

Habitat preference rather than predation risk determines the distribution patterns of filefish *Rudarius ercodes* in and around seagrass habitats

Masahiro Horinouchi^{1,*}, Naoki Mizuno², Yuka Jo², Masashi Fujita², Yuzuru Suzuki², Futoshi Aranishi¹, Mitsuhiko Sano³

¹Research Center for Coastal Lagoon Environments, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 6908504, Japan

²Fisheries Laboratory, Graduate School of Agricultural and Life Sciences, University of Tokyo, 2971-4 Bentenjima, Maisaka, Hamamatsu, Shizuoka 4310214, Japan

³Laboratory of Aquatic Conservation, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 1138657, Japan

ABSTRACT: Using field and laboratory experiments, this study examined the mechanisms that determine the distribution patterns of the filefish *Rudarius ercodes*, one of the most dominant seagrass habitat fishes in temperate Japan. Few individuals were preyed on in a field tethering experiment, suggesting a relatively low predation risk, even in the open bare sand and mud areas around seagrass habitats. In the laboratory, predator presence or absence did not affect filefish distribution, with filefish mostly staying in the area planted with seagrass, even when predators were absent in the open bare area. Filefish showed a strong preference for the structure provided by seagrass, staying significantly longer in the seagrass area (despite a lack of food) than in the area with food but devoid of seagrass. Faced with the choice of both seagrass and food, and seagrass without food, even satiated filefish, which showed lower foraging activity, remained significantly longer in the seagrass area with food, suggesting an additional preference for seagrass with abundant epiphytic food. Accordingly, habitat preference may be the most important factor determining the distribution patterns of filefish, which may primarily select seagrass habitats via an intrinsic preference for the structure provided by seagrass, with their distribution pattern subsequently being modified by food availability via a preference for seagrass with abundant epiphytic food.

KEY WORDS: Seagrass fish distribution pattern · Habitat preference · Predation risk · Food availability

—Resale or republication not permitted without written consent of the publisher—

INTRODUCTION

It is generally thought that seagrass habitats support large numbers of fish species and individuals, and provide nursery habitats for juveniles of many species, whereas unvegetated bare substrata are usually characterized by fewer species and individuals (e.g. Kikuchi 1974, Sogard et al. 1989, Edgar & Shaw 1995). Habitat complexity, structured by seagrass, has been considered as one of the major fac-

tors responsible for such a difference in habitat use, with greater habitat complexity providing shelter from predation and/or strong water movement, increased microhabitat availability and abundant food (e.g. Crowder & Cooper 1982, Diehl & Eklöv 1995). For example, predation sometimes generates habitat-specific abundance patterns, either directly by the removal of prey species or indirectly by inducing habitat selection in prey species. This attribute is especially important for small-sized fishes, such as

*Email: hori@soc.shimane-u.ac.jp

juveniles, because of their vulnerability to predation (Sogard 1997); thus, they sometimes select seagrass habitats to reduce predation risks (e.g. Sogard & Olla 1993, Gotceitas et al. 1997, Jordan et al. 1997). However, fish abundance is not always lower in areas with lower habitat complexity—a large number of juveniles of some species sometimes occur in open areas adjacent to seagrass habitats in temperate Japanese waters but seldom appear within the complex seagrass structure (Horinouchi 2009). Such a phenomenon suggests that, in fact, dense seagrass does not always improve prey survival, because it does not favor some anti-predator tactics (Horinouchi 2007a). Early recognition of a predator beyond its strike range, or at an early attack stage, may enable a prey species to employ anti-predator behavior, by which means it may successfully avoid predation (e.g. FitzGibbon 1989, Lingle & Wilson 2001).

In a complex habitat, however, prey may sometimes be delayed or fail completely in detecting the presence and/or approach of predators because the complex structure obstructs their view. This often results in predation. In other words, a complex habitat structure favors some predator types, such as ambush and stalk-and-attack predators. In fact, increased habitat complexity may improve the foraging efficiency of ambush predators by providing more sites from which to attack (Janes 1985). In a study of cryptic reef fish assemblages in kelp beds and barren areas, Willis & Anderson (2003) found that in some cases fish densities were higher in the barren areas, and suggested that the kelp canopy may assist predators by providing cover from which to ambush small fishes. Laurel & Brown (2006) suggested that the predator *Myoxocephalus scorpius* takes advantage of the visual impairment of prey by seagrass, and Horinouchi et al. (2009) found that the mortality rate of goby *Gymnogobius heptacanthus* juveniles due to seagrass-resident predator *Pseudoblennius cottoides* increased in denser seagrass and concluded that this may explain the greater abundance of goby juveniles in an adjacent open area, compared with dense seagrass. Therefore, for small prey fishes, a complex habitat may result in a potential predation risk. Accordingly, this study re-evaluated the predation risk pattern across an open sand and mud area surrounding a seagrass habitat. In addition, this study examined the relative importance of predation, habitat preference and food availability in generated fish distribution patterns to provide a better understanding of the functions of a seagrass habitat.

In the present study, the mechanisms determining the distribution patterns of a small permanent resi-

dent seagrass fish *Rudarius ercodes* were examined by field and laboratory experiments. Three specific questions were asked. (1) Do predation rates on this small seagrass fish differ between seagrass and bare sand and mud areas? (2) Does this fish exhibit changes in microhabitat use in the presence or absence of seagrass-resident and/or open-area predators? (3) Does this fish show preferences for the seagrass habitat and/or abundant food, and does hunger level play a role in the habitat choice?

MATERIALS AND METHODS

Study sites

This study was carried out in Lake Hamana (a saltwater-influenced lake opening directly to the sea), Shizuoka Prefecture, Japan (Fig. 1). The field experiments were conducted at 2 sites (A and B in Fig. 1). At each site, there were large *Zostera marina* beds (ca. 0.2 and 0.8 ha at Sites A and B, respectively) in the flat sand and mud areas at depths between ca. 1.0 and 1.5 m at high tide. Water temperature and salinity at the study sites ranged from about 12 to 31°C and about 27 to 30, respectively.

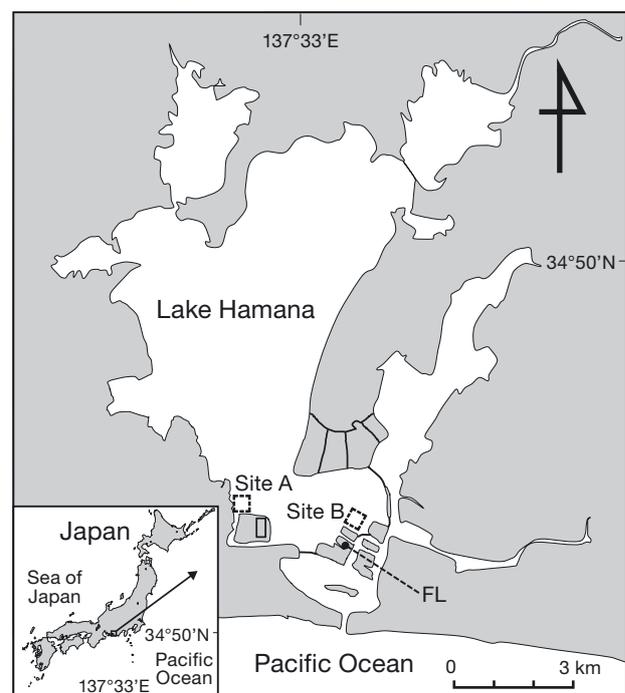


Fig. 1. Study sites (Sites A and B) and the Fisheries Laboratory, Graduate School of Agricultural and Life Sciences, University of Tokyo (FL), in Lake Hamana, Shizuoka Prefecture, Japan

At the study sites, at least 25 fishes with piscivorous tendencies, e.g. *Dasyatis akajei* and *Paralichthys olivaceus*, were recorded during qualitative underwater observations conducted between 1998 and 2000 (M. Horinouchi pers. obs.).

Target species

The monacanthid *Rudarius ercodes* (hereafter, filefish) is a common coastal diurnal species of small body size (up to about 60 mm in standard length [SL]) with cryptic body coloration similar to the substrate, i.e. green, gray or gray-brown. They inhabit mainly seagrass beds in temperate waters around Japan (Matsuura 1984) and are sometimes the most dominant species in such a habitat (e.g. Koike & Nishiwaki 1977, Shiobara & Suzuki 1985). The restricted distribution of filefish to mostly vegetative habitats such as seagrass beds and their infrequent occurrence in open bare sand and mud areas has been well documented (e.g. Koike & Nishiwaki 1977, Horinouchi 2009). The distribution pattern at the present study sites is in accordance with the above general pattern, e.g. average individual numbers per 10 m²: 4.38 ± 6.28 SE in seagrass habitat and 0.08 ± 0.14 SE in bare sand and mud habitat (M. Horinouchi pers. obs. during summer and autumn in 1998 and 1999). The species is usually solitary, occupies the water column among the seagrass leaves and feeds mainly on small epiphytic invertebrates (Horinouchi & Sano 2000). During the day, they swim slowly or remain stationary, sometimes picking at the surface of a seagrass leaf or at the bottom substrate.

In the laboratory experiment (described below in 'Laboratory Expt 1'), the serranid *Lateolabrax japonicus* (hereafter, Japanese seabass or seabass) and the blenniid *Pseudoblennius cottoides* (hereafter, sculpin) were employed as predators of 2 different types (i.e. open-bare-habitat and seagrass-habitat predators, respectively). In the temperate region of Japan, including Lake Hamana, Japanese seabass that reach sizes of ca. 90 cm SL reside mainly in nearshore areas and inner bays (Nakabo 2000). Young and adult individuals (ca. >13 cm SL) occur outside seagrass habitats, and feed mainly on mobile animals, especially fishes (Hatanaka & Sekino 1962). Although Japanese seabass may be most active at dawn and dusk, it also actively captures prey animals during the day when it has an opportunity (M. Horinouchi pers. obs.). As seabasses actively chase and capture their prey, they are one of the most important target fishes in the leisure lure fishing industry in Japan. The species is

common in the sand and mud areas at the study sites, with their yearly catch being the highest of all species taken, contributing over 22% of the total fish catch in Lake Hamana (Shizuoka Prefectural Research Institute of Fishery Hamanako Branch 2007). There are no reports that describe wild seabasses preying on filefish in the field, perhaps because, as described above, young and adult seabasses reside outside a seagrass habitat while filefish are restricted to such a habitat, and thus, they seldom encounter each other under natural conditions. Nevertheless, seabasses were employed in the experiments as an open-water predator because they are voracious predators and, in the pre-experiment trial, although in quite rare cases, they captured filefish in the tank lacking vegetation. Sculpins, reaching sizes of ca. 13 cm SL, occur throughout the temperate coastal region of Japan, including Lake Hamana, and reside in seagrass habitats (Nakabo 2000) throughout the year. Sculpins usually employ ambush and stalk-and-attack tactics for prey capture, usually fishes, in dense seagrass habitats (Horinouchi & Sano 2000, Horinouchi et al. 2009). Horinouchi & Sano (2000) studied the food habits of seagrass fishes and found that small fish, including *Rudarius ercodes*, were the main food of sculpin, although the species names of prey fishes were not described in the manuscript.

Collection and maintenance of fishes

Filefishes were collected with a small beach seine and hand nets in *Zostera marina* habitats near the study sites just before each trial (see 'Laboratory Expt 1' and 'Laboratory Expt 2'). Immediately after collection, they were transported to the Fisheries Laboratory, the University of Tokyo, on Benten Island (Fig. 1). In the laboratory, they were maintained in continuous flow-through tanks supplied with filtered ambient seawater pumped from Lake Hamana. Similar size individuals (ca. 20 to 30 mm total length [TL]) were used in all of the trials to avoid possible size-related differences in predator-avoidance abilities. Each specimen was used for a single trial only; further field sampling was conducted several times to obtain new fishes. The filefishes, except ones employed as 'hungry filefish' (see explanation in 'Laboratory Expt 2'), were sufficiently fed with epiphytic invertebrates collected from seagrass leaves and krill.

A total of 10 seabasses (ca. 260 to 320 mm TL), captured in commercial set nets on bare sand and mud flats and landed at Washizu fishing port, and 10 sculpins (ca. 90 to 110 mm TL), collected from sea-

grass habitats using small seine or hand nets, were employed in the laboratory experiments. In the laboratory, individuals of each species were maintained for 2 to 7 d until each trial in continuous flow-through tanks that were supplied with filtered ambient seawater pumped from Lake Hamana. They were fed with small live fishes until 2 d before the trials, and after that not at all.

Tethering experiment

Tethering experiments were conducted in the field to investigate predation risk patterns for filefishes across the open sand and mud area surrounding the seagrass habitat. Such experiments can only measure relative predation rates rather than actual rates (Peterson & Black 1994). As several other drawbacks of this method, including possible changes in fish behavior and escape response, have also been suggested (e.g. Curran & Able 1998, Manderson et al. 2004), the results obtained required careful interpretation.

To improve the reliability of the results, a preliminary investigation, using 10 filefishes of ca. 20 to 30 mm TL that were collected from seagrass habitats near the study sites in February 2009, was conducted in the laboratory as follows. A thin 1 m long transparent monofilament tether (about 0.1 mm in diameter) was attached to the tough skin just behind the lower end of the pelvic girdle of each individual. Immediately after attachment, each individual was released into a cylindrical tank (0.5 m diameter, 0.8 m depth) containing filtered ambient seawater and several seagrass leaves, and its behavior was observed. Just after attachment and release, they showed zigzag swimming, but soon (within 1 min) became calm and behaved normally. Examination after 24 h confirmed that all individuals had remained strong, and none had broken free from the tether. After the trial, the tethers were removed and the fishes released alive to the sea.

A tethering experiment was conducted at each site (A and B in Fig. 1) in winter (February), spring (May), summer (July) and autumn (October) 2009. On each occasion, filefishes of ca. 20 to 30 mm TL were collected from seagrass habitats and maintained until just before the trial following the above procedure. After transportation to each tethering site,

each individual was tethered as described above. In each seagrass bed at each site, 3 quadrats of 30×2 m were randomly established (see Fig. 2). In addition, at each site 3 quadrats of 30×2 m were randomly established on the area adjacent to the outer border of the seagrass bed (sand and mud area), and also in the sand and mud area within 3 to 5 m from the outer border of the seagrass bed (i.e. 9 quadrats in total per site). Immediately after being tethered, 10 filefishes were transported to each quadrat and gently released into the water column (above the canopy for the seagrass habitat quadrats, because, if they were put within the canopy just after being tethered, they may have become entangled with seagrass leaves). After the fish had become calm, the free ends of their tethers were attached to the seafloor by a snorkeler using 20 cm long iron pegs buried completely in the sediment. The position of each attachment was at least 3 m distant from its nearest neighbors. These procedures were conducted between 09:00 and 12:00 h at the tether sites on 4 successive days in each season. Each tether was retrieved 3 h after attachment, and filefish presence or absence noted. Absence was regarded as evidence of predation. At the end of each trial, all survivors were detached from the tethers, and released alive to the sea.

The filefish is diurnal and restricted mostly to vegetative habitats, such as seagrass beds, in the day (and also in the night). It is reasonable to assume that the daytime distribution pattern of diurnal fish is influenced more strongly by daytime factors than by nighttime factors, e.g. predation risk from dusk to dawn. Nevertheless, the nighttime predation risk

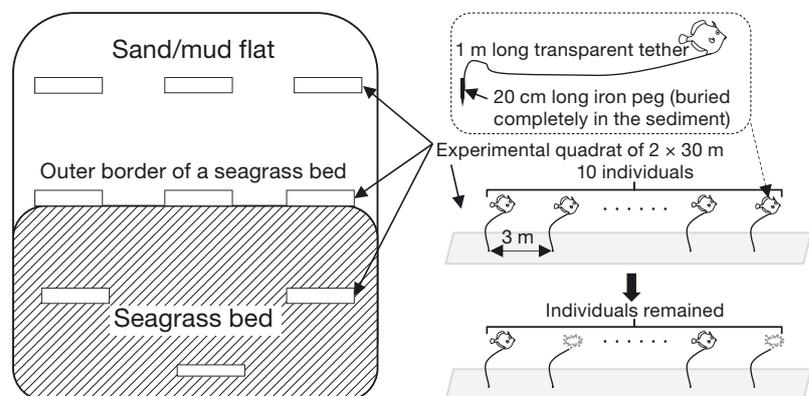


Fig. 2. Tethering experiment. A total of 9 quadrats were established at each site (3 on the seagrass area, 3 on the outer border of the seagrass bed and 3 on the sand and mud area). After being tethered using a thin transparent monofilament tether, 10 filefish *Rudarius ercodes* were transported to each quadrat and the tethers buried in the seafloor via an iron peg. The position of each attachment was at least 3 m distant from its nearest neighbors. After 3 h, tethers were retrieved and filefish absence or presence was noted

patterns were also examined. In March 2013, an additional tethering experiment was conducted at each site from dusk to dawn, following the same procedure (except the experiment time) employed in the day trials. Attachment of tethered fish to the seafloor was conducted between 16:00 and 19:00 h, and survivor counts between 06:00 and 09:00 h the next morning.

The mean length of *Zostera marina* leaves and the number of shoots per 1 m² in each month when the tethering was conducted at the sites are shown in Table 1.

Laboratory Expt 1: Microhabitat choice with predators present and absent

To examine the effects of predator presence on prey fish distribution patterns, the following laboratory experiment was conducted in October 2009.

Seagrass and open sand and mud habitats were established in cylindrical tanks (1.0 m in diameter and 0.8 m depth) as follows (see also Fig. 3). A ca. 5 cm thick layer of treated sand and mud sediment was spread over the bottom of each tank and divided into 2 semi-circular areas. Treated *Zostera marina* shoots were then planted in one of the semi-circular areas at intervals of 7 cm (which corresponds to natural shoot density in the seagrass beds, i.e. about 150 to 200 shoots m⁻²). Both the sand and mud sediment and *Z. marina* shoots were taken from natural seagrass beds and treated by washing out all organic matter with running fresh water. Epiphytic animals and plants attached to the seagrass were scraped off using razors. The mean length of *Z. marina* leaves was 48.2 (± 6.7 SD) cm. Each experimental tank was filled with filtered ambient seawater (20.7 to 20.8°C) to a depth of ca. 70 cm.

Filefish microhabitat choices in the presence and absence of predators were examined in 4 treatments: (1) seabass (open-water predator) present and sculpin (seagrass predator)

absent, (2) seabass absent and sculpin present, (3) both predators present, and (4) both predators absent.

In each case, a single filefish was placed into a transparent cylindrical holder (20 cm diameter, 30 cm long), which was dipped into the surface water at the center of each tank. The bottom of the holder was

Table 1. Mean seagrass height and shoot density (\pm SD) of the study beds in each month when the tethering experiment was conducted at Sites A and B

Date	Site A		Site B	
	Seagrass height (cm)	Shoot density (m ⁻²)	Seagrass height (cm)	Shoot density (m ⁻²)
February 2009	53.0 \pm 2.4	162 \pm 26	62.8 \pm 2.6	148 \pm 35
May 2009	68.8 \pm 10.3	172 \pm 19	79.2 \pm 5.3	196 \pm 16
July 2009	73.4 \pm 13.5	148 \pm 43	66.6 \pm 10.4	166 \pm 22
October 2009	48.4 \pm 4.1	136 \pm 21	52.0 \pm 6.1	146 \pm 15
March 2013	39.0 \pm 8.2	172 \pm 19	32.6 \pm 5.6	174 \pm 15

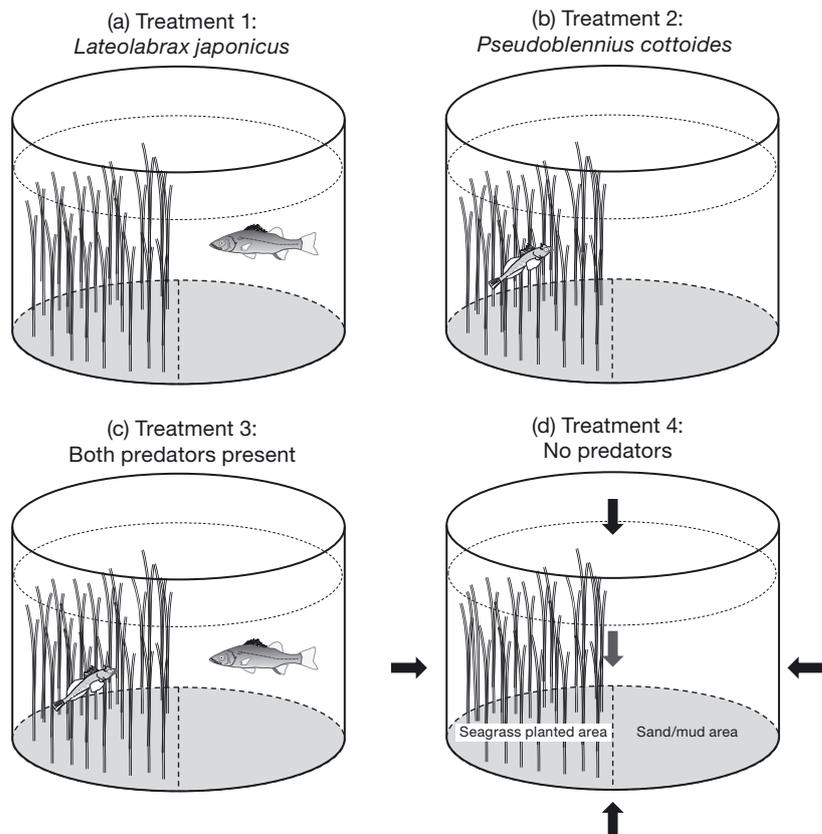


Fig. 3. Laboratory Expt 1. Seagrass and open sand and mud habitats were established in each cylindrical tank. Filefish *Rudarius ercodes* microhabitat choices in the presence and absence of 2 predators, seabass *Lateolabrax japonicus* (predator in open area around seagrass habitats) and sculpin *Pseudoblennius cottoides* (predator in seagrass habitats), were examined in 4 treatments (a–d). Observations were conducted from the point where the fish position could be most precisely recognized, i.e. either from directly above or from 1 of 4 equidistant positions around the tank (see arrows in d)

then opened gently to release the filefish into the tank, and the holder removed. For the treatments with predators, 5 min after the release of the filefish, a predator (either 1 seabass, 1 sculpin or both) was gently introduced into each experimental tank. Observations commenced 15 min after the release of the filefish, long enough for the fishes to acclimate to the conditions, and the time the filefish spent in seagrass and open sand and mud areas in the tank was measured for 30 min using a stopwatch. During the whole trial period (30 min = 1800 s), an observer (M. Horinouchi) continuously recorded the position of the fish from a point where the fish position could be most precisely recognized, i.e. either from directly above or from 1 of 4 equidistant positions around the tank (see Fig. 3d). Observations were conducted very carefully so as not to disturb the fishes. For each treatment, 5 trials were conducted between 09:00 and 16:00 h, providing 5 replicates for each treatment. Following each trial, each tank was washed and set up again following the above procedure. The order of the trials was determined randomly.

Laboratory Expt 2: Microhabitat choice with seagrass and/or food present and absent

This experiment was conducted to establish whether habitat preference and food availability are important determinants of filefish distribution patterns. The procedures were conducted in November 2009.

The cylindrical tanks used in Expt 1 were also employed here. First, a sediment layer was prepared as in Expt 1, and 2 semi-circular areas were established. A ca. 10 cm zone, in which no treatments were done, was then established across the middle of the tank to provide 2 experimental areas with a no-treatment (buffer) zone between them (see Fig. 4). Each tank was filled with filtered ambient seawater (17.9 to 19.7°C) to a depth of ca. 70 cm.

Filefish microhabitat choices in the presence and absence of the structure provided by the seagrass and/or food were examined in 6 treatments (see Fig. 4). In each treatment, 2 experimental microhabitats were established in each tank: (1) area with seagrass and no food versus open sand and mud area (no structure) with no food, (2) area with both seagrass and food versus open sand and mud area with food, (3) area with both seagrass and food versus open sand and mud area without food, (4) area with seagrass and no food versus open sand and mud area with food, (5) area with both seagrass and food versus seagrass without food, and (6) area with sand/mud with food versus sand/mud without food.

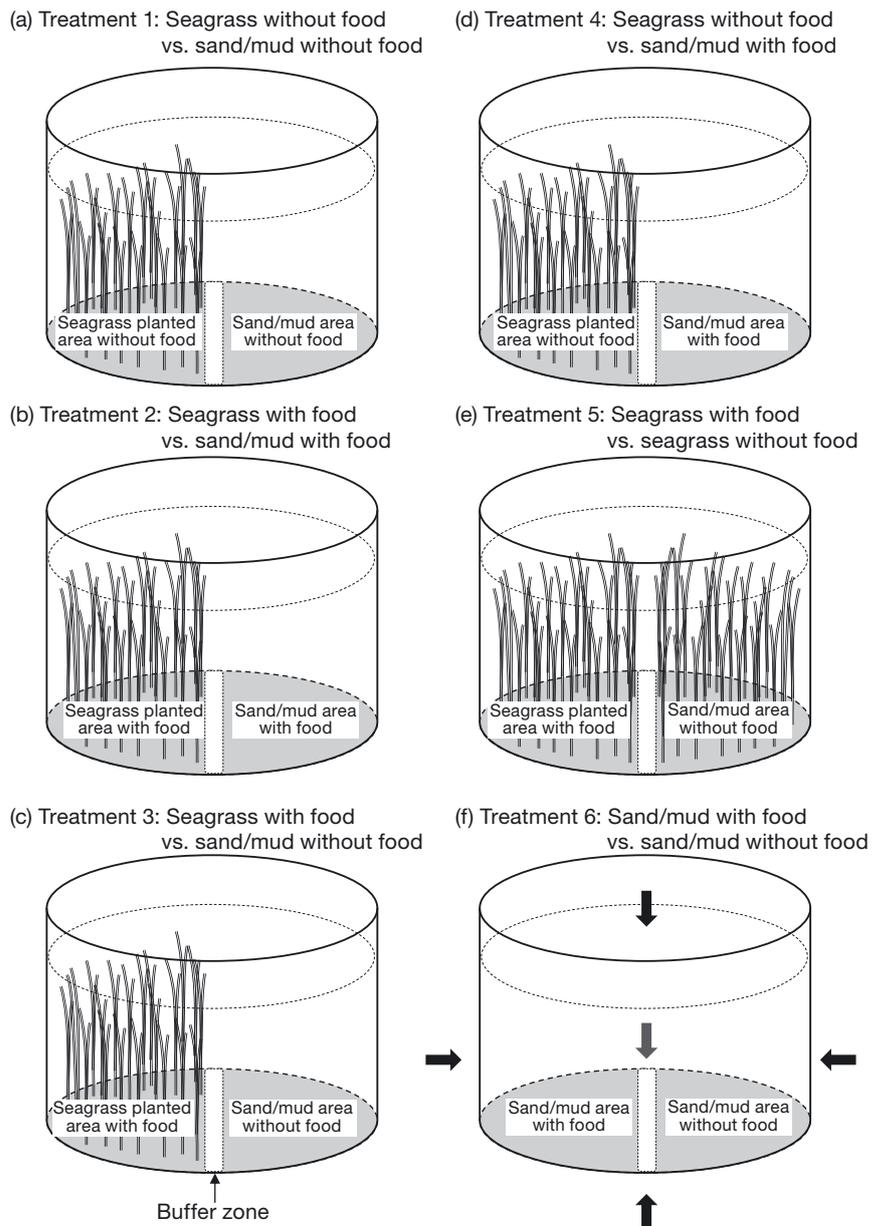


Fig. 4. Laboratory Expt 2. In each experimental cylindrical tank, 2 microhabitats and a no-treatment (buffer) zone were established. Filefish *Rudarius ercodes* microhabitat choices in the presence and absence of the structure provided by the seagrass and/or food were examined in 6 treatments (a–f) for both satiated and hungry filefishes. Observations were conducted from the point where the fish position could be most precisely recognized, i.e. either from directly above or from 1 of 4 equidistant positions around the tank (see arrows in f)

(3) area with seagrass and food versus open sand and mud area with no food, (4) area with seagrass and no food versus open sand and mud area with food, (5) area with seagrass and food versus area with seagrass and no food, and (6) open sand and mud area with food versus open sand and mud area with no food.

In the area with both seagrass and food, 60 fresh natural *Zostera marina* shoots with attached epiphytic objects were planted as in Expt 1. In the trial with seagrass and no food, 60 treated (see explanation in 'Laboratory Expt 1') *Zostera marina* shoots were planted in the same manner. The seagrass shoots employed (mean leaf length 48.6 [\pm 9.8 SD] cm) were collected from seagrass habitats near the study sites 1 d before each trial and maintained in holding tanks. The food added to the open sand and mud area comprised epiphytic animals and plants, which had been scraped from 60 natural *Z. marina* shoots using a razor and frozen with a small volume of seawater at -20°C . The frozen seawater containing food was transferred into a cylindrical steel mesh cage (20 cm diameter, 5 cm long), which was then carefully placed on the sediment surface in the appropriate area in the tank. After the ice had thawed, the bottom of the cage was opened gently to release, drop and spread the food over the sediment surface of the treatment area, and the cage was then removed. As the water flow was negligible within the tank, the defrosted food was not washed away to no-food areas. Furthermore, the buffer zone between the treated areas within the tank kept the experimental condition of each area consistent.

In each case, a single filefish was released into the tank as in Expt 1. Observations commenced 15 min after the release of the filefish, and the time the filefish spent in each treatment area in the tank was measured for 30 min using a stopwatch. Observations were conducted as in Expt 1. For each treatment, 5 trials were conducted between 09:00 and 16:00 h, providing 5 replicates for each treatment. Following each trial, each tank was washed and set up again following the above procedure. The order of the trials was determined randomly. These observations were conducted for 'satiated' (fed up to trial) and 'hungry' (starved for 1 d before the trial) filefishes.

Statistical analysis

To examine whether the microhabitat use pattern may change over time, for both Laboratory Expts 1 and 2, the trial period (1800 s) was divided into 3 time segments, i.e. 0–600, 600–1200 and 1200–1800 s, and Friedman's test was employed to examine whether

the time filefish spent in the experimental areas differed among the time segments for each treatment. As the microhabitat use pattern did not change over time (see 'Results' and Figs. 5 & 6), data were pooled and the overall time filefish spent in experimental areas in each treatment was compared using the Wilcoxon test. In addition, for Expt 1, the time filefish spent in the seagrass area was compared between the predator presence and absence treatments using the Kruskal-Wallis test to clarify whether the predator presence or absence influenced the filefish microhabitat use pattern. For Expt 2, a comparison of the time spent in the areas by satiated and hungry individuals was also conducted using Mann-Whitney *U*-test, because, if foraging activities are mainly responsible for the length of time spent in the areas with food, it is expected that the hungry individuals will spend longer in the areas with food (even in the area with food, but lacking above-ground structure) compared with satiated individuals. The significant level was set at $p \leq 0.05$.

Although the replicate numbers of the laboratory experiments were relatively small ($n = 5$), post hoc power analysis, conducted using G*Power 3.1 (Faul et al. 2007) found the obtained results to be sufficiently reliable (for treatments in Expt 1, Power >0.95 ; for treatments in Expt 2, Power >0.77).

All of the statistical analyses except post hoc power analysis were conducted using SPSS version 18J.

RESULTS

Predation risk pattern between open sandy and seagrass habitats

Out of 720 filefishes employed in the tethering experiments conducted in the day, only 5 individuals at Site A in the autumn trial were preyed on (line break). Out of these, 3 individuals were in the open sand and mud area and 2 in the area adjacent to the outer border of the seagrass habitat. No fishes were preyed on within the seagrass habitats at either site.

In the night tethering experiment conducted with 120 filefishes in March 2013, no fish were preyed on, even in the open sand and mud areas.

Microhabitat choice with predators present and absent

The times spent by filefish in seagrass and open sand and mud areas in the presence or absence of

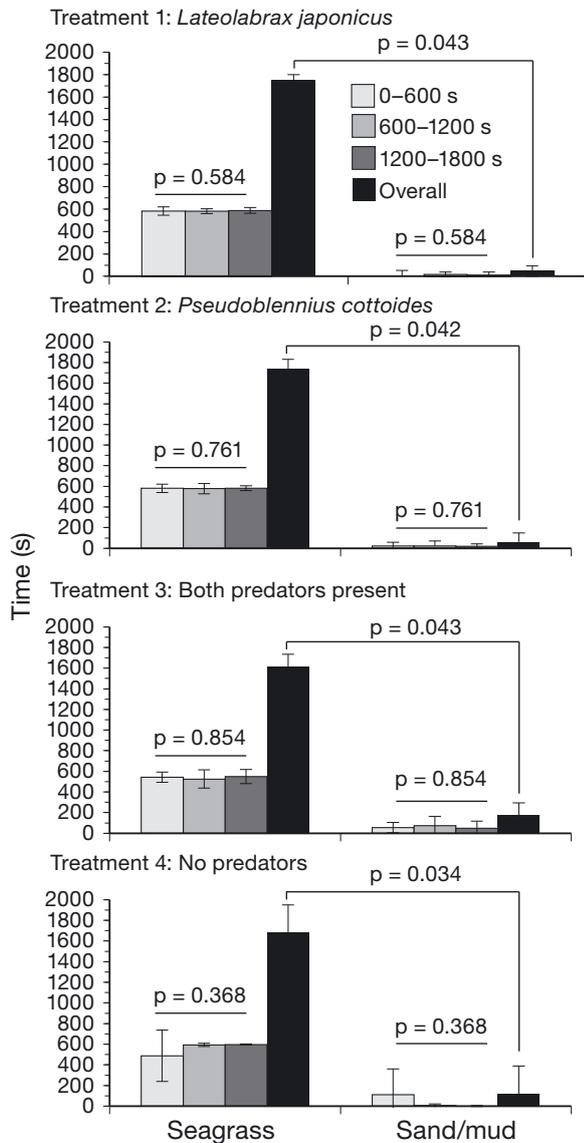


Fig. 5. Laboratory Expt 1. Time spent by filefish *Rudarius ercodes* in each area in the presence/absence of seabass *Lateolabrax japonicus* (predator in open area around seagrass habitats) and/or sculpin *Pseudoblennius cottoides* (predator in seagrass habitats) during time segments 0–600 (start of trial), 600–1200 and 1200–1800 s (end of trial). The overall time spent in each area is also shown. The p-values on the horizontal bar over the 3 time segments indicate the results of the Friedman test examining whether or not microhabitat-use patterns change over time. The p-values on the horizontal bar over overall time indicate the results of the Wilcoxon test comparing the overall times that filefish spent in each area in the treatments

seabass and sculpin are shown in Fig. 5. The microhabitat-choice pattern of the filefish did not change over time (see Fig. 5). Regardless of predator presence or absence, filefish remained significantly longer in the area with seagrass, even when an open-

water predator (seabass) was absent in the open bare area and/or a seagrass predator (sculpin) was present in the seagrass area. Within the seagrass area, filefish were relatively inactive. They sometimes swam a short distance slowly and then stopped and stayed in that position, but seldom showed continuous swimming. The filefish sometimes moved to the open sand and mud area, but soon returned to the seagrass area. In all the trials, no filefish were preyed on.

The time spent in the seagrass area did not differ among treatments (Kruskal-Wallis test, $p = 0.195$).

Microhabitat choice with seagrass and/or food present and absent

Filefish showed similar habitat-use patterns, regardless of whether they were hungry or not (Mann-Whitney test, $p > 0.17$ for all comparisons between satiated and hungry fish), which did not change over time (see Fig. 6). When both seagrass and open sand and mud areas were available, filefish (both satiated and hungry) consistently spent a significantly longer time in the seagrass area regardless of food presence or absence in the alternative habitats (Fig. 6, Treatments 1 to 4).

However, when seagrass areas with and without food were available, both satiated and hungry filefish spent a longer time in the area with food (Fig. 6, Treatment 5). When only open sand and mud areas with and without food were available, both satiated and hungry filefish utilized both areas equally (Fig. 6, Treatment 6). In the treatments with seagrass, filefish showed behavior similar to that observed in Expt 1. However, in the treatment with no seagrass in the tank (Treatment 6), filefish sometimes roamed around nervously, such behavior being rarely observed in other treatments.

DISCUSSION

The present study suggested that a preference for the structure provided by the seagrass is the primary determinant of habitat use of small filefish *Rudarius ercodes*, which are abundant within seagrass habitats but seldom occur in open bare sand and mud areas. Predation risks apparently play a less important role. Out of 720 individuals employed in the tethering experiments conducted in the day, only 3 individuals (0.42% of total) in the surrounding open sand and mud area and 2 (0.28%) in the area adjacent to the outer border of the seagrass habitat were preyed

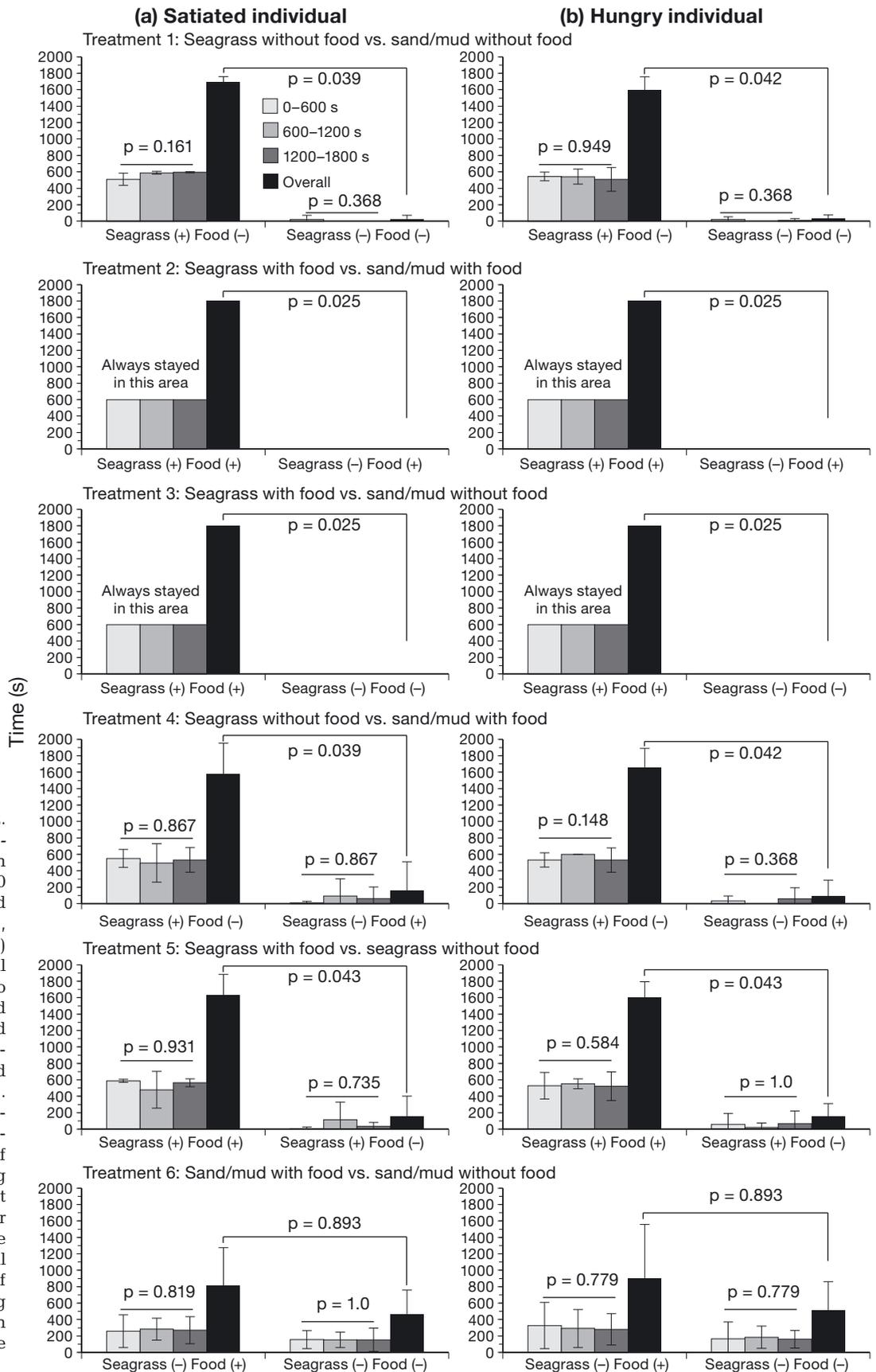


Fig. 6. Laboratory Expt 2. Time spent by filefish *Ruda-rius ercodes* in each area in each treatment during 0–600 (start of trial), 600–1200 and 1200–1800 s (end of trial), for both (a) satiated and (b) hungry filefishes. The overall time spent in each area is also shown. +: seagrass or food present; -: seagrass or food absent. For details, see explanation in ‘Materials and methods: Laboratory Expt 2’. The p-values on the horizontal bar over the 3 time segments indicate the results of the Friedman test examining whether or not microhabitat use patterns change over time. The p-values on the horizontal bar over overall time indicate the results of the Wilcoxon test comparing the overall times that filefish spent in each area in the treatments

on. In the night tethering trial conducted from dusk to dawn using 180 individuals, no individuals were preyed on, even in the bare sand and mud areas, though many piscivorous fishes are thought to be active during such period (e.g. Holbrook & Schmitt 2002). These results strongly suggest that predation of filefish is probably relatively low, even in open bare sand and mud areas.

In the laboratory experiment, the presence or absence of predators did not affect microhabitat choice—the filefish mostly remained in the seagrass area. The filefish spent a significantly longer time in the seagrass area even in the absence of a predator (seabass) in the open bare area. It is implausible that the wild-reared filefish did not perceive the presence or absence of the open-water predator in the relatively small experimental microhabitats. While it should also be noted that the results of the small-scale laboratory experiments might not be completely translatable to natural conditions, such a preference for seagrass was quite clear and consistent, not changing over time, even in the predator-free conditions. Although lower predation risks due to the sheltering effects of complex seagrass is often considered to be an especially important factor responsible for higher fish densities in seagrass habitats (e.g. Sogard & Olla 1993), the present results suggest that this is not the case for this species.

In fact, a low level of predation risk for *Rudarius ercodes* within seagrass habitats should be the norm, because permanent seagrass resident fishes usually have efficient tactics against seagrass-resident predators and thus can coexist with these predators within seagrass habitats (Horinouchi 2007a). This was supported by the results of the laboratory experiment, in which filefish co-occurred with a predator (sculpin) within the seagrass habitat in the experimental tank. It was, however, unexpected that the predation risk for this small seagrass fish was relatively low in the open bare sand and mud area.

Several explanations can be advanced for the fact that few individuals were preyed on in the open sand and mud areas. It is generally believed that small-sized fishes are particularly vulnerable to predation (e.g. Sogard 1997). Such a generalization, however, may not always be the case, and it is plausible that the small body size of this species contributes to the lower predation risk. A similar phenomenon was reported by Horinouchi (2007b), who conducted a tethering experiment over seagrass and bare sand and mud habitats and found a low predation risk for small goby juveniles in the bare habitat. Small body size per se may make them less detectable to predators.

Furthermore, such small-sized fishes might be much less valuable in terms of energy gain and therefore less attractive to larger predators in the bare sand and mud area, resulting in relatively low predation risk. In addition, the laterally compressed rhombus-shaped body of *Rudarius ercodes*, accompanied by a green-gray-brown body color and inactive tendency, may possibly have a camouflaging effect, giving the appearance of a drifting leaf and/or algae and thus reducing predation risks at least to some degree, even in an open bare area. Similar mimicry in other small monacanthid fishes was reported by Randall & Randall (1960). Alternatively, predators in open areas may not recognize filefish as prey because they seldom encounter each other in natural situations and/or because the filefish has unappetizing appearance.

Whatever the mechanisms responsible for the relatively low predation risk for this species in open bare areas, the fact is that filefishes seldom appear in open bare areas, being mostly restricted to seagrass habitats in natural situations (e.g. Horinouchi 2009). In addition, in the laboratory experiment, they seldom occurred over the bare sand and mud area, even when no predators were present. We concluded that predation risks, therefore, probably do not affect the habitat-use patterns of this species. Several studies on seagrass fishes have demonstrated a similar relatively low importance of predation in determining fish distribution patterns. Bell & Westoby (1986) manipulated seagrass densities in the presence and absence of predators and found no difference in prey animal response to such alteration, suggesting that their abundance patterns in seagrass may have been due to habitat preference, rather than predation. Levin et al. (1997) conducted predator-exclusion experiments using cages and found that the impact of predation on *Lagodon rhomboids* recruits was equivalent in both sand and vegetated habitats, suggesting that predation may not explain the greater abundance of recruits in the vegetated habitat.

The results of Laboratory Expt 2 also suggested that a preference for the structure provided by seagrass is a primary determinant of habitat use by *Rudarius ercodes*. When both seagrass and bare sand and mud areas were available, filefish always selected the seagrass area, regardless of food presence or absence. This pattern was consistent, even when food was absent in the seagrass area but present in the sand and mud areas, and did not change over time. This suggested that the existence of seagrass is the most important factor in the habitat choice of this species and that other factors, including the trade-off between the predation risk and energy gain, are rather ineffective,

though such a trade-off sometimes influences fish distribution patterns strongly (e.g. Borcharding 2006, Hammerschlag et al. 2010). That is, in Laboratory Expt 2, there was no predation risk; nevertheless, the filefish always chose the area with seagrass rather than the bare area even when the seagrass area contained no food (with no benefit) while the sand and mud area contained food (with energy gain benefit). In addition, in the treatment of seabass absent and sculpin present in Laboratory Expt 1, in which no food was employed (no energy gain benefit), the filefish chose the seagrass area even though it contained a potential predation risk and lacked any benefit. By considering these results along with the quite low predation mortality over bare sand and mud areas observed in the field tethering experiment, we conclude that an intrinsic preference for seagrass habitat, which may probably be 'a ghost of past predation', i.e. evolved through past predation risk patterns but not directly linked to the risk at present, is mainly responsible for the filefish distribution patterns. Exploration of the evolutionary aspects of such habitat preference acquisition is beyond the scope of the present study. Nevertheless, a potential advantage, such as faster growth (e.g. Levin et al. 1997, Stallings et al. 2010) due to a greater abundance of food (usually expected in seagrass habitats; see, e.g. Nakamura & Sano 2005), thereby allowing fish to attain a greater size and thus be relatively free from predation more quickly, may also have been a driving force.

The distribution pattern of *Rudarius ercodes* is apparently determined primarily by their preference for seagrass, with the pattern possibly being modified secondarily by food availability. In Laboratory Expt 2, when seagrass areas with and without food were available, the fish spent a significantly longer time in the seagrass area, suggesting that once the filefish have entered a seagrass habitat, they may redistribute, seeking seagrass with abundant epiphytic food. Greater food availability is known to have merits, such as increasing growth rates, and may therefore play an important role in modifying fish distribution patterns (e.g. Connolly 1994, Levin et al. 1997, Jenkins & Hamer 2001, Horinouchi 2007b). It should be noted that foraging activities alone may not fully explain longer periods in seagrass with food. Even satiated fishes, which had been fed sufficiently just before the trial and accordingly showed decreased foraging activities, preferred seagrass with abundant epiphytic food, as did hungry fishes. Preference for seagrass with epiphytic food rather than foraging activities may therefore be more responsible for their microhabitat-use pattern.

It has not yet been clarified how fishes discriminate between seagrass with epiphytic food and seagrass lacking food, but it may be based at least partly on visual keys, owing to different surface coloration. Seagrass leaves with rich epiphytic animal and plant assemblages have patches with white-brown coloration on their surface, such as, e.g. white spots of 1 to 2 mm diameter created by small snail-worms, which are one of the food items of the filefish. Seagrass leaves lacking such epiphytes, as is often the case with young leaves, usually have few such patches, being mostly green in color overall.

In summary, the present study indicated that the distribution patterns of the small monacanthid *Rudarius ercodes*, a dominant species in temperate seagrass habitats in Japan, may be determined primarily by an intrinsic preference for seagrass habitat, with secondary modification according to preference for seagrass with abundant epiphytic food. Furthermore, predation risks do not play an important role in producing habitat-use patterns. Further study is needed to clarify the relative importance of other factors, including water current attenuation, in addition to the mechanisms responsible for microhabitat choice within seagrass habitats, to fully understand the functions of a seagrass habitat.

Acknowledgements. We are grateful to T. Takami, K. Urushibata, A. Kurata, J. Miyajima and the staff of the Fisheries Laboratory, University of Tokyo, for their generous assistance with field and laboratory work. Constructive comments on the manuscript from G. S. Hardy, C. Birkeland and anonymous reviewers were much appreciated. We thank the fishermen's unions in Lake Hamana for their permission to collect experimental fish.

LITERATURE CITED

- Bell JD, Westoby M (1986) Abundance of macrofauna in dense seagrass is due to habitat preference, not predation. *Oecologia* 68:205–209
- Borcharding J (2006) Prey or predator: 0+ perch (*Perca fluviatilis*) in the trade-off between food and shelter. *Environ Biol Fishes* 77:87–96
- Connolly RM (1994) Removal of seagrass canopy: effects on small fish and their prey. *J Exp Mar Biol Ecol* 184:99–110
- Crowder LB, Cooper WE (1982) Habitat structural complexity and the interaction between bluegills and their prey. *Ecology* 63:1802–1813
- Curran MC, Able KW (1998) The value of tethering fishes (winter flounder and tautog) as a tool for assessing predation rates. *Mar Ecol Prog Ser* 163:45–51
- Diehl S, Eklöv P (1995) Piscivore-mediated habitat use in fish: effects on invertebrate resources, diet and growth of perch, *Perca fluviatilis*. *Ecology* 76:1712–1726
- Edgar GJ, Shaw C (1995) The production and trophic ecology of shallow-water fish assemblages in southern Aus-

- tralia. I. Species richness, size-structure and production of fishes in Western Port Victoria. *J Exp Mar Biol Ecol* 194:53–81
- Faul F, Erdfelder E, Lang AG, Buchner A (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 39:175–191
- FitzGibbon CD (1989) A cost to individuals with reduced vigilance in groups of Thomson's gazelles hunted by cheetahs. *Anim Behav* 37:508–510
- Gotceitas V, Fraser S, Brown JA (1997) Use of eelgrass beds (*Zostera marina*) by juvenile Atlantic cod (*Gadus morhua*). *Can J Fish Aquat Sci* 54:1306–1319
- Hammerschlag N, Heithaus MR, Serafy JE (2010) Influence of predation risk and food supply on nocturnal fish foraging distributions along a mangrove–seagrass ecotone. *Mar Ecol Prog Ser* 414:223–235
- Hatanaka M, Sekino K (1962) Ecological studies on the Japanese sea-bass, *Lateolabrax japonicus*. 1. Feeding habit. *Bull Jpn Soc Sci Fish* 28:851–856 (in Japanese with English abstract)
- Holbrook SJ, Schmitt RJ (2002) Competition for shelter space causes density-dependent predation mortality in damselfishes. *Ecology* 83:2855–2868
- Horinouchi M (2007a) Review of the effects of within-patch scale structural complexity on seagrass fishes. *J Exp Mar Biol Ecol* 350:111–129
- Horinouchi M (2007b) Distribution patterns of benthic juvenile gobies in and around seagrass habitats: effectiveness of seagrass shelter against predators. *Estuar Coast Shelf Sci* 72:657–664
- Horinouchi M (2009) Horizontal gradient in fish assemblage structures in and around a seagrass habitat: some implications for seagrass habitat conservation. *Ichthyol Res* 56:109–125
- Horinouchi M, Sano M (2000) Food habits of fishes in a *Zostera marina* bed at Aburatsubo, central Japan. *Ichthyol Res* 47:163–173
- Horinouchi M, Mizuno N, Jo Y, Fujita M, Sano M, Suzuki Y (2009) Seagrass habitat complexity does not always decrease foraging efficiencies of piscivorous fishes. *Mar Ecol Prog Ser* 377:43–49
- Janes SW (1985) Habitat selection in raptorial birds. In: Cody ML (ed) *Habitat selection in birds*. Academic Press, New York, NY, p 159–188
- Jenkins GP, Hamer PA (2001) Spatial variation in the use of seagrass and unvegetated habitats by post-settlement King George whiting (Percodae: Silliginidae) in relation to meiofaunal distribution and macrophyte structure. *Mar Ecol Prog Ser* 224:219–229
- Jordan F, Bartolini M, Nelson C, Patterson PE, Soulen HL (1997) Risk of predation affects habitat selection by the pinfish *Lagodon rhomboids* (Linnaeus). *J Exp Mar Biol Ecol* 208:45–56
- Kikuchi T (1974) Japanese contributions on consumer ecology in eelgrass (*Zostera marina* L.) beds, with special reference to trophic relationships and resources in inshore fisheries. *Aquaculture* 4:145–160
- Koike K, Nishiwaki S (1977) Seasonal change of fish fauna in the *Zostera* zone in Shimoda Bay and Nabeta Cove, the Izu Peninsula. *Jpn J Ichthyol* 24:182–192 (in Japanese with English abstract)
- Laurel B, Brown JA (2006) Influence of cruising and ambush predators on 3-dimensional habitat use in age 0 juvenile Atlantic cod *Gadus morhua*. *J Exp Mar Biol Ecol* 329:34–46
- Levin P, Petrik R, Malone J (1997) Interactive effects of habitat selection, food supply and predation in recruitment of an estuarine fish. *Oecologia* 112:55–63
- Lingle S, Wilson WF (2001) Detection and avoidance of predators in white-tailed deer (*Odocoileus virginianus*) and mule deer (*O. hemionus*). *Ethology* 107:125–147
- Manderson JP, Pessutti J, Hilbert JG, Juanes F (2004) Shallow water predation risks for a juvenile flatfish (winter flounder; *Pseudopleuronectes americanus*, Walbaum) in a northwest Atlantic estuary. *J Exp Mar Biol Ecol* 304:137–157
- Matsuura K (1984) Family Monacanthidae. In: Masuda H, Amaoka K, Araga C, Uyeno T, Yoshino T (eds) *The fishes of the Japanese Archipelago*. Tokai University Press, Tokyo (English text and plates)
- Nakabo T (2000) Family Cottidae. In: Nakabo T (ed) *Fishes of Japan with pictorial keys to the species*, 2nd edn. Tokai University Press, Tokyo, p 628–650
- Nakamura Y, Sano M (2005) Comparison of invertebrate abundance in a seagrass bed and adjacent coral and sand areas at Amatori Bay, Iriomote Island, Japan. *Fish Sci* 71:543–550
- Peterson CH, Black R (1994) An experimentalist's challenge: when artifacts of intervention interact with treatments. *Mar Ecol Prog Ser* 111:289–297
- Randall JE, Randall HA (1960) Examples of mimicry and protective resemblance in tropical marine fishes. *Bull Mar Sci Gulf Caribb* 10:444–480
- Shiobara Y, Suzuki K (1985) An ecological study on the fish community in *Zostera* bed at the Uchiura Coast, Suruga Bay, Japan. *J Fac Mar Sci Technol* 221:129–143 (in Japanese with English abstract)
- Shizuoka Prefectural Research Institute of Fishery Hamanako Branch (2007) Catch data of fish species landed at fishing ports in Lake Hamana. http://fish-exp.pref.shizuoka.jp/hamanako/1_kaiyo/catch_ha.html
- Sogard SM (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bull Mar Sci* 60:1129–1157
- Sogard SM, Olla BL (1993) The influence of predator presence on utilization of artificial seagrass habitat by juvenile walleye Pollock, *Theragra chalcogramma*. *Environ Biol Fishes* 37:57–65
- Sogard SM, Powell GVN, Holmquist JG (1989) Utilization by fishes of shallow, seagrass-covered banks in Florida Bay: 1. Species composition and spatial heterogeneity. *Environ Biol Fishes* 24:53–65
- Stallings CD, Coleman FC, Koenig CC, Markiewicz DA (2010) Energy allocation in juveniles of a warm-temperate reef fish. *Environ Biol Fishes* 88:389–398
- Willis TJ, Anderson MJ (2003) Structure of cryptic reef fish assemblages: relationships with habitat characteristics and predator density. *Mar Ecol Prog Ser* 257:209–221

Editorial responsibility: Charles Birkeland,
Honolulu, Hawaii, USA

Submitted: December 6, 2012; Accepted: April 30, 2013
Proofs received from author(s): July 19, 2013