Mem. Fac. Fish., Kagoshima Univ.
Vol. 29 pp. 275–291 (1980)

Culture Experiments on the Harpacticoid Copepod, *Tisbintra elongata* Mori, and Evaluation of that Species as a Food Organism for Milkfish Larvae*1

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Abstract

The mass culture of the harpacticoid copepod, *Tisbintra elongata*, indigenous to Panay Island were conducted, feeding them on several kinds of food materials. Salinity tolerance as physiological parameter was also examined. At the same time their efficiency as food for milkfish larvae was evaluated.

The highest density of 10.5 individuals/*ml* was obtained in copepods fed on rice bran and fermented fish solubles at rates of 0.125 to 0.25 and 0.16 mg/indiv./day respectively. The provision of shelter as habitat was also supplementary for growth. This species of copepod was found to be euryhaline, and could grow to high densities in waters hypersaline to their natural habitat.

Statistically, no significant difference of growth was observed between the milkfish larvae fed on this species and *Artemia* nauplius. However, comparatively stable results were obtained using *Tisbintra* as food. In this rearing of milkfish larvae, those larvae whose size was 12.3 to 13.5 mm in body length were considered to be just prior to morphological change.

Introduction

As seed production of several species of fin fish and crustaceans is established (Chaudhuri et al., 1978), the search for appropriate food organisms for their mass culture is essential. Harpacticoid copepods which dwell even in small tide pools thus having wide tolerance to environmental changes, are included in these feeding organisms. *Tigriopus japonicus* living in rock pools along the sea coast in warm regions of Japan, has much resistance against environmental change, and is also considered a suitable feeding organism for mass production of fish frys (Takano, 1968). In addition, this species can be cultured with not only living food, i.e. diatom and other phytoplanktons, but also with some kinds of foodstaff and by-products from food processing (Koga, 1970; Takano, 1971).

The present experiment was conducted to discover a native harpacticoid copepod at Panay Island which can be easily cultured, and to establish a mass culture method for this animal feeding it on simple foods. A second objective was to test the efficiency of this copepod as food for milkfish larvae, whose mass breeding method is presently

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being established (LIAO, 1979), compared with the more frequently utilized *Artemia* nauplius.

Materials and Methods

These experiments were carried out at the Aquaculture Department of SEAFDEC (Southeast Asian Fisheries Development Center), in the Philippines, from June 1978 to November 1978. The species used in the copepod culture experiments was *Tisbintra elongata* MORI, collected from the waterway of the milkfish reserve tank at SEAFDEC. Milkfish, *Chanos chanos* FORSKAL, reared for the evaluation of feeding organisms, were caught along the coast in the vicinity of SEAFDEC.

This study was undertaken as follows:

I. Culture experiments of *T. elongata.*
   (1) Body length frequency of *T. elongata* from its natural habitat.
   (2) Effectivity of different food materials on the growth; and the determination of optimum feeding levels.
   (3) Outdoor mass culture.
   (4) Salinity tolerance as a physiological parameter.

II. Feeding experiments of *T. elongata* for milkfish larvae.

I. Culture experiments of *T. elongata.*

(1) Body length frequency of *T. elongata* from its natural habitat.

The specimens collected from the place described above were fixed with formalin; and the body lengths of 500 randomly selected individuals were measured under light microscopy, using an eye piece micrometer.

In the following observations, developmental stage, i.e. nauplius, copepodid, or adult stage of each individual, was determined and frequencies catalogued.

(2) Effectivity of different food materials on the growth; and the determination of optimum amount of food.

Five kinds of food materials for the copepods were provided, namely: rice bran, cow dung, bread yeast, fermented fish solubles, and *Spirulina.* Rice bran, cow dung, bread yeast, and *Spirulina* were mixed with fresh water and filtered through a small mesh-size net after weighing. Fermented fish solubles were prepared by heavily aerating a mixture of 7.7 kg of trash fish, and fresh water which made the volume to 20 *l.* This was fermented at least for two weeks.

In this experiment, the effectiveness of those food materials was examined by feeding them to the copepods at a rate of approximately 0.01 mg dry weight (excluding the remainings on the net) per individual for copepodid and adult stages (ITOH, 1973). Then optimum feeding amounts of the food materials which demonstrated best feeding efficiencies was determined.

Rearing was accomplished by two methods. In one, 200 *ml* glass beakers were used as culture vessels. In the other, 30 *l* plastic pails were utilized. In the former,

50 *ml* of sea water was placed in each beaker along with ten gravid females from the field. Three replications of each treatment were prepared. Culture was continued for 1 week, without aeration. Food materials were supplied every morning at the rate mentioned above. The densities were counted after mixing with formalin. In conjunction, body length of 100 individuals, which had developed into copepodid and adult stage, were measured under a dissecting microscope. These culture vessels were kept at room temperature (27°C), and the salinity adjusted to 34‰ every morning.

In the latter, 20 *l* of sea water was filled into 30 *l* capacity plastic pails, provided with aeration. Two replications of these were prepared. The initial density of copepods was 0.4 to 0.6 indiv./*ml*. Counting was carried out every morning, prior to feeding, to determine the density. These cultures were continued for 16 days at 26°C to 27°C.

Determination of the optimum food amounts was carried out in 30 *l* pails, as in the latter case. The feeding amount was tested only for rice bran and fermented fish solubles, on the basis of growth rate and the amount of food supply, i.e. the amount fed in the initial period of the exponential growth phase. 0.125, 0.125×5 and 0.125×10 mg/indiv./day of rice bran (include the remainings on the filter) and 0.27, 0.27×5 and 0.27×10 mg/indiv./day of fermented fish solubles, were established. Rearing was continued for 9 days at 26°C to 28°C.

(3) Outdoor mass culture.

Initially, the effectiveness of both rice bran and fermented fish solubles for outdoor culture of copepods were compared. At the same time, the efficiency of shelter as an attachment substratum was examined. Secondly, the copepod density was observed feeding on both foods in a mixture.

In the first case, this experiment was conducted as shown in Table 1. In all tanks, aeration was provided. After *Chaetoceros* sp. or *Skeletonema* sp. was cultured up to 1.0 to 2.0×10^6 cells/*ml*, being supplied with fermented fish solubles, copepods were inoculated at a density of 0.05 indiv./*ml*. Simultaneously with the inoculation of copepods, feeding exclusively on rice bran was begun in 1 ton tanks, while fermented fish solubles was fed into 350 *l* tanks. The feeding amounts calculated on the basis of the above experiment were 0.4 mg/indiv./day of rice bran and 0.27 mg/indiv./day of fermented fish solubles, but on this feeding, the maximum feeding amount were limited to 100 g for rice bran per 1 ton and 135 g for fermented fish solubles per 350 *l*. The shelter provided in the tanks were the leaves of a coconut palm called "Nipa". These tanks were set outdoors, at 29°C to 30°C and a salinity of 25 to 29‰.

In the latter experiment, a 1 ton tank was used, providing shelter and aeration. As in the former experiment, copepods were inoculated at 0.26 indiv./*ml* after the blooming of *Chaetoceros* had occurred. Feeding amount of each kind of food was reduced to less than one half that calculated on the basis of optimum amount, considering the condition of water quality, i.e. 0.125 to 0.250 mg/indiv./day of rice bran and 0.160 mg/indiv./day of fermented fish solubles. This tank was also set

Table 1. Experimental groups in outdoor mass culture of *T. elongata* with 1 ton and 350 liters tanks.

1 ton tank		350 *l* tank	
Feeding on rice bran		Feeding on fermented fish solubles	
Shelter	no Shelter	Shelter	no Shelter

outdoors at 31°C.

(4) Salinity tolerance as one of the physiological parameters.

The specimens which had grown under 2 kinds of growth conditions were used. These were: the copepods transferred immediately from their natural habitat (Trial I–II); and those pre-cultured for one month in indoor tanks (Trial III–IV). The adjusted salinity is shown in Table 2. Adjustment of salinity to the desired levels was made by addition of distilled water or artificial sea salts. Three replications were provided for each treatment.

Table 2. Test salinities for examination of salinity tolerance of *T. elongata*.

Trial	I	II	III	IV
Salinity	7 ppt	13 ppt	5 ppt	25 ppt
	10	19	12	31
	34	25	18	37
	51	31	26	44
	67	37	32	49

Glass vessels of 200 *ml* capacity, containning 50 *ml* of each experimental salinity water, were used. Ten copepods with egg sack were transferred into each vessel from the natural habitat or from stock culture tanks; at salinities of 32 and 28‰, respectively. The experiments which were carried out indoors at 27 to 28.5°C, without aeration, lasted for one week. Rice bran was supplied at the rate of 1.25 mg/indiv./day (including the remainings on the filter net) per vessel. Salinity was checked prior to feeding daily with a refractometer.

II. Feeding experiment of *T. elongata* for milkfish larvae.

Milkfish larvae used in this experiment, whose mean body length and body weight were 12.3 to 13.5 mm and 7.6 mg, respectively, were collected from the sea. Very little food materials were contained in the stomachs of these larvae. After these frys were kept in a 60 *l* fiber glass tank for one day, fed on *Brachionus*, 25 individuals were transferred into 20 *l* aquaria, containing 10 *l* of sand filtered sea water. *Tisbintra* or *Artemia* were fed to the fry twice a day, in the morning and evening, at rates of 4, 20, and 40 times larval saturation levels, i.e. 8 individuals of *Tisbintra* and 7 individuals

of *Artemia* (Table 3). These feeding amounts were increased after checking the remaining food. *Tisbintra* was collected from a 1 ton culture tank, and concentrated with a plankton net. Newly hatched *Artemia* nauplii were separated from their cyst

Table 3. The amount of food supplied per treatment (individuals of copepodid and adult stage/fry/day) and remaining food density in each aquarium before feeding (indiv./*ml*).

Culture Period (days)	*Tisbintra* Feeding			*Artemia* Feeding		
	I	II	III	I	II	III
1	32	160	320	28	140	280
2	32	160	320	28	140	280
3	32	160	320	28	140	280
4*	32	160	480	28	140	280
5	32	160	450	28	140	280
6	62	318	1270	28	140	280
7**	62	318	1280	28	140	280
8	62	318	1280	28	140	280
9	182	960	3840	56	280	560
10	182	960	3840	56	280	560
11	182	960	3840	84	420	840
12***	163	819	3269	95	476	952
13	173	868	3456	101	504	1008
14	182	912	3456	101	506	917
*	0	0	0	0	0	20
**	0	0	0	0	0	5
***	0	0	0	0	0	0

* ** *** Remaining food in the morning

shells, then washed with fresh sea water.

Four-fifths of the rearing water was changed and the remaining feed removed, in all aquaria, every morning before feeding. This experiment was continued for two weeks, with aeration, at the wet laboratory. Two replicates were provided in each treatment. The body weight and length of all surviving fry were measured at the end of this experiment.

Results

I. Culture experiment of *T. elongata.*

(1) Body length frequency of *T. elongata* from its natural habitat.

Fig. 1 shows the body length frequency of *T. elongata.* Considering that the body length of one stage group shows a normal distribution, and that the presence of egg

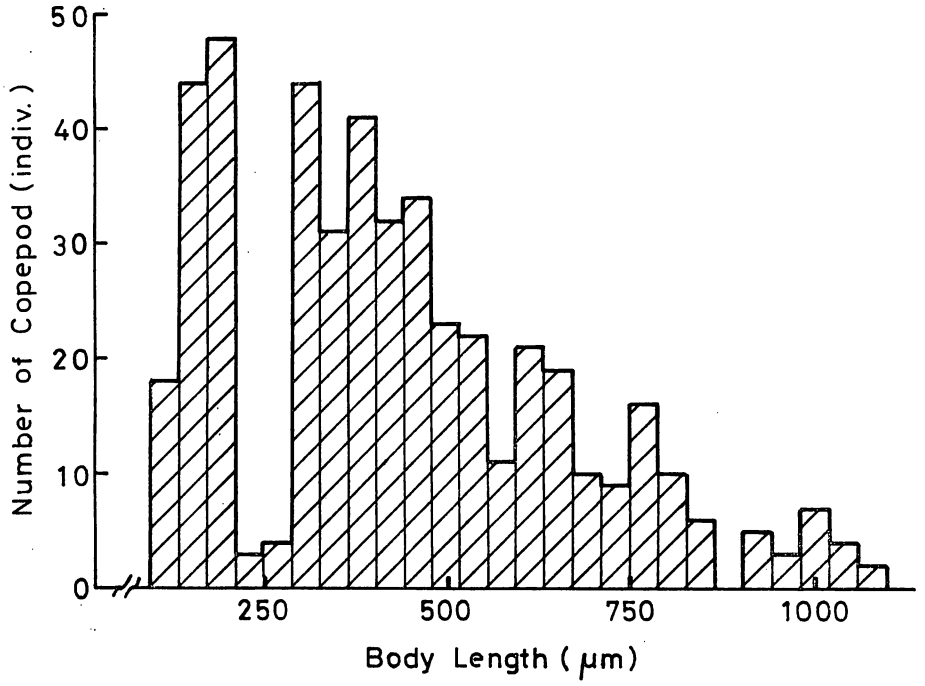

Fig. 1. Body length frequency of *Tisbintra elongata* collected from natural habitat.

sack was observed on the individuals of 710 μm body length, 700 μm was set as the "boundary" size between copepodid and adult stages.

(2) Effectivity of different food materials on the growth; and the determination of optimum feeding levels.

In the 200 *ml* beaker cultures, the highest density of 16.2 indiv./*ml* was obtained in the rice bran feeding treatments. *Spirulina* and bread yeast feeding yielded similar densities of 14.6 and 13.6 indiv./*ml*, respectively. Other kinds of food materials, i.e. fermented fish solubles and cow dung, showed little efficiency (Table 4). Body length frequency in each treatment is shown in Fig. 2. In addition, the mean body lengths of copepodid and adult were calculated. As for the effectiveness of those food materials, the tendency was towards similar densities and mean body lengths,

Table 4. Density of *Tisbintra* cultured in 200 *ml* glass beakers fed on 5 kinds of food materials (individuals/*ml*).

	Rice bran	*Spirulina*	Bread Yeast	Fermented Fish solubles	Cow Dung
Nauplius	0.6	0.1	1.6	0.1	0.02
Copepodid & Adult	15.6	14.5	12.0	5.7	2.2
Total	16.2	14.6	13.6	5.8	2.2

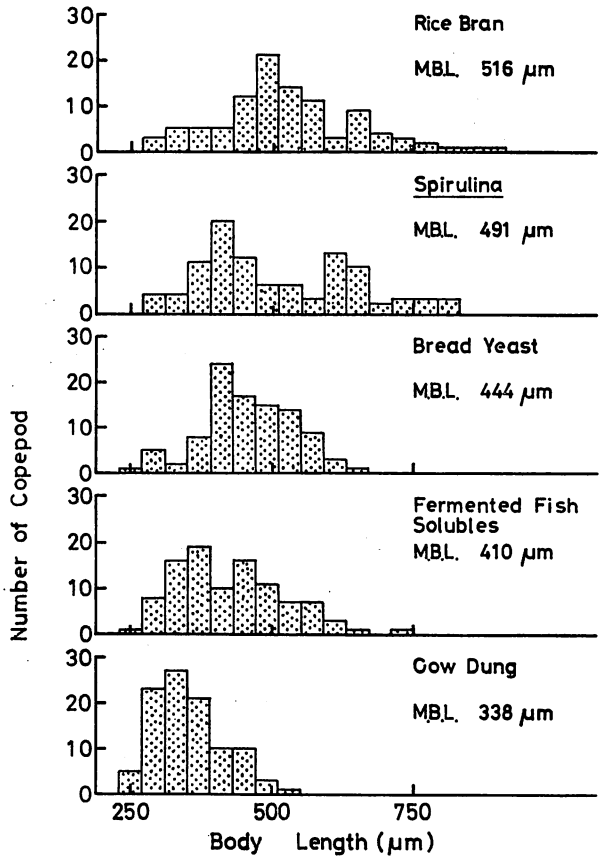

Fig. 2. Body length frequency in each food treatment conducted in 200 *ml* beakers.

however, the distribution of the size was wider in rice bran and *Spirulina* feeding treatments than that of other food materials.

On the culture in 30 *l* plastic pails, the highest density of 3.92 indiv./*ml* was obtained with bread yeast feeding (Fig. 3). Rice bran showed a similar efficiency, at 3.70 indiv./*ml*. The growth curve of rice bran feeding was more stable, but this time similar efficiencies for *Spirulina*, rice bran and bread yeast were not obtained.

From the result mentioned above and consideration of the cost of food materials, rice bran and fermented fish solubles* were selected as foods for mass culture of *T. elongata*.

In the case of the experiment on optimum feeding rates, rice bran fed at the rate of 0.125 mg/indiv./day yielded an increase in density to 2.8 indiv./*ml* 7 days after inoculation (Fig. 4). The growth rate of copepods at this rate was a little slower than at the other two rates. In the case of fermented fish solubles, the density increased to 1.0 indiv./*ml* at the feeding rate of 0.27 mg/indiv./day, about 2.5 times as

* The reason why fermented fish solubles was selected for mass culture is explained in the discussion.

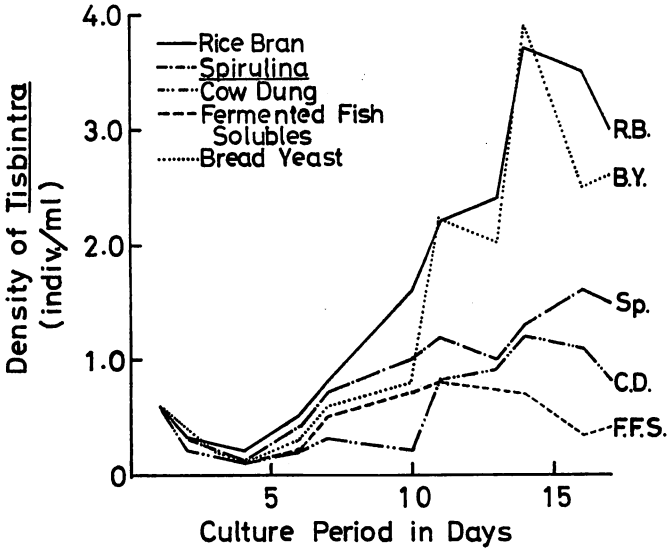

Fig. 3. Comparison of feeding efficiencies of five kinds of food materials in 30 *l* plastic pails.

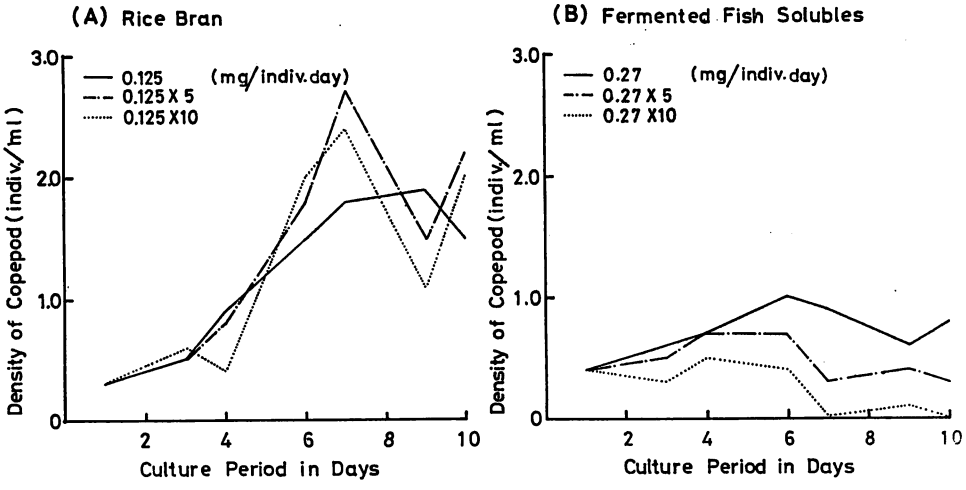

Fig. 4. Population growth of *Tisbintra* feeding on different amounts of rice bran and fermented fish solubles.

much as the initial density, however, the density in the other treatments gradually decreased.

Optimum feeding rates of rice bran and fermented fish solubles were, thus, desired at 0.125 to 0.625 and 0.27 mg/indiv./day, respectively; and were employed in the outdoor mass culture series.

(3) Outdoor mass culture.

a. The cultures, fed on rice bran or fermented fish solubles exclusively; and the

examination of the efficiency of shelter.

In the 1 ton tank, *T. elongata* fed on rice bran increased rapidly to 9.5 indiv./*ml* by the 12th day of the culture period; in the shelter setting tank (Fig. 5). However, in the tank without shelter, rapid increase in density was not observed. Instead, density decreased gradually after reaching a peak of 1.9 indiv./*ml*. On the other hand, in the 350 *l* tanks fed on fermented fish solubles, remarkable increase in density was observed in both tanks during the first half of the culture period. The density at peak growth was higher in the shelter setting tank than in that without. These densities were 10.0 and 8.0 indiv./*ml*, respectively. During the latter half of the culture period, the copepod density declined rapidly in both tanks.

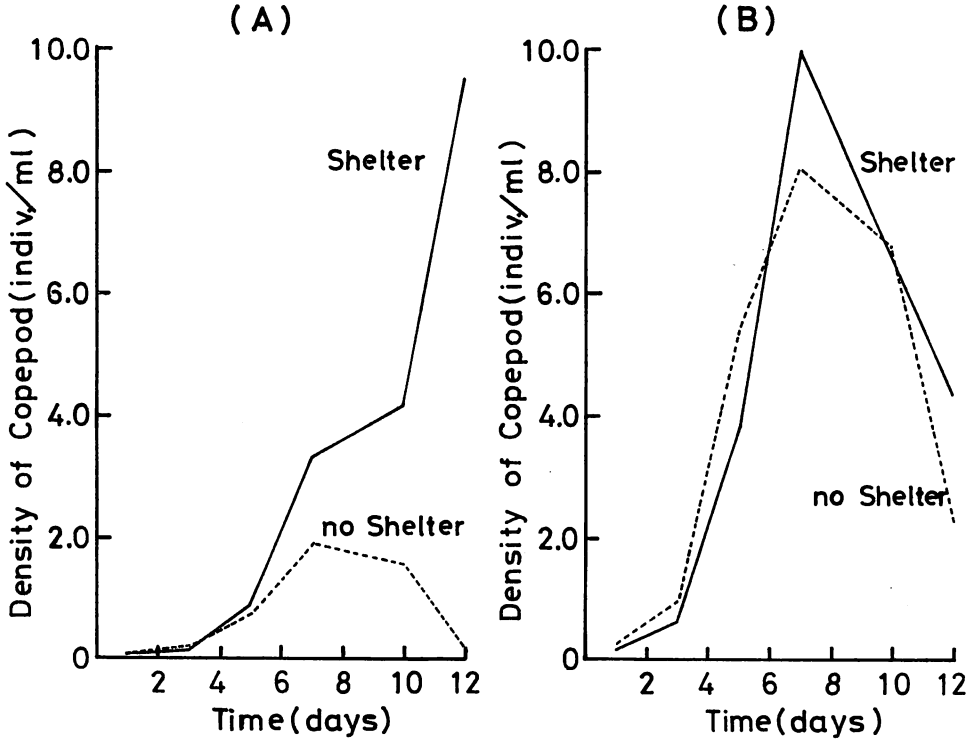

Fig. 5. Outdoor mass culture in 1 ton and 350 liters tanks examinning the effect of shelter as the attaching substratum of copepods fed on rice bran or fermented fish solubles exclusively.
A: 1 ton tank with rice bran feeding
B: 350 liters tank with fermented fish soluble feeding

b. The culture fed on the mixture.

The density increased to 10.5 indiv./*ml* 6 days after inoculation and the population was composed of about 80% by copepodid and adult stages (Fig. 6). Those individuals suddenly disappeared the next day, before noon. *Chaetoceros* density of 255×10^3 cells/*ml* was obtained 2 days after inoculation; but the density reduced

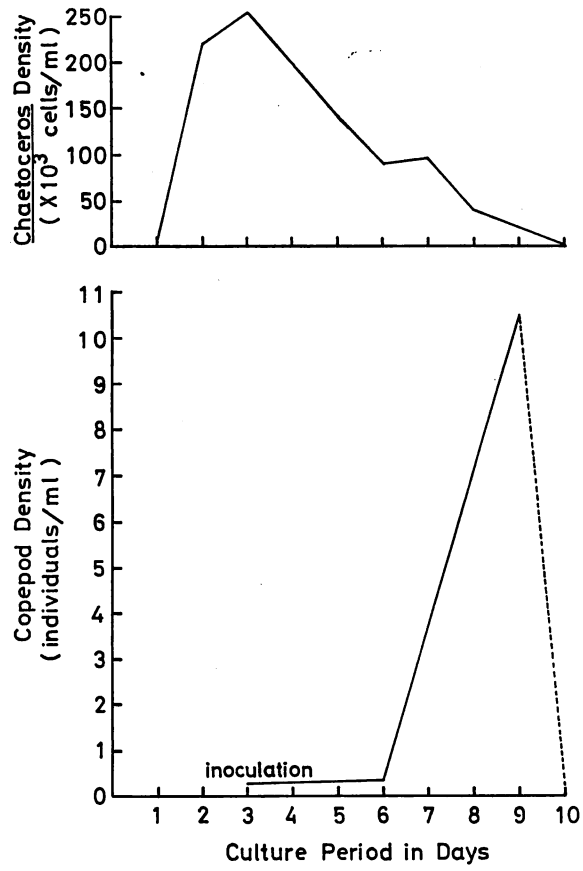

Fig. 6. Outdoor mass culture in 1 ton tank provided with shelter, fed on the mixture of rice bran juice and fermented fish solubles.

rapidly following the growth of the copepods.

(4) Salinity tolerance as one of the physiological parameters.

a. Specimens transferred immediately from their natural habitat.

In the first trial, active reproduction was observed in 34, 51, and 67‰ saline water (Fig. 7). Especially in 51‰ the total density increased about 70 times, including nauplius, copepodid, and adult. The adult density was 5 times that of the initial number, by one week time; however, the major portion of the population was occupied by copepodid, except in the 67‰ treatment where nearly one half of the density was occupied by nauplii. In the second trial, between the salinity range of 13 to 37‰, reproduction of 33 to 37 times was observed, uniformly. Those populations were composed of mainly the copepodid stage.

b. Specimens pre-cultured in indoor tank as stock culture.

In both trials, remarkable growth was not obtained at lower salinity than 25 to 26‰ and at 49‰ (Fig. 8). In other treatments the density increased about 21 times

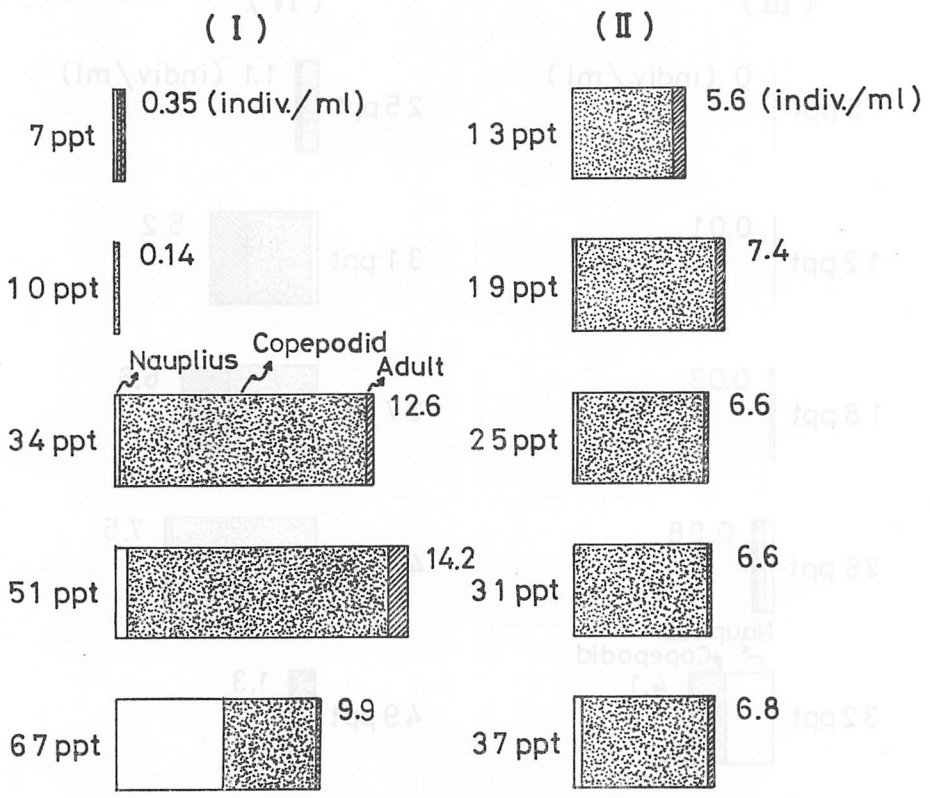

Fig. 7. Salinity tolerance examination of *Tisbintra* transferred immediately from natural habitat.

to 38 times. The results being closely related were observed in the third and fourth trials. Different compositions of population between the third trial of the 32‰ and the fourth trial of the 31‰ treatment were even noted. More than 1/3 of the population in 31 and 37‰ was occupied by the adult stage. On the other hand in 44‰, almost all the population was composed of the copepodid stage.

II. Feeding experiment of *T. elongata* for milkfish larvae.

The survival rates of milkfish larvae in all treatments were higher than 85% (Fig. 9). The specimens fed on sufficient food amounts showed the highest survival rates of 92 to 100%. Mean body weight and length in each treatment are shown in Fig. 10. The mean body weight at low, middle, and high feeding rates were 9.3 to 12.8, 22.5 to 28.8, and 42.0 to 53.6 mg, respectively, showing approximately a two fold increase among groups. In the case of body length, however, no big difference was observed among them. As for the mean body length, analysis of variance testings were performed between those replicates or treatments (Table 5). Between those replicates, no significant difference was observed except in the *Artemia* feeding at the

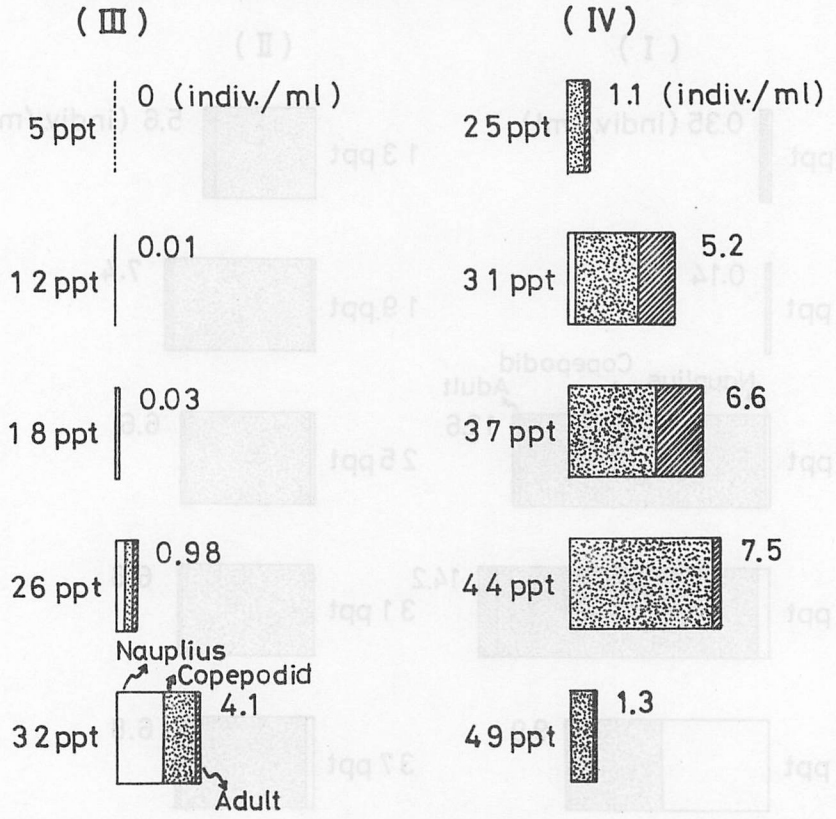

Fig. 8. Salinity tolerance examination of *Tisbintra* precultured in indoor tank as stock culture.

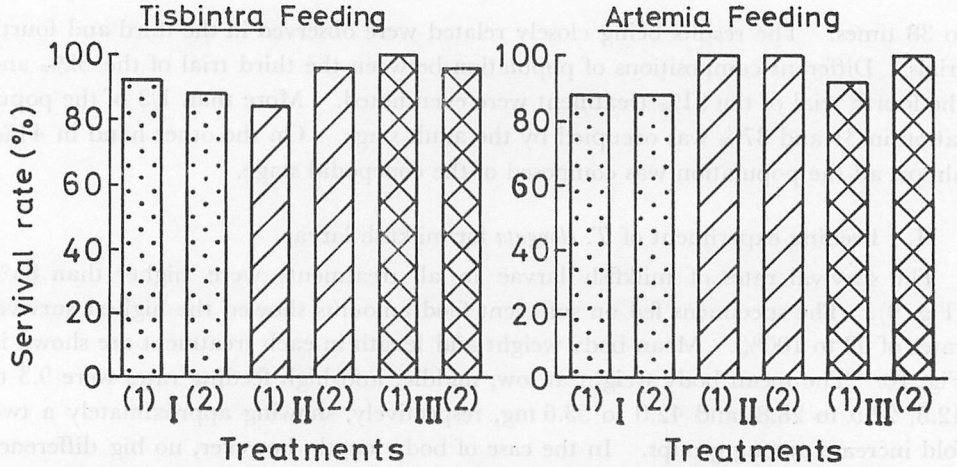

Fig. 9. Survival rate of milkfish larvae fed on *Tisbintra* and *Artemia* in 10 *l* aquaria.

I : Treatment at low feeding rate
II : Treatment at medium feeding rate
III: Treatment at high feeding rate

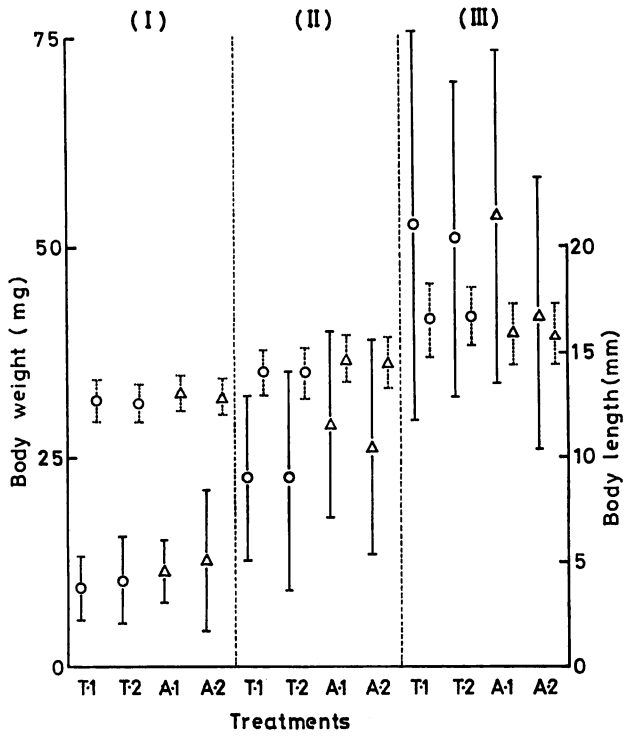

Fig. 10. Mean body weight and length of milkfish fry in each treatment.
——○—— Body weight of *Tisbintra* fed fry
——△—— Body weight of *Artemia* fed fry
……○…… Body length of *Tisbintra* fed fry
……△…… Body length of *Artemia* fed fry

Table 5. Analysis of variance testing of body length between treatments.

Treatments	Probability	Treatments	Probability
T-III-1 : T-III-2	0.40 >P>0.35	A-III-1 : A-III-2	0.025 >P>0.01
T-II-1 : T-II-2	0.45 >P>0.40	A-II-1 : A-II-2	0.25 >P>0.20
T-III : T-II	0.0005>P	A-III-1 : A-II	0.0005>P
T-III : A-III-1	0.35 >P>0.30	T-II : A-II	P>0.45

highest rate; and no significant difference between *Tisbintra* and *Artemia* feeding at each similar rate was observed.

During the rearing period, the changing of the larval shape was observed, as shown in Fig. 11. The condition coefficient was 0.35 at the beginning of this experiment when the body length was 12.8 mm. At the end its coefficient reached to 4.4 to 5.6 on the body length of 12.6 to 13.0 mm, even at a low feeding rate. It also increased rapidly following the growth of body length.

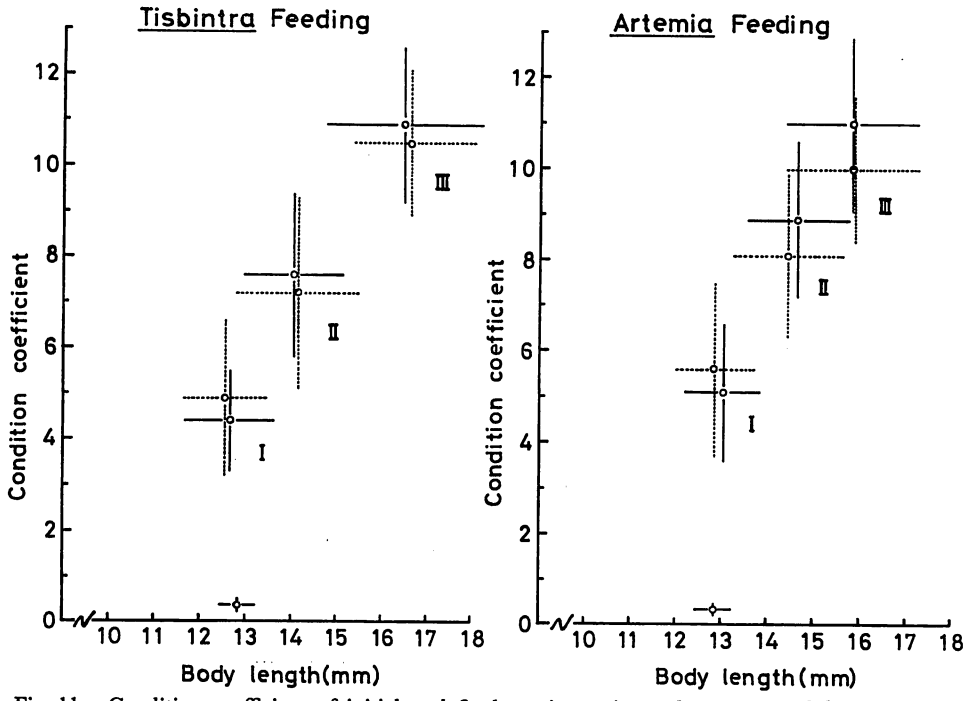

Fig. 11. Condition coefficient of initial and final specimens in each treatment fed on *Tisbintra* and *Artemia*.

Discussion

The body length of this copepod species was a little shorter than that of *Tisbintra jonesi* in its young developmental stage, as reported by UMMERCUTTY (1960); where the size was 140 to 150 μm in the 6th nauplius stage and 208 μm in 1st copepodid stage. While it can be supposed that the body lengths of the 6th nauplius and 1st copepodid are 200 to 250 μm and 250 to 290 μm, respectively, for *T. elongata*; but in the case of adults these sizes, in both observations, may be assumed to show the same body length distribution, i.e. 640 μm for male and 1,100 μm for largest female of *T. jonesi*.

As one of the effective foods for *T. elongata* culture, fermented fish solubles was selected, even though little efficiency was obtained with direct feeding of it in indoor culture. Nevertheless, it was found that high densities of diatoms could be observed, as shown in Fig. 12; and many kinds of diatoms are useful as the effective food for harpacticoid copepods (TAKANO, 1971). Therefore, fermented fish solubles were supplied to the outdoor mass culture tank as fertilizer for diatom culture, primarily before inoculation of the copepods. It was mentioned also by TAKANO (1971) that the combination of live algae with non-living materials could be supplied so that the density fed in mixture increased faster for 2 to 6 days than that fed on rice bran and fermented fish

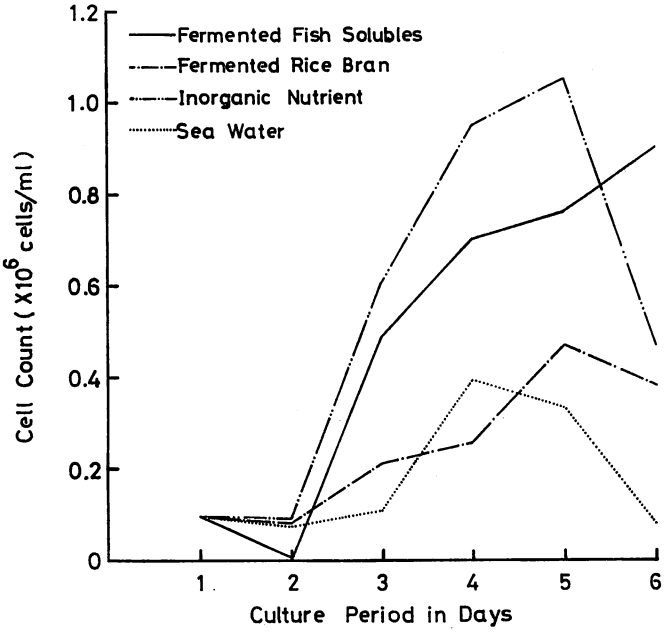

Fig. 12. Nutritional effect of fermented fish solubles on *Chaetoceros*.

solubles exclusively.

As for the type of effective food for copepods, KAHAN (1979) reported that many kinds of harpacticoid copepod could perforate the epidermal cuticle of vegetables and consumption of bacteria was mentioned by RIEPER (1978). In addition, the possibility of dissolved glucose utilization by *Cyclops* and *Halectinosoma* was suggested by GYLLENBERG et al. (1978). It can be considered diatoms and small particles of rice bran are eaten directly and as detritus type together with adherring bacteria.

From the 10th day of the culture period, *Enteromorpha* sp. was beginning to grow on the sides of the outdoor culture tank. Since the copepod density on those parts was higher than other parts of the tank, it was assumed that *Enteromorpha* made the environment favorable for copepods. On the role of *Enteromorpha*, oxygen is supplied and toxic compounds of nitrogen and other elements are absorbed through the process of photosynthesis (HARLIN, 1978). It also becomes a food supplying source directly (ROTHBARD, 1976); and indirectly as the substratum of adhesive diatoms and organic particle accumulation. In addition, the efficiency shown by "Nipa" leaves, as shelter, should not be overlooked (ITAMI, 1977).

On the observation of salinity tolerance, the specimens transferred immediately from their natural habitat showed a wider tolerance from 13 to 67‰ than those pre-cultured in indoor tank with a range of 31 to 44‰. This difference was considered to be caused by the differences between the two habitat conditions. The salinity in their natural habitat fluctuates often by evaporation and dilution with

rainfall. On the other hand, the highest density was obtained at the salinity of about 1.6 times as high as that where the specimens dwelled or were cultured. According to MATUTANI (1961) when *Tigriopus* cultured in normal sea water (100% of sea water) was tested about heat resistance in 100 to 150% of sea water, highest heat resistance was shown in 150% sea water. Between these two results there may be some common physiological relation. It can be considered that this species is euryhaline and has the ability to adapt itself to especially higher salinities.

In the feeding efficiency test of *T. elongata*, no significance was observed between *Tisbintra* and *Artemia*. On the other hand, in the seed production of *Chrysophrys major* and *Plecoglossus altivelis* it was reported that *Artemia* mono-specific feeding often caused death in large quantities (TAKAMI et al., 1976; FUSHIMI, 1968); but in the case of combination feeding with *Artemia* and other zooplankton, i.e. copepod or rotifer, those phenomena did not appear (TAKAMI, 1968). In addition, mono-specific feeding on *Artemia* had little effect on the survival of *P. altivalis*, after the total length became 28.1 mm (TATEISHI and TAKAMI, 1968). In this feeding experiment on milkfish larvae, it also can be concluded that no difference was observed on growth because of consumption of natural zooplankton in the sea prior to capture.

As for the behavior of food organisms and feeding efficiency, FUSHIMI and HASHIMOTO (1969) reported that *Tigriopus*, also a harpacticoid copepod, was superior food for red sea bream (*C. major*) larvae due to the fact that *Tigriopus* creep on the tank side or bottom besides swimming, so that feeding time can be greatly extended.

The nauplius stage of *T. elongata*, whose size is shorter (100 to 200 μm) than *Artemia*, will probably provide an effective food source for the early developmental stages of many kinds of fish and crustacean larvae.

Acknowledgement

This experiment was supported by the Aquaculture Department of SEAFDEC (Southeast Asian Fisheries Development Center) and JICA (Japan International Co-operation Agency).

The author gratefully thanks Dr. T. ITOH of Hokkaido Univ. for identification of this species, and Dr. H. HIRATA, Dr. N. HOSHINO, Dr. C. T. VILLEGAS and Dr. J. V. JUARIO for their invaluable advice. And the author thanks Mr. W. NAGATA for his proofreading of the manuscript.

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