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

Vye, Siobhan; Dick, Jaimie T. A.; Emmerson, Mark C.; O'Connor, Nessa E.

Marine Ecology Progress Series

DOI:
10.3354/meps12529

Published: 26/04/2018

Peer reviewed version

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
Vye, S., Dick, J. T. A., Emmerson, M. C., & O'Connor, N. E. (2018). Cumulative effects of an invasive species and nutrient enrichment on rock pool communities. *Marine Ecology Progress Series*, *594*, 39-50. https://doi.org/10.3354/meps12529

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

Running head: Cumulative effects of multiple stressors

Authors: Siobhan R. Vye[1, 2, 3] *, Jaimie T.A. Dick[1, 2, 4], Mark C. Emmerson[1, 2, 4], Nessa E. O'Connor[1, 2, 4, 5]

[1]School of Biological Sciences, Medical Biology Centre, Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, UK.

[2]Queen's University Marine Laboratory, 12-13 The Strand, Portaferry, Co. Down, Northern Ireland, BT22 1PF, UK.

[3]Present address: School of Ocean Sciences, Bangor University, Askew Street, Menai Bridge, Isle of Anglesey, LL59 5AB, UK.

[4]Institute for Global Food Security, Queen's University Belfast, 18-30 Malone Road, Belfast, BT9 5BN, UK.

[5]Present address: School of Natural Sciences, Trinity College Dublin, Dublin 2, Ireland.

*Corresponding author: Siobhan R. Vye, Email: s.vye@bangor.ac.uk

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.

Materials and methods

Experimental site

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

Experimental design

A field experiment was designed to test for the individual and cumulative effects of the presence of an invasive species and nutrient enrichment on rock pool assemblage structure and functioning. Based on an orthogonal experimental design, the experiment allowed mensurative (i.e. natural presence vs. natural absence of invader) and manipulated comparisons (i.e. natural presence vs. manipulated removal of invader, Hurlbert 1984). The factorial experiment had two fixed factors: presence of an invader (three levels: present, 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 ± S.E.: length = 118.6 ± 4.5 cm; width = 66.4 ± 3.1 cm; and depth = 14.4 ± 0.8 cm), within the same shore height and exposure. The initial percentage cover of *S. muticum* in all invaded pools was 7.3 ± 1.7 % (mean ± S.E.), increasing to 18.9 ± 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 g l^{-1} and 3 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 ± 0.12 µm l^{-1} DIN and 0.17 ± 0.01 µm l^{-1} phosphate. Intermediate nutrient enrichment treatments contained 19.22 ± 3.67 µm l^{-1} DIN and 3.11 ± 0.62 µm l^{-1} phosphate, and high nutrient enrichment treatments contained 29.99 ± 5.64 µm l^{-1} DIN and 4.25 ± 1.01 µm 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 ± 12.76 µm l^{-1}) approximately doubled and phosphate concentrations (35.58 ± 11.23 µm l^{-1}) increased by a magnitude of ten over five hours. In high nutrient enrichment treatments DIN (111.66 ± 57.74 µm l^{-1}) approximately quadrupled and phosphate concentrations (78.24 ± 28.27 µm 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 (>1cm) 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^2 each) were taken randomly from the base of the pools using a benthic fluorometer (BenthoTorch, 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. mutium* 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.

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

Analysis of variance (ANOVA) was used to test hypotheses relating to species richness, Shannon-Wiener diversity and Pielou’s evenness using a similar design with two fixed 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 ≠ Present, t = 1.793, P = 0.008, Fig. 1) and from pools where it was removed experimentally (Absent ≠ 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* on benthic assemblage structure (MS = 1748.4, pseudo-$F_{2, 32}$ = 2.320, P = 0.042) and no effect of nutrient enrichment (MS = 275.61, pseudo-$F_{2, 32}$ = 0.366, P > 0.05). Post-hoc tests again identified differences in assemblages without *S. muticum* compared to rock pools where *S. muticum* was present (Absent ≠ Present, t = 1.689, P = 0.046) or had been removed (Absent ≠ Removed, t = 2.240, P = 0.007). SIMPER analyses showed that these differences in benthic assemblage structure were driven primarily by algal taxa, which were dominant in both richness and abundance, rather than faunal taxa, and therefore, algal taxa were focused on for the rest of the analysis. There was a greater proportion of the canopy alga, *Fucus serratus*, the turf and sub-canopy algae (*Ceramium* spp. and *Chondrus crispus)*, and all ephemeral green algae (*Chaetomorpha* sp., *Ulva* spp. and *Cladophora rupestris)* in treatments without *S. muticum* compared to treatments where it was present or had been removed (Table S2 in Supplementary Material). In addition, there was an effect of the presence of *S. muticum* on variation in benthic assemblage structure (PERMDISP: $F_{2, 38}$ = 4.886, P = 0.016, Fig. 1), however, post-hoc tests could not identify conclusively where differences among treatments lay because pools with and without *S. muticum* had similar variation in assemblage structure to rock pools where *S. muticum* had been removed experimentally. There was also an effect of the presence of *S. muticum* on species richness (MS = 34.15, $F_{2, 32}$ = 3.4165, P = 0.045, Fig. 2), however, post-hoc tests could not identify where differences lay because although species richness differed, i.e. was lower when *S. muticum* was present than when it was absent, both these treatments were similar to the rock pools where *S. muticum* was removed experimentally (Fig. 2). Nutrient enrichment did not affect species richness (MS = 8.45, $F_{2, 32}$ = 0.848, P > 0.05). Also, there was no effect of the presence of *S. muticum* (MS = 0.052, $F_{2, 32}$ = 2.267, P > 0.05), nor nutrient enrichment (MS = 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

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

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

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

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

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

Acknowledgements

We thank Brian Stewart of the Agri-Food and Biosciences Institute for nutrient analysis, Nadescha Zweschke, Alice Goward Brown and Katherine Oliver for assistance with the experiment, and the staff of Carna research station, NUIG, for provision of facilities. We also thank four anonymous reviewers whose comments greatly improved this manuscript. This study was completed as part of a PhD funded by the Department for Employment and Learning Northern Ireland with additional funding from the Challenger Society for Marine Science and a Royal Society Research Grant (RG 120432).

Literature cited

Anderson MJ (2001) Permutation tests for univariate or multivariate analysis of variance and regression. Can J Fish Aquat Sci 639:626–639

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E Ltd, Plymouth, UK

Arenas F, Sánchez I, Hawkins SJ, Jenkins SR (2006) The invasibility of marine algal assemblages: role of functional diversity and identity. Ecology 87:2851–61

Atalah J, Crowe TP (2010) Combined effects of nutrient enrichment, sedimentation and grazer loss on rock pool assemblages. J Exp Mar Bio Ecol 388:51–57

Baer J, Stengel DB (2010) Variability in growth, development and reproduction of the non-native seaweed *Sargassum muticum* (Phaeophyceae) on the Irish west coast. Estuar Coast Shelf Sci 90:185–194

Baiser B, Olden JD, Record S, Lockwood JL, McKinney ML (2012) Pattern and process of biotic homogenization in the New Pangaea. Proceedings Biol Sci 279:4772–7

Benedetti-Cecchi L, Bertocci I, Vaselli S, Maggi E (2006) Temporal variance reverses the impact of high mean intensity of stress in climate change. Ecology 87:2489–2499

Bird NL, Chen LCM, McLachlan J (1979) Effects of temperature, light and salinity on growth in culture of *Chondrus crispus*, *Furcellaria lumbricalis*, *Gracilaria tikvahiae* (Gigartinales, Rhodophyta), and *Fucus serratus* (Fucales, Phaeophyta). Bot Mar 22:521–528

Boyd P, Hutchins D (2012) Understanding the responses of ocean biota to a complex matrix of cumulative anthropogenic change. Mar Ecol Prog Ser 470:125–135

Boyer KE, Kertesz JS, Bruno JF (2009) Biodiversity effects on productivity and stability of marine macroalgal communities: the role of environmental context. Oikos 118:1062–1072

Britton-Simmons KH (2004) Direct and indirect effects of the introduced alga *Sargassum muticum* on benthic, subtidal communities of Washington State, USA. Mar Ecol Ser 277:61–78

Britton-Simmons KH (2006) Functional group diversity, resource preemption and the genesis of invasion resistance in a community of marine algae. Oikos 3:395–401

Bracken MES, Friberg SE, Gonzalez-Dorantes CA, Williams SL (2008) Functional consequences of realistic biodiversity changes in a marine ecosystem. Proc Natl Acad Sci U S A 105:924–8

Bustamante R, Branch G (1996) Large scale patterns and trophic structure of southern African rocky shores: the roles of geographic variation and wave exposure. J Biogeogr 23:339–351Campbell V, Murphy G, Romanuk TN (2011) Experimental design and the outcome and interpretation of diversity-stability relations. Oikos 120:399–408

Carpentier C, Dahlhaus A, Giesen N van de, Maršálek B (2013) The influence of hard substratum reflection and calibration profiles on in situ fluorescence measurements of benthic microalgal biomass. Environ Sci Process Impacts 15:783–93

Clarke K, Gorley R (2006) PRIMER v6: User Maunal/Tutorial. PRIMER-E Ltd, Plymouth

Clarke K, Warwick R (2001) Changes in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E Ltd, Plymouth

Cottingham KL, Brown BL, Lennon JTJT (2001) Biodiversity may regulate the temporal variability of ecological systems. Ecol Lett 4:72–85

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecol Lett 11:1304–1315

Crowe T, Frost N, Hawkins S (2011) Interactive effects of losing key grazers and ecosystem engineers vary with environmental context. Mar Ecol Prog Ser 430:223–234

Crowe TP, Bracken MES, O’Connor NE (2012) Reality check: issues of scale and abstraction in biodiversity research, and potential solutions. In: Paterson DM, Aspen RJ, Solan M (eds) Marine Biodiversity and Ecosystem Functioning: Frameworks, Methodologies and Integration. Oxford, p 185

Darling ES, Côté IM (2008) Quantifying the evidence for ecological synergies. Ecol Lett 11:1278–86

Davis MA, Grime JP, Thompson K, Davis A, Philip J (2000) Fluctuating resources in plant communities : a general of invasibility theory. J Ecol 88:528–534

Delgado O, Lapointe BE (1994) Nutrient-limited productivity of calcareous versus fleshy macroalgae in a eutrophic, carbonate-rich tropical marine environment. Coral Reefs 13:151–159

Dixon PS, Irwine L. (Eds) (1977) Seaweeds of the British Isles, Volume 1 Part 1: Rhododphyta. Introduction, Nemaliales, Gigartinales. British Museum (Natural History), London

Donohue I, Petchey OL, Montoya JM, Jackson AL, McNally L, Viana M, Healy K, Lurgi M, O'Connor NE, Emmerson MC (2013) On the dimensionality of ecological stability. Ecol Lett 16:421–9

Donohue I, Hillebrand H, Montoya JM, Petchey OL, Pimm SL, Fowler MS, Healy K, Jackson AL, Lurgi M, McClean D, O'Connor NE, O'Gorman EJ, Yang Q (2016) Navigating the complexity of ecological stability (F Adler, Ed.). Ecol Lett 19:1172–1185

Elmqvist T, Folke C, Nystrom M, Peterson G, Bengtsson J, Walker B, Norberg J (2003) Response diversity, ecosystem change, and resilience. Front Ecol Environ 1:488–494

Firth LB, Crowe TP (2010) Competition and habitat suitability: small-scale segregation underpins large-scale coexistence of key species on temperate rocky shores. Oecologia 162:163–174

Folke C, Carpenter S, Walker B, Scheffer M, Elmqvist T, Gunderson L, Holling CS (2004) Regime shifts, resilience, and biodiversity in ecosystem management. Annu Rev Ecol Evol Syst 35:557–581

Folt CL, Chen CY, Moore M V, Burnaford J (1999) Synergism and antagonism among multiple stressors. Limnol Oceanogr 44:864–877

Gordillo FJL, Dring MJ, Savidge G (2002) Nitrate and phosphate uptake characteristics of three species of brown algae cultured at low salinity. Mar Ecol Prog Ser 234:111–118

Gribben PE, Byers JE, Wright JT, Glasby TM (2013) Positive versus negative effects of an invasive ecosystem engineer on different components of a marine ecosystem. Oikos 122:816–824

Griffin J, Noël L, Crowe T, Burrows M, Hawkins S, Thompson R, Jenkins S (2010) Consumer effects on ecosystem functioning in rock pools: roles of species richness and composition. Mar Ecol Prog Ser 420:45–56

Hawkins SJ, Proud SV, Spence SK, Southward AJ (1994) From the individual to the community and beyond: water quality, stress indicators and key species in coastal waters. In: Sutcliffe DW (ed) Water quality & stress indicators in marine and freshwater systems: linking levels of organisation. Freshwater Biological Association, Ambleside, UK, p 35–62

Hiscock S (1986) A Field Guide to the British Red Seaweeds. Field Studies Council

Hurlbert S (1984) Pseudoreplication and the Design of Ecological Field Experiments. Ecol Monogr 54:187–211

Huston MA (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. Oecologia 110:449–460

Ives AR, Carpenter SR (2007) Stability and diversity of ecosystems. Science 317:58–62

Jänes, H, Kotta, J, Pärnoja, M, Crowe, TP, Rindi, F, Orav-Kotta, H (2017) Functional traits of marine macrophytes predict primary production. Funct Ecol 31, 975–986.

Kahlert M, McKie B (2014) Comparing new and conventional methods to estimate benthic algal biomass and composition in freshwaters. Environ Sci Process impacts 16:2627–2634

Karez R, Engelbert S, Kraufvelin P, Pedersen MF, Sommer U (2004) Biomass response and changes in composition of ephemeral macroalgal assemblages along an experimental gradient of nutrient enrichment. Aquat Bot 78:103–117

Knight M, Parke M (1950) A Biological Study of *Fucus Vesiculosus* L. and *F. Serratus* L. J Mar Biol Assoc United Kingdom 29:439

Lapointe BE, Barile PJ, Matzie WR (2004) Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. J Exp Mar Bio Ecol 308:24–58

Little C, Williams GA, Trowbridge CD (2009) The biology of rocky shores. Oxford University Press

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294:804–8

Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC, Bay M (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312:1806–1809

Lotze HK, Worm B (2002) Complex interactions of climatic and ecological controls on macroalgal recruitment. Limnol Oceanogr 47:1734–1741

Masterson P, Arenas FA, Thompson RC, Jenkins SR (2008) Interaction of top down and bottom up factors in intertidal rockpools: Effects on early successional macroalgal community composition, abundance and productivity. J Exp Mar Bio Ecol 363:12–20

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82:290–297

Molinos JG, Donohue I (2010) Interactions among temporal patterns determine the effects of multiple stressors. Ecol Appl 20:1794–1800

Mrowicki RJ, Maggs CA, Connor NEO (2014) Does wave exposure determine the interactive effects of losing key grazers and ecosystem engineers? J Exp Mar Bio Ecol 461:416–424

Mrowicki RJ, Maggs CA, O’Connor NE (2015) Consistent effects of consumer species loss across different habitats. Oikos 124:1555–1563

Mrowicki RJ, O’Connor NE, Donohue I (2016) Temporal variability of a single population can determine the vulnerability of communities to perturbations (C Thornber, Ed.). J Ecol 104:887–897

Murphy RJ, Underwood AJ, Pinkerton MH, Range P (2005) Field spectrometry: New methods to investigate epilithic micro-algae on rocky shores. J Exp Mar Bio Ecol 325:111–124

O’Connor NE (2013) Impacts of sewage outfalls on rocky shores : Incorporating scale , biotic assemblage structure and variability into monitoring tools. Ecol Indic 29:501–509

O’Connor NE, Bracken ME, Crowe TP, Donohue I (2015) Nutrient enrichment alters the consequences of species loss. J Ecol 103:862–870

O’Connor NE, Crowe TP (2005) Biodiversity loss and ecosystem functioning : distinguishing between number and identity of species. Ecology 86:1783–1796

O'Connor NE, Crowe TP, McGrath D (2006) Effects of epibiotic algae on the survival, biomass and recruitment of mussels, *Mytilus* L. (Bivalvia : Mollusca). J Exp Mar Bio Ecol 328:265–276

Olden JD, Rooney TP (2006) On Defining and Quantifying Biotic Homogenization. Glob Ecol Biogeogr 15:113–120

Paine RT, Tegner MJ, Johnson EA (1998) Compounded perturbations yield ecological surprises. Ecosystems 1:535–545

Parker IM, Simberloff D, Lonsdale WM, Goodell K, Wonham M, Kareiva PM, Williamson MH, Holle B Von, Moyle PB, Byers JE, Goldwasser L (1999) Impact: toward a framework for understanding the ecological effects of invaders. Biol Invasions 1:3–19

Petersen JE, Hastings A (2001) Dimensional approaches to scaling experimental ecosystems: designing mousetraps to catch elephants. Am Nat 157:324–33

Piazzi L, Ceccherelli G (2006) Persistence of biological invasion effects: Recovery of macroalgal assemblages after removal of Caulerpa racemosa var. cylindracea. Estuar Coast Shelf Sci 68:455–461

R Development Core Team (2011) R: a language and environment for statistical computing (RDC Team, Ed.). R Found Stat Comput 1:409

Russell BD, Connell SD (2012) Origins and consequences of global and local stressors: incorporating climatic and non-climatic phenomena that buffer or accelerate ecological change. Mar Biol 159:2633–2639

Sanchez I, Fernandez C (2006) Resource availability and invasibility in an intertidal macroalgal assemblage. Mar Ecol Ser 313:85–94

Sandulli R, Pinckney J (1999) Patch sizes and spatial patterns of meiobenthic copepods and benthic microalgae in sandy sediments : a microscale approach. J Sea Res 41:179–187

Sharp J (1983) The distributions of inorganic nitrogen and dissolved and particulate organic nitrogen in the sea. In: Elsevier (ed) Nitrogen in the marine environment.p 1–35

Stachowicz JJ, Best RJ, Bracken MES, Graham MH (2008) Complementarity in marine biodiversity manipulations: reconciling divergent evidence from field and mesocosm experiments. Proc Natl Acad Sci U S A 105:18842–7

Strayer DL (2012) Eight questions about invasions and ecosystem functioning. Ecol Lett 15:1199–1210

Thomsen MS, Wernberg T, Olden JD, Griffin JN, Silliman BR (2011) A framework to study the context-dependent impacts of marine invasions. J Exp Mar Bio Ecol 400:322–327

Thomsen MS, Wernberg T, South PM, Schiel DR (2016) To include or not to include (the invader in community analyses)? That is the question. Biol Invasions 18:1515–1521

Thomsen MS, Wernberg T, Stæhr PA, Pedersen MF (2005) Spatio-temporal distribution patterns of the invasive macroalga *Sargassum muticum* within a Danish Sargassum-bed. Helgol Mar Res 60:50–58

Thresher RE, Kuris AM (2004) Options for managing invasive marine species. Biol Invasions 6:295–300

Underwood AJ (2009) Components of design in ecological field experiments. Ann Zool Fenn 46:93–111

Uyà M, Maggi E, Mori G, Nuccio C, Gribben PE, Bulleri F (2017) Carry over effects of nutrient addition on the recovery of an invasive seaweed from the winter die-back. Mar Environ Res

Vaz-Pinto F, Olabarria C, Arenas F (2013) Role of top-down and bottom-up forces on the invasibility of intertidal macroalgal assemblages. J Sea Res 76:178–186

Vaz-Pinto F, Olabarria C, Arenas F (2014) Ecosystem functioning impacts of the invasive seaweed Sargassum muticum (Fucales, Phaeophyceae). J Phycol 50:108–116

Vieira R, Pinto IS, Arenas F (2017) The role of nutrient enrichment in the invasion process in intertidal rock pools. Hydrobiologia 797:183–198

Viejo RM (1997) The effects of colonization by *Sargassum muticum* on tidepool macroalgal assemblages. J Mar Biol Assoc United Kingdom 77:325–340

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104:451–457

Vitousek PM, D'Antonio CM, Loope L, Rejmanek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global environmental change. N Z J Ecol 21:1–16

Vye SR, Emmerson MC, Arenas F, Dick JTA, O'Connor NE (2015) Stressor intensity determines antagonistic interactions between species invasion and multiple stressor effects on ecosystem functioning. Oikos 124:1005 – 1012

Vye SR, Emmerson MC, Dick JTA, O'Connor NE (2017) Cumulative effects of multiple stressors: An invasive oyster and nutrient enrichment reduce subsequent invasive barnacle recruitment. J Exp Mar Bio Ecol 486:322–327

Warwick RM, Clarke KR (1993) Increased variability as a symptom of stress in marine communities. J Exp Mar Bio Ecol 172:215–226

Wernberg T, Thomsen MS, Stæhr PA, Pedersen MF (2000) Comparative Phenology of *Sargassum muticum* and *Halidrys siliquosa* (Phaeophyceae : Fucales) in Limfjorden, Denmark. Bot Mar 43:31–39

White LF, Shurin JB (2011) Density dependent effects of an exotic marine macroalga on native community diversity. J Exp Mar Bio Ecol 405:111–11

Williams G, Davies M, Nagarkar S (2000) Primary succession on a seasonal tropical rocky shore: the relative roles of spatial heterogeneity and herbivory. Mar Ecol Prog Ser 203:81–94

Worm B, Reusch TBH, Lotze HK (2000) In situ nutrient enrichment: Methods for marine benthic ecology. Int Rev Hydrobiol 85:359–375

Zavaleta ES, Hobbs RJ, Mooney HA (2001) Viewing invasive species removal in a whole-ecosystem context. Trends Ecol Evol 16:454–459

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 ($P < 0.05$).

Source	df	MS	*F*	*P*
		a) Total algal biomass		
Invasion (=Inv.)	2	2970 (2198)	4.491 (3.504)	**0.019** (**0.042)**
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)	**0.012** (**0.026)**
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	**0.027**
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

Inv. x Nut.	4	19.688	1.663	0.183
Residual	32	0.740		
f) Green ephemeral				
Invasion (=Inv.)	2	7.333	4.369	**0.021**
Nutrient (=Nut.)	2	1.725	1.028	0.369
Inv. x Nut.	4	4.526	2.696	**0.048**
Residual	32	1.679		
g) Microalgal biomass				
Invasion (=Inv.)	2	2.791	9.099	**0.001**
Nutrient (=Nut.)	2	0.291	0.949	0.398
Inv. x Nut.	4	0.303	0.988	0.428
Residual	32	0.307		

Figure titles

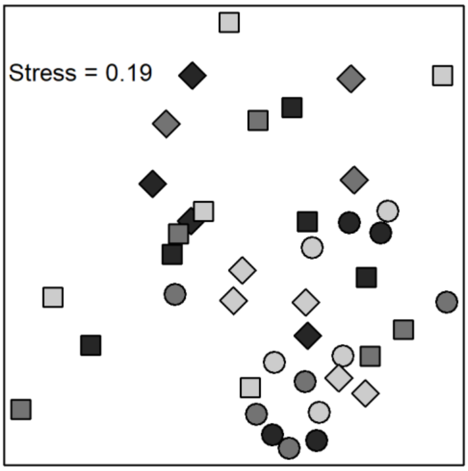

Figure 1. Non-metric multidimensional scaling plot (nMDS) of rock pool assemblages (percentage cover of macroalgae and abundance of slow moving or sessile invertebrates >1cm) in experimental treatments at the end of the experiment based on a Bray-Curtis similarity matrix (data are square-root transformed).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

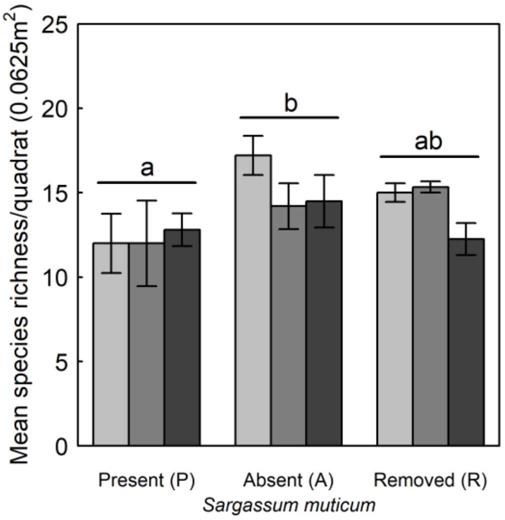

Figure 2. Mean species richness (± S.E.) of rock pools in experimental treatments. Light grey bars = ambient nutrient enrichment, mid grey bars = intermediate nutrient enrichment and dark grey bars = high nutrient enrichment (*n* = 3: invader removed, intermediate nutrient enrichment; *n* = 4: invader 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).

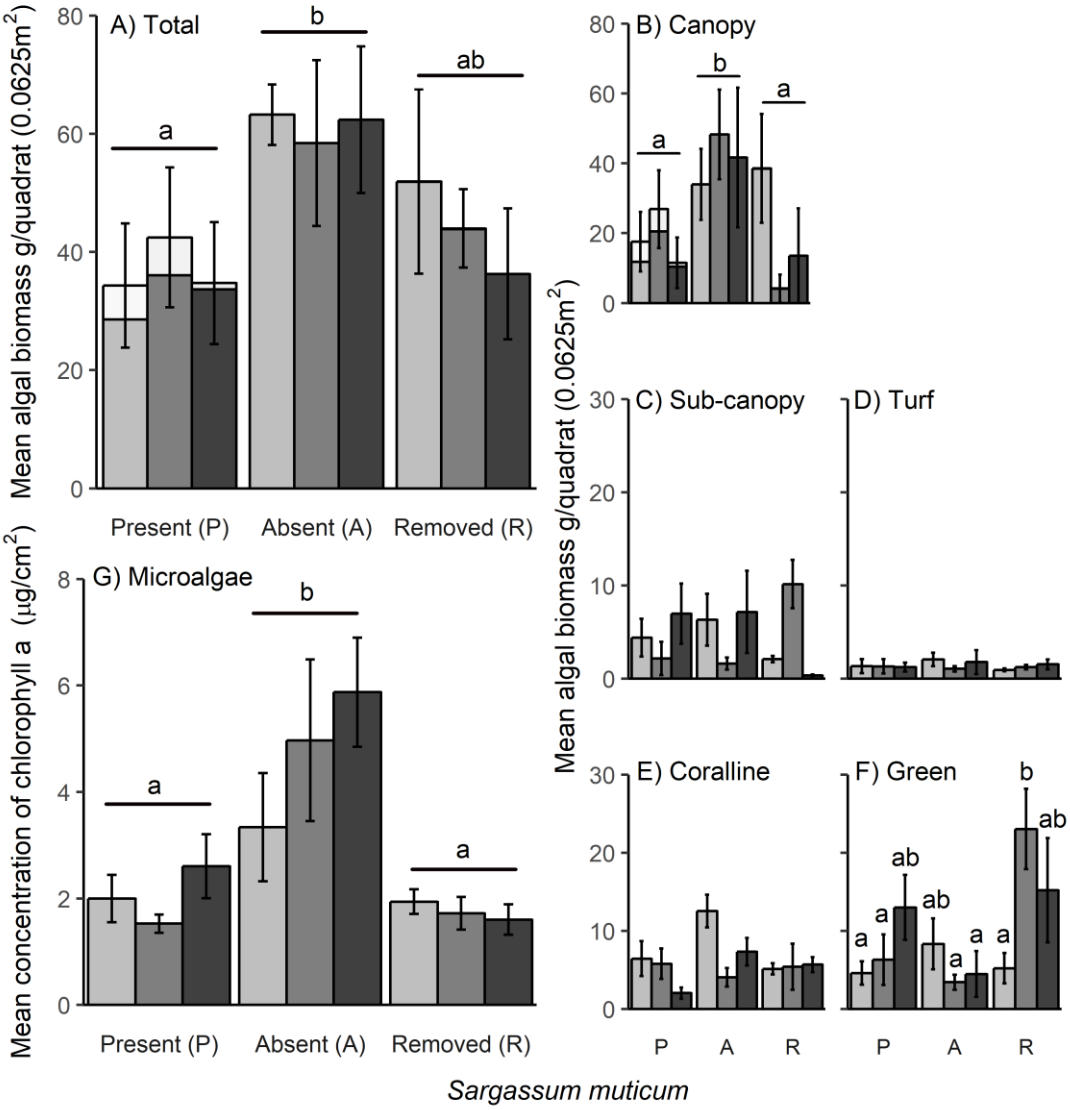

Figure 3. Mean biomass (± S.E.) of: A) total macroalgal biomass; B) canopy algal biomass; C) sub-canopy algal biomass; D) turf algal biomass; E) coralline algal biomass, F) ephemeral green algal biomass; and G) total microalgal chlorophyll *a* concentration (± S.E.), in rock pools where *S. muticum* 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 ($P < 0.05$).