

RESEARCH ARTICLE

Experimental evaluation of the anti-attachment effect of microalgal mats on grazing activity of the sea urchin *Strongylocentrotus nudus* in oscillating flows

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SUMMARY

Algal mats can hinder the adhesion of the tube feet of sea urchins. This leads to the hypothesis that the restriction of sea urchin feeding activity by wave action can potentially be enhanced by the presence of algal mats, which will facilitate the survival of kelp recruits at sites with wave action in urchin barrens. To evaluate the potential anti-attachment effect of algal mats on sea urchins, a laboratory tank experiment was performed on the movement of *Strongylocentrotus nudus* sea urchins and their grazing on juvenile kelp plants at the center of 30×30 cm flat test substrates with or without a thin-layer microalgal mat at four levels of oscillatory flow (maximum orbital velocity: 10, 20, 30 and 40 cm s⁻¹). The grazing loss of kelp slightly increased with increasing velocity up to 30 cm s⁻¹ in the absence of microalgal mats, while in contrast the loss substantially decreased at 30 cm s⁻¹ in their presence. Sea urchins were dislodged more frequently at 20 cm s⁻¹ or higher velocities in the presence of microalgal mats. Mats were frequently abraded by scraping by the adoral spines during urchin movement at high velocities (30 and 40 cm s⁻¹) but were subject to no or only slight urchin grazing in most cases. The results indicate that the overall decrease in grazing loss of kelp within the microalgal mats was attributable to the anti-attachment effect on urchins during incursions rather than due to urchins grazing on the mats.

Supplementary material available online at <http://jeb.biologists.org/cgi/content/full/215/9/1464/DC1>

Key words: algal mat, anti-attachment effect, movement, sea urchin.

INTRODUCTION

Movement of benthic marine animals is affected by water motion and the properties of the substratum onto which they attach (Kawamata, 1998; Laur et al., 1986; Martinez, 1996; Martinez, 2001). This may in turn influence not only their spatial distributions but also, as a consequence, their interactions with other organisms (Bruno and Bertness, 2001). In most shallow rocky habitats, water motion is primarily caused by waves. Wave-induced oscillatory water motion imposes hydrodynamic forces including drag, lift and acceleration forces on attached organisms (Denny et al., 1985). The adhesive strength required to withstand moving water may be affected by the substratum properties. Substrata vary considerably in surface morphology and texture. The rock surface may be smooth and flat with few holes and depressions or it may be ridged or deeply pitted. The physical properties of the surface may also be modified by attached organisms. Dense cover by sessile invertebrates including sponges, sea anemones and ascidians as well as algal mats may make the substratum difficult for motile animals to crawl over. In addition, movement in animals is likely to be susceptible to water motion at a level much lower than the critical level at which the animals become dislodged, because adhesive force decreases substantially during movement (Branch and Marsh, 1978; Denny, 1984; Miller, 1974). Consequently, animals may avoid areas of specific surface properties unless the water motion is slower than some critical level. However, no studies have examined such effects of substratum properties on the movement of benthic animals in moving water.

In many rocky subtidal areas, sea urchins are dominant herbivores and their mobility, which allows them to graze, is of great importance for determining the algal community structure. Large aggregates of sea urchins can denude the rock surface of all erect macroalgae, forming 'barrens', areas covered with little more than crustose coralline algae (Lawrence, 1975). Sea urchins can subsist and reproduce in food-limited barrens and so urchin barrens tend to be extensive and persistent. However, kelp beds can be established at wave-exposed shallow depths where the foraging activity of urchins is frequently inhibited by wave action (Kawamata 1998; Mann, 1982). Shallow kelp beds exclude and repulse sea urchins by the wave-driven sweeping motion of the fronds (Gagnon et al., 2004; Konar, 2000; Konar and Estes, 2003). However, wave conditions vary greatly over time. During calm conditions sea urchins may aggregate at the lower edge of the kelp beds to graze, resulting in the beds receding (Himmelman et al., 1983; Lauzon-Guay and Scheibling, 2007). In addition, kelp is more vulnerable to grazers when it is small than when it is large (Lubchenco and Gaines, 1981), making it difficult to recruit it to urchin barrens. Thus, whether kelp recolonizes deeper urchin barrens is a key issue for the persistence of kelp beds, but the mechanism allowing recolonization is poorly understood.

On the Sea of Japan coast of southwestern Hokkaido and the Pacific coast of northeastern Honshu, Japan, the sea urchin *Strongylocentrotus nudus* (A. Agassiz 1863) is extremely abundant and forms extensive urchin barrens (Fujita, 1998; Kawamata, 1998; Muraoka, 2008), whereas kelps, such as *Laminaria religiosa*,

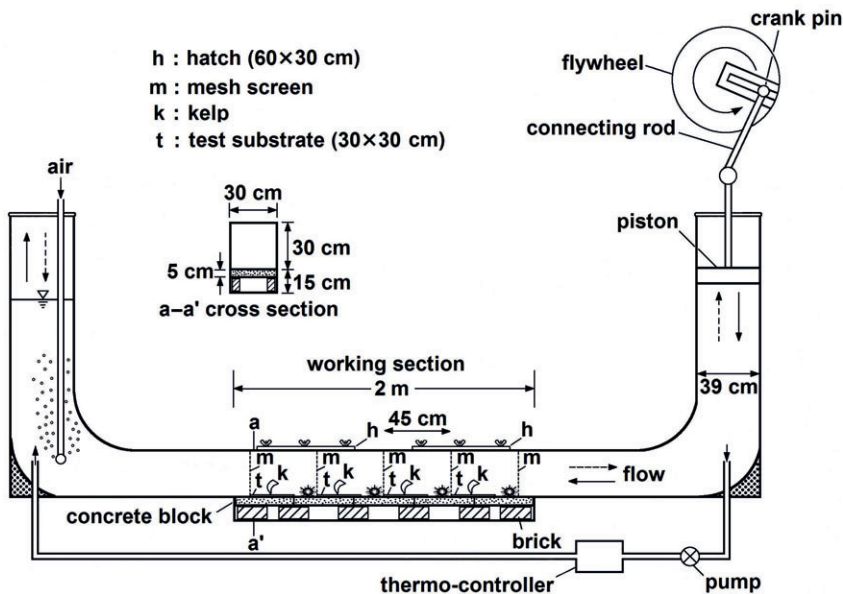


Fig. 1. Illustration of the oscillating flow tank, showing test substrates each with a juvenile kelp plant attached at the center and sea urchins placed initially in the right-hand part in four test sections (45 cm long) separated by mesh screens.

Undaria pinnatifida and *Eisenia bicyclis*, are common at wave-exposed shallow subtidal reefs. Shallow kelp beds recede or disappear from late summer to early winter as most annual macroalgae seasonally disappear. At the same time, *S. nudus* grazing fronts advance, turning the area barren. In winter, however, the urchin grazing fronts recede from the area as it begins to be covered by thin layers of algal mats, in which kelp occurs later (Kawamata, 2001; Kawamata, 2010). A laboratory experiment with a velocity gradient in an oscillating flow tank showed that at low temperatures (<13°C), the foraging area of *S. nudus* was limited to a maximum wave orbital velocity of $\sim 40 \text{ cm s}^{-1}$ (Kawamata, 2001), which is comparable to the upper velocity limit for their feeding on foliose macroalgae (Kawamata, 1998). However, the wave orbital velocity at the lower edges of algal mats is frequently lower than the velocity limit, suggesting that the presence of algal mats may reduce the velocity limit for urchin movement (Kawamata, 2001). For example, in Oshoro Bay, on the Sea of Japan coast of Hokkaido, where urchin grazing fronts reached the uppermost level of the subtidal zone in October–November and then receded from newly established algal mats until February, the overall average of the ‘significant wave orbital velocity’ (defined as the average of the top third highest amplitudes of orbital velocities in analogy to significant wave height) at the fronts was estimated to be as low as 14 cm s^{-1} (Kawamata, 2001).

The locomotion of sea urchins in water motion relies mainly on their adhesion to the substratum by their tube feet. A tube foot has a small, distal sucker that has viscoelastic properties and adapts its surface to the substratum roughness (Santos et al., 2005). Sea urchins can probably move over any rigid surface at relatively high velocities by means of their highly flexible tube feet. For example, *S. nudus* of 5 cm test diameter can climb a thin upright rod of 3 mm diameter in oscillatory flows even at an orbital velocity of 40 cm s^{-1} (Kawamata, 2008). These observations lead to the hypothesis that flexible and fine structures of algal mats may inhibit the firm attachment of the tube feet, thus deterring sea urchins from moving on them. If this hypothesis is true, the above mechanism may be particularly important for maintaining structures of near-shore algal communities because the substratum can be covered by small, fast-growing algae such as diatoms and filamentous algae (Breen and Mann, 1976; Duggins, 1981; Himmelman et al., 1983; Kawamata,

2001; Scheibling et al., 1999; Witman, 1987). However, little attention has been paid to such effects of algal mats on sea urchin foraging activity, although Hay described the combined effects of algal mats and wave action on sea urchin behaviors (Hay, 1981).

In the present study, a laboratory experiment was performed to examine how the presence of even a thin algal mat affects the invasive movement and grazing of *S. nudus* in wave-induced oscillatory flows. The experiment simulated juvenile kelp that colonize substrata covered by algal mats adjacent to *S. nudus*-dominated barren areas. Algal mats (or often referred to as algal turfs) are typically dominated by densely packed filamentous and finely branched algae but may vary in morphology and species composition (Miller et al., 2009). In this study, I used extremely thin mats of microalgae (mostly diatoms), which apparently had the least effect on urchin movement, as a typical example of ubiquitous mats of early successional algae.

MATERIALS AND METHODS

This study was conducted at a laboratory of the National Research Institute of Fisheries Engineering, Ibaraki, Japan. An oscillating flow tank experiment was performed with 64 *S. nudus* (mean \pm s.d. test diameter, 53.2 ± 9.0 mm; range, 34–70 mm) collected haphazardly on 4 November 2009 from 2–5 m depth at Kashima, Ibaraki, on the northeastern Pacific coast of Honshu, Japan ($35^{\circ}55'24''\text{N}$, $140^{\circ}42'6''\text{E}$). The sea urchins were kept in a large recirculating flow tank (for details, see Kawamata and Hasegawa, 2006) without food (but remained vigorous) under a moderate oscillatory flow (maximum orbital velocity, 20 cm s^{-1}), to adapt them to oscillatory flows, for at least 7 days prior to experiments. Water temperature was maintained at 15°C throughout the experiments.

Experimental apparatus

The oscillating flow tank used in this study was a U-shaped pipe in which regular oscillatory flows in the 2 m long working section were produced by driving the piston up and down in the right-hand arm of the U-pipe (Fig. 1). The maximum orbital velocity in the working section was adjusted by the amplitude and cyclic period of the piston (Kawamata, 1998). The working section had Plexiglas® windows in the front and back as well as two large rectangular acrylic hatches (60×30 cm) in the ceiling, while the remaining parts of the tank were

made of stainless steel coated with polyvinyl chloride (PVC). The working section had a 30×30 cm cross-section and a 15 cm deep cavity below it. Five concrete blocks (40×30×5 cm) were embedded in the cavity to create a false bottom flush with the flume bed. The tank was equipped with a thermo-control system as well as a recirculating pump, producing a minimal (1.3 cm s^{-1}), left-to-right flow in the working section to maintain a constant temperature. White fluorescent lights were installed behind the rear windows to illuminate the entire working section. However, in order to eliminate possible biases due to darkness at both the ends, the 180 cm long central portion of the section was separated with 1 cm mesh screens to form four replicate, 45 cm long test sections. The screens were supported by attaching them to one edge of the 29.5×29.5 cm square frames made of stainless steel bars (3 cm wide×5 mm thick) set parallel to the flow. In each test section, a thin PVC bar (20 cm×2 cm×5 mm) was attached to the bottom, 30 cm away from the frame of the left-hand screen so the test substrate could be fixed between them, separating out the remaining right-hand area to allow an urchin to be placed in it. This deployment may enhance urchin foraging activity because a previous study (Kawamata, 2008) indicated that in the working section *S. nudus* tended to move towards the slow, left-to-right recirculating flow, so the urchins more readily found left-hand (i.e. upstream) food than right-hand food.

Test substrate

Test substrates consisted of a microalgae-covered or microalgae-free (control) PVC settlement plate (30 cm×30 cm×2 mm) with a 'loop' of Scotch® Magic™ Tape attached to the center of the upper surface, allowing quick and firm attachment of a small PVC chip (4 cm×3 cm×2 mm) with a palatable juvenile kelp (*E. bicyclis*) and a 'hook' of the tape attached on the upper and lower surfaces, respectively. Juvenile *E. bicyclis* sporophytes (<7 cm), consisting of a single flat blade, stipe and holdfast, were collected intact from the rocky shore of Choshi, Chiba (35°43.1'N, 140°52.4'E) in early August 2009. The plants were glued at the holdfast to the PVC chip with an instant adhesive gel (GEL-10, Toagosei Co., Ltd, Tokyo, Japan) so that they stood upright, and they were kept fresh in a 100 l recirculating aquarium at 15°C under 10h:14h light:dark cycles with fluorescent lights until experimental use. The upper surface of the settlement plate was roughened with coarse abrasive scrub cloth to enhance the attachment of microalgae. The microalgal-mat coverage was achieved by placing the settlement plates, together with cobbles collected from the rocky shore of Choshi for seeding, in a unidirectional flow of sand-filtered seawater in another large recirculating flow tank, at 15°C under irradiance provided by 150 W metal halide bulbs, until the top surface of the plates was covered entirely by thin, dark brown microalgal mats. Prior to use in experiments, filamentous microalgae, if elongated (>~5 mm), were removed with tweezers so that the mats were of even thickness (see Fig. 2). Almost all mats were composed of diatoms, and pooled samples removed from microalgal mats (100 cm² in total) with a scraper showed that single celled diatoms *Amphora* sp. and Pennales were dominant, accounting for 55% and 30% of the cell number, while the chain-forming diatom *Melosira* sp. accounted for 11%. The mean ± s.d. biomass of the mats was $57.2 \pm 34.4 \text{ mg dry mass } 25 \text{ cm}^{-2}$ ($N=9$).

Experimental procedures

The experiment was carried out between 22 November and 9 December 2009. Essentially, I used a between-subjects factorial design with two factors: microalgal treatment (microalgal mat-covered vs control substrate) and maximum orbital velocity (10, 20,

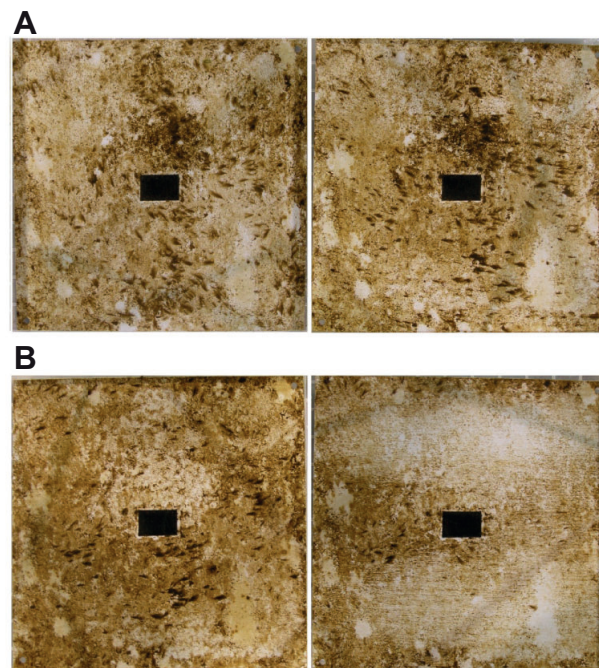


Fig. 2. Typical examples of changes in vegetation of microalgal mats on 30×30 cm settlement plates before (left) and after (right) the experimental use of low (A, 10 cm s^{-1}) and high (B, 30 cm s^{-1}) velocity treatments. All settlement plates are shown in the same orientation as in the oscillating flow tank. Note, the abraded-like reduction in microalgal mats shown in the lower right-hand photograph resulted from repeated movement (especially along the Plexiglas® walls) by the sea urchin.

30 and 40 cm s^{-1}). These velocities ranged from a low velocity, which was predicted to have little inhibitory effect on urchin feeding, to a critical one at which their feeding on kelp would begin to be completely inhibited (Kawamata, 1998). Each of eight treatment combinations was repeated eight times with 16 experimental runs, each of which had two microalgal mat-covered and two control substrates simultaneously assigned to the four test sections of the same oscillating flow tank in the following manner. (1) To minimize the possibility of bias over time, a series of experimental runs with the four levels of orbital velocity was repeated four times in a random order. (2) To counterbalance the possible effect of animal size, 64 test urchins were divided into four size groups, and eight individual urchins (two from each group) were assigned to each of the eight treatment combinations. (3) To minimize the possible effect of the location of test sections, the two levels of microalgal treatment and four size groups were equally assigned to each of the four test sections.

Each experimental run was initiated as follows: four sets of juvenile *E. bicyclis* sporophytes attached to the PVC chips were randomly selected and trimmed 10 cm from the proximal end to ensure sea urchins in the test sections were unable to reach the sporophytes unless they moved onto the areas of the test substrate away from the surrounding objects (i.e. Plexiglas® walls, the frame of the left-hand mesh screen and the right-hand PVC bar). The kelp plants were blotted dry with tissue paper and weighed (mean ± s.d., $2.36 \pm 0.76 \text{ g wet mass}$), and were pressed between thick acrylic boards with mesh lines and then photographed to measure the planform area of the blade (mean ± s.d., $27.03 \pm 5.06 \text{ cm}^2$) on a computer. The working section of the oscillating flow tank was filled with fresh seawater in advance.

In each test section, the test substrate was installed in the left-hand part as described above and then an urchin was placed in the right-hand part. The urchins were stimulated by rubbing them with steel rods until they firmly attached to the substratum; the upper hatches were immediately bolted and fresh seawater was added within 10 min to commence production of a given orbital velocity of oscillating flow. The cyclic period was a constant 5 s as in previous experiments (Kawamata, 1998; Kawamata, 2008). Each experimental run lasted ~24 h under illumination in 12 h:12 h light:dark cycles with white fluorescent lights. This duration was minimally required for examining less frequent urchin incursions into the test substrate at high orbital velocities and for eliminating possible biases due to the diurnal rhythm in urchins. The test sections were cleaned using tap water every 7 days to eliminate the effects of biofouling organisms. The behavior of sea urchins in the test sections was recorded at 1 min intervals during the experiment with three digital video cameras (DCR-HC90 NTSC, Sony, Tokyo, Japan) using the time-lapse photography function and automatic flashlight. After each experimental run, the juvenile sporophytes together with the substratum chips were retrieved, and the blotted dry mass and blade area were measured again to determine the grazing loss. Microalgal mats on the test substrates were also photographed before and after experimental runs to determine the area of mats grazed by sea urchins. The mats were reduced or thinned not only by urchin feeding but also by abrasion by the crawling urchins (for details see Discussion). Thus, grazed areas were carefully (but roughly) estimated by checking urchin behaviors with the aid of the video images described below (sea urchins became sluggish and moved in a zigzag pattern to graze, while they abraded mats by moving relatively fast and straight).

Behavior analysis

A sequence of time-lapse images was combined into an Audio Video Interleave file for the video analyses of sea urchin behaviors. Sea urchins tended to move on the edge of the bottom against the Plexiglas® walls or mesh screens, or climbed up the Plexiglas® walls or on the ceiling. Therefore, they mostly invaded the area of test substrates along the edges from the right-hand bottom or from the surface of the Plexiglas® walls and then sporadically departed from the walls into the inner part of the area. In this study, a move from the surrounding objects entirely or partly into the area of the test substrates was referred to as an 'incursion'. Sea urchins that invaded the area often moved along the Plexiglas® walls without grazing or stopping. The locomotion rates during such movements were determined by averaging two to four measurements of displacement during 1–10 min for all available (59 out of 64) individual urchins. Sea urchins were occasionally dislodged by oscillatory flows while moving on test substrates or feeding on kelp. The classification of the dislodged sea urchins was an unusually long displacement (>15 cm) in 1 min intervals with the body tilting or in upside-down posture, observed with the time-lapse images. The number of incursions and dislodgements was counted based on the video sequences. The mat-grazing time (defined as the ratio of the total time spent grazing on mats to the experimental duration) was recorded to examine how microalgal grazing potentially in turn affected grazing on kelp.

Statistical analyses

The effect of treatment combinations of microalgae and velocity on urchin movement and feeding was tested in terms of the five response variables: (1) the number of incursions, (2) the 'climbing time', defined as the ratio of the total length of time spent climbing

the Plexiglas® walls or ceiling to the experimental duration, (3) the locomotion rate, (4) the number of dislodgements, and (5) the 'grazing loss', defined as the ratio of the lost blade area to the initial one. The grazing loss was used here as a better indicator of the grazing impact than the weight-based feeding amount or relative feeding rate (the ratio of the feeding amount to urchin mass), because sea urchins occasionally grazed on the base of the frond, causing the blade to drift away and because urchin feeding for thin blades is more closely related to the area of the blade than to the mass (S.K., unpublished). From preliminary analyses of the response variable using 2-way and 3-way analyses of variance (ANOVA) and general linear model (GLM), it was found that inclusion of urchin size as a continuous factor resulted in the most powerful analysis. Thus, data for all response variables were analyzed using the GLM that included independent variables for microalgal treatment (2-level categorical variable: the presence or absence of microalgal mats), velocity treatment (4-level categorical variable: 10, 20, 30 and 40 cm s⁻¹) and urchin size (continuous variable) and all interactions. The assumptions of homogeneity of variance and normality were checked by residual analysis.

To allow for further interpretation, the effect of microalgal mats on the number of incursions was tested using 2-way analysis of covariance (ANCOVA) with velocity (continuous variable) as a covariate. The homogeneity of regression slopes was checked by the interaction term of the ANCOVA ($P=0.69$). In addition, Games–Howell multiple comparisons were performed to test for differences in the response variables between microalgal treatments for each velocity level. The correlation between the response variable and size for each treatment combination was tested using the Spearman rank correlation test.

For data on microalgal grazing, GLM analysis was performed to test for the effects of velocity and urchin size on grazed mat area, whereas mat-grazing time was analyzed only in terms of its correlation to urchin size for separate velocities using the Spearman rank correlation test because a normal error structure in the GLM could not be assumed even with $\log(x+1)$ -transformation. For all tests the significance level was held at 0.05, although marginal values ($0.05 < P < 0.1$) were also discussed following Stoehr (Stoehr, 1999). Statistical analyses were performed in R (R Development Core Team, 2009), with the aid of the add-on package 'MASS' (Venables and Ripley, 2002).

RESULTS

Sea urchins actively moved over all surfaces including the Plexiglas® walls and ceiling at the lowest velocity treatment (10 cm s⁻¹), as indicated by the highest number of incursions and greatest climbing time (Fig. 3A,B). However, movement decreased with increasing orbital velocity, resulting in a significant decrease in the number of incursions with increasing velocity (ANCOVA, $P < 0.001$). The overall climbing time also significantly decreased with increasing velocity (Table 1, $P < 0.001$), with one exceptional increase in the presence of microalgal mats at 40 cm s⁻¹ (Fig. 3B). The number of incursions did not significantly differ between the presence and absence of microalgal mats (ANCOVA, $P=0.42$), indicating that movement of sea urchins was not much affected by the presence of the mats (Fig. 3A). However, the climbing time was shorter (and thus sea urchins spent more time on the bottom) in the presence of microalgal mats than in their absence, up to an orbital velocity of 20 cm s⁻¹. This was indicated by the significant interaction effect between microalgal and velocity treatments (Table 1, $P < 0.05$). In the presence of microalgal mats at 40 cm s⁻¹, sea urchins were observed trying to invade the mats but then they frequently moved

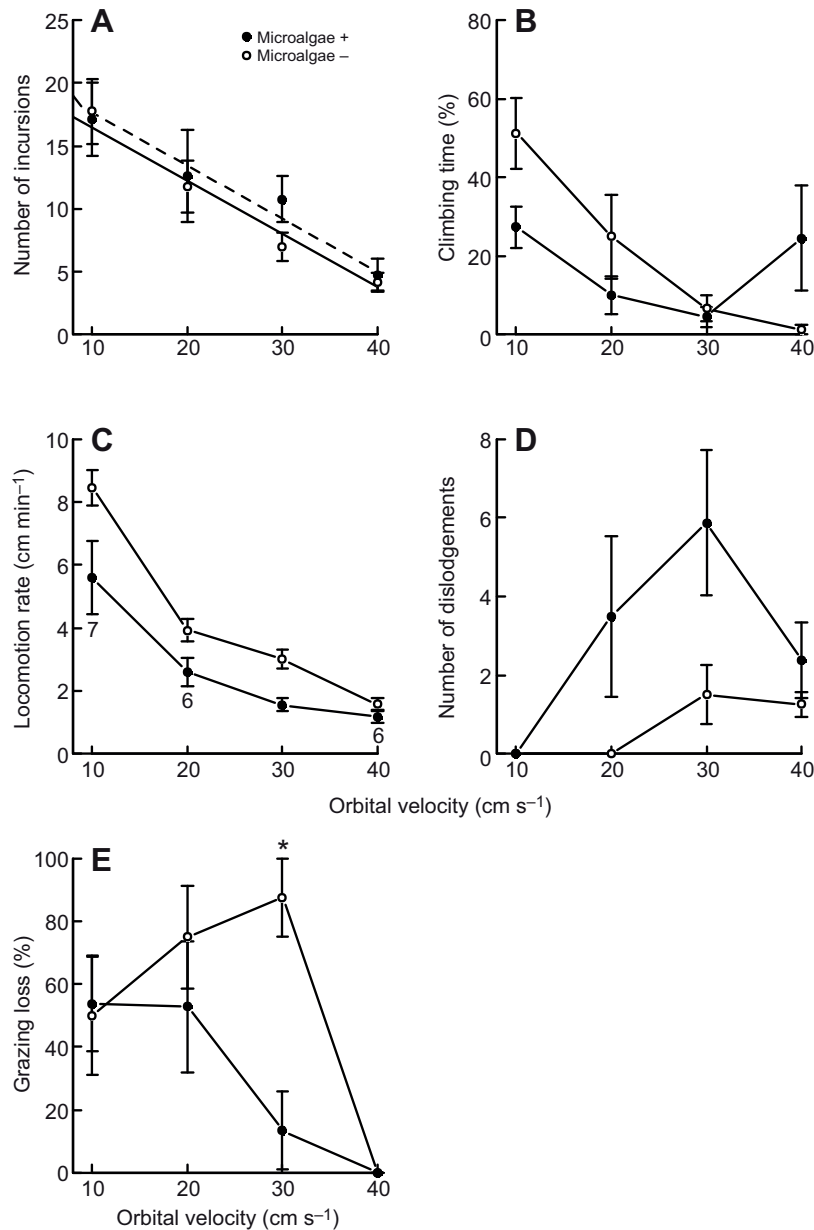


Fig. 3. Variation (means \pm 1 s.e.m.) in the number of incursions (A), climbing time (B), locomotion rate (C), number of dislodgements (D) and grazing loss (E) with velocity and microalgal treatments. ANCOVA for the effects of velocity (covariate) and microalgal treatment (factor) on the number of incursions showed a statistical significance for velocity ($P < 0.001$) but not for microalgal treatment ($P = 0.42$) and regression equations $y = 21.89 - 0.423x$ (dashed line) and $y = 20.73 - 0.423x$ (solid line) for the presence and absence of microalgal mats, respectively. An asterisk indicates that there was a significant difference between microalgal treatments for the velocity level (Games–Howell multiple comparisons, $P < 0.05$). Sample numbers other than $N = 8$ are shown below error bars.

to the surface of the Plexiglas® walls, resulting in the increase in climbing time. The number of incursions increased in a marginally significant way with urchin size (Spearman rank correlation test, $P < 0.1$) for the separate treatment combinations except at 30 and 20 cm s^{-1} without microalgal mats. This resulted in a significant effect of velocity and an interaction effect between velocity and size on the number of incursions (Table 1, $P < 0.05$).

The rate of locomotion over the test substrates was reduced by the presence of microalgal mats as well as with the increasing velocity (Fig. 3C), as indicated by significant effects of microalgal and velocity treatments on the locomotion rate (Table 1, $P < 0.05$). No significant or marginally significant correlations between locomotion rate and urchin size were detected for separate treatment combinations (Spearman rank correlation test, $P > 0.1$). Note that locomotion over microalgal mats was not observed in five trials, so the values of locomotion rate were conditional on invasion occurring.

Sea urchins began to be dislodged on microalgal mats even at an orbital velocity of 20 cm s^{-1} , while on the control substrate urchins were dislodged less frequently at higher velocities (Fig. 3D).

Dislodgement in the presence of microalgal mats at 30 cm s^{-1} occurred more frequently (see supplementary material Movie 1) than that in the absence of mats and then decreased at 40 cm s^{-1} , as indicated by the highly significant differences between microalgal treatments and among velocity treatments from the GLM analysis (Table 1, $P < 0.005$). Although Games–Howell multiple comparison tests failed to detect any significant differences ($P > 0.05$) between treatment combinations for the number of dislodgements, the GLM analysis showed a marginally significant interaction effect between microalgal and velocity treatments (Table 1, $P = 0.094$). In addition, the number of dislodgements showed a marginally significant interaction between velocity and urchin size ($P = 0.053$) and a significant positive correlation to urchin size in the presence of microalgal mats at 40 cm s^{-1} (Spearman rank correlation test, $r_s = 0.82$, $P < 0.05$). This, coupled with the previously mentioned marginally significant positive correlations between the number of incursions and urchin size at 40 cm s^{-1} , suggested that larger urchins made more attempts to move over the test substrates, resulting in a higher risk of dislodgement in the presence of microalgal mats.

Table 1. Results of the GLM analyses testing for the effects on five response variables of microalgal and velocity treatments, urchin size and all interactions

Source of variance	d.f.	MS	F	P
Number of incursions				
M	1	21.39	0.82	0.368
V	3	480.81	18.53	<0.001
S	1	436.52	16.82	<0.001
M × V	3	21.01	0.81	0.495
V × S	3	74.24	2.86	0.047
M × S	1	10.64	0.41	0.525
M × V × S	3	4.67	0.18	0.909
Residual	48	25.95		
Climbing time				
M	1	315.0	0.72	0.400
V	3	3382.6	7.74	<0.001
S	1	48.0	0.11	0.742
M × V	3	1668.4	3.82	0.016
V × S	3	412.0	0.94	0.336
M × S	1	14.0	0.03	0.992
M × V × S	3	96.9	0.22	0.881
Residual	48	437.2		
Locomotion rate				
M	1	32.47	23.05	<0.001
V	3	94.83	67.32	<0.001
S	1	3.27	2.32	0.135
M × V	3	4.42	3.14	0.035
V × S	3	2.76	1.96	0.134
M × S	1	4.51	3.20	0.081
M × V × S	3	6.51	4.62	0.007
Residual	43			
Number of dislodgements				
M	1	81.00	11.06	0.002
V	3	36.29	4.96	0.004
S	1	1.79	0.25	0.623
M × V	3	16.49	2.25	0.094
V × S	3	20.12	2.78	0.053
M × S	1	1.55	0.21	0.648
M × V × S	3	10.44	1.43	0.247
Residual	48	7.32		
Grazing loss				
M	1	8606.7	6.19	0.016
V	3	12844.3	9.23	<0.001
S	1	28.0	0.02	0.888
M × V	3	5190.7	3.73	0.017
V × S	3	810.5	0.58	0.629
M × S	1	8476.6	6.09	0.017
M × V × S	3	1308.9	0.94	0.428
Residual	48	1391.4		

Response variables: number of incursions, climbing time (%), locomotion rate (cm min^{-1}), number of dislodgements and grazing loss (%).

M, microalgae; V, velocity; S, size.

P-values in bold are significant at <0.05 .

The grazing loss showed discrepancies between microalgal treatments in the moderate velocity range (20 and 30 cm s^{-1}) but not at the two extreme velocities (Fig. 3E). In the absence of microalgal mats, the grazing loss increased with increasing velocity up to 30 cm s^{-1} , while in the presence of mats, the loss decreased with increasing velocity, resulting in the significant difference between microalgal treatments at 30 cm s^{-1} (Games–Howell multiple comparison, $P < 0.05$). At 30 cm s^{-1} , 7 of 8 kelp plants were killed (grazed at the base of the blade and thus unable to grow any more) by sea urchin grazing in the absence of microalgal mats, while in the presence of mats, 7 of 8 plants survived with no or only slight grazing loss. When the velocity increased at 40 cm s^{-1} , which has

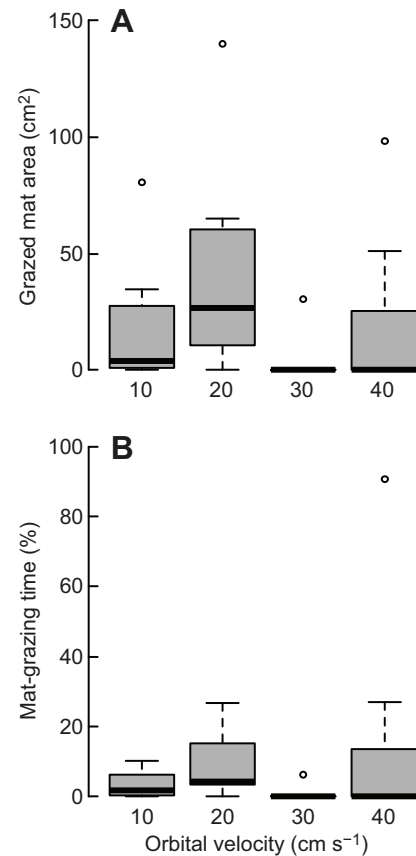


Fig. 4. Variation in (A) grazed areas of microalgal mats and (B) mat-grazing time. The horizontal thick line indicates the median value. Box shows 25th to 75th percentiles. Single point represents outliers. Note that even the largest grazed area (140 cm^2) at 20 cm s^{-1} is equivalent to only 16% of the initial mat area ($\sim 900 \text{ cm}^2$) or $0.32 \text{ g dry mass day}^{-1}$ in feeding rate estimated based on the mean of initial densities of microalgae ($57.2 \text{ mg dry mass } 25 \text{ cm}^{-2}$).

been considered as the upper velocity limit for *S. nudus* to feed on foliose macroalgae (Kawamata, 1998), sea urchins were almost completely prevented from grazing on juvenile kelp with or without microalgal treatments. The above relationships resulted in significant microalgal and velocity treatment effects as well as significant interaction effects between them (Table 1, $P < 0.05$).

Grazing on microalgal mats by sea urchins, in most cases, was infrequent and transient, as indicated by the small grazed areas of mats and in the short mat-grazing time, with one exception at 40 cm s^{-1} , where the smallest sea urchin (test diameter, 34 mm) continued to graze on the mat during most ($\sim 90\%$) of the experiment (Fig. 4). Significant variability in the grazed mat area was detected only for velocity (Table 2, $P < 0.05$) and there were no significant correlations between mat-grazing time and urchin size for separate velocities (Spearman rank correlation test, $P > 0.05$).

DISCUSSION

The results provide quantitative evidence that algal mats, even when very thin, may have anti-attachment effects on sea urchins in moderately high oscillatory flows. The more frequent dislodgement on the microalgal mats in moderate oscillatory flows ($20\text{--}30 \text{ cm s}^{-1}$) suggests that the tube feet suckers might fail to attach effectively

Table 2. Results of the GLM analysis testing for the effects of velocity, urchin size and their interaction on log(x+1)-transformed grazed areas of microalgal mats

Source of variance	d.f.	MS	F	P
V	3	10.420	4.36	0.014
S	1	4.855	2.03	0.167
V × S	3	3.580	1.50	0.240
Residual	24	2.389		

V, velocity; S, size.
P-value in bold is significant at <0.05.

to the microalgal mats. However, this does not indicate an inability of sea urchins to attach to the mats. A preliminary experiment in a recirculating flow tank showed that *S. nudus* could withstand much faster oscillatory flows (an orbital velocity of 1.4 ms^{-1} with an oscillation period of 10s) even on microalgal mats if they were stationary (S.K., unpublished).

Tenacity in sea urchins obviously decreases to a great extent when they move, and it is likely to further decrease as the speed of locomotion increases. Thus, the locomotion rate is reduced with increasing orbital velocity to resist the increasing hydrodynamic force, as shown in Fig. 3C. Similarly, the reduced locomotion rate on microalgal mats (Fig. 3C) was attributable to the reduction in tenacity by the mats. Dislodgement sometimes occurred, suggesting that the observed movement rate might be close to the maximum rate at which the urchins were able to move. This implies that the difficulty in movement might be assessed by the decrease in the locomotion rate. In other words, the increased difficulty in movement due to the presence of algal mats might compare with the increased velocity in the absence of mats where the locomotion rate would decrease to the same level as in the presence of mats. Thus, the results in Fig. 3C suggest that, for example, the presence of algal mats at 20 and 30 cm s^{-1} might parallel the velocity increase up to about 34 and 40 cm s^{-1} , respectively, in the absence of mats.

The results of this experiment also provide direct evidence that the presence of algal mats may enhance survival of juvenile kelp at orbital velocities lower than the upper limit for urchin feeding on erect macroalgae. A previous experiment with similar-shaped (single blade) but larger kelp plants (blade length, 30–50 cm) showed that grazing by *S. nudus* substantially decreased at an orbital velocity of 30 cm s^{-1} and almost ceased at 40 cm s^{-1} (Kawamata, 1998). For the smaller kelp plants used in this study, grazing loss by *S. nudus* on control substrates slightly increased rather than decreased at 30 cm s^{-1} , although their grazing was negligible at 40 cm s^{-1} as previously described (Kawamata, 1998). These results are consistent with previous speculation that sea urchin grazing may vary with the size or morphological structure of plants at velocities lower than the upper limit, which is a constant $\sim 40\text{ cm s}^{-1}$, strictly controlled by mechanical constraints (Kawamata, 1998). Interestingly, the grazing loss slightly increased with velocity up to 30 cm s^{-1} for the control substrates. There are two possible explanations for this result. (1) At low velocities, sea urchins actively moved around over all the surfaces, but mostly made only short visits to the edge of test substrate areas, while at high velocities, the animals were less active but stayed on the bottom for longer time periods, thus increasing the chances of finding kelp. (2) The increased water flow might enhance the diffusion of water-borne stimuli from kelp plants or the flow-induced motion of the fronds, therefore stimulating sea urchins to forage or facilitating their search for plants (Mann et al., 1984). It is noteworthy that at 30 cm s^{-1} ,

despite the increased urchin feeding activity, a limited area of microalgal mats largely prevented *S. nudus* from grazing on the kelp plants. Furthermore, the lower limit of this effective velocity range may be reduced if algal mats are thicker or denser, as estimated in the field (Kawamata, 2001).

Sea urchin feeding on microalgal mats was, in most cases, infrequent and transient even at low velocities (Fig. 4) at which urchins frequently visited the mats (Fig. 3A). Thus, it can be concluded that, overall, microalgal feeding had only minor effects on kelp grazing. Persistent apparent grazing on a mat was observed only for the smallest sea urchin (34 mm in test diameter). Microalgae such as diatoms may be the major food source for small sea urchins (Kharlamenko et al., 1995). Perhaps persistent or selective grazing on microalgal mats, if it occurs, is limited to smaller urchins because the mats seem too thin for efficient food intake by adult urchins, although small amounts of microalgae were found in gut contents of sea urchins in the field (Chapman, 1981; Vadas, 1977). Furthermore, such grazing is likely to be limited to the edge of the mats in the presence of moderately high oscillatory flows, as observed in the present study.

The reduction in microalgal mats was also due to abrasion by crawling urchins (see supplementary material Movie 1). The reduction was characterized by thinned areas of microalgae with scratches running along the periphery of the test substrate, and occurred only at velocities $\geq 30\text{ cm s}^{-1}$ (Fig. 2). This could be largely interpreted as follows: at high velocities, sea urchins invaded microalgal mats but moved in close contact with the substrate (thus scratching mats with their adoral spines) to avoid being dislodged, while at low velocities urchins moved by attaching themselves loosely to the substrate without abrading the mats. Taking into account the reduction in microalgae during the experiment, the finding that the limited area of microalgae provided by the thin mats reduced sea urchin grazing on kelp is even more surprising.

The present experiment was performed with only smooth flat plates, although in the field sea urchins can acquire more resistance by bracing their spines against the rugged bottom when stationary. Nevertheless, the substratum roughness is unlikely to increase the mobility of sea urchins, especially over algal mats, because only the adhesive force of the tube feet can resist the lift force and bidirectional dynamic loading due to oscillatory flows.

The above findings may lead to an awareness of a previously unnoticed but important role of algal mats on the maintenance of kelp beds adjacent to urchin barrens. Algal recolonization of barrens proceeds from the formation of mats by early successional small algae, such as diatoms and filamentous algae, which are replaced by canopy-forming kelps later in succession (Breen and Mann, 1976; Harris et al., 1984; Himmelman et al., 1983; Scheibling, 1986; Scheibling et al., 1999; Witman, 1987). In most of the previous studies, this type of succession was attributed to the mass mortality of sea urchins. However, the early successional algae can grow rapidly enough to establish thin but dense mats in urchin-dominated barren sites in a short time period (2 or 3 weeks) without a decline in the sea urchin population (Duggins, 1981; Harrold and Reed, 1985; Kawamata, 2001). Such a situation can occur in a period when environmental conditions become favorable for high algal recruitment and growth and/or unfavorable for sea urchin feeding (e.g. high nutrients, low temperature and storm waves) (Harrold and Reed, 1985). Therefore, the consequent formation of algal mats may remove sea urchins from areas less exposed to wave action (e.g. deeper barren sites), as found previously (Kawamata, 2001), facilitating the later successional recolonization by macroalgae of overgrazed barrens. This facilitation model needs empirical testing,

because early successional small algae may inhibit not only urchin movement but also kelp recruitment (Dayton et al., 1984; Kennelly, 1987).

The anti-attachment effect of algal mats was tested here only on sea urchins, but the mechanism is common for crawling animals (including snails, limpets and starfishes) inhabiting wave-exposed rocky beds. The anti-attachment effect may lead to a common small-scale distribution pattern of herbivorous gastropods on rock surfaces at wave-exposed shallow sites. In Miyagi, northeastern Japan, for example, common gastropods, such as the abalone *Haliotis discus hannai* and the snail *Omphalius rusticus*, occur abundantly at wave-exposed shallow sites with high cover of algal mats, but their habitats are largely limited to smooth patches of crustose coralline algae (S.K., unpublished). However, to date there have been no systematic studies supporting the anti-attachment hypothesis for other animals. Further studies are needed to test the hypothesis.

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