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Interactions of microorganisms and their use as biocontrol agents in aquaculture

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Abstract : The function and activity of microorganisms are the key factors in understanding the quality of the aquacultural environment. In fact, both harmful and useful microbes exist in aquaculture water that directly affect fish growth. Among these microorganisms, useful bacteria that can repress pathogenic microbes in the process of microbial antagonism and are utilized in biological production are called biocontrol (biological control) agents (BCAs). BCAs were applied to aquaculture production in this study to prevent bacterial and viral diseases in fishes and crustaceans. This paper describes the results of a study of biocontrol for fish and crustacean production, and also reviews research reports on the use of microorganisms as BCAs in aquacultural processes.

Keywords : *biocontrol, probiotics, antagonism, microorganisms, virus, aquaculture, fish, crustaceans*

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The main purpose of this study was to find and utilize microorganisms that promote fish growth, while at the same time repressing the growth of pathogenic microorganisms. The results are presented here, in addition to a review

of the use of microorganisms as biological control (biocontrol) agents (BCAs) in aquaculture.

Antagonism among microbes is a naturally occurring phenomenon, through which pathogens can be killed or reduced in numbers. In order to apply such biocontrol to the aquacultural environment, BCAs that 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, the bacterial strain that promoted the growth of the prawn larvae being isolated (MAEDA and 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 and

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flatfish were also isolated (MAEDA, 1999).

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 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 vibrio-static 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 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 and NOGAMI, 1989; NOGAMI and 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 vibriostatic 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, probably because of nutrient deficiency. These data suggest that the use of the BCA might improve the physiological state of the larvae by serving as a nutrient source for growth (NOGAMI and MAEDA, 1992).

One of the bacterial strains used in aquacultural processes showed an ability to prevent infection of fish larvae by Striped Jack Nervous Necrosis Virus, baculo-like viruses and irido virus. When this 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 strengthening of the immune system. 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.

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.

Through these studies, the author determined that a bacterium exists that promotes the growth of microalgae, rotifers and fishes at the same time. Since microalgae – rotifers – fish are linked in the food chain, this bacterium functions well as a growth promoter on each component, and this microbial process is termed the “microbial line” in aquaculture.

Following is a review of the interactions of microorganisms and their use as biocontrol agents in aquaculture.

2. Biocontrol Agents (BCAs) and Probiotics

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 (LANDIS *et al.*, 2000). The method is especially familiar in the example of the bacterium, *Bacillus thuringiensis*, which infects the mouth of pathogenic insects and eventually kills them (KERR, 1972 and 1980), the process having been commercialized in Europe and North America where several thousands of tons of the former are used in agriculture. The results have encouraged further studies on the use of viruses, fungi and protozoa as biocontrol agents to eliminate pathogenic organisms. The three main biocontrol methods include classical (augmentative), conservation and integrated biocontrol. In the classical method, exotic

agents are used against pathogens being released or those released augmentatively (GURR and WRATTEN, 1999). The use of conservation biocontrol in conjunction with classical or augmentative methods, is called integrated biocontrol. As a method of habitat manipulation, conservation and integrated biocontrol include actions with multiple functions, such as sowing one plant near another in order to release materials, whilst having a negative influence on the plant's natural enemies. Thorough research of the ecosystem and careful management is required to manage these trade-offs.

LILLY and STILLWELL (1965) coined the term “probiotics” to describe substances produced by one protozoan which stimulated another. Subsequently the term became used to describe animal feed supplements that had a beneficial effect on the host animal by affecting its gut flora (PARKER, 1974). FULLER (1989) used “probiotic” to describe live microbial feed supplements that beneficially affected the host animal by improving its intestinal microbial balance. The best evidence for such a protective effect on gut flora stems from the observation that germ-free animals are more susceptible to disease than animals with several intestinal flora. NURMI and RANTALA (1973) showed that young chickens reared under modern husbandry practices and orally pretreated with a diluted faecal solution or mixture of intestinal microorganisms obtained from healthy adult birds, developed resistance to the establishment of *Salmonella infantis* in the intestine. They attributed this increased resistance to competition between the newly-established intestinal flora and the invading pathogen, *S. infantis*. According to GOMEZ-GIL *et al.* (2000), a probiotic ought not to be classified as a biological control agent in the strictest sense, since a probiotic microorganism does not necessarily attack the noxious agent (pathogen) (i.e. a natural enemy of the latter), but it merely prevents damage to the host caused by the pathogen. Such prevention, usually through competition, may produce substances that inhibit the growth or attachment of the harmful microorganism. Neither should probiotics be classified as growth promoters, since their actions are not confined to improved growth but are

also associated with general improvements in health.

Based on these views, biocontrol agents (BCAs) can be considered as natural enemies that kill or repress the growth of pathogenic organisms, and are beneficial or at least not harmful to the plants and animals cultivated.

3. Biocontrol of Bacterial Pathogens in Aquaculture

Following DOPAZO *et al.* (1988), the activity of antibiotic-producing marine bacteria was assayed against bacterial pathogens (*Vibrio*, *Aeromonas*, *Pasteurella*, *Edwardsiella*, *Yersinia* and *Pseudomonas*) of fishes with the aim of evaluating the possible use of these marine strains for controlling epizootics in aquaculture. Inhibition tests on solid media showed that the majority of fish bacteria were highly sensitive to the strains tested, only two strains (*Edwardsiella tarda* and *Pseudomonas aeruginosa*) being resistant to all of the antibiotic-producing strains. TANASOMWANG *et al.* (1998) isolated *Vibrio*-inhibiting marine bacteria from a black-tiger shrimp hatchery. MORIARTY (1998) reported that as a result of using a *Bacillus* species for over 160 days at about 10^4 to 10^5 cells/ml, *Vibrio* numbers, especially those of luminous *Vibrio*, were low in prawn ponds where the *Bacillus* sp. was maintained in the water column. *Vibrio* numbers were also low in the sediments and luminous *Vibrio* absent. Since this report gave no data for fluctuations of *Bacillus* sp. concentrations in the pond, it is unclear whether or not the species added could grow in seawater. One strain of *Alteromonas* showed greater antagonism against fish and shrimp bacterial pathogens than other strains (JAYANTH *et al.*, 2001). In addition, RUIZ *et al.*, (1996) found antagonisms of *Alteromonas* sp. to a large number of bacteria in the aquaculture biotope. The inhibitory effect of *Vibrio alginolyticus* against a *Vibrio harveyi* strain was greater in seawater at 10 ppt. of salinity compared with 20 and 30 ppt. (RUANGPAN *et al.*, 1998). According to RICO-MORA *et al.* (1998), a bacterial strain (SK-05), selected for its active growth in organic-poor substrates and inoculated into a *Skeletonema costatum* culture in late exponential growth, prevented

the establishment of *Vibrio alginolyticus*, purposely introduced into the diatom culture. Since SK-05 has no bacteriostatic or antibiotic activity against *V. alginolyticus*, they concluded that it had the effect of competitive exclusion, due to its ability to utilize the exudates of *S. costatum*, which maintained an organic-poor environment within the culture, unsuitable for *Vibrio* growth.

These observations indicate a need for further studies of how individual microbes inhibit or promote fish health and growth, several reports next having indicated beneficial effects to fishes.

JÖBORN *et al.* (1997) reported the production of a growth inhibitor against two common fish pathogens *Vibrio anguillarum* and *Aeromonas salmonicida* by *Carnobacterium* sp., such being demonstrated *in vitro* in mucus and fecal extract. The *Carnobacterium* cells remained viable in the gastrointestinal tract for several days, no detrimental effects on the fish being observed as a result of the presence of the bacterium. SMITH and DAVEY (1993) noted that fluorescent *Pseudomonas* was capable of inhibiting the growth of *Aeromonas salmonicida* in culture media, such inhibition being probably due to the siderophore effect, resulting in competition for free iron. They found that the strain used was also capable of excluding *A. salmonicida* from the fish with stress-inducible infections, and suggested that as the strain did not significantly invade the fish following bath treatment, the effect must have been generated from external sources. Siderophore is a microbial iron (III)-transport agent that sequesters a limited supply of iron (III) and limits its availability to pathogens, ultimately suppressing their growth. Also, as a siderophore effect, GATESOUBE (1997) described the proportion of *Vibrio* sp., dominant in healthy turbot larvae, as being artificially increased in a rotifer enrichment medium and rotifers fed to the larvae. The main purpose of such *Vibrio* enrichment was to improve the resistance of larval turbot challenged by a pathogenic strain of *Vibrio splendidus*. GATESOUBE (1997) concluded that the biocontrol effect of the *Vibrio* sp. used may have been at least partly due to competition for iron with the pathogen.

ANDLID *et al.* (1995) reported 3.8×10^4 to 2.3×10^9 viable yeast cells per gram of intestine or feces in fish. Although the concentration of yeast in their experimental fish tank water never exceeded 10^3 viable cells per milliliter, no indication of fish sickness as a result of the high yeast colonization was recorded during any of the colonization experiments. During the period of their experiments, the concentrations of intestinal aerobic bacteria were lower than the intestinal yeast concentration.

Biocontrol effects and the manner in which biocontrol agents affected the growth of shellfish were reported by NAKAMURA *et al.* (1999). They showed that 12 strains of 51 isolates had inhibitory effects on the growth of 3 vibrios (*V. alginolyticus* and others) tested by a smear technique on an agar plate. One strain, which demonstrated the greater inhibitory effect, had no harmful effects on oysters. The challenge test of *V. alginolyticus* with this bacterium indicated greater than 70% survival of shellfish, while only about 8% survived without the bacterium. This report presents biocontrol agents that promoted the survival rates of shellfish. However, identification of biocontrol agents is still necessary, because of their possible harmful effects on humans.

Several studies have focused on the identification and use of bacteria as biocontrol agents in aquaculture. RIQUELME *et al.* (1996) noted that a culture supernatant of *Alteromonas haloplanktis* stationary-phase cells delayed the growth of pathogens, although the supernatant of the same strain from early and middle log phase growth stages did not negatively affect the growth of pathogenic *Vibrio* spp. Their larval scallop survival experiments showed that preconditioning of larvae with the bacterium for a short time (1 h) was effective in providing larval protection against such *Vibrio* spp. Pre-incubation for 24 h resulted in no significant differences from the control, the most effective result being obtained with the 1-h bath and the addition of the pathogen at 10^3 cells/ml. However, at a concentration of 10^6 cells/ml of the pathogen, the protective effect decreased. This technique, termed "bacterization" in agriculture, wherein bacterial inoculation of seeds or roots leads to changes in

plant growth, sometimes yields positive effects and the biological control of some plant pathogens (BROWN, 1974). AUSTIN *et al.* (1995) showed that following the addition of freeze-dried culture supernatant of the BCA, *Vibrio alginolyticus*, to *Vibrio ordalii*, there was a rapid decline of *V. ordalii* numbers (compared to the controls) occurred within 3 h. Following similar treatment, *Aeromonas salmonicida* and *Vibrio anguillarum* counts decreased steadily over 24 h, whereas *Yersinia ruckeri* increased in numbers and did not appear to be adversely affected by the BCA supernatant. These authors also applied the BCA to Atlantic salmon, which led to a reduction in mortality due to *Aeromonas salmonicida*, and to a lesser extent to *Vibrio anguillarum* and *V. ordalii*. GRAM *et al.* (1999) reported that sterile-filtered culture supernatants from iron-limited (0.1 mM) *Pseudomonas fluorescens* inhibited the growth of *Vibrio anguillarum*, whereas sterile-filtered supernatants from iron-replete cultures of *P. fluorescens* did not. *P. fluorescens* inhibited the growth of *V. anguillarum* during culture, independently of iron concentration, when the initial count of the antagonist was 100 to 1,000 times greater than that of the fish pathogen. GRAM *et al.* (1999) also tested the BCA effect *in vivo* by exposing rainbow trout to *Pseudomonas fluorescens* at a density of 10^5 CFU/ml for 5 days before a challenge with *Vibrio anguillarum* at 10^4 to 10^5 CFU/ml for 1 h. Some fish were also exposed to *P. fluorescens* at 10^7 CFU/ml during the 1-h infection. The combined BCA treatment resulted in a 46% reduction in calculated accumulated mortality; accumulated mortality was 25% after 7 days at 12°C in the BCA-treated fish, compared with 47% in fish not treated with the BCA.

ROBERTSON *et al.* (2000) showed that feeding salmonids with diets containing a probiotic (*Carnobacterium* sp.), being antagonistic against several pathogens, revealed that the strain remained viable in the gastrointestinal tract and that after 14 days of feeding challenge by cohabitation demonstrated its effectiveness in reducing disease caused by *Aeromonas salmonicida*, *Vibrio ordalii* and *Yersinia ruckeri*, but not *Vibrio anguillarum*. NIKOSKELAINEN *et al.* (2001) administered

Lactobacillus bacterium, *Lactobacillus rhamnosus* at different doses (10^9 and 10^{12} CFU/g-feed) to rainbow trout for 51 days. After sixteen days the fish were challenged with *Aeromonas salmonicida* spp. *salmonicida*, which normally causes furunculosis. However, the administration of *Lactobacillus rhamnosus* resulted in a significant reduction in fish mortality, from 52.6% in the control to 18.9% and 46.3% in the 10^9 CFU/g feed and the 10^{12} CFU/g feed groups, respectively.

GIBSON *et al.* (1998) studied the BCA ability of a bacteriocin-like inhibitory substance-producing *Aeromonas media* strain A199 by assessing its action on the survival of oyster larvae (*Crassostrea gigas*), challenged with *Vibrio tubiashii*. The larvae challenged with *Vibrio* died within 5 days, whereas the presence of the pathogen and BCA strain together did not affect the viability of the larvae over the same time period. The viability of larvae challenged with strain A199 alone was also unaffected. In addition, that BCA exhibited antagonistic activity against a wide range of fish/shellfish pathogens *in vitro*.

Introduction of the spores of *Bacillus* sp. into a culture medium of rotifers, which filtered more than 90% of the spores in 1 h, greatly altered the associated flora of the rotifers. After 5 days of culture, a species of the family Vibrionaceae was dominant in the control rotifers, whereas the spore-fed rotifers had very diverse flora. The mean weight of turbot at day 10 was significantly improved with the spore-fed rotifers, their survival rate also increasing (GATESOUBE, 1991).

Studies of MAEDA (1988), MAEDA and NOGAMI (1989), MAEDA and LIAO (1992), MAEDA *et al.* (1992), NOGAMI and MAEDA (1992), MAEDA and LIAO (1994), NOGAMI *et al.* (1997), MAEDA *et al.* (1997) and MAEDA (1999) have reported biocontrol ability in isolated bacteria, *Thalassobacter utilis* and *Pseudoalteromonas undina*, in promoting the growth of fishes and crustaceans, and inhibiting the growth of pathogens. The use of these bacteria for larval production of *Portunus trituberculatus* (crab), *Penaeus monodon* (prawn), and *Caranx delicatissimus* (fish), not only resulted in high survival rates of all but also repressed

diseases caused by bacteria and viruses. In addition, *Thalassobacter utilis* inhibited the growth of pathogenic fungi as well as bacterial pathogens (NOGAMI *et al.*, 1997).

4. Biocontrol of Viral Pathogens

Several viral diseases have had serious implications for the fish rearing industry. Similarly, the culture of penaeid shrimps (*Penaeus monodon* and *P. japonicus*) has been infected by baculo-like viruses. In Taiwan, the production of *P. monodon* decreased from about 90,000 metric tons in 1987 to 30,000 in 1988, dropping further to 20,000 in 1989. SINCE 1993, the *P. japonicus* rearing industry in Japan has been seriously affected by a virus infection, many nursery ponds in the western part of Japan having stopped production. Other viruses exist which are also significant pathogens of finfish. These include infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV) (which infect salmon), Hiramé rhabdovirus (HIRRV) (flounder), the yellowtail ascites virus (YAV) (yellowtail), striped jack (Sima-Aji) nervous necrosis virus (SJNNV) (mainly striped jack) and an iridovirus (sea bream). All of these can cause serious harm to aquaculture.

In the marine environment, viruses that are obligate parasites of cellular organisms, which are usually specific to certain hosts, are abundant. Virus infections have been found in almost all organisms, although it appears that most of the viruses present in seawater infect bacteria and are responsible for about 10–40% of total bacterial mortality. In addition, the release of dissolved organic matter (DOM) during the lysis of microbes is thought to stimulate the activity of other bacterial components in the water column. Viruses are also involved in genetic transfer and can influence species compositions (FUHRMAN, 2000).

FAURÉ-FREMIET *et al.* (1963) and BERGH *et al.* (1989) reported that the use of direct counting methods with an electron microscope resulted in virus counts that were much higher than those previously reported from natural aquatic environments (based on counts of plaque-forming units using various host bacteria) (SPENCER 1955). According to BERGH *et al.*

Table 1. Biocontrol agents used in aquaculture

Biocontrol agents	Pathogens tested	Fishes reared	Resources
<i>Thalassobacter utilis</i>	<i>Vibrio anguillarum</i>	shrimp (<i>Penaeus monodon</i>)	Maeda & Nogami (1989)
<i>Pseudoalteromonas undina</i>	IHNV	crab (<i>Portunus trituberculatus</i>)	Nogami & Maeda (1992)
	SJNNV	striped jack (<i>Caranx delicatissimus</i>)	Maeda & Liao (1994)
	baculo-like virus	sea-bream (<i>Pagrus major</i>)	Maeda <i>et al.</i> (1997)
	irido virus		Maeda (1999)
<i>Bacillus</i> sp. through rotifers	Vibrionaceae	turbot (<i>Scophthalmus maximus</i>)	Gatesoupe (1991)
<i>Pseudomonas</i> sp.	<i>Aeromonas salmonicida</i>	brown trout (<i>Salmo trutta</i>)	Smith & Davey (1993)
		Atlantic salmon (<i>Salmo salar</i>)	
yeast	intestine bacteria	trout (<i>Salmo gairdneri</i>)	Andlid <i>et al.</i> (1995)
<i>Vibrio alginolyticus</i>	<i>Vibrio ordalii</i>	Atlantic salmon (<i>Salmo salar</i>)	Austin <i>et al.</i> (1995)
	<i>Vibrio anguillarum</i>		
	<i>Aeromonas salmonicida</i>		
<i>Alteromonas haloplanktis</i>	<i>Vibrio alginolyticus</i>	scallops (<i>Argopecten purpuratus</i>)	Riquelme <i>et al.</i> (1996)
	<i>Vibrio anguillarum</i>		
<i>Vibrio</i> sp. through rotifers	<i>Vibrio splendidus</i>	turbot (<i>Scophthalmus maximus</i>)	Gatesoupe (1997)
<i>Carnobacterium</i> sp.	<i>Vibrio anguillarum</i>	rainbow trout (<i>Oncorhynchus mykiss</i>)	Jöborn <i>et al.</i> (1997)
	<i>Vibrio salmonicida</i>	Atlantic salmon (<i>Salmo salar</i>)	
<i>Thalassobacter utilis</i>	<i>Haliphthoros</i> sp. (fungus)	crab (<i>Portunus trituberculatus</i>)	Nogami <i>et al.</i> (1997)
<i>Aeromonas media</i>	<i>Vibrio tubiashii</i>	Pacific oyster (<i>Crassostrea gigas</i>)	Gibson <i>et al.</i> (1998)
<i>Pseudomonas fluorescens</i>	<i>Vibrio anguillarum</i>	rainbow trout (<i>Oncorhynchus mykiss</i>)	Gram <i>et al.</i> (1999)
bacteria not identified	<i>Vibrio alginolyticus</i>	Pacific oyster (<i>Crassostrea gigas</i>)	Nakamura <i>et al.</i> (1999)
	<i>Vibrio tubiashii</i>		
<i>Aeromonas</i> spp.	IHNV	masu salmon (<i>Oncorhynchus masou</i>)	Yoshimizu & Ezura (1999)
<i>Vibrio</i> spp.	OMV	barfin flounder (<i>Verasper moseri</i>)	
	BF-NNV		
<i>Carnobacterium</i> sp.	<i>Aeromonas salmonicida</i>	Atlantic salmon (<i>Salmo salar</i>)	Robertson <i>et al.</i> (2000)
	<i>Vibrio ordalii</i>	rainbow trout (<i>Oncorhynchus mykiss</i>)	
	<i>Yersinia ruckeri</i>		
<i>Lactobacillus rhamnosus</i>	<i>Aeromonas salmonicida</i> spp. salmonicida	rainbow trout (<i>Oncorhynchus mykiss</i>)	Nikoskelainen <i>et al.</i> (2001)
<i>Pseudomonas</i> sp.	<i>Vibrio harveyi</i>	Shrimp (<i>Penaeus monodon</i>)	Chythanya <i>et al.</i> (2002)
	<i>Vibrio fluvialis</i>		
	<i>Vibrio</i> <i>parahaemolyticus</i>		
	<i>Vibrio damsela</i>		
<i>Vibrio</i> spp.	<i>Vibrio harveyi</i>	Shrimp (<i>Penaeus vannamei</i>)	Gullian <i>et al.</i> (2004)
<i>Bacillus</i> spp.			

IHNV: Infectious Hematopoietic Necrosis Virus

SJNNV: Striped Jack Nervous Necrosis Virus

OMV: *Oncorhynchus masou* Virus

BF-NNV: Barfin Flounder Nervous Necrosis Virus

(1989), viral concentrations changed from 10^4 to 10^8 virus particles/ml, which indicates a possible effect of anti-viral microorganisms on the presence of virus particles in seawater. In fact microorganisms in both sea and fresh water commonly inactivate viruses (see below), being responsible for great fluctuations in viral concentrations in the aquatic environment. In addition, water borne viruses have the ability to

transfer from one infected organism to another. If anti-virus bacteria dominate the aquatic environment, virus transfer among fish communities could be repressed to a large extent. Based on this concept, anti-viral bacteria are now used in larval rearing procedures in commercial aquaculture (MAEDA *et al.*, 1997; MAEDA, 1999; YOSHIMIZU and EZURA, 1999).

The bacterial strain VKM-124, *Pseudoaltero-*

monas undina, when used in aquaculture, prevented fish larvae from being infected by SJNNV, baculo-like viruses and iridovirus. When added to water at a concentration of about 10^6 cells/ml in rearing containers of *Penaeus* prawn and striped-jack (*Caranx delicatissimus*) larvae, the survival rates of the larvae were much greater with the bacteria than in its absence. In fact, without the addition of the bacteria, all fish and prawn larvae died due to viral infection (MAEDA and LIAO 1994; MAEDA *et al.*, 1997; MAEDA, 1999). According to HERRMANN *et al.* (1974), WARD and ASHLEY (1976), CLIVER and HERRMANN (1972) and HERRMANN and CLIVER (1973), viral inactivation is mainly due to degradation of the viral capsid by proteinases. Thus, biocontrol agents, for example proteinase-producing bacteria, may inhibit the multiplication of viruses among fishes.

According to YOSHIMIZU and EZURA (1999), fish intestinal bacteria, such as *Aeromonas* spp. and *Vibrio* spp., producing anti-viral substances, were isolated from masu salmon (*Oncorhynchus masou*), Japanese flounder (*Pararichthys olivaceus*) and barfin flounder (*Verasper moseri*). The *Aeromonas* strains produced anti-infectious hematopoietic necrosis virus substances, and the *Vibrio* strains showed anti-IHNV, *Oncorhynchus masou* virus (OMV) and barfin flounder Nervous necrosis virus (BF-NNV) activities. When *Aeromonas* spp. strains were mixed with feed pellets and fed to rainbow trout (*O. mykiss*) and masu salmon, the bacteria became dominant in the intestinal microflora and anti-IHNV activity was observed in homogenates of the intestinal contents. The rainbow trout and masu salmon that were fed the *Aeromonas* spp. showed more resistance to the artificial IHNV challenge test. Barfin flounder fed on *Vibrio* sp. strain with *Artemia salina* showed anti-OMV and anti-BF-NNV activities in the intestinal contents.

5. Virus Distribution and Survival in Seawater

Several reports have considered the abundance of viruses (although in some cases, short-term inactivated) in seawater. FAURE-FREMIET *et al.* (1963) found that the ciliated

protozoan, *Zoothamnium alternans*, was attached to and formed a considerably large community on the body surface of the crab, *Cancer pagurus*. Bacteria attached to the surface of the ciliate showed enormous numbers of bacteriophages (two kinds apparent) inside the cell under an electron microscope examination. BERGH *et al.* (1989) also reported virus counts in the range of 10^4 – 10^8 virus particles/ml of water using a direct counting method with an electron microscope. Distribution patterns of virus particles in seawater have also been reported by HELDAL and BRATBAK (1991), WEINBAUER *et al.* (1995), BRATBAK *et al.* (1996) and STEWARD *et al.* (1996).

According to GERBA *et al.* (1977), significant concentrations of human viruses occurred in water and sediments of a coastal canal into which secondarily treated sewage was discharged. LABELLE *et al.* (1980) found that viruses existed in greater numbers in sediment than in overlying seawater and SMITH *et al.* (1978) reported that viruses survived for longer periods in sediment, than in overlaying estuarine water. LABELLE and GERBA (1979) studied the adsorption and elution characteristics of several enteroviruses and a rotavirus in estuarine sediments under varying conditions of pH, salinity and presence of soluble organic matter. More than 99% of the added poliovirus, coxsackievirus, echovirus and rotavirus were adsorbed into the sediment. Under similar conditions, some viruses were attached significantly less than the poliovirus. GERBA and SCHAIBERGER (1975a) investigated the loss of viral titers of *Escherichia coli* B bacteriophage in natural seawater without kaolinite and with 500 mg kaolinite/l, in which the virus activity was protected by the presence of kaolinite. Viruses accumulated in sediments near the shore could be easily released into seawater by simple mechanical shaking (FLORA *et al.*, 1975). During feeding, bivalves (oysters, mussels and clams) can accumulate pathogenic human enteric viruses from sewage-polluted seawater. Enteric viruses, such as polio, echo, coxsackie and reo viruses, have been detected in shellfish, field and laboratory studies having indicated that enteric viruses can survive in shellfish for long periods (GERBA and GOYAL, 1978).

METCALF and STILES (1965) reported coxsackie and other enteric viruses from the eastern oyster, *Crassostrea virginica*, which were located in estuarine waters at distances as great as 4 miles from the nearest raw sewage outlet. The virus remained relatively stable within oyster tissues stored at 5°C for at least 28 days. Of all the tissues examined, the digestive gland showed the greatest retention of the virus, but it was not possible to demonstrate the occurrence of virus multiplication in any of the oyster tissues examined. METCALF and STILES (1965) suggested that the vector potential of oysters resulted from the stability of the virus within oyster tissues following ingestion from environmental seawater. SUZUKI *et al.* (2001) showed that marine birnavirus (MABV), a member of aquabirnavirus, an opportunistic pathogen in eukaryotic marine organisms with a broad host range in wild and cultured fish and shell-fish, was widely distributed in coastal and pelagic seawater as well as in samples of zooplankton collected from the Pacific Ocean.

6. Inactivation of Viruses in Seawater

A number of studies have considered the inactivation of viruses in seawater. PLISSIER and THERRE (1961) stated that poliovirus was inactivated to a significant degree after several weeks in seawater. According to MATOSSIAN and GARABEDIAN (1967), surface seawater was found to inactivate poliovirus type 1 in some six to nine days. However, boiling of seawater or filtration through a Seitz filter removes the virucidal properties of the former. TORANZO and HETRICK (1982) investigated the survival rates of two fish viruses (infectious pancreatic necrosis virus, and infectious hematopoietic necrosis virus) pathogenic to young salmonids and poliovirus type 1 using untreated fresh, estuarine and sea water samples held at 15 and 20°C. The results indicated longer survival of the salmonid viruses than the poliovirus in saline water, whereas in fresh water, the poliovirus was the most stable. They also noted that at 20°C, the inactivation rate for each virus was independent of salt concentrations in both estuarine and seawater samples. BAUDOY (1976) found that the cyto-infectious power of

the infectious pancreatic necrosis virus subsisted for at least 300 days at 4°C, and for 60 days at 14°C in less highly mineralized water. Comparatively, the virus strength decreased more slowly at 4°C in more highly mineralized river water. When filtered, the same river water maintained its infectious power better than untreated homologues.

Physical factors in the aquatic environment also affect the activity of viruses. PIETSCH *et al.* (1977) showed that salinity distinctly affected viral survival rates. DENIS *et al.* (1977) investigated the stability of twenty strains of DNA and RNA viruses in natural, heated and synthetic seawater over a period of 400 days. Their studies, under controlled laboratory conditions, indicated temperature as a critical factor affecting viral inactivation in seawater. Differences were noted between viral groups, serotypes, and also between strains of the same serotype. LO *et al.* (1976) also showed that temperature, rather than salinity, was the critical factor affecting viral stability, in that the higher the temperature, the more rapid was the loss of viral infectivity. O'BRIEN and NEWMAN (1977) have reported that inactivation of the viruses was exponential, and the rates of inactivation appearing to be affected principally by water temperature. BERRY and NORTON (1976) investigated the stability of T2 bacteriophages in seawater under laboratory conditions and in the natural waters of a bay, and reported that inactivation was temperature-dependent, being enhanced by sunlight and sewage pollution. WEINBAUER *et al.* (1995) and WEINBAUER *et al.* (1997) subsequently described the role of sunlight in the removal and repair of viruses in seawater.

According to GERBA and SCHAIERGER (1975b), extensive aggregation, including that of viruses, takes place in artificial seawater. Viral clumps formed in both natural and artificial seawater might be disaggregated by an increase in the amount of organic matter or a decrease in salinity. GERBA and SCHAIERGER (1975b) have suggested that aggregation might play a role in the initial decline of viral titers in seawater, as well as reducing the number of "infectious foci" present in seawater.

7. Virus Inactivation by Microorganisms in Seawater

MAGNUSSON *et al.* (1966) reported that heating seawater to temperatures above 45°C for one hour destroyed its virus inactivating capacity. This function of seawater required a NaCl concentration of 0.1 M or higher, although not directly caused by salinity, suggesting that the presence of marine bacteria inactivates viruses which require salt for their growth. TORANZO *et al.* (1982) stated that in estuarine water and sediment, the stability of poliovirus type 1 showed a 2-log reduction in virus titer at 15°C, occurring within 6–7 days in water samples taken from estuarine waters of Rita of Pontevedra and Chesapeake Bay in the Atlantic Ocean. They also indicated that bacterial extracellular products appeared to be involved in the virus-inactivation process, including coxsackie and other enteric viruses. According to LABELLE and GERBA (1980), the time required to inactivate 99% of poliovirus increased from 1.4 days in seawater alone to 6.0 days when the virus was adsorbed into sediment at a relatively non-polluted site. TORANZO *et al.* (1982) indicated that the addition of sediment to natural seawater containing poliovirus increased the length of virus survival to over three times that in seawater alone. Although this effect was not attributed to virus adsorption into sediment particles, thereby aiding virus survival in some way, a similar result was not found under sterile conditions, suggesting that the sediment can protect viruses from inactivation by marine microflora. TORANZO *et al.* (1983b) reported that virus-inactivation rates in infectious pancreatic necrosis virus differed significantly in untreated and filtered (or autoclaved) estuarine water samples. In untreated water, the time required for a 90% reduction in IPNV infectivity was only 9 days, whereas it took over 35 days in autoclaved water. IPNV viability was also favored in filter-sterilized water, where it survived nearly four times longer than in untreated estuarine water. Interestingly, the period of the most rapid viral inactivation was correlated with the highest bacterial numbers in untreated water, which suggested that autochthonous microbial flora played an important role in the virus inactivation

process. FUJIOKA *et al.* (1980) found that the time for 90% reduction of poliovirus type 1 at 24°C in seawater in Hawaii ranged from 24 to 48 h, complete inactivation occurring within 72 to 98 h. In fact, their accumulated evidence strongly indicated the presence of virus-inactivating agent(s) of a microbiological nature in both clean and sewage-polluted seawater. Antiviral activity was lost when the seawater samples were subjected to boiling, autoclaving or filtration through a 0.22- or 0.45 μm , but not through a 1.0 μm pore size membrane filter. Other enteric viruses, such as coxsackie virus and echo virus, were also shown to be inactivated in seawater. Before these studies, GUNDERSEN *et al.* (1967) had already reported the effect of marine bacteria in restoring the virus inactivating capacity (VIC) of seawater, depleted of such by heating and filtration. The bacterium responsible for this inactivation was identified as *Vibrio* spp. MAGNUSSON *et al.* (1967) found that *Vibrio marinus* possessed certain antiviral properties. Characteristically, this property of the bacterium was maintained only if the latter was subcultured at a low temperature (4–12°C), whereas the antiviral property disappeared after a number of subcultures at 25°C. KAMEI *et al.* (1987) also reported the presence of anti-virus bacteria in estuarine and seawater. DIREKBUSARAKOM *et al.* (1998) described several strains of *Vibrio* as showing the antiviral activities to IHNV and *Oncorhynchus masou* virus, as measured by plaque reduction rates. On the contrary, SUTTLE and CHEN (1992) suggested that most bacteria were not responsible for the decay of viruses in seawater.

8. Substances which Inactivate Viruses

TORANZO *et al.* (1983a) investigated the mechanism of enterovirus inactivation by marine bacteria using poliovirus type I as a model virus, with strains of *Pseudomonas* and *Vibrio* spp. isolated from the marine environment. Treatment of the virus, with a cell-free filtrate from late log phase bacterial cultures, seemed to produce alterations in the viral capsid, as shown by a reduction in efficiency of adsorption to host cells, increased sensitivity to ribonuclease, and by the release of ribonucleic

acid from the treated virions. In fact, filtration of a ^{14}C -labelled virus sample through 25-nm pore size filters revealed that the majority of the isotope (85–96%) passed through the filters, indicating extensive capsid disruption.

Several reports have indicated that proteases may alter the infectivity of a few enteroviruses. Because many viruses possess a protein capsid, they should be susceptible to at least some proteolytic enzymes. HERRMANN *et al.* (1974) suggested that proteases play a role in inactivating viruses by degrading their protein coat and reported more rapid inactivation of two enteroviruses in a natural lake than in sterile lake water. WARD and ASHLEY (1976) also indicated that the mechanism of inactivation of a virus in sludge involved cleavage of viral proteins, followed by nicking of the encapsulated RNA. CLIVER and HERRMANN (1972) reported that the inactivation mechanism by proteolytic bacteria (notably *Pseudomonas aeruginosa*) could be distinguished from adsorption or aggregation of virus particles because ^{14}C labels from the virus coat protein, but not ^{32}P from the viral nucleic acid, was taken up by the bacterial cells.

HERRMANN and CLIVER (1973) investigated the means by which coxsackievirus type A9 (CA9) was inactivated by proteolytic enzymes. After the reaction of ^{14}C -leucine-labeled CA9 with the protease, only free leucine was liberated, not the infective viral RNA. However further treatment with 1% sodium dodecyl sulfate at pH 7.0 promoted viral RNA release. Sodium dodecyl sulfate treatment of CA9 that was not reacted with the protease did not inactivate the virus or cause viral RNA release. These results demonstrated that the primary means by which protease-sensitive enteroviruses were inactivated was by the degradation of the virus capsid, with subsequent release of viral RNA.

Evidence exists, however, that some viruses are highly resistant to protease action. In fact, LERNER and MIRANDA'S (1968) study of the interactions of a number of hemagglutinating enteroviruses, reovirus type 2 and poliovirus type 1 after treatment with sodium borohydride, several proteases, or carbohydrases, showed that the hemagglutinating activity of virus particles was destroyed by sodium boro-

hydride and certain glycosidases, but was not altered by a number of proteases. MATHEKA *et al.* (1962) have also reported that some enteroviruses from cattle and swine were stable in the presence of protease, but that the coxsackievirus was inactivated by the latter.

Biologically active materials have been reported as inactivating viruses in the marine biotope. EHRESMANN *et al.* (1977) found that ten members of Rhodophyta (algae) from seawater contained substance(s) which caused a greater than 2 log reduction in the infectivity of herpes simplex virus types 1 and 2. In addition, anti-coxsackie B₃ virus activity was detected in extracts of *Constantinea simplex* Satchelt. DEIG *et al.* (1974) reported that extracts from two species of marine red algae, *Cryptosiphonia woodii* and *Farlowia mollis*, specifically inhibited *in vitro* herpes simplex virus replication. GERBER *et al.* (1958) mentioned that extracted polysaccharides from *Gelidium cartilagenium* (seaweed) and carrageenin showed a marked inhibitory effect on the growth of influenza B and mumps viruses. KATHAN (1965) stated that a preparation from crude kelp inhibited bacterial and viral neuraminidases, and also the multiplication of some influenza viruses in embryonated eggs. The inhibitory mechanism of kelp extract might be due to the prevention of penetration of the virus into host cells by direct binding of the virus or by inhibiting the viral enzyme, since the inhibitory effect occurred when eggs were treated with kelp extracts prior to inoculation with the infective virus. RICHARDS *et al.* (1978) tested extracts of two species of marine algae, *Constantinea simplex* and *Farlowia mollis*, for antiviral activity in tissue culture and in experimental infections of mice. Treatment of confluent mouse embryo fibroblast cell mono-layers with either compound before viral inoculation was effective in inhibiting the replication of the herpes simplex virus, types 1 and 2, and vesicular stomatitis virus, but not encephalomyocarditis virus, semliki forest virus, or murine cytomegalovirus.

9. Antagonism of bacteria and microalgae

Burgess *et al.* (1999) isolated over 400 strains of surface-associated bacteria from various

species of seaweed and invertebrates from Scottish coastal waters, and found 35% of them to be producing antimicrobial compounds. This was considered much higher than the free living marine proportion or soil bacteria producing antimicrobial agents. They also reported that many strains which did not normally produce antibiotics could be induced to do so by exposing them to small amounts of live cells, supernatants from other bacterial cultures or certain chemicals.

Gil-TURNES *et al* (1989) reported that embryos of the shrimp *Plalaemon macrodactylus* were remarkably resistant to infection by the fungus *Lagenidium callinectes*, a recognized pathogen of many crustaceans. An *Alteromonas* sp. bacterial strain, consistently isolated from the surface of the embryos, produced 2,3-indolinedione (isatin), a compound that inhibited the pathogenic fungus. When exposed to the fungus, bacteria-free embryos quickly died, whereas similar embryos reinoculated with the bacteria or treated only with 2,3-indolinedione survived. Gil-TURNES and FENICAL (1992) also reported the resistance of American lobster (*Homarus americanus*) embryos to infection by the pathogenic fungus, *Lagenidium callinectes*. The surfaces of healthy lobster embryos were found to be covered almost exclusively by a single, Gram-negative bacterium, which grew in a dense mosaic pattern. The bacterium produced 4-hydroxyphenethyl alcohol (tyrosol), an antibiotic substance known to be produced by terrestrial fungi to inhibit the growth of the pathogenic fungus.

10. Probiotic Effects and Antagonisms of Intestinal Microorganisms of Fish

According to the review paper of OLAFSEN (2001), mucus in the gastrointestinal tract of fishes is known to serve as a source of nutrients, and enhance colonization by serving as an initial attachment site for bacteria or as a matrix for permanent bacterial attachment. Conversely, the mucus layer in some instances may serve as an effective barrier, providing protection against penetration by invading microorganisms. Evidence of a high proportion of bacteria growing as attached forms in the gastrointestinal tract of several larval groups was

considered beneficial for both bacteria and host, where the internal bacteria prevent the colonization and proliferation by pathogens.

Accordingly, the gastrointestinal tract of fishes provides a nutrient-rich habitat for microbial growth, most data indicating that like mammals, fishes have an indigenous intestinal microbiota, at least so far as having autochthonous gastrointestinal microorganisms of different composition from these in the surrounding water. Some authors, however, claim that the intestinal biota mostly reflect the feeding and drinking habits of the animal, and are therefore influenced by the external environment. GATESOUBE (1999) asserted that it was unclear whether or not the intestinal microbiota of aquatic animals changed rapidly with the intrusion of microbes from water, but noted that the influence of food has been clearly demonstrated in larval and juvenile fishes, the influence of bacteria introduced via live food organisms being particularly dramatic during first feeding.

According to a review paper by GOMEZ-GIL *et al.* (2000), the ingestion of bacteria by cold-water fishes at the yolk sac stage resulted in the establishment of a primary intestinal microflora, which persisted beyond first feeding. This was followed by a bacterial succession until the adult microflora was established. It is therefore important to add potential probiotics as soon as possible after hatching, in order to effectively colonize the larval gut before the introduction of live food.

GRAM (1993) found that one-third (67 strains) of the total number of bacterial strains isolated from the intestinal gut of fishes inhibited the growth of one or several of six target pathogenic organisms. The inhibitory action was most pronounced among those strains producing siderophores; mediated competition for iron may explain the inhibitory activity of these strains because the addition of iron eliminated the inhibitory activity of two-thirds of the strains tested. On the other hand, the antibacterial action of 21 strains was not completely eliminated by iron supplementation, such possibly being attributable to the production of several different bacterial inhibitors, such as antibiotics and bacteriocins.

Commensal bacteria with inhibitory activity against pathogens have been isolated from the mucosal surfaces of healthy fishes by several researchers (WESTERDAHL *et al.*, 1991; Olsson *et al.*, 1992; BERGH, 1995). WESTERDAHL *et al.* (1991) found that most of the inhibitory bacteria occurred in the rinse and mucus fractions of the gastrointestinal tract. Of the isolates from the gut with an inhibitory effect against *Vibrio anguillarum*, 60% had an inhibitory effect on five other fish-pathogenic *V. anguillarum* serotypes. Inhibitory effects of the isolates were also shown against *Aeromonas salmonicida* and *Aeromonas hydrophila*. SUGITA *et al.* (1998) isolated *Bacillus* sp. from a dragonet (*Callionymus* sp.), which had an inhibitory activity against other bacteria, although they presented no data on the proportion of *Bacillus* spp. among the gut microbial population. As indicated by SAKATA (1990) and ONARHEIM and RAA (1990), *Bacillus* spp. are not dominant intestinal microorganisms in fishes. OLAFSEN (2001) reported that lactic acid bacteria produced growth inhibiting factors that could inhibit various *Vibrio* spp., especially *Vibrio anguillarum*. Although some reports have described the effect of gut microorganisms on fish health, some of the former presented only the composition of bacterial flora (SUGITA *et al.*, 1996). Since some bacterial strain isolates from the gut inhibited fish growth (ROSS and TOTH, 1974), when distributed at high concentrations, the growth of fishes in the presence of bacteria, even if gut isolates, should be assayed before the application of a probiotic or BCAs.

Many investigators have reported that lactic acid bacteria play an important role in the beneficial biological functions of industrial animals. Accordingly the effects of lactic acid bacteria as a growth promoter in fishes have been studied, resulting in commercial probiotics apparently improving the dietary value of rotifers for flatfish larvae (GATESOUBE *et al.*, 1989). ROBERTSON *et al.* (2000) reported that feeding salmonids with diets containing a probiotic (*Carnobacterium* sp.), which showed antagonism against several pathogens, revealed the viability of an isolate of *Carnobacterium* sp. in the gastrointestinal tract. NIKOSKELAINEN *et al.* (2001) administered *Lactobacillus*

rhamnosus at different doses to rainbow trout before the fish were exposed to *Aeromonas salmonicida* ssp. *salmonicida*, thereby reducing fish mortality significantly. This effect could be attributed to the immune system improvement of the fish, the stimulation of the immune system of terrestrial animals by lactic acid bacteria having already been demonstrated. However, in spite of their beneficial effects, lactic acid bacteria formed a minor component of the adherent intestinal microflora of salmonid fry in seawater (OLAFSEN, 2001).

On the other hand, ROSS and TOTH (1974) reported that pathological conditions of rainbow trout were associated with a species of *Lactobacillus*. RINGØ and GATESOUBE (1998) also stated in their review paper that pathogenic lactic acid bacteria, such as *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Carnobacterium* and *Lactococcus* spp., have been detected in the ascites, kidneys, livers, hearts and spleens of fishes.

11. Prebiotics

GIBSON and ROBERFROID (1995) have proposed that prebiotics are significant for establishing intestinal flora. Probiotics have been used to change the composition of colonic microbiots, but the possibility of such changes being transient has limited the implantation of exogenous bacteria. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of one or a limited number of bacterial species already resident in the colon, thus attempting to improve the health of the host. Nondigestible oligosaccharides in general, and fructooligosaccharides in particular, are types of prebiotics, having been shown to stimulate the growth of endogenous bifidobacteria, which after a short feeding period of these materials became predominant in human feces, for example.

RINGØ (1993) showed that the total counts of viable colonies in the feces of Arctic charr fed linoleic acid supplemented diet, were higher than in fish fed an unsupplemented diet. The Gram-positive bacteria species (*Lactobacillus* and coryneforms) found in the faeces of fish fed the unsupplemented diet were not present

in feces of the linoleic acid group, linoleic acid having a stimulatory effect on the growth of *Aeromonas* sp., *Pseudomonas* sp. and *Vibrio* sp., whereas the growth of *Lactobacillus* sp. was inhibited. However, the role of these transformed flora in fish fed with linoleic acid has not yet been clearly demonstrated.

12. Conclusions

More than million microorganisms per milliliter inhabit the aquaculture environment and affect each other, both through the substances they produce and emit, as well as through the various ways they come into contact with one another (EDDY and JONES, 2002). These microorganisms in an aquaculture environment cannot be eliminated by sterilization with ultraviolet radiation or ozone treatment, or even by filtration. Bacterial counts decrease with these treatments, but quickly recover to their original levels because microorganisms abound in the surrounding environment. In the case of sterilization using drugs, the number of drug-resistant microorganisms increases in water to levels much higher than those in non-sterilized water, because the chemicals destroy the microbial interactions of the antagonists. In fact, the growth rates of the specific microorganisms increase without antagonists. Furthermore, no one can foresee which bacterial species may occupy the niche vacated by these treatments. Throughout the world, many fish diseases are spreading in aquaculture systems as a result of pathogens that dominate the microbial community. This in turn has resulted in more chemicals used and further increases in pathogens (ABDELZAHER and ELNAGHY, 1998; DIJKSTERHUIS *et al.*, 1999). As described in this review, a number of reports have been published on the inactivation of virus infectivity in seawater, and the presence of anti-virus microorganisms. The growth of these anti-virus bacteria should be almost certainly repressed with antibiotics.

Biocontrol agents (BCAs) that promote fish growth as well as inhibiting pathogens, belong to a variety of bacterial genera that include *Pseudomonas*, *Vibrio*, *Pseudoalteromonas*, *Alteromonas*, *Carnobacterium*, *Lactobacillus*, *Aeromonas* and *Thalassobacter*. The antago-

nistic effect of these BCAs on pathogens is due to the production of bacteriocins, siderophores and enzymes, as well as niche exclusion. Several reports have suggested that niche exclusion among microbial populations can possibly eliminate pathogens, even if the microorganisms used do not exert antimicrobial effects on the pathogens directly. In addition, certain bacteria have been found to repress viral pathogens in aquaculture, but the virus inactivation processes generated by these bacteria have not been elucidated, although several reports cited have suggested degradation of the viral capsid by proteases.

Due to the risks of chemical intervention in aquaculture and concerns about the safety of aquacultural fishes as food sources, the use of drugs as a mainstream response to pathogens is likely to be increasingly constrained by regulations, and consumer and environmental pressures. Thus, alternative methods for disease management such as biocontrol should remain as one of the key strategies. In addition, an increasing need for biocontrol agents seems highly likely, owing to global climate changes combined with increasing free trade most likely leading to an expansion of a range of diseases, from tropical and subtropical to northern countries.

MALAKOFF (1999) described biocontrol as "fighting fire with fire," in which exotic enemies that have been released against pathogens become harmful and disturb the natural ecosystem, and HOWARTH (2000) warned that the administration of non-indigenous agents could potentially result in an irreversible impact on the natural environment, although most of the environmental harm caused by native agents would normally be reversible. In similar vein, WRATTEN and GURR (2000) noted that after a first target of pathogens was reduced in number, further biological control programs would have to be developed in a potentially never ending cycle of adventive organisms exploiting anthropogenic or natural resources, forming a "biological control treadmill".

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Direct Current Measurements with special reference to Velocity Fields of Mesoscale Eddies in the Shikoku Basin

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Abstract : We have made two direct current measurements at 29.50°N and 135.25°E in the Shikoku Basin over the periods from 20 February to 16 April in 1999 at a depth of 470 m and from 17 April to 9 December in 1999 at a depth of 730 m. Main results of these direct current measurements are presented in this paper. It is shown that variations in the velocity is essentially due to the inertial oscillation with a period of a day. It is also shown that the inertial oscillation is added over the mean flow of mesoscale eddies estimated from the sea surface height observed by the TOPEX/POSEIDON pass the observational station and it commonly coincides with the deepening of the current meter by the larger dragging effect of the larger horizontal velocity. The mesoscale eddies have a horizontal velocity of about 15 cm sec⁻¹ in a middle layer with a depth of 500–700 m, which indicates the mesoscale eddies detected from the satellite altimeter have a vertical structure and it has a possibility to give a dynamical influence on the path dynamics of the Kuroshio.

Keywords : *Inertial oscillation, mesoscale eddy, direct current measurement*

1. Introduction

Recently, some long-term direct current measurements have been carried out (e.g., FUJIO *et al.*, 2000; IMAWAKI *et al.*, 2001) and various velocity variations are detected. In particular, general deep layer circulation in the Shikoku Basin has been observed by some direct current measurements (TAIRA and TERAMOTO, 1981, 1985; NISHIDA and KURAMOTO, 1982; ISHIZAKI *et al.*, 1983; FUKASAWA *et al.*, 1986; TAIRA *et al.*, 1990). On the other hand, influence of mesoscale eddies on the path variation of the Kuroshio has been discussed by some authors (YOSHIKAWA *et al.*, 1998; ICHIKAWA, 2001; EBUCHI and HANAWA, 2003). It is commonly pointed out that the anticyclonic

eddy formed in the Kuroshio Extension propagated southwestward and collided with the main Kuroshio flow in the south of Kyushu, and triggered a small meander off Kyushu. Furthermore, EBUCHI and HANAWA(2003) showed that the cyclonic eddy propagated to the south of Shikoku and collided with the Kuroshio to form a small meander path of the Kuroshio.

Several numerical experiments have been performed to see the dynamical effect of an anticyclonic eddy inserted artificially in ocean models on the Kuroshio path variation (AKITOMO and KUROI, 2001; MITSUDERA *et al.*, 2001; ENDOH and HIBIYA, 2001). They commonly showed that the anticyclonic eddy given in the numerical model yields a meander path of the Kuroshio.

Here, we should notice that the mesoscale eddies discussed in these studies are essentially detected from the sea surface height estimated by the satellite observation of TOPEX/POSEIDON (e.g., KURAGANO and KAMACHI, 2000), the vertical velocity structure of mesoscale eddies has not been well observed by

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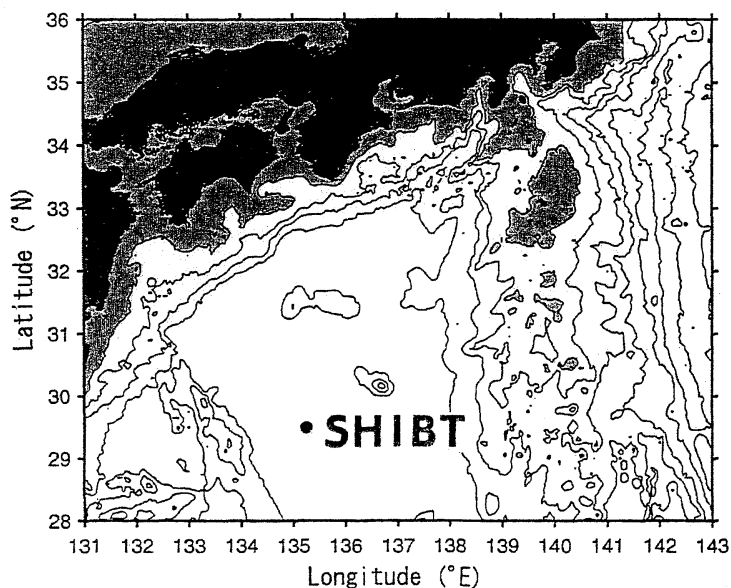


Fig. 1 Location of the station (SHIBT) of the direct current measurements of the present study. Isoleth of depth with a contour interval of 1000 m is also shown and the regions with the depth shallower than 1000 m are stippled.

direct current measurements. Therefore, the assumption of the vertical structure of the mesoscale eddies inserted in the numerical model should be checked by use of the observational data.

In order to see this problems, we have made a direct current measurements at Station SHIBT (**SHI**ikoku **B**asin **T**ime series; 135.25°E, 29.50°N) in the Shikoku Basin shown in Fig. 1 (Li *et al.*, 2004). The observational station is in the interior region where mesoscale eddies have a tendency to pass over this latitudes (MITUDERA *et al.*, 2001; EBUCHI and HANAWA, 2003). Even though a small meander of the Kuroshio off Kyushu is generated, the path of the small meander can not develop to the observational point. The first observation was made over the periods from 20 February to 16 April 1999 and the current meter is set at a depth of 470 m. The second observation was made from 17 April to 9 December 1999 at a depth of 730 m.

The main results of the current observation is presented in this paper. In the following, details of the observation will be mentioned in the next section. Results of the direct current measurements will be noted in the section 3. Summary and discussion will be made in

section 4.

2. Observations

Direct current measurements were made by use of a doppler current meter (AANDERAA RCM-9) deployed at 20 m above the upper sediment trap of a sediment trap mooring system at 500 m and 4000 m (Li *et al.*, 2004). The first mooring system with the current meter at a depth of 470 m at 135.251°E and 29.507°N with a ocean depth of 4511 m was set on 20 February 1999 by Research Vessel "Yokosuka" of JAMSTEC and was recovered on 16 April 1999 by use of the Training Vessel "Seisui-maru" of Mie University. The observed velocity data at every 30 minutes in this period is hereafter referred to as the first mooring case.

The second mooring system with a current meter at a depth of 730 m at 135.234°E and 29.473°N with a ocean depth of 4610 m was set on 17 April 1999 by use of "Seisui-maru" and recovered on 9 December 1999 by use of the Research Vessel "Tansei-maru" of Ocean Research Institute of University of Tokyo, of which observed velocity data at every 60 minutes is referred to as the second mooring case. The difference in the current meter depth

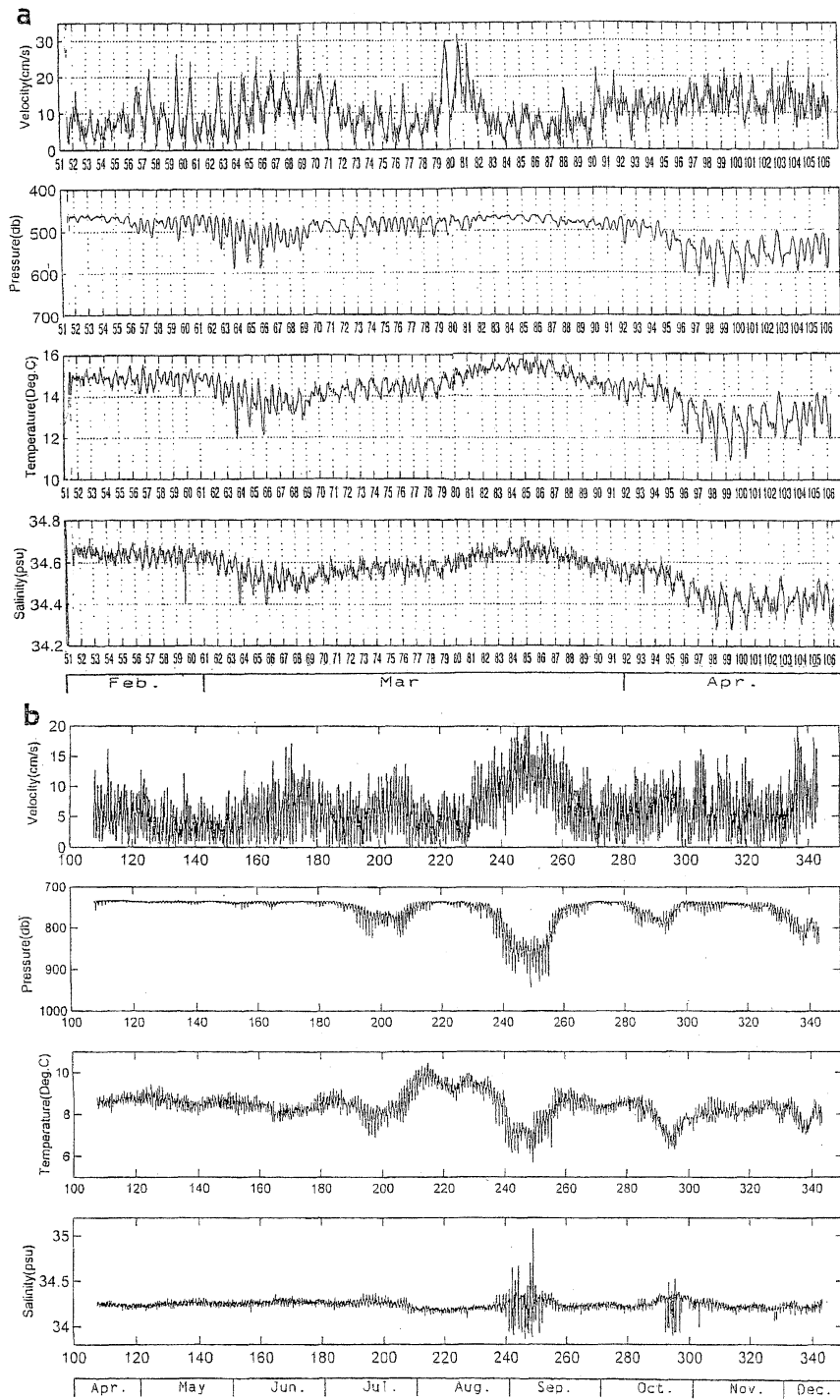


Fig. 2 Time variation in the observed current speed (absolute velocity) (top), pressure (upper middle) temperature (lower middle) and salinity (bottom), during (a) first mooring case and (b) second mooring case. Numbers along the axis of abscissas are days from a new year (Julian days).

between the two mooring cases was due to that of sea floor depth and to intertwining of mooring ropes during the second mooring case. Type of the sinker and the buoyancy force of the mooring system by use of the sixteen buoys are same in both the observations, in which the weight of sinker is 650 kg (565 kg in situ) and the actual buoyancy force of the buoys is 140 kg.

3. Observational results

In order to see the observational condition of the mooring system, the observed pressure of the current meter is shown in Fig. 2 together with the observed absolute velocity, temperature and salinity. In the first mooring case (Fig. 2a), semidiurnal variation of the pressure is observed in early March, while the semidiurnal variation is relatively weak in last 10 days in March. Two periods of increase in the pressure are observed in early March and in April. Periodic vertical change of the pressure is observed in early March and the amplitude of the pressure variation exceeds 100 db, in which the current meter moves in the depth range from 470 m to 580 m. A long period downward shift of the current meter with a diurnal variation is observed in April. As the salinity minimum layer formed by the North Pacific Intermediate Water exists in the depths of 800 m in this region (e.g., SEKINE, 2002a), low temperature and low salinity are essentially observed when the current meter shifts to a deeper level in the first mooring case. As the current meter shifts to a deeper level than 800 m, salinity is slightly increased in the second mooring case, whereas some noises are also observed.

More prominent downward shift of the current meter is seen in the second mooring case (Fig. 2b). Here, please notice that the scales of the ordinates and abscissas of Fig. 2b is different from those of Fig. 2a. During early September, the current meter moves in the depth range from 720 m to 910 m. Other two downward shifts of the current meter are detected in middle July and October and low temperature and high salinity are observed in these periods. Another prominent downward shift of the current meter is seen in the last period of the observation, complete observational data during

the downward shift have not been obtained.

It should be noted that a larger absolute velocity is commonly observed in the periods with the downward shift of the current meter. It is suggested that the larger velocity induces a large horizontal drawing force on the mooring system and it forces the mooring system to shift to the downstream area. Since the length of the mooring system from the sinker to the current meter is constant, the large horizontal drawing force induces the downward shift of the current meter.

We should correct the observational data by the influence of downward shift of the current meter. However, because the vertical velocity difference estimated from the geostrophic flow estimation is not so large in the depth range with the vertical shift of the current meter (SEKINE, 2002b) and because we will not discuss the temperature, salinity and velocity variation at a fixed depth in this range, the revision of the observational data by the vertical shift of the current meter is not carried out.

Stick diagrams of the observed velocities at every 30 minutes (60 minutes) observed by the first and second mooring cases are shown in Fig. 3 (Fig. 4). In general, velocity oscillation with a period of one day essentially dominated. However, some periods with almost uniform larger velocities are also detected and a mechanical velocity variation is inferred.

To see the velocity variations more quantitatively, rotary spectrum of the observed velocity is shown in Fig. 5. In both cases, a prominent peak is found in a period of 24 hours. Another peak with a period of 12 hours is also commonly seen, however there exist no other common peaks between the two mooring cases. Rotary coefficient of the rotary spectrum is shown in Fig. 6. For both cases, positive coefficient showing the anticyclonic (clockwise) circulation is significantly large for the peak with periods of 24 hours and 12 hours. Velocity variations with the period of 24 hours is caused by the inertial oscillation induced by the Coriolis force with the period of a day (T); $2\pi/f = T/(2\sin(\phi)) \sim T$, where f and ϕ are the Coriolis parameter and the latitude of 29.50° in this case. The velocity variations with the period of 12 hours are due to the tidal flow

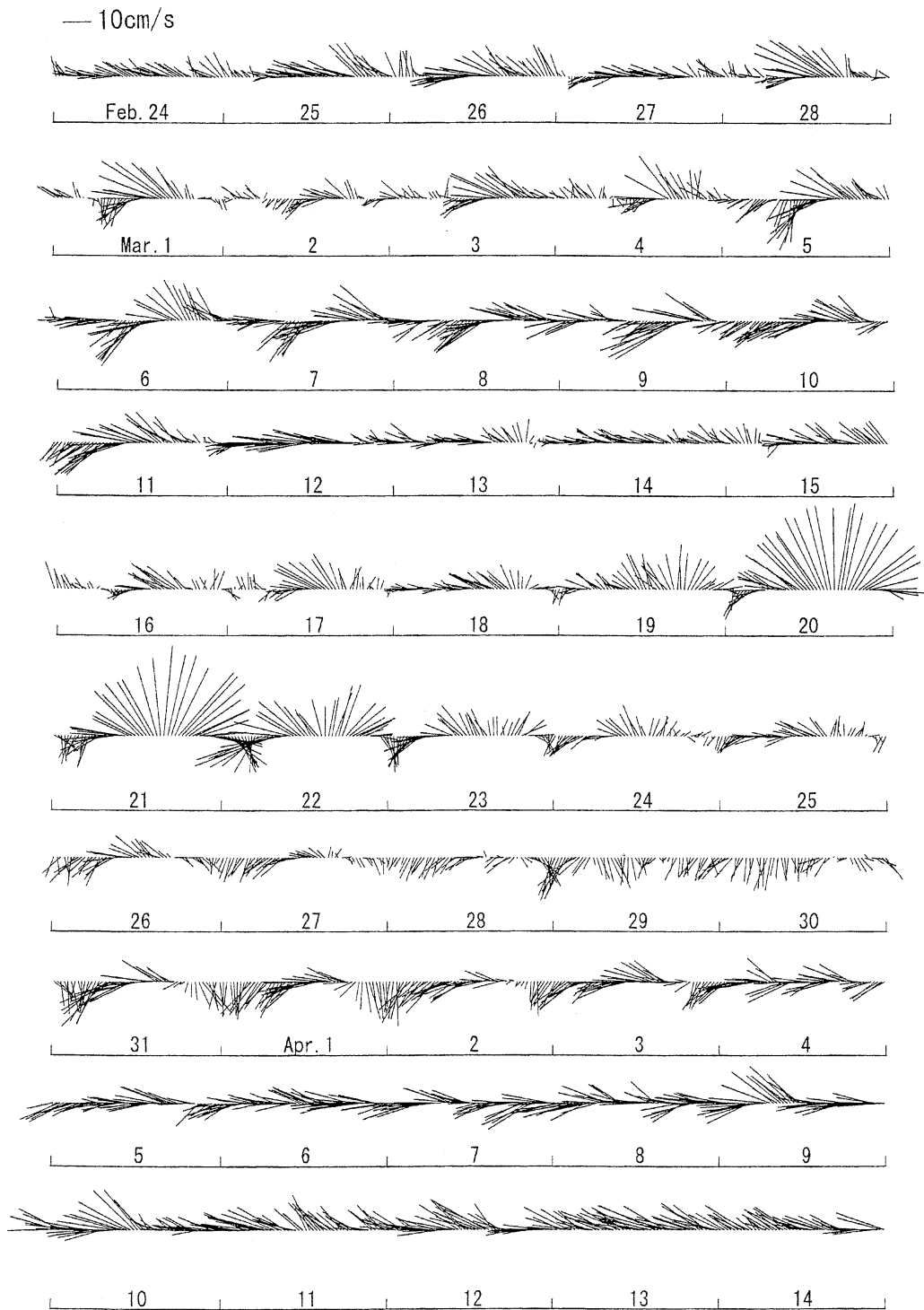


Fig. 3 Northward stick diagram of the observed velocity vectors in the first mooring case.

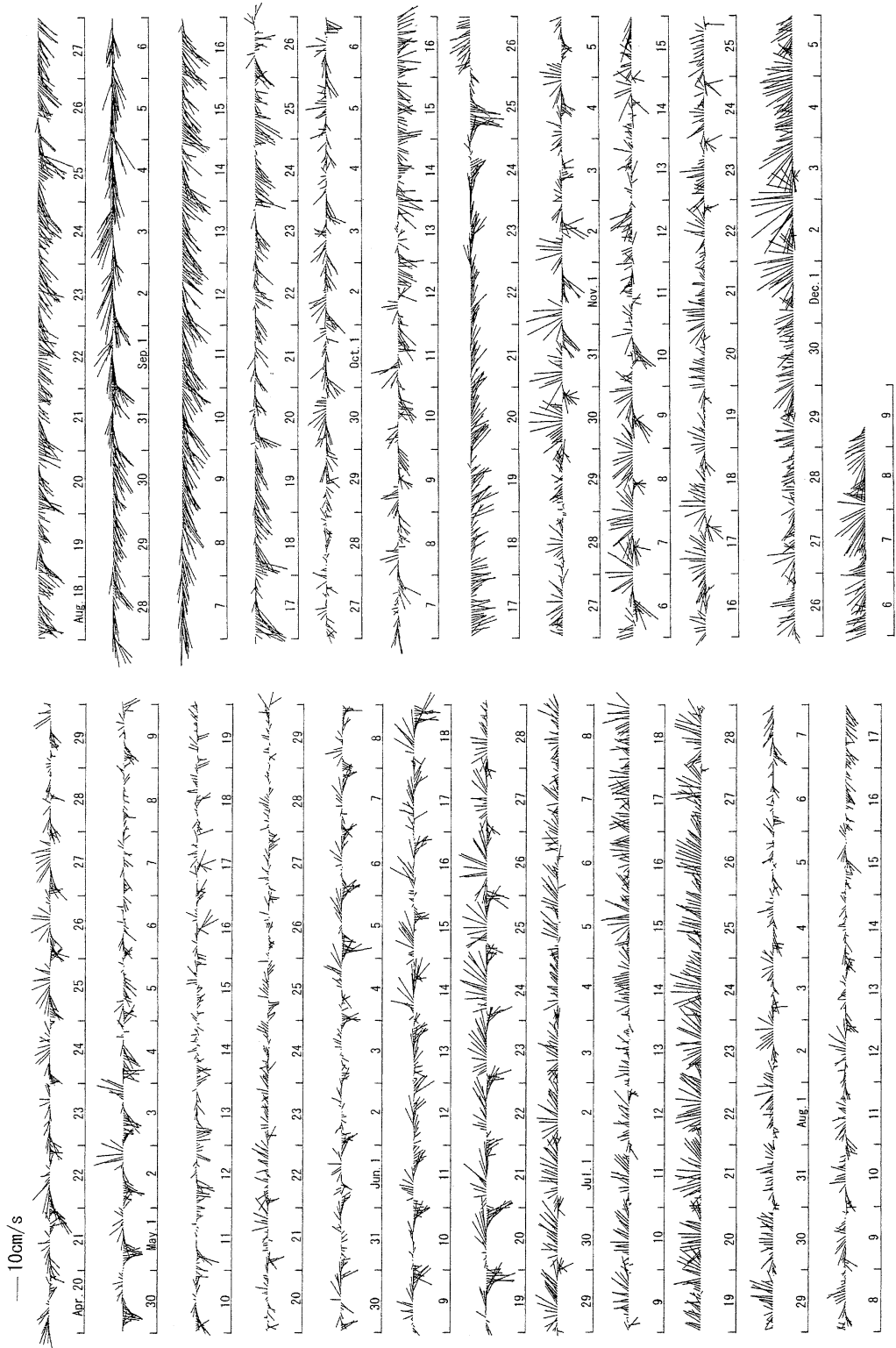


Fig. 4 Northward stick diagram of the observed velocity vectors in the second mooring case.

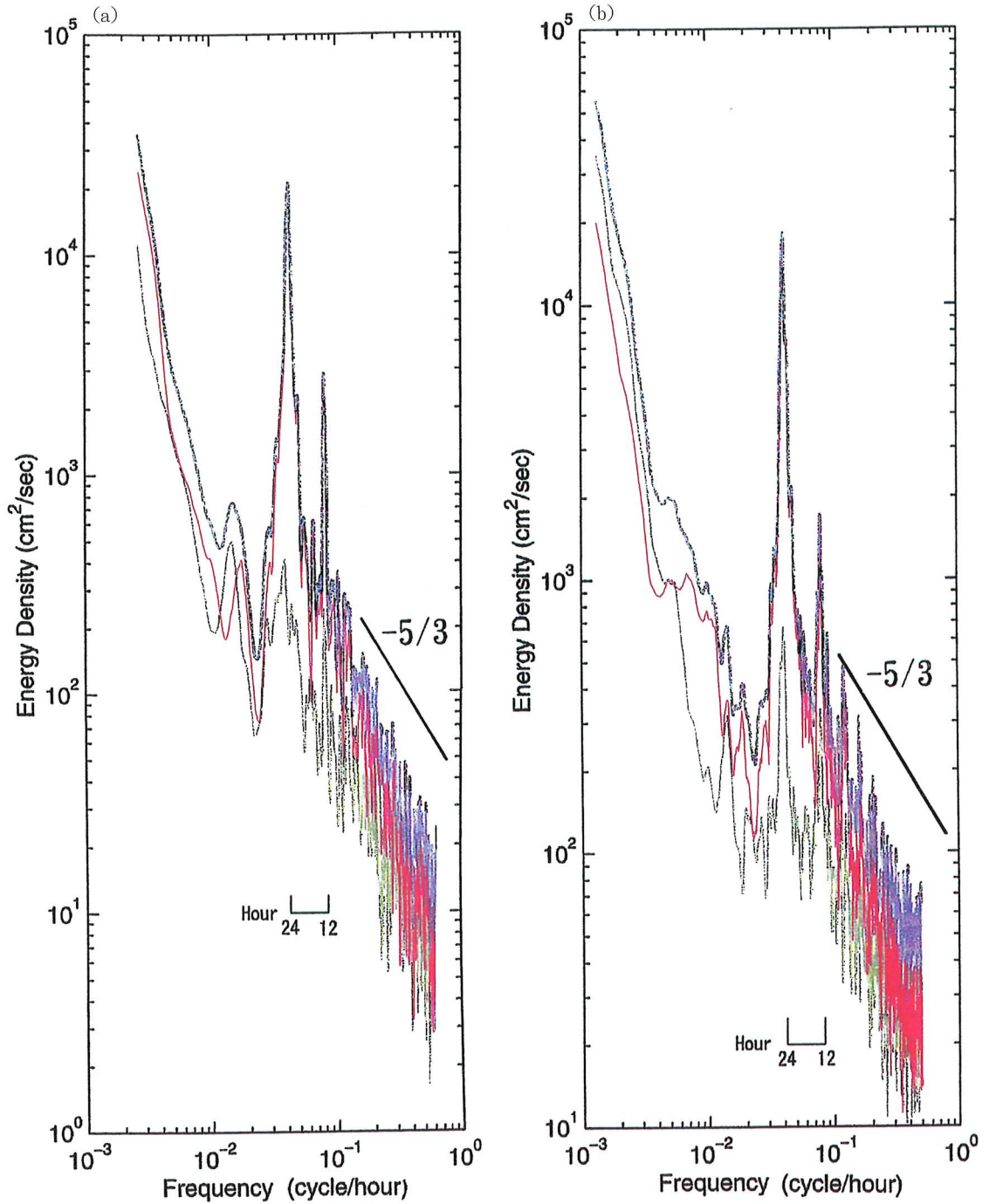


Fig. 5 Rotary spectrum of the observed velocity. (a) First mooring case and (b) second mooring case. Blue lines show the total spectrum, red (green) line represents the anticyclonic clockwise (cyclonic counterclockwise) circulation.

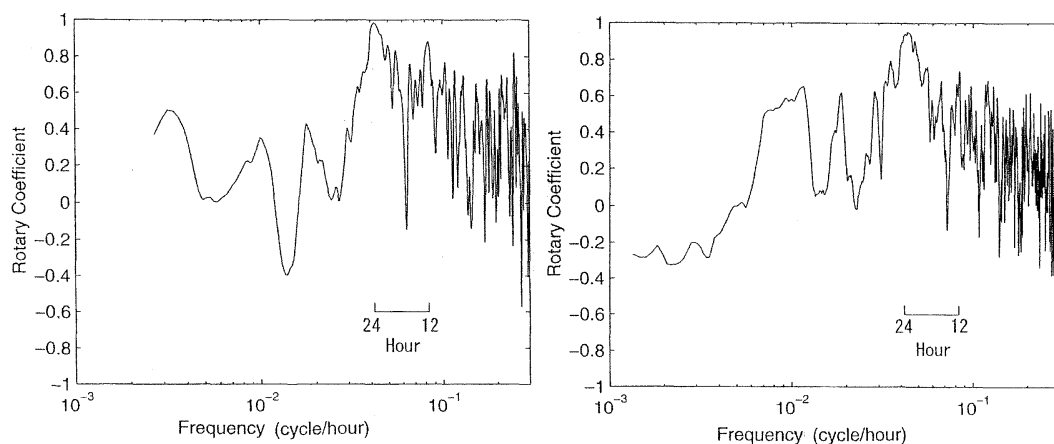


Fig. 6 Rotary coefficient of the rotary spectrum. First mooring case (left panel) and the second mooring case (right panel).

oscillation. Detailed analyses on the diurnal variations including the inertial oscillation, K_1 with the period of 23.93 hours and O_1 with 25.82 hours, and on the half diurnal variations including M_2 (12.42 hours) and S_2 (12.00 hours) seem to be possible by use of the long-term observational data. However, because FFT analyses by use of hourly data yield some inevitable error, results of the detailed analyses are not shown.

Mean slope (gradient) of the rotary spectrum (Fig. 5) in the shorter periods between 10 hours and 4 hours is -2.1 for the first mooring case and -2.3 for the second mooring case. These slopes are larger than the $-5/3$ power law of the Kolmogorov Spectrum (OZMIDOV, 1965) and relatively well approximated by the -2 power law of the GARRETT and MUNK Spectrum (GARRETT and MUNK, 1971, 1975), which indicates that the short period velocity variation is mainly due to internal waves. However, the slope of the spectrum is decreased if the estimated range is shifted to shorter period. Therefore, the slope of the spectrum has a tendency to represent the inertial subrange of the $-5/3$ power law of the Kolmogorov Spectrum in more shorter periods.

Based on the dominant inertial oscillation shown by the rotary spectrum (Fig. 5), progressive velocity vector diagram of the second mooring case is shown in Fig. 7. It is clearly seen that the clockwise inertial oscillation with a period of 24 hours is dominant in the

observed velocity fields. Here, it should be noticed from Fig. 7a that the inertial oscillation is relatively weak and it is not clearly seen in some time periods; from 190 days to 210 days, 240 days to 260 days, 280 days to 300 days and 320 days to 340 days, while a large horizontal shift is commonly detected in these periods. In these periods, northward progress, southwestward progress, a cyclonic southward progress and northwestward progress are suggested from Fig. 7a, respectively. Mean velocities during these periods are 5.8 cm sec^{-1} , 11.4 cm sec^{-1} , 6.3 cm sec^{-1} and 6.2 cm sec^{-1} . These periods commonly correspond to the downward shift of the current meter shown in Fig. 2b. The power spectrum during the large horizontal shift is shown in Fig. 8. Here, period of 5.3 days during the large velocity with coherent direction is adopted from Fig. 4. It is shown from Fig. 8 that the peak of 24 hours are still clear, while its intensity is not so clear as that in total period shown in Fig. 5. The constant of the FFT analyses of these case is large in comparison with the total period shown in Fig. 5. Therefore, the inertial oscillation with the bias of the mean flow is suggested in these periods.

In order to see the oceanic condition of the larger velocity periods accompanied by the downward shift of the current meter, sea surface velocity estimated from the sea surface height observed by the satellite observation of TOPEX/POSEIDON (KURAGANO and KAMACHI, 2000) is shown in Fig. 9. Together

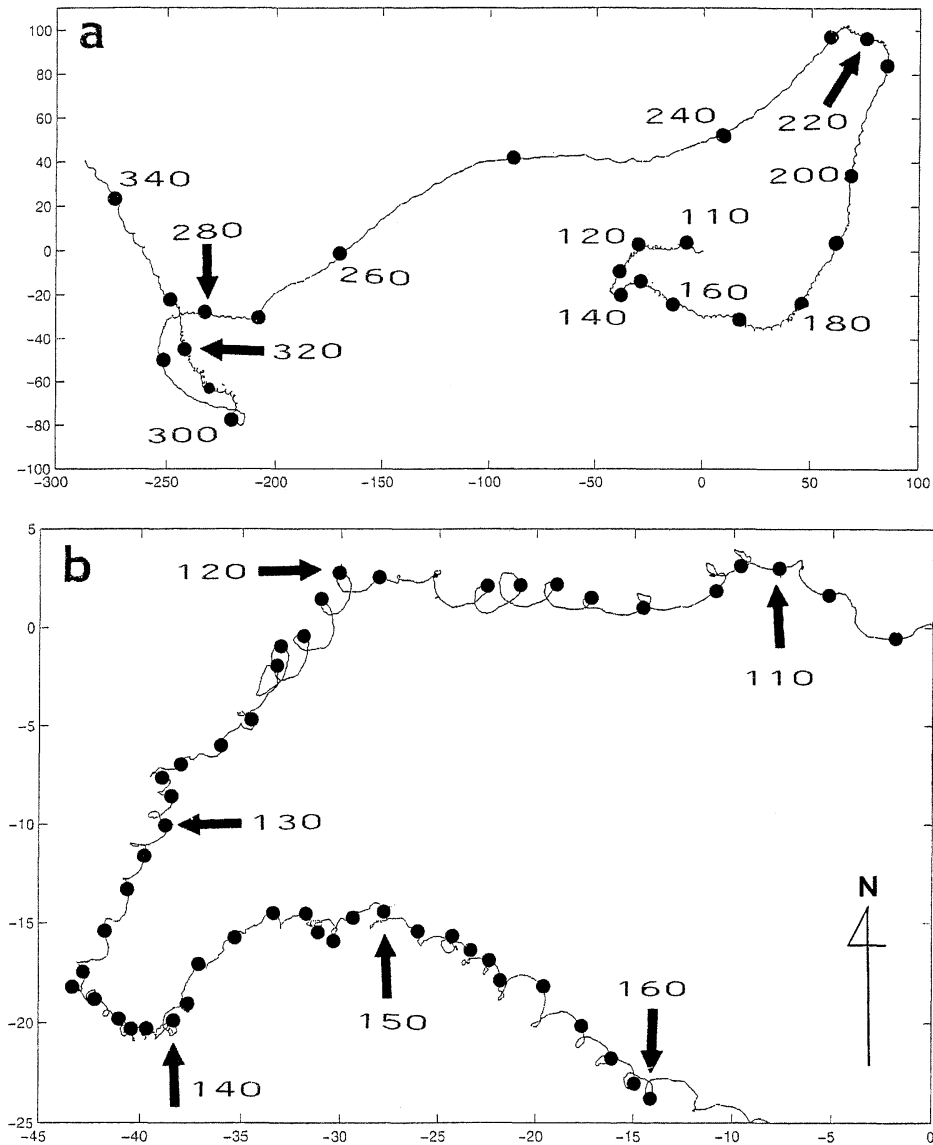


Fig. 7 (a) Progressive velocity vector diagram in the second mooring system. (b) Enlargement of the period from the initial stage to 160 Julian days. Numbers show the Julian days as were shown in Fig. 2b.

with Figs. 4 and 7a, it is found that the north-eastward flow during the period of 190 days to 210 days is caused by the approach of the anticyclonic eddy with the northeastward flow in its western part (Fig. 9a). During the period of 240 days to 260 days, the southwestward flow is also suggested by the approach of the anticyclonic eddy and cyclonic eddy (Fig. 9b). Southward flow from 280 days to 300 days and

northwestward flow from 320 days to 340 days are also explained by the coupled anticyclonic eddy and cyclonic eddy (Fig. 9c) and approach of the anticyclonic eddy (Fig. 9d), respectively. As for the downward shift of the current meter in early April in the first mooring case (Fig. 2a) with larger westward flow (Fig. 3), the approach of a cyclonic eddy south of the observational point and an anticyclonic eddy in

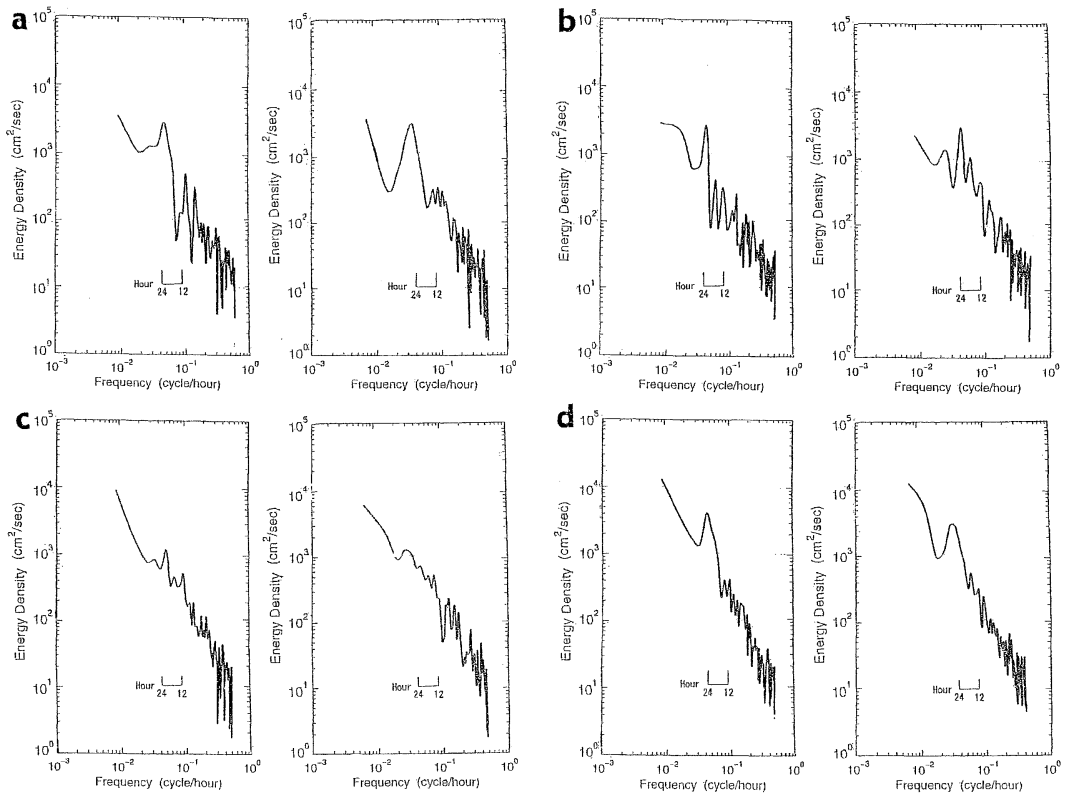


Fig. 8 Power spectrum in the periods of large horizontal shifts with coherent direction during 5.3 days from (a) 21 July, (b) 13 September, (c) 13 October and (d) 2 December. The left (right) panel shows the power spectrum of eastward (northward) velocity.

northern region are seen in the surface velocity fields shown in Fig. 10.

Since mean velocities of the westward shift of the mesoscale eddies are about 6.8 cm sec^{-1} (EBUCHI and HANAWA, 2000), the monthly westward shift of a mesoscale eddy are about 180 km, which is not so fast and the almost uniform velocity has a possibility to be maintained in a horizontal distance shown in Fig. 7. It is thus resulted that mesoscale eddies have a vertical structure deeper than 700 m with a mean velocity of 15 cm sec^{-1} . Together with larger surface velocity shown in Figs. 9 and 10, a possible dynamical influence of mesoscale eddies on the path dynamics of the Kuroshio is suggested.

4. Summary and discussion

We have made two direct current measure-

ments at 29.50°N and 135.25°E in the Shikoku Basin (Fig. 1) over the periods from 20 February to 16 April in 1999 at a depth of 470 m and from 17 April to 9 December in 1999 at a depth of 730 m. Main results of these direct current measurements are summarized as follows.

(1) Inertial velocity oscillation with a period of one day is dominated in the velocity variation and the clockwise circulation is seen in the progressive velocity vector diagram. However, some periods of large uniform velocity are also observed and the inertial oscillation is added over the bias of uniform velocity in this period.

(2) During the periods of some almost uniform velocities, downward shift of the current meter and the large velocity are commonly observed. It is suggested that the larger uniform velocity yields a larger dragging force on the mooring system, which results in the downward shift of

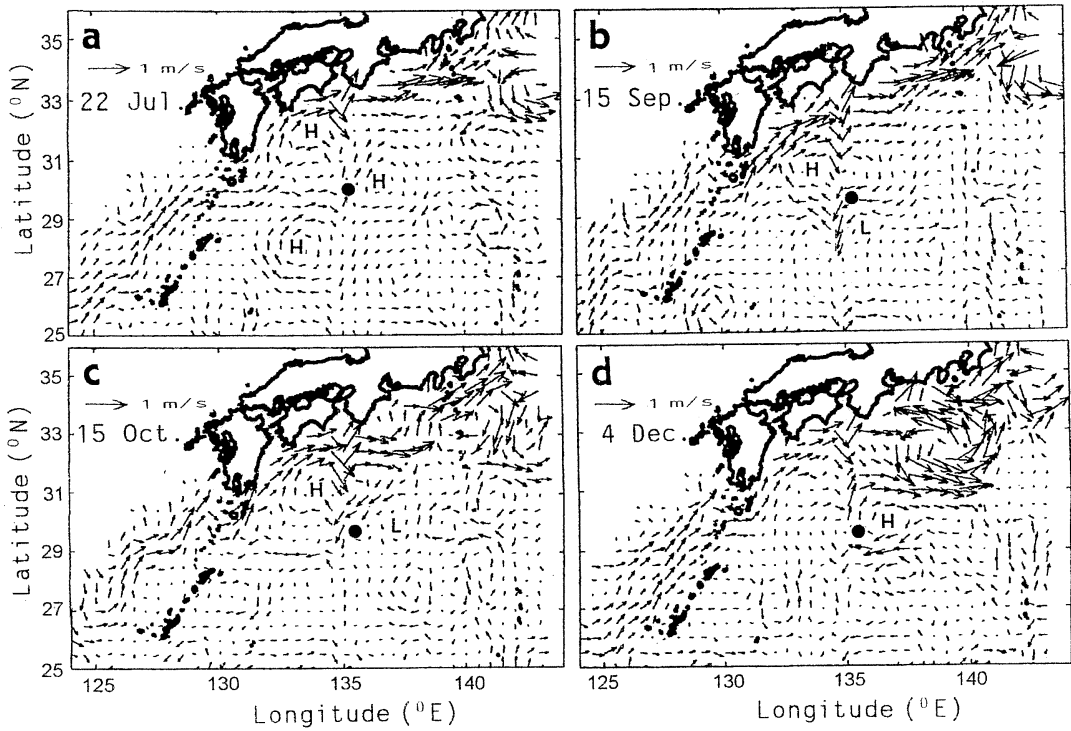


Fig. 9 Sea surface velocity estimated from the sea surface height observed by the satellite TOPEX/POSEIDON (KURAGANO and KAMACHI, 2000) during the second mooring case. Date of the observational and reference velocity are shown in left top of each panel. The observational point of the mooring system is also shown by closed circle.

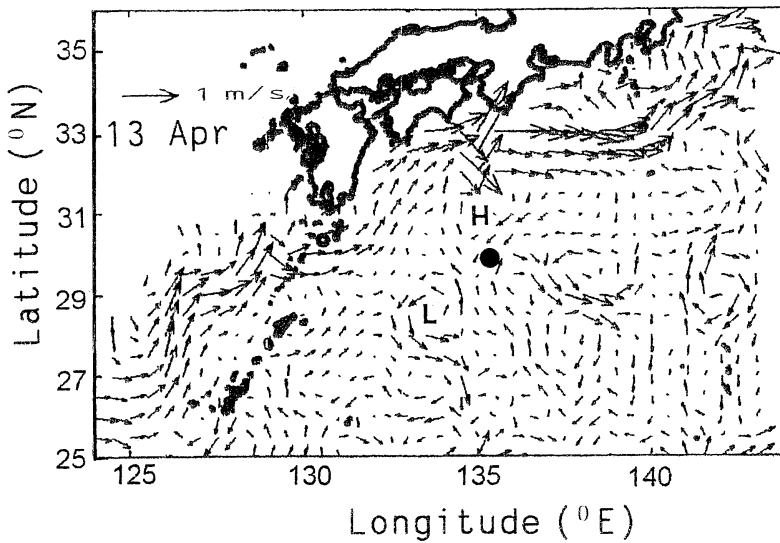


Fig. 10 Same as in Fig. 9, except for 13 April 1999 in period of the first mooring case.

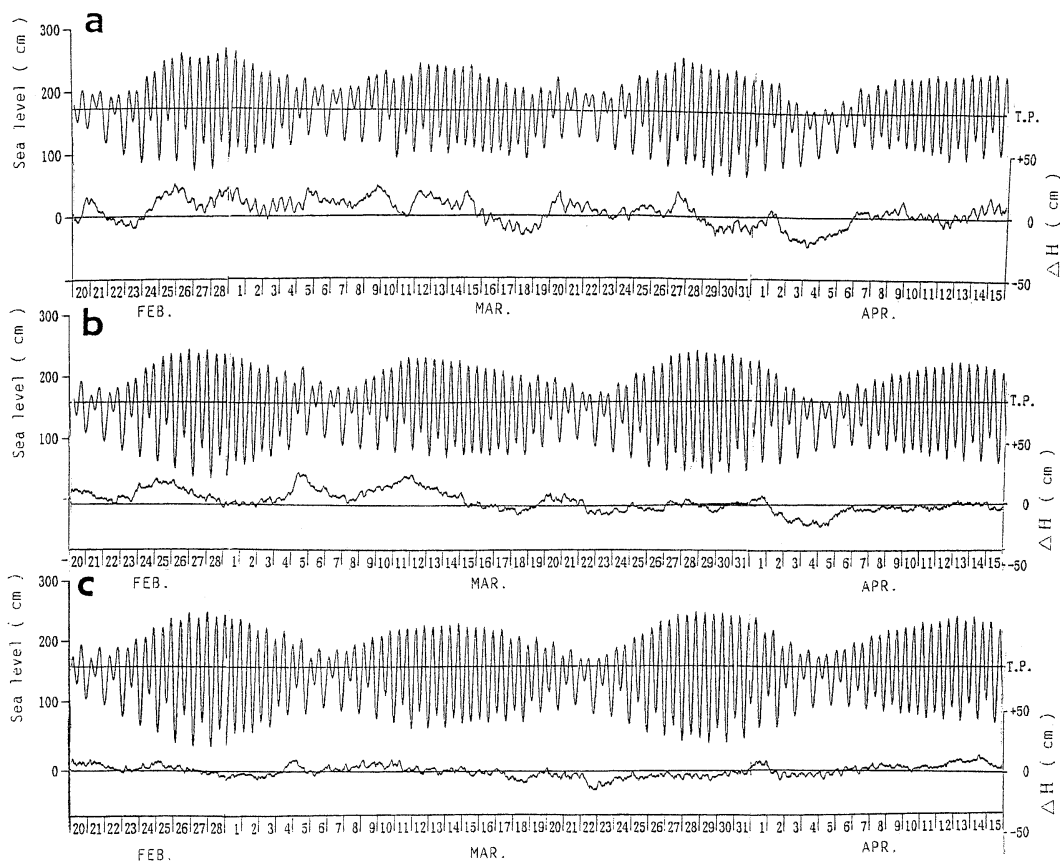


Fig. 11 Hourly sea level variation (upper) and their deviation from the predicted sea level (lower) based on the Climate and Marine Department of the Japan Meteorological Agency (1999). (a) Kushimoto, (b) Aburatsu and (c) Naha.

the current meter by the constraint of constant length of the mooring system from sinker to the current meter.

(3) The larger uniform velocity noted in (2) is caused by the approach of mesoscale eddies, which were detected by the satellite sea surface level. It is resulted that the mesoscale eddies have a vertical structure deeper than 700 m and a mean velocity of 15 cm sec^{-1} at an observed depth.

Larger velocity with an inertial oscillation is observed in 20–23 in March (Fig. 3), which is also shown by the large absolute velocity in Fig. 2. Because no significant atmospheric disturbance such as typhoon was observed in this observational period, it is inferred that the large inertial velocity may be related to the

large sea level difference during the spring tide. So, the sea level variations at Kushimoto, Aburatsu and Naha are shown in Fig. 11. It is seen from Fig. 11 that the period of 20–23 in March is in the neap tide and the amplitude of the sea level variation is rather small. However, since the diurnal tide is essentially dominant in this period (Fig. 11), the resonant phenomenon between the tidal current and the inertial oscillation is suggested in this case. Similar large amplitude velocity oscillations in 23–25 June (Fig. 4), and 30–31 October are also observed in the neap tide with a diurnal tide variation (not shown). A resonant effect between the inertial oscillation and the diurnal tide is inferred, however its detailed dynamics will be left out for future study.

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東京湾内湾の人工海浜 2 地点（葛西臨海公園と八景島海の公園） の仔稚魚相

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Larval and juvenile ichthyofauna in artificial beaches facing Tokyo Bay

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Hiroshi KOHNO* and Kouki KANOU**

Abstract : A total of 95,804 fishes of 35 species and 8,008 fishes of 38 species were collected from Kasai and Hakkei-jima artificial beaches, respectively, in the inner Tokyo Bay by monthly seine-net (mesh size 1.0mm) samplings from July 2002 to June 2003. Cluster analyses based on species and individual numbers collected from each station and sampling month showed that each month by stations were classified into seven groups such as 1) early summer in Kasai, 2) summer to autumn in Kasai, 3) summer to autumn in Hakkei-jima, 4) winter in both stations, 5) spring in Kasai, 6) spring in Hakkei-jima, and 7) June in Hakkei-jima. Comparisons of fish species collected from the two sites suggested that the difference of fish assemblage between the both sites would be caused partly by the salinity tolerance of the species and partly by the geographic condition of the sites, Kasai being located in the bottom, whereas Hakkei-jima near the mouth, in the bay.

Keywords : Tokyo Bay, artificial tideland, fish fauna, juveniles

1. 緒言

東京湾内湾（富津と観音崎を結んだ線より北の海域）は、かつて遠浅の干潟や浅瀬が全域に広がり豊かな海域であったが、1960年代からの埋め立てにより、自然の干潟や浅瀬は著しく減少した（小倉，1993）。しかし1980年代から、干潟や浅海域が生物にとって重要であることが見直され、失われた干潟や浅海域を復元する機運が高まり、人工的に海浜などが造られ始めた。なかでも、葛西臨海公園の人工渚や八景島海の公園の人工砂浜などはその代表的な例である。

両地点とも魚類相のモニタリングは盛んで、葛西では東京都環境局環境評価部（2002）、桑原ら（2003）などが、八景島では田辺・林（1999）や工藤ら（2002）などが研究を行っている。工藤ら（2002）は、八景島海の公園とそれに隣接する自然海浜の野島海岸の魚類相が異なることを示し、その原因が人工海浜か否かではなく、汽水の影響や地理的要因の可能性もあると推測している。桑原ら（2003）は、既往の文献に基づいて東京湾内湾の13地点の魚類相を比較し、八景島海の公園の人工砂浜の魚類相が東京湾内湾の他の地点と大きく異なることを指摘している。しかし、東京湾を代表する人工海浜である八景島海の公園と葛西臨海公園での、出現魚種の季節変化などの詳細な比較はなされていない。

本研究では、2002年7月から2003年6月までの1年間にわたり、八景島海の公園と葛西臨海公園で、地曳網による同一の方法で魚類の採集を行い、魚類相の比較を行うとともに、魚類相の違いに影響する環境要因を推測した。

2. 材料と方法

採集は東京湾内湾湾奥部に位置する葛西臨海公園の西

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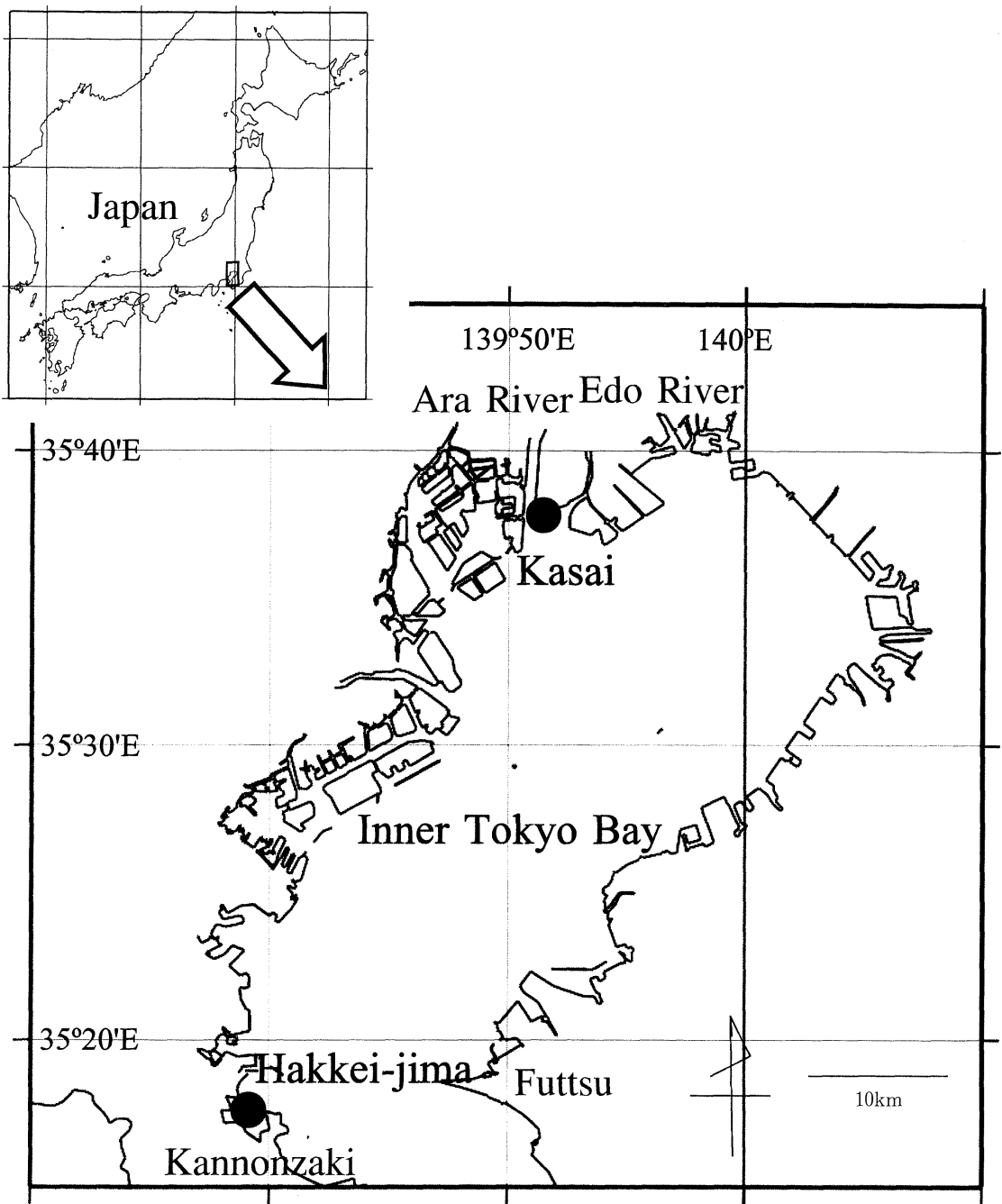


Fig. 1. Map of sampling sites in the inner Tokyo Bay.

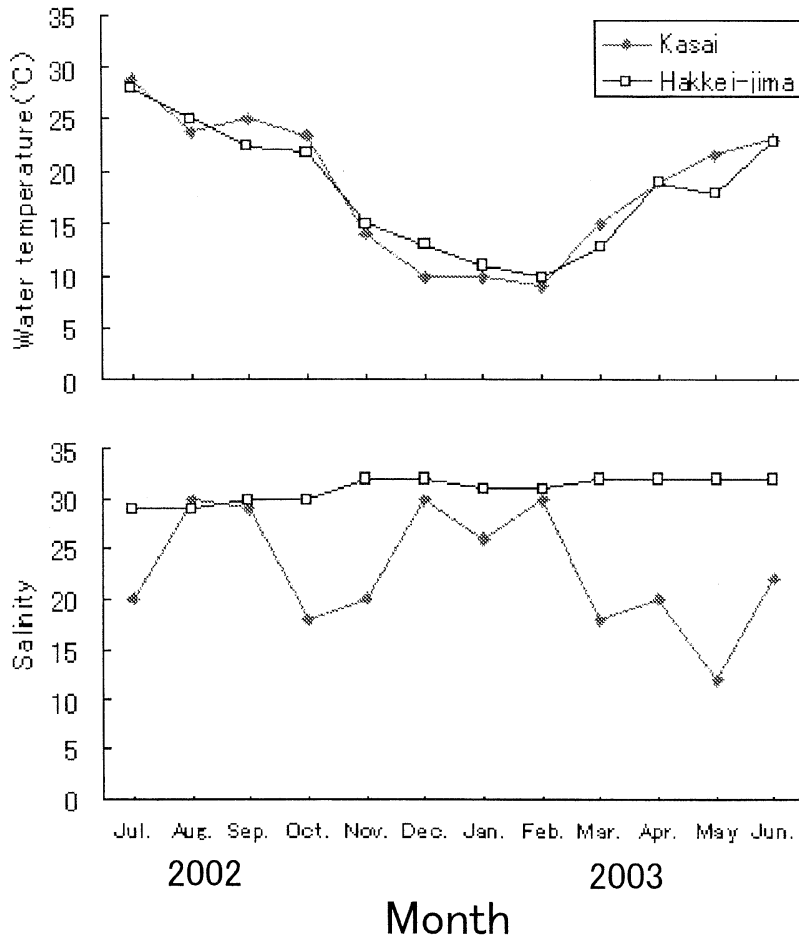


Fig. 2. Monthly changes of water temperature ($^{\circ}\text{C}$) and salinities at Kasai and Hakkei-jima artificial beaches in the inner Tokyo Bay.

渚と横浜八景島海の公園の人工海浜で行った (Fig. 1)。葛西臨海公園は、1970年代末に造成された人工海浜で、荒川と江戸川の河口にはさまれている。八景島海の公園は、1980年に竣工された延長約1 kmの人工海浜である。両地点とも海岸タイプはSHORT and WRIGHT (1983) に従うと反射型の海岸に分類される。

両地点ともに2002年7月から2003年6月にかけて、毎月一回大潮前後の昼間の干潮時に採集を行った。採集には小型地曳網 (袖網長4 m、高さ1 m、目合い1 mm) を用い、水深1 m以浅の場所を汀線とほぼ平行に約50 m、3回ずつ曳網した。採集物は現場でただちに10%海水ホルマリンで固定し研究室に持ち帰った。また、魚類の採集と同時に棒状水銀温度計とアタゴ社製海水濃度屈折計で水温と塩分をそれぞれ計測した。採集された魚類については、種同定と個体数の計数、標準体長の測定、および発育段階の観察を行い、それぞれの種の生活史型 (加

納ら、2000) (海水魚、河口魚、両側回遊魚、遡河回遊魚) と発育段階 (仔魚: 鱗条が未完成; 稚魚: 鱗条が完成し鱗も出現するが、体形、模様は成魚と異なる; 若魚: 鱗、模様がほぼ完成、体形が成魚に近いが未成熟; 成魚: 成熟しているもの) を特定した。リストの科の配列はNELSON (1994) に、和名と学名は中坊 (編) (2000) に従った。

両地点の各月に採集された魚類の種ごとの個体数に基づき、類似度をもとめクラスター分析を行なった。類似度指数にはBray-Curtis指数 (PS_2) を、クラスター連結には群平均法を用いた (小林, 1995)。なお、本研究で採集された魚類の個体数は種間の差が大きく、類似度が個体数の多い種に影響されやすい。したがって、各種の個体数を対数変換し、個体数が少なかった種の貢献度を高めた。なお、クラスター分析において、 PS_2 が0.3以上のクラスターを群集構造がよく似たグループとした。

Table 1. Fish collected from artificial beaches of Kasai and Hakkei-jima in Tokyo Bay from July 2002 to June 2003

Family	Species	Kasai			Hakkei			Life type	Species code
		Number	Size rang (SL, mm)	Developmental stage	Number	Size rang (SL, mm)	Developmental stage		
Gymnuridae	<i>Gymnura japonica</i>	1	225	A	0		-	M	
Clupeidae	<i>Sardinella zunasi</i>	8399	4.4-103.7	L-Y	150	6.7-46.9	L-J	M	SZ
	<i>Konosirus punctatus</i>	2966	5.4-72.8	L-Y	174	5.5-60.6	L-J	M	KP
Engraulidae	<i>Engraulis japonicus</i>	8	8.0-32.7	L-J	2	9.3-27.0	A	M	EJ
Plotosidae	<i>Plotosus lineatus</i>	0		-	2	28.5-128.7	A	M	
Plecoglossidae	<i>Plecoglossus altivelis al tivelis</i>	1181	4.4-56.1	L-Y	1006	16.1-45.1	L-J	Am	PA
Osmeridae	<i>Hypomesus nipponensis</i>	1	37.7	L	0		-	An	
Atherinidae	<i>Hypoatherina valenciennei</i>	3	11.0-12.5	J	30	5.6-84.7	J-A	M	HV
Scorpaenidae	<i>Sebastes inermis</i>	1	38.1	J	0		-	M	
Platycephalidae	<i>Platycephalus</i> sp.2	60	6.2-45.1	Y	0		-	M	PS
Cottidae	<i>Pseudoblennius cottoides</i>	0		-	36	14.7-52.8	Y-A	M	PbC
Liparidae	<i>Liparis tessellatus</i>	0		-	1	23.4	A	M	
Moronidae	<i>Lateolabrax japonicus</i>	500	10.7-365	J-A	1	125.4	A	M	LJ
Teraponidae	<i>Rhyncopelates oxyrhynchus</i>	4	10.9-14.8	J	18	9.9-28.1	J	M	RO
Sillaginidae	<i>Sillago japonica</i>	63	12.4-70.0	J-Y	152	8.3-32.6	J-A	M	SJ
Leiognathidae	<i>Leiognathus nuchalis</i>	1586	5.8-34.4	L-Y	52	5.0-24.9	J-Y	M	LN
Gerreidae	<i>Gerres equulus</i>	0		-	1198	10.3-23.8	J	M	GE
	<i>Gerres filamentosus</i>	2	15.4-16.2	J	0		-	M	
	<i>Gerres japonicus</i>	1	10.3	J	0		-	M	
Sparidae	<i>Acanthopagrus Schlegelii</i>	1	11.1	J	2	10.3-50.8	-	M	
	<i>Acanthopagrus latus</i>	3	10.3-11.8	J	0		-	M	ApL
Sciaenidae	<i>Pennahia argentata</i>	11	15.3-39.3	J	0		-	M	
Embiotocidae	<i>Ditrema temmincki</i>	0		-	33	40.0-133.4	Y-A	M	DT
Mugiliformes	<i>Mugil cephalus</i>	20	21.7-30.0	J	0		-	M	MC
	<i>Chelon affinis</i>	0		-	1	13.4	J	M	
Pholidae	<i>Pholis nebulosa</i>	0		-	10	37.8-112.2	Y	M	PN
	<i>Pholis crassispina</i>	0		-	31	39.6-68.9	Y	M	PhC
Blenniidae	<i>Omobranchus punctatus</i>	2	3.7-5.1	Y	0		-	E	
	<i>Omobranchus fasciolatocep</i>	12	2.9-12.7	Y	0		-	E	
Stichaeidae	<i>Dictyosoma rubrimaculatum</i>	0		-	1	14.9	Y-A	M	
Callionymidae	<i>Repomucenus beniteguri</i>	0		-	1	55.7	Y	M	
Gobiidae	<i>Luciogobius guttatus</i>	8	6.8-15.2	-	2	10.1-10.8	J	E	
	<i>Luciogobius</i> sp.	0		-	4	4.3-8.5	J	-	
	<i>Eutaeniichthys gilli</i>	20	5.0-34.2	J	5	4.2-6.9	J	E	
	<i>Gymnogobius urotaenia</i>	623	5.2-30.0	L-J	3	15.3-17.5	J	AM	GU
	<i>Gymnogobius macrognathos</i>	9911	7.6-44.0	L-A	0		-	E	GM
	<i>Gymnogobius heptacanthus</i>	5	7.7-34.3	J	4097	5.0-49.0	L-A	E-M	GH
	<i>Gymnogobius breunigii</i>	3761	5.4-39.7	L-Y	29	5.3-13.0	L	E	GB
	<i>Acanthogobius flavimanus</i>	64691	10.1-52.8	J-Y	249	11.3-172.4	J-Y	E	A
	<i>Acanthogobius lactipes</i>	52	5.3-43.4	J-A	0		-	E	AL
	<i>Favonigobius gymnauchen</i>	13	14.6-53.1	J-A	67	13.4-54.5	J-A	E	FG
	<i>Acentrogobius pflaumii</i>	0		-	28	12.1-45.7	J-A	M	AgP
	Gobiidae spp.	<i>Tridentiger</i> spp.	1745	4.2-15.6	L-J	563	4.3-33.1	L-J	-
Gobiidae sp.		0		-	1		J	-	
Gobiidae spp.		25	5.3-9.9	L-J	17	4.4-8.8	L-J	-	
Siganidae	<i>Siganus fuscescens</i>	0		-	1	15.6	Y	M	
Centrolophidae	<i>Psenopsis anomala</i>	1	13.2	J	0		-	M	
Pleuronectidae	<i>Kareius bicoloratus</i>	7	9.5-11.9	J	1	52.8	J	M	KB
Triacanthidae	<i>Triacanthus biaculeatus</i>	117	5.4-38.4	L-Y	18	5.9-42.8	L-J	M	TB
Monacanthidae	<i>Rudarius ercodes</i>	0		-	11	6.3-35.6	J	M	RE
Tetraodontidae	<i>Takifugu niphobles</i>	0		-	10	6.2-137.4	J-Y	M	
	Tetraodontidae sp.	0		-	1	12.1	J	M	

Developmental stage : A, adult ; J, juvenile ; L, larva ; Y, young. ; and -, absent. Life type : Am, amphidromous fishes ; An, anadromous fishes ; E, estuarine fishes ; M, marine fishes ; and -, indeterminate. Species codes being used in Fig.4.

また、各地点の各月の群集の多様度を計量するために、Shannon-Weaver diversity index (H') (木元, 1993) を求めた。

3. 結果

3.1 水温と塩分

両地点の水温は7月に最高を示し、28.9°C (葛西臨海公園, 以下葛西と略す) と28.0°C (八景島海の公園, 以下八景島と略す) であった (Fig.2)。それ以降は下降し、2月に最低になった (10.0°Cと9.0°C)。塩分は、八景島では年間を通じてほぼ一定 (29~32) で推移した。一方、葛西では調査月ごとに大きく変動したが、概ね夏季に高く、冬季に低い傾向を示した。最高は8月と12月の30、最低は5月の12であった。

3.2 種数と個体数

葛西では合計35種 (2未同定種を含む) 95,804個体で、生活史型で分けると、海水魚21種, 13,759個体, 河口魚9種, 78,470個体, 両側回遊魚2種, 1,181個体, 遡河回遊魚1種, 1個体, 不明2種, 1,770個体が採集された (Table 1)。一方、八景島では合計38種 (4未同定種を含む) 8,008個体で、海水魚27種, 6,062個体, 河口魚5種, 352個体, 両側回遊魚1種, 1,009個体, 不明4種, 585個体が採集された。

葛西のみに出現した種は14種10,078個体 (海水魚: 9種, 100個体; 河口魚: 4種, 9,977個体; 遡河回遊魚: 1種, 1個体) であった。一方、八景島のみに出現した種は17種1,933個体 (海水魚: 15種, 1,365個体; 不明: 3種, 568個体) であった。

3.3 優占種

葛西の最優占種はマハゼ (64,691個体, 葛西全体の個体数の67.5%) で、次いでエドハゼ (10.3%), サッパ (8.8%), ビリンゴ (3.9%), コノシロ (3.1%), チチブ属 spp. (1.8%), ヒイラギ (1.7%), アユ (1.2%), ウキゴリ (0.6%), スズキ (0.5%) の順で、この上位10種で全体の99.5%であった。特に春期のマハゼとエドハゼの仔稚魚で全体の77.8%を占めていた。

八景島の最優占種はニクハゼ (4,097個体, 八景島全体の個体数の51.2%) で、次いでクロサギ (15%), アユ (12.6%), チチブ属 spp. (7%), マハゼ (3.1%), コノシロ (2.2%), シロギス (1.9%), サッパ (1.9%), ヒメハゼ (0.8%) で、これら10種で全体の96.2%を占めていた。とくにニクハゼとクロサギ, アユの3種で全体の78.6%を占めていた。

3.4 種数と個体数, 多様度の経月変化

種数は、葛西では7月と8月が最多で15種であった (Fig.3)。それ以降は減少し、12月に1種と最も少なくなり、その後、月を追うごとに増加した。一方八景島では、

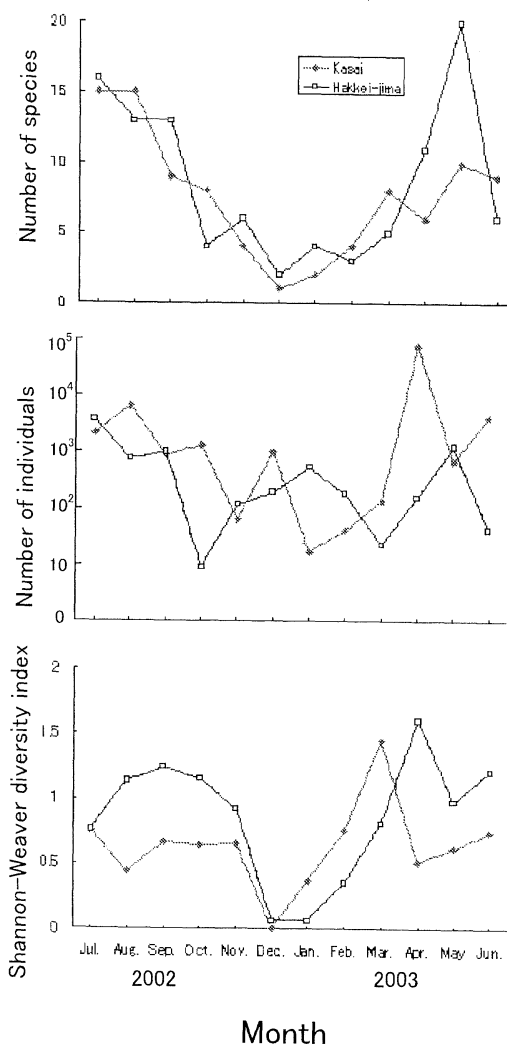


Fig. 3. Monthly changes of numbers of species and individuals and Shannon-Weaver diversity index (H') of fish larvae and juveniles collected at Kasai and Hakkei-jima artificial beaches in the inner Tokyo Bay from July 2002 to June 2003.

5月に最多で20種であったが、それ以外の経月変化は夏から冬にかけて減少し、冬から春に向けて増加した。最少は12月の2種であった。八景島の5月の急激な種数の増加を除けば、両地点ともほぼ同様の傾向であった。

採集個体数は、葛西では、4月が最多で78,715個体, 1月が最少で17個体であった (Fig.3)。一方八景島では、7月が最多で3,709個体で、10月が最少で9個体であった。

多様度は、葛西では、3月が最大で1.44, 12月が最小で0であった (Fig.3)。一方八景島では、4月が最大で

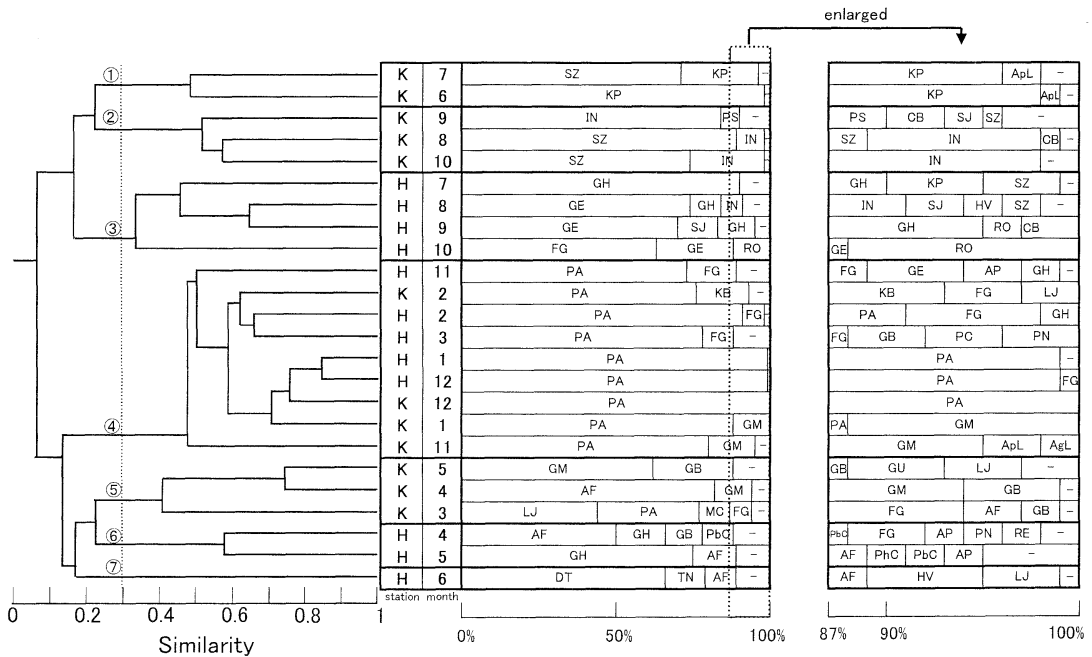


Fig. 4. Dendrogram based on similarity index among sampling months of each station in species compositions of fishes collected from Kasai(K) and Hakkei-jima(H) artificial beaches in the inner Tokyo Bay from July 2002 to June 2003. Species codes are given in Table 1.

1.72, 最小は12月と1月の0.06であった。両地点を比べると1～3月に葛西が八景島を上回る以外は、八景島の方が高い多様度を示した。

3.5 両地点の各月の魚類相の類似性

クラスター分析の結果、葛西と八景島の各月は7つのグループに分けられた (Fig.4)。すなわち、①初夏 (6, 7月) の葛西, ②夏～秋 (8～10月) の葛西, ③夏～秋 (7～10月) の八景島, ④冬 (11～2月あるいは3月) の両地点, ⑤春 (3～5月) の葛西, ⑥春 (4, 5月) の八景島, ⑦6月の八景島である。

3.6 各グループの優占種

前項で得られた各グループの優占種は以下のとおりである。

①初夏の葛西 (6, 7月)

両月にコノシロが、7月にはサッパが多く出現した (Fig.4)。両種とも東京湾全域に広く出現する浮遊生活を送る仔稚魚である (加納ら, 2000; 荒山ら, 2002)。また、それ以外に、キチヌなども比較的多く出現した。

②夏～秋の葛西 (8～10月)

サッパ、ヒイラギなどの東京湾全域に広く出現する浮遊生活を送る仔稚魚 (加納ら, 2000; 荒山ら, 2002) や、9月には東京湾内湾に広く出現するマゴチ (加納ら, 2000) などが出現した。

③夏～秋の八景島 (7～10月)

ニクハゼ、ヒメハゼ、アミメハギ、クロサギ、シロギス、シマイサキなど、比較的高塩分のところに多く出現する種が多く採集された。

④冬の両地点 (葛西: 11～2月, 八景島: 11～3月)

アユが優占していた。また、アユ以外には、八景島には高塩分のところにも比較的多く出現するヒメハゼが、葛西にはエドハゼなどの汽水域特有の種が出現した。

⑤春の葛西 (3～5月)

マハゼ、エドハゼ、ウキゴリ、ビリンゴ、スズキ、アユなどの、仔稚魚期に汽水域に出現する種 (藤田, 1998; 加納ら, 2000) が優占していた。この内、マハゼとビリンゴは東京湾内湾に最も広く分布する種である (桑原ら, 2003)。

⑥春の八景島 (4, 5月)

ニクハゼ、アサヒアナハゼなどの比較的高塩分域に出現する種とともに、葛西にも出現した汽水魚のマハゼやビリンゴなども多く出現した。

⑦6月の八景島

春の八景島 (4, 5月) に引き続き、汽水魚のマハゼが多く出現したが、それ以外に、ウミタナゴなどの海産魚やクサフグなどの内湾にはあまり出現しない種も多く出現した。

4. 考察

葛西は、春先に河口魚のハゼ科魚類の仔稚魚の個体数が多くなるという特徴をもつ（グループ⑤, Fig. 4）。また、海水魚でも広塩性魚類であるスズキやボラなどが出現した。一方八景島では、春先にはマハゼも多く出現するが、海水魚の仔稚魚も種数、個体数ともに多くなる（グループ⑥と⑦, Fig. 4）。夏～秋には、葛西では仔稚魚期に東京湾全域に出現するサッパやコノシロ、ヒイラギ（加納ら, 2000; 荒山ら, 2002）や東京湾内湾の河口干潟域に広く出現するマゴチ（加納ら, 2000）が多く出現した（グループ①と②, Fig. 4）。一方八景島では、これらの魚種に加えて、アミメハギ、クロサギ、シロギスといった海産魚の仔稚魚や、ニクハゼ、ヒメハゼなどの比較的高塩分を好む種（加納ら, 2000）によって特徴づけられた（グループ③, Fig. 4）。

以上のような比較から、春から夏、さらには秋にかけての両地点の魚類相の違いは、主に塩分による出現魚種の違いに起因するものと考えられた。実際の塩分記録でも、八景島では常に30前後の安定した塩分を保っているが、江戸川と荒川の河口にはさまれた葛西では、夏の一時を除いて、春から秋にかけては12から20の範囲で変動した（Fig. 2）。さらに、八景島の近隣で塩分の比較的低い平潟湾や野島海岸の調査（工藤ら, 2002）では、マハゼ、スズキ、ボラ、マゴチなど、葛西と同じような魚種が採集されている。このような出現魚種の違いは、多様度にも反映されている。すなわち、春から秋にかけては両地点とも高い多様度を示し、さらに4月以降は八景島の方が葛西よりも高い値を示している（Fig. 3）。

一方冬季には、両地点ともアユが優占した（グループ④, Fig. 4）。このため、両地点の多様度も、とくに12月から2月にかけては小さい値を示した（Fig. 3）。なお、この時期の塩分は、葛西の11月を除けば25から30前後と高かった（Fig. 2）。アユは夏から秋に河川から流下した後、冬の河川への遡上を前にして、沿岸の汀線域に集積することが知られている（SENTA and KINOSHITA, 1985; 塚本, 1988）。したがって両地点とも、アユにとって冬季の一時滞在の場を提供していると判断される。なお、工藤ら（2002）は八景島より野島海岸の方がアユが多いとしているが、これは11月のみの調査で、冬季に採集を行っていないためであると考えられる。

葛西にのみ出現した種の個体数は河口魚のエドハゼやアシシロハゼが大部分をしめた（Table 1）。また、葛西にのみ出現した海水魚はマゴチやボラなどで、これらは比較的広塩性であるとされている（藤田, 1998）。一方、八景島にのみ出現した種はすべて海水魚で（Table 1）、クロサギ、アサヒアナハゼ、ウミタナゴ、スジハゼ、タケギンボ、アミメハギ、クサフグなどであった。これらの魚種は、葛西でも塩分が30近くになる時期がある（Fig. 2）にもかかわらず、葛西では出現しなかった。さらにこれらの魚種は、汽水域や湾奥では出現が稀であ

るとされている（加納ら, 2000）。したがって、上で述べた塩分という要素以外にも、八景島がより東京湾の湾口に近く、葛西が湾奥に位置するという、両地点の地理的な条件が出現する魚種に影響を与えているものと考えられる。

以上のように、東京湾の内湾に位置する人工海浜である葛西と八景島は、冬はアユの優占という共通した特徴をもつが、春から秋にかけては、異なる生活様式をもつ魚類が出現することにより魚類相が大きく異なることが判明した。さらにその原因については、両地点の塩分と地理的立地条件が関わっていると考えられた。今後はさらに、東京湾の湾奥から外湾にかけてのさまざまな立地条件および水環境の場所で調査を行い、どのような環境がどの魚種に利用されているのかを詳しく調査する必要がある。

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文献

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Characteristics of Tidal Currents Observed at Five Mooring Stations in Sagami Bay

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Abstract : Tidal currents in Sagami Bay were investigated by the long-term records of current and temperature obtained in the surface and deep layers at five mooring stations during the period from July to November, 1996. The time series and power spectra of the current and temperature records showed the predominance of the semidiurnal period fluctuations at all stations. The semidiurnal current was high correlation to the temperature at the same depth at all stations. The semidiurnal current amplitudes observed at the station in the inner region of the bay were dependent on the depth, and was very larger than tidal current amplitudes to be due to the surface mode estimated by the numerical experiments. These results indicate the observed tidal current to be due to the internal tides mostly. The predominance of the semidiurnal internal tide supports the key idea that the internal tides observed in Sagami Bay are mostly generated at the northern part of the Izu Ridge (OHWAKI *et al.* 1994, KITADE and MATSUYAMA 1997). The current and temperature data obtained at three depths (10m, 35m and 60m depths) near the northwest coast show that the tidal current amplitude with the semidiurnal period had a maximum at 10m depth and gradually decreased with depth. Its current amplitudes were variable with the change of the stratification. The phase relation between the current and temperature with the semidiurnal period indicates the internal wave propagation in the upper layer along the coast to be right the hand. These results are consistent to the results obtained by both the temperature measurements along the coast and the numerical experiment (KITADE and MATSUYAMA, 1997).

Keywords : tidal current, internal tides, inertial period, internal Kelvin wave, internal inertia gravity wave, vertical structure of the tidal current

1. Introduction

Sagami Bay is located in the central Japan, facing to the Pacific Ocean and its length from the mouth to head is about 55km and the width is about 60km (Fig.1). The Oshima island is located at the bay mouth and divides into two channels, *i.e.*, the Oshima West Channel and East Channel. The Sagami Trough extends to the northwest region of the bay head through the Oshima East Channel, and the shallow wa-

ter region is only found off the northeast coast of the bay head. The Kuroshio flows south of Sagami Bay and part of the Kuroshio water usually inflows through the Oshima West Channel and flows out through the Oshima East Channel (TAIRA and TERAMOTO, 1986). The anticlockwise current circulation in the bay is formed by this current and the strength of the circulation is closely related to the location of the Kuroshio path (IWATA and MATSUYAMA 1989, KAWABE and YONENO 1987).

Tidal phenomena in Sagami Bay have been observed by some methods, and the observational results have represented some interesting features. TERAMOTO (1971) observed the surface current by GEK on the ship and the volume transport by the Electronic Potential

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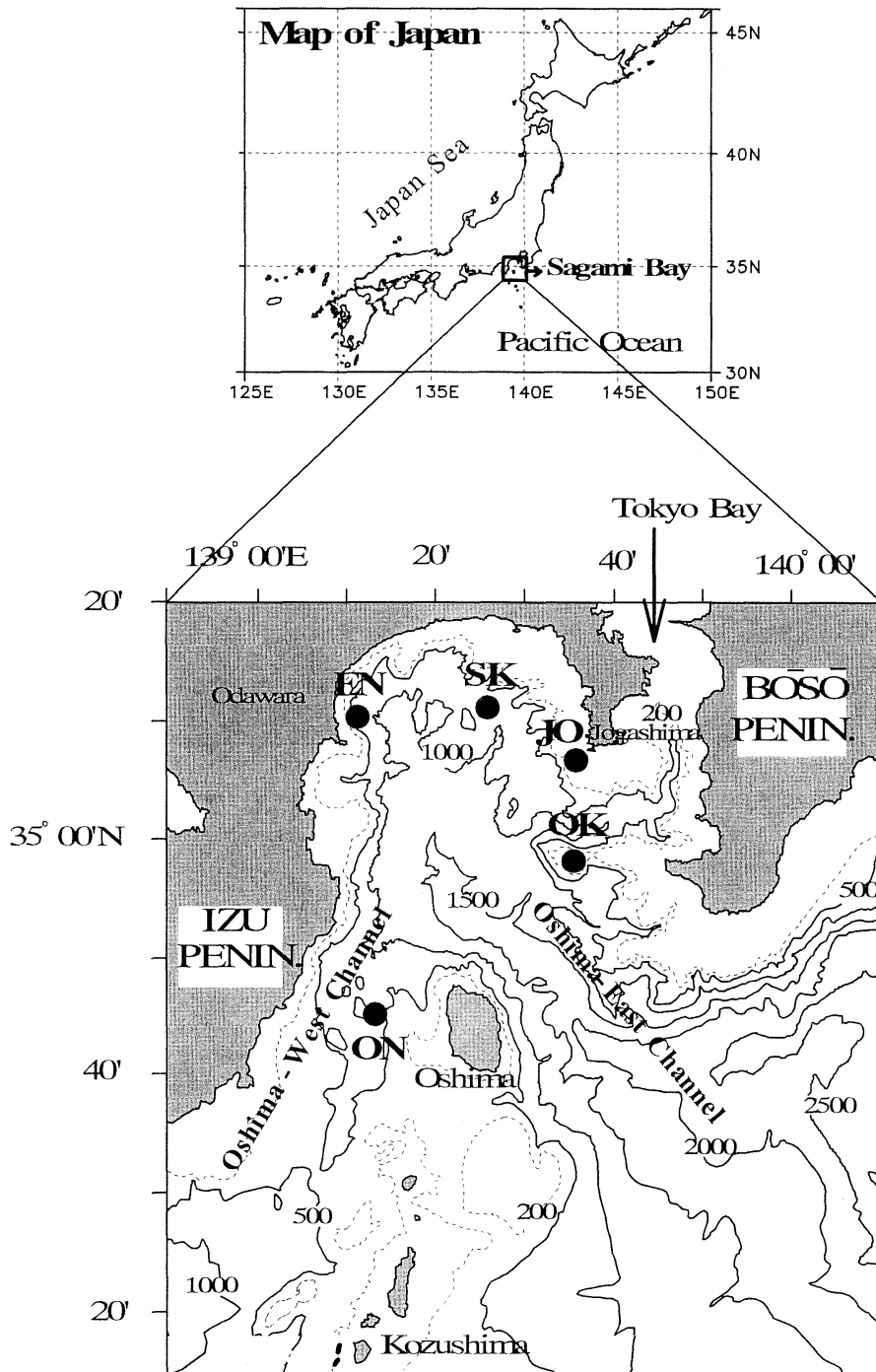


Fig. 1. Locations of current measurement Numerals on the bottom contour are in meter.

Measurements through the Oshima West Channel. He reported the strong current with semidiurnal period in spite of the small volume transport across the channel. KONAGA *et al.* (1979) carried out the current measurement at the mooring buoy near Oshima Island in the same channel and found the strong tidal currents and the predominance of the semidiurnal period, especially M2 constituent. However, the characteristics of these tidal currents were not sufficiently explained by them. MATSUYAMA and IWATA (1985) made the current and temperature measurements at 30m and 60m depths on the shelf off Jogashima (JO shown in Fig.1) and reported the existence of internal tides on the shelf. MATSUYAMA *et al.* (1988) also showed the existence of the internal tides from the temperature measurements by the thermistor chain near the northwest coast of the bay (near EN in Fig.1). OHWAKI *et al.* (1991) showed that the tidal current due to the surface tides is smaller than 1 cm s^{-1} in the main part of the bay, estimated by the numerical experiments. They also indicated the observed tidal current in the upper layer at the five mooring stations of the inner region of the bay to be much larger than the tidal current due to the surface mode. Then they documented the observed tidal current to be mainly due to the internal tides in the upper layer of the inner region of the bay. KITADE *et al.* (1993) and KITADE and MATSUYAMA (1997) clarified the predominance and behavior of the semidiurnal internal tide by the temperature measurements at seven mooring stations in the upper layer of the inner region of the bay and the numerical experiments by using a two-layer model.

The current measurements of the previous studies were made only in the upper layer of the bay, and the temperature measurements were made at some depths, which were shallower than 60m depth (MATSUYAMA *et al.* 1988, KITADE and MATSUYAMA 1997). Therefore the existence and characteristics of the internal tides were clarified to be mostly due to analysis of the temperature measurements in the upper layer near the coast. The current measurements in multiple layers are required to investigate vertical structure with the tidal current at the mooring stations in not only the

shallow shelf region but also deep waters to confirm the previous studies and to verify the predominance of semidiurnal internal tide in the deep water.

The data set obtained from the mooring stations during the period from the summer to fall of 1996, was used to study the tidal current variations and verify the predominance of the semidiurnal internal tide.

2. Tidal current due to the surface tides in Sagami Bay

It is very difficult to extract the tidal current due to the surface tides from the observational current data for including the internal tidal current in the records and for the large water depth in Sagami Bay. Then OHWAKI *et al.* (1991) estimated the tidal current amplitudes and phase for M2 and K1 constituents of the surface tides in Sagami Bay and Suruga Bay by the numerical modeling. Figure 2 shows the horizontal distributions of the length of major axis of the tidal ellipses for both constituents. In Sagami Bay, the length of the major axis of both constituents is very small in the inner region of the bay. The numerical calculation was carried out with square grids of 2km spacing, so it is not so sufficient to clarify the local phenomena of the surface tides in the complicated bottom topography region, *i.e.*, the small bank such as Okinoyama Bank nearby the mouth of Tokyo Bay. But, the characteristics of the surface tides in the most part of the bay are considered to be grasped from the numerical experiments. In this numerical model results, the current amplitude due to the surface tides in Sagami Bay is very weak, below 1 cm s^{-1} , except around Oshima Island for both M2 and K1 constituents, while the current amplitude for M2 constituent is over 10 cm s^{-1} in the Oshima West Channel. These results will be useful for the analysis of the current data in this study.

3. Current Measurements and Data

The observations were made during the period from July to November, 1996. The mooring stations, as shown in Fig.1, were located in the Oshima West-Channel (ON), on small bank of Okinoyama facing to the mouth of Tokyo Bay (OK), at the eastern coast off

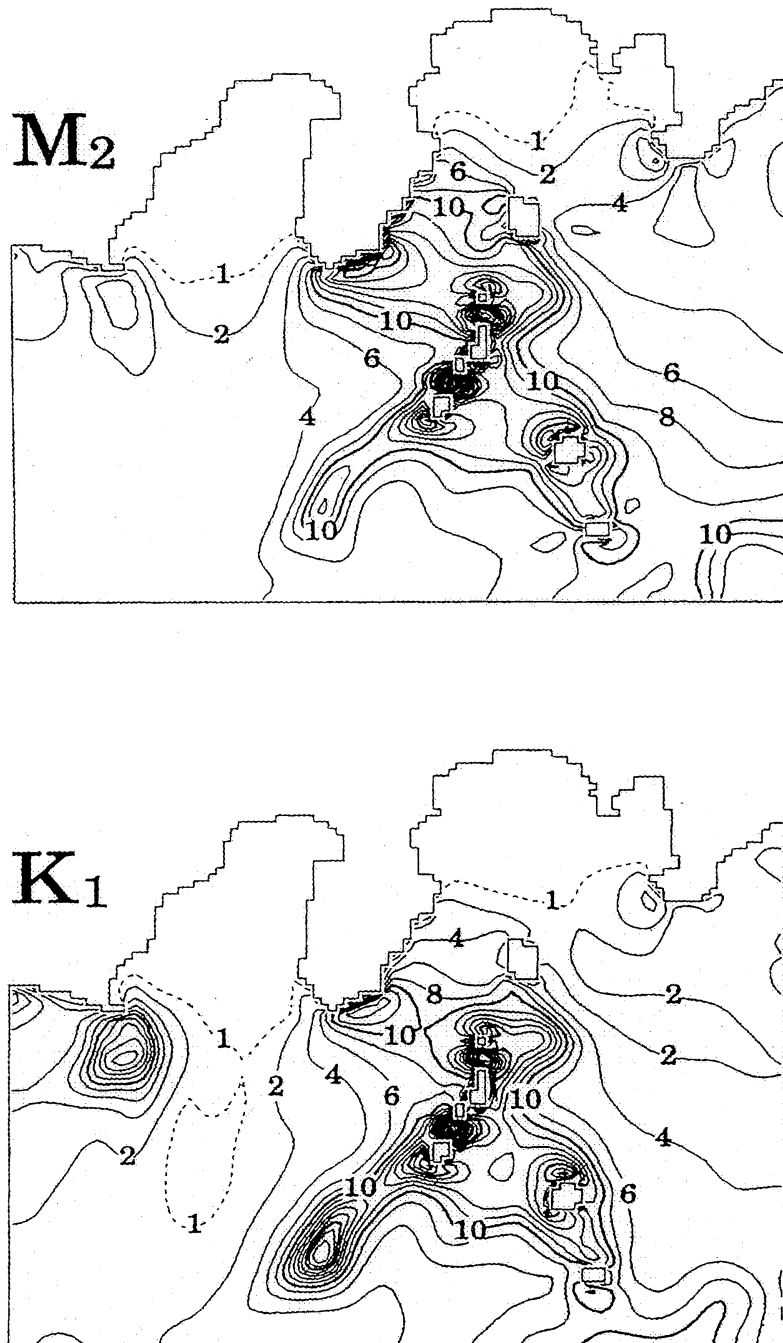


Fig. 2. Tidal current amplitudes for the M_2 and K_1 constituents for the surface tides estimated by numerical experiments. Contour numerals are in cm s^{-1} (after OHWAKI, *et al.*, 1991)

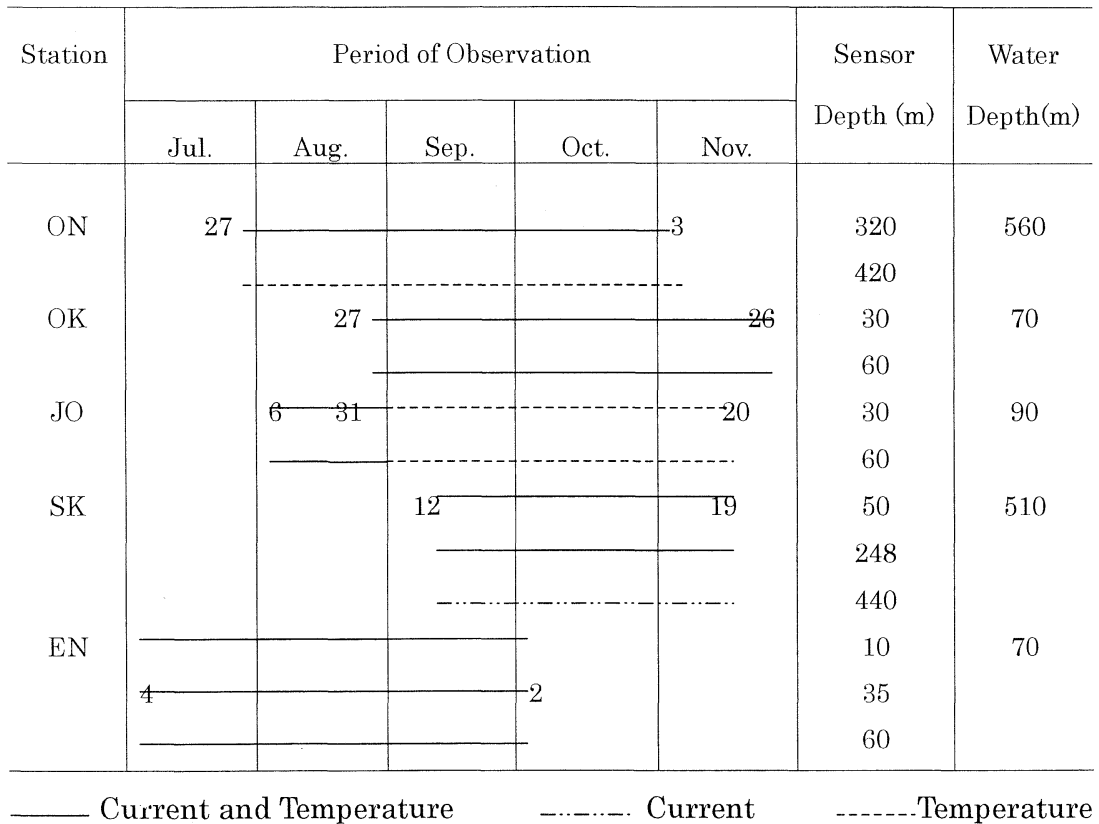


Fig. 3. Mooring information at five stations in Sagami Bay.

Jyogashima (JO), near the center of the bay (SK) and at the northwestern coast off Odawara (EN). The mooring periods and sensor depths for each station are represented in Fig.3. Both ON and SK were located in the deep water, while other three stations were located in the shallow and/or coastal regions. The sampling interval was 30 minutes for ON, OK, JO, SK and 10 minutes for EN. Each record length was different among the stations, so that the starting time and the record length at each station are shown in Fig. 3.

4. Results

Current and Temperature Fluctuations

Although the observation periods were different among the stations, as shown in Fig.3, the observations at the same time were made in the latter half of September, 1996 except JO. Then, for comparison to each other, we show

the time series of the current and temperature fluctuations at four mooring stations at the same time during the period from September 15 to September 29 (Figs. 4 and 5). We can find the predominance of tidal period fluctuations in both the current and temperature records at all stations. The temperature at 60m depth abruptly increased at OK, JO and EN on September 22, so that the temperature difference between 30m and 60m depths at these stations became small, *i.e.*, the change of the vertical stratification in the upper layer (Fig.5). As the results, the temperature variations with the tidal periods at 60m depth at OK and JO were slightly small in the latter half of the record in comparison with those in the former half.

The tidal currents in the deep layer of Sagami Bay have rarely reported from the long-term measurements except the study of TAIRA and TERAMOTO (1985). Even they focused on

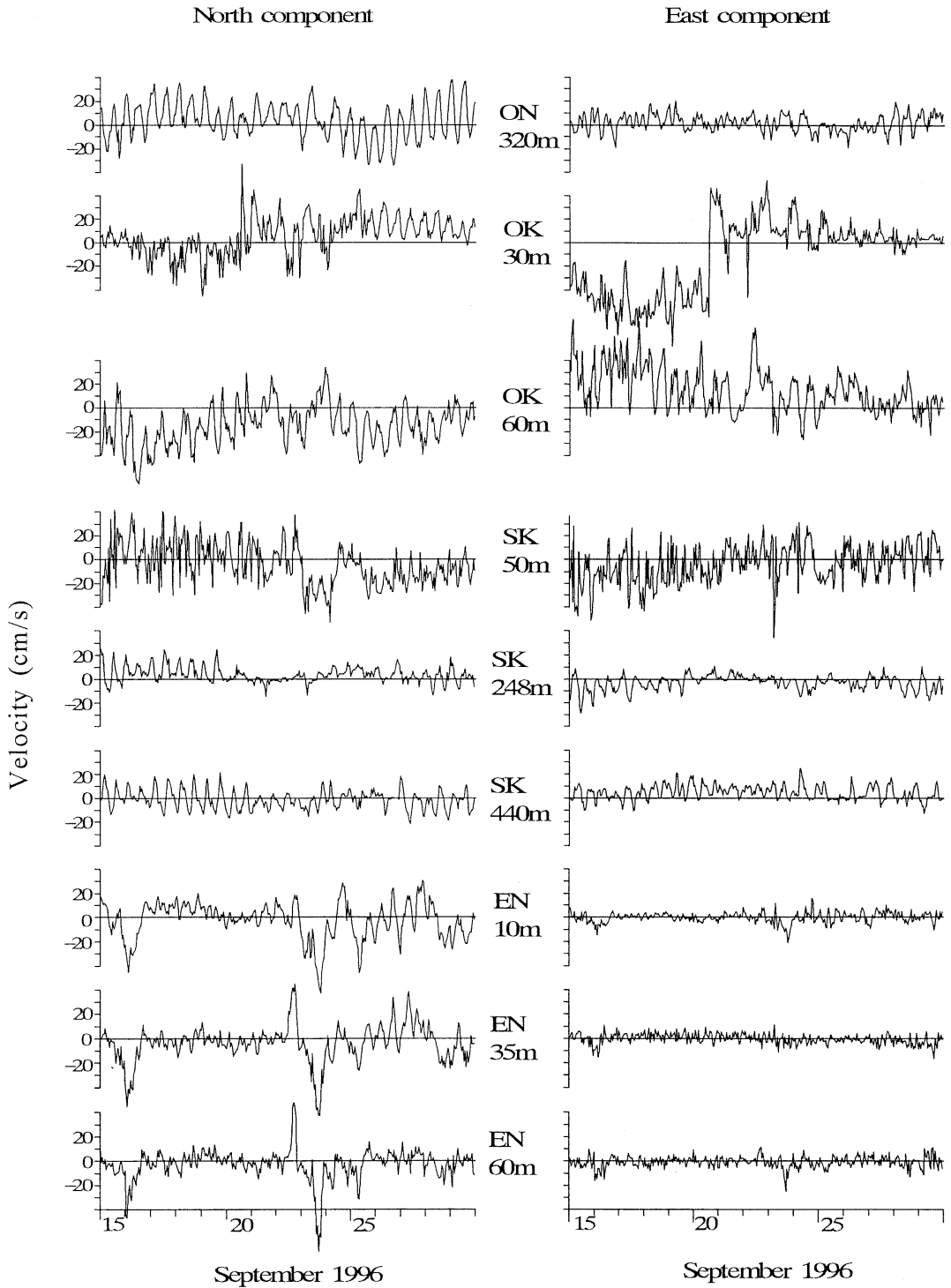


Fig. 4. Time variations of the east and north components of current at four stations during the period from September 5 to September 29, 1996.

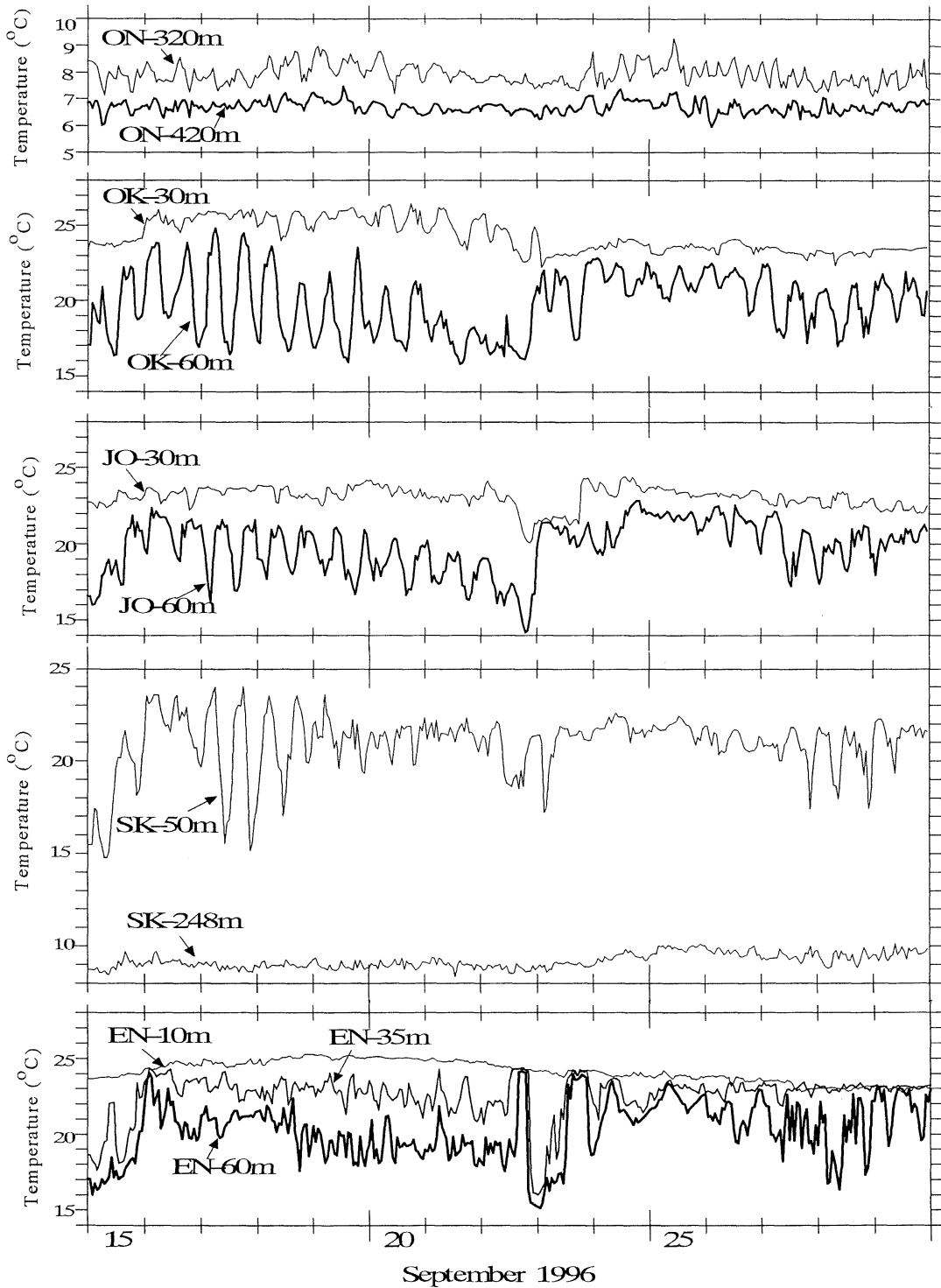


Fig. 5. Time variations of temperature at five stations during the period from September 15 to September 29, 1996.

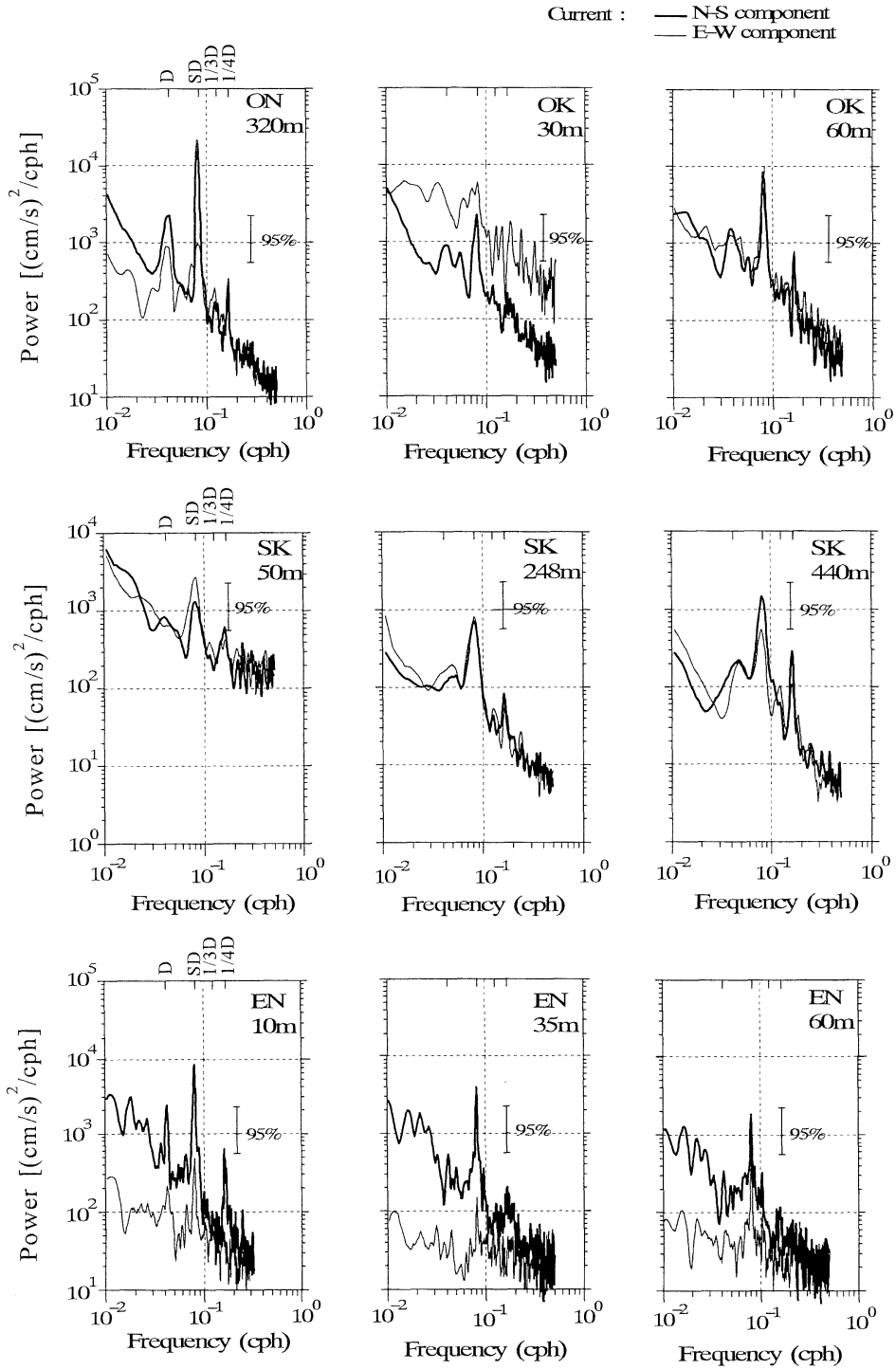


Fig. 6. Power spectra of the current at ON, OK, SK and EN for each period of observation.

the bottom current in the Sagami Trough. Therefore, the current data observed in the deep layer at ON and SK are expected to supply the information of tidal current characteristics. The current data at ON were obtained at 320m depth, but were not obtained for the meter trouble at 440m depth unfortunately. The tidal current at 320m depth at ON was usually over 10 cm s^{-1} and the north component of the current dominated over the east component, so the orientation of the tidal current ellipse was almost north-south direction, *i.e.*, the along-channel direction. The temperature records were obtained both at 320m and 420m depths in the same period as the current records. The temperature in the deep layer at ON also shows the remarkable tidal fluctuations, and the high frequency fluctuations also existed at 320m depth during the period from September 24 to 30, 1996.

At SK, located near the center of the bay, the current data were obtained at three depths, *i.e.*, at 50m, 248m and 440m depths and the tidal current amplitude at 50m depth was larger than those at 248m and 440m depths. The current amplitudes were not so different between 248m and 440m depths, and their variations closely resemble each other in this observational period. The temperature fluctuations with the tidal periods at 50 m depth were found through the record, and at times had very large amplitude, while they were very smaller at 248m depth than at 50m depth. The depth dependence of the current amplitude and partial intensification of the temperature fluctuations with the tidal periods suggest the predominance of the internal tides at SK.

The tidal current fluctuations at the station on the bank (Okinoyama Bank) near the bay mouth, OK, had the large amplitude. The current fluctuations included both the several-day period and high frequency period fluctuations together with tidal periods, so the more detailed analysis is required to grasp the characteristics of the tidal current fluctuations. The temperature fluctuations with tidal periods had the large amplitude at 60m depth, while they were not so clear at 30m depth. At JO (Fig.1), as shown in Fig. 3, the current data were not useful for the current meter trouble

except those in August, but the temperature data were obtained at both 30m and 60m depths. The temperature records with the tidal periods at 60m depth resemble those at the same depth at OK, but the amplitude at JO was clearly smaller than OK. The amplitude difference of the temperature at the same depth between both stations was expected to be difference of the internal tidal amplitude because the basic stratification was little difference between both stations.

The current and temperature fluctuations at the northwestern part of the bay head, EN, were also shown in Figs. 4 and 5, respectively. The tidal current fluctuations were the significant phenomena at the three depths, and the north component of the current was dominant over the east one. The north component of the current almost agrees with the alongshore direction at EN, so the results indicate the predominance of the alongshore component. The tidal amplitude of the north component of the current gradually decreased from the upper layer (10m depth) to lower layer (60m), that is, the current included the characteristic of baroclinic mode. In addition, the temperatures at both 35m and 60m depths also fluctuated with the tidal periods, so that both temperature records also suggested the existence of the internal tides. The tidal period fluctuations were rarely found in the temperature record at 10m depth at a glance, because the temperature sensor at 10m depth was already set in the surface mixed layer with a weak stratification in the latter half in September.

Statistical Properties

Power spectra were calculated for the current and temperature records at four stations, ON, OK, EN and SK to detect the predominant periods in more detail. Figure 6 shows the power spectra for both east and north components of current at four stations. The current data were obtained at the two or three depths at these stations except at ON. The significant peaks are found at the semidiurnal period in all records, while the peaks of the diurnal period existed at 320m depth at ON, and at 10m depth at EN. The weak peaks at the inertial period (about 20.9 hour) are found at 248m and 440m

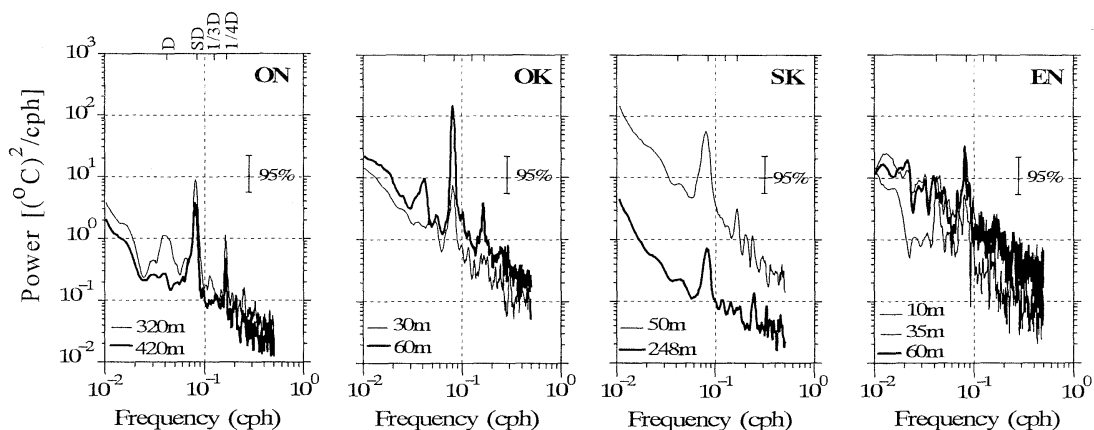


Fig. 7. Power spectra of the temperature at ON, OK, SK and EN for each period of observation.

depths at SK. The phenomenon is expected to be relation to the near-inertial motion. The peak at 1/4 day period existed in all spectra, and was especially remarkable at 320m depth at ON, at 60m depth at OK, at 440m depth at SK and 10m depth at EN.

The current records were obtained at each station in the different observation period, but the records at the different depth at the same station were obtained for the same time. Therefore, it is useful for the comparison of the kinetic energy among the records at the same station. At EN, the energy level of the north component of current with the semidiurnal period gradually decreased with the depth increase from 10m to 60m depths. The peaks at diurnal period and 1/4 day period existed at 10m depth, while it is not found at the two deeper depths at EN. For the semidiurnal period at SK, the east component of current dominated over the north component at 50m depth, but vice versa at 440m depth, and both components were almost equal at 248m depth. The energy level of the semidiurnal current was a minimum at 248m depth among the three observation depths. The results suggest the complexity of the vertical distribution of the semidiurnal current at SK.

Figure 7 shows the temperature spectra at the four stations. The semidiurnal period peak is found in all records, but the energy levels are different from each other at this period. The diurnal period peak is found only at 60m depth at

OK, at 10m depth at EN and at 320m depth ON, but is not found at all three depths at SK, located near the center of the bay. The reason of no peaks at diurnal period at the station near the center of the bay is explained as follows; the diurnal internal wave have the characteristic of the internal Kelvin wave, and distance between the station and coast is longer than the internal radius of Rossby deformation (5 to 7km). In addition to it, the diurnal internal wave does not have the large amplitude in Sagami Bay (OHWAKI *et al.* 1994, and KITADE and MATSUYAMA 1997).

The power spectra of both the current and temperature fluctuations indicate the predominance of the semidiurnal period in all records. Then it implies the semidiurnal period fluctuations to be deep relation to the internal tides. The spectra in some records have the peaks at the diurnal period as well. So, it is expected to be a closely relation between the current and temperature fluctuations with not only semidiurnal but diurnal periods.

Table 1 shows the coherence between north and east components of the current and temperature for the diurnal and semidiurnal periods at the same depth at all stations. The coherence at the semidiurnal period is higher than the value of the 95 % confident limit in all case. This result indicates that the current with the semidiurnal period is a closely relation to the temperature, as expected. So the predominance of the semidiurnal internal tide is

Table 1. Coherence between current and temperature.

Station	Observation Period	Depth	Semidiurnal Constituent		Diurnal Constituent	
			east comp.	north comp.	east comp.	north comp.
ON	Jul.27 to Oct.21	320m	0.64	0.82	0.37	0.38
OK	Aug.27 to Nov.26	30m	0.21	0.66	0.43	0.43
		60m	0.79	0.84	0.35	0.35
SK	Sep.12 to Nov.13	50m	0.66	0.19	0.52	0.52
		248m	0.42	0.35	0.29	0.29
EN	July 4 to Oct.2	10m	0.43	0.35	0.52	0.52
		35m	0.62	0.61	0.37	0.37
		60m	0.80	0.78	0.38	0.38

Confidence limit of 95% is 0.39

clarified in the inner region of the bay.

The other hands, the diurnal period is high coherence at 30m depth at OK, 50m depth at SK and 10m depth at EN, so that the diurnal period shows the high coherence in the record of the upper layer only. We do not have the more data to explain the reason of the phenomenon in this paper.

5. Discussion

Predominance of the semidiurnal internal tide

OHWAKI *et al.* (1994) and KITADE and MATSUYAMA (1997) indicated most of the internal wave observed in Sagami Bay are generated at the northern part of the Izu Ridge and propagated into the bay by the numerical experiments as follows. The semidiurnal internal wave are propagated into the bay head, having the characteristic of the internal inertial gravity wave, while the diurnal one can not be propagated into the bay head for having the characteristic of the internal Kelvin wave. As a result, the semidiurnal internal tide dominates over the diurnal period. Both studies also demonstrated the predominance of the semidiurnal internal tide by the observational results in the upper layer in the inner region of the bay.

In this study, the power spectra of the current and temperature data illustrated the predominance of the semidiurnal period in not only the surface layer but also deep layer at all stations as shown in Figures 6 and 7. The predominance of the semidiurnal internal tide implies that the internal tides observed in the inner region of the bay are generated on the Izu Ridge and propagated into Sagami Bay. Our

results support the key idea of the internal tides generation on Izu Ridge and propagation into Sagami and Suruga Bays indicated by OHWAKI *et al.* (1994) and KITADE and MATSUYAMA (1997).

Characteristics of tidal current in Oshima West Channel

In order to comprehend the characteristic of the tidal current ellipse, we calculated the major and minor axis, and orientation of the current ellipse of four major constituents, *i.e.*, M2, S2, K1 and O1 for 29.5 days record at 320m depth at ON. The harmonic constants of two segments, *i.e.*, from August 27 to September 25 and from September 22 to October 21, are shown in Table 2. The M2 constituent is predominant over the other constituents, and the length of the major axis and orientation for M2 constituent were less variable between the two segments. In addition, the other three constituents are also not large different between the two segments. The tidal currents due to the surface tides (Fig. 2) are slightly smaller than the observed current amplitude. These results suggest that the tidal currents observed at 320m depth in the Oshima West Channel are not so strongly affected by the internal tides. The internal tidal currents are expected to have a large amplitude in the Oshima West Channel as indicated by TERAMOTO (1971). Unfortunately, we have no information of the detailed vertical structure of the tidal current in the bay. We have measured the vertical structure of the tidal current near the center of the bay by the mooring ADCP to investigate the structure of the internal tides.

Table 2. Harmonic constants of tidal current ellipses at ON for 29.5 days period

Period	Depth (m)	Const.	Maj. Axis (cms ⁻¹)	Min. Axis (cms ⁻¹)	Orientation (deg.)
Aug.27 to Sep.25	320m	M2	13.2	1.1	7
		S2	5.8	0.1	13
		K1	2.7	0.1	51
Sep.22 to Oct. 21	320m	O1	2.9	0.7	31
		M2	13.1	0.9	5
		S2	7.7	0.6	24
		K1	4.4	0.6	27
		O1	3.2	0.2	34

Tidal currents near the northwest coast of the bay

We obtained the current and temperature records at three depths, *i.e.*, 10m, 35m and 60m depths at EN at the same time during about three month from early July to early October 1996. We do not yet have the data in the three layers except the above ones in this bay, so it is important to investigate the vertical structure of tidal currents. The observations were carried out during the existence of the seasonal thermocline, but the thermocline depth varied for this three months. The observation was made at the steep slope sits near the northwest coast of the bay (Fig. 1). The power spectra of the current and temperature at EN with the semidiurnal period had the significant peaks in all records (Figs.6 and 7). However, the energy level of both current and temperature was different among depths, that is, the current and temperature were the highest energy at 10m depth and 60m depth, respectively. Then we investigate the vertical structure and behavior of the semidiurnal internal wave near the coast, using the data at the three depths at EN.

Figure 8 shows the 25-hours running mean records of temperature at each depth at EN. The temperature records show the abrupt change of the vertical distribution, so that we firstly examine how the difference of the vertical density distribution affects on the characteristics of the semidiurnal internal waves. At second, we investigate the behavior of the semidiurnal internal wave near the coast. Then, we separate the records into three segments as shown in Fig.8, and calculate the harmonic constants of the tidal currents for each segment, which is the record length of 29.5 days.

Figure 9 shows the amplitude of the north and east components of current for the four major constituents, *i.e.*, M2, S2, K1, and O1 constituents. As expected, the M2 constituents are dominant over the other three constituents. We will focus on the M2 constituent. The north component of current was predominant over the east component at each depth. The current amplitude was a maximum at 10m depth in all segments as indicated in the power spectra. The north component of current was the maximum for Period II from early August to early September. Period II agrees with that of maximum value of the mean temperature difference between 10m and 60m depths. The results suggest the magnitude of the current amplitude in the upper layer to be related to the strength of the stratification.

Figure 10 shows the coherence and phase difference between the temperature at 60m depth and the north component of current at each depth. The temperature is useful as an index for expressing the vertical displacement of seasonal thermocline. The temperature at 60m depth was high coherent with the alongshore current at 10m, 35m and 60m depths, and the phase difference was about 180 degrees, that is, the northward current in the upper layer was out of phase to the subsurface temperature. This relation implies the southward current with the downward displacement of the seasonal thermocline, that is, the phase relation implies the southward propagation of the internal waves. Therefore the semidiurnal internal waves propagated to be right the land, near the northwest coast of the bay head. KITADE and MATSUYAMA (1997) indicated the behavior of the semidiurnal internal tide in Sagami Bay by the numerical experiments using a two-

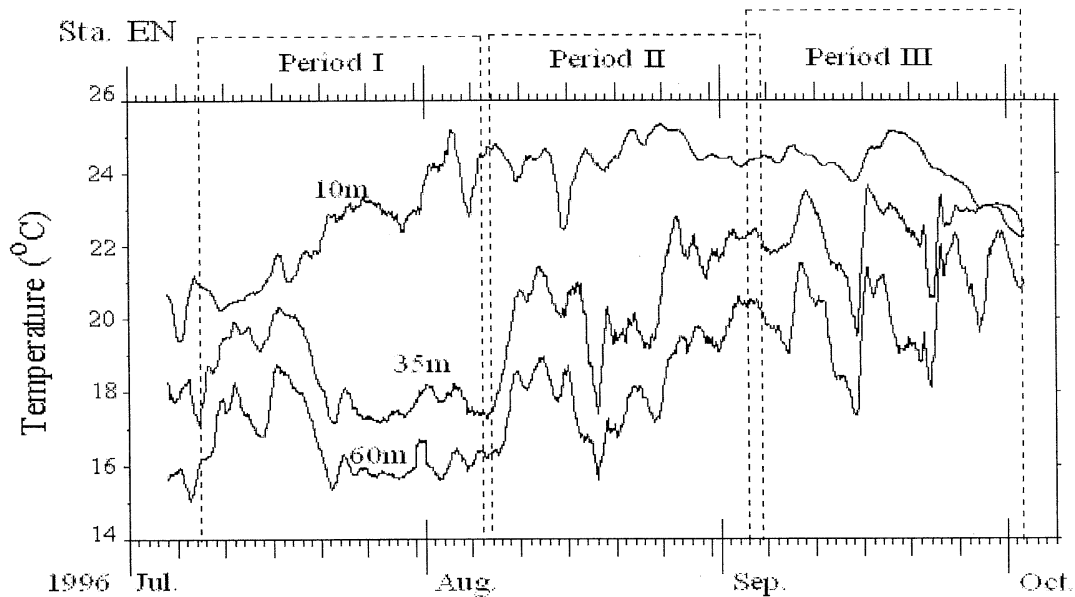


Fig. 8. Time series of 25-hour running averaged of temperature fluctuation at 10m, 35m and 60m depths at EN, during the period from July 4 to October 2, 1996. Divided in three segments : Period I : July 8 to August 6, Period II : August 7 to September 5, Period III : September 3 to October 2, 1996.

layer model. They showed the internal wave to behave an internal Poincare wave near the coast for the reflection at the coast, and for the phase of the internal wave to propagate along the coast as the above results. We can summarize that the behavior of the semidiurnal internal wave observed at EN agree with those of the numerical experiments using the layer model.

The coherence for the diurnal period was high between the north component of current at 10m depth and temperature at 60m depth, and the phase difference was about 180 degrees. As the same as the semidiurnal period, the diurnal period fluctuation also propagated the southward along the coast to be right the land in the upper layer. The diurnal period is over inertial period at this latitude, so the phenomenon behaved as the internal Kelvin wave at EN.

6. Summary

Tidal currents in Sagami Bay were investigated by the long term records of current and temperature obtained at five mooring stations during the period from July to November, 1996. The current data were obtained both in

the surface and deep layers at the same time. The time series of the temperature and current records at all stations showed the remarkable fluctuations with tidal periods and power spectra of these records clearly expressed the peaks at tidal periods, especially semidiurnal period. The semidiurnal period fluctuation was predominant over the diurnal one in the current records at all records. The semidiurnal current was high correlation to the current in the all observation depths, while the high correlation between the current and temperature for the diurnal period was limited in the upper layer. The semidiurnal current amplitude depended on the depth. The tidal current amplitudes for the M2 constituent are very larger than ones of the tidal current due to the surface tides (less than 1cm s^{-1}) estimated by the numerical experiments in the inner region of the bay (OHWAKI *et al.*, 1991). Therefore the observed tidal current is considered to be mainly due to the internal tides.

The current and temperature data obtained at three depths (10m, 35m and 60m depths) at EN shows that the tidal current amplitude with semidiurnal and diurnal periods had a

Sta. EN



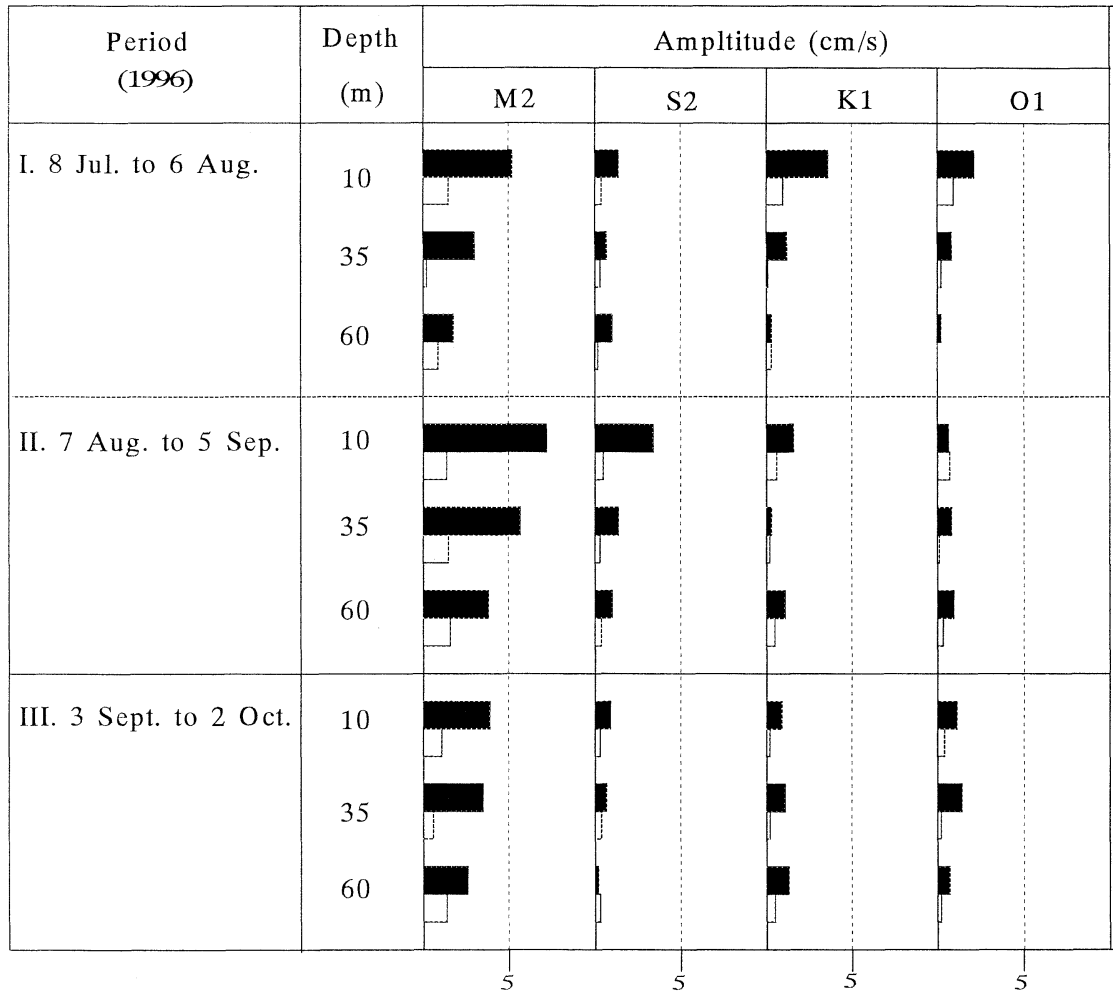
 North component 
 East component 


Fig. 9. Amplitude of the north and east components of current for four major constituents at EN for three segments.

maximum at 10m depth and gradually decreased with depth, while the maximum amplitudes for temperature fluctuations appeared at 60m depth. The seasonal thermocline existed slightly under at 60m depth, so that the current fluctuations show high correlation with the temperature fluctuations at 60m depth. The phase relation between the current in the upper layer and temperature in the subsurface layer indicates the internal wave propagation in the upper layer along the coast to be right a hand.

These results are consistent with the results obtained by the temperature measurements along the coast and the numerical experiment with a two-layer model (KITADE and MATSUYAMA, 1997). At EN, the diurnal period fluctuations also indicated to be high coherence and phase lag of about 180 degrees between alongshore current at 10m depth and temperature at 60m depth. The diurnal internal wave also propagated the southward along the coast to be right the land in the upper layer, as

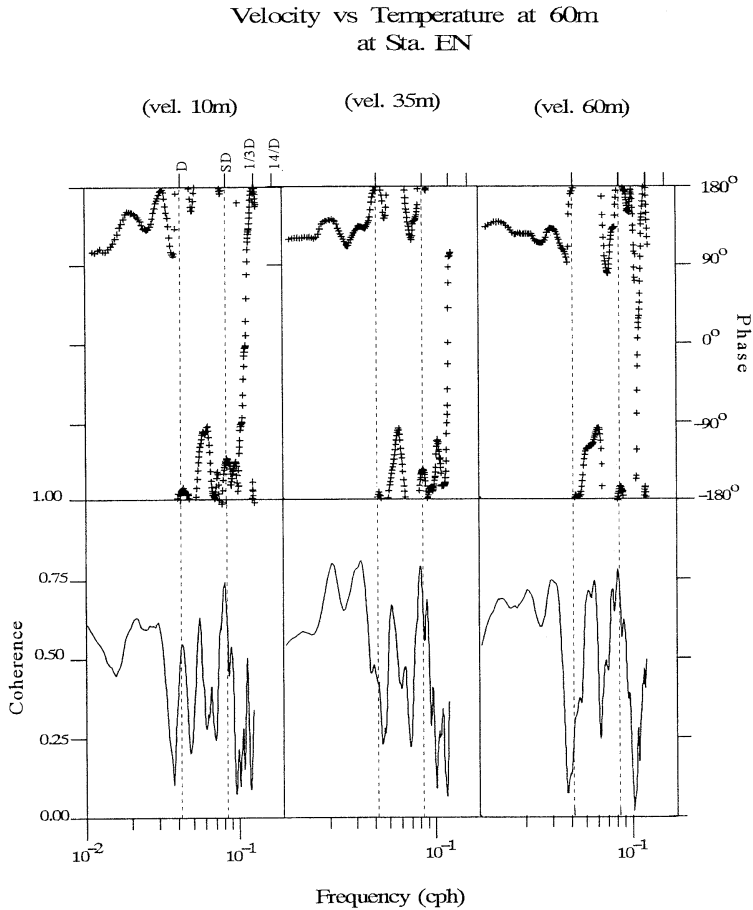


Fig. 10. Coherence and phase difference between the north component of current at 10m, 35m and 60m depths and temperature at 60m depth at EN, during the period from July 5 to October 2, 1996.

having a characteristic of the internal Kelvin wave.

TAIRA and TERAMOTO (1985) observed the currents at the depth of 7m above the sea bottom in the Sagami Trough (water depth of more than 1500m) and showed the tidal current of about 5 cms^{-1} and the energy concentration around the semidiurnal period. The strong deep current due to the internal tides were observed in the bottom layer in the Suruga Trough (MATSUYAMA *et al.* 1993). We are also interested in the bottom tidal current in relation to the internal tides such as Suruga Trough. In addition, we have examined the observations to take the vertical structure of the tidal currents at mooring stations by the ADCP

measurements to investigate the detailed characteristics of the internal tides. In near future, the observation results will be offered. We will also examine the numerical experiment with the continuously stratified model both to explain the observational results and to clarify the characteristics of the internal tides in Sagami Bay.

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資 料

第 42 卷第 1 号掲載欧文論文要旨

日仏海洋学会賞記念論文

前田昌調：増養殖環境における微生物の相互作用と利用

水産増養殖水に生息する微生物の組成および機能は、魚介類生産環境の適性をはかる重要な指標になるとともに、これら微生物群集中の魚介類成長促進細菌種の存在は、養殖生産の可否にかかわる重要な要素となる。筆者らは、これら有用微生物の中で微生物間拮抗作用をあらわす細菌株を使用して、病原菌の増殖を抑制するバイオコントロール（生物防除）方法を開発し、増養殖環境水中の病原細菌およびウイルスの防除を行った。さらに本論文では、水産増養殖における微生物利用研究報文をレビューした。

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関根義彦^{*1}・増澤敏行^{*2}：四国海盆での直接測流観測

東経135度15分、北緯29度30分の四国海盆において、1999年2月20日より4月16日まで水深470m、4月17日より12月9日まで水深730mで直接測流を行った。測定された流速の変動過程についての解析結果をまとめた。流速の変動はほぼ一日の慣性周期で振動する慣性波が基本的に卓越している。しかし、中規模渦が観測点を通る際には中規模渦の流れが卓越し、それに乗った形で慣性振動が存在する。中規模渦は15cm/秒程度の流れを持ち、観測水深まで流れの構造が存在することが示された。現在 TOPEX/POSEIDON などの衛星による海面高度計から推定される中規模渦の海面での流れと、この直接流速測定から得られた顕著な大きい流れの期間のそれが一致し、人工衛星から観測される中規模渦が500m-700m程度のかかなり深い構造を持ち、そのために黒潮流路に接近した場合にはその影響が無視できないことが示唆された。

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Ping Astony Angmalisang^{*1}, 松山優治^{*1}, 石戸谷博範^{*2}, 北出裕二郎^{*1}, 岩田静夫^{*3}：相模湾内の5係留点で観測された潮流の特性

1996年7月から11月にかけて相模湾内の5測点に係留系を設置し、上層および下層の流速と水温を測定した結果を用いて潮流の特性を調べた。流速記録と水温記録および両記録のパワースペクトルは全測点で半日周期成分の卓越を示した。湾内の4測点の半日周期潮流は同一深度の水温と高い相関を示し、その振幅は深さに依存しており、いずれも外部潮汐の潮流振幅よりはるかに大きい事が分かった。この結果は湾内では上層、下層共に内部潮汐が卓越し、且つ観測される半日周期内部潮汐は伊豆海嶺北部で生成されたものであるとの研究 (OHWAKI *et al* 1994, KITADE and MATSUYAMA 1997) を支持するものである。湾奥西部の測点では上層で潮流振幅が最大となり深さと共に減少した。また流速振幅は成層の変化とも連動しており、流速と水温との位相関係から、内部波は岸を右手に見ながら伝播する様子が伺えた。この結果は筆者らが数値実験と水温観測から得られた結果と一致する (KITADE and MATSUYAMA 1997)。

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学 会 記 事

1. 2003年11月20日～2004年1月30日 Eメールを媒介として、評議員から推薦のあった学会賞及び論文賞受賞候補者について審議の結果、学会賞受賞候補者 磯田豊会員（北海道大学）、論文賞受賞者 藤村弘行会員（琉球大学）と決定し、会長に報告することとした。

2. 2004年4月26日（月）東京海洋大学9号館（海洋環境棟）会議室において幹事会が開かれた。

主な審議事項は下記通りである

- ・水研連・海研連 審査委員候補者 選出
- ・日仏理科教育シンポ
- ・東京湾 委員会 アンケート

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(1) 論文の場合

有賀祐勝, 前川行幸, 横濱康継 (1996): 下田湾におけるアラメ群落構造の経年変化. うみ, **34**, 45-52.

YANAGI, T. T. TAKAO and A. MORIMOTO (1997): Co-tidal and co-range charts in the South China Sea derived from satellite altimetry data. *La mer*, **35**, 85-93.

(2) 単行本分載論文（単行本の一部引用の場合）

村野正昭 (1974): あみ類と近底層プランクトン. 海洋学講座10 海洋プランクトン (丸茂隆三編), 東京大学出版会, 東京, p.111-128.

WYNNE, M. J. (1981): Pheophyta: Morphology and classification. *In* the Biology of Seaweeds. LOBBAN, C. S. and M. J. WYNNE (eds.), Blackwell Science, Oxford, p.52-85.

(3) 単行本の場合

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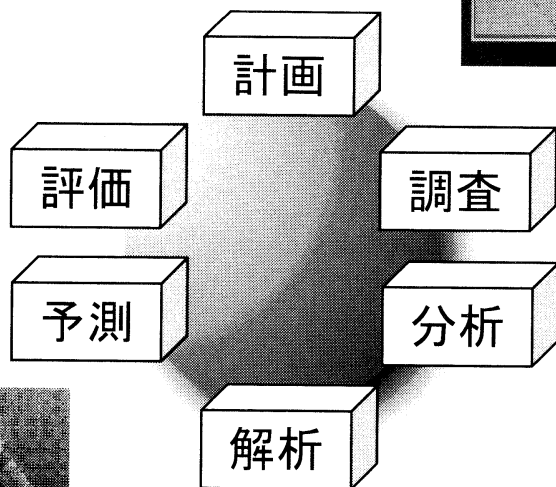
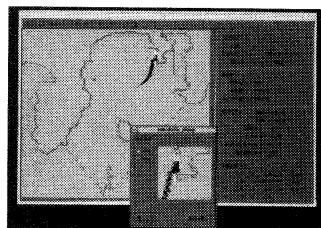
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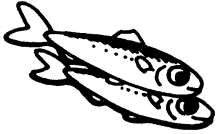
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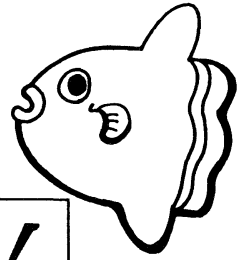
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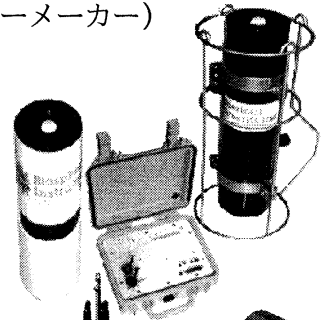
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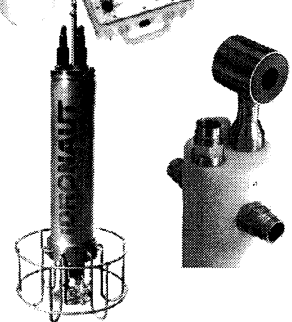
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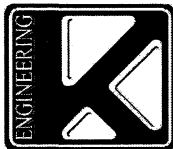
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