

Food preferences and related behavior of the browsing sea urchin *Tripneustes gratilla* (Linnaeus) and its potential for use as a biological control agent

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Received: 30 June 2005 / Accepted: 4 January 2007 / Published online: 13 February 2007
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Abstract The short-spined toxopneustid sea urchin *Tripneustes gratilla* feeds on a wide variety of algal species and on sea grasses. However, the urchin does show preferences when offered a selection of macroalgal species, which it encounters in nature. Preferences among macroalgae were evident in field-collected urchins exposed to pair-wise tests where the variable was either the consumption rate of the algae or observation of which algal species the urchins chose to touch with their lantern teeth. Exposure of lab-housed urchins to one of five species of macroalgae for 5 months did not seem to alter preferences of urchins in three of the exposure groups, but those exposed to *Padina sanctaecrucis* seemed to show an enhanced preference for this species when offered a choice of the five species of macroalgae at the end of the exposure period, and those exposed to *Gracilaria salicornia* seemed to avoid the species when offered the choice of the five species. Perhaps more ecologically important than their preferences were two other observations on these urchins: first, when offered only a single species of algae, the urchins on four of five diets ate the same quantity per day. Second, when simultaneously offered the choice among the five macroalgal species, the urchins consumed more macroalgae per day than when offered only one species. These urchins move about a meter a day. They probably encounter food resources in a relatively coarse-grained fashion and have evolved to eat

what is available. Because of their limited movements, their habitat overlap with grazing fishes, their acceptance of a wide variety of macroalgae and their preference for macroalgae, these native urchins are thought to have the potential to serve as biological control agents of alien and invasive macroalgae, which have come to dominate some reef zones normally occupied by corals in Hawaii.

Introduction

Large herbivorous marine invertebrates and fishes have a very different relationship to the algal community than do terrestrial invertebrate herbivores (Knowlton 1992). Specialization and the co-evolutionary development of defenses and detoxification are less common in the marine community, although algae have an array of compounds that have been shown to deter particular herbivores (Hay et al. 1987; Bolser and Hay 1996; Cronin et al. 1997).

In the tropics, grazing urchins appear to operate as generalists (Hay 1984a; Ogden et al. 1989; Klumpp et al. 1993; Beddington and McClintock 1999), although there are also examples of urchins showing preferences or behaving as specialists (Carpenter 1981; Hay 1984b; Himmelman and Nedelec 1990; Klumpp et al. 1993; De Loma et al. 2002; Vaitilingon et al. 2003). While preferences can be demonstrated in laboratory studies of tropical sea urchin diet (Hay 1984a), there are questions about the ecological importance of these preferences, because there is also evidence that these grazers often feed indiscriminately on what is available in the field (Birkeland 1989; Maharavo et al. 1994). This breadth of diet might be related to the

Communicated by P.W. Sammarco.

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uncertainty associated with patchy recruitment and survival (Ebert 1983), in combination with the limited dispersal abilities displayed by adults.

The purpose of this study was to examine the diet and feeding ecology of the Indo-Pacific sea urchin *Tripneustes gratilla*, specifically; its rate of movement, the extent to which it has preferences among various species of macroalgae, its ability to be “trained” to eat a particular macroalgal species and the growth rates of individuals on each of a number of single-species algal diets. We chose to investigate these aspects of the behavior of *T. gratilla* because it has the potential to be used as a biological control agent, to increase herbivory on reefs, which have a high cover of alien and invasive macroalgae. At least three species of alien red algae have become established on reefs in Hawaii and now compete with corals for space: *Acanthophora spicifera*, *Gracilaria salicornia*, and *Kappaphycus* sp. A fourth species, the native macroscopic green alga *Dictyosphaeria cavernosa*, has become invasive in coral-rich habitats. The current level of herbivory by fishes and invertebrates is evidently lower than necessary to control the growth of these macroalgae. Since *T. gratilla* (Chen and Run 1988; Shokita et al. 1991) and other urchin species (Agatsuma and Momma 1988; Tegner 1989; Wolcott and Messing 2005) have been successfully cultured and out-planted (Junio-Menez et al. 1998), it appears possible to use them as biological control agents. This study was designed to investigate the movement and feeding behavior of this native species in anticipation of such an application.

Methods

Field observations of feeding

Field observations of grazing by *T. gratilla* were made on groups of individuals, which occur naturally on patch reefs of Kaneohe Bay, Oahu (Fig. 1), or on urchins which were introduced to urchin-free reefs in the Bay from nearby more wave-exposed sites in the Sampan Channel (Fig. 1). Individual urchins were slowly lifted and rolled to see which algae or substratum their lantern teeth were contacting. Frequently scrape marks on rock surfaces or damaged algal surfaces indicated urchin feeding activity.

Movement

Which algae the urchins feed on and the number of species they have to choose from is partly a function of the extent and direction of their movements. The rates

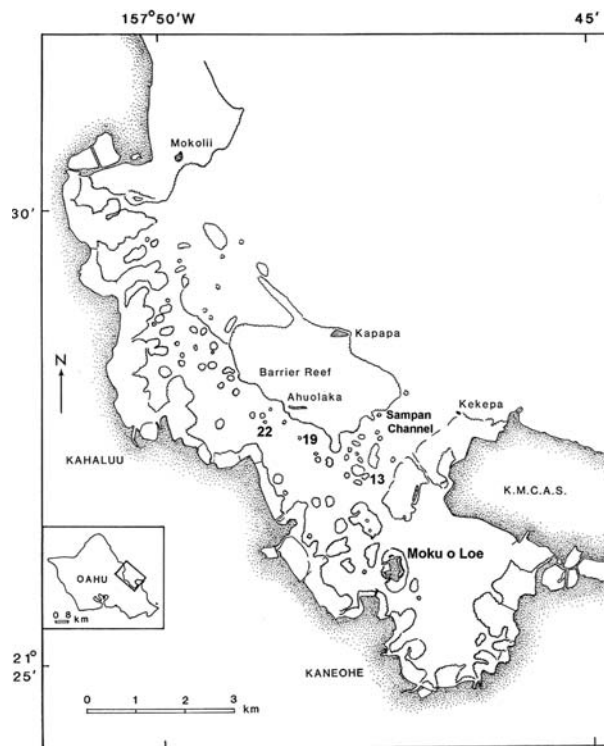


Fig. 1 Kaneohe Bay showing the location of patch reefs (13, 19, 22), the Sampan Channel and the windward (NE) fringing reef of Moku o Loe (Coconut Island)

of movement of individual urchins and groups of urchins were monitored to determine the rate at which urchins can sample their environment and resources. One method for assessing rate of movement was to measure the distance moved by relatively isolated urchins (<0.1 urchin/m²) in the 24 h after their individual starting positions were marked. These urchins were followed on a large reef (Reef 13, Fig. 1) with a substratum consisting of a mixture of coral, sand, rubble, turf algae, and macroscopic algae. The second method for assessing movement was to measure the distance and direction of movement of a group of individuals in the days following their release at a reference point on a reef flat. Direction of movement was determined by taking compass headings to the reference stake; distance was measured as the straight line distance between each urchin and the stake. These urchins were monitored in either a habitat rich in *G. salicornia* on the windward (NE) outer reef flat of Moku o Loe (Fig. 1), or on small patch reefs ~30 m in diameter (Reefs 19 and 22, Fig. 1). The flat-topped patch reefs have the form of truncated cones and have a high cover of *D. cavernosa* on the outer reef flat and upper reef slope. Urchins used in studies of movement were 7–9 cm in test diameter.

Food preferences

Food preferences were examined in outdoor flow-through laboratory tanks where food availability could be controlled. These preference experiments utilized alien and native macroalgal species, which were abundant, convenient for experimentation, and were accessible to and fed on by urchins in the field. The urchins used in preference tests were 7–8 cm in test diameter.

Three types of preference tests were performed. The first type used the wet weight of algae consumed as a measure of preference. Pre-weighed algal thalli (~30 g) (Stimson et al. 2001) were offered to each urchin in all possible pair-wise combinations and the weight of each alga consumed was measured 24 h later. Five species of macroalgae were used in these preference tests: *A. spicifera*, *D. cavernosa*, *G. salicornia*, *Kappaphycus* sp., and *Padina sanctae-crucis*. Urchins were individually housed in 20 l aquaria supplied with running sea water and aeration. Each aquarium was screened from full sunlight (25% reduction). Every urchin was offered all possible pair-wise combinations over a course of days equal to the number of possible combinations. The sequence in which combinations were offered to any one urchin was randomized. Every pair-wise offering to an urchin consisted of fresh algal material attached with rubber bands to $8 \times 8 \times 1 \text{ cm}^3$ well-seasoned cement blocks. Algae were attached to blocks with rubber bands in order to maintain the algae in a coherent mass, and prevent particular species from floating and becoming less accessible. For all preference tests fresh algae were collected approximately every other day from the same area. Algae were cleaned to remove any epiphytes or animals visible to the naked eye. Supplies of algae were kept in outdoor tanks supplied with running sea water and screened to reduce irradiance by 25%. The change in weight of control algal thalli in tanks without urchins was also measured and found to be positive, but small, about 5% of the absolute value of weight losses due to grazing.

The second type of preference test was based on instantaneous observations of contact between urchins and the thalli of test algae. This “touch test” was performed at pre-specified intervals after the introduction of the set of test algae to the urchins’ tanks. An urchin was defined as touching one of the test algae if its lantern-teeth were on or over a thallus. The test algae weighed about 30 g, were attached to $8 \times 8 \times 1 \text{ cm}^3$ cement blocks and were placed in the bottom of 20 l tanks.

The touch-tests were performed both to assess preferences among macroalgal species and to assess the preference for turfs versus macroalgae. Turfs (Stuercke

and McDermid 2004) constitute an important component of the diet of urchins at some sites (Harmelin-Vivien et al. 1992; Williams and Carpenter 1997). In this study the turfs consisted primarily of filamentous algae less than 3 mm in length, which seemed to create a matrix, which accumulated fine sediments. The “touch-test” was used when comparing the preference for turf on reef-rock to the preference for macroalgae because it is impractical to assess the weight change of turf algae resulting from feeding by the sea urchins. On five dates, independent sets of ten urchins were offered all possible pair-wise combinations of five potential foods: two turfs and three macroalgal species. The turfs, growing on rubble pieces, came from either a reef where the urchins naturally occurred (Reef 13, Fig. 1) or the windward reef flat of Moku o Loe, a site where transplanted urchins have lived and grown for over 3 years, but which did not initially have any urchins. The rubble pieces were selected to be of about the same shape and volume as the blocks, which held the macroalgae samples used in the experiment. Every urchin received all ten possible pair-wise combinations over the period of the experiment. This assessment of “touching” was made each 15 min for 75 min after placing the test algae in the urchin’s tank. If the thallus of a particular species of algae was touched with the teeth on two or more consecutive observations, the species was recorded as preferred. The touch-tests were performed by monitoring individual urchins housed in 20 l laboratory tanks

The third type of preference study was an experiment performed to test whether individual urchins could be induced to prefer or reject particular macroalgal species if they had long exposure to that species. The corollary in the field was thought to be that the limited dispersal displayed by individual urchins could keep them in association with a particular macroalga which characterized a site and that this might then “train” them to feed on that alga and to seek it out as abundance declined. Thirty urchins were used to assess the effect of protracted exposure on preferences. Each of six urchins was fed daily for 5 months with one of five species of macroalgae (August–December); at the end of the 5-month period their preferences were tested once by simultaneously offering each of the 30 urchins all five of the experimental algal species and measuring the weight of each alga consumed. The urchins were all of about the same size, ~7.5 cm in test diameter; no growth was detected for these 30 urchins over the 5-month exposure/pre-test period. The consumption rates, of these 30 urchins when simultaneously offered five test species was compared to the rates of 16 control or naïve urchins offered the same set of five algal species. These 16 urchins were collected in

August from a site where there were no macroscopic algae and all urchins were feeding on coralline algae. Their preferences were tested in August.

During the 5-month exposure period to a single algal species the urchins' consumption rates were intermittently measured by providing the urchins with pre-weighed thalli of the species on which they had been feeding. At the end of 24 h of feeding, all uneaten algae was recovered from each urchin's tank, and re-weighed to estimate the consumption rate of the urchins. These consumption rate values were compared to the consumption rate values of the same urchins when they had five species of algae available (December) and were also used to examine what they indicated about preferences.

Growth rate

A comparison was made of the growth rate of small urchins less than 1 year old, which were kept on single species diets for 21 days. The urchins were fed ad libitum one of six species of macroalgae: *D. cavernosa*, *G. salicornia*, *Kappaphycus* sp., *P. sanctae-crucis*, *Ulva* spp. (*U. expansa* or *U. taeniata*), and *Sargassum echinocarpum*, and maintained in 20 l aerated aquaria supplied with running sea water delivered at a rate of 2 l/min. Up to four urchins were housed in each aquarium, and a total of three aquaria were used for each alga. A range of urchin sizes were placed in each tank spanning diameters from 2.5 to 4 cm. These urchins were small enough that they showed significant growth in the 21-day period. Initial and final diameters of the urchin tests were measured three times with stainless steel calipers and the three values on each date were averaged.

Results

Observations of grazing in the field

At field sites in Kaneohe Bay and around Oahu, *T. gratilla* have been observed feeding on a variety of

macroscopic algae, coralline algae, endolithic algae and turfs. Endolithic algae could be seen in voids in the limestone where the urchins had removed the overlying carbonate material. The macro-algae consumed include the native species, *D. cavernosa*, *Dictyosphaeria vershluisii*, *Dictyota* sp., *Galaxaura* sp., *Microdictyon* sp., *P. sanctae-crucis*, *Porteria hornemanii*, *Turbinaria ornata*, and crustose coralline algae. A number of alien species were also consumed including: *A. spicifera*, *G. salicornia*, and *Kappaphycus* spp.. No urchins were found feeding on corals. Three particular patch reefs differ dramatically in which species of macroalga dominates. On each of these reefs, the predominant alga was the alga being grazed by the largest number of urchins (Table 1).

Movement

This species of urchin is not restricted to crevices or burrows, nor does it move out from and return to refuges; individuals appear to move independently of one another through the day and night. Twenty-four hours after marking the initial position of urchins on a large patch reef, each urchin had moved about 1 m. When groups of 50 urchins were released at a base point in the center of the reef flat of a patch reef, they dispersed across the width of the reef flat, then the distance from the base point stabilized as urchins reached the edge of the reef flat (Fig. 2). The dispersal on Reefs 19 and 22, was more rapid than that on the reef of Moku o Loe (Fig. 2), probably because the area in the center of Reefs 19 and 22 where they were released had a higher proportion of sand and virtually no macroalgae. Their dispersal on reefs 19 and 22 slowed when the urchins reached the edge of the reef flat, possibly because there was more macroalgae (*D. cavernosa*) and more coral. The live coral appears to act as a partial barrier to movement. Very few of the urchins descended the reef slope (~10 m from the reef center) despite the high cover of macroalgae on the upper reef slope. On the fringing reef of Moku o Loe the urchins were released

Table 1 Among-reef differences in macroalgal consumption by *Tripneustes gratilla*

Reef	Predominant macroalga on the reef	Urchins feeding on predominant alga (%)	Urchins feeding on macroalgae (%)	Number of observations
Moku o Loe	<i>Gracilaria salicornia</i>	40	53	15
Reef 19	<i>Dictyosphaeria cavernosa</i>	53	58	80
Reef 22	<i>Dictyosphaeria cavernosa</i>	40	46	181
Reef 29	<i>Kappaphycus</i> sp.	100	100	13

Reef locations are given in Fig. 1

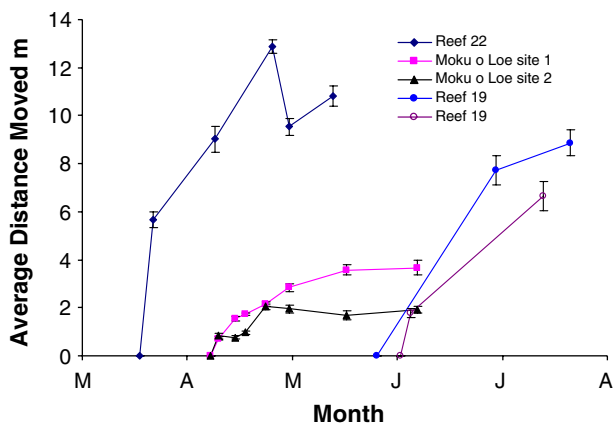


Fig. 2 Dispersal of groups of *Tripneustes gratilla* through time from reference points on small patch reefs (Reefs 19 and 22) and at two sites on the fringing reef of Moku o Loe

on the windward reef flat just shoreward of a 10–20 m wide coral/limestone platform, which characterized the outer reef flat. This zone has a high cover of *G. salicornia*. These urchins dispersed slowly parallel to the inner margin of the platform and stayed within the *G. salicornia* band (Fig. 2). They tended not to venture shoreward into more sandy habitat or toward the reef crest across living coral. No urchins were encountered on living coral.

Preferences

Tripneustes gratilla offered pair-wise choices of a set of algae displayed some significant preferences (Table 2), but the significant preferences always involved *D. cavernosa* as the less preferred species. *Kappaphycus* sp. was significantly preferred over *D. cavernosa* in two of the three tests, *G. salicornia* was preferred over *D. cavernosa* in one of the three tests and *P. sanctae-crucis* was preferred over *D. cavernosa* in one of two tests.

The touch-test of preferences among turfs and macroalgae showed that the urchins tended to move onto macroalgae more frequently than onto turfs (Table 3). A randomized blocks design ANOVA performed on the data of Table 3 showed that significantly more urchins moved onto *P. sanctae-crucis* than onto the turf algae collected from two reefs. In the analysis, dates were blocks, types of algae (species of macroalgae or sources of turfs) were treatments, and the variable was the number of urchins touching one of the choices at a specific time.

The 5-month exposure to a single species of macroalgae had a limited effect on preferences when the urchins' preferences were tested at the end of the 5-month period. In December, each of the 30 urchins was

Table 2 Results of pair-wise tests of preferences of *Tripneustes gratilla* for different macroalgal species. Preferences were assayed by measuring the wet weight of each alga consumed by an urchin in 24 h

July 2002, 12 urchins, three algal species				
K		G		
K	>	G	D	
		G	D	
Feb. 2003, 12 urchins, four algal species				
K	P			
K		G		
K			D	
	P	G		
	P		D	
	P			D
		G	>	D
March 2003, six urchins, four algal species				
	K	G		
	K	>		D
P	K			
P			G	
P		>		D
		G		D

Within each test, the algal species are ranked by the amount of the species eaten by *T. gratilla*. If a greater than sign appears on a line, then the comparison is significant at the $P < 0.05$ level by a paired *t*-test. Abbreviations for the algal species: *K* *Kappaphycus* spp, *G* *Gracilaria salicornia*, *P* *Padina sanctae-crucis*, *D* *Dictyosphaeria cavernosa*

simultaneously offered approximately equal weights of each of the five test algal species. Only the test group fed for 5 months on *P. sanctae-crucis* displayed a markedly greater feeding rate (15.79 g per 24 h) on the alga to which it had been exposed (Fig. 3, Table 4), and only one test group, those fed for 5 months on *G. salicornia*, had a markedly depressed feeding rate (0.25 g per 24 h) on the species to which it had been exposed (Fig. 3, Table 4). This conclusion is drawn by comparing the figure for each algae-exposure group (set of six urchins) with the figure for the control or naïve group of 16 urchins (Fig. 3), or by comparing the two sets of means at the bottom of Table 4. Despite the prolonged exposure to single species the average consumption of each of the six test species (Table 4, values 1.21–8.80) suggest a similar preference hierarchy to the one presented in Table 2: *P. sanctae-crucis* and *Kappaphycus* sp. are more preferred than *G. salicornia* and *D. cavernosa*. The control or naïve group (Fig. 3) also showed relative consumption rates similar to those found in the pair-wise tests of preferences (Table 2). A lack of independence in the observations precludes a statistical analysis of preferences in this experiment (each urchin was able to feed on any of the five test algal species in the 24-h period, and the more it fed on one alga, the less it could feed on another).

Table 3 Relative preference for turfs and macroalgae based on the touch-test criterion. Variable is number of touches per species

	Number of sets of ten urchins	Moku o Loe Turf	<i>Padina sanctae-crucis</i>	Reef 13 Turf	<i>Gracilaria salicornia</i>	<i>Dictyosphaeria cavernosa</i>
June 24	3	0	2	1	1	6
June 27	5	1	3	5	3	5
July 1	6	2	12	5	6	6
July 9	10	4	14	2	6	11
July 11	10	9	24	12	14	10
Average number of touches		3.2	11	5	6	7.6

Source	df	MS	F	P
Alga	4	43.54	4.47	0.013
Blocks (dates)	4	105.04		
Error	16	9.74		
Total	24			

Sets of ten individually housed urchins were offered the ten possible pair-wise combinations of the five algae. Two types of turfs and three species of macroalgae were offered. Results on each date are from independent sets of ten urchins. An urchin could have contributed no contacts if it was located on the wall or floor of its tank at the time of the assessment. Results were analyzed by a randomized blocks ANOVA in which dates (sets of ten urchins) were blocks and species were treatments

A posteriori test: Touches on *P. sanctae-crucis* exceed those on the two turfs by the *Q*-test (Snedecor and Cochran 1967) at $\alpha = 0.05$

The urchins in all the exposure-groups had about the same total consumption during the test period (Table 4), although those exposed for 5 months to *Kappaphycus* sp. ate slightly less than the urchins in the other four groups.

The touch-test measure of preference was also applied in this December choice experiment and also showed the existence of a preference for *P. sanctae-crucis* and *Kappaphycus* sp. (Table 5).

The consumption rates of the 30 urchins in the 5-month exposure experiment were measured on five dates during the exposure period to determine how the average consumption rates on the five algal species compared to the preference results of other experiments (Fig. 4). The relative consumption rates of these algae produce a very different ranking than that obtained by offering individual urchins pairs of algae (Table 2) or sets of five algae (Table 4). *Kappaphycus* sp was the least quickly consumed and *D. cavernosa* was the most quickly consumed (Table 6); the consumption rate of the urchins feeding on *Kappaphycus* sp. (g macroalgae consumed per 24 h per urchin) was found to be significantly lower than the consumption rates for urchins feeding on *D. cavernosa* (Fig. 4). These same urchins had the lowest consumption rate among the five sets of urchins during the December test period when they were exposed to all five species (Table 4). This result shows how the method of testing preferences can influence the conclusions about preferences. Basing preferences on the rate of consumption of algae when only one species is presented at a time

gave a different result than the three determinations of preference in which multiple species were offered at once (Tables 2, 4, 5). It also shows that despite the low preference displayed for *D. cavernosa*, if it is the only alga available, the rate of consumption is high.

The consumption rate of urchins (total g/urchin/day) in laboratory trials was influenced by the number of algal species offered. The consumption rate of urchins in the 5-month exposure experiment was measured on November 14 and 22, 2003 (3 and 2 weeks before the end of the 5-month period, December 6) while they were on a single-species diet. These consumption rate values were compared to the consumption rates in the test period at the end of the 5-month exposure period when each urchin had five species simultaneously available. The comparison showed that when offered five species simultaneously, the urchins in the five single-species exposure groups ate more per day than they had earlier when on a single-species diet (Table 7).

Growth on different algal diets

Small *T. gratilla* fed on *D. cavernosa* for 21 days grew less than the urchins maintained on some of the other five single-species diets (Table 8). A one-way ANOVA performed on the mean growth of individuals from each tank (four individuals per tank, three tanks per algal species) showed there were significant differences in growth on the different diets ($F = 5.47$, $df 5.12$, and $P < 0.01$). A set of *a posteriori* tests (SNK) showed that growth on a diet of *D. cavernosa* was significantly less

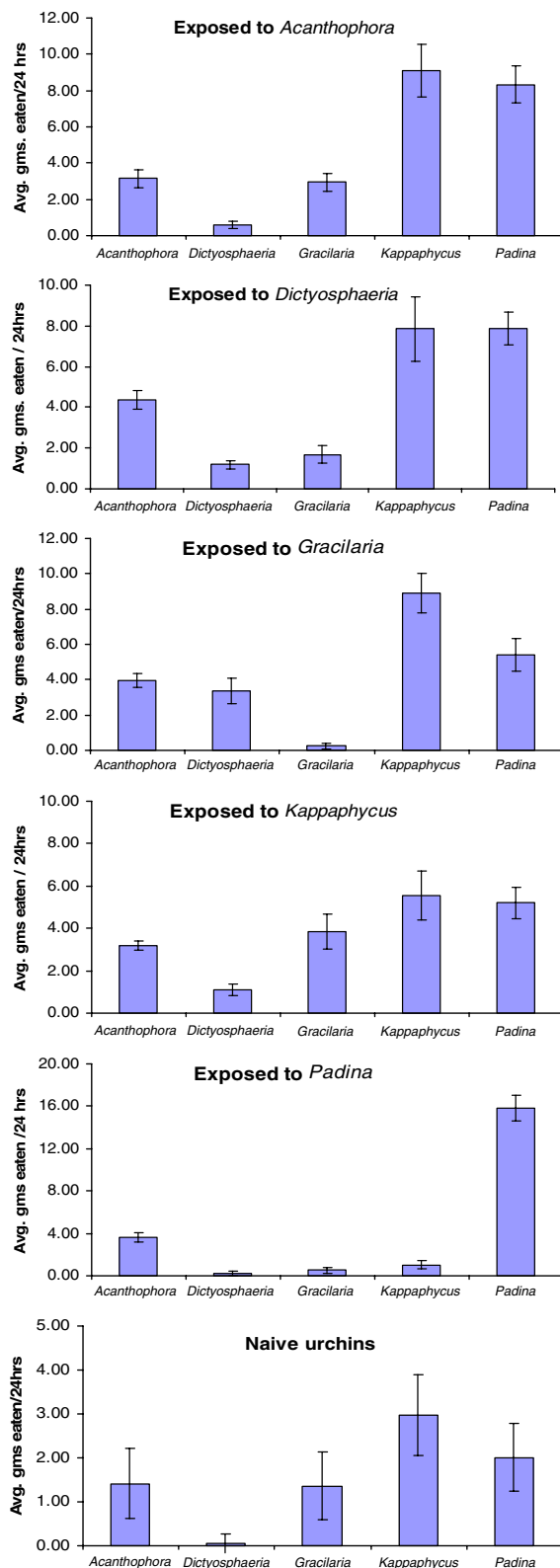


Fig. 3 Algal preferences expressed by *Tripneustes gratilla* at the end of a 5-month exposure period during which they received one of the five species. Note that Y-axes on figures vary and that the Y-axis of the *Padina*-exposed figure is more compressed than the others. Naïve urchins were collected from a reef with no fleshy macroalgae

than the growth on *S. echinocarpum* and *P. sanctaecrucis* ($P < 0.05$).

Discussion

Tripneustes gratilla appear to be general and opportunistic in their feeding; an observation that has been made concerning this (Ogden et al. 1989) and other species of urchins (Lawrence 1975; Ayling 1978; De Ridder and Lawrence 1982; Ogden et al. 1989; Beddington and McClintock 1999). This lack of feeding specialization in *T. gratilla* correlates with its presence in a wide variety of habitats which contain very different algal species (Lawrence and Agatsuma 2001), and with its ability to grow to a test diameter of 8–9 cm in areas where algal biomass is either low (turfs), or high (stands of macroalgae). On the island of Hawaii, small *T. gratilla* in their first months of life have been seen in tidepools. On Oahu, large urchins have been found on boulders on sections of coast periodically subject to heavy surf, they have been observed on terraces in 15 m of water, and on reef flats exposed to either periodically heavy surf or virtually no surf. At other tropical sites they have been found feeding on sea grasses (Dy et al. 2002; Vaitilingon et al. 2003), which suggest quiet water habitats. Given the wide range of habitats into which they apparently settle, it is not surprising that they are generalist herbivores.

The view that these urchins eat what is available is supported by the consumption rate experiment. When the consumption rate of the urchins exposed to a single species for 5 months was assessed during this exposure period by measuring their consumption of a single species, it was found that they generally ate about the same amount of algae per day, regardless of species (Fig. 4). The one exception was *Kappaphycus* sp., which was fed upon at a lower rate, even though in other experiments involving choice (Tables 2, 3) it was a preferred alga. Prince and LeBlanc (1992) and Dy et al. (2002) have also observed equivalent feeding rates among groups of urchins each receiving one of three different macroalgal species. The view that urchins eat what is available has also been proposed by Paine and Vadas (1969) and Beddington and McClintock (1999), and is possibly related to the observation that “tropical herbivores are more resistant than temperate herbivores to seaweed chemical defenses” (Cronin et al. 1997).

Tripneustes gratilla does show preferences in laboratory experiments (Hay 1984a, this study), but it is unclear what the basis is for these preferences. It has been suggested that preferences are a response to

Table 4 Comparison of the average consumption rates (g/urchin/24 h) of the five test species at the end of the 5-month exposure period

	Test species					Total
	<i>Acanth. spicifera</i>	<i>Dictyosph. Cavernosa</i>	<i>Gracil. salicornia</i>	<i>Kappaphycus sp</i>	<i>P. sanctae-crucis</i>	
Urchins exposed to:						
<i>A. spicifera</i>	3.14	0.59	2.95	9.12	8.34	24.14
<i>D. cavernosa</i>	4.37	1.19	1.58	7.85	7.85	22.84
<i>G. salicornia</i>	3.96	3.37	0.25	8.91	5.42	21.91
<i>Kappaphycus sp.</i>	3.18	1.09	3.85	5.55	5.19	18.86
<i>P. sanctae-crucis</i>	4.10	−0.01	0.44	1.20	15.79	21.53
Average	3.73	1.21	1.94	6.63	8.80	
Std. Dev.	2.16	2.12	2.94	6.94	6.29	
Average consumption of test species by experimental urchins exposed to test species	3.14	1.19	0.25	5.55	15.79	
Std. Dev	2.47	1.12	0.91	6.37	6.58	
Average consumption of test species by experimental urchins not exposed to test species	3.97	1.24	2.25	6.63	6.76	
Std. Dev.	2.07	2.29	3.05	7.18	4.70	

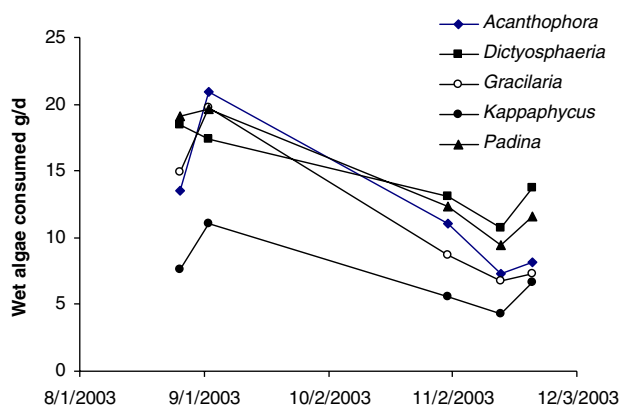
Values in the body of the table are the average consumption rate in grams/urchin/day with six urchins in each exposure group

Table 5 Preference for five species of macroalgae assayed by determining which algal species an urchin was touching with the teeth of its lantern

Algae urchin had lantern teeth on:	2 PM	3 PM	4 PM
<i>Kappaphycus sp</i>	11	11	10
<i>P. sanctae-crucis</i>	12	11	12
<i>Acanthophora spicifera</i>	0	2	1
<i>Gracilaria salicornia</i>	0	0	1
<i>Dictyosphaeria cavernosa</i>	0	0	0
Analysis of positions at 2 PM			
χ^2	23.08		
<i>P</i>	<001		

The assay was performed 1 h after introducing the test algae by examining all 30 urchins at three times 1 h apart. Null hypothesis for chi-square test of the data collected at 2 PM is that each algal species had an equal chance of being selected by the 23 urchins, which had moved onto an alga. Not all 30 urchins were clearly in contact with one of the test algae at each time

macroalgal defenses (Hay 1997; Grando and Caballero 2001; Van Alstyne et al. 2001). The consumption of encrusting corallines and the preference for the lightly calcified *P. sanctae-crucis* (Tables 2, 4) suggest that the carbonate defenses of these species (Paul and Hay 1986) do not always deter this herbivore. The preferences displayed by the urchins may also be a response to the morphology of particular algal species. The low preference shown for *A. spicifera* may be the result of its long fine branches which may be more difficult for urchins to handle and ingest than the rigid morphologies of *D. cavernosa*, *G. salicornia* and *Kappaphycus spp.* In contrast, herbivorous reef fishes show a preference for

**Fig. 4** Comparison of the consumption rates of the 30 urchins kept on single species macroalgal diets for 5 months

A. spicifera (Stimson et al. 2001). *D. cavernosa* may possess a different type of defense against urchins; once the large fluid-filled vesicles of this alga are punctured, much of the contents of the vesicles are probably lost and the tissue becomes flaccid. Even though urchins removed a considerable wet-weight of this alga when only offered one species (Fig. 4), the actual amount of tissue ingested by an urchin has probably been overestimated, because of the loss of the fluid in the vesicles. Because *D. cavernosa* is not known to be strongly defended (Paul and Hay 1986), the low preference for this species (Tables 2, 5) and the low growth achieved when feeding exclusively on this species (Table 7), may be the result of the low actual ingestion rate of this alga per unit wet-weight we measured that it had consumed.

Table 6 Comparison of the consumption rate (g/urchin/24 h) of urchins maintained on five single species diets

Untransformed consumption rates	<i>Acanthophora spicifera</i>	<i>Dictyosphaeria cavernosa</i>	<i>Gracilaria alicornia</i>	<i>Kappaphycus</i> spp.	<i>Padina sanctae-crucis</i>
Average	10.1	13.3	8.2	6.0	12.1
Standard deviations	2.6	35.4	18.9	28.8	15.8
Number of urchins	6	6	6	6	6
ANOVA (on log transformed consumption rates)					
Source	df	MS	F	P	
Alga	4	1.14	3.62	0.018	
Error	25	0.31			
Total	29				

Consumption was rate was measured on three dates in November on each of the 30 urchins in the 5-month exposure experiment. The rates for each urchin on the three dates were averaged and the natural logs of these averages were the variable in the analysis

Kappaphycus sp. was consumed at a lower rate than *D. cavernosa* on the basis of a *Q*-test (Snedecor and Cochran 1967) at $\alpha = 0.05$

Table 7 Comparison of consumption rate on a single-species diet versus a multi-species diet

Treatment	Average consumption per day per urchin (g)	Standard deviation	Sample size	
Five species offered simultaneously 12/7/04	22.3	6.0	28	
One species offered 11/14/03	9.6	6.3	28	
One species offered 11/22/03	7.8	6.0	28	
ANOVA				
Source	df	MS	F	P
Treatments	2	1753	64.6	<0.001
Blocks	27	57		
Error	54	27		
Total	83			

Each urchin constituted a block in the ANOVA. One urchin was feeding very little and another died by the time of the December preference test reducing sample size to 28. Blocks were individual urchins. Consumption when five species were offered was significantly greater than when one species was offered by SNK at $P < 0.05$

There is evidence in this study that the preferences reported here could be influenced by recent past experience, because the 5-month exposure study (Fig. 3) did seem to generate one case of enhanced preference and one of avoidance among the five species tested. Exposure to *P. sanctae-crucis* induced an even greater preference for *P. sanctae-crucis* when offered a choice from among five species (Fig. 3) and exposure to *G. salicornia* may have caused urchins to subsequently avoid the species when offered a choice among five species. Poore and Hill (2006) found that in amphipods, past diet did seem to influence subsequent preferences;

Table 8 Comparison of the growth rates of *Tripneustes gratilla* fed different diets in the lab for 21 days. Sample size refers to total number of urchins

Algal species	Average growth in diameter (cm)	Variance	Sample size
<i>Dictyosphaeria cavernosa</i>	0.46	0.016	12
<i>Gracilaria salicornia</i>	0.94	0.060	12
<i>Kappaphycus</i> sp.	0.93	0.044	11
<i>Padina sanctae-crucis</i>	1.07	0.024	12
<i>Sargassum echinocarpum</i>	1.22	0.029	11
<i>Ulva</i> spp. ^a	0.93	0.042	12

^a *U. expansa* and *U. taeniata*

individuals avoided algal species on which they had been feeding and they selected alternate species.

The use of a wide variety of foods by these urchins and the consumption of a greater weight of algae per day if a mixture of algae was available (Table 7) could be an example of the use of complementary resources as proposed by Pennings et al. (1993). According to this hypothesis, consuming a broader variety of algae could either render individual defensive compounds less effective or bring about some nutritional advantage. At the end of the 5-month exposure period, urchins (except for those exposed to *P. sanctae-crucis*) ate some of each of the five test algal species which were simultaneously offered to them, despite their prolonged exposure to a single species of macroalgae (Fig. 3), and they ate more total weight of algae than when kept on single-species regimens (Table 7). These results are consistent with this “complementary” hypothesis, but this study does not separate between the nutritional advantage and attenuation of chemical defenses alternatives.

It is possible that the breadth of the diet and lack of specialization observed in this study is an artifact of the algae used in these experiments. These are all species (with the exception of *Ulva* spp.) which can occur in a single habitat and which *T. gratilla* would encounter at sites where the urchin naturally occurs. These algae may have similar overall levels of chemical defense even if the defensive compounds or mechanisms are different. This level of defense may be associated with the intensity of herbivory, which prevails in the habitat in which the algae are found and were collected. We did not test species that live in very protected environments and might have fewer chemical defenses, as found by Hay (1984a).

Finally, the use of a wide variety of foods could be related to the observations that food preferences in other urchin species have been found to change with season, location and size of the urchins (Lewis 1964; Randall et al. 1964; Atkinson et al. 1973; Lawrence 1975; Carpenter 1981; DeRidder and Lawrence 1982; Ogden et al. 1989). Further testing could examine these relationships.

There are very few similarities between the grazing preferences of these urchins and those of the common herbivorous fishes (acanthurids and scarids) on the reefs of Kaneohe Bay. Like fish, the urchins often ranked other macroscopic algae ahead of *D. cavernosa*, despite the fact that they ate large amounts of this alga when it was the only alga available (Fig. 4) and despite the accessibility (semi-rigid, unbranched thallus) of this alga. Both acanthurids and scarids prefer *A. spicifera* (Stimson et al. 2001), an alien alga, which is generally confined to the inner-most parts of the reef flats far from the center of activity of the herbivorous fishes. This species was not preferred by *T. gratilla* on the basis of either the rankings developed from consumption rate or the touch-test (Tables 4, 5), possibly because of the mechanical difficulty of dealing with the long, thin (<1 mm diameter) branches. Both acanthurids and scarids ranked *Kappaphycus* sp. almost as low as *D. cavernosa*, but the urchins appeared to give this alga a high ranking in the analysis of preference by the touch test and consumption tests (Tables 2, 5). The preference hierarchy shown by herbivorous fishes corresponds to the distribution of macroalgae across the reef flat in Kaneohe Bay (Stimson et al. 2001). Their least preferred alga is *D. cavernosa*, which is abundant on the outer reef flat and especially the reef slope. These are the two habitats with the highest densities of herbivorous fishes. *Kappaphycus* sp. and *G. salicornia* become common 5–15 m onto the reef flat where fish densities are lower. *P. sanctae-crucis* and *A. spicifera* become abundant in the sandy region in the center of

the reef flat, well away from the highest densities of herbivorous fishes. The fact that urchins have a different ranking of algae than do the fishes suggests their effect as biological control agents would be somewhat complementary to the effect of herbivorous fishes. This result contrasts with the results of Hay (1984a), which suggested that the preferences of urchins and fishes were similar. This difference may be the result of the inclusion in this study of alien algal species introduced into the local flora in the last 30 years.

The feeding preferences, consumption rates, movements, growth rates and field feeding habits of *T. gratilla* suggest it could aid in controlling alien algae on reefs within Kaneohe Bay. Other urchin species have also been proposed as control agents of macroalgae (Vasserot 1992). Among the most important characteristics of this species is the fact that these animals are generalists, which eat a wide variety of algae, including alien and invasive species. Further, these urchins rank at least one macroalgal species as significantly more preferred than turfs (Table 3). This is consistent with the result that macroalgae seem to be the major food source for these urchins in Kaneohe Bay despite the generally greater availability of turfs and corallines. The heavy usage of macroalgae and the browsing habit of this urchin (as opposed to a grazing habit in which the urchin would abrade the substrate, Birkeland 1989) suggest this particular species would have less impact on the limestone reef framework than other species if released in large numbers. Second, individuals of this species have a high consumption rate; individuals of 7–8 cm in test diameter eat about 7–22 g wet weight of algae per 24 h in the laboratory (Table 7). The lower value is comparable to the feeding rate in laboratory tanks by grazing fishes of the average size encountered in Kaneohe Bay (<10 m total length) (J. Stimson, unpublished data). Third, individual urchins dispersed slowly from the release points at the sites we introduced them, and so can be introduced to areas of heavy algal biomass and can be expected to maintain a relatively high density in the area of the introduction. Sand and live coral act as partial barriers to this urchin species' movements; sand has been found to be at least a partial barrier to movement of other urchin species (Laur et al. 1986). These urchins have never been observed feeding on coral in the field. The effectiveness of sand means that the native species, such as *P. sanctae-crucis*, which was preferred by the urchins in this study, will be relatively safe from urchins on reefs in Kaneohe Bay, because *P. sanctae-crucis* generally occurs on the inner reef flat, which is primarily a sandy area. Urchins introduced to the outer reef flat on Moku o Loe into a zone of high cover of *G. salicornia*, have generally stayed in

this zone and have moved parallel to the crest rather than shoreward into sandy areas. Fourth, individuals of this species evidently grow quickly, reach a test diameter of 7 cm in 1 year (Junio-Meñez et al. 1998; Shokita et al. 1991) and can attain a test diameter of 13 cm (this study). Both of these factors mean that this species can represent a significant contribution to herbivory on reefs. Finally, in contrast to classic biological control agents, these urchins are native to Hawaii and they are conspicuous since they remain in the open and graze night and day. Conspicuousness is important because it means the urchins can be easily enumerated and because it makes it possible to retrieve them if they have cleared an area of invasive macroalgae.

The ability to utilize a wide range of foods is important when considering the possible use of cultured *T. gratilla* to control invasive macroalgae. Specialists are often selected as biological control agents because they will concentrate their efforts on the pest species and cause little damage to closely related but non-pest species. In the case of invasive macroalgae in Hawaii, three species of red macroalgae have been introduced either intentionally or accidentally, have spread rapidly (Rodgers and Cox 1999), and are now pests because they can occupy habitats where they compete with corals for space. As a potential biological control agent, *T. gratilla* would evidently feed heavily on all of these. It would also feed on natives, which are not invasives, but because native macroalgal species are more common on inner reef flats protected by expanses of sand, this is unlikely to happen.

Acknowledgments We gratefully acknowledge the Hawaii Institute of Marine Biology for access to its facilities and the Univ. of Hawaii, Dept. of Zoology for use of equipment and facilities. We wish to thank Meghan Dailer, Shino Ogawa, and Rudolf Pan for assistance with experiments and with data collection. The manuscript has benefited from comments by Drs. Karla McDermid and Charles Birkeland. Support for this research came from the Hawaii Coral Reef Initiative. The study complies with the laws in the State of Hawaii and the United States.

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