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Do the threats of predation and competition alter larval behaviour and selectivity at settlement under field conditions?

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ABSTRACT

The temporary ability of larvae to select optimal substrata during settlement can strongly influence the success of sedentary benthic invertebrates. Larval responses to cues from algae, epilithic microbial assemblages and conspecific species are well recognised. Comparatively, little is known about how the threat of predation or of competition influences settlement. We used a manipulative field experiment to test if the presence of a predator or spatial competitor affects settlement patterns of two intertidal mussel species, *Perna perna* and *Mytilus galloprovincialis*. By deploying treated artificial units of settlement habitat (larval collectors) in 'no choice' and 'choice' combinations, we determined whether the observed settlement patterns resulted from active larval choices (preference/avoidance). Among treatments where no choice was offered, settlement was generally greater on the predator treatment, lower on treatments with epilithic biofilm or competitor presence, and least on collectors without biofilm. In all but one case, the observed proportions of settlement in the 'choice' tests were significantly different from that expected by random association based on the 'no choice' tests. Choices related primarily to the absence of biofilm and to the threat of predation, with little influence of competitor presence. Although active preferential behaviour was confirmed, patterns of selectivity differed between the two study sites. Settlers of both species preferred the predator treatment at one site, possibly suggesting a predator swamping strategy. In contrast, settlers at the second site avoided the presence of predators. This suggests that settlement behaviour may be contingent on site-specific factors and demonstrates a greater degree of flexibility in settlement behaviour than expected.

1. Introduction

Predation and competition are well recognised as being among the principal biological variables influencing the distributions of marine benthic invertebrates (Connell, 1961; Connolly and Roughgarden, 1999; Dayton, 1971; Menge, 1976). Given the direct effects of predation and competition on the survival and fitness of benthic species, behaviours and phenotypic traits that enable organisms to avoid or to mitigate these factors are ecologically relevant (e.g. Cotton et al., 2004; Griffiths and Richardson, 2006; Rochette et al., 1997).

In adults of various taxa, physical defences or deterrents such as thickened shells, stronger musculature and allelopathy (toxic deterrence) are used in response to predators or competitors (e.g. Cheung et al., 2004; Freeman and Byers, 2006; Freeman, 2007; Koh and Sweatman, 2000; Sammarco and Coll, 1992). Similarly, avoidance behaviours, such as increasing burrowing depth in the presence of a predator are employed by other species (Griffiths and Richardson, 2006). Larvae and recently settled individuals have fewer possibilities for physical (phenotypic) deterrence against predators and potential competitors, and seem mainly to use behavioural strategies, which may include active avoidance, habitat associations that result in avoidance (e.g. use of spatial refuges), delayed metamorphosis, or synchronised settlement to 'swamp' predators (e.g. Boudreau et al., 1993; Grosberg, 1981; Johnson and Strathmann, 1989; Manríquez et al., 2013; Metaxas and Burdett-Coutts, 2006; Vail and McCormick, 2011; Welch et al., 1997; Young and Chia, 1981).

Larval responses to cues are expected to be particularly important in the case of sessile or sedentary species, for which settlement affords a brief window of selectivity, resulting in the potential for substantial selective pressure (Manríquez et al., 2013; Rittschof et al., 1998; Webster, 2002). While it is known that settling larvae and juveniles of many taxa respond to positive and/or negative waterborne cues from sources such as conspecifics, bacterial communities, algae and other extracts (Dobretsov, 1999; Keough and Raimondi, 1995; Soares et al., 2008; Zhao and Qian, 2002), less is understood of the role that the threat of predation and competition plays in this context. Vail and McCormick (2011) highlighted that avoidance behaviours are inherently stage-specific in nature, and argued that knowledge of these sorts of

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behaviours in transitional life stages, such as during settlement, is important and widely lacking. In this sense, cues from resident predators or competitor species could have strong effects on initial settlement patterns and potentially, on the longer-term success of settling organisms (Boudreau et al., 1993; Grosberg, 1981; Rittschof et al., 1998; Vail and McCormick, 2011; Webster, 2002; Welch et al., 1997).

In the field, settlement and recruitment patterns are the result of variations in processes such as larval delivery, settler habitat preference, early mortality and post-settlement movement (Caselle and Warner, 1996; Connell, 1985; Crowe and Underwood, 1998). Distinguishing among these influences is particularly difficult. Moreover, avoidance behaviour of settling larvae (in response to cues) can take the form of relatively large scale movements such as larval descent from surface waters to avoid water-column predators (e.g. McKelvey and Forward, 1995; Metaxas and Burdett-Coutts, 2006), but can also occur at small scales (cm to m). For example, settling crab megalopae discriminate over small scales between predator-inhabited and predator-free treatments (Rittschof et al., 1998; Welch et al., 1997).

Avoidance responses to the threat of predation have been exhibited by larvae and juveniles of several invertebrate taxa in laboratory (Boudreau et al., 1993; Manríquez et al., 2013; Metaxas and Burdett-Coutts, 2006; Welch et al., 1997) and field experiments (e.g. Johnson and Strathmann, 1989; Welch et al., 1997). Generally, responses in these studies are seen as movement away from the source of predator cues or increased settlement on cue-free substrata. Findings concerning the influence of competitor presence are more variable, and are not consistent or ubiquitous among taxa or experimental conditions. For example, several studies have identified some form of competitor avoidance by settling invertebrates (Grosberg, 1981; Petersen, 1984; Sweatman, 1988; Young and Chia, 1981), while some found no effect of competitor presence on settlement patterns (Bullard et al., 2004; Denley and Underwood, 1979; Vail and McCormick, 2011), and yet others have found increased rates of metamorphosis in response to the presence of competitors (Durante, 1991).

Although previous field studies demonstrate that predator and, in some cases, competitor presence can alter settlement rates, it is not known whether the observed patterns are due to active behaviour (preference or avoidance) or other causes, such as differential mortality or an unknown habitat association (Crowe and Underwood, 1998; Jennings and Hunt, 2010; Mercier et al., 2000). To address this uncertainty it is important to test correctly for behavioural preference using a two-stage “choice experiment” that can separate associations from active behaviour (Olabarria et al., 2002; Underwood et al., 2004). This method has been used in a variety of studies to identify preferences of invertebrates for different habitats and prey (Cole et al., 2012; Crowe and Underwood, 1998; Liszka and Underwood, 1990).

Because the applicability of laboratory findings to field conditions remains unknown, particularly when behaviour is the variable being investigated (Crowe and Underwood, 1998; Olabarria et al., 2002), field experiments were designed for the present study. We tested the hypothesis that settling intertidal mussels, *Perna perna* and *Mytilus galloprovincialis*, have preferences among four treatments of manipulated artificial habitat (untreated, biofilm conditioned, predator-inhabited and competitor-inhabited). Using the choice/no-choice design, we determined whether the threat of predation or of competition influenced settlement patterns of intertidal mussels and whether the observed patterns resulted from active larval preference behaviour.

2. Methods

To determine if *Perna perna* and *Mytilus galloprovincialis* displayed preferential settlement behaviour in response to predator or competitor-inhabited substrata, we adapted the laboratory methodology of Olabarria et al. (2002) as a field experiment. This was done by deploying clusters or ‘rosettes’ of artificial habitat (plastic mesh pot-scourers) which offered the different experimental treatments as either choice combinations or single treatment (no choice) rosettes. This choice-type method quantifies the inherent settlement patterns when no choice of treatments is provided, and uses these data to calculate a null expectation. Observed settlement from assays where a choice of treatments is provided is then tested against the null expectation. By making this comparison it is possible to test statistically if behaviour differs between situations where a choice is offered and those where no choice is offered.

2.1. Pre-treatment and preparation

During the austral autumn of 2011 (16th to 18th April), four treatments were prepared using plastic pot scourers (e.g. King et al., 1990; Menge, 1992). Three treatments involved modification of the scourers so as to introduce natural biofilm (B), biofilm plus a competitor (BC) or, biofilm plus a predator (BP), while the fourth treatment comprised untreated scourers (N). The natural biofilm treatment was used as a positive control as it is known that mussels respond to the presence of biofilm on experimental scourers (von der Meden et al., 2010). To allow the development of natural microbial and microalgal communities constituting biofilm, all scourers to be used for manipulated treatments (B, BC, BP) were soaked in an aerated tank of filtered (50 μm) seawater for 4 d; the water was collected from the study sites and changed daily.

Dogwhelks (*Nucella* spp.) are among the main predators of mussels, particularly of juveniles of 1–5 mm in shell-length (e.g. Dye, 1991; Moreno, 1995). A locally abundant species, *Nucella dubia*, was selected for the experiment. A common limpet species, *Scutellastra cochlear* was selected as a relevant competitor. Limpets have a range of disruptive effects on competing sessile organisms, including bulldozing, dislodgement and crushing (Denley and Underwood, 1979; Menge et al., 2010). Although such interactions can be mediated by wave exposure, established patellid limpet populations are known to compete with encroaching adult mussel beds and prevent mussel recruitment by disrupting settlement, particularly on semi-exposed shores (Steffani and Branch, 2003, 2005). During the third day of preparation, live dogwhelks (*N. dubia*) and limpets (*S. cochlear*) were collected. They were maintained in aerated aquaria and the limpets were allowed to attach to a thin flexible PVC sheet that had been placed on the bottom of the aquarium. This minimised disturbance to the animals during transfer to the treatments as the sheet could be cut around each limpet to separate individuals. The sheet also allowed limpets to seal naturally against a surface, protecting them during the experiment and preventing their desiccation and the release of unusual cues (e.g. distress). The pot-scourers are made of tubes of coarse plastic mesh rolled to form a pad. Scourers to be used for competitor and predator treatments were unrolled slightly to allow a single animal (either competitor or predator) to be glued with a fast drying 2 part epoxy into the scourer's centre using the animal's shell as the contact point. Scourers were then re-rolled and stitched closed. For the fourth treatment, designated as ‘New’ (N), scourers were unmanipulated.

The effect of the epoxy glue used in the predator and competitor treatments was tested at one of the experimental sites. No significant differences in settlement were found between collectors with glue and those without for either *P. perna* ($t_{crit} = 2.45$, d.f. = 6, P = 0.92) or for *M. galloprovincialis* ($t_{crit} = 2.45$, d.f. = 6, P = 0.76).

2.2. Field experiment

Two study sites, separated by approximately 800 m, were selected for the experiment at Brenton-on-Sea on the south coast of South Africa (−34.075° S; 23.0204° E). To test for preference, ‘choice’ and ‘no-choice’ rosettes of scourers were assembled and deployed on the evening low tide of the fourth day of preparation (Fig. 1). The ‘no-choice’ rosettes were made by attaching four scourers of a single treatment to an eye-bolt using small plastic cable-ties threaded through each scourer,