GRAZING OF SELECTED GENERA OF GREEN, RED AND BROWN MACROALGAE

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Abstract. Macrobenthic algae and grazers like gastropods, amphipods etc. eventually form a key component of intensified grazing ecosystem in rocky intertidal area. Grazing gastropods suppresses the abundance of canopy forming algal species, thus lead to a reduced algal diversity in the long term. We conducted feeding trials with single and mixed diet during a 28–Days indoor experiment to examine the grazing intensity among green, red and brown algae namely Ulva lactuta, Enteromorpha linza, Gelidium and Padina tetrastromatica following a gut content analysis of experimental gastropods such as Euchelus asper and Nerita oryzarum. Feeding experiments revealed that Euchelus asper consumed 70% Enteromorpha, 10% Ulva and about 10% Gelidium whereas, Nerita oryzarum consumed 45% Ulva and 10% Enteromorpha during single diet experiment. Euchelus asper showed its preference towards all experimental diet where as Nerita preferred Ulva to a greater extends than Enteromorpha and rest other combined diet. Our results showed a higher grazing pressure exerted by Euchelus asper might pose a threat for algal culture and form competition in animals of same trophic level intern affecting their population. Similar experiment may be useful to control mass canopy forming algal growth and habitat studies for grazing animals will also be attributed.
Keywords: *grazer, herbivory, seaweed*

Introduction

Macrograzers strongly affect the abundance and distribution of seaweed in both temperate and tropical communities (Hay, 1985). Lubchenco and Cubit (1980) have suggested that the upright and boring stages of ephemeral macroalgae are adapted for high growth rates and reproduction due to lower grazing pressure, and on the other hand they are adapted for surviving during high grazing pressure. *Littorina littorea* is the most prevalent herbivore in the intertidal zone which shows a specific preference towards *Enteromorpha intestinalis* than *Chondrus crispus* among the algal foods (Lubchenco, 1978). A comparative grazing experiment conducted by Underwood and Jernakoff (1981) brought out the differential grazing features of *Siphonaria denticulate* on established algal growth and *Cellana tramoserica* on early sporeling stages of algae. Grazers maintain algal diversity by depressing the abundance of dominant canopy forming species, and that

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despite an initial increase in number of algal species exclusion of grazers reduced algal diversity in the long term. Herbivores can be a determining factor in seaweed distribution directly through grazing which has been proved by Forslund et al. (2012). Result showed that the range of the herbivore *Idotea balthica* is known to decrease the abundance of fucoids *Fucus radicans* through grazing along the Swedish coast. Hence the present investigation has been conducted to examine the grazing preference of *Euchelus asper* and *Nerita oryzarum* during a 28 - days feeding experiment.

Materials and Methods

Indoor grazing experiment set up

Two gastropods namely *Nerita oryzarum* (Recluz) and *Euchelus asper* (Gml) were chosen to conduct laboratory experiment for measuring rates of grazing and seaweed consumption in single feeding trials. Seaweeds used for feeding experiments were *Ulva lactuta* Linnaeus (1753*), Enteromorpha linza* Linnaeus (1753) *and Gelidium* Lamouroux (1813), *Padina tetrastromatica* Hauck (1887) which were collected from intertidal zone along Bandra (19^o 02’41. 58”N, 72^{o}49’08. 80”E) and Colaba (18^{o}54’08. 40”N, 72^o 48’12. 22”E) region of Maharashtra coast of India.

Collected animals were starved and acclimatized for 24 hours in filtered seawater at temperature 25ºC and at salinity 30-32 ppt. These conditions were similar for both the experiments conducted with a period of 28-days. A group of ten gastropods (consisting 5 nos. *Nerita oryzarum* and 5 nos. *Euchelus asper*) were placed in the plastic tub filled with 10 liter filtered seawater. In experiment -1, four sets of treatments in triplicate (comprising first with *Ulva lactuta* as feed, second with *Enteromorpha linza* and the third with *Gelidium*; and the fourth one with *Padina tetrastromatica*) were set. The second experiment was the repetition of the previous. During 28-days treatment phase, seaweeds were exposed to gastropod. Rate of grazing in 7-days interval were calculated by loss of algal fresh weight. Gastropods were dissected after 12 hours interval of grazing to examine the gut content. Single factor ANOVA was performed separately for treatments and replicates of both the experiments to find out the significant difference in feeding preference and grazing intensity in the experimental gastropods.

Gut Content Analysis

The experimental gastropods (*Euchelus asper* and *Nerita oryzarum)* were dissected in laboratory and their gut content had been examined for the availability of seaweed fragments using stereoscope and compound microscope (Hund, Germany) and images were taken using Nikon digital camera (Nikon 310) attached to the microscope. Grazing preference of the gastropod was estimated using Chi-Square test.

Results

Single factor analysis of variance was performed in within treatment to compute the significant difference in feeding preferences of the gastropods, while similar analysis was

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carried out within replicates to find out the significance difference in grazing intensity of gastropods (*Table 1*). Experiment-I revealed a significant difference in feeding preferences of *Nerita oryzarum* as this organism has shown preference towards algal diets, while there is no significant difference in grazing interval since the feeding intensity was almost similar during the study period. On the other side, *Euchelus asper* showed significant difference within the replicates as it shows similar attraction towards all kind of seaweed except *Padina*. During experiment-II, *Euchelus asper* had shown significant difference in feeding preferences within the treatment and on the other hand within replicates there was no significance as the organisms preferred all types of alga except *Padina.* Whereas, *Nerita* did not show any significant difference within the treatment and within the replicates since *Nerita* had fed upon only in *Ulva* and most of the time they were found with empty gut.

***Table 1**. Analysis of Variance showing significance difference in grazing intensity of the experimental organisms (* indicates significance)*

Source of Variation	SS	df	MS	F
Experiment - 1				
Nerita oryzarum				
Within Treatments	2.379456	4	0.594864	8.204568*
Within Replicates	0.394296	4	0.098574	0.573899
Euchelus asper				
Within Treatments	0.577464	4	0.144366	0.982469
Within Replicates	1.532824	4	0.383206	3.863976*
Experiment - 2				
Nerita oryzarum				
Within Treatments	0.474944	4	0.118736	2.580769
Within Replicates	0.396784	4	0.099196	1.987259
Euchelus asper				
Within Treatments	1.92692	4	0.48173	7.301816*
Within Replicates	0.35052	4	0.08763	0.605205

Feeding experiment set up (as shown in *Figure 1*) was installed to estimate the feeding preference of the experimental grazers (*Figure 2*) for 28-days. Loss of individual algal weight was noted down with an interval of 7 days and was represented graphically to find out rate of grazing. The graph interpreted that *Enteromorpha* was being grazed much faster than the other three algae as it losed much weight within similar period of time (*Figure 3*). The overall rate of grazing was observed higher in *Enteromorpha* than *Ulva*, *Gelidium* and *Padina*. In both the experiments *Padina* was not grazed by the gastropods which may be due to presence of phenolic compounds which shows antiherbivory defense mechanism among all other seaweed given as feed to the grazer.

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***Figure 1**. Grazing Experimental Set up*

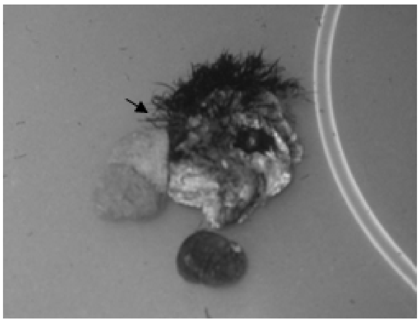

***Figure 2**. Gelidium being Grazed by Euchelus asper*

Gut Content Analysis

A total of 20 gastropods (*Euchelus asper* and *Nerita oryzarum*) from each of four single diet treatments (Treatment I – *Ulva lactuta*, Treatment II – *Enteromorpha linza*, Treatment III - *Gelidium* and Treatment IV – *Padina tetrastromatica*) were dissected. The experimental organisms were dissected after 12 hours of grazing and gut content were observed under microscope for presence of algal fronds. The analysis had shown semidigested *Enteromorpha linza* frequently in the gut of gastropods during feeding experiments. The bar diagram (*Figure 4*) revealed that *Euchelus asper* preferred *Enteromorpha linza* since their stomach contained algal fronds very often. They consumed 70% of *Enteromorpha linza*, 10% *Ulva lactuta* and about 10% *Gelidium. Nerita oryzarum* were observed with only 45% of *Ulva lactuta* and 10% *of Enteromorpha linza*. Gut contents of *Nerita* were examined empty on very frequent observations. In fact *Euchelus*

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asper stretched out its preference towards all experimental diet where as *Nerita oryzarum* confined towards *Ulva* in a greater extends than *Enteromorpha linza*. Fronds of *Padina tetrastromatica* were absent totally during gut content analysis of both *Euchelus asper* and *Nerita oryzarum* which may be due to presence of extractive phenolic compound and also may be due to the hardy nature of the thalli.

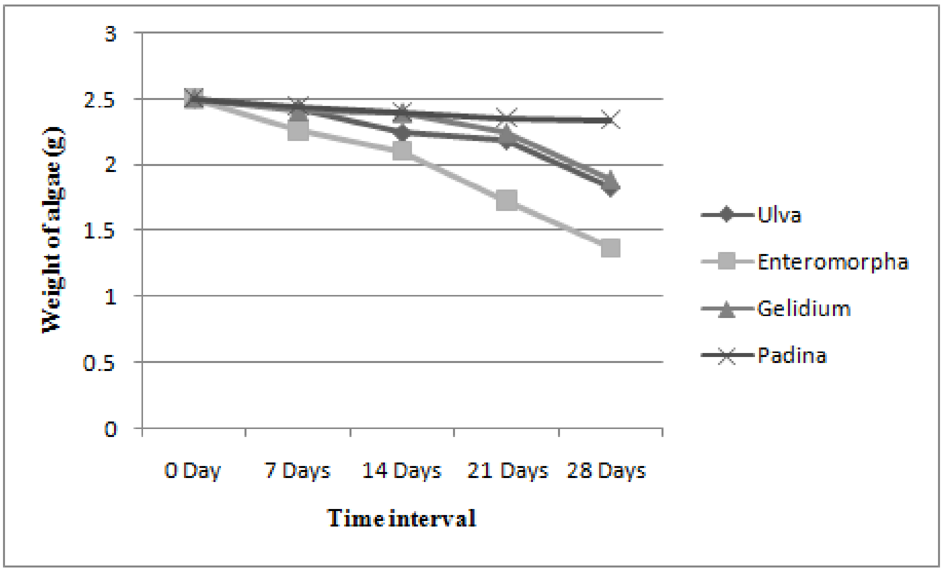

Figure 3. *Loss of algal weight in single diet experiment in a week interval*

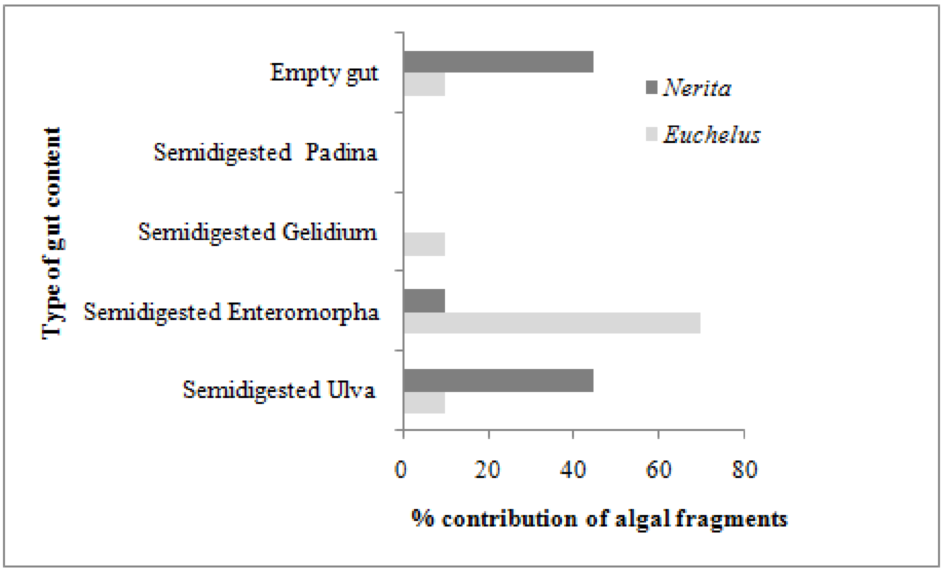

Figure 4. *Percentage contribution of algal fragments in gut of Nerita and Euchelus*

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Semidigested algal fragments were observed in the gut of *Euchelus asper* on frequent basis (*Figures 5 and 6*) as compared to *Nerita oryzarum*. A Chi-square test was carried out for each treatment to check the type of gut content in organisms in relation to their food preference (*Table 2*). In experiment-I, among all of the single diet treatment *Enteromorpha linza* had been ingested at a significantly greater rate (P<0.05) than the other algae, whereas *Ulva lactuta, Gelidium* and *Padina tetrastromatica* did not show any significance within the treatments. On the other hand, during experiment-II, except *Padina* all three other algae show significance within the treatment.

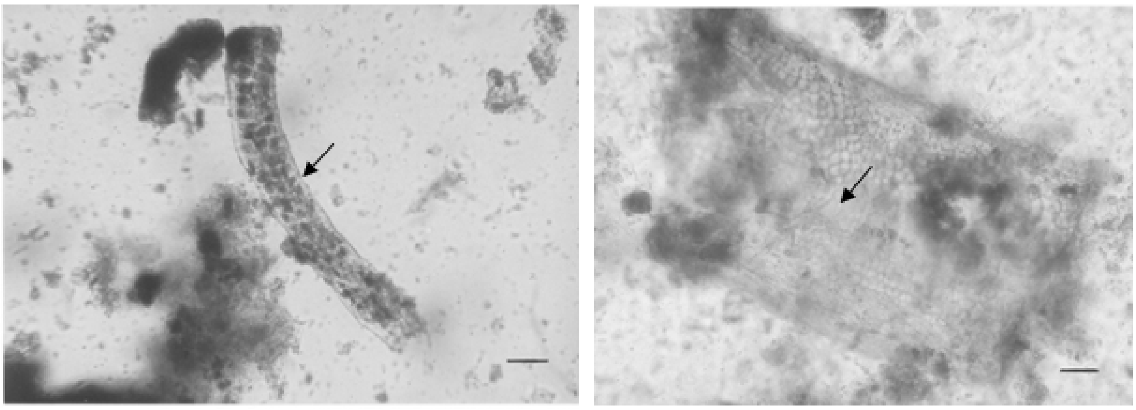

Figure 5. *Semi digested Enteromorpha and Ulva fragments in the Gut of Euchelus in Single Diet Experiment (Scale bar = 50 µm)*

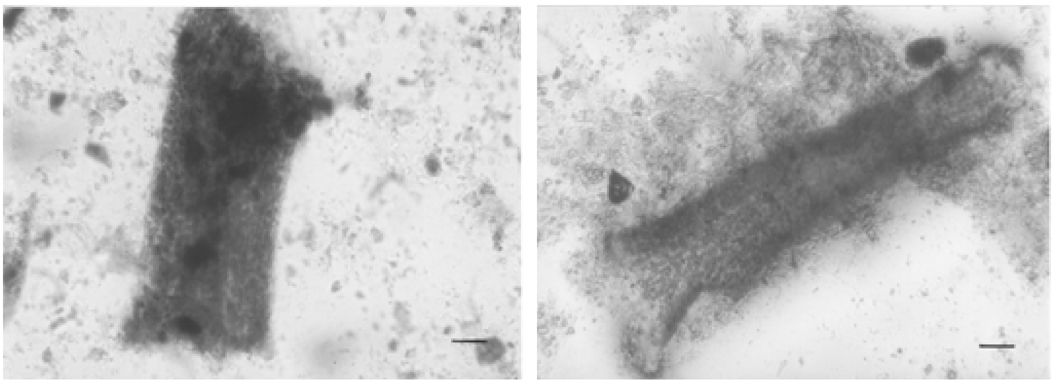

Figure 6. *Semi Digested Gelidium Thalli in the Gut of Euchelus During Single Diet Experiment (Scale bar = 0.2 mm)*

Discussion

The algal turf is reduced to dominance of species like *Gelidiopsis*, small turfs of *Ulva* and *Enteromorpha*, *Centroceras* – a filamentous algae. Lubchenco and Cubit (1980) showed direct relationship between grazers algal abundance. The Lubchenco - Cubit model has been developed to justify the heteromorphic life histories in marine algae. Their

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experiment on cleaning the area off grazers proved that ephemeral fronds do grow in absence of grazer supports present findings.

***Table 2**. Chi-Square test showing feeding preferences of the grazing gastropods among the algal diets (* indicate significance)*

Experiment 1	
Treatment	Chi^2
Ulva lactuta	2.1429
Enteromorpha linza	10.8*
Gelidium	0.83333
Padina tetrastromatica	0.2608
Experiment 2	
Treatments	Chi^2
Ulva lactuta	10.995*
Enteromorpha linza	16.425*
Gelidium	20*
Padina tetrastromatica	0.2857

In a grazing experiment, Vadas (1985) had suggested direct assessment of grazing by removal or addition of grazers, physical manipulation and algal manipulation. In the present study, *Euchelus asper* showed wide significance within algal treatment (diet) and grazing intensity as they might have fed on the respective diet with similar interval of time while *Nerita oryzarum* feds on algal diet but not in similar time interval as differ significantly within the treatments.

The result of the single diet experiment has been unveiled in terms of loss of algal fresh weight varied among experiments related to the studies of Hambrook and Sheath (1987). Grazing rate calculated from single diet feeding experiment in present study has concluded in greater rate of grazing. Similar observation was noticed by Nicotri (1980) where he studied the comparative attractiveness of algal species against artificial plants towards isopods and amphipods and by Lawrence (1975) for sea urchin grazing. Various scientific studies have also justified the present outcomes where algae offered singly have been consumed at faster rate than in combined form (Leighton, 1971; Vadas, 1977). Abundant colonization of green algae *Enteromorpha intestinalis*, *Ulva* have been observed within period of six month and finally appearance of perennials as Sargassaceans, *Padina,* Corallinaceans have become dominant in the tidal pools without the grazer snails, *Nerita albicilla* in the lower intertidal rocky shore (Arai and Arai, 1984).

Comparative analysis of gut content of both *Euchelus asper* and *Nerita oryzarum* have shown the occurrence of semidigested algal diet in their gut. Maximum number of *Euchelus* has been recorded with semidigested algal fronds in gut as compared to *Nerita oryzarum*. Results of Chi-square test between four algal treatments have given a detail account of occurrence of algal fragments in *Euchelus asper* and *Nerita oryzarum*. The analysis has demonstrated that treatment I (*Ulva*) differs in greater significance than treatment II (*Enteromorpha*), treatment III (*Gelidium*) and treatment IV (*Padina*) in single diet

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experiment. In bar diagram representation *Euchelus asper* pulls out maximum preference towards *Enteromorpha linza* which may be considered to be a highly preferred seaweed followed by *Ulva lactuta* and *Gelidium* as they show lower level of significance in Chi-square test.

The feeding experiment has also emphasized that *Euchelus asper* and *Nerita oryzarum* together prefer higher percentage of green algae (*Enteromorpha linza* and *Ulva lactuta*) than red algae i.e. *Gelidium*. Present study has also brought into light the fact that *Euchelus asper* and *Nerita oryzarum* have fully avoided *Padina tetrastromatica* during the study period which may be due to the presence of phlorotannins (Ragan and Glombitza, 1986). These polymers of phloroglucinol units hold an important position when plant-herbivore interactions and anti-fouling studies are being conducted (Hay et al., 1987; Cronin, 2001; Targett and Arnold, 2001; Amsler and Fairhead, 2006). And it is also proved that phaeophytes are resistant to meso-hervivores owing to concentrations of phlorotannins (Targett and Arnold, 1998; Wikstro ¨m et al., 2006; Jormalainen and Ramsay, 2009) which also supports the present study. High rate of grazing pressure by *Euchelus asper* may pose a threat to algal culture and may build up a competition for particular food item to other grazer intern affecting the trophic food chain. The hardy cell wall structure and polyexctractives compounds of *Padina* may prevent the grazer from not being consumed during the feeding experiment which was also supported by Arai and Arai (1984). Feeding experiment in this investigation has concluded that grazing gastropods prefer more of green and red macroalga in comparison with brown algae such as *Padina* due to presence of polyextractive compounds. Hence, present study addresses threats of cultivating edible seaweed like *Ulva* in mass scale production system.

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