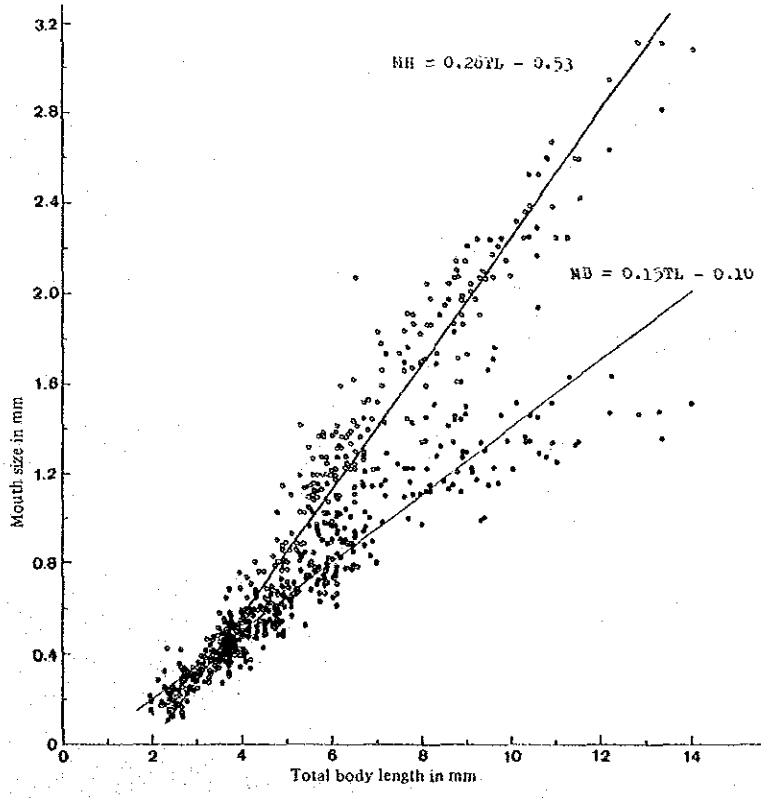


Fig. 8. Changes in the mouth size of *Epinephelus malabaricus*.
Open circles = mouth height (MH) and solid circles = mouth breadth (MB).

Size composition of consumed rotifer at the beginning of feeding

It has been said that rotifer, *Brachionus plicatilis*, may not be suitable as beginning food for grouper larvae, because the size of rotifer has been considered too big for them (e.g.: Hussain, 1980 and JASFA, 1983). JASFA (1983) has actually used fertilized eggs and larvae of oyster, *Crassostrea gigas* and small rotifer *B. plicatilis* (lorica length: 100–180 μm) as the beginning food for the larvae of *E. akaara*. In the present study, we checked on the size composition of rotifers consumed by the larvae of *E. malabaricus*, just beginning to feed, compared with that of rotifers distributed in the rearing tank.

Fig. 9 represents the lorica length and width of rotifers consumed by the larvae and those of rotifers distributed in the rearing tank. The mouth height (MH) of the sampled larvae was 169.7 ± 16.1 μm. The averages of lorica length were 140.7 ± 23.1 μm and 154.3 ± 19.4 μm for the consumed rotifers and for the rotifers in the rearing tank, respectively. The mode of the lorica length of consumed rotifers was identical to that in the rearing tank (mode: 151–160 μm). The size composition of consumed rotifers was slightly inclined to sizes smaller than this mode, while the size composition of rotifers in the rearing tank represented nearly a normal distribution.

The averages of the lorica width were 113.6 ± 19.1 μm and 119.8 ± 16.2 μm for the consumed rotifers and for the rotifers in the tank, respectively. The mode of the lorica width of consumed rotifers was much smaller (91–100 μm) than that in the rearing tank (131–140 μm). Nevertheless, all the sizes of rotifers in the rearing tank were taken by the larvae at the beginning of feeding.

Although the averages of lorica length and width of consumed rotifers were slightly smaller than those of rotifers in the rearing tank, and the size composition of consumed rotifers was inclined to the smaller sizes, the size composition and its mode were similar in both the rotifers. It seems, consequantly, that the distributed rotifers in the rearing tank were not so over large as the beginning food for this fish species. Actually, most of the sampled larvae had some rotifers in their digestive tract.

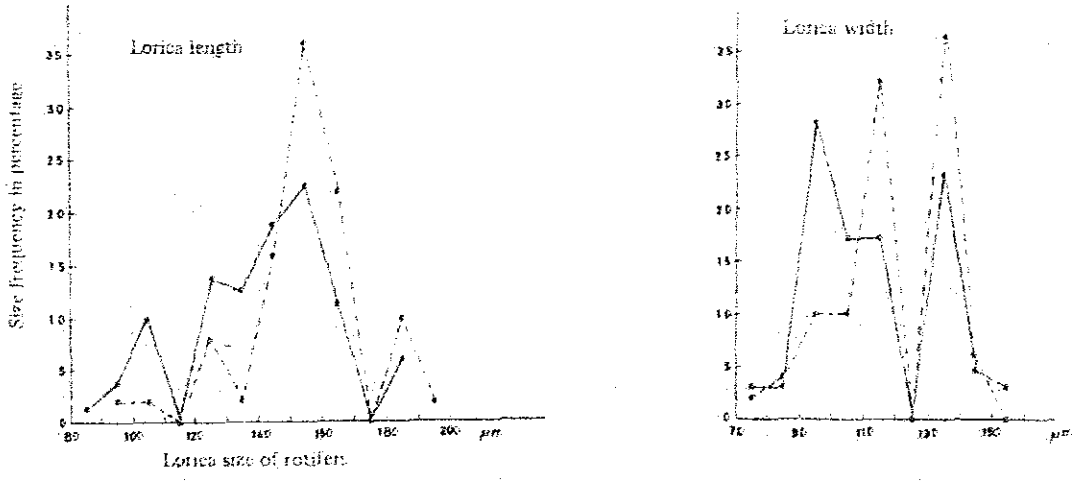


Fig. 9. Size composition of rotifers consumed by *Epinephelus malabaricus* larvae at the beginning of feeding (continuous line) and that of rotifers distributed in the rearing tank (dotted line).

### Amount of consumed rotifer (Fig. 10)

The number of rotifers in digestive tract increased exponentially as the larvae grew (1.7 ± 1.5 to 71.5 ± 14.8 rotifers for 2 to 11 day old larvae). From 12 days after hatching, *Artemia* nauplius was added to the rearing tanks. So, the number of consumed rotifers decreased suddenly. From the following days, copepod *Tigriopus japonicus* was again given to the one rearing tank, while sole *Artemia* nauplius was added to the other tank. And so the difference of the number of consumed rotifers appeared between the larvae of these two tanks; the larvae fed on rotifer plus *Artemia* nauplius and *T. japonicus* consumed a smaller amount of rotifers than the larvae fed on rotifers plus *Artemia* nauplius. This might have been due to the difference of digestibility between *Artemia* nauplius and *T. japonicus*; the latter may be less digestible and be retained a longer time in the digestive tract of the larvae than the former.

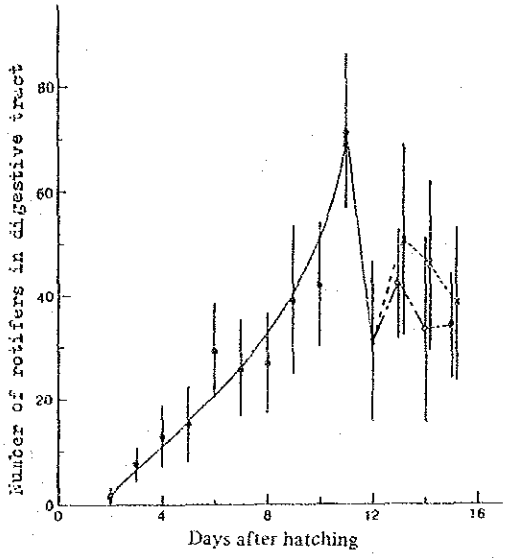


Fig. 10. Amount of rotifers consumed by *Epinephelus malabaricus*, at each feeding scheme; ● Rotifer, x Rotifer plus *Artemia* nauplius, ○ Rotifer plus *Artemia* nauplius and *Tigriopus japonicus*. Vertical bars = standard deviation.

## CONCLUSION

During the rearing trials, turbidity of seawater was very strong, and so we could not rear the larvae in running water throughout the rearing trials. Moreover, water exchange took place in a short time (within two hours per tank) in the morning and the newly changed water was previously treated with a medicine ($CaOCl_2$). Therefore, the rearing condition was not in any sense good.

After several trials of larval rearing of *E. malabaricus*, we observed and clarified some difficulties for mass production as follows; The larvae, until about 2 weeks, had a strong mucus on the epidelmis of their bodies. So, the larvae had always a danger of dying from suffocation in a group caused by the phototaxis of the larvae. The larvae had a long dorsal and two long ventral spines. These spines appeared at about 6 days old and became normal spines of dorsal and ventral fins at the age of about 2 months. The larvae were very weak and died easily as opening these long spines

by physical shocks during these two months. Moreover, the cannibalism of the larvae began at about 20 days after hatching, but we could not sort the larvae in size because of the spines and the weakness of the larvae.

For resolving these problems, we have been trying to improve the rearing method, but have not yet found the most effective method of larval rearing. In future studies, the nutritional requirement of the larvae should be examined in terms of EFA (essential fatty acid) which is considered to be a very important factor for larval rearing of marine fish. On the other hand, the larval weakness of this species might be related to the quality of eggs which must be effected by the quality of spawner fish. It would also be necessary to check this point.

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# Study on development of larva and juvenile of seabass, *Lates calcarifer*

Dusit Tunvilai, Putth Songsangjinda and Yongyut Predalumpaburt

## INTRODUCTION

A knowledge of the morphology and development of fish larvae is important for rearing them in the hatchery. This study involves the examination of the morphology, fin development and some osteology of 2–30 days old seabass, *Lates calcarifer*, larvae.

## MATERIALS AND METHODS

About 40 samples of seabass larvae and junveniles were collected each day from 2 to 30 days old in the NICA hatchery. The water salinity was 20–30 ‰ and the water temperature was approximately 28–30°C. The sample was divided into two parts for the study; one fixed with formalin, and one treated by calcareous stain with Alizarin Red and Alician Blue.

Morphometrics were taken by objective micrometer. The pigmentation of the fish was observed, and sketched using a drawing tube. The measurement was done according to Johnson and Loesch (1983), and Mori (1983), as follows:

Total length (TL): Tip of snout to end of caudal finfold complex in preflexion larvae, and to end of the longest superior procurrent caudal ray in flexion and post flexion larvae.

Notocord-standard length (SL): Tip of snout to tip of notocord in preflexion, and early flexion larvae. Tip of snout to posterior end of hypural plate in late flexion and postflexion larvae.

Preanal length (PAL): Tip of snout to end of anus measured along the middle of body.

Head length (HL): Tip of snout to posterior margin of auditory vesicle in early preflexion larvae. Tip of snout to posterior margin of opercular membrane in larvae at later stages.

Eye diameter (ED): Horizontal diameter between anterior and posterior edges of the fleshy orbit.

Snout length (SN): Tip of snout to anterior margin of fleshy orbit of the eye.

Body depth (BD): Vertical height of the body measured at base of pectoral fin.

Maxillary length (MX): The length of maxillary bone.

Lower jaw (LJ): The length of mandible bone.

Mouth width (MW) can be calculated by an equation: $MW = \sqrt{MX^2 + LJ^2}$

## RESULTS

### Morphometrics

The relations of various morphometric measurements to the standard length are expressed by linear regressions with high coefficients of determination as follows:

| | | | |
|---|---|---|---|
| Total length | : | TL = –0.48 + 1.29SL | $r^2$ = 0.9916 |
| Preanal length | : | PAL= -0.56+0.755SL | $r^2$ = 0.9908 |
| Head length | : | HL = –0.40 + 0.45SL | $r^2$ = 0.9809 |
| Eye diameter | : | ED = –0.01 + 0.13SL | $r^2$ = 0.9677 |
| Snout length | : | SN = –0.06 + 0.09SL | $r^2$ = 0.9378 |
| Body depth | : | BD = –0.40 + 0.45SL | $r^2$ = 0.9821 |
| Mouth width | : | MW = – 0.27 + 0.28SL | $r^2$ = 0.9561 |

The proportions of these morphometric measurements to standard length changed by age as shown in Fig. 1.

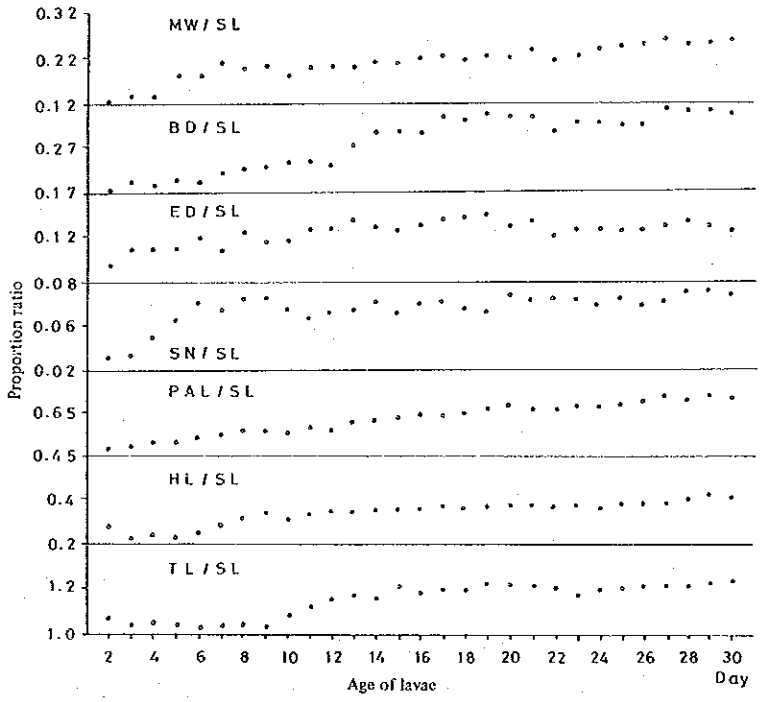


Fig. 1. The proportion ratio of each morphometric measurement to the standard length (SL) of seabass larva and juvenile. TL: total length, HL: head length, PAL: preanal length, SN: snout length, ED: eye diameter, BD: body depth, MW: mouth width.

### Pigmentation

In 2 day old larvae (SL: 1.9 mm), few melanophores appear at the base of the anal fin fold and there is no pigment in the eyes. In 4 day old larvae (SL: 2.0 mm), with dark eyes, a little more pigment accumulates at the base of the anal fin fold. By the time larvae are 25 days old (SL: 9.0 mm), pigment is distributed throughout most of the body, except the operculum, and has

accumulated at the base of every fin. The pigment pattern of larvae 26–30 days old is similar to that of 25 day old larvae (Fig. 2).

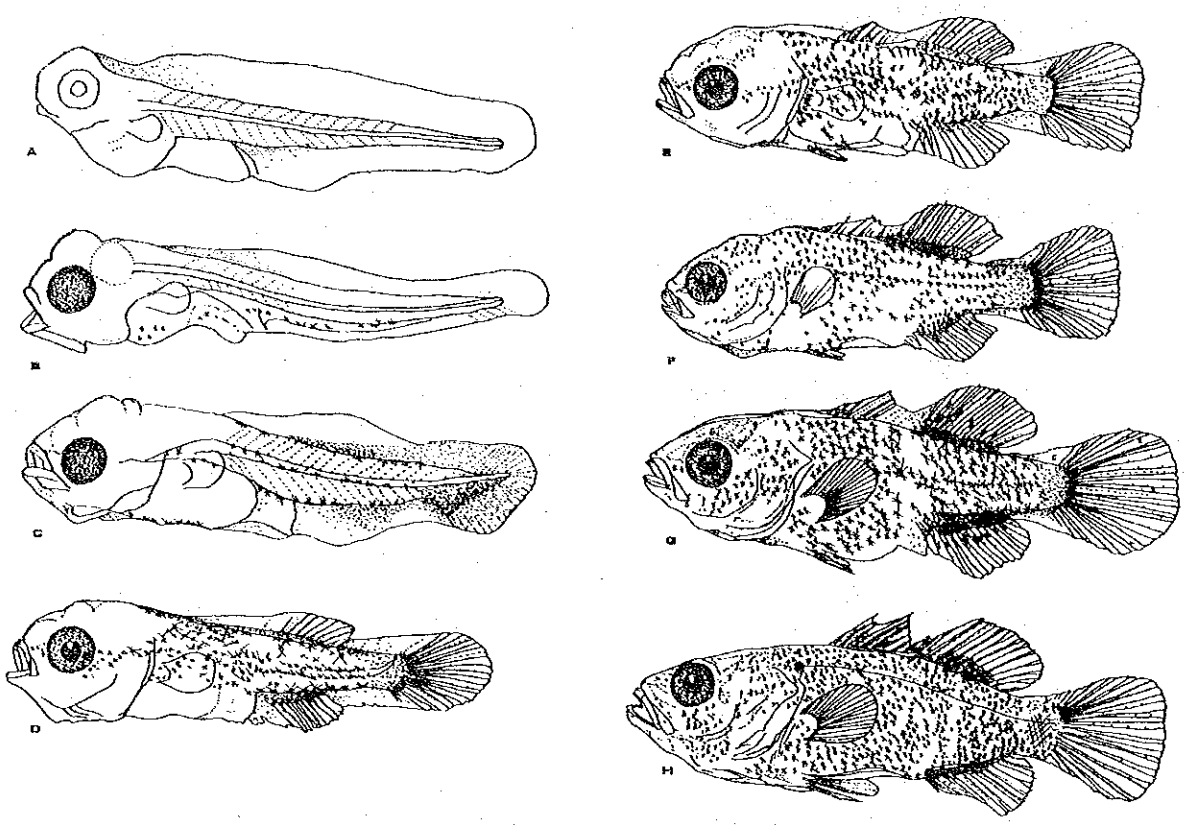

Fig. 2. Pigmentation of seabass larva. A, early preflexion larva 2 days old (1.90 mm SL); B, preflexion larva 4 days old (2.10 mm SL); C, late preflexion larva 8 days old (2.90 mm SL) and D, early flexion larva 11 days olds (3.15 mm SL).
E, flexion larva 14 days old (3.45 mm SL); F, late flexion larva 16 days old (5.10 mm SL); G, postflexion larva 19 days old (5.65 mm SL) and H, late postflexion larva (juvenile) 25 days old (9.50 mm SL).

## Fin development

Caudal fin development is divided into 3 stages according to Johnson and Loesch (1983):

1. Preflexion notochord–The straight notochord stage was observed in 2–9 day old larvae. Soft fin rays can be counted in the last preflexion notochord stage.

2. Flexion notochord–In this stage, the end of the notochord bends from the horizontal line. The structures in the upper part of the hypural and the parypural plate are bone. The 5th hypural plate and uroneurals with cartilaginous structures can be observed in the last flexion notochord stage.

3. Postflexion notochord–In this stage, the parypural and hemal spine separate. Epurals can also be observed. In the late postflexion (juvenile) stage, larvae more than 24 days old, and about 9.45 mm in SL, all structures become bone (Fig. 3).

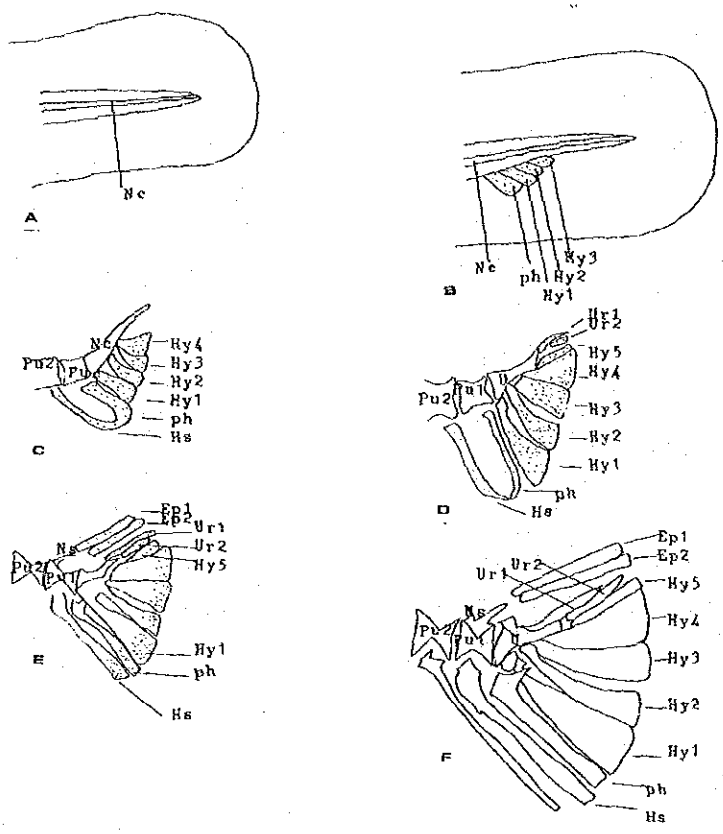


Fig. 3. Development of the caudal fin osteology in larvae and juvenile seabass. Fin raysare omitted to clearly show support osteology; (A) early preflexion 1.90 mm SL; (B) late preflexion 2.65 mm SL; (C) flexion 3.15 mm SL; (D) late flexion 4.25 mm SL; (E) post flexion 5.67 mm SL; (F) juvenile 9.45 mm SL; Hy (1–5): hypural plastes; U: ural vertebra; Ur (1–2): Uroneurals; Pu: preural vertebra; Ep (1–2): Epurals; Hs: hemal spine; Nc: notochord; Ns:neural spine; Ph: parhypural.The stippled areas indicated uptake of Alician Blue.

The dorsal, anal and caudal fins initially have fin folds from between the eyes to the posterior of the anus. The completion of the dorsal, anal and ventral fins takes 15 days (D.VII, 10–11), 15 days (A. III, 8), and 13 days (V.I, 5), respectively (Table 1).

The vertebral column can be counted from 10 days old. The count becomes stable at 23 + 1 or 24 + 1 at 18 days old.

Table 1. Fin formation and number of vertebral column and branchial arch of seabass larvae and juveniles.

| Age (Day) | No. | SL (mm) | Dorsal fin (mm) | | | | | | Anal fin (mm) | | | | | Pectoral fin (mm) | | | | Ventral fin | | Caudal fin | | | Vertebral column | | | | | B.A. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | VII | VIII | 9 | 10 | 11 | 12 | II | III | 7 | 8 | 9 | 16 | 17 | 18 | 19 | 1 | 5 | 8 | 9 | 7 | 22 | 23 | 24 | 25 | 26 | 3,4 |
| 10 | 12 | 3.2917 | | 2.1950 ± 0.7633 | | | | | | 1.4889 ± 0.6162 | | | | | 0.5675 ± 0.2825 | | | | -- | | 6 | 6 | 2 | 4 | 3 | -- | -- | 5 |
| 11 | 18 | 3.1528 | | 1.2350 ± 0.6483 | | | | | | 0.7550 ± 0.5777 | | | | | 0.4971 ± 0.0662 | | | | -- | | 11 | 11 | 6 | 8 | -- | -- | -- | 13 |
| 12 | 12 | 3.5417 | | 1.1000 ± 0.1472 | | | | | | 0.375 ± 0.1500 | | | | | 0.3958 ± 0.1424 | | | | -- | | 12 | 12 | -- | 4 | 4 | 4 | -- | 12 |
| 13 | 16 | 3.6500 | -- | -- | -- | 4 | 10 | 1 | 3 | -- | 2 | 10 | 3 | | 0.4769 ± 0.1467 | | | 16 | 16 | -- | 16 | 16 | 1 | 4 | 6 | 5 | -- | 16 |
| 14 | 8 | 3.4625 | -- | 1 | 1 | 3 | 2 | -- | 1 | -- | -- | 7 | 1 | | 0.3917 ± 0.0367 | | | 8 | 8 | -- | 8 | 8 | 1 | 7 | -- | -- | -- | 8 |
| 15 | 18 | 4.2389 | -- | 18 | -- | 6 | 12 | -- | -- | 18 | 2 | 16 | -- | | 0.5056 ± 0.0472 | | | 18 | 18 | -- | 18 | 18 | -- | 4 | 8 | 6 | -- | 18 |
| 16 | 16 | 5.1156 | -- | 16 | -- | 6 | 10 | -- | -- | 16 | 3 | 13 | -- | | 0.5625 ± 0.0659 | | | 16 | 16 | -- | 16 | 16 | -- | 1 | 8 | 7 | -- | 16 |
| 17 | 17 | 4.9524 | -- | 17 | -- | 4 | 13 | -- | -- | 17 | 2 | 15 | -- | | 0.5533 ± 0.0905 | | | 17 | 17 | -- | 17 | 17 | 1 | 6 | 7 | 3 | -- | 17 |
| 18 | 15 | 5.1333 | -- | 15 | -- | 2 | 13 | -- | 1 | 14 | -- | 15 | -- | | 0.6571 ± 0.0791 | | | 15 | 15 | -- | 15 | 15 | -- | 4 | 5 | 6 | -- | 15 |
| 19 | 17 | 5.6735 | 2 | 15 | -- | 2 | 15 | -- | -- | 17 | 1 | 15 | 1 | -- | -- | -- | -- | 17 | 17 | -- | 17 | 17 | -- | -- | 12 | 5 | -- | 17 |
| 20 | 18 | 7.0583 | 4 | 14 | -- | -- | 18 | -- | -- | 18 | -- | 18 | -- | -- | -- | -- | -- | 18 | 18 | -- | 18 | 18 | -- | 1 | 10 | 7 | -- | 18 |
| 21 | 15 | 6.8567 | 4 | 11 | -- | 2 | 12 | 1 | -- | 15 | 1 | 14 | -- | -- | -- | -- | -- | 15 | 15 | -- | 15 | 15 | 1 | 3 | 8 | 3 | -- | 15 |
| 22 | 16 | 8.0813 | -- | 16 | -- | 3 | 13 | -- | -- | 15 | 2 | 14 | -- | 3 | 3 | 1 | -- | 16 | 16 | -- | 16 | 16 | -- | 1 | 5 | 10 | -- | 16 |
| 23 | 16 | 3.7813 | -- | 16 | -- | 3 | 13 | -- | -- | 16 | 1 | 15 | -- | -- | -- | -- | -- | 16 | 16 | -- | 16 | 16 | -- | 3 | 4 | 9 | -- | 16 |
| 24 | 16 | 9.4500 | -- | 16 | -- | 3 | 13 | -- | -- | 16 | -- | 15 | 1 | -- | -- | -- | -- | 16 | 16 | -- | 16 | 16 | -- | -- | 4 | 12 | -- | 16 |
| 25 | 20 | 9.4600 | -- | 20 | -- | 5 | 15 | -- | -- | 20 | 2 | 17 | 1 | 5 | 2 | -- | -- | 20 | 20 | -- | 20 | 20 | -- | 4 | 6 | 10 | -- | 20 |
| 26 | 15 | 9.7833 | -- | 15 | -- | 2 | 13 | -- | -- | 15 | -- | 15 | -- | 3 | 3 | -- | -- | 15 | 15 | -- | 15 | 15 | -- | 2 | 5 | 8 | -- | 15 |
| 27 | 17 | 10.5265 | -- | 17 | -- | 3 | 14 | -- | -- | 17 | -- | 16 | 1 | 7 | 3 | 1 | -- | 17 | 17 | -- | 17 | 17 | -- | -- | 6 | 9 | 2 | 17 |
| 28 | 17 | 10.5588 | -- | 17 | -- | 3 | 14 | -- | -- | 17 | 2 | 14 | 1 | 3 | 7 | -- | -- | 17 | 17 | 5 | 12 | 17 | -- | 1 | 6 | 8 | 2 | 17 |
| 29 | 20 | 10.5225 | -- | 20 | -- | 3 | 17 | -- | -- | 20 | 1 | 14 | 5 | 11 | 3 | -- | -- | 20 | 20 | -- | 20 | 20 | -- | 1 | 10 | 8 | 1 | 20 |
| 30 | 20 | 12.6534 | -- | 20 | -- | 2 | 18 | -- | 1 | 19 | 5 | 15 | -- | 5 | 6 | 1 | 1 | 17 | 17 | -- | 20 | 20 | -- | 4 | 2 | 14 | -- | 20 |

B. A.: Branchial arch

## DISCUSSION AND CONCLUSION

### Morphometrics

TL/SL ratio remains low until 9 days old. From 10 to 15 days old the ratio increases until it reaches about 1.2 at 16 days old. Later on, the ratio remains constant. These changes of TL/SL ratio correspond to the formation of the caudal fin which develops from early flexion stage at 9 days old to late flexion stage at 15–16 days old.

HL/SL ratio is rather high at 2 days old but it drops to a lower level at 3–5 days old. The ratio increases from 6 to 9 days old and remains rather constant until 27 days old when it again seems to rise. These changes in ratio may reflect the development of the fish's mouth, brain and gills.

PAL/SL ratio increases steadily during 2–19 days old and, after that, it remains more or less constant. Limsuwan (1985) stated that a 2 day old larva had a short, straight intestine, which increased its length until 11–13 days old. Development of stomach was observed to start around 11–13 days old. Digestive system was almost completed at 20–27 days old. The steady increase of PAL/SL ratio until 19 days old and constant PAL/SL ratio after 20 days old observed in the present study may be reflecting this completion of the development of the digestive system of the fish.

MW/SL ratio increases from 2 to 7 days old rather rapidly, and from 7 to 21 days old it remains more or less constant. After that, it increases again. In NICA's feeding schedule for seabass seed production, seabass larvae are fed with rotifer at 2–15 days old, brine shrimp nauplius at 8–25 days old, water flea at 20–25 days old and chopped fish meat from 25 days old. The first increase in MW/SL ratio corresponds to the period of exclusive rotifer feeding, and constant MW/SL ratio corresponds to the rotifer, and later to the brine shrimp nauplius, feeding period. The ratio increases when fish grow to the stage in which they are fed with water flea and chopped fish meat.

**Fin development**

Pechmanee at al. (1985) reported that the amount of rotifer consumed by 4–14 day old seabass larvae increased when larvae were older, if fed with the same rotifer density. This may indicate that the ability to consume rotifer relates to fin development which influences the locomotion of the larvae. The present study shows that dorsal and anal fins are completed at 15 days old, caudal fin at 20 days old, and ventral fin at 23 days old.

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# Net cage culture of seabass in Songkhla Lake

Coastal Aquaculture Survey Division Team

## INTRODUCTION

The seabass, *Lates calcarifer,* is one of the commercially important fishes in Thailand. The culture of seabass has been practiced for a long years by using wild fry or juveniles. In 1973, artificial propagation of seabass was first conducted and first mass production of seabass fry was achieved at Songkhla Fisheries Station (presently NICA) in 1975. At present, seabass culture by using hatchery bred fry is a common practice along the coastal water area of Thailand and its total production in 1981 was close to 300 tons (Sirikul, 1982).

In the present study, an experimental net cage culture was carried out in a low salinity water area to see growth and survival rate of the fish under a low salinity condition.

## MATERIALS AND METHODS

1. Experimental fish

The fish used for this experiment were produced in NICA hatchery and were nursed in floating net cages set in the open sea for 3 months before the experiment. Average size of the fish at the start of the experiment was 13.0 (standard deviation, 1.4) cm in total length and 30.0 (s. d., 9.9)g in body weight. During the nursing period, they were fed with minced trash fish meat.

2. Net cage

Two net cages were used to culture the fish throughout the experiment period. The size and mesh size of the net cages were 4x4x2.5m and 6mm, respectively, for the first month of the experiment. After one month, they were replaced with the net cages of the same size, with 2.5cm mesh. These cages were placed inside the outer net cages of 5x5x2m for the protection from possible damages. Both experimental and outer net cages were supported by wooden and bamboo frames.

3. Location

Net cages were set in the Songkhla Lake at Pakrore, Songkhla Province (Fig. 1).

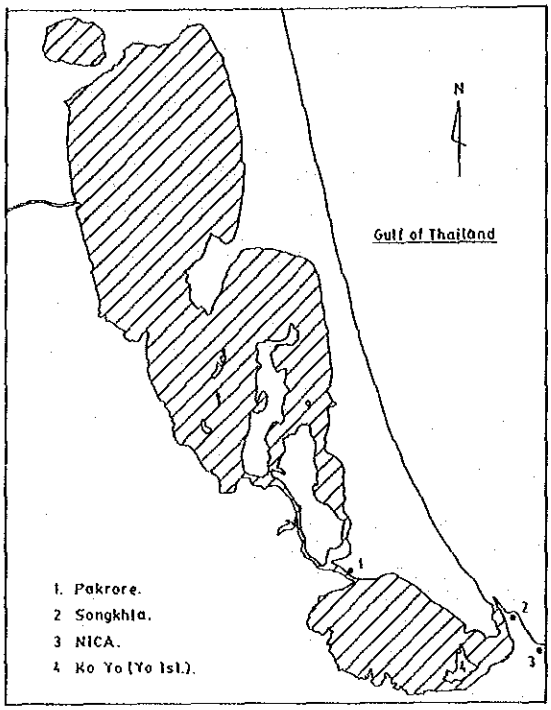


Fig. 1. Map of Songkhla Lake.

4. Stocking density

Initial stocking density of the fish in the net cages was 330 fish/cage or 13.8 fish/$m^3$.

5. Feed and feeding

Fish were fed with chopped meat of fresh trash fish composed of many species, normally twice a day.

6. Management and sampling

Routine culture management was undertaken by a fish farmer. Monthly sampling of fish and checking of water conditions were done by the staff of the Coastal Aquaculture Survey Division.

## RESULTS

Water conditions during the culture were shown in Table 1 which were summarized as follows:

| | | |
|---|---|---|
| Water temperature: | Average 29.9°C | Range 28.9–31.5°C |
| Salinity: | Average 4.1 ‰ | Range 0.0–8.0 ‰ |
| Dissolved oxygen: | Average 6.6ppm | Range 5.6–7.3ppm |

At the end of the experiment, fish attained 33.5 (s.d. 2.1)cm in total length and 544.0 (s.d. 107.6)g in body weight with mean survival rate of 51.5% (Table 2). As total weight gain was 165.1kg against 1,624.3kg of total feed given (Table 3), food quotient (FQ = Feed given/Weight gain) was 9.84.

Relationships between total length and body weight of the fish are shown in Fig. 2, and days of culture and total length and days of culture and body weight are shown in Fig. 3. Fig. 4 shows survival rate by period of culture.

Table 1. Water condition of Pakrore.

| Date | Culture days | pH | Water temperature (°C) | Dissolved oxygen (ppm) | Conductivity (μΩ/cm) | Turbidity (ppm) | Salinity (‰) |
|---|---|---|---|---|---|---|---|
| 7/17/84 | 1 | – | – | – | – | – | 7.0 |
| 8/16 | 31 | 7.60 | 31.5 | 7.0 | 9.5 | 32.0 | 5.0 |
| 9/11 | 57 | 7.70 | 28.9 | 5.6 | 39.5 | 25.0 | 2.4 |
| 10/16 | 92 | 7.50 | 30.3 | 6.9 | 15.0 | 14.0 | 8.0 |
| 11/13 | 120 | 7.55 | 31.0 | 6.0 | 12.6 | 41.0 | 6.0 |
| 12/14 | 151 | 7.25 | 28.9 | 6.9 | 1.2 | 79.0 | 0.0 |
| 1/18/85 | 186 | 6.90 | – | 7.3 | 0.3 | 24.0 | 2.0 |
| 2/16 | 215 | 7.75 | 28.9 | 6.8 | 4.2 | 44.0 | 2.0 |
| Average | | 7.46 | 29.92 | 6.64 | 11.76 | 37.00 | 4.05 |
| Standard deviation | | 0.27 | 1.07 | 0.56 | 12.45 | 19.63 | 2.66 |

Table 2. Growth of seabass at Pakrore.

| Number of fish | Survival (%) | Total length (cm) | | | | | Body weight (g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Average | s.d. | Min | Max | Gain/Day | Average | s.d. | Min | Max | Gain/Day |
| 660 | 100.0 | 13.01 | 1.41 | 10.5 | 16.1 | – | 30.03 | 9.89 | 14.2 | 53.4 | – |
| 572 | 86.7 | 19.58 | 2.99 | 12.9 | 26.0 | +0.212 | 105.42 | 46.73 | 24.2 | 225.0 | +2.432 |
| 382 | 57.9 | 21.86 | 1.89 | 16.4 | 25.3 | +0.088 | 144.00 | 35.93 | 55.0 | 225.0 | +1.481 |
| 374 | 56.7 | 26.38 | 1.98 | 20.9 | 31.0 | +0.129 | 274.60 | 61.96 | 120.0 | 450.0 | +3.731 |
| 343 | 52.0 | 29.41 | 2.36 | 21.3 | 34.2 | +0.108 | 381.30 | 83.90 | 145.0 | 610.0 | +3.811 |
| 377 | 57.1 | 32.54 | 2.16 | 27.5 | 37.2 | +0.101 | 504.71 | 105.39 | 280.0 | 770.0 | +3.981 |
| 310 | 47.0 | 32.51 | 1.75 | 28.5 | 37.5 | -0.001 | 489.79 | 86.28 | 250.0 | 700.0 | -0.426 |
| 340 | 51.5 | 33.53 | 2.14 | 29.5 | 39.0 | +0.036 | 544.00 | 107.57 | 300.0 | 800.0 | +1.869 |

Table 3. Feeding condition and growth of seabass at Pakrore.

| Days | Total weight (kg) | Weight gain (kg) | Feeding (kg) | | FQ* | Feeding rate (% BW) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Feed/day | Total feed | | Start | End | Mean |
| 31 | 19.82 | + 40.48 | 4.96 | 153.7 | + 3.80 | 25.03 | 8.23 | 16.63 |
| 26 | 60.30 | - 5.29 | 6.27 | 163.1 | -30.83 | 10.40 | 11.40 | 10.90 |
| 35 | 55.01 | + 47.69 | 8.01 | 280.4 | + 5.88 | 14.56 | 7.80 | 11.18 |
| 28 | 102.70 | + 28.09 | 9.51 | 266.3 | + 9.48 | 9.26 | 7.27 | 8.27 |
| 31 | 130.79 | + 59.49 | 9.61 | 297.8 | + 5.01 | 7.35 | 5.05 | 6.20 |
| 35 | 190.28 | - 38.45 | 7.37 | 257.9 | - 6.71 | 3.84 | 4.84 | 4.36 |
| 29 | 151.83 | + 33.13 | 7.07 | 205.1 | + 6.19 | 4.66 | 3.82 | 4.24 |
| 215 | 184.96 | +165.14 | – | 1,624.3 | + 9.84 | – | – | – |

* FQ = Feed given / Weight gain

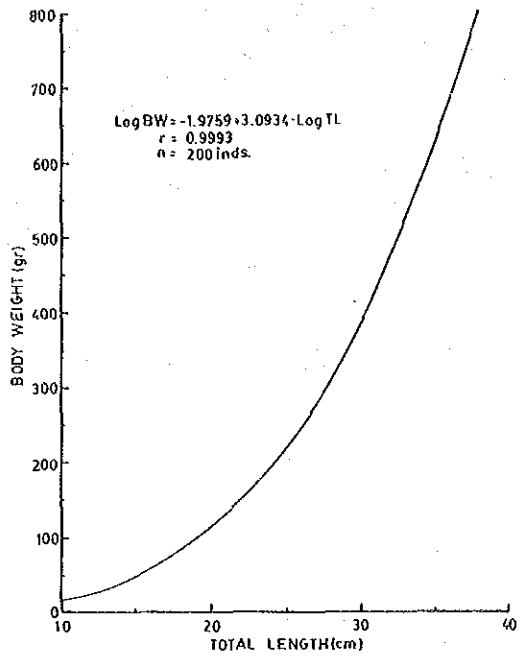


Fig. 2. Total length-body weight relationship of cultured seabass at Pakrore.

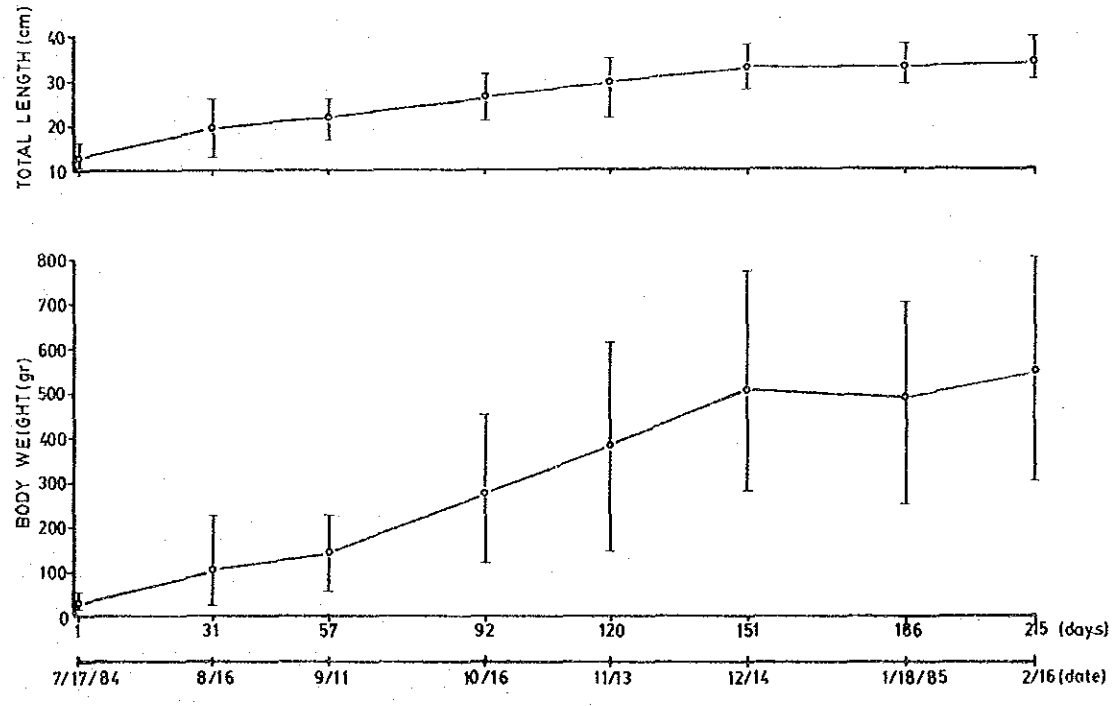


Fig. 3. Growth of cultured seabass at Pakrore.

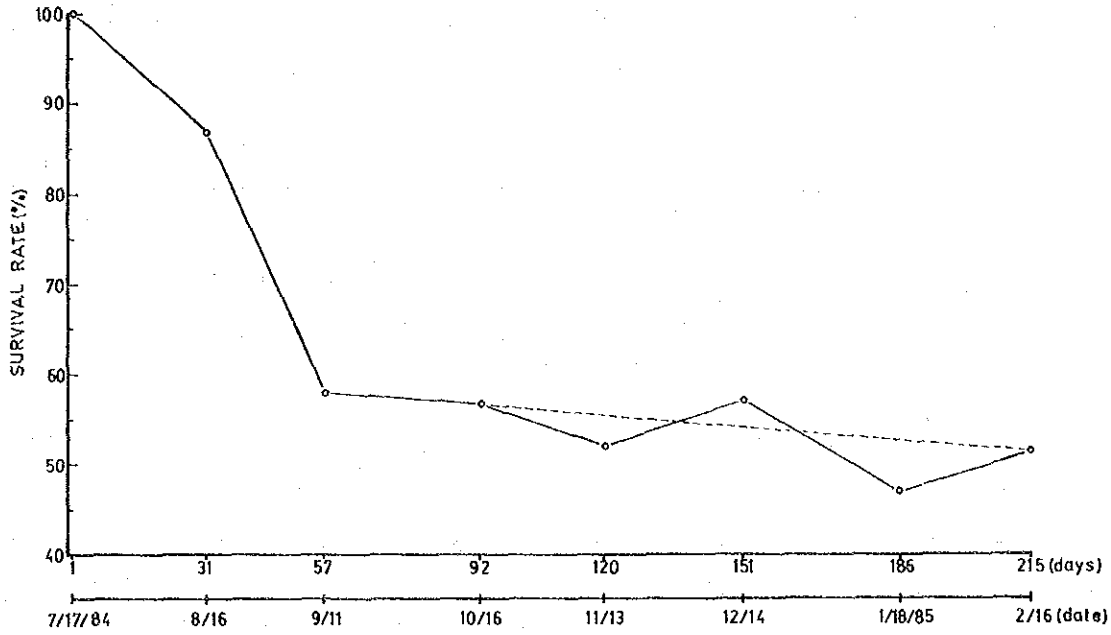


Fig. 4. Survival rate of cultured seabass at Pakrore.

## DISCUSSION

Seabass cultured in brackishwater of a higher salinity attain 350g and 500g in 6 and 8 months, respectively, with an initial body weight of 50g. FQ value of trash fish is 7–10 (Sirikul, 1982). Results of this study were satisfactory in body weight gain and FQ value, but inferior in production per unit volume due to the low survival rate. The cause of the low survival rate was high mortality of the fish occured one month after the start of the culture when the net cages were changed. More than 100 fish were trapped in the mesh openings of the net cages and died within a few days. For this reason, mesh size of the net cage should be carefully selected depending to the size of the fish.

The low salinity of 0 to 2‰ did not cause mortality of the fish but growth was retarded due to loss of appetite of the fish. Though the present experiment was not sufficient to deduce final conclusion, salinity level of 5 to 8‰ is estimated to be minimal fo the normal growth of seabass in the culture.

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# Progress report on floating cage culture of red snapper, *Lutjanus argentimaculatus*

Sujin Maneewong, Tanan Tattanon and Yoshibumi Yashiro

## INTRODUCTION

Red snapper, *Lutjanus argentimaculatus*, is one of the commercially important species in Thailand, but commercial scale culture of this species has not been established yet.

Net cage culture from the juvenile stage was conducted using wild juveniles at Bokhen sub-station of Songkhla Brackishwater Fisheries Station (presently NICA) in 1976 (Panichsuk and Chungyampin, 1976). Experimental seed production and study on larval development were conducted in 1983 (Wudthisin and Maneenawa, 1983 and Wudthisin *et al.* 1983). The first successful induced spawning was achieved, and the larvae obtained were reared to juvenile stage (60 days) in 1984 at Rayond Marine Fisheries Station (Wudthisin, 1984).

At NICA, larval rearing of red snapper was attempted using naturally spawned eggs from Boken sub-station in 1985, but this was not successful due to problems with larval stage foods. However, 206 juveniles were obtained from the brood stock tank of the red snapper the same year (on 29th Aug., 123 juveniles, 3.95±0.77cm in total length and 2.60±0.54g in body weight, and on 22nd Oct., 83 juveniles of 7.97±1.48cm in total length and 10.95±6.01g in body weight). These juveniles were thought to be spawned in the tank by the brood stock and grew naturally in the tank.

The present report deals with the result of experimental culture of those juveniles in cage nets.

## MATERIALS AND METHODS

90 juveniles (8.88±1.09cm in total length and 14.33±5.19g in body weight) were selected on 30th October, 1985 from the naturally spawned group which was collected from the fish brood stock culture tank.

During the first month, from 30th October to 29th November, juveniles were cultured in a floating net cage made of mosquito net, then transferred to a bigger net cage made of polyethylene. The sizes of the net cages were 1.0x0.8x0.8m (0.5m$^3$) and 4.0x4.0x2.5m (32m$^3$), respectively. Initial stocking densities in each net were 180/m$^3$ and 2.75/m$^3$, respectively. The net cage was set in a fish brood stock culture tank under running water conditions with a water exchange rate of 40%/day.

During the first month, juveniles were fed with minced, fresh fish meat, then chopped fresh

fish meat was given until the end of the study period.

## RESULTS

Water conditions during the study were 28.8°C average water temperature (range, 26.5–31.2°C) and 28.0‰ average salinity (range, 24.5–32.0‰) (Table 1).

Table 1. Water conditons in rearing tank.

| No. | Date | Days | WT (°C) | Sal.(‰) |
|---|---|---|---|---|
| 1 | Oct. 30, 85 | 1st | 31.2 | 32.0 |
| 2 | Nov. 29 | 31st | 30.8 | 30.0 |
| 3 | Dec. 25 | 57th | 27.2 | 24.5 |
| 4 | Jan. 27, 86 | 90th | 26.5 | 25.0 |
| 5 | Feb. 26 | 120th | 28.4 | 28.5 |

During 120 days of culture, fish gained 8.25cm in mean total length and 81.52g in mean body weight. Sruvival rate was 85.6% (Table 2).

Table 2. Growth of red snapper.

| No. | Fish | | Total length (cm) | | | Body weight (g ) | | |
|---|---|---|---|---|---|---|---|---|
| | inds | SR (%) | m ± SD | Min | Max | m ± SD | Min | Max |
| 1 | 90 | 100.0 | 8.88 ± 1.09 | 7.3 | 11.2 | 14.33 ± 5.19 | 7.47 | 26.80 |
| 2 | 88 | 97.8 | 11.19 b 1.40 | 9.0 | 13.9 | 27.86 ± 11.03 | 13.04 | 50.78 |
| 3 | 88 | 97.8 | 12.48 ± 1.61 | 9.0 | 15.2 | 37.00 ± 12.98 | 15.00 | 60.00 |
| 4 | 77 | 85.6 | 14.35 ± 1.41 | 12.2 | 17.4 | 54.75 ± 15.37 | 35.00 | 90.00 |
| 5 | 77 | 85.6 | 17.13 ± 1.87 | 13.6 | 19.8 | 95.85 ± 28.06 | 50.00 | 135.00 |

Total weight gain was 6.09kg against 52.85kg of total weight of feed given to the fish. Food quotient (FQ) value (Total weight of feed/total weight gain of fish) was 8.68 (Table 3).

Relationship of total length and body weight, culture days and body weight, and culture days and total length are shown in Figs. 1, 2 and 3, respectively.

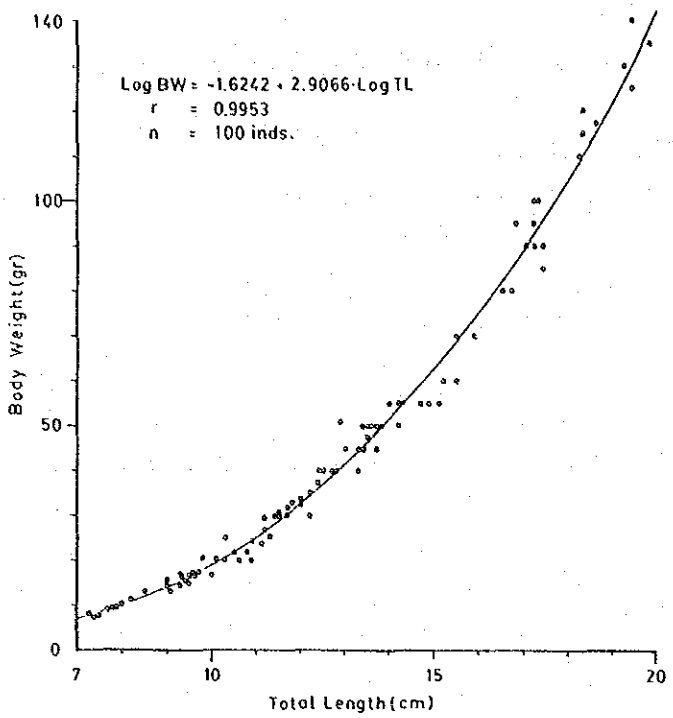


Fig. 1. Total length – body weight relationship of juvenile red snapper.

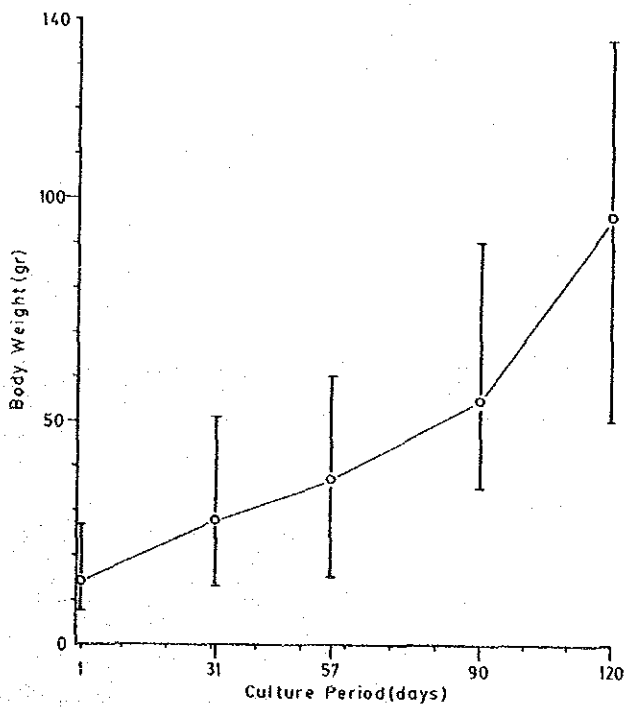


Fig. 2. Growth of juvenile red snapper in body weight.

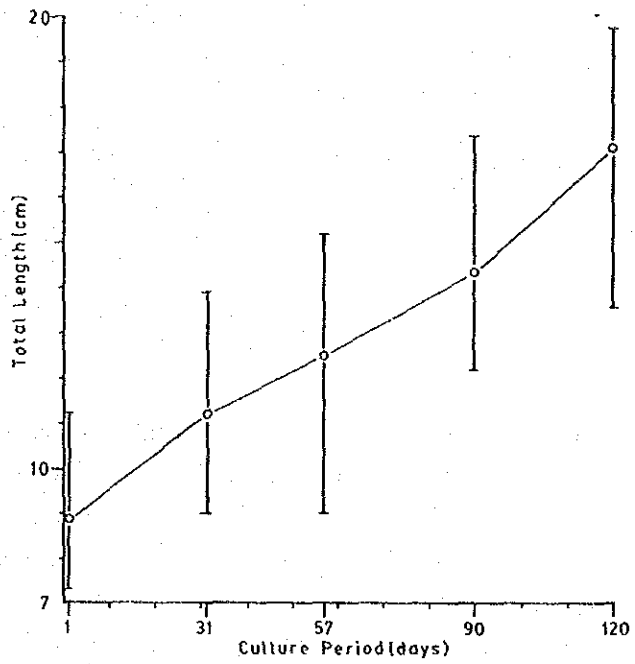


Fig. 3. Growth of juvenile red snapper in total length.

## DISCUSSION

The objective of this study was to collect basic data on the growth of red snapper and the results showed that the growth rate was relatively high and indicated a high possibility of the fish as a suitable culture species.

However, it is too early to conclude the suitability of this species as a new culture species and further studies are needed in the fields of mass production of seed, nutrition requirement, ecology and physiology of the fish.

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# Experiment on culture of copepod, *Tigriopus japonicus*, with four kinds of food

Tida Pechmanee and Sunit Rojanapittayakul

## INTRODUCTION

In Japan, *Tigriopus japonicus* is the most promising species for mass culture because it endures considerable change in environmental conditions and also reproduces vigorously (Kitajima, 1973). The most practical technique for the production of the copepod, *Tigriopus japonicus*, is in combination culture with the rotifer, *Brachinous plicatilis*, fed with baker's yeast (Fukusho, 1980). However, many kinds of living and non-living foods, such as diatom, yeasts, synthetic fish food and soy cake could be used for this copepod (Kitajima, 1973 and Takano, 1971). It is important to determine which kind of food is suitable for *Tigriopus japonicus* cultured in Thailand to support the reasonable way to produce this copepod as food for marine fish larvae.

## MATERIALS AND METHODS

Seeds of *Tigriopus japonicus* were collected from a rotifer tank in laboratory at Nansei Regional Fisheries Research Laboratory, Hiroshima, Japan in 1982 and they were stocked in laboratory of National Institute of Coastal Aquaculture, Songkhla, Thailand until 1985, then this experiment was carried out. Four kinds of food were used : baker's yeast, rice-bran, fish meal, and a mixture of wheat flour and soya flour, 1 to 1 in weight ratio.

The experiment was performed in 8, 30 ℓ tanks, each filled with 25 ℓ of water. The copepods, *Tigriopus japonicus*, were put into each tank with aeration. They were fed with 0.3 g of food per tank every day for 24 days without changing water. The density of the copepods, including nauplius, copepodite, and adult, was observed under the microscope every 3 days.

At the same time, a copepod female with eggs was separated to rear in 100 ml beakers, after they gave nauplii, the mother was taken out and the time for the growth from nauplius up to mating was recorded.

## RESULT

After 14 days, there was plenty of sediment in the experimental tanks fed with the mixture of wheat flour and soya flour, so feeding was stopped for 3 days.

After 17 days, 2 ℓ of fresh water was added to each tank to maintain the level of salinity.

The water temperature in each tank ranged from 26.5 to 27.5 °C. The results showed that the nauplius of copepod, *Tigriopus japonicus* grew to mating stage within 10 days with every kind of food. Density of the copepod fed with each kind of food is shown in Fig. 1. Nauplius numbers are always bigger than copepodite and adult numbers in every kind of food. In general, the results with rice-bran and fish meal were better than with yeast or flour mixture. The peak density of copepods during this experiment was highest in the tank of copepods fed rice-bran.

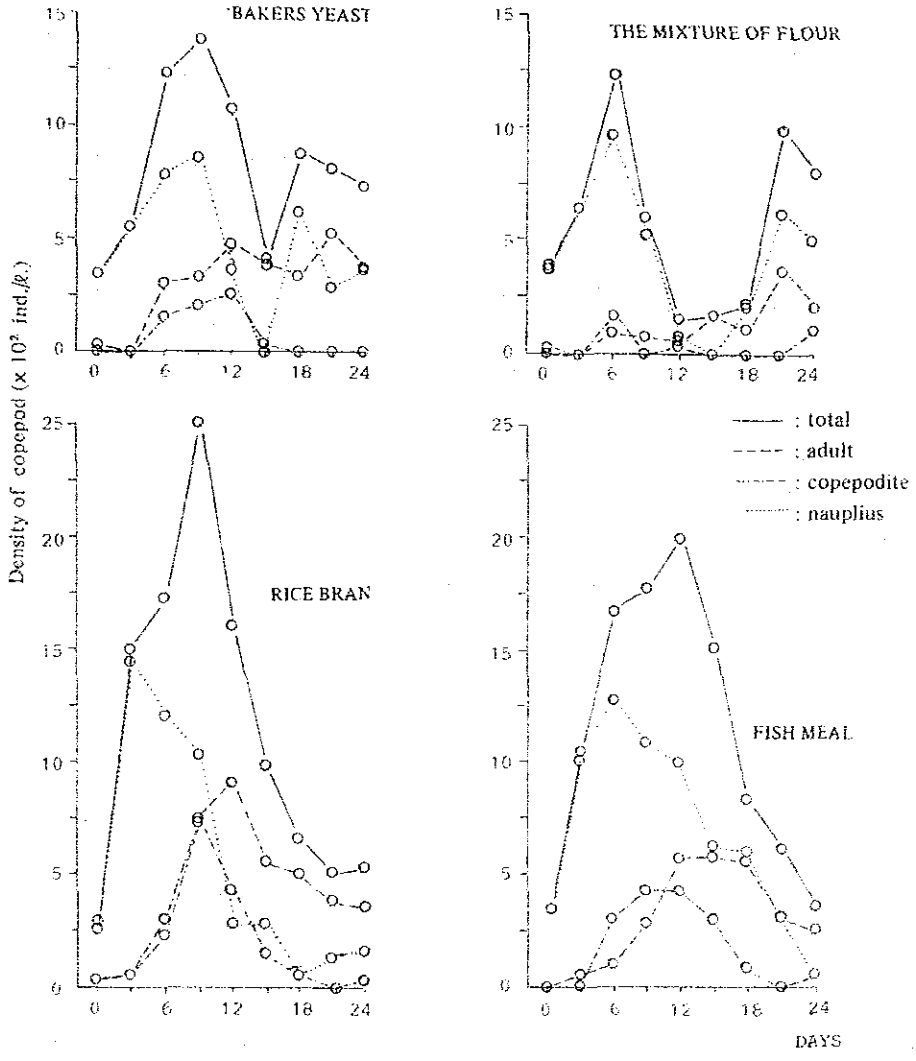


Fig. 1. Change of individual numbers of *Tigriopus japonicus* supplied with baker's yeast, rice-bran, fish meal and the mixture of wheat flour and soya flour.

## DISCUSSION AND CONCLUSION

The nauplii of *Tigriopus japonicus* grew to mating stage within 10 days, the same as those reported by Takano (1971). However, the density of nauplii fed with a mixture of wheat flour and soya flour was less than those fed with other kinds of food in this experiment, while Takano (1971) reported that this kind of flour mixture could be used for 12 generations *Tigriopus japonicus* in laboratory.

The fact that the density of copepod in this experiment was rather low might be accounted for by several reasons. First, the amount of food may not have been suitable and, because of the small amount involved, no adjusting was done during the experiment. Second, because only non-living food was used, the water quality became bad more easily than when using living food. In this case Takano (1971) suggested that a combination of a mixture of cereal flour and cultivated microalgae seemed to be the best to feed the copepod for a long time. Finally, the lower density may simply be due to the tank capacity, since the results of culture differe depending on the capacity of tank (Kitajima, 1973). These problems are important to examine in future. However, among the four kinds of food used in this experiment, it seems that rice-bran is most profitable to use as a non-living food for the copepod, *Tigriopus japonicus*, because it had the lowest price and, in general, it had good effects on population growth of the copepod.

Table 1. Food prices for feeding the copepod, *Tigriopus japonicus* in the experiment.

| Food | Prices/kg (Baht) |
|---|---|
| Rice-bran | 4 |
| Wheat flour | 18 |
| Soy Bean | 12 |
| Fish meal | 12 |
| Baking yeast | 116 |

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# Study on food and stocking density for brine shrimp, *Artemia salina*, culture

Paitoon Akkayanont and Sujin Maneewong

## INTRODUCTION

Nauplius larva of brine shrimp, *Artemia salina*, is a popular and important living food organism for fry rearing of fish and shellfish. Since the nauplius larva is small in size, the price of brine shrimp nauplius per unit weight is very high.

If brine shrimp nauplii are cultured for a certain period to a larger size, price of brine shrimp per unit weight will be lowered, and therefore, the cost to produce fish and shellfish fry can be lowered at stages when fish and shellfish fry are large enough to eat larger size brine shrimp.

Many experiments have been conducted to find the best culture conditions for brine shrimp (Hutasing 1978, Sorgeloos 1979 and 1980, Brisset et al. 1981, Pechmanee 1984 and etc.). Fujita et al. (1980) reported that the brine shrimp nauplius has generally low nutritional value in relation to highly unsaturated fatty acids (HUFA). If the nauplii are cultured with a suitable food, the HUFA content can be increased and thus survival rate of fry will be raised.

The present experiment was conducted to compare the efficiency of three kinds of food and to find a suitable culture density for effective and economical production of brine shrimp.

## MATERIALS AND METHODS

1. Experiment to compare three kinds of food

The experiment was conducted in fibreglass tanks of $1m^3$ capacity. Three kinds of food, rice bran, *Tetraselmis* sp., and rice bran + *Tetraselmis* sp., were used for this experiment. Two tanks were prepared for each kind of food. Tank water was circulated in the tank with an air lift. Great Wall brand brine shrimp eggs imported from China were incubated for 36 hours, and hatched nauplii were used for the experiment. The stocking density of nauplius was 1,500 inds/ℓ for each tank.

Feeding for brine shrimp nauplii was started from the 2nd day of the experiment. 50% of the tank water was changed daily from the 3rd day throughout the experiment period.

The rice bran was processed into a micronized solution by the method of Sogeloos (1979). One kg of rice bran and one kg of salt were processed into 7.53 ℓ of the rice bran solution. *Tetraselmis* water was at a density of $4–6 \times 10^4$ cells/ml. Amount of food given to each tank is as follows:

| | |
|---|---|
| Tanks 1 and 2 | *Tetraselmis* water, 0.5m$^3$/tank/day |
| Tanks 3 and 4 | Rice bran solution, 400ml/tank/day on 2nd and 3rd days, and 800ml/tank/day from 4th to 6th day. |
| Tanks 5 and 6 | *Tetraselmis* water, 0.5m$^3$/tank/day plus rice bran solution, 400ml/tank/day on 2nd and 3rd days, and 800ml/tank/day from 4th to 6 th day. |

2. Experiment on culture density

Culture vessels, water circulation and exchange regime and origin of brine shrimp nauplii were the same as for the former experiment. The experiment was carried out in two parts.

The first part

Stocking densities of brine shrimp were set at 3,000, 4,000 and 5,000 inds/ℓ, with 2 tanks for each density. Culture period was 14 days. Rice bran solution, containing 0.15kg rice bran and 0.15kg salt per litre, and *Tetraselmis* water at a density of 4–6 x $10^4$ cells/ml were given to tank. The amount given was 0.5m$^3$/tank/day of *Tetraselmis* water and 400ml/tank/day of rice bran solution from the 2nd to 4th day. The amount of rice bran solution was doubled on the 5th and 6th days.

The second part

Stocking densities of brine shrimp were set at 1,500, 3,000 and 5,000 inds/ℓ, with 2 tanks for each density. Culture peri d was 14 days. Rice bran solution, containing 0.15kg rice bran and 0.15kg salt per one litre, and *Tetraselmis* water at a density of 4–6x$10^4$ cells/ml were given to each tank. The amount given was 0.5m$^3$/tank/day of *Tetraselmis* water, and 400ml/tank/day of rice bran solution from the 2nd to 5th day. The amount of rice bran solution was doubled from the 6th to 13th day.

## RESULTS AND CONCLUSIONS

1. Experiment to compare three kinds of food

Brine shrimp cultured with rice bran and *Tetraselmis* showed the fastest growth and the highest survival rate and production (Table 1). Production of brine shrimp with rice bran and *Tetraselmis* was 2.45 times as high as rice bran alone and 6.75 times as high as *Tetraselmis* alone.

There were statistically significant differences in growth and production of brine shrimps cultured with three different kinds of food. There, however, was no significant difference in survival rate between brine shrimps cultured with rice bran alone and rice bran and *Tetraselmis*. Survival rate of brine shrimp culture only with *Tetraselmis* was significantly lower than those cultured with other two kinds of food.

The unit cost of production of brine shrimp was cheapest when cultured with rice bran and *Tetraselmis*, being ฿0.020/g only (Table 2).

Table 1. Results of the experiments on *Artemia* cultured with three kinds of food in 7 days.

| Teat | Kind of food | No. of *Artemia*/ $m^3$ | Initial TL (mm)± SD | Final TL (mm)± SD | Survival no. | Survival rate (%) | Production (g) | pH | Temp. (°C) | Salinity (‰) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Tetraselmis* sp. | 1.5 × $10^6$ | 0.634 ± 0.198 | 1.73 ± 0.48 | 0.708 × $10^6$ | 47.2 | 120 | 7.74/ 8.55 | 27.0/ 28.0 | 34.7 |
| 2 | Rice bran | 1.5 × $10^6$ | 0.634 ± 0.198 | 2.35 ± 0.36 | 1.04 × $10^6$ | 69.3 | 330 | 7.46/ 8.35 | 27.0/ 28.0 | 35.1 |
| 3 | Rice bran + *Tetraselmis* sp. | 1.5 × $10^6$ | 0.634 ± 0.198 | 4.16 ± 0.66 | 1.06 × $10^6$ | 70.7 | 810 | 7.29/ 8.33 | 27.0/ 29.0 | 35.1 |

Table 2. Cost of production/$m^3$ for *Artemia* fed on 3 different food.

| Type of cost | *Tetraselmis* sp. | | Rice bran | | *Tetraselmis* sp. Rice bran | |
|---|---|---|---|---|---|---|
| | Amount | Cost (baht) | Amount | Cost (baht) | Amount | Cost (baht) |
| *Tetraselmis* | 2.5 ton | 0.45 | – | – | 2.5 ton | 0.45 |
| Rice bran | – | – | 432 g | 1.7 | 432 g | 1.7 |
| Salt | – | – | 432 g | 2.3 | 432 g | 2.3 |
| *Artemia* | 12 g | 11.5 | 12 g | 11.5 | 12 g | 11.5 |
| Total cost | – | 11.95 | – | 15.5 | – | 15.95 |
| Production/$m^3$ | – | 120 g | – | 330 g | – | 810 g |
| Unit cost/g | – | 0.100 | – | 0.047 | – | 0.020 |

As to the nutritional value of brine shrimp cultured with the rice bran and *Tetraselmis*, Rojanapitayagul and Akkayanont (1985) reported the rearing of shrimp, *Penaeus merguiensis*, from postlarva 5 to postlarva 25 fed with brine shrimps which had been cultured with rice bran and *Tetraselmis* for 7–14 day. The result showed that the survival rate was about 90% and the total length of these shrimps was nearly 2 times as large as that of shrimp reared with squid.

2. Experiment on culture density and period

The results of the first part of the experiment are shown in Table 3. These results do not show any statistically significant differences between stocking densities in growth, survival rate and production. The unit cost of production of brine shrimp, however, was significantly lower when cultured at the lowest density of 3,000 inds./ℓ (Table 4).

Table 3. Results of experiments on *Artemia* cultured in 7 days at 3 different stocking densities.

| Test | No. of *Artemia*/ $m^3$ | Initial TL(mm)± SD | Final TL(mm)± SD | Survival no. | Survival rate (%) | Production (g) | pH | Temp. (°C) | Salinity (‰) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 x $10^6$ | 0.521 ± 0.041 | 2.26 ± 0.382 | 1.62 x $10^6$ | 54 | 690 | 7.17/ 8.71 | 26.2/ 27.8 | 33.7 |
| 2 | 4 x $10^6$ | 0.521 ± 0.041 | 2.0 ± 0.244 | 2.57 x $10^6$ | 64 | 650 | 7.36/ 8.15 | 26.1/ 27.7 | 34.1 |
| 3 | 5 x $10^6$ | 0.521 ± 0.041 | 1.728 ± 0.255 | 2.99 x $10^6$ | 60 | 695 | 7.33/ 8.88 | 26.3/ 28 | 33.6 |

Table 4. Cost of production/$m^3$ for *Artemia* cultured in 7 days of 3 different stocking densities.

| Type of cost | 3000 ind/ℓ | | 4000 ind/ℓ | | 5000 ind/ℓ | |
|---|---|---|---|---|---|---|
| | Amount | Cost (baht) | Amount | Cost (baht) | Amount | Cost (baht) |
| *Tetraselmis* | 2.5 ton | 0.45 | 2.5 ton | 0.45 | 2.5 ton | 0.45 |
| Rice bran | 374 g | 1.50 | 374 g | 1.50 | 374 g | 1.50 |
| Salt | 374 g | 1.87 | 374 g | 1.87 | 374 g | 1.87 |
| *Artemia* | 24 g | 23.0 | 32 g | 30.7 | 40 g | 38.4 |
| Total cost | – | 26.8 | – | 34.5 | – | 42.2 |
| Production/$m^3$ | – | 690 g | – | 650 g | – | 695 g |
| Unit cost/g | – | 0.039 | – | 0.053 | – | 0.061 |

The results of the second part of the experiment are shown in Table 5. These results, too, do not show any significant differences between stocking densities in growth, survival rate and production. The unit cost of production of brine shrimp was, again, lowest at the lowest culture density of 1,500 inds./ℓ (Table 6).

Table 5. Results of experiments on *Artemia* cultured in 14 days at 3 different stocking densities.

| Test | No. of *Artemia*/ $m^3$ | Initial TL(mm)± SD | Final TL(mm)± SD | Survival no | Survival rate (%) | Production (g) | pH | Temp. (°C) | Salinity (‰) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | $1.5 \times 10^6$ | 0.504 ± 0.017 | 4.99 ± 0.775 | $0.332 \times 10^6$ | 21.1 | 565 | 7.14/ 8.08 | 26.0/ 30.5 | 34 |
| 2 | $3.0 \times 10^6$ | 0.504 ± 0.017 | 4.558 ± 0.833 | $0.760 \times 10^6$ | 25.3 | 760 | 7.11/ 8.04 | 26.0/ 28.0 | 34.2 |
| 3 | $5.0 \times 10^6$ | 0.504 ± 0.017 | 4.153 ± 0.434 | $0.640 \times 10^6$ | 12.8 | 465 | 7.16/ 7.97 | 25.8/ 28.5 | 34.4 |

Table 6. Cost of production/$m^3$ for *Artemia* cultured in 14 days at 3 different stocking densities.

| Type of cost | 1500 ind/ℓ | | 3000 ind/ℓ | | 5000 ind/ℓ | |
|---|---|---|---|---|---|---|
| | Amount | Cost (baht) | Amount | Cost (baht) | Amount | Cost (baht) |
| *Tetraselmis* | 6 ton | 1.1 | 6 ton | 1.1 | 6 ton | 1.1 |
| Rice bran | 936 g | 3.7 | 936 g | 3.7 | 936 g | 3.7 |
| Salt | 936 g | 4.7 | 936 g | 4.7 | 936 g | 4.7 |
| *Artemia* | 12 g | 11.5 | 24 g | 23.0 | 40 g | 38.4 |
| Total cost | – | 21.0 | – | 32.5 | – | 47.9 |
| Production/$m^3$ | – | 565 g | – | 760 g | – | 465 g |
| Unit cost/g | – | 0.037 | – | 0.043 | – | 0.103 |

Comparing the results of 7 days and 14 days, rearing at 1,500 inds./ℓ in density with *Tetraselmis* and rice bran in Tables 1 and 5, it can be said the production is much higher when cultured only for 7 days than for 14 days, since the survival rate was much higher in the former than in the later.

From the results obtained in these experiments, it can be concluded that mixed feeding of *Tetraselmis* sp. and rice bran gives much better production of brine shrimp than feeding of *Tetraselmis* sp. alone or rice bran alone. As for the culture density, 1,500 inds./ℓ gives a higher production than higher culture densities. And as for the cultured period, 7 days culture gives higher production than 14 days culture.

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# Study on the relationship between transparency and density of 2 kinds of phytoplankton in outdoor tanks

Tida Pechmanee and Niwes Ruangpanit

## ABSTRACT

This study was done in order to discover the relationship between transparency and density of phytoplanktons, *Tetraselmis* sp. and *Chlorella* sp., cultured in outdoor tanks for feeding rotifer, *Brachionus plicatilis*, which was used as feed for seabass and marine shrimp larvae. This study was carried out from March 6 to July 16, 1984 and the following equations were obtained:

$$D_1 = 21.44 - 0.19\ TR_1$$
$$D_2 = 15.0 - 0.15\ TR_2$$

Where $D_1$ = Density of *Tetraselmis* sp. ($\times 10^4$ cells/ml.)
$D_2$ = Density of *Chlorella* sp. ($\times 10^6$ cells/ml.)
$TR_1$ = Transparency in Tetraselmis tank (cm.)
$TR_2$ = Transparency in Chlorella tank (cm.)

## INTRODUCTION

The 50% harvesting method is one of the effective methods for the mass culture of rotifer in Thailand (Pechmanee, et al., 1984). Density of phytoplankton as food for rotifer is one factor that can indicate rotifer production. This study was done to find a convenient method for measuring the density of 2 phytoplanktons, *Tetraselmis* sp. and *Chlorella* sp., that are always used for feeding rotifer at NICA.

## MATERIALS AND METHODS

Culturing *Tetraselmis* sp. and *Chlorella* sp. in 26 ton tanks started at the spawning season of seabass and marine shrimp. A few days after inoculation they bloomed. Then they were harvested and a reculture was started.

The chemicals for the phytoplankton used in each 26 ton tank were:
$(NH_4)_2SO_4$ 1200 g, calcium superphosphate 120 g and urea 60 g.

Density and transparency, were recorded every day in the morning (9.00–10.00) from March 6 to July 16, 1984 by checking the number of phytoplanktons under a microscope and measuring transparency with a secchi disc (diameter 15.5 cm).

During this study the salinities of water in Chlorella tanks and Tetraselmis tanks were 22–28 and 28.0–32.5 ‰ respectively. The temperature in both cases was between 26.5 and 29.5°C.

## RESULTS

*Tetraselmis* sp. grew through at the study period, while *Chlorella* sp. could not be grown during some period of time. Hence, *Tetraselmis* data were collected 549 times and only 173 times for *Chlorella* sp.

Transparency is related to density of *Tetraselmis* sp. and *Chlorella* sp. as in Figures 1 and 2. The relation can be shown by the following equations :

$$D_1 = 21.44 - 0.19\ TR_1$$

$$D_2 = 15.0 - 0.15\ TR_2$$

Where $D_1$ = Density of *Tetraselmis* sp. ($\times 10^4$ cells/ml )

$D_2$ = Density of *Chlorella* sp. ($\times 10^6$ cells/ml )

$TR_1$ = Transparency in Tetraselmis tank (cm )

$TR_2$ = Transparency in Chlorella tank (cm )

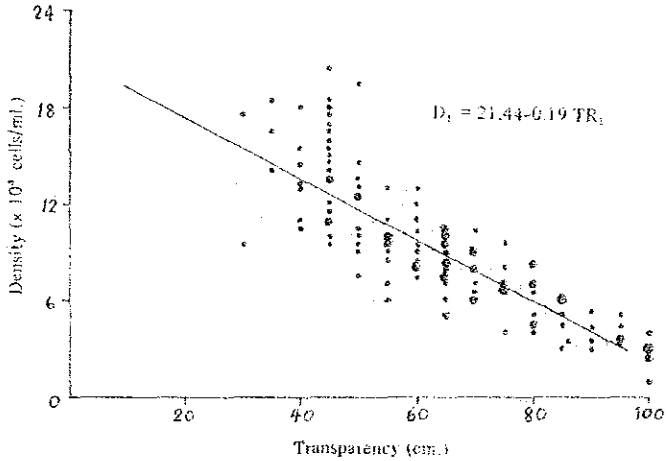


Fig. 1 The relationship between transparency and density of *Tetraselmis* sp.

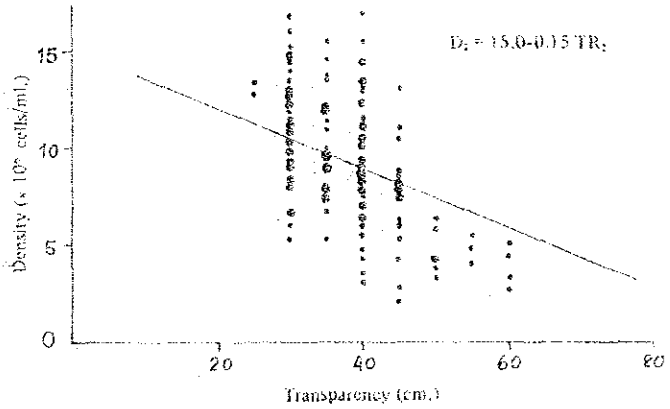


Fig. 2 The relationship between transparency and density of *Chlorella* sp.

## DISCUSSION

Because phytoplankton density effects the population growth of rotier (Hirayama et al., 1973), it is necessary to know phytoplankton density for combination with another factors for estimating rotifer production. Transparency measurement is a convenient method for estimating phytoplankton density and it is suitable for using in routine work.

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# Study on the effects of adding animal feed supplement when feeding fish meat to juvenile seabass, *Lates calcarifer*

Pairat Kosutarak

## INTRODUCTION

This experiment was carried out after we knew that juvenile seabass which were fed only with minced fish meat suffered from a poor growth rate and such symptoms as loss of equilibrium, blackening of the body and loss of caudal fin before finally dying.

This is a big problem for the fishfarmers who rear seabass in Songkhla lake and other areas. Therefore, this experiment was carried out to study the effects of mixing in animal feed supplement (2%) with the minced fish meat for feeding seabass. The animal feed supplement used can be easily obtained by the fishfarmers.

## MATERIALS AND METHODS

### Experimental fish

1,500 juvenile seabass aged 20 days (T.L. – 1.0 cm.) were obtained in September 1984, from the Marine Aqua Seed Company. They were kept in a 1,000 ℓ tank and maintained on minced fish meat for 17 days, then sorted at the start of experiment.

### Feeding and testing method

18 glass aquariums (497 x 257 x 295 mm) were used. The volume of seawater (salinity 33–34 ‰., temp. 26~28°C) in each tank was about 30 ℓ, and it was aerated sufficiently. 15 fish were put into each tank and fed twice a day (10.00 a.m., 2.00 p.m.) except on measuring day when there was no feeding. Feeding was continued until fish were satiated. At 4.00 p.m. the waste was siphoned out and about 25 ℓ of the seawater was removed, then the tanks were filled with new seawater until there was 30 ℓ in each. Fish were weighed individually at intervals of 1 week by anesthetizing with a 20 ppm quinaldine solution. The feeding experiment was continued for 9 weeks.

Test Diet

*Decaptures russelli* was used as the basis of the test diets during the entire experimental period. This experiment had 6 treatments and 3 replications in each treatment. The test diets (Table 1) were prepared every day by using a meat chopper. After the morning feeding, the rest of the test diets were kept in the refrigerator at 5°C for use in the 2.00 p.m. feeding.

Table 1. Composition of test diets.

| Diet | Composition |
|---|---|
| A | Minced fish meat included vertebra + 2% Animal feed supplement A |
| B | " + 2% " B |
| C | " + 2% " C |
| D | " + 2% " D |
| F | Minced fish meat included vertebra |
| M | Minced fish meat not including vertebra |

Note: Compositions of animal feed supplements A, B, C and D are shown in Appendices 1, 2, 3 and 4.

## RESULTS

The body weight of fish at the beginning and end of the experiment, the percent gain in body weight, feed efficiency, food conversion rate and mortality are all summarized in Table 2 and Figs. 1 and 2.

Week 1–2

Fish fed diet-M had fin hemorrhages. Fish fed diet-F had same phenomenon but seemed to be more serious. However, hemorrhages did not appear in fish which were fed diets-A, B, C or D (with animal feed supplement added).

Week 2–3

Fish fed diet-M decreased in food intake, lost equilibrium as shown by swimming activity, blackened in color, had a few fin hemorrhages and a few had bent back-bones. The first mortalities occured in this period. Fish fed diet-F had the same phenomena as fish in diet-M but the back-bones were normal. Fish fed diets-A,B,C and D appeared relatively healthy.

Week 3–4

For fish fed diet-M, the phenomena were the same as during week 2–3. For fish fed diet-F, the phenomena were the same, but the caudal fin was damaged little by little in some fish. Jaw and fin hemorrhages were observed. Fish fed diets-A, B, C and D again appeared relatively healthy.

Week 4–7

The results of observation were the same. Growth rate was very poor in fish fed diets-M and F, but in fish fed diets-A,B,C and D it was satisfactory.

Week 7–9

A recovery test was done on fish fed diets-M and F (two replications for each treatment) by switching them to diet-C.

After fish ate diet-C only one day, they recovered little by little to good condition. Swimming activity increased and body color was lighter. After 2 weeks of the recovery test, those caudal fins which were damaged regenerated little by little.

On the other hand, fish which did not undergo the recovery test remained in bad condition and their mortality rate was still high.

After the 9-week experiment, difference in average body weight, percent gain in body weight, feed efficiency among treatment groups were tested for significance ($P<0.05$) by Duncan's multiple range test.

The fish fed diets-F and M showed no significant differences in average body weight, percent gain in body weight and feed efficiency. But the results for fish fed diets-F or M were significantly different from those for fish fed diets with animal feed supplement added (A, B, C and D).

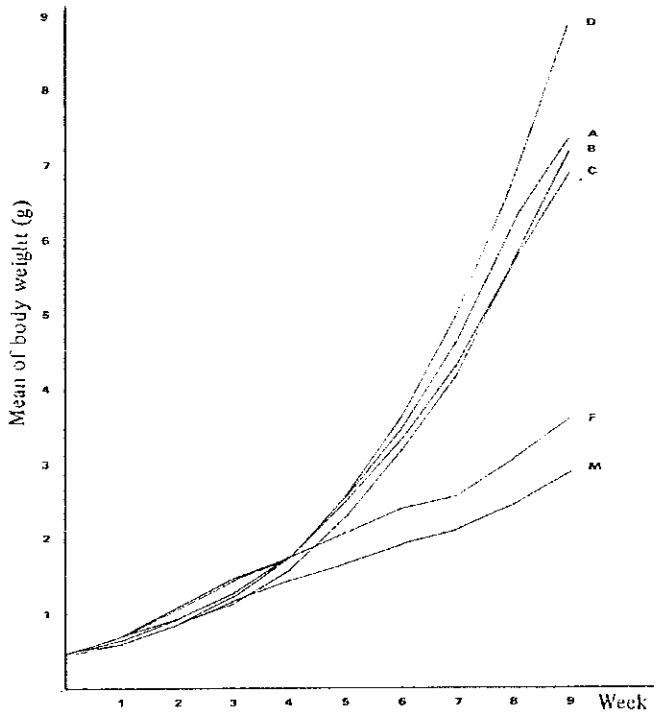


Fig. 1. The growth of juvenile seabass in 9 weeks.

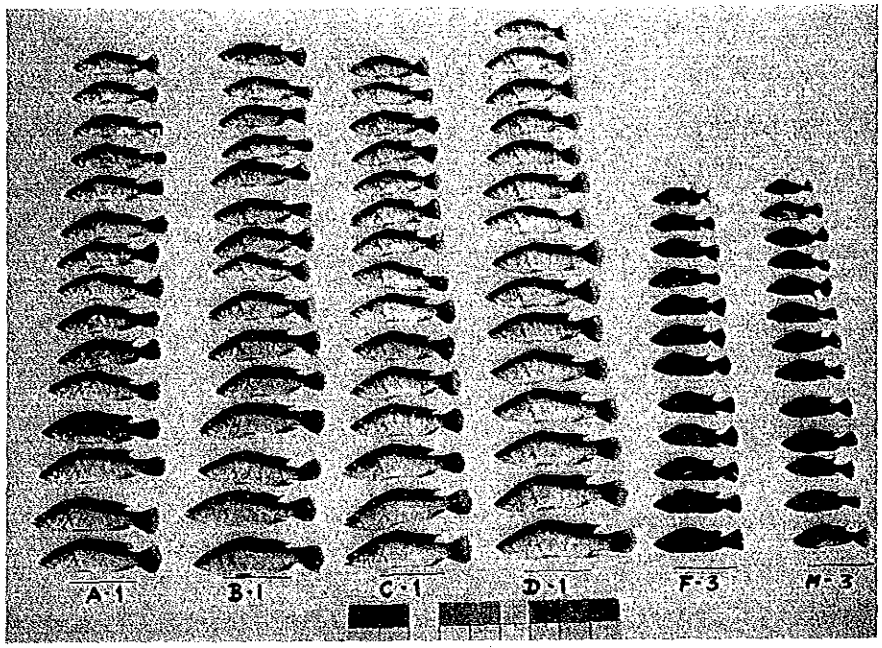


Fig. 2. Results of the 9-week feeding experiment.

Table 2. Results of the 9-week feeding experiment.

| | A | B | C | D | F | M | S.E.M. |
|---|---|---|---|---|---|---|---|
| Average body weight (g) | | | | | | | |
| Initial | 0.50 | 0.47 | 0.48 | 0.46 | 0.48 | 0.45 | 0.05 |
| Final | $7.38^{b}$ | $7.19^{b}$ | $6.9^{b}$ | $8.92^{b,c}$ | $3.60^{a}$ | $2.88^{a}$ | 1.22 |
| Feed efficiency (%) | $32.03^{b}$ | $32.6^{b}$ | $29.73^{b,c}$ | $35.0^{b,d}$ | $25.8^{a}$ | $27.5^{a}$ | 1.99 |
| Percent gain (%) | $1386.2^{b}$ | $1427.0^{b}$ | $1326.7^{b}$ | $1840.1^{b,c}$ | $656.7^{a}$ | $505.5^{a}$ | 212.11 |
| Food conversion rate | $3.12^{a}$ | $3.16^{a}$ | $3.36^{a}$ | $2.89^{a}$ | $4.89^{c}$ | $4.33^{b}$ | 0.59 |
| Mortality (%) | 0 | 4.5 | 0 | 2.2 | 26.7 | 20 | |

All values are average values from triplicate tanks each containing 15 fish. Values within a given line followed by the same letter superscript are not significantly different ($P > 0.05$)

S.E.M.: Standard error of a treatment.

## DISCUSSION

This experiment showed that juvenile seabass which were fed with minced fish meat had to have animal feed supplement added for normal growth. Using the 2% animal feed supplement mixed with the minced fish meat as shown in this experiment gave a percent gain in body weight of 1326–1840% in 9 weeks, while using only minced fish meat produced only about 505–656% in body weight gain.

After the recovery test was done during weeks 7–9, the mean body weight of fish in the two replications formerly fed diet-F increased from 2.19 and 2.52 g to 3.46 and 3.83 g, respectively,

while the replications not undergoing the recovery test increased from 2.93 to 3.51 g. The mean body weight of fish formerly fed diet-M increased from 2.09 and 1.91 g to 2.99 and 2.75 g, respectively, while the replications not undergoing the recovery test increased from 2.34 to 2.90 g. This shows that fish are able to recover in a short time if given an animal feed supplement.

There were some differences in results between fish fed minced fish meat including vertebra (diet-F) and those fed only minced fish meat (diet-M). The fish in diet-F showed a higher mean body weight than the fish in diet-M, but the mortality was also high. These results call for further study.

Finally the qualitative and quantitative requirements of seabass for vitamins should also be studied by using a purified test diet.

## ACKNOWLEDGEMENTS

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Appendix 1. Composition of animal feed supplement A (Adiuvit).

Each Kg contains:

| | |
|---|---|
| Vitamin A | 1,500,000 I.U. |
| " $D_3$ | 200,000 I.U. |
| " E | 400 mg |
| " K | 50 mg |
| " $B_1$ | 10 mg |
| " $B_2$ | 300 mg |
| " $B_6$ | 10 mg |
| " $B_{12}$ | 2,000 mcg |
| " PP | 2.5 g |
| Calcium Pantothenate | 2 g |
| Choline chloride | 35 g |
| DL-Methionine | 5,000 me |
| Chlortetracycline | 2.5 g |
| Iron | 3.8 g |
| Magnesium | 3.4 g |
| Zinc | 2.6 g |
| Manganese | 1.6 g |
| Iodine | 244 mg |
| Copper | 53 mg |
| Cobalt | 23.5 mg |

Appendix 2. Composition of animal feed supplement B (Biofac-200).

Each Pound contains:

| | |
|---|---|
| Vitamin A | 4,000,000 I.U. |
| " $D_3$ | 1,000,000 I.U. |
| " E | 600 I.U. |
| Riboflavin | 850 mg |
| Vitamin $B_3$ | 1,100 mg |
| " $B_4$ | 7,000 mg |
| Choline chloride | 125,000 mg |
| Vitamin $B_{12}$ | 1 mg |
| " $B_1$ | 40 mg |
| " K | 400 mg |
| " $B_6$ | 10 mg |
| " H | 0.02 mg |
| Inositol | 160 mg |
| Folic acid | 20 mg |
| BHT | 100 mg |
| Methionine | 5,000 mg |
| Manganese | 3,520 mg |
| Iron | 1,760 mg |
| Iodine | 80 mg |
| Copper | 6.6 mg |
| Cobalt | 9.1 mg |
| Zinc | 886 mg |
| Para-amino benzoic acid | 0.8 mg |

Appendix 3. Composition of animal feed supplement C (Stamix).

Each Kg Contains:

| | |
|---|---|
| Vitamin A | 1,200,000 I.U. |
| " $D_3$ | 240,000 I.U. |
| " E | 840 mg |
| " $K_3$ | 100 mg |
| " $B_1$ | 30 mg |
| " $B_2$ | 600 mg |
| " $B_6$ | 10 mg |
| Niacin | 3,600 mg |
| Pantothenic acid | 2,000 mg |
| Vitamin $B_{12}$ | 4 mg |
| Folic acid | 10 mg |
| Biotin | 2 mg |
| Choline | 5,000 mg |
| BHT | 200 mg |
| Cobalt | 4 mg |
| Copper | 2,400 mg |
| Iron | 7,200 mg |
| Iodine | 120 mg |
| Manganese | 9,600 gm |
| Zinc | 24,000 mg |
| Verginia Mycin | 4,000 mg |
| Furasolidone | 20,000 mg |

Appendix 4. Composition of animal feed supplement D (Ovimin).

Each Kg. Contains:

| | |
|---|---|
| Vitamin A | 20,000,000 I.U. |
| " K | 3,000 mg |
| " $D_3$ | 5,000,000 I.U. |
| Pyridoxine ($B_6$) | 3,600 mg |
| Vitamin E | 5,500 I.U. |
| *Folic Acid* | 400 mg |
| Riboflavin ($B_2$) | 3,500 mg |
| Thiamine ($B_1$) | 2,000 mg |
| Pantothenic acid ($B_5$) | 6,600 mg |
| Vitamin $B_{12}$ | 20 mg |
| Niacin ($B_3$) | 20,000 mg |
| Vitamin C | 20,000 mg |
| Methionine | 10,000 mg |

# Optimum level of protein in a purified diet for mullet, *Mugil dussumieri* (Valenciennes).

Pairat Kosutarak

## INTRODUCTION

At present, most of the mullet that are sold in the market come from natural fishing grounds. As a result, in the near future the amount of mullet will certainly decrease. To solve this problem, the culture of mullet should be examined.

This experiment was carried out to study the optimum level of protein to include in a purified diet for mullet *Mugil dussumieri* (Valenciennes). The levels of protein studied were: 0%, 13.4%, 22.3%, 31.2%, 40.1% and 49.0% of a purified diet, respectively.

## MATERIALS AND METHODS

### Experimental fish

Fingerling sized mullet were obtained from Songkhla Lake in April, 1984. They were kept in tanks and maintained first on a formula diet, then on a purified diet. About 1,000 fish, weighing more than 1.3g each, were fed a diet, then on a purified diet. About 1,000 fish, weighing more than 1.3g each, were fed a diet containing 40.1% protein as in Table 1, for one week, then sorted before the start of the experiment.

### Feeding and testing

Six, 500 ℓ plastic tanks were used. These were supplied with well seawater (salinity 30–32 ‰, temp. 26–28°C at a rate of 0.8 to 1.0 ℓ/min, and aerated sufficiently. 90 fish were put into each tank for each experimental diet. Feeding was done twice a day, 9 a.m. and 3 p.m., except on measuring day when there was no feeding. Fish were fed 10% of their body weight per day. The food was in the form of a paste which was rolled into a ball and put into a small basket. Fish were weighed individually, at 2 week intervals, by anesthetizing them with an 18 ppm quinaldine solution. The feeding experiment contained for 8 weeks.

### Diet

The diets used in this experiment are shown in Table 1. New supplies of the diets were prepared every week and stored in a refrigerator at –3° C until used.

Table 1. Composition of test diets.

| Ingredients \ Lot No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Vitamin-free casein | 55 | 45 | 35 | 25 | 15 | 0 |
| Dextrin | 16 | 26 | 36 | 46 | 56 | 71 |
| Cellulose powder* | 4 | 4 | 4 | 4 | 4 | 4 |
| Mineral mixture** | 7 | 7 | 7 | 7 | 7 | 7 |
| Oil*** | 8 | 8 | 8 | 8 | 8 | 8 |
| C.M.C. | 10 | 10 | 10 | 10 | 10 | 10 |
| Water | 100 | 100 | 100 | 100 | 100 | 100 |
| Crude protein (%) ++ | 49.0 | 40.1 | 31.2 | 22.3 | 13.4 | 0 |

* Vitaminized cellulose powder. The amount of vitamine mixture added to each diet was the same as reported by Halver (1957).

** USPXII, Salt mixture No.2 plus trace metals (Halver 1957).

*** A mixture of corn oil and cod liver oil in 2:1.

++ Expressed on dry basis and calculated from nitrogen content of casein (Nx6.25).

## RESULTS AND DISCUSSION

The body weight and number of fish at the beginning and end of the experiment, the percent gain in body weight, daily feed intake, feed efficiency, and food conversion rate are all summarized in Table 2. In Fig. 1, the percent gain in body weight is shown with regard to the different protein levels of the various diets. The mullet showed a remarkable decrease in body weight when kept on a non-protein diet, while diets containing more than 13.4% protein showed a positive weight gain. The best growth rates were for diets of 49.0%, 40.1%, 31.2%, 22.3%, and 13.4% protein, in that order. However, there was no significant difference in growth among the 49.0%, 40.1%, 31.2%, and 22.3% protein groups (P = 0.001).

Table 2. Results of feeding experiment for 8 weeks.

| Lot No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Number of fish | | | | | | |
| at beginning (No) | 90 | 90 | 90 | 90 | 90 | 90 |
| at end (N) | 90 | 87 | 83 | 88 | 90 | 90 |
| Average body weight (g) | | | | | | |
| at beginning (Wo) | 1.65 | 1.61 | 1.56 | 1.51 | 1.51 | 1.47 |
| SD. | 0.54 | 0.47 | 0.47 | 0.50 | 0.46 | 0.45 |
| at end (W) | 3.30 | 3.12 | 2.96 | 2.76 | 2.33 | 1.20 |
| SD. | 1.21 | 1.32 | 1.24 | 1.70 | 1.15 | 0.40 |
| Percent gain* | 100.0 | 93.8 | 89.7 | 82.8 | 54.3 | -18.4 |
| Daily feed intake (%)** | 8.7 | 8.8 | 8.8 | 8.6 | 8.8 | 9.6 |
| Feed efficiency (%) *** | 12.8 | 12.2 | 11.7 | 11.3 | 8.1 | -3.5 |
| Food conversion rate **** | 7.8 | 8.4 | 8.6 | 8.8 | 12.4 | 00 |

* Percent gain $= \frac{100\,(W-Wo)}{Wo}$

** Daily feed intake (%) $= \frac{400F}{(W+Wo)\,(N+No)\,D}$

*** Feed efficiency (%) $= \frac{50\,(W-Wo)\,(N+No)}{F}$

**** Food conversion rate $= \frac{\text{wet weight of feed}}{\text{wet weight gained}}$

Remarks: F = Total amount of feed intake (g)
D = Days

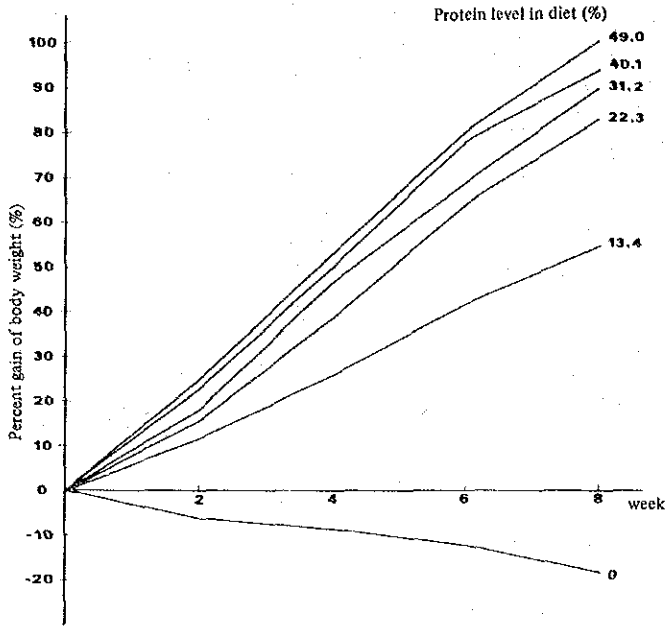


Fig. 1. The growth of mullet in a 8--week feeding experiment.

The results show that the highest percentage of protein (49.0%) gives a better growth rate, narrower distribution of size and higher food conversion rate than 4.1%, 31.2%, or 22.3% protein. However, if the economic cost of the diets is taken into consideration, the best choice may be the diet with 22.3% protein.

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# Nutritional evaluation of feedstuffs made in Thailand.

Pairat Kosutarak and Hiroshi Ogata*

Five feedstuffs made in Thailand, fish meal, squid meal, shrimp meal, soy bean meal and rice bran meal were evaluated in nutrition value in March–April 1985, at National Research Institute of Aquaculture, Mie, Japan.

These feedstuffs were bought in the market in Songkhla province except the squid meal which was made by using only head-part.

Crude protein was measured by the micro-Kjeldahl method, ash by combustion in a furnace at 550–600°C, moisture by drying at 105°C, crude fat by extraction with ethyl ether (Ex-fat: Laboman Geneco), gross energy (GE) value by a bomb calorimeter (Shimadzu CA-3), fatty acid compositions by gas chromatograph and amino acid compositions by an automatic amino acid analyzer (Hitachi, Model 835).

The results were summarized in Table 1, 2 and 3.

Table 1. Proximate compositions of feedstuffs made in Thailand.

| | Fish meal | Squid meal | Shrimp meal | Rice bran meal | Soy bean meal |
|---|---|---|---|---|---|
| Moisture (%) | 11.7 | 8.8 | 15.2 | 14.5 | 9.8 |
| Crude protein (%) | 54.3 | 68.9 | 32.6 | 11.6 | 46.7 |
| Total lipid (%) | 8.1 | 8.1 | 3.0 | 8.0 | 4.3 |
| Crude fat (%) | 5.7 | 5.6 | 1.3 | 7.3 | 1.8 |
| Crude ash (%) | 26.7 | 11.3 | 38.5 | 10.4 | 7.0 |
| Gross energy (cal/g ) | 3678 | 4192 | 2482 | 3022 | 3869 |

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Table 2. Amino acid composition and A/E ratio of feedstuffs made in Thailand.

| Amino acids | Composition (g/100 g dry meal) | | | | | A/E ratio | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Shrimp meal | Fish meal | Squid meal | Soy meal | Rice bran meal | Shrimp meal | Fish meal | Squid meal | Soy meal | Rice bran meal |
| Arginine | 2.033 | 3.571 | 3.425 | 3.872 | 0.656 | 126 | 132 | 149 | 239 | 145 |
| Histidine | 0.641 | 1.282 | 0.937 | 0.986 | 0.259 | 40 | 47 | 41 | 61 | 57 |
| Isoleucine | 1.195 | 2.001 | 1.839 | 1.113 | 0.343 | 74 | 74 | 80 | 69 | 76 |
| Leucine | 2.021 | 3.747 | 3.201 | 2.165 | 0.629 | 125 | 138 | 140 | 134 | 139 |
| Lysine | 3.842 | 5.741 | 4.119 | 1.823 | 0.557 | 238 | 212 | 180 | 112 | 123 |
| Methionine | 0.612 | 1.451 | 1.284 | 0.294 | 0.171 | 38 | 54 | 56 | 18 | 38 |
| Cystine | 0.410 | 0.589 | 0.728 | 0.613 | 0.350 | 25 | 22 | 32 | 38 | 78 |
| Met + Cys | | | | | | (63) | (76) | (88) | (56) | (116) |
| Phenylalanine | 1.293 | 1.993 | 1.566 | 1.577 | 0.349 | 80 | 74 | 68 | 97 | 77 |
| Tyrosine | 1.166 | 1.719 | 1.682 | 1.246 | 0.318 | 72 | 63 | 73 | 77 | 71 |
| Phe + Tyr | | | | | | (152) | (137) | (141) | (174) | (148) |
| Threonine | 1.314 | 2.291 | 1.847 | 1.059 | 0.343 | 82 | 85 | 81 | 65 | 76 |
| Tryptophan | 0.176 | 0.333 | 0.446 | 0.032 | 0.025 | 11 | 12 | 19 | 2 | 6 |
| Valine | 1.420 | 2.407 | 1.874 | 1.445 | 0.517 | 88 | 89 | 82 | 89 | 114 |
| Alanine | 1.520 | 3.398 | 2.419 | 1.492 | 0.510 | | | | | |
| Aspartic acid | 2.509 | 3.962 | 3.615 | 3.013 | 0.618 | | | | | |
| Glutamic acid | 4.049 | 6.947 | 6.029 | 5.733 | 1.029 | | | | | |
| Glycine | 1.851 | 3.787 | 2.653 | 2.435 | 0.473 | | | | | |
| Proline | 1.175 | 2.467 | 1.773 | 1.557 | 0.384 | | | | | |
| Hydroxyproline | 0 | 0.951 | 0.801 | 0.448 | 0 | | | | | |
| Serine | 1.295 | 2.154 | 1.761 | 1.662 | 0.411 | | | | | |
| Taurine | 0.386 | 0.758 | 2.203 | 0 | 0 | | | | | |
| Ammonia | 0.512 | 0.496 | 0.320 | 0.623 | 0.137 | | | | | |
| Total | 29.420 | 52.045 | 44.522 | 33.188 | 8.079 | | | | | |

Table 3. Fatty acid compositions of feedstuffs made in Thailand (%).

| Fatty acid | | Fish meal | Squid meal | Shrimp meal | Rice bran meal | Soybean meal |
|---|---|---|---|---|---|---|
| Myristic | 14:0 | 4.6 | 2.4 | 2.4 | 1.3 | 0.5 |
| | 15:0 | 16.6 | 1.3 | 4.1 | 0.4 | |
| | 15:1 | 0.4 | 0.2 | 1.3 | | |
| Palmitic | 16:0 | 26.0 | 32.6 | 29.4 | 10.7 | 7.5 |
| Palmitoleic | 16:1 | 4.6 | 2.3 | 4.3 | 2.5 | 1.1 |
| | 17:0 | 1.5 | 2.9 | 4.8 | 0.9 | 0.4 |
| | 17:1 | 2.5 | 0.8 | 3.9 | | 3.6 |
| Stearic | 18:0 | 9.8 | 21.4 | 16.9 | 11.4 | 3.8 |
| Oleic | 18:1 | 11.4 | 9.4 | 14.1 | 5.5 | 20.4 |
| Linoleic | 18.2 | 0.3 | 0.3 | 0.4 | 32.2 | 19.5 |
| Linolenic | 18:3 | | | | | |
| & Eicosenoic | 20:1 | 0.7 | 4.1 | 2.9 | 19.8 | 2.3 |
| Octadecatetraenoic | 18:4 | 0.4 | | | | |
| | 19:0 | 0.9 | | | | |
| Eicosatrienoic | 20:3w6 | | | 1.6 | | 0.4 |
| Arachidonic | 20:4w6 | | | | | |
| & Docoseoic | 22:1 | 2.8 | 6.5 | 3.5 | 2.0 | |
| Arachidonic | 20:4w3 | 0.8 | | | | |
| Eicosapentaenoic | 20:5w3 | 3.4 | 4.6 | 2.2 | 0.8 | |
| Docosatetraenoic | 22:4w6 | 1.6 | 1.2 | 1.9 | 0.3 | 1.0 |
| Docosapentaenoic | 22:5w6 | 1.0 | 0.8 | | 0.3 | 1.9 |
| Docosapentaenoic | 22:5w3 | 1.0 | 0.7 | 0.9 | | |
| Docosahexaeoic | 22:6w3 | 7.7 | 6.7 | 0.9 | 0.2 | 0.5 |
| Unknown 1 | | | | | | 4.2 |
| Unknown 2 | | | | | | 28.8 |

# Haematological study on kidney disease in seabass, *Lates calcarifer*

Yaowanit Danayadol and Jaruratt Boonranapanichagit

## INTRODUCTION

In November, 1983, kidney disease broke out in seabass, *Lates calcarifer,* in three areas of southern Thailand and was the subject of an earlier report (Danayadol and Boonranapanichagit, 1984). This report described the bacteriological examination performed and noted the histological changes which occurred in some organs, especially the kidney. The most important at observation made concerned the needle-type crystals which were found on smear slides of nodules taken from the kidneys and which could be disolved in either acid or alkaline solutions. Because of this, it was supposed that the crystals were caused by a mineral imbalance or an overdose of one mineral. When further examination showed that the crystals could not be stained by Alizarin s., it was reported that the crystals were compounds of P. mineral.

The current experiment was carried out to further investigate the kidney disease, both through haematological examination and through a check of the calcium (Ca) and phosphorus (P) balance present in the kidneys and in the muscles.

## MATERIALS AND METHODS

Sample Fish:

A control group of 10 normal seabass, *Lates calcarifer,* about 2.5 months in age, 5.1–12.6 cm in length, and 3.45–45.55 g in weight were taken from the wet laboratory of the National Institute of Coastal Aquaculture. They were kept in running seawater of 32 ‰ salinity and about 28°C. Their diet consisted of raw fish, mostly sardines, including bone and meat. They were fed twice a day.

Eight diseased fish, 4.0–6.5 cm in length and 5.0–19.5 g in weight were brought in from the culturing area in Natap, Songkhla province. They were cultured in water of 32 ‰ salinity and about 29°C. Their diet consisted only of muscle of raw fish, mostly sardines, which they were fed twice a day.

Pathological Anatomical Study:

External and internal examinations were made of both normal and diseased fish unaided and under the microscope. Slide smears of the kidney were also examined.

Preparation of Blood Samples:

Blood was collected in heparinized capillary tubes by tail cut from fish which were not anesthetized. An attempt was made to keep the effects of handling to a minimum. Haematocrit levels were measured by Hematocrit accessory after centrifuging at 12,000 rpm for 5 minutes. Plasma total protein levels were measured by hand protein refractometer.

Ca and P Level Tests:

Ca and P levels were analysed using a pooled sample from the kidneys and muscles of about 5 fish. The kidney or muscle tissue was homogenized and diluted 10–100 times in 0.1 N HCl solution after being weighed. Ca levels were measured by the OCPC method, and P levels by the modified Lowry-Lopetz method using a Ra BA machine (Chugai Pharmaceutical Co. Ltd., Japan).

## RESULTS

General Symptoms:

Most of the symptoms found in the diseased fish in this study were the same as those found in the fish with kidney disease in the earlier report.

Haematocrit Levels:

As shown in Table 1, the haematocrit level in healthy fish ranged from 36.0% to 45.5% with a mean level of 40.39%, while the level in diseased fish varied from 5.0% to 19.5% with a mean level of 9.3%. The haematocrit level in most of the diseased fish was lower than 10%, or about ¼ of the normal level, and the range in the level for these fish was very wide.

Plasma Total Protein:

In healthy fish, the plasma total protein level varied from 3.5 to 5.0 g/100 ml, with a mean level of 4.6, while the level in diseased fish ranged from 2.0 to 5.3 g/100 ml, with a mean level of 3.4. The range of levels in diseased fish was very wide. Some levels were similar to those of healthy fish while some were very low, about half the normal level.

Ca and P Levels:

The Ca level in kidneys of the healthy fish varied from 30.9 to 45.3 mg/100 mg of tissue, with a mean of 41.2, while the P level ranged from 36.7 to 59.5 mg/100 mg tissue, with a mean level of 48.3. P per mg of Ca varied from 1.11 to 1.22 with a mean of 1.17.

The Ca level in muscle tissue of healthy fish ranged from 2.7 to 10.6 mg/100 mg tissue, with a mean level of 10.15, while the P level ranged from 112.1 to 122.7 mg/100 mg tissue, with a mean level of 117.4. P per mg of Ca was 10.58 and 12.65 with a mean of 41.62.

The Ca level in kidneys of diseased fish ranged from 38.7 to 72.5 mg/100 mg tissue, with a mean level of 53.2, while the P level ranged from 69.6 to 135.3 mg/100 mg tissue, with a mean

level of 113.4. P per mg of Ca varied from 2.80 with a mean of 2.16.

In general, the Ca level was the same in both diseased and healthy fish, but the P level and P:Ca ratio were twice as high in diseased fish. The Ca and P levels were lower for less serious cases of the disease than for serious cases, but the P:Ca ratio remained the same. The amount of P present correlated with the severity of the symptoms.

## DISCUSSION

The data in Table 1 show that the level of the haematocrit was lower in diseased fish than in healthy ones. This means that there was anemia present in diseased fish. Which may have been caused by iron deficiency or lack of vitamin $B_{12}$. This is supported by the data obtained from observation of fish farming methods; i.e., none of the fish were given internal fish organs as part of their diet, non were they given Vitamin $B_{12}$ supplements. The decrease in anterior kidney function is related to lower haematocrit levels because it reduces the blood forming tissue's ability to produce new blood.

Table 1. Haematocrit level and plasma total protein level of normal and diseased seabass, *Lates calcarifer*.

| | B.L. (cm.) | B.W. (gm.) | Ht (%) | T.P. (g/100 ml.) |
|---|---|---|---|---|
| Normal fish | | | | |
| No. 1 | 12.6 | 49.99 | 36.0 | 4.1 |
| No. 2 | 12.6 | 48.87 | 40.4 | 4.8 |
| No. 3 | 6.5 | 7.34 | 37.5 | 4.4 |
| No. 4 | 7.3 | 8.80 | 40.0 | 5.0 |
| No. 5 | 8.1 | 11.46 | 39.0 | 4.6 |
| No. 6 | 6.5 | 7.33 | 45.5 | 4.4 |
| No. 7 | 5.1 | 3.95 | 43.0 | 4.6 |
| No. 8 | 8.8 | 18.97 | 40.5 | 5.0 |
| No. 9 | 6.7 | 8.60 | 40.5 | 4.8 |
| No. 10 | 6.0 | 5.03 | 41.5 | 3.9 |
| mean | 8.02 | 17.03 | 40.39 | 4.6 |
| Diseased fish | | | | |
| No. 1 | 6.4 | 5.43 | 19.5 | 5.3 |
| No. 2 | 4.2 | 1.73 | 9.0 | – |
| No. 3 | 6.4 | 4.57 | 10.0 | 2.8 |
| No. 4 | 6.9 | 6.02 | 6.0 | 2.6 |
| No. 5 | 5.2 | 2.54 | 5.5 | 2.0 |
| No. 6 | 6.9 | 5.42 | 9.5 | 4.8 |
| No. 7 | 5.9 | 3.44 | 5.0 | 3.8 |
| No. 8 | 4.0 | 1.07 | 10.0 | 2.3 |
| mean | 5.2 | 3.18 | 9.3 | 3.4 |

The data in Table 2 show that the P level and the P:Ca ratio were twice as high in the kidneys of diseased fish as in those of healthy fish, while tha Ca level remained relatively the same in each. Since it is known that fish farmers generally use fish muscle as food for culturing seabass and that fish muscle contains a high level of P, it may be that the formation of nodules in the kidneys of these seabass was caused by an excessive amount of P in their diet.

It can be concluded, that there were two diseases found in the examined fish. First, kidney disease caused by a decrease in the function of the posterior kidney due to the formation of nodules caused by an imbalance of P and Ca levels. Second, anemia caused by a decrease in haematocrit levels. The presence of anemia meant that fish with only mild symptoms of kidney disease sometimes died.

Table 2. Ca and P level in Kidney or muscle of normal and diseased seabass, *Lates calcarifer.*

| | | | Ca | P | P:Ca ratio |
|---|---|---|---|---|---|
| Normal fish | Kidney | No. 1 | 49.3 | 59.9 | 1.22 |
| | | No. 2 | 43.5 | 40.3 | 1.11 |
| | | No. 3 | 30.9 | 36.7 | 1.19 |
| | mean | | 41.2 | 48.3 | 1.17 |
| | Muscle | No. 1 | 10.6 | 112.1 | 10.58 |
| | | No. 2 | 9.7 | 122.7 | 12.65 |
| | mean | | 10.15 | 117.4 | 11.62 |
| Diseased fish | | | | | |
| | Kidney | No. 1 | 38.7 | 69.6 | 1.8 |
| | | No. 2 | 48.3 | 135.3 | 2.8 |
| | | No. 3 | 72.5 | 135.3 | 1.87 |
| | mean | | 53.2 | 113.4 | 2.16 |

Notes: Unit of Ca or P level is mg/100mg of wet tissue.
No. 1 of diseased fish is of light symptoms.
No. 2 & No. 3 of diseased fish are of serious symptoms.

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