REPORT OF THAILAND AND JAPAN JOINT COASTAL AQUACULTURE RESEARCH PROJECT

(APRIL 1981 — MARCH 1984)
No. 1

SEPTEMBER 1984

JAPAN INTERNATIONAL COOPERATION AGENCY

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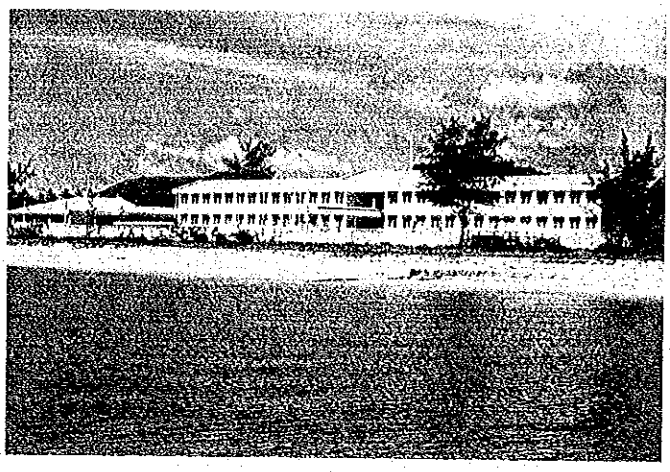
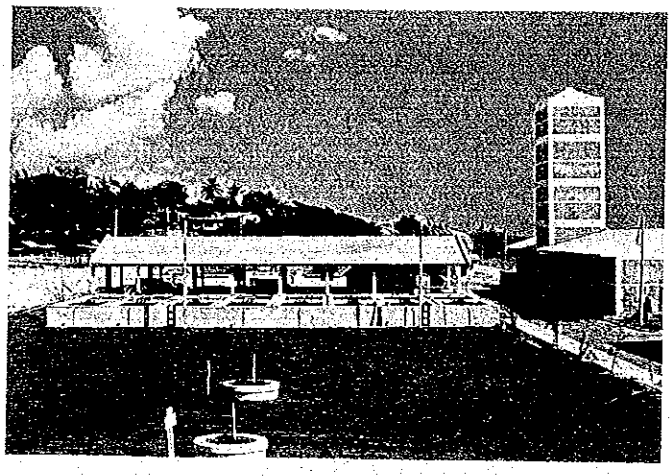
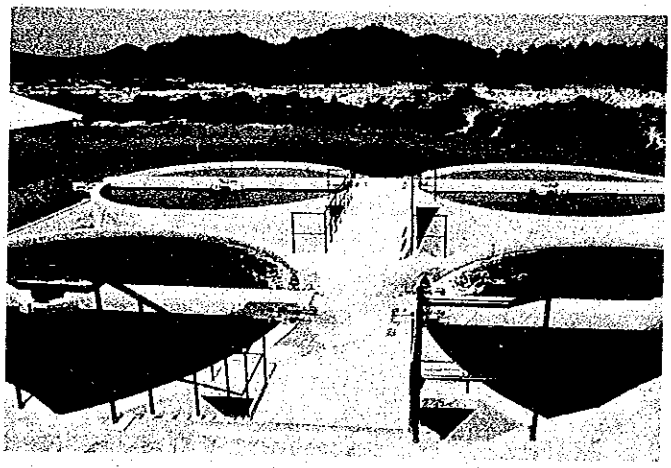
National Institute of Coastal Aquaculture



Main building

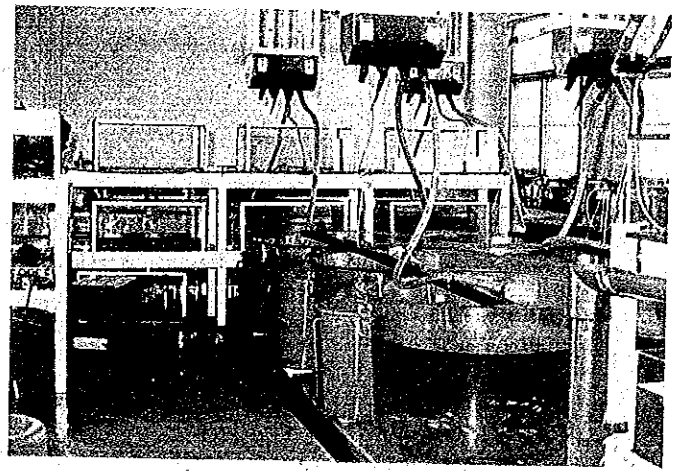
Seed production facilities



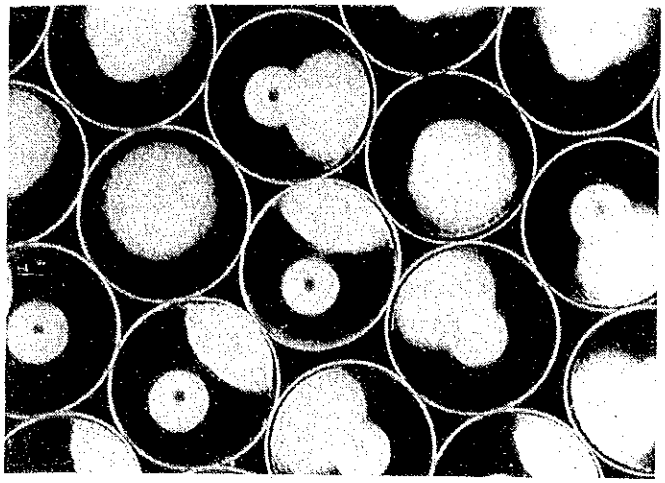


Parent fish tank

Wet laboratory

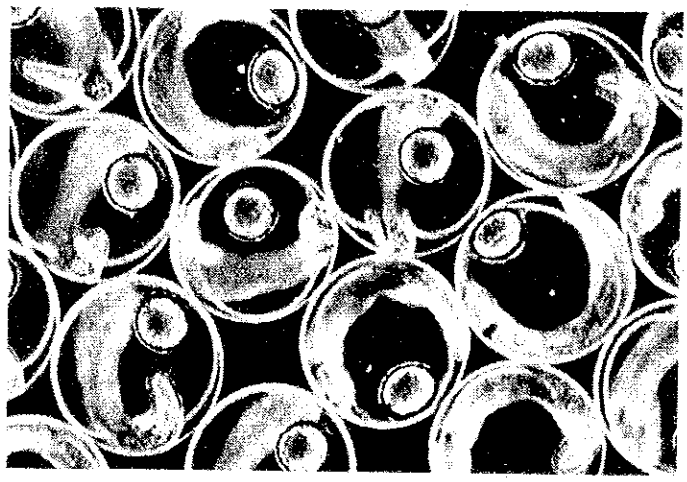


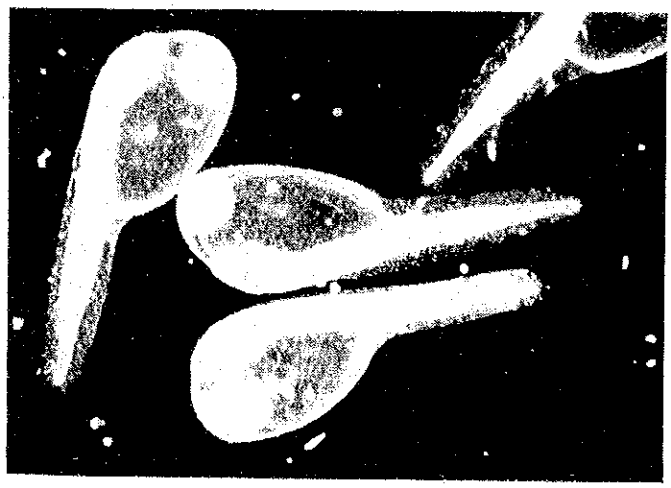
Egg and early fry of seabass, *Lates calcarifer*



Fertilized egg

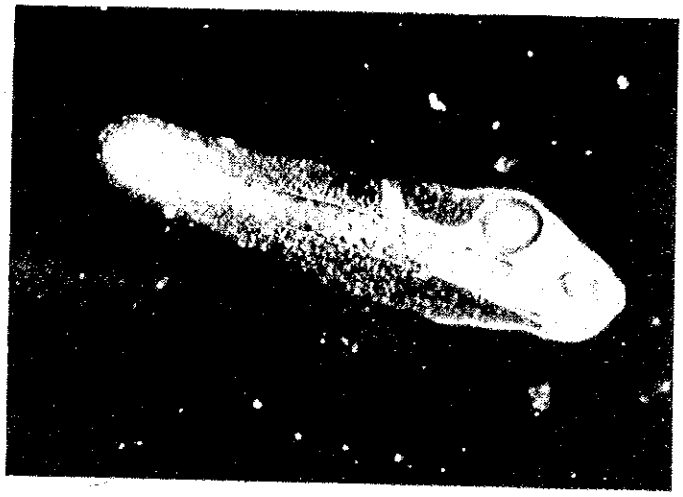
Developed egg





Hatched fry

Fry



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Progress of Thailand and Japan Joint Coastal Aquaculture Research Project (April 1981 - March 1984)

Munekazu Masuo

(1) Introduction

The Japanese Basic Design Survey Team, organized by the Japan International Cooperation Agency (hereinafter referred to as JICA) and headed by Dr. M. Fujiya, visited Bangkok in October, 1979.

With Commander Swarng Charernphol, Director General, Department of Fisheries, Ministry of Agriculture and Cooperatives, they discussed the establishment of a Coastal Aquaculture Research Center at Songkhla and, on October 5, 1979, signed "Minutes of Discussion on the Construction Project of the Songkhla Coastal Aquaculture Center, the Kingdom of Thailand", concerning a Thailand-Japan Joint Coastal Aquaculture Research Project.

A second mission, the Japanese Implementation Survey Team, also organized by JICA and headed by Dr. M. Fujiya, visited Bangkok in October 1980 and discussed the coastal aquaculture development project. On October 27, 1980, they signed "The Record of Discussions between the Japanese Implementation Survey Team and the Authorities Concerned of Thailand on the Japanese Technical Cooperation for the Coastal Aquaculture Development Project".

Since then, both governments have cooperated with each other in implementing the coastal aquaculture development project for the purpose of developing coastal aquaculture in Thailand. This will help to promote the production of protein sources in the coastal area, as well as increasing employment opportunities for the Thai people.

The construction of a building for this center started in May 1980 and was completed in March 1981.

The project itself started in April 1981 and, since then, research has been carried out for the purpose of improving technology and knowledge.

Note: The name of the Songkhla Coastal Aquaculture Center was changed to the National Institute of Coastal Aquaculture (NICA) in September 1981.

(2) The schedule of the Coastal Aquaculture Development Project.

The duration of the project is five years from April 1981: the project consists of the following subjects.

From 1st year to 3rd year.

1. Seed production procedures.
   Brood stock rearing.
   Egg and larva rearing.
   Food organism rearing.
2. Intensive culture procedures.
   Growing procedures.
   Fish nutrition.
   Fish health control.
3. Field development procedures.
   Survey of fish culture field.
   Survey of fish distribution.
   Survey of major fish spawning ground.

From 4th to 5th year.

1. Practical modification of seed production technique.
2. Practical modification of fish growing technique.
3. Practical modification of field survey technique.

(3) The research work by the Japanese experts.

As mention above, Japanese experts, Mr. M. Masuo, Team Leader, and Mr. T. Yokokawa arrived in Songkhla in April 1981.

Later on Mr. T. Watanabe arrived in August 1981. The short term experts who take part in research cooperation at the National Institute of Coastal Aquaculture in Songkhla are; Dr. S. Arai, fish nutrition, Dr. K. Fukusho, seed production, Mr. T. Matsusato, fish health control, Mr. K. Okubo, chemical analysis, Dr. Y. Taki, fish taxonomy, Mr. T. Sumita, marine environment, Mr. H. Konno, egg and larva taxonomy, Mr. T. Shimizu, fish taxonomy.

These Japanese experts carry out cooperative research work with the Thai researchers in NICA.

In October 1982, a Japanese Technical Guidance Team, organized by JICA and headed by Dr. Y. Taki visited Thailand and discussed with the NICA staff members the problems of the project. The Team presented a "Brief Report" in which several problems with the development of the project were pointed out.

In November 1983, the same team visited Songkhla, this time headed by Dr. M. Fujiya. Discussions were held with NICA staff members and the team presented a "Brief Report" which was mainly on the extension and expansion of the project in future.

(4) Research facilities and equipment.

Initially, in 1980, the Coastal Aquaculture Research Center building, the dormitory, the fish rearing facilities and much equipment were all donated by Japanese Government.

During the period 1981 - 1983, a lot of machinery and equipment as well as a new survey boat were also donated by JICA.

A summary of the budget for the building, facilities, equipment and other things supplied by the Japanese Government and JICA:

(unit: thousand Yen)

Center building	1980	680,000
Equipment	"	120,000
"	1981	35,000
"	1982	90,000
"	1983	80,000
Total		1,005,000

(5) Research activities

Since Japanese experts arrived in Songkhla in 1981, they have done their best, with their Thai counterparts, to improve research techniques and to create new techniques for coastal aquaculture.

They have been conducting laboratory as well as field experimentation cooperatively each season, based on mutual respect and understanding.

During this period of cooperation, more Thai staff members were recruited and trained. Improvement of the laboratory, with machinery and apparatus donated by JICA, has gradually made it possible for more elabo-

rate experimentation to be conducted in the laboratory as well as in the field.

In spite of the deficient condition of the laboratory and the shortage in number of researchers in the cooperative research field in NICA at the beginning of the project, the project has steadily developed, through the mutual understanding and cooperative efforts betweem NICA staff members and the Japanese project team, and with the continuing encouragement of Japanese project team, and with the continuing encouragement of the authorities concerned in Thailand and Japan.

From the Thai government, more budget for site development was contributed, resulting in the construction of an officials' residence, a road, a fence and the other facilities on the NICA premises.

Field trips have been made and observation work done by the Japanese experts and their counterparts. This opportunity was also taken to exchange opinions and cultural background between the two nations.

Most of the Thai junior staff members visited Japan for a short term training period of four months. Which helped to upgrade their research capabilities.

With better facilities and a better trained staff of researchers, more sophisticated research can be conducted which produces new findings leading to basic solutions to fundamental and practical problem in protein food crop production.

(6) Future views.

In November 1983, a Japanese Technical Guidance Team, organized by JICA and headed by Dr. M. Fujiya, visited Thailand.

They exchanged views with, and had a series of discussions with, the NICA staff members and Japanese experts concerned on the progress in research activities and on future views. From this exchange of views and discussions, the team produced a "Brief Report".

Seed Production

The results of the seabass seed production techniques were highly praised. In Future, it is desirable that this research plan prepared by NICA be put into effect to the fullest extent. In view of the importance of experimental data for the establishment of seed production system suit-

able for local conditions, it is suggested that, conjunction with the operation of mass production of seed, data necessary for the above purpose should be recorded for future analyses.

Brood Stock

The rearing of Grouper for brood stock has met with considerable difficulties due to seasonal changes in environmental conditions. In the near future, NICA should have facilities for this brood stock on the premises.

Food Organisms

In NICA staff concerned has planned to make as much use as possible of local resource organisms for the production of food for fish larvae, in addition to using some introduced organisms. In future, more research work should be done on food organism rearing.

Intensive Culture

The research subjects taken up in the first year concerning intensive fish culture should be continued in the future, in order to make experimental data more reliable and hence more useful by accumulation.

Fish Health Control

The study section concerning fish disease and health control should be reinforced both in terms of biologists and study level.

Field Development

The survey field should be extended to coastal waters adjacent to the lake. Survey of the inner part of the lake is also desirable, since the environmental condition of the outer part cannot be comprehensively understood without data on the inner part as well as coastal areas.

Surveys

Biological information which can be secured from surveys of fish distribution and spawning is of great importance as reference data for the rearing and propagation of fish and the procurement of natural seed.

In future, these researches will be more extensive than they are now.

(7) Effect of Tropical Monsoon on Seabass Culture.

Mainly, this report describes the problems of seabass culture technique. During the past three years, we have studied the seed production, food organisms and spawning ground environment of the seabass, because this fish easily adapts to tropical monsoon areas.

During monsoon season in the tropical region, the coastal area becomes a brackish-water area, while water in the lagoons and at the mouths of rivers changes to pure, fresh water. Even during the dry season, if there is a heavy rain squall in the mountains, the water at the mouths of rivers becomes fresh immediately. The winds and waves of monsoon season are very high; the highest wind over 30 knots. Therefore, the net cages and other fish culture equipment cannot be used in the coastal area except during dry season, and the fish culture area is limited only to lagoons and river mouths.

Because of these limitations, selection of fish which can bear low salinity levels is preferable to the selection of marine fish. The seed production of seabass has proceeded at the National Institute of Coastal Aquaculture for three years because this is the most suitable culture fish under these conditions. This is attested to by the fact that the growth rate of this fish is good in water of 20-22 ‰. salinity, and the fingerling (30 days from hatch-out, 2 cm length) is soon adapted to fresh water.

Recently, many farmers have cultured this fish in Songkhla Lake, as well as in estuary bay and other river mouths. Because the environment in each of these areas is very different, we should have more research data on environmental factors effecting each area. This will lead, in turn, to future development and expansion of the cultivation of seabass.

Fry Production of Seabass, *Lates calcarifer*, at National Institute of Coastal Aquaculture in 1983.

Niwes Ruangpanit, Sujin Mancewong and Tida Pechmanee

Introduction

Seed production of seabass, *Lates calcarifer*, is one of the main projects of the National Institute of Coastal Aquaculture (NICA). It has been carried out in order to sell seabass to both private hatcheries and fish farmers and to provide seabass for other governmental projects. The remaining seabass are released into the sea in order to preserve resources. Reported here are the procedures and results of seed production at NICA in 1983.

Materials and Methods

Broodstock of about 4 kg in body weight were taken from the netcage and raised in 2 concrete tanks of 10 m in diameter and 2 m in depth for the 2 weeks prior to spawning season. Forty nine fish were placed in one tank and 30 in the other. During spawning season, broodstock were fed 1% of their body weight with good quality trash fish daily.

Eggs and newly hatched larvae were collected from the spawning tanks on the morning following spawning and reared in 26 ton tanks for 12 - 15 days. The different densities present in the tanks depended on the number of tanks available after each spawning as well as the number of eggs spawned. Data was recorded from 1 or 2 selected tanks of each spawning group, a total of 9 sample tanks in all.

After 12 - 15 days, seabass fry begin to show a wide range in body size and are ready to begin cannibalism. Therefore, around that time larvae bigger than about 6 mm began to be transferred to 12 ton tanks, with an initial density of from 2,167 to 2,583 individuals per ton. This grading, using a bucket with pores through which larvae of a certain size could move, was continued every 3 - 5 days. During the entire nursing period, 5 buckets with gradually larger pore sizes were used. Nursing continued until the larvae size ranged from 1.5 to 3.0 cm. Data was recorded from 3 or 4 sample tanks of each spawning group; a total of 23 samples in all.

Larvae were given rotifer, *Brachionus plicatilis*, from the 2nd day to about the 10th to 15th day after hatching. Rotifer were cultured in 26 ton tanks and fed with *Chlorella* sp., *Tetraselmis* sp. or unidentified blue green algae grown in separate 26 ton tanks. Nauplii of brine shrimp, *Artemia salina*,

were fed to the larvae from about the 8th to 10th day until the 30th day. Water flea, *Moina* sp. or *Daphnia* sp. cultured in 12 and 50 ton tanks were collected with a net and given to the fish to supplement the brine shrimp feeding during the period from the 20th to the 25th day. From the 25th day after hatching, minced fish meat was given to the fish as food.

From 1.1 to 2.2 tons of water containing *Chlorella* sp. or *Tetraselmis* sp. was added to the fish rearing tanks daily to stabilize water quality and to enhance propagation of rotifer in the fish rearing tanks.

Thirty to fifty percent of the water in the fish rearing tanks was replaced daily with fresh sea water, and dirt deposited on the tank bottoms was siphoned out.

Water temperature in the tanks ranged from 28.5°C to 30.5°C.

Results

The first spawning of seabass took place at the end of March and the last in September. Spawning occured twice in May and there were 7 spawnings in all.

Initial number of eggs or newly hatched larvae stocked, number of fish harvested and survival rate in each 26 ton sample tanks are shown in Table 1.

Table 1. Survival rate of 15 day old larvae of seabass cultured in 26 ton tanks.

Tank	Initial stocking number	Harvested number	Survival rate %
1	332,482	150,000	45.1
2	831,600	472,000	56.8
3	1,755,000	770,000	43.9*
4	1,000,000	600,000	60.0
5	984,000	570,000	57.9
6	910,000	470,000	47.8
7	1,150,000	708,000	61.6
8	1,200,000	350,000	29.2
9	1,104,000	594,000	53.8
Average	939,010	489,250	52.1

* Data for sample tank 3 are excluded in the calculation of averages since its harvested number includes those larvae harvested before the 15th day after hatching.

The survival rate ranged from 29.2% to 61.6%. No clear tendency was observed in the relationship between stocking number and survival rate, though a high initial stocking number of 1.2 million resulted in the highest mortality rate (Fig. 1). On average, with 939,010 as the initial stocking number, 489,250 fifteen day old fry were produced with an average survival rate of 52.1%.

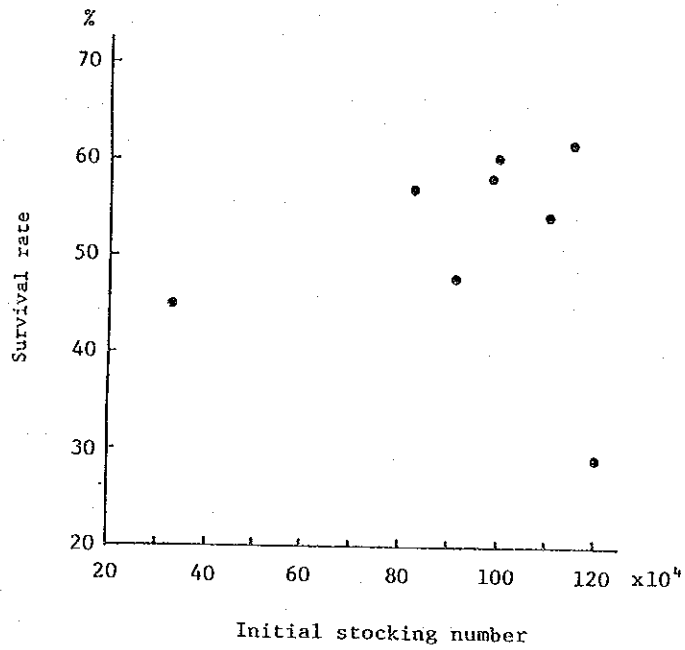

Fig. 1. Relationship between survival rate and initial stocking number of larval seabass.

The survival rate for the 23 samples which were reared from 15 day old to a total length of 1.5 cm to 2.5 cm was 68.2%.

Amount of rotifer and brine shrimp fed daily and total length of fish are shown in Table 2 for three sample tanks.

In tank 1, for fish fry of 332,480 in initial stocking number, a total of 4.54×10^9 rotifer were given as feed in 15 days of rearing. Brine shrimp were fed to the fish from the 9th day and the total amount was 3.45×10^8. After the 15 days of rearing, fish fry attained an averaged total length of 8.6 mm.

In tank 2, a total of 5.86×10^9 rotifers were fed to fish of 831,600 in initial stocking number. Brine shrimp feeding was started on the 9th day and the total amount was 2.2×10^8. Fish fry attained an average total length of 6.5 mm in the same period.

Table 2. Daily record of rotifer and brine shrimp feeding and growth of seabass larvae in 26 ton tanks.

	Tank 1			Tank 2			Tank 3		
Initial stocking	332,480			831,600			1,755,000		
Age of larvae (days)	Amount of rotifer (x 10)	Amount of brine shrimp (x 10)	Total length of larvae (mm)	Amount of rotifer (x 10)	Amount of brine shrimp (x 10)	Total length of larvae (mm)	Amount of rotifer (x 10)	Amount of brine shrimp (x 10)	Total length of larvae (mm)
2	130			150			300		
3	100			350			300		
4	150			200			400		
5	150			300			500		
6	150		3.28	400		3.19	450		2.91
7	200			500			600		
8	400			500			600	20	
9	300	20		600	10		600	10	
10	400	10		600	20		600	20	
11	400	25	4.34	600	35	3.87	700	20	3.49
12	300	35		500	60		650	20	
13	300	60		600	95		620		
14	600	95		560			380	200	
15	560	100					300	200	
Total	4,540	345	8.61*	5,860	220	6.51*	7,000	490	5.76*

* Total length of larvae when harvested on the 16th day.

In tank 3, a total of 7×10^9 rotifers were fed to fish of 1,755,000 in initial stocking number. Brine shrimp feeding was started on the 8th day and the total amount was 4.9×10^8. Fish attained an average total length of 5.7 mm. From the 11th day to 14th day 20,000 to 200,000 larvae were harvested daily as the tank became overcrowded.

Comparing these results, it can be said that fish fry grew faster at a lower stocking density, as indicated by the fact that tank 1 showed the fastest growth of fish. The numbers of rotifer used to produce one, 15 day old fry were 30,000 and 12,000 for tanks 1 and 2 respectively, while those of brine shrimp were 2,300 and 466. In tank 2, the amounts of rotifer and brine shrimp per produced fry were much smaller than in tank 1. Further study is required, however, to decide the most suitable stocking density and best feeding method to bring about the optimum growth rate.

Table 3 shows the number of produced seabass fry according to age and size. A total of 12.7 million fry was produced in 1983, with 8 - 15 day old fry of 3 - 8 mm in total length as the largest group, followed by 2 - 7 day old fry of 2 - 3 mm.

Table 3. Production of seabass fry by age and by size from NICA hatchery in 1983.

Age (days)	Size of larvae (total length in mm)	Number	Ratio (%)
2 - 7	2 - 3	5,560,000	43.7
8 - 15	3 - 8	6,036,200	47.5
25 - 35	10 - 15	1,013,000	8.0
36 - 45	15 - 25	101,367	0.8
	Total production	12,710,567	

Of the amount fry produced, 52.8% were sold to private hatcheries and 16.1% were distributed to government hatcheries, mainly at younger stages for further rearing. Twenty-nine percent of the fry were stocked in the sea. The remaining 2% of the fry of larger than 1.5 cm in total length were distributed to fishermen to promote the government's Poor village Fisheries Development Project (Table 4).

Table 4. Ratio of seabass fry produced for selling, distribution, restocking, and Poor Village Fisheries Development Project (PVFDP).

Purpose	Number of fish	Ratio (%)	Size of fish (total length in mm)
Selling	6,714,367	52.8	2-5 mm and 10-25 mm
Distribution to government hatcheries	2,046,700	16.1	3-6 mm
Restocking	3,694,500	29.1	8 mm
PVFDP	255,000	2.0	15-25 mm
Total	12,710,567	100.0	

Studies on the Seed Production of the Seabass, *Lates calcarifer*. I. Present Status of Production and Some Results of Rearing in 1982 at National Institute of Coastal Aquaculture, Thailand.

Sujin Maneewong, Tanan Tattanon and Tatsuo Watanabe

Introduction

On October 16, 1981, the Thailand and Japan Joint Committee for the Coastal Aquaculture Development Project in Thailand was held at the National Institute of Coastal Aquaculture (NICA), Songkhla, and a five-year research program was agreed upon. A study for the establishment of a technical manual for seed production of the seabass, *Lates calcarifer* (Bloch), aiming at a constant supply of seed, was listed as one of the research subjects to be undertaken in the program. Inasmuch as seed production of this species had already been conducted on a considerably large scale at NICA and, on other scales, at other governmental fisheries stations as well as at several private hatcheries, I expected that a standardized method of production had already been established. During my participation in the seed production of the fish and the culture of food organisms at NICA in April - July 1982, however, I faced several problems and difficulties, and came to realize that not a few technical problems remained to be worked out. In this paper, I will report on these problems, describe the results of the production of seed and food organisms, and discuss the ways to solve the problems.

Production of *L. calcarifer* seed

1) Rearing procedures

Since the methods of seed production of *L. calcarifer* being employed at NICA have already been reported by Manewongsa et al. (1981), only a brief description is made of spawning and rearing procedures observed in 1982.

On March 11, 1982, seven-year-old spawners, which had been stocked at the Boa-Keng substation located on the outer part of Songkhla Lake and which measured 7.2 kg in mean body weight, were transferred into three spawning tanks at NICA. The tanks were round, concrete ponds of 150 ton capacity. The number of spawners introduced into each tank was 24, and their sex ratio was estimated at about 1 : 1.

Spawning took place from evening to midnight for three to five consecutive days, starting from three to five days after full moon. Eggs were collected on the following morning with fine-meshed nets and placed in rearing tanks, passing through 1 mm mesh screen in order to remove floating algae and other foreign substances.

The process of larval rearing at NICA can be divided into two steps. The first step, which is called "primary rearing" in this paper, extends from hatching to a larval size of 4 - 6 mm in total length (TL), 10 - 15 days after hatching. Larvae produced in this step are distributed to private hatcheries and middlemen for export. The second step, termed "secondary rearing", covers the following period, up to 10 - 25 mm sizes. Juvenile of these sizes are distributed to local fish farmers for the production of marketable fish.

For primary rearing in 1982, larvae were placed in three to six 30-ton concrete tanks with filtered sea water which was partly replaced with new water every morning, at an exchange rate of 10 - 50% of total volume of tank water. Water temperature ranged from 27°C to 29°C, and salinity from 30‰ to 32‰. Feeding with the rotifer, *Brachionus plicatilis*, was commenced on the second day after hatching, corresponding with the completion of mouth opening which was generally observed in the afternoon of the second day. Green water with fully propagated phytoplankton (*Chlorella* sp., *Tetraselmis* sp., and a species of bluegreen algae) was added to the tanks during the period of rotifer feeding. Larvae of about 4 mm TL and larger were fed *Artemia* nauplii together with rotifer.

For the secondary rearing, 15-ton concrete tanks were used. Rearing water was changed every other day at a rate of about 80% of the total volume of rearing water. Larvae and juveniles were fed *Artemia* nauplii, *Moina* sp., and minced fish meat.

2) Results of rearing

From April to July, spawning took place once a month, and seed production was conducted in four series. Since my work in these series was mainly on the production of food organisms and, hence, restricted mostly to the primary rearing, the following observations are limited mostly to the results of the four series of primary rearing.

In the first-series primary rearing (April 14 - May 4), eggs were stocked in five, 30-ton tanks. The number of larvae on the first day after

hatching was estimated at about 0.8 million each in two tanks, and about 2 million each in the other three tanks (Table 1). The growth of the larvae was smooth in the former two tanks with the low stocking density; whereas, in the latter three tanks, the growth of the larvae was retarded particularly between 3 mm and 4 mm TL (Fig. 1). Although an attempt was made to recover their retarded growth by transferring part of the larvae from two of the latter three tanks into two other tanks on the 7th and 8th days respectively, their growth still remained at a low rate. In one of these tanks, in particular, the larvae were much smaller than in other tanks, with a mean TL of 3.15 mm on the 13th day. Rearing in this tank was therefore cancelled.

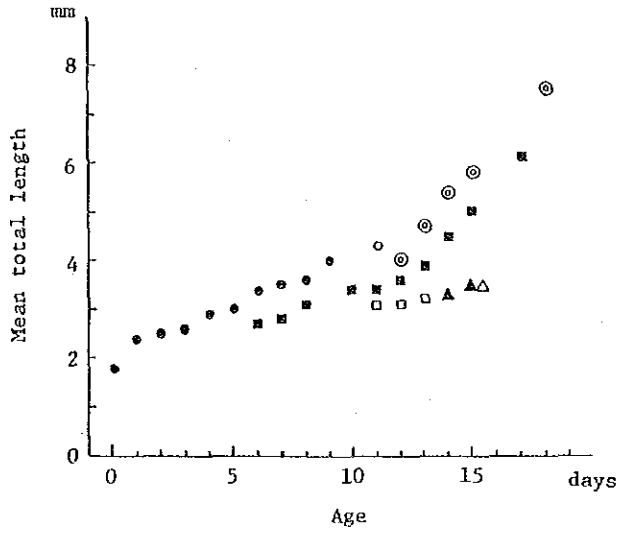

Fig. 1. Growth of seabass larvae in the first-series primary rearing.

○ : Tank No.1; ● : No.2; ▲ : No.3;
△ : No. 3-D; ■ : No.4; □ : No.4-D; ◎ : No.5.

In the second-series primary rearing (May 1 - June 1), we attempted to stock larvae in a graded series of stocking densities in six tanks, i.e. 10 larvae/ℓ water, 20/ℓ, 30/ℓ, 40/ℓ (two tanks), and 50/ℓ. The initial densities of larvae were estimated by the number of eggs calculated in the count of eggs in 100 mℓ of water out of 1 ton of water containing eggs. However, we failed to make on accurate count of eggs and, hence, estimation was made again of the number of larvae on the first day after hatching, based on the counts of larvae in 1 ℓ of rearing water from 9 different points around the rearing tank (Table 2). Decrease in growth rate was noted, again in a size range of 3 mm to 4 mm TL, in this case at all density levels (Fig. 2), most

Table 1. Number of larvae at 1 day after hatching and number, age and mean total length of larvae at harvest in the first-series primary rearing (April 14 - May 4).

Tank	1	2	3	4	5
Number of larvae at day 1 ($\times 10^4$)	79	77	190	200	200
Harvest					
Number of larvae (10^4)	33	30	60		38 + *
Age (days)	11	9	14 - 19		13
Mean total length (mm)	4.34 ± 0.43	3.98 ± 0.31	4 - 5		4.71 ± 0.40

* Number of larvae distributed and stocked for secondary rearing.

Table 2. Number of larvae after hatching and number, age and mean total length of larvae at harvest in the second-series primary rearing (May 15 - June 1).

Tank	1	2	3	4	5	6
No. of larvae (x 10^4)						
Day 1	84	41 29	52	112	102	122
Day 2	36	28	57	83	83	101
Day 3	-	25	54 (42)*	99 (73)	-	99 (93)
Harvest						
No. of larvae (x 10^4)		Total number**	65 +			
Age (days)	14	13	15	14	14	16
Mean total length (mm)	5.56 ± 0.44	4.82 ± 0.52	5.37 ± 0.41	4.57 ± 0.39	5.24 ± 0.44	5.28 ± 0.71

* Number of larvae were estimated at night again, since larvae became not to distribute uniformly at day time.

** Total number showing number of larvae distributed to private hatcheries and middle men + number of larvae used as seen for secondary rearing and others.

probably indicating insufficiency in the amount of rotifer given to the larvae. *Artemia* was then fed to the larvae to supplement rotifer from the 8th - 11th days after hatching.

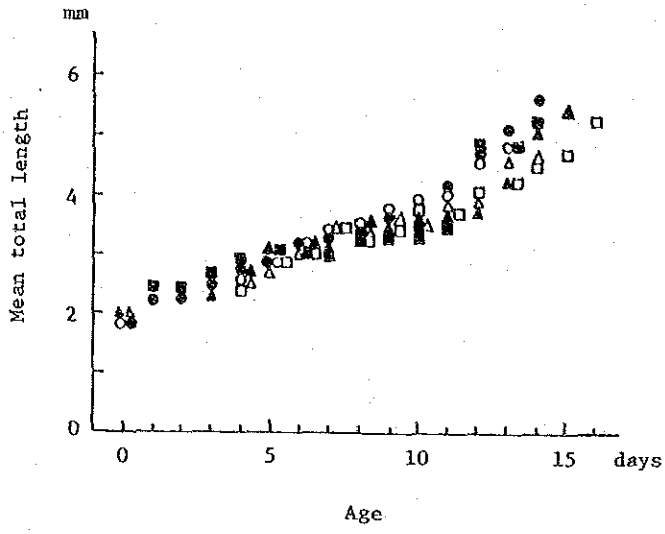

Fig. 2. Growth of seabass larvae in the second-series primary rearing.

● : Tank No.1; ○ : No.2; ▲ : No.3;
△ : No.4; ■ : No.5; □ : No.6.

Despite the fact that the larvae at that time were 3.3 - 3.7 mm in mean TL and only larger individuals seemed capable of preying on *Artemia*, the TL of larvae in these tanks showed a marked increase after they were fed with *Artemia*. This suggests the occurrence of a high mortality rate among smaller larvae, though actual survival rates could not be estimated because records of harvest were lost. If my memory is correct, there was no significant difference in survival rates among stocking densities.

In the third-series primary rearing (June 11 - 30), larvae were reared at an initial stocking density ranging from 10/ℓ to 85/ℓ (Table 3). In view of the results of the second-series, we attempted to provide the larvae with an adequate and sufficient amount of food, counting the number of rotifer and *Artemia* remaining in the rearing water before feeding as well as the number in the food supply (Table 4). Counting was made using a profile projector because counting with the naked eye was difficult due to the amount of suspended matter which increased day by day. In this series, the larvae showed smooth growth between 3 mm and 5 mm TL, but a

Table 3. Number of larvae after hatching and number, age and mean total length at harvest in the third - series primary rearing (June 11 - 30).

Tank	1	2	3	4	5
Number or larvae (x 10^4)					
Day 1	25	194	220	63	45
Day 2	19	98	91	37	35
Harvest					
No. of larvae (x 10^4)	5	52	37	26	21
Age (day)	19	18	19	18	19
Mean total length (mm)	7.79 ± 1.42	5.72 ± 0.91	5.67 ± 0.58	6.06 ± 0.82	6.40 ± 0.72

Table 4. Counts of rotifer (x 10^8) and/or *Artemia* (x 10^6) fed to seabass larvae and in rearing water before feeding (in parentheses) in the third-series primary rearing. Day 2-day 11: counts of rotifer; day 12 - day 18: counts of rotifer (upper) and of *Artemia* (lower).

Days after hatching \ Tank	1	2	3	4	5
2	2.5	2.5	2.5	2.5	2.5
3	1.2	2.4	2.4	2.0	2.0
4	0.6(3.4)	1.3(2.3)	1.3(1.8)	1.3(2.3)	1.3(2.9)
5	1.0(2.3)	2.0(1.6)	2.0(1.3)	2.0(1.3)	2.0(2.1)
6	- (4.9)	1.4(2.3)	1.4(2.1)	0.7(3.6)	0.7(4.2)
7	1.4(3.6)	1.4(3.6)	2.7(0.8)	2.7(1.3)	1.4(4.7)
8	1.3(4.7)	2.5(3.9)	2.5(2.9)	2.5(2.9)	2.5(3.4)
9	1.1(4.4)	5.4(0.5)	2.7(3.4)	2.2(5.2)	2.2(4.4)
10	- (7.0)	2.9(2.6)	2.9(1.3)	1.9(1.6)	1.9(1.8)
11	- (3.9)	2.9(1.0)	2.9(0.5)	1.5(2.6)	1.5(2.1)
12	- (0.8)	1.6(0.8)	3.2(0.3)	0.8(0.3)	1.6(0.8)
	4.6	27.5	17.0		
13	- (-)	- (-)	- (0.3)	- (-)	1.9(0.3)
	5.0(-)	20.0(-)	20.0(-)	15.0(-)	
14			0.6(-)		2.0(1.8)
	9.6(-)	38.4(-)	28.8(-)	19.2(-)	
15					1.7(1.3)
	14.0(-)	52.0(-)	52.0(-)	26.0(-)	
16					2.4(0.3)
	14.0(-)	52.0(-)	52.0(-)	26.0(-)	
17					3.8(0.3)
	14.0(-)	52.0(-)	52.0(-)	26.0(-)	
18					1.7(0.3)
	14.0(-)		52.0(-)		26.0

decrease in growth rate was noticed at around 5 mm TL (about the 14th day) onward (Fig. 3). This growth retardation was considered to have been caused by an insufficient supply of *Artemia*, which was given to the larvae from the 12th day after hatching, due to the fact that the *Artemia* given daily at 10:00 - 11:00 hours was consumed completely by 15:00 - 16:00 hours. The results of rearing for 18 - 19 days are given in Table 3.

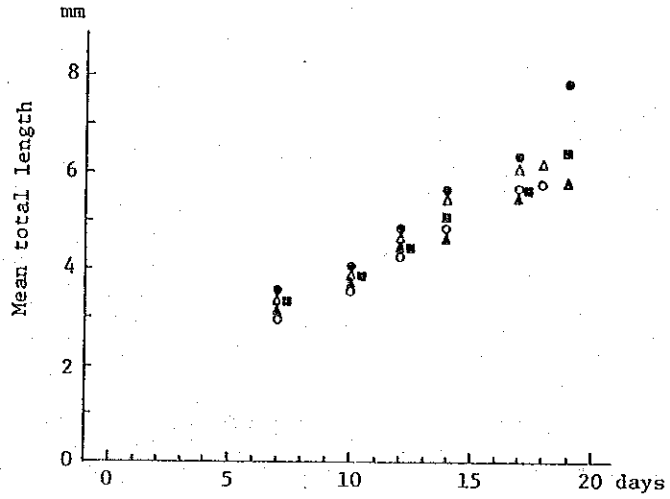

Fig. 3. Growth of seabass larvae in the third series primary rearing.
●: Tank No.1; ○: No.2; ▲: No. 3;
△: No.4; ■: No.5.

In the fourth-series primary rearing (July 11 - 29), only three rearing tanks were used because the production target in this series was only 300,000 larvae and a sufficient and constant supply of food organisms seemed possible. Table 5 shows stocking conditions and harvest results in these three tanks, and Table 6 indicates rotifer counts in rearing water checked every morning with a profile projector. Larvae in tank No.1 were fed with both rotifer and *Artemia* from the 10th day after hatching, while only rotifer was fed to the larvae in the other two tanks up to the 15th day. In all tanks larvae grew normally, attaining a mean TL of 4 mm by the 11th day. In tank No.1, a change in the color of the digestive tract of larvae into orange on the 13th day indicated that they came to prefer *Artemia* to rotifer from that day (mean TL: 4.79 ± 0.26 mm). On the other hand, the growth rate of larvae in the remaining tanks began to decline at 4.5 - 5 mm TL. *Artemia* was therefore supplied to these tanks from the 16th day.

Table 5. Number of larvae after hatching and number, age and mean total length at harvest in the fourth-series primary rearing (July 11 - 29).

Tank	1	2	3
Number of larvae (x 10^4)			
Day 1	127	181	85
Day 2	103	77	42
Harvest			
No. of larvae (x 10^4)	41	33	11
Age (days)	16	18	17
Mean TL (mm)	6.10 ± 0.71	6.07 ± 0.88	5.88 ± 0.98

The early growth of *L. calcarifer* was traced also during the secondary rearing in the fourth-series till the 30th day after hatching (Fig. 4). About 330,000 larvae were transferred from tank No.1 to six, 15-ton tanks, stocking 50,000 - 60,000 larvae in each tank. Size grading was conducted twice or thrice a week in order to prevent cannibalism. Specimens were sampled out from a size-group containing the largest number of individuals, except for the 30th day material which were from all size-groups, i.e. large, medium, small, and smallest groups. Though exact composition in number of individuals of the four groups was not known, it was estimated approximately at 1:3:5:1, showing the so-called "shoot phenomenon" (great variation of growth).

Production of food organisms

1) Production of phytoplankton (green water) for rotifer feeding

Cultures of phytoplankton, i.e. *Chlorella* sp., *Tetraselmis* sp., and a species of one-to-four-cell bluegreen algae, were being cultivated at NICA with which to feed rotifer. Water with propagated phytoplankton is called "green water" due to its color.

Mass production of green water was carried out using 15 tanks of 30 ton capacity and of the same structure as the larvae rearing tanks. Production was started with the culture of "starter" on a one-liter scale.

Table 6. Count of rotifer (x 10^8) and/or *Artemia* (x 10^7) fed to seabass larvae and in rearing water before feeding (in parentheses) in the fourth-series primary rearing. Day 1 - day 9: counts of rotifer; day 10 - day 16: counts of rotifer (upper) and of *Artemia* (lower).

Age (days) \ Tank	1	2	3
1	1.9	1.9	1.9
2	2.0(0.3)	2.0(1.8)	2.0(1.0)
3	2.8(1.6)	2.8(2.3)	2.8(1.8)
4	2.3(1.8)	2.3(2.3)	2.3(2.9)
5	1.9(3.1)	1.9(3.1)	1.9(2.1)
6	3.0(2.1)	3.0(2.3)	2.0(3.9)
7	5.0(0.8)	4.0(2.1)	3.0(1.6)
8	4.4(1.6)	3.3(2.6)	3.3(2.6)
9	3.2(5.2	3.2(2.3)	3.2(1.8)
10	3.0(3.4)	4.0(1.8)	2.0(3.9)
	4.0		
11	4.0(0.5)	5.0(1.8)	3.0(1.8)
	5.0(-)		
12	4.0(1.8)	5.0(1.8)	2.0(3.1)
	5.0(-)		
13	4.0(0.8)	5.0(1.8)	3.0(1.6)
	6.7((-)		
14	4.0(0.8)	6.0(0.5)	2.0(1.3)
	8.0(-)		
15	- (0.3)	6.0(0.3)	2.6(-)
	10.0(-)		
16		6.0(0.3)	2.5(-)
		4.0	2.7

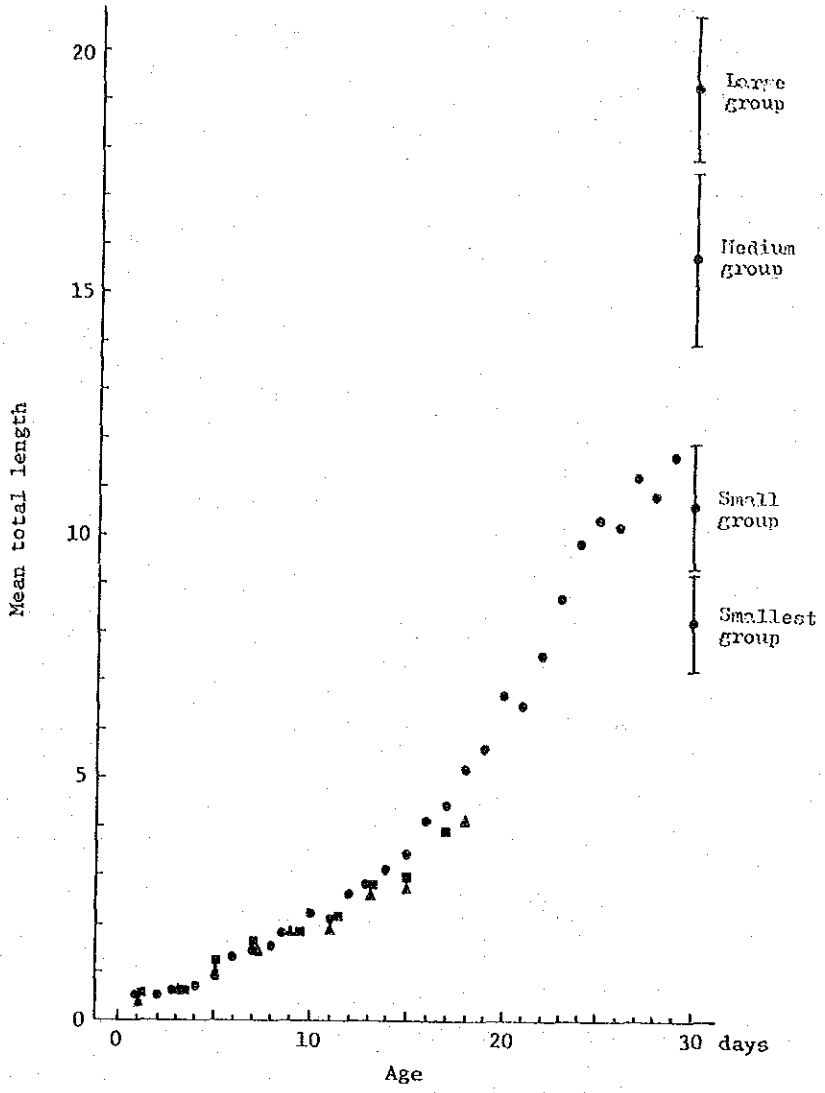

Fig. 4. Growth of larvae and juvenile of seabass in the fourth-series primary and secondary rearing.
●: Tank No.1; ▲: No.2; ■: No.3.

The scale of culture was then gradually increased up to a 24-ton scale. In this final step of mass culture, 5 to 6 tons of starter green water was inoculated into 18 - 19 tons of filtered sea water at salinity levels of 30 - 32‰, with fertilizers added as shown in Table 7. The measurement of cell density by means of a blood cell counter as conducted in April turned out to require a long time. This method was therefore abandoned and transparency of water was employed for the estimation of density, measuring transparency by using a white disc, 15 cm in diameter, and estimating cell density based on the relationship between transparency and cell density obtained from experiments made in May (Fig. 5).

Table 7. The amount of fertilizer for the green water production

Fertilizer \ Period	April	May - July
Ammoniumsulfide	17 g/ton	63 g/ton
	400 g/tank	1500 g/tank
Supercalciumphosphate	0.8 g/ton	
	20 g/tank	
Urea	-	6 g/ton
		150 g/tank
16-20-0	-	6 g/ton
		150 g/tank

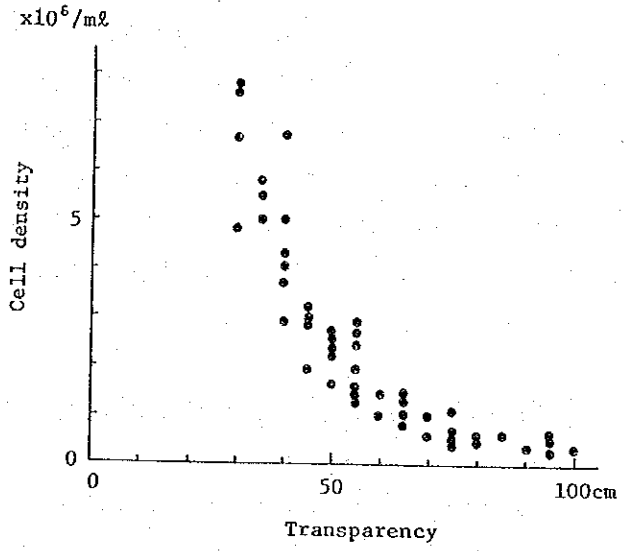

Fig. 5. Relationship between transparency and cell density in bluegreen algae culturing water.

The quantity of fertilizers used for the mass production of phytoplankton in April was the same as thus far commonly applied at NICA, which was far smaller than in common practice in Japan. Cell density reached its peak within 3 - 4 days, with density of 1 - 2 million cells/mℓ for *Chlorella* sp. and 60,000 - 120,000/mℓ for *Tetraselmis* sp. The culture of *Tetraselmis* was quite successful compared with *Chlorella*, which was usually

contaminated with a bluegreen algae, so that mainly *Tetraselmis* was fed to the rotifer. However, *Tetraselmis* cultures became contaminated with other phytoplankton (probably Xanthophyceae) from the end of April, and the production of green water became unstable.

From May, an N:P:K = 16:20:0 mixture of fertilizers for agricultural use was employed as a phosphate source instead of supercalciumphosphate. This mixture had been applied in SEAFDEC in the Philippines (Nukiyama, 1980) and was easily available in Songkhla. The dosage of the fertilizer was decided at two-thirds that used in Japan, considering that, while 7 to 10 days are required to attain a peak cell density in Japan, green water culture of NICA could be done in a 4-day interval, consuming green water in three tanks for feeding rotifer and leaving half a tank for the starter of new stock. Since the propagation of *Chlorella* and *Tetraselmis* was not very successful, green water was produced mainly with a species of bluegreen algae. The cell density of the bluegreen algae at peak was 5 - 8 million cells/mℓ.

2) Production of rotifer for seabass larvae feeding

Mass production of rotifer was carried out in seven to eight, 30-ton tanks similar in structure to those used for phytoplankton and seabass larvae production. Because the nets used for collecting rotifer in April and May were about 100 μ in mesh, young rotifer were lost through the mesh, making it impossible to concentrate rotifer for feeding with green water or to prepare starter. This problem was solved by adopting the following production methods:

1. introduce rearing water of rotifer and green water in production ponds,
2. add green water daily to maximum water level,
3. harvest rotifer from 1/3 to 2/3 total water volume,
4. add green water after harvesting rotifer.

From July 12 nets of 63 μ in mesh size became available for the collection of rotifer.

The density of rotifer was estimated every morning from counts of three to five, 1 mℓ samples using a profile projector. Number of rotifer harvested daily for the seed production of seabass from April to July is shown in Fig. 6.

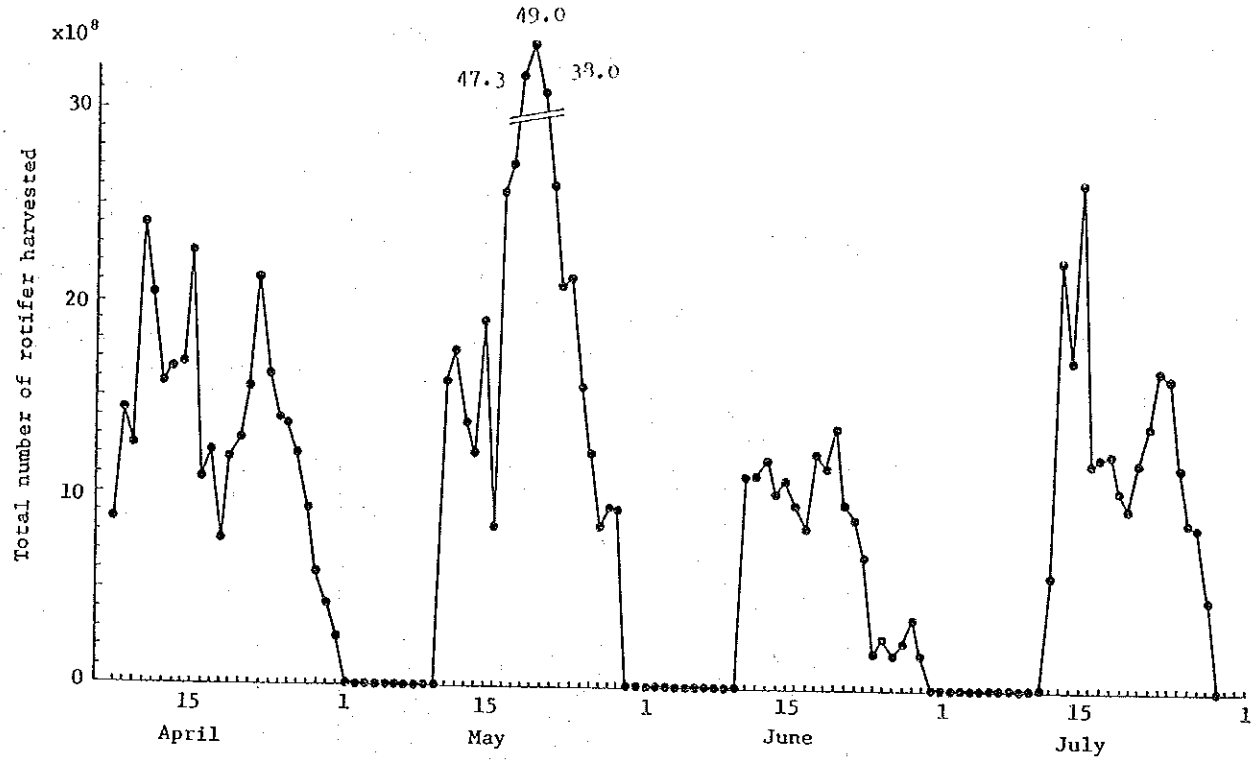

Fig. 6. Total number of rotifer at daily harvest during a period of seabass seed production.

The main item of food for the rotifer culture in April was *Tetraselmis*. Dry baker's yeast was supplemented from April 17 to compensate for lack of green water. Details of an example of the rotifer production in April are given in Fig. 7. In the beginning of May, green water in the mass culture tanks became nearly extinct, and a new cycle of green water production from starter became necessary. During this period, May 1 - 9, rotifer were sustained by only dry baker's yeast.

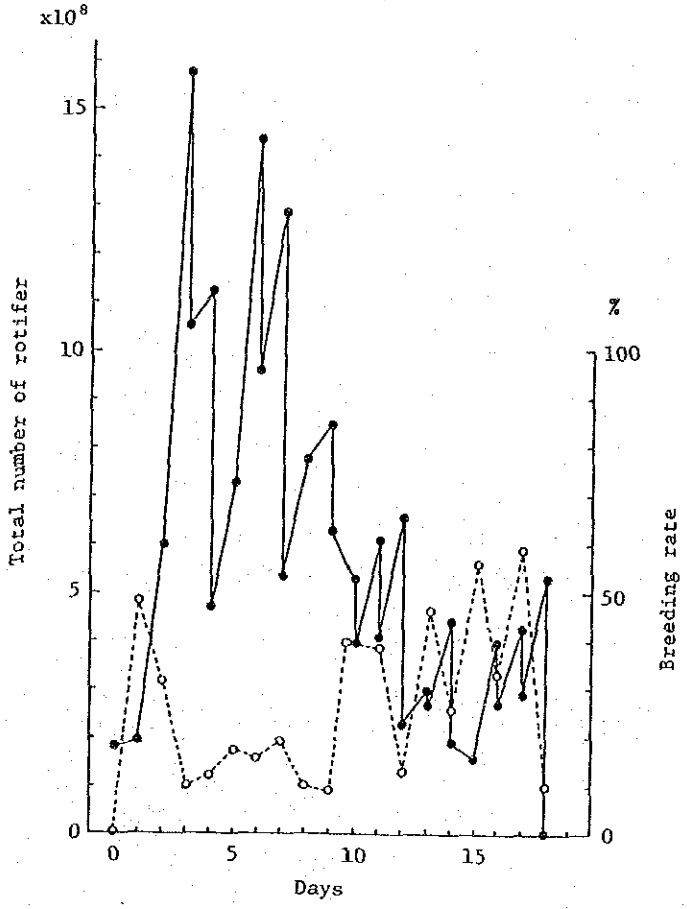

Fig. 7. An example of rotifer production using green water containing mainly *Tetraselmis* sp. during April 8 - 26.

●: total number of rotifer;
o: breeding rate.

Mainly bluegreen algae was fed to the rotifer in May - July, with supplementary feeding of baker's yeast in May. Details of an example of rotifer production in this period with dry baker's yeast and green water are shown in Fig. 8. While the density of rotifer was about 10 - 30 individuals/mℓ in the culture with green water only, it ranged from about 80 - 130/mℓ when baker's yeast was added.

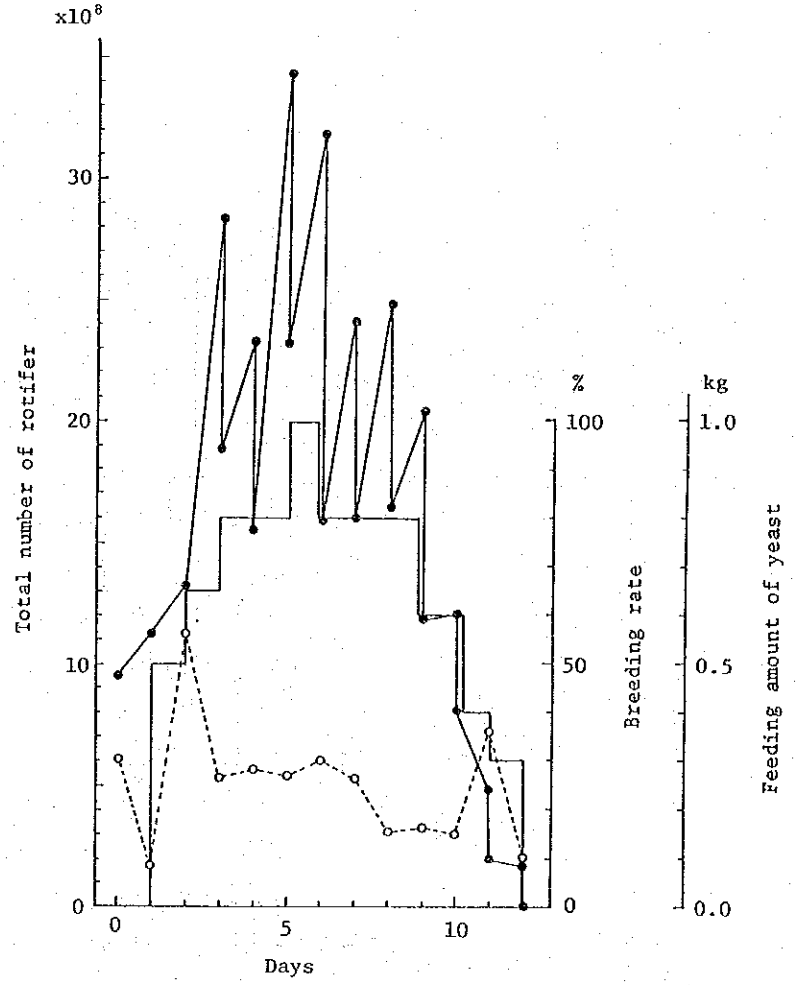

Fig. 8. An example of rotifer production using dry baker's yeast and bluegreen algae during May 14 - 26.

●: total number of rotifer; ○: breeding rate;
–: feeding amount of dry baker's yeast.

Encouraged by the above results, further trials of rotifer culture using baker's yeast were made in June in preserving rotifer for starter for the following cycle, anticipating that this method would be practicable in the mass production of rotifer. However, this plan had to be abandoned, since the results were not as promising as in the preceding month, with the density of rotifer not reaching 100 individuals/mℓ. Thus we came back to the green-water method, with which the constant level of density was 10 - 40/mℓ in June and 20 - 50/mℓ in July (Fig. 9).

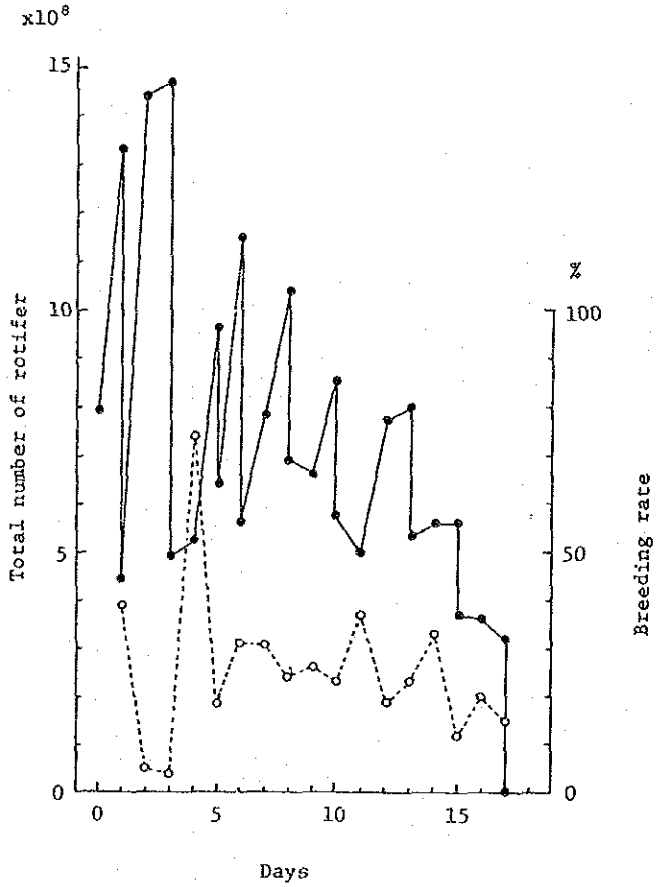

Fig. 9. An example of rotifer production using bluegreen algae during July 10 - 27.

●: total number of rotifer;
○: breeding rate.

Discussion

1) Seed production of *L. calcarifer*

In the rearing of early-stage larvae (primary rearing) of the seabass in 1982, retardation of growth at 3 - 4 mm TL occurred in the first and second-series of seed production. This problem of retarded growth was solved in the following series by increasing the quantity of rotifer given to the larvae. Similarly, the retardation of growth in larvae of 5 mm TL and larger seen in the third series was successfully improved in the fourth series by increasing the amount of *Artemia* nauplii fed to the larvae. These results indicate that the quantity of food for larvae thus far used in the seed production of *L. calcarifer* at NICA may often have been insufficient to expect a maximum efficiency in seed production. The "shoot phenomenon" observed in the secondary rearing in the fourth series can also be attributable to a failure in feeding management. It should be stressed that rational feeding management is required to achieve a constant production of seabass seed at NICA.

It has been stressed that success in the mass production of fish seed depends largely on the production of food organisms (e.g. Japan Fisheries Resource Conservation Association, 1977; Yamaguchi, 1978; Fukusho, 1981). This can be readily understood when we consider the general concept of "pyramid of number" in the trophic relationship within an ecosystem. In order to achieve a constant seed production, it is essential to know the capacity of food supply and the amount of food required by larvae. Table 8 gives an example of the ratio of water quantity required for the production of green water, rotifer and larvae in the seed production of the red sea seabream, *Pagrus major*, in Japan, reported by Japan Fisheries Resource Concervation Association (1977). Kumamoto Fisheries Experimental Station in Japan uses only *Chlorella* for rotifer culture, and the above-mentioned ratio of water quantity at the station is 1 : 1.8 : 6.3. This exemplifies how large a water quantity is necessary to produce rotifer constantly when only green water is used.

In the seed production of *L. calcarifer* conducted at NICA in 1982, the green water : rotifer : fish larvae ratio was changed in each series of rearing (Table 9), and the ratio in the fourth series, which was managed successfuly compared with former series, was 1 : 2.4 : 4.6. In this case the proportion of water quantity for rotifer production is greater, and that for green water production is smaller, than in the above-noted case

Table 8. Present status of water volume ratio for seed production of the red seabream and food organisms in the several prefectual fisheries experimental stations in Japan (1976).
(from Japan Fisheries Resource Conservation Association: 1977).

Station	Larval rearing	Rotifer	*Chlorella*	Ratio	Kinds of Rotifer*
Yamaguchi	300 ton (50 x 6)	158 ton	460.8 ton	1 : 0.5 : 1.5	E.R.
Nagasaki	300 (100 x 3)	160	340	1 : 0.5 : 1.1	M.R.
Kumamoto	100 (100 x 1)	180	625	1 : 1.8 : 6.3	G.R.
Kagoshima	240 (60 x 4)	120	330	1 : 0.5 : 1.4	E.R.
Oita	80 (40, other)	140	154	1 : 1.8 : 1.9	E.R.
Hiroshima	150 (25 x 6)	360	320	1 : 2.4 : 2.1	M.R.

E.R. : Rotifer is fed with baker's yeast, then it is enriched by *Chlorella* before feeding to larvae.

M.R. : It is fed with baker's yeast and *Chlorella* together.

G.R. : It is fed with *Chlorella* only.

Table 9. Water volume ratio for the seabass seed production and food organisms during April to July in 1982.

Period	Larval Rearing	Rotifer	Green Water	Ratio	Kind of G.W.
April	130 ton (26 x 5)	182	360	1 : 1.4 : 2.8	*Tetraselmis*
May	156 (26 x 6)	208	360	1 : 1.3 : 2.3	Blugreen + B.Y.*
June	130 (26 x 5)	208	360	1 : 1.6 : 2.8	Bluegreen algae
July	78 (26 x 5)	208	360	1 : 2.7 : 4.6	Bluegreen algae

* B.Y. : dry baker's yeast

at Kumamoto Station. Such differences can be interpreted as resulting from the lower productivity of green water at NICA, which in turn resulted in a low productivity of rotifer; the peak cell density of green water is 10 - 20 million/mℓ and attained in 7 - 10 days in Japan, while it is 5 - 8 million/mℓ and reached in 3 - 4 days at NICA.

Although the exact level of food requirement of *L. calcarifer* larvae remains unknown, the results of the fourth-series of rearing suggest that 300 - 400 million of rotifer are required for one, 26 ton rearing tank containing 400,000 - 500,000 larvae when *Artemia* is supplemented from a 4.5 mm TL size.

2) Production of food organisms

There are many problems to be solved to achieve a constant supply of phytoplankton (green water) to maintain the rotifer production at a constant level. Among others, the culture of *Chlorella* sp. was not very successful, usually contaminated with bluegreen algae. Although the bluegreen algae can also serve as rotifer food, their nutritive value is unknown and their productivity was lower than *Tetraselmis* in terms of quantity of fertilizers used. While the production level of rotifer was similar to these two items of food (Fig. 6, 7, and 9), the amount of fertilizers used for the bluegreen algae was 3.75 times as much as that used for *Tetraselmis*. From this standpoints *Tetraselmis* will make a useful food item for rotifer. Further studies are required to determine suitable conditions for its culture.

Supplementary use of dry baker's yeast resulted in a higher production of rotifer in a few cases. However, the results were not always successful and, moreover, its high cost (75 baht/450 g) makes it difficult to use for the mass production of rotifer. The possibility of utilizing some other materials, such as microbial flock or marine yeast produced as a by-product from sugar or alcohol industry, should be studied from both technical and economic viewpoints. Nevertheless, this by no means implies that rotifer can be cultured solely with yeasts. It has been reported that yeast-fed rotifer lacks in some highly unsaturated fatty acids and is of lower nutritive value compared with *Chlorella*-fed rotifer (Watanabe, 1979), and *Pagrus major* larvae fed with yeast-fed rotifer shows a high mortality rate (Japan Fisheries Resource Conservation Association, 1977; Yamaguchi, 1978; Fukusho, 1981). All these point to the importance, and necessity of NICA, of the establishment of techniques that enable a constant, sufficient and efficient production of rotifer.

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Notes on Development of Larval and Juvenile Stages of Seabass, *Lates calcarifer*

Pairat Kosutarak and Tatsuo Watanabe

Introduction

We observed about 350 individuals in larval and juvenile stages to discover the process of development of seabass, *Lates calcarifer*. The work has not been completed for the juvenile to young stage, so we have prepared only the notes on the development of the larval to juvenile stage.

Materials and Methods

The samples were collected from the seed production tank and fixed with a 5% formalin solution every day from July 11 to August 10, 1982. The samples were cleared by enzyme (trypsin) and stained by alizarin red S and alcian blue in November, then ten individuals from the samples of each day were observed.

Results

1) Observation of fish:

- Day 0 (TL: 1.60 ±0.04 mm) (Fig. 1-a)

It took 12-14 hours for the eggs to hatch out in 30 - 32°C water. A light brownish chromatophore was observed behind the eyes and in the body. The oil globule was sited in the front part of the yolk sac, and it had a light brownish chromatophore. The number of myomere was 6-8 + 14-16. The newly hatched larvae were floating on the water surface.

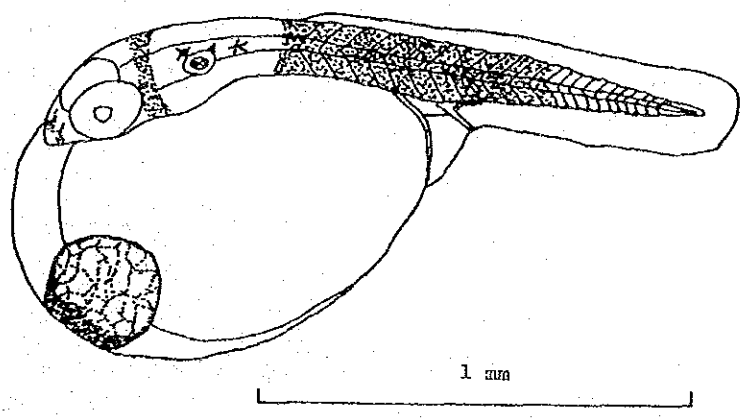

Fig. 1-a. Newly hatched larva of seabass, *Lates calcarifer*.

- Day 1 (TL: 2.20 ±0.08 mm) (Fig. 1-b)

The much part of yolk was absorbed, but the mouth was not opened yet. The larvae distributed uniformly in the rearing tank.

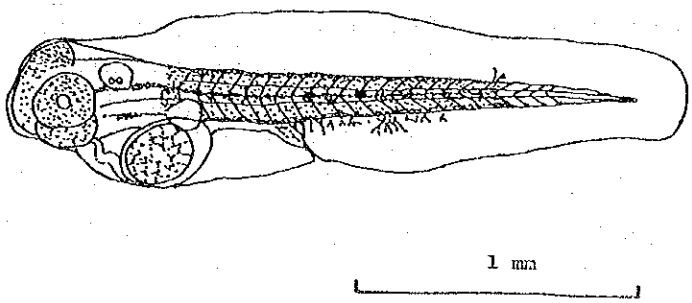

Fig. 1-b. Larva of seabass, *Lates calcarifer*, on day 1.

- Day 2 (TL: 2.52 ±0.06 mm) (Fig. 1-c)

The yolk was almot absorbed, and the mouth was opened. The average length of upper jaw was 0.201 mm (range 0.186 - 0.227 mm). The larvae gathered near aeration or in the direction of light.

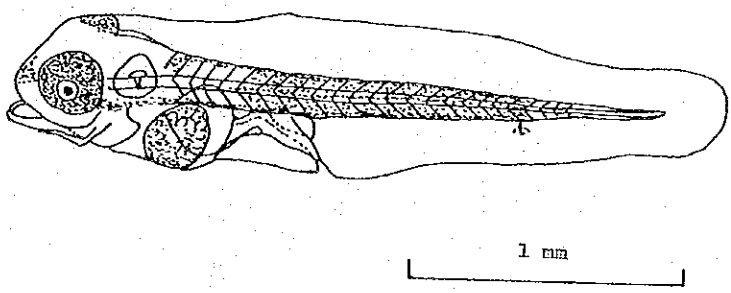

Fig. 1-c. Larva of seabass, *Lates calcarifer*, on day 2.

- Day 3 (TL: 2.61 ±0.08 mm)

The air-bladder appeared in some larvae. The yolk had disappeared, but the oil globule was still observed.

- Day 4 (TL: 2.78 ±0.15 mm)

The oil globule had almost disappeared.

- Day 5 (TL: 3.08 ±0.09 mm)

Teeth appeared in the upper jaw.

- Day 6 (TL: 3.10 ±0.13 mm)

The under part of caudal end became whitish.

- Day 7 (TL: 3.44 ±0.09 mm) (Fig. 1-d)

The rudiments of a dorsal and an anal fin appeared. The serrated teeth appeared in preoperculum which numbered one or two in the anterior margin and two in the posterior margin. The melanophore strongly appeared from snout to tail.

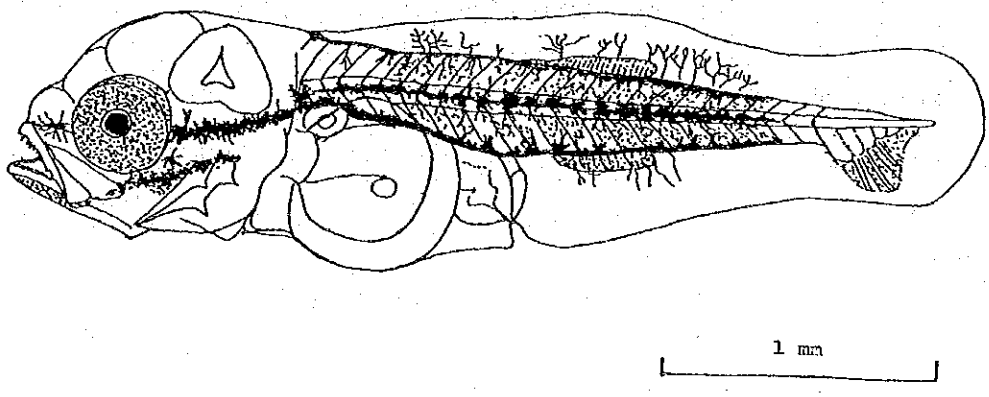

Fig. 1-d. Larva of seabass, *Lates calcarifer*, on day 7.

- Day 8 (TL: 3.58 ±0.13 mm)

Teeth appeared in the lower jaw.

- Day 9 (TL: 3.49 ±0.26 mm)

The caudal end of the notochord was bent. The soft ray part of the caudal fin became clear.

- Day 10 (TL: 3.81 ±0.27 mm)

The number of serrated teeth in the posterior margin of the preoperculum grew to three. Segmented soft rays appeared in the caudal fin. Part of the posterior margin of the dorsal and anal fins were cut in the larval membrane. The distribution of melanophore was expanded to the belly.

- Day 11 (TL: 3.87 ±0.24 mm)

The posterior margins of the dorsal and anal fins were deeply cut, and the larval membrane in front of the anal remained small. The number of serrated teeth in the posterior margin of the preoperculum became three - four.

- Day 12 (TL: 4.41 ±0.29 mm)

Segmented soft rays appeared in the dorsal fin.

- Day 13 (TL: 4.58 ±0.17 mm)

The number of serrated teeth in the posterior margin of the preoperculum grew to four. The larval membrane in front of anal disappeared. The shape of the myomere became "3".

- Day 14 (TL: 4.75 ±0.32 mm) (Fig. 1-e)

The dorsal and anal fins were separated from the caudal fin, and the rudiment of a pelvic fin appeared. The cordal skeleton became well developed and the vertebrae could be counted (11 + 14). The distribution of melanophore was expanded to the whole belly and also to the dorsal and anal fins, but it did not distribute over the center of the body. A white line was distinguishable from the center of the dorsal fin to the anal with the naked eye.

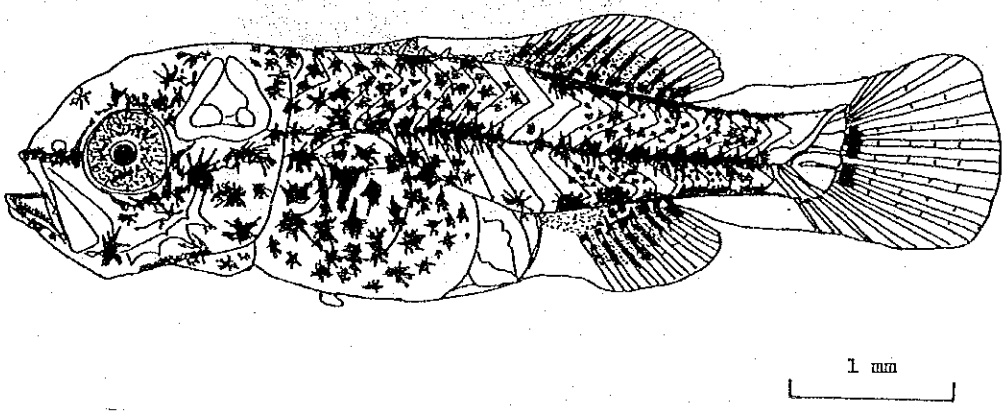

Fig. 1-e. Larva of seabass, *Lates calcarifer*, on day 14.

- Day 15 (TL: 5.41 ±0.50 mm)

The spine and soft rays of the dorsal and anal fins were clearly distinguishable. One or two serrated teeth in the upper part of the posterior margin of the preoperculum appeared.

- Day 16 (TL: 6.56 ±0.56 mm)

Each fin was completely separated, and the total number of spines and soft rays of the dorsal and anal fins became constant (19 and 11 respectively). The serrated teeth of the anterior margin of the preoperculum disappeared.

- Day 21 (TL: 8.91 +1.19 mm) (Fig. 1-f)

The number of spines and soft rays of the dorsal and anal fins became constant (VIII.11 and III.8 respectively). Scales appeared in the mid-lateral surface above tha anal fin. The body colour changed from black to pale brown.

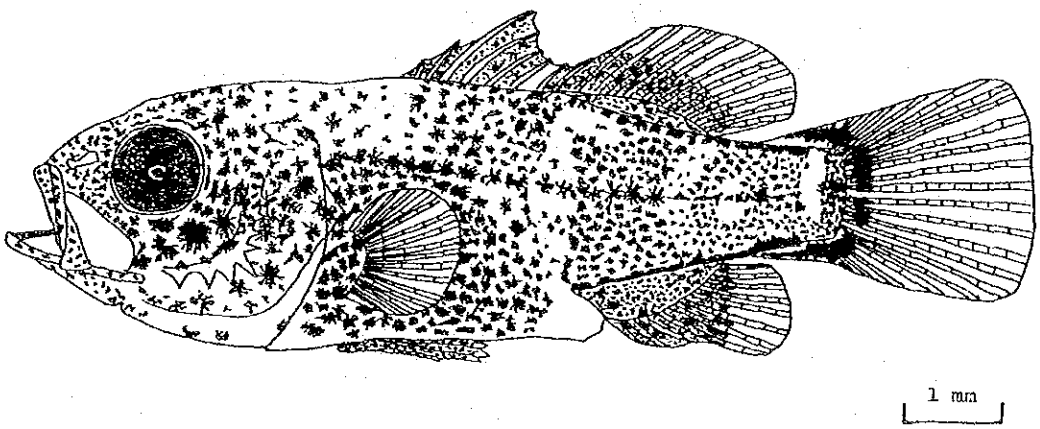

Fig. 1-f. Juvenile seabass, *Lates calcarifer*, on day 21.

2) Formation of fin rays.

The number of spines and soft rays in each fin, except the pectoral fin, is shown in Figs. 2-a ∿ -d.

In the dorsal fin (Fig. 2-a), the first to seventh spines were formed at about 6 mm in TL, but the eighth spine was still like a soft ray. It became a spine at about 8 - 12 mm in TL, so that the number of fin rays

became constant (VIII.11).

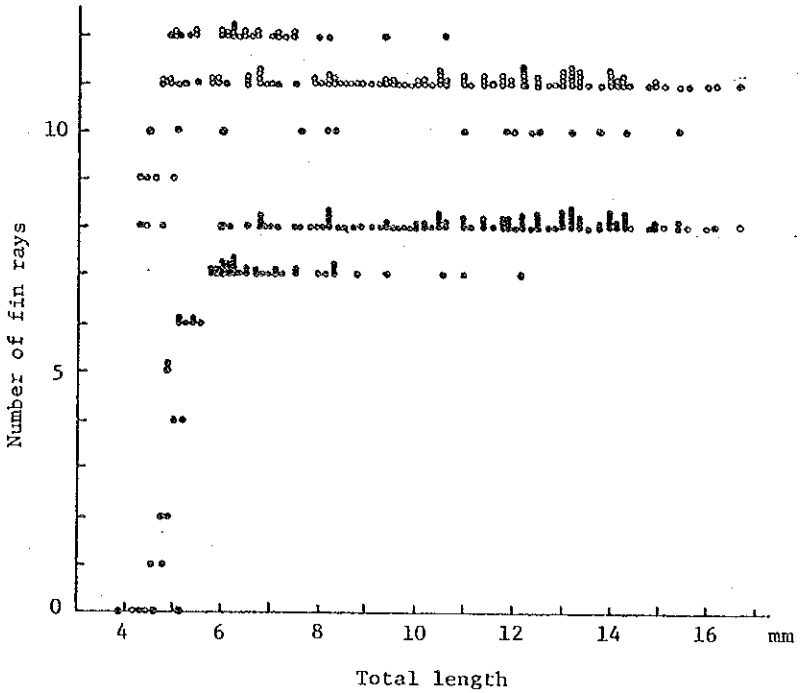

Fig. 2-a. Formation of spines (closed circle) and soft rays (open circle) in dorsal fin of larval and juvenile seabass.

In the anal fin (Fig. 2-b), the first and second spines were formed at about 6 mm in TL, but the third one was still like a soft ray. It became a spine at about 8 - 11 mm in TL, so that the number of fin rays became constant (III.8) just the same as the dorsal fin.

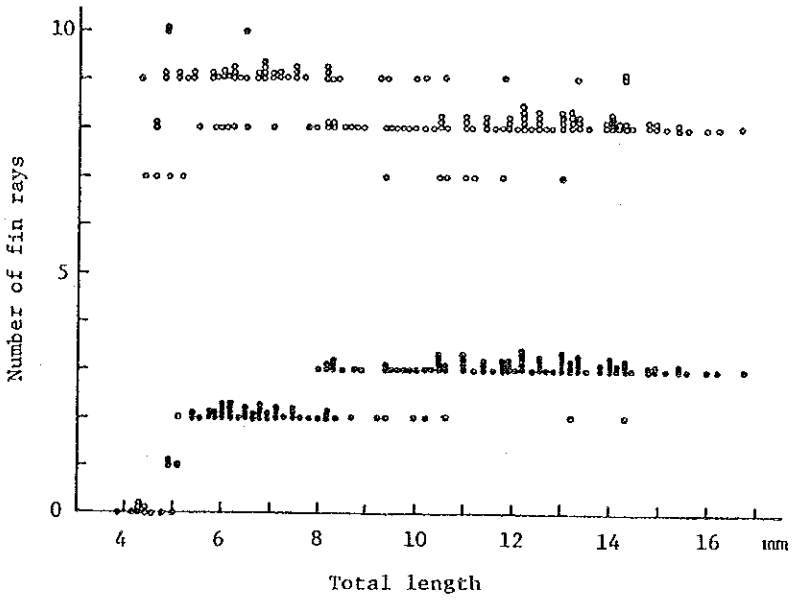

Fig. 2-b. Formation of spines (closed circle) and soft rays (open circle) in anal fin of larval and juvenile seabass.

The pelvic fin (Fig. 2-c) developed rapidly from about 5 mm in TL, then the number of fin rays became constant (I.5) at about 8 mm in TL.

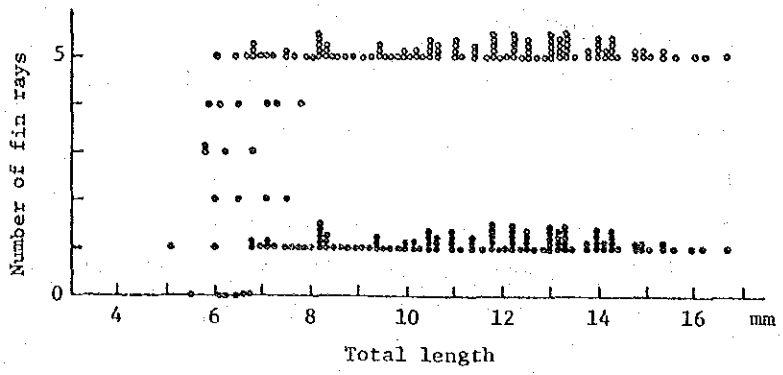

Fig. 2-c. Formation of spine (closed circle) and soft rays (open circle) in pelvic fin of larval and juvenile seabass.

In the caudal fin (Fig. 2-d), the soft rays started to develop from 3 - 4 mm in TL, the total number, which contained rudimentary soft rays, became constant (30 - 33) at about 11 - 12 mm in TL.

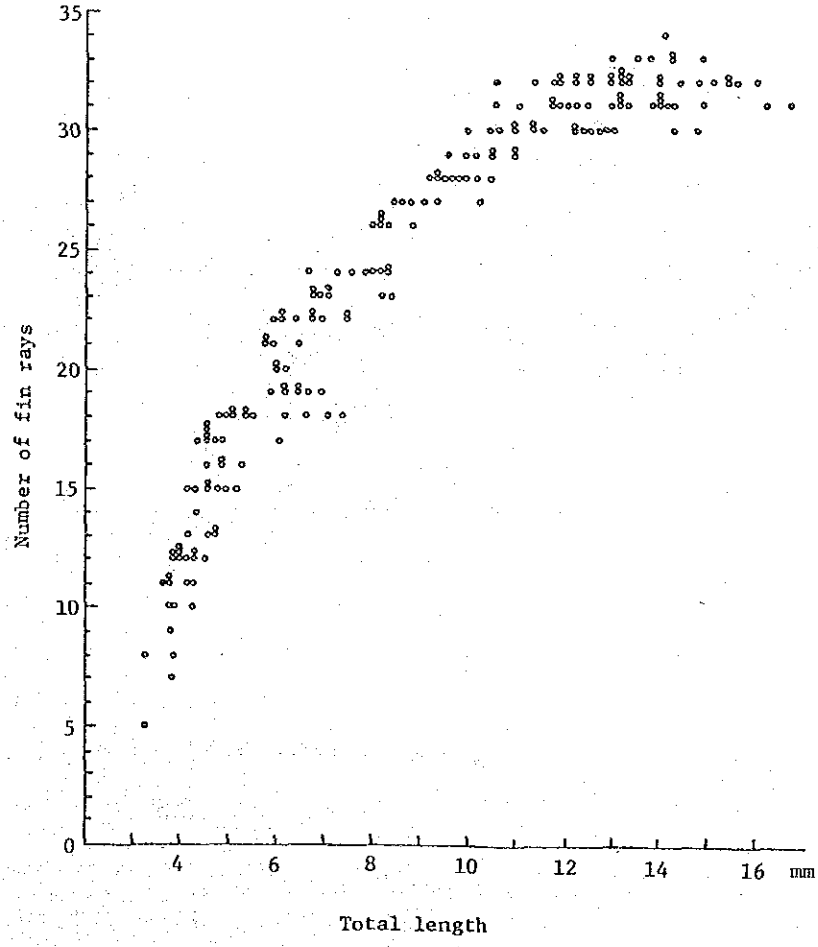

Fig. 2-d. Formation of soft rays in caudal fin of larval and juvenile seabass, including rudimental soft rays.

The number of segmented and branched soft rays are shown in Figs. 3-a ∿ -d.

In the dorsal fin (Fig. 3-a), the segmentation was started from about 5 mm in TL, then completed at 6 -16 mm in TL. The branching was started from 20 mm in TL, but it was still not completed at 44 mm TL.

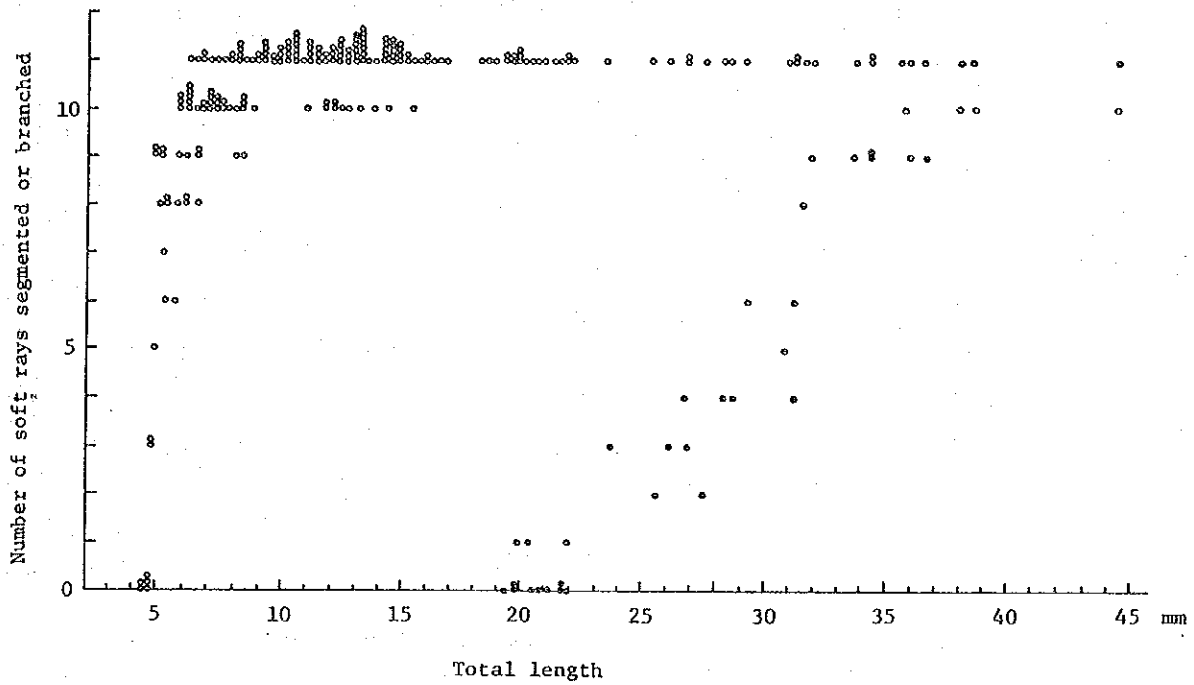

Fig. 3-a. Segmentation (open circle) and branching (closed circle) in dorsal fin of larval and juvenile seabass.

In the anal fin (Fig. 3-b), the segmentation was just the same as in the dorsal fin, but branching was completed at 34 - 38 mm in TL.

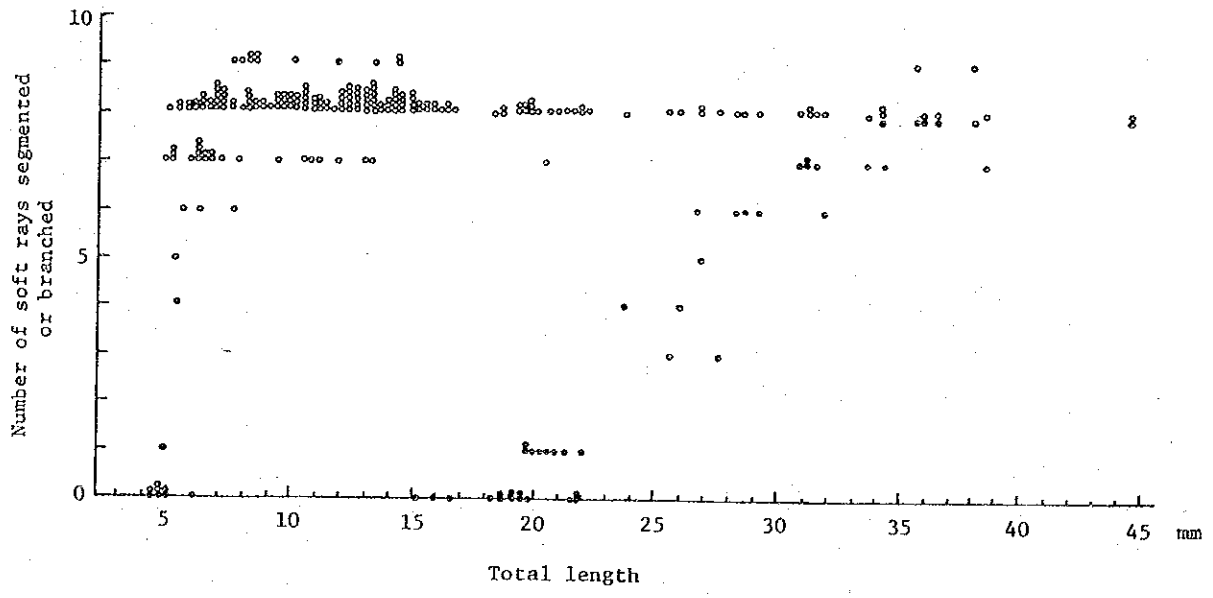

Fig. 3-b. Segmentation (open circle) and branching (closed circle) in anal fin of larval and juvenile seabass.

In the pelvic fin (Fig. 3-c), the segmentation was started at 7 mm, then completed at 9 - 10 mm in TL. The branching was started from 10 - 11 mm, then completed at 12 - 16 mm in TL.

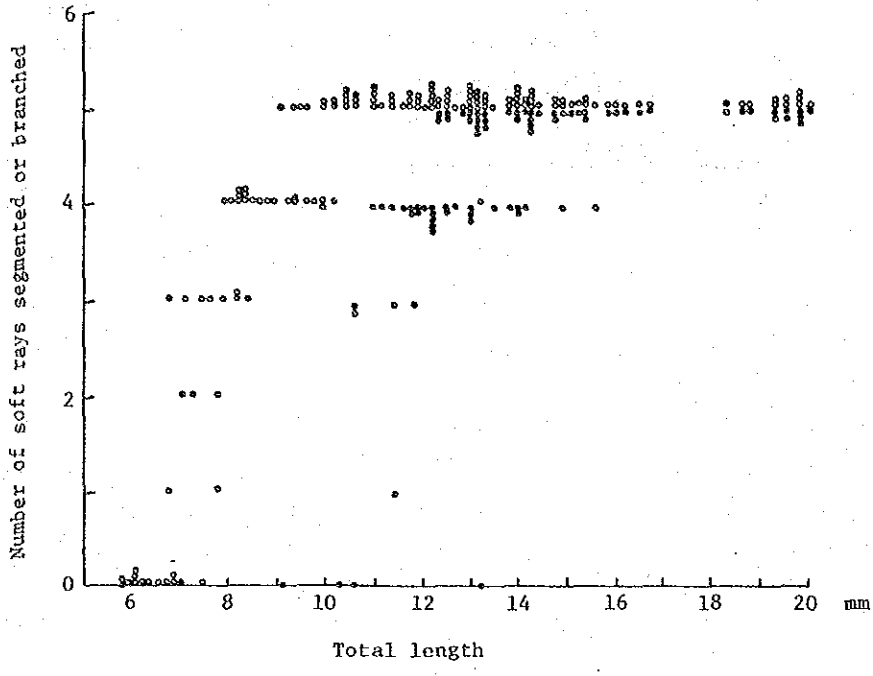

Fig. 3-c. Segmentation (open circle) and branching (closed circle) in pelvic fin of larval and juvenile seabass.

In the caudal fin (Fig. 3-d), the segmentation was started at 3 - 4 mm, then completed at 12 - 15 mm in TL. The branching was started from 12 - 17 mm, then completed at 31 - 35 mm in TL.

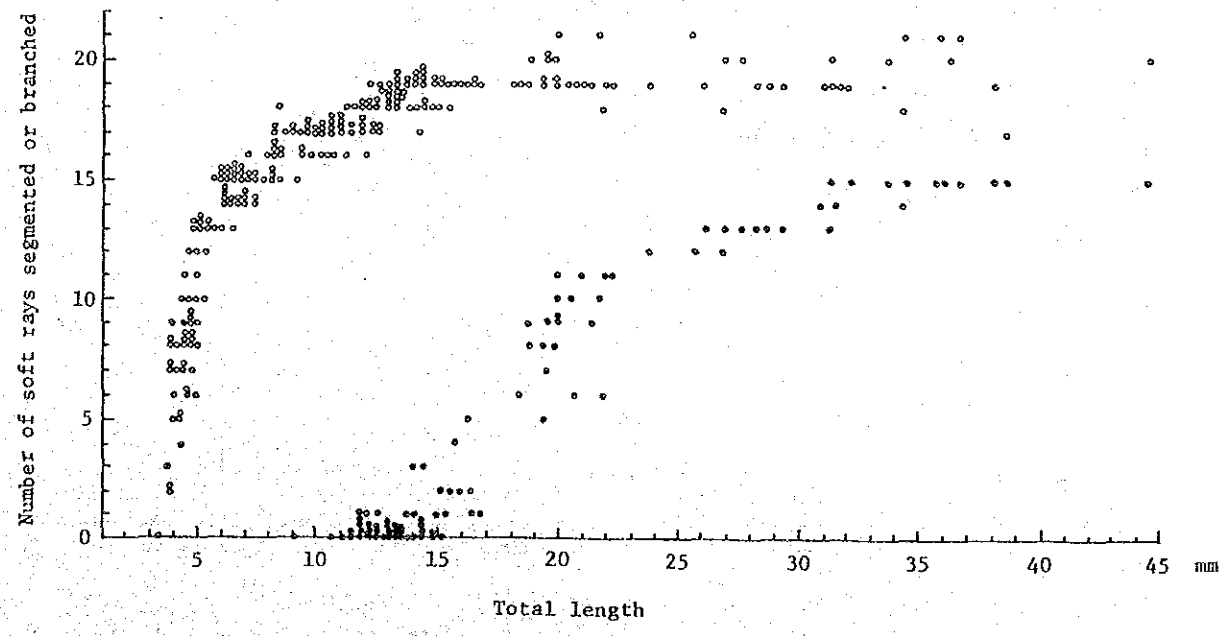

Fig 3-d. Segmentation (open circle) and branching (closed circle) in caudal fin of larval and juvenile seabass.

Discussion

The number of fin ray of each fin became constant at 8 - 12 mm in TL, so that it is clear that larvae become juvenile at that size. Furthermore, branching of soft ray in all fins except pectral fin might be completed before 35 - 50 mm, so that it seems that the juvenile may become the young stage at that size.

Record of Spawning and Hatching of Eggs of Seabass, *Lates calcarifer*, in Concrete Tanks

Sujin Maneewong and Tatsuo Watanabe

Introduction

This work has been done to discover the spawning time of seabass, *Lates calcarifer*, and to measure the hatching rate and size of eggs as a method for evaluating the quality of eggs.

Materials and Methods

The parent fish were transferred from the Boa-keng substation to two 150 ton spawning tanks in NICA on March 21, 1983. Forty nine fish (body weight 3.8 ±1.0 kg.) were stocked in No.1 tank, and 56 fish (7.4 ±1.2 kg.) in No.2 tank. The fish in No.2 did not spawn, so they were exchanged for 30 fish of new stock on May 24, (body weight 3.2 - 5.4 kg.).

The observation of spawning was made from 19:30 to 21:30 on April 1 - 4 and May 2 - 4, but, after that, only confirmation of spawning was done, at 21:00. The time required for hatching and water temperature were observed at the same time.

The hatching rate was estimated in the laboratory. The eggs were collected from spawning tanks during the 21:00 - 22:00 hour by a hand net (63 μ mesh), then brought to the laboratory. About 100 floating eggs were put in a 1/3 liter beaker, then the number of hatched larvae were counted on the next day.

The size of 30 eggs from each group was measured in fresh sample by the profile projector at 50 magnifications.

Results

The record of spawning and hatching of eggs is shown in Tables 1-a ∿ 1-f, and Tables 2-a ∿ 2-d.

The spawning was confirmed to take place during the 20:00 - 21:00 hour in April and May, and it was also done before 21:00 in later months. In every observation, the time of sunset was around 19:00 hour, and the time of moonrise was after 21:00, so that it seems that seabass spawn in the dark between sunset and moonrise. The spawning behaviour could not be observed because of the dark condition. However, the spawning could be confirmed by the sound of hitting surface of water by parent fish.

The time of hatching was during 8:00 - 11:00 on the next day, so that the time needed for hatching was 12 - 15 hours in 29.5 - 31°C water.

The hatching rates were 75.6 - 99.0% in tank No.1 and 28.2 - 93.1% in tank No.2. No clear tendency was observed in the relationship between hatching rate and quantity of newly hatched larvae.

The range in diameter of egg was 0.68 - 0.89 mm, but most were 0.74 - 0.80 mm. The range in diameter of oil globule was 0.20 - 0.28 mm, but most were 0.23 - 0.26 mm. And the ratio of diameter of egg and oil globule ranged from 27.4 - 36.8%, but most were 30.9 - 33.1%. It was observed that the size of eggs became bigger month by month.

The parent fish of first stocking in No.2 tank did not spawn. However, it could not been confirmed whether it was caused by the effect of age (8 - 12 years old) or high stocking density (2.8 kg/ton).

Table 1-a. Record of spawning and hatching time, hatching rate, egg size and quantity of hatched larvae in the No.1 spawning tank during March 31 - April 4. (The day of full moon was March 29).

Date & Time		Temp.	Hatching Rate	Egg diameter (mm)	Oil globule diameter (mm)	B/A x 100	Quantity of *
Spawning	Hatching	(°C)	(%)	A	B		hatched larvae
Mar. 31	Apr. 1	31	-	-	-	-	++
Apr. 1 20:00 ∫ 21:00	Apr. 2 10:00 ∫ 11:00	31	91.2	0.76 ± 0.018 (0.73 - 0.80)	0.24 ± 0.009 (0.23 - 0.25)	30.9 ± 1.5 (28.8 - 33.3)	+++
Apr. 2 20:15	Apr. 3 8:30 ∫ 9:00	31	94.1	0.77 ± 0.016 (0.75 - 0.80)	0.24 ± 0.010 (0.23 - 0.25)	31.0 ± 1.3 (28.8 - 33.3)	++++
Apr. 3 20:20 ∫ 20:25	Apr. 4 8:00 ∫ 9:00	31	98.0	0.77 ± 0.018 (0.75 - 0.80)	0.24 ± 0.012 (0.23 - 0.28)	31.4 ± 1.4 (29.5 - 35.0)	+++
Apr. 4 20:24 20:25 20:40 20:41 20:44	Apr. 5 9:30 ∫ 10:30	30	82.0	0.75 ± 0.019 (0.73 - 0.78)	0.23 ± 0.012 (0.20 - 0.25)	30.9 ± 1.4 (27.4 - 33.3)	++

* : + : few
++ : several handred thousand
+++ : about one million
++++ : several million
+++++ : many

Table 1-b. Record of spawning and hatching time, hatching rate, egg size and quantity of hatched larvae in the No.1 spawning tank during May 1 - May 4 and May 18 - 20. (The day of full moon was April 27).

Date & Time		Temp.	Hatching Rate	Egg diameter (mm)	Oil globule diameter (mm)	B/A x 100	Quantity of
Spawning	Hatching	(°C)	(%)	A	B		hatched larvae
May 1	May 2	32	-	-	-	-	+
May 2 20:25 ~ 20:40	May 3 9:00 ~ 10:00	32	95.0	0.74 ± 0.024 (0.68 - 0.77)	0.24 ± 0.010 (0.23 - 0.26)	32.8 ± 1.2 (30.3 - 35.2)	+++
May 3 20:18	May 4 8:00 ~ 9:00	32	93.1	0.75 ± 0.010 (0.74 - 0.78)	0.24 ± 0.006 (0.23 - 0.25)	32.0 ± 0.7 (30.7 - 33.3)	++
May 4 19:55 ~ 20:50	May 5 8:30 ~ 9:30	31.5	96.2	0.78 ± 0.011 (0.76 - 0.79)	0.25 ± 0.007 (0.23 - 0.27)	32.4 ± 0.8 (29.9 - 33.3)	++
May 18	May 19 9:00 ~ 10:00	-	-	-	-	-	+++
May 19	May 20	-	-	-	-	-	++
May 20	May 21	-	-	-	-	-	++

Table 1-c. Record of spawning and hatching time, hatching rate, egg size, and quantity of hatched larvae in the No.1 spawning tank during May 30 - June 3. (The day of full moon was May 27).

Date & Time		Temp.	Hatching Rate	Egg diameter (mm)	Oil globule diameter (mm)	B/A x 100	Quantity of
Spawning	Hatching	(°C)	(%)	A	B		hatched larvae
May 30	May 31	30	-	-	-	-	+
May 31 20:25 ~ 20:26	Jun. 1 8:00 ~ 9:00	30	95.0	0.76 ± 0.028 (0.68 - 0.82)	0.24 ± 0.009 (0.22 - 0.26)	32.2 ± 1.0 (30.0 - 34.2)	+++
Jun. 1 before 21:00	Jun. 2 9:00 ~ 10:00	30	89.1	0.76 ± 0.018 (0.73 - 0.79)	0.25 ± 0.009 (0.23 - 0.26)	32.0 ± 1.1 (29.9 - 33.8)	++++
Jun. 2 before 20:40	Jun. 3 before 8:30	30	87.0	0.77 ± 0.017 (0.73 - 0.82)	0.24 ± 0.007 (-.23 - 0.25)	31.5 ± 1.1 (29.3 - 34.2)	+++
Jun. 3 before 21:00	Jun. 4	30	-	-	-	-	+ (many dead eggs)

Table 1-d. Record of spawning and hatching time, hatching rate, egg size, and quantity of hatched larvae in the No.1 spawning tank during June 28 - July 2. (The day of full moon was June 25).

Date & Time		Temp.	Hatching Rate	Egg diameter (mm) A	Oil globule diameter (mm) B	B/A x 100	Quantity of hatched larvae
Spawning	Hatching	(°C)	(%)				
Jun. 28	Jun. 29 before 8:30	30	-	-	-	-	++
Jun. 29 before 21:00	Jun. 30 before 8:30	30	82.7	0.78 ± 0.017 (0.76 - 0.82)	0.25 ± 0.008 (0.24 - 0.27)	32.4 ± 0.9 (30.9 - 34.2)	+++++
Jun. 30 before 21:00	Jul. 1 8:30 ~ 9:30	30	82.5	0.78 ± 0.032 (0.72 - 0.89)	0.25 ± 0.009 (0.22 - 0.26)	31.9 ± 1.2 (28.1 - 34.7)	+++
Jul. 1 before 21:00	Jul. 2 9:00 ~ 10:00	30	78.8	0.78 ± 0.020 (0.74 - 0.83)	0.25 ± 0.012 (0.23 - 0.27)	32.4 ± 1.2 (29.9 - 34.6)	+++
Jul. 2	Jul. 3 8:00 ~ 9:00	30	-	-	-	-	++

Table 1-e. Record of spawning and hatching time, hatching rate, egg size and quantity of hatched Larvae in the No.1 spawning tank during July 26 - 31. (The day of full moon was July 25).

Date & Time		Temp.	Hatching Rate	Egg diameter (mm)	Oil globule diameter (mm)	B/A x 100	Quantity of
Spawning	Hatching	(°C)	(%)	A	B		hatched larvae
Jul. 26	Jul. 27 9:30 ~ 10:30	30	-	-	-	-	+
Jul. 27	Jul. 28	30	-	-	-	-	+
Jul. 28 before 21:00	Jul. 29 9:30 ~ 10:30	30	99.0	0.78 ± 0.015 (0.75 - 0.81)	0.26 ± 0.008 (0.24 - 0.28)	33.1 ± 0.9 (31.6 - 35.4)	++++
Jul. 29 before 21:00	Jul. 30 after 9:00	30	97.2	0.78 ± 0.016 (0.76 - 0.80)	0.26 ± 0.009 (0.24 - 0.28)	33.0 ± 1.3 (30.0 - 36.8)	+++
Jul. 30	Jul. 31 9:00 ~ 10:00	-	-	-	-	-	+++
Jul. 31	Aug. 1 after 8:30	-	-	-	-	-	++

Table 1-f. Record on spawning and hatching time, hatching rate, egg size, and quantity of hatched larvae in the No.1 spawning tank during August 24 - 30. (The day of full moon was August 23).

Date & Time		Temp.	Hatching Rate	Egg diameter (mm)	Oil globule diameter (mm)	B/A x 100	Quantity of
Spawning	Hatching	(°C)	(%)	A	B		hatched larvae
Aug. 24	Aug. 25 before 8:30	30	-	-	-	-	+
Aug. 26 before 21:00	Aug. 27	30	93.0	0.80 ± 0.016 (0.77 - 0.84)	0.26 ± 0.007 (0.24 - 0.28)	32.3 ± 0.8 (30.8 - 34.6)	++
Aug. 27	Aug. 28	30	-	-	-	-	+
Aug. 28 before 21:00	Aug. 29 9:00 ~ 10:00	29.5	75.6	0.79 ± 0.019 (0.76 - 0.83)	0.26 ± 0.007 (0.24 - 0.27)	32.5 ± 0.6 (31.2 - 33.3)	++++
Aug. 29	Aug. 30	29.5	-	-	-	-	++
Aug. 30	Aug. 31	29.5	-	-	-	-	++

Table 2-a. Record of spawning and hatching time, hatching rate, egg size and quantity of hatched larvae in the No.2 spawning tank during May 31 - June 3. (The day of full moon was May 27).

Date & Time		Temp.	Hatching Rate	Egg diameter (mm) A	Oil globule diameter (mm) B	B/A x 100	Quantity of hatched larvae
Spawning	Hatching	(°C)	(%)				
May 31 before 20:15	Jun. 1 8:00 ~ 9:00	30	88.0	0.74 ± 0.017 (0.70 - 0.77)	0.24 ± 0.010 (0.22 - 0.25)	32.4 ± 1.1 (30.1 - 34.2)	+++
Jun. 1 before 20:30	Jun. 2 9:00 ~ 10:00	30	83.3	0.76 ± 0.015 (0.73 - 0.79)	0.24 ± 0.006 (0.23 - 0.25)	31.9 ± 0.9 (30.4 - 33.8)	++++
Jun. 2 before 20:40	Jun. 3 before 8:30	30	78.2	0.77 ± 0.016 (0.72 - 0.79)	0.25 ± 0.009 (0.22 - 0.26)	32.0 ± 1.1 (29.5 - 34.2)	++
Jun. 3 before 21:00	Jun. 4 10:00 ~ 11:00	30	28.2	0.77 ± 0.013 (0.74 - 0.79)	0.24 ± 0.005 (0.23 - 0.25)	31.5 ± 0.8 (29.5 - 33.8)	++

Table 2-b. Record of spawning and hatching time, hatching rate, bgg size and quantity of hatched larvae in the No.2 spawning tank during June 28 - July 2. (The day of full moon was June 25).

Date & Time		Temp.	Hatching Rate	Egg diameter (mm) A	Oil globule diameter (mm) B	B/A x 100	Quantity of hatched larvae
Spawning	Hatching	(°C)	(%)				
Jun. 28	Jun. 29 before 8:30	-	-	-	-	-	++
Jun. 29 before 21:00	Jun. 30 before 8:30	30	84.2	0.78 ± 0.016 (0.74 - 0.80)	0.25 ± 0.008 (0.23 - 0.26)	32.0 ± 0.9 (30.0 - 33.8)	++++
Jun. 30 before 21:00	Jul. 1 9:00 ~ 10:00	30	76.8	0.77 ± 0.015 (0.74 - 0.81)	0.25 ± 0.008 (0.23 - 0.26)	32.1 ± 0.9 (30.0 - 34.2)	++++
Jul. 1 before 21:00	Jul. 2 9:00 ~ 10:00	30	81.0	0.78 ± 0.020 (0.75 - 0.83)	0.26 ± 0.008 (0.24 - 0.28)	32.8 ± 0.9 (30.9 - 34.7)	+++
Jul. 2	Jul. 3 8:00 ~ 9:00	30	-	-	-	-	++

Table 2-c. Record of spawning and hatching time, hatching rate, egg size, and quantity of hatched larvae in the No.2 spawning tank during July 27 - 31. (The day of full moon was July 25).

Date & Time		Temp.	Hatching Rate	Egg diameter (mm)	Oil globule diameter (mm)	B/A x 100	Quantity of
Spawning	Hatching	(°C)	(%)	A	B		hatched larvae
Jul. 27	Jul. 28 9:30 ~ 10:30	30	-	-	-	-	++++
Jul. 28 before 21:00	Jul. 29 before 8:30	30	93.1	0.76 ± 0.024 (0.72 - 0.80)	0.25 ± 0.007 (0.24 - 0.27)	33.2 ± 1.1 (31.3 - 35.1)	+++
Jul. 29 before 21:00	Jul. 30 before 9:00	30	89.7	0.77 ± 0.022 (0.72 ± 0.81)	0.25 ± 0.008 (0.23 - 0.26)	32.5 ± 1.1 (30.8 - 34.7)	+++
Jul. 30	Jul. 31 8:30 ~ 9:30	30	-	-	-	-	++
Jul. 31	Aug. 1 before 8:30	-	-	-	-	-	+

Table 2-d. Record of spawning and hatching time, hatching rate, egg size, and quantity of hatched larvae in the No.2 spawning tanks during August 26 - 30. (The day of full moon was August 23).

Date & Time		Temp.	Hatching rate	Egg diameter (mm)	Oil globule diameter (mm)	B/A x 100	Quantity of
Spawning	Hatching	(°C)	(%)	A	B		hatched larvae
Aug. 26 before 21:00	Aug. 27 before 8:00	30	89.0	0.79 ± 0.015 (0.76 - 0.82)	0.26 ± 0.007 (0.24 - 0.27)	32.3 ± 0.7 (31.2 - 33.3)	+++
Aug. 27	Aug. 28	30	-	-	-	-	++
Aug. 28 before 21:00	Aug. 29 9:00 ~ 10:11	29.5	73.0	0.79 ± 0.020 (0.76 - 0.84)	0.26 ± 0.010 (0.24 - 0.28)	33.0 ± 1.1 (31.2 - 34.6)	+++++
Aug. 29	Aug. 30	29.5	-	-	-	-	++
Aug. 30	Aug. 31	29.5	-	-	-	-	++

Survival and Growth of Early Stage of Seabass, *Lates calcarifer*, under Different Conditions of Salinity

Pairat Kosutarak, Tida Pechmanee and Tatsuo Watanabe

Introduction

The seabass, *Lates calcarifer*, is well known as one of the brakish water fish. Therefore, it is important to know its potential for survival and growth under various salinity conditions for management in seed production and also for biological information.

Materials and Methods

Seven series of experiments were carried out in the laboratory.

1) Experiment 1

The hatching rate of eggs under different salinity conditions, i.e. 100%, 80%, 60%, 40%, 20% and 0% of sea water, was examined. The eggs were collected from the spawning tank at night on June 29, 1983. About 100 floating eggs were put into a 2 litre glass cylinder, about 12 cm in diameter and, 20 cm in depth, and kept 15 - 18 hours without aeration. Then the number of newly hatched larvae were counted.

2) Experiment 2

The survival rate of larvae for 24 hours under different salinity conditions, which were set the same as for Experiment 1, was examined. The newly hatched larvae were collected on June 30, then 15,000 larvae were stocked and reared in a 0.5 ton polycarbonate tank for the experiment. However, they all died on July 10, for reasons which were not clear, so that several thousand larvae, which were of the same egg group, were stocked again from the seed production tank. The experiment was carried out every day till 2 days after hatching, and then once in two days till day 24. The number of larvae put into each glass cylinder, same size as experiment 1, was 82 - 109 individuals in day 0 - 2, 82 - 109 in day 4 - 16, and 20 - 34 in day 18 - 24. Then they were kept for 24 hours without aerations. The size of larvae on each day was estimated by measurement of total length of 30 fish collected from the stocking tank. The measurement was done on fresh samples anesthetized by 20 ppm. of quinaldin solution.

3) Experiment 3

The survival rate of larvae at different salinity levels, i.e. seawater, 20% seawater, 10% seawater and fresh water, was examined. The newly hatched larvae were collected on July 28 and about 15,000 larvae were stocked in a 0.5 ton polycarbonate tank. The experiment was carried out from 11 days to 21 days after hatching, once every two days. The number of larvae put into the glass cylinder was 47 - 51 individuals on day 11; 39 - 42 on days 13 and 15; and 30 on days 17 - 21. They were kept for 24 hours without aeration. The number of survivors was counted. The size of larvae was estimated by the same method as in experiment 2.

4) Experiment 4

The survival and growth rates of newly hatched larvae at different levels of salinity, i.e. 100%, 75%, 50% and 20%, seawater, were examined for 12 days. The newly hatched larvae were collected on August 27, then 500 larvae were put into each rectangular glass tank (26x50x30 cm, water volume 24 ℓ) with weak aeration. The rearing water was exchanged 16 - 50% every day from 2 days after start of experiment. They were fed with rotifers (5 - 15 rotifer/mℓ) in the morning. At 15:30 the water temperature was taken and the salinity was measured by using the refract meter "ATAGO".

5) Experiment 5

The survival and growth rates of larvae sized 4.54 mm in mean total length (range: 3.36 - 5.46 mm) at different salinity levels, i.e., 100%, 50%, 25%, 12.5% seawater, were examined for 7 days. The newly hatched larvae were stocked in a 0.5 ton polycarbonate tank on August 29, then they were reared till 12 days after hatching and used for the experiment. 100 larvae were put into a rectangular glass tank for 2 replications on September 10. They were fed the nauplii of *Artemia* (1 - 4/mℓ). The other methods were same as in former experiments.

6) Experiment 6

The survival and growth rates of larvae sized 8.5 ±1.2 mm in total length, which is the transitional stage between larva and juvenile, were examined for 7 days at different levels of salinity, i.e., 100%, 66.7%, 33.3% and 0% seawater in 2 replications.

About 4,000 fish aged 17 days were transferred from the tank of seed production to the 0.5 ton polycarbonate tank, then 60 fish aged day 22 were put into each glass tank on September 18. They were fed with the nauplii of *Artemia* (1-2.5 individuals/mℓ). Other methods were the same as in former experiments.

7) Experiment 7

The survival and growth rates of juvinile larvae sized 15.8 ±1.9 mm in total length, at salinity levels the same as in experiment 6, were examined for 12 days. They were the same crop as in experiment 6, but they were treated with 50 ppm of "Dimeton Soda" for 7 days, from September 18 to Sept. 24, because some dead fish were found in the stock tank. Since dead fish did not appear on September 27, 30 fish aged day 31 were put into the glass tanks and the experiment was started. The fish were fed *Moina* sp. and other fresh water zooplankton (500 - 600 individuals/fish/day), which was divided for 5 - 6 times/day feeding. The 30 ℓ of water was exchanged 80% every day. Other methods were the same as in former experiments.

Results

1) Hatching rate at different salinity levels (Exp. 1)

The hatching rates at different salinity levels are shown in Table 1. The eggs hatched out well at 100%, 80%, 60% seawater, however the hatching rates at lower salinity levels were inferior, and they did not hatch out at all in fresh water. The eggs at 100% seawater (29‰of salinity) did not float on the surface of the water but were suspended in the middle layer in this experiment. However, the eggs floated on the surface of the water of 32 - 34‰ in salinity and sank in low salinity water of probably less than about 20 - 25‰.

Table 1. Hatching rates (%) of eggs of *Lates calcarifer* under different salinity conditions in experiment 1.

% of S.W.** / Replication	100 (29)*	80 (23)	60 (16)	40 (11)	20 (7)	0 (0)
1	84.2	82.0	88.3	42.7	1.0	0.0
2	82.7	43.1	71.7	44.2	9.5	0.0

* Salinity (‰) was measured by refract meter of ATAGO.
** S.W.: sea water

2) Survival of larvae for 24 hours at different salinity levels (Exp. 2 and 3)

The survival rates for 24 hours at different salinity levels in experiments 2 and 3 are shown in Table 2-a and Table 3-a, and the size of larvae on each day in both experiments was shown in Table 2-b and Table 3-b.

The newly hatched larvae showed good survival even in low salinity such as in 20% sea water, but they could not survive in fresh water till day 10 when their size in total length was 3.26 - 4.64 mm. However, some of them showed tolerance to fresh water at least 24 hours from day 12 when their size in TL was 4.40 - 6.35 mm in experiment 2. They showed the same tolerance from day 13, which their size in TL was 4.75 - 6.05 mm, in experiment 3. Although 100% survival in fresh water was not observed in experiment 2, good survival was noted (more than 70%) from day 20. In experiment 3, 100% survival was observed from day 19 when total length was 6.05 - 8.75 mm.

3) Growth and survival of newly hatched larvae at different salinity levels (Exp. 4)

The total length and number of surviving larvae at the end of experiment 4 are shown in Table 4. When the newly hatched larvae were put into different salinity levels, they showed different actions as follows:

100% sea water:	larvae were floating on the surface of the water or suspended in higher layers.
75% sea water:	they were suspended in lower layers.
50% sea water to 25% sea water:	they were lying on the bottom of the tank or sometimes swimming in an upwards direction and sinking down.

On day 1, larvae were evenly distributed in 100% seawater, but almost all larvae were lying on the bottom at less than 75% sea water. On day 2 they kept their bodies horizontal and started swimming and gathering in the direction of light in all tanks. Then many larvae died at 25% sea water on day 5, and only a few larvae were seen on day 7. The water temperature was 28 - 29.5°C, and the salinity was 33 - 34‰ in 100%, 23 - 26% in 75%, 16 - 17‰ in 50%, and 8 - 9‰ in 25% sea water.

At the end of the experiment, the size and survival of larvae at 100% and 75% sea water were almost the same. The size at 50% sea water was

Table 2-a. Survival rate (%) of larvae of *Lates calcarifer* on each day after hatching under different salinity conditions in experiment 2.

% of sea water \ Days after hatching	0	1	2	4	6	8	10	12	14	16	18	20	22	24
100	100.0	21.9	98.1	87.3	62.3	85.2	96.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0
80	98.1	45.5	98.1	88.7	79.2	81.8	98.2	100.0	97.9	100.0	100.0	100.0	100.0	100.0
60	100.0	77.0	100.0	96.2	72.7	92.5	91.7	98.0	100.0	100.0	100.0	100.0	100.0	100.0
40	100.0	78.6	97.1	87.7	77.4	96.1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
20	90.1	82.2	100.0	98.2	87.3	88.5	100.0	96.0	100.0	100.0	100.0	100.0	100.0	100.0
0	0.0	(65.1)*	0.0	0.0	0.0	0.0	0.0	30.4	8.6	18.6	44.1	76.7	70.0	95.0

* Salinity was 3‰.

Table 2-b. Size of larvae (in total length) used in experiment 2 and surviving under fresh water conditions.

Days after hatching	T.L. of larvae on each day (mm) mean ± s.d.	range	Range of T.L. of larvae surviving in F.W. (mm)
4	2.99 ± 0.09	2.86 - 3.18	-
6	3.32 ± 0.18	2.84 - 3.62	-
8	3.69 ± 0.20	3.36 - 4.08	-
10	3.88 ± 0.33	3.26 - 4.64	-
12	4.44 ± 0.42	3.52 - 5.54	4.40 - 6.35
14	5.76 ± 0.47	5.10 - 6.95	5.20 - 6.40
16	6.88 ± 0.62	5.35 - 8.80	6.45 - 8.40
18	7.50 ± 0.77	6.10 - 8.75	7.50 - 9.10
20	9.03 ± 0.91	6.90 - 11.05	8.40 - 10.50
22	10.06 ± 0.99	8.20 - 11.30	8.60 - 13.40
24	10.32 ± 1.04	7.90 - 12.65	8.40 - 12.50

Table 3-a. Survival rate (%) of larvae on each day after hatching under different salinity conditions in experiment 3.

% of sea water \ Days after hatching	11	13	15	17	19	21
100	98.0	97.6	100.0	100.0	100.0	-
20	97.9	97.6	100.0	100.0	100.0	-
10	90.2	100.0	100.0	100.0	100.0	-
0	0.0	12.5	2.8	46.7	100.0	100.0

Table 3-b. Size of larvae (in total length) used in experiment 3 and surviving under fresh water conditions.

Days after hatching	T.L. of larvae on each day (mm) mean ± s.d.	range	Range of T.L. of larvae surviving in F.W. (mm)
11	4.01 ± 0.43	3.04 - 4.70	-
13	4.76 ± 0.69	3.35 - 6.35	4.75 - 6.05
15	5.88 ± 0.92	3.55 - 7.15	6.60
17	6.25 ± 0.85	4.30 - 7.65	5.90 - 8.65
19	7.78 ± 0.79	6.00 - 9.60	6.05 - 8.75
21	7.73 ± 0.69	6.00 - 8.95	6.00 - 8.95

Table 4. Mean and standard deviation of total length of larvae and number of surviving larvae under different salinity conditions at end of experiment 4.

Replication \ % of S.W.*	100%	75%	50%	25%
1	4.98 ± 0.96 (32)**	4.82 ± 1.27 (66)	3.56 ± 0.60 (35)	4.38 ± 1.38 (2)
2	4.59 ± 0.85 (71)	5.17 ± 1.17 (50)	3.97 ± 0.70 (79)	5.24 ± 1.08 (12)
Total	4.72 ± 0.90 (103)	4.97 ± 1.24 (116)	3.84 ± 0.69** (114)	5.12 ± 1.11 (14)

* S.W. : sea water.

** number of larvea surviving.

considerably smaller, although the survival rate was almost the same as at higher salinity. Only a few bigger larvae survived at 25% sea water.

4) Growth and survival of larvae at different salinity levels (Exp. 5)

The total length and number of surviving larvae at the end of experiment 5 are shown in Table 5. The size and survival of larvae were almost the same under all conditions, although the size at 25% sea water was a little bigger than at other levels. The water temperature was 27.5 - 29°C and the salinity was 33 - 34‰ at 100%, 16 - 18‰ at 50%, 8 - 9‰ at 25%, and 4 - 5‰ at 12.5% sea water.

Table 5. Mean and standard deviation of total length of larvae and number of surviving larvae under different salinity conditions at the end of experiment 5.
(Total length at start of experiment was 4.54 mm in mean)

% of S.W. / Replication	100%	50%	25%	12.5%
1	10.62 ± 1.87 (92)	10.66 ± 1.64 (98)	11.13 ± 1.60 (96)	10.87 ± 1.76 (89)
2	10.43 ± 1.69 (94)	10.82 ± 1.36 (100)	10.99 ± 1.52 (93)	10.84 ± 1.49 (95)
Total	10.53 ± 1.78 (186)	10.74 ± 1.50 (198)	11.07 ± 1.56 (189)	10.85 ± 1.62 (184)

5) Growth and survival of fish in the transitional stage between larva and juvenile at different salinity levels. (Exp. 6)

The total length and number of fish at the end of this experiment are shown in Table 6. The growth and survival rates of fish at 100% - 33.3% seawater were not different, but those for fresh water were inferior to the others. The fish just put into fresh water showed weak swimming at the surface, and many dead fish were found on the first two days in both replications (43 and 34 individuals). The water temperature was 27 - 28.5°C and the salinity was 33‰ at 100%, 22‰ at 66.7%, 11 - 12‰ at 33.3%, and 0 - 1‰ at 0% sea water.

Table 6. Mean and standard deviation of total length of larvae and number of surviving larvae under different salinity conditions at the end of experiment 6. (Total length at start of experiment was 8.53 mm in mean)

% of S.W. / Replication	100%	66.7%	33.3%	0%
1	13.58 ± 2.84 (50)	13.92 ± 2.57 (55)	15.08 ± 2.55 (44)	10.98 ± 1.21 (11)
2	14.23 ± 2.61 (50)	14.13 ± 2.53 (56)	14.23 ± 2.90 (54)	10.90 ± 2.65 (20)
Total	13.91 ± 2.73 (100)	14.02 ± 2.54 (111)	14.64 ± 2.77 (98)	10.93 ± 2.23 (31)

6) Growth and survival of juveniles at different salinity levels (Exp. 7)

The total length and number of fish at the end of this experiment are shown in Table 7. The growth of fish in all tanks was not different, but the survival rate in fresh water was inferior to others. The survival rate for one replication at 66.7% sea water was rather low, but that was caused by cannibalism. Some fish put into freshwater showed weak swimming, and seven and five fish died in replications 1 and 2 respectively.

Table 7. Mean and standard deviation of total length of larvae and number of surviving larvae under different salinity conditions at the end of experiment 7. (Total length at start of experiment was 15.8 mm in mean)

% of S.W. / Replication	100%	66.7%	33.3%	0%
1	25.4 ± 2.9 (29)	26.6 ± 4.7 (18)	26.6 ± 2.9 (30)	25.5 ± 3.8 (23)
2	24.7 ± 3.3 (30)	25.8 ± 3.6 (29)	24.8 ± 4.3 (28)	23.9 ± 3.8 (25)
Total	25.1 ± 3.1 (59)	26.1 ± 4.0 (47)	25.7 ± 3.7 (58)	24.6 ± 3.8 (48)

Discussion

The egg requires high salinity for hatching (probably more than 15‰), but it requires higher salinity (more than 30‰) for floating in the surface layer of water. The newly hatched larvae can tolerate low salinity, but they require higher salinity (probably more than 20‰) for normal growth. However, they can grow well at low salinity (4 - 5‰) within the larval stage, at which their TL was 4.5 mm in this experiment.

Tolerance for fresh water was found starting at 4.4 mm in TL in this experiment, but the growth and survival rates of fish in the transitional stage (8.5 ±1.2 mm in this experiment) were still inferior to those of fish in saline water. At the juvenile stage (15.8 ±1.9mm in this experiment) fish begin to grow well in fresh water as in saline water.

The survival rate of larvae in fresh water was different in experiments 2 and 3, and also the survival rate of fish in fresh water in experiments 6 and 7 was lower than expected. Dead fish were found in the stocking tank during those experiments, so the reason for such results seems to be the fact that the fish used for the experiments were not perfectly healthy.

Value of Rotifer, *Brachionus plicatilis*, Fed with Microbial Flock for Rearing Larvae of Seabass, *Lates calcarifer*

Tida Pechmanee and Tatsuo Watanabe

Introduction

The microbial flock, which is composed mainly of marine yeast, has been used as supplemental food for the mass production of rotifer. However, the value of rotifer fed with microbial flock for rearing seabass, *Lates calcarifer*, larvae has been doubted due to recent find that lack of highly unsaturated fatty acid occurs in rotifers fed with baker's yeast for rearing larvae of marine fishes. Therefore, experiments were carried out to discover their value and also the effect of enrichment of them by cod liver oil.

Materials and Methods

Three series of experiments were carried out in the wet laboratory using a rectangular glass tank (26x50x30 cm) with weak aeration. Rearing water was exchanged every day from 3 days after start of experiment when larvae started to feed. The period of experiments was four weeks, but only rotifer was fed to larvae during the first two weeks; nauplius of *Artemia* and commercial formula food were fed later. The size of larvae in total length was measured in fresh samples anesthetized by 20 ppm of quinaldin solution.

In experiment 1, four kinds of rotifers were fed to larvae, i.e., A: those fed with green water (G.R.), G: fed with microbial flock (Y.R.), C: kept Y.R. for one hour in green water (G.Y.R.), and D: kept Y.R. for one hour in suspension of cod liver oil (O.Y.R.). G.R. was produced in the outdoor 26 ton concrete tanks, Y.R. was produced in 0.5 ton of polycarbonate tank in the laboratory, and G.Y.R. and O.Y.R. were prepared in 2 ℓ glass cylinders with strong aeration. The cod liver oil (1.5 g/ℓ) was emulsified by fresh egg yolk. The newly hatched seabass larvae were collected from the spawning tank on May 31, 1983, then 500 larvae were stocked in each tank.

In experiment 2, two kinds of rotifers, i.e., G.R. and Y.R., were compared by the same method as in experiment 1. The newly hatched larvae were collected and stocked on June 29.

In experiment 3, two kinds of rotifers were compared, i.e., G.R. and rotifers fed with microbial flock enriched by cod liver oil (3 g/1.5 ℓ) for 24 hours before feeding to them (E.Y.R.). The newly hatched larvae were collected and stocked on July 28.

Results

1) Experiment 1.

The survival and growth rates of larvae at two weeks after start of experiment are shown in Table 1, and the record of rearing for this period is shown in Table 2. The larvae fed with G.R. showed rather a higher survival rate (19.4% in A-1 and 28.2% in A-2) compared to others, although the survival rate in all tanks was low. Many larvae disappeared just a few days after the start of the experiment in all tanks, but many dead larvae were found in B-2 from day 8. These dead larvae showed weak pigmentation, swelling of the air-bladder, redish heart and greenish gall-bladder. The size of larvae fed with G.R. was bigger than of those fed with Y.R. The rearing was continued from day 14 in A-1, A-2, B-1, D-2 (Tables 3 ∿ 6). Good survival and growth was observed in A-1, A-2, B-1, but many dead larvae were found starting from day 17 in D-2.

2) Experiment 2

The results in experiment 1 were not clear due to a high mortality in all groups, so the comparison of G.R. and Y.R. was carried out again. The survival rate and size of larvae at each week is shown in Table 7 and the record of rearing for each interval is shown in Tables 8 ∿ 10.
The larvae showed better survival in all tanks compared with the former experiment. However, many dead larvae were found in Y.R. tanks from day 7, which was the same tendency as the former experiment. The size of larvae fed with Y.R. were also smaller at day 14. The growth and survival after that showed the same tendency under both feeding conditions.

3) Experiment 3

The effect of enrichment by cod liver oil to Y.R. was examined. The survival rate and size at each week are shown in Table 11, and the records of rearing for each period are shown in Tables 12 ∿ 14. The survival rates in all tanks were nearly the same (25.8 - 35.6%) and the size of larvae were also not so different at day 14. Only one dead larva was found during the experiment. Therefore, the effect of enrichment was recognized.

Discussion

In experiments 1 and 2, the larvae fed with rotifers produced by microbial flock (Y.R.) showed a slower growth rate. They also showed high mortality in case by case. High mortality of larvae in feeding Y.R. occured especially within the first two weeks, but they showed good survival and growth later. Therefore, it is considered that the larvae in early stages become weaker when Y.R. is fed to them.

On the other hand, larvae fed with rotifer produced by microbial flock enriched by cod liver oil showed nearly the same growth and stable survival compared with those fed with rotifers produced by green water in experiment 3.

With this in mind, it is considered that the larvae of seabass in the early stage may require highly unsaturated fatty acid. Therefore, it seems necessary to enrich microbial flock for rotifer feed by cod liver oil for seed production of seabass.

Table 1. The survival and growth rates of larvae of *Lates calcarifer* at two week after start of experiment 1.

Kinds of food		G.R.		Y.R.		G.Y.R.		O.Y.R.	
Replication		A-1	A-2	B-1	B-2	C-1	C-2	D-1	D-2
Number of larvae	Day 0	500	500	500	500	500	500	500	500
	Day 14	97	141	48	2	0	4	13	111
Survival rate (%)		19.4	28.2	9.6	0.4	0.0	0.8	2.6	22.2
Mean total length (mm) on day 14		5.53	6.52	5.02	4.51	-	7.04	6.91	6.29
Standard deviation		1.19	1.04	0.66	0.35	-	0.65	0.57	1.35
Number of samples measured		10	10	10	2	-	4	13	10

Table 2. The record of rearing of larvae of *Lates calcarifer* for the first two week in experiment 1.

Day after hatching	Number of rotifer fed (/mℓ)	Water exchange (%)	Water temp. (°C)	Remarks
0	-	-	-	stocked 500 newly hatched larvae
1	-	-	-	
2	10	-	29	open mouth
3	10	20	30	only few fish survived in C-2
4	10	50	29	only few fish survived in C-1, D-1
5	10	50	29	only few fish survived in no feeding condition.
6	10	50	29.5	all fish died in no feeding condition
7	10	50	29	
8	10	50	29	dead fish found: 1 in A-1, 3 in B-2
9	10	50	29	dead fish found: 1 in B-1, all fish died in C-1
10	15	50	28.5	
11	15	50	28.5	dead fish found: 15 in B-2
12	20	50	28.5	dead fish found: 31 in B-2
13	20	50	29	dead fish found: 18 in B-2, 1 in C-2
14	first measurement			dead fish found: 5 in B-2

Table 3. The survival and growth rates of larvae of *Lates calcarifer* at three week after start of experiment 1.

Kinds of food		G.R. (A-1)	G.R. (A-2)	Y.R. (B-1)	O.Y.R. (D-2)
Number of larvae	Day 14	87	131	38	101
	Day 21	83	128	35	19
Survival rate (%)		95.4	97.7	92.1	18.8
Mean TL ± s.d. (mm) on day 21		10.41±2.16	11.66±1.74	11.04±1.85	11.01±1.66
Number of sample		34	38	35*	19

* fish were measured in anesthetized condition

Table 5. The survival and growth rates of larvae of *Lates calcarifer* at end of experiment 1.

Kinds of food		G.R. (A-1)	G.R. (A2-1)	G.R. (A2-2)	Y.R. (B-1)
Number of larvae	Day 21	50	45	45	34
	Day 28	50	45	44	33
Survival rate (%)		100.0	100.0	97.8	97.1
Mean TL ± s.d. (mm) on day 28		15.62±1.49	16.04±1.36	16.25±1.63	17.00±2.56
Number of sample		50	45	44	33

Table 4. The record of rearing of larvae of *Lates calcarifer* for third week in the experiment 1.

Day after hatching	Number of food organisms (mℓ) rotifer	*Artemia*	Water exchange (%)	Water temp. (°C)	Remarks
14	20	-	100	29	continued rearing in A-1, A-2, B-1, D-2
15	20	0.5	50	29	
16	20	1 - 1.5	50	29	dead fish found: 1 in B-1
17	15	1.5 - 2	50	28	dead fish found: 1 in A-2 1 in D-2
18	15	2 - 3	50	28	dead fish found: 7 in D-2
19	15	2.5 - 4	50	28	dead fish found: 24 in D-2
20	15	1 - 4	50	28	dead fish found: 1 in B-1 30 in D-2
21	second measurement				dead fish found: 11 in D-2

Table 6. The record of rearing of larvae of *Lates calcarifer* for last week in the experiment 1.

Day after hatching	Feeding amount *Artemia* (/mℓ)	formula-food (g)	Water exchange (%)	Water temp. (°C)	Remarks
21	4	-	100	29	continued rearing in A-1, A2-1, A2-2, B-1
22	3 - 4	-	75	28.5	
23	2	0.5	75	28.5	
24	2	1.5	75	28.5	
25	1	1.5	75	28.5	
26	1	1.5	75	29	
27	1	1.5	75	29	
28	end of experiment				

Table 7. The survival and growth of larvae of *Lates calcarifer* at each week in experiment 2.

	Kind of food		G.R.			Y.R.		
	Replication		A-1	A-2	A-3	B-1	B-2	B-3
Week 2	Number of larvae	Day 0	500	500	500	500	500	500
		Day 14	156	210	145	200	80	134
	Survival rate (%)		31.2	42.0	29.0	40.0	16.0	26.8
	Mean total length (mm)		6.30	6.58	6.74	5.42	6.25	5.97
	Standard deviation		0.96	0.85	1.18	0.97	1.02	0.58
	Number of sample		30	30	30	30	30	30
Week 3	Number of larvae	Day 14	126	180	115	170	50	104
		Day 21	115	147	102	130	41	91
	Survival rate (%)		91.3	81.7	88.7	76.3	82.0	87.5
	Mean total length (mm)		12.61	11.87	13.38	11.10	13.15	12.28
	Standard deviation		1.29	1.01	1.43	2.57	1.87	2.14
	Number of sample		30	30	30	30	30	30
Week 4	Number of larvae	Day 21	85	117	72	98	41	61
		Day 28	73	93	2(57)*	82	36	46
	Survival rate (%)		85.9	79.5	-	83.7	87.8	75.4
	Mean total length (mm)		15.55	14.75	15.92	13.94	16.28	14.80
	Standard deviation		1.88	2.66	2.49	2.09	2.78	2.11
	Number of sample		73	93	59	82	336	46

* 57 fish died at night on day 27.

Table 8. The record of rearing of larvae of *Lates calcarifer* for first two week in experiment 2.

Day after hatching	Number of rotifer fed (/mℓ)	Water exchange (%)	Water temp. (°C)	Remarks
0	-	-	28	stocked 500 newly hatched larvae
1	0	-	-	
2	5	-	28	
3	10	50	28	
4	10	50	28	
5	10	50	28	
6	10	50	28	
7	15	50	28	dead fish were observed in Y.R. tanks
8	15	50	28	
9	20	50	28	
10	20	50	28	dead fish found: 2 in B-1 15 in B-3
11	25	50	28	dead fish found: 21 in B-3
12	25	50	28	dead fish found: 16 in B-3
13	25	50	28	dead fish found: 1 in B-1 11 in B-3
14	first measurement			dead fish found: 5 in B-3

Table 9. The record of rearing of larvae of *Lates calcarifer* for third week in the experiment 2.

Day after hatching	Number of food organisms (/mℓ) rotifer	*Artemia*	Water exchange (%)	Water temp. (°C)	Remarks
14	30	-	100	28	continued rearing in all tanks
15	20-30	0.5	75	28	
16	15-25	1-1.5	75	28	dead fish found: 1 in A-3
17	10-15	1.5-2	75	28	
18	10-15	2-3	75	27.5	
19	10-15	3-4	75	27.5	dead fish found: 1 in B-3
20	10-15	3-4	75	27	dead fish found: 1 in A-1 2 in B-1
21	second measurement				

Table 10. The record of rearing of larvae of *Lates calcarifer* for last week in the experiment 2.

Day after hatching	Feeding amount *Artemia* (/mℓ)	formula-food (g)	Water exchange (%)	Water temp. (°C)	Remarks
21	4	-	100	28	continued rearing in all tanks
22	3-4	0.5	75	29	dead fish found: 2 in A-3 1 in B-3
23	1.5-2	1.0	75	28.5	dead fish found: 1 in A-3, B-3, 5 in B1, 3 in B-2
24	0.7-1	1.0	75	28	
25	0.5	1.0	75	-	dead fish found: 2 in B-1 1 in B-3
26	-	1.0	75	29	dead fish found: 1 in A-1 B-1
27	-	1.0	75	28	
28	end of experiment				almost all fish (57/59) died in A-3

Table 11. The survival and growth of larvae of *Lates calcarifer* at each week in experiment 3.

	Kinds of food		G.R.			O.Y.R.		
	Replication		1	2	3	1	2	3
Week 2	Number of larvae	day 0	500	500	500	500	500	500
		day 14	129	152	178	153	166	170
	Survival rate (%)		25.8	30.4	35.6	30.6	33.2	34.0
	Mean TL (mm)		6.20	6.55	6.75	5.97	6.33	6.21
	Standard deviation		1.73	0.81	0.63	1.01	0.95	1.08
	Number of sample		29	32	58	33	46	50
Week 3	Number of larvae	day 14	100	120	120	120	120	120
		day 21	90	101	113	105	108	108
	Survival rate (%)		90.0	84.2	94.2	87.5	90.0	90.0
	Mean TL (mm)		11.7	12.5	12.6	12.0	12.4	12.0
	Standard deviation		2.12	2.26	1.87	2.46	2.22	1.88
	Number of sample		30	41	53	45	48	48
Week 4	Number of larvae	day 21	60	60	60	60	60	60
		day 28	50	46	52	56	59	51
	Survival rate (%)		83.3	76.7	86.7	93.3	98.3	85.0
	Mean TL (mm)		19.1	19.2	18.7	18.4	17.6	18.9
	Survival deviation		2.80	2.98	3.07	2.63	2.24	2.45
	Number of sample		50	46	52	56	59	51

Table 12. The record on rearing of larvae of *Lates calcarifer* for first two week in experiment 3.

Day after hatching	Number of fotifer fed (/mℓ)	Water exchange (%)	Water temp. (°C)	Remarks
0	-	-	28	stocked 500 newly hatched larvae
1	-	-	28	
2	5	-	28.5	
3	10	40	28.5	
4	10	50	28.5	
5	10	50	28	
6	10	50	27.5	
7	10	50	27	
8	15	50	26.5	
9	15	50	27.5	
10	15	50	28	dead fish found: 1 in B-3
11	20	50	28	
12	20	50	28	
13	25	50	28	
14	first measurement			

Table 13. The record on rearing of larvae of *Lates calcarifer* for third week in the experiment 3.

Day after hatching	Number of food organisms (/mℓ) rotifer	*Artemia*	Water exchange (%)	Water temp. (°C)	Remarks
14	25	-	100	28	
15	25	0.5	70	28	
16	20	1.0	70	28.5	
17	15	2.0	80	26.5	
18	15	2.0	80	26	
19	10	3.0	80	27	
20	5	4.0	80	28	
21	second measurement				

Table 14. The record on rearing of larvae of *Lates calcarifer* for last week in the experiment 3.

Day after hatching	Feeding amount *Artemia* (/mℓ)	formula-food (g)	Water exchange (%)	Water temp. (°C)	Remarks
21	4	-	100	28.5	
22	4	-	85	28.5	
23	4	-	85	28	
24	3	0.5	85	28.5	
25	2	0.5	85	28.5	
26	1	0.5	85	29	
27	1	0.5	85	29.5	
28	end of experiment				

Growth and Survival of Newly Hatched Larvae of Seabass, *Lates calcarifer*, in Starved Condition

Pairat Kosutarak and Tatsuo Watanabe

Introduction

It is important to know when the newly hatched larvae start to eat, for then the time to start feeding is grasped. Therefore, the growth of larvae under feeding and not-feeding conditions was compared, and the process of survival in starved condition was also examined for the seabass, *Lates calcarifer*.

Materials and Methods

Newly hatched larvae were collected from the spawning tank on the morning of April 1, 1983, for examination of their growth in a starved condition. About 2,000 larvae were kept in the 30 ℓ polycarbonate tank (20 ℓ of water volume) without aeration. Eleven to thirty larvae were sampled for the time to time and kept in a 5% formalin solution till 120 hours after hatching. Meanwhile, larvae fed with rotifer were sampled from the seed production tank, stocked with the same egg group, from 54 hours to 125 hours after hatching. Then their total length was measured by the profile projector at 50 magnifications on September 15 - 20.

The survival of newly hatched larvae in starved condition was examined from the night of April 3. Two hundred eggs were stocked in a 2 ℓ glass beaker, then dead larvae were counted.

Results

The growth rate comparison of newly hatched larvae, those which were fed with rotifers and those not fed at all, is shown in Fig. 1. The water temperature was 27 - 30°C. The larvae started to grow from just after hatching (total length 1.60 ± 0.04mm), then they grew fast till about 24 hours after hatching (2.34 ± 0.07mm). The egg yolk was almost absorbed by 24 hours, so that they grew little from 24 hours to about 50 hours when the mouth opened (2.45 ± 0.05mm). After that, they did not grow, and their total length began to shrink at about 120 hours after hatching (2.37 ± 0.07mm) when many larvae started to die. Meanwhile, the larvae fed with rotifer started to grow fast again after opening the mouth, and the difference in body size was apparent 3 days after hatching.

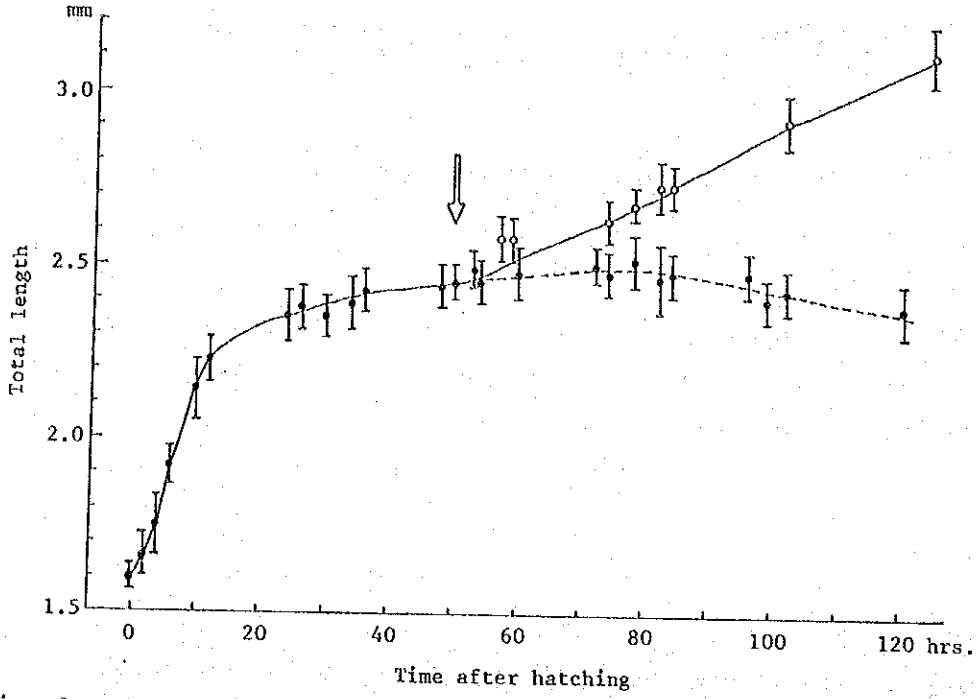

Fig. 1. Growth in total length of newly hatched larvae of seabass fed with rotifer (open circle) or kept without food (closed circle). Vertical bar represents the standard deviation of total length. Arrow shows the time when mouths of all larvae opened.

The process of survival of newly hatched larvae is shown in Fig. 2. High mortality was observed at 100 - 120 hours (4 - 5 days) after hatching, which was just the same time as the oil globule was almost absorbed.

Therefore, proper time to start feeding is from 2 days after hatching, when the mouths of larvae open.

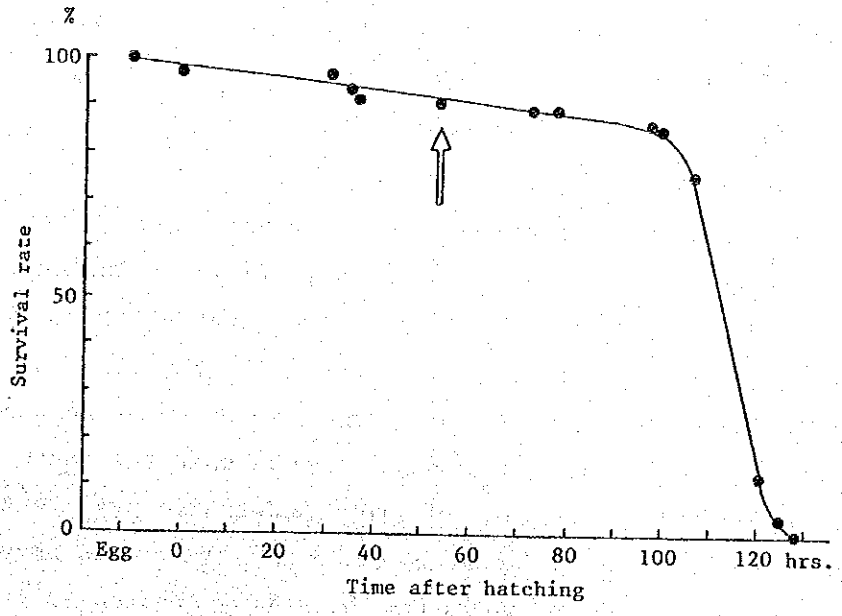

Fig. 2. Survival rate of newly hatched larvae of seabass in starved condition. Arrow shows the time when mouths of all larvae opened.

Shrinkage of Body by Preservation in Formalin Solution in Egg and Larval Stages of Seabass, *Lates calcarifer*

Pairat Kosutarak and Tatsuo Watanabe

Introduction

In studying the growth or development of the larval stage, it is convenient to observe formalin samples. However, it is necessary to determine the size of larvae in a fresh condition. Therefore, the ratio of shrinkage by 5% of formalin solution was examined for egg and larvae of seabass, *Lates calcarifer*.

Materials and Methods

From May 31 to June 20, 30 individual samples of fresh eggs and larvae were measured, when anesthetized by 20 ppm of quinaldin, and preserved in 5% of formalin solution. Then they were measured again on September 20 - 23.

Results

In comparing fresh eggs to eggs preserved a 5% formalin solution, the diameter was 0.74 ± 0.02 mm in fresh samples, but 0.76 ± 0.02 mm in preserved samples. The swelling was 2.7% and this difference is statistically significant.

In the case of preserved larvae, the ratio of shrinkage in total length was changed depending on growth and development. From 0 to 12 days after hatching when total length is less than 40 mm, 8.5% shrinkage was recognized, from 13 to 16 days after hatching when total length is between 4.0 mm and 6.0 mm, 5.5% and from 17 to 20 days after hatching, when total length is more than 6.0 mm, 3.5% shrinkage was recognized (Table 1, and Fig. 1.). The difference in total length of fresh samples and preserved samples is statistically significant for fry younger than 12 days old.

Table 1. Total length (mm) of fresh and preserved samples of larvae of seabass.

Day after hatching	Fresh samples			Size of sample	Reserved samples			% of shrinkage
	mean	s.d.	range		mean	s.d.	range	
0	2.11	0.06	1.98 - 2.24	29	1.92*	0.06	1.80 - 2.04	9.0
1	2.34	0.07	2.20 - 2.46	30	2.23*	0.06	2.10 - 2.36	4.7
2	2.50	0.05	2.36 - 2.50	11	2.31*	0.06	2.20 - 2.40	7.6
3	2.62	0.09	2.40 - 2.84	-	-	-	-	-
4	2.71	0.12	2.40 - 2.90	27	2.36*	0.17	2.80 - 2.74	12.9
5	2.86	0.11	2.66 - 3.20	30	2.65*	0.11	2.42 - 2.92	7.3
6	3.01	0.21	2.54 - 3.28	30	2.76*	0.18	2.30 - 3.02	8.3
7	3.22	0.25	2.66 - 3.80	29	2.89*	0.24	2.26 - 3.40	10.2
8	3.29	0.23	2.76 - 3.68	30	2.99*	0.18	2.62 - 3.32	9.1
9	3.50	0.23	2.92 - 3.90	30	3.19*	0.21	2.76 - 3.56	8.9
10	3.79	0.34	2.76 - 4.36	29	3.50*	0.33	2.60 - 4.06	7.7
11	3.73	0.23	3.36 - 4.22	28	3.42*	0.23	2.98 - 3.80	8.3
12	4.01	0.35	3.32 - 4.70	30	3.69*	0.33	3.08 - 4.34	8.0
13	4.34	0.54	3.30 - 5.76	30	4.10	0.51	3.12 - 5.34	5.5
14	4.55	0.54	3.45 - 5.60	30	4.31	0.55	3.20 - 5.28	5.3
15	5.05	0.59	3.85 - 6.45	30	4.77	0.57	3.64 - 6.00	5.5
16	5.52	0.65	4.00 - 6.70	30	5.21	0.67	3.70 - 6.45	5.6
17	6.03	0.58	4.75 - 7.00	30	5.78	0.57	4.55 - 6.90	4.1
18	6.46	0.68	4.35 - 7.65	30	6.21	0.67	4.30 - 7.50	3.9
19	7.01	0.89	5.20 - 8.60	30	6.77	0.92	4.95 - 8.80	3.4
20	7.36	0.92	5.65 - 9.10	30	7.13	0.97	5.30 - 9.00	3.1

*: Statistically significant difference in comparison with mean total length of fresh and stored samples.

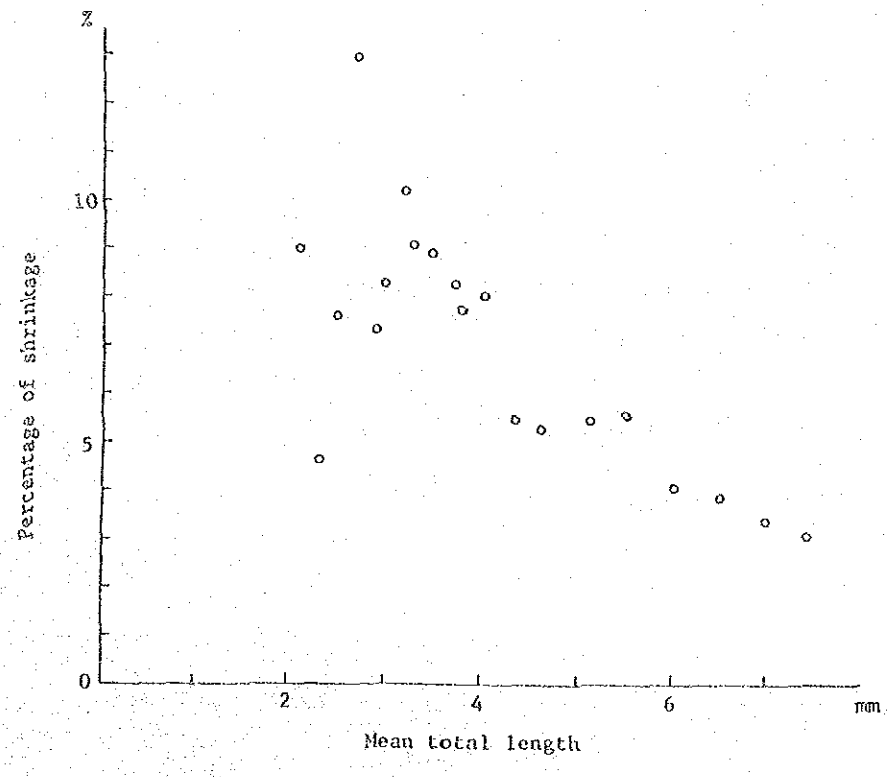

Fig. 1. Relationship between mean total length of fresh samples and percentage of shrinkage of preserved sample of larvae of seabass.

Effect of Handling on Survival in Larval Stage of Seabass, *Lates calcarifer*

Tida Pechmanee and Tatsuo Watanabe

Introduction

When the larvae of seabass, *Lates calcarifer*, were collected, high mortality was observed depending on the size of larvae and the methods of handling. Therefore the experiment was carried out to discover the effects of handling and the size of larvae which can tolerate handling.

Materials and Methods

About 70,000 newly hatched larvae were stocked in a 0.5 ton polycarbonate tank on June 1, 1983, then larvae were taken out by three kinds of handling methods every day till 20 days after hatching. The handling methods were as follows:

A) Gathered larvae slowly in 63μ mesh handling net then ladled them with water into small beaker.

B) Gathered by same means as method A, but collected in 100μ mesh small hand net, then put into a one liter beaker immediately.

C) Gathered and collected by same means as method B, but taken out of water as much as possible with small hand net, then exposed for 15 seconds before putting into beaker.

40 - 150 larvae were collected by each method and kept in one liter beakers for 3 - 4 hours, then the number of dead and surviving larvae were counted. The total length of 30 individuals, which were collected by method A, were measured when anesthetized by 20 ppm of quinaldin to confirm the size of larvae.

Results

The mortality rates by different methods of handling larvae each day are shown in Fig. 1, and the size of larvae are shown in Table 1.

When the larvae were touched with a net and exposed in air, their bodies bent, then died. In the case of method A, the larvae were always collected with water, so that they showed low mortality (0 - 8%). In the case of method B, the mortality was lower (0 - 42%) than method C, because water still remained in

the hand net for the short time of handling. However, in the case of method C, high mortality (47 - 98%) occurred during the 2 - 6 days after hatching. In every method, highest mortality was recognized during 2 - 6 days after hatching (TL 2.5 - 3.0 mm), so that careful handling is required during that period. The mortality of every method is still high during 7 - 12 days after hatching, but after that (TL more than 4 mm), larvae could tolerate every method.

According to these results, it is better to handle larvae on day 0 - 1 or later than 7 days after hatching.

Table 1. Body size in total length of larvae of *Lates calcarifer* on each day after hatching during experiment

Day after hatching	Size of larvae in total length(mm) mean	s.d.	range
0	2.11	0.06	1.98 - 2.24
1	2.34	0.07	2.20 - 2.46
2	2.50	0.05	2.36 - 2.50
3	2.62	0.09	2.40 - 2.84
4	2.71	0.12	2.40 - 2.90
5	2.86	0.11	2.66 - 3.20
6	3.01	0.21	2.54 - 3.28
7	3.22	0.25	2.66 - 3.80
8	3.29	0.23	2.76 - 3.68
9	3.50	0.23	2.92 - 3.90
10	3.79	0.34	2.76 - 4.36
11	3.73	0.23	3.36 - 4.22
12	4.01	0.35	3.32 - 4.70
13	4.34	0.54	3.30 - 5.75
14	4.55	0.54	3.45 - 5.60
15	5.05	0.59	3.85 - 6.45
16	5.52	0.65	4.00 - 6.70
17	6.03	0.58	4.75 - 7.00
18	6.46	0.68	4.35 - 7.65
19	7.01	0.89	5.20 - 8.60
20	7.36	0.92	5.65 - 9.10

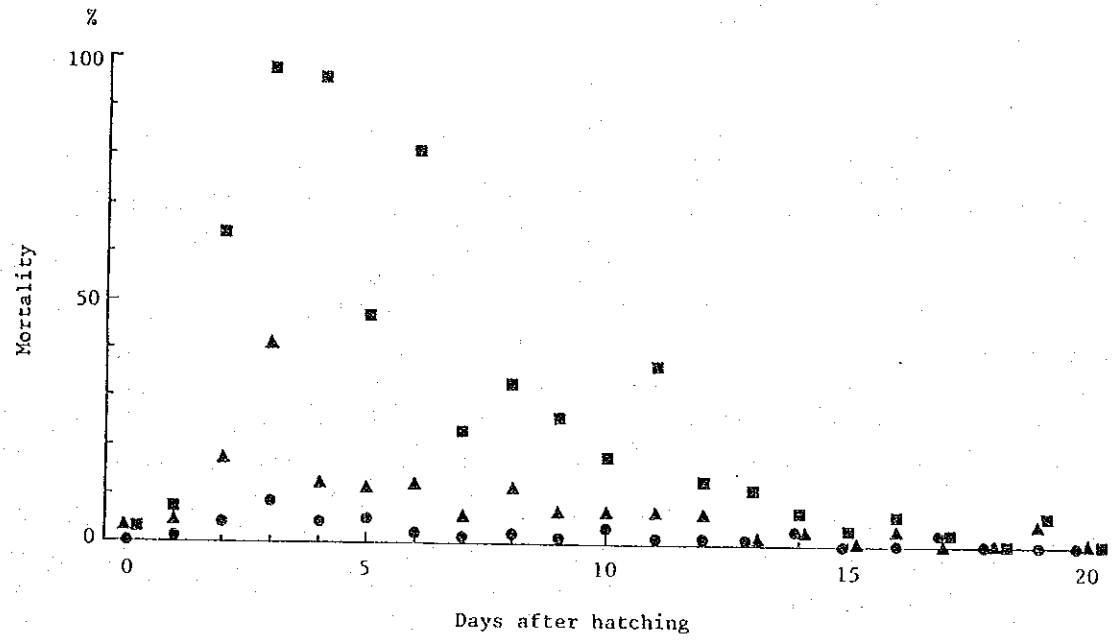

Fig. 1. Mortality of larvae of seabass for different methods of handling. Circle: collected with water in small beaker. Triangle: collected with water by 100μ mesh net. Square: collected by 100μ mesh net and kept out of water for 15 seconds before put into beaker.

Effect of Supplemental Vitamins on Feeding Fish Meat to Juvenile Seabass, *Lates calcarifer*

Pairat Kosutarak

Introduction

Up to the present, fishfarmers rearing seabass, *Lates calcarifer*, in Songkhla and other areas have faced many problems. One of the big problems is the high mortality of juvenile seabass during their nursery rearing. Juveniles are fed with minced fish meat. However, their feeding activity usually becomes lower after rearing for several weeks and they finally die.

As a cause of this problem, we supposed that some vitamins may be lacking in fish meat, depending on the quality of the meat itself or on its method of preservation. Therefore, we carried out an experiment on rearing juvenile seabass by feeding them minced fish meat with vitamins added (2% vitamin mixture, Halver 1957) and without vitamin added.

Materials and Methods

Experimental Fish: About 300 juvenile seabass were obtained in September 1983 from the hatchery at NICA. They were kept in a 500 ℓ tank and maintained on minced fish meat for one week, then sorted again at the start of experiment.

Feeding and Testing Method: A glass aquarium, 497 x 257 x 295 mm, was used, which was supplied with well seawater at the rate of 0.28 - 0.35 ℓ/min. and aerated sufficiently. The volume of seawater in each aquarium was about 32 ℓ. Twelve fish were put into each aquarium and fed twice a day, except on measuring day when they were fed only once. Feeding was continued until fish were satiated. The waste was siphoned off before each feeding. 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. Range of water temperature and salinity in aquariums in each week are shown in Table 1.

Test Diet: The diets consisted mainly of minced fish meat which was prepared every day. The details of the diets are shown in Tables 2 and 3.

Table 1. Range of temperature and salinity in aquariums in each week.

(Temperature: °C, Salinity: ‰)

Week	1	2	3	4	5	6	7	8	9
Temperature at 930-1000	26.0-27.9	26.2-28.0	27.1-27.9	26.8-28.0	26.9-27.8	27.0-28.0	27.2-28.0	27.0-27.5	26.0-27.0
Temperature at 1530-1600	27.6-29.8	27.4-29.1	28.1-29.3	28.0-29.4	28.0-28.9	28.0-29.2	28.8-29.6	28.0-28.9	27.0-28.5
Salinity	34	33-34	33-34	33-34	33-34	33	33	28-33	26-29

Table 2. The details of diets in each lot.

Lot No.	week 0-1	week 1-2	week 2-3	week 3-4	week 4-5	week 5-6	week 6-7	week 7-8	week 8-9
F-1	×	×	×	×	×	●	●	●	■
F-2	×	×	×	×	×	×	×	×	●
V-1	●	●	●	●	●	●	●	●	○
V-2	●	●	●	●	●	●	●	●	●

× minced fish meat

● minced fish meat +2% vitamin mixture (Halver., 1957)

■ minced fish meat +2% vitamin mixture (Halver., 1957) mineral mixture (USP XII Salt mixture No.2., Halver 1957)

○ minced fish meat +2% vitamin mixture (Halver., 1957) +2% cod liver oil.

Table 3. The kind of fish used for feeding

Date	Name of fish
23 Sept. - 10 Oct. '83	*Caranx (Selaroides) leptolepis*
11 Oct. - 24 Oct. '83	*Lutjanus vitta*
25 Oct. - 26 Oct. '83	*Sauride tumbil*
27 Oct. - 3 Nov. '83	*Lutjanus vitta*
4 Nov. '83	*Scolopsis dubiosus*
5 Nov. - 7 Nov. '83	*Lutjanus vitta*
8 Nov. - 9 Nov. '83	*Scolopsis dubiosus*
10 Nov. - 18 Nov. '83	*Saurida tumbil*
19 Nov. - 21 Nov. '83	*Scolopsis dubiosus*
22 Nov. - 24 Nov. '83	*Rastrelliger brachysoma*

Results and Discussions

The effect of the supplement of vitamins of the growth of the fish is already observable from 2 - 3 weeks after the experiment was started (Table 4). The phenomena of vitamin deficiency is as follow:

1) food intake and swimming activity decrease
2) body color becomes black
3) caudal fin loosens
4) dies

Table 4. Mean and standard deviation of body weight (g).

Lot No.	Initial	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9
F-1	0.25±0.03	0.56±0.10	0.93±0.18	1.19±0.26	1.47±0.31	2.12±0.48	3.26±0.69	4.55±0.96	6.53±1.43	8.50±1.83
F-2	0.26±0.04	0.56±0.10	0.92±0.17	1.24±0.32	1.44±0.45	1.78±0.43	2.01±0.51	2.25±0.65	2.63±0.89	3.27±1.08
V-1	0.27±0.05	0.60±0.12	1.12±0.15	1.91±0.31	2.67±0.41	3.62±0.51	5.00±0.82	6.38±1.20	8.23±1.52	10.23±2.20
V-2	0.26±0.06	0.52±0.13	0.93±0.28	1.62±0.47	2.34±0.66	3.27±0.89	4.54±1.28	5.97±1.73	7.45±2.21	9.01±2.65

A recovery test was done for F-1 at the beginning of week 5. The fish became more healthy in a short time. Growth rate was increased very quickly, there was a good conversion rate and no mortality. For F-2, the recovery test was done at the beginning of week 8 and the results were the same (Fig. 1).

We tried to add a 2% mineral mixture in F-1 and 2% cod liver oil in V-1 at the beginning of week 8, but the results were not very clear so we must experiment again.

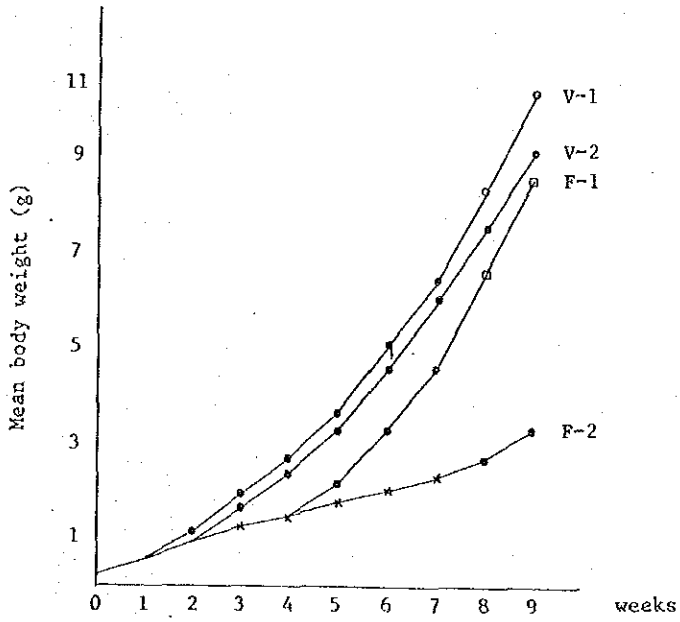

Fig. 1. The growth of juvenile seabass fed with feed of different composition.

—o— 2% vitamin mixture
—x— no vitamin added
—□— 2% vitamin mixture + 2% mineral mixture
—o— 2% vitamin mixture + 2% cod liver oil

Table 5. Food conversion rate.

Lot No.	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9
F-1	2.14	2.98	4.58	6.36	3.40	2.98	3.44	2.84	3.37
F-2	2.54	2.88	4.25	7.09	17.33	**	**	**	4.62
V-1	2.43	2.66	3.31	3.74	3.43	3.02	3.51	6.68	5.13
V-2	2.61	2.88	3.47	3.68	3.49	3.12	3.49	4.19	4.23

** weight gain is minus.

Table 6. Survival rate (%)

Lot No.	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9
F-1	100	100	100	91.7	91.7	91.7	91.7	91.7	91.7
F-2	100	100	100	100	75	66.7	50	50	50
V-1	100	100	100	100	100	100	91.7	83.3	83.3
V-2	100	100	100	100	100	100	100	100	100

Effect of the Protein Level in Formula Diet for Seabass, *Lates calcarifer*

Pairat Kosutarak

Introduction

The optimum protein content is one of the most essential factors in developing an economical and efficient diet formula for fish cultures. The present experiment was carried out to study the effects of various protein levels in diet formulae for seabass, *Lates calcarifer*.

Materials and Methods

Experimental fish: About 300 fish were obtained in October, 1983, from the hatchery at NICA. They were kept in a 500 ℓ tank and maintained on trash fish and then formula diet in that order. After the fish became accustomed to the formula diet (43.6% protein diet as shown in Table 1) for one week, they were sorted again for the start of the experiment.

Feeding and testing method: A glass aquarium, 51 x 27 x 30 cm, was used, which was supplied with well seawater at the rate of 0.25 - 0.36 ℓ/min. and aerated sufficiently. Ten fish were put into each aquarium for each experimental diet and fed twice a day, except on measuring day when they were fed only once. The pasty mixture was extruded into the tank as a moist, fine noodle from a 12 mℓ nylon syringe without a needle. The waste was siphoned off before each feeding. Fish were weighed individually at intervals of 1 week by anesthetizing with a 20 ppm quinaldine solution. The feeding experiment was continued for 8 weeks.

Test diet: The diets used in this experiment are shown in Table 1. Six kinds of diet with different crude protein content ranging from 10.8% to 62.3% were tested. The level of protein content was adjusted by changing the mixing rate of fish meal, casein and dextrin.

New mixture were prepared every week. After preparation, the mixtures were stocked in a refrigerator at 5°C until used.

Table 1. Composition of test diets

Ingredients/Lot No.	1	2	3	4	5	6
Fish meal	21	50	73	55	36	18
Casein	54	25	–	–	–	–
Dextrin	–	–	2	20	39	57
C.M.C	6	6	6	6	6	6
Vitamin mixture*	4	4	4	4	4	4
Mineral**	7	7	7	7	7	7
Cod liver oil	3	3	3	3	3	3
Corn oil	5	5	5	5	5	5
Water	140	140	140	140	140	140
Crude protein(%)	62.3	50.3	43.6	33.1	22.6	10.8

* Vitamin mixture as reported by Halver (1957)

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

Results

The initial and final body weight, percent gain of body weight, mortality during the experiment and approximate composition of experimental fish are shown in Table 2. Growth curves of fish in the experiment period are shown in Fig. 1 for six kinds of diet. It is obvious that the diet with the highest crude protein content produced the fastest growth of fish. Growth becomes poorer as the protein content becomes lower. The mortality rate does not show any relation to the protein content of the diet. Crude protein content seems to be a little higher in fish fed with a high protein diet than in fish fed with a low protein diet, though lots No. 1 and 2 show reverse results.

The food conversion rates of each diet at the end of each week are shown in Table 3. The diet of highest protein content always shows the smallest food conversion rate. The food conversion rate almost always becomes larger as protein content becomes lower.

As a result of the experiment, it can be said that a crude protein content as high as 62.3% seemes to be required for the fastest growth of the fish with the lowest food conversion rate.

Table 2. Results of feeding experiment for 8 weeks

Lot No.	1	2	3	4	5	6
Mean body weight (g):						
Initial	0.88	0.84	0.89	0.90	0.93	0.91
Standard error	0.12	0.08	0.12	0.09	0.11	0.10
Final	12.11	11.07	7.91	6.09	3.69	1.51
Standard error	4.46	2.90	3.97	2.11	1.33	0.46
Percent gain (%)	1276	1218	789	577	297	66
Mortality (%)	10	0	0	0	0	10
Approximate composition of fish:						
Moisture (%)	74.0	73.3	74.2	73.9	72.9	74.1
Crude fat (%)	13.9	15.0	15.3	15.1	20.4	16.8
Crude protein (%)	16.7	17.2	16.3	16.1	15.7	15.2

Table 3. Food conversion rate*

Lot No.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
1	3.18	2.56	2.29	2.62	2.84	2.37	2.85	3.09
2	4.93	4.16	4.10	3.54	2.99	2.89	2.80	3.12
3	9.55	8.43	9.20	7.86	6.62	5.68	5.90	6.47
4	8.75	9.54	8.20	8.48	7.74	7.49	6.46	7.97
5	12.67	14.56	11.79	14.71	13.94	11.29	10.48	12.35
6	45.00	226.88	81.96	40.40	120.24	33.07	40.53	33.75

* Food conversion rate = $\frac{\text{Feed intake}}{\text{weight gain}}$

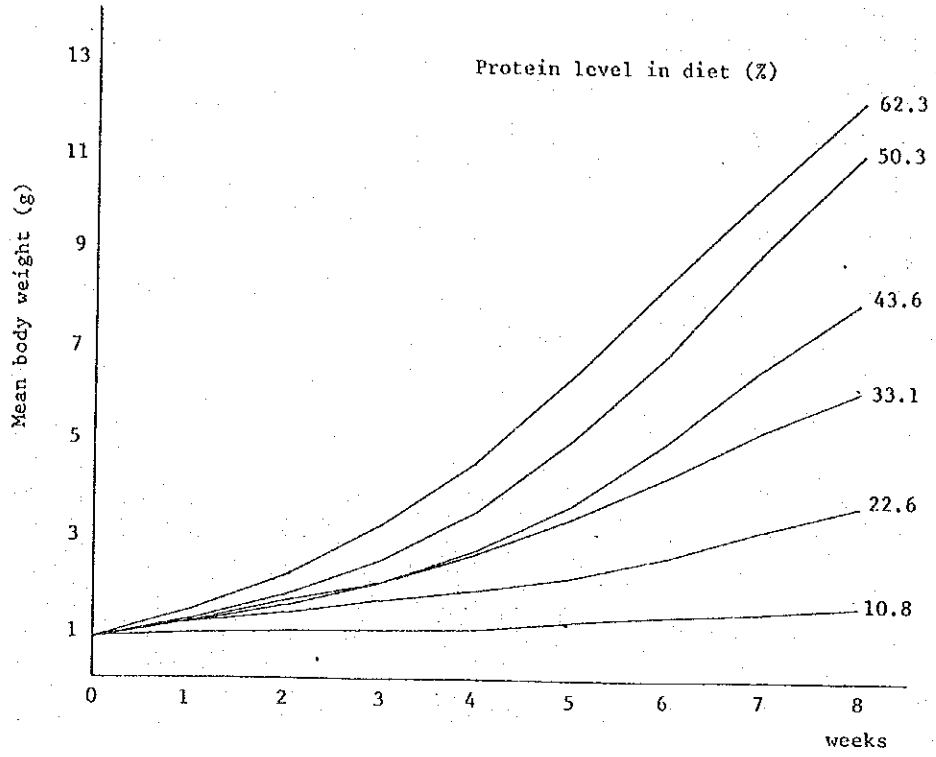

Fig. 1. The growth of seabass fed with diets of different protein content.

Feeding Experiments on Rearing the Early Stage of Seabass, *Lates calcarifer*, Using Rotifer, *Brachionus plicatilis*, Frozen Rotifer and Boiled Egg Yolk as Food

Tida Pechmanee, Prakit Kraisingdecha and Niwes Ruangpanit

Introduction

The normal food for rearing seabass, *Lates calcarifer*, larvae just starting to eat is rotifer, *Brachionus plicatilis*, but it is not a perfect food source. Its production depends on a wide variety of factors. It is rather difficult to produce enough rotifer if the conditions are not good and, like all live food supplies, it takes time to produce. This experiment was carried out to study the possibility of using nonliving food to rear seabass larvae, instead of the rotifer.

Materials and Methods

The experiment was performed twice, in 30 ℓ plastic tanks, each containing 500 larvae in 25 ℓ of water. In the first experiment larvae 4 days old were used, and in the second experiment, newly hatched larvae. Live rotifer, boiled egg yolk of chicken, and frozen rotifer were fed to seabass larvae in the experimental tanks, and 50% of the water was changed each day.

Food Preparation: The rotifer was first filtered, then put into water containing *Tetraselmis* for 15 minutes. Afterwards, it was checked for density and the amount needed for the day's feeding was calculated. To prepare frozen rotifer a certain number of rotifer prepared in the same way were packed in plastic bags and frozen. The egg yolk was first boiled, then forced through a 105 μ sieve. Afterwards, it was washed, checked for density, and the amount needed was calculated.

Results

Growth in the first experiment: The experiment was finished in 6 days when the larvae were 10 days old. Food supplied in experimental tanks are shown in Table 1. The growth of larvae fed with the 3 different kinds of food is shown in Table 2 and Fig. 1. Larvae fed with living rotifer increased 0.970 mm. in total length, with egg yolk 0.315 mm, and with frozen rotifer 0.205 mm. The growth of larvae fed with live rotifer and of those fed with egg yolk or frozen rotifer were significantly different.

Table 1. Food supply in experimental tanks for the first experiment.

Tank	Food density (per mℓ) in each day					
	1	2	3	4	5	6
Tank R	20	20	20	20	25	30
Tank F	30	37	25	25	25	25
Tank E	30	37	25	20	30	35

Tank R: Larvae fed with living rotifer.
Tank F: Larvae fed with frozen rotifer.
Tank E: Larvae fed with boiled egg yolk.

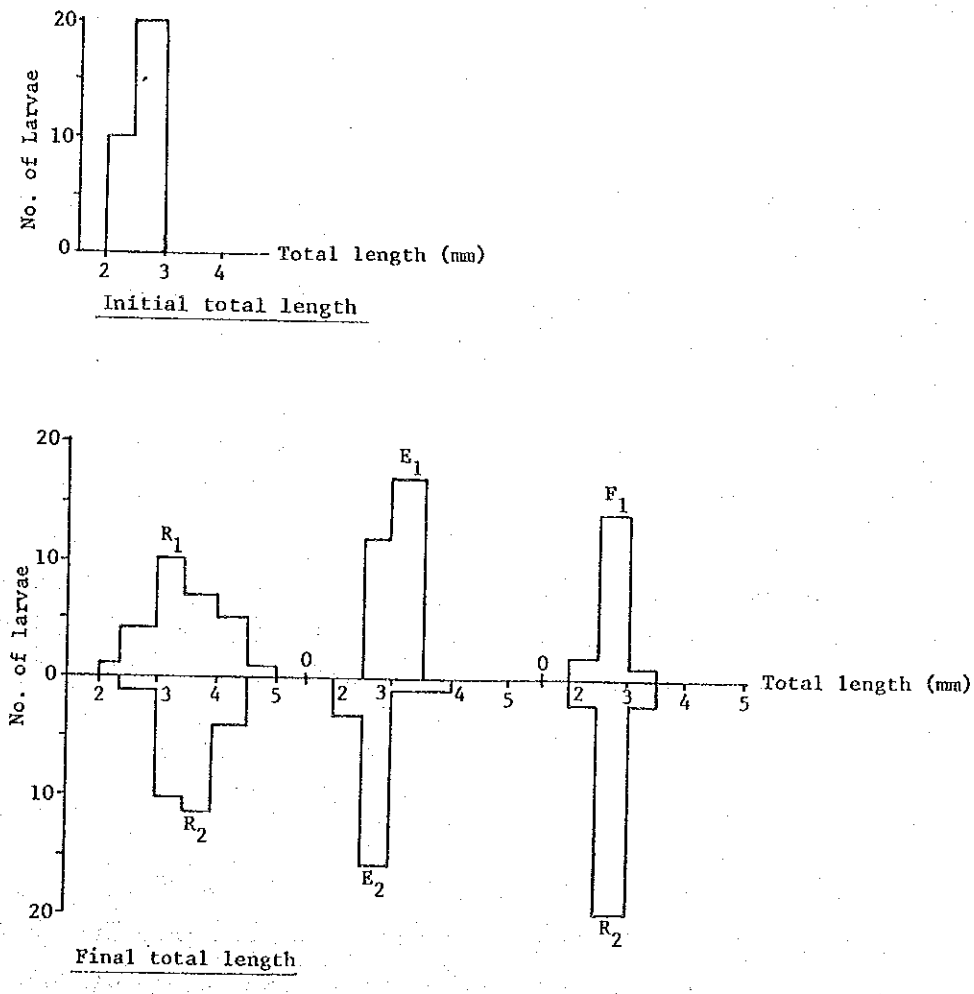

Fig. 1. Size distribution of experimental larvae in the first experiment.

Table 2. Sizes and survival rates of experimental larvae for the first experiment.

Tank	Total length (mm) at different age (days)				Increasing of TL.(mm)	Survival rate (%)
	4	6	8	10		
Tank R_1	2.64	2.96	3.20	3.58	0.94	52.0
Tank R_2	2.64	2.93	3.35	3.64	1.00	44.0
Tank F_1	2.64	2.69	2.86	2.79	0.15	4.0
Tank F_2	2.64	2.76	2.80	2.90	0.26	11.4
Tank E_1	2.64	2.67	3.05	3.12	0.48	48.4
Tank E_2	2.64	2.72	3.08	2.79	0.15	19.8

Growth in the second experiment: Food supplied in the experimental tank are shown in Table 3. After 7 days of this experiment, when the larvae were 9 days old, larval growth (Table 4, Fig. 2) was 0.87 mm - 1.16 mm for those fed with live rotifer, 0.17 mm - 0.25 mm for frozen rotifer and 0.01 mm - 0.16 mm for egg yolk. Again, the growth of larvae fed with living rotifer and of those fed with egg yolk or frozen rotifer were significantly different. The growth of larvae of 12 days and 14 days old were also very poor when fed with egg yolk or frozen rotifer.

Table 3. Food supply for larvae during second experiment

Tank	Food density (per mℓ) in each day												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Tank R	5	7	12	20	25	30	30	30	35	35	35	40	40
Tank F	5	10	15	20	20	25	30	30	30	30	-	-	-
Tank E	5	7	12	12	15	20	25	20	25	25	-	-	-

Survival rate in the first experiment: The highest survival rate (48.0%) was for larvae fed with live rotifer, and the lowest (7.7%) was for those fed with frozen rotifer (Table 2).

Survival rate in the second experiment: After 7 days in this experiment, the highest survival rate (64.7%) was for larvae fed with live rotifer, and the survival rates for those fed frozen rotifer and egg yolk were 50.8% and 24.4%, respectively (Table 4). After 10 days, the survival rate for larvae fed with egg yolk had dropped to 11%, and for those fed frozen rotifer (Tank F_1) to 6.8%. After 12 days, when the larvae were 14 days old, the survival rate for larvae fed with live rotifer was 56.5% and for those fed with frozen rotifer (Tank F_2) 36%.

Table 4. Sizes and survival rates at different ages (days) of experimental larvae started with total length 2.52±0.08 mm in second experiment.

Tank	Total length ± S.D. (mm) 9	12	14	Increasing of T.L. (9 days)	Survival rate (%) 9	12	14
Tank R_1	3.39±0.5	-	6.30±0.7	0.87	57.0		45
Tank R_2	3.68±0.4	-	6.30±0.7	1.16	72.4		68
Tank F_1	2.77±0.5	2.70±0.2	-	0.25	33.2	6.8	-
Tank F_2	2.69±0.2	-	3.2 ±0.2	0.17	68.4	-	36
Tank E_1	2.68±0.2	2.80±0.4	-	0.16	16.8	11.0	-
Tank E_2	2.51±0.2	3.10±0.5	-	-0.01	32.0	11.0	-

Water Quality: The quality of the water in the experimental tanks was nearly the same for both trials. For the first experiment, salinity was between 30.0‰ and 33.1‰ , pH values varied from 8.10 to 8.52, and the water temperature ranged from 28.1°C to 32.9°C. For the second experiment, salinity was between 29‰ and 34‰ , pH values varied from 7.80 to 8.05, and water temperature ranged from 26.5°C to 29.5°C.

Discussion

The seed production of marine fish depends greatly on food organisms, at least for the first 2 - 3 weeks (Girin, 1979). This may be because most economical marine fish larvae are smaller than freshwater larvae that are produced (Nash, 1977). Artificial food has been tried as a substitute for live food for marine fish larvae before, but the techniques for successfully using this kind of feeding remains a big problem (Girin, 1979). Similarly, the results here show high growth and survival rate only for the larvae fed with live rotifer. The growth of larvae fed with egg yolk and frozen rotifer was very slow. It might be that egg yolk lacks growth factors necessary for marine fish, such as fatty acid 20:5 w 3. Also, perhaps because of its minute size, rotifer quickly loses most of its nutritional value when frozen. So, the problem remains of how to maintain the growth and survival rate of seabass larvae using artificial or frozen foods.

The experiment did show, however, that, in case of a lack of rotifer or other live food supply suitable for 2 - 9 day old larvae, frozen rotifer or egg yolk could be used to keep the larvae alive for a short period.

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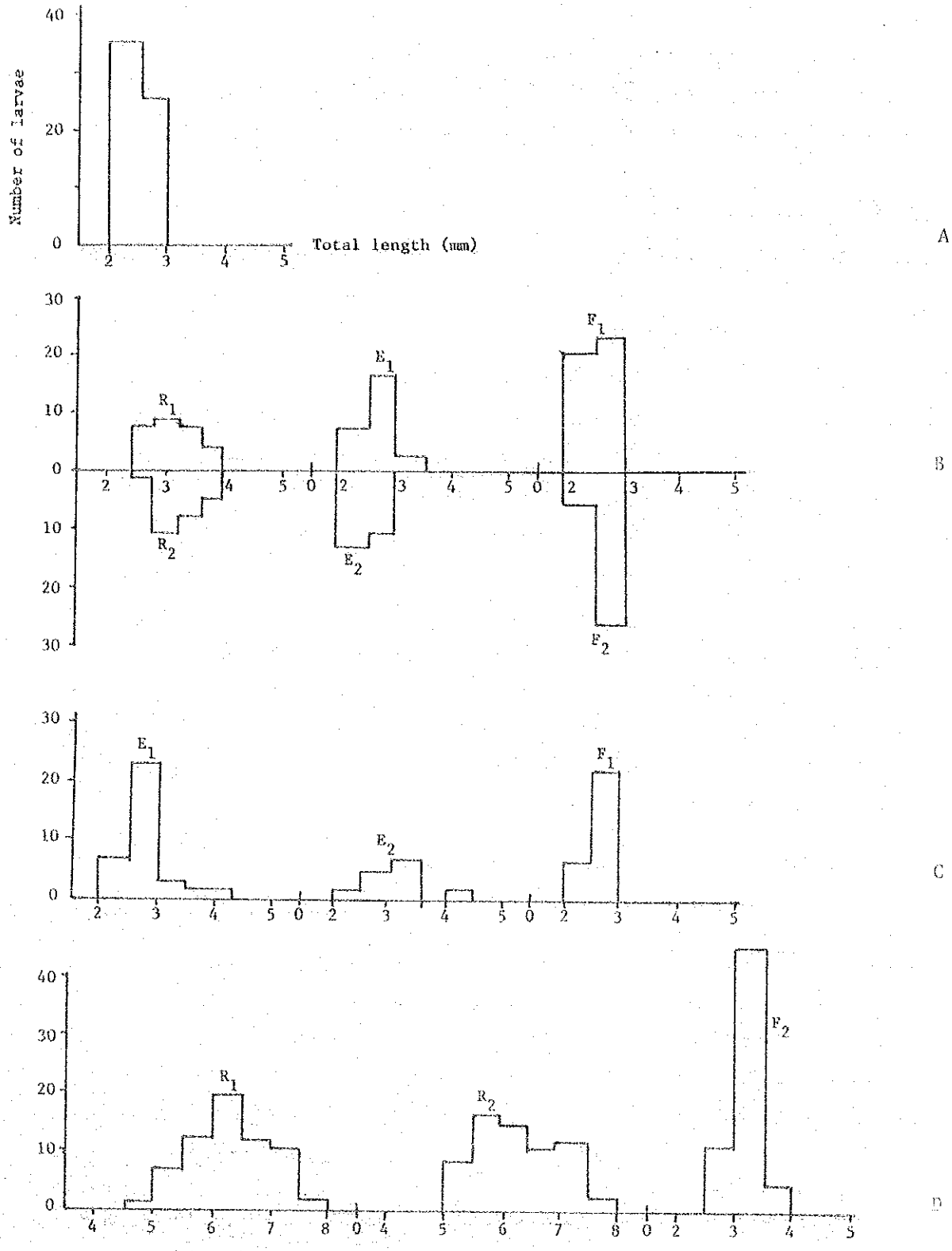

Fig. 2. Size distribution of experimental larvae in the second experiment. A: Initial total length (2 days old). B: On the 8th day of the experiment. C: On the 11th day of the experiment. D: At the end of the experiment.

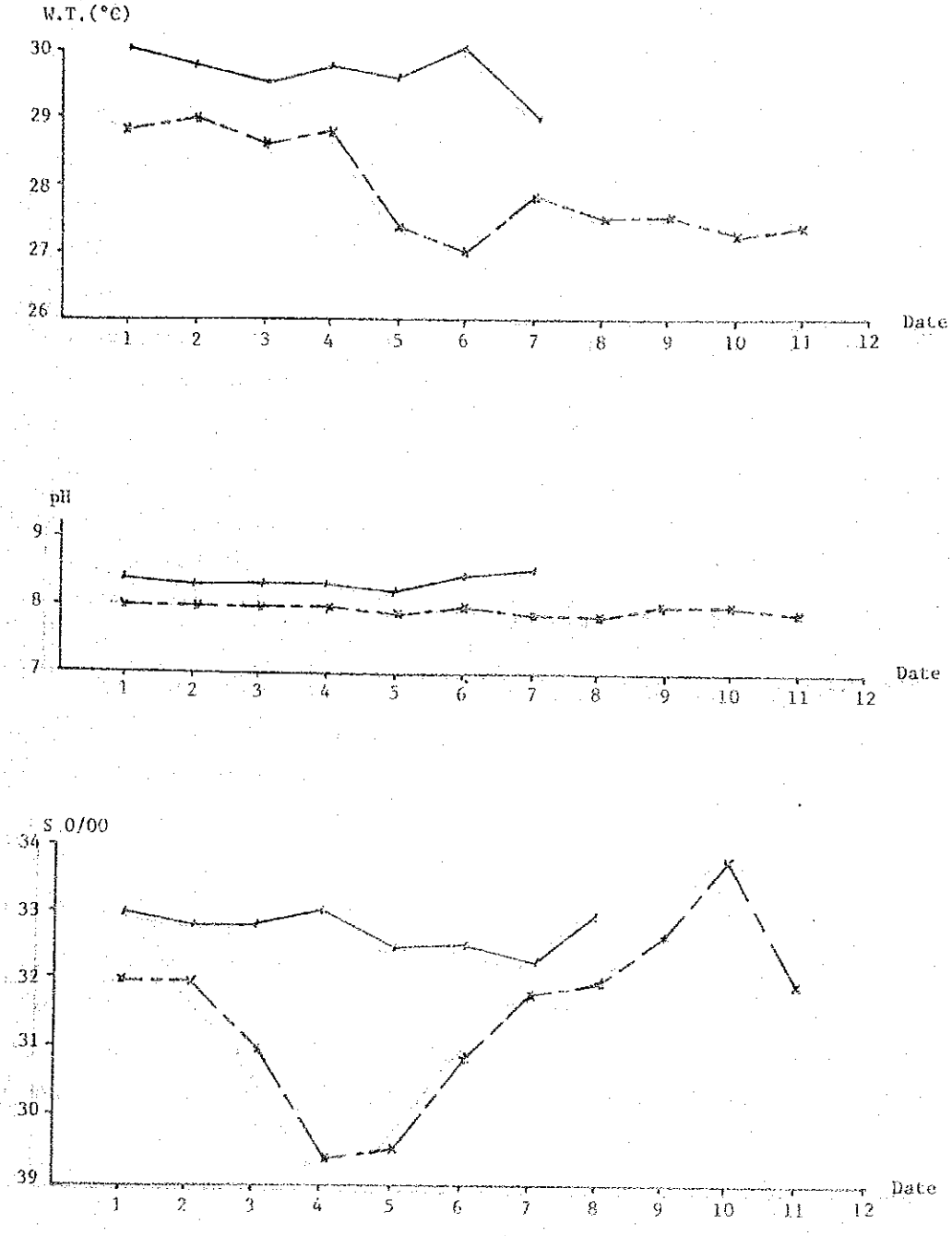

Fig. 3. Environmental conditions of experimental tanks.
——— : First experiment. – – – – – : Second experiment.

Feeding Experiments on Rearing, of 11 - 28 Day Old Seabass Larvae, *Lates calcarifer*, Using Artificial Plankton, Boiled Egg Yolk, and Formula Food

Tida Pechmanee, Paitoon Ugkayanon, and Tanan Tattanon

Introduction

An important factor in the successful seed production of fish is the type of food organism fed to the larvae, especially in the first month. There has always been a problem with a lack of food organisms or their high cost, so attempts have been made to switch larvae to non-living food as early as possible. Artificial food was successfully used to rear 2 - 3 mg, marine shrimp larvae, while European seabass, *Dicentrachus labrax*, were trained to eat artificial food when they were 10 mg of body weight (Girin, 1979). *Pagrus major* are normally fed rotifer, *Brachionus plicatilis*, from day 3 to 20; total length 3.1 - 8.1 mm, but, when there is a lack of rotifer, artificial food is used for larvae of 6.0 to 8.0 mm (Fujita, 1979). Seabass larvae, *Lates calcarifer*, have been trained to eat fish meat when they are 25 days old, or about 10 mm (Sujin et. al., 1978). The present experiment was carried out to study the possibility of using non-living foods instead of nauplius of, brine shrimp, *Artemia salina* for 11 - 28 day old seabass larvae.

Materials and Methods

The experimentwas divided into two trials, each in a series of 30 ℓ plastic tanks containing 100 larvae in 25 ℓ of water. The water temperature and the number of dead larvae were recorded every day. At the end of the experiment, the number of larvae remaining and the size in total length of each larvae were measured.

Trial 1: 11 day old seabass larvae (TL 4.15 mm) were fed with only one kind of food, but at 2 levels. The experimental food was Artificial Plankton B.P., a product of Nippon Formula Food Mfg. Co., Ltd., Japan. Each gram consists of 7.5×10^6 particles, 30 - 130 μ in size. The feeding schedule was: Tanks $P_{1.1}$, $P_{1.2}$ fed 0.03 g/tank/day; Tanks $P_{2.1}$, $P_{2.2}$ fed 0.06 g/tank/day. seventy percent of the water in each tank was changed each day.

In Control tank A, larvae were fed once per day for the first 2 days with rotifers plus nauplius of brine shrimp, at densities of 3 and 1 individuals/mℓ, respectively. Later, brine shrimp, only, was used, once per day, at a density of 2 individuals/mℓ. In control tank N, larvae were not fed. In both control tanks, 70% of the water was changed each day.

Trial 2: Twenty one day old seabass larvae (TL 7.8mm) were fed 3 kinds of food: Artificial Plankton B.P., egg yolk, and Commercial Formula Food for red sea bream. The egg yolk was first boiled, then forced through a 177 μ sieve and washed in a 100 μ scoop net. The size of the egg yolk particles was 59 - 276 μ. The density was checked and the amount needed for feeding was calculated daily. The commercial food was a product of Nihon - Nosan - Kogyo Co., Ltd., Japan. Particle size ranged from 74 - 246 μ, and each gram consisted of 580×10^3 particles.

The feeding schedule was: Tanks P_1, P_2 fed Artificial Plankton B.P., 0.12g/tank/day; Tanks E_1, E_2 fed egg yolk, 30 particles/mℓ; Tanks F_1, F_2 fed Commercial Formula Food No. 1, 0.5 g/tank/day.

Control tank A was fed nauplius of brine shrimp, density 3 individuals/mℓ, one time per day. All other tanks were fed 4 times daily. All tanks had a 70% water change each day.

Results

Trial 1: The water temperature in each tank renged from 27.0°C to 28.5°C, and the larvae for the experiment were 4.15 mm in total length. The results are shown in Tables 1 and 2, and Fig. 1.

All the larvae in the control tanks fed nothing died within 4 days. Those in the control tank fed brine shrimp had a 69.0% survival rate. The tanks fed Artificial Plankton B.P. had a 36.0% rate (in the tank fed 0.03g/day a 28.5% rate, and in the tank fed 0.06g/day a 43.5% survival rate).

Larvae fed with Artificial Plankton B.P. grew very little: 4.40 mm and 4.45 mm for those fed 0.03 g and 0.06 g/day, respectively. Larvae in the control tank fed brine shrimp grew to 8.77 mm in total length.

Table 1. Number of dead larvae each day in the first experiment.

Tank	Number of dead larvae each day							
	1	2	3	4	5	6	7	8
Tank N	10	7	25	33	25	–	–	–
Tank A	13	4	8	2	0	3	1	–
Tank $P_{1.1}$	5	12	14	22	11	3	3	–
Tank $P_{1.2}$	4	19	3	17	20	5	3	2
Tank $P_{2.1}$	8	13	3	10	8	5	0	7
Tank $P_{2.2}$	5	9	4	20	12	5	1	3

Table 2. Survival rate and final total length of the larvae that have an initial total length of 4.15±0.3mm in the first experiments.

Tank	Survival rate (%)	Final total length(mm) ± standard diviation	Range of temperature (°C)
Tank N	0	–	27.0 - 28.5
Tank A	69	8.77 ± 1.2	27.0 - 28.5
Tank $P_{1.1}$	30	4.50 ± 0.4	27.0 - 28.5
Tank $P_{1.2}$	27	4.30 ± 0.3	27.0 - 28.5
Tank $P_{2.1}$	46	4.50 ± 0.4	27.0 - 28.5
Tank $P_{2.2}$	41	4.40 ± 0.5	27.0 - 28.5

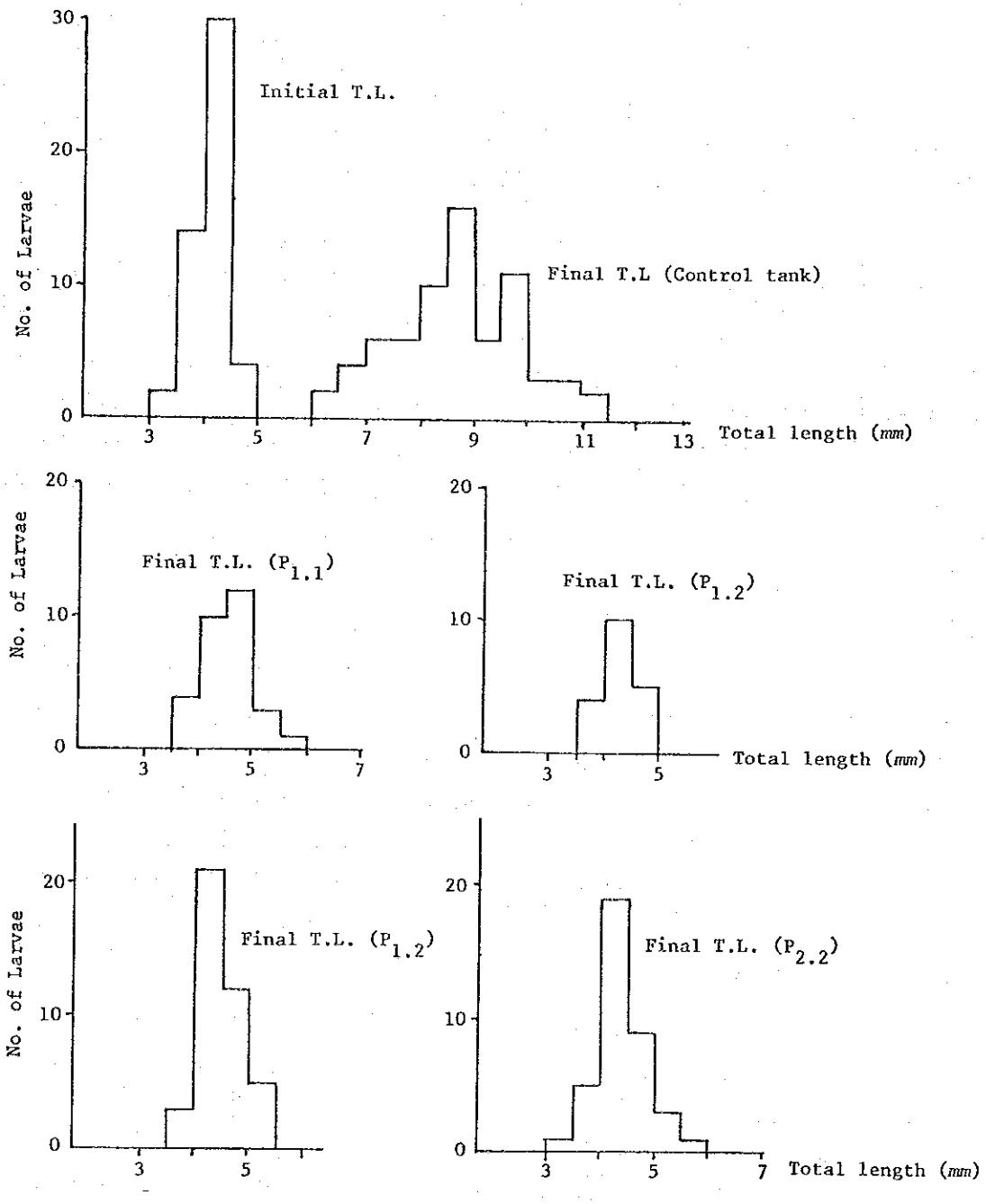

Fig. 1. Distribution of total length of the experimental larvae of seabass at the start and the end of the first experiment.

Trial 2: The water temperature in each tank ranged from 25.5°C to 29.0°C. Larvae for the experiment were 7.8 mm in total length at the start. The results are shown in Tables 3, 4 and in Fig. 2.

During the first two days of the trial, the larvae swam rapidly all the time and did not eat. After that, they recovered and began to eat. At tne end of the experiment, some larvae remained in every tank.

The survival rate in the control tank was 76% and in the tank fed egg yolk 65%. The survival rate of those fed Artificial Plankton B.P. was very different between tanks, with a mean rate of 39.5%. The rate for those fed Commercial Formula Food No. 1 was 24.5%.

The growth of larvae fed with each kind of trial food was similar and very low. At the end of the experiment, the total length of larvae fed with brine shrimp in the control tank was 13.2 mm, compared to the growth of those fed Commercial Formula Food No. 1, 8.4 mm, Artificial Plankton B.P., 7.85 mm, and egg yolk, 7.85 mm. The total length of larvae fed with Artificial Plankton B.P. in tank P_1 and of larvae fed with egg yolk in tank E_2 were equal to each other and less than the total length of larvae at the start.

Discussion

The three kindsof food used in this experiment were smaller in size than the food normally given to larvae of seabass of this age and size. When seabass larvae are 4-10mm in total length, they like to eat nauplius of brine shrimp, which are about 500 μ in size. However, we used Artificial Plankton B.P., 30 - 130 μ, egg yolk, 59 - 276 μ, and Commercial Formula Food No. 1, 74 - 246 μ because we wanted to encourage the larvae to accept these foods so that we could observe the results of Trial 2 clearly. After not eating for 2 days, the larvae tried to eat without spitting out.

Results of Trial 2 show that the total length of larvae fed with Artificial Plankton B.P. in tank P_1 was 7.6 mm, or less than the total length of 7.8 mm the larvae started at. This was because the survival rate for this tank was only 27% and most of the larger larvae died (Fig. 2). In the same way, the total length of larvae fed with egg yolk in tank E_2 was less than at the start.

The results of this experiment show that 11 - 28 day old seabass larvae can be trained to eat non-living food, as seen in the relatively high survival rates which occurred in some treatments, compared to the unfed larvae which died within 4 days (Trial 1). However, the problem of how to maintain growth still remains.

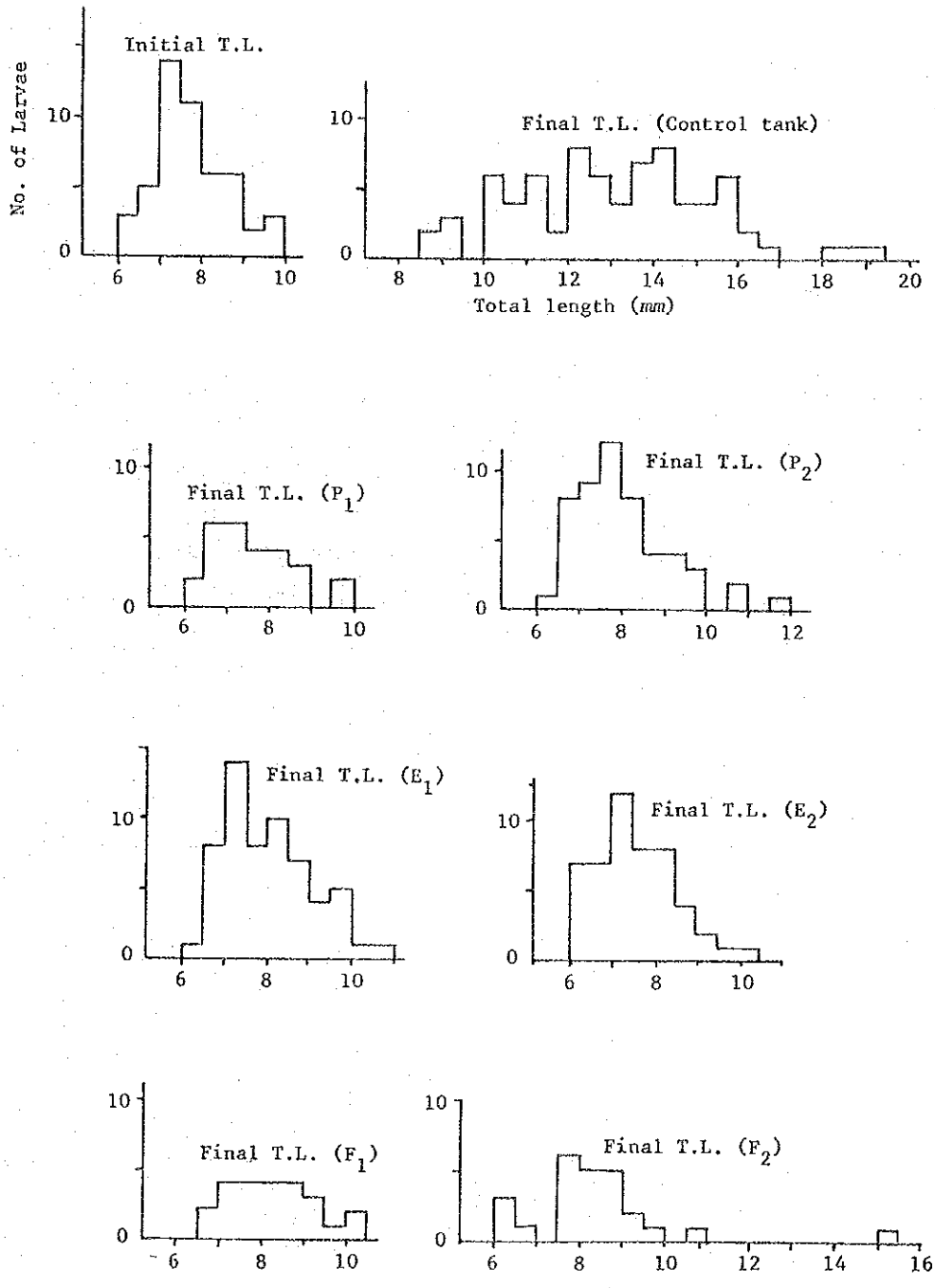

Fig. 2. Distribution of total length of the experimental larvae of seabass at the start and the end of the second experiment.

Acknowledgements

We wish to express our deep gratitude to Mr. Pairoj Brohmanonda, Director of NICA. Our thanks are also due to Mr. Tatsuo Watanabe, expert from Japan, who gave suggestions and helped to plan this experiment.

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Mass Production of Rotifer, *Brachionus plicatilis*, at NICA in 1983

Tida Pechmanee, Sujin Maneewong and Niwes Ruangpanit

Introduction

The culture of microorganisms as food for larvae of fish and shrimp is very important. The success of the production of fish fry depends on the potential growth of the microorganisms to a level that can be collected continuously to supply the larvae. Rotifer, *Brachionus plicatilis*, is one of the important species that are able to grow at the high density level needed for feeding shrimp and fish larvae. At 2 days old, seabass larvae, *Lates calcarifer*, begin eating food; especially rotifer, which is suitable in both size and nutritional value. In addition, rotifer is also suitable for feeding mysis larvae of shrimps, *Penaeus monodon* and *Penaeus merguiensis*. Every year, NICA produces a lot of rotifer using phytoplankton as food; in 1983, marine yeast was used as well.

Material and Methods

Phytoplankton culture: Three kinds of phytoplankton, *Chlorella* sp., *Tetraselmis* sp., and blue green alga, were cultured in 26-ton rectangular tanks. The amounts of fertilizer applied to the culture tanks were: 1200 g of $(NH_4)SO_4$, 120g of agriculture fertilizer formula 16-20-0, and 60g of Urea. It took about 2-5 days for *Tetraselmis* sp. to grow to harvest level, and 3-7 days for *Tetraselmis* sp. to grow to harvest level, and 3-7 days for *Chlorella* sp. and blue green alga.

Marine yeast culture: Marine yeast seed was collected from the fish culture water. The nutrient used for growing marine yeast was one litre of water added to 15g sugar, 1g of $(NH_4)_2SO_4$, 1g of K_2HPO_4. One millilitre of HCl concentrate was added to produce a pH level of 4. The yeast was reared for 2-3 days, then transferred to grow in a 10 litre vessel for 2 days. The nutrients added then were the same but without the HCl. The yeast was then transferred to a 500 ℓ tank, the sugar supply was reduced to 8g/ℓ, and, 24 hrs. later, it could be used for feeding rotifer.

Rotifer culture: Rotifer was cultured in 26-ton tanks. First, 1-2 tons of fresh water were added to 11 tons of phytoplankton (*Chlorella* sp. density, 10 x 10^6 cells/mℓ; *Tetraselmis* sp., 10 x 10^4 cells/mℓ), in order to adjust salinity to 25-30‰. Rotifer seed was then introduced at a density of 10-20/mℓ. After 2 days, this initital food supply was exhausted, and the density or rotifer has risen to 40-100/mℓ. At this point, an additional 13 tons of food and water mixture (same as initial batch) was added to the tank to a total of 25 tons. The next day, the rotifer density remained at 40-100/mℓ. It was then ready for harvesting by draining the water from the rotifer tanks through 63 μ mesh bags, leaving half of the original volume to serve as starter for the next batch. Then phytoplankton, or phytoplankton plus marine yeast (0.5 x 10^6 cell/mℓ), or 25-30‰ seawater and only marine yeast (1 x 10^6 cell/mℓ) was added to the rotifer culture tanks to the 26 ton level in order to grow the next batch of rotifer. Each rotifer tank was used for from 10 to 20 days.

Results

In 1983, only *Tetraselmis* sp. was able to grow throughout the season. In May, however, even it did not grow well, so most of the rotifers were fed marine yeast that month. Filamentous blue green algae are the dominant groups of phytoplankton that cause contamination in phytoplankton culture tanks. The maximum density of *Tetraselmis* sp. was 14 x 10^4 cells/mℓ in May. The maximum density of marine yeast in the 500 ℓ tank was 700 x 10^6 cells/mℓ, and the minumum was 100 x 10^6 cells/mℓ.

In all, 44 x 10^{10} rotifers were produced. Table 1 shows samples of population growth of rotifer in each month. The highest density of rotifer recorded in one tank in February was 230 individuals/mℓ. This tank was fed *Chlorella* sp. plus marine yeast. In May, while larvae were fed rotifer, many seabass larvae became diseased and high mortality occurred. The worst conditions for culturing rotifer appeared in August.

Discussion

Filamentous blue green algae and diatoms always cause contamination in phytoplankton culture tanks. If filamentous blue green algae contaminate a *Terraselmis* tank, the problem can be solved by stopping aeration for a period of time. The filamentous blue green algae will then sink, while the *Tetraselmis* comes to the surface and grows.

Tetraselmis can grow throughout the season, but in May it does not grow well, so most of the food for the rotifer that month is marine yeast. At that time, a lot of seabass larvae fed with rotifer die. This is probably related to the nutritional value of rotifer fed with marine yeast. This problem was further investigated by Tida Pechmanee and T. Watanabe, 1983 (see Value of rotifer, *Brachionus plicatilis*, fed with microbial flock for rearing larvae of seabass, *Lates calcarifer*, in this volume).

The worst conditions for culturing rotifer appeared in August, during that time water salinity and temperature were high. But enough amount of rotifer could be fed to larvae in the month. In 1983, 44 x 10^{10} rotifers were produced, which was enough to feed the seabass and shrimp larvae.

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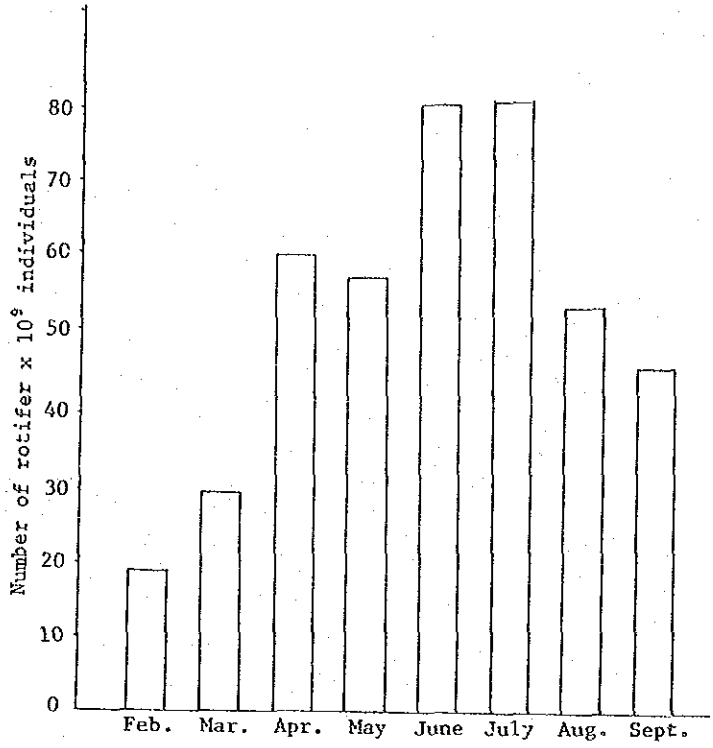

Fig. 1 Production of rotifer, *Brachionus plicatilis* in 1983

Table 1. Sample of population growth of rotifer, *Brachionus plicatilis*, in 26 ton tank (from the 3rd day of culture harvested a half of total amount of rotifer every day).

(Unit: individuals/ml)

Date	1	2	3	4	5	6	7	8	9	10	Food
Feb.	10(6)	15(9)	30(14)	61(19)	120(26)	146(19)	162(6)	187(17)	187(16)	230(13)	*Chlorella* sp. + yeast
Mar.	9(2)	18(7)	80(27)	107(10)	92(14)	73(17)	58(12)	72(9)	70(8)	70(7)	*Tetraselmis* sp.
Apr.	8(0)	14(10)	65(20)	83(19)	81(13)	48(23)	71(6)	33(20)	35(21)	41(15)	*Tetraselmis* sp.
May	13(6)	21(2)	44(11)	121(3)	78(25)	72(8)	49(23)	39(13)	33(4)	25(3)	Yeast
June	18(2)	36(15)	91(14)	89(12)	62(20)	56(24)	72(20)	52(12)	54(14)	43(18)	*Tetraselmis* sp.
July	20(2)	13(8)	32(9)	33(12)	46(18)	44(20)	42(6)	40(20)	27(6)	25(9)	*Tetraselmis* sp.
Aug.	12(9)	20(7)	21(4)	13(4)	10(4)	5(4)	8(2)	14(6)	26(11)	27(12)	*Tetraselmis* sp.
Sept.	13(6)	43(18)	91(13)	48(5)	43(14)	39(10)	64(1)	38(6)	32(7)	18(8)	*Tetraselmis* sp.

Figure in brankets shows number of rotifer with egg.

Table 2. Maximum density of phytoplankton (green water) in 26 ton tank in 1983.

Month	Environment			Contaminating algae		Maximum density (cells/mℓ)	
	Temperature (°C)	Salinity (‰)	pH	group	abundance	*Chlorella* sp.	*Tetraselmis* sp.
Feb.	27.0-29.0	30.0	7.8-9.4		-	10×10^6	10×10^4
Mar.	28.0-30.5	32.0	8.0-9.5	blue green diatom	++ +	10×10^6	10×10^4
Apr.	30.0-32.5	35.0	8.0-9.0	blue green	+++	5×10^6	10×10^4
May	27.0-32.5	33.5	8.0-9.0	blue green diatom	+++ ++	4×10^6	4×10^4
June	28.0-32.0	32.0	8.0-9.4	blue green diatom	++ +++	2×10^6	12×10^4
July	29.0-32.0	32.0	7.5-9.4	blue green protozoa	+ +	7×10^6	14×10^4
Aug.	26.5-33.0	34.5	7.0-9.8	blue green diatom	+ +	7×10^6	14×10^4
Sept.	28.0-31.0	32.0	8.0-9.0	blue green diatom	+ +	-	10×10^4

\+ low
++ medium
+++ abundant

Table 3. Condition of 26 ton rotifer tank in 1983.

Month	Number of rotifer tanks	Temperature (°C)	Salinity (‰)	pH	Number of phyto-plankton tanks	Remarks
Feb.	4	27.5-29.0	25.0-28.0	6.5-9.1	10	
Mar.	5	28.0-30.5	23.0-28.0	6.9-9.7	11	
Apr.	5	28.0-32.5	22.0-29.0	6.4-9.5	11	Plenty of filamentous blue green in culture tank
May	6	26.0-33.0	23.0-29.0	6.6-9.1	12	Most of food was marine yeast
June	6	27.0-32.0	23.0-29.5	6.9-8.7	11	Plenty of diatom in culture tank
July	6	27.0-32.0	26.3-31.0	7.3-9.1	11	
Aug.	6	26.0-33.0	27.0-34	6.1-9.9	10	Salinity was more than 30‰ almost all time due to lack of fresh water supply
Sept.	6	27.5-30.0	26.0-32.0	8.2-9.4	10	