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Microbial Processes in Aquaculture Environment and Their Importance for Increasing Crustacean Production

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Abstract
Based on the photosynthesis of microalgae mainly, it was clarified that bacteria, protozoa and other microorganisms form microbial food assemblages using the organic matter produced by the algae and that these assemblages play a significant role in the aquatic food chain. Based on this concept, larvae of the prawn, *Penaeus monodon* and of the crab, *Portunus trituberculatus* were reared. The growth of the larvae and their production were markedly promoted by the introduction of certain bacterial strains such as PM-4 and NS-110. When the ciliated protozoon, *Strombidium sulcatum*, isolated in a prawn culture pond was used as feed for the larvae of Penaeus *monodon*, the growth of the larvae was also promoted.

Discipline: Fisheries/Aquaculture
Additional key words: crab, food chain, larvae, microorganisms, prawn, protozoa

Introduction

There are three main procedures to obtain marine biological resources as follows: 1) collection of naturally grown animals and plants, 2) stocking and rearing of young fish or plants of a certain size in enclosed areas by feeding and fertilization of water, and 3) release of larvae and recovery of grown fishes after a certain period of time in the sea. Since there is a growing demand for animal protein, harvesting of biological resources by using advanced technology has extended to wider areas of the sea, resulting in the rapid depletion of marine resources. Mari- and aquaculture was developed in the 1960s to supplement and eventually replace wild catches. Today, the production of such fishes as yellow-tail and seabream, under rearing procedures, exceeds the amount captured in the sea in Japan. Furthermore, the harvest of the prawn, *Penaeus japonicus*, now extensively cultured in Japan, is equivalent to that in the sea. Cultured fish and prawn account for more than 10% of total yield and about 25% of total profit in the Japanese fisheries industry.

One of the major problems in the culture of fish is the development and rearing of larvae. Fish larvae often die in less than 24 hours if adequate food is not available, since fry have small toothless mouths and are not enough mobile to catch food. For this reason, food size should be smaller than their mouth parts and food needs to be located near the fish. If fish larvae could be kept in enclosures away

from their predators and receive an adequate supply of food, their survival rates would be much higher than those in the sea.

In this paper, several processes involved in the formation of microbial food assemblages in the food chain of the sea are described. According to this new concept of microbial food chain, fish production increased *in situ* in aquaculture using bacteria and protozoa as live feeds.

Formation of microbial food assemblages in the sea

It is currently assumed that in the food chain of the sea primary producers, which fix light energy and yield organic materials, are consumed by the zooplankton as soon as they are produced.

Calanus, a dominant genus among the zooplankton communities has been considered to be a typical herbivore that feeds on detritus and bacteria[10]. Since protozoa occur abundantly in the sea, for example, the numbers of ciliates and colorless flagellates exceed 10^3 cells/m*l* and 10^5 cells/m*l* in seawater, respectively, in the coastal waters of Japan, and the zooplankton tends to gather in the water layer where the bacterial populations are abundant[7], nutrient transfer from microorganisms through protozoa to zooplankton is considerably larger and more important in the lower trophic levels of the marine food chain.

In terrestrial ecosystems few of the primary producers are directly utilized by predators as food. In the forest ecosystems, for example, a small portion of the leaves is eaten by animals. A major part of the carbon is transferred from fallen leaves through bacteria, fungi, protozoa, small animals and small plants to middle-sized animals. This energy transfer is referred to as detritus food chain, but a large number of microorganisms attached to detritus are the main nutrient source for the predator[2]. Thus, this food pathway could be designated as microbial food chain instead of detritus food chain. It is preferable to define primary producers not utilized as food, as builders of "microbial food assemblages" using exudates, debris and faeces from microalgae or through the processes of zooplankton feeding. These assemblages are the starting point of the energy flow in the lower trophic levels of the food chain.

It was reported that when the copepods *Eurytemora* sp., *Scottolana* sp., or *Heteropsyllus* sp. ingested detritus or ciliated protozoa, they produced a larger number of eggs and broods compared to when microalgae were used as food[3,11]. Thus microbial foods play a significant role in determining the quantity and quality of the food chain in the sea.

Use of microorganisms in aquaculture

Microbial food assemblages are important in aquaculture as well. Table 1 shows the prey organisms currently used in mariculture. Larvae of prawns, shellfish and sea urchins digest diatoms, green algae and phytoflagellates, although the young stages of prawn and crab prefer other microorganisms to diatoms[9]. There are surprisingly few organisms that can be used as feed for fish larvae as they do not ingest

Table 1. Prey organisms frequently used in aquaculture

Prey organisms		Predators
Diatoms	*Skeletonema* *Chaetoceros* *Nitzschia* *Navicula*	Penaeid prawn, crab, abalone
Flagellates	*Monochrysis* *Isochrysis* *Tetraselmis*	Penaeid prawn, oyster
Rotifers	*Brachionus*	Fish larvae
Crustacea	*Artemia* *Tigriopus*	Prawn mysis, young fish
Bivalve eggs	*Osteria* *Mytilus*	Fish larvae

phytoalgae. Even if microalgae are ingested by the larvae, they can not digest them and eventually die. Currently only the rotifer *Brachionus* sp. can be used for fish larvae because bivalve and sea urchin eggs can only be obtained seasonally. Due to its large size, the rotifer cannot be eaten by fish larvae immediately after hatching. In fact, many more fishes could be reared if micro-food of a size less than that of rotifers could be identified and cultured easily. Artificial encapsuled food was reported to be suitable only if there were bacteria associated with it[5]. Under these circumstances in aquaculture, preparation and treatment of water using fertilizer and microalgae markedly affect the survival rates of fish larvae. Even if they can survive in these early stages under unsuitable water conditions, larval feeding rates decrease and eventually death occurs due to diseases and other factors such as insufficient storage of nutrients in the larval body. Thus it is recommended that in water treatment prior to stocking, microbial food assemblages should be cultured so as to provide a more appropriate source of food for the larvae.

Two examples of the use of microbial food assemblages are described as follows. The crab, *Portunus trituberculatus*, is usually reared for 1–2 months and released into the sea, mainly the Seto Inland Sea, Japan. The following year, the crabs collected by fishermen contribute to the increase of profit. An increase in the survival rates of crab larvae was observed when the culture water was fertilized with organic material, although it was considered that the larvae fed on detritus rather than on microorganisms.

1) Naturally grown microbial food

To a container with 200 m^3 seawater filtered with sand grains (size about 400 μm), 20 *l* of a microbial culture (4.0×10^8 cells/m*l*) naturally grown in seawater by supplying glucose and urea as main organic nutrients[4] was added twice, before the crab larvae (Zoea 1 stage) were transferred. Five *l* of these microbial assemblages and an amount of 1×10^9 individuals of the rotifer were inoculated everyday after the transfer of the larvae to the containers. By the addition of microbial assemblages, the numbers of bacteria, diatoms and flagellates increased gradually in the 200 m^3 seawater at the beginning of the experiment. Soon after the larvae were transferred, the bacterial numbers decreased, while the numbers of diatoms and flagellates continued to increase for a 2-day period. At the Zoea II stage of the crab larvae, the flagellate population decreased, followed by the decrease of the diatom population. These data suggest that the crab larvae fed on bacteria, protozoa and diatoms, successively[9].

2) Addition of identified bacterial strains

Instead of using naturally grown microbial food assemblages, we added the bacterial strains, PM-4 and NS-110, which promote the growth of the larvae of the prawn, *Penaeus monodon* and larvae of the crab, *Portunus trituberculatus*. To 200 m^3 of seawater previously sterilized with sodium hypochlorite followed by neutralization with sodium thiosulphate, 15 *l* of the bacterial culture solution was added once a day for 7 days. Initial bacterial concentrations amounted to about 10^6 cells/m*l*. Crab larvae (about 28,000 ind./m^3), diatoms (1,200 cells/m*l*), and rotifers (5,000 ind./*l*) were added to the culture water on the first day of the experiment.

As shown in Plate 1, the bacterial strains were eaten by the crab larvae, since stained bacteria with a fluorescent dye were observed inside the digestive organ of the larvae by examination under a fluorescence microscope. In 7 trials in 1990, the survival rates of the crab larvae in a 200 m^3 container were 27.2% (mean value) when bacterial strain PM-4 was added. In 6 out of 9 trials in which strain PM-4 was not added, no larvae grew into adults, resulting in an average survival rate of only

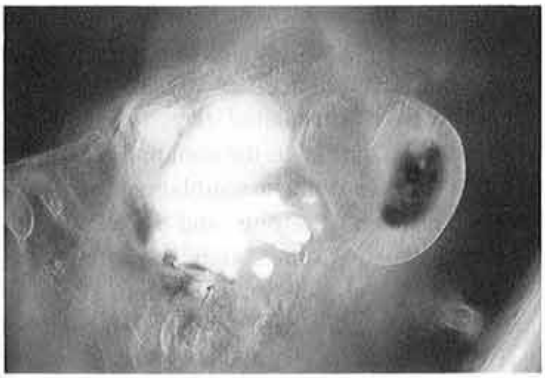

Plate 1. Bacterial clump, stained with a fluorescent dye, inside the digestive organ of the larva of the crab *Portunus trituberculatus*

6.8%. The same findings were obtained in the production of crab larvae in 1991. The average production of the crab larvae in 1990 and 1991 is indicated in Fig. 1. The method was also applied for the culture of the larvae of the prawn, *Penaeus monodon*. With the addition of the bacterial strain NS-110 in this case, 57% of the larvae survived after 13 days (Post-larva V growth stage). On the other hand, in the absence of bacteria, the larvae died at the growth-stage of mitosis I (5-day growth after hatching out from the egg) (Fig. 2).

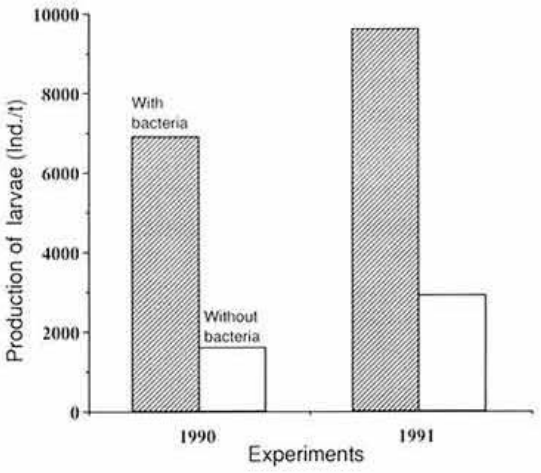

Fig. 1. Production of larvae of the crab *Portunus trituberculatus*, with and without the bacterial strain PM-4 in 1990 and 1991

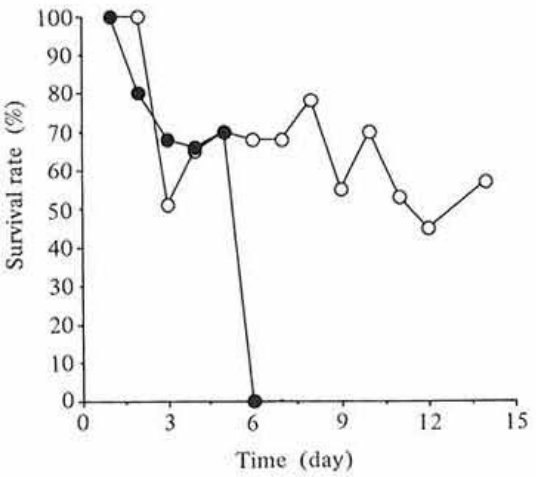

Fig. 2. Survival rates of the larvae of the prawn, *Penaeus monodon*, with (○) and without (●) the bacterial strain

Protozoa as live feeds for prawn larvae

We isolated ciliated protozoa and determined whether the larvae of the prawn, *Penaeus monodon*, could feed on them. Protozoa in the prawn culture pond were isolated with a micropipette under a stereoscopic microscope and transferred to a petri dish, 28 mm in diameter, in which a droplet of solid agar at the bottom of the dish and 2 m*l* of sterilized seawater were added. The solid agar droplet contained 1.5% (w/v) agar and 0.1% (w/v) malt extract from Difco Co. Ltd. which were autoclaved for 15 min at 121°C and placed at the bottom of the dish before solidifying. The number of protozoa increased in the dish by feeding on bacteria which grew using the nutrients exuded from the agar. A diatom, *Navicula* sp. was also used as feed for this protozoon.

As shown in Fig. 3, the protozoa isolated were about 60 μm long, round in shape and slightly elongated posteriorly. The characteristic protuberance was located in the apical top area surrounded by the adoral zone of membranelles (AZM), large and short. At the end

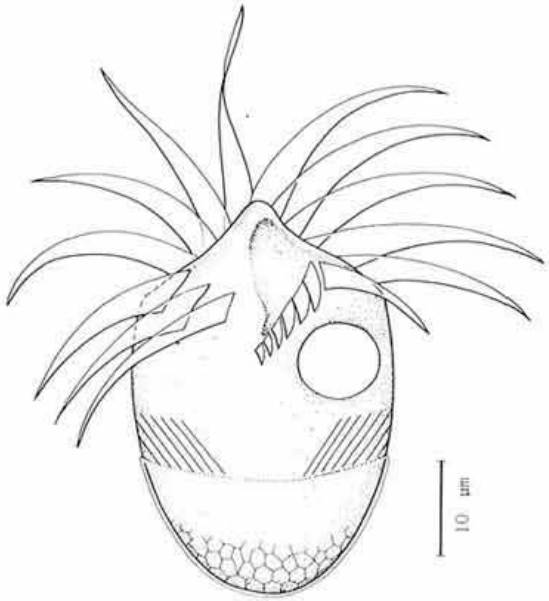

Fig. 3. Ciliated protozoon, *Strombidium sulcatum* Claparède and Lachmann, 1858

of the line of short membranelles of AZM, the cytostome was open. The line of trichite was observed in the center of the body. Polygonal cortical platelets were attached in the posterior half of the body. Based on this observation the protozoon was identified as *Strombidium sulcatum* Claparède and Lachmann, 1858[1] based on taxonomical references[6,8].

When *Strombidium sulcatum* was added as feed to the rearing container of prawn larvae (Protozoea I stage) at the concentration of 1,000 cells/*l* with 10^4 cells/m*l* of *Navicula* sp. and 100 ind./*l* of larvae of *Penaeus monodon*, the survival and molting rates of the larvae of *P. monodon* were much higher than when other protozoa, *Strobilidium* sp. (ciliate), and *Oxyrrhis marina* (dinoflagellate), were present (Fig. 4).

Through this work we conclude that the use of microbial food assemblages may enable to develop more profitable farming practices in aquaculture.

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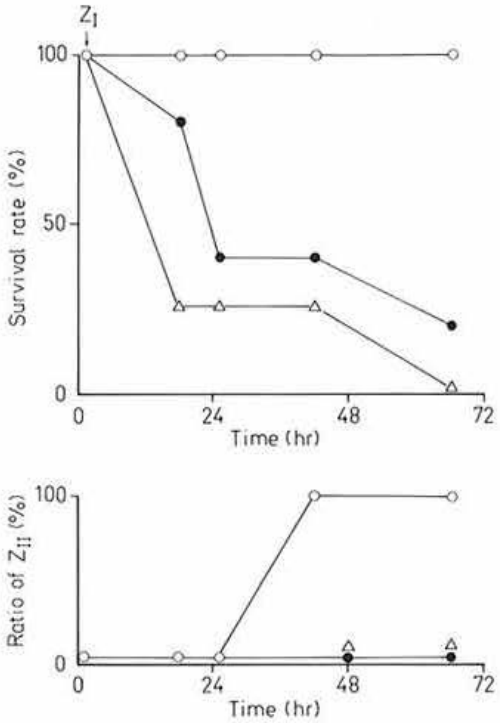

Fig. 4. Survival rates of the larvae of the prawn, *Penaeus monodon*, in the presence of protozoa
○ : *Strombidium sulcatum*,
● : *Strobilidium* sp.,
△ : *Oxyrrhis marina*.

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