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# Interaction of top down and bottom up factors in intertidal rockpools: Effects on early successional macroalgal community composition, abundance and productivity

P. Masterson<sup>a,\*</sup>, F.A. Arenas<sup>a,b</sup>, R.C. Thompson<sup>c</sup>, S.R. Jenkins<sup>a,d</sup>

<sup>a</sup> Marine Biological Association of the UK, Citadel Hill, Plymouth, PL1 2PB, UK

<sup>b</sup> Laboratory of Coastal Biodiversity, ClIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas 289, 4050-123 Porto, Portugal

<sup>c</sup> Marine Biology and Ecology Research Group, University of Plymouth, Plymouth PL4 8AA, UK

<sup>d</sup> School of Ocean Sciences, Bangor University, Menai Bridge, Anglesey, LL59 5AB, UK

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## ABSTRACT

Increasing levels of eutrophication in coastal waters and recognition of natural variation in supply of nutrients, through for example variations in upwelling intensity, have led to an increasing demand to understand the role of bottom up forcing in coastal communities. Rocky intertidal habitats are highly disturbed and are composed of a patchy mosaic of assemblages in different successional stages. The role of consumer control of algae in such habitats is well recognised but the way consumer control interacts with variation in resource supply is still debated. We examined, using a factorial manipulative experiment, the way top down control by molluscan grazers interacts with nutrient supply to determine the community composition and productivity of early successional communities in intertidal rockpools. The experiment was run over a six-month period, through the summer months, when nutrients were potentially limiting. Rockpools were cleared of biota and nutrient supply enhanced using slow release fertiliser, while the level of molluscan grazing was reduced through regular removals. Macroalgal colonisation was rapid. Positive effects of nutrient enrichment on macroalgal cover were generally lower than the negative effects of grazer control, although at the peak of algal cover, nutrient effects were apparent irrespective of grazer treatment. There were distinct differences in the identity of dominant species among nutrient treatments with green Ulva spp. dominating under enhanced nutrient conditions and red Ceramium spp. dominating under ambient conditions. These differences contributed to a mismatch between effect estimates based on cover and those based on direct measures of productivity. The use of percentage cover as a surrogate of biomass in intertidal studies is discussed. The results indicate that in a disturbed environment enhanced nutrient supply can stimulate macroalgal blooms despite strong top down control by intertidal grazers.

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# 1. Introduction

Intertidal rocky shores are highly productive components of coastal ecosystems but the pathways of energy flow vary significantly depending on community structure (Raffaelli and Hawkins, 1996). Shores dominated by macroalgae may contribute relatively little to local food-webs, since the majority of fixed carbon is transported away from the shore as detritus by water movement (Duarte and Cebrian, 1996). In contrast shores dominated by sessile filter feeders such as barnacles and mussels are net importers of energy, mainly through phytoplankton. Numerous physical and biological factors influence the balance between macroalgal and secondary consumer dominated communities and hence the role of rocky shores as net importers or exporters of energy. Top down control of macroalgal development on rocky shores through the action of a diverse suite of grazers has been recognised for decades (Jones, 1948; Lodge, 1948; Southward and Southward, 1978), but more recently the role of

\* Corresponding author. E-mail address: pamas@mba.ac.uk (P. Masterson). bottom up factors such as nutrient supply has received increasing attention (e.g. Worm et al., 2002; Menge et al., 2003).

There is an extensive literature on the consumer mediated control of macroalgae on rocky shores. Experimental work has frequently demonstrated that intertidal grazers have the ability to control almost completely the species composition, distribution and dynamics of algal communities (Lubchenco and Gaines, 1981; Paine, 2002). In NW Europe on shores exposed to wave action the dominant grazers, patellid limpets, control the development of macroalgae through consumption of microscopic stages (Hawkins and Hartnoll, 1983; Jenkins et al., 2005) and in some cases adult stages (Davies et al., 2007). Through doing so, they provide a suitable habitat for filter-feeding secondary producers (such as barnacles and mussels). Such 'top down' control of community structure declines with increasing latitude in Europe (Ballantine, 1961; Jenkins et al., 2005; Coleman et al., 2006) and is almost absent in sheltered areas (Jenkins et al., 1999), but its relative effectiveness determines the character and functioning of rocky shores.

Bottom up control of macroalgal development and production on rocky shores through nutrient supply is increasingly recognised both in

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observational studies across natural gradients in nutrients (Bustamante et al., 1995; Nielsen and Navarrete, 2004) and experimental work where nutrients are artificially enhanced (Metaxas and Scheibling, 1996; Nielsen, 2001; Worm et al., 2002). Increased recognition of the role of bottom up control arises in part from concern at the rate at which humans are disrupting the natural nutrient balance in both terrestrial and aquatic ecosystems (Vitousek et al., 1997). This concern is especially high in coastal ecosystems, at the interface between terrestrial and aquatic environments. Here human disposal or run off from the land can result in high local concentrations of nitrogen and phosphorous (Nixon, 1995; Jickells, 1998).

Enhanced nutrient supply to coastal systems is generally considered to result in enhanced algal growth and primary productivity, particularly in fast growing opportunistic ephemeral taxa (Pedersen and Borum, 1996; Worm et al., 2000; Bokn et al., 2003; Karez et al., 2004). Such changes may also have knock on effects to higher trophic levels through the elevated production of palatable undefended tissue (Kraufvelin et al., 2002, 2006a). Both theoretical and empirical evidence also suggest a relationship between resource supply and algal diversity (e.g. Worm et al., 1999, 2002; Hillebrand, 2003). However while bottom up resources are potentially important, top down control can have strong mediating effects. The way in which top down and bottom up control interact to determine community structure, biomass and diversity in coastal marine systems has been the subject of numerous recent studies (Worm et al., 2000, 2002; Lotze et al., 2001; Nielsen, 2001; Diaz-Pulido and McCook, 2003; Hillebrand, 2003). It appears that the extent to which top down consumer control of macroalgal abundance can nullify the effects of enhanced supply of resources, shows enormous variation dependent on numerous factors including the nutrient status of the system (Worm et al., 2002), seasonal variation of nutrient supply and grazers (Lotze et al., 2001), life history and life stage of dominant biota (Lotze et al., 2001; Diaz-Pulido and McCook, 2003), level of exposure to wave action (Nielsen, 2001; Kraufvelin, 2007) and the stability and composition of local communities (Bokn et al., 2003; Kraufvelin et al., 2006b; Eriksson et al., 2006a,b).

We aimed to assess the interactive effect of herbivore abundance and nutrient enrichment on early successional macroalgal community composition, abundance and productivity on intertidal rocky shores. It is likely that nutrient effects will be most apparent in early successional communities, made up of fast growing ephemeral algae which are typically more limited by nutrients in summer months when the availability of dissolved organic nutrients is low, than slow growing perennials (Pedersen and Borum, 1996). Additionally such highly productive, generally palatable taxa with a short life span are functionally important, contributing towards energy flow both within and outside the intertidal habitat (O'Connor and Crowe, 2005). In order to examine nutrient and grazer effects on early succession we removed existing biota from experimental plots. We hypothesise that in a nutrient limited (summer time) disturbed environment where dominant perennial species are removed, nutrient enrichment will have a positive effect on ephemeral macroalgal abundance and productivity irrespective of grazer control. Our experimental system consisted of intertidal rockpools for two main reasons. Firstly unlike freshwater habitats such as lakes and ponds, and terrestrial habitats, most marine habitats except those in very sheltered areas are subject to extensive water movement and hence manipulation of the nutrient regime is logistically difficult. The mesocosm-like features of rockpools at low water periods provide a very tractable system for nutrient manipulation on open coasts (e.g. Methratta, 2004). Secondly these features also facilitate effective measures of productivity during low water periods (Nielsen, 2001).

# 2. Methods

# 2.1. Description of study site

Experiments were undertaken at Wembury Point, a moderately exposed shore in the south-west of England (50° 18.26N, 04° 04.45W)

and part of a Voluntary Marine Reserve. Tides are semi-diurnal and tidal range is approximately 6 m. The area of shore chosen for experimentation is semi-sheltered owing to protection from the prevailing weather by a small offshore island, the Mewstone. The area is composed of Lower Devonian Slate which presents a much-eroded marine platform with naturally abundant rockpools with a rich algal flora. Rock pools are dominated by a range of macroalgae with differing morphology including encrusting coralline algae (Phymatolithon spp. and Lithophyllum incrustans), turf forming species (predominantly Corallina officinalis), 'secondary space-holding' species (e.g. Chondrus crispus and Mastocarpus stellatus) and canopy forming species (e.g. Halidrys siliquosa, Sargassum muticum and other Fucales). In addition, ephemeral opportunistic algae, such as Ulva spp. are common although variable in their distribution in space and time. The common grazer species encountered within pools are the trochids Gibbula umbilicalis and Osilinus lineatus, the littorinid Littorina littorea and the limpet Patella ulyssiponensis. Other grazers abundant on emergent substrata which may forage within pools are the limpets Patella vulgata and Patella depressa.

## 2.2. Experimental design and set up

The experiment manipulated two factors, grazing intensity and level of nutrients in order to determine the effects of top down and bottom up forcing on early successional development of rock pool flora following disturbance. A factorial experiment was established with each factor having two levels; grazers were either left at natural densities or reduced, whilst nutrients were either left at ambient levels or artificially enhanced.

The experiment was established in May 2004. Twenty-four similarly sized natural rock pools were selected at mid-tide level (2.5 m– 3 m above Chart Datum) at the experimental site. Pools were chosen at least 3 m apart and within relatively well defined size limits (area between 0.25 and 0.7 m<sup>2</sup>; depth between 15 and 25 cm). Such small pools were relatively common on the shore used. All existing biota except limpets were removed using a paint scraper and a wire brush. Encrusting species such as *Lithophyllum incrustans* and *Phymatolithon* spp. were difficult to remove. These were left in place, hence the disturbance applied may be considered as establishing a basis for secondary rather than primary succession. Two fixed quadrats, 15×15 cm in size, were marked in each rock pool, using screws inserted into drilled holes.

The four treatments were randomly assigned to the 24 rock pools. In order to reduce grazing intensity in the appropriate treatments all molluscan grazers (Patella vulgata, Patella ulyssiponensis Gibbula umbilicalis, Osilinus lineatus, Littorina littorea and Littorina saxatilis) were removed from inside, as well as from an area of approximately one meter around each rock pool. Re-invading molluscs were removed every 2 days over the 6 month experimental period. Limpet re-invasion was rare but the mobility of trochids and littorinids meant that they accumulated in pools unless regularly removed. This approach avoided the necessity of fences or cages and the need for artefactual controls. Non-molluscan grazers such as amphipods and isopods were rare in the experimental pools during low water periods. While these mesoherbivores could potentially have invaded plots when immersed (e.g. Ingolfsson and Agnarsson, 2003) they were not considered here. Nutrient concentration was manipulated using the technique recommended by Worm et al. (2000). Slow release fertiliser (Osmocote® Miracle-Gro Granules : NPK formulation 14-13-13) was placed in dispensers made using two 5 cm long and 3 cm diameter plastic cylindrical tubes attached to a PVC tile and screwed to the bottom of the rock pool. Dispensers had sixty 2.2-mm holes distributed over their surface to allow effective nutrient release. They were filled with 60 g of fertilizer (packaged in fine mesh bags) in those rock pools which were assigned elevated nutrient concentrations. Empty nutrient dispensers were installed in the ambient nutrient rock pools as procedural controls. Nutrient dispensers were added to pools on May 10th and were refilled

with fertilizer twice during the experimental period, at the beginning of July and end of August.

## 2.3. Sampling

# 2.3.1. Macroalgal cover

Sampling of algal abundance was carried out four times during the six months of the experiment. Percentage cover of macroalgae was estimated using the point-intercept method by placing a plexiglas sampling grid of  $15 \times 15$  cm with 64 random points onto each of the two fixed quadrats. Two quadrats were used in this way to increase the accuracy of estimates of cover and the average value from both used as a single estimate of cover for each species. Macroalgae present in the quadrat but not recorded by this method were assigned a cover of 1%. Algae were identified in the field when possible, but in case of doubt, small samples were collected for further identification in the laboratory.

## 2.3.2. Primary Productivity

Community productivity was measured in August using an 'open system' method described by Kinsey (1985) and modified recently by Nielsen (2001) and Martins et al. (2007). Such measures allow estimates of community production in a field setting under natural environmental conditions. Measurements were taken under conditions of light wind to minimise diffusive loss of oxygen at the air;water interface. Water samples were taken three times over the low tide period using 300 ml Winkler bottles. The first sample was taken just after pool emersion and, immediately afterwards, each pool was covered with an opaque plastic screen to create an artificial darkness. After 2 hours the screens were removed and a second water sample was taken. The pools were then left uncovered for another 2-hour period after which a third water sample was taken. Dissolved oxygen in water samples was fixed in the field immediately after each water sample was taken using manganous sulphate followed by alkaline iodide-azide. Water samples were analysed for oxygen concentration in the laboratory over the next 24 hours, following the Winkler titration procedure (Grasshoff et al., 1999). Gross primary productivity (GPP) per unit area of substratum in each pool was calculated as: GPP per unit area =  $[(N+R) \times V]/SA$ , where N is the change in oxygen concentration over the light period (net primary production), R is the change in oxygen concentration over the dark period (respiratory demand), V is pool volume and SA is estimated area of substratum in the pool. Use of GPP rather than NPP was made since this accounts for potential variations in the level of respiratory demand due to differing animal abundance in pools. Full details of this open incubation procedure as applied to rockpools are described in Martins et al. (2007).

## 2.3.3. Grazing activity

The level of grazing activity of molluscan grazers was assessed using the method developed by Thompson et al. (1997). This utilises the rasping marks left by microphagous grazers in discs of dental wax to provide an index of grazing pressure. Jenkins et al. (2001) verified the effectiveness of this technique by showing that grazers showed little or no modification of grazing behaviour when encountering the wax. Five wax discs (diameter 14 mm) were placed in pre-formed holes in the surface of each rock pool during two periods of 14 days in June and August. Following each 14-day period all discs were returned to the laboratory. The grazing marks of trochids and limpets were recognized following the description of Thompson et al. (1997). The area of wax surface scraped on each disc was assessed under a dissecting light microscope. A transparent circular grid of 25 dots was overlaid on each wax disc and the number of dots overlying grazing marks was determined to provide an estimate of the percentage of grazed surface.

# 2.3.4. Nutrient concentration

Laboratory trials of the nutrient methodology showed it to be effective at elevating nutrient levels in mesocosms over a typical 6hour low water period. Field measurements of the nutrient concentrations in the experimental rock pools were carried out once during the experiment, on the 9th of July 2004, just under 2 months after nutrients were added to pools. The first sample was taken just after pool emersion and the second one 5 hours later, just before the incoming tide covered the pools again. Before taking the sample the water in the rock pools was stirred gently. Samples were collected 10 cm below the water surface at least 10 cm away from the nutrient dispenser using 60 ml polypropylene syringes and filtered through 45 GF/F filters into 60 ml acid washed bottles (10% HCl). The samples were placed immediately in a dark cool-box and transported to the laboratory where they were frozen within 2 hours of collection for subsequent nutrient analysis. Concentrations of nitrate+nitrite (subsequently referred to as nitrate) and phosphate were measured within 2 weeks using a Bran and Luebbe AAIII Segmented Flow Colorimetric Autoanalyzer.

## 2.4. Data analysis

Repeated measures ANOVA was used to assess change in treatment effects on macroalgal cover over time using SPSS. The assumption of compound symmetry was tested using Mauchly's test of sphericity. Following a significant treatment by time interaction, separate two way ANOVAs were performed at each of the four sampling dates to determine the effects of nutrient concentration and grazing level (both fixed factors). Following Moran (2003) analyses were not corrected for multiple testing and the associated increase in probability of type I errors. In this way we retained reasonable power in analyses. Prior to ANOVA, data were examined for heterogeneity of variance using Cochran's test and heterogeneous data transformed using square root or log transformations. Where heterogeneity existed despite transformation, a higher level of significance (P<0.01) was required. A posteriori multiple comparisons of treatment means were done using Student-Newman-Keuls (SNK) tests. All analyses, unless otherwise stated, were carried out using WinGmav5.

Multivariate analysis was performed on the macroalgal abundance data. A similarity matrix was calculated on square root transformed data and a multidimensional scaling ordination (MDS) produced using Primer v6. Distance-based permutational multivariable analysis of variance (PERMANOVA, Anderson, 2001) was used to analyze the multivariate assemblage data for the two-factor design including crossed factors Nutrients and Grazing. Finally, a SIMPER procedure was used to determine the contributions of different taxa to treatment differences (Clarke and Warwick, 2001). Where grazing effects only were apparent, SIMPER was used to determine which algae contributed to differences between the two grazer treatments, while for the significant interaction, pairwise comparisons were made among the 4 different treatments in an analogous way to multiple comparisons of a significant interaction term in ANOVA.

## 3. Results

#### 3.1. Efficacy of experimental manipulations

#### 3.1.1. Grazing activity

Analysis of wax discs allowed discrimination between the grazing marks left by *Patella* spp. which were relatively distinct and those of the other abundant grazers (*Gibbula umbilicalis, Osilinus lineata, Littorina littorea*) which were grouped as 'other grazers'. The index of grazing intensity provided by the wax discs clearly showed that manual grazer removal was successful in significantly reducing the level of grazing in manipulated pools, from both patellid limpets and 'other grazers' (Fig. 1). Two-way ANOVA showed a significant effect of grazer groups (June: patellid limpets F <sub>1,20</sub> 124.7, P<0.001; 'other grazers' F <sub>1,20</sub> 10.3, P<0.01; August: patellid limpets F <sub>1,20</sub> 109.2,



Fig. 1. Indices of grazing intensity indicated by use of wax discs in June and August 2004 in the four experimental treatments for patellid limpets alone, all other grazers (topshells and littorinids) and all grazers combined. Values are mean percentage cover of wax discs +1 SE.

P<0.001; 'other grazers' F  $_{1,20}$  27.6, P<0.001). Patellid limpets showed negligible levels of grazing following their removal reflecting the ease with which manual removal controlled their activity in pools. Manual removal of trochids and littorinids did not completely eliminate these taxa from pools but there was a 48% and 40% reduction in grazing intensity in June and August respectively (Fig. 1). Overall there was a 71% and 59% reduction in grazing intensity from all grazers combined



**Fig. 2.** Change in estimated total percentage cover of macroalgae in four experimental treatments over the time course of the experiment. Error bars  $\pm 1$  SE. Lines between sampling points are included to enhance clarity but do not indicate any measured pattern of change.

in June and August respectively in grazer removal treatments. There was no effect of nutrient addition on the level of grazing activity and no interaction between nutrient addition and grazer removal.

# 3.1.2. Nutrient enrichment

At the point of emersion, when rockpools were isolated from the sea, there was no significant difference in nitrate levels between ambient nutrient pools and elevated nutrient pools (mean±SE;  $1.11\pm$  0.15 versus  $1.23\pm0.19 \mu$ mol/L respectively). However there was a significant difference (one way ANOVA F  $_{1,20}$ =7.66; p=0.012) in phosphate levels, with double the concentration in the elevated nutrient pools (mean±SE; 0.15±0.03 versus 0.30±0.04 µmol/L).

#### Table 1

Repeated measures ANOVA of total algal cover under nutrient (Nu) and grazer (Gr) manipulations over four sampling dates

Source	df	MS	F	Р
Between subjects				
Grazing	1	19494.9	37.56	< 0.001
Nutrients	1	2167.5	4.18	0.054
Gr×Nu	1	137.7	0.26	0.61
Residual	20	519.1		
Within subjects				
Time	3	21097.16	73.72	< 0.001
Time×Gr	3	3728.78	13.03	< 0.001
Time×Nu	3	2111.12	7.37	< 0.001
Time×Gr×Nu	3	524.04	1.83	0.151
Residual	30	286.17		

All data untransformed. McCauly's test of sphericity non significant.

#### Table 2

Two way ANOVA of total algal cover under nutrient and grazer manipulations at each of four sampling dates

		June	July		August		October		
Source	df	F	Р	F	Р	F	Р	F	Р
Grazing	1	3.92	0.062	14.62	0.001	43.20	< 0.001	3.99	0.06
Nutrients	1	0.01	0.913	6.78	0.017	10.19	0.005	1.68	0.210
Gr×Nu	1	0.01	0.918	4.06	0.058	0.15	0.702	0.94	0.344
Residual	20								

All data untransformed. Cochran's test indicates homogenous variances for dates 1, 2 and 4, but heterogeneous for date 3.

After five hours of isolation over the period of low water, mean levels of nitrate and phosphate had increased dramatically to 440.5 ( $\pm$ 97.4) and 76.65 ( $\pm$ 20.06) µmol/L respectively in nutrient enhanced pools. While our sampling methodology did not determine the extent to which nutrient gradients built up in pools, it did show that nutrient dispensers were effective at raising mean nutrient concentrations to very high levels during the low water period. The low levels observed at the point of emersion indicated that during high water periods diffusion due to water movement ensured only slight local enhancement of phosphate levels.

# 3.2. Treatment effects on macroalgal cover and productivity

# 3.2.1. Total algal cover

Total algal cover increased over time in all treatments until the third sampling date in August (Fig. 2), after which there was a decline in all treatments except in the control, where grazers and nutrients were left un-manipulated. The rate of change in macroalgal cover differed among the four treatments; this is reflected in the significant treatment×time interactions in the repeated measures ANOVA (Table 1). Consequently treatment effects were examined separately at each sampling date. At the first sampling date, four weeks after establishment of the experiment, total algal cover was low and showed little difference between treatments (Table 2; Fig. 2). Large differences between treatments became apparent in July when total algal cover showed a large response under a regime of high nutrients and reduced grazing (SNK of marginally

## Table 3

Two way PERMANOVA of square root transformed algal community data at each of four sampling dates

		June		July		Augus	t	Octob	er
Source	df	F	Р	F	Р	F	Р	F	Р
Grazing	1	10.85	0.001	6.44	0.001	6.56	0.002	2.27	0.017
Nutrients	1	3.30	0.021	1.79	0.098	3.82	0.003	1.79	0.062
Gr×Nu	1	2.03	0.112	0.94	0.466	2.28	0.043	0.94	0.497
Residual	20								
Pairwise comparisons of significant interaction at Date 3:									
Ambient No grazer≠Grazers					Grazers present: Ambient nutrients =				
nutrients: present			Nutrients added.						
Nutrients No grazer≠Grazers			No grazers: Ambient nutrients≠Nutrients						
added:		present			added.				

non-significant interaction). Approximately four weeks later in August there was a significant effect of both grazing and nutrients, although these factors did not interact. Release from grazing pressure allowed high levels of algal cover, and nutrient addition had a positive effect on algal cover whether grazers were present or absent. The magnitude of the overall grazing effect (134%) was almost three times that of the nutrients (48%). The additive effect of the two factors resulted in the highest algal development where nutrients were added and grazing levels reduced. At the final sampling date in October treatments had converged. All treatments except the control showed a reduction in algal cover as the bloom of algae which had developed over the summer died back. Thus in October there was no longer an effect of nutrient addition and a marginally non-significant effect of grazing (P=0.06).

# 3.2.2. Algal cover and species composition

Differences in the community composition of treatments at each sampling date were highlighted by MDS plots (Fig. 3). PERMANOVA showed a significant effect of both grazing and nutrients on community composition even at the first sampling date in June although at the second date in July there was no evidence of effects of nutrients, only of grazing (Table 3). At the third sampling date in August when the peak of algal cover was reached, PERMANOVA showed an interaction between the two treatments. Pairwise comparisons indicated that the effect of



Fig. 3. Multi-dimensional scaling plots of algal community composition for each of 4 sampling points (t1-t4).



Fig. 4. Summary of percentage cover of dominant algae in each of four experimental treatments at the third sampling point in August 2004 at the peak of algal cover. Error bars + 1 SE.

nutrients on community composition was dependent on the grazing regime, with significant effects of nutrients only when algae were released from grazing (Table 3). At the final sampling date there was a significant effect only of grazing.

At the first sampling date in June, generally higher abundances of *Ulva* spp. and soft encrusting red algae (predominantly *Hildenbrandia* spp.) in ungrazed treatments and higher levels of the encrusting species *Ralfsia verrucosa* in grazed treatments contributed most toward significant grazing effects (SIMPER analysis). These same taxa also contributed most to the significant effect of nutrients (with higher levels of both *Ralfsia verrucosa* and soft reds without nutrients). In July, the combined effects of greater abundances of *Ulva* spp., *Spongomorpha* and *Polysiphonia* spp. in ungrazed treatments contributed almost 50% to the dissimilarity between grazer treatments. In August when algal cover peaked, *Ulva* spp. clearly dominated assemblages when nutrients were added, but was virtually absent at ambient nutrient levels, even when grazing was reduced (Fig. 4).

Under ambient nutrient levels *Ceramium* spp. dominated when grazing pressure was reduced. This difference is reflected in the output of SIMPER with *Ulva* spp. contributing almost one third toward

## Table 4

Two way Al	NOVA of macroalga	l productivity in	August 2004

Source	df	MS	F	Р		
Grazing	1	7.48	37.37	< 0.001		
Nutrients	1	12.21	61.02	< 0.001		
Gr×Nu	1	5.51	27.56	< 0.001		
Residual	20	0.20				
SNK pairwise co						
Ambient nutriei	nts:	No grazer=Gra	No grazer=Grazers present			
Nutrients added	l:	No grazer>Grazers present				
Grazers present: Ambient nutrients=Nutrients added						
No grazers: Ambient nutrients < Nutrients added						
D ( ) (						

Data untransformed with homogenous variances.



**Fig. 5.** Gross primary productivity in August 2004 in each of four experimental treatments. Error bars +1 SE.

dissimilarity between the two nutrient levels under reduced grazing. Also, under ambient nutrient levels *Ceramium* spp. contributed most to differences between grazing treatments whilst Ulva spp. contributed most when nutrients were added. In many other ways the community assemblies were quite similar with for example moderate levels of Ralfsia verrucosa and soft encrusting red algae across all treatments. At the final sampling date in October, Ulva spp. contributed most to differences among grazing treatments, although overall cover had declined. Ceramium spp. abundance was generally low, and both Ralfsia verrucosa (higher abundance in grazed treatments) and Fucus spp. (higher abundance in ungrazed treatments) contributed more towards differences. In addition to those taxa described above a diverse array of rarer taxa which showed little or no apparent pattern among treatments was observed. Additional taxa recorded were Corallina officinalis, Colpomenia peregrina, Halopteris spp., Leathesia difformis, Gelidium spp., Phymatolithon spp., Gigartina spp., Champia parvula, Laurencia spp., and Cladophora spp.

## 3.2.3. Primary Productivity

Gross primary productivity measured at the peak of algal cover in August demonstrated a clear interaction between the nutrient and grazing regime (Table 4; Fig. 5). Productivity was only enhanced where nutrients were added to pools in the absence of grazers; where grazers were present, nutrient addition had no effect on production.

# 4. Discussion

Manipulation of grazing and nutrient levels was effective at enhancing resource supply and reducing top down control. Analysis of wax discs showed that manual removal of grazers resulted in an overall reduction of grazing activity of approximately 65%. This treatment may be a more realistic simulation of localised reduction of grazer control, through for example human collection, natural predation or storm driven disturbance events than total grazer exclusion. Analysis of nutrient levels in the laboratory and field showed the slow release fertiliser was highly effective at elevating nutrient levels in enclosed rock pools during low water periods. Background ambient levels of DIN in August were around 1 µM, a level expected to result in nitrogen levels below concentrations for optimal growth for many ephemeral algal species. Typically 2-6 µM of DIN are required for optimal growth (Pedersen and Borum, 1997). Manipulation of nutrients produced extremely high and variable values in experimental pools, up to 400 times the natural ambient summer levels. Such levels only occasionally occur in coastal and estuarine waters when run-off from fertilised land is concentrated in sheltered areas (e.g. Sharp, 1983) and it was not our intention to simulate such high levels. However our manipulation will have ensured that nutrients were not limiting macroalgal growth and evidence from investigations into the use of intertidal algae in the treatment of waste water indicate that high levels are not typically harmful (Gordillo et al., 2002). Another aspect of our nutrient manipulation common to all studies which have utilised rockpools in this way (e.g. Nielsen, 2001; Methratta, 2004) is that enhanced levels of nutrients were delivered during low water when pools are isolated from the sea, whereas at high water significant dilution of the slow release fertiliser will have occurred. Such variability could potentially have an important effect on the response variables measured (e.g. Benedetti-Cecchi, 2003) but was not addressed in our study.

In parallel with many previous studies on rocky shores, nutrients and grazing had strong but opposing effects on macroalgal biomass and productivity. Nutrient enrichment of intertidal rock pools and a lowering of grazing intensity both resulted in an increase in macroalgal cover in an early successional community over the 6month period of study. The magnitude of grazing and nutrient effects, and the extent to which the effects of nutrient enrichment were dependent on the grazing regime, varied through the course of succession. In July, 2 months after establishment of the experiment, nutrient effects were only apparent when grazing was reduced. Hence at this stage strong top down control limited nutrient effects. In contrast, at the peak of algal cover in August an enhanced nutrient regime resulted in increased cover irrespective of grazer treatment. It was apparent that despite obvious nutrient effects the effect of grazers was approximately three times larger, emphasising the strong role of consumer control on exposed rocky shores (see Hawkins and Hartnoll, 1983 for review). However like Worm and Lotze (2006) we showed that despite strong grazer control, nutrient effects can result in algal blooms on intertidal rocky shores.

The question of the extent to which strong top down control can determine the outcome of an elevation of nutrient levels will determine the character of coastal communities subject to eutrophic conditions. Bokn et al. (2003) concluded that nutrient enrichment in itself may not be enough to stimulate structural changes in rocky shore communities. They observed little impact of substantial nutrient enrichment over a three year period on total algal biomass, community structure and primary production suggesting that littoral communities on moderately exposed rocky shores are resistant to local eutrophication. However further study on the same system recorded a rapid community shift after 5 years of nutrient addition with decline in canopy species and domination by green algae (Kraufvelin et al., 2006b). Other studies on rocky shores demonstrate conflicting conclusions and indicate outcome of manipulations may be strongly context dependent (Lotze et al., 2001; Nielsen, 2001; Worm et al., 2002; Bokn et al., 2003; Diaz-Pulido and McCook, 2003). For example Eriksson et al. (2006a) showed that the presence of canopy forming macroalgae limited experimental nutrient effects on biomass production presumably through light limitation. Other work has demonstrated that the nutrient status of the system under study has important implications when considering the extent to which nutrient effects are observed (Worm et al., 2002).

We showed obvious nutrient effects on early successional assemblages after clearing mature communities from pools. Our experimental set-up was not designed to replicate natural events, since complete loss of biota from pools is unlikely. Our aim was to provide space for colonisation, without competition from established perennial algae. These are ideal conditions for annuals to flourish in the absence of nutrient limitation. Localised loss of individual canopy plants and loss of understorey algae and molluscan grazers through the direct or indirect effects of wave action will open up patches of space which may be utilised by opportunistic algae. These conditions may increase owing to predicted loss of canopy algae as a result of reductions in water quality in coastal ecosystems (Benedetti-Cecchi et al., 2001; Gorgula and Connell, 2004) and under expected climate change scenarios which predict an increased frequency of storms (e.g. Bromirski et al., 2003) and hence enhanced disturbance regimes. In a highly disturbed environment such as intertidal rocky shores, enhanced nutrient levels may be expected to stimulate ephemeral colonisation and hence enhance coastal macroalgal productivity where grazing control is reduced and other perennial species are lost through physical disturbance or wide spread reductions in water quality.

In addition to changes in algal cover, there was a simultaneous change in algal species composition which was most apparent in August at the peak of algal abundance. The green ephemeral algae Ulva spp. dominated in nutrient enriched treatments whereas the red ephemeral alga Ceramium spp. dominated under ambient nutrient levels where grazing control was relaxed. These differences reflect the morphological and physiological characteristics of these algae. In general algal species with a high surface area to volume ratio tend to grow faster, require more nutrients and have higher nutrient uptake rates per unit time (Nielsen and Sandjensen, 1990; Pedersen and Borum, 1996; Taylor et al., 1998) than thicker algae with a lower surface area to volume ratio. Thus, thin foliose species such as Ulva spp. and Porphyra spp. and uniserate filamentous species such as *Cladophora* spp. and *Ectocarpus* spp. are expected to out perform thin corticated or siphonal filamentous algae such as *Polysiphonia* spp. and *Ceramium* spp. with a lower surface area to volume ratio under nutrient enrichment regimes (Karez et al., 2004). Our results support these expectations and the observations of Karez et al. (2004) and Bokn et al. (2003) who showed that the growth of corticated filamentous red algae such as *Ceramium* spp. was unaffected by nutrients.

Accumulation of biomass (or a surrogate of biomass such as percent cover) is used frequently as a response variable in investigations of ecological processes. More recently it has been used as a surrogate of productivity in studies of ecosystem functioning (e.g. Bruno et al., 2005) despite taking no account of biomass loss through senescence or consumption. We compared the pattern of macroalgal cover at its peak in August with a measure of community productivity. There was an apparent mismatch between the two measures. Relaxation of grazing control led to enhanced production only in the presence of elevated nutrient conditions, whereas macroalgal abundance was high both where nutrients were enhanced (dominated by Ulva spp.) and at ambient levels (dominated by Ceramium spp.). While differences in the identity of the dominant algae between treatments may have contributed to this mismatch through differences in species specific levels of productivity, initial investigations suggest not. In open incubations in replicate mesocosms there was no difference between levels of productivity per unit biomass in samples of Ulva spp. and Ceramium spp. collected from the same shore (Jenkins, unpublished data). An alternative reason may be the inadequacy of percentage cover estimates as surrogates of biomass. Percentage cover is a useful technique to assess abundance or biomass through non-destructive means. However it takes no account of differing thallus thickness or other morphological differences and hence in comparisons among different taxa it may be somewhat deficient. This point is rarely made, yet nondestructive sampling of macroalgae forms the basis of many studies in macroalgal ecology. For example O'Connor and Crowe (2005) measured both percentage cover and biomass of macroalgae in an investigation of consumer diversity effects on ecosystem functioning, yet discussion was predominantly based on significant percentage cover estimates. While our estimates of productivity were only made at one point in time they do suggest that relying solely on estimates of percentage cover may not reveal the complete picture with respect to energy flow and rates of ecosystem functioning.

In conclusion we showed that the way in which top down control, through molluscan grazing, and bottom up control, through nutrient supply, interacted in intertidal rockpools varied over the period of early succession. However there were clear nutrient effects on early successional assemblages, although these were of a lower magnitude than grazing effects. Manipulation of the nutrient regime resulted in assemblages which differed in the identity of dominant taxa. These differences may have contributed to a mismatch between estimates of abundance and productivity. Such observations are not only relevant for understanding of eutrophication in coastal systems but also for decisions on appropriate response variables of ecosystem functioning. Here we show that the relationship between top down and bottom up control of macroalgal production may depend on whether productivity is measured directly or indirectly through some measure of abundance or biomass accumulation.

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