Trophic Value of the Unicellular Diatom *Phaeodactylum tricornutum* for Larvae of Kuruma Prawn, *Penaeus japonicus*

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Key words: *Phaeodactylum tricornutum*, *Chaetoceros gracilis*, *Penaeus japonicus*, trophic value, larval rearing experiment, large-scale culture, larval production

Abstract

The trophic value of the unicellular and silica-less diatom, *Phaeodactylum tricornutum* for the larvae of kuruma prawn, *Penaeus japonicus,* was evaluated in uni-algal culture, nutritive analyses and larval rearing in this experiment. The algal strain of *Ph. tricornutum* used in this experiment had been selected by more than 100 repeat colony screenings using an agar medium under 30° and light conditions of 50-80μmol/m^2/s. From the results of this repeat selection, the alga grew well at temperatures from 25° to 30°. The limiting temperature and salinity conditions for its growth were from 15° to 35° and from 10 to 40ppt, respectively. Thus, the alga was eurythermal and euryhaline. The optimal reagent medium modified Guillard F medium was Guillard 4F medium without vitamins and silica. Moreover, fertilizers could be used instead of reagents. The algal growth and stationary phases continued for more than 21 days under temperatures lower than 30°. Therefore, the alga was found to be appropriate for culture easily using outdoor, large-scale culture tanks.

The feeding experiment showed that the survival rate of *P. japonicus* zoeal and mysis larvae fed with *Ph. tricornutum* approximated that of larvae fed with *Chaetoceros gracilis,* which is extensively used as a nutritive food organism in the rearing of prawn larvae in Japan. Moreover, the progress of metamorphosis of larvae fed with *Ph. tricornutum* was faster than that of larvae fed with *Ch. gracilis*. In the case of mixed feeding of both algae, the progress of metamorphosis was fast and the survival rate significantly increased in comparison with the sole feeding of each alga. Moreover, *Ph. tricornutum* contains much eicosapentaenoic acid (EPA), crude protein and total lipid. So, the algal nutritive value is evaluated as high. Therefore, *Ph. tricornutum* can be used as a good live food for the larval production of *P. japonicus*.

Introduction

The larval rearing techniques for the kuruma prawn, *Penaeus japonicus*, have been developed actively in Japan. During the spawning season, from June to August, two or three times hatchery cycles are usually carried out. However, the survival rate of prawn larvae at the zoeal and mysis stages is unstable. This uncertainty of production has been attributed to low quality eggs, deterioration of water quality (Okauchi *et al*., 1995; Chuntapa *et al*., 2003), and insufficient feeding. The diatom, *Chaetoceros gracilis*, is extensively used as a nutritive food organism in the rearing of prawn larvae (Chu, 1989). Moreover, the high-temperature tolerant strain has been selected by colony screening (Okauchi, 2002), and algal automated production systems have been developed by some private companies. Stable algae

production has been realized in Japan, and the demand for algae has increased. *Chaetoceros* sp. Concentrate has come onto the market recently from some private companies, but it is too expensive (about $160 US dollars per 20 liters at a density of about 50 million cells/mL) for most users, and its long-term preservation under refrigeration beyond one month in a refrigerator is difficult. The culture of additional species of algae for prawn larvae is important to increase larval shrimp productivity. Moreover, it was reported that the mixed feeding of several algae species is effective for the improvement of survival and growth rates of four larval shrimp species (Kuban *et al*., 1985). *Phaeodactylum tricornutum* does not have a siliaceous valve and is a unicellular diatom. Its reproductive and gliding movement are described well (Lewin, 1958). The large-scale cultivation (Ansell *et al*., 1963) and fatty acid component of the alga (Reitan *et al*., 1994) were already reported, however, the alga is not commonly used as live food of invertebrate larvae. The alga is used as a live food for artemia, *Artemia salina*, and rotifers, *Brachionus plicatilis* and *B. rotundiformis*, to enrich their eicosapentaenoic acid (EPA) content. If the high-density culture techniques were developed for *Ph. tricornutum* and the nutritive value was evaluated as high, this species could be used as a live food for shrimp larvae like *Ch. gracilis*. Therefore, the algal growth rate and productivity were investigated under various temperature and salinity conditions, and the optimal media, using reagents or fertilizers, were investigated by uni-algal and axenic culture experiments. The effect of mixed feeding of both *Ph. tricornutum* and *Ch. gracilis* was evaluated and compared to the sole feeding of these algae.

Materials and Methods

Alga

Phaeodactylum tricornutum used in this study was received from Dr. Takano in Tokai Regional Fisheries Research Laboratory in 1987. After that, colonies have been cultured under temperatures of 30° and 20° continuous light condition (50-80μmol/m^2/s). During the past 15 year period colonies held under each temperature condition screened using an agar medium more than 100 times. Hereafter, the strains selected under 30° or 20° are designated high-temperature selected strain (H-strain) and low-temperature selected strain (L-strain), respectively. The alga was sterilized using antibiotics.

Uni-algal culture experiment in various temperature conditions

Both H-strain and L-strain of *Ph. tricornutum* were cultured at 5 temperature conditions (15, 20, 25, 30, 35°) using 100mL flat-bottom flasks with 100mL Guillard F medium (Guillard and Ryther, 1962). The culture was batch style, and was carried out under continuous light (80μmol/m^2/s) with sufficient air supply for 21 days. The cell density was estimated using a Coulter Counter or a hemocytometer on the 7^{th}, 14^{th}, and 21^{st} day after inoculation. Quadruplicate flasks under each temperature were used, and average and standard deviation (SD) were calculated. Assuming that the alga increase logarithmically for 7 days after inoculation, the growth rate (k) was calculated using the formula; k (divisions/day) = 1/7 X Log_2 (N_7/N_0). Where N_7 = cell density at 7^{th} day, and N_0 = cell density at inoculation. Moreover, supposing that its population growth changes from growth phase to stationary phase at about the14^{th} day, the cell density at the 14^{th} day seems to be

approaching the maximum. Decreasing rate (d) was calculated using the formula; d (%/day) = (N_{21}-N_{14})/7X100. Where N_{21} = cell density at 21st day, and N_{14} = cell density at 14th day.

Uni-algal culture experiment in various salinity conditions

The H-strain of *Ph. tricornutum* was cultured under 8 step-wise salinity conditions (5, 10, 15, 20, 25, 30, 35, 40ppt). Four 500mL flat-bottom flasks with various salinities of 400mL GuillardF medium, were used in each salinity group. Their salinity was controlled with distilled water or boiled seawater. The culture was continued for 7 days, and the growth rate calculated using the above-mentioned formula.

Uni-algal culture experiment to make an optimum fertilizer medium for large-scale culture

Three trials were conducted. First, the suitable reagent medium was determined by modifying GuillardF medium (Trial-1). Except for silica and vitamins (Vitamin B_{12}, Biotin, and Thiamin Hydrochloride), other nutrients, which composed GuillardF medium, were added at one to ten times the quantity of those into autoclaved seawater. Each medium was abbreviated GuillardF to 10F. Then, the additive amount of each fertilizer required for optimal growth of the alga was investigated in Trial-2. Potassium nitrate (KNO_3), Calcium dihydrogenphosphate ($Ca(H_2PO_4)_2$) and Clewat32, which is metal powder on sale from Teikoku Kagaku Sangyo Co., were selected as fertilizers. As test groups (n = 4 each), H-strain of *Ph. tricolnutum* was cultured for 10 days in seawater with eight step-wise addition of each fertilizer (the quantity of KNO_3; 178.5, 356.8, 535.0, 713.5, 892.0, 1070.5, 1248.8, and 1427.3μg/mL, that of $Ca(H_2PO_4)_2$; 9.2, 18.4, 27.6, 36.8, 46.0, 55.2, 64.4, and 73.6μg/mL, that of Clewat32; 40, 80, 120, 160, 200, 240, 280, and 320μg/mL). The alga was cultured in Guillard4F as a control group (n=4). In Trial-3, effectiveness of the addition of vitamins and silica on the growth of the alga was evaluated. H-strain of the alga was cultured in the optimum reagent and fertilizer media, which were selected in Trial-1 and 2, with or without vitamins and silica.

All cultures were carried out using 500mL flat-bottom flasks with 400mL of each medium in light and temperature controlled room at 60 to 80μmol/m^2/s (24 light) and 25° , respectively. Cultures were continuously aerated at a rate of about 1L/min/vessel. The alga inoculated grew well in the pre-culture using Erd-Schreiber medium. These trials continued for 10 days. An estimate of the cell density of all cultures was made at the end of each trial using a Coulter Counter. The productivities in various media were calculated from the formula; p ($X10^4$ cells/day) = (N_{10}-N_0)/10. Where N_{10} = cell density at 10th day, and N_0 = cell density at inoculation. The average productivity and SD (n=4) of a particular group was calculated from the results of quadruplicate vessels.

Feeding experiment to kuruma prawn larvae

Penaeus japonicus nauplii used in this experiment were hatched from eggs obtained from a single female. Some hours after hatching, vigorous nauplii were collected using a pipette and a Petri dish, and were divided into 12 groups of 1,500 larvae each. A group was held in a 5L plastic circular tank containing 5L of autoclaved seawater. Four tanks were used for each treatment. The larvae were reared from nauplii to mysis-3 stage or post

larval-1 stage for 14 days by feeding *Ph. tricornutum* only (Test I group; PH-1 to 4), mixed feeding both *Ph. tricornutum* and *Ch. gracilis* (Test II group; PH&CH-1 to 4), or feeding *Ch. gracilis* only (Control group; CH-1 to 4). Initial density of algae in Test I, Test II, and Control groups was $16X10^4$ cells/mL (*Ph. tricornutum*), $8X10^4$ cells/mL (*Ph.tricormutum*) and 10^5 cells/mL (*Ch. gracilis*), and $20X10^4$ cells/mL (*Ch. gracilis*), respectively. Algal cell densities in every tanks were examined daily under a microscope with hematocytometers, and they were maintained at $12\text{-}18X10^4$ cells/mL (*Ph. tricornutum*) in Test I group, $5\text{-}10X10^4$ cells/mL (*Ph. tricornutum*) and $8\text{-}15X10^4$ cells/mL (*Ch. gracilis*) in Test II group, and $15\text{-}22X10^4$ cells/mL (*Ch. gracilis*) in the Control group by either diluting rearing water with seawater or by supplementing cultured algae. Daily estimate of the survival larval number in every tank was made by counting the vigorous swimming larvae in 500mL rearing water using 500mL measuring cylinders and watch glasses, and the average and SD in each group (n=4) were calculated. Moreover, 100 larvae each were randomly sampled from every treatment group and fixed with 1% formalin to determine the larval metamorphosis stage using a stereoscopic microscope. The larval rearing experiment was carried out in a temperature and light controlled room at 25° and 50μmol/m^2/s (14L:10D), and sufficient air was supplied continuously. During the experiment, 1L of rearing water in every tank was drained out and autoclaved fresh seawater was added once a day. *Phaeodactylum tricornutum* and *Ch. gracilis* were cultured using 10L glass carboys in Guillard4F medium.

Contents of crude protein, total lipid, and ash of feeding *Ph. tricornutum* and *Ch. gracilis,* which were randomly collected four times during the larval rearing experiment, were measured using common methods. Moreover, the fatty acids component of total lipid in *Ph. tricornutum*, which were cultured in Guillard4F medium, was analyzed by a gas-liquid chromatography.

Results

Morphological feature

Figure 1 shows the *Ph. tricornutum* which have increased vigorously in the growth phase. Most cells are the fusiform, and some cells are trigonometrical and ovoid types. Apical axis and transapical axis of the fusiform cell are 12-18μm and 3-5μm, respectively. Packed cell volume is about 55μm^3. Its chloroplast grows remarkably well and the transapical axis extends in a eutropic medium, however, it shrinks in an oligotropic medium (**Fig.2**).

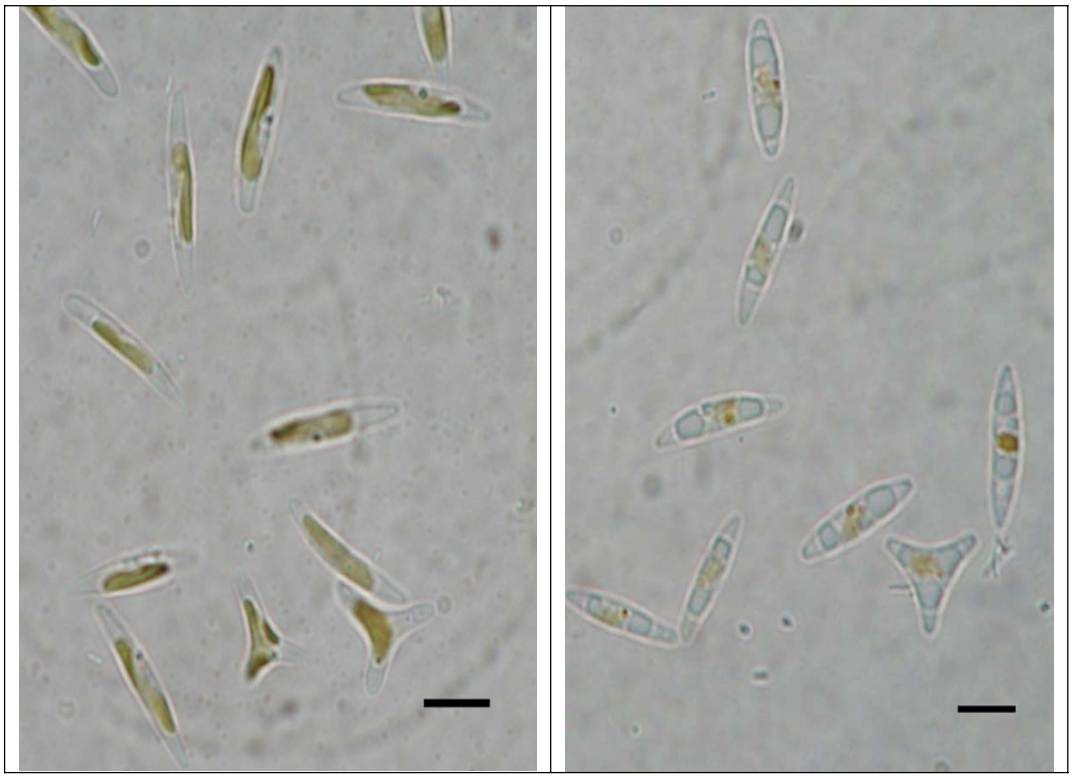

Figure 1 (on the left). *Phaeodactylum tricornutum* increased vigorously in Guillard 4F medium. Most cells are fusiform, and some cells are trigonometrical and ovoid forms. Chloroplasts grew remarkably well. Bar shows 5 μm.

Figure 2 (on the right). *Phaeodactylum tricornutum* which increased in an oligotropic medium which lacks some heavy metals. Chloroplast in the middle part of the cell is reduced in size. Bar shows 5 μm.

Growth under various temperature

The growth rate of the H-strain, which was selected under 30°, was 0.6 divisions/day under 20° to 30° (25° =30° =20° >15° =35° ; $P<0.05$). While, that of L-strain, which was selected under 20°, was 0.6 divisions/day under 20° to 25° (25° =20° >30° =15° >35° ; $P<0.05$) (**Fig.3-A**). In comparing both strains, the growth rates of H-strain under 30° and 35° were significantly higher than those of L-strain ($P<0.05$ and $P<0.01$, respectively). The cell density at the 14th day after inoculation is shown in **Fig.3-B**. The cell density of H-strain under 25° and 30° was significantly higher than that under other temperature conditions (25° =30° >20° >15° >35° ; $P<0.05$). The density of the L-strain under 25° and 20° was significantly higher than that under other temperature conditions (25° =20° >30° >15° >35° ; $P<0.05$). The density of H-strain under 25°, 30° and 35° was significantly higher than that of L-strain ($P<0.05$, $P<0.01$, and $P<0.01$, respectively). Moreover, the decreasing rate, which calculates from the

difference between the cell density of 14th day and that of 21st day, is shown in **Fig.3-C**. The decreasing rate at 35° , was a minus value under the other temperature conditions. The decreasing rate was clearly low under 15° in both strains. While, the rate of L-strain under 35° was significantly higher than that of H-strain ($P<0.01$).

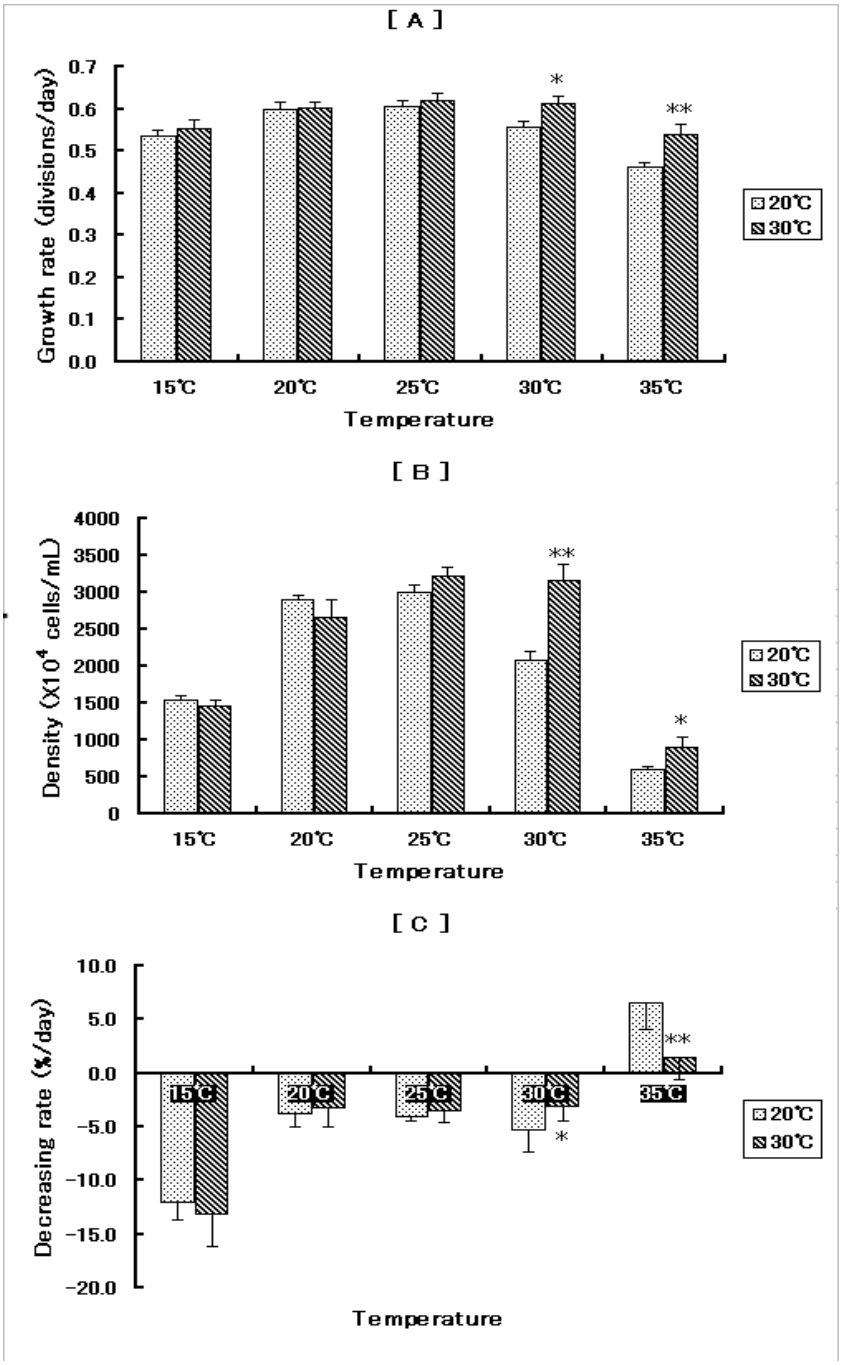

Figure 3. Growth features of the two *Ph. tricornutum* strains which were made by repeat colony selection using agar medium under 20°C or 30°C. Growth rates, which are calculated from the cell densities at inoculation and the 7th day, are shown in [A], cell

densities on the 14th day after inoculation are shown in [B], and decreasing rate which are calculated from the difference between cell density on the 14th day and that on the 21st day are shown in [C]. Dotted and obliquely striped bars in [A], [B],and [C] show average values (n=4) of strains selected 20°C and 30°C respectively. Vertical bars indicate SD (n=4). Statistical tests were carried out between the two strains, which were cultured under the five stepwise temperature conditions. Single asterisk and double asterisks indicate significant differences between the two strains at $P<0.05$ and $P<0.01$, respectively.

Growth in various salinity media

The algal growth rates in various salinity media are shown in **Figure 4**. No growth was observed in 5 ppt medium. In 10 ppt medium, the alga could increase with lower growth rate (k = 0.33). While, the algal growth rates in 15 to 40 ppt media were 0.62 to 0.65, and there was no significant difference between them ($P>0.05$).

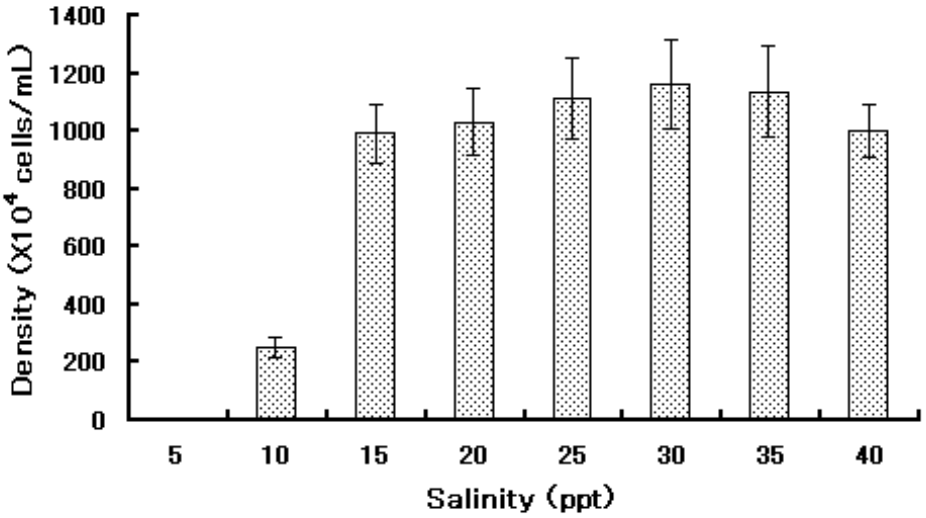

Figure 4. Average growth rates of *Ph. tricornutum* in various salinity media. Vertical bars indicate SD (n=4).

Growth in modified GuillardF medium

The productivity of *Ph. tricornutum* H-strain, which was cultured in GuillardF to 10F medium for 10 days, is shown in **Figure 5**. The value of the alga cultured in Guillard4F, 5F and 6F medium was about $480X10^4$ cells/mL/day and was significantly higher than that in other media except for Guillard8F. From the result of this experiment, Guillard4F was selected as a suitable reagent medium, and it was used as the control medium in Trial-2 and 3.

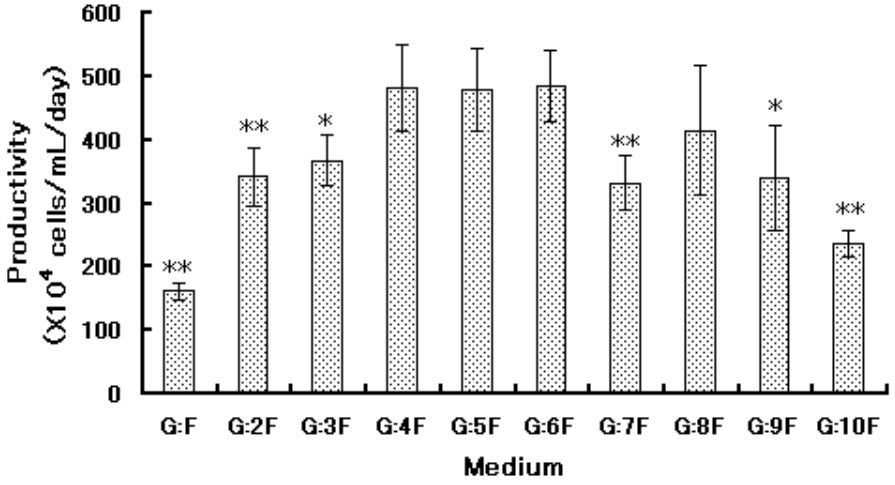

Figure 5. Average growth rates of *Ph. tricornutum* in modified Guillard F medium without silica and vitamins (vitamin B_{12}, biotin, and thiamin hydrochloride). Guillard 2F to 10F means 2 to 10 times contents of nutrients which are contained in Guillard F medium. Vertical bars indicate SD (n=4), and asterisks indicate significant differences between the control group (Guillard 4F) and other groups (**; $P<0.01$, *; $P<0.05$).

Growth in various concentration of fertilizer medium

Productivity of *Ph. tricormutum* cultured in media, with eight stepwise concentrations of KNO_3, $Ca(H_2PO_4)_2 \cdot H_2O$ and Clewat32, are shown in **Figure 6**. No significant difference was detected between the productivities in the media containing 713.5μg/mL or 892.0μg/mL of KNO_3 compared that in the control medium; Guillard4F ($P>0.05$) (**Fig.6-A**). Therefore, the suitable concentration of KNO_3 was considered to be 713.5μg/mL to 892.0μg/mL. The contents of NO_3-N in 713.5μg/mL and 892.0μg/mL of KNO_3 were almost equal those of Guillard4F and 5F media. In case of $Ca(H_2PO_4)_2 \cdot H_2O$, the productivity of the alga in the media that contained 9.2μg/mL to 27.6μg/mL of that fertilizer, were significantly lower than that in control medium; Guillard4F ($P<0.05$). However, the productivity of media containing 36.8μg/mL to 73.6μg/mL of $Ca(H_2PO_4)_2 \cdot H_2O$ were the same as that in control medium ($P>0.05$) (**Fig.6-B**). Therefore, 36.8μg/mL of $Ca(H_2PO_4)_2 \cdot H_2O$ regarded as a suitable concentration. The content of PO_4-P in 36.8μg/mL of $Ca(H_2PO_4)_2 \cdot H_2O$ was almost equal with that of Guillard4F medium. While, productivity of the alga in the media with 80μg/mL to 320μg/mL of Clewat32 were almost equal that of Guillard4F as control ($P>0.05$) (**Fig.6-C**). In comparing the productivity of 120μg/mL medium and that of 80μg/mL medium, significant difference was detected between them ($P<0.05$). Thus, the suitable concentration of Clewat32 was 120μg/mL to 320μg/mL.

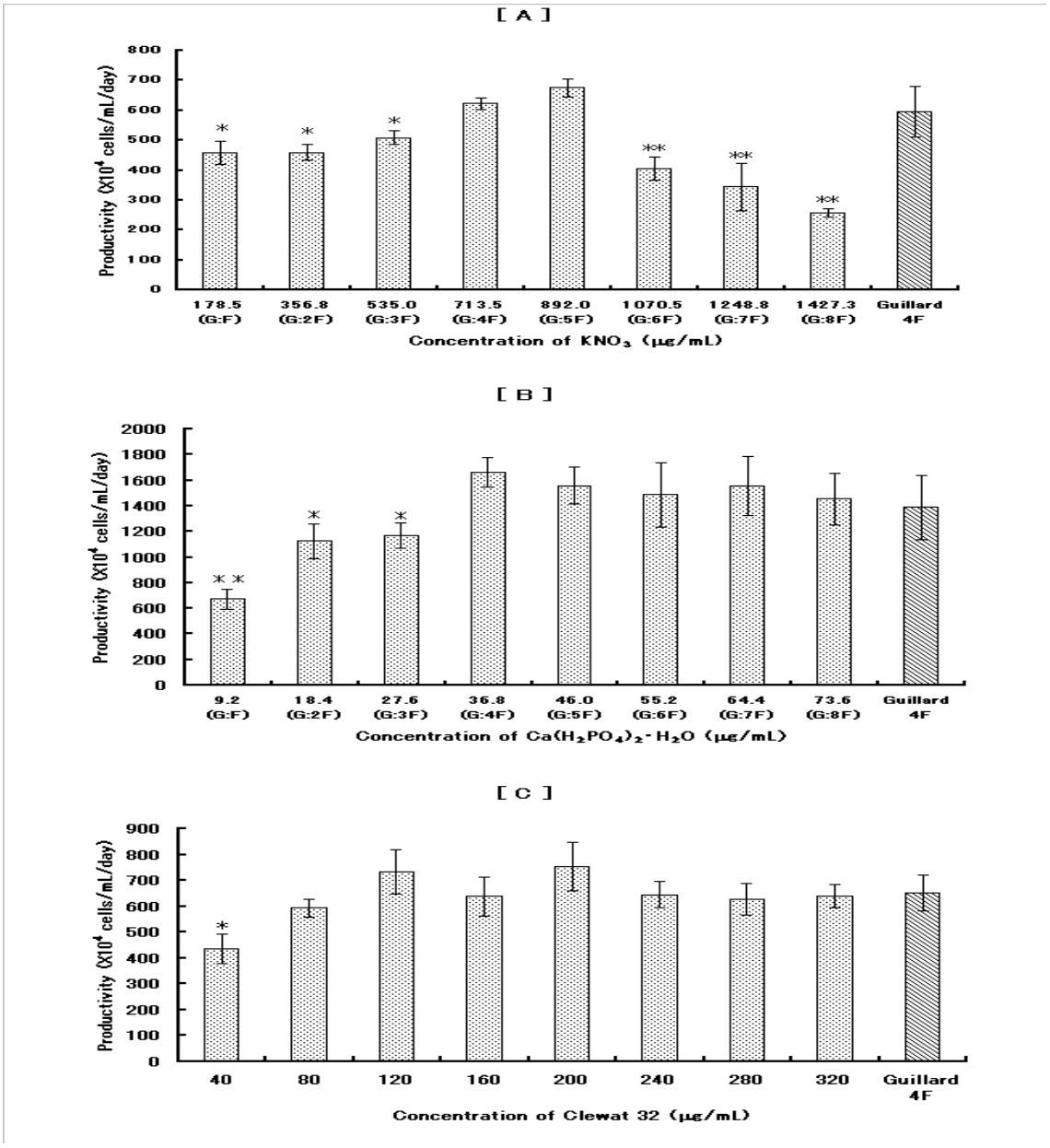

Figure 6. Productivity of *Ph. tricornutum* cultured for ten days in fertilizer media containing various quantities of Potassium nitrate (KNO_3); [A], Calcium dihydrogenphosphate monohydrate ($Ca(H_2PO_4)_2 \cdot H_2O$); [B], and Clewat 32 (heavy metal compound which is sold from Teikoku Kagaku Sangyo Co.); [C]. Productivity in each fertilizer medium was compared with that in Guillard 4F medium as the control. Marks in parentheses are for Guillard F to Guillard 8F (G:F – G:8F) media which includes the same quantity of NO_3-N or PO_4-P as in the various fertilizer media. Vertical bars indicate SD (n=4), and asterisks indicate significant differences between the control group (Guillard 4F) and others (**; $P<0.01$, *; $P<0.05$).

Growth in Guillard4F and fertilizer media with or without vitamins and silica

Productivities of *Ph. tricornutum* in Guillard4F and fertilizer media with or without vitamins and silica are shown in **Figure 7**. In spite of reagent medium and fertilizer medium, no significant difference was detected between them (P>0.05).

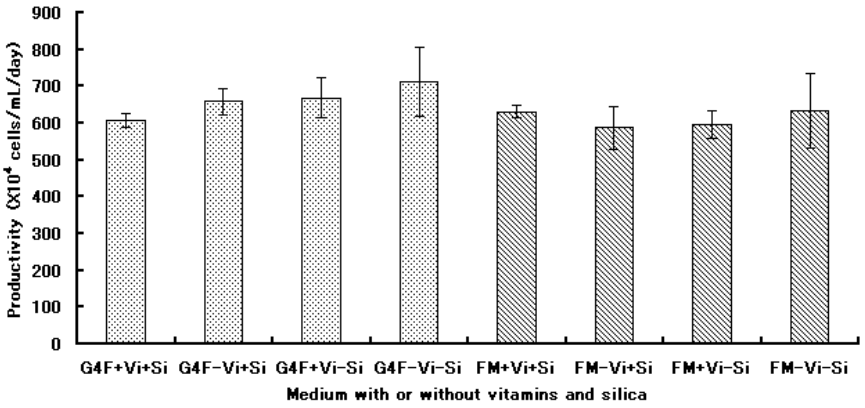

Figure 7. Productivity of *Ph. tricornutum* in Guillard 4F and fertilizer media with or without vitamins and silica.

Survival and growth of prawn larvae fed with Phaedactylum and/or Chaetoceros

The change of the number of live larvae and progression of the metamorphic stage of larvae fed with *Ph. tricornutum* and/or *Ch. gracilis* are shown **Figures 8, 10, 11**. The number of larvae fed with *Ph. tricornutum* alone decreased remarkably at zoeal–1 stage (**Fig.8; PH-1**). At day 9, about 80% of the larvae grew to post-larvae and survival rate was 80% (**Fig.8; PH-2**). **Figure 9** shows the zoeal and mysis larvae fed with *Ph. tricornutum*. Their digestive tracts are filled with the alga, and vigorously swimming larvae with long feces were observed frequently. Advance of metamorphosis of the larvae fed with *Ch. gracilis* only was slow in comparison with the larvae fed with *Ph. tricornutum*. About 80% of the larvae changed to post-larvae at day 13(**Fig.10; CH-2**), and the survival rate at day 13 was 65% (**Fig.10; CH-1**). During zoea-2 stage to mysis-3 stage, the survival rate of the larvae decreased remarkably (**Fig.10; CH-1**). The larvae fed with both *Ph. tricornutum* and *Ch. gracilis* survived well, and their survival rate at day 10, when about 80% of the larvae changed to post-larvae (**Fig.11; PH&CH-2**), was 92% and there was only a small reduction in the survival rate during the experiment (**Fig.11; PH & CH-1**). Moreover, the larvae seemed to eat both algae without selection.

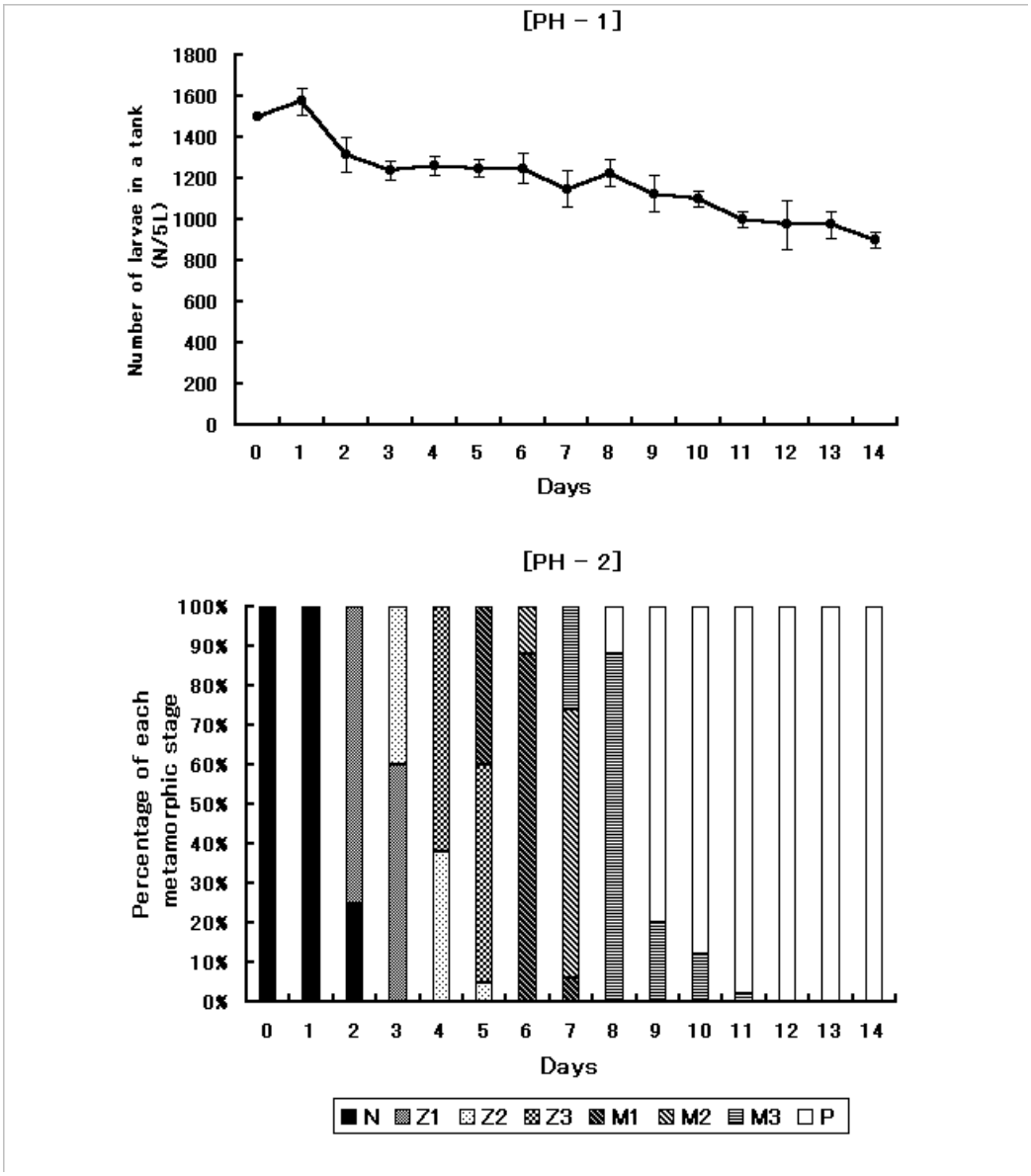

Figure 8. Change in the average number of living larvae, which were fed with *Ph. tricornutum,* in four tanks [PH-1], and the progress of the metamorphic stages of larvae fed with the alga [PH-2]. Vertical bars in [PH-1] indicate SD (n=4), and N, Z1, Z2, Z3, M1, M2, M3, and P means nauplius stage, zoea-1 stage, zoea-2 stage, zoea-3 stage, mysis-1 stage, mysis-2 stage, mysis-3 stage, and post larvae, respectively.

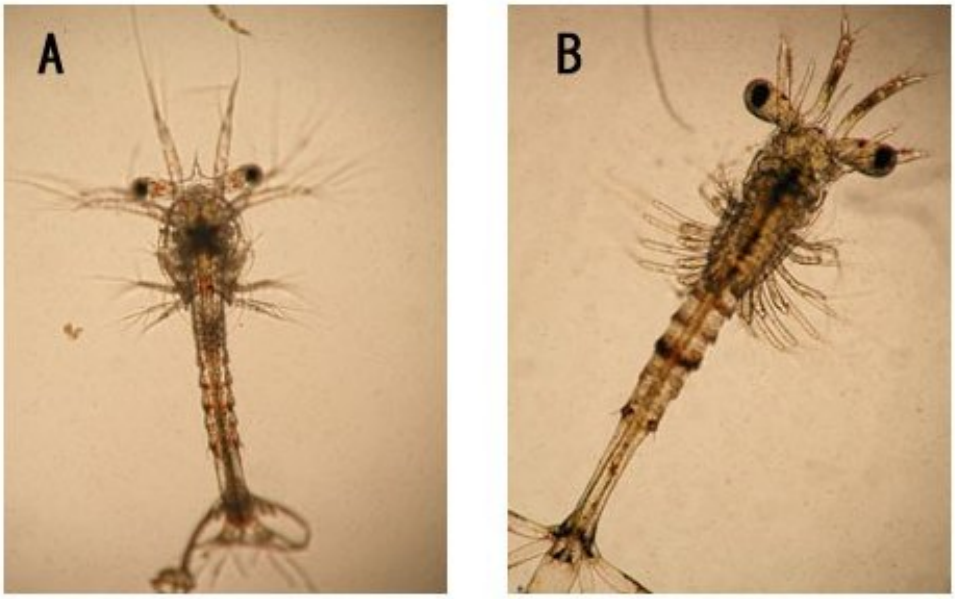

Figure 9. Zoeal [A] and mysis [B] larvae fed with *Ph. tricornutum*

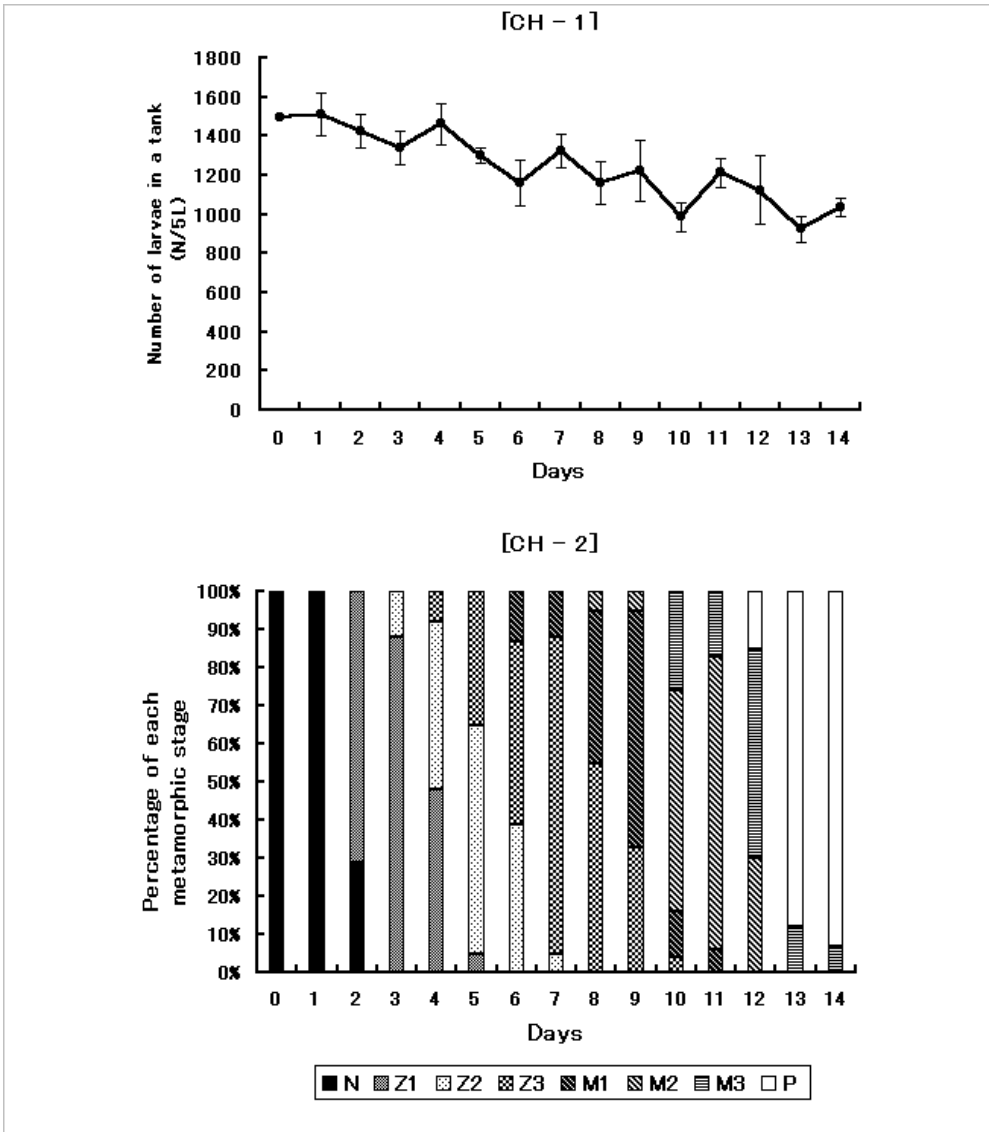

Figure 10. Change of the average number of living larvae, which were fed with *Ch. gracilis* in four tanks [CH-1], and the progress of metamorphic stages of larvae fed with the alga [CH-2]. For further explanation see **Figure 8**.

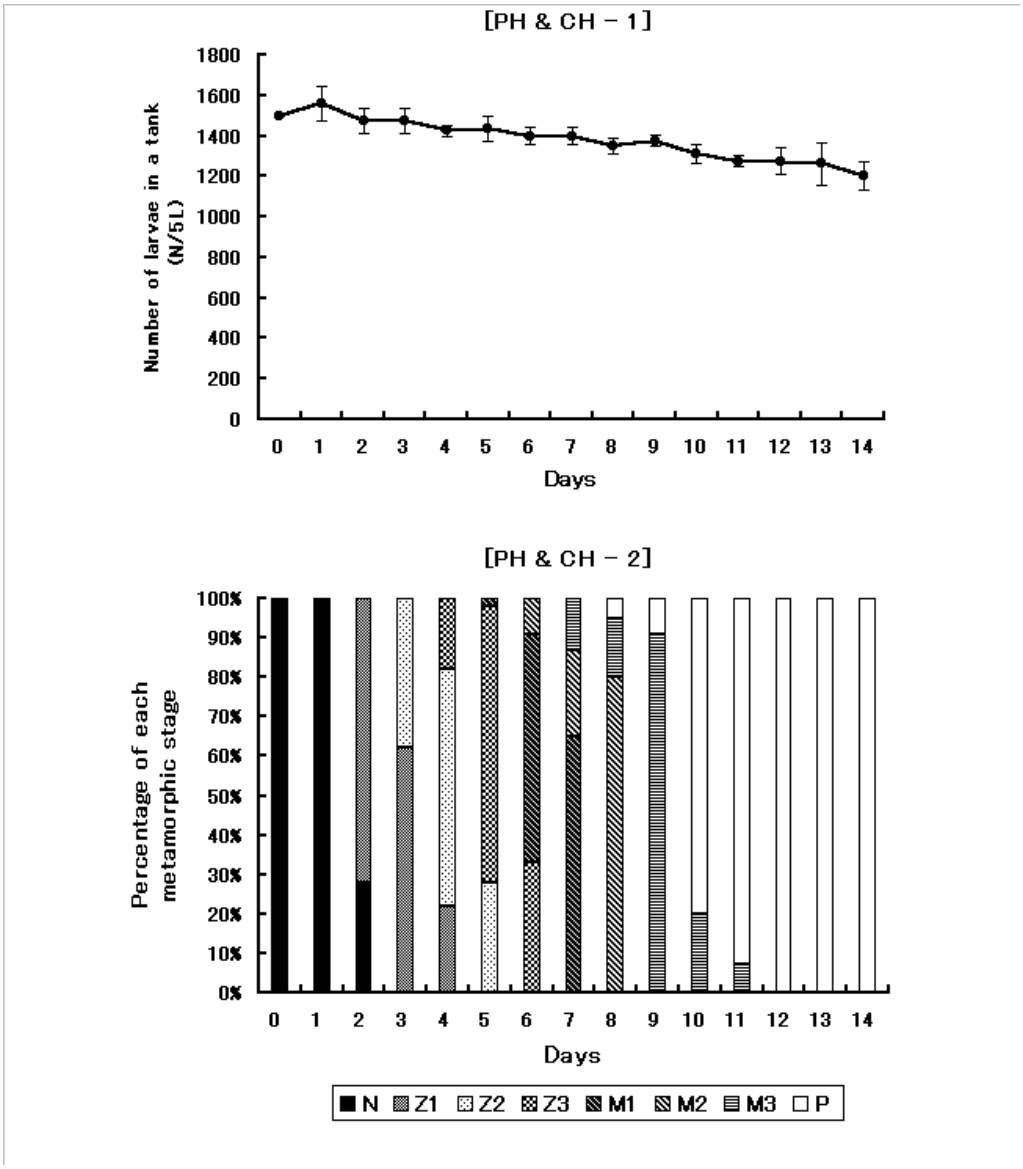

Figure 11. Change of the average number of living larvae, which were fed with both *Ph. tricornutum* and *Ch. gracilis*, in four tanks [PH & CH-1], and the progress of metamorphic stages of larvae fed with both algae [PH & CH-2]. For further explanation see **Figure 8**.

Contents of crude protein, total lipid, and ash in *Ph. tricornutum* compared with those in *Ch. gracilis* are shown in **Table 1**. The average contents of these components in *Ph. tricornutum* were 56.6%, 22.8% and 11.1%, respectively, while those contents in *Ch. gracilis* were 35.7%, 16.2% and 24.0%, respectively. The fatty acid component of total lipid in *Ph. tricornutum* is shown in **Table 2**. The alga contained 12.5% of 20:5 fatty acids.

Table 1. Proximate composition of *Ph. tricornutum* and *Ch. gracilis* which were collected

	Phaeodactylum tricornutum				*Chaetoceros gracilis*			
	1	2	3	4	1	2	3	4
Crude protein *	59.8	62.4	55.3	48.8	40.6	38.8	32.4	31.0
Total lipid *	23.5	25.5	21.8	20.5	15.5	14.5	16.9	17.8
Crude ash *	11.8	10.5	10.1	12.0	27.8	24.6	22.3	21.2

* On a dry matter basis

Table 1

Table 2. Fatty acid composition of total lipid of *Ph. tricornutum* which was collected on the 14th day after inoculation.

Fatty acid	Area %
14 : 0	7.0
14 : 1	0.0
16 : 0	20.5
16 : 1	43.3
16 : 2	3.1
16 : 3	7.6
18 : 0	6.4
18 : 1	2.2
18 : 2	0.0
20 : 4	0.0
20 : 5	9.9

Table 2

Discussion

Characteristics of the growth of Ph. tricornutum H-strain and L-strain

When both strains were cultured under 30° and 35° , the difference of growth characteristics was apparent. The growth rate and the cell density at day 14 of H-strain were significantly higher than those of L-strain. The difference was considered to be induced by the repeat selection for higher temperature tolerance under 30° , and the H-strain would adjust to higher temperature conditions. The difference was minimal betweenr 15° to 25°. Thus, the H-strain can grow well under the low temperature conditions the same as the L-strain, and it is a eurythermal strain. Moreover, the decreasing rates of both strains were negative values between 15° to 30°. The growth rates and the cell density at day 14 for both strains and were remarkably lower under 35° and the decreasing rates were a positive value. This tendency was clearly seen in the L-strain. So the limiting upper temperature was 35°. The negative value for decreasing rate means that the alga continuously increased until day 21 and the stationary phase was long. Therefore, *Ph. tricornutum* could be increased continuously when grown between 15° to 30° for 21 days. Because semi-continuous culture was possible, and its growth phase continued for a long term, the alga is suitable for mass

cultivation as a live food organism.

The salinity condition for good growth of H-strain was from 10 to 40ppt, so that strain is euryhaline, too. This character is desirable as a food organism, because the alga can be cultured in a wide range of salinity, which is appropriate for the good growth of the target culture species. Thus, *Ph. tricornutum* H-strain is suitable as a live food organism, in terms of accommodation to salinity.

Morphological feature of Ph. tricornutum H-strain

As shown in **Figure 1**, the algal cells, which grew in a medium that includes sufficient nutrients, was fusiform, and their prevalence and transapical axes were long. The algal chloroplast grew well in the nutrient medium and its nutritive value estimates are high. Thus, the algal nutritive value can be estimated roughly by observation using a photomicroscope. As the size of apical axis is longer than that of *Chaetoceros*, there was concern whether the prawn larvae could eat them easily.

Suitable reagent medium for Ph. tricornutum H-strain culture

Guillard4F, 5F, and 6F were concluded to be suitable reagent media for the alga because the difference quantities of nutrients are small between these levels of media. Guillard4F was selected as the most suitable medium in this experiment. This medium level is recommended for use in small-scale culture, which is lower than 1,000mL.

Possibility of using fertilizers substitute for reagents to make a suitable medium

If fertilizers could be used as nutrients instead of reagents, the cultivation is economical and easy, especially in outdoor large-scale culture. In this experiment, KNO_3, $Ca(H_2PO_4)_2 \cdot H_2O$, and Clewat32, which are used agriculture, were selected as N-source, P-source, and heavy metal nutrients source respectively. From the result of Trial-2, it is clear that the alga increased as well in fertilizer medium as it did in reagent medium. The efficiency of Clewat32 was very conspicuous in that the algal chloroplast was well developed.

Moreover, from the result of Trial-3, it was proved that silica and vitamins were not essential nutrients for the good growth of the alga. High levels of silica induces white sediments in seawater, and vitamins are expensive and induce propagation of bacteria. The use of these components should be avoided. *Phaeodactylum tricornutum* does not require them in outdoor large-scale culture or indoor culture.

Food value of Ph. tricornutum to prawn larvae

From the result of larval rearing experiment, *Ph. tricounrutum* is considered to be a suitable food organism for kuruma prawn zoeal and mysis larvae. For larvae fed on *Ch. gracilis* alone, the number of larvae clearly decreased during zoeal-1 and zoeal-2 stages. The reason seems to be that *Ph. tricornutum* is bigger than *Ch. gracilis*, so that some larvae eating the latter starved to death. After the zoeal-2 stage, the number of larvae fed *Ph. tricornutum* did not decline noticeably. The larvae fed on *Ph. tricornutum* metamorphosed

faster than those fed on *Ch. gracilis*. At day 9 , about 80% larvae fed on *Ph. tricornutum* alone grew to post larvae, while most larvae fed on *Ch. gracilis* were only at mysis-1 or mysis-2 stage. One of the reasons for the difference in metamorphosis progress was considered to be the nutritive difference between *Ph. tricornutum* and *Ch. gracilis*. *Phaeoductylum tricornutum* used in this experiment contained 20:5 fatty acid. From the results of the algal fatty acid composition reported (Ackman *et al*. 1968, Moreno *et al*. 1979), the 20:5 fatty acid seems to be EPA and fatty acids component of both diatoms are similar. The crude protein and total lipid contents in *Ph. tricornutum* were about 1.2 to 1.5 times higher than those of *Ch. gracilis*, so that the nutritive value of *Ph. tricornutum* was evaluated high compared with *Ch. gracilis*. However, the contents of crude protein and total lipid usually change under various culture conditions using batch style culture (James *et al*. 1989, Okauchi *et al.* 1990). Therefore, more detailed research on these algal nutrient changes relative to culture conditions will be required.

Effect of mixed feeding of Ph. tricornutum and Ch. Gracilis

It is already shown in bivalve larval rearing experiments that mixed feeding of some algae species, whose nutritive values are evaluated as high, is superior to sole feeding of each alga (Davis and Guillard, 1958). In this experiment, the survival rate of the prawn larvae fed on both algae was high compared with that of sole feeding trials. The larval survived in a tank was little changed during the mixed feeding experiment. Moreover, the progress of metamorphosis was almost same as that of larvae fed on *Ph. tricornutum*. Such a mixed feeding of some algae is effective not only for survival and growth of bivalve larvae, but also those of prawn larvae. Kuruma prawn larval rearing is usually carried out during the rainy and hot-temperature season, using outdoor large-scale tanks, in Japan. Under bad weather conditions, it is difficult to prepare enough *Ch. gracilis*. Therefore, culture of some species of algae with high nutritive value and growth capacity, such as *Ph. tricornutum*, *Isochrysis* sp.(Tahiti) (Okauchi et al. 1997), *Tetraselmis tetrathele* (Okauchi and Hirano, 1988), and *Tetraselmis chuii* (Tobias-Qunitio and Villegas, 1982) is important to avoid insufficient food supply. Moreover, the mixed feeding of these algae is effective to stabilize prawn larval production.

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