Original Article

Effect of Water Flow on Grazing by the Sea Urchin (*Strongylocentrotus Nudus*) in the Presence of Refuge Habitat

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ABSTRACT

Effect of water disturbance on grazing by the sea urchin with the refuge habitat was estimated. We tested the hypothesis that sea urchins ceased feeding and remained in refuge habitat for protection against disturbance even though water flows were not high to inhibit their grazing. Fecal density from sea urchins was positively related to their residence time under the undisturbed condition. This result suggested that the fecal density is a useful criterion for understanding the residence time of sea urchins in the calm condition. Five types of structure (L-shaped, cave shaped, crack-shaped, smaller and larger rectangular structure) as refuge habitats were deployed in the undisturbed tank with sea urchins and the fecal densities at each structure were measured to examine the habitat forms which sea urchins prefer. This experiment indicated that the crack-shaped form was used for sea urchins as the refuge habitat. Feeding rate of sea urchins inversely corresponded to water flow. Feeding rates with the refuge habitat were lower than in the absence of habitat, although water disturbances were almost the same. The presence of refuge habitat seemed to be a factor responsible for the reduction of their grazing, because sea urchins sheltered to avoid water disturbances.

Keywords: grazing, refuge habitat, sea urchin, water flow

INTRODUCTION

Macroalgal habitats such as *Eisenia bicyclis* and *Saccharina japonica* are highly productive components in temperate coastal ecosystems, and support diverse faunal assemblages [1, 2]. They provide suitable habitats for many commercial fishes and benthic animals [1, 3, 4]. However, subtidal macrophyte habitats around the world have declined due to human pollutants [5, 6], overgrazing of sea urchins [4, 7–9] and herbivorous fishes [10], as well as natural disturbances [11–13]. Sea urchins are important agents of disturbance and are frequently regarded as proximate determinants of community structure and abundance of macroalgal habitats [14]. In Vestfjorden, northern Norway, overgrazing by the sea urchin *Strongylocentrotus droebachiensis* has resulted in the decrease of large kelp forests and has remained a barren ground dominated by crustose coralline algae [7]. Losses of *Eisenia bicyclis* habitat in Miyagi Prefecture, Japan, have been ascribed to the heavy grazing by the sea urchin *Strongylocentrotus nudus* [9].

Wave-induced benthic water flow is well known to inhibit the feeding behaviors of sea urchins [4, 14, 15]. High water velocities prevent the active grazing of sea urchins and restrict their distributions by dislodgment. In a laboratory study using a flume tank, the feeding activity of the sea urchin, *S. nudus*, markedly reduced in the peak velocity exceeding 0.3 m/s [15]. In field surveys, however, there are many areas where *S. nudus* do not approach algal communities and macroalgal habitats have remained with water velocities less than 0.3 m/s, even which were around $0.1 - 0.2$ m/s [4]. The difference in the water velocities required to prevent grazing by sea urchins might be attributed to changes in their behaviors by having access to refuge habitats or not. We considered a hypothesis that there are two kinds of water disturbances which prevent sea urchin grazing. One is the water flow which inhibits feeding behavior of sea urchins

and the second is the flow which was lower than the velocity limit for feeding, but sea urchins hesitated to receive it and remained in the refuge habitat as preferred shelter, although it was possible to carry out the grazing. In the field, sea urchins are often found in refuge habitats, e.g. cracks, pockets and crevices, for the avoidance against water disturbances, and we assumed that sea urchins ceased grazing due to reluctance and remain in refuge habitats even though water disturbances were not sufficiently high to inhibit their grazing. On the other hand, in the laboratory experiment, since the sea urchin had been placed in the flume tank without refuge habitat, the sea urchin was continuously affected by the water disturbance. As a result, it was thought the higher velocity that inhibited their grazing was estimated.

In the present study, we tested the hypothesis that the influence of water flow on the grazing behavior of sea urchin differs in the presence or absence of refuge habitat. The sea urchin *S. nudus* is probably the dominant herbivore in the subtidal hard-bottom regions of southwestern Hokkaido and the northeastern Pacific Coast of Honshu, Japan. We addressed 2 main questions: What kind of refuge habitat structure is preferred and used by sea urchins as the shelter against water flows? And, do the feeding behaviors of sea urchins reduce in relation with the avoidance to the refuge habitat due to reluctance to the water disturbances?

MATERIALS AND METHODS

Test animals

Sea urchins, *S. nudus*, were collected from the barren ground in Otsuchi Bay (38°20'N, 141°56'E), Pacific Coast of Northern Honshu, Japan. Sea urchin samples were fed with the rehydrated kelp (20.0 cm × 5.0 cm) for 1 day in aquaria and then remained for a few days before the trail observation and selection experiment of preferred habitat, and starved for more than 2 weeks before the feeding experiment mentioned below. Test diameter of the sea urchin was 6.4 ± 0.2 cm width and 3.4 ± 0.2 cm height (n = 3). These sea urchin sizes were often observed at the barren ground in Otsuchi Bay [16].

Trail observation of sea urchin using feces density

To assess what kind of structure sea urchin prefers as a refuge habitat, it was necessary to figure out how long the sea urchin was in the place. When sea urchins were placed in an aquarium under the calm water condition, there were more feces where the sea urchin remained. Therefore, in this study, the fecal density of sea urchins was used as an index for the monitoring of the residence time of sea urchins in the calm water condition. Prior to using this index, we had confirmed whether the fecal density was related to the residence time of the sea urchin or not. Sea urchin was placed in a 1.2 L cylindrical container (bottom area: 132.7 cm^2) filled with artificial sea water with a salinity of 30 at 20°C under the undisturbed water condition. Fecal particles from sea urchin in the container were counted to estimate the fecal density. The residence time of sea urchin was from the time it was placed in the container until the time it was retrieved. Changes in the fecal density were recorded with the increase of the residence time of the sea urchin (n = 3; total number of sea urchins tested), and the results were statistically examined using regression analysis.

Selection of preferred refuge habitat

Several types of structure were deployed in the tank in order to examine the habitat forms which sea urchins prefer (**Fig. 1A**). The tank consists of a doughnut shaped form with a 3.69 m waterway length × 0.30 m width × 0.25 m height. Five types of structure as refuge habitats were prepared with the reference to the place where sea urchins hid in the field (**Fig. 2**): (1) L-shaped, consisted of two blocks (0.10 m length × 0.06 m width × 0.10 m height) at right angles, in which one side was aligned with the side wall of the tank; (2) cave-shaped, prepared by laying 0.17 m of plastic cylinder with 0.15 m height along the waterway; (3) crack-shaped, prepared by placing a block that was attached with a 45-degree angle in a side wall of the tank and covered. The length of the block was 0.15 m with 0.06 m width and 0.20 m height; (4) smaller rectangular structure, consisted of two blocks (0.10 m length × 0.10 m width × 0.12 m height) that were aligned with the side wall of the tank and were placed with an interval of 0.15 m; and (5) larger rectangular structure, consisted of two blocks (0.15 m length × 0.12 m width × 0.15 m height) aligned with the side wall of the tank and were placed with an interval of 0.15 m. The bottom areas of L-shaped, cave shaped, crack-shaped, smaller and larger rectangular structures were 0.01, 0.04, 0.01, 0.02 and 0.02 m^2. Three sea urchins and these refuge habitats were installed in the tank with sand-filtered sea water at 19 − 20°C under the undisturbed condition for 1 day and then the fecal density from the sea urchins at each refuge habitat was recorded (n = 3), because the fecal density indicated the residence time of sea urchin as mentioned later. As the control, the fecal density on the waterway in the absence of refuge habitat was measured at random using the quadrat of 0.1 m × 0.1 m (n = 15). After this trial, we selected the upper three refuge habitats in which high density of the feces was confirmed and then experimented with the same
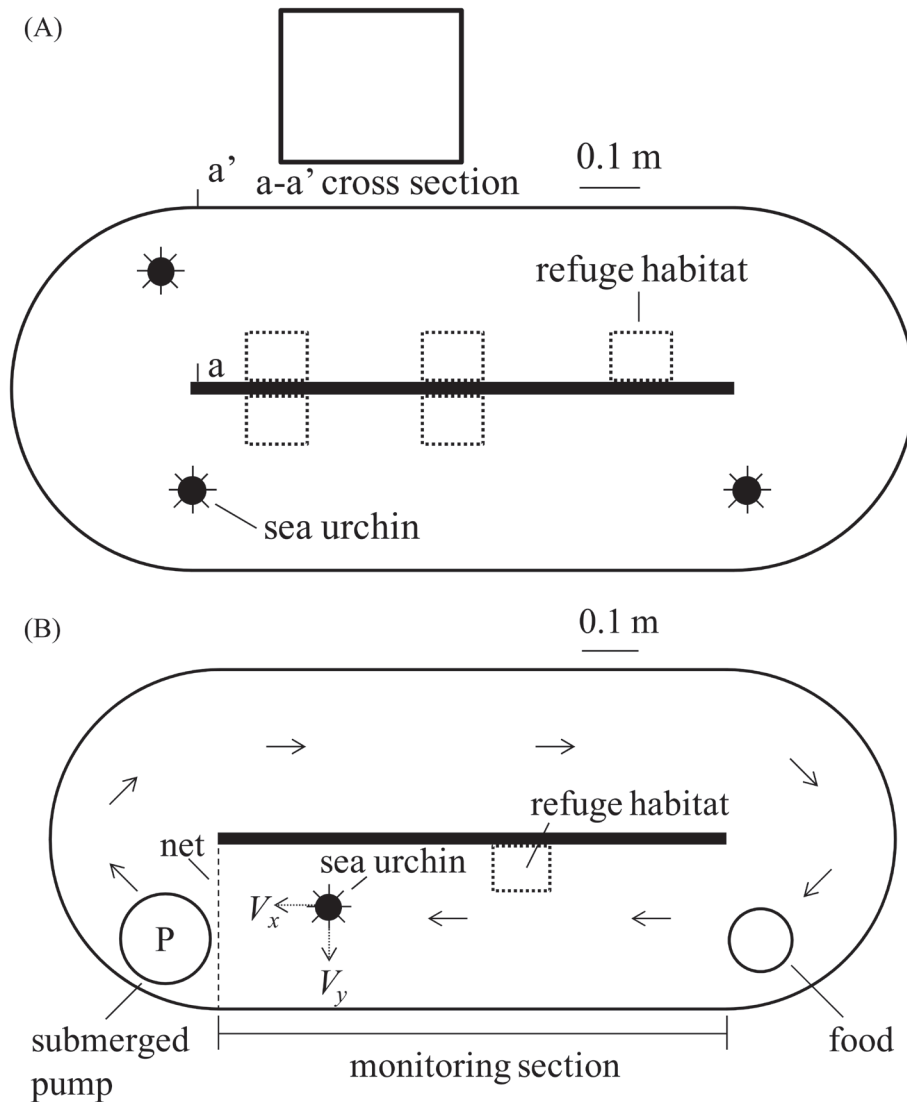
Fig. 1 Sketch of the doughnut-shaped flow tank. (A) The selection experiment of preferred habitat. The refuge habitat is shown by the square of dashed line. (B) The feeding experiment. The flow velocity was controlled by the submerged pump from 0 to 0.35 m/s. The directions of the water flow from the food to the sea urchin (V_x) and from an inside wall of the tank (V_y) were defined as positive values. The main current direction is shown by the arrows.

manipulation to examine the habitat forms which sea urchins prefer.

Effect of water flow on grazing by sea urchin in the presence of the refuge habitat

Feeding experiments were conducted in the doughnut-shaped tank described above (**Fig. 1B**). The monitoring section was prepared between both ends of the longer axis of the waterway. Food for sea urchin was placed at one end of the waterway and at the opposite end, a submerged pump

(SK-53210, KOSHIN Co. Ltd, Nagaokakyo, Japan) was installed. The flow velocity was controlled by the pump from 0 to 0.35 m/s. The main current direction of water flow was adjusted from the area where food was placed to the pump. Both ends of the tank were rounded to reduce turbulence. Dried blades of kelp *Saccharina japonica* were used as the food. The dried blades were rehydrated in fresh water for 40 min and then were trimmed to 20.0 cm × 5.0 cm. A soaked dried blade was anchored by a weight and was placed in the tank filled with sand-filtered seawater at 19 − 20°C. Since sea
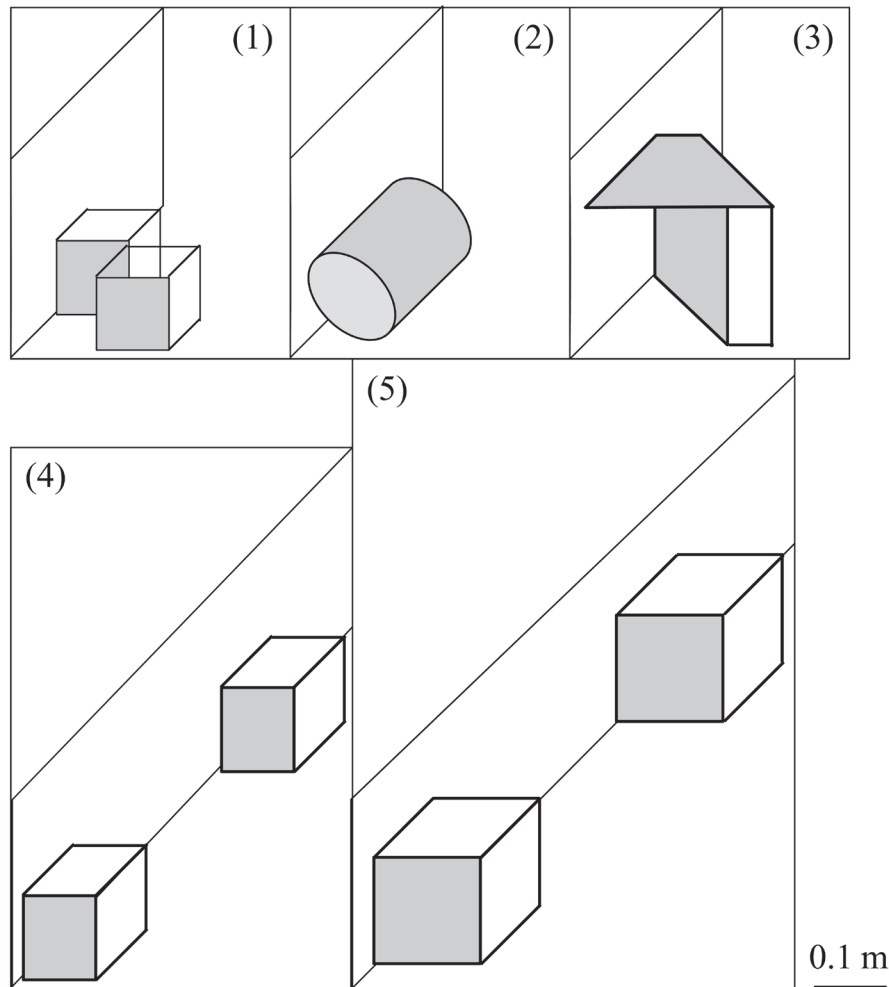
Fig. 2 Diagram showing the refuge habitats used in this study. (1) L-shaped form, (2) cave-shaped form, (3) crack-shaped form, (4) smaller rectangular structure, (5) larger rectangular structure.

urchins tended to prefer the crack-shaped form of the refuge habitat as mentioned later, this refuge habitat was deployed $0.3 - 0.4$ m away from the kelp in the monitoring section. A starved sea urchin was also installed with 0.7 m away from the kelp in the monitoring section. In the presence or absence of the refuge habitat, feeding experiments were conducted under various flow conditions for 1 day and carried out for a total of three times. Feeding rates on the rehydrated dried kelp were determined by the modified procedure reported by Kawamata [15]. The amount of consumption by the sea urchin was estimated from:

amount of consumption (g w.w./(sea urchin·d)) =
$$w\,(r_1 r_2 A_i - A_f) \tag{1}$$

where w is the wet mass per unit blade area for the food and, r_1 and r_2 are the ratio of particular length and width of the remaining food to the corresponding sizes of the initial one after soaking in seawater for 1 day. The planform areas of the food before (A_i) and after (A_f) experiments were measured using a photocopy. Since the decrease in wet mass of food before and after the experiment is not available for the index of feeding rate because the wet mass often changed significantly probably due to absorption of seawater or loss of dissolved mucus substances, this calculation procedure based on the wet mass density was believed to give a reasonable estimation of consumption [15].

At the end of an experiment, after retrieving the sea urchin from the tank, water velocities in 60 seconds with 1 Hz at 0.05 m above the bottom were recorded between 0.1 m and 0.7 m away from the food with the intervals of 0.1 m using a velocity meter (COMPACT-EM, JFE Advantech Co. Ltd., Nishinomiya, Japan). Averaged water velocities were deter-

mined in each flow condition in order to examine the effect of water flow on grazing by sea urchin in the presence or absence of the refuge habitat.

Hydrodynamic force against the object (sea urchin) along the direction of flow was calculated as the sum of the drag and the accelerational force by following the Morison equation [17].

$$f_u = f_d + f_a \tag{2}$$

where, f_u is the total force on the object (N), f_d is the drag (N), and f_a is the accelerational force in the direction of flow (N). In this study, the sea urchin was assumed as a sphere and then, the drag reaction in the direction of the flow was estimated from the following equation.

$$f_d = 1/2 \times \rho \, S_p \, C_d \, u_n \times |u_n| \tag{3}$$

where, ρ is seawater density (= 1.03×10^3 kg/m^3), S_p is the area of the object projected in the direction of flow (= $\pi / 4 \times D^2$ D: body width of sea urchin (m)), C_d is the drag coefficient (= 0.47) that was reported by Denny [17], and u_n is the water velocity (m/s) at 0.05 m above the bottom. We calculated the water flow from the food to the sea urchin (V_x) was defined as the value in positive in which prevented their grazing (**Fig. 1B**). A flow of water from an inside wall of the tank (V_y) was also counted as a plus. The accelerational force in the direction of flow was estimated from the following equation.

$$f_a = \rho \, C_m \, V \, (\Delta u_n/\Delta t) \tag{4}$$

where, C_m is the inertia coefficient of a sphere (= 1.5) that

was reported by Denny [17], V is the volume of the object (= $\pi / 6 \times D^3$) and $\Delta u_n/\Delta t$ is the acceleration of the fluid which acts on the u_n vector. When the water velocity (u_{n-1}) changed in u_n one second later, the acceleration $\Delta u_n/\Delta t$ was calculated as follows:

$$\Delta u_n/\Delta t = u_n - u_{n-1} \times \cos(\theta_2 - \theta_1) \tag{5}$$

where, θ_1 and θ_2 are the radians between the direction of flow of V_y and flow directions of u_{n-1} and u_n (rad). When the flow direction was located at the axis of V_y or V_x, the radians were defined as 0 or $\pi / 2$.

Data analysis

Changes in the fecal density from sea urchin were tested by a 1-way analysis of variance (ANOVA) using the type of refuge habitat as fixed factors. In cases where significant variations were detected by ANOVA, *post hoc* comparisons were carried out using Tukey's HSD tests. Relationship between the feeding rate of sea urchin and water flow in the presence or absence of the refuge habitat was tested by the regression analysis. All statistical analyses were carried out with the SPSS version 23 statistical computer software (IBM Japan Co. Ltd., Tokyo, Japan).

RESULTS AND DISCUSSION

Trail observation of sea urchin using fecal density

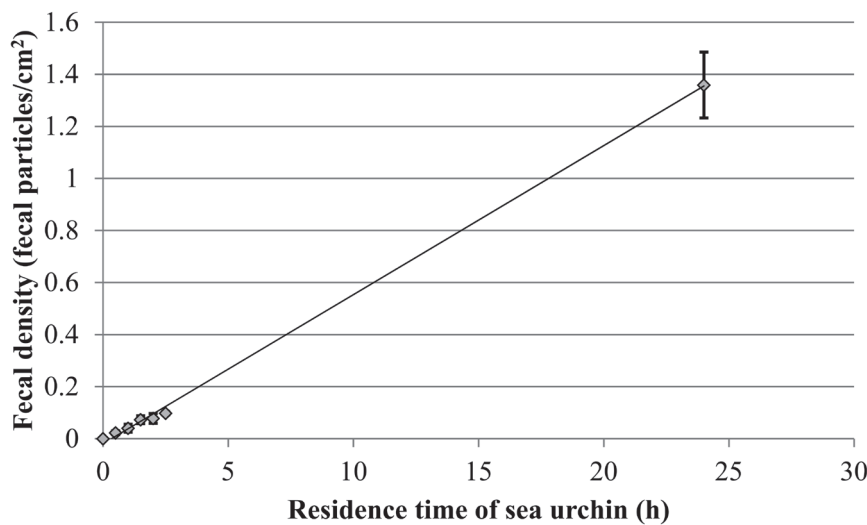**Figure 3** shows the relationship between the residence

Fig. 3 Relationship between the residence time of the sea urchin, *Strongylocentrotus nudus*, and the fecal density under the undisturbed water condition. Error bars indicate ± 1 standard deviation.

time of the sea urchin and their fecal density under the calm water condition. Several fecal particles from sea urchin were found at 0.5 hour later after installing. Trail observation revealed that fecal density was positively related to the residence times in the place of the sea urchin ($r = 0.999$, $p < 0.01$). According to this examination, the fecal density from the sea urchin was determined as a useful criterion for understanding the residence time of sea urchins under the calm water condition.

Selection of preferred refuge habitat

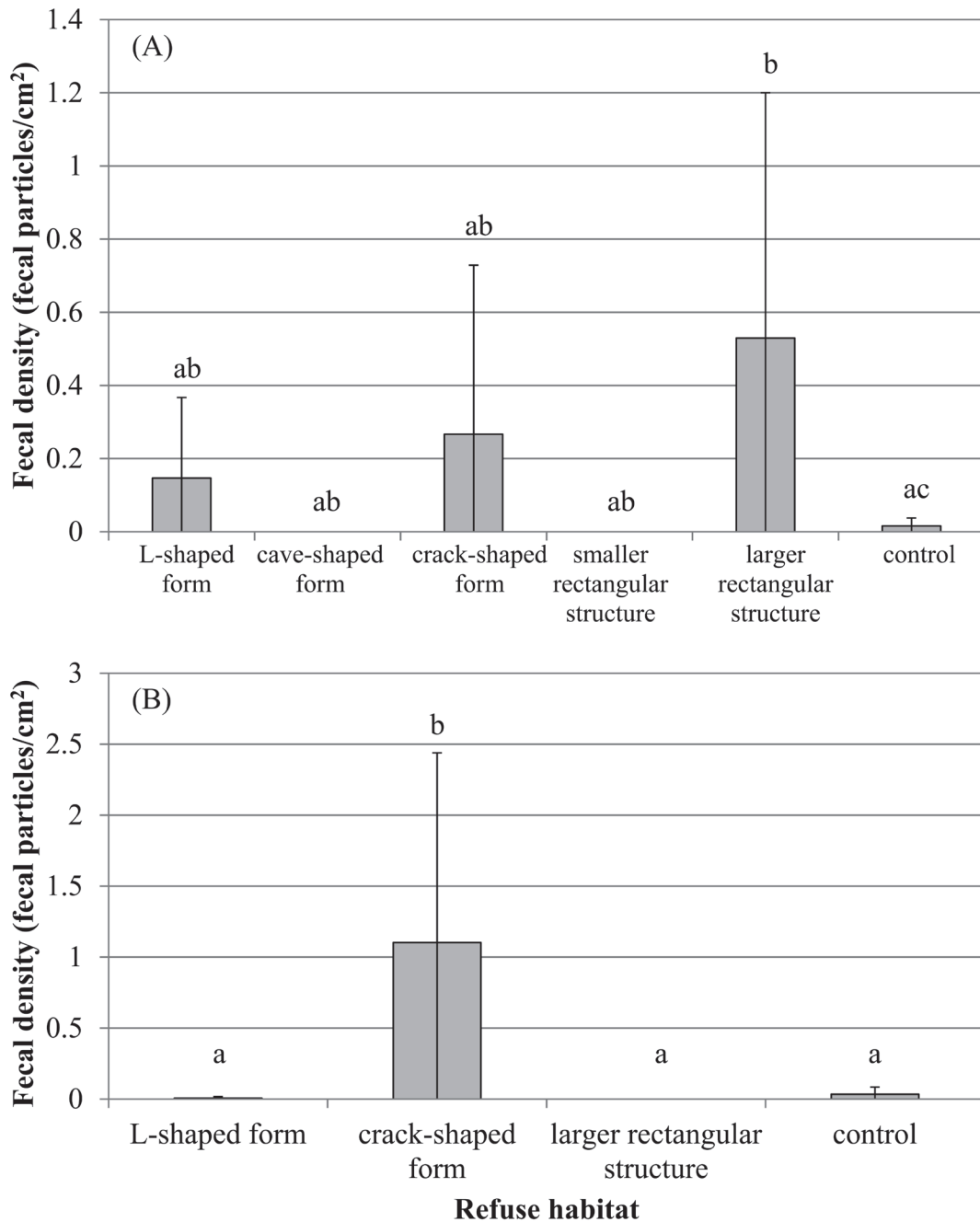**Figure 4A** shows changes in the fecal density from the

Fig. 4 Change in the fecal density from the sea urchin in each type of refuge habitat. (A) First trial with five types of refuge habitat, (B) Second trial using the upper three refuge habitats in which high density of the feces was confirmed at the first trial. Error bars indicate ± 1 standard deviation. Differing letters denote significant differences by *post hoc* comparison ($p < 0.05$).

sea urchins in each type of refuge habitat at no flow condition. Although *post hoc* comparison indicated that the fecal density at the larger rectangular structure was higher than those at the control, there were no significant differences among the other five types of refuge habitat (Tukey's HSD test, $p > 0.05$). The averaged fecal densities at the L-shaped, crack-shaped and larger rectangular structure were higher than those at the cave-shaped and smaller rectangular structure habitat. These results indicate that sea urchins remained resident for a long time in these kinds of habitat. The change in the fecal densities in the second trial with the selected upper three refuge habitats (L-shaped, crack-shaped and larger rectangular structure) is shown in **Fig. 4B**. In the second trial, *post hoc* comparison revealed that large amounts of feces at the crack-shaped form were found when compared to those at the other habitat (Tukey's HSD test, $p < 0.05$). Trail observation also confirmed that sea urchins frequently hid in the crack-shaped form in the tank. Imai & Kodama [18] investigated the behavior of the sea urchin, *Anthocidaris crassispina*, in the field and indicated sea urchins seemed to choose cliff-shaded and small areas like a crevice or groove on the rocky shore as its microhabitat. As a result, there was a possibility that the sea urchin, *S. nudus*, also selected the crack-shaped form which was structured by dark and narrow. Although the investigation of preferred refuge habitat for sea

urchin was carried out at calm water, this result might seem to give reasonable estimation under water flow condition, because *S. nudus* which hides at cracks are often observed in the field. Therefore, it was thought that the crack-shaped form was preferred and used for the sea urchin as the refuge habitat in this study. Unfortunately, we could not find any clear reasons for the reduction of fecal density in the larger rectangular structure in the second trial even though a large amount of feces was found when the five types of structure were deployed.

Effect of water flow on grazing by the sea urchin in the presence of refuge habitat

Figure 5 shows the change in feeding rates of sea urchins against water flow in the presence or absence of the crack-shaped form refuge habitat. Regression analysis revealed that water flow was inversely related to the feeding behavior of the sea urchin with and without the refuge habitat ($p < 0.05$). Decrease in the feeding rate at higher velocities indicates that the feeding or the movement for grazing by sea urchin was restricted by water disturbance. Feeding rates were also influenced by the refuge habitat. Feeding rates of the sea urchin with the refuge habitat were lower than those in the absence of the habitat, although water disturbances were almost the same. Feeding rates of the sea urchin were also
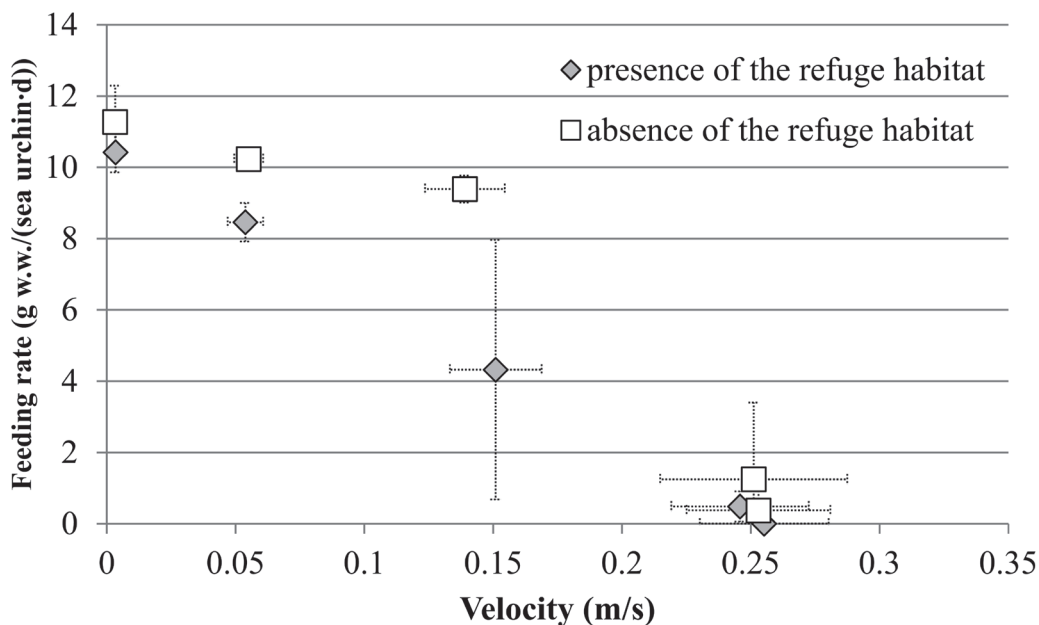

Fig. 5 Effect of water flow on grazing by the sea urchin in the presence or absence of the refuge habitat. The crack-shaped form was deployed as the refuge habitat. Error bars indicate ± 1 standard deviation.
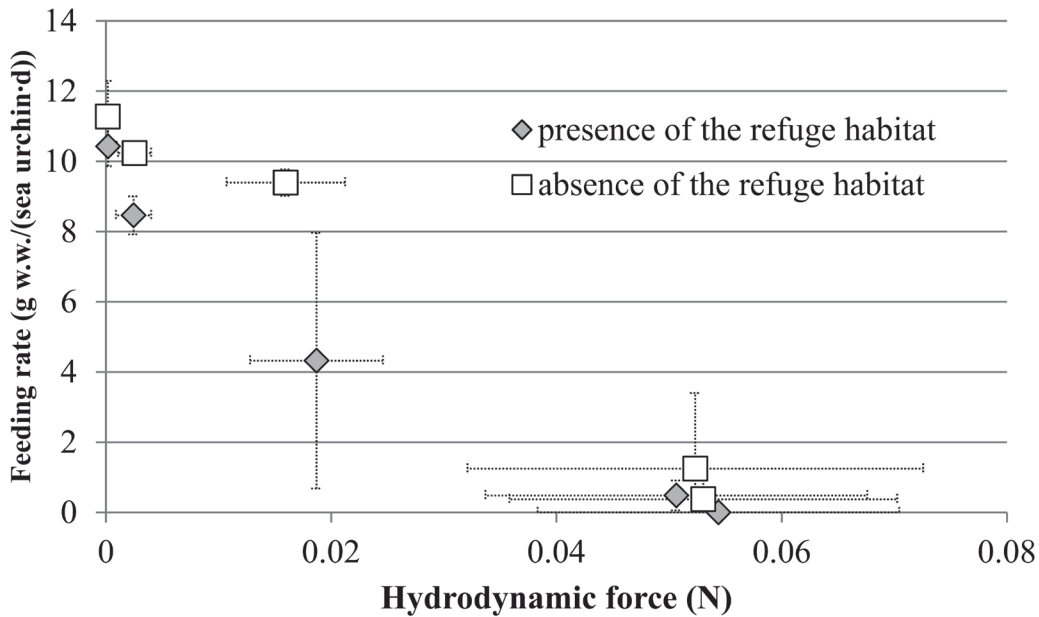
Fig. 6 Effect of the hydrodynamic force on grazing by the sea urchin in the presence or absence of the refuge habitat. The crack-shaped form was deployed as the refuge habitat. Error bars indicate ± 1 standard deviation.

inversely related to the hydrodynamic force and the presence of the refuge habitat (**Fig. 6**). The drag coefficient is actually a function of the Reynolds number. Although there was the possibility of increasing the drag coefficient when the Reynolds number was low under the calm water condition, it was thought that increasing this coefficient had almost no influence to the hydrodynamic force calculation, because the drag force in itself was small at the lower water velocities. The data sets of the effect of the water flow on grazing by the sea urchin with the refuge habitat allows us to conclude that the feeding behavior of sea urchins was changed by the presence of the refuge habitat; i.e. sea urchins ceased grazing because of reluctance to the water flow and remained in the refuge habitat even though water disturbances were not sufficiently high to inhibit their grazing. This result can also be supported by the observation that sea urchins hide in the refuge habitat more frequently, when water velocities were higher than 0.1 m/s. Because the relationship between the feeding rate of the sea urchin in the presence of the refuge habitat and water flow is a linear function, the equation is expressed as follows:

Feeding rate of sea urchin with the refuge habitat (g w.w./(sea urchin·d)) = $-41.4\ u_n + 10.6$ ($r = -0.9999$, $p < 0.01$)　　(6)

The water flow in the presence of refuge habitat that is required to reduce the feeding rates of the sea urchin by half was estimated to be about 0.13 m/s. This velocity that decreased the feeding rate by half was almost equivalent with the water flow in which sea urchin, *S. nudus*, did not approach algal communities and macroalgal habitats have remained in the field [4].

CONCLUSIONS

In the present study, we examined that the influences of water flow on the grazing behavior of sea urchins changed in the presence of refuge habitat or not using a water flow tank. Fecal density excreted by sea urchins was positively related to their residence time in the place under the undisturbed water condition. This result indicates that the fecal density is a useful criterion for understanding the residence time of sea urchins in the calm water condition. Five types of structure (L-shaped, cave shaped, crack-shaped, smaller and larger rectangular structure) were deployed in the undisturbed water tank with sea urchins and then their respective fecal densities were measured for the examination of the habitat forms which sea urchins prefer. Because a large amount of feces from sea urchins at the crack-shaped form were found, feeding experiments with flow control were conducted in the presence or absence of the refuge habitat that was structured by the crack-shaped form. Feeding rate of sea urchins inversely corresponded to the water flow and were

influenced by the refuge habitat. The feeding rates with the refuge habitat were lower than those in the absence of the habitat, although water disturbances were almost the same. The data sets of the effect of the water flow on grazing by the sea urchin in the presence of the refuge habitat allows us to conclude that sea urchins seemed to cease feeding because of reluctance to water flow and they remain in refuge habitat even though water disturbances were not sufficiently high to limit their grazing.

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