The disruption of habitat isolation among three *Hexagrammos* species by artificial habitat alterations that create mosaic-habitat

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Abstract In coastal areas in Japan, three species of greenling (*Hexagrammos* spp.) can hybridize. In a natural reef setting we showed that *Hexagrammos agrammus* and *H. octogrammus* established their breeding territories in a shallow area where seaweed was abundant, whereas *H. otakii* established breeding territories in a deep area that was sparsely covered with seaweed. This difference in habitat use resulted in *H. otakii* being distributed separately from the other two species, thereby reducing the potential for hybridization. However, all the three species co-occurred in an artificial area near a breakwater. This area is characterized by steep slopes and complex stacked concrete structures, which create a mosaic-habitat consisting of a shallow environment with seaweed and a deep environment with sparse seaweed, allowing the three species to breed within a single area. Our results suggest that man-made structures can create an artificial mosaic-habitat that can disrupt habitat isolation and promote hybridization between species.

Key words Coastal fish; Breakwater; Human disturbance; Hybridization; Microhabitat use

Introduction

Habitat isolation is a reproductive isolating mechanism in which gene exchange among species are prevented because frequencies of heterospecific encounters are reduced due to differential mating habitats (Mayr 1963; Coyne and Orr 2004; Nosil et al. 2005). This isolation can be disrupted by artificial alterations of habitat, which can promote hybridization between related species that are naturally isolated (Arnold 1997). Anthropogenic increases of the hybridization rates among species can result in extinctions of many plant and animal populations (Levin et al. 1996; Rhymer and Simberloff 1996; Wolf et al. 2001; Seehausen et al. 2008).

Scribner et al. (2001) reviewed some general mechanisms that disrupt habitat isolation through habitat alteration by human activity. In case that two parental species show distinct habitat preferences in their reproductive sites, they hardly have chances to hybridize each other. However, when human-activities remove one habitat that was preferred by one species, the species would be compelled to reproduce in a less favorable habitat that is preferred by the other species. Such habitat shift may lead to hybridization between species. This process called *habitat loss* (Scribner et al. 2001) has been reported in freshwater fishes (Eisenhour and Piller 1997) and in frogs (Lamb and Avise 1986; Schlefer et al. 1986).

Hexagrammos fishes commonly occur in coastal waters of the North Pacific (Rutenberg 1970). One boreal species *H. octogrammus* (masked greenling) and two temperate species *H. otakii* (fat greenling) and *H. agrammus* (spottybelly greenling) co-occur in the northern Japanese coastal areas of Tohoku and southern Hokkaido (Rutenberg 1970; Fig. 1a; Kanamoto 1976b; Amaoka 1984). *H. agrammus* and *H. octogrammus* inhabit shallow seaweed beds, while *H. otakii* inhabits comparatively deeper water (Gorbunova 1970; Kanamoto 1976a; Kanamoto 1976b). Because of such habitat differences, it was thought that habitat isolation would inhibit hybridization between *H. otakii* and two shallow water species. However, frequent hybridization between *H. otakii* and either of the two shallow water species have been reported (Munehara et al. 2000; Balanov et al. 2001; Crow et al. 2007). These studies suggest that the habitat isolation between *H. otakii* and the two shallow water species may be disrupted at some spawning sites.

During the breeding season, males of *Hexagrammos* species establish breeding territories and multiple females visit males' territories for spawning (Crow et al. 1997; Munehara 2001). The three species utilize seaweeds (Oshima and Nakamura 1942; Yamamoto and Nishioka 1948) or sessile benthos such as bryozoans (M.R. Kimura personal observations) as spawning substrates in male breeding territories. The biota of sessile organisms is generally different between shallow and deep water. If a steep slope of artificial breakwater approximates shallow and deep environments, a mosaic distribution of shallow and deep biota would be observed within a single area. As a result, the three species might establish their breeding territories close to each other in artificial breakwater area. In a previous study using underwater video camera, females from shallow water species were observed to spawn in the territories of the deep water species which were established on concrete blocks along a breakwater (Munehara et al. 2000). To explore the cause of hybridization, we hypothesize that construction of artificial structures such as breakwaters with steep slopes would produce a mosaic habitat consisting of shallow and deep environments and increase the encounter rates between shallow and deep water species. In such artificial areas, shallow and deep water species may encounter each other more frequently than in natural reefs.

In this study, to test the hypothesis that artificial steep slopes cause a disruption of habitat isolation among the three *Hexagrammos* species, we examined 1) the distribution of the three *Hexagrammos* species in a natural reef and an artificial area (near a breakwater), 2) the differences in biota in breeding territories among the three species, and 3) the distribution of biota in a natural reef and an artificial area.

Materials and Methods

Overview of Research Area

The study was conducted off the Usujiri Fisheries Station (41° 57’ N, 140° 58’ E), Field Science Center for Northern Biosphere, Hokkaido University, in southern Hokkaido, Japan, where all the three *Hexagrammos* species occur and breed (Fig. 1a), from September to November in 2003. The station is located on the tip of a headland surrounded by a natural reef. West of the headland, there is a 500-m man-made breakwater. We established three research areas on the natural seafloor around the headland and one research area along the artificial breakwater (Fig. 1b): Shallow Rocky Area (depth range = 1 - 9.5 m), Deep Rocky Area (depth range = 4 - 19 m), Deep Sandy Area (depth range = 19 - 20.5 m, a large number of concrete pipes are scattered on the sandy floor as a habitat for reef fish), and Breakwater Area (depth range = 0-9.5 m). Because the research areas contained heterogeneous seafloor structures, the areas were further divided into several habitat types according to depth and seafloor structure. The research areas on the natural seafloor (Shallow Rocky Area, Deep Rocky Area, and Deep Sandy Area) were divided into three habitat types: *upper reef*, inshore (depth < 3 m) area with relatively flat rock shelf; *lower reef*, offshore rock shelf with moderate slopes; *sandy floor*, sandy or sparse boulder floor around the reef. The Breakwater Area was divided into two habitat types: *concrete blocks* (depth range = 0-8.5 m), and *net base* (depth range = 6.5-9.5 m). In a typical breakwater in Japanese coast, a concrete wall is constructed on a base, and outside of the wall, many concrete blocks are stacked on a base to reduce wave power (Takayama 2004). In the artificial Breakwater Area in this study site, big nets containing stones were laid under the concrete blocks as a base.

Distribution of the *Hexagrammos* species in each research area

The distributions of the three *Hexagrammos* species were investigated by visual transects using SCUBA. In the Shallow Rocky Area and the Deep Rocky Area (each approximately 0.5 ha), transect lines (70 m) were set perpendicular to the isobaths. *Hexagrammos* species found within 1.5 m on both sides of the transect line were counted, and the depth and the habitat types (*upper reef*, *lower reef*, and *sandy floor*) were recorded to compare the distribution patterns of the three species. The census was conducted 22 times in the Shallow Rocky Area and 12 times in the Deep Rocky Area. Transect lines were randomly reset in each census. In the Breakwater Area, a 25 × 100 m census area was set. The census was conducted 13 times in the same manner as above.

During breeding season, mature territorial males of *Hexagrammos* species demonstrate nuptial coloration variation including the following: the ventral and anal fins of *H. agrammus* and *H. octogrammus* males turn into black, and the bodies of *H. otakii* male turn into bright yellow (Munehara et al. 2000). In each species, a territorial male guards eggs that are deposited in its territory (Munehara et al. 2000). We distinguished territorial males from non-territorial individuals (mature females, sneaker males, and immature individuals) by their nuptial coloration and nest guarding behavior. Because breeding territories were not moved or abandoned throughout breeding season if once they were established, we identified territories which had been already counted with marking tapes and did not recount. But all non-territorial individuals which were observed during a census were counted at every census, because we could not know whether an individual had been already counted or not. In the Deep Sandy Area, only territorial males within a census area (approximately 0.5 ha) were counted and the position and depth were recorded on underwater map during 12 censuses.

We compared the average depth of the distributions of the three species using a Kruskal-Wallis test, and statistical significance ($p < 0.05$) was further examined by Steel-Dwass test. We tested whether the three species distribute randomly on each habitat type by extended

Fisher's exact test using R for windows version 2.7.2.

Biota in the breeding territories and the research areas

To determine biota preferred by each *Hexagrammos* species as the breeding substrates, sessile organisms in the breeding territories was sampled with quadrats (25 × 25 cm) (territory sampling). Distributions of biota in each research area were also investigated with the same method (area sampling). All sessile organisms including both plants (seaweeds) and animals collected within each quadrat were identified and weighed after being wiped with paper.
Territory sampling: quadrats were placed on the seafloor so that egg masses in breeding territories were located in the center of each quadrat. A total of 31 territories were examined: nine for *H. agrammus* (three in the Shallow Rocky Area and six in the Breakwater Area), six for *H. octogrammus* (all in the Shallow Rocky Area), and 16 for *H. otakii* (seven in the Deep Sandy Area and nine in the Breakwater Area) territories.
Area sampling: a total of 38 quadrats were placed on the seafloor at regular intervals. In the Shallow Rocky Area and the Deep Rocky Area, eight quadrats were set at 10-m intervals on the 70-m lines, which were set perpendicular to the isobaths from the shore to deep water in each research area. In the Deep Sandy Area, five quadrats were set on five randomly chosen concrete pipes. In the Breakwater Area, 17 quadrats were set at 2.5-m intervals on the two lines, which were set perpendicular to the breakwater from surface to the bottom. The habitat types (*upper reef*, *lower reef*, *sandy floor*, *concrete blocks*, and *net base*) within each quadrat were recorded.

To see differences in preferred biota as a territory sites among the three species and association with the distributions of biota in the research areas, cluster analysis was conducted using data for the 11 most abundant species in territory sampling (n = 31) and area sampling (n = 38). Similarities among quadrats were represented by the Euclidean distance, and a dendrogram was drawn using the unweighted cluster average strategy. The differences of biota among the territories of the three species and research areas were tested by extended Fisher's exact test with the number of quadrats classified into each cluster using R for windows version 2.7.2.

Results

Distribution of the three *Hexagrammos* species in each research area

The distributions of *H. agrammus* and *H. octogrammus* overlapped but were segregated from that of *H. otakii* in the natural reef, while the distribution of the three species overlapped in the artificial Breakwater Area. This segregation in the natural reef and the overlapping in the Breakwater Area were observed in both non-territorial individuals (Fig. 2a) and territorial males (Fig. 2b).

In the Shallow Rocky Area, 11 *H. agrammus* and 23 *H. octogrammus* non-territorial individuals were observed on the *upper reef*, and non-territorial individuals of three *H. agrammus* and one *H. octogrammus* were observed on the *lower reef* (Fig. 2a). Non-territorial individuals of *H. otakii* (n = 12) were observed only on the *sandy floor*. In the Deep Rocky Area, only *H. otakii* (n = 33) occurred on the *lower reef*. Although there was no data about the distribution of non-territorial individuals in the Deep Sandy area in this study, no *H. agrammus* and *H. octogrammus* but *H. otakii* has been frequently collected around this area with gill net sampling in following research (T. Nakamura unpublished data). In those natural reef research areas, the depth of the distributions of the two shallow water species (*H. agrammus* and *H. octogrammus*) and the deep water species (*H. otakii*) differed significantly and did not overlap (*H. agrammus*: range = 0.5-3.5 m, n = 14; *H. octogrammus*: range = 0.5-3.0 m, n = 24; *H. otakii*: range = 6.5-19.0 m, n = 45; Kruskal-Wallis test, $p < 0.001$, *H. otakii* v.s. the other two species were significantly different in Steel-Dwass test). The observed distribution pattern was significantly different from random distribution (Fisher's test, $p < 0.001$). In the Breakwater Area, 28 *H. agrammus*, one *H. octogrammus* and 21 *H. otakii* non-territorial individuals were observed on the *concrete blocks*, and 10 *H. agrammus* and 21 *H. otakii* on the *net base*. In this artificial areas, the depth of the distributions of the three species did not differ significantly and largely overlapped (*H. agrammus*: range = 1.6-8.6 m, n = 38; *H. octogrammus*: 2.6 m, n = 1; *H. otakii*: range = 1.5-9.3 m, n = 42; Kruskal-Wallis test, $p = 0.115$), although the observed distribution pattern was slightly different from random distribution (Fisher's test, $p = 0.049$).

In the Shallow Rocky Area, territories of nine *H. agrammus* and six *H. octogrammus* were observed on the *upper reef*, and one *H. agrammus* and two *H. octogrammus* were found to have their territories on the *lower reef* (Fig. 2b). No territories were found on the *sandy floor* in the Shallow Rocky Area and on any of the habitat types in the Deep Rocky Area. In the Deep Sandy Area, the territories of only *H. otakii* (n = 12) were observed. In those natural reef research areas, the depth of the territories of the shallow and deep water species differed significantly and did not overlap (*H. agrammus*: range = 1.0-3.0 m, n = 10; *H. octogrammus*: range = 0.3-4.5 m, n = 8; *H. otakii*: range = 19.0-20.5 m, n = 12; Kruskal-Wallis test, $p < 0.001$, *H. otakii* v.s. the other two species were significantly different in Steel-Dwass test). The observed distribution pattern was significantly different from random distribution (Fisher's test, $p < 0.001$). In the Breakwater Area, the territories of 18 *H. agrammus*, one *H. octogrammus*, and six *H. otakii* were found on the *concrete blocks*, but only *H. otakii* males (n = 7) had territories on the *net base*. The depth of the territories of three species differed significantly also in this artificial area, but the range of depth overlapped largely (*H. agrammus*: range = 1.5-4.0 m, n = 18; *H. octogrammus*: 3.0 m, n = 1; *H. otakii*: range = 2.0-9.5 m, n = 13; Kruskal-Wallis test, $p = 0.002$). The observed distribution pattern of the territories was significantly different from random distribution (Fisher's test, $p = 0.001$).

Biota in the breeding territories and in the research areas

In territory sampling (33 quadrats) and area sampling (38 quadrats), 53 species (44 plants and 9 animals; Appendix 1) and 46 species (42 plants and 4 animals; Appendix 2) of sessile organisms were collected, respectively. The cluster analysis of the 69 quadrats classified the data into six groups (cluster I – VI; Table 1). Clusters I, II, and III were characterized by small red algae *Gelidium elegans*, *Chondrus* spp. and *Tichocarpus crinitus*, respectively. Cluster IV was characterized by the sea grass *Phyllospadix iwatensis*. Cluster V was characterized by bryozoans *Phidolopora elongata* and *Microporina articulata*. In cluster VI, neither seaweeds nor sessile benthos was found abundantly within the quadrats.

The biota collected in territory sampling significantly differed among the three *Hexagrammos* species (Table 2; Fisher's exact test, $p < 0.001$). All the quadrats in territories of *H. agrammus* were classified into cluster I where seaweed were abundant, and those of *H. octogrammus* were classified into clusters I, II, III, and IV, where seaweed and sea grass were abundant. The quadrats in territories of *H. otakii* were all classified into cluster V where bryozoans were abundant or cluster VI where few sessile organisms were found, except for one quadrat in the Breakwater Area that was classified into cluster II.

The biota collected in area sampling differed greatly among research areas and habitat types within a research area (Table 3; Fisher's exact test, $p < 0.001$). On the *upper reef* in the Shallow Rocky Area and the Deep Rocky Area, small red algae (clusters I - III) were abundant, while small amounts of seaweed (cluster VI) grew on the other habitat types. In the Deep Sandy Area, bryozoans (cluster V) were dominant. However, on the *concrete blocks* in the Breakwater Area, the biota comprised both small red algae (clusters I - III) frequently found in the territories of *H. agrammus* and *H. octogrammus* and few sessile organisms (cluster VI) preferred by *H. otakii*.

Discussion

In this study, the distributions of the two shallow water species *H. agrammus* and *H. octogrammus* and a deep water species *H. otakii* were segregated in a natural reef. The biota in breeding territories distinctly differed between the two shallow water species and the deep water species. The shallow water species *H. agrammus* and *H. octogrammus* established their breeding territories on areas covered with thick seaweed or sea grass, while the deep water species *H. otakii* established its territory on areas covered with bryozoans. The habitat isolation among the three species might be retained by the differences in biota preferred by each species. In natural reef, the biota of sessile organisms in shallow water and that in deep water were different. The different distribution of biota might lead the spatial isolation between the two shallow water species (*H. agrammus* and *H. octogrammus*) and the deep water species *H. otakii* in a natural reef. Spatial isolation caused by such differences of preferred habitat has been reported in many taxa: e.g. butterfly in open secondary forest and that in closed-canopy forest (Estrada and Jiggins 2002), and rock and shell-bed dwelling cichlid fish (Takahashi et al. 2001).

In contrast, the two shallow water species and the deep water species were found to co-occur in the artificial Breakwater Area. The Breakwater Area contained a typical shallow water environment with thick seaweed as well as a typical deep water environment with sparse seaweed density. The coexistence of different biota in the Breakwater Area is thought to be generated by steep slopes and complex structure of the breakwater. Because abundance of seaweed is largely dependent on sunlight, some stacked concrete blocks were mostly covered with seaweed, and some were scarcely covered with seaweed when they were shaded by others. The big nets containing stones under the concrete blocks were also scarcely covered with seaweed. Thus, a steeply sloping breakwater with complex stacked concrete blocks approximate typical shallow water and deep water environments in closer proximity than in natural reefs and create a mosaic distribution of different environments that are usually far apart. Consequently, both *H. agrammus* and *H. octogrammus*, which prefer a thick seaweed environment, and *H. otakii*, which prefers a thin seaweed environment, might be able to find its most suitable spawning sites in this artificial area. This could explain the reason why all the three *Hexagrammos* species co-occur and establish their territories in the artificial Breakwater Area.

Such mosaic structures of habitat are frequently observed in natural hybrid zones. In ground crickets, *Allonemobius socius* live in a warm, moist and heavily grazed habitat, while *A. fasciatus* live in a cool, dried and lightly grazed habitat. Some pastures contain both habitats mosaically, and consequently, hybrid zones of the two species are formed in such pastures (Ross et al. 2008). Such mosaic hybrid zones which are structured by a patchwork of habitats which are preferred by different species are known in, for example, *Bombina* toads (Yanchukov et al. 2006), *Gryllus* ground crickets (Ross and Harrison 2002), *Chorthippus* grasshoppers (Bridle et al. 2001), and *Mytilus* mussels (Bierne et al. 2003). In the case of the three *Hexagrammos* species, we show that steep and complex breakwater structures may make an artificial mosaic hybrid zone containing two types of habitat in a single area, leading to a disruption of habitat isolation. Munehara et al. (2000) found a highly frequent crossbreeding rate (19 of 26 matings, 73%) between male of *H. otakii* and the other two species in the same area as the Breakwater Area in this study from observations for over 600 hours with using an underwater video camera. In addition, Crow et al. (2007) reported that 25 (31%) of the 76 specimens randomly sampled off the Usujiri Fisheries Station (same area as this study) were hybrids or backcrosses using genetic markers. It is not known why hybridization and introgression of three *Hexagrammos* species occurs so frequently in Usujiri, but it is possible that artificially modified mosaic habitat may play a role. There have been many examples showing that species which are naturally isolated hybridize only in artificial habitat (Arnold 1997). In this study, we suggest that artificial mosaic habitat provides a habitat for breeding between different species which would normally be segregated spatially.

The *Hexagrammos* generic species is one of the most popular fish which distribute widely in a coastal area around the North Pacific (Rutenberg 1970; Amaoka 1984). Coastal areas in Japan have been often altered by human activities during the construction of fishing ports and land reclamation. This study suggested that coastal fish fauna may be altered by artificial interference to isolation mechanism among closely related species.

Acknowledgments: The authors thank Drs. Masakado Kawata, John Bower, Satoshi Awata, Yoko Iwata, Karen Crow and Ayumi Tezuka for reading this manuscript and providing valuable comments, Drs. H. Yasui, H. Mizuta and T. Kawagoe for advice on the classification of seaweeds, Dr. S. Mawatari for identification of bryozoans. This work was supported by a grant in aid for scientific research from the Ministry of Education.

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Figure Legends

Fig. 1. Overview of research area. (a) Distributions of the three *Hexagrammos* species in Japan. These *Hexagrammos* species occur sympatrically from Tohoku to southern Hokkaido. (b) The location of the research areas. The line shown north and west of the fishing port represents breakwaters. The star denotes Usujiri Fisheries Station. SR: Shallow Rocky Area; DR: Deep Rocky Area; DS: Deep Sandy Area; BW: Breakwater Area.

Fig. 2. Distribution patterns of non-territorial individuals (a) and territorial males (b) of the three *Hexagrammos* species in each research area. Solid lines in each graph represent the maximum and minimum depth of transect lines and census areas. N.D. means no data.

Table 1. Composition of sessile organisms in quadrats (n = 69 in both territory sampling and area sampling) classified into each cluster. The average wet weights (g) per quadrat (25 cm × 25 cm) are shown. For clarity, zeros are omitted. Bold type indicates the representative species of each cluster.

Species of sessile organisms		Cluster number					
		I	II	III	IV	V	VI
Red algae	***Gelidium elegans***[a]	**127.9**	6.0	7.4	1.3		0.1
	***Chondrus* sps.**	19.6	**111.5**	10.6	2.5		0.5
	Odonthalia corymbifera	3.3	4.2				5.7
	Tichocarpus crinitus			**277.4**			0.6
	Corallinaceae	10.9	7.2	1.9	1.5	0.3	2.0
	Neohypophyllum middendorfii					21.5	4.2
	Callophyllis adnata					2.3	
Brown algae	*Sargassum horneri*	4.2	0.9	0.2			3.6
Other algae		33.7	15.7	21.5	118.5	4.3	32.8
Sea grass	***Phyllospadix iwatensis***				**778.3**		
Bryozoans	***Phidolopora elongata***					**135.7**	
	Microporina articulata					**53.4**	0.2
	other bryozoans	0.2				17.8	0.0
Other animals						5.3	0.2

[a] *Symphyocladia latiuscula* attached to *Gelidium elegans* are included.

Table 2. Differences in biota in breeding territories. The numbers of quadrats classified into each cluster are shown. The character and the representative sessile organisms of each cluster numbers were showed in Table 1 (see text also). For clarity, zeros are omitted.

Territorial male	Research area	Cluster					
		Seaweed			Sea grass	Bryozoans	Bare
		I	II	III	IV	V	VI
H. agrammus	Shallow Rocky Area	3					
	Breakwater Area	6					
H. octogrammus	Shallow Rocky Area	1	2	2	1		
H. otakii	Deep Sandy Area					7	
	Breakwater Area		1				8

Table 3. Biota in each research area. The numbers of quadrats classified into each cluster are shown. The character and the representative sessile organisms of each cluster were showed in Table 1 (see text also). For clarity, zeros are omitted.

Research area	Habitat type	Cluster					
		Seaweed			Sea grass	Bryozoans	Bare
		I	II	III	IV	V	VI
Shallow Rocky Area	*upper reef*	2		1			
	lower reef	1					2
	sandy floor						2
Deep Rocky Area	*upper reef*	1					
	lower reef						7
Deep Sandy Area	*sandy floor*						2
Breakwater Area	*concrete blocks*	3		2		3	7
	net base						5

Appendix 1. Sessile organisms collected in territory sampling. Average wet weights (g) of benthos species per quadrat (25 × 25 cm) are shown. For clarity, zeros are omitted. SR: Shallow Rocky Area; DR: Deep Rocky Area; DS: Deep Sandy Area; BW: Breakwater Area.

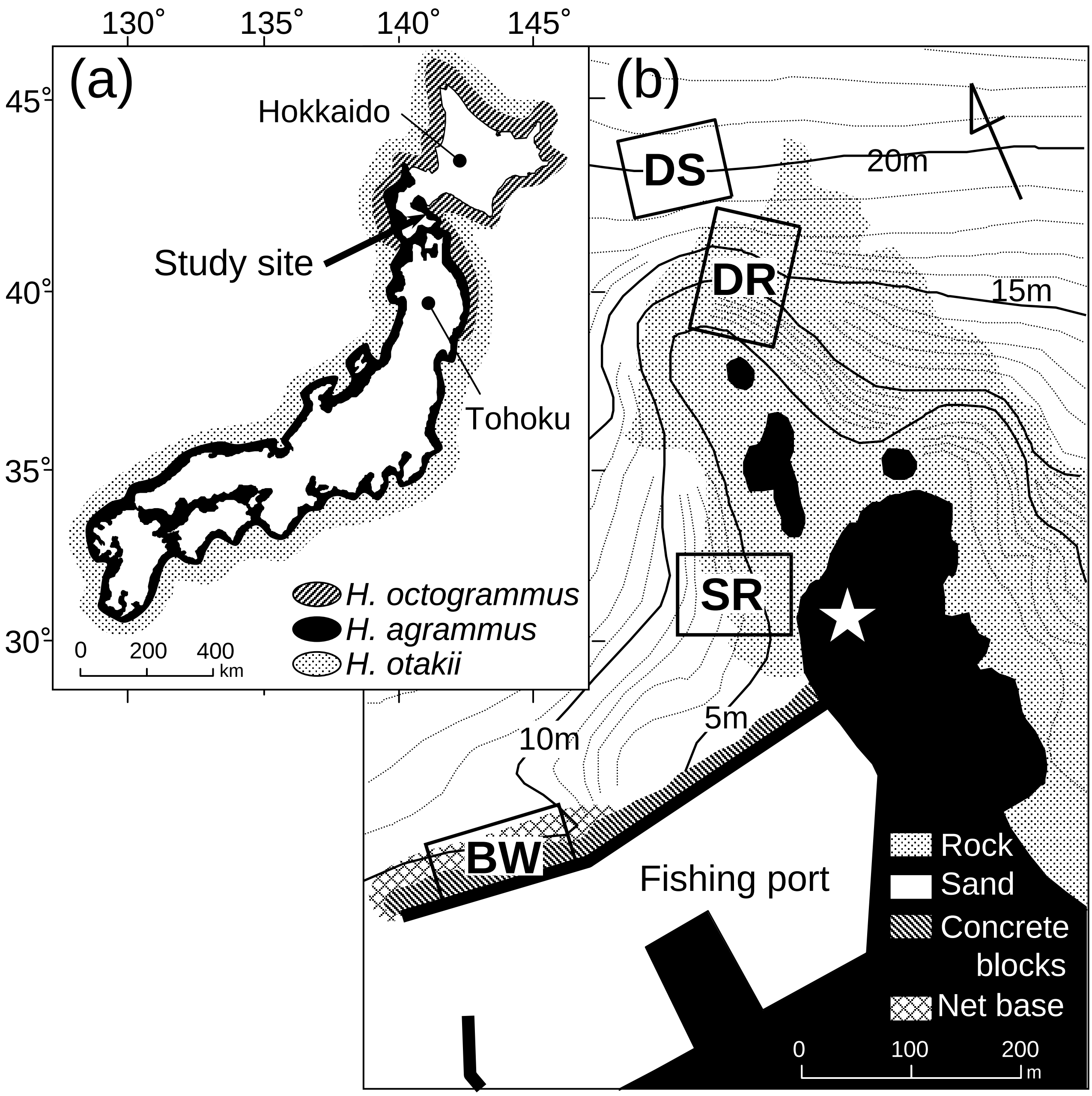
Species of sessile organisms		*H. agrammus*		*H. octogrammus*	*H. otakii*	
		SR	BW	SR	DS	BW
Red algae	*Gelidium elegans*	80.44	103.75	4.76		0.69
	Odonthalia corymbifera	5.48	1.40			1.55
	Tichocarpus crinitus			75.16		
	Symphyocladia latiuscula	83.22	0.29	11.07		0.23
	Neodilsea yendoana	15.61	0.11	8.93		
	Chondrus yendoi	15.28	20.07	39.33		3.96
	Chondrus occellatus			9.38		0.09
	Chondrus nipponicus			0.83		
	Chondrus pinnulatus		0.63	1.93		0.22
	Chondrus elatus			0.66		
	Corallina pilulifera	5.23	4.76	11.88		0.01
	Bossiella cretacea	1.61	0.15	1.05	0.39	
	Calliarthron yessoense			0.53		
	Neohypophyllum middendorfii				17.76	0.26
	Callophyllis adnata				1.47	
	Sparlingia pertusa					0.42
	Gracilaria textorii			0.82		
	Carpopeltis affinis			0.88		
	Grateloupia filicina	0.67		0.24		
	Ptilata filicina				0.01	0.02
	Dasya sessilis					0.00
	Heterosiphonia japonica				0.27	0.07
	Heterosiphonia pulchra		0.04			0.00
	Aglaothamnion callophyllidicola					0.00

	Pterothamnion yezoense		0.13		0.04	
	Acrosorium yendoi	3.94	0.12	0.19	0.01	0.44
	Ceramium kondoi			0.66		0.00
	Ceramium japonicum			0.04		0.03
	Campylaephora crassa	0.01				0.01
	Lomentaria hakodatesis	0.06	0.36			0.01
	Champia parvula	0.11				
	Gloiopeltis furcata	0.01				
Brown algae	*Laminaria japonica*	7.49	4.07			
	Alaria crassifolia	0.75	16.15	19.49		
	Agarum clathratum				0.28	
	Undaria pinnatifida		0.78			0.67
	Costaria costata					0.08
	Sargassum horneri		11.35			3.94
	Sargassum confusum		2.00			0.75
	Sargassum thunbergii			5.57		
	Dictyota dichotoma			0.06		
Green algae	*Ulva pertusa*	0.02	1.45	7.53		0.46
	Bryopsis plumosa		0.01			
Sea grass	*Phyllospadix iwatensis*			129.72		
Bryozoans	*Phidolopora elongata*				145.44	
	Microporina articulata				71.46	
	Celleporina incrassata				12.33	
	Other bryozoans		0.40		0.97	0.10
Other animals					1.56	0.36
(Total wet weight)		(220.0)	(168.0)	(330.7)	(252.0)	(14.4)
No. of quadrat		3	3	3	7	9

Appendix 2. Sessile organisms collected in area sampling. Average wet weights (g) of benthos species per quadrat (25 × 25 cm) are shown. For clarity, zeros were omitted. SR: Shallow Rocky Area; DR: Deep Rocky Area; DS: Deep Sandy Area; BW: Breakwater Area.

Species of sessile organisms		SR			DR		DS	BW			
		upper reef	*lower reef*	*sandy floor*	*upper reef*	*lower reef*	*sandy floor*	*concrete blocks*			*net base*
Red algae	*Gelidium elegans*	14.96	17.63		145.56			111.46	0.06		
	Odonthalia corymbifera		14.96			16.91			14.23		
	Tichocarpus crinitus	119.57						115.50	4.70		
	Symphyocladia latiuscula	42.98	27.07					0.99			
	Neodilsea yendoana	1.69						1.71	0.90		
	Chondrus yendoi	32.45	1.96					13.56	3.22		
	Chondrus occellatus	1.46							0.54		
	Chondrus nipponicus	8.69									
	Chondrus pinnulatus	0.40									
	Chondrus armatus		0.03								
	Corallina pilulifera	6.54	0.17		2.65			2.78	1.13		
	Bossiella cretacea	4.21	12.25		37.12	1.79	2.16	0.06			
	Neohypophyllum middendorfii					8.87	32.77				
	Congregatocaepus pacificus						0.52			0.87	
	Callophyllis adnata						2.62				
	Hideophyllum yezoence						6.30				
	Sparlingia pertusa										0.02
	Gracilaria textorii		5.47								
	Chrysymenia wrightii			15.24							
	Grateloupia turuturu								0.03		
	Carpopeltis affinis		1.51								
	Grateloupia filicina		0.01	0.10							
	Mastocarpus pacificus	0.23									
	Ptilata filicina					0.05		0.35	0.50		
	Dasya sessilis			1.13				1.12			

	Heterosiphonia japonica						0.89				0.01
	Pterothamnion yezoense		0.03			0.03					
	Acrosorium yendoi	2.87	0.98								0.11
	Ceramium kondoi								0.02		
	Ceramium japonicum		0.07								
	Campylaephora crassa										0.08
	Lomentaria hakodatesis		0.06					0.28			
	Champia parvula	1.80	0.04								
	Gracilaria vermiculophylla	0.50			0.55				0.05		
	Gloiopeltis furcata		0.04								
Brown algae	*Laminaria japonica*		69.07						40.05		
	Alaria crassifolia	3.86			61.09			10.42			
	Agarum clathratum					85.11	21.72				
	Undaria pinnatifida		0.04	1.06				0.10	3.91		0.06
	Sargassum horneri		2.30					0.89	19.91		
	Sargassum confusum		1.12					1.26	4.65		
Green algae	*Ulva pertusa*	0.13	1.88	0.44				4.73	2.30		1.50
Bryozoans	*Phidolopora elongata*						67.86				
	Microporina articulata					0.80	7.08				
	other bryozoans				1.16		16.92				
Other animals							8.80				
(Total wet weight)		(242.3)	(156.7)	(18.0)	(248.1)	(113.6)	(167.6)	(265.2)	(96.2)	(0.9)	(1.8)
No. of quadrat		3	3	2	1	7	5	7	2	3	5
Dominant sessile organism		red algae	brown algae	bare ground	red algae	brown algae	bryozoans	red algae	brown algae	bare ground	bare ground



130°
135°
140°
145°
45°
40°
35°
30°
(a)
Hokkaido
Study site
Tohoku
0
200
400
km
H. octogrammus
H. agrammus
H. otakii
(b)
DS
20m
DR
15m
SR
5m
10m
BW
Fishing port
Rock
Sand
Concrete blocks
Net base
0
100
200
m

Fig. 1

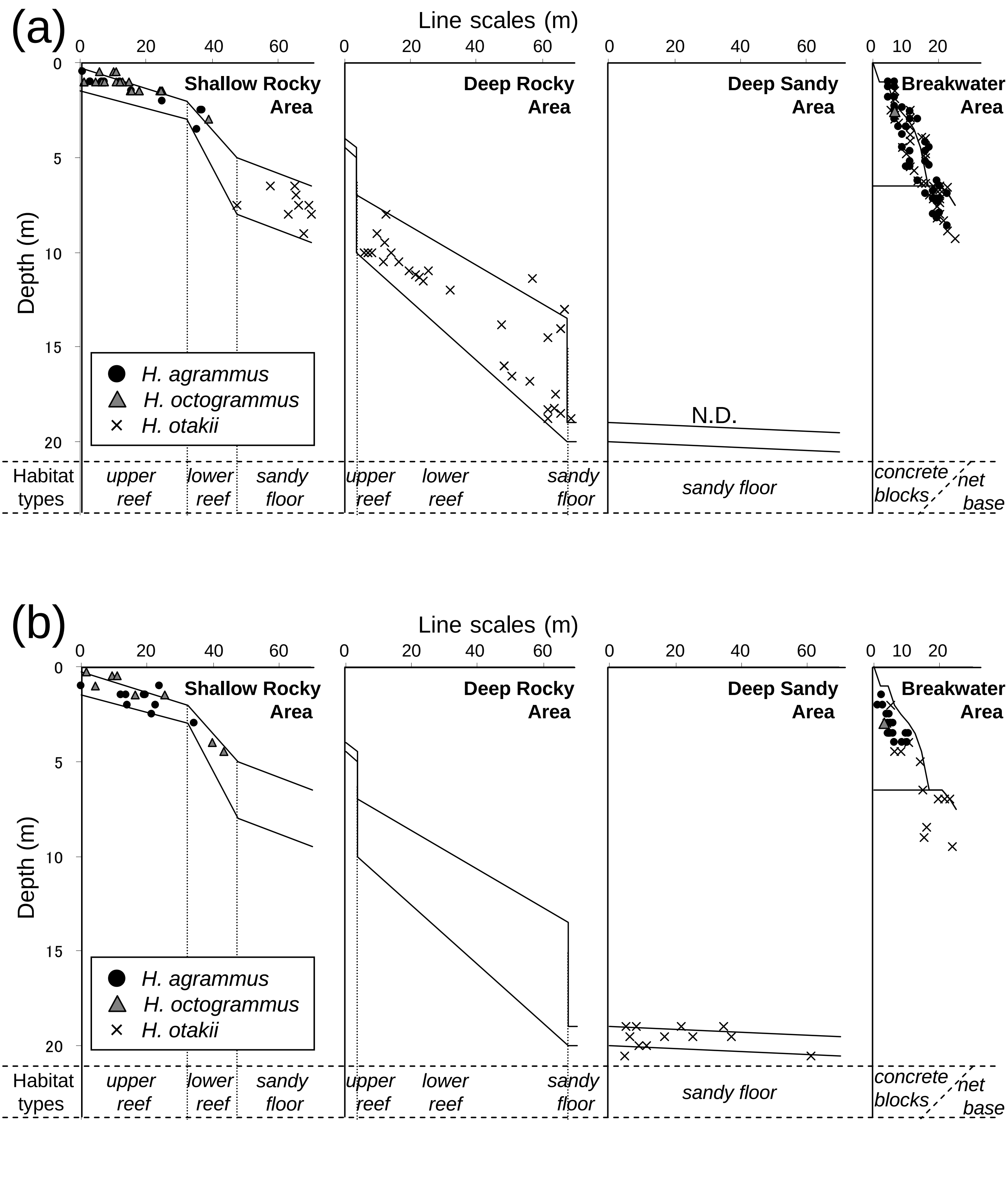

(a)
Line scales (m)
Depth (m)
Shallow Rocky Area
Deep Rocky Area
Deep Sandy Area
Breakwater Area
H. agrammus
H. octogrammus
H. otakii
N.D.
Habitat types
upper reef
lower reef
sandy floor
concrete blocks
net base
(b)
Line scales (m)
Depth (m)
Shallow Rocky Area
Deep Rocky Area
Deep Sandy Area
Breakwater Area
H. agrammus
H. octogrammus
H. otakii
Habitat types
upper reef
lower reef
sandy floor
concrete blocks
net base

Fig. 2