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Cumulative effects of multiple stressors: An invasive oyster and nutrient enrichment reduce subsequent invasive barnacle recruitment

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ABSTRACT

Studies identifying interactions between biological invasions and other stressors have generally focussed on quantifying their cumulative effects on mature species assemblages. In benthic systems, however, early life history processes are key determinants of assemblage structure and functioning. This study tested whether the presence of an invasive species affected early life history processes of two common barnacle species and whether this was affected by a second common stressor, nutrient enrichment. The results of a field experiment identified and characterised the effects of an invasive oyster, *Crassostrea gigas*, on the early life history processes of the two barnacle species under ambient and enriched nutrient conditions. In the presence *C. gigas*, the invasive barnacle *Austrominius modestus*, had a lower recruitment rate, however, there was no effect of the presence of *C. gigas* on native barnacle, *Semibalanus balanoides*, recruitment. Nutrient enrichment also reduced the recruitment rate of *A. modestus*, however, there was no evidence of synergistic or antagonistic interactions between these stressors, indicating their cumulative effects were additive. There was no effect of nutrient enrichment on native barnacle recruitment. Our results show that the presence of an invasive oyster and nutrient enrichment altered the recruitment of another non-native benthic species. These findings emphasise the importance of considering early life history processes when assessing effects of multiple stressors on communities.

1. Introduction

Identifying and quantifying the impacts of multiple anthropogenic stressors, such as invasive species and nutrient enrichment, is a research priority in order to understand and predict potential detrimental effects on ecosystems (Crain et al., 2008; Sutherland et al., 2009; Strayer, 2012). Interactions between invasive species and other anthropogenic stressors can lead to cumulative effects that are additive or are greater than (synergistic) or less than (antagonistic) the sum of the individual effects (Folt et al., 1999; Crain et al., 2008). Synergistic cumulative effects on communities are thought to be the most common (Sala and Knowlton, 2006) and their occurrence has been supported by several empirical studies. For example, Piazzi et al. (2005) showed a decline in percentage cover of erect algal species when exposed to the invasive green algae *Caulerpa racemosa* var. *cylindracea* in increased sedimentation regimes. Conversely, antagonistic interactions have also been identified, such as the ability of the invasive freshwater zebra mussel, *Dreissena polymorpha*, to negate the effects of nutrient enrichment on algal biomass (Dzialowski and Jessie, 2009), and the presence of *Sargassum muticum*, an invasive fucoid algae, mediating the effects of nutrient enrichment and warming on algal biomass (Vye et al., 2015).

To date, studies have focussed on the context-dependent impacts of biological invasions on the diversity and functioning of mature communities (e.g. Queiros et al., 2011; Green and Crowe, 2014). In benthic ecosystems, the structure and functioning of a mature community can be determined by early life history processes, such as larval settlement and post-settlement mortality (Connell, 1985; Gaines and Roughgarden, 1985; Hunt and Scheibling, 1997; Aguilera and Navarrete, 2012). Settlement, defined as the permanent attachment of larvae to the substratum (Connell, 1985), is often determined by larval supply and a range of settlement cues that indicate habitat suitability and resource availability, such as the presence of free space and biofilm abundance (Strathmann et al., 1981; Rodriguez et al., 1993). Early post-settlement mortality may be driven by predation, disturbance or physiological stress (Menge and Sutherland, 1987). Both settlement and early post-settlement mortality can constrain recruitment into the adult population and, therefore, are important components of benthic species population dynamics (Gosselin and Qian, 1997; Delany et al., 2003; Jenkins, 2005). The relative importance of these early life history processes in structuring communities can be context specific. Early post-settlement mortality is generally

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more important in determining population structure in species with high recruitment rates, such as barnacles (Connell, 1961a; Gosselin and Qian, 1996), whereas populations of species with a lower larval supply, such as some species of corals (Hughes et al., 2000), crustaceans (Wahle and Incze, 1997) and echinoderms (Balch and Scheibling, 2000), are more likely to be affected by differences in settlement rates (Connell, 1961a). Invasive species, in combination with other stressors, such as nutrient enrichment or warming, may drive changes in settlement and post-settlement mortality by altering physical conditions, such as substratum type and hydrological regimes, and biological interactions, such as competition and predation (Gutierrez et al., 2003; Wilkie et al., 2013).

In coastal ecosystems, bivalve molluscs are common invasive species. Outside of its native range, the Pacific oyster, *Crassostrea gigas*, has wide-ranging and context-dependent effects on recipient communities, including driving shifts in native species assemblage structures (Kochmann et al., 2008), differences in ecosystem functioning rates (Green et al., 2012), and the co-introduction and facilitation of other invaders (Ruesink et al., 2005). Often the impacts of *C. gigas* increase in intensity as invasion progresses and the density of the oysters increases (Yokomizo et al., 2009; Green and Crowe, 2013). Although the impacts of *C. gigas* on mature communities are well documented (e.g., Padilla, 2010), little is known about the potential interactions between *C. gigas* and native or invasive species at early life history stages (Wilkie et al., 2012).

C. gigas forms feral populations in inlets and estuaries, where eutrophication is a common co-occurring stressor that can affect the impacts of biological invasions on recipient communities (Lotze et al., 2006; Gennaro and Piazzi, 2011; Vaz-Pinto et al., 2013). Thus, testing whether the presence and density of *C. gigas* interacts with nutrient enrichment to affect settlement and recruitment processes is a realistic scenario from which to identify the context-dependent effects of invasive species. A field experiment was designed to test for the separate and cumulative effects of the presence of *C. gigas* and nutrient enrichment on benthic species settlement and recruitment rates. Specifically, the hypothesis tested were: (1) the presence of invasive *C. gigas* and nutrient enrichment will affect the identity and abundance of other benthic species settlers and recruits; (2) these putative effects will interact, such that the effect of the presence of the invasive oyster on other benthic species settlement and recruitment will differ between ambient and enriched nutrient conditions; (3) the cumulative effects of the presence of the invasive oyster and nutrient enrichment on other benthic species will be determined by oyster density.

2. Material and methods

2.1. Study site

The field experiment ran from February through to August 2013 at Ballygreen, a sheltered intertidal sedimentary shore on the south western shore of Lough Swilly, Co. Donegal, Ireland (55° 2′ 31.54″ N, 7° 33′ 36.06″W). At this site, boulders are common and scattered on sediment comprised of sandy mud, pebbles and shell fragments. Tides are semidiurnal and have a maximal range of approximately 4.5 m. The study was conducted at mid shore where boulders were colonised primarily by the native barnacle *Semibalanus balanoides* and the non-native barnacle *Austrominius modestus* (formerly *Elminius modestus*), the fucoid algae *Fucus vesiculosis*, the honeycomb worm *Sabellaria alveolata* and the keel worm, *Pomatoceros triqueter. Austrominius modestus* has spread rapidly since its introduction to the UK and Ireland in the 1940s and may compete with native barnacle species (Bishop, 1947; Crisp, 1958; Lawson et al., 2004). Lough Swilly is a relatively unpolluted estuary compared to other more densely populated coastal areas of Ireland that have been classified as eutrophic in assessments of water quality (Bradley et al., 2015).

2.2. Experimental design and set up

To quantify benthic species recruitment under manipulated conditions, forty grey opaque Perspex® settlement plates (210 mm × 148 mm × 5 mm) were attached to the side of boulders (one per boulder), which had been selected randomly along approximately 40 m × 10 m of mid shore dominated by barnacles and *Fucus vesiculosis*. Grey Perspex**®** was chosen to represent natural conditions based on the colour of the bedrock to minimise any differences in thermal regime between the settlement plates and boulders (Lathlean and Minchinton, 2012). Each plate was sanded for 30 s using coarse sand paper to ensure suitable rugosity for settlement (Jara et al., 2006). Plates were attached to boulders at least 2 m apart using stainless steel screws (Stachowicz et al., 2002; Canning-Clode et al., 2008).

An orthogonal experimental design included two fixed factors: (i) presence of the invasive *C. gigas* at four levels: absent, 1 individual (ind.) per plate, 4 ind. or 8 ind. (equivalent to approximately 0, 32, 129, and 515 individuals per m^2); and (ii) nutrient enrichment at two levels: ambient conditions and nutrient enriched. Each treatment was replicated five times, yielding 40 experimental units. Settlement plates were allocated randomly to treatments. Non-reproductive triploid oysters (Guo and Allen, 1994) from a local aquaculture facility were used to minimise effects on the feral oyster population. Juvenile oysters (spat) were used in the experiment and were six months old and 36 ± 0.5 mm in length, similar to the age and size of naturally settled spat at the time the experiment commenced. Spat were attached to the front surface of the settlement plates using Milliput® epoxy putty (Dolgellau, Wales). Previous work showed that there were no differences in assemblages associated with *C. gigas* attached using this method compared to those with *C. gigas* attached naturally (Vye, unpublished results).

Localised nutrient enrichment was achieved by attaching nutrient diffusers (drilled 50 ml sample tubes) to each plate. Diffusers were filled with 140 g of Everris Osmocote® Exact (Geldermalsen, Netherlands) slow release fertilizer pellets (11N:11P:18K) similar to previous studies (e.g., Hall et al., 2000; Minchinton and McKenzie, 2008; O'Connor and Donohue, 2013). Ambient treatments had diffusers filled with shell fragments to limit potential experimental artefacts. Analysis of water samples from within a 15 cm radius of experimental plates 8 weeks after the addition of fertilizer pellets using the same method indicated that nutrient enrichment was effective (ambient total oxidised nitrogen (mean ± S.E.): 10.54 ± 0.81 μm l^{-1}, enriched total oxidised nitrogen: 14.24 ± 1.44 μm l^{-1}, ANOVA: $F_{1,\,14} = 5.014$, $P = 0.042$).

The top surface of each plate was monitored every two to four weeks to ensure treatments were maintained and photographed at eight weeks and 24 weeks (Fig. 1). Abundance of all species on each plate

Fig. 1. Experimental plate showing settlement and recruitment of barnacles after 24 weeks.