Sustainable Aquaculture in the Estuary Area

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Abstract An estuary is the widening part of a river where it meets the sea, and is an interface between agriculture, forestry, animal husbandry, civilian life etc., where mangrove trees grow in the tropical and subtropical area. The leaves of mangrove and seaweeds, which grow close to the mangrove, are decomposed, adding enormous quantities of detritus to the estuary ecosystem. Because most of the energy flow through estuary food chains is derived from detritus, mangrove and seaweeds are major contributors to the productivity of the estuary. For example, the estuary place where the ratio of mangrove forest area/river area is high, the quantity of fish catch is much larger than the place where the ratios are low, and also bivalves are dominant in the area of the high ratio, but the polychaetes are dominating, in stead, in the low ratio area.

Based on these results, the microbial (detritus) food chain was adopted for the production of fish; that is, the bacteria which promoted the growth of fish are added with the conventional feed line in situ aquaculture. The bacterial strains were also selected to have the function to repress the pathogenic bacteria, fungi and viruses. With this method, the production rates of many fishes, prawn and crabs increased and also they were protected from pathogens. This method, in which microorganisms repress the pathogens, is called biological control or biocontrol and the useful microorganisms adopted are termed as biocontrol agents (BCAs).

Following the feeding of an artificial compound feed (ACF)/BCAs mixture to fish, the BCAs contained in residual ACF and feces after digestion sank to the sediment. Eventually, these bacteria degraded organic materials in the sediment.

Key Words: aquaculture, estuary, microorganisms, mangroves, organic

1. Introduction

Increased population and economic growth accelerates the development of coastal areas in tropical and sub-tropical countries, particularly mangrove forests along the sea coast. Urbanization, resort development, expansion of farmland and aquaculture in recent years have resulted in water pollution, soil erosion, runoff of soil into the sea, and the occurrence of red tides worldwide, especially in the estuary brackish water area. Since the over-intensive utilization of brackish water areas, a result of inadequate information on the production mechanisms of these ecosystems, has resulted in the destruction of many of their functions, it is necessary, therefore, to carry out comprehensive studies to evaluate the biological productivity of brackish water ecosystems, with the main emphasis placed on developing strategies for their sustainable exploitation. In this report, the significances of the mangrove ecosystem are described for the production of fish and bivalves in the estuary. Because the high production of the mangrove area is based on the microbial food chain (so-called detritus food chains), in this research microbial procedure is also adopted in the aquaculture system; that is, utilization of microorganisms as feed and also as the biocontrol agent in situ in the aquaculture site.

2. Characteristic features of the estuary

An estuary is the widening part of a river where it meets the sea, and is frequently chosen by urban populations as the nearest natural location or amenity site. It is an interface between agriculture, forestry, animal husbandry and civilian life but also place most at risk from pollution.

The part of an estuary which contains diversified living species is the area that changes in both time and space: the transition zone between the worlds of fresh and salt water. The estuary has many partial ecosystems created by salinity gradations. It is a very demanding environment, and few species are adapted to survival in this fluctuating environment, where salt concentration varies erratically between 0.5 and 3.5.

Estuarial species include various polychaete worms, clams, oysters, gastropods, crabs, shrimps and fishes. The number of species living in the estuarine environment is considerably smaller than the number of species in the neighboring marine and fresh water habitats. In estuaries, species richness, or diversity, decreases with salinity at the mouth of the river.

When fresh water is mixed with ocean water, the rise in salinity causes fine particles of silt to flocculate and settle. Salts promote the aggregation of the particles. Some dissolved nutrients in fresh water
became insoluble as salinity increase, causing the estuary to be a natural nutrient trap. Most nitrates and silicates come from river water and phosphates are dissolved in seawater. A tidal marsh or wetland is formed in this area which is favorable to mud snails, bloodworms, blue claw crabs, striped bass, and flounder. Mangrove roots and stems also retain the sediment particles which settle on the sea bottom and promote the formation of wetlands. In addition the concentration of salts in the interstitial water in the sediment shows less fluctuation than the water above in the estuary, and marine organisms in the sediment can thus penetrate further the upstream.

Mangrove trees that grow mainly in brackish water areas are utilized for fuel, and are an important source of wood chips, timber, medicinal products, etc. They protect the coast from erosion and from the damage by hurricanes and cyclones. Mangrove forests also serve as breeding grounds for fish species, making them valuable for fish production.

Mangrove promote the growth of colonies of seaweeds in the soft, sandy mud in the estuary. Seaweed beds contain complex communities of plants and animals present in the subtidal zone of the estuary, making them ecologically important in the estuary ecosystem in many ways. For example, seaweeds help to stabilize the fine sediments in the estuary. The entangled roots bind the loose sediments, and the long green leaves slow water currents, allowing additional sediment particles to settle. The major ecological role of seaweeds and mangrove roots is to provide support for a variety of benthic organisms. Dense growths of microalgae and animals cover the ribbon-like blades of seaweed. The microscopic plants and bacteria etc. attached to eelgrass and mangroves produce large amounts of food, which is eaten by snails and other benthic grazers. Thus, the primary productivity of the estuary is dramatically increased by the mangrove and seaweed beds. Moreover, the solid substrate provided by seaweed leaves and mangrove leaves is necessary for the survival of organisms such as economically valuable scallops. Other organisms that benefit by living attached to mangrove and seaweeds include sponges, bryozoans, small worms, and tunicates.

These mangrove and seaweed leaves are decomposed, adding enormous quantities of detritus to the estuary ecosystem. Because most of the energy flowing through estuary food chains is derived from detritus, mangrove and seaweeds are major contributors to the productivity of the estuary, because very few organisms feed directly on seaweed and mangrove leaves.

3. Fish and bivalves production in the Matang, Merbok and Lumut mangrove areas in Malaysia

The research project “Productivity and sustainable utilization of brackish water mangrove ecosystems” were carried out by Japan International Research Center for Agricultural Sciences (JIRCAS) with the collaboration of the Malaysian Department (Fisheries Research Institute, Forest Research Institute, Department of Fisheries, Department of Forestry, University of Malaya), in which the author was the project leader at that time. The research results of the project especially on the fish and bivalves production are shown below. The locations of the survey was Matang, Merbok and Lumut in Malaysia (Fig. 1) and as shown in Fig. 2, there are many rivers inside the mangrove forest.

In these three survey sites, fishes were collected using several tackles (beam-trawling, push-net, otter-trawling etc.), which are described in the project report “Productivity and sustainable utilization of brackish water mangrove ecosystems” published by JIRCAS in 2000. In the Matang mangrove estuary area, where the ratio of mangrove forest area/river area is 4.7, the quantity of fish catch is larger than that in Merbok (ratio: 2.1) and Lumut (ratio: 1.1). In case of the diversity index of fish, however, shows a higher value in Merbok than in the other two mangrove areas. In Lumut, the volume of fish catch and diversity index is the lowest of the three areas (Tables 1 and 2). Based on these results, it should be suggested that the value of the ratio of mangrove forest area/river area is a useful index, with a value of between 4.7 and 2.1 being a precondition for sustainable fish production in mangrove estuary areas.

Macrobenthos species in Matang and Lumut are representedly polychaetes, amphipods, isopods, decapods, gastropods, bivalves and ophiuroids; that of the former showed increasing abundance toward the sea. The dominant group in Matang was the bivalves, accounting for 47% of the total abundance, followed by crustaceans making up 43% and polychaetes of 9%. In the Lumut water, polychaetes were the clearly dominant group, making up about 46% of the total macrobenthos population (Table 1). This was followed by ophiuroids, bivalves and crustaceans. As bivalves such as Anadara granosa were found mainly in sediment with higher silt content, as in Matang, the
more sandy environment may be less conducive to these animals. On the other hand, polychaetes, being largely burrowing forms, may find the sandier and less anoxic environment less stressful.

4. Microbial food chain in the estuary

Energy flow studies in this research, it can be concluded that (1) there is a mangrove leaf-based organic matter flux in the water, running into the sea through the river, (2) fishes showed the highest possibility to feed on the many food organisms which are basically originated mangrove leaf-carbon and nitrogen.

For years, the food chain in seawater was not clearly understood, and consequently, aquaculturists have often been misdirected in their endeavors. Until recently, scientists thought that the primary producers of the food chain (microalgae, which fix light energy and yield organic materials) were consumed by zooplankton as soon as they were produced. Recent studies, however, have proven that several other food transfer pathways exist as well. For example, it has been reported that the dominant species in zooplankton communities, which were considered to be typical herbivores, feed on detritus and bacteria (Paffenhofer and Strickland 1970). Seki and Kennedy (1969) also describe the significant role of bacterial aggregates as food for zooplankton.

In terrestrial ecosystems, very few of the primary producers are directly utilized as food by predators. In the forest ecosystem, for example, only a small portion of the leaves are eaten by animals, while a major portion of the nutrients from fallen leaves are transferred through bacteria, fungi, and protozoa, as well as through small plants and small- to middle-sized animals (Odum, 1971). This energy transfer is thought of as a detritus food chain, but a large number of microorganisms attached to the detritus are in fact the main nutrient source for predators (Harrison and Mann 1975; Uchida et al., 1997). Thus, this food pathway could be designated as a microbial food chain instead of a detritus food chain (Maeda, 1988). It is

<table>
<thead>
<tr>
<th>Sites</th>
<th>Fish catch (Kg)</th>
<th>Diversity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matang</td>
<td>5.6</td>
<td>2.8</td>
</tr>
<tr>
<td>Merbok</td>
<td>1.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Lumut</td>
<td>1.0</td>
<td>1.6</td>
</tr>
</tbody>
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Table 2 Diversity index and catch quantities of fish in the three research sites of Peninsular Malaysia

From the project report “Productivity and sustainable utilization of brackish water mangrove ecosystems” by JIRCAS in 2000.
therefore more useful to define primary producers as builders of a dissolved organic matters (DOM) pool. Based on this DOM pool, which mainly originates from exudates of microalgae, for instance, as reported by Fogg et al., (1965) and Williams (1981), “microbial food assemblages” are formed. These assemblages are the starting point of the energy flow in the lower trophic levels of the food chain in the aquatic environment (Maeda 1988), although the effect of viruses on these microorganisms, suggested by Fuhrman (1992) are not considered (Fig. 3).

Many protozoa feed on bacteria and thus help the bacteria to contribute to higher trophic levels (Porter et al., 1985). In fact, protozoa are considered to be a significant food source for fish and zooplankton, as Ryder (1881) and Hirano and Ohshima (1957) suggest. Furthermore, Robertson (1983) increased the feeding rate of Acartia by using tintinnids as food at a concentration of 10³ cells/L. It is interesting to note that the copepod Scottolana canadensis was capable of producing eggs more frequently when ciliated protozoa were given as food than when only microalgae were available (Heinle et al., 1977). ZoBell and Feltham (1937), Azam et al. (1983) and Sherr and Sherr (1988) have also stressed the significance of the food value of bacteria and protozoa.

In aquaculture, the concept of the microbial food chain can be adopted. In fact, the addition of low concentrations of organic matter promotes the growth of fish. For example, in cases where phytoplankton is used as the main live food, certain species of bacteria may be added with the algae (Maeda et al., 1992). Treatments such as this can cause survival rates to increase significantly (Maeda and Liao, 1992). It is therefore considered preferable to feed microbes to the fish, shellfish, prawn etc. along with algae. Gall et al. (1997) also reported the significant role of protozoa as live feeds to the oyster, Crassostrea gigas. Microbial food assemblages formed by microorganisms, microalgae, and other plankton, thus, play significant roles as live food in aquaculture water (Fig. 2).

5. Use of microorganisms in aquaculture

Since the above studies on the mangrove ecosystem, significances of microorganisms are elucidated to decompose mangrove detritus which contribute to the production of fish and bivalves. We adopted these microorganisms (bacteria) for the stable and more amount of production in aquaculture. Besides the growth promotion effect, bacteria show the antagonistic activity to other microorganisms (bacteria, fungi, viruses etc.) and the method to repress the growth of pathogenic microorganisms by other microbes is called as biological control (biocontrol). Biological control (biocontrol) utilizes the naturally occurring antagonism between organisms, having been frequently used to enhance the activity of natural antagonists to repress growth or kill pathogenic organisms in agriculture. For this purpose, bacterial strains which promote the fish growth and at the same time inhibit the pathogenic bacteria, fungi and viruses, in terms of the antagonism among the microbial community were studied in many fish rearing-sites.

In order to apply such biocontrol to the aquaculture environment, biocontrol agents (BCAs) bacteria of which can repress the growth of pathogenic bacteria and viruses were sought. Initially, microorganisms that promote fish and crustacean growth were isolated, since BCAs should not be harmful to them. Prawns (Penaeus monodon) were cultured with and without soil extracts (the source of organic matter). Higher survival and molt rates of prawn larvae were obtained in the experiment that contained soil extracts and the bacterial strain that promoted the growth of the prawn larvae being isolated (Maeda & Liao, 1992). The same bacterial strains also promoted the growth of a crab (Portunus trituberculatus) (Maeda et al., 1992; Maeda, 1999). Following this procedure, several other bacterial strains that promoted the growth of fishes, such as striped jack, sea bream, flatfish, Ayu fish, eel etc. were also isolated (Maeda, 1999; Noguchi et al., 2006; Itoh et al., 2006).

These useful bacteria were marked with a fluorescent dye following the method of Sherr et al. (1987), and fed live to rotifers (Brachionus plicatilis) and crab larvae (Portunus trituberculatus). Under an epifluorescent microscope, the stained bacteria could
be seen inside the guts of the rotifers and crab larvae. Thus, the utility of bacteria as live food can be determined from the survival data of their predators, as well as through direct observation of the former following ingestion (Maeda and Liao, 1994; Maeda, 1999).

*Vibrio anguillarum*, *Edwardsiella tarda*, *Flavobacterium psychrophilum* and infectious hematopoietic necrosis virus (IHNV) were used as microbial pathogens to test whether or not isolated microbial strains could repress the growth of those pathogens. Accordingly, several bacterial strains that strongly repressed the growth of the pathogenic microorganisms were obtained. Determination of anti-viral activity in isolated bacteria indicated that bacteria that showed the anti-bacterial pathogen activity were also able to repress the infectious activity of the virus (Maeda et al., 1997).

One of these BCAs was applied to the culture of crab (*Portunus trituberculatus*) larvae that were found to be infected by a pathogenic *Vibrio* sp. Before this application, crab culture methodology included the addition of several antibiotics to the larval rearing water, such treatment being initially able to repress the growth of pathogenic *Vibrio* sp.; however, the appearance of resistant microbes (mainly fungi) killed all of the larvae within a few days. Infection of larvae by pathogens interfered significantly with larval production wherein whole batches of diseased larvae were abandoned and a new production cycle initiated. Although the shortcomings of antibiotic use were apparent, few if any alternative means for controlling disease were known. It was therefore essential that new approaches should be adopted, wherein the antagonism of certain microorganisms could be used to repress other pathogenic microbes in aquaculture systems.

Subsequently, the addition of a bacterial strain as a biocontrol agent, instead of antibiotics, to the *Portunus trituberculatus* larval culture facility was found to improve growth and protect larvae from pathogens. Among the bacterial assemblages monitored, the added bacterial strain dominated the bacterial populations, *Vibrio* spp. counts decreasing or becoming undetectable in seawater. In this way, production of crab larvae was greatly increased (Maeda & Nogami, 1989; Nogami & Maeda, 1992; Maeda, 1999). Two possible explanations for the reduction in concentrations of *Vibrio* spp. when the BCA was added are: (1) the production (although not high) of vibrio-static reagents by the BCA, and (2) niche exclusion between the zymogenous bacteria and BCA. The latter is particularly important in controlling microbial communities. In experiments not involving the addition of a BCA, survival rates of larvae from zoea I to zoea IV were high, larvae not always being infected with pathogenic microbes. However, the larvae died on reaching the megalopa I growth stage in many experiments without BCA, probably because of nutrient deficiency. These data suggest that the use of the BCA should improve the physiological state of the larvae by serving as a nutrient source for growth (Nogami & Maeda, 1992).

As well as the above results, BCAs were also applied to eel (Fig. 4), Ayu fish and others, and the high survival rates of these fishes with the inhibition of pathogens were obtained (Maeda, 1999; Noguchi et al., 2006; Itoh et al., 2006; Itoh and Maeda, 2007).

One of the bacterial strains used in aquacultural processes showed an ability to prevent infection of fish larvae by SJNNV, baculo-like viruses and irido virus. When the former strain was added to water containing the larvae of the *Penaeus* prawns, striped jack and sea bream, the survival rates of these larvae were much higher than those without the bacterial strain, all fish larvae dying due to viral disease in the latter experiments (Maeda et al., 1997; Maeda, 1999). Viruses spread from infected fish to healthy fish through the seawater, thereby reducing the fish numbers gradually or rapidly. However, BCAs could help to inhibit the spread of viruses among fishes. In addition, if fish fed on such BCAs, a probiotic effect might be the strengthening of the immune system of the former. With such useful effects and features, BCAs could prove to be effective in protecting fish from the spread of bacterial and viral diseases in aquaculture.

Fig. 4 Cumulated mortality of eel with addition of BCA and without BCA.
Following the feeding of an artificial compound feed (ACF)/BCA mixture to fish, the bacteria contained in residual ACF and feces after digestion sank to the sediment. Eventually, bacteria, including BCAs, degraded the organic matter in the sediment (Maeda, 1999). In the sediment, the many benthic animals that feed on detritus, microorganisms and other small animals, move and agitate particles, thereby allowing greater penetration of oxygen-rich water into the sediment (bioturbation). In heavily stagnant and eutrophicated sediments, if such BCAs are added and grow well, the bacteria stimulate the growth of benthic animals, resulting in accelerated bioturbation and material processing, which in turn stimulates the growth of various other microorganisms and animals. In this manner, the activity levels of these organisms at various trophic levels is accelerated and an improved sedimentary environment evolves.

References


Noguchi, K., I. Iwata and M. Maeda 2006. The antagonistic bacterium that repress the growth of eel pathogens, Edwardsiella tarda. La Mer 44: 157-160.


(*) in Japanese with English abstract, figures and tables)
要旨 河川が沿岸域に流入して構成される水域は、農林畜水産業、工業および都市（文化）生活との接点となっており、この地域は、これらの産業および人間生活の影響を同時に受ける。

熱帯亜熱帯地域では、この水域にマングローブが繁茂し、またマングローブの地先には海草藻群落が分布する。そして、この水域における、魚類、甲殻類（エビ、カニ）、貝類などの主要な食物源は、マングローブ葉や海草藻由来の有機物（デトリタス）であるため、マングローブとマングローブ林中を流れる河川の面積比（マングローブ林面積／河川面積）の大きい、すなわち、マングローブがよく繁茂する水域では、魚類や貝類の漁獲量が飛躍的に多くなる。

デトリタスには、微生物が被着するように付着しているため、デトリタス（すでに分解の進行している有機物）の栄養価の大半は、微生物に依存する。このため、デトリタスに由来する食物連鎖は、微生物（デトリタス）食物連鎖とよばれている。

この微生物食物連鎖を水産養殖に適応するために、魚類の成長を促進する微生物（細菌）を探索、分離した。さらに、これらの微生物株のなかで、病原微生物（病原細菌、真菌、ウイルスなど）を抑制する機能を持たせる株を選択した。そして、これらの有用微生物を飼料に混合して養殖魚に与えたところ、魚類の生産率が向上し、かつ疾病防止の効果が得られた。このような微生物によって病原微生物を防除（排除）する方法、生物防除（バイオコントロール）は、自然に備わった機能であり、その有効利用によって、環境と調和した（薬剤を使用しない）安定した養殖生産が可能となる。

キーワード：オーガニック、水産業、微生物、マングローブ、養殖