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2,4-Decadienal: Exploring a novel approach for the control of polychaete pests on cultured abalone

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ABSTRACT

Infestations by shell-boring polychaete worms represent a threat to the continued growth of the abalone culture industry. The South African cultivated abalone, *Haliotis midae*, is affected by 3 species; the spionids *Boccardia proboscidea* and *Polydora hoplura* and the sabellid, *Terebrasabella heterouncinata*. Currently there are no effective treatments that do not cause undue harm to the abalone host while proving effective against the worms. Here we report on the efficacy of the diatom-derived polyunsaturated aldehyde 2,4-decadienal as a potential chemotherapeutic agent against shell-infesting polychaetes. Exposure to decadienal had pronounced dose- and time-dependent negative effects on larval mortality, growth and developmental rate. There was a variation in the response to treatment in relation to larval strategy, with planktotrophic *B. proboscidea* larvae more susceptible than adelphophagic larvae, and species, with lecithotrophic *T. heterouncinata* generally more sensitive than planktotrophic *B. proboscidea* larvae. Emergent larvae were more susceptible than encapsulated larvae. The egg capsule appears to provide an effective barrier to decadienal exposure. Decadienal could therefore prove effective as a control agent at several life history stages by reducing the number of larvae that survive and are able to settle, and also the fitness of the larvae that do survive and settle.

1. Introduction

The international abalone industry has grown significantly over the last few decades, with cultured abalone production increasing from 689 metric tonnes in 1989 to 8696 metric tonnes in 2002 (Gordon and Cook, 2004). Accompanying this increase in production has been an increase in the incidence of shell-infesting polychaetes which, through compromising abalone growth, can lead to substantial revenue loss (Lleonart et al., 2003; Moreno et al., 2006; Simon et al., 2006; Sato-Okoshi et al., 2008). The South African cultivated abalone, *Haliotis midae*, is infested by 3 species of problematic, shell-infesting polychaete worms; the spionids include the non-indigenous *Boccardia proboscidea* and possibly cryptogenic *Polydora hoplura* and the endemic sabellid, *Terebrasabella heterouncinata* (Simon et al., 2004, 2006; Simon and Booth, 2007). All 3 species are intra-tubular brooders with lecithotrophic or adelphophagic larvae emerging at an advanced developmental stage, while *B. proboscidea* simultaneously produces planktotrophic larvae that emerge at an early developmental stage (Wilson, 1928; Gibson, 1997; Fitzhugh and Rouse, 1999; Simon et al., 2010). In a farm situation these early recruiting larvae may contribute to the maintenance of local populations (Strathmann, 1990) thereby potentially exacerbating the pest burden on farms.

High levels of infestation in molluscs often lead to decreased growth, flesh condition and increased host mortality (Bailey-Brock and Ringwood, 1982; Lleonart et al., 2003; Simon et al. 2006; Moreno et al., 2006; Sato-Okoshi et al., 2008), prompting the assessment of techniques intended to reduce infestation burden. Exposure to air, hyper- or hypo-saline water, heat and various chemical treatments have been trialled with varying degrees of success (Bailey-Brock and Ringwood, 1982; Nel et al., 1996; Oakes and Fields, 1996; Caceres-Martinez et al., 1998; Leighton, 1998; Lleonart et al., 2003; Dunphy et al., 2005). While several of these treatments have proven reasonably successful for oysters, few effective treatments for abalone have been found. Lleonart et al. (2003) found that although aerial exposure significantly reduced spionid infestations, abalone growth could be impaired. Similarly, heat treatment against *T. heterouncinata* was only effective for tropical abalone species with naturally higher tolerance to elevated water temperatures (Leighton, 1998). In general, the worms tend to be more resistant to the treatment than the abalone (Leighton, 1998), presumably because worms can retreat to the relative safety of their burrows. Effective administration would therefore be most likely at a stage when the worms are most susceptible, i.e., as larvae or juveniles prior to forming the protective burrows. However, a treatment that could simultaneously inhibit the development of the larvae before emergence from the parental burrow would greatly increase the efficacy.

Chemical treatments have to date proved unsuccessful (Lleonart et al., 2003), although crenulacital-C, a secondary compound produced by the

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brown alga, *Dictyota dichotoma,* has been shown to kill larvae of *Polydora websteri* which infests oysters (Takikawa et al., 1998). There is growing interest in exploiting natural products for targeted therapeutics. A number of studies have evaluated the potential for phyco-derived compounds for both disease and fouling management (e.g., Lakshmi et al., 2004; Plouguerné et al., 2010). Among the attractions of a natural products-based approach is the potential for effective, non-bioaccumulating and environmentally benign compounds. Here we present preliminary data on the efficacy of the diatom-derived aldehyde, 2,4-decadienal as a potential biocidal treatment against polychaete infestation. Decadienal is one of a suite of polyunsaturated aldehydes that are synthesised by numerous diatom taxa including many of the major bloom forming species (Miralto et al., 1999; Wichard et al., 2005). Formation is wound activated and under enzymatic control via a number of lipid peroxidation pathways (e.g. Pohnert 2002, 2005). The polyunsaturated aldehydes and similar lipid peroxidation products (c.f. Fontana et al., 2007) belong to a family of biologically active compounds known collectively as oxylipins (see Caldwell 2009; Ianora and Miralto 2010 for review). They, and latterly, reactive oxygen species and fatty acid hydroperoxides, have been implicated in negatively impacting copepod fecundity, hatching success, larval development and survival with no or limited effect on the diatom-feeding adults (Miralto et al., 1999; Ianora et al., 2004; Taylor et al., 2007; Fontana et al., 2007). In addition, decadienal also inhibits larval survival, fertilisation, hatching success and larval development in certain polychaete and echinoderm species, with certain polychaete species appearing more vulnerable to the toxic effects than other marine invertebrates (Caldwell et al., 2002; Lewis et al. 2004; Caldwell et al., 2005). Furthermore, sensitivity to decadienal decreases with an increase in developmental stage (Caldwell et al., 2005), and presumably body size (Taylor et al., 2007). Decadienal has an environmental half life approximating to 24 h (Caldwell unpublished data) and is readily oxidised to non-toxic products. This suggests that decadienal may be a candidate chemical treatment against infesting worms without negatively impacting abalone health.

The aim of this study was therefore to establish the range of sensitivity of larval *B. proboscidea* and *T. heterouncinata*, the two most prevalent polychaete pests on cultivated abalone in South Africa, to decadienal. This study presents an initial investigation into the efficacy of decadienal as an environmentally benign treatment for polychaete infestation of cultured abalone.

2. Methods and materials

2.1. Experimental animals

Abalone shells infested with *T. heterouncinata* and *B. proboscidea* were provided by the abalone farm Abagold Ltd (Pty) in Hermanus, South Africa (34°25″30′S, 19°13″30′E). The infested shells were maintained in a recirculating seawater system in a constant environment room, between 17 and 18 °C and 12:12 L:D photoperiod. Larval survival trials were conducted at the School of Marine Science and Technology, Newcastle University while development and growth trials were conducted at the Department of Botany and Zoology, Stellenbosch University.

Larvae of *T. heterouncinata* were removed by crushing the shells, and leaving the fragments in seawater for 1 to 2 h to allow the larvae to escape. Only pre-emergent larvae (i.e. actively crawling larvae with eye spots) were used for assays. *B. proboscidea* brood capsules containing fertilised but as yet unhatched eggs or larvae at different developmental stages were removed from tubes on the shell surface. Larvae were staged according to the number of chromatophores (pigmented bars) on the dorsal surface (see supplementary material, Appendix B). The eggs were subsequently transferred to filtered seawater (FSW) and kept in a constant environment room until they reached the required developmental stage. Sample sizes were small owing to the difficulty in finding sufficient larvae at the same developmental stage to complete a full set of treatments.

2.2. Chemical preparation

Commercial grade (Sigma-Aldrich) 2,4-decadienal was dissolved in 200 µl dimethyl sulphoxide (DMSO). A stock solution of 100 µg ml^{-1} was made by addition to 25 ml FSW.

2.3. Effects of decadienal exposure on larval survival

Two types of survival bioassays were conducted; on 'free' and encapsulated larvae. The protocol for the 'free' larvae bioassays was adapted from Takikawa et al. (1998) who aimed to determine the concentration that resulted in more than 80% mortality within 3 h. In the current study the aim was to determine the concentration which caused more than 60% mortality within 3 h, although mortality was also monitored after 6 and 24 h.

Seven pre-emergent *T. heterouncinata* larvae were placed into individual wells of 24-well microplates containing 2 ml of decadienal at concentrations of 1.5, 3, 4 and 5 µg ml^{-1} or FSW (Trial 1). Two acute assays (Trial 2) were conducted on *B. proboscidea*, using either planktotrophic or pre-emergent adelphophagic larvae removed from capsules that no longer contained nurse eggs ('free' larvae): A) between 5 and 8 'free' planktotrophic 4-chaetiger *B. proboscidea* larvae were placed in 24-well microplates containing 2 ml of decadienal at concentrations of 1.5, 3, 4 and 5 µg ml^{-1} or FSW; B) either 8 or 9 12-chromatophore *B. proboscidea* larvae were placed in 24-well microplates containing 2 ml of decadienal at concentrations of 3, 4, 5, 6 and 7 µg ml^{-1} or FSW. All treatments were performed in triplicate, randomised and scored blind. Survival was monitored after 3, 6 and 24 h.

Two trials (=Trials 3 and 4) were conducted on encapsulated larvae that had up to 4 or 5 chromatophores. Individual capsules from a single brood were placed in 24-well microplates containing 2 ml decadienal or FSW. Six capsules were used for each treatment. The concentrations used were 0, 2.5, 5 and 10 µg ml^{-1} and 0, 2, 4 and 8 µg ml^{-1} decadienal respectively. Survival (as percentage alive within each capsule) was determined after 24 h.

2.4. Effects of decadienal exposure on larval growth and number of chromatophores (larval development) of B. proboscidea

Four separate trials (2 short term and 2 of longer duration) were conducted to measure the effect of decadienal (0.5, 1, 2 µg ml^{-1}) on the growth rate and development of encapsulated larvae. Each trial was conducted with a single brood as inter-brood variability was higher than intra-brood variability (unpublished data). Trials 5 and 6 were short term. In Trial 5, embryos at a very early developmental stage (some had developed as far as 2 eyespots) were used, while older embryos with 4 eyespots and dark pigment on the pygidium were used in Trial 6. One capsule was placed in each well with 2 ml of decadienal or FSW which was replaced daily until the end of the trials. There were 6 replicates per treatment. Incubations were suspended after 3 and 4 days for Trials 5 and 6, respectively, by replacing the decadienal or water with 4% seawater formalin. After 10 to 20 min, the capsules were opened and the larvae mounted on slides with Aquamount mounting medium.

The 2 longer duration trials used larvae with 2 eyespots at the beginning of the trials. They were conducted as before, but after 3 days decadienal was replaced with FSW and the capsules were left for an additional 3 days (Trial 7), or the capsules were left in decadienal for an additional 4 days (Trial 8). Trial 7 was suspended due to the mortality of a capsule in the control.

The number of chromatophores per larva was recorded in all four trials. Growth of siblings was not synchronous. To determine the