Feeding Ecology of Settled Japanese Flounder in Nursery Grounds in Eastern Hiuchi-Nada, the Seto Inland Sea, Japan*

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瀬戸内海燧灘東部海域の砂浜域におけるヒラメ着底仔稚魚の摂餌生態

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Japanese flounder *Paralichthys olivaceus* is widely distributed around coastal areas of Japan, Korea and China. Food organisms and feeding habits of juvenile Japanese flounder are different by area, and juveniles probably adopt feeding tactics to accommodate to abiotic and biotic factors at various sandy beaches. In the Seto Inland Sea, the annual catch of this species comprised approximately 15% of the total catch in Japan (6,500 t) in recent years, and more than 4 million hatchery-reared juveniles have been released annually. In order to determine release timing and size of hatchery-reared flounder, it is essential to elucidate mechanism of forming nursery grounds. For the purpose of clarifying the mechanism in the Seto Inland Sea, I examined density of larval and juvenile Japanese flounder, catch efficiency of a sampling gear, feeding ecology of dominant fish species, their food organisms and growth rate of juvenile flounder at the sandy beaches in eastern Hiuchi-Nada, the Seto Inland Sea.

Density and catch efficiency of a 2-m beam trawl net of juvenile Japanese flounder

To estimate density and catch efficiency of a two-meter beam trawl net (Seikai National Fisheries Research Institute [SNFRI] type net) for juvenile Japanese flounder, I carried out diving and fishing surveys at Ohama Beach. Direct counting was conducted by divers and then a density of flounder was estimated. A fishing survey in which SNFRI net was towed along 200 m at a speed of 0.5-1.0 knot was conducted. Catch efficiency was calculated by number of fish collected by the net (individuals [inds]/100 m^2)/density of fish (inds/100 m^2) × 100. The density ranged from 0.5 to 42 inds/100 m^2 and then the mean density in June was 12.6 inds/100 m^2. The mean catch efficiency (95% confidence interval) was 16.1% (11.0-21.2 %).

Occurrence and feeding ecology of Japanese flounder and food organisms

Distribution of Japanese flounder and demersal fish species were examined at the seven sandy beaches. A total of 6,412 individuals of 17 species representing 12 families were collected with 242 hauls of the 2-m beam trawl net. Larval and juvenile flounder occurred from late May to August, and were mainly collected at a depth of 2-5 m. The dominant fish species were *Favonigobius gymnauchen* (56.0% in number), *Tarphops oligolepis* (8.1%), *Rudarius ercodes* (7.8%), Japanese flounder *P. olivaceus* (7.3%), *Repomucenus* spp. (7.0%) and *Heteromycteris japonica* (3.3%). The number of flounder per haul differed among the beaches and months, but the trend of variations was similar among the beaches.

Abundance of food organisms and feeding habits of flounder were examined from May to August at Ohama Beach. The food organisms collected by a sledge net consisted of 40 families from 18 orders, dominated by mysids, crangonids and gammarids. The mean density of mysids, crangonids, gammarids and fishes was 2.74 inds/m^2, 6.74 inds/m^2, 2.91 inds/m^2 and 0.15 inds/m^2, respectively. Most of crangonids were small (< 14 mm in body length), and so large crangonids, which could prey on newly settled flounder, were not abundant. Larval metamorphosing flounder settled at Ohama Beach from late May to late June when mysids were abundant. Juveniles mainly consumed mysids and small crangonids. Crangonids were not important as a predator for

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18

flounder but as prey. Fishes were observed in the stomach of flounder larger than 50 mm in total length, but cannibalism was not found. Flounder preferred epifaunal mysids, *Nipponomysis ornata* and *Anisomysis ijimai*, to sand-burrowing mysids, *Iiella ohshimai*, and avoided crangonids.

Feeding ecology of dominant fish species

Feeding habits of the dominant fish species *F. gymnauchen*, *Repomucenus* spp. and *T. oligolepis* were examined at Ohama Beach. All the fish species consumed mainly small crustaceans, and not fishes and polychaetes. *F. gymnauchen* and *Repomucenus* spp. consumed mainly mysids and small crangonids. In contrast, *T. oligolepis* actively selected only epifaunal mysids *N. ornata* and avoided crangonids. Newly settled Japanese flounder were not observed in the guts of the three fish species. The diet of juvenile Japanese flounder was similar to those of the three species. From these results, it appears that these three fish species are not predator but competing species for juvenile flounder in this area.

To estimate the daily ration of Japanese flounder *P. olivaceus*, *T. oligolepis* and *F. gymnauchen* and to clarify diel variation in food availability, I conducted diel sampling over a 24-hour period. Demersal fishes and small crustaceans were collected with the 2-m beam trawl net and a two-layer sledge net, respectively. The exponential model established by Elliot and Persson was used to calculate daily ration. Gastric evacuation rates for the three fish species were estimated from the decrease in the stomach content index (SCI: wet stomach weight per wet body weight) from 21:00 to 3:00, assuming no feeding activity at night. The mean total length and mean wet body weight BW of *P. olivaceus*, *T. oligolepis*, and *F. gymnauchen* were 32.6 mm (0.35 g), 23.7 mm (0.13 g) and 50.2 mm (1.03 g), respectively. The mean SCI and percentage of stomachs with food were high during the day and low at night, indicating that the species are daytime-feeders. The mean daily ration estimate (95% confidence intervals) for *P. olivaceus*, *T. oligolepis* and *F. gymnauchen* made with the bootstrap method was 18.1 (9.3-31.3) %BW, 13.2 (7.9-24.3) %BW and 3.7 (1.7-7.4) % BW, respectively. The daily mysids consumption was greater in *P. olivaceus* (7.8 mg/m^2) than in *T. oligolepis* (3.1 mg/m^2) and *F. gymnauchen* (2.2 mg/m^2). The density of mysids in the near-bottom layer was higher during the day than at night, whereas in crangonids an opposite trend was observed. Gammarids and isopods were abundant at night.

Daily growth estimation of juvenile Japanese flounder

Daily growth rate of juvenile Japanese flounder was analyzed using an otolith microstructure. The absolute growth rate (AGR) and the relative growth rate (RGR) of the juveniles (18.3-56.0 mm in total length [TL]) range from 0.41 to 1.84 mm/day and from 0.86 to 5.12 %/day. With simple regression analysis, the relationship was found between AGR and TL, whereas there was not the significant relationship between RGR and TL. The mean RGR was greatest in early June, and gradually decreased after late June. It seems that large juveniles migrate form nursery grounds, when their RGR falls below a threshold.

Tactics of Juvenile Japanese flounder in the nursery grounds in eastern Hiuchi-Nada

The settling of Japanese flounder was synchronized with abundance of mysids. Juveniles selected mysids, but consumed also small crangonids because of low density of mysids. The daily ration for Japanese flounder was lower than that observed in rich food condition, indicating that the mysids availability might be not sufficient for juveniles in this area. In this area, the size of shift from mysids to fishes as their main food and emigrant to offshore area was small compared with Igarashihama Beach, where mysids were abundant. Judging from these results, juveniles might adopt a feeding tactics, in which they do not depend on mysids as much as possible in the nursery grounds in this area.

Contents

Chapter 1. General introduction

Japanese flounder *Paralichthys olivaceus*, one of the highest-priced commercial fish in Japan coastal fisheries, is mainly caught by trawl nets, gill nets and set nets, and has yield approximately 6,500 t in 2003[1]. The production of the hatchery-reared Japanese flounder increased with improvements of rearing technique, and more than 26 million juvenile flounder have been released annually into shallow waters[2,3]. For establishment of more effective stock enhancement, many researchers have studied the occurrence, density, feeding habits, food organisms, daily growth rate and recruitment mechanisms of larval and juvenile flounder in the nursery grounds (e. g. Minami, 1982[4]; Goto et al., 1989[5]; Tanaka et al., 1989[6]; Hirota et al., 1990[7]; Fujii and Noguchi, 1996[8]; Noichi, 1997[9]; Tanaka et al., 1997[10]; Tanaka et al., 1998[11]). Therefore, the outline of the early life history was clarified as follows. Spawning starts in early spring and continues until early summer. The duration of pelagic stage as eggs and larvae varies between one and two months depending on water temperatures[5,12], and metamorphosing larvae then settle at sandy beaches[4-6]. Main prey of juvenile flounder were only mysids and fishes in the nursery grounds[13-16]. The daily growth varied between approximately 0.5 and 2.0 mm/day[8,10,11,16], and fluctuated according to abundance and species composition of food organisms, especially density of mysids[8,17]. Large juveniles migrate to offshore area a few months later from settlement[18].

As stated before, Japanese flounder is widely distributing including in subtropical and subarctic zones[19,20], then the physical environmental conditions are greatly different between the northern and southern area. In addition, food organisms and feeding habits of juveniles were different by area[10,14]. For example, in the Sea of Japan, the abundance of mysids was different between northern and southern areas and the density of settled juveniles in the north was one-digit smaller than that in the south[10]. At Igarashihama Beach in the northern Sea of Japan, juveniles fed chiefly on mysids until 80-100 mm in total length (TL) and then shifted to fishes in their diet[8]. In contrast, the shift from mysids to fishes occurred at 30-50 mm in TL[15] and juveniles larger than 60 mm emigrated to offshore area[21] at Wada Beach in the central Sea of Japan. The size of flounder emigra-tion became smaller in the nursery grounds where abundance of mysids was lower than those in the northern Pacific Ocean coast[14]. Moreover, the daily ration fluctuated according to food availability especially mysids biomass[21]. The daily growth of wild juveniles was approximately 2.0 mm/day at Igarashi-hama Beach where mysids were abundant[8], in contrast, that was approximately 0.6 mm/day at Wada Beach[16]. From these observations, I expected settled flounder juveniles adopted feeding tactics to accommodate to food availability at various sandy beaches.

The cause of the depletion of juvenile Japanese flounder is partially depending on predation in shallow waters[22-24], while starvation is considered to have great influence on predation[25]. Starved fish would be preyed by predators easily and slow growth rate implies prolonged exposure to predation. Thus, feeding condition probably is a major factor, which determine the survival rates during juvenile period.

Many fish species including Japanese flounder and crustaceans utilize sandy beaches as their habitats (e. g. Senta and Kinoshita, 1985[26]; Senta et al., 1988[27]; Brown and McLachlan, 1990[28]; Gibson et al., 1993[29]; Takahashi and Kawaguchi, 1997[30]; Senta and Kinoshita, 1998[31]; Layman, 2000[32]; Hanamura and Matsuoka, 2003[33]). Previous studies have revealed that flatfishes and gobies predominated at the beaches in Japan[23,34-38]. Most of the dominant fish species consumed mainly small crustaceans such as mysids, copepods, gammarids and decapods[22,39-42], and also preyed on newly settled flounder at beaches where flounder were greatly abundant[22,23]. Therefore, dominant fish species influence on the vulnerability of newly settled flounder as a competitor and/or predator. However, little information on feeding ecology of dominant fish species is still available. Especially, quantitative analyses of food organisms consumed by dominant fish species including flounder are limited. Estimation of daily food consumption is essential to elucidate a role of these fish species in ecosystem and construct a model of the ecosystem in nursery grounds.

In Kagawa Prefecture, the central Seto Inland Sea, release of hatchery-reared flounder started since 1984, and the annual landing increased with number of released flounder (Fig. 1-1). In the Seto Inland Sea, approximately 4 million hatchery-reared juveniles have been released, and the annual catch of Japanese flounder is approximately 15% of the total catch in
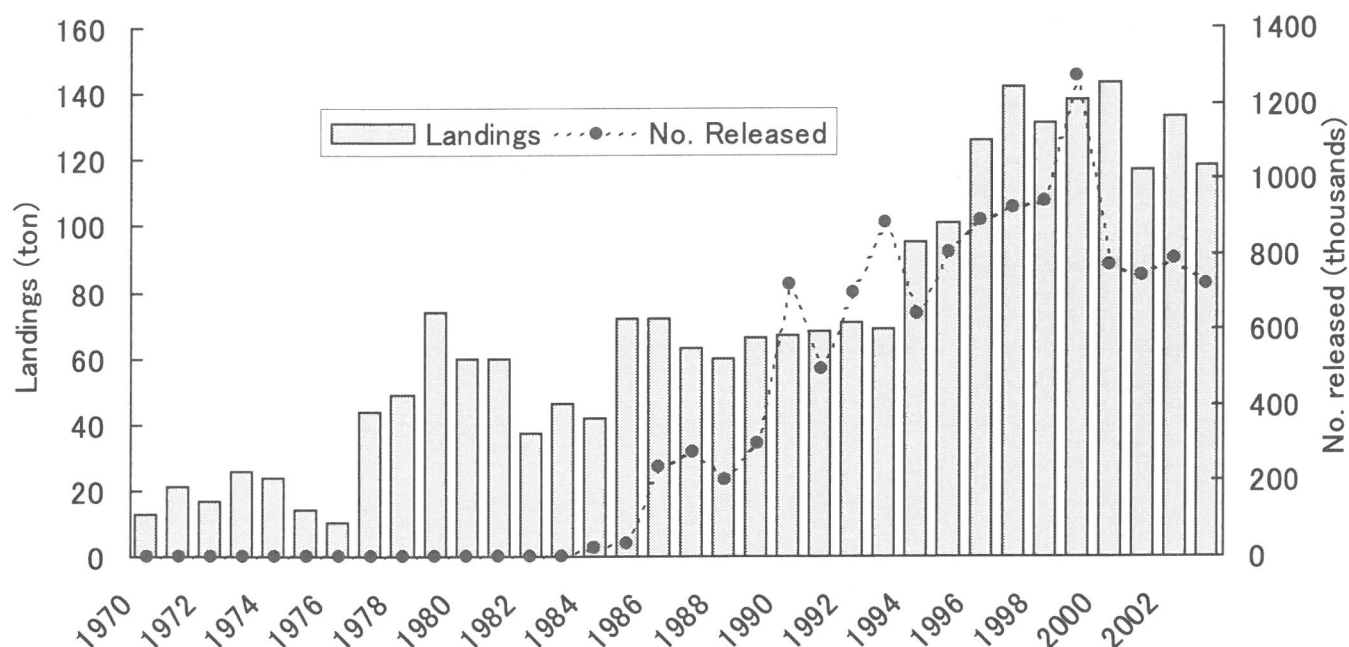
Fig. 1-1 Changes in annual landings and numbers of hatchery-reared juveniles released for Japanese flounder in Kagawa Prefecture.

Japan. It is necessary to estimate the optimum release number of hatchery-reared flounder. For this purpose, feeding ecology during juvenile stage and density of juvenile are the important element to estimate carrying capacity. Maehara[43] have studied the biology of juvenile Japanese flounder in southwestern Hiuchi-Nada, the Seto Inland Sea, and noted that newly settled flounder occurred from late May to early July at the sandy beaches, mysids were not abundant and the dominant prey were small crangonids and larval Japanese anchovy beside for mysids in the nursery grounds. These results suggested that the food condition in the nursery grounds was worse in the Seto Inland Sea than those in the Sea of Japan, the East China Sea and the Pacific Ocean where most of the previous study on early life history of Japanese flounder were carried out. Nevertheless the stock of Japanese flounder is abundant also in the Seto Inland Sea[20,40]. I think there may be a peculiar mechanism of forming nursery grounds in the Seto Inland Sea. For clarifying the mechanism, it is essential to elucidate feeding condition of settled Japanese flounder. Thus, I make the main object of my doctoral thesis to be a study on feeding ecology of settled Japanese flounder in nursery grounds in eastern Hiuchi-Nada, the Seto Inland Sea.

In order to reveal the mechanism of forming nursery in eastern Hiuchi-Nada, I refer to previous studies (e. g. Fujii and Noguchi, 1996[8]; Yamashita et al., 1996[45]; Noichi 1997[9]; Maehara, 1997[43]; Tanaka et al., 1997[10]; Tanaka et al., 1999[15]; Tominaga 2001[21]) and set up hypotheses of the early life history as follows: 1) larval and juvenile Japanese flounder immigrate from offshore to shallow waters and settle at sandy beaches in early summer when availability of food items especially mysids is high (hypothesis 1); 2) newly settled flounder prefer mysids, however, decrease in density of mysids with increasing temperature makes juveniles diet diversify (hypothesis 2); 3) dominant fish species and crangonids are not predators for juvenile Japanese flounder (hypothesis 3); 4) Daily food consumption of flounder is the greatest among those in dominant fishes, namely juvenile Japanese flounder can use the large amount of mysids among the members of the sympatric demersal fishes (hypothesis 4); 5) When juvenile flounder settle in their nursery grounds in early June, their food organisms are abundant and consequently their growth rate is relative high. However, their food availability becomes bad in late June, they can not take the enough amount of food and migrate from their nursery grounds to offshore area, where they can shift their food to abundant fish juveniles, mainly Japanese anchovy (hypothesis 5).

To establish these hypotheses, I examined occurrence, feeding ecology and growth rate of larval

and juvenile Japanese flounder, food organisms and feeding ecology of dominant demersal fishes from spring to summer at the sandy beaches in eastern Hiuchi-Nada, the Seto Inland Sea. In addition, catch efficiency of a 2-m beam trawl net was estimated. This thesis is divided into four parts(chapters 2-5). In chapter 2, density of Japanese flounder and catch efficiency of the Seikai National Fisheries Research Institute(SNFRI)type net for juveniles is estimated. In chapter 3, occurrence and feeding habits of Japanese flounder and food organisms is revealed for the verification of hypothesis 1 and 2. Chapter 4, which describes feeding ecology of dominant fish species, consists of two sections: feeding habits of *Favonigobius gymnauchen*, *Repomucenus* spp. and *Tarphops oligolepis* are shown for the verification of hypothesis 3, and daily rations of *P. olivaceus*, *T. oligolepis* and *F. gymnauchen* and diel variation in food availability to them for the verification of hypothesis 4. Finally, in chapter 5, daily growth is measured using otolith microstructure analysis for the verification of hypothesis 5.

Chapter 2. Estimation of density and catch efficiency of a two-meter beam trawl for Japanese flounder

Introduction

For stock enhancement, mass releases of hatchery-reared Japanese flounder juveniles have been conducted in shallow waters since the 1980[3]. Estimation of juvenile density is indispensable in order to clarify mechanism of forming nursery grounds. However, there is little information on density of juveniles in the Seto Inland Sea. Moreover, it was reported that few catch efficiency of the Seikai National Fisheries Research Institute (SNFRI) type net[46-50], with which I collected demersal fish species.

In this chapter, I estimate catch efficiency of the SNFRI type net at Ohama Beach in eastern Hiuchi-Nada, the Seto Inland Sea.

Materials and methods

Fishing surveys for juvenile Japanese flounder were carried out together with diving surveys for 12 days from June to August in 1998, 1999 and 2000 at Ohama Beach, where juvenile were abundant in eastern Hiuchi-Nada (Fig. 2-1; see Chapter 3A). The maximum tidal range is approximately 3 m and the sediment at depths greater than approximately 10 m is silty clay[51].
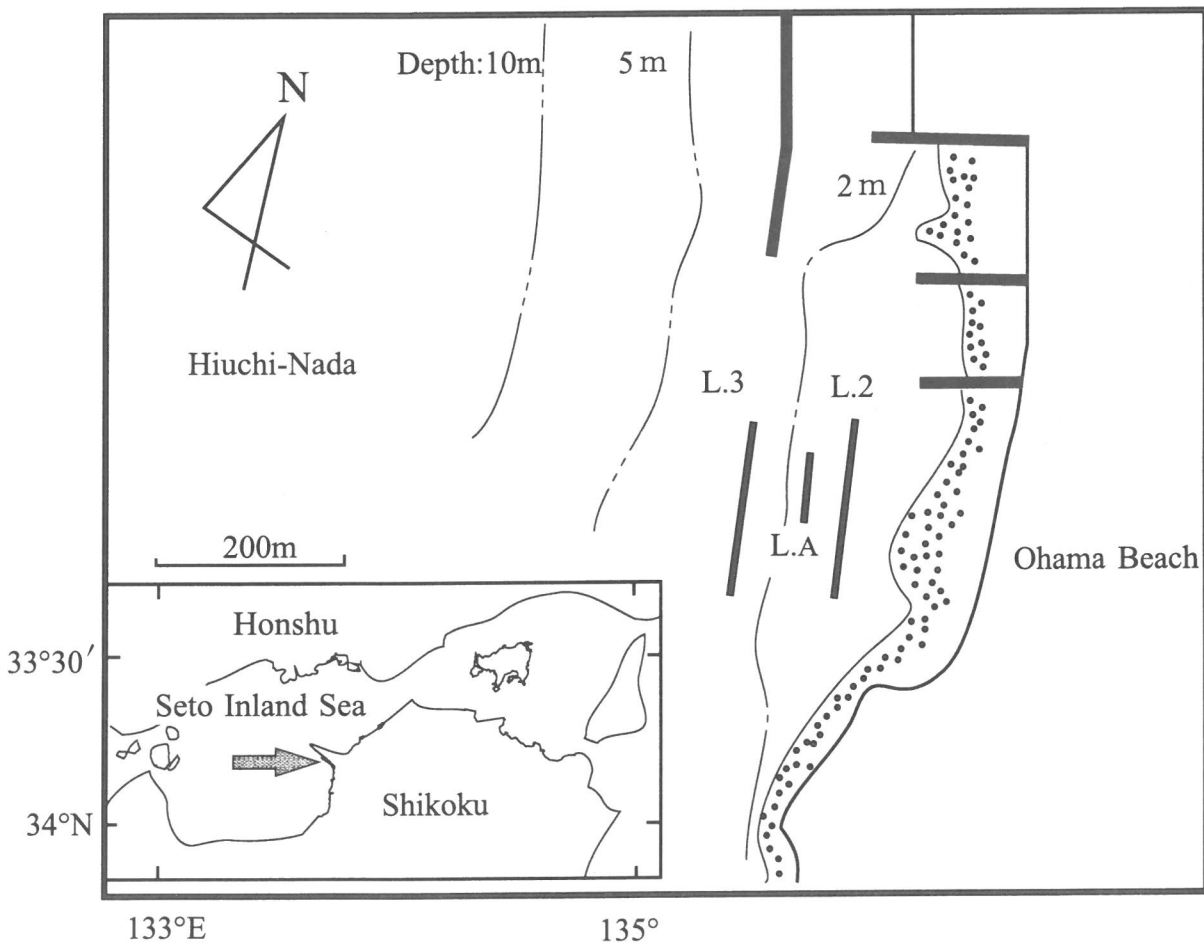

Fig. 2-1 Lines for fishing and diving surveys at Ohama Beach in eastern Hiuchi-Nada, the Seto Inland Sea.

Table 2-1 Number of fish collected by the trawl net, density of fish by diving survey and catch efficiency

Date	Fishing survey					Diving survey			Catch efficiency (%)
	No. of haul	Depth (m)	No. of fish	Mean TL (mm)	CPUE* (inds/100m^2)	Swept area (m^2)	No. of fish counted	Density of fish (inds/100m^2)	
1998									
June 25	2	3.2, 5.5	34	30.0	4.25	100	42	42.0	10.1
July 8	2	3.4, 4.9	7	40.1	0.88	100	22	22.0	4.0
July 22	2	3.6, 5.0	3	40.8	0.38	100	6	6.0	6.3
1999									
June 29	2	2.5, 3.9	8	47.0	1.00	150	7	4.7	21.4
July 7	2	1.6, 2.5	4	47.0	0.50	200	6	3.0	16.7
July 22	2	1.9, 2.9	1	52.4	0.13	200	1	0.5	26.0
Aug. 6	2	1.7, 2.8	1	68.8	0.13	200	3	1.5	8.3
2000									
June 15	2	3.2, 4.9	7	25.4	0.88	200	10	5.0	17.5
June 21	3	1.8 − 3.8	13	29.6	1.08	200	12	6.0	18.1
June 29	4	1.1 − 3.9	32	32.1	2.00	200	11	5.5	36.4
July 6	3	2.3 − 4.4	9	39.7	0.75	250	19	7.6	9.9
July 17	3	3.2 − 4.8	7	46.9	0.58	100	3	3.0	19.4
Mean									16.1

* Number of fish collected per swept area

The fishing survey was conducted with the SNFRI type net (net mouth 0.3 m high and 2 m width, mesh size 2.1 mm; weight in air, approximately 22 kg[48]) during the daytime. The net with 30 m towing rope was towed 200 m by a boat along the beach on Line 2 and Line 3 at a speed of 0.5-1.0 knot based on landmarks, and start and end time of the operation was recorded. The mean depth on Line 2 and Line 3 was 2.4 m (range: 1.1-3.0 m) and 4.1 m (3.0-5.0 m), respectively (Table 2-1). Collected fishes were immediately fixed in 10% seawater formalin. Wild Japanese flounder were measured their total length (TL) to nearest 0.1 mm with a caliper. Assuming that the net perfectly touched the bottom (swept area: 400 m^2, catch per unit effort (CPUE: number of flounder collected per swept area, individuals [inds]/100 m^2 was calculated. Additionally, to compare the density in the 12 nursery grounds by Tanaka et al.[52] with my result, I converted the density indexes by Tanaka et al.[52] (number of flounder collected by a 1.5-m beam trawl net per minute, inds/min) into CPUE (inds/100 m^2) based on towing speed (36 m/min).

The diving survey was conducted on Line A (Fig. 2-1). Two divers with threatening instruments (Fig. 2-2) swam along rope installed on the ground (swept area: 100-250m^2 and counted directly escaping flounder[53]. Density of flounder (inds/100 m^2) was calcu-

lated by swept area and number of fish counted.

Previous studies[46-48,54] have estimated the catch efficiencies of the 2-m beam trawl net base on assumption that the net caught the flounder irrespective of their density. However, there is a possibility of underestimation in catch efficiency because of density-dependent effects at high density. I therefore compared two models of density-independent (model 1) and density-dependent (model 2) models as follows:

Model 1 : $Y = aP$,

Model 2 : $Y = (a + bP)P = aP + bP^2$,

where Y is CPUE and P is density of fish. The parameters, a and b, were determined by the least squares method using solver on Microsoft-EXCEL. A better-fit model was statistically selected by Akaike
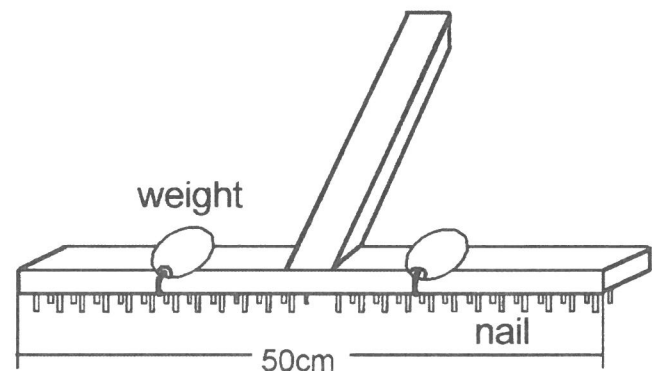

Fig. 2-2 Threatening instruments for diving survey.

Information Criterion (AIC)[55]. The model with the smaller AIC is the better-fit model. Then, based on the accepted model, catch efficiency of the SNFRI type net (CE, %) was obtained using the following equation: $CE= Y/P \times 100$.

Two samples of bottom sediments were collected at Line 2 and Line 3 by SCUBA on 17 July 2000 for grain size analysis. The sample was classified into five classes (gravel and coarse, particle diameter [Pd] > 0.5 mm; medium sand, 0.5 > Pd > 0.25 mm; fine sand, 0.25 > Pd > 0.125 mm; very fine sand, 0.125 > Pd > 0.063 mm; mud, Pd < 0.063 mm) with sieves, and then medium particle diameter was calculated by the weight composition[28].

Table 2-2 Particle diameters (Pd) at Line 2 and Line 3 (%)

	Line2	Line3
Gravel and coarse (Pd>0.5 mm)	1.0	1.2
Medium sand (0.5 > Pd > 0.25 mm)	42.8	20.7
Fine sand (0.25 > Pd > 0.125 mm)	53.5	73.7
Very fine sand (0.125 > Pd > 0.063 mm)	0.4	2.2
Mud (Pd < 0.063 mm)	2.3	2.2

Table 2-3 Relationship between density of juvenile Japanese flounder and number of flounder collected by the net

	a	b	AIC
Model 1 ($Y=aP$)	0.0953	–	28.6
Model 2 ($Y=aP+bP^2$)	0.1018	-0.00187	30.6

Y, number of fish collected by the net per swept area
P, density of fish by diving survey

Results

The medium particle diameter of the sediments were 0.23 mm at Line 2 and 0.20 mm at Line 3, respectively (Table 2-2), showing the bottom sediments mainly consisted of sand, especially fine sand at Ohama Beach.

The CPUE (Y) varied between 0.13 and 4.25 inds/100 m^2, and was higher in June than in July and August (Table 2-1). The CPUE in June was 1.84 inds/100 m^2. The mean total lengths of flounder ranged from 25.4 to 68.8 mm. The densities of fish (P) by the diving surveys varied between 0.5 and 42.0 inds/100 m^2, and the mean density in June was 12.6 inds/100 m^2. The divers seldom observed juveniles larger than 60 mm.

The parameter estimates obtained by the two models are shown in Table 2-3. The values of AIC were calculated to be 28.6 in the model 1 and 30.6 in the model 2, indicating that the model 1 was the better-fit model. Thus, I calculated catch efficiency of the SNFRI type net (CE) based on the density-independent model (model 1) and then the catch efficiency of each day varied between 4.0 and 36.4% ($n=12$; Table 2-1). The mean estimate (95% confidence intervals) was 16.1% (11.0-21.2%).

The density indexes by Tanaka et al. (2006; inds/min) were converted into CPUE (inds/100 m^2), and then the results are shown in Table 2-4. The CPUE varied between 0.76 and 8.55 inds/100 m^2.

Table 2-4 CPUE derive from the data* by Tanaka et al. (2006)

Site	Prefecture	Area	CPUE (inds/100 m^2)
Miyako	Iwate	The northern Pacific Ocean	0.44
Onagawa	Miyagi	The northern Pacific Ocean	1.80
Yoichi	Hokkaido	The northern Sea of Japan	0.76
Ajigasawa	Aomori	The northern Sea of Japan	1.31
Igarashi	Niigata	The northern Sea of Japan	0.41
Naoetsu	Niigata	The northern Sea of Japan	1.24
Kokubu	Toyama	The central Sea of Japan	4.28
Mikuni	Fukui	The central Sea of Japan	2.35
Funaiso	Tottori	The southern Sea of Japan	2.70
Hagi	Yamaguchi	The southern Sea of Japan	8.56
Oseto	Nagasaki	The East China Sea	2.78
Kazusa	Nagasaki	The East China Sea	3.80

* : number of collected by a 1.5-m beam trawl net per minute

Discussion

Tanda[56] observed that ability of burring into sand by hatchery-reared juvenile flounder (size: approximately 20-100mm in TL) was higher on medium and fine sand (0.125 < Pd < 0.5 mm) than on mud and coarse sand in the laboratory experiment. Wild juveniles occurred mainly on fine sand area in the coast of Chiba Prefecture[57]. Therefore, this study beach has comfortable bottom sediments for larvae and juveniles.

At Ohama Beach, the mean CPUE and density of flounder was 1.84 inds/100 m^2 and 12.6 inds/100 m^2, respectively. The CPUE at Ohama Beach was higher than those at Miyako and Onagawa in the northern Pacific Ocean and at Yoichi, Ajigasawa, Igarashi and Naoetsu in the northern Sea of Japan, but lower than those at other sites in the central and southern Sea of Japan and the East China Sea (Table 2-4). The density was lower at Ohama than in the coast of Totori Prefecture (14-81 inds/100 m^2)[48], at Yanagihama Beach (86-420 inds/100 m^2)[58] and at Taniura Beach (40-222 inds/100 m^2)[46] in Nagasaki Prefecture. These results indicated that the abundance of juveniles at Ohama Beach was higher than those in the northern Pacific Ocean and the northern Sea of Japan, but lower than those in the central and southern Sea of Japan and the East China Sea.

The mean catch efficiencies of the SNFRI type net have been estimated to be 26.7% (range: 24.5-30.8%) in Sijiki Bay, Nagasaki Prefecture[46], and 21.6% (9.6-41.4%) in the coast of Tottori Prefecture[48]. These values were higher than the mean estimate in this chapter. Catch efficiency should be influenced by size of flounder and towing speed[47]. Catch efficiency decreases with an increase in size of flounder collected, because swimming ability increase with an increase in size. The mean total length of flounder was smaller in Shijiki Bay (mode: 11 mm in standard length)[46] than in this study.

Few juveniles larger than 60 mm TL were collected by the SNFRI type net. In the diving survey, few juveniles larger than 60 mm were also observed. At the sandy beach in southwestern Hiuchi-Nada, the same type net collected juvenile approximately 100 mm. These results suggest that there might be not net avoidance of large juveniles.

Kimoto at el.[47] improved catch efficiency of the National Research Institute of Fisheries Engineering (NRIFE) I type net by lengthening a tickler chain and ground chain and added weight to the net. The catch efficiency of the modified net (the NRIFE II type net) was higher than that of the SNFRI type net[47]. However, the NRIFE II type net could not be towed in the Seto Inland Sea, because NRIFE II type net was too heavy (weight in air, approximately 36 kg)[47] and then buried into bottom[59]. Therefore SNFRI type net would be useful for collecting demersal fishes at sandy beaches in the Seto Inland Sea.

Chapter 3. Occurrence and feeding habits of settled Japanese flounder and food organisms

A : Distribution of larval and juvenile Japanese flounder

Introduction

Japanese flounder is an important species in commercial fisheries and stock enhancement in Japan. To establish a more effective fisheries management and stock enhancement, studies on the early life history have been carried out in the Sea of Japan and the East Chaina Sea (e.g. Kato, 1987[60]; Fujii et al., 1989[46]; Ikewaki and Tanaka, 1993[61]; Furuta, 1999[62]), and the Pacific Ocean (e.g. Ishida et al., 1977[57]; Yamada et al., 1998[14]; Takahashi et al., 1999[41]). Previous reports demonstrated that the occurrence, distribution and size of migrating from nursery grounds differed among survey areas (e.g. Minami, 1989[18]; Tanaka et al., 1997[10]; Noichi, 1997[9]). Information on general biology for flounder in the Seto Inland Sea, where the annual catch comprised approximately 15% of the total catch in Japan, has been accumulated[20], but the early life history is still not fully understood with the exception of the seasonal occurrence of pelagic larvae[63,64].

The objective of this chapter was to describe occurrence, distribution and size of larval and juvenile Japanese flounder at sandy beaches in the eastern Hiuchi-Nada.

Materials and methods

Sampling area

Eastern Hiuchi-Nada, the central Seto Inland Sea, has sandy tidal flats of approximately 220 ha (Fig. 3-1). Although the bottom sediment at Line 4 at Ohama Beach (Fig. 3-1C) consisted of silty clay (median particle diameter [Mdϕ]: 4.4), those at other sampling stations were fine sand (Md ϕ : 2.0-3.2). A residual current circulates counterclockwise around Ibuki-jima Island at a constant velocity of 10-20 cm/s[51].
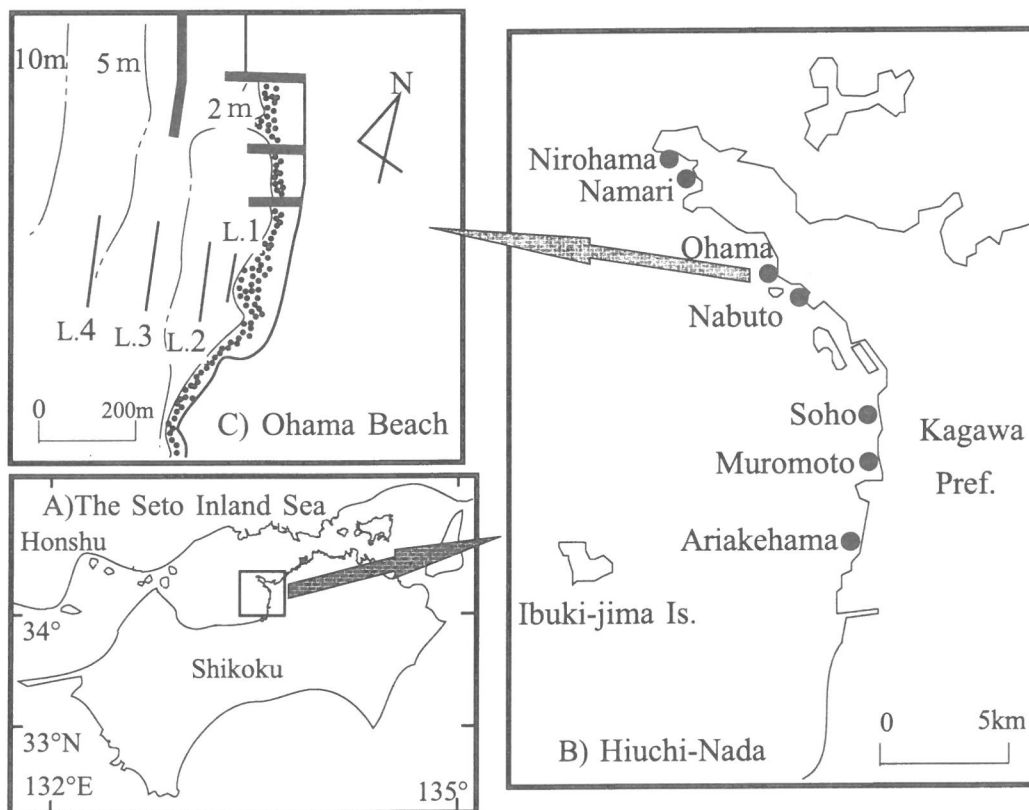

Fig. 3-1 Sampling stations for larval and juvenile Japanese flounder.

Geographical distribution of Japanese flounder and other demersal fishes

Sampling surveys of demersal fishes were carried out 22 times from May to September in 1995-1997 at the seven beaches (Fig. 3-1B; Table 3-1). The sampling line at Ohama Beach was only Line 2

Table 3-1 Collection records of larval and juvenile Japanese flounder at the sandy beaches in the central Seto Inland Sea from 1995 to 2001

Geographical distribution			○	○		○			○	○	○	○
Depth distribution					○	○	○	○				
Water depth (m)*			ca. 1.0	ca. 1.0	ca. 0.4	ca. 1.0	ca. 2.4	ca. 6.3	ca. 0.9	ca. 1.0	ca. 1.0	ca. 1.4
Year	Month	Day	Measured water depth (m)** and tide									
			Nr	Nm	Line 1	Line 2	Line 3	Line 4	Nb	Sh	Mr	Ar
1995	May	30	ND, e	ND, e		ND, e			ND, e	ND, e	ND, e	ND, e
	June	14	ND, f	ND, f		ND, f			ND, e	ND, e	ND, e	ND, e
	June	26, 27	ND, e	ND, e		ND, e			ND, e	ND, e	ND, e	ND, e
	July	10, 13	ND, e	ND, e		ND, e			ND, f	ND, e	ND, e	ND, e
	July	26, 27	ND, f	ND, f		ND, e			ND, e	ND, e	ND, e	ND, e
	Aug.	7, 8	ND, e	ND, e		ND, e			ND, e	ND, e	ND, e	ND, e
	Sep.	7, 8	ND, f	ND, f		ND, e			ND, e	ND, e	ND, e	ND, e
1996	May	16, 17	ND, e	ND, e		ND, e	ND, e	ND, e	ND, e	ND, e	ND, e	ND, e
	May	30, 31	4.0, e	1.8, e		1.5, e	4.1, e	6.5, e	1.6, e	3.0, e	2.7, e	3.8, e
	June	11, 12	2.4, e	2.4, e		1.1, f	3.8, f	6.3, f	1.1, f	2.8, e	1.5, e	3.0, e
	June	24, 25	1.8, e	1.0, e		1.7, f	3.0, f	7.3, f	3.2, f	2.2, e	1.1, e	1.8, e
	July	8, 9	1.2, f	1.1, f		1.0, f	3.2, f	6.3, f	1.3, f	3.2, f	2.0, f	3.1, f
	July	22, 23	1.5, f	1.3, f		2.2, f	5.6, f	8.3, f		2.5, e	2.3, f	2.8, f
	Aug.	5	1.3, f	0.9, f		1.4, f	3.4, f	6.5, f	3.3, f	2.7, f	2.5, e	3.9, e
	Aug.	20	2.0, f	1.5, f		2.3, f	5.0, f	9.0, f		2.6, f	2.1, e	3.2, e
1997	May	12, 13	2.7, f	1.5, f		2.7, e	4.0, e	7.5, e		2.0, f	1.4, f	3.8, f
	June	3, 4	2.7, e	1.9, e		1.0, e	2.6, e	6.3, e		1.4, e	2.6, e	
	June	16, 17	2.2, e	1.1, e		1.3, f	3.3, f	6.6, f		2.2, e	2.1, e	1.8, e
	June	30	1.3, e	1.0, e		1.3, e	3.0, e	6.4, e		1.3, f	2.1, f	3.1, f
	July	14	1.1, e	1.0, e		1.8, e	4.1, e			1.2, f	1.7, f	
	July	29	2.1, e	1.5, e		1.5, e	2.8, e	6.5, e		2.0, f	1.5, f	
	Aug.	19	3.4, f	2.7, f		4.0, e	4.7, e	9.0, e		2.2, f	2.6, f	
1998	May	21				2.0, e	3.2, e	9.6, e				
	June	11				3.0, f	5.0, e	8.4, e				
	June	25				3.2, f	5.5, e	9.0, e				
	July	8				3.4, e	4.9, e	9.3, e				
	July	22				3.6, e	5.0, e	9.5, e				
1999	May	25				2.0, e	3.2, e					
	June	11				2.3, e	3.5, e					
	June	29				2.5, f	3.9, e					
	July	7				1.6, f	2.3, f					
	July	22				1.9, e	2.9, e					
	Aug.	6				1.7, e	2.8, e					
	Aug.	26				3.4, e	5.1, e					
2000	May	31			0.5, e	2.5, e	3.2, e					
	June	15			0.5, e	3.2, e	4.9, e					
	June	21				2.0, f	3.7, f					
	June	29			0.5, e	2.1, e	3.9, e					
	July	6				2.3, f	4.4, f					
	July	17				3.2, e	4.5, e					
	July	26				1.5, e	3.1, e					
2001	May	22			0.5, e	1.3, e	3.1, e					
	June	6			0.5, e	1.6, e	3.4, e					
	June	19			0.5, e	1.3, e	2.4, e					
	July	3			0.5, e	1.1, e	2.4, e					
	July	24				3.9, e	4.6, e					
	Aug.	28			0.5, f	1.4, f	3.5, f					

Nr, Nirohama; Line 1-Line 4, Ohama; Nb, Nabuto; Sh, Soho; Mr, Muromoto; Ar, Ariakehama; ND, No data on measured water depth; e, ebb tide; f, flood tide

* : the distance from the mean low water springs to the bottom
** : the distance from the surface to the bottom in the beach at sampling

(Fig. 3-1C). The mean measured water depth of the beaches was 2.1 m (depth range of 0.9-4.0 m; Table 3-1). Demersal fishes were collected by the SNFRI type net (Chapter 1). The net was towed approximately 200 m by a boat along the beach at a speed of 0.5-1.0 knot during the daytime. The water temperature at the surface and water depth were recorded every sampling station.

To compare abundance of flounder among depth, sampling were carried out in 1996-2001 at Ohama Beach (Fig. 3-1C; Table 3-1). The mean measured depths in Line 1, Line 2, Line 3 and Line 4 was 0.5, 2.1, 3.8 and 7.8 m, respectively. On Line 2, Line 3 and Line 4, the 2-m net was towed 200 m along the beach based on landmarks (swept area: 400 m², whereas, on Line 1, samplings were conducted using a 1.5-m beam trawl net (net mouth 0.3 m high and mesh size 2.0 mm)[65] during low tide, as most of larval and juvenile flounder were caught near the shoreline during low tide at Yanagihama Beach, Nagasaki Prefecture[65]. The 1.5-m net was towed 150 m by two persons along the beach at a speed of approximately 1.0 knot (swept area: 225 m²). To compare catch per unit effort (CPUE, individuals [inds]/haul) of the 2-m net, I modified the CPUE by multiplying 1.78 in CPUE of the 1.5-m nets.

Sample treatments

Fishes were sorted from samples preserved in 10% formalin. Japanese flounder were divided into wild and hatchery-reared fish based on abnormal pigmentation on the blind side. Data from the hatchery-reared fish was not used for analysis in this chapter. Total length (TL) of wild flounder was measured to the nearest 0.1 mm except in 1995 and 1998. The developmental stages of flounder were classified after Minami[4] and Goto et al.[5].

Statistical analysis

For comparisons of CPUE among sampling stations, data were log transformed and then analyzed using Tukey-Kramer multiple comparison tests. Difference in size among sampling lines was examined using Mann-Whitney U test or Tukey-Kramer tests[66].

Results

Number of larval and juvenile Japanese flounder collected

A total of 963 individuals were collected from 242 hauls at the seven beaches from 1995 to 2001. The number of wild and hatchery-reared Japanese flounder was 959 and 4, respectively.

Occurrence and distribution of Japanese flounder
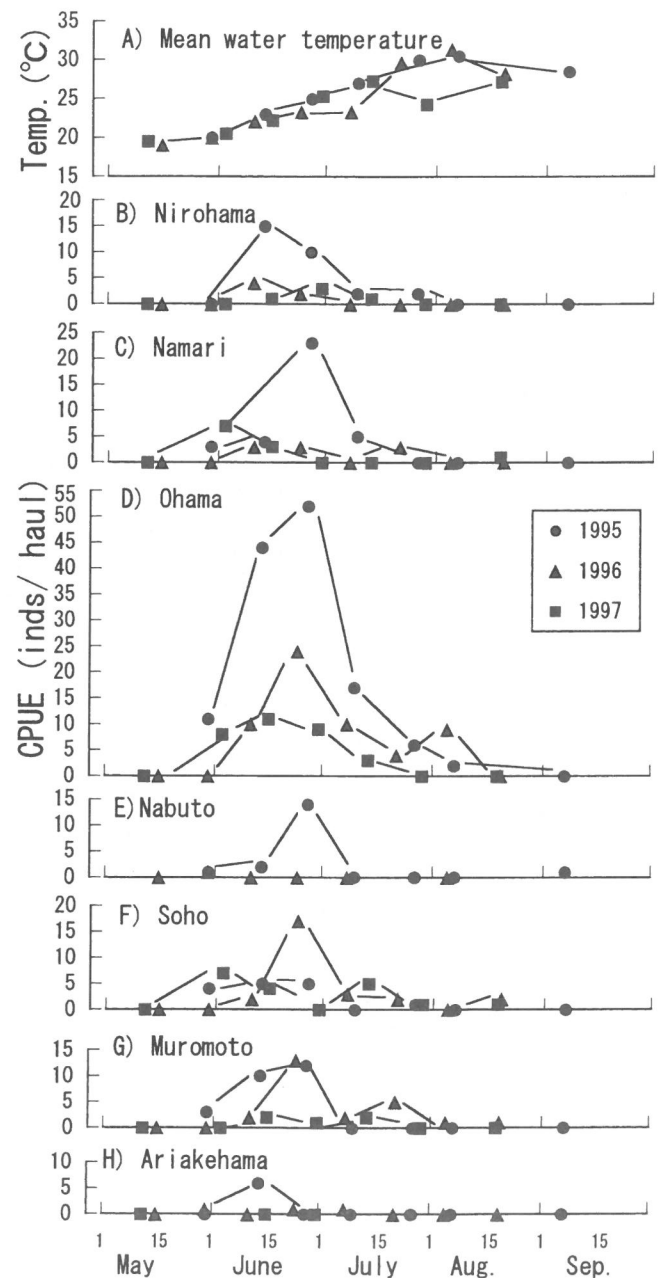
The mean surface water temperature at the seven

Fig. 3-2 Seasonal changes in (A) mean sea surface temperature and (B-H) catch per unit effort (CPUE) of Japanese flounder at the seven beaches.

beaches ranged between 20.0 and 30.5 ℃ (Fig. 3-2). Larval and juvenile flounder were collected from late May to early September. The CPUE of flounder was high in June at all the beaches, but was different among the beaches. The CPUE at Ohama Beach was greater than those at the other beaches. At Ohama Beach, the mean CPUE was 22.6 inds/haul (standard deviation: 18.3) in June, whereas those at the other beaches were less than 6.1 inds/haul. The CPUE fluctuated by year, and was higher in 1995 than in 1996 and 1997.

Demersal fishes in nursery grounds

A total of 6,412 individuals including more than 17 species in 12 families were collected by the 2-m beam from 1995 to 1997 (Table 3-2). Most of the fishes were juveniles. The CPUE was significantly higher at Nabuto than at Muromoto (Tukey-Kramer test, $P < 0.05$; Fig. 3-3). The dominant fish species were *Favonigobius gymnauchen*, *Repomucenus* spp. and *Tarphops oligolepis* at all beaches. Especially, at Nirohama, Nabuto and Ariakehama, the percentage of *F. gymnauchen* was higher than 70%. The dominant species was *Rudarius ercodes* at Ohama Beach and Soho. *Acanthopagrus schlegeli* was abundant only at Soho.

Size and stage composition of Japanese flounder

Seasonal changes in total length and stage composition of flounder collected in 1996 and 1997 are
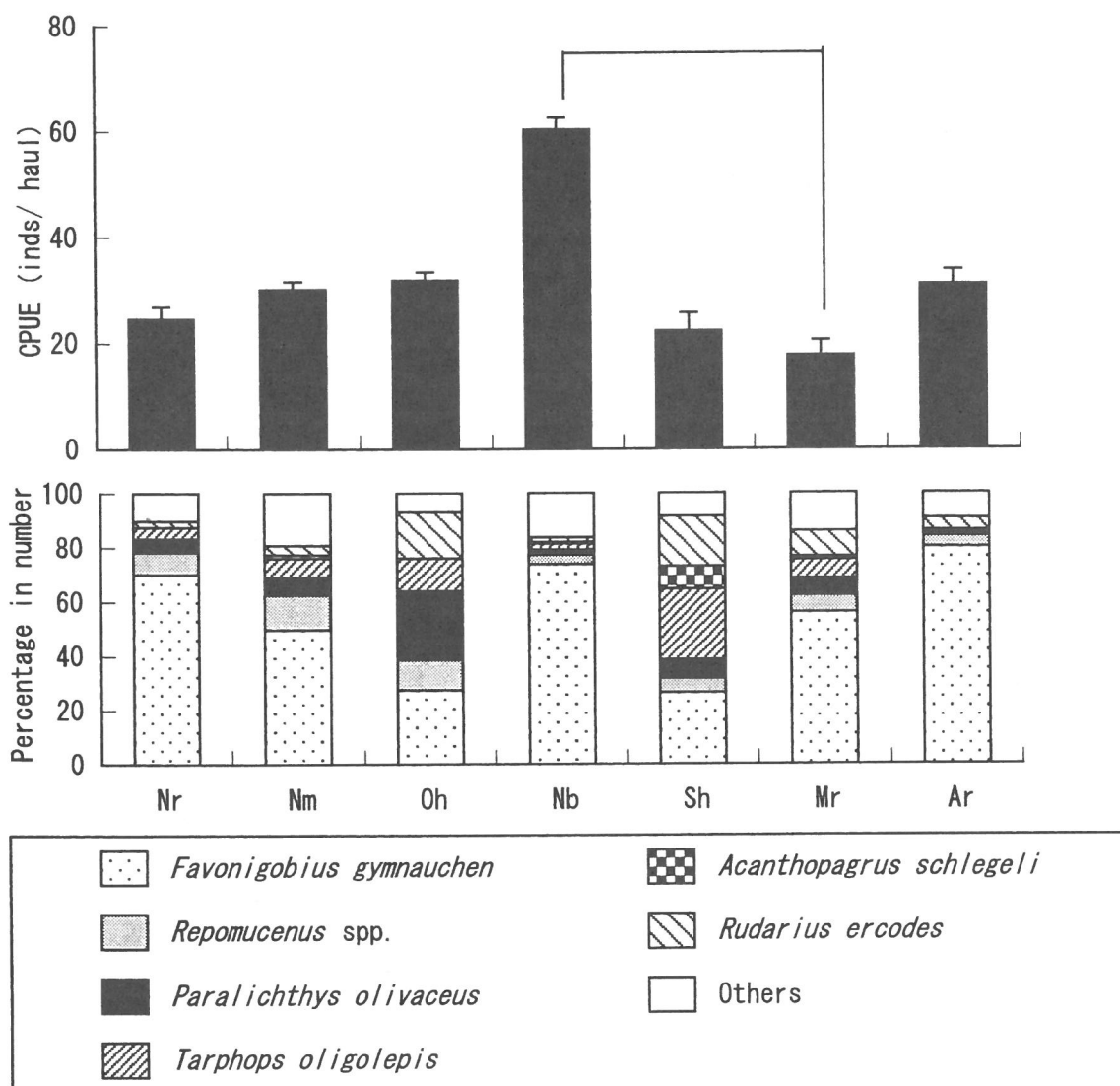

Fig. 3-3 CPUE (top) and composition of demersal fishes (bottom) by beach from 1995 to 1997. Data connected line and vertical lines indicate significant difference (Tukey-Kramer test, $P < 0.05$) and standard error, respectively. Ar, Ariakehama; Mr, Muromoto; Nb, Nabuto; Nm, Namari; Nr, Nirouhama; Oh, Ohama; Sh; Siho.

**Table 3-2** Demersal fish species collected by the 2-m trawl at the seven beaches from 1995 to 1997

Family	Species	Nirohama	Namari	Ohama	Nabuto	Soho	Muromoto	Ariake
	Number of hauls	22	22	22	13	22	22	18
Sparidae	*Acanthopagrus schlegeli*	0	9	0	7	73	8	5
	Pagrus major	0	5	1	0	10	2	2
Labridae	*Halichoeres poecilopterus*	5	10	0	1	4	36	2
Ammodytidae	*Ammodytes personatus*	0	1	0	19	0	3	0
Gobiidae	*Favonigobius gymnauchen*	574	418	237	826	234	482	821
	Gobiidae	8	14	3	129	8	5	10
Tetrarogidae	*Hypodytes rubripinnis*	16	8	1	0	4	2	16
Hexagrammidae	*Hexagrammos agrammus*	0	1	0	0	0	0	0
Cottidae	*Pseudoblennius cottoides*	3	21	1	0	0	0	9
Callionymidae	*Repomucenus spp.*	67	108	96	38	47	53	42
Pleuronectidae	*Paralichthys olivaceus*	40	55	220	21	61	55	9
	(wild)	(40)	(55)	(220)	(20)	(59)	(54)	(9)
	(hatchery-reared)	(0)	(0)	(0)	(1)	(2)	(1)	(0)
	Tarphops oligolepis	35	59	104	24	229	60	7
	Kareius bicoloratus	0	0	0	2	1	2	1
	Limanda yokohamae	1	1	1	10	1	2	6
Soleidae	*Heteromycteris japonica*	31	68	35	7	22	39	7
Monacanthidae	*Rudarius ercodes*	18	30	145	19	163	81	47
Tetraodontidae	Tetraodontidae sp.	0	0	1	1	0	0	4
Others		26	32	17	13	25	30	39
Total		824	840	862	1,117	882	860	1,027

shown in Fig. 3-4. Size ranged from 9.8mm to 92.6 mm TL. The number of large juveniles (> 60 mm TL) was small (4.3%). There were 29 and 226 individuals in the H-I_3 stages (from late metamorphosing to nearly metamorphosed stage) and I_4 stage (completely metamorphosed stage), respectively. Settling larvae (H-I_3 stages) occurred from late May to late June. The two modes shifted from 10-15 and 25-30 mm TL in early June, through 25-30 and 35-40 mm TL in late June, to 30-35 and 45-50 mm TL in early July, respectively. Judging from these results, the daily growth rate was estimated to be 0.4-1.0 mm/day.

Difference in abundance by depth of Japanese flounder at Ohama Beach

Fig. 3-5 shows the CPUE by depth (mean water depth: Line 1 [0.5 m] < Line 2 [2.1 m] < Line 3 [3.8 m] < Line 4 [7.7 m]) at Ohama Beach from 1996 to 2001. The CPUE was significantly greater on Line 2 and Line 3 than on Line 4 (Tukey-Kramer test, $P < 0.05$). Significant differences in CPUE were not found among Line 1, Line 2 and Line 3, but CPUE on Line 2 (9.23 inds/haul) and on Line 3 (9.26 inds/haul) was more than sixfold that on Line 1 (1.26 inds/haul). These results demonstrate that settled flounder was abundant on Line 2 and Line 3.
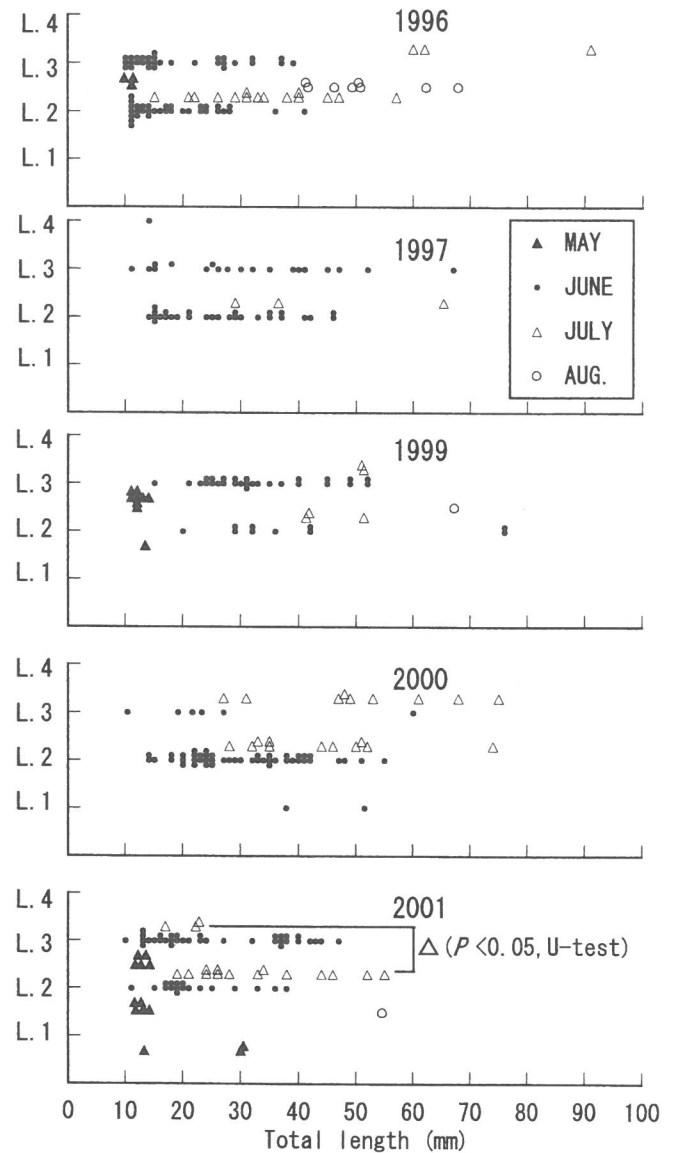
Size distributions by depth at Ohama Beach in
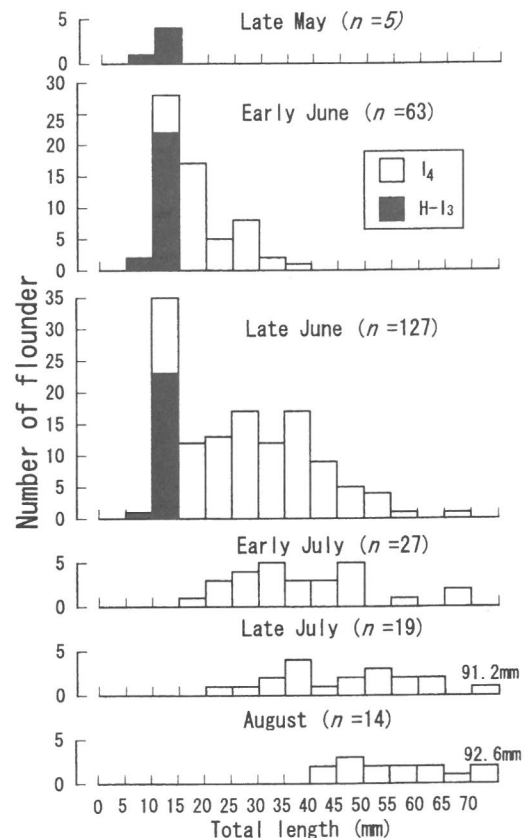
Fig. 3-4 Seasonal changes in size and stage composition of Japanese flounder collected at the seven beaches in 1995 and 1996. Stages: H-I_3, from the late metamorphosing to the nearly metamorphosed stage; I_4, completely metamorphosed stage.
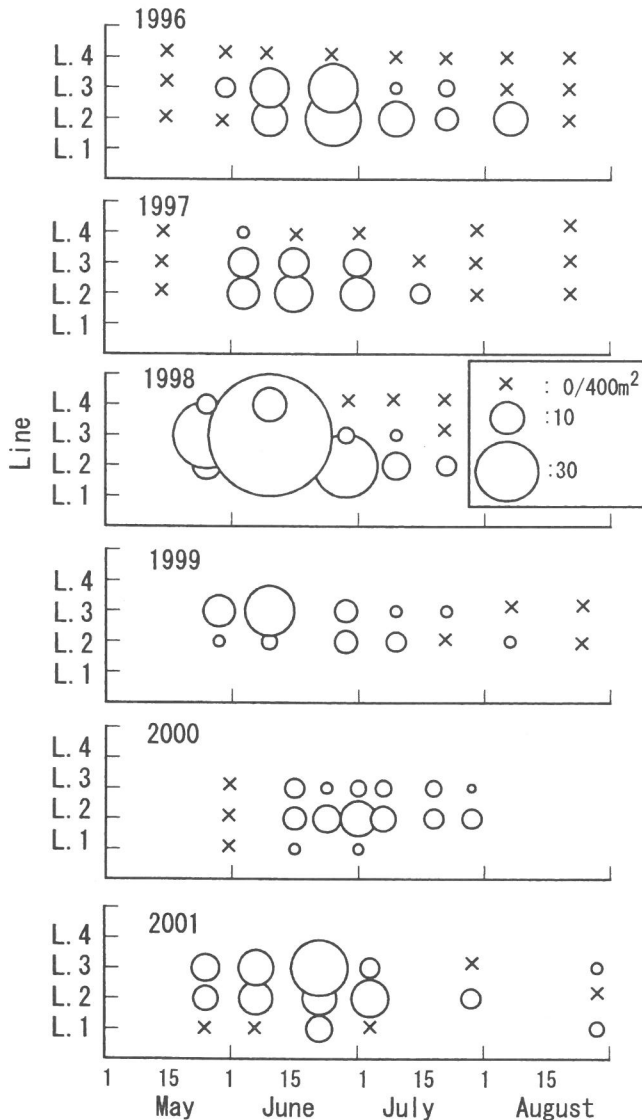
Fig. 3-5 CPUE by depth at Ohama Beach from 1996 to 2001. Mean depths on Line 1, Line 2, Line 3 and Line 4 was 0.5, 2.1, 3.8 and 7.7 m, respectively.

Fig. 3-6 Size distribution of larval and juvenile Japanese flounder in total length (TL) by water depth at Ohama Beach in 1996, 1997, 1999, 2000 and 2001. Mean depth on Line 1, Line 2, Line 3 and Line 4 was 0.5, 2.1, 3.8, and 7.7 m, respectively.

1996, 1997, 1999, 2000 and 2001 is shown in Fig. 3-6. In July 2001, mean total length(33.5 mm TL)on Line 2 was significantly larger than that on Line 3(20.6 mm TL; Mann-Whitney U-test, $P < 0.05$), whereas in other months, significant differences in size were not observed among water depths. The range of sizes was wide on the both Line 2 and Line 3. The major part of distribution did not move to shallower/deeper waters with growth.

Discussion

Time of settlement of larval Japanese flounder

Metamorphosing flounder larvae settled at the sandy beaches at a depth of 2-5 m from late May to late June (water temperature: 19-23 ℃). The seasons of settlement in Japan are as follows: March-June(water temperature: 15-25 ℃)in the eastern East China Sea, Nagasaki Prefecture[5,65]; May-June(15-24 ℃)in the central Sea of Japan, Wakasa Bay[10]; June-July(18-25 ℃)in the northern Sea of Japan, Niigata Prefecture[8]; late July-

September (19-23 ℃) in the northern Pacific Ocean, Iwate Prefecture[14]. Although season of settlement differs among the areas, metamorphosing flounder larvae would occur in spring/summer when water temperature ranges from 15 to 25 ℃ in the nursery grounds.

Demersal fishes in nursery grounds

F. gymnauchen, Repomucenus spp. and *T. oligolepis* were abundant at the beaches. Noichi et al.[22,23] reported that these three fish species consumed newly settled flounder, mysids, amphipods, polychaetes and decapods. Therefore, these fish species might be a competitive species or predator for larval and juvenile Japanese flounder. In Chapter 4, I examined their feeding ecology and discuss food web in this area.

Growth of Japanese flounder

The daily growth rate of juveniles is high in rich food conditions[8,17]. In the nursery ground, where mysids were abundant, large juveniles were collected[8,67]. At Igarashihama Beach, the northern Sea of Japan, where mysids were abundant, large juveniles (> 100 mm TL) were collected and the daily growth rate was high (1.5-2.0 mm/day)[8]. However, at beaches with poor food conditions, large juveniles were seldom collected[67] and the daily growth rate was low[8,21]. At Ohama Beach, a few large juveniles were colleted and the daily growth rate was less than 1.0 (mm/day). These results suggest that food availability was lower in the Seto Inland Sea than in the northern Sea of Japan. Therefore I examined abundance of food organisms and feeding habit of flounder in next chapter (Chapter 3B), moreover, in Chapter 5, the daily growth rate was estimated using an otolith microstructure in detail.

Distribution of Japanese flounder by depth

Kinoshita[68] proposed that tidal range was closely related to habitat depth of larval and juvenile flounder at sandy beaches. Larval and juvenile Japanese flounder were abundant at a depth of 3-10m in the Sea of Japan[8,60,62], where tidal range is low (< 1 m), whereas fish was observed at 0.5-1.0 m of Yanagihama Beach[65], Nagasaki Prefecture, and Yatsushiro Sea[67], where tidal range is high (> 3 m). Because, in eastern Hiuchi-Nada, the maximum tidal range is approximately 3 m[51], I expected that flounder might be abundant near the shoreline at Ohama Beach. However, the main nursery ground was found at a depth from 2 to 5 m at Ohama Beach. At beaches in eastern Hiuchi-Nada, abundance of mysids was very low near the shoreline, but mysids were relatively abundant at depths greater than 2 m[69]. These observations suggest that abundance of mysids might be closely associated with the main habitat of flounder.

Migration from nursery grounds

There were few juveniles larger than 60 mm after late June at the sandy beach. According to the tagging experiments in this area from late June and early July[70], the tagged flounder ranging from 80 to 160 mm in TL were not collected at the release beach, but recaptured offshore on the day subsequent to the release. Besides, wild juveniles approximately 100 mm TL were caught offshore in Hiuchi-Nada in middle July[43]. These observations suggest that large juveniles (> approximately 60 mm TL) began to emigrate from nursery grounds after late June.

B : Food organisms and feeding habits of Japanese flounder

Introduction

Generally, larval metamorphosing Japanese flounder settled at sandy beaches in early summer, when mysids were abundant, and juvenile flounder feed chiefly on mysids and shift to fishes as their main food source with growth (e. g. Imabayashi, 1980[13]; Ishida et al., 1977[57]; Noichi, 1997[9]; Tanaka et al. 1997[10]; Tanaka et al., 1999[15]). However, the major item of diet became amphipods and copepods in the nursery grounds, where mysids were not abundant[67]. In Chapter 3A, the result of daily growth rate proposed that food availability was low at the sandy beaches in eastern Hiuchi-Nada. Taking into consideration of these reports, I hypothesized that the peak in settlement of flounder is synchronized with that in abundance of mysids (hypothesis 1) and flounder juveniles eat a variety of diet in this area (hypothesis 2).

In order to make the verification of the hypotheses, I examined density of food organisms and feeding habits of larval and juvenile flounder at the sandy beach in eastern Hiuchi-Nada. Besides, the predator-prey relationship between juveniles and crangonids (a part of hypothesis 3 as follows: dominant fish species and crangonids are not predators for flounder) was discussed.

Materials and methods

Field Sampling

Sampling was carried from May to August in 1999-2001 at Ohama Beach (Fig. 3-7). Japanese flounder and food organisms were collected with the 2-m SNFRI type net (Chapter 2) and a sledge net (net mouth 0.3 m high and 0.6 m wide, mesh size 0.76 mm)[14], respectively. The beam trawl and sledge net were towed 200 m and 50 m by a boat during the daytime along the beach, respectively. Juveniles collected by the beam trawl net were preserved in 70% ethanol after 10% formalin fixation, and food organisms collected by the sledge net were preserved in 10% formalin.

Laboratory analysis

The food organisms in 1999 and 2000 were identified to the lowest taxonomic level as possible as I could, but the samples in 2001 were classified into major taxa, and the number of individuals and weight of each major taxa were recorded. Crangonids were composed of three species, *Crangon affinis*, *C. cassiope* and *C. uritai* in the Seto Inland Sea[71]. The dominant species was *C. uritai* at the sandy beaches in Hiuchi-Nada[33]. Density was calculated as weight
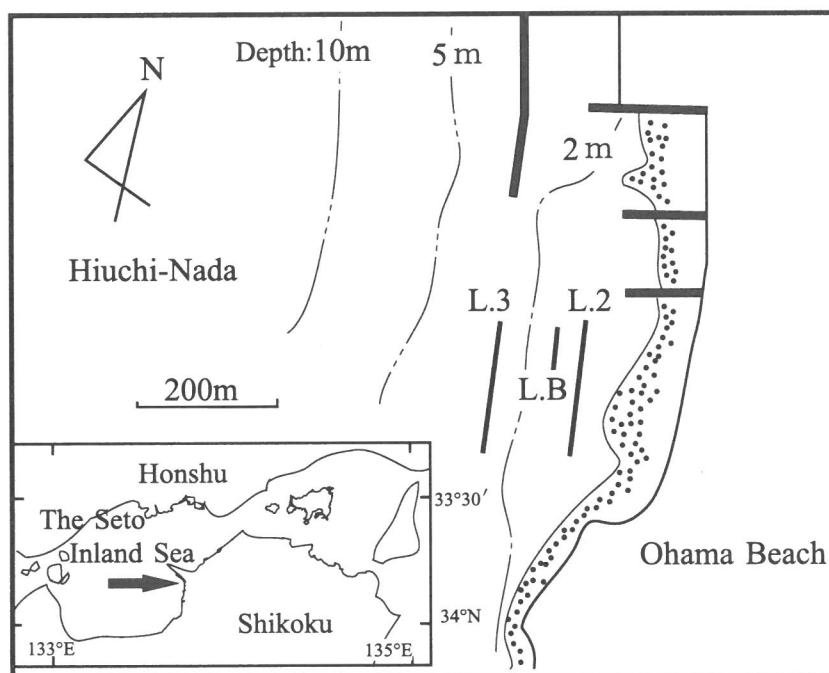

Fig. 3-7 Survey line for larval and juvenile Japanese flounder (Line 2 and Line 3) and food organisms (Line B) from 1999 to 2001.

and/or number of individuals in each major taxa per swept area(30 m²). Here, I did not take into consideration of catch efficiency. Body length of mysids and crangonids was measured with a caliper. When more than 100 individuals were obtained, we measured 100 individuals extracted at random from the sample. Additionally, in order to compare body width of mysids with that of crangonids in the same body size, body length(BL: the level of the posterior margin of the orbit to the tip of the telson)and body width (greatest body width)of both mysids and crangonids were measured, and regression lines between body length and body width were calculated.

Japanese flounder were sorted from the samples collected by the beam trawl net. Total length(TL) was measured to the nearest 0.05 mm with a caliper. Stomachs of flounder were dissected under a stereomicroscope. Stomach contents were classified into major taxa and mysids were identified to the level of species. Prey were enumerated and their wet weights were measured. The body lengths of mysids, crangonids and gammarids were measured.

Analyses of stomach contents

The relative importance(%*IRI*)[72]of a prey item was determined by frequency occurrence(%), number(%)and weight(%). An *IRI* value for each prey item was calculated as follows:

$$IRI_i = (\%nn_i + \%ww_i) \times \%F_i,$$

where %nn_i is the number of each prey item i as a percentage of the total number of all prey items identified, %ww_i is the percentage in wet weight of each prey item i to the total wet weight of all prey items identified, and %F_i is the frequency occurrence for each prey item i in the total number of guts examined. The *IRI* of each prey was standardized to % *IRI* :

$$\%IRI_i = 100 \times IRI_i / \Sigma IRI_i$$

Diet breadth was analyzed with the Simpson diversity[73], since this index, 1 - *D*, is not only unbiased, but also has a small standard deviation in the case of small samples[74]. The Simpson diversity of each predator was computed as follows:

$$1 - D = 1 - \Sigma N_i(N_i - 1)/N(N - 1),$$

where N_i is the number of each prey item i and N is the total number of all prey items.

To assess preference, an selective index, Chesson's *a* selectivity[75]for main prey items, was calculated

for each day. The selectivity, a_i, was as follows:

$$a_i = (r_i/n_i)/\Sigma(r_i/n_i), \quad i = 1, \cdots m,$$

where r_i and n_i are the proportions by number of prey i in the diet and in the environment, respectively. This index varies between 0 and 1 with values above $1/m$ indicating a positive preference, and those less than $1/m$ indicating a negative preference. The value of a that represents a neutral preference is 0.125 for $m = 8$.

Results

Abundance of food organisms in the nursery area

The food organisms in 1999 and 2000 consisted of 18 orders in 10 classes, being dominated by mysids,
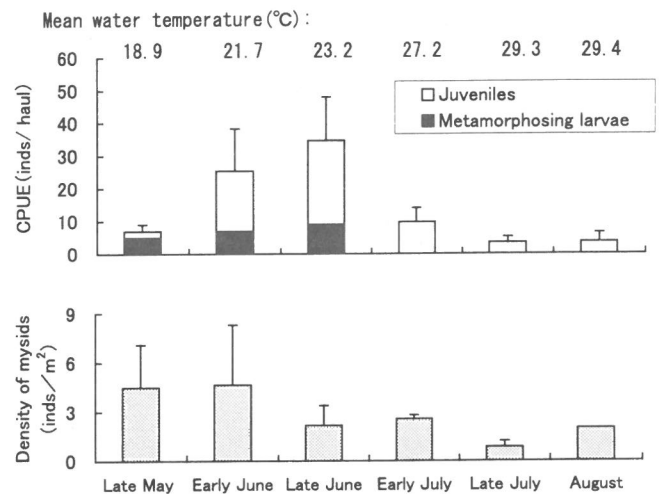

Fig. 3-8 Seasonal changes in CUPE of Japanese flounder (top) and density of mysids (bottom) at Ohama Beach. Vertical lines indicate standard error.
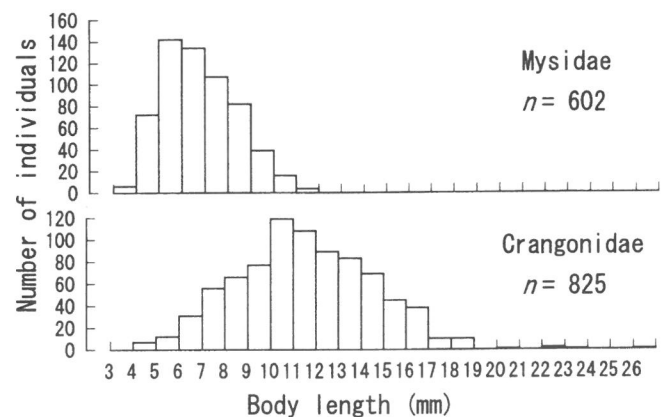

Fig. 3-9 Body lengthe distribtion of mysids and crangonids collected by the sledge net at Ohama Beach from 1999 to 2000.

Table 3-3 List of food organisms collected by the sledge net at Ohama Beach in 1999 and 2000

Class	Order	Family	Species	No. of Individuals	Weight (g)	%F
GASTROPODA	DISCODODA	Naticidae	*Glassaulax didyma*	3	0.16	20
	CEPHALASPIDEA	Philinidae	*Philine argentata*	22	0.09	40
BIVALVIA	VENEROIDA	Mactridae	*Mactra chinensis*	47	1.55	90
		Veneridae	*Ruditapes philippinarum*	1	0.01	10
CEPHALOPODA	SEPIOIDEA	Sepiolodae	*Euprymna morsei*	3	0.05	30
POLYCHAETA	PHYLLODOCIDA	Glyceridae	*Glycera sp.*	4	0.01	20
		Nereididae	*Platynereis bicanaliculata*	7	0.05	30
		Nephtyidae	*Nephtys neopolybranchia*	19	0.12	60
		Polynoidae	*Harmothoe imbricata*	2	0.02	10
	SPIONIDA	Spionidae	*Spiophanes bombyx*	1	+	10
OSTRACODA	MYODOCOPIDA		*Vargula hilgendorfii*	41	0.07	80
			OSTRACODA spp.	17	0.05	20
MALACOSTRACA	MYSIDACEA	Mysidae	*Iiella ohshimai*	300	0.88	100
			Nipponnimysis ornata	418	1.00	100
			Anisomysis ijimai	1	+	10
	AMPHIPODA	Amphithoidae	*Ampithoe lacertosa*	14	0.07	30
		Aoridae	*Aoroides sp.*	11	+	20
		Corophiidae	*Corophium acherusicum*	4	+	30
		Ischyroceridae	*Ericthonius pugnax*	24	0.01	50
			Jassa slatteryi	3	+	20
		Dexaminidae	*Paradexamine micronesica*	8	+	40
		Pontogeneiidae	*Pontogeneia sp.*	39	0.02	50
		Pleustidae	*Pleustes panoplus*	1	+	10
		Lysianassidae	*Anonyx sp.*	12	0.04	60
		Melitidae	*Melita japonica*	1	0.01	10
		Oedicerotidae	*Synchelidium lenorostralum*	612	0.62	100
		Urothoidae	*Urothoe sp.*	12	0.02	70
		Hyalidae	*Hyale sp.*	1	+	10
		Phtisicidae	*Protomima imitatrix*	2	+	20
		Csprellidae	*Caprella scaura*	7	0.01	10
			C. subtilis	5	+	10
			C. gigantochir	3	+	10
	ISOPODA	Cirolanidae	*Metacirolana sp.*	35	0.08	80
	CUMARIDA	Bodotriidae	*Bodotria sp.*	19	0.01	40
		Diastylidae	*Diastylis tricincta*	3	+	30
			D. sp.	3	+	30
	DECAPODA	Pasiphaeidae	*Leptochela gracilis*	14	0.22	20
		Hippolytidae	*Eualus sp.*	60	0.08	20
		Crangonidea	*Crangon spp.*	858	18.18	100
		Callianassidae	*Callianassa japonica*	26	0.04	70
		Diogenidae	*Diogenes nitidimanus*	1	0.12	10
		Portunidae	*Portunus pelagicus*	2	0.08	20
ASTEROZOA	FORCIPULATIDA	Asteriidae	*Asterias amurensis*	1	0.03	10
OPHIUROIDEA	MYOPHIURIDA	Ophiuridae	*Ophiuridae sp.*	336	1.12	50
ECHINOIDEA	CLYPEASTEROIDA	Astriclypeidae	*Astriclypeus manni*	284	3.77	100
OSTEICHTHYES	PERCIFORMES	Gobiidae	*Gobiidae sp.*	32	0.36	60
	GOBIESOCIFORMES	Callionymidae	*Repomucenus sp.*	5	0.91	30
	PLEURONECTIFORMES	Paralichthyidae	*Paralichthys olivaceus*	5	1.07	30
			Total	3,329		

%F, frequency occurrence; +, less than 0.005

amphipods, decapods, myophiurids and clypeasteroids (Table 3-3). *Nipponomysis ornata, Iiella ohshimai, Synchelidium lenorostralum* (gammarids), crangonids (*Crangon* spp.) and *Asterias amurensis* were always collected and these species were abundant. The mean density of mysids, gammarids, crangonids and fishes was 2.74, 2.91, 6.74 and 0.15 individuals [inds] /m², respectively (Table 3-4). The density of mysids was higher in late May and early June than after late June, although there was not significant difference among seasons (Fig. 3-8). Mysids were not abundant at a temperature higher than 23 ℃.

The body length of mysids and crangonids ranged from 2.4 to 10.6 mm and from 3.3 to 34.2 mm, respectively (Fig. 3-9). The mean length of crangonids (10.6 mm) was significantly greater than that of mysids (5.8 mm; t-test, $P < 0.01$). However, most of crangonids were smaller than 18mm in BL, and there was few crangonids larger than 25 mm, which could prey on newly settled flounder.[76] The liner regression equations for relationship between body length (L: in mm) and body width (W: in mm) are expressed as follows (Fig. 3-10) :

Mysids : $W = 0.13L + 0.23$ ($n=50$; F-test, $P<0.01$),
Crangonids: $W = 0.19L + 0.20$ ($n=50$; F-test, $P<0.01$).

Table 3-4 Mean density of food organisms at Ohama Beach from 1999 to 2001

Contents	inds/m²	mg/m²	Mean body weigh (mg/ind)
Mysidacea	2.74	7.56	2.76
(*Iiella ohshimai*)	(1.38)	(4.53)	(3.3)
(*Nipponomysis ornata*)	(1.28)	(2.87)	(2.2)
(*Anisomysis ijimai*)	(0.08)	(0.16)	(2.0)
(unidentified Mysides)	(−)	(−)	
Gammaridea	2.91	4.42	1.52
Crangonidea	6.74	106.49	15.78
Callianassidae	0.06	0.16	2.7
Brachyura	0.09	0.47	5.2
Fishes	0.15	6.20	
Others	3.12	29.62	

−, less than 0.005
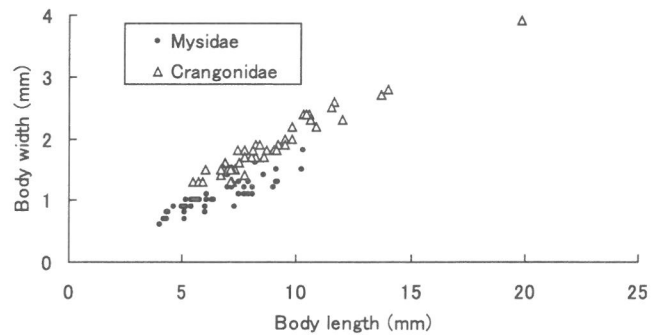
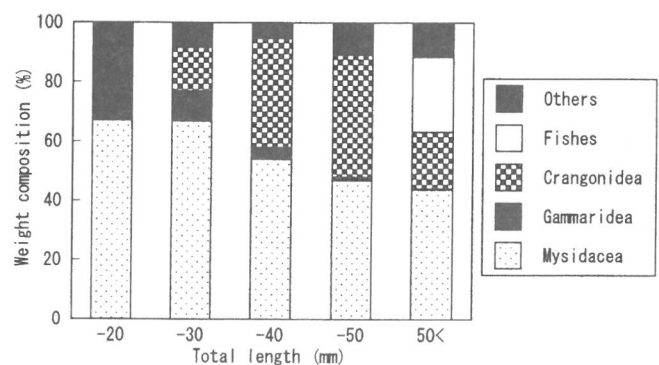**Fig.3-10** Relationship between body length and body width of mysids and crangonids.

The regression lines were significantly different between species (F-test, $P < 0.01$), and then the body width of crangonids was larger than that of mysids.

Seasonal changes in CPUE of Japanese flounder

The catch per unit effort (CPUE, inds/haul) of Japanese flounder is shown in Fig. 3-8. The CPUE increased from late May, peaked in late June, and decreased after July. Metamorphosing flounder larvae were collected from late May to late June.

Stomach content of flounder

The stomach contents of 202 wild flounder ranging from 9.80 to 75.95 mm in TL were examined. The feeding incidence (percentage of stomachs with food) was 82.7% (Table 3-5). The prey items consisted of copepods, mysids, gammarids, decapods and fishes, and the dietary diversity was 0.77. The most dominant prey items were mysids (%nn, 76.7; %ww, 51.7; %F, 58.9; %IRI, 73.8) including *N. ornata*, *I. ohshi-*

Fig.3-11 Stomach content composition in weight (%ww) by the size of larval and juvenile Japanese flounder collected at Ohama Beach from 1999 to 2001.
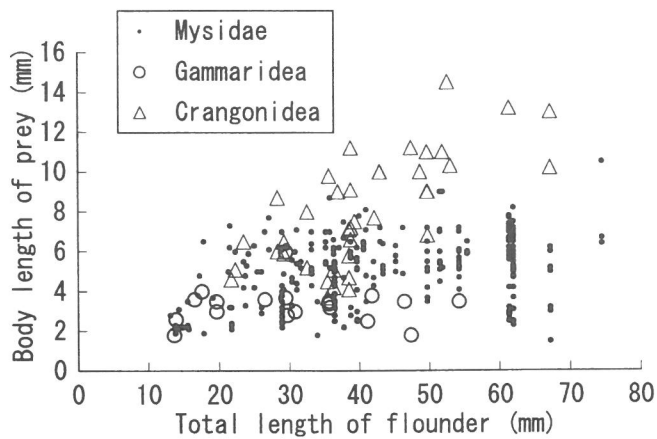
Fig.3-12 Size relationship between prey (body length) and flounder (total length).

mai and *Anisomysis ijimai*. Crangonids were the second most dominant prey taxon(%*nn*, 9.1; %*ww*, 27.0; %*F*, 12.6; %*IRI*, 17.2). Gammarids were the third most dominant prey taxon by number and frequency of occurrence(%*nn*, 7.7; %*ww*, 4.1; %*F*, 12.2; %*IRI*, 4.7). Conversely, fishes were the third most dominant prey taxon by weight, but were low in numerical importance(%*nn*, 1.3; %*ww*, 8.5; %*F*, 4.0; %*IRI*, 0.3). Copepods and brachyura were minor prey. Mysids and gammarids predominated in the stomachs of flounder smaller than 20 mm TL, but with growth, the percentage of gammarids decreased, whereas that of crangonids increased(Fig. 3-11). Flounder juveniles larger than 50 mm TL consumed fishes.

The size relationship between Japanese flounder and their prey is shown in Fig. 3-12. Body length of mysids consumed by flounder increased with the total length of the predator up to 30 mm. Then, the maximum size of the prey did not increase to larger than approximately 10 mm BL. The maximum size of crangonids consumed increased with growth of the predator, and the flounder 52.6 mm TL fed on prey 14.5 mm BL. The size of gammarids consumed ranged from 1.8 to 4.0 mm BL(mean: 3.3 mm). The mean body lengths were 5.0 mm(range: 1.5-10.5 mm) in mysids and 8.0 mm(3.7-14.5 mm)in crangons. The mean individual body weights of mysids and crangonids(*BW*: in mg)calculated from body length(*L*: in mm)using the following equations[69] were 1.00 mg and 9.29 mg, respectively:

Mysids(*N. ornata*) : $BW=0.010L^{2.862}$,

Crangonids(*C. uritai*) : $BW=0.013L^{3.162}$.

Table 3-5 Feeding incidence, dietary diversity, number(%nn), weight(%ww), frequency occurrence (%F) and index of the relative importance (IRI) of prey items of larvae and juvenile Japanese flounder collected at Ohama Beach from 1999 to 2001

Number		202		
Range of total length (mm)		9.80 − 75.95		
Feeding Incidence (%)		82.7		
Dietary diversity		0.77		
Contents	*%nn*	*%ww*	*%F*	*%IRI*
Copepoda	0.1	0.1	0.4	−
Mysidae	76.7	51.7	58.9	73.8
(*Iiella oshimai*)	15.3	7.8	12.2	10.6
(*Nipponomysis ornata*)	41.3	26.8	16.5	42.5
(*Anisomysis ijimai*)	11.9	6.8	5.7	4.0
(*Misidae fragments*)	8.2	10.3	23.9	16.7
Gammaridea	7.7	4.1	12.2	4.7
Crangonidea	9.1	27.0	12.6	17.2
Callianassidae	1.8	2.7	3.9	0.7
Brachyura	0.1	0.1	0.4	−
Fishes	1.3	8.5	4.0	0.3
(Callionymidae)	0.3	2.2	0.9	0.1
(Gobiidae)	0.6	3.9	0.9	0.1
(Fish fragments)	0.4	2.4	1.3	0.1
unidentified	3.1	6.1	9.1	3.2

−, less than 0.05

Selective predation

The selectivity(Chesson's *a*)on major prey items is shown in Table 3-6. Only the selectivity on *N. ornata* was significantly higher than neutral preference (*a* =1/*m*=0.125; *m*, number of the major food item; Willcoxon signed-rank test, *P* < 0.05). The mean selectivities on *A. ijimai*(0.567)and gobiidae(0.167) were higher than neutral preference, but significant differences were not observed. The mean selectivity on *I. ohshimai* was 0.106. Japanese flounder preferred *N. ornata* and *A. ijimai* to *I. ohshimai*. Crangonids were avoided(Willcoxon signed-rank test, *P* < 0.05).

Table 3-6 Selectivities（Chesson's α ）on the major food items *

Contents	Mean	Range
Mysidae		
Iiella ohshimai	0.106	0.003-0.0377
Nipponomysis ornata	0.753	0.383-1
Anisomysis ijimai	0.567	0.137-0.997
Gammarids	0.076	0.001-0.223
Crangonidea	0.011	0.0001-0.032
Callianassidae	0.097	0.008-0.234
Fishes		
Callionymidae	0.076	0.363-0.117
Gobiidae	0.167	0.012-0.322

*The neutral preference was 0.125

Discussion

Abundance of food organisms in the nursery area

The mean density of mysids and crangonids was 2.74 and 6.74 inds/m^2, respectively. The density of mysids in the Sea of Japan[8,10,15], Ono Bay and Miyako in the northern Pacific Ocean[77] were 100-200, 800-2700 and 90 inds/m^2, respectively. The density of crangonids in the Sea of Japan was 0.5-4.0 inds/m^2 [10,78]. These results indicate that mysids were not more abundant in this area than in the Sea of Japan and the Pacific Ocean, while crangonids were more abundant in this area than in the Sea of Japan.

The density of fishes in this area was 6.20 mg/m^2, and was lower than that（16.2-124.4 mg/m^2）in exposed areas of Iwate Prefecture[14]. In addition, Japanese anchovy *Engraulis japonicus* was dominant prey of flounder in Iwate Prefecture[14], Chiba Prefecture[57] and the Sea of Japan[15,62], but were not found in the stomachs of flounder in this area. This species was dominant in the shallow waters of Tottori Prefecture[79] and Iwate Prefecture[80], and in the surf zone of Kashima-Nada[81]. However, the abundance of the species would be low at sandy beaches in this area, because they were not observed in food organisms and the samples collected by a small triangle net in the surf zone of Hiuchi-Nada[82].

Characteristics diet of juveniles in this area

The IRI of the prey taxa ranked in the order mysids > crangonids > gammarids > fishes. Generally, the main prey of juvenile flounder were only mysids and fishes, and the abundance of crangonids is remarkably low in the nursery grounds（e. g. Imabayashi, 1980[13]; Fujii and Noguchi, 1996[8]; Tanaka et al., 1999[15]）. However, in this area, the major prey was characterized by crangonids, and flounder juveniles ate a variety of diet. This result supports the hypothesis on the diet（hypothesis 2）.

Relationship between settlement of metamorphosing larvae and abundance of mysids

Metamorphosing flounder larvae occurred from late May to late June, when abundance of mysids was high. These results indicate that settlement of larvae was synchronized with abundance of mysids. This observation is consistent with the hypothesis 1.

Predation pressure on larval and juvenile Japanese flounder from large crangonids

Most of crangonids were at less than 18mm BL, and few large crangonids（> 25 mm BL）were collected. This result suggests that there might be not high predation pressure on newly settled flounder from large crangonids, and supports only part on crangonids in the hypothesis 3.

Verification of hypotheses

In this chapter, I proved the hypothesis 1, hypothesis 2 and a part of the hypothesis 3 true.

Chapter 4. Feeding ecology of dominant fish species
A : Feeding habits of *Favonigobius gymnauchen*, *Repomucenus* spp. and *Tarphops oligolepis*

Introduction

At the beaches in the present study, flatfishes, gobies and dragonets predominated (Chapter 3A). These dominant fish species preyed on newly settled flounder at Yanagihama Beach in Nagasaki Prefecture[22,23]. Thus, predation by these fishes might influence the stock of Japanese flounder[9]. However, in the Seto Inland Sea, the stock of Japanese flounder is abundant[20,44], nevertheless the abundance of juvenile flounder was relative low (Chapter 3A). Therefore, in order to examine predation pressure on newly settled flounder from dominant fishes in this area, I hypothesized as follows: dominant fish species and crangonids are not predators for juvenile Japanese flounder (hypothesis 3). Here, only part on crangonids in this hypothesis was proved true in Chapter 3B.

For the verification of the hypothesis 3, the present chapter describes the feeding ecology of *Favonigobius gymnauchen*, *Repomucenus* spp. and *Tarphops oligolepis* at the sandy beach in eastern Hiuchi-Nada. In addition, I discussed the interspecific relationships among the dominant demersal fishes.

Materials and methods

Field sampling

Sampling surveys were carried out for 15 days during the period from May to August in 1999, 2000 and 2001 at Ohama Beach (Fig. 4-1). The SNFRI type net was towed 200 m by a boat along the shoreline. The samples were immediately fixed in 10% formalin. The sampling procedure is described in more detail in Chapter 3B.

Sample treatments

Fishes, mysids and crangonids were sorted from the samples in the laboratory. Numbers of individuals and wet weights in each species were measured, and subsequently these samples were preserved in 70% ethanol. Two species of dragonets, *Repomuce-*
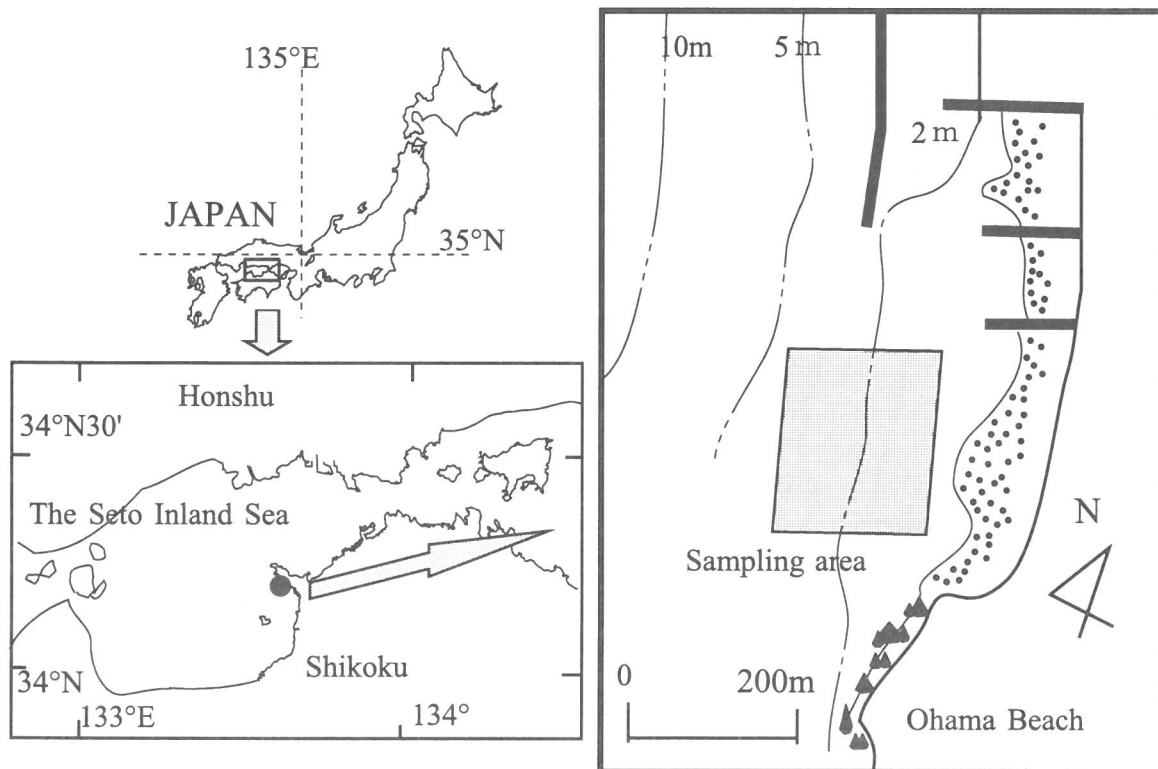

Fig.4-1 Eastern Hiuchi-Nada showing the sampling area. Water depths indicate the distance from the surface to the bottom at the mean low spring tide.

nus richardsonii and R. beniteguri, were treated as Repomucenus spp. due to difficulty in the identification of small fishes.

Total length (TL) and upper jaw length (UJL: linear distance from anteriormost point of premaxilla to posteriormost point of maxilla) or mouth width (MW: linear distance between right and left most posterior points of maxilla) of F. gymnauchen, Repomucenus spp. and T. oligolepis were measured to the nearest 0.1 mm with a caliper. When more than 10 individuals of F. gymnauchen and Repomucenus spp. were obtained in a sampling day, we measured 10 individuals (>20 mm in TL) chosen at random from the samples. Additionally, to compare of mouth size between Japanese flounder and T. oligolepis, I measured total length and upper jaw length of Japanese flounder collected with these three fish species, and then deference of correlation coefficients of the regression line was determined by analysis of covariance (ANCOVA).

Gut contents (F. gymnauchen and T. oligolepis: stomach; Repomucenus spp.: fore quarter of gut) of the specimens were classified into major taxa and mysids were identified to the species level under a stereomicroscope. Number of individuals in each prey were counted and their wet weights were measured to the nearest 0.1 mg (AEX-200B, Shimadzu Corporation, Japan). Body lengths (mysids and shrimp: from the level of the posterior margin of the orbit to the tip of the telson; gammarids: from the rostrum to the tip of the tellson) of undigested mysids, crangonids and gammarids were measured to the nearest 0.1 mm under a stereomicroscope.

Data analysis

Relationship between feeding incidence (percentage of stomach with food) and fish total length examined using Spearman's rank correlation coefficient test. The relative importance of each prey item (%IRI; Cortes)[72] was calculated using the following equation:

$$IRI_i = (\%nn_i + \%ww_i) \times \%F_i,$$

where $\%nn_i$ is the percentage in number of each prey item i, $\%ww_i$ is the percentage in wet weight of each prey item i, and $\%F_i$ is the percentage frequency occurrence for each prey item i. The IRI of each prey item was standardized to %IRI:

$$\%IRI_i = 100 \times IRI_i / \Sigma IRI_i.$$

Diet breadth was analyzed with the Simpson diversity[73] as follows:

$$1 - D = 1 - \Sigma N_i(N_i - 1)/N(N - 1),$$

where N_i is the number of each prey item i and N is the total number of all prey items.

To assess preference, an selective index, Chesson's a selectivity[74] for main prey items, was calculated. The selectivity, a_i, was as follows:

$$a_i = (n_i/r_i) / \Sigma (n_i/r_i), \quad i = 1, \cdots m,$$

where n_i and r_i are the proportions of the number of prey i in the diet and in the environment, respectively. The r_i values used data of Chapter 3B. The selective index varies between 0 and 1 with values greater than $1/m$ indicating a positive preference, and those less than $1/m$ indicating a negative preference. The value of a that represents neutral preference is 0.20 for $m = 5$.

Dietary overlaps among dominant species including Japanese flounder at Ohama Beach were measured for each sampling day with Pianka's O index[83], which was as follows:

$$O_{12} = \Sigma p_{1i} p_{2i} / \Sigma (p_{1i}^2 p_{2i}^2)^{(1/2)}$$

where p_{1i} and p_{2i} are the proportions of the number of prey item i in predator 1 and predator 2, respectively. This index ranges from 0 (no overlap) to 1 (complete overlap). Here, I used the data on the stomach contents of Japanese flounder in Chapter 3B (Table 3-5).

Results

Abundance of fishes, mysids and crangonids

A total of 4,460 individual fishes including more than 25 species in 15 families were collected by 35 operations of the trawl net at Ohama Beach (Table 4-1). In terms of individual numbers, F. gymnauchen (50.7%), Rudarius ercodes (26.1%), Repomucenus spp. (7.0%), Heteromycteris japonica (4.7%), Japanese flounder P. olivaceus (4.3%) and T. oligolepis (2.7%) were dominant. Of which F. gymnauchen, Repomucenus spp., P. olivaceus and T. oligolepis comprised 64.7% of the total number and 95.6% of the total weight of fishes collected. Since sizes of R. ercodes and H. japonica were small (major size: <15 mm in TL), the stomachs of two species were not examined. Although large dragonets (> approximately 40 mm TL) were composed of R. richardsonii and R. beniteguri (mostly R. beniteguri), smaller individuals were not identified.

Table 4-1 Demersal fish species collected at Ohama Beach from May to August in 1999, 2000 and 2001

Family	Species	No. of Inds.	Weight (g)
Clupeidae	*Konosirus punctatus*	3	0.1
Syngnathidae	*Syngathus schlegeli*	3	0.2
	Hippocampus mohnikei	3	0.4
Scorpaenidae	*Sebastes inermis*	1	1.8
	Inimicus japonicus	2	6.8
Congiopodidae	*Hypodytes rubripinnis*	18	3.9
Platycephalidae	*Platycephalidae* spp.	80	3.2
Cottidae	*Pseudoblennius cottoides*	2	1.6
Sparidae	*Pagrus major*	7	0.5
	Acanthopagrus schlegeli	31	2.2
Labridae	*Halichoeres poecilopterus*	3	94.2
Blenniidae	*Scartella emarginata*	2	0.1
Callionymidae	*Repomucenus* spp.	313	214.7
Gobiidae	*Sagamia genetonema*	5	2.0
	Favonigobius gymnauchen	2262	5612.7
	Tridentiger trigonocephalus	2	0.1
	Gobiidae spp.	24	0.4
Paralichthyidae	*Paralichthys olivaceus*	190	73.2
	Tarphops oligolepis	122	72.4
Soleidae	*Heteromycteris japonica*	211	122.0
	Pseudaesopia japonica	1	–
Monacanthidae	*Rudarius ercodes*	1166	33.9
	Thamnaconus modestus	1	0.6
Tetraodontidae	*Takifugu niphobles*	2	0.1
Unidentified		6	0.7
	Total	4460	6247.9

－ : less than 0.05g

Besides fishes, 32,235 mysids (wet weight: 166.1 g) and 120,491 crangonids (3591.9g) were also collected. The dominant mysids were *Iiella ohshimai* and *Nipponomysis ornata*.

Gut contents

Of 150 individuals of *F. gymnauchen* examined, which ranged from 20.7 to 71.4 mm in TL, 76.7% contained food (Table 4-2). In *Repomucenus* spp., 84.4% of 90 individuals examined contained food. Although size of this species (n= 313) ranged from 20.1 to 107.4 mm TL, there was few individuals ranging from 30 to 40 mm (n= 5). The feeding incidence of 122 individuals of *T. oligolepis* ranging from 9.0 to 75.4 mm TL was 87.7%. The feeding incidences of these three fish species were not significantly correlated with body size (Spearman's rank correlation coefficient test, $P > 0.3$).

The main prey items of the three fish species were small crustaceans such as mysids, gammarids, and decapods, but larvae and juvenile Japanese flounder were not observed in the guts of any fishes examined (Table 4-2). *F. gymnauchen* consumed mysids (%*IRI*: 67.9%) and crangonids (26.0%). The %*IRI* of *I. ohshimai* (30.5%) was higher than that of *N. ornata* (20.4%). Other prey items were gammarids, brachyurans, fishes and eggs of *Hemiramphus sajori*, but these %*IRI* values were very low. In the gut of *Repomucenus* spp., mysids and crangonids predominated, and sand grains were often observed (frequency occurrence: approximately 40%). *T. oligolepis* consumed mainly mysids, particularly *N. ornata*. The dietary diversity (Simpson diversity index, 1-D) of *F. gymnauchen* and *Repomucenus* spp. of 0.81 and 0.80, respectively, were higher than that of *T. oligolepis*, 0.66.

Relationship between mouth size and fish length

Mouth size (upper jaw length and/or mouth width) was significantly correlated with total length (Table 4-3). The slopes of the regression lines were signifi-

Table 4-2 Feeding incidence, dietary diversity, number (%*nn*), weight (%*ww*), frequency occurrence (%*F*) and index of relative importance (%*IRI*) of the three dominant fish species at Ohama Beach in 1999, 2000 and 2001

	Favonigobius gymnauchen				*Repomucenus* spp.				*Tarphops oligolepis*			
Number	150				90				122			
Range of TL (mm)	20.7 – 71.4				20.1 – 107.4				9.0 – 75.4			
Feeding Incidence (%)	76.7				84.4				87.7			
Dietary diversity	0.81				0.80				0.66			
Contents	%*nn*	%*ww*	%*F*	%*IRI*	%*nn*	%*ww*	%*F*	%*IRI*	%*nn*	%*ww*	%*F*	%*IRI*
Polychaeta					1.0	9.3	3.3	0.9				
Ostracoda [*Vargula hilgendorfii*]	0.9	0.1	1.3	–								
Copepoda									2.3	0.4	5.7	0.2
Mysidae	69.2	48.8	49.3	67.9	68.4	44.0	46.7	71.1	84.3	71.5	67.2	92.6
(*Iiella ohshimai*)	30.2	23.0	18.7	30.5	18.6	14.1	13.3	11.3	18.8	17.5	24.6	14.0
(*Nipponomysis ornata*)	22.0	16.3	17.3	20.4	11.3	7.5	12.2	5.9	54.4	47.6	45.9	73.4
(*Anisomysis ijimai*)	1.8	1.4	2.0	0.2	1.3	1.3	3.3	0.2	2.5	2.0	6.6	0.5
(*Mysidae fragments*)	15.3	8.2	23.3	16.8	37.2	21.1	35.6	53.7	8.5	4.4	23.8	4.8
Gammaridea	5.3	2.3	8.0	1.9	7.0	1.4	10.0	2.2	5.2	3.7	9.8	1.4
Caprellidea	0.6	0.3	1.3	–	2.0	0.5	1.1	0.1				
Flabellifera					0.7	0.2	2.2	–				
Tanaidacea	0.6	0.0	0.7	–								
Cumacea									0.3	0.4	0.8	–
Penaeidea	0.3	0.2	0.7	–								
Crangonidea	14.1	41.2	15.3	26.0	11.0	27.2	16.7	16.5	3.7	15.5	12.3	3.7
Callianassidae					0.3	0.6	1.1	–	2.0	3.5	9.0	0.8
Brachyura	1.8	1.3	2.0	0.2	1.3	0.8	4.4	0.2				
Fishes	1.2	1.6	2.7	0.1	0.7	4.6	2.2	0.2	0.3	1.0	1.6	–
(*Repomucenus spp.*)					0.3	1.5	1.1	0.1	0.1	0.1	0.8	–
(*Favonigobius gymnauchen*)	0.9	1.3	2.0	0.1	0.3	3.1	1.1	0.1	0.1	0.9	0.8	–
(*Tarphops oligolepis*)	0.3	0.3	0.7	–								
Egg of Hemiramphus sajori	0.6	0.4	0.7	–	1.7	1.0	4.4	0.3				
Unidentified	5.6	4.0	12.7	3.7	6.0	10.4	20.0	8.5	2.0	4.1	13.1	1.3

– ; less than 0.05%

Table 4-3 Relationships between upper jaw length (*UJL* : in mm)/ mouth width (*MW* : in mm) and total length (*TL* : in mm) of *Favonigobius gymnauchen*, *Repomucenus* spp., *Tarphops oligolepis* and *Paralichthys olivaceus*

	Equation[*]	df	r^2	F	P[*]
F. gymnauchen	$UJL = 0.0523 TL + 0.209$	49	0.81	207	<0.01
	$MW = 0.0913 TL - 0.00068$	49	0.83	251	<0.01
Repomucenus spp.	$MW = 0.0646 TL + 0.149$	49	0.94	766	<0.01
T. oligolepis	$UJL = 0.0678 TL + 0.600$	29	0.90	273	<0.01
P. olivaceus	$UJL = 0.103 TL - 0.0704$	29	0.97	1034	<0.01

[*]Regression equations and significance levels were shown

cantly different between *P. olivaceus* and *T. oligolepis* (ANCOVA, $P < 0.01$), and the mouth size of *P. olivaceus* was larger than that of *T. oligolepis* at the same total length larger than 15.0 mm.

Diet shift with growth

The reverse trends of diet shift with growth were observed in mysids and crangonids in Diets of *F. gymnauchen* shifted from mysids to crangonids in weight composition as its growth (Fig. 4-2). The diet
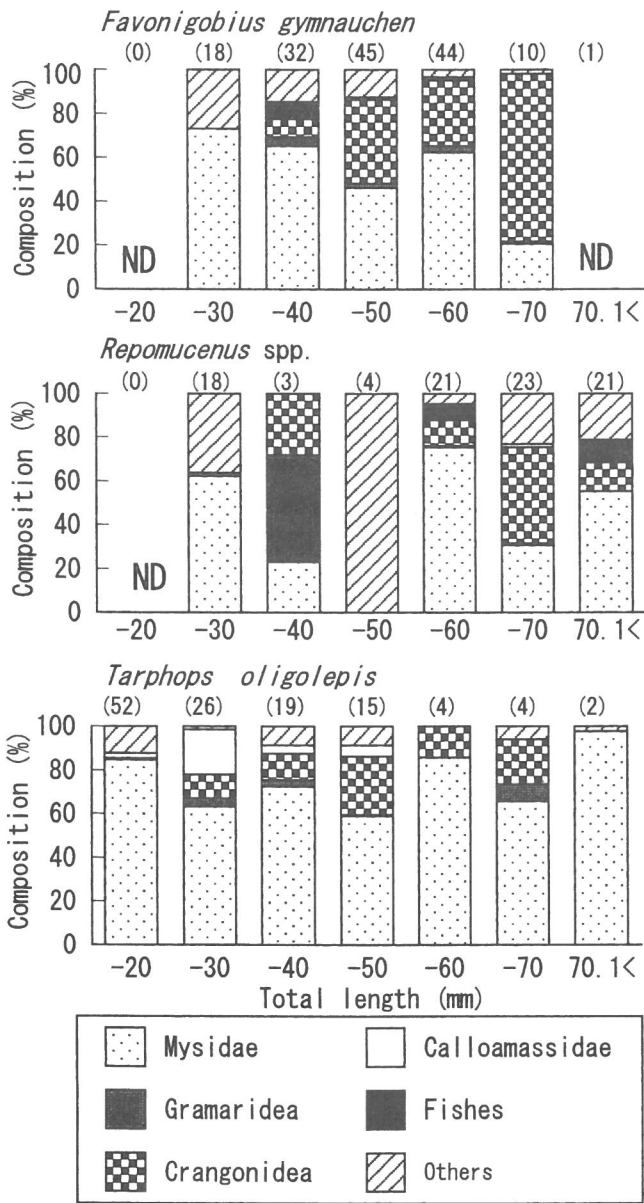
Fig.4-2 Wet weight composition of stomach contents by size for *Favonigobius gymnauchen*, *Repomucenus* spp. and *Tarphops oligolepis*. Numerals above bars indicate the number of individuals examined.

of small *Repomucenus* spp.(>40 mm TL)was composed of mysids, gammarids and crangonids. With an increase in size, the percentage of gammarids decreased, whereas that of fishes increased. Large *Repomucenus* spp. consumed mysids, crangonids and fishes. The stomach contents of *T. oligolepis* consisted primarily of mysids at all sizes and no ontogenetic shift in the feeding habits was observed.

The size relationships among the three fish species and their major prey items are shown in Fig. 4-3. In *F. gymnauchen* until 40 mm, *Repomucenus* spp. until 50 mm and *T. oligolepis* until 30 mm, the body length(BL)of mysids consumed increased with the total length of the predators. The maximum size of mysids eaten by *F. gymnauchen*, *Repomucenus* spp. and *T. oligolepis* was 9.3 mm(mean: 5.9 mm in BL), 10.0 mm(6.4 mm)and 8.3 mm(4.7 mm), respectively. These maximum sizes(approximately 9 mm BL) was consistent with that of mysids in the environment(Chapter 3B). The maximum size of crangonids eaten increased with the growth of the predators, and the mean body lengths observed in the guts of *F. gymnauchen*, *Repomucenus* spp. and *T. oligolepis* were 8.3 mm(range: 5.2-11.8 mm), 8.5 mm(5.0-11.3 mm) and 7.7 mm(3.8-10.2 mm), respectively. In gammarids, it was not possible to analyze the size relationship between predator and their prey due to lack of prey body length. However, the mean body length of gammarids eaten by *F. gymnauchen*, *Repomucenus* spp. and *T. oligolepis* were 2.3 mm(range: 0.8-3.8 mm), 1.3 mm(0.8-1.8 mm)and 2.2 mm(0.8-4.2 mm), respectively.

Prey selection
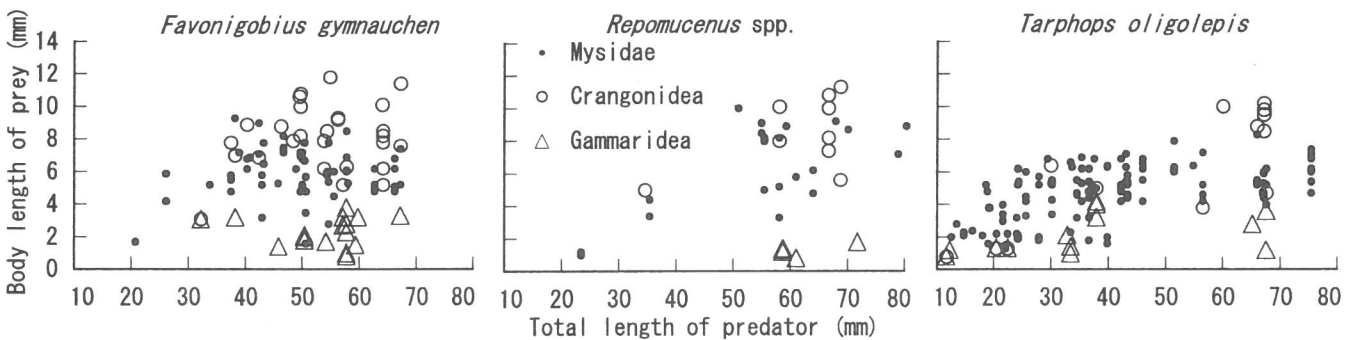
The selectivity, Chesson's *a*, on the major prey

Fig.4-3 Size relationship between major prey taxa (body length) and predator (total length) for *Favonigbius gymnuchen*, *Repomuceus* spp. and *Tarphops oligoleis*.

Table 4-4 Selectivity (Chesson's a) on the major food items for *Favonigobius gymnauchen*, *Repomucenus* spp. and Tarphops oligolepis at Ohama Beach in 1999, 2000 and 2001*

Contents	*F. gymnauchen* Mean	(range)	*Repomucenus* spp. Mean	(range)	*T. oligolepis* Mean	(range)
Mysidae						
Iiella ohshimai	0.303	(0.004-0.899)	0.242	(0-0.84)	0.156	(0.006-0.558)
Nipponomysis ornata	0.576	(0.083-1)	0.524	(0.247-0.732)	0.747	(0.241-1)
Anisomysis ijimai	0.402	(0.153-0.847)	0.460	(0.180-0.740)	0.203	(0-0.541)
Gammaridea	0.093	(0-0.217)	0.203	(0-1)	0.037	(0-0.178)
Crangonidea	0.221	(0-1)	0.150	(0-0.637)	0.015	(0-0.089)

*The neutral preference was 0.20

items is shown in Table 4-4. In *F. gymnauchen*, only the selectivity on *N. ornata* was significantly higher than neutral preference (Wilcoxon signed-ranks test, $P < 0.05$), although those for the other mysids, *I. ohshimai* (0.30) and *A. ijimai* (0.40), and crangonids (0.22) were greater than neutral preference (0.20). *Repomucenus* spp. selected *N. ornata* significantly ($P < 0.05$), and consumed other prey items at random ($P > 0.1$). *T. oligolepis* had positive selectivity on *N. ornata* and negative selectivity on gammarids and crangonids ($P < 0.01$). The trends of the selectivity of *Repomucenus* spp. and *F. gymnauchen* were similar each other, whereas *T. oligolepis* differed from the other two fishes, with a higher preference for *N. ornata*. *T. oligolepis* had the most strong diet selectivity and specialized diet among the three fish species.
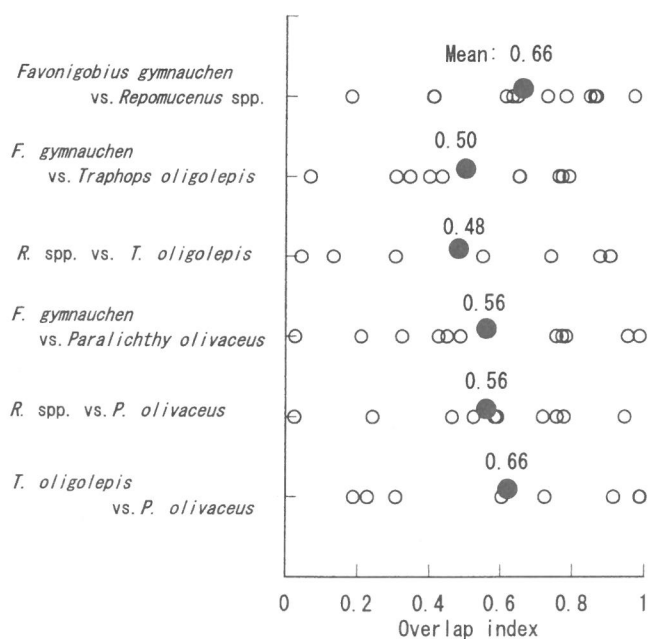
Diet overlap

All of the mean overlap values, Pianka's O index, for pair species from *F. gymnauchen*, *Repomucenus* spp. *T. oligolepis* and *P. olivaceus* ranged from 0.48 to 0.66, although the value of each sampling day greatly fluctuated (Fig. 4-4). Those for pairs of *F. gymnauchen* and *Repomucenus* spp., *T. oligolepis* and *P. olivaceus* showed relatively higher values.

Discussion

Gut contents and prey selection

F. gymnauchen consumed mysids, crangonids and small fishes including this species. At Yanagihama Beach in Nagasaki Prefecture, the eastern East China Sea, the prey items of *F. gymnauchen* were decapods, gammarids, polychaetes, mysids and fishes[22]. These results indicate that this species was carnivo-

rous and a benthos feeder.

T. oligolepis actively selected the epifaunal mysids *N. ornata*. *P. olivaceus* also preferred *N. ornata* than sand-burrowing mysids (Chapter 3B). The importance of mysids as food for *P. olivaceus*, however, was relatively low in this area compared to Japan Sea due to the low density of mysids (Chapter 3B), although newly settled flounder generally fed chiefly on mysids[7,13,15]. In contrast, *T. oligolepis* mainly consumed mysids of all sizes in this area. The results

Fig.4-4 The overlap values, Pianka's O index, between pair of two fish species from *Favonigobius gymnauchen*, *Repomuceus* spp., *Tarphops oligilepis* and *Paralichys olivaceus*. Open and closed circles indicate the value of each dampling day and the mean values, respectively. Data on the Japanese flounder after Chapter 3B.

indicate that the preference for mysids was higher in *T. oligolepis* than in *P. olivaceus* irrespective of abundance of mysids.

Diet overlap

The mean overlap values among *F. gymnauchen*, *Repomucenus* spp., *T. oligolepis* and *P. olivaceus* were relatively high, since the four fish species fed on crustaceans such as mysids and crangonids (Fig. 4-5). The mean body length of mysids, crangonids and gammarids in the stomach of *P. olivaceus* in this area was 5.0 mm (range: 1.5-10.5 mm), 8.0 mm (3.7-14.5 mm) and 3.3 mm (1.8-4.0 mm), respectively (Chapter 3B), showing the size of prey in *P. olivaceus* to be similar to that of the three fish species. From these results, I propose that there would be interspecific competition among the four fish species. Additionally, since crangonids consumed mysids in this area[69], there might be exploitative competition between crangonids and the four fish species. A more detail study on daily rations and feeding rhythms of these species is necessary in order to estimate food condition in nursery grounds (Chapter 4B).

Predation on newly settled Japanese flounder by the three dominant fish species

Generally, the major cause of mortality of larval and juvenile Japanese flounder would be predation by fishes and crustaceans[9]. Noichi et al.[22,23] have reported that many fishes including the three fish species preyed on newly settled flounder at Yanagihama Beach in Nagasaki Prefecture. However, far more serious predators[4,22,23], *Trachinocephalus myops*, *Pseudoblennius percoides*, *P. cottoides* and *Chelidonichthy spinsus*, were not abundant in this area (Table 4-1). The three dominant fishes preyed on small *F. gymnauchen*, *Repomucenus* spp. and *T. oligolepis*, but these %*IRI* values were very low. Moreover, newly settled flounder was not found in the guts of the fishes examined. These results suggest that predation pressure on newly settled Japanese flounder by the three fish species might not be high in this area, and support a part of the hypothesis 3 on the interspecific composition between dominant fish species.

Verification of hypothesis

In this chapter, the result is consistent with a part of the hypothesis 3 on the interspecific composition between dominant fishes. Then, the results in this chapter and chapter 3B proved the hypothesis 3 true.
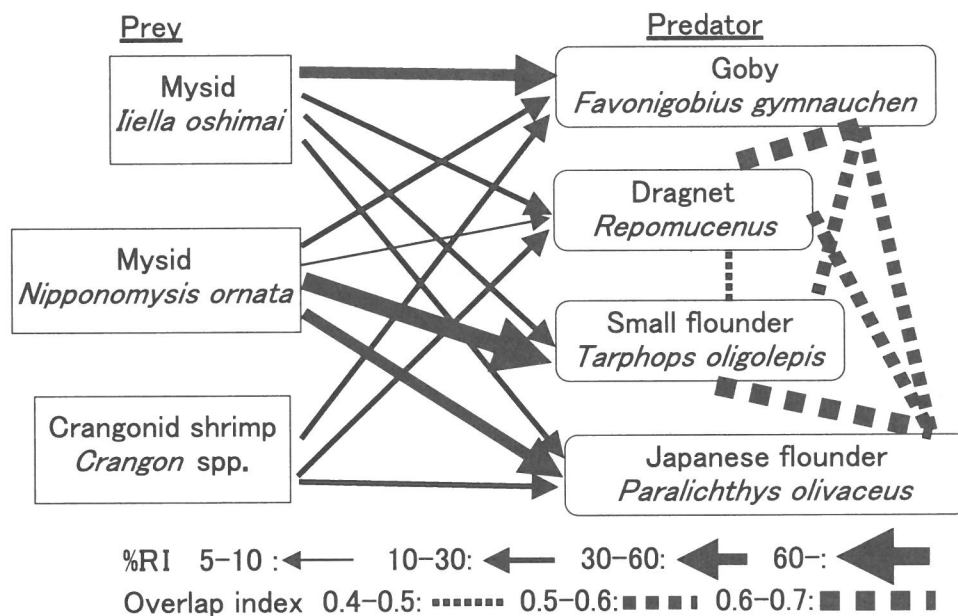
Fig.4-5 Schematic diagram of food habits of *Favonigobius gymnauchen*, *Repomucenus* spp., *Tarphops oligolepis* and *Paralichthys olivaceus*. Diet overlaps and predator-prey relationship are shown.

B : Daily rations of Paralichthys olivaceus, Tarphops oligolepis and Favonigobius gymnauchen and diel variation in food availability

Introduction

Daily ration of juvenile Japanese flounder was affected by food availability[8,17,84], and is one of the important elements to estimate feeding conditions in nursery ground. Flatfishes, gobies and dragonet were sympatrically distributed with Japanese flounder in eastern Hiuchi-Nada(Chapter 3A and 4A). The dominant fishes including flounder consumed mysids (Chapter 3B and 4A), of which density was low in this area(Chapter 3B), suggesting that sufficient mysids might not be available for flounder. However, growth rate of flounder is probably greatest among those in demersal juvenile fishes at sandy beaches. In consideration on these observations, I set up a hypothesis concerning daily ration as follows: daily food consumption of flounder is greatest among those in dominant fishes, namely juvenile Japanese flounder can use the largest amount of mysids among the members of the inmate demersal fishes(hypothesis 4).

In this chapter, to establish the hypothesis 4, I estimated the daily rations of the three dominant fish species, *Paralichthys olivaceus*, *Tarphops oligolepis* and *Favonigobius gymnauchen* using the Elliott and Person model[85], and also clarified diel variation in food availability at the sandy beach.

Materials and methods

Sampling

A total of 11 sampling surveys were carried out at 1.5 or 3-hours interval over a 24-hours period beginning at 12:00 on 26 June, 2003 at Ohmana Beach (Table 4-5, Fig. 4-1). In this chapter, 'day' and 'night' refer to the period between 6:00 and 18:00, and between 21:00 and 3:00, respectively(sunset, 19:20; sunrise, 4:35). For collection of fishes, the 2-m beam trawl net was towed 200 m(swept area: 400 m^2)by a boat along the beach at a speed of approximately 1.0 knot. One to two hauls of the beam trawl net were taken during each sampling to obtain sufficient samples. The collected fishes were packed on ice immediately and frozen at –20℃ within 30 minutes. Food organisms were collected with a two-layered sledge net(net mouth 0.3 + 0.3 m high and 0.6 m wide, mesh size 0.33 mm)for 30 m, and was immediately preserved in 10% seawater formalin. Surface water temperature was measured with a digital thermometer(SK-250WP, Sato Keiryoki Corporation, Japan) and water depth with a digital sounder(PS-7, Honda electronics Corporation).

Table 4-5　Collection records of fishes and food organisms at Ohama Beach on 26-27 June 2003

Time	WT (℃)	Tide[a]	Sledge net No. of hauls	Depth (m)[b]	Beam trawl No. of hauls	Depth (m)	Number of sample P.olivaceus	T. oligolepis	F. gymnauchen
12:00	25.7	ebb	1	2.8	2	2.8	17	21	16
15:00	26.0	ebb	1	2.8	2	2.4	30	26	13
18:00	25.7	flood	1	1.6	1	3.0	32	21	4
19:30	25.2	flood	1	2.6	1	4.1	13	6	12
21:00	25.0	flood	1	4.4	2	4.2	32	21	22
0:00	24.0	ebb	1	2.5	1	3.0	16	10	22
3:00	23.4	ebb	1	2.0	2	2.1	29	40	20
4:30	23.0	flood	1	2.5	1	2.0	18	22	9
6:00	23.2	flood	1	2.4	1	3.3	13	10	4
9:00	24.7	flood	1	3.3	2	3.6	18	14	10
12:00	24.3	ebb	1	2.4	2	3.3	14	20	23
mean	24.6	–		2.7	–	3.0	Total 232	211	155

[a] The moon age was 25.9 (low tide, 15:04 and 4:15; high tide, 22:04 and 9:08).

[b] The distance from the surface to the bottom at sampling

Sample treatments

Fishes collected by the beam trawl net were classified into species. Total length (TL) was measured to the nearest 0.1 mm with a caliper and wet body weight (BW) to the nearest 0.1 mg with a digital balance (AEX-200B, Shimadzu Corporation, Japan), respectively. Stomachs of the three dominant fish species, *P. olivaceus* ($n = 232$), *T. oligolepis* ($n = 211$) and *F. gymnauchen* ($n = 155$), were extracted and preserved in 10% buffered formalin (Table 4-5). Stomach contents were identified to the lowest possible taxonomic level and number of individuals in each prey taxa was determined under binoculars. Wet weight of prey taxa (G) was measured to the nearest 0.1 mg after blotting with filter paper. The mean stomach content index (SCI) for each prey group i at sampling time j (S_{ij}) was calculated as follows:

$$S_{ij} = \Sigma\, (G_{ij}/W_{ij})/n \times 100 \tag{4-1},$$

where G_{ij} is the wet weight of each prey group i of each fish sampled at time j, W_{ij} is the wet body weight excluding total stomach content weight of each fish sampled at time j and n is the number of individuals sampled at time j.

Food organisms were identified to the lowest possible taxonomic level. Wet weight in each taxon was measured, and density was calculated as weight per swept area (18 m^2). Here, catch efficiency of the sledge net was not considered.

Point estimates of daily ration and instantaneous gastric evacuation rate

Daily rations of the three fish species were estimated in terms of percent wet body weight (%BW) from the Elliot and Persson model[85]:

$$C_i = (S_{iT} - S_{i0}e^{-R_iT})R_iT/(1 - e^{-R_iT}) \tag{4-2},$$

where C_i is the consumption of major prey group i during the time interval from 0 to T (hours), S_{i0} and S_{iT} are the mean S of major prey group i at time 0 and T, and R_i is the instantaneous gastric evacuation rate (R) of major prey group i. The daily ration for each fish (C) expressed as a percentage of body weight is determined by

$$C = \Sigma\, \Sigma\, C_{ij} \tag{4-3},$$

where j is the number of sampling surveys (j=11) and i is the number of major prey groups (i=2 or 3).

Assuming that feeding does not occur from t to $t+T$, depletion of S from t to $t+T$ was described by the exponential model:

$$S_{it+T} = S_{it}e^{-RT} \tag{4-4},$$

which, in its logarithm form, is

$$\mathrm{Ln}\, S_{it+T} = \mathrm{Ln}\, S_{it} - RT \tag{4-5},$$

where S_{it} and S_{it+T} are the mean stomach content indexes of major prey group i at the beginning and end of time interval T (hours), respectively. The instantaneous gastric evacuation rate R of major prey group i for each fish was estimated as the slope of the relationship between Ln S_{it} and time.

Simulated daily ration and estimated gastric evacuation rate

To estimate the mean and 95% confidence intervals (CI) associated with the instantaneous gastric evacuation rate R and daily ration C of each major prey group, we used a simulation technique based on the bootstrap method[86-88].

The bootstrap method designed to estimate the mean and 95% CI associated with the R and C values proceeded in two steps. First, we computed pseudo-values of the mean stomach content index S of each major prey group in each sampling survey j (j=11). The S values were selected at random by sampling with replacement from the observed samples. When the sample size was n, these procedures were repeated n times. Second, we calculated R and C by using the slope of equation (4-5) and equations (4-2) and (4-3), respectively, with the pseudo-values of SCI. When the R value estimated with equation (4-5) was a negative value, daily ration C was calculated using the point estimates of R. This procedure was repeated 1,000 times and produced 1,000 pseudo-values of the R and C values. The pseudo-values were log transformed because of skewed distribution, and the results are expressed as geometric mean values. The 95% CI of the two values were taken as the 2.5 and 97.5 percentiles of the corresponding sets of 1,000 pseudo-values. Estimates of daily rations and the instantaneous gastric evacuation rate obtained with the bootstrap method were declared significant when their 95% CI did not overlap.

Daily food consumption

The daily ration estimate was converted into daily food consumption (mg/ind) using wet body weight of the fish. The densities (inds/m^2) of the three fish species were estimated using the catch per unit effort (CPUE; inds/haul [400 m^2]) and the estimated catch

efficiency (16.1%; Chapter 2) of the beam trawl net. Then, daily food consumption of fish per square meter (mg/m^2) was estimated using the daily food consumption of fish and the density values of fish.

Results

Abundance of food organisms and fishes

The abundance of food organisms was much greater in the near-bottom layer (within 30 cm from the bottom) than in the middle layer (30 to 60 cm above the bottom; Table 4-6). Mysids, gammarids and crangonids were dominant prey, and their density in the near-bottom layer was 7.8 mg/m^2, 5.8 mg/m^2 and 153.0 mg/m^2, respectively, during the day. The dominant mysids species was *I. ohshimai*, whereas *N. ornata*, which flounder prefer (Chapter 3B), was not abundant. Most crangonids were less than 15 mm in body length (BL) and no crangonids larger than 25 mm in BL, which could prey on juvenile flounder[76], were caught. The density

of *I. ohshimai* in the near-bottom layer was significantly higher during the day than at night, while the opposite was true in the middle layer (Mann-Whitney U test, $p<0.05$). The abundance of crangonids, isopods and gammarids was relatively high at night in both layers. Ghost shrimp (*Callianassa* sp.) appeared at night.

A total of 908 individuals of fishes were collected by 17 hauls of the beam trawl (Table 4-7). The numbers of individuals (density, inds/m^2) were 252 (0.23 inds/m^2) in *F. gymnauchen*, 232 (0.21 inds/m^2) in *P. olivaceus*, 211 (0.19 inds/m^2) in *T. oligolepis* and 112 (0.10 inds/m^2) in *Repomucenus* spp. Since *Repomucenus* spp. were small (mean TL: 16.1 mm), their stomachs were not examined.

Diet and diel feeding periodicity

P. olivaceus and *F. gymnauchen* consumed mainly mysids, crangonids and gammarids (Table 4-8, Fig. 4-6). Fishes and polychaetes was also important prey

Table 4-6 Mean density (±standard error) of food organisms collected by the two-layer sledge net at Ohama Beach

Contents	Middle layer[a] (mg/m^2)		Near-bottom layer[b] (mg/m^2)	
	Day	Night	Day	Night
Philine argentata			0.59 ± 0.39	6.21 ± 2.41
Polychaeta			0.03 ± 0.03	0.18 ± 0.18
Ostracoda				0.25 ± 0.25
Copepoda	0.02 ± 0.01			
Mysidacea	0.15 ± 0.07	1.84 ± 0.84	7.78 ± 0.92	3.65 ± 0.87
(*Archaeomysis japonica*)			0.05 ± 0.05	
(*Iiella ohshimai*)	0.10 ± 0.07	1.79 ± 0.78	7.59 ± 0.88	3.33 ± 0.87
(*Nipponnimysis ornata*)		0.06 ± 0.06	0.12 ± 0.07	0.16 ± 0.07
(unidentified mysids)	0.04 ± 0.03		0.03 ± 0.03	0.16 ± 0.14
Gammaridea	0.14 ± 0.08	0.83 ± 0.38	5.79 ± 0.60	11.21 ± 3.94
Cappellida	0.01 ± 0.01		0.05 ± 0.02	0.04 ± 0.04
Isopoda	0.01 ± 0.01	3.13 ± 1.76	0.10 ± 0.10	2.04 ± 2.04
Caridea	0.01 ± 0.01	0.83 ± 0.27	0.07 ± 0.05	0.30 ± 0.30
Crangonidea	0.05 ± 0.05	1.23 ± 0.86	152.98 ± 30.06	251.10 ± 16.14
(-5 mm BL)			0.77 ± 0.37	0.25 ± 0.05
(-10 mm BL)	0.05 ± 0.05	0.42 ± 0.23	37.12 ± 6.95	59.17 ± 11.22
(-15 mm BL)		0.80 ± 0.80	102.86 ± 21.25	181.81 ± 9.01
(-25 mm BL)			12.22 ± 5.23	9.87 ± 4.05
Callianassa sp.		1.48 ± 0.43		0.15 ± 0.15
Brachyura	0.11 ± 0.04	1.16 ± 0.31	0.10 ± 0.07	0.04 ± 0.04
Fishes	0.08 ± 0.04	0.60 ± 0.19	7.60 ± 6.35	8.48 ± 8.07
(-10 mm TL)	0.08 ± 0.04	0.30 ± 0.11	0.14 ± 0.07	0.16 ± 0.09
(>10 mm TL)		0.30 ± 0.30	7.46 ± 6.38	8.32 ± 8.07
Total	0.57 ± 0.12	11.08 ± 2.48	175.08 ± 33.18	283.64 ± 14.61

[a] 30 to 60 cm above the bottom; [b] within 30 cm from the bottom

Table 4-7 Demersal fish species collected by the beam trawl net at Ohama Beach on 26-27 June 2003

Family	Species	No. of inds.	Weight (g)	Size (mm TL)
Syngnathidae	*Syngathus schlegeli*	5	0.53	55.5-91.7
Scorpaenidae	*Sebastes inermis*	2	3.20	43.3-50.0
Congiopodidae	*Hypodytes rubripinnis*	7	16.56	6.9-72.2
Triglidae	*Chelidonichthys spinosus*	1	0.21	26.5
Cottidae	*Pseudoblennius cottoides*	1	1.27	52.2
Sparidae	*Pagrus major*	12	2.14	10.8-28.3
	Acanthopagrus schlegeli	22	0.57	12.3-16.4
Sillaginidae	*Sillago japonica*	1	0.02	20.3
Labridae	*Halichoeres poecilopterus*	1	20.50	119.8
Ammodytidae	*Ammodytes personatus*	2	2.26	73.4-78.0
Blenniidae	*Omobranchus elegans*	1	0.07	23.6
Callionymidae	*Repomucenus beniteguri*	3	42.84	112.6-160.3
	Repomucenus spp.	112	3.77	8.5-27.0
Gobiidae	*Favonigobius gymnauchen*	252	244.32	33.3-71.5
Paralichthyidae	*Paralichthys olivaceus*	232	80.05	17.1-59.6
	Tarphops oligolepis	211	27.53	10.1-28.7
Soleidae	*Heteromycteris japonica*	25	35.54	7.4-115.1
Monacanthidae	*Rudarius ercodes*	10	0.04	6.0-10.3
Tetraodontidae	*Takifugu niphobles*	8	161.52	7.3-125.0
	Total	908	642.93	

Table 4-8 Feeding incidence (percentage of stomachs with food) and percentage in weight (%ww) of prey items for *Paralichthys olivaceus, Tarphops oligolepis* and *Favonigobius gymnauchen* at Ohama Beach

	P. olivaceus	*T. oligolepis*	*F. gymnauchen*
Number	232	211	155
Mean total length (mm)	32.6	23.7	50.2
Mean body weight (mg)	345.1	130.5	1031.6
Feeding incidence (%)	74.6	75.8	80.3
Contents	%ww	%ww	%ww
Polychaeta			11.7
Copepoda		2.9	0.1
Mysidacea	26.0	68.5	13.4
(*Iiella oshimai*)	4.0	3.6	4.4
(*Nipponomysis ornata*)	14.9	9.1	2.3
(*Misidacea fragments*)	7.1	55.8	6.7
Gammaridea	7.1	2.6	11.6
Isopoda			0.2
Crangonidae	27.4	4.5	40.0
Callianassa sp.	0.2	21.6	7.9
Caridea	0.6		
Fishes	38.6		6.6
Egg of *Hemiramphus sajori*			8.6

for *P. olivaceus* and *F. gymnauchen*, respectively. The diet of *T. oligolepis* consisted exclusively of mysids. I regarded mysids and crangonids as important prey items for these fish species, and estimated the food consumption of these and other prey groups.

Generally, the mean stomach content indexes for the three fish species were high during the day and low at night(Fig. 4-7). Clear peaks in the SCIs of mysids and others for *P. olivaceus* were found around dusk and/or dawn, whereas two peaks in the SCI
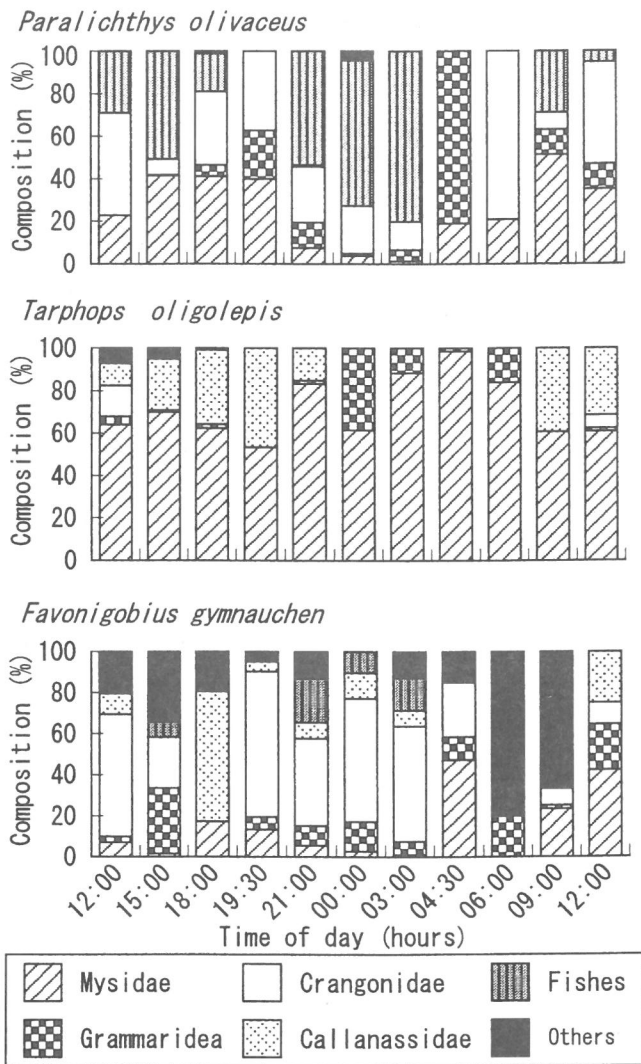
Fig.4-6 Diel changes in wet composition of each prey item of the three fish species, *Paralichthys olivaceus*, *Tarphos oligoleps* and *Favonigobius gymnauchen* and, at Ohama Beach on 26-27 June 2003.
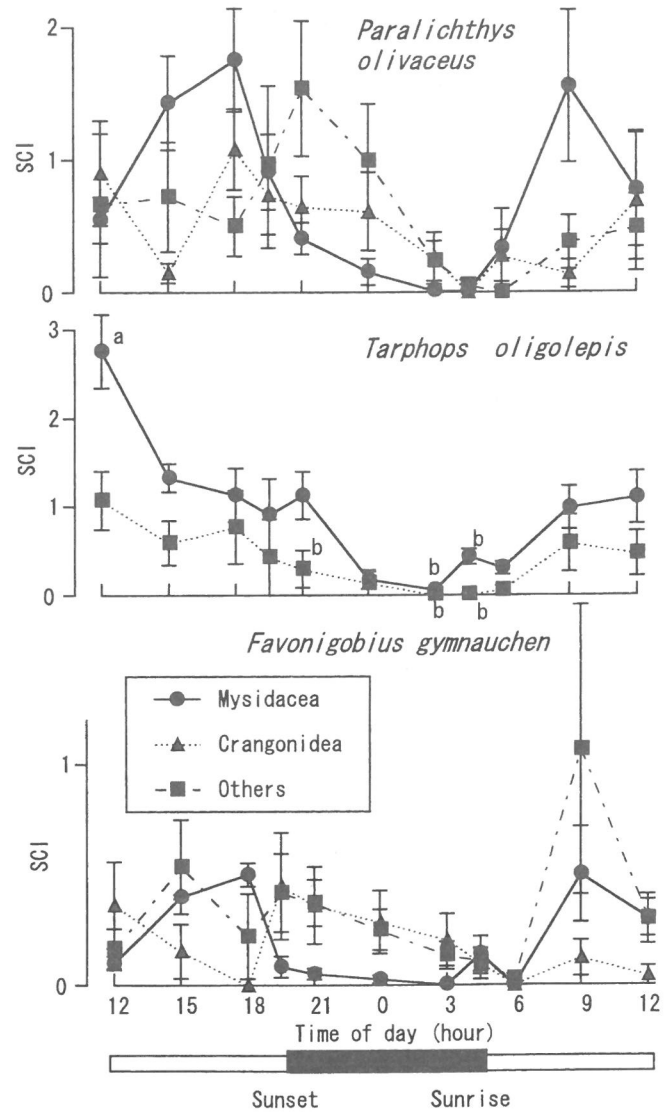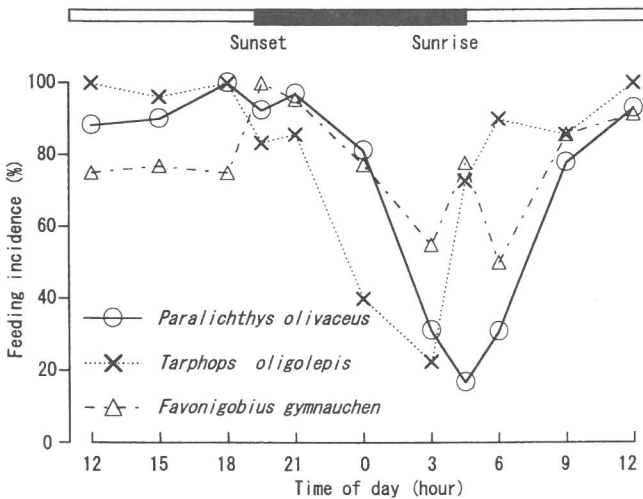

Fig.4-7 Diel changes in the mean stomach content index (SCI) of major prey groups in the three fish species, *Paralichthys olivaceus*, *Tarphops oligolepis* and *F. gymnauchen* at Ohama Beach on 26-27 June 2003. Different superscript are significantly different at P< 0.05 (Scheffs F test). Vertical bars indicate standard error.

of crangonids were found at noon and after dusk. In *T. oligolepis*, both of the SCIs increased from dawn, reached a maximum at noon and decreased throughout the afternoon and night to reach a minimum at around dawn. In *F. gymnauchen*, the peaks of the three SCIs were observed at around dusk and/or dawn. Diel variation in feeding incidences (percentage of stomachs with food) were similar among the three species (Fig. 4-8), increasing during the day, reaching a maximum at dusk and decreasing throughout the nigh to reach a minimum at dawn.

Estimation values of gastric evacuation rates and daily ration

All the three fish species demonstrated a gradual decrease in SCI throughout the night (Fig. 4-7). I therefore considered that these species feed only during the day, and thus calculated the instantaneous gastric evacuation rate R as the slope of depletion Ln S_t from 21:00 to 3:00. Although there were no sig-

Fig.4-8 Diel changes in feeding incidences (percentage of stomachs with food) in the three species, *Paralichys olivaceus*, *Tarphops oligolepis* and *Favonigobius gymnauchen* on 26-27 June 2003.

nificant differences among the simulated estimates of R in three fish species, the R value of mysids was the highest among that of the major prey groups and that of crangonids was most lowest in *P. olivaceus* and *F. gymnauchen*(Table 4-9). In contrast, in *T. oligolepis*, R of mysids was slightly lower than that of the other prey group.

The estimates of instantaneous consumption of each time interval(C_{ij}; %BW per hour)were high during the day and negligible at night in all the three species(Fig. 4-9). Instantaneous consumption presented negative, as well as positive, values. However, the negative values were considerably lower than the positive values and the numbers of occurrences was small. Therefore, estimated daily ration were derived by summing the amount of food consumed during each interval, including negative values. The estimated total daily ration for *P. olivaceus*(16.7 %BW)was higher than that for *T. oligolepis*(13.6 %BW)and *F.*

Table 4-9 Point and simulated estimates of evacuation rates for three fish species, *Paralichthys olivaceus*, *Tarphops oligolepis* and *Favonigobius gymnauchen*

| Species | Point estimates | | | Simulation estimates | | | | | |
| | | | | Mysidacea | | Crangonidea | | Others | |
	Mysidacea	Crangonidea	Others	Mean	95%CI	Mean	95%CI	Mean	95%CI
P. olivaceus	0.59	0.15	0.31	0.59	0.19-0.94	0.25	0.01-0.92	0.33	0.11-0.65
T. oligolepis	0.49	–	0.62	0.50	0.37-0.65	–		0.55	0.08-1.82
F. gymnauchen	0.50	0.09	0.17	0.39	0.03-0.98	0.18	0.01-0.60	0.19	0.03-0.50

Table 4-10 Point and simulated estimates of daily rations (%BW) for three fish species, Paralichthys olivaceus, Tarphops oligolepis and Favonigobius gymnauchen

| Species | Point estimates | | | | Simulation estimates | | | | | | | |
| | | | | | Mysidacea | | Crangonidea | | Others | | Total | |
	Mysidacea	Crangonidea	Others	Total	Mean	95%CI	Mean	95%CI	Mean	95%CI	Mean	95%CI
P. olivaceus	11.25	1.16	4.33	16.74	10.67[a]	3.78-21.35	2.09	0-10.39	4.17	1.03-9.94	18.14[a]	9.26-31.26
T. oligolepis	8.43	–	5.19	13.62	8.76[a]	5.74-12.45			3.84	0-15.03	13.23[a]	7.94-24.30
F. gymnauchen	2.05	0.09	1.62	3.76	1.31[b]	0.33-3.31	0.51	0-2.53	1.62	0.28-4.52	3.65[b]	1.66-7.43

Different superscripts within the same column are significantly different at $P < 0.05$(Scheffe's F test).

Table 4-11 Daily consumption (mg/m^2) in the three fish species, *Paralichthys olivaceus*, *Tarphops oligolepis* and *Favonigobius gymnauchen*

| Species | Mysidacea | | Crangonidea | | Others | | Total | |
	Mean	95%CI	Mean	95%CI	Mean	95%CI	Mean	95%CI
P. olivaceus	7.75	2.7-15.5	1.51	0-7.5	0.92	0.8-7.2	13.15a	6.7-22.7
T. oligolepis	2.21	0.1-3.1	–	–	0.97	0-3.8	3.36b	0.1-6.1
F. gymnauchen	3.13	0.8-7.9	1.23	0-6.1	3.87	0.7-10.8	8.75	4.0-17.8

Different superscripts within the same column are significantly different at $P < 0.05$(Scheffe' s F test).
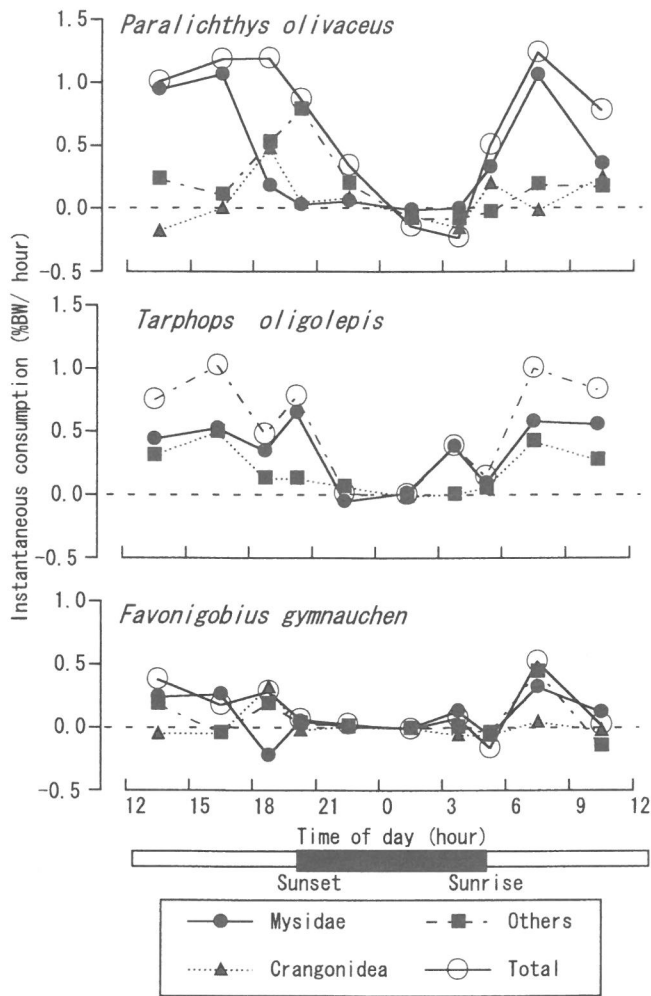
Fig.4-9 Diel changes in instantaneous consumption (%wet body weght per h) of the three fish species, *P. olivaceus*, *Tarphops oligolepis* and *F. gymnauchen* collected at Ohama on 26-27 June 2003.

gymnauchen(3.8 %BW; Table 4-10). The geometric mean total daily ration(95 %CI) for *P. olivaceus*, *T. oligolepis* and *F. gymnauchen* determined by simulation were 18.1(9.3-31.3)%BW, 13.2(7.9-24.3)%BW and 3.7(1.7-7.4)%BW, and those in *P. olivaceus* and *T. oligolepis* were significantly higher than in *F. gymnauchen*($P < 0.05$). The daily rations of mysids were high in all three fish species, although significant differences were not observed among prey groups.

The mean daily food consumption(95%CI, mg/m^2) was 13.2(6.7-22.7) in *P. olivaceus*, 3.4 (0.1-6.1) in *T. oligolepis* and 8.8(4.0-17.8) in *F. gymnauchen*, that in *P. olivaceus* was significantly higher than in *T. oligolepis*($P < 0.05$, Table 4-11). The mysids consumption in *P. olivaceus*(7.8 mg/m^2)was approximately three times greater than in *T. oligolepis*(3.1 mg/m^2)and *F.*

gymnauchen(2.2 mg/m^2)although there were no significant differences, indicating that *P. olivaceus* might be the major consumer of mysids in the food web of the sandy beach. The total daily mysids consumption in the three fish species was 13.1 mg/m^2.

Discussion

Abundance of food organisms

Many isopods and gammarids have circadian rhythms in their swimming activities, more active at night[28,89]. This chapter found that isopods and gammarids were more abundant at night than during the day, which supports the findings of the previous reports[28,89]. The abundance of *I. ohshimai* in the near-bottom layer decreased at night, demonstrating a counter-trend in the middle layer. These results indicate that *I. ohshimai* emerged into the water column from sand bottom at night. A similar diel migration of this species is known to occur at a beach in Otsu-chi Bay, northeastern Japan[30].

Diel periodic feeding rhythm

The difference in the instantaneous food consumption for *P. olivaceus* between day and night was obvious, indicating that juvenile *P. olivaceus* was day-feeders in this area. In this species, larvae and juvenile in the laboratory experiment[84,90,91]and juvenile[7,84,92] and young[93,94]in the field also actively consumed during the day. Therefore, *P. olivaceus* is a day-feeder through the larval, juvenile and young stages. Diel changes in feeding incidences and instantaneous food consumption for *T. oligolepis* and *F. gymnauchen* were similar to those for *P. olivaceus*, suggesting that the three fish species are day-feeders and have the same feeding rhythms.

Gastric evacuation rate and daily ration

The relationship between instantaneous gastric evacuation rate R and water temperature was described by exponential or linear models[95,96], and the R value for winter flounder *Pseudopleuronectes americanus* [96], bluefish *Pomatomus saltatrix*[97]and Japanese Spanish mackerel *Scomberomorus niphonius*[98]decreased as water temperature decrease. In this study, assuming no feeding during night time, I estimated R at night when the water temperature was lower(Table 4-5). Therefore, the R value might

have been underestimated. The instantaneous gastric evacuation rate R may be underestimated under fasting conditions[97].

I calculated means and 95% CI of daily ration C and instantaneous gastric evacuation rate R estimates using a simulation technique derived from the bootstrap method. Worobec[96], and Boisclair and Leggett[86] and Boisclair and Marchand[87] estimated the C and R value using algebraic formulae and simulation techniques, respectively. The former technique is logically difficult, while the later is easy but requires many calculations. However, because one can reduce the workload with a personal computer (Microsoft EXCEL including Visual Basic 6.0 for application), I recommend the simulation techniques for estimating variation.

The prey availability – especially mysids biomass – had a profound effect on the daily ration for *P. olivaceu*[84]. Tominaga et al.[84] demonstrated that the daily ration of food, that was mostly composed of mysids, for juvenile *P. olivaceus* (range of mean total length: 38.4-55.9 mm) ranged between 1.5 %BW and 22.2 % BW at Wada Beach where the density of mysids varied between 20 and 220 mg/m^2. Seikai et al.[17] reported that when juvenile *P. olivaceus* were fed sufficient mysids in the laboratory experiments (water temperature, 22 ℃), the daily ration in dry weight was 6.4 %BW. We converted the dry weight of 6.4 %BW into wet weight of 32.0 %BW based on moisture content (80%[17]). In this chapter, although the daily ration for *P. olivaceus* was 18.1%BW, that of mysids was 10.7% BW, was lower than that observed in those studies[17,84]. Besides, the density of mysids in this study beach was lower than in Wada Beach[84]. Judging from these results, the mysids availability was a limiting factor of the daily ration.

Yamada et al.[14] demonstrated that it was advantage that juveniles larger than 50 mm TL consumed fishes rather than mysids for growth when the latter were not abundant. The size of *P. olivaceus* moving out from the nursery grounds was 120 mm TL and juveniles smaller than 100 mm TL mainly consumed mysids at Igarashihama Beach where mysids were abundant[8,18]. In contrast, juveniles larger than 50 mm TL shifted their main food from mysids to fishes and then migrated to offshore after late June in this area (Chapter 3). Therefore, the importance of mysids in the diet for juveniles was lower in this area than at Igarashihama Beach. Thus, feeding habits of juveniles varies geographically, probably with the change of the density of mysids in nursery grounds. Plasticity in juvenile feeding characteristic of this species may be the outcome of feeding tactics depending on mysids local availability.

Verification of hypothesis

The daily mysids consumption of Japanese flounder was the greatest among those in the three fish species, although sufficient mysids were not available for flounder in this area. The result proved the hypothesis 4 true.

Chapter 5. Daily growth estimation of juvenile Japanese flounder using otolith microstructure analysis

This chapter describes the seasonal variations in the daily growth rate and the relative daily growth rate of wild juveniles using otolith microstructure analysis[99].

Introduction

The previous chapters (Chapter 3B and 4B) have described that abundance of mysids was low and the daily ration of mysids was also low for juvenile Japanese flounder in the nursery grounds in eastern Hiuchi-Nada. There was the positive relationship between daily growth rate and daily ration, and then feeding condition could be estimated using daily growth rate[21]. From these observations, the daily growth rate is expected to be low in this area. In addition, since emigration from nursery grounds was caused by a drop of daily food consumption[13], I set up a hypothesis as follows: when juvenile flounder settle in their nursery grounds in early June, their food organisms are abundant and consequently their growth rate is relative high. However, their food availability becomes bad in late June, they can not take the enough amount of food and migrate from their nursery grounds to offshore area, where they can shift their food to abundant fish juveniles, mainly Japanese anchovy (hypothesis 5).

Materials and methods

Field sampling

Juvenile Japanese flounder were collected by the 2-m beam trawl net from June to July in 2003 at Ohama Beach (Table 5-1; Fig. 5-1). All samples except for 26 June were fixed in 5% neutral formalin within 2 hours and preserved in 90% ethanol. The flounder collected on 26 June were packed in ice immediately and frozen at –20 ℃ within 30 minutes. Water temperature at surface range from 22.0 to 25.0 ℃ (Table 5-1).

Table 5-1 Collection records and size of juvenile Japanese flounder collected at Ohama Beach in 2003

Date	Water temperature (℃)	Mean total length (mm)
9 June	22.0	19.8
23 June	23.5	30.6
26 June	24.6	32.6
7 July	25.0	38.6
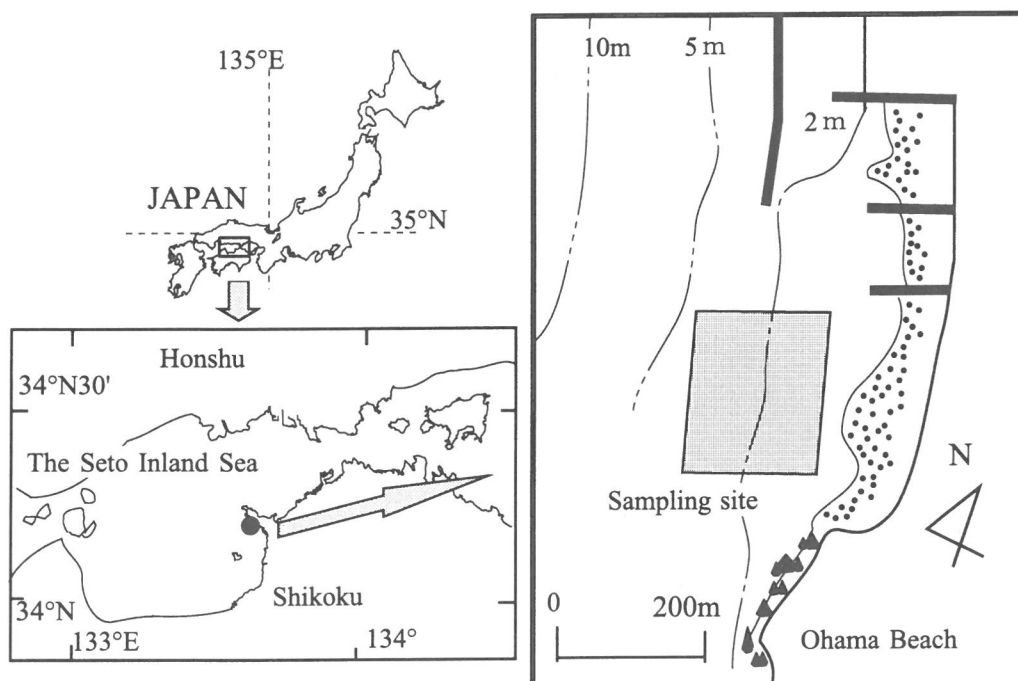
Fig. 5-1 Sampling site for juvenile Japanese flounder in eastern Hiuchi-Nada, the Seto Inland Sea.

Growth analysis

Total length(TL)of flounder was measured with a caliper. Otolith(lapillus)of the ocular side was extracted and mounted in epoxy resin(N.T. epoxy-coat, TOHO, Japan)with its lateral side, and then were observed under a microscope at 400×magnification. When we could not recognize the microstructure (rings and nucleus)of otolith clearly, the lapillus was polished using fine abrasive paper and diamond paste. The ring of lapillus formed daily in this species[16,21,100]. The increments on lapillus were traced on paper with a camera lucida, and then the otolith radius at capture and fifth outermost increment were measured along the longest axis.

The relationship between otolith radius and total length at capture obtained from a total of 105 individuals could be expressed by allometric formula (Fig. 5-2). I used the biological Intercept method[99] for back-calculation of total length at age(day). The equation is given by the expression:

$$L_{c-5} = (L_c - L_a) \cdot (O_{c-5} - O_c) \cdot (O_c - O_a)^{-1} + Lc \ ,$$

where $L_{c-5} = \ln$(TL at 5 days before capture), $L_c = \ln$(TL at capture), $L_a = \ln$(TL at the biological intercept), $O_{c-5} =$ otolith radius at 5 days before capture, $O_c =$ otolith radius at capture and $O_a =$ otolith radius at biological intercept. In this chapter, I used the TL (14.8 ± 1.4 mm)and otolith radius(68.8 ± 8.8 μ m)of the smallest fish collected at Ohama Beach in 2003 as L_a(ln 14.8)and O_a(68.8), respectively.

Recent growth rate for 5 days before capture was express as the absolute growth rate(AGR; mm/day) and the relative growth rate(RGR; %/day):

$$AGR = (TL_c - TL_{c-5})/5,$$
$$RGR = AGR / TL_c = (TL_c - TL_{c-5})/5TL_c \ ,$$

where TL_c and TL_{c-5} are total length at capture and at 5 days before capture.

The relation between the growth rate(AGR or RGR)and total was examined using simple regression analysis. To compare mean daily growth rates among sampling days, the data were analyzed by Scheff's F test.

Results

Relation between growth rate and total length

The total length(TL), the absolute growth rate (AGR)and the relative growth rate(RGR)ranged from 18.3 to 56.0 mm, from 0.41 to 1.84 mm/day and from 0.86 to 5.12 %/day, respectively(Fig. 5-3). The average of the AGR and RGR was 0.92 mm/day and 2.88 %/day, respectively. The AGR related positively with TL on 9, 23 and 26 June(Table 5-2; $P < 0.05$), but not related on 7 July($P > 0.5$). On the other hand, there were no significant relationships between TL and RGR in all the sampling days($P > 0.1$).

Fig.5-3 Absolute growth rate (a) and relative growth rate (b) of jvenile flounder collected in 2003.

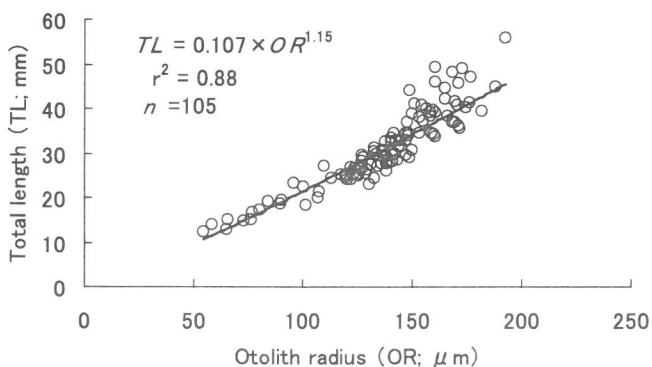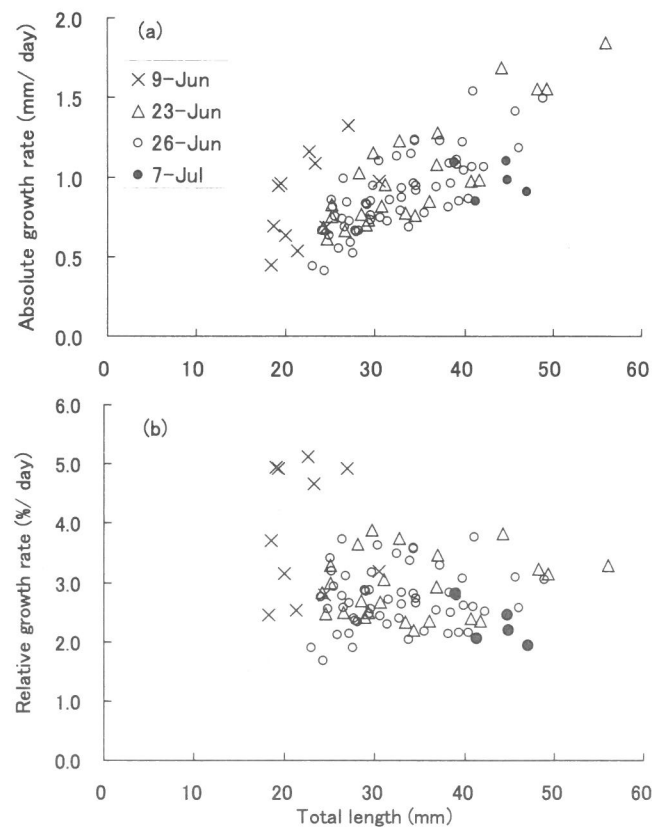**Fig.5-2** Relationship between total length and otolish radius of juvenile Japanese flounder collected at Ohama Beach in 2003.

Table 5-2 Relationships between total length and absolute growth rate or between total length and relative growth rate of juvenile Japanese flounder in 2003

Date	n	Absolute growth rate		Relative growth rate	
9 June	11	$r = 0.52$	$P < 0.05$	$r = 0.02$	$P = 0.47$
23 June	24	$r = 0.85$	$P < 0.01$	$r = 0.18$	$P = 0.19$
26 June	57	$r = 0.77$	$P < 0.01$	$r = 0.12$	$P = 0.17$
7 July	5	$r = -0.24$	$P = 0.65$	$r = -0.68$	$P = 0.90$

Seasonal changes in growth rate

The seasonal changes in the mean AGR and RGR are shown in Fig. 5-4. The mean total length increased from 22.2 to 43.4 mm. The mean AGR ranged between 0.86 and 1.01 mm/day, whose ranges were relatively narrow (mean ± standard deviation [SD] : 9 June, 0.86 ± 0.28 mm/day; 23 June, 1.01 ± 0.35 mm/day; 26 June, 0.90 ± 0.25 mm/day; 7 July, 0.99 ± 0.11 mm/day). Significant differences in AGR were not observed among the days ($P > 0.1$, Scheff's F test). However, the mean RGR gradually
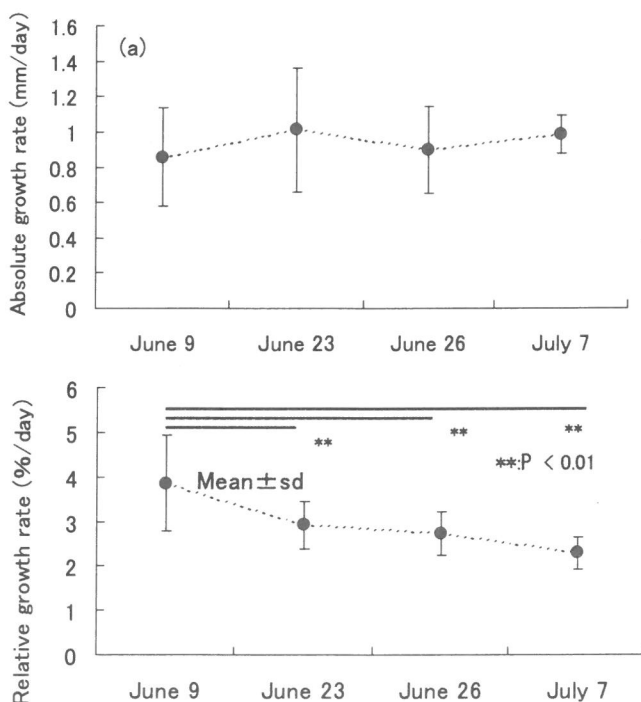
Fig.5-4 Seasonal changes in absolute growth rate (a) and relative growth rate (b) of juvenile Japanese flounder at Ohama Beach in 2003. Vertical bars and asterisks indicate standard deviation and significant difference (Sheffe's F test, $P < 0.01$), respectively.

decreased (mean ± SD : 9 June, 3.85 ± 1.07 %/day; 23 June, 2.92 ± 0.53 %/day; 26 June, 2.72 ± 0.49 %/day; 7 July, 2.29 ± 0.35 %/day). Moreover, the RGR on 9 June was significantly greater than those on the other days ($P < 0.01$, Scheff's F test) and was approximately 1.5 times as large as that in 7 July.

Discussion

Index for comparing growth rate

There was not the relationship between RGR and TL, whereas the positive relationship between AGR and TL. In this chapter, because size of juveniles was not taken into consideration, significant difference in AGR was not shown among the sampling days (Fig. 5-3). Comparison of AGR should be examined using the same size of juveniles. However, it is difficult to obtain the sufficient flounder of same size throughout several months, due to rapid growth of juvenile flounder. Therefore, RGR would be suitable as an index for comparing seasonal variation in the growth rate.

Relationship between daily growth rate and daily ration

The average AGR was 0.92 mm/day, and was half of 2.0 mm/day Igarashihama Beach, the northern part of Japan Sea, where mysids were abundant[8]. The mean RGR (2.7 %/day) on 26 June was lower than that (3.4 %/day) in the laboratory experiments, where juveniles consumed sufficient live mysids[17]. Equally, the daily ration of mysids was lower on 26 June (10.7 %BW) than in the laboratory experiment (32.0 %BW; Chapter 4B). The low daily growth rate may be explained by the low daily ration.

Factor of the depletion of the RGR

The RGR peaked on 9 June and then gradually decreased after late June. Growth rate generally has a close relation to food quality and quantity, water temperature, dissolved oxygen (DO) and salinity[101]. Decreases in DO below a critical threshold reduce growth of flatfishes, and Japanese flounder grow better at high salinities[101]. The optimum temperature was reported to be 20-25 ℃[102,103]. The abundance of food organisms especially mysids had a significantly positive correlation with growth rate of juvenile flounder[8,21]. The DO at Ohama Beach is probably high, because this beach is exposed to open inshore

58

area. The fluctuation of the salinity was relatively small(28.7-33.7 psu; Yamamoto, unpublished), since there is no big river near the beach. The temperature ranged from 20 to 25 ℃. It is suggested that the DO, salinity and temperature therefore did not affected greatly on the depletion of the RGR. At Ohama Beach, density of mysids decreased with the increaseding temperature after June(Chapter 3B). From these results, it appears that a main factor of the depletion of RGR is repressesd food consumption due to decrease of mysids availability.

Cue for emigration to offshore

Juveniles larger than 60 mm TL migrated from the sandy beach to offshore area after late June(Chapter 3A). The RGR after late June was lower than that in early June. Thus, large juveniles might leave for the nursery grounds, when RGR falls below a threshold. The decrease of the food intake might be one of the triggers of the flounder emigration. However, environmental factors including salinity, DO, predator and light intensity, development of organs such as muscle and digestive system and hormonal secretion must also affect their emigration, the emigrant mechanism from sandy beach is complex. Because fish size and timing of emigration affect on survival rate and recruitment success to adult stocks, studies on emigrant mechanism of juveniles are crucial.

Deference of food condition between sandy beach and offshore area

On one hand, food availability was not sufficient for flounder juveniles in the nursery ground in this are after late June, because abundance of mysids was low(Chapter 4B). On the other hand, in offshore area, juveniles consumed mainly fishes such as Japanese anchovy[104,105], of which abundance are very high[106,107]. Moreover, daily growth rate was relatively high(1.0-1.6 mm/day)[43,104]. Therefore, feeding condition might be better in the offshore area than that at the sandy beach.

Verification of hypothesis

The RGR of newly settled flounder was the highest, but when food consumption becomes insufficient under poor feeding conditions, the RGR decreases and juveniles emigrate from the nursery grounds to offshore area. In offshore area, large juveniles con-

sumed mainly abundant fishes. These results proved the hypothesis 5 true.

Chapter 6. General discussion

Recruitment of larval flounder to sandy beaches

Metamorphosing Japanese flounder larvae settled at the 2-5 m depths sandy beaches from late May to late June when mysids were relatively abundant (water temperature: 19-23 ℃). This result is consistent with the hypothesis on that the settlement timing of larval flounder occurs under mysids rich conditions (hypothesis 1). Metamorphosing flounder larvae have been reported to immigrate from offshore area to sandy beaches when mysids were abundant[10], and the survival rate of newly settled flounder increased with the increase of mysids[67]. Because newly settled flounder prefer mysids to crangonids and gammarids (Chapter 3B), and mysids are better food items for them[6,17,67], recruitment to nursery ground that is synchronized with the peak in abundance of mysids would be advantageous for growth and survival of flounder.

Relationship between stomach contents of Japanese flounder juveniles and their food organisms in the field or in situ food organisms

Mysids in this area are less abundant than those in the coasts of the Sea of Japan and the Pacific Ocean, whereas crangonids are more abundant in this area. Generally, the main prey items of juveniles were only mysids and fishes, and the importance of crangonids was very low in their diet[7,9,15]. However, in this area, the index of the relative importance (IRI) of the prey taxa ranked in the order mysids > crangonids > gammarids > fishes, and flounder juveniles ate a variety of diets in this area (Chapter 3B). This result proved the hypothesis 2 true.

Predation on newly settled flounder

Generally, the major cause of mortality among larval and juvenile Japanese flounder is predation by fishes and crustaceans[9]. Newly settled flounder have been reported to be preyed by many fishes including, Japanese flounder, *F. gymnauchen*, *Repomucenus* spp. and *T. oligolepis* at Yanagihama Beach, Nagasaki Prefecture[22,23]. In addition, large crangonids, *Crangon crangon* and *C. affinis*, consumed newly settled flatfishes and then the predation have a strong effect on the their stocks[45,108]. In the present study, the four dominant fish species preyed on small fishes, but the IRI values were very

low. Few large crangonids (>25 mm BL) were caught from May to August. Thus, there may be low predation pressure on newly settled flounder by the four dominant fishes and crangonids in this area. This result supports the hypothesis as follows dominant fish species and crangonids are not predator for juveniles (hypothesis 3). Recently, cuttlefish and crustaceans such as swimming crab *Charybadis japonica* were listed as serious predators for hatchery-reared flounder[109,110]. The more study of predator will be necessary to reveal the cause of mortality.

Interaction among four dominant fish species in this study area

Mean daily food consumption in Japanese flounder, *T. oligolepis* and *F. gymnauchen* was 13.2, 3.4 and 8.8 mg/m^2, respectively (Chapter 4B). This result indicated that the consumption in juvenile flounder was the greatest among those of the dominant fishes, and then these evidences proved the hypothesis 4 (juveniles can consume the greatest amounts of diets in this area among dominant fishes) true.

The mean overlap values among the four dominant fish species were relatively high, because the four fishes consumed mainly small crustaceans such as mysids and/or crangonids (Chapter 4A). Furthermore, because the size of major prey items of the flounder was similar to those of other dominant fishes, there exist an interspecific competition among the four fish species.

Advantage of emigration to offshore

In this area, juveniles larger than 60 mm TL migrated to offshore area (Chapter 3A), when the RGR was less than the certain value (Chapter 5). In addition, the shift to fishes in their food occurred at size larger than 50 mm in TL in this area, where mysids were not abundant. In contrast, the size of emigration from the nursery grounds was 120 mm TL[18] and juveniles smaller than 100 mm TL consumed mainly mysids[8] at Igarashihama Beach, where mysids were abundant. Therefore, it was suggested that the size of emigration varies according to mysids availability in nursery grounds.

The RGR of newly settled flounder in early June was the highest, but that of juveniles emigrating from nursery grounds to offshore area decreased due to the small amount of food consumption as a result

of low food availability after late June (Chapter 5). The hypothesis on growth rate (hypothesis 5) is consistent with this observation. To compare of growth rate between sandy beach and offshore area, I discuss water temperature and food condition. The water temperature often exceeds 25 ℃ after late June at the sandy beach and reaches approximately 30 ℃ in July and August, whereas that at bottom layer in offshore area (<25 ℃ ; Kagawa Pref. Exp. Sta., unpublished) is lower than at sandy beach. Since the optimum temperature for growth was observed around 20-25 ℃ [17,102,103], the temperature condition would be better in the offshore area than at the sandy beach. In feeding condition, abundance of food organisms decreased after late June at the sandy beach, but Japanese anchovy are abundant in offshore area [106,107]. Large juvenile flounder consumed mainly fishes such as Japanese anchovy offshore and daily growth rate of them was relatively high [104,105]. Therefore, the food condition might be better in the offshore area than in the nursery grounds.

Future perspectives

It is considered that juvenile flounder adopted the feeding tactics, in which small juveniles did not depend on only mysids in nursery grounds in this area, where mysids were not abundant, whereas larger juveniles (>approximately 60 mm) consumed mainly abundant fishes such as offshore Japanese anchovy to keep high growth rate in this area. In the Seto Inland Sea, the catch of Japanese flounder increased with the increase in released large size hatchery-reared juveniles (Fig. 1-1). In Tokyo Bay, the survival rate and growth rate of released flounder (60-70 mm TL) were high, because they utilized abundant goby [111]. The high stocking efficiency might be explained by the richness of fishes offshore in the Seto Inland Sea. In addition, larger release size proved to be more successful in surviving until recruitment size [112]. Thus, for more effective stock enhancement in the Seto Inland Sea, large juvenile flounder larger than 50 mm TL should be released from late May to July when larval and juvenile Japanese anchovy is abundant.

The trend of the CPUE at Ohama Beach was very similar to those at other beaches (Chapter 3A). Hiuchi-Nada has large sandy beach area, and many larval flounder seem to settle at sandy beaches. Positive correlation between the CPUE at Ohama Beach in June and the catch of the cohort in Kagawa Prefecture was observed (Yamaoto M, unpublished). In order to forecast the stock size of Japanese flounder in the Seto Inland Sea, information on the CPUE at the beach must be more effective.

This study described the feeding ecology of settled juvenile flounder in the nursery grounds in eastern Hiuchi-Nada, the Seto Inland Sea. Though the feeding ecology is important, this is a part of information on the life history trait. Natural mortality rate of eggs and pelagic larval stage of this species is extremely high and variable for starvation [113,114] and predation by fishes, jellyfish and crustaceans [115-117] and greatly affect recruitment of settled larvae to sandy beach. Natural mortality decreases with an increase in growth, and then becomes lower at large juvenile (young) and adult stage. However, fishing mortality becomes high in young and adult stage due to the high-priced commercial fish. Fishery has strong effect on the stock abundance. Additionally, since the annual average water temperature increased 0.81 ℃ from 1966 to 2002 (37years) by the global warming in the Seto Inland Sea [118], I worry that the coast ecosystem might change by the increased temperature. Hereafter, information on survival characteristic of egg and larval stage and long-term stock assessment must be necessary in order to maintain the high stock abundance.

Summary in Japanese

はじめに

ヒラメ*Paralichthys olivaceus*は沖縄を除く日本列島周辺に広く分布し，産卵盛期は鹿児島では1月から3月，瀬戸内海や若狭湾では4月から5月，北海道では7月から8月と北部海域で遅い。仔魚は1〜2ヵ月の沖合での浮遊期を経て，変態しながら次第に浅海域に移動し砂浜域へ着底する。浮遊仔魚は尾虫類とカイアシ類を主に摂餌するが，着底後に食性が大きく変化し，小型甲殻類のアミ類mysidsが主要餌生物になる。その後，成長に伴いエビ類や魚類に移行する。着底後の稚魚の日間成長量は餌生物の種類と豊度に強く依存して約0.5〜2.0mm/日の範囲を示し，着底して数ヶ月後には全長5cmから10cm程度に成長して浅海域の成育場から沖合へ移出する。

本種は，日本で最も重要な栽培漁業対象種であり，近年全国で2,500万尾以上の人工種苗が放流されている。そのため，これまで多くの海域の成育場で天然稚魚の着底時期，食性，餌料環境，成長，砂浜への加入機構などが研究されてきた。しかしながら，本種の成育場の物理環境（例えば，水温，塩分，潮汐差）や生物環境（餌料生物の組成や密度，捕食者の密度，砂浜域の魚類相）が多様であるため，ヒラメ稚魚がそれぞれの海域の環境に適合した摂餌生態を示すことが考えられ，必ずしも共通した摂餌・成長様式を示すとは限らない。このことは成育場の成立要因を検討するときに，各海域の環境特性を十分に把握しておく必要があることを意味する。

瀬戸内海は，全国のヒラメ漁獲量の約15%を占め，約400万尾の人工種苗ヒラメを放流しているにもかかわらず，本海域における摂餌生態に関する知見は極めて不十分である。今後，最適な放流量や放流時期を決定するために成育場の餌料環境やそれに対応した摂餌生態の情報が不可欠である。瀬戸内海の砂浜域は餌料環境や捕食者・競争種との相互関係によって，必ずしも一般的に認められている成育場としての優良性をもっていないと考えられている。それにもかかわらず，瀬戸内海ではヒラメ資源が高い豊度を示しており，特有の成育場形成機構が働いていることが予想された。そこで本研究では効率的な成育場の成立要因としてヒラメ稚魚の摂餌様式と競争種の存在に関して，次のような仮説を立てた。ヒラメ仔稚魚はアミ類の豊度が高い時期に砂浜海岸に着底する（仮説1）。着底直後のヒラメ稚魚はアミ類を選択して食べるが，相対的にアミ類の密度が低くなるとアミ類以外の小型甲殻類や仔魚を消費する（仮説2）。エビジャコcrangonidsおよび砂浜海岸に優占する魚類は着底直後のヒラメにとって重要な捕食者ではない（仮説3）。砂浜域においてヒラメは他の優占する魚類よりも多くアミ類を消費する（仮説4）。ヒラメ稚魚は着底直後には豊度の高いアミ類を利用でき比較的良好な成長をするが，餌料環境が低下してヒラメ稚魚の成長が大きく抑制されると成育場から移出する（仮説5）。上記の5つの仮説を検証するために，瀬戸内海の砂浜域において，成育場としての餌料環境，ヒラメ着底仔稚魚の出現パターンと摂餌生態および成長，さらに同所的に分布する優占種のアラメガレイ*Tarphops oligolepis*，ヒメハゼ*Favonigobius gymnauchen*およびネズッポ類*Repomucenus* spp.との餌料をめぐる競争関係を検討した。

方　法

1995〜2003年の5〜8月に瀬戸内海の中央部に位置する燧灘（ひうちなだ）東部の砂浜海岸（底質，細砂；干満差，約3m；水深，0.5m〜9.5m）において調査を実施した。餌料生物は広田式桁網（幅0.6m，高さ0.3m，目合0.33mm，50m曳網）によって採集し，10%ホルマリンで固定した。そして，実験室において種査定を行ない，種ごとの個体数と湿重量を測定した。ヒラメ稚魚の捕食者の可能性が指摘されているエビジャコについては，体長を測定した。砂浜域に分布する底生魚類は西水研型桁網（幅2m，高さ0.3m，目合2.1mm，200m曳網）によって採集し10%ホルマリンで固定した後，70%エタノールで保存した。ヒラメ，ヒメハゼ，ネズッポ類，アラメガレイは全長を測定した後，胃内容物の種査定を行いアミ類はできる限り種まで分類した。各餌生物の出現頻度（% F），個体数組成（% N），重量組成（% W）から餌料重要度指数IRI（Index of relative importance）の百分率組成を算出した。それぞれの餌への嗜好性を判定するため，Chesson（1978）の餌選択係数を算出した。ヒラメ稚魚，ヒメハゼ，ネズッポ類，アラメガレイの食性の重なり具合は，Pianka（1973）の重複度指数を用いて評価した。また，ヒラメ稚魚，ヒメハゼ，アラメガレイの1日あたりの餌消費量（日間摂餌量）を推定するために，1.5〜3時間間隔で24時間にわたってこれら3魚種を連続採集し，Elliott and Persson（1978）の方法で日間摂餌量を算出した。さらに，ヒラメ稚魚の成長の季節変動を調べるためにBiological Intercept法（Campana 1990）を用いて耳石（有眼側の礫石）日周輪幅から採集日5日前の全長を逆算し，日間成長率（mm/日）と日間相対成長率（%全長/日）を推定した。

結果および考察

調査海域では瀬戸内海の砂浜海岸における優占魚類は，ヒメハゼ，アミメハギ *Rudarius ercodes*，アラメガレイ，ヒラメ，ネズッポ属，ササウシノシタ *Heteromycteris japonica*，クロダイ *Acanthopagrus schlegeli* が優占しており，そのほとんどは稚魚であった。ヒラメの着底は水深2～5mの砂浜域において5月下旬（水温：19℃）から6月下旬（23℃）まで続き，そして，6月下旬から全長約60mm以上に成長した稚魚が砂浜域から移出した。餌料生物は，アミ類，エビジャコ，ヨコエビ類 gammarids が優占し，特に体長14mm以下のエビジャコ *Crangon* spp.の密度が高かった。アミ類の豊度は水温23℃以下の5月下旬から6月下旬に高かったことから，ヒラメ稚魚の出現時期と同調しており，仮説1を支持する結果が得られた。しかしながら，日本海や太平洋におけるヒラメ稚魚の成育場に比べて，本海域のアミ類の現存量は非常に少なく，それに対して体長14mm以下の小型のエビジャコの現存量は高かった。

当海域におけるヒラメ稚魚は，2種のアミ類，*Iiella ohshimai* と *Nipponomysis ornata* および体長14mm以下の小型のエビジャコを優占して摂餌していた。これらのうちアミ類は着底直後の全長10mmから50mmのヒラメ稚魚にとって最も重要な餌であったが，エビジャコは全長30mm以上のヒラメ稚魚が主に利用していた。また，アミ類に対してヒラメ稚魚は，潜砂性のアミの *Iiella ohshimai* よりも表在性のアミの *Nipponomysis ornata* や *Anisomysis ijimai* を選択して摂餌する傾向が認められた。一方，小型のエビジャコに対する餌選択係数は非常に低い値を示した。成育場から移出し始める全長50mm以上のヒラメ稚魚はヒメハゼやネズッポ類の稚魚を摂餌し，魚食性に移行していた。成育場でのヒラメ稚魚は表在性のアミ類に対する嗜好性が強いが，アミ類の密度が低いことから成育場に豊富に分布する小型のエビジャコを摂餌していた。対照的に，日本海新潟沿岸域のようなアミ類が豊富な他海域の成育場では，ヒラメ稚魚はアミ類を専食している。このように幅広い食性は，アミ類が豊富な海域と異なっており，多様な餌生物を利用することで餌料水準を補償するという仮説2を支持した。また，ヒラメの着底直後の仔稚魚を捕食すると考えられる体長25mm以上のエビジャコの密度は非常に低く，捕食者としての影響は低いものと考えられた。したがって，当海域においてエビジャコはヒラメの捕食者ではなく，被食者（餌生物）として関係していると考えられた。

ヒメハゼ，ネズッポ類，アラメガレイは主にアミ類やエビジャコ，ヨコエビなどの小型甲殻類を摂餌する点で食性が一致していたが，ヒメハゼとネズッポ類がアミ類や小型のエビジャコを，アラメガレイがヒラメ稚魚と同じく表在性のアミである *Nipponomysis ornata* を好んで摂餌する点で異なっていた。これら3魚種の主な餌料生物は同海岸に出現するヒラメ稚魚と共通しており，餌生物の大きさもほぼ同様であった。しかし，これら3魚種の胃内容物からはヒラメをはじめとする魚類の仔稚魚がほとんど観察されなかったことから，これら3魚種はヒラメ稚魚の捕食者ではなく，競争種として機能することが示された。この結果と大型のエビジャコが少ないという結果は仮説3を支持するものであった。

ヒラメ，アラメガレイ，ヒメハゼの日間摂餌量は体重あたりで18.14％BW，13.23％BW，3.65％BW，1個体あたりでは62.6mg/個体，17.3mg/個体，37.7mg/個体と推定され，3魚種の中でヒラメの摂餌量が最も高かった。この結果は砂浜域でヒラメは最も多くアミ類を摂餌するという仮説4を支持し，ヒラメ稚魚が競争種に対して優位にアミ類を利用できることを示した。しかしながら，本海域のヒラメの日間摂餌量は，飼育実験によって推定された最大日間摂餌量に比較して低い値であった。

全長18.3～56.0mmのヒラメ稚魚の耳石を用いて推定した各個体の日間成長率と日間相対成長率はそれぞれ0.41～1.84mm/日と0.86～5.12％/日であった。日間成長率と採集全長の間には正の相関があったが，日間相対成長率と全長には有意な相関が認められなかった。日間成長率の平均値は0.92 mm/日で，日本海新潟沿岸域における成長（約2.0mm/日）の約1/2であった。日間相対摂餌率は，着底仔魚の加入最盛期である6月上旬（3.85％/日）をピークに成育場からヒラメ稚魚が移出する7月上旬には2.29％/日まで低下した。日間相対摂餌率は餌生物密度の変化と符号しており，餌不足による摂餌量低下が成長率の減少を引き起こし，砂浜域から沖合域への移出のシグナルになっている可能性が考えられた。これらの結果はヒラメの移出機構に関する仮説5を支持していた。

成育場からの移出および魚食性への移行サイズを本海域とアミ類の密度が高い日本海新潟沿岸域を比較する。ヒラメ稚魚が沖合域へ移出するサイズは，新潟沿岸域の全長120mm程度に対し，同60mmと小さかった。また，新潟沿岸域のヒラメが全長100mm程度でもアミ類を専食しているのに対して，本海域では50mm程度から魚食性に移行する傾向が認められた。アミ類の分布量が少ない海域では，全長50mm程度で沖合域に移出して魚食性に移行する方が成長に有利であると報告されており，本海域においてもヒラメ稚魚

は成育過程でアミ類への依存をできるだけ小さくし，沖合に豊富に存在する魚類を利用することで成長を良好に保つ戦術を選択しているものと考えられた。

Acknowledgements

List of publications

The chapter (Ch.) 2, Ch. 3A, Ch. 3B Ch. 4A and Ch. 4B are published by Yamamoto and Makino[119], Yamamoto et al.[120], Yamamoto et al.[121], Yamamoto and Tominaga[122] and Yamamoto and Tominaga[123], respectively.

References

1) Fisheries Agency: 2005, *Statistical annual report on fishery and aquaculture production H15* (in Japanese). Fisheries Agency of Japan, Tokyo, 42－45.

2) Fisheries Agency and Fisheries Research Agency: 2004, *Annual report on seedling production and release for sea farming* (in Japanese). Fisheries Agency of Japan and Fisheries Research Agency of Japan, Tokyo, 8－11.

3) Howell BR, Yamashita Y: 2005, Aquaculture and stock enhancement. In: Gibson RN (Ed.). *Flatfishes*, Blackwell, Oxford, 347－371.

4) Minami T: 1982, The early life history of a flounder *Paralichthys olivaceus* (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **48**, 1581－1588.

5) Goto T, Sudo H, Tomiyama M, Tanaka M: 1989, Settling period of larvae and juveniles of Japanese flounder, in Shijiki Bay, Hirado Island (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **55**, 9－16.

6) Tanaka M, Goto T, Tomiyama M, Sudo H: 1989, Immigration, settlement and mortality of flounder (*Paralichthys olivaceus*) larvae and juveniles in a nursery ground, Shijiki Bay, Japan. *Neth. J. Sea Res.* **24**, 57－67.

7) Hirota Y, Koshiishi Y, Naganuma N: 1990, Size of mysids eaten by juvenile flounder *Paralichthys olivaceus* and diurnal change of its feeding activity (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **56**, 201-206.

8) Fujii T, Noguchi M: 1996, Feeding and growth of Japanese flounder (*Paralichthys olivaceus*) in the nursery ground. In: Watanabe, Y Yamashita, Y Oozeki Y (Eds.). *Survival strategies in early life stages of marine resources*. A.A. Balkema, Rotterdam, 141－151.

9) Noichi T: 1997, Early life history (in Japanese), In: Minani T, Tanaka M (Eds). *Biology and stock enhancement of Japanese flounder*. Kouseisha-kouseikaku, Tokyo, 25－40.

10) Tanaka M, Ohkawa T, Maeda T, Kinoshita I, Seikai T, Nishida M: 1997, Ecological diversities and stock structure of the flounder in the Sea of Japan in relation to stock enhancement. *Bull. Natl. Res. Inst. Aquacult., Suppl.* **3**, 77－85.

11) Tanaka M, Seikai T, Yamamoto E, Furuta S: 1998, Significance of larval and juvenile ecophysiology for stock enhancement of the Japanese flounder, *Paralichthys olivaceus*. *Bulletin of Marine Science*. **62**, 551－571.

12) Seikai T, Tanangonan JB, Tanaka M: 1986, Temperature influence on larvalagrowth and metamorphosis of the Japansese flounder *Paralichthys olivaceus* in the laboratory. *Nippon Suisan Gakkaishi* **52**, 977－982.

13) Imabayashi H: 1980, Feeding activities of juvenile and young bastard halibut. *Paralichthys olivaceus*, in the biotic community-Ⅰ, Intraspecific relationships in the population (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **46**, 427－435.

14) Yamada H, Sato K, Nagahora S, Kumagai A, Yamashita Y: 1998, Feeding habits of the Japanese flounder *Paralichthys olivaceus* in Pacific coastal waters of Tohoku district, Northeastern Japan (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **64**, 249－258.

15) Tanaka Y, Tominaga O, Tsusaki T, Nogami K, Fushimi H, Tanaka M: 1999, UJNR experimental release of Japanese flounder at Wada Beach, Wakasa Bay: Feeding habits of wild and released juveniles. *Bull. Natl. Res. Inst. Aquacult. Suppl.* 1, 125 – 133.

16) Tanaka Y, Yamaguchi H, Gwak WS, Tominaga O, Tsusaki T, Tanaka M: 2005, Influence of mass release of hatchery-reared Japanese flounder on the feeding and growth of wild juveniles in a nursery ground in the Japan Sea. *J. Exp. Mar. Ecol.* 314, 137 – 147.

17) Seikai T, Takeuchi T, Park GS: 1997, Comparison of growth, feed efficiency, and chemical composition of juvenile flounder fed live mysids and formula feed under laboratory conditions. *Fish. Sci.* 63, 520 – 526.

18) Minami T: 1989, Early life history of flatfishes - XⅡ, nursery ground (2) (in Japanese, with English Abstr.). *Aquabiology* 11, 449 – 453.

19) Ochiai A, Tanaka M: 1985, Ichthyography (in Japanese). Kouseisha-kouseikaku, Tokyo, 1075 – 1082.

20) Minami T: 1997, Life history (in Japanese). In: Minani T, Tanaka M (Eds.). *Biology and stock enhancement of Japanese flounder.* Kouseisha-kouseikaku, Tokyo, 9 – 24.

21) Tominaga O: 2001, Evalution of fish quality of seedings for release (in Japanese). In: Fisheries Agency of Japan (Ed.), *Rreport on the improvement of Japanses flounder quality for stock enhancement H8-12.* Fisheries Agency of Japan, Tokyo, 165 – 194.

22) Noichi T, Kusano M, Ueki D, Senta T: 1993, Feeding habit of fishes eating settled larval and juvenile Japanese flounder (*Paralichthys olivaceus*) at Yanagihama beach, Nagasaki Prefecture (in Japanese, with English Abstr.). *Bull. Fac. Fish. Nagasaki Univ.* 73, 1 – 6.

23) Noichi T, Kusano M, Kanbara T, Senta T: 1993, Predation by fishes on larval and juvenile Japanese flounder at Yanagihama Beach, Nagasaki, Japan. *Nippon Suisan Gakkaishi* 59, 1851 – 1855.

24) Yamashita Y, Yamamoto K, Nagahora S, Igarashi K, Ishikawa Y, Sakama O, Yamada H, Nakamoto Y: 1993, Predation by fishes on hatchery-rered Japanese flounder *Paralichthys olivaeus*, fry in the coastal waters of Iwate Prefecture, northeastern Japan (in Japanese, with English Abstr.). *Suisanzoshoku.* 41, 497 – 505.

25) Yamashita Y: 2005, Variation in recruitments of larval and juvenile flatfish (in Japanses). In: Watanabe Y (Ed.). *Bioresources in the ocean.* Tokai University Press, Kanagawa. 272 – 285.

26) Senta T, Kinoshita I: 1985, Larval and juvenile fishes occurring in the surf-zone of western Japan. *Transactions of the American Fisheries Society.* 114, 609 – 618.

27) Senta T, Amarullah MH, Yasuda M: 1988, Invitation to the study juvenile fishes occurring in surf zone. In: Go YB (Ed.). *Proceedings of Symposium on Development of Marine Resources and International Cooperation in the Yellow Sea and the East China Sea.* Mar. Res. Inst. Cheju Nat. Univ., Korea, Cheju. 131 – 146.

28) Brown AB, McLachlan A: 1990, *Ecology of sandy shores.* Elsevier, Amsterdam, 328 pp.

29) Gibson RN, Ansell AD, Robb L: 1993, Seasonal and annual variations in abuandance and specoes commposition of fish and macrocrustacean communities on a Scottish sandy beach. *Mar. Ecol. Prog. Ser.* 98, 89 – 105.

30) Takahishi K, Kawaguchi K: 1997, Diel and tidal migrations of the sand-burrowing mysids, *Archaemysis kokuboi, A. japonica and Iiella ohshimai,* in Otsuchi Bay, northeastern Japan. *Mar. Ecol. Prog. Ser.* 148, 95 – 107.

31) Senta T, Kinoshita I, (Eds.): 1998, *Biology of larval and juvenile fishes in sandy beaches* (in Japanese). Kouseisha-kouseikaku, Tokyo, 136pp.

32) Layman CA: 2000, Fish assemblage structure of the shallow ocean surf-zone on the eastern shore of Virginia Barrier Island. *Estuarine, Coastal and Shelf Science* 51, 201 – 213.

33) Hanamura Y, Matsuoka M: 2003, Feeding habits of the sand shrimp, *Crangon uritai* Hayashi and Kim, 1999, in the central Seto Inland Sea, Japan. *Crustaceana* 76, 1017 – 1023.

34) Minami T: 1983, The early life history of a flounder *Tarphops oligolepis* (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* 49, 533 – 539.

35) Minami T, Tanaka M: 1992, Life history cycles in flatfish from the northwestern Pacific, with particular reference to their early life histories. *Neth. J. Sea Res.* 29, 35 – 48.

36) Hibino M, Ohta T, Kinoshita I, Tanaka M: 2002, Fish larvae and juveniles occurring in the littoral zone of a tidal flat, in the bottom of Ariake Bay (in Japanese, with English Abstr.). *Jpn. J. Ichthyol.* 49, 109 – 120.

37) Suda Y, Inoue T, Uchida H: 2002, Fish communities in the surf zone of a protected sandy beach at Doigahama, Yamaguchi Prefecture, Japan. *Estuarine, Coastal and Shelf Science* 55, 81 – 96.

38) Omi H, Kusakabe T, Nabeshima Y: 2003, Fishes occurring on artificial beach of southern coast in Osaka Bay (in Japanese). *Bull. Osaka Pref. Fish. Exp. Sta.* 14, 57 – 70.

39) Minami T, Nakabo T, Uozumi Y, Kiyono S: 1976, Benthic fish fauna off the river month of Yura, Wakasa Bay (in Japanese). *Showa 50 nendo Annu. Rep. Kyoto Pref. Fish. Exp. Stn.* 74 – 100.

40) Eda H: 1994, *Life history of callioymid fishes in Omura Bay, Nagasaki, Japan* (in Japanese, with English Abstr.). PhD Thesis, Nagasaki University, Nagasaki.

41) Takahashi K, Hirose T, Kawaguchi K: 1999, The importance of intertidal sand-burrowing peracarid crustaceans as prey for fish in the surf-zone of a sandy beach in Otsuchi Bay, northeastern Japan. *Fish. Sci.* 65, 856 – 864.

42) Inoue T, Suda Y, Sano M: 2005, Food habits of fishes in the surf zone of a sandy beach at Sanrimatsubara, Fukuoka Prefecture, Japan. *Ichthyol. Res.* 52, 9 – 14.

43) Maehara T: 1997, Ecology of Japanese flounder juvenile at shallow water of southwestern Hiuchi-Nada (in Japanese). *Report on the workshop of resources and oceanography in the Seto Inland Sea,* 3, 39 – 47.

44) Tokimura M, Matsuoka M: 2006, The stock assessment evaluation of Japanese flounder in the Seto Inland Sea in 2005 (in Japanese). In: Fisheries Research Agency (Ed.), *Rreport on the stock assesment of important commercial fishes in the water of Japan in 2005 (H17).* Fisheries Agency of Japan,

Tokyo. 1181 – 1191.

45) Yamashita Y, Yamada H, Malloy KD, Targett TE., Tsuruta Y: 1996, Sand shrimp predation on settling and newly-settled stone flounder and its relationship to optimal nursery habit selection in Sendai Bay, Japan. In: Watanabe Y, Yamashita Y, Oozeki Y (Eds). *Survival strategies in early life stages of marine resources*. A.A.Balkema, Rotterdam. 271 – 283.

46) Fujii T, Sudo H, Azeta M, Tanaka M: 1989, Settling process of laevae and juveniles of Japansese flounder, in Shijiki Bay, Hirado Island (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **55**, 17 – 23.

47) Kimoto K, Fujita K, Noguchi M, Koshiishi Y: 2001, Development of the two-meter beam trawl net (NRIFE II type) and estimate of the catch efficiency for juvenile Japanese flounder (in Japanese, with English Abstr.). *Bull. Nat. Res. Inst. Fish. Eng.* **22**: 67 – 90.

48) Kimoto K, Fujita K, Noguchi M, Koshiishi Y: 2001, Comparison of the catch efficiency of the three types of two-meter beam trawls juvenile flatfish (in Japanese, with English Abstr.). *Bull. Nat. Res. Inst. Fish. Eng.* **22**: 91-98.52) Furuta S, Watanabe T, Yamada H, Nishida T, Miyanaga T: 1997, Changes in distribution, growth and abandance of hatchery-reared Japanese flounder *Paralichthys olivaceus* released in the coasal area of Tottori Prefecture (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **63**, 877 – 885.

49) Minami T: 1990, Early life history of flatfishes - X IV, sampling gear for juvenile (in Japanese, with English Abstr.). *Aquabiology* **12**, 143 – 146.

50) Yamashita Y, Koshiishi Y, Minami T: 1998, Present state and problem of collection method for demersal juvenile in the fisheries research institutions (in Japanese). *Contrib. Res. Fish. Eng.* **7**, 1 – 26.

51) Takeoka H: 1985, Seto Inland Sea – II. Hiuch-Nada (in Japanese). In: The Oceanograpical Society of Japan (Ed).. *Coastal oceanography of Japanese Island*. Tokai University Press, Tokyo. 694 – 697.

52) Tanaka Y, Ohkawa T, Yamashita Y, Tanaka M: 2006, Geographical differentces in stomach contents and feeding intensity of juvenile Japanese flounder *Paralichthys olivaceus* (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi.* **72**, 50 – 57.

53) Furuta S, Watanabe T, Yamada H, Nishida T, Miyanaga T: 1997, Changes in distribution, growth and abandance of hatchery-reared Japanese flounder *Paralichthys olivaceus* released in the coasal area of Tottori Prefecture (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **63**, 877 – 885.

54) Yasunaga Y, Koshiishi Y: 1981, Basic studies of problems on the propagation of plaice, *Paralichthys olivaceus* II, a consideration of relation between distribution density of juvenile plaice and of mysids (in Japanese, with English Abstr.). *Bull. Jap. Sea. Reg. Fish. Res. Lab.* **32**, 9 – 26.

55) Kitagawa G, Ishiguro M: 1983, Regression model (in Japanese). In: Sakamoto T, Ishiguro M, Kitagawa G (Eds.). *Statistics of information quantity*. Kyoritsu, Tokyo. 127 – 154.

56) Tanda M: 1988, Ability of creeping into sand by hatchery-reared "Hirame" *Paralichthys olivaceus* (in Japanese, with English Abstr.). *Suisanzoshoku.* **36**, 21 – 25.

57) Ishida O, Tanaka K, Sato S, Shoji Y: 1977, The biological research of Japanese flounder (*Paralichthys olivaceus*) – II, the ecology of the juveniles in Tateyama Bay (in Japanese). *Bull. Chiba Pref. Fish. Exp. Sta.* **36**, 23 – 31.

58) Senta T, Sakamoto F, Noich T, Kanbara T, 1990. The R-H II push-net, gears for studing distribution patterns of jvenile flatfishes along the beach. *Bull. Fac. Fish. Nagasaki Univ.* **68**, 35 – 41.

59) Sako H, Maehara T, Takaba M, Fujita K: 1998, Seine test of National Research Institute of Fisheries Engineering (NRIFE) I type and NRIFE II type net in the Seto Inland Sea (in Japanese). *Contrib. Res. Fish. Eng.* **7**, 73 – 77.

60) Kato K: 1987, Study on resource management of flounder (*Paralichthys olivaceus*) in the northern coast of Niigata (in Japanese). *Bull Niigata Pref. Fish. Exp. Sta.* **12**, 27 – 41.

61) Ikewaki Y, Tanaka M: 1993, Feeding habit of Japanese flounder (*Paralichthys olivaceus*) larvae in the western part of Wakasa Bay, the Japan Sea (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **59**, 951 – 956.

62) Furuta S: 1999, Seasonal changes in abundance, length distribution, feeding condition and predation vulnerability of juvenile Japanese flounder, *Paralichthys olivaceus*, and prey mysid density in the Tottori coastal area (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **65**, 167 – 174.

63) Mito S: 1965, Studies on the fish eggs and larvae found in the Seto Inland Sea- II, a list of species by some morphological and ecological notes (in Japanese). *Naikai-suiken-kankoubutsu C kan.* Bull. Naikai Reg. Fish. Res. Lab., Hiroshima . **4**: 1 – 17.

64) Shoji J, Maehara T, Takechi A, Tanigawa T, Murata N, Tanaka M: 2002, Larval fishes collected in Hiuchi-nada, the central Seto Inland Sea, Japan (in Japanese, with English Abstr.). *Nippon Suisan Gakkaishi* **68**, 835 – 842.

65) Amarullah MH, Subiyanto, Noichi T, Shigemitsu K, Tamamoto Y, Senta T: 1991, Settlement of larval Japanese flounder (*Paralichthys olivaceus*) along Yanagihama beach, Nagasaki Prefecture. *Bull. Fac. Fish. Nagasaki Univ.* **70**, 7 – 12.

66) Sokal RR, Rohlf A: 1995, *Biometry*. W. H. Freeman and Company, New York, 887 pp.

67) Subiyanto Hirata I, Senta T: 1993, Larval settlement of the Japanese flounder on sandy beaches of the Yatsushiro Sea, Japan. *Nippon Suisan Gakkaishi* **59**, 1121 – 1128.

68) Kinoshita I: 1998, Significance as nursery of sandy beaches (in Japanses). In: Senta T, Kinoshita I (Eds). *Biology of larval and juvenile fishes in sandy beaches*. Kouseisha-kouseikaku, Tokyo. 122 – 133.

69) Hanamura Y, Matsuoka M Suzuki N, 2001. food organisms and predation relation of Japanese flounder juveniles in nursay grounds in the central Seto Inland Sea (in Japanses). In: Fisheries Agency of Japan. (Ed.), *The basic research of fishing grounds productivity model in Hiuch-Nada and Bingo-Geiyo-Nada*. National reserch Institute of fisheries and Environment of Inland Sea, Hiroshima, 82 – 98.

70) Yamamoto M, Makino H, Kagawa T: 2001, The charactristic ecology of Japanese flounder juveniles in shallow water in eastern Hiuch-Nada, Kagawa Prefecture (in Japanses). In: Fisheries Agency of Japan. (Ed.), *The basic research of fishing grounds productivity model in Hiuch-Nada and Bingo-Geiyo-*

Nada. National reserch Institute of fisheries and Environment of Inland Sea, Hiroshima, 51 – 71.

71) Hayashi K, Kim JN: 1999, Revision of the East Asian species *Crangon* (Decapoda: Caridea: Crangonidae). *Crustacean Res.* **28**, 62 – 103.

72) Cortes E: 1997, A critical review of method of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Can. J. Fish. Aquat. Sci.* **54**, 726 – 738.

73) Simpson EH: 1949, Measurement of diversity. *Nature* **163**, 688.74)

74) Lande R: 1996, Statistics and partitioning of species diversity, and similarity among multiple communities. *Oikos* **76**, 5 – 13.

75) Chesson J: 1978, Measuring preference in selective predation. *Ecology* **59**: 211 – 215.

76) Seikai T, Kinoshita I, Tanaka M: 1993, Predation by crangonid shrimp on juvenile Japanese flounder under laboratory conditions. *Nippon Suisan Gakkaishi* **59**, 321 – 326.

77) Yamada H, Nafahori S, Sato K, Musashi T, Fujita T, Nihira A, Kageyama Y, Kumagai A, Kitagawa D, Hirota Y, Yamashita Y: 1994, Specices composition and distribution pattern of Mysidacea in Pacific Ocean Coast of Japan (in Japanese, with English Abstr.). *Bull. Tohoku Natl. Fish. Res. Inst.* **56**, 57 – 67.

78) Mori J: 1998, Ecology of crangonid shrimp as predator (in Japanese). In: Senta T, Kinoshita I (Eds.). *Biology of larval and juveniles fishes in sandy beaches*. Kouseisha-kouseikaku, Tokyo, 65 – 77.

79) Kimoto K, Higano J, Adachi K, Takagi N, Arai K, Terashima H, Yokoyama Y, Nagahata T: 1996, Quantitative sampling method of small demersal fish and mysids using the SCUBA net, result of sampling on sandy bottoms in shallow water in Japan (in Japanese, with English Abstr.). *Tech. Rept. Nat. Res. Inst. Fish. Eng.* **18**: 45 – 57.

80) Yamada H, Endo Y, Musashi T, Yamashita Y: 1998, Distribution of mysids and larval anchovy as food organisms for juvenile flounder around artificial flounder reefs (in Japanese, with English Abstr.). *Suisanzoshoku* **46**, 487 – 494.

81) Suda Y, Gomyo M: 1995, Physical environment and larval and juvenile fishes collected in the surf zone of sandy beach (in Japanese). *Contrib. Res. Fish. Eng.* **1**, 39 – 52.

82) Shoji J, Tanaka M: 2002, Larval and juvenile fishes collected in the surf zone of Hiuchi-nada, the central Seto Inland Sea, Japan (in Japanese, with English Abstr.). *Suisanzoshoku* **50**, 123 – 128.

83) Pianka ER: 1973, The structure of lizard communities. *Annu. Rev. Ecol. Syst.* **4**, 53 – 74.

84) Tominaga O, Seikai T, Nogami K, Tsusaki T, Hondo Y, Murakami N, Tanaka Y, Tanaka M: 2003, Daily ration of hatchery-reared Japanese flounder *Paralichthys olivaceus* as an indicator of release place, time and fry quality. In situ direct estimation and possibility of new methods by stable isotope. *UJNR Tec. Rep.* **30**, 7 – 23.

85) Elliot JM, Persson L: 1978, The estimation of daily rates of food consumption for fish. *Journal of Animal Ecology.* **47**, 977 – 991.

86) Boisclair D, Legget WC: 1988, An in situ experimental evaluation of the Elliot and Persson and the Eggers models for estimating fish daily ration. *Can. J. Fish. Aquat. Sci.* **45**, 138 – 145.

87) Boisclair D, Marchand F: 1993, The gut to estimate fish daily ration. *Can. J. Fish. Aquat. Sci.* **50**, 1969 – 1975.

88) Efron B, Tibshirani RJ: 1993, *An introduction to the bootstrap.* Chapman and Hall, New York, 436 pp.

89) Sudo H, Azuma M, Azeta M: 1987, Diel changes in predator-prey relationship between red sea bream and gammaridean amphipods in Shijiki Bay. *Nippon Suisan Gakkaishi* **53**, 1567 – 1575.

90) Yasunaga Y: 1971, Study on the feeding habit and growth of the plaice, Paralichthys olivaceus, in the larval stage (in Japanese, with English Abstr.). *Bull. Tokai Reg. Fish. Res. Lab.* **68**, 31 – 43.

91) Dou S, Seikai T, Tsukamoto K: 2000, Feeding behaviour of Japanese flounder larvae under laboratory conditions. *J. Fish Biol.* **56**, 654 – 666.

92) Koshiishi Y, Nakanishi T, Akamine T, Tanaka K, Naganuma N: 1982, Basic studies of problems on the propagation of sinistral flounder, *Paralichthys olivaceus* III, diurnal feeding periodicity of 0-group flounder (in Japanese, with English Abstr.). *Bull. Jap. Reg. Fish. Res. Lab.* **33**, 67 – 80.

93) Nashida K, Tominaga O: 1987, Studies of ground communities in the coastal water of northern Niigata Prefecture II, seasonal changes of feeding habit and daily rations of young flounder, *Paralichthys olivaceus* (in Japanese, with English Abstr.). *Bull. Jap. Reg. Fish. Res. Lab.* **37**, 39 – 56.

94) Nashida K, Tominaga O, Miyajima H, Ito M: 1984, Studies of ground communities in the coastal water of northern Niigata Prefecture I, estimation of daily food consumption of young flounder, *Paralichthys olivaceus* (in Japanese, with English Abstr.). *Bull. Jap. Reg. Fish. Res. Lab.* **31**, 1 – 17.

95) Durbin EG, Duribin AG, Langton RW, Rowman RE: 1983, Stomach contents of silver hake, *Merluccius bilinearis*, and Atlantic cod, *Gadus morhua*, and estimation of their daily rations. *Fish. Bull.* **81**, 437 – 454.

96) Worobec MN, 1984, Field estimates of the daily ration of winter flounder, *Pseudopleuronectes Americanus* (Walbaum), in a southern New England salt pond. *J. Exp. Mar. Biol. Ecol.* **77**, 183 – 196.

97) Theilacker GH: 1987, Feeding ecology and growth energetic of larval northern anchovy, *Engraulis mordax*. *Fishery Bulletin* **85**, 213 – 228.

98) Shoji J, Tanaka M, 2005, Prey size and daily ration of juvenile piscivore Japanese Spanish mackerel. *J. Fish Biol.* **67**, 1107 – 1118.

99) Campana SE: 1990, How reliable are growth back-calculations based on otolish? *Can. J. Fish. Aquat. Sci.* **47**, 2219 – 2227.

100) Noichi T, Matsuo T, Senta T: 1994, Hatching dates of the Japanese flounder settling at Yanagihama beach in Nagasaki Prefecture, Japan. *Fish. Sci.* **60**, 369 – 372.

101) Yamashita Y, Tanaka M, Miller JM: 2001, Ecophysiology of juvenile flatfish in nursery grounds. *J. Sea Res.* **45**, 205 – 218.

102) Iwata N, Yamano K, Honda H, Kiyono M, Kurokura H: 1994, Effects of temperature on the growth of Japanese flounder. *Fish. Sci.* **60**, 527 – 531.

103) Fonds M, Tanaka M, Van der Veer HW: 1995, Feeding and

growth of juvenile Japanese flounder *Paralichthys olivaceus* in relation to temperature and food supply. *Neth. J. Sea Res.* **34**, 111 – 118.

104) Takechi A, Maehara T: 2001, The charactristic ecology of Japanese flounder juveniles in shallow water in western Hiuchi-Nada, Ehime Prefecture (in Japanses). In: Fisheries Agency of Japan. (Ed.), *The basic research of fishing grounds productivity model in Hiuch-Nasa and Bingo-Geiyo-Nada*. National reserch Institute of fisheries and Environment of Inland Sea, Hiroshima, 26 – 50.

105) Shibata R, Arima G, 2001, The ecology of yong and adult Japanese flounder and other demersal fishes and benthos in Hiuchi-Nada (in Japanses). In: Fisheries Agency of Japan. (Ed.), *The basic research of fishing grounds productivity model in Hiuch-Nasa and Bingo-Geiyo-Nada*. National reserch Institute of fisheries and Environment of Inland Sea, Hiroshima, 99 – 120.

106) Nagai T: 1991, Trends on catch and stock abundance indices of the Japanese anchovy in the Seto Inland (in Japanese, with English Abstr.). *Bull. Nansei Natl. Fish. Res. Inst.* **24**, 1 – 26.

107) Shoji J, Maehara T, Tanaka M, 1999, Short-term occurrence and rapid growth of Spanish mackerel larvae in the central waters of the Seto Inland Sea. *Fish. Sci.* **65**, 68 – 72.

108) Van der Veer HW, Bergman MJN: 1987, Predation by crustaceans on a newly settled 0-group plaice Pleuronectes platessa population in the western Wadden Sea. *Mar. Ecol. Prog. Ser.* **35**, 203 – 215.

109) Watanabe K, Takagaki M, Saitoh K, Nakayama K, Yamashita Y, Tanaka M: 2005, Search of the potential predators for released Japanese flounder *Paralichthys olivaceus* in Wakasa Bay. In: *Six international symposium on flatfish ecology*, Kyoto.

110) Sudo H, Kajihara N, Fujii T: 2005, Predation by the swimming crab and piscivorous fish: a major mortality factor in hatchery-reared juvenile Japan flounder released into Mano Bay. In: *Six international symposium on flatfish ecology*, Kyoto.

111) Nakamura Y: 1996, Fishery and release of flatfishes (in Japanese). *Bull. Japan Soc. Fish. Oceangr.* **60**, 271 – 275.

112) Yamashita Y, Nagahora S, Yamada H, Kitagawa D: 1994, Effects of release size on survival and growth of Japanese flounder Paralichthys olivaceus in coastal waters off Iwate Prefecture, northeastern Japan. *Mar. Ecol. Prog. Ser.* **105**, 269 – 276.

113) Houde ED: 1987, Fish early life dynamics and recreitment variability. *Amer. Fish. Soc. Symp.* **2**, 17 – 29.

114) Kimura R: 1994, Starvation (in Japanses). In: Tanaka M, Watanabe Y (Eds). *Studies on early life mortality of fishes*. Kouseisha-kouseikaku, Tokyo. 47 – 59.

115) Yamashita Y: 1994, Predation (in Japanses). In: Tanaka M, Watanabe Y (Eds). *Studies on early life mortality of fishes*. Kouseisha-kouseikaku, Tokyo. 61 – 71.

116) Purcell JE, Sturdevant MV: 2001, Prey selection and dietary overlap among zooplanktivorous jellyfish and juvenile fishes in Prince William Sound, Alaska. *Mar. Ecol. Prog Ser.* **210**, 67 – 83.

117) Terazaki M: 2005, Role of carnivorous plankton (in Japanses). In: Watanabe Y (Ed). *Marine resource*. Tokai University Press, Kanagawa. 259 – 59.

118) Yamamoto M: 2003, The long-term variations in water temperture and Salinity in Bisan-Seto, the central Seto Inland Sea (in Japanese, with English Abstr.). *Bull. Jpn. Soc. Fish. Oceangr.* **67**, 163 – 167.

119) Yamamoto M, Makino H: 2002, Estimation of catch efficiency of the two-meter beam trawl for juvenile Japanese flounder in Hiuchi-nada Sea (in Japanese, with English Abstr.). *Saibai Giken.* **30**, 31 – 35.

120) Yamamoto M, Makino H, Kagawa T, Tominaga O: 2004, Occurrence and distribution of larval and juvenile Japanese flounder *Paralichthys olivaceus* at sandy beaches in eastern Hiuchi-Nada, the central Seto Inland Sea, Japan. *Fish. Sci.* **70**, 1089 – 1097.

121) Yamamoto M, Makino H, Kobayashi J, Tominaga O: 2004, Food organisms and feeding habits of larval and juvenile Japanese flounder *Paralichthys olivaceus* at Ohama Beach in Hiuchi-Nada, the central Seto Inland Sea, Japan. *Fish. Sci.* **70**, 1098 – 1105.

122) Yamamoto M, Tominaga O: 2005, Feeding ecology of dominant demersal fish species, *Favonigobius gymnauchen*, *Repomucenus* spp. and *Tarphops oligolepis*, at a sandy beach where larval Japanese flounder settle in the Seto Inland Sea, Japan. *Fish. Sci.* **71**, 1332 – 1340.

123) Yamamoto M, Tominaga O: 2007, Daily rations of Japanese flounder *Paralichthys olivaceus*, small flounder *Tarphops oligolepis* and sand goby *Favonigobius gymnauchen* and diel changes in the availability of food at a sandy beach in the central Seto Inland Sea. *Fish. Sci.* **73**, 314 – 323.