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Feeding preferences of *Tripneustes gratilla* (Linnaeus) (Echinodermata: Echinodea) and the prospect for sea urchin aquaculture in South Africa

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Abstract

Sea urchin aquaculture for human food is a major industry, and there are prospects for the industry in South Africa. Feeding preferences of the sea urchin *Tripneustes gratilla* were tested by two different methods; a touch-preference test and a 48-hour consumption test. A touch-preference is established when the sea urchins' lantern teeth touched an algal species. The 48-hour consumption test was performed with paired combinations using four species of fresh algae. It was hypothesized that the preferred species would be similar in the touch-preference test and the consumption test. Five algal species were used in the touch-preference test; namely, *Ecklonia maxima* (kelp), *Gigartina polycarpa, Grateloupia capensis, Porphyra capensis,* and *Ulva rigida*. Four of these species were used in the paired consumption test; namely, *E. maxima, G. polycarpa, P. capensis* and *U. rigida.* We found that *E. maxima* was most preferred in the touch-test followed by *P. capensis* and *U. rigida.* This result did not correlate with our consumption test. Here, *U. rigida* was significantly most preferred followed by *E. maxima.* Overall, the result is significant in relation to the prospect of echinoculture in South Africa as *Ulva* may provide a valuable alternate feed or supplement to this new industry. Due to the increase in time provided for the paired consumption test, we found that the paired consumption test was more conclusive in providing a preference for *Tripneustes gratilla.* Our results agree with literature which suggests that *Tripneustes gratilla* is a generalist feeder; however, *Tripneustes gratilla* shows definite preferences. *Ulva spp.* has been grown on a number of abalone farms in South Africa. Integrated aquaculture using *T. gratilla* and *U. rigida* as a biofilter and alternate feed is a very promising idea for echinoculture in South Africa.

Key words: touch-preference, *Tripneustes gratilla,* aquaculture, *Ulva spp.*

Introduction

Sea urchin aquaculture

Commercial harvesting of sea urchins is a major fishing industry in many countries, particularly in Japan, the United States, Canada, Chile and Russia. The major market for sea urchin products is Japan which accounts for in excess of 75% of world consumption (Krause 2003). There are two types of sea urchin aquaculture (echinoculture). The first type is full life cycle grow-out where adults are collected, and larvae raised in a hatchery until their gonads reach export size and quality. The second type is gonad enhancement from wild populations, where adults are collected from wild populations, maintained in captivity and fed artificial and/or natural diets to increase gonad mass and quality. This has, however, contributed to the drastic decline in wild populations (Andrew *et al.* 2002; Daggett *et al* 2005). Sea urchin aquaculture is an emerging practice in many well-established aquaculture countries around the world in pursuit of developing the best quality roe. High quality roe is a firm, mango-orange colour gonad with little to no gametes present (Shpigel *et al.* 2005). Sea urchin roe is marketed and available in several forms: fresh, frozen, steamed, baked and frozen and salted (Lawrence 2007). The most popular form, of highest value, is chilled fresh roe (which is known as uni in the sushi industry) (Pearce *et al.* 2002). The demand for high quality roe continues to increase and the reliance of large imports from other countries increases regularly (Pearce *et al.* 2002). Global production of sea urchins in general, has increased drastically from 25 000 metric tons in 2002 to 60 852 metric tons in 2004 and leads the list of the top ten species in terms of growth in production (FAO 2006). Production in Japan has decreased for some time now and stands currently at 10,000MT per year. Thus, Japan now imports about 80-85% of its uni supply (Krause 2003). Some countries harvest sea urchins and then feed them for a short period on a diet that promotes a rapid increase in gonad size and improves gonad colour and taste, before they are processed for export (Woods *et al.* 2008). Many studies have investigated the potential of various sea urchins as aquaculture species fed a range of diets for gonad enhancement (McBride *et al.* 1997; Pearce *et al.* 2002; Shpigel *et al.* 2005; James 2008)

There are a range of edible sea urchins found around the world, many of which are harvested for their prize roe, some of these species include; *Centrostephanus rodgersii (*A Agassiz), *C. coronatus* (Verrill), *Diadema mexicanum* (A Agassiz), *D. savinyi* (Michelin), *Loxechinus albus* (Molina), *Paracentrotus lividus* (Lamarck), *Echinometra mathaei* (Blainville), *Heliocidaris erythrogramma* (Valenciennes), *Tripneustes gratilla* (Linnaeus), *T. ventricosus* (Lamarck) and *Strongylocentrotus droebachiensis* (O. F. Müller) and many others (see Lawrence 2007 for full list). The urchin concerned in this project is *Tripneustes gratilla* (Linnaeus). It is one of three extant species in the genus. *T. gratilla* is cultured in Okinawa, Japan currently (Lawrence 2007).

Tripneustes gratilla is a common sea urchin found in shallow waters although may be found at 75m and *T. ventricosus* at 30m (Lawrence 2007). *T. gratilla* is a circumtropical sea urchin and its distribution extends into the subtropics as far south as East London on the southeast coast of South Africa (Day 1968). It *T. gratilla* and *T. ventricosus* occur on a variety of habitats including seagrass and algal beds, sand with rubble, rock, and coral reef flats (Lawrence 2007).

Tripneustes gratilla is limited in its poleward distribution by seawater temperature. Evidence for this was found by Tokioka (1966) when *T. gratilla* almost completely disappeared from shallow waters at Seto, Japan after water temperatures decreased to <20°C from mid November to mid-April 1965, however, they are known to tolerate lower temperatures in culture (pers. obs.). The minimum mean monthly seawater temperature near East London, South Africa was recorded at 19°C (Bolton 1986) and decreases rapidly southwards, which explains this species distribution limit into the cooler warm temperate waters of the south coast of South Africa.

Urchin diets

Echinoids are known to be generalist herbivores feeding on a range of marine plants and seagrasses (de Loma *et al.* 2002; Vaïtilingon *et al* 2003). There is evidence of sea urchins showing preferences or behaving as specialists although this is often related to food availability, nutritional values of food types and/or the presence of chemical substances in food which attract or repel sea urchins (Vadas 1977; Vaïtilingon *et al* 2003; Stimson *et al.* 2007). The nature of food ingested is a combination of food availability and food preference (de Loma *et al.* 2002). Many studies have provided evidence of *T. gratilla's* generalist foraging behavior. De Loma *et al.* (2002) provided evidence of a feeding preference of *Tripneustes gratilla* in Réunion Island (Indian Ocean). A strong preference was noted for the fucoid macroalga *Turbinara ornata.* The gut contents of *T. gratilla* were a direct indication of what was in the field, to such an extent that the proportion of algal species in the gut was reflected in algal species available in the wild. Even though the diet mainly consisted of algae, *T. gratilla* fed on detritus as well. Although adult sea urchins may show signs of avoidance, especially to calcareous and turf algae, juveniles usually do not show any signs of avoidance (de Loma *et al.* 2002) and therefore show a generalist feeding behavior. Studies provide evidence of some feeding patterns in the wild; however, this is largely due what is present during that particular time of the year (Vaïtilingon *et al* 2003). Vaïtilingon *et al.* (2003) found that *Tripneustes gratilla* preferentially fed on *Syringodium isoetifolium,* the dominant seagrass species that was found in the study area. Maharova *et al.* (1994) showed that on the NW coast of Madagascar (Indian Ocean) *Tripneustes gratilla* grazed on seagrass leaves of the species *Thallasiodendron ciliatum, Syringodium isoetifolium* and less frequently on *Thallassia hempricci* and *Halodule uninervis.* They found that their food preference was a function of the availability, accessibility and leaf characteristics (toughness and chemical composition). Grazing in different habitats also leads to the role of sea urchins in maintaining biodiversity on many rocky shores (Vaïtilingon *et al* 2003). The evidence for a selective feeding behavior seems contradictory and the feeding behavior seems directly related to the geographic location of the study.

Feeding behavior of marine herbivores with limited motility often depends on chemosensory signals that either attract, repel and/or stimulate feeding of a particular algal species. It is suggested by Akakabe and Kajiwara (2007) that feeding behavior of lower herbivores, such as gastropod snails and sea urchins are divided into three steps; (1) searching marine algae, (2) taking into a mouth, and (3) consuming the algae. Akakabe and Kajiwara (2007) provided evidence for chemosensory attractants in the green alga, *Ulva pertusa,* using a local gastropod, *Lunella coronata,* where a preference test with six other algae were performed. They suggest that the gastropod preferentially fed on *U. pertusa* compared to the other marine algae, and recognized chemoreception compounds from alga but not their structural or morphological differences. They also fed the gastropod freeze-dried *U. pertusa* added to an agar-diet to assess the possibility that morphology may play a role in preference. However, the gastropod responded positively to both the fresh and agar feed (Akakabe and Kajiwara 2007).

Artificial diets are very common in the aquaculture industry. There are several components that make up an artificial diet that is required by sea urchins for optimal growth, texture and quality of gonads. Ingredients such as, fish meal, soybean meal, wheat starch, linseed oil are used in artificial urchin feeds (see Dworjanyn *et al.* 2007). Artificial diets used on farms often contain a range of components obtained from various sources to meet the dietary requirements of proteins, carotenoids, fats, carbohydrates, and vitamins. The ratio of these dietary constituents is the underlying factor that enhances growth and quality of the gonad. More importantly is the ability of the animal to incorporate these compounds into the body.

Carotenoid pigments are mainly responsible for colouration of gonads in echinoids, particularly echinenone which is synthesized from β-carotene in sea urchins (Shpigel *et al.* 2005). Sphigel *et al.* (2005) showed that synthetic β-carotene added to diets were not as effective as natural sources of β-carotene. This is undoubtedly very important to reach export quality, as pale colour gonads are unacceptable. A combination of growth and colouration is vital for export quality gonads. Protein is the most important component of diet that contributes to growth rate. Dworjanyn *et al.* (2007) showed that juvenile *Tripneustes gratilla* grew quickly on a Sargassum-diet, with an 8-12% weight increase per week. The sea urchins fed Sargassum-diets that were 19% protein and 14.8 MJ/kg in energy. In culturing these animals, protein levels are often related to growth rates but do not necessarily have a significant effect on food selectivity (Dworjanyn *et al.* 2007). Artificial diets are produced with the aim to reach a balance between growth and quality (Fleming *et al.* 1996). Many studies investigate the potential for new or improving existing artificial diets (eg. Pearce *et al.* 2002; Shpigel *et al.* 2005; Dworjanyn *et al.* 2007). Thus preference tests are conducted to investigate this potential. Many tests aim to obtain essential compounds from a natural source, such as adding algae to existing diets (Dworjanyn *et al.* 2007) or developing an algae-only diet of mixed species (Shipgel *et al. 2005)*, in an attempt to improve the colour, size and taste of sea urchin gonads.

Dietary preference tests with sea urchins

Preference tests in aquaculture are often performed to create new or enhance existing knowledge of artificial diets. One aspect of these tests that has received much attention in recent years is the improvement of palatability of artificial diets for sea urchin aquaculture (Dwojanyn *et al* 2007).

Dworjanyn *et al.* (2007) found that when the sea urchin *Tripneustes gratilla* was offered seven seaweed species in the fresh state, it showed a strong preference for kelp, *Ecklonia radiata,* the green alga, *Ulva lactuca,* and the brown alga, *Sargassum linearifolium*. Similarly, when these three algae were dried and added to an existing artificial diet, this addition increased the palatability of these artificial diets. Ultimately, growth rates and consumption rates increased substantially. Vadas (1977) showed strong algal preferences in laboratory studies using *Strongylocentrotus drobachiensis* and *S. franciscanus.* Algae were ranked from most to least preferred; *Nereocystis luetkeana, Costaria costata, Laminaria saccharina, L. groenlandica, Monostroma fuscum, Opuntiella californica, Agarum fimbriatum* and *A cribrosum*. He also showed that substances released by *N. luetkeana* attracted the urchins while substances released by *A. fimbriatum* repelled or were not detected by the urchins. The detection of certain chemical substances released by the algae appears to be the underlying factor of attraction or repulsion to or from a particular algal species (Sakata *et al.* 1989).

Shpigel *et al.* (2005) performed an experiment aimed particularly to enhance gonadal growth and quality which is essential for export. They found that an algal diet consisting of *Ulva* and *Gracilaria* produced a mango-orange gonad colour but with a low Gonadal-somatic index (GSI). GSI is calculated as gonad mass (wet)/whole urchin mass (wet). The artificial diet produced a large gonad which was pale in colour. However, the artificial diet fed for eight weeks followed by an *Ulva* and *Gracilaria* algal diet for four weeks produced the best combination of desired gonad colour and GSI. Daggett *et al.* (2005) performed similar tests on juvenile and adult sea urchins and found that algal diets produced the best growth rates. The use or addition of algae to existing diets thus appears to have many benefits, although seaweeds are not common components of artificial aquafeeds. In South Africa kelp (*Ecklonia*) is now added to some artificial abalone feed (Robertson-Andersson *in press*).

Seaweed cultivation and mariculture has been practiced for a several years now and seaweeds play a vital industrial role in the world for phycocolloid usage (Ohno and Critchley 1993; McHugh 2003). The industry today is estimated to be valued at US$ 5.6 billion for all products with the largest share attributed to food products (McHugh 2003). Traditionally seaweeds were collected from natural stocks or "wild" populations, but these are declining due to over-harvesting. The main cultivated seaweeds are *Laminaria japonica* – kombu, a Japanese kelp (Phaeophyta), *Undaria pinnatifida* – wakame, also a Japanese kelp (Phaeophyta) and *Porphyra tenera*- nori (Rhodophyta) (FAO2006). China is the largest producer of edible seaweeds, about 5 million tones of which is kombu (*Laminaria japonica*) which accounts for the most of this value (FAO 2006). However, the growing of algae species for feed as part of the diet of cultured animals is not as widely practiced, but has been substantially investigated (Shpigel *et al.* 2005; Robertson-Andersson *et al. in press*). However, the technique is being practiced in several aquaculture farms. Integrated aquaculture such as growing seaweed in aquaculture effluent on the farm can be used for a number of reasons. Some examples of these are; feed for abalone (Naidoo *et al.* 2006; Smit *et al.* 2007; Robertson-Andersson *et al. in press*) where Robertson-Andersson (*in press*) found that an integrated system using 25% recirculation as opposed to a flow through unit with abalone (*Haliotis midae*) and the green alga, *Ulva lactuca*, had no negative impact on the farmed animals. They also found no significant differences between flow-through and re-circulated *Ulva* systems in dissolved nutrient concentration, particle concentration, abalone specific growth rate, abalone health, bacterial and mesoherbivore abundance in an 18-month period. Dubber and Harder (2008) showed that seaweed extracts from *Ceramium rubrum, Mastocarpus stellatus* and *Laminaria digitata* had anti-bacterial activity against various prominent fish bacteria. Strains of bacteria were lysed during exposure to seaweed extracts and bacterial growth was inhibited. Pang *et al.* (2006) found that polyculture of seaweed alongside animal fed aquaculture was an environmentally friendly way of avoiding eutrophication problems in both land-based and sea-based monoculture systems. They found that when *Gracilaria textorii* was cultured in a dual tank system with juvenile abalone, *Haliotis discus hannai*, the levels of *Vibrio* had decreased and prevented propagation of 2 purified strains of bacteria. Algae are also used in fish diets in aquaculture. Farmed fish have no access to carotenoid-rich feed and cannot be synthesized *de novo* by the fish; therefore these are needed to be added to their diet (Barbosa *et al.* 1999; Chatzifotis *et al.* 2005). Barbosa *et al.* (1999) and Chatzifotis *et al.* (2005) showed that cells of the green microalga *Haematococcus pluvialis*, when grown under certain stress conditions such as high irradiance and nitrogen limitation, formed cysts and accumulated large amounts of the carotenoid astaxanthin. This was added to the diet of cultured fish, (eg. trout and pink salmon) and was responsible for the natural pigmentation of the fish skin.

In addition to growing seaweeds for the human food industry, there is sufficient evidence that suggests growing plants on an aquaculture farm from animal effluent is a good idea. The collection of fresh algae for feed would be the alternative to growing your own plants. There are many problems associated with fresh collection, such as the limited sources of suitable macroalgal species and temporal variation in quantity and quality of algae available (Troell *et al.*2006). To alleviate this problem, we need to consider what is being cultured in South Africa at the moment and whether or not growing seaweed is feasible or not.

The aquaculture production in South Africa is very low compared to the rest of Africa. One reason that is seldom stated is the lack of water to support it and the lack of sheltered-marine sites available for aquaculture (Bolton 2006). Though abalone (*Haliotis midae*) production and export in South Africa is a great success (FAO 2006), it is believed that part of this success is that abalone is low in food chain (Bolton 2006). Similarly is the success of seaweed cultivation (*Eucheuma* for carrageenan production) in Zanzibar. Seaweeds and filter feeders are farmed because they are nutritious, tasty and relatively easy to grow (Bolton 2006).

Aims of the project

Recent developments indicate that there are many ongoing experiments to test the possibility of *Tripneustes gratilla* as a culture species in South Africa. The desired goal is to produce export quality uni to Asia, particularly Japan, where the market is very big. In order to have a successful practice of this nature, the culture animals' biology must be understood. To identify which diet optimizes growth and quality may help aquaculture operations produce market-size, good quality sea urchins in the least amount of time, and ultimately maximize profitability. South African individuals of *Tripneustes gratilla* are found in a temperate rocky shore environment, where the available food present is seaweed (not seagrasses as opposed to the population found further north in Mozambique). Experiments to culture South African specimens of *Tripneustes gratilla* are under way (M. Cyrus pers. comm.). The aim of this project was to test the dietary preferences of South African *T. gratilla* using locally available seaweeds, and to compare preferences with seaweed ingestion with the more rapid touch test method. We test the hypotheses that *Tripneustes gratilla* has no preference for various seaweed species and that touch-preference experiments give similar results to feeding preference experiments.

Methods

Experiments were carried out at Marine and Coastal Management Research Aquarium, Seapoint, Cape Town (33.9207°S; 18.3806°E). Data were collected on two occasions. The touch preference test was performed from the 18th to the 21st September and the consumption test performed from the 08th October to 12th October 2008. *Tripneustes gratilla* sea urchins were housed at the aquarium and range from the youngest (≈1 years) to the oldest (≈2.5 years) individuals. These were spawned in the aquarium from adults collected in the wild at Haga Haga (32.7505°S; 28.2754°E) near East London, South Africa. All experimental tanks were constantly aerated and supplied with sea water at 21°C with a flow rate of $8.7 ml.s^{-1}$. The sea water is warmed in a geyser alongside the housing tanks from 15°C to 21°C before it flows into the housing tanks. The water flows through a closed system that is regularly replenished with fresh seawater every hour. The dimensions of the housing tanks are 269.7 X 69.8 X 53 cm^3 with water depth of approximately 40-45cm with 4 housing baskets per tank containing approximately 20-30 individuals (Figure 1). All sea urchins were fed kelp (*Ecklonia maxima*) regularly prior to experiment.

Touch preference test

The urchins were starved for 7 days before tests begun. The individuals used in this study were 70-90mm in test diameter with a mean diameter of 84.1mm±4.2. Following Stimson *et al.* (2007), individual sea urchins were placed in the centre of a tank and surrounded by the five algal species at equal distances apart. Five algal species were used namely; *Ecklonia maxima* (Osbeck) Papenfuss, *Gigartina polycarpa* Kuetzing, *Grateloupia capensis* De Clerck, *Porphyra capensis* Kuetzing and *Ulva rigida* C. Agardh, (Stegenga *et al.* 1997). A photograph was taken to monitor movement every 15mins for 75mins (Stimson *et al.* 2007). Individual sea urchins were placed in the centre of a round 63cm plastic tank that was 17.8cm deep with five algal species placed around the sea urchin (Figure 2). The seaweeds were placed 25.9cm apart from one another on the bottom of the tank and was each 20cm away from the sea urchin. The reason for using shallow round tanks is because it was easier to photograph and monitor urchin movement. The sequence in which combinations were offered was randomized for every replicate without repetition to avoid directional placement of specific algae. A touch preference is established when the sea urchins' lantern teeth touched an algal species. Four replicates were used in this test.

Paired preference test

Of the five species used in the touch preference test, four algae were used in the pair-wise preference test (*E. maxima, G. polycarpa, P. capensis* and *U.rigida*). Individual sea urchins were placed into rectangular tanks (51.4 X 35.8 X 41.2) that were 41.2cm deep with two algal species in each tank (Figure 1). Algal thalli were offered fresh in every test. All species were allowed to drip dry in a crate for 10mins. All algal species were placed into a salad spinner and spun for 30 seconds to remove excess water. Pre-weighed fresh algal thalli (≈50g fresh weight) were offered to each individually placed urchin per tank in all possible combinations (six) and the weight of each alga consumed was measured 48 hours later. Every pairwise offering to an urchin was attached to rubber band which was subsequently attached to long and flat lead fishing sinkers (≈115g). Algae were attached to these sinkers with rubber bands to maintain a coherent block and to prevent any species from floating and becoming less accessible. All algae species used were collected at low spring tide from the intertidal zone outside the aquarium at Seapoint for each test performed. They were cleaned to remove any animals visible to the naked eye.

Data analysis

Transition matrices were used to display individual movement between time periods of the touch-preference test. T-tests were performed to test for significant differences in consumption between pair-wise combinations. Nonparametric chi-squared tests were performed on the touch-preference data to test for significant differences in each time period. We test the null hypothesis that the sea urchins are evenly spread among all the algal species. One – Way Analysis of Variance (ANOVA) was performed across overall consumption followed by Tukey HSD to test for homogenous groups.

Figure 1. Apparatus used for preference test. The water supply is heated and re-circulated in a closed system. A fresh supply of sea water enters the heating cylinder regularly. Water then supplies housing tanks and the 18 tanks needed for this experiment on the floor next to the housing tank. The supply was split into four points and each point was further split into four, except the strongest two which was split into five so that every tank had its own water supply. The main water supply was increased to maintain sufficient to supply all experimental tanks as well as housing tanks with water.

Figure 2. Round tanks were used for the touch-preference test. It was easier to monitor and photograph the urchins using these tanks.

Results

Touch-preference test

The touch-preference test showed that most observations over a 75 minute interval were made when urchins were on *Ecklonia* (46 observations) (Table 1). This is also shown in Table 1.

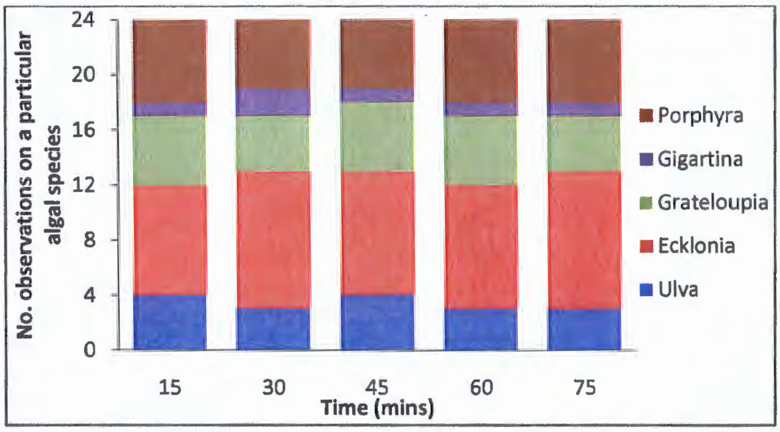


Figure 3. Results from touch-preference test, showing preferences made by 24 sea urchins over a 75 minute period.

From the results displayed in Figure 3 and Table 1, it is clear that *Ecklonia* was most preferred in the touch-test. Thereafter, *Porphyra* was preferred, followed by *Ulva*. The observations obtained over 75 minutes suggest a stable trend across the time interval allocated in the experiment. Figure 4 expands on Table 1 showing individual movement (where possible) from species to species.

Table 1. Total number of observations on each species after 75mins

Species	**15**	**30**	**45**	**60**	**75**	**Total**
Ulva rigida	4	3	4	3	3	**17**
Ecklonia maxima	8	10	9	9	10	**46**
Grateloupia capensis	5	4	5	5	4	**23**
Gigartina polycarpa	1	2	1	1	1	**6**
Porphyra capensis	6	5	5	6	6	**28**
Total	**24**	**24**	**24**	**24**	**24**	**120**

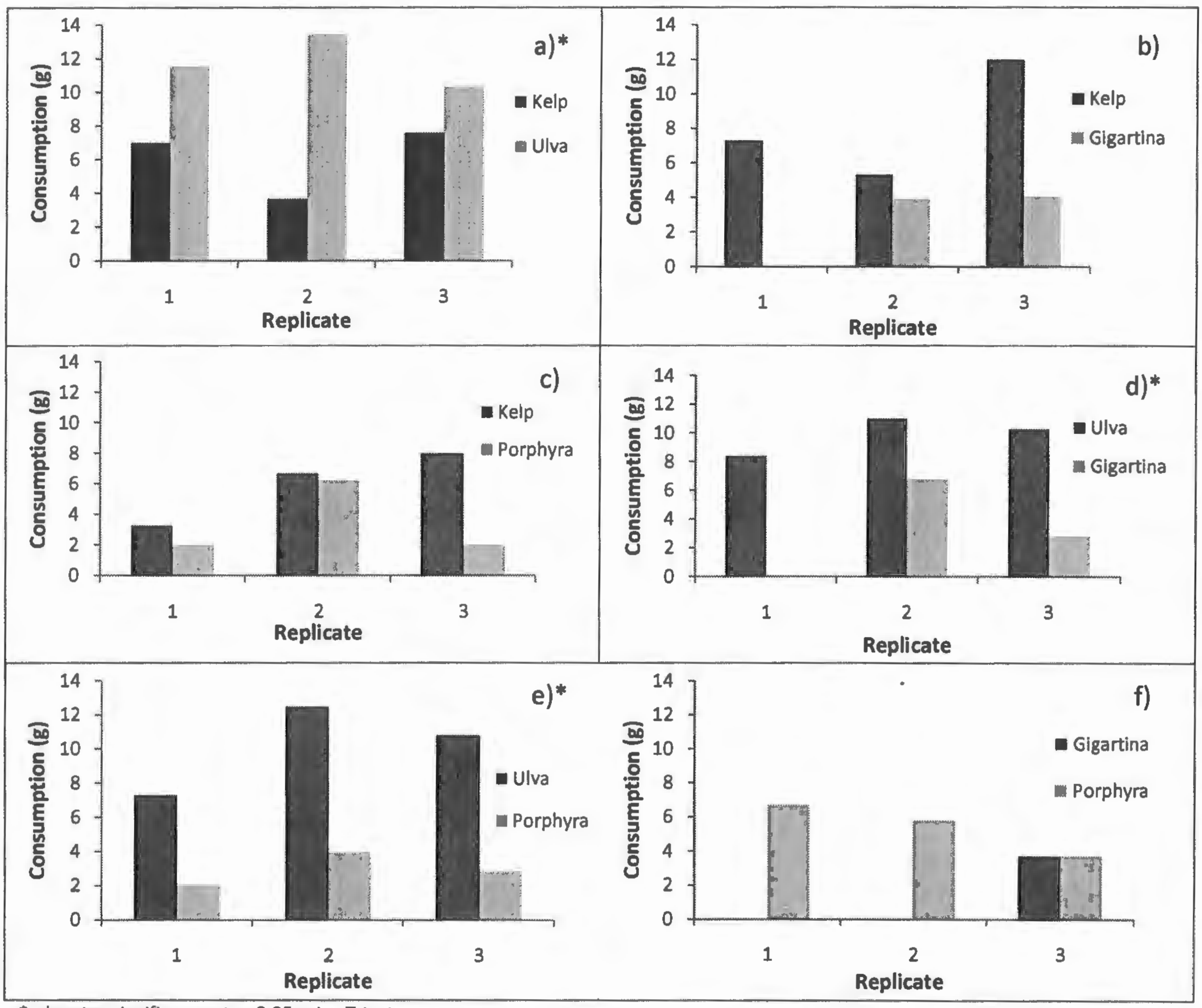
	Time (mins)						Time (mins)					
	15-30	UR	EM	GC	GP	PC	**30-45**	UR	EM	GC	GP	PC
Species	UR	3			1		UR	3				
	EM		8				EM		9	1		
	GC		1	4			GC			4		
	GP				1		GP	1			1	
	PC		1			5	PC					5
	45-60	UR	EM	GC	GP	PC	**60-75**	UR	EM	GC	GP	PC
	UR	3		1			UR	3				
	EM		9				EM		9			
	GC			4		1	GC		1	4		
	GP				1		GP				1	
	PC					5	PC					6

Table 2. Transition matrices of each time interval for 24 sea urchins. On the Y-axis is time 1 and the X-axis is time 2 given in the top-left corner of the grid. Movement direction is read from Time 1 to Time 2.

The transition matrix is read from left ($Time_1$) to the right ($Time_2$). It represents the number of individuals moving from species to species between time intervals. The matrices provide an indication of an average preference after and between time intervals. The data collected during the touch test indicated that 83.3% (20 individuals – See Appendix) of the urchins remained on first species that they encountered. With the exception of individuals on *G. polycarpa,* most of the choices made during the touch tests were evenly distributed, there was however a slight preference for kelp (38.3%) (Table 1). From time 0 to 60 minutes, urchins are not evenly distributed among algae. At the 75 minute period, the distribution of individuals among the algae were significantly different (χ^2= 9.75 df=4 p=0.04). However, we do not know which algal species is responsible for this difference. The number of individuals moving between time intervals decreased after 45 minutes. The initial preference for *Ecklonia* might be related to the fact that these individuals have been fed kelp regularly since they were collected from the wild.

Consumption Test

In the paired consumption test over a 48 hour period, *Ulva rigida* was the most consumed algae species (Figure 5), followed by Ecklonia *maxima,* then *Porphyra capensis* and *Gigartina polycarpa* which was least preferred.



*- denotes significance at p<0.05 using T-test

Figure 4. Graphs (a) – (f) showing pair-wise tests of all possible algal combinations. Consumption values of 3 replicates are present on the X-axis. T-tests were performed to detect significant differences between consumption.

Graph (a) produced the highest consumption of *Ulva rigida* (35.2g) for both algal species. There is a significant difference between *Ecklonia maxima* and *Ulva rigida* consumption, even though both species were readily consumed. Graph (b) shows a preference for *Ecklonia maxima* over *Gigartina polycarpa*. Graph (c) shows that two of the three replicates consumed similar amounts of *Ecklonia maxima* and *Porphyra capensis*. Graph (d) shows a significant difference between *Ulva rigida* and *Gigartina polycarpa* consumption, with an overwhelming preference for *U. rigida* shown by the amount consumed. Graph (e) provides an indication of two species that were relatively preferred in the touch-preference test. Here, *U. rigida* is significantly preferred over *P. capensis*. Graph (f) shows that in two replicates, *G. polycarpa* was not consumed, showing a clear avoidance for the species and the preference for *P. capensis* over *G. polycarpa*.

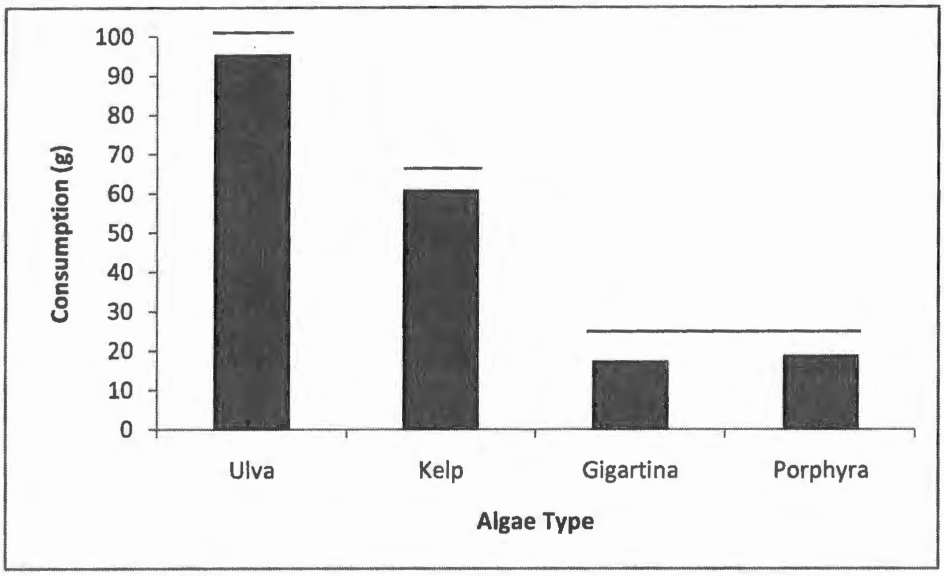


Figure 5. Overall algae consumption over a 48-hour period. Solid line across algal species denotes no statistical difference between species preferred according to Tukey HSD test.

Each algal species was consumed in the experiment. 60.9g of kelp (*E.* maxima), 95.5g of *U. rigida*, 17.4g of *G. capensis* and 18.9g *P. capensis* was consumed in total. The values observed in the figure above are a true representation of the rank of the preferred alga. Post-hoc homogenous tests reveal that *Gigartina polycarpa* and *Porphyra capensis* are similarly the least preferred species of the 4 algae, followed by *Ecklonia maxima* and *Ulva rigida* which was the most preferred.

Discussion

Touch-preference test

The observed behavior of *Tripneustes gratilla* in this experiment agrees with previous research and shows the generalist feeding behavior of this species (Vaïtilingon *et al.* 2003; Stimson *et al.* 2007) as every algal species was consumed and encountered in both experiments. This implies that there was sufficient reason for any particular urchin to investigate what algal species were placed inside the tank. Not all urchins encountered every species, but cumulatively each algal species was encountered at least once in the experiment (Table 1) and this is an indication of the generalist feeding behavior. The relatively even distribution of urchins' individual choice throughout the experiment is an indication of this animals' generalist feeding behavior, and may be an indication that they stay on the first alga they touch (Table 2). Almost 40% of the urchins had a touch preference for kelp (*Ecklonia maxima*) but could not be statistically proven. However, *E. maxima* was the only alga that had to be cut in order to meet the method requirements and it is possible that the chemosensory signal perceived by the urchin attracts the individual to the *E. maxima,* stronger than the signal released by *Ulva rigida* or *Gigartina polycarpa* for that matter. Cut kelp exudes mucilage from mucilage ducts in the tissues. This may have explained some of the initial attraction to kelp. On the other hand some of the other species (eg. *U. rigida, P. capensis*) had to be cut up at occasions to meet the required weight (≈50g) but the extent of this was much less than with the kelp. In addition, they do not extrude mucilage, having no mucilage ducts (Stegenga *et al.* 1997). Akakabe and Kajiwara (2007) suggest that volatile compounds found within essential oils of algae play an important role in feeding behaviors of the lower herbivores, and they have already found that long-chain aldehydes that have marine odours, were major components in the essential oil found in algae. These were increased by mechanical wounding, which might explain why kelp was more attractive in the touch-preference test.

Chemosensory signals (e.g. feed stimulants) detected by urchins are believed to attract or repel individuals to particular algae (Akakabe and Kajiwara 2007; Dworjanyn *et al.* 2007). The chemical signal given off by the severed kelp may have aided in that choice, however we did not test for this effect in this experiment. The touch-preference test also showed that *Porphyra capensis* (23.3%) was slightly more preferred than *Ulva rigida* (14.1%). Overall, the touch-preference performed shows a preference for kelp but this does not correlate with the consumption test.

Vadas (1977) provided evidence for algal preferences in the laboratory and showed how species may be attracted or repelled by chemicals released by algae. The first four preferred species that he found, *Nereocystis luetkeana, Costaria costata, Laminaria saccharina, L. groenlandica,* were kelps. The *Monostroma fuscum* is similar to *Ulva* but slightly thinner in morphology, and the *Opuntiella californica* is a red alga related to *Eucheuma*. The kelp may have acted as a feeding stimulant in this experiment as suggested by Dworjanyn *et al.* (2007), however similarly to Vadas (1977) and Dworjanyn *et al* (2007), and the current results, the sea urchins disliked the red algae. Evidence for feeding stimulants has been documented where glycerolipids of the kelp *Eisenia bicyclis* stimulate feeding in the sea urchin *S. intermedius* (Sakata *et al.* 1989). Should this be the case in this touch-preference test, it may support the results from our consumption test as more time was allowed in that test.

It must be noted that the touch-preference test methodology as followed by Stimson *et al.* (2007) was flawed in the analysis of time-series data for a preference in the laboratory. The generalist feeding behavior displayed by sea urchins showed us that when an algal species was encountered, the majority (83% in this case) of individuals remained there for the entire duration of the experiment. And as most went to kelp, they remained there for 75 minutes. It is possible that 75 minutes as followed by Stimson *et al.* (2007) is too short. They too did not analyze their touch-preference data sufficiently by performing an ANOVA, which I think is insufficient as it does not take the dependence of every observation (per 15 minutes) on each other into account. Observations made every 15 minutes are dependent on the observations of the previous time period. Therefore, this needs to be taken into account when doing the data analysis of time-series data. An improvement might be to add a behavioral aspect into this method. The 48 hour consumption test using paired combinations was of more use in providing a preference for a specific alga. Not only is the time period lengthened, but there is a consistent hierarchy of what is eaten.

Consumption test

Over a 48 hour period *Ulva rigida* was consumed the most (Figure 5) and thus most preferred. In addition, only significant differences in consumption were detected when *U. rigida* was one of the species of the paired combination (Figure 4). Every species was consumed to some extent in all pair-wise combinations performed, which indicates the generalist feeding behavior of *Tripneustes gratilla*. With reference to the touch-preference test, kelp (*E. maxima*) was readily consumed, even when paired with *U. rigida*. *Ulva spp.* are known to have a high percentage of protein (a maximum of 36-44%, Robertson-Andersson *et al. in press*) compared to *Ecklonia maxima* which has a crude protein content of 11-12% of dry weight all year round (M. Smith and J.J. Bolton, unpublished data). However, in this combination, *U. rigida* was consumed more.

The fact that *Ulva* was preferred is of importance in terms of integrated aquaculture industry for South Africa. Algae have been shown to be very valuable in feeding regimes and as alternative feeds (Shpigel *et al.* 2005; Naidoo *et al.* 2006; Dworjanyn *et al.* 2007; Smit *et al.* 2007; Robertson-Andersson *et al. in press*) and the prospect of a new aquaculture industry needs to take this advantage into account. *Ulva spp.* have been grown on a number of farms using abalone (*Haliotis midae*) effluent in South Africa and have been recorded to reach proteins levels of 44% of dry weight (Robertson-Andersson *et al. in press*). Almost 1000 metric tonnes of *Ulva* are produced on South African abalone farms per annum currently (Bolton 2006).

Similarly to our consumption test, Dworjanyn *et al.* (2007) also used a kelp species (*Ecklonia radiata*) and an *Ulva spp.* (*U. lactuca*) in their consumption experiment and found the opposite relationship to what our data suggests with relation to these species mentioned. From their consumption test, kelp (*E. radiata*) was most preferred followed by *Ulva lactuca*. However, in comparison with the results of Dworjanyn *et al.* (2007), there was a general dislike for red algae used (*G. polycarpa* in this experiment) and individuals rarely ate this species. It is possible that the palatability or texture of *G. polycarpa* does not suit *Tripneustes gratilla* feeding habits. In addition, although not measured in this experiment, there is the possibility that chemical deterrents may be released by *G. polycarpa* which are not attractive. Overall, the results from this consumption experiment partly agree with those of Dworjanyn *et al.* (2007), in that there is a general preference for *Ulva* and *Ecklonia*, although the species differed in the two sets of experiments, and no preference for the red alga (*Gracilaria sp.* in Dworjanyn *et al.* 2007) which was a similar outcome for our red algae used in this experiment.

In light of this information, it appears that *Tripneustes gratilla* might show feeding preference based on its biogeographic location, rather than a general global preference. The population found at Haga Haga is an example of a population that exists at the lower temperature limits of its distribution and the area is associated with a rich macroalgal community that is similar to the area where Dworjanyn *et al.* (2007) collected their animals from. Studies provide evidence of *T. gratilla* readily consuming a range of seagrass species (Maharova *et al.* 1994; Vaïtilingon *et al.* 2003) and algal species (de Loma *et al.* 2002, Dworjanyn *et al.* 2007). Dworjanyn *et al.* (2007) also used a seagrass (*Zostera capricorni*) in his experiment, but the sea urchins disliked this species. Whether this species prefers seagrasses over macroalgae in general is not clear.

An important aspect of sea urchin aquaculture is the production of export quality gonads (uni). Though gonad enhancement was not tested in this experiment, literature suggests that animals fed *Ulva spp.* in addition to artificial feed produce export quality gonads (Shpigel *et al.* 2005). The preference for *U. rigida* by *Tripneustes gratilla* suggests that export quality gonad production may be produced using *Ulva rigida* as an addition to artificial feed. *T. gratilla* has been identified as an excellent candidate for sea urchin aquaculture with its fast growth rates and gonad enhancing potential (Dworjanyn *et al.* 2007; Lawrence 2007), and the beginning of a new industry in South Africa appears to be very promising and may contribute a great portion to aquaculture production values in the country. The seawater temperature on the majority of

South Africa's coast may not meet the requirements for this species echinoculture. The great part of South Africa's coast (West and South) experiences sea water temperatures that are too low (11°C-16°C, obtained from Branch *et al.* 1999). I suggest that should this industry go into practice, the farm should be situated on the East Coast, where seawater temperatures are higher (21°C -23°C, obtained from Branch *et al.* 1999), close to where this species occurs naturally in South Africa. Overall, our touch-preference test partially correlates with our consumption test; a fair amount of *Ecklonia maxima* was consumed, but not as much as *Ulva rigida*. In both tests we observed a clear dislike for *Gigartina polycarpa*. These results are valuable steps while investigating the potential farming practices of *Tripnuestes gratilla* in South Africa.

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