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FULL PAPER

Horizontal gradient in fish assemblage structures in and around a seagrass habitat: some implications for seagrass habitat conservation

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Abstract Horizontal gradients in fish assemblage structures in and around a seagrass habitat were evaluated by visual observations. The assemblage structures clearly differed between open microhabitats lacking seagrass, i.e., nearby sand and the area adjoining the outer margin of the seagrass bed (referred to as "outer gap"), and microhabitats with seagrass within the overall seagrass bed, although not differing among the latter microhabitats, including both edge and core portions. Such open microhabitats were found to be not always inferior, but nearly equal to or even sometimes greatly superior in fish species' diversity and/or abundance to the microhabitats with seagrass. In particular, the outer gap was always ranked first in total species' number and had outstanding abundance in spring. Similar open microhabitats adjoining seagrass walls facing the sand patch within the seagrass bed (referred to as "inner gap") in spring were also characterized by higher fish species and individual numbers. The 11 most abundant fishes showed four discrete distribution patterns (three recognized herein and one implied by precedent studies), such contributing to a horizontal gradient in the fish assemblage structure. While no fishes showed a preference for the edge or core of the seagrass bed, group-forming juveniles of several species favored gap microhabitats, suggesting that, in addition to the traditionally recognized edge and core microhabitats, the concept of gap microhabitats should be included in seagrass conservation ecology.

Keywords Seagrass-fish assemblage structure · Edge · Core · Gap · Conservation

Introduction

It is generally believed that seagrass habitats support large numbers of fish species and individuals, and provide nursery habitats for juveniles of many species, compared to unvegetated substrata, which are usually characterized by fewer species and fewer individuals (e.g., Kikuchi 1974; Beckley 1983; Pollard 1984; Sogard 1989; Connolly 1994; Edgar and Shaw 1995; but see also, e.g., Horinouchi 2005).

In recent years, extensive losses of seagrass habitats have occurred in coastal marine ecosystems around the world (Shepard et al. 1989; Short and Wyllie-Echeverria 1996; Hauxwell et al. 2003; Orth et al. 2006). Such losses may involve several transitional phases. Partial disturbance, for example, by boat-propeller scarring, in a large seagrass habitat may reduce seagrass height/density or remove seagrass leaves in some portions of the bed, which may in turn lead to the fragmentation of the latter habitat into several smaller patches. Their sizes may be further reduced by other factors, including erosion of substrate. On the other hand, even when anthropogenic disturbance factors are absent, small seagrass habitats are not uncommon, the sizes of seagrass habitats being extremely variable in nature, ranging from <1 m^2 to a hectare or more. Whatever the situation, a decrease in the size of a habitat is accompanied by an increase in the relative amount of its outer edge portion (for a detailed explanation of edge and other microhabitats recognized in this study, see Fig. 1).

The edge of a habitat is often focused upon, especially in conservation ecology, because species' interactions, e.g., predation, are often enhanced in that potentially vulnerable

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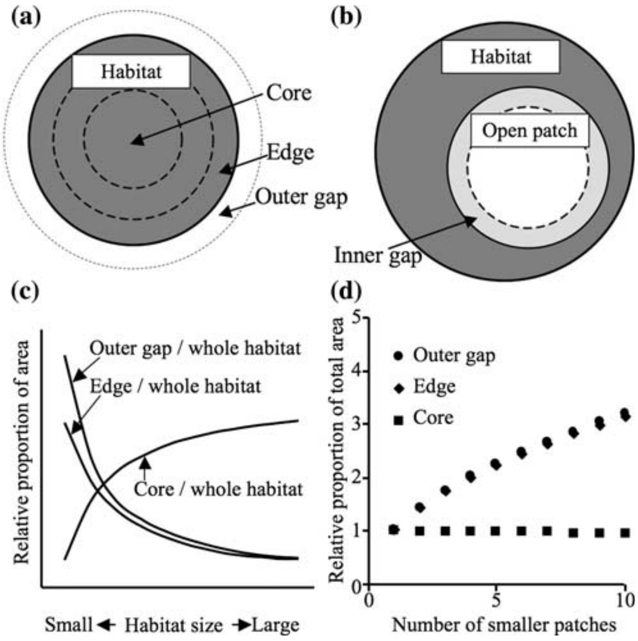


Fig. 1 Conceptual diagrams (viewed from above) of core, edge, and outer gap microhabitats (**a**), of an inner gap (*brighter shaded*) in and around a given habitat (*darker shaded*) (**b**), and simplified examples of relationships of the relative proportions of their areas (except the inner gap) (**c**) and of the proportion of total area of each microhabitat in multiple smaller patches to the areas of corresponding microhabitat at a single large habitat of the same total area as the patches (**d**). Relationships in **c** based on a round habitat with edge and outer gap microhabitats constantly the same range, and the microhabitat core diameter monotonically increasing in accordance with the whole habitat diameter. Relationships in **d** based on assumptions that the large (diameter 10 m) and smaller habitats were round, all of the latter having identical size, the ranges of both edge and gap being 1 m, and the core diameter monotonically decreasing in accordance with a decrease in habitat/patch diameter. The relationships of habitat size or patch numbers and proportional abundance of fish preferring each microhabitat show a basically similar pattern with the corresponding microhabitat. Note that all of the above relationships would be modified by various internal and external factors

region (see Saunders et al. 1991; Fagan et al. 1999). For example, in a terrestrial forest system, songbird nest predators utilizing areas bordering isolated forest patches sometimes significantly reduce egg survival at patch edges, the survival rates of eggs often being lowest in small patches with a high edge proportion (Paton 1994). In a seagrass system, Laurel et al. (2003) found the lowest survival rate of age-0 cod (*Gadus* spp.) in the smallest patch, suggesting that the closer proximity to the "dangerous" edge was responsible. However, habitat edge is not always unfavorable. Holt et al. (1983), for example, found that small recruits of *Sciaenops ocellatus* concentrated on the edge portions of *Halodule wrightii* seagrass beds, and Uhrin and Holmquist (2003), who examined differences in fish abundance among horizontal zones in and around seagrass habitats, found only a slight increase in total fish abundance in the interior portions. Such differential results suggest that edge significance may vary among species, the preference for any horizontal microhabitats being species-specific.

Clarification of any horizontal (across-edge-to-core directional) gradient in the fish assemblage structure and identification of the species responsible for that gradient should be crucial for predicting a response of the assemblage to habitat disturbance such as fragmentation. Smaller seagrass habitats may basically support more individuals of edge-preferring fishes and fewer core-preferring fishes, the opposite being true for larger seagrass habitats (see Fig. 1c). Such information should be useful for testing traditional issues of the SLOSS concept ("Single Large Or Several Small" protected areas) for the conservation of biodiversity (McNeil and Fairweather 1993) (see Fig. 1d). They should also be applicable for habitat restoration: the construction of many small seagrass habitats rather than a single large habitat may be suitable for edge-preferring target species, vise versa for core-preferring species (see Fig. 1d). However, such a study has not been conducted to date in a Japanese coastal system.

Accordingly, I examined the horizontal gradient of the fish assemblage structure in and around a *Zostera marina* seagrass bed at Aburatsubo. In addition to the concepts of "edge" and "core" microhabitats, an open space near the outer border of the seagrass bed (hereafter referred to as "outer gap," a conceptual diagram being included in Fig. 1a) was also included in the study. The relationship of the latter to whole habitat size was similar to that of the edge. Although most of the former studies on edge versus core issues of seagrass habitats have taken no account of this microhabitat (see reviews of Bell et al. 2001 and Connolly and Hindell 2006), which may be due to the sampling techniques often employed (netting) barely discerning between the microhabitat and the edge, an observational study conducted by Horinouchi and Sano (1999) suggested that such microhabitats sometimes supported a relatively large number of fish individuals. Additionally, in spring, inner open spaces adjacent to surrounding seagrass in sand patches within the seagrass bed (hereafter referred to as "inner gap," a conceptual diagram being included in Fig. 1b) were studied specifically, because they also suggested that group-forming juveniles of some fish species, which frequented the study site in spring, may prefer such a microhabitat. The specific questions addressed in this study were: (1) Does a gradient in fish assemblage structure exist from the outer open space near the border to the core portion of the seagrass bed? (2) Do any seasonal differences exist in such a gradient? (3) Which fish species contributed significantly to such a gradient? In addition, I aimed to draw some implications for seagrass-fish conservation from the acquired information.

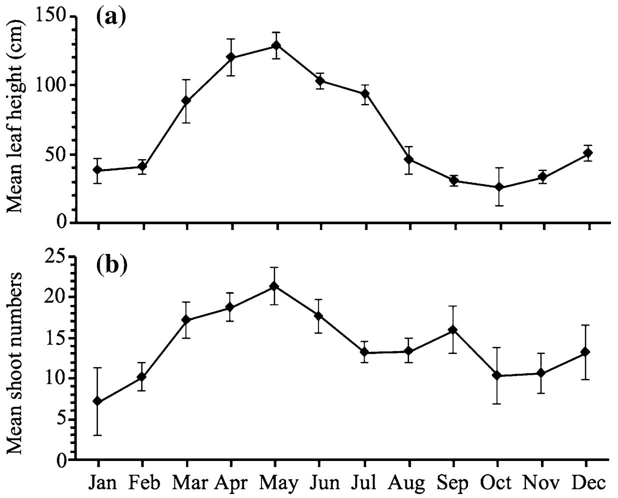


Fig. 2 Mean seagrass leaf height (cm) (**a**) and shoot numbers per 0.1 m^2 (**b**) in each month. *Vertical bars* indicate standard deviation. $n = 5$

Methods

Study site. The study was carried out in and around a monospecific *Zostera marina* seagrass bed (ca. 2 ha) in Moroiso Bay at Aburatsubo (35°09′N, 139°37′E) on the southwestern side of Miura Peninsula, Kanagawa Prefecture, Japan, during January through December 2001. A map of the study site was given by Horinouchi (2007a). Understory algae were negligible within the seagrass bed and on the surrounding bare sand substratum. Mean seagrass leaf height and shoot numbers per 0.1 m^2 in each study month are shown in Fig. 2.

Visual census of the distribution patterns of fishes. To document horizontal (across-edge-to-core directional) patterns of fish assemblage structure, visual censuses using strip-transects 1 m wide and 20 m long were conducted in the seagrass bed at depths between about 1.0 and 2.0 m at low tide monthly, between 10:00 and 16:00 h. At each census time, five transects were employed within the seagrass bed. Prior to each census of each transect within the seagrass bed, a starting point was randomly established at the border on the surrounding sand substratum (see Fig. 3), the SCUBA diver (M. Horinouchi) then swimming toward the center of the bed at a speed of about 1.0 m min^{-1}, all the while observing the area within strips some 0.5 m on either side of the center path (for the relationships of census efficiency and strategy, see Horinouchi et al. 2005). Each transect was at least 10 m apart from other transects. To identify horizontal patterns of fish assemblage structure within the seagrass bed, each transect was divided into ten sections at an interval of 2 m from the starting point (Fig. 3) and fish within each section (1 × 2 m) recorded. Fish species names followed Nakabo (2002). In addition, to document fish occurrence in the open space near the outer border of the seagrass bed and also on the sand substratum, five quadrats 1 m wide and 2 m long were established on the adjacent area along the outer border of the seagrass bed, i.e., outer gap, and the area within 2–3 m from the outer border of the seagrass bed, respectively (latter area hereafter referred to as "sand"; see Fig. 3). All fish within each quadrat were recorded by visual census.

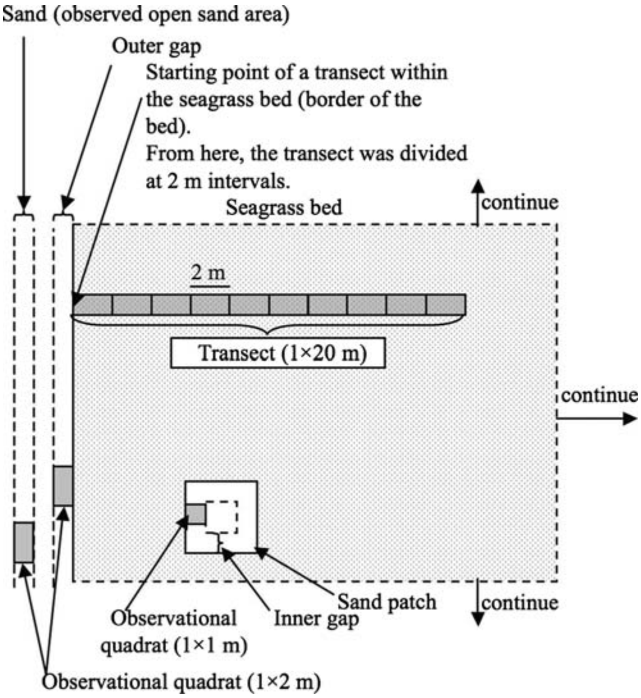


Fig. 3 Diagrams (viewed from above) of the microhabitats, transects, and observational quadrats in and around the seagrass bed and a sand patch within the seagrass bed. Open spaces near the outer border of the seagrass bed and the inner seagrass border in the sand patch within the seagrass bed identified as *outer gap* and *inner gap*, respectively (see text), and sand substratum within 2–3 m area from the outer seagrass border as *sand*

During April through June, five randomly chosen sand patches within the seagrass bed (ca. 4–9 m^2) were also observed. Within each sand patch, a 1-m^2 quadrat was established on the adjacent area along the inner seagrass border facing the patch, i.e., inner gap (see Fig. 1b). All fish within each quadrat were recorded by visual census.

At each census, whenever possible, the total length (TL) of each individual recorded was estimated, with a transparent ruler, to the nearest 5 mm for less mobile species and to the nearest 10 mm for active swimmers.

Statistical analyses. During the study period, the bottom water temperature at the study site was lowest in February (10°C) and highest in August (30°C). Based on such water temperature fluctuation pattern at the present study site, data were pooled for each season, i.e., winter: January–March (range of water temperature measured at the censuses, 10–13°C); spring: April–June (16–24°C); summer:

July–September (26–30°C); autumn: October–December (13–22°C), such providing 15 replications for each microhabitat in each season.

Total mean fish species and individual numbers per 2 m^2 were compared among microhabitats, i.e., sand substratum, outer gap, and each section of the transect within the seagrass bed (hereafter referred to as seagrass microhabitats), using two-way analysis of variance (ANOVA) with microhabitat and season as factors. Because microhabitat × season interactions were significant (see "Results"), fish species' numbers and densities were again compared among microhabitats in each season, using one-way ANOVA.

In order to examine possible horizontal gradients in fish assemblage structure from the outer sand to the core of the seagrass bed, densities of component species were compared among microhabitats using two-way MANOVA with microhabitat and season as factors. Because microhabitat × season interactions were significant (see "Results"), fish densities were again compared among microhabitats in each season, using one-way MANOVA. In addition, the same procedures were conducted only on seagrass microhabitat data, so as to check whether or not the fish assemblage structure differed between the edge and core portions of the seagrass bed.

In spring, the inner edge was also studied. Accordingly, the data for spring were subjected to separate statistical analysis, after adjustment (per 1 m^2). Total mean fish species and individual numbers were compared among microhabitats, including the inner gap, using one-way ANOVA. MANOVA was also conducted to compare fish assemblage structures among microhabitats.

In the case of differences in mean species or individual numbers being recognized by the above analyses, post hoc Games–Howell tests were employed to check for differences between all pairs of microhabitats.

Prior to the above analyses, density data were log-transformed. Such transformation, however, did not produce homogeneous variances in some cases. In such cases, to compensate for the increased likelihood of Type 1 errors (the rejection of a true null hypothesis), the significance level was set at $\alpha = 0.01$ (Underwood 1981).

Because the distribution patterns of abundant species should contribute largely to any gradients in the fish assemblage structures, following the above MANOVA, post hoc Games–Howell tests were conducted to check for differences in the densities of 11 abundant species (>5% of the total individual number) between microhabitat pairs. In addition, to clarify whether or not these abundant species showed discrete distribution patterns, a cluster analysis was conducted. The similarity of the distribution patterns across microhabitats between the species pairs was evaluated using the Bray–Curtis similarity index (Krebs 1998) based on the fish density data. Subsequently, a similarity matrix was generated and subjected to an average linkage clustering method in order to generate a similarity phenogram for fish distribution patterns. Less abundant species were not included in these analyses.

To graphically represent possible gradients in fish assemblage structure, a cluster analysis and multidimensional scaling (MDS) were applied. Initially, the Bray–Curtis similarity index was calculated based on density data as the similarity of the assemblage structure between all the microhabitat pairs. Then, a similarity matrix was generated and subjected to an average linkage clustering method in order to generate a similarity phenogram for the microhabitat, and also to MDS in order to check the grouping of microhabitat by plotting them in a two-dimensional ordination.

The statistical analyses were carried out on SPSS ver. 12.0 J (SPSS, Tokyo, Japan).

Results

A total of 42 fish species were recorded from the microhabitats, the majority being of small body size (Table 1).

Total fish species numbers always tended to be highest in the outer gap, which therefore had the highest overall species' numbers (Fig. 4). Two-way ANOVA detected significant differences in mean species' numbers among microhabitats and seasons ($F = 3.77$, $P < 0.001$ for microhabitat; $F = 111.33$, $P < 0.001$ for season; $F = 2.95$, $P < 0.001$ for microhabitat × season). The following post hoc tests revealed that, in general, the mean species' number was higher in the outer gap compared with sand (Games–Howell test, $P = 0.005$), but was not different between all other microhabitat pairs at a significance level of 0.01 (Fig. 4), although the strong interactions indicated the existence of seasonally different patterns. In each season, except winter, the mean species' number differed significantly among microhabitats (one-way ANOVA: $F = 1.16$, $P = 0.320$ for winter; $F = 4.71$, $P < 0.001$ for spring; $F = 2.73$, $P = 0.003$ for summer; $F = 4.21$, $P < 0.001$ for autumn). In spring, the mean species' number in the outer gap was significantly higher than those in all other microhabitats (Games–Howell test, $P \leq 0.005$ for all pairs in seagrass microhabitats; $P \leq 0.001$ for pairs on sand). For other pairs in each season, post hoc tests seldom detected significant differences.

Mean total fish density also differed significantly among microhabitats and seasons (two-way ANOVA, $F = 10.43$, $P < 0.001$ for microhabitat; $F = 96.07$, $P < 0.001$ for season; $F = 11.33$, $P < 0.001$ for microhabitat × season). Overall, the outer gap tended to support the highest number of individuals, although the post hoc test detected no

Table 1 Fish species (codes used in Figs. 7, 8) observed within the transects in each microhabitat in each season (body size ranges in mm TL)

Family	Season	Microhabitat			
Species (code)		Sand	Outer gap	Seagrass	Inner gap[a]
Ophichthidae					
Ophisurus macrorhynchus (1)	Spring		L.A.		
Pisodonophis zophistius (2)	Spring		L.A.	L.A.	
	Summer	L.A.	L.A.	L.A.	
Aulorhynchidae					
Aulichthys japonicus (3)	Spring		16–20		16–20
Fistulariidae					
Fistularia commersonii (4)	Autumn		L.A.		
Syngnathidae					
Syngnathus schlegeli (5)	Winter			241–250	
	Summer			290–300	
Mugilidae					
Mugil cephalus cephalus (6)	Spring			L.A.	
	Summer			L.A.	
Scorpaenidae					
Sebastes inermis (7)	Winter			15–100	
	Spring		26–30	26–100	26–30
Sebastes hubbsi (8)	Spring			146–150	
	Autumn			115–120	
Tetrarogidae					
Hypodytes rubripinnis (9)	Winter			36–40	
	Spring			36–50	
	Summer			26–80	
	Autumn			45–80	
Platycephalidae					
Inegocia japonica (10)	Summer			196–200	
Hexagrammidae					
Hexagrammos agrammus (11)	Spring			66–120	
Cottidae					
Pseudoblennius cottoides (12)	Spring		26–30	16–20	26–30
	Summer		96–100	96–100	
	Autumn			96–100	
Pseudoblennius percoides (13)	Spring			36–50	
	Summer			66–70	
	Autumn			96–100	
Apogonidae					
Apogon semilineatus (14)	Summer			66–70	
Scombropidae					
Scombrops boops (15)	Spring		61–70		
Gerreidae					
Gerres equulus (16)	Winter	16–20		36–40	
	Spring	36–40	26–30		
	Summer	16–20	16–20	26–30	
	Autumn	26–50	26–50	26–30	
Sparidae					
Acanthopagrus schlegelii (17)	Summer			31–100	
	Autumn		151–200	151–200	

Table 1 continued

Family	Season	Microhabitat			
Species (code)		Sand	Outer gap	Seagrass	Inner gap[a]
Sillaginidae					
Sillago japonica (18)	Summer	16–150	16–150		
	Autumn	46–60	46–60		
Mullidae					
Upeneus tragula (19)	Summer			56–60	
Upeneus japonicus (20)	Spring			191–200	
	Summer		26–30	16–20	
	Autmn			56–60	
Cheilodactylidae					
Goniistius zonatus (21)	Spring				
	Summer		46–50	46–50	
Embiotocidae					
Ditrema temmincki (22)	Winter			L.A.	
	Spring	91–100	41–50	41–50	
	Summer			91–100	
Opegnathidae					
Oplegnathus fasciatus (23)	Summer			91–100	
Labridae					
Halichoeres poecilopterus (24)	Winter			L.A.	
	Spring			41–50	
	Summer	L.A.	90–100	16–20	
	Autumn			151–200	
Blenniidae					
Petroscirtes breviceps (25)	Winter			56–60	
	Spring			56–60	
Callionymidae					
Paradiplogrammus enneactis (26)	Summer			26–100	
	Autumn	66–70		66–70	
Repomucenus curvicornis (27)	Winter		146–150		
	Summer	96–100			
	Autumn	196–200	196–200		
Repomucenus beniteguri (28)	Spring		141–145		
Gobiidae					
Chaenogobius gulosus (29)	Winter		11–20		
	Spring		11–20	11–20	11–20
Gymnogobius heptacanthus (30)	Winter		46–50		
	Spring		11–20		11–20
Sagamia geneionema (31)	Winter	96–100	76–80		
	Spring	71–75			
	Summer	61–65	56–70		
	Autumn	56–100		96–100	
Acanthogobius flavimanus (32)	Summer	66–70	96–100	96–100	
	Autumn		96–100	146–150	
Pterogobius zonoleucus (33)	Spring		16–20	16–20	16–20
Pterogobius elapoides (34)	Winter		11–20	11–20	
	Spring			26–30	26–30

Table 1 continued

Family	Season	Microhabitat			
Species (code)		Sand	Outer gap	Seagrass	Inner gap[a]
Favonigobius gymnauchen (35)	Winter	46–50	46–50	46–50	
	Spring	31–80	31–80		
	Summer	11–60	11–60	46–50	
	Autumn	26–50	26–50	26–50	
Acentrogobius sp. (36)	Winter		46–50	35–75	
	Spring	36–50	36–40	31–60	36–40
	Summer		46–50	11–60	
	Autumn		46–50	11–80	
Pleuronectidae					
Pleuronectes yokohamae (37)	Summer			66–70	
Monacanthidae					
Brachaluteres ulvarum (38)	Spring			36–40	
Rudarius ercodes (39)	Winter			11–40	
	Spring			11–60	41–45
	Summer		41–45	5–60	
	Autumn		41–45	16–50	
Tetraodontidae					
Canthigaster rivulata (40)	Spring			96–100	
Takifugu pardalis (41)	Autumn	196–200			
Takifugu niphobles (42)	Winter	96–100	96–100		
	Summer	146–150			

L.A. indicates large adults whose body size not measured

[a] Note that the inner gap was studied only in spring

differences between any microhabitat pairs at the 0.01 significance level (Fig. 5). The mean densities differed among microhabitats in spring and autumn, but not in winter and summer (one-way ANOVA: $F = 1.24$, $P = 0.263$ for winter; $F = 21.22$, $P < 0.001$ for spring; $F = 1.12$, $P = 0.347$ for summer; $F = 3.29$, $P < 0.001$ for autumn). In spring, total fish density was highest in the outer gap due to the occurrence of group-forming juveniles, compared with other microhabitats, but similar for all other pairs (Games–Howell test, $P \leq 0.001$ for all pairs in the outer gap in spring; $0.619 \leq P \leq 1.00$ for other pairs). In autumn, the mean densities in seagrass microhabitats seemed to be slightly higher than those in the outer microhabitats, but the post hoc tests did not detect any significant differences ($0.057 \leq P \leq 0.887$).

Mean species numbers differed significantly between all pairs of seasons (Games–Howell test, $P < 0.01$), being highest in summer and lowest in winter (Fig. 6). Mean total fish density also differed between most season-pairs ($P < 0.01$) except spring–autumn pair ($P = 0.98$), being highest in spring and lowest in winter (Fig. 6).

Two-way crossed MANOVA, including all microhabitats except the inner gap, detected spatial and seasonal differences in fish assemblage structures, in addition to significant interactions between the factors (for the statistical results, see Table 2) (Fig. 7). One-way MANOVA also detected significant difference among all the microhabitats in each season. Notwithstanding, additional two-way and one-way MANOVAs, which were restricted to data for seagrass microhabitats, revealed that fish assemblage structure did not differ among seagrass microhabitats, including the edge and core portions of the seagrass bed (see Table 2).

In spring, a large number of group-forming juveniles occurred in the inner gap (Fig. 8), the mean species and individual numbers being significantly higher in that microhabitat than in the sand and seagrass microhabitats, although not differing with the outer gap. One-way crossed MANOVA, including the inner gap, detected spatial differences in fish assemblage structure (see Table 2).

Several fishes were abundant seasonally or year-round in this system, their differential horizontal distribution patterns, sometimes with seasonal occurrence patterns, contributing largely to a horizontal gradient in assemblage structure and also to season × microhabitat interactions (Figs. 7, 8). Based on their distribution patterns across the

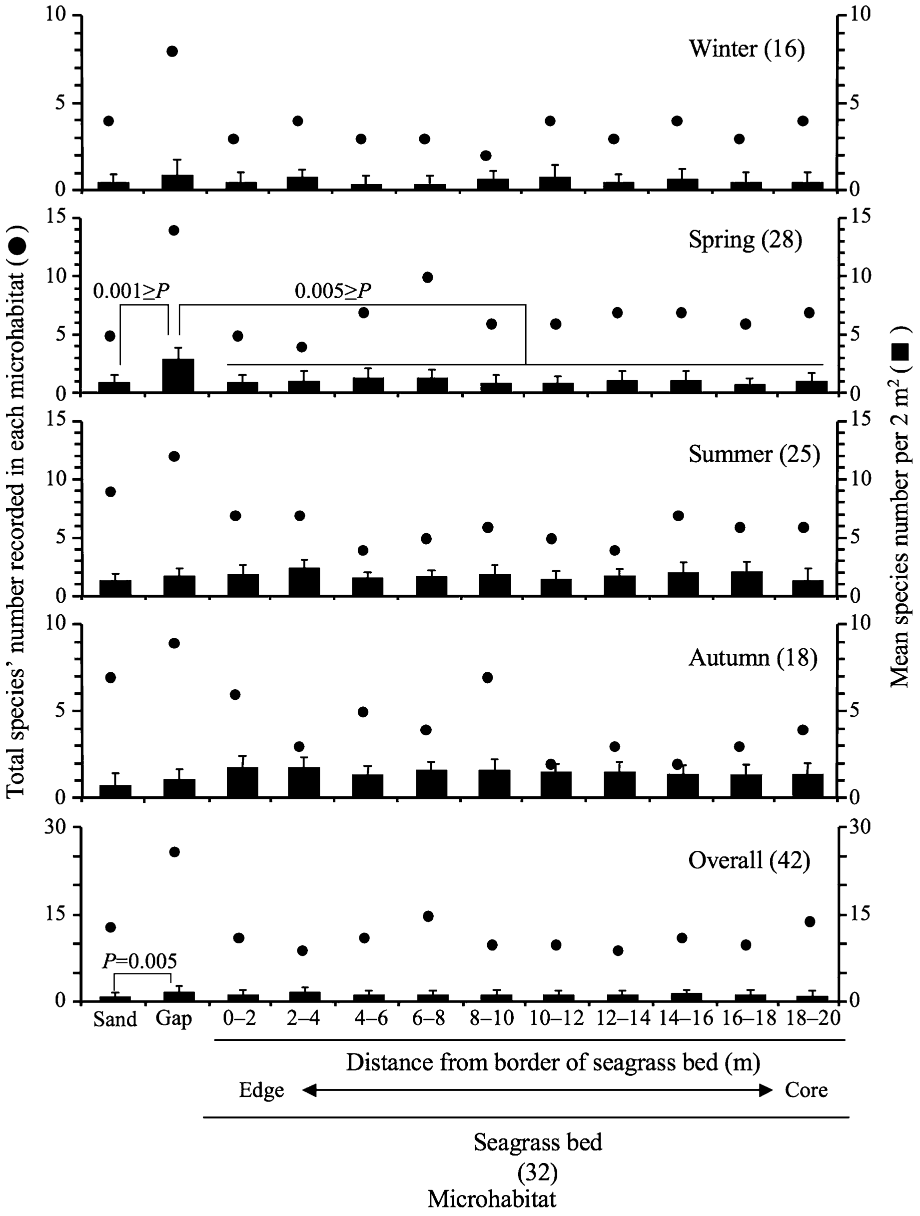


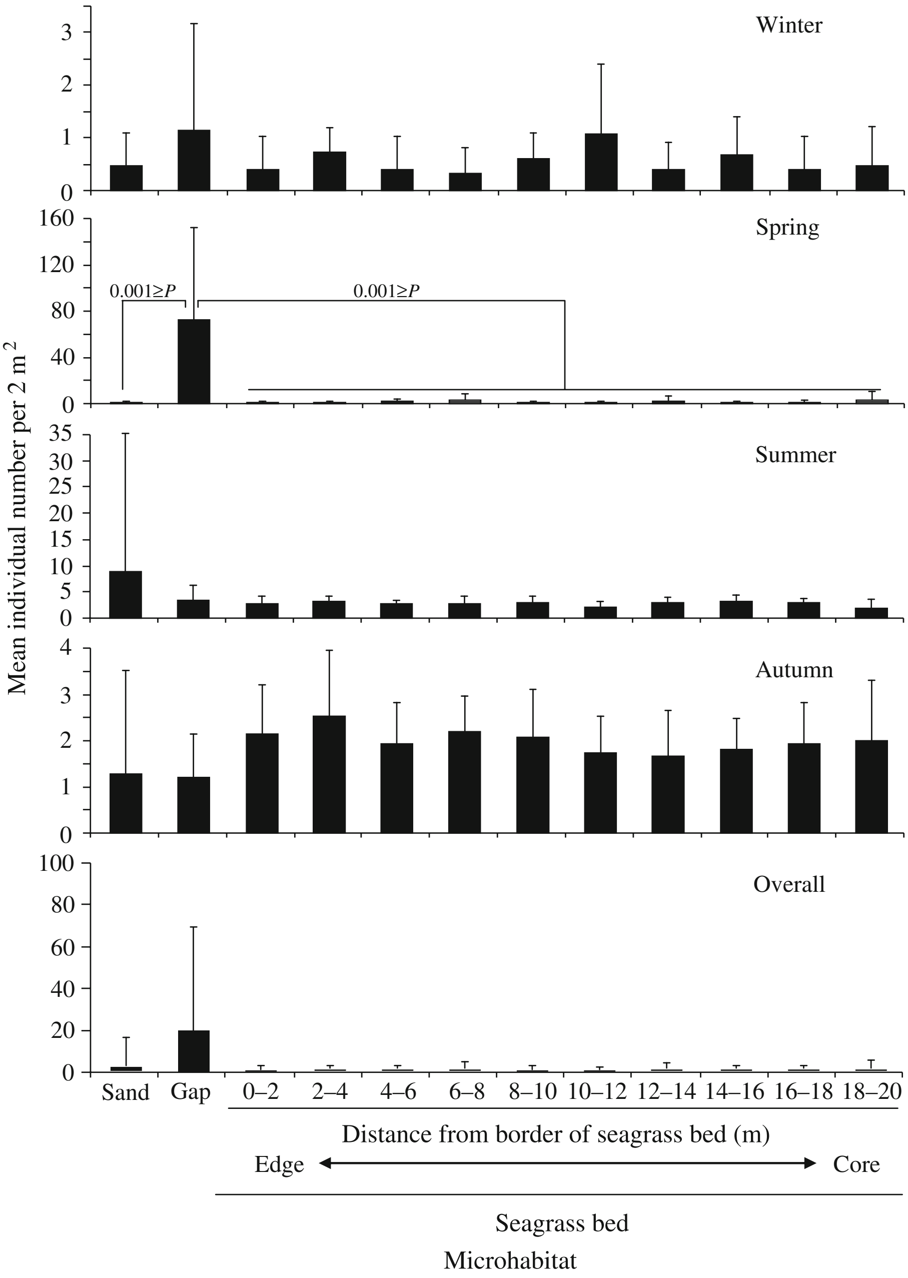
Fig. 4 Total and mean species' numbers in each microhabitat in each and all seasons. *Number* in *parentheses* following season indicates total species' numbers observed in that season. *Number* in the *parentheses* below "*Seagrass bed*" indicates total species' number observed in all sections of the seagrass transects. *Vertical bars* indicate standard deviations. *P* values on *horizontal bars* indicate results of post hoc Games–Howell test following ANOVA

microhabitats, these species could be divided, more or less, into three categories at a similarity-index level of 40 (Fig. 9). Their distribution patterns are described below. Those of the remaining (lower density) species are shown in Figs. 7 and 8 (see also Table 1), but are not dealt with specifically in the text.

Favonigobius gymnauchen, *Sagamia geneionema*, *Gerres equulus*, and *Sillago japonica*: The densities of these species were apparently higher in both the sand and outer gap, compared with seagrass microhabitats (Fig. 7), although the Games–Howell test did not always detect significant differences (probably, at least for the latter two species, due to their highly fluctuations in density owing to their group-forming characteristics). The three former species sometimes intruded into the seagrass bed, whereas the last-named was restricted to the outside area (Fig. 7).

Juveniles of *Chaenogobius gulosus*, *Pterogobius zonoleucus*, *Gymnogobius heptacanthus*, *Aulichthys japonicus*, and *Sebastes inermis*: In spring, these group-forming juveniles were extremely abundant in the water column at the outer and inner gaps, thereby contributing to the extraordinarily high total fish densities at these gaps (Figs. 7, 8). They never appeared over sand, although occasionally occurring in microhabitats within the seagrass bed. Differences in their densities between the gap and seagrass microhabitats were clearly apparent (see Figs. 7, 8), although density fluctuations resulted in non-significant differences (Table 3).

Fig. 5 Mean fish individual numbers observed in each microhabitat in each and all seasons. *Vertical bars* indicate standard deviation. *P* values on *horizontal bars* indicate results of post hoc Games–Howell test following ANOVA



Acentrogobius sp. and *Rudarius ercodes*: *Acentrogobius* sp. and *R. ercodes* were both dominant in the seagrass bed throughout the year, their densities remaining unchanged among the transect sections within the seagrass bed (see Table 3). This contributed largely to the similar assemblage structure among the edge and core portions of the seagrass bed (Fig. 7). These species, despite a few individuals occurring at the gap, seldom or never appeared over sand.

A cluster analysis and the MDS provided graphical representation supporting the results of MANOVA. The phenogram of the similarity in fish assemblage structure showed a clear grouping of all of the seagrass microhabitats, including both edge and core portions of the seagrass bed, at a similarity-index level of 40 (intermediate similarity) (Fig. 9). A tendency toward sub-grouping by season was apparent, except for a cluster of four seagrass microhabitats in spring where some group-forming juveniles appeared (Fig. 10a, see also Fig. 7). The microhabitats within the seagrass bed were at no time grouped together with the gaps and sand. The outer gap and sand were grouped together in summer and autumn, but not in winter and spring (Fig. 10a). The outer and inner gaps in spring were grouped together. The MDS ordination described similar patterns, all of the microhabitats within the seagrass bed, except for a group of four seagrass microhabitats in spring, being convergent, but separated from the other microhabitats (Fig. 10b). The group of outer and inner gap microhabitats in spring was the most distant from the others.

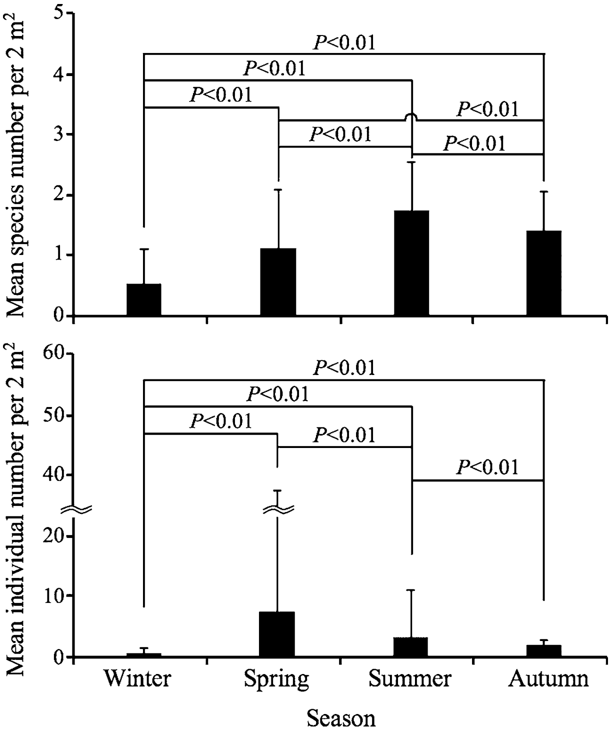


Fig. 6 Mean species' and individual numbers per 2 m^2 observed in each seasons. Inner gap data excluded. *Vertical bars* indicate standard deviations. *P* values on horizontal bars indicate results of post hoc Games–Howell test following ANOVA

Discussion

The present study revealed that the fish assemblage structure clearly differed between open microhabitats lacking seagrass (i.e., sand and outer and inner gaps) and microhabitats with seagrass in the overall seagrass bed, although not differing among the latter microhabitats including both edge and core portions. Additionally, the open microhabitats were found to be nearly equal to and sometimes well superior in fish species' diversity and/or abundance to the microhabitats with seagrass, thereby contrasting with the generally held notion of higher diversity and abundance in the more structurally complex seagrass habitat (e.g., Connolly 1994; Edgar and Shaw 1995). In particular, the gap microhabitat was always ranked first in total species number, having particularly high abundance in spring.

A horizontal gradient in the fish assemblage structure can be determined from the distribution patterns of the component species of the fish assemblage in and around the seagrass bed. Based on the density patterns across the microhabitats observed in the present study, abundant fishes fell basically into three types, with discrete distribution patterns. Of them, one type can be further divided into two separate types, according to their responses to habitat complexity as determined in earlier studies (e.g., Horinouchi and Sano 2001). Therefore, fishes occurring in and/or around a seagrass habitat can be categorized into at least four types with discrete distribution patterns: (1) species abundant in both sand and outer gap microhabitats but relatively scarce within the dense seagrass area (e.g., *Favonigobius gymnauchen*, *Sagamia geneionema*, *Gerres equulus*, and *Sillago japonica*), (2) species almost exclusively occurring at the outer and inner gaps (e.g., juveniles of *Chaenogobius gulosus*, *Pterogobius zonoleucus*, *Gymnogobius heptacanthus*,

Table 2 Summary of results of two-way and one-way MANOVAs examining differences in fish assemblage structure among seagrass only and all microhabitats in each and all seasons

	All microhabitats			Seagrass microhabitats		
	Lawley–Hotelling trace	*F*	*P*	Lawley–Hotelling trace	*F*	*P*
All seasons						
Habitat	2.702	3.680	_<0.001_	0.622	1.006	0.058
Season	1.649	8.238	_<0.001_	1.499	8.237	_<0.001_
Habitat × season	4.708	2.137	_<0.001_	1.649	1.006	0.447
Each season						
Winter	1.470	1.262	0.015	0.683	0.884	0.780
Spring	6.582	3.107	_<0.001_	1.505	0.997	0.500
	4.177*	1.904*	_<0.001*_			
Summer	6.091	3.007	_<0.001_	1.630	1.080	0.240
Autumn	2.446	1.843	_<0.001_	1.137	1.130	0.166

Values with *asterisk* indicate results for data including inner gap in spring. Note that the inner gap was studied only in spring. *Italic face* indicates significant difference at $\alpha = 0.01$

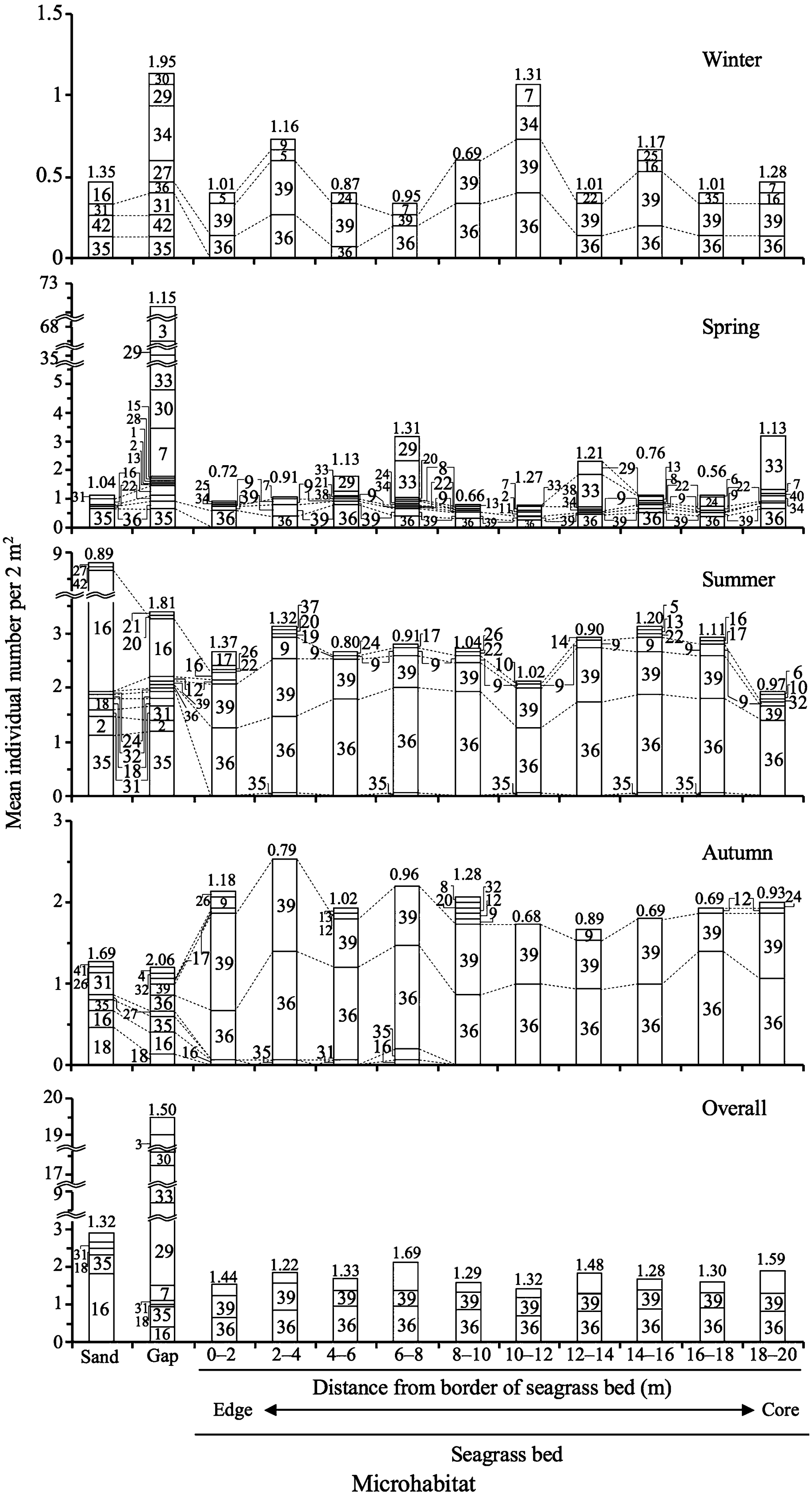


Fig. 7 Mean individual numbers of each species per 2 m^2 in each microhabitat observed in each and all seasons. For species' codes, see Table 1. For overall seasons, mean individual numbers of less abundant species (<5% of the total) were pooled in the blank cells. *Number* on each bar indicates Shannon–Wiener species diversity index *H*′

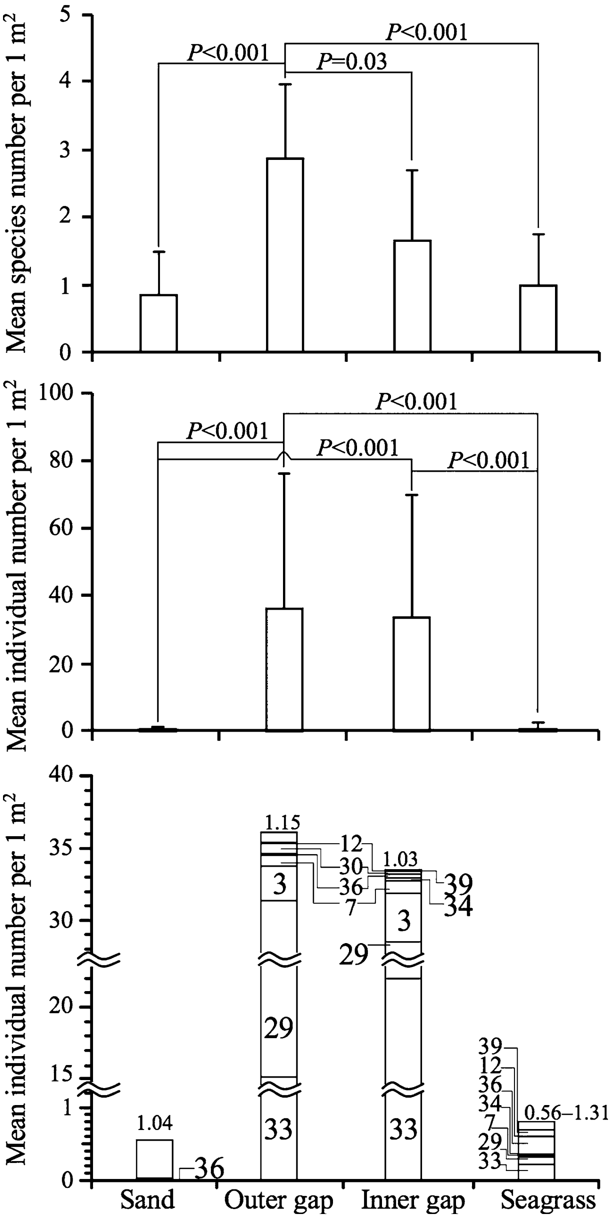


Fig. 8 Mean fish species' numbers (*top*), density (*middle*), and relative abundance of each species (*bottom*) per 1 m^2 in sand, outer and inner gaps, and seagrass microhabitats in spring. *Vertical bars* indicate standard deviation. Microhabitats with seagrass were lumped together. Significant *P* values on *horizontal bars* in the *top* and *middle* figures indicate the results of post hoc Games–Howell test following ANOVA. Species code given in Table 1. Densities of fishes not occurring at the inner gaps pooled in the bottom figure (*blank cells*). Note partially differential scale used in bottom figure

Aulichthys japonicus, and *Sebastes inermis*), (3) species, although sometimes occurring in outer and inner gap microhabitats restricted almost entirely to dense seagrass (e.g., *Rudarius ercodes*), and (4) species occurring (potentially) also in all seagrass bed microhabitats including gap and sand microhabitats (e.g., *Acentrogobius* sp.). Although, in the present study, *Acentrogobius* sp. was restricted to the seagrass bed, earlier field experiments conducted in the present seagrass bed had shown that seagrass presence/absence had no proximate influence on the distribution pattern of that species (e.g., Horinouchi and Sano 2001). Moreover, in Aburatsubo Bay, adjacent to the present study area in Moroiso Bay, this goby was abundant also on open sand (Horinouchi 2007a). Therefore, such a habitat-generalist fish should be discriminated from seagrass specialists. Clearly, the observed horizontal gradient in the fish assemblage structure resulted from the combination of these distribution patterns, the density patterns of the category 1 species providing the common base of fish assemblage structures in the sand and outer gap microhabitats, while seasonal occurrences of category 2 species added a large modification to the assemblage structure at the latter, enhancing the species diversity there. The occasional occurrence of category 3 and 4 species also contributed to the high diversity at the outer gap. The assemblage characteristic in the inner gap in spring was similar to that at the outer gap, although category 1 species seldom occurred in the former. The horizontally even distribution of category 3 and 4 species within the dense seagrass contributed to the similarity in assemblage structure across the edge to the core portions of the seagrass bed.

Several explanations can be advanced for the above distribution patterns. Because predation is a major factor influencing fish distribution patterns, one of the major benefits for small-sized fishes attributed to a vegetated habitat is reduced predation risk due to structurally complex vegetation (e.g., Savino and Stein 1982; Anderson 1994). Fishes which occur in the sand and/or gap microhabitats (i.e., those falling into categories 1, 2, and 4), however, may have anti-predator tactics independent of seagrass structure such as camouflage body coloration similar to the sand/mud substratum (e.g., Horinouchi 2007a), fleeing speedily in response to the presence or approach of predators, burying themselves in the sediments (e.g., Kruuk 1963), and school/shoal formation (e.g., Pitcher and Parrish 1993). Fishes which occur in the sand and/or gap microhabitats may employ one, or a combination of these tactics, in order to avoid predation in substratum lacking above-ground structures.

Dense seagrass, however, is unfavorable for such anti-predator tactics, except when used for camouflage. Dense seagrass roots may sometimes prevent small fishes from burying themselves in the sediment. Weaving speedily through dense seagrass leaves and stems is apparently difficult even for fishes with strong swimming ability. Dense seagrass also prevents the formation of a larger group (Horinouchi and Sano 1999), which in turn reduces the effectiveness of that tactic. Because of such inconvenience, fishes which rely largely on these anti-predator tactics may not favor dense seagrass.

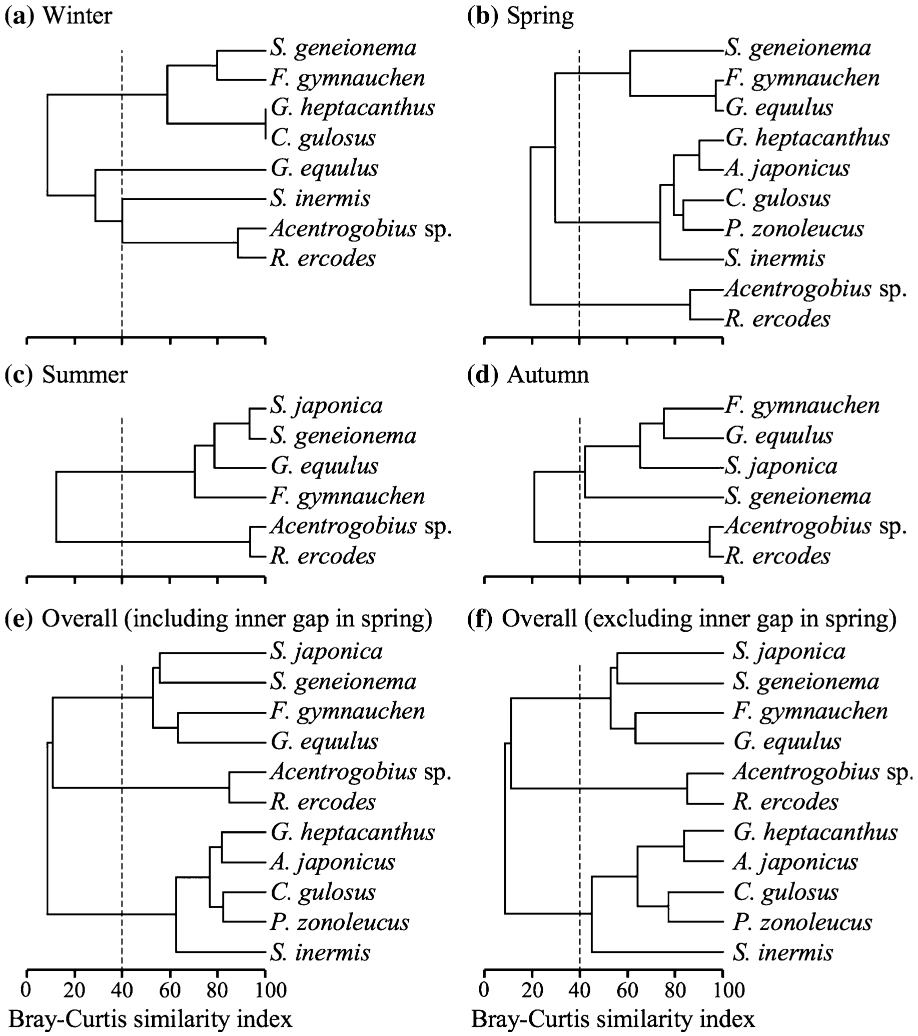


Fig. 9 Phenogram of abundant fishes, i.e., *Sillago japonica*, *Sagamia geneionema* *Favonigobius gymnauchen*, *Gerres equulus*, *Acentrogobius* sp., *Rudarius ercodes*, *Chaenogobius gulosus*, *Pterogobius zonoleucus*, *Gymnogobius heptacanthus*, *Aulichthys japonicus,* and *Sebastes inermis*, obtained from the cluster analysis based on the Bray–Curtis similarity estimates between species' pairs calculated from fish density data for each microhabitat in each and all seasons. An average linkage clustering method was employed in the analysis. Inner gap was studied only in spring. *Broken lines* indicate similarity-index level of 40

In addition, because seagrass habitats are characterized by a constant potential predation risk induced by ambush predators such as *Pseudoblennius cottoides* permanently residing in the present seagrass bed, such fishes do not always stay within dense seagrass. A detailed explanation of this idea has been provided in a review by Horinouchi (2007b). Briefly, within a seagrass canopy, active swimming significantly increases the encounter rate with ambush predators. In addition, forming a large sometimes conspicuous group may also run the risk of being noticed by predators within a seagrass canopy. Therefore, prey fish with such behavioral traits are less likely to favor dense seagrass.

On the other hand, fish restricted almost entirely to a seagrass canopy, such as *R. ercodes* in the present study, often have cryptic body coloration and/or shape similar to seagrass leaves or stems, in addition to being solitary and inactive. Such behavioral traits may be effective in decreasing their detection by or encounters with ambush predators in a dense seagrass, enabling the former to exist within a seagrass canopy subject to constant potential predation risks.

Group-forming juveniles of several species frequented the water column at outer and inner gaps, despite not having strong swimming ability. In the case of such fishes being attacked by transient chase-and-attack predators in an open water column, fleeing or group-maneuvering (see Pitcher and Parrish 1993) outside the seagrass microhabitat is likely to be less effective for avoidance of such predators. However, because of the greater difficulty for chase-and-attack predators of larger body size to penetrate the small spaces among seagrass leaves and stems, such predators may seldom penetrate dense seagrass (see, e.g., Harris et al. 2004) (which probably leading to no differences in predation risk and thus assemblage structure between edge and core portions of the seagrass bed), the avoidance success rate of small prey juveniles therefore may be high within the latter (e.g., Harris et al. 2004). Clearly, earlier recognition of predators, one of the merits of the group-forming anti-predator tactic (e.g., Godin and Morgan 1985; Godin et al.

Table 3 Summary of post hoc Games–Howell tests comparing densities of abundant species between microhabitat pairs

Species	*P*						
	Microhabitat pairs						
	Sand			Outer gap		Inner gap	
	Outer gap	Inner gap	Seagrass	Inner gap	Seagrass	Seagrass	Seagrass pairs
Sand and outer gap occupants							
Favonigobius gymnauchen (5)	1.000		0.003–0.007		≤0.001		1.000
Sagamia geneionema (1)	1.000		0.374		0.853		
Gerres equulus (5)	1.000		0.881–0.967		0.498–0.778		0.997–1.000
Sillago japonica (0)	1.000						
Gap occupants[a]							
Chaenogobius gulosus (4)				0.997	0.926–0.944	0.786–0.845	0.982–1.000
Pterogobius zonoleucus (5)				1.000	0.927–0.952	0.433–0.495	0.994–1.000
Gymnogobius heptacanthus (0)				1.000			
Aulichthys japonicus (0)				1.000			
Sebastes inermis (4)				1.000	0.997–0.999	0.997–0.998	0.993–1.000
Seagrass occupants							
Acentrogobius sp.	0.247	0.940	<0.001	1.000	<0.001	0.971–1.000	0.807–1.000
Rudarius ercodes				0.997	<0.001–0.037	0.960–1.000	0.134–1.000

For body sizes, see Table 1. Blank cells indicate no occurrence at one or both of pair microhabitats. Numbers in parentheses following sand and gap occupants and gap occupants indicate number of seagrass microhabitats in which they occurred

[a] Group-forming juveniles

1988), may provide the prey juveniles with a margin for retreating successfully into a seagrass canopy. Predation risks caused by transient predators are sporadic, being dependent upon predators' movements. For example, in the present study site in spring, young piscivorous *Scombrops boops* sometimes appeared in the open water column along the margin of seagrass habitats, although never occurring within the dense seagrass. This chase-and-attack predator remained on the move while searching for prey, intermittently occurring near seagrass habitats. In the absence of such predators, open areas near seagrass habitats may be of lower predation risk, thus being occupied by prey fish which can successfully retreat into seagrass after predator recognition. This may also be true for solitary fishes in which recognition of predators might be somewhat delayed compared with that of group-forming species, but compensated for by strong swimming abilities. Even for relatively weak swimming species within a seagrass canopy, there may exist a narrow safe zone, thereby explaining their occasional occurrence in gap microhabitats. Such a safe zone should be one of the factors responsible for higher fish species richness/abundance at the gap microhabitat.

Food availability sometimes strongly influences fish distribution patterns. Group-forming gobiid juveniles, which frequent gap and sparse seagrass microhabitats, feed mainly on planktonic copepods (Horinouchi and Sano 2000), which are probably much easier to detect and catch in a less complex habitat. The availability of seagrass epiphytic fauna may be one reason why some fishes are restricted almost entirely to dense seagrass. Although, sediment-related invertebrate animals were more abundant in the present seagrass bed compared with the surrounding sand habitat (Horinouchi 2007a), some fish species restricted to the seagrass canopy in the present study, may potentially exist over open sand. Horinouchi (2007a) reported that *Acentrogobius* sp. were abundant on the open sand (which included a rich benthic prey fauna) in Aburatsubo Bay, suggesting that food availability was a major determinant of distribution of this goby.

It is clear that no single factor can explain all fish distribution patterns. For example, although the benthic goby *F. gymnauchen* had a dietary preference similar to *Acentrogobius* sp. (Horinouchi and Sano 2000), the former seldom appeared within the seagrass canopy, suggesting that the other factors rather than food availability were critical. In fact, distribution patterns of many fishes are determined by a complex combination of multiple factors, e.g. amount of prey and/or resting area, anti-predator tactics, habitat quality as a refuge, and inter- and/or intraspecific competition, although the relative importance of each factor is most likely species-specific.

Implications for conservation. The results of the present study provided important implications for conservation, including habitat-restoration, indicating that while

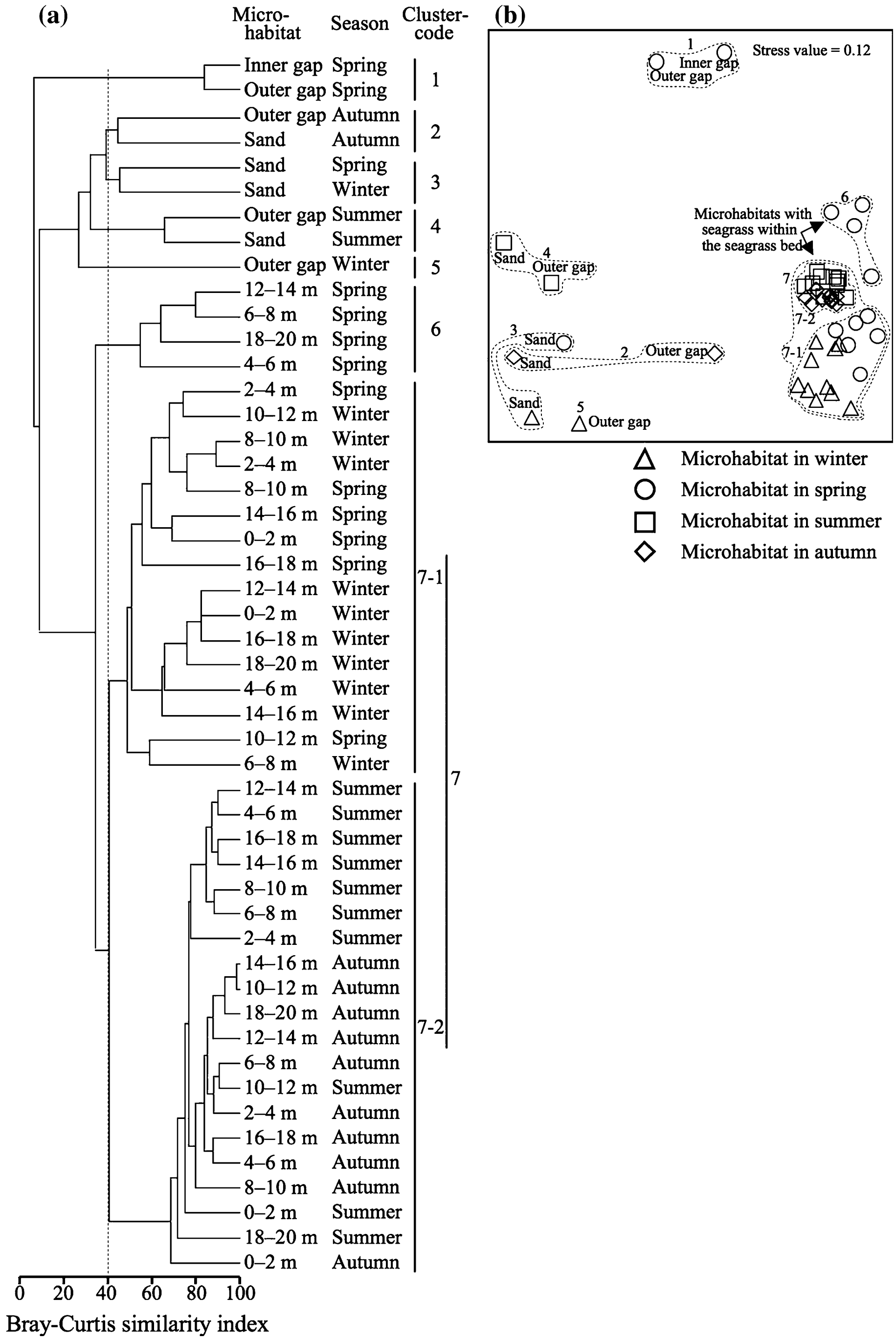


Fig. 10 Phenogram of microhabitats obtained from the cluster analysis (**a**) and a two-dimensional ordination of microhabitats from MDS (**b**) based on the Bray–Curtis similarity estimates between microhabitat pairs calculated from fish density data. In the former analysis, an average linkage clustering method was employed. At a similarity-index level of 40, the microhabitats were divided into seven clusters (coded 1–7). Microhabitats gathered in one (sub-) cluster in the phenogram (**a**) are *circled* by a *broken line* in the two-dimensional map (**b**). A number on each *symbol* or *circle* in the map denotes the corresponding cluster. Inner gap was studied only in spring

no fish showed any apparent preference for edge or core microhabitats, large numbers of group-forming juveniles of several species occurred at the outer and inner gaps of the seagrass bed. Moreover, fish species' diversity was sometimes significant at the latter microhabitats, the concept of which should therefore be included together with traditionally recognized edge and core microhabitats (see review of Bell et al. 2001) in seagrass conservation ecology. The relative abundances of gap-loving fishes and also overall fish species' diversity would probably increase in seagrass habitats with a greater amount of gap microhabitats (see Fig. 1c). In the case of a seagrass habitat restoration intended to raise the stock levels of such gap-preferring species and/or whole biodiversity in the target area, it may be a better approach to design the restored habitats so as to include a higher proportion of gap microhabitats. Similarly, for the preservation of species which utilize gap microhabitats during their juvenile stage and/or of high biodiversity in the area, preservation of seagrass habitats containing higher proportions of gaps might be more effective. In both cases, as a matter of course, other factors should be considered concurrently.

For example, the construction of many small habitats is clearly one way to increase the proportion of such microhabitat (see Fig. 1d). Small artificial patches, however, might be easily removed by natural disturbances, such as strong water motion. Therefore, in areas subject to possible disturbance, a better approach may be to create seagrass habitats as large as possible, designed so as to have uneven perimeters and/or sand patches within them, in order to increase the proportion of gaps. Similar approaches may be suitable for edge-preferring species (if present) because their response to microhabitats is likely to be similar to those of gap-preferring species. However, they are unlikely to be suitable for core-preferring species (if present). Notwithstanding, before any development of conservation and/or restoration treatment strategies for local areas, broad spectrum ecological research, including detailed clarification of animal distribution patterns in the target area, as done in this study, should be conducted.

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