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Use of extract from adults of the triangle barnacle, *Balanus trigonus*, for reducing fouling in mussel farms

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ABSTRACT

Many millions of dollars are lost each year in the green-lipped mussel (*Perna canaliculus*) aquaculture industry in New Zealand due to fouling with the triangle barnacle (*Balanus trigonus*). A novel approach to reducing the numbers of barnacle cyprids settling on the cultured mussels would be to promote their settlement onto sacrificial materials placed in the vicinity of the mussels. An initial field experiment found settlement plates treated with an extract from adult *B. trigonus* (AE) increased the settlement of conspecific cyprids by 4.6 times. Subsequently, AE-treated material placed near cultured mussels reduced the overall number of barnacles on the mussels by around 4.5 times and increased the number of barnacle-free mussels by almost a third. These results suggest this novel approach to using natural settlement cues to remove barnacle cyprids from the vicinity of valuable shellfish may have potential in controlling these major biofouling pests in shellfish aquaculture.

1. Introduction

Biofouling in aquaculture production systems can have major impacts on aquaculture structures and cultured organisms (Dürr and Watson, 2010). With the recent rapid global expansion of aquaculture the associated costs due to biofouling are also increasing (FAO, 2012). Estimates of between 5 and 10% of total aquaculture production costs are attributed to managing biofouling (Lane and Willemsen, 2004), amounting to a total estimated impost of US$1.5 to 3 billion per year for the global aquaculture industry (Fitridge et al., 2012). The molluscan shellfish aquaculture industry in the United States is estimated to spend more than US$21 million a year on controlling biofouling (Adams et al., 2011). In Scotland, the estimated cost of fouling on cultured mussels is up to €750,000 (US$900,000) per year for each farmer (Willemsen, 2005). Cultured shellfish can be directly affected by fouling organisms which establish on their shells. The fouling organisms can physically damage the shell, interfere with the shell function, affect feeding ability, and directly compete for resources, such as food and space (Dürr and Watson, 2010; Willemsen, 2005). All these effects can influence the growth, condition, and aesthetics of the shellfish, and ultimately reduce their market value (Dürr and Watson, 2010; Fitridge et al., 2012). Biofouling of aquacultured shellfish is dominated by four marine invertebrate groups; sponges, barnacles, ascidians, spirorbid and other serpulid tube worms (Dürr and Watson, 2010). Among these four groups, barnacles are probably the most problematic for aquaculture operations (Christie and Dalley, 1987).

In 2009 there was a dramatic increase in fouling of green-lipped mussels, *Perna canaliculus*, by the triangle barnacle, *Balanus trigonus*, in mussel farms over an extensive area of the Hauraki Gulf, in north-eastern New Zealand. Subsequently, high levels of barnacle fouling have continued to cause substantial losses in export revenue from mussel aquaculture due to the difficulties in machine processing and marketing mussels with shells fouled with barnacles. A novel approach to reduce the numbers of barnacle cyprids settling on the cultured mussels would be to attract them to settle onto sacrificial settlement materials deployed in the vicinity of the mussels within the farm. Even a slight reduction in the number of barnacles settling onto cultured mussel shells as a result of them choosing to settle on the sacrificial materials has the potential to greatly benefit mussel farm production. A thorough examination of the scientific and aquaculture industry literature suggests there are no published accounts or reports of the existing use of sacrificial settlement material to control barnacle fouling or any other marine biofouling species.

Settlement cues for barnacle larvae have been used for about 60 years to study the settlement behaviour of cyprids (Crisp and Meadows, 1962, 1963; Dreanno et al., 2006a, 2006c; Knight-Jones and Stevenson, 1950). The most common and effective settlement cue used has been a crude extract of conspecific adults, prepared by simply grinding live adult barnacles in seawater and then filtering the resultant mixture to produce what is known as adult extract (AE). Studies on

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Amphibalanus amphitrite revealed the presence of a contact pheromone in the mantle and shell cuticle of adult barnacles of this species (Matsumura et al., 1998a). This pheromone, known as "settlement-inducing protein complex" (SIPC), is a glycoprotein belonging to the alpha(2)-macroglobulins family (Dreanno et al., 2006c; Matsumura et al., 1998a, 1998c). Studies on other barnacle species belonging to other major families of barnacles, such as Balanoidea, Tetraclitoidea and Chthamaloidea, have also demonstrated the presence of SIPC-like proteins (Kato-Yoshinaga et al., 2000), suggesting that these proteins are likely to be ubiquitous among barnacles (Dreanno et al., 2007). The SIPC is present in AE, as well as extracts prepared from cyprids and juveniles, indicating that it is also expressed during all of the lifecycle of barnacles (Dreanno et al., 2006b).

The gregarious settlement behaviour, characteristic of all the obligate cross-fertilizing barnacles, has been consistently shown to be due to the presence of this pheromone (Clare, 2011; Dreanno et al., 2007), which is detected by the antennular chemosensors of cyprids whilst they are actively searching surfaces as a suitable settlement location (Bielecki et al., 2009). The cyprids are able to discriminate between conspecific and allospecific SIPC, making the AE an ideal settlement cue for attracting a target species (Crisp and Meadows, 1962; Dreanno et al., 2007; Kato-Yoshinaga et al., 2000; Larman and Gabbott, 1975). The SIPC must be adsorbed onto the settlement surface where it is available to be detected by the cyprids that come into contact with the chemically active surface (Crisp, 1990; Crisp and Meadows, 1963; Matsumura et al., 1998b). The SIPC is responsible for the induction of settlement in conspecific cyprids, but other molecules released by barnacles may also attract larvae from the water column to the surface where the adult conspecific is attached. For example, a waterborne pheromone with a molecular size of 32 kDa and different from the SIPC, has been isolated from *A. amphitrite* and found to be highly effective in inducing settlement in conspecific cyprids (Elbourne and Clare, 2010; Endo et al., 2009).

The boiled AE of *B. trigonus* has been successfully used in laboratory experiments to induce settlement of cyprids (Mishra et al., 2001), but there are no published accounts of the chemical being deployed in field experiments. The cyprids belonging to other barnacle species, such as *Semibalanus balanoides* and *A. improvisus*, have been attracted and induced to settle to surfaces treated with conspecific AE in their natural environment (Berntsson et al., 2004; Hills et al., 1998; Prendergast et al., 2008). For example, in *A. improvisus*, pits in artificial tiles made of transparent polycarbonate treated with AE showed about four times higher settlement when compared with the untreated control pits (Hills et al., 1998), while in *S. balanoides*, regardless of the surface texture, there were always significantly more settlers on the surfaces treated with AE than the untreated ones (Prendergast et al., 2008).

Therefore, the aim of this current study is to test whether or not the AE of *B. trigonus* is able to attract and induce cyprids to settle on sacrificial material placed in the vicinity of mussels within a farm, and in so doing, reduce the number of cyprids settling on the adjacent mussel shells.

2. Materials and methods

2.1. Preparation of adult barnacle extract

Adults of *B. trigonus* were collected from shells of green-lipped mussels harvested from a mussel farm in Papakarahi Bay (36°48′25.39″S; 175°26′17.42″E) in the Hauraki Gulf, northeastern New Zealand. The live barnacles were detached from the mussel shells with a scalpel, gently washed with freshwater and all other fouling organisms on the barnacles were carefully removed.

The crude adult extract (AE) from the barnacles was prepared using a modified method of Rittschof et al. (1984). An aliquot of 100 g (wet weight) of adult barnacles were crushed with mortar and pestle slowly adding distilled water to make up a final total volume of 100 ml. The mixture was then centrifuged at 3000*g* for 10 min, the supernatant removed and filtered through Whatman paper (1.2 μm, filter number: 1822 055). The final aqueous AE was then stored at − 20 °C until further use. The protein content of the AE was determined by using the method of Lowry et al. (1951).

2.2. Experiment 1; confirmation of AE as settlement cue

The purpose of the first field experiment was to confirm that the AE from *B. trigonus* induced cyprid settlement in a field situation when deployed on artificial settlement surfaces with two different degrees of surface roughness.

Thirty plates (290 × 280 × 6 mm) were cut from two sheets of fibre-reinforced cement board (HardieFlex™, James Hardie Industries PLC) and used as the experimental settlement material for barnacles. Each plate had a smooth and a rough textured side with the smooth side having a roughness width (the distance parallel to the nominal surface between successive peaks or ridges) ranging from 0.01 to 0.21 mm, and the rough side from 0.06 to 1.04 mm (i.e., ~five times rougher). Ten of the plates were randomly selected and soaked for 5 min in distilled water (control treatment). A further two sets of 10 plates were soaked in a solution of AE either at a concentration of 50 μg ml^{-1} (Treatment 1 or T1) and 100 μg ml^{-1} (Treatment 2 or T2) for 5 min. After removal from their respective baths all of the plates were left to dry overnight and deployed into the field on the next day (17 February 2014). The plates were deployed onto a green-lipped mussel farm in Papakarahi Bay in the Hauraki Gulf belonging to North Island Mussels Ltd (NIML). This site was selected as an experimental site because previous studies revealed a high settlement rate of *B. trigonus* and very low settlement of other barnacle species, such as *Austrominius modestus* (Zazzaro, unpublished data). Each of the 30 plates was attached to a 5 m length of polypropylene rope (Ø = 6 mm) and then each rope was secured to the backbone lines of mussel farm which are also used to suspend the dropper lines of cultured mussels (Jeffs et al., 1999). Each plate was held down in a vertical position by attaching two 1.5 kg lead dive weights to the bottom edge of the plate. The 30 plates were distributed randomly in the farm by assigning five plates per alternate mussel backbone line to ensure a spacing between plates of at least 20–25 m.

After 8 days the plates were retrieved and returned to the laboratory for cleaning and analysis of the attached barnacles. All the fouling organisms except the barnacles were removed by soaking each plate for 1–2 min in a solution of tap water and commercial bleach (ratio 1:3; final concentrations: NaClO = 14 g L^{-1} and NaOH = 1.3 g L^{-1}). The examination of the removed material confirmed that no barnacles were removed during the cleaning process, including recently attached cyprids. High resolution digital images of both sides of each plate were taken with a CANON EOS 40D attached to a photographic stand and using an EF 50 mm f/2.5 compact macro lens. All the images were processed using ImageJ software (Schneider et al., 2012), and for each image all of the barnacles attached were counted on a fixed area of 624 cm^2, which excluded an area around the edges of the plate (2 cm top and bottom, 1.5 cm right and left) to avoid any edge effects. A binocular microscope (Wild Heerbrugg M8) was used to identify the barnacle species when the identification was uncertain from the digital image.

To compare the barnacle abundance among treatments and between the smooth and rough surfaces of the plate, a generalized linear mixed model (GLMM) accounting for differences between the sides of the plates (i.e., smooth and rough), was fitted using a log-link function and Poisson probability mass function. Adjustments were made for multiple comparisons between means using the Tukey-Kramer method. All analyses were performed using SAS software v9.3 (SAS Institute Inc., 2012).