

## **Cumulative effects of an invasive species and nutrient enrichment on rock pool communities**

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**Title:** Cumulative effects of an invasive species and nutrient enrichment on rock pool communities

**Running head:** Cumulative effects of multiple stressors

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## Abstract

Most ecosystems are affected by multiple anthropogenic stressors simultaneously, however, there is a lack of information describing the cumulative effects of many common stressor pairs. Consequently, we have but a rudimentary understanding of the roles that stressor characteristics and environmental context play in determining interactions among stressors. Nutrient enrichment often affects coastal ecosystems that may have already been affected by invasive species. To identify the effects of nutrient enrichment on communities under different invasion scenarios, the presence of the invasive fucoid algae, *Sargassum muticum*, and nutrient conditions were manipulated in the field to test for their independent and cumulative effects. Their combined effects on the diversity and functioning of rock pool communities were quantified. Rock pools with *S. muticum* contained fewer species, lower macroalgal and microalgal biomass, and their overall benthic assemblage structure differed from pools without *S. muticum*. Both the presence of *S. muticum* and nutrient enrichment affected different functional groups of algae differently. Their cumulative effects, however, did not differ with increasing intensity of nutrient enrichment. Furthermore, invaded communities from which *S. muticum* had been removed manually, tended towards greater species richness following removal than pools where *S. muticum* remained present, indicating a potential for recovery. These findings highlight the importance of identifying the cumulative effects of multiple stressors on the responses of individual functional groups, alongside effects on overall assemblage structure, in order to fully understand the consequences for ecosystems.

**Keywords:** *Sargassum muticum*, eutrophication, community ecology, biodiversity, multiple stressors, coastal ecology

## Introduction

An emerging field of research aims to disentangle the impacts of multiple anthropogenic stressors and to better predict their cumulative effects on ecosystems (Crain et al. 2008, Darling & Côté 2008, Russell & Connell 2012, Boyd & Hutchins 2012). Among the most common anthropogenic stressors in marine ecosystems are invasive species (Crain et al. 2008). Invasive species often occur in coastal and estuarine environments that are already affected by one or more anthropogenic stressors, which may affect invasion success and impact (Vitousek et al. 1997, Lotze et al. 2006). Predicting the impacts of invasive species under multiple stressor scenarios is limited by our lack of understanding of how environmental or experimental context determines their effects in natural communities and similarly, how invasive species alter the impacts of other stressors. The introduction of a secondary stressor, such as nutrient enrichment, to a system that is already under stress from an invasive species, may lead to stress-induced tolerance or stress-induced sensitivity of the community (Vinebrooke et al. 2004). Stress-induced sensitivity may occur when the effects of a stressor leads to a community that is less resilient towards the second stressor and the cumulative impact of the stressors is synergistic or greater than the sum of the individual effects. Alternatively, stress-induced tolerance may occur when the effects of the initial stressor drives the community to be more resilient to the effects of the second stressor and thus, the second stressor has a reduced impact and the cumulative impact of the two stressors is antagonistic (Folt et al. 1999, Vinebrooke et al. 2004). Where the combined effects of multiple stressors do not interact, their cumulative effect is additive, or equal to the sum of the individual effects (e.g. Vye et al. 2017).

Coastal ecosystems that are influenced by invasive species are often exposed to both press and pulse nutrient enrichment from a range of sources, such as land run-off and sewage outfalls (Lotze & Worm 2002, Lapointe et al. 2004). Nutrient enrichment may influence the

invasion process in some invasive seaweeds (Sanchez & Fernandez 2006, Vaz-Pinto et al. 2013, Uyà et al. 2017, Vieira et al. 2017). For example, nutrient enrichment has been shown to increase the invasion success of *Sargassum muticum*, a common invasive macroalga in Europe (Vieira et al. 2017), suggesting not only could nutrient enrichment affect the native community independently, but it could also increase the impacts of the invader on the native community structure and functioning. Therefore, the cumulative effect of these stressors would be synergistic. Alternatively, *S. muticum* could reduce the impacts of nutrient enrichment itself on the native community by exploiting the excess resource in nutrient enriched ecosystems, as outlined in the fluctuating resources theory presented by Davis et al. (2000). In this scenario, there may be effects of increased invasion success of the invader on the community, however, the interactive effect may be antagonistic as the effect of nutrient enrichment on community structure and functioning would be lessened.

The majority of experimental studies to date have focused primarily on the introduction of both nutrient enrichment and invasive species simultaneously (Vye et al. 2015, Vieira et al. 2017). Nutrient enrichment events, however, often occur in systems where invasive species are already established (Lotze et al. 2006). Testing how an established invasive species alters the impacts of a nutrient enrichment event on the native community is important to enable us to understand the cumulative effects of the stressors in a realistic scenario (Strayer 2012). Furthermore, a common management approach to invasive species is to undertake manual removal of individuals either for eradication or population control (Thresher & Kuris 2004). If the invasive species is modulating the impacts of another stressor, such as nutrient enrichment, indirect effects of removing the invader from the system can be complicated and unpredictable. As such, there is a need for experimental studies that allow a better understanding of the future consequences of invasive species management in coastal ecosystems influenced by multiple stressors (Zavaleta et al. 2001).

In addition to the presence or absence of stressors, other stressor characteristics, such as intensity or temporal variability, may also have a role in determining the cumulative effects of multiple stressors (Benedetti-Cecchi et al. 2006, Molinos & Donohue 2010, O'Connor et al. 2015). Stressor intensity has been shown previously to determine the cumulative impacts of invasive species and other stressors (Vye et al. 2015). Specifically, a recent study identified an antagonistic cumulative effect of the presence of the invasive fucoid, *S. muticum*, and nutrient enrichment on total algal biomass accumulation in an assembled rock pool community, but only at certain levels of nutrient enrichment (Vye et al. 2015). Such shifts in algal biomass production are an important proxy for energy flow and although not analogous with primary productivity *per se*, are indicative of an implicit change in ecosystem functioning (O'Connor & Crowe 2005, Masterson et al. 2008).

The aim of this study, therefore, was to identify and characterise the effects of nutrient enrichment on communities in rock pools that have been invaded by *S. muticum* and compare them with rock pool communities that have not been invaded by *S. muticum*. We also removed *S. muticum* manually from pools and compared these communities to those with and without *S. muticum*. This removal treatment was an essential control for the potentially confounding influences of factors that may covary with the presence of *S. muticum*. It also allowed us to assess the potential impacts of invader management by means of targeted removal over the peak growth season. The hypotheses tested were that: 1) the presence of an invasive species will modulate the effects of nutrient enrichment on benthic assemblage structure and diversity; 2) the presence of invasive species and nutrient enrichment will have different cumulative effects on different algal functional group biomass; and 3) the level, or intensity, of nutrient enrichment will determine the cumulative impacts of the presence of an invader and nutrient enrichment on benthic assemblage structure, diversity and functional group biomass.

## 115    **Materials and methods**

### 116    *Experimental site*

117    The experiment was conducted in intertidal rock pools on an exposed rocky shore at  
118    Muighinis, Co. Galway, Ireland (53°17'39.46"N, 9°51'2.87"W) between April and June 2014.  
119    The shore is comprised of exposed granite bedrock and has a tidal range of approximately  
120    four metres (Firth & Crowe 2010). These rock pool assemblages were comprised of many  
121    different morphological forms of algae including fucoids, such as *Fucus serratus* and  
122    *Halidrys siliquosa*, ephemeral green algae, including *Ulva* spp. and *Cladophora rupestris* and  
123    many species of red algae, both encrusting (e.g. *Lithothamnium* sp. and *Mesophyllum*  
124    *lichenoides*) and branched (e.g. *Gelidium* spp. and *Polysiphonia* spp.). Invertebrate  
125    communities in the pools included gastropod grazers, such as the limpet, *Patella*  
126    *ulyssiponensis*, the topshell, *Gibbula umbilicalis* and the periwinkle, *Littorina littorea*  
127    (O'Connor & Crowe 2005). Within the past two decades, a large proportion of the rock pools  
128    have also been colonised by the invasive fucoid *Sargassum muticum* (Baer & Stengel 2010),  
129    which increases in percentage cover during summer (February to July locally; Baer & Stengel  
130    2010).

### 131    *Experimental design*

132    A field experiment was designed to test for the individual and cumulative effects of the  
133    presence of an invasive species and nutrient enrichment on rock pool assemblage structure  
134    and functioning. Based on an orthogonal experimental design, the experiment allowed  
135    mensurative (i.e. natural presence vs. natural absence of invader) and manipulated  
136    comparisons (i.e. natural presence vs. manipulated removal of invader, Hurlbert 1984). The  
137    factorial experiment had two fixed factors: presence of an invader (three levels: present,  
138    absent and removed) and nutrient enrichment (three levels: ambient, intermediate, high), and

tested for all interactions among treatments. All nine treatments were replicated five times, each in separate rock pools with a minimum distance of two metres apart, yielding 45 experimental units. Fifteen rock pools without *Sargassum muticum* and thirty rock pools with *S. muticum* present were selected randomly on the mid shore. *Sargassum muticum* was removed carefully (using a flat chisel to ensure the whole of the holdfast was removed, preventing plant regrowth) from half of the rock pools with *S. muticum* present (15 pools). This removal treatment was important to test for and assess any potentially confounding variables that may have co-varied with the presence of *S. muticum* (O'Connor et al. 2006, Underwood 2009) and to identify nutrient enrichment effects on recovering communities after invader removal. All rock pools were similar in size (mean  $\pm$  S.E.: length =  $118.6 \pm 4.5$  cm; width =  $66.4 \pm 3.1$  cm; and depth =  $14.4 \pm 0.8$  cm), within the same shore height and exposure. The initial percentage cover of *S. muticum* in all invaded pools was  $7.3 \pm 1.7$  % (mean  $\pm$  S.E.), increasing to  $18.9 \pm 6.4$  % cover by the end of the experiment (peak growth period).

Rock pools were assigned randomly to treatments ( $n = 5$ ). To manipulate nutrient concentrations in nutrient enriched pools, Everris Osmocote® Exact (Geldermalsen, Netherlands) slow release fertiliser pellets (11N: 11P: 18K) were used (Worm et al. 2000, Atalah & Crowe 2010, O'Connor et al. 2015). Fertiliser was contained within mesh cases attached to the base of each pool. Ambient treatments contained an empty mesh bag to control for potential artefact effects of the presence of the bag. Intermediate and high nutrient enrichment treatments contained  $1 \text{ g l}^{-1}$  and  $3 \text{ g l}^{-1}$  of fertiliser pellets respectively. At the end of the experimental period, water samples were taken immediately after the emersion of the pools at low tide and were analysed using an autoanalyzer for dissolved inorganic nitrogen (DIN) and phosphate to confirm the efficacy of the nutrient enrichment treatments. Ambient treatments contained  $1.00 \pm 0.12 \text{ } \mu\text{m l}^{-1}$  DIN and  $0.17 \pm 0.01 \text{ } \mu\text{m l}^{-1}$  phosphate. Intermediate



nutrient enrichment treatments contained  $19.22 \pm 3.67 \mu\text{m l}^{-1}$  DIN and  $3.11 \pm 0.62 \mu\text{m l}^{-1}$  phosphate, and high nutrient enrichment treatments contained  $29.99 \pm 5.64 \mu\text{m l}^{-1}$  DIN and  $4.25 \pm 1.01 \mu\text{m l}^{-1}$  phosphate (DIN: MS = 38.12,  $F_{2,40} = 20.15$ ,  $P < 0.05$ , SNK post-hoc: A < N+ < N++; phosphate: MS = 6.64,  $F_{2,40} = 7.60$ ,  $P < 0.05$ , SNK post-hoc: A < N+ < N++). As the nutrient treatment represented a pulse of nutrient enrichment similar to that of land based run-off into the intertidal zone (Sharp 1983, O'Connor et al. 2015), a further subset of samples were taken 5 hours after initial emersion to identify how much nutrient flux occurred in the pools during the emersion period. In intermediate nutrient enrichment treatments, DIN ( $40.09 \pm 12.76 \mu\text{m l}^{-1}$ ) approximately doubled and phosphate concentrations ( $35.58 \pm 11.23 \mu\text{m l}^{-1}$ ) increased by a magnitude of ten over five hours. In high nutrient enrichment treatments DIN ( $111.66 \pm 57.74 \mu\text{m l}^{-1}$ ) approximately quadrupled and phosphate concentrations ( $78.24 \pm 28.27 \mu\text{m l}^{-1}$ ) increased by a magnitude of twenty. These concentrations are unlikely to limit macroalgal growth (Gordillo et al. 2002) and are similar to levels achieved in previous nutrient enrichment studies in intertidal systems (Atalah & Crowe 2010, O'Connor et al. 2015).

The experiment ran between April and June 2014 to focus on the peak growth period of the invasive species, *S. muticum* (Baer & Stengel 2010), which tends to die back in the winter months. This duration also reflects the length of similar multiple stressor experiments in artificial systems to allow for broad comparisons of findings (Boyer et al. 2009, Vye et al. 2015).

#### *Response variables*

Benthic assemblage structure (percentage cover of macroalgae and abundance of slow moving or sessile invertebrates >1cm) was quantified using a 25 cm x 25 cm quadrat with 64 intersections prior to the application of nutrient enrichment treatments and before *S. muticum*

was removed from the removal treatments, to test for any initial differences between invaded and non-invaded communities. One quadrat for each pool may have reduced statistical power, however, we have based our analyses on means of each pool (replicate) for each treatment. This size was chosen as it allowed a standard random sample to be taken in all pool shapes including the narrowest pools in the range. Quantification of benthic assemblage structure was repeated at the end of the experiment, similarly using a randomly placed 25 cm x 25 cm quadrat with 64 intersections in each rock pool. All species present in the quadrat, but not beneath an intersection, were recorded as 0.5 % cover. Algal taxa were identified to the lowest practicable taxonomic level using taxonomic keys (e.g. Dixon & Irwine 1977, Hiscock 1986) and epiphytes, which are common in this system, were not distinguished from other epilithic algae for analyses. Abundance of slow moving and sessile invertebrates (>1 cm) was also estimated at the same time. There were no initial differences in benthic assemblage structure between pools assigned to each treatment, including invaded and non-invaded communities (PERMANOVA:  $MS = 0.1869$ , pseudo- $F_{2,36} = 1.383$ ,  $P > 0.05$ ) based on an extensive survey prior to treatment allocation at the start of the experiment and before the anticipated *S. muticum* seasonal growth period.

At the conclusion of the experiment, species richness ( $N$ ), Shannon-Wiener diversity ( $H'$ ) and Pielou's evenness ( $J$ ) indices were also estimated for each pool based on percentage cover and abundance data. Furthermore, all benthic taxa were classified into functional groups and analysed to test for more general trends based on potential functional traits (Jänes et al. 2017, Table S1 in Supplementary Material). Algal taxa were assigned to functional groups based on their expected response to treatments and functional role in the rock pool community (Arenas et al. 2006). These included turf-forming algae (taxa typically < 5 cm vertical height) and sub-canopy space-holding algae, which were hypothesised to be affected by shading by *S. muticum* (Britton-Simmons 2004, Olabarria et al. 2009). Canopy algae were hypothesised to

be affected by competition for resources, such as space, by *S. muticum* (Viejo 1997). Green ephemerals and coralline algae were also classified into different functional groups (distinct from turf-forming taxa) because these groups have been shown to have different responses to nutrient enrichment (Hawkins et al. 1994, Delgado & Lapointe 1994, Karez et al. 2004, O'Connor 2013, Vieira et al. 2017). Invertebrate functional groups included grazing gastropods (e.g. winkles, *Littorina littorea*), suspension feeders (e.g. beadlet anemone, *Actina equina*) and mobile predators (e.g. dog whelk, *Nucella lapillus*; Little et al. 2009).

At the end of the experiment, destructive samples of algae were taken to quantify the effects of the presence of *S. muticum* and nutrient enrichment on total algal biomass and the biomass of each algal functional group (canopy, sub-canopy, turf, coralline, and green ephemerals, Table S1 in Supplementary Material). Algae were collected from within each quadrat, sorted into taxa, except for encrusting coralline algae (e.g. *Lithothamnium* sp.) and dried to a constant mass (at 60°C). Up to 80% of coralline algae consists of calcium carbonate and thus dry biomass of coralline algae was adjusted by a conversion factor of 0.2 (Griffin et al. 2010, Mrowicki & O'Connor 2015). Microalgal biomass was also estimated *in situ* by quantification of chlorophyll *a* concentration of biofilm in the rock pools (Murphy et al. 2005, Carpentier et al. 2013, Kahlert & McKie 2014, Mrowicki et al. 2014). Three measurements (1 cm<sup>2</sup> each) were taken randomly from the base of the pools using a benthic fluorometer (BenthosTorch, bbe Moldaenke®). The mean of these measurements was used in analyses to incorporate potential effects of small-scale variability (Sandulli & Pinckney 1999, Murphy et al. 2005). Three pools that had *S. muticum* removed and one pool from the treatment where it was thought *S. muticum* was absent were excluded from the analysis because the presence of the invader was noted (>0.5 % cover) at the end of the experiment, suggesting the experimental treatment was not effective in these pools.

237 Permutational multivariate analysis of variance (PERMANOVA; Anderson 2001, McArdle &  
 238 Anderson 2001) was used to test hypotheses relating to benthic assemblage structure  
 239 (percentage cover of macroalgae and abundance of slow moving or sessile invertebrates  
 240 >1cm) at the end of the study, with a similar factorial experimental design (two fixed factors:  
 241 the presence of *S. muticum* [three levels]; and nutrient concentration [three levels]). Non-  
 242 metric multi-dimensional scaling (nMDS) was used to visualise differences among  
 243 assemblages based on dissimilarities of their assemblage structure. Similarities of percentages  
 244 (SIMPER) analyses were used to identify which taxa contributed most to differences in  
 245 assemblage structure among treatments (Clarke & Warwick 2001). All multivariate analyses  
 246 were conducted on Bray-Curtis dissimilarity matrices with 9,999 permutations of residuals  
 247 under the reduced model and tested for all possible interactions among treatments. Data were  
 248 square-root transformed to reduce the influence of the highly abundant canopy species  
 249 (Clarke & Warwick 2001). The percentage cover estimates of *S. muticum* were not included  
 250 in analyses of assemblage structures to prevent confounding independent (manipulated) and  
 251 dependent (response) variables (Huston 1997) and to identify the impact of *S. muticum* on the  
 252 rest of the assemblage (Thomsen et al. 2016). Post-hoc pairwise *t*-tests were used to identify  
 253 differences between levels of significant terms. Post-hoc PERMDISP routines were  
 254 conducted on significant terms, after inspection of the nMDS plots, to detect differences in  
 255 the assemblages caused by treatment effects on the variation in assemblage structure rather  
 256 than shifts in assemblage structure. Percentage cover and abundance of taxa were classified  
 257 into functional groups and reanalysed to test for effects of the presence of *S. muticum* and  
 258 nutrient enrichment on functional diversity (Table S1 in Supplementary Material).  
 259 Analysis of variance (ANOVA) was used to test hypotheses relating to species richness,  
 260 Shannon-Wiener diversity and Pielou's evenness using a similar design with two fixed  
 261 factors: *S. muticum* (three levels) and nutrient concentration (three levels). *Sargassum*

*muticum* was not included in this analysis in order to identify effects on native diversity. In addition to the multivariate tests on functional group abundance data, a univariate approach was also undertaken to identify the biomass response of individual algal functional groups to *S. muticum* and nutrient concentration. This approach was considered prudent because algal biomass may be more indicative of different algal functional group contributions to energy flow and productivity than percent cover estimates (Masterson et al. 2008, Atalah & Crowe 2010, Crowe et al. 2011, White & Shurin 2011). To further disentangle the effect of the invader, biomass results for total macroalgae and canopy algae were analysed with and without *S. muticum* biomass (Thomsen et al. 2016). Data were first tested for normality and homogeneity of variances using Shapiro-Wilk and Levene's tests and were transformed where assumptions were not met. Shannon-Wiener diversity, green ephemeral, turf, coralline and canopy algal biomass were square-root transformed, microalgal biomass data were natural-log transformed and Pielou's evenness data were arcsine transformed. Student-Newman-Keuls post-hoc procedures were used to make comparisons among levels of significant terms. Post-hoc test results presented are with *S. muticum* biomass included. All analyses were undertaken in R 3.1.0 (R Development Core Team 2011) and PRIMER v6 (Clarke & Gorley 2006, Anderson et al. 2008).

## Results

At the end of the experiment, there was an effect of the presence of *S. muticum* on benthic assemblage structure (MS = 4056.5, pseudo- $F_{2,32} = 2.397$ ,  $P = 0.006$ ), however, there was no effect of nutrient enrichment (MS = 771.9, pseudo- $F_{2,32} = 0.456$ ,  $P > 0.05$ ). Post-hoc tests showed that assemblage structure in rock pools without *S. muticum* differed from those where it was present (Absent  $\neq$  Present,  $t = 1.793$ ,  $P = 0.008$ , Fig. 1) and from pools where it was removed experimentally (Absent  $\neq$  Removed,  $t = 1.731$ ,  $P = 0.004$ , Fig. 1). When all taxa were classified into functional groups, there remained an effect of the presence of *S. muticum*

287 on benthic assemblage structure ( $MS = 1748.4$ , pseudo- $F_{2,32} = 2.320$ ,  $P = 0.042$ ) and no  
 288 effect of nutrient enrichment ( $MS = 275.61$ , pseudo- $F_{2,32} = 0.366$ ,  $P > 0.05$ ). Post-hoc tests  
 289 again identified differences in assemblages without *S. muticum* compared to rock pools where  
 290 *S. muticum* was present (Absent  $\neq$  Present,  $t = 1.689$ ,  $P = 0.046$ ) or had been removed  
 291 (Absent  $\neq$  Removed,  $t = 2.240$ ,  $P = 0.007$ ). SIMPER analyses showed that these differences  
 292 in benthic assemblage structure were driven primarily by algal taxa, which were dominant in  
 293 both richness and abundance, rather than faunal taxa, and therefore, algal taxa were focused  
 294 on for the rest of the analysis. There was a greater proportion of the canopy alga, *Fucus*  
 295 *serratus*, the turf and sub-canopy algae (*Ceramium* spp. and *Chondrus crispus*), and all  
 296 ephemeral green algae (*Chaetomorpha* sp., *Ulva* spp. and *Cladophora rupestris*) in  
 297 treatments without *S. muticum* compared to treatments where it was present or had been  
 298 removed (Table S2 in Supplementary Material). In addition, there was an effect of the  
 299 presence of *S. muticum* on variation in benthic assemblage structure (PERMDISP:  $F_{2,38} =$   
 300  $4.886$ ,  $P = 0.016$ , Fig. 1), however, post-hoc tests could not identify conclusively where  
 301 differences among treatments lay because pools with and without *S. muticum* had similar  
 302 variation in assemblage structure to rock pools where *S. muticum* had been removed  
 303 experimentally. There was also an effect of the presence of *S. muticum* on species richness  
 304 ( $MS = 34.15$ ,  $F_{2,32} = 3.4165$ ,  $P = 0.045$ , Fig. 2), however, post-hoc tests could not identify  
 305 where differences lay because although species richness differed, i.e. was lower when *S.*  
 306 *muticum* was present than when it was absent, both these treatments were similar to the rock  
 307 pools where *S. muticum* was removed experimentally (Fig. 2). Nutrient enrichment did not  
 308 affect species richness ( $MS = 8.45$ ,  $F_{2,32} = 0.848$ ,  $P > 0.05$ ). Also, there was no effect of the  
 309 presence of *S. muticum* ( $MS = 0.052$ ,  $F_{2,32} = 2.267$ ,  $P > 0.05$ ), nor nutrient enrichment ( $MS$   
 310  $= 0.006$ ,  $F_{2,32} = 0.264$ ,  $P > 0.05$ ) on Shannon-Wiener diversity, nor were there any effects of

the presence of *S. muticum* (MS = 0.001,  $F_{2, 32} = 0.447$ ,  $P > 0.05$ ) or nutrient enrichment (MS = 0.001,  $F_{2, 32} = 0.341$ ,  $P > 0.05$ ) on Pielou's evenness.

There was an effect of the presence of *S. muticum* on total macroalgal biomass (Table 1a, Fig. 3A), however post-hoc tests were not fully conclusive because, although algal biomass was greater in the pools where *S. muticum* was present compared to pools where it was absent, both these treatments were similar to the treatment from which *S. muticum* was removed experimentally. There was no effect of nutrient enrichment on total macroalgal biomass (Table 1a, Fig. 3A). In terms of responses of individual functional groups of algae, pools where *S. muticum* was absent had a greater biomass of canopy algae than pools with *S. muticum* and pools where *S. muticum* had been removed, but there was no effect of nutrient enrichment (Table 1b, Fig. 3B). Furthermore, the impacts of *S. muticum* on total and canopy algal biomass did not differ depending on whether *S. muticum* biomass was included in the analysis or not (Table 1a & b, Fig. 3A & B). The effect of the presence of *S. muticum* on the biomass of sub-canopy algal species differed with nutrient enrichment (as indicated by the significant interaction between presence of *S. muticum* and nutrient enrichment, Table 1c), however, post-hoc tests were unable to identify where differences among treatments lay (Fig. 3C). The effects of the presence of *S. muticum* on the biomass of green ephemeral algae also differed with nutrient enrichment, however, post-hoc tests could not determine where precisely differences among treatments lay (Table 1f, Fig. 3F). There was no effect of the presence of *S. muticum* or nutrient enrichment on coralline or turf algal biomass (Table 1d & e, Fig. 3D & E). Microalgal biomass was lower in rock pools where *S. muticum* was present or removed compared to pools without *S. muticum* (Table 1g, Fig. 3G) and there was no effect of nutrient enrichment on microalgal chlorophyll *a* concentration (Table 1g, Fig. 3G).

## Discussion

335 This study found that pools where *Sargassum muticum* was present had different benthic  
336 assemblage structure from those without *S. muticum*, where the former tended to have a lower  
337 abundance of canopy algae and reduced benthic species richness. Surprisingly, none of the  
338 effects on assemblage structure were modulated by nutrient enrichment. Our findings did  
339 show, however, that nutrient enrichment had variable interactive cumulative effects with the  
340 presence of *S. muticum* on the biomass of some algal functional groups. There was no clear  
341 effect of intensity of nutrient enrichment, which suggests that, contrary to our hypotheses, the  
342 intensity of this stressor was not important in determining the cumulative effects in this rock  
343 pool system.

344 The invasion-driven shift in benthic assemblage structure coincided with the peak algal  
345 growth period. Unlike many native algal species, *S. muticum* undergoes senescence during  
346 the winter months, where it loses a large proportion of biomass and percentage cover, re-  
347 growing during spring and reaching peak biomass during summer months (Wernberg et al.  
348 2000, Baer & Stengel 2010). When taxa were classified into functional groups and re-  
349 analysed, the overall assemblage structure still differed where *S. muticum* was present, which  
350 shows that the effect of *S. muticum* was apparent on whole functional groups of algae not  
351 based on individual species responses. The increase in percentage cover of *S. muticum* during  
352 the experiment may have reduced light penetration to the understory species (Britton-  
353 Simmons 2004), and reduced space available for other canopy species, such as *F. serratus*  
354 (Viejo 1997, White & Shurin 2011). Furthermore, increased light intensity, daylight duration  
355 and temperature during the summer stimulates the growth of many native sub-canopy algal  
356 species, such as *Chondrus crispus* and *Furcellaria lumbricalis* (Bird et al. 1979), which may  
357 increase competition for primary resources with *S. muticum* (Britton-Simmons 2004). Thus,  
358 seasonal macroalgal growth, in combination with increases in percentage cover of *S.*



359 *muticum*, may have led to seasonal differences in benthic assemblages between the invaded  
360 and non-invaded communities (Thomsen et al. 2005).

361 The differences among benthic assemblages were characterised by a tendency towards  
362 increased variability of assemblage structure and towards decreased species richness in the  
363 presence of *S. muticum*. High spatial variability has been suggested to be a symptom of  
364 stressed communities when exposed to anthropogenic disturbances (Warwick & Clarke 1993,  
365 Cottingham et al. 2001, Ives & Carpenter 2007, Donohue et al. 2013). Although the findings  
366 of this study are contrary to others that identified an invasive species-driven homogenisation  
367 of communities (Olden & Rooney 2006, Baiser et al. 2012), increased variability may be  
368 expected owing to the non-linearity and context-dependency of invasion impacts (Parker et  
369 al. 1999, Thomsen et al. 2011, Vaz-Pinto et al. 2014). Our invaded assemblages also tended  
370 to be less species rich, which may contribute to increased variability within invaded rock  
371 pools (Loreau et al. 2001, Campbell et al. 2011). Increases in community variability driven  
372 by invasion could decrease the predictability of the response of communities to biological  
373 invasions. This could lead to ‘ecological surprises’ becoming more common and  
374 complicating management decisions (Paine et al. 1998).

375 Benthic assemblage structure was less variable and tended towards greater species richness  
376 and total algal biomass in assemblages where *S. muticum* had been removed, compared to  
377 assemblages where *S. muticum* remained present. Although some of these responses were not  
378 conclusive, this does indicate some potential for recovery. This finding provides some initial  
379 evidence that the removal or management of *S. muticum* could allow intertidal communities  
380 to begin to recover within a relatively short time period. However, there was no evidence of  
381 the recovery of canopy algal biomass, which may be owing to the slow growth rates  
382 associated with the primary native canopy species, *Fucus serratus* (Knight & Parke 1950).

383 The temporal duration of this study, designed to look at impacts during the main growth

season of *S. muticum*, may not have been long enough for these slow growing species to show detectable signs of recovery. Other invasive macroalgal removal studies have found a lag of five or more months before some species or functional groups of algae recover from the impacts of an invasive macroalgae, which can depend on other perturbations in the system as well as the original biomass of the invader (Piazzi & Ceccherelli 2006, Gribben et al. 2013). Although this study occurred over the main period of *S. muticum* growth, and therefore the main period of expected impact, further work should follow the trajectory of macroalgal communities over a longer duration to assess the full potential for community recovery after *S. muticum* removal.

Previous studies have identified antagonistic cumulative effects of the presence of *S. muticum* and nutrient enrichment on total algal biomass in artificial assemblages (Vye et al. 2015). The current study however, showed that the effects of *S. muticum* on total macroalgal and microalgal biomass were not determined by nutrient enrichment. Here, the presence of *S. muticum* was characterised by less total algal biomass regardless of whether *S. muticum* was included in the analysis, indicating that the presence of *S. muticum* drove decreases in the biomass of native species, such as the native canopy alga, *F. serratus*. Furthermore, there was also less total microalgal biomass in treatments where *S. muticum* had been removed suggesting that effects on total microalgal biomass were persistent even after removal of the invasive species. This could be due to a number of mechanisms, for example, the increase in algal biomass could indicate increased competition for resources with microalgae, such as light and space, inhibiting recovery (Williams et al. 2000). Natural communities, such as the rock pools in this study, are more complex and provide greater potential for species interactions than the artificially assembled communities used in mesocosm experiments (Bracken et al. 2008, Stachowicz et al. 2008, Crowe et al. 2012, Vye et al. 2015). Such species interactions may moderate the cumulative impacts of stressors and reduce the

sensitivity of communities to changes in stressor characteristics, such as intensity (Petersen & Hastings 2001). Furthermore, our previous mesocosm-based study, introduced both stressors simultaneously (Vye et al. 2015), whereas this field study introduced nutrient enrichment to pools where *S. muticum* was already established. The introduction of a second stressor to an already stressed or destabilised system may determine the interactive effect of the stressors (Mrowicki et al. 2016, Donohue et al. 2016). Our findings highlight the importance of testing multiple stressor hypotheses in different of environmental contexts, i.e. field as well as mesocosm experiments, in order to better understand the cumulative impacts of different communities and assemblages. This study has an obvious limitation in that it was only conducted on a single shore. Replicating multiple stressor studies at multiple sites would incorporate spatial variation in environment context which could then be tested explicitly (Bustamante & Branch 1996, Mrowicki et al. 2014).

Functional diversity has been shown previously to be an important determinant of the invasibility of a community (Britton-Simmons 2006, Arenas et al. 2006). Our findings suggest that species that use primary resources, such as light and space, differently may also respond differently to the cumulative effects of invasion and nutrient enrichment. The cumulative effects identified in this study were not consistent across all algal functional groups present, because nutrient enrichment affected invasion effects on green and sub-canopy algae, but not on canopy algae or turf algae. Mechanisms behind this interactive effect are unclear, however, it is possible that *S. muticum* may have limited light penetration to other algae (Britton-Simmons 2004), possibly offsetting nutrient enrichment effects on growth. Differences in the cumulative effects among functional groups may lead to further consequences for communities, including decreased resistance and resilience to other invasive species and anthropogenic stressors (Elmqvist et al. 2003, Folke et al. 2004). To conclude, this study shows complex cumulative effects of nutrient enrichment on

communities under different invasion scenarios, including the removal of the invader from the system. These findings highlight the importance of identifying the cumulative effects of multiple stressors on the responses of individual functional groups, alongside effects on overall assemblage structure, in order to fully understand the consequences for ecosystems.

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**Table 1.** ANOVA of effects of the presence of *S. muticum* and nutrient enrichment treatments on the biomass of algae: a) total algal biomass (values in brackets represent algal biomass with *Sargassum muticum* included); b) canopy algal biomass (values in brackets represent algal biomass with *Sargassum muticum* included); c) sub-canopy algal biomass; d) turf algal biomass; e) coralline algal biomass; f) green ephemeral biomass g) microalgal biomass. Significant values are in bold ( $P < 0.05$ ).

a) Total algal biomass				
Source	df	MS	<i>F</i>	<i>P</i>
Invasion (=Inv.)	2	2970 (2198)	4.491 (3.504)	<b>0.019 (0.042)</b>
Nutrient (=Nut.)	2	50 (103)	0.075 (0.165)	0.928 (0.849)
Inv. x Nut.	4	166 (154)	0.251 (0.246)	0.907 (0.910)
Residual	32	661 (627)		
b) Canopy algal biomass				
Invasion (=Inv.)	2	44.505 (30.340)	5.151 (4.099)	<b>0.012 (0.026)</b>
Nutrient (=Nut.)	2	5.780 (7.235)	0.669 (0.977)	0.519 (0.387)
Inv. x Nut.	4	10.918 (11.560)	1.264 (1.562)	0.305 (0.208)
Residual	32	8.639 (7.400)		
c) Sub-canopy algal biomass				
Invasion (=Inv.)	2	2.350	0.094	0.911
Nutrient (=Nut.)	2	1.160	0.046	0.955
Inv. x Nut.	4	78.740	3.154	<b>0.027</b>
Residual	32	24.960		
d) Turf algal biomass				
Invasion (=Inv.)	2	0.164	0.465	0.632
Nutrient (=Nut.)	2	0.065	0.185	0.832
Inv. x Nut.	4	0.106	0.302	0.874
Residual	32	0.351		
e) Coralline algal biomass				
Invasion (=Inv.)	2	2.127	2.875	0.071
Nutrient (=Nut.)	2	1.872	2.530	0.095

692	Inv. x Nut.	4	19.688	1.663	0.183
693	Residual	32	0.740		
694	f) Green ephemeral				
695	Invasion (=Inv.)	2	7.333	4.369	<b>0.021</b>
696	Nutrient (=Nut.)	2	1.725	1.028	0.369
697	Inv. x Nut.	4	4.526	2.696	<b>0.048</b>
698	Residual	32	1.679		
699	g) Microalgal biomass				
700	Invasion (=Inv.)	2	2.791	9.099	<b>0.001</b>
701	Nutrient (=Nut.)	2	0.291	0.949	0.398
702	Inv. x Nut.	4	0.303	0.988	0.428
703	Residual	32	0.307		

# Figure titles

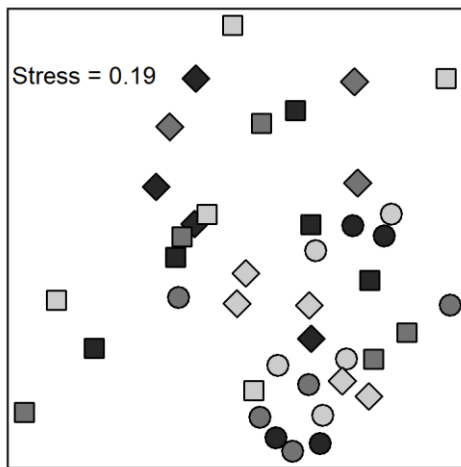


Figure 1. Non-metric multidimensional scaling plot (nMDS) of rock pool assemblages (percentage cover of macroalgae and abundance of slow moving or sessile invertebrates >1cm) in experimental treatments at the end of the experiment based on a Bray-Curtis similarity matrix (data are square-root transformed).  $\diamond$  = *S. muticum* removed;  $\bigcirc$  = *S. muticum* absent;  $\blacksquare$  = *S. muticum* present. Light grey symbols = ambient nutrient concentrations, mid grey symbols = intermediate nutrient concentrations and dark grey symbols = high nutrient concentrations.

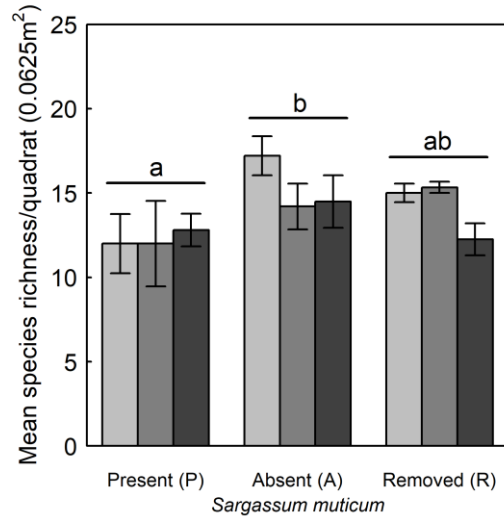


Figure 2. Mean species richness ( $\pm$  S.E.) of rock pools in experimental treatments. Light grey bars = ambient nutrient enrichment, mid grey bars = intermediate nutrient enrichment and dark grey bars = high nutrient enrichment ( $n = 3$ : invader removed, intermediate nutrient enrichment;  $n = 4$ : invader removed, high nutrient enrichment and invader absent, high nutrient enrichment;  $n = 5$ : all other treatments). Lower case letters indicate significant differences among treatment levels or groups or groups of treatments ( $P < 0.05$ ).



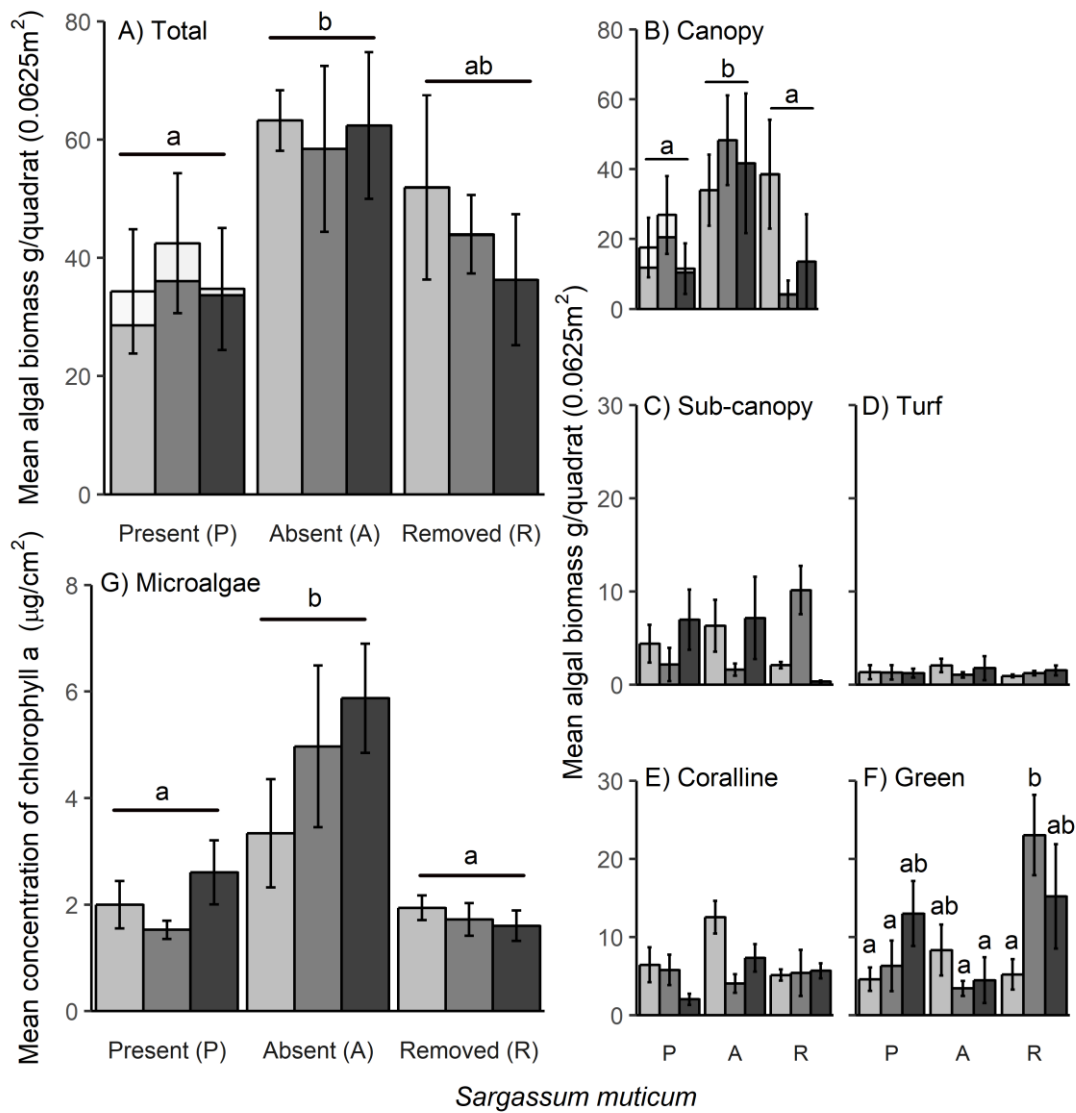


Figure 3. Mean biomass (± S.E.) of: A) total macroalgal biomass; B) canopy algal biomass; C) sub-canopy algal biomass; D) turf algal biomass; E) coralline algal biomass, F) ephemeral green algal biomass; and G) total microalgal chlorophyll *a* concentration (± S.E.), in rock pools where *S. muticum* was present, absent or removed. Light bars = ambient nutrient enrichment, mid grey bars = intermediate nutrient enrichment and dark grey bars = high nutrient enrichment, open section of bars = *S. muticum* biomass. Lower case letters indicate significant differences among treatment levels or groups or groups of treatments ( $P < 0.05$ ).