Journal of Experimental Marine Biology and Ecology 486 (2017) 93–97

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Effects of coral extracts on survivorship, swimming behavior, and settlement of *Pocillopora damicornis* larvae

Jeric P. Da-Anoy, Ronald D. Villanueva, Patrick C. Cabaitan, Cecilia Conaco *

Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, Philippines

ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 6 October 2016
Accepted 8 October 2016
Available online xxxx

Keywords:
Allelopathy
Recruitment
Porites cylindrica
Sarcophyton glaucum

ABSTRACT

Coral larvae released into the water column encounter different dissolved chemical cues secreted by organisms on the substratum that are engaged in spatial competition. These cues initiate vital biological processes in the larvae. In this study, the effects of crude extracts from the scleractinian corals *Pocillopora damicornis* and *Porites cylindrica*, and the soft coral *Sarcophyton glaucum*, on the survivorship, swimming behavior, and settlement of *Pocillopora damicornis* larvae were evaluated. High concentrations of heterospecific coral extracts caused a significant decline in both larval survivorship and settlement whereas conspecific extracts did not result in any larval mortality. Exposure to heterospecific extracts resulted in repulsion of larvae as indicated by a transient change in larval morphology and sustained swimming in the upper water column. In contrast, the conspecific extract enhanced larval exploration of both the water column and substratum and was able to induce larval settlement. These results suggest that chemical cues emanating from conspecifics and other coral species are capable of altering the biology of coral larvae, thus potentially influencing settlement behavior and species distribution on the reef.

1. Introduction

Most extant benthic marine organisms have a biphasic life cycle characterized by a morphologically distinct planktonic larva that is dispersed in the water column before settlement and metamorphosis into a benthic adult (Degnan and Degnan, 2010). The biphasic life cycle involves the spawning of gametes, development of competent planktonic larvae, selection of a suitable microhabitat, settlement, and metamorphosis. It is estimated that 55 to 85% of all benthic marine invertebrate species produce long-lived planktotrophic larvae (spending weeks to months in the plankton), 5% produce short-lived planktotrophic larvae (spending hours to days in the plankton), and about 10% produce lecithotrophic larvae (Thorson, 1950, 1966). Successful recruitment requires survival of the larvae through each developmental stage, settlement on an appropriate substrate, and continued growth. The factors that influence larval release, dispersal, and settlement thus play a key role in determining the patterns of distribution and community structure of these organisms.

Transition from the planktonic to the benthic life history stage is dependent on settlement cues that signal habitat suitability and induce larval settlement. These cues may come in the form of environmental parameters or chemical compounds that convey information about the post-settlement habitat (Chia, 1978). Larvae of sessile organisms respond either positively or negatively to settlement cues (Fleck and Fitt, 1999; Hadfield and Pennington, 1990; Kato et al., 1975; Pawlik, 1992; Rodriguez et al., 1993). Coral larvae, in particular, will cease swimming, attach to the substrate, and develop into the primary polyp upon encountering the appropriate cues (reviewed in Ritson-Williams et al. (2009)). Notably, in the absence of settlement cues, many marine invertebrate propagules delay or cease settlement altogether (Graham et al., 2008; Qian et al., 2007), indicating a crucial role for these cues in marine benthic communities.

Settlement cues are typically associated with or secreted by crustose coralline algae (CCA), microbes in biofilms, or other organisms on the benthos (Ettinger-Epstein et al., 2008; Hadfield, 2011; Harrington et al., 2004; Heyward and Negri, 1999). Chemicals able to induce settlement and metamorphosis have been isolated from CCA (Tebben et al., 2015). Coral larvae are also known to settle and metamorphose in response to microbial biofilms or bacterial strains isolated from marine biofilms (Negri et al., 2001; Tran and Hadfield, 2011; Webster et al., 2004). Tetrabromopyrrole, which was isolated from a marine bacterium, induces spontaneous metamorphosis without attachment (Tebben et al., 2011). Interestingly, extracts derived from adult corals themselves can affect the settlement of coral larvae. For example, an extract derived from the *Goniastrea* skeleton is able to induce settlement of *Acropora millepora* (Heyward and Negri, 1999).

On the other hand, many sponge, coral, bryozoan, and ascidian species are rarely overgrown, indicating that these species may synthesize potential allelochemicals that prevent competition from other sessile organisms (Degnan and Johnson, 1999). Biologically active metabolites have been isolated from marine invertebrates, like sponges, soft or

* Corresponding author.
E-mail address: cconaco@msi.upd.edu.ph (C. Conaco).

http://dx.doi.org/10.1016/j.jembe.2016.10.006
0022-0981/

scleractinian corals, bryozoans, and colonial ascidians that inhibit settlement and subsequent metamorphosis of different species of marine invertebrate larvae (Bakus et al., 1986; Faulkner, 2000; Fearon and Cameron, 1996; Fearon and Cameron, 1997; Paul, 1992; Zimmer and Butman, 2000). Some compounds involved in allelopathic interactions range in action from repellents to toxins and vary in the distances over which they act (Hadfield and Scheuer, 1985; Slattery et al., 1997). Allelochemicals thus affect the health, growth, behavior, or population biology of organisms of other species (Whittaker and Feeny, 1971). For example, extracts derived from various scleractinian corals have been shown to exhibit toxicity against the larvae of other coral species (Fearon and Cameron, 1996; Fearon and Cameron, 1997).

The scleractinian coral, *Pocillopora damicornis*, is a simultaneous hermaphrodite that releases brooded planula larvae each month, with peaks during the warmer months (Richmond and Jokiel, 1984; Villanueva et al., 2008). At the present study site in the Bolinao-Anda Reef Complex (BARC), adult colonies of *P. damicornis* can be found in the same reef area but not in close proximity to another scleractinian coral, *Porites cylindrica*, or the soft coral, *Sarcophyton glaucum*. *P. damicornis* grows in smaller patches whereas *P. cylindrica* tends to form huge stands (Gomez et al., 2014). Few coral recruits can be found near *S. glaucum* in situ (Maida et al., 1995a). The processes influencing these observed distribution patterns presumably start at the larval stage, but empirical data to support this claim is limited. While the effects of extracts from various coral species have previously been tested on the survival of larvae of *P. damicornis* (Fearon and Cameron, 1996; Fearon and Cameron, 1997), the effect of heterospecific and conspecific chemicals on larval behavior and settlement induction remains to be investigated. This study thus explores the effects of crude ethanolic extracts derived from *P. damicornis* and *P. cylindrica*, as well as from the soft coral, *S. glaucum*, on (1) larval survivorship, (2) larval swimming behavior, and (3) settlement of *P. damicornis* larvae. The results suggest that chemical compounds from adult coral colonies can influence population processes at the larval stage.

2. Materials and methods

2.1. Coral colony and larvae collection

Colonies of *P. damicornis*, *P. cylindrica*, and *S. glaucum* were collected in April 2015 from a reef flat at about 4 m depth in Cangaluyan, Bolinao, Pangasinan, Philippines (N 16°22.923′, E 120°00.228′) and transported to the Bolinao Marine Laboratory. Coral identification was verified based on descriptions by Veron (2000). Colonies ($n = 5$) from which planulae were derived were transferred separately to individual translucent plastic buckets (25 cm in diameter and 25 cm in depth) supplied with constant aeration and flow-through sand-filtered seawater. Seawater flow was stopped at 1800 h and the seawater was filtered at 0900 h through a nylon screen (215 μm mesh) to collect larvae. Larvae were pooled and maintained in a glass dish with filtered seawater (0.45 μm, FSW). Larvae used for the experiments were approximately three to four hours post-release. All experiments were conducted within a temperature range of 26–28 °C and a 12:12 light:dark regime under a fluorescent lamp with irradiance level of ca. 60 μE m^{-2} s^{-1}. Coral collection, spawning, and larval culture were adapted from Villanueva et al. (2008).

2.2. Extract preparation

Extraction methods for scleractinian corals were modified from Gunthorpe and Cameron (1990). Soft tissues from five colonies each of *P. damicornis* and *P. cylindrica* were dissociated from corallites using a water pick. Each colony was approximately 16 cm in diameter with an estimated total surface area of about 500–600 cm^2. Tissues obtained from all five colonies for each species were pooled and suspended in 500 ml absolute ethanol. Three hundred grams of *S. glaucum* tissue was homogenized in 500 ml of absolute ethanol. Tissue homogenates were incubated at 4 °C for 72 h. The ethanolic extracts were filtered twice through a double thickness of Whatman number 1 filter paper, concentrated under vacuum, and freeze-dried. The freeze-dried powder was resuspended in filtered seawater to obtain the test concentrations of 1, 10, 100, and 1000 μg/ml.

2.3. Larval survivorship assay

Ten actively swimming *P. damicornis* larvae were introduced into 10 ml of filtered seawater containing variable concentrations of extract (1, 10, 100, 1000 μg/ml) from each of three coral species in a 50 ml polystyrene tube. Filtered seawater was used as the control. The experiment was conducted with 5 replicates per treatment. Surviving and dead larvae were counted after 24 h. Mortality was defined by movement arrest with leaking gastrovascular material and marked tissue degeneration (Epstein et al., 2000; Goh, 1991). The lethal concentration of ethanolic extracts resulting in 50% mortality of *P. damicornis* larvae (LC50) was determined using the dose effect analysis tool of XLSTAT.

2.4. Swimming behavior assay

Ten actively swimming *P. damicornis* larvae were introduced into 10 ml of filtered seawater containing 1 μg/ml or 1000 μg/ml of extract from each of three coral species in a 50 ml polystyrene tube. Filtered seawater was used as the control. The experiment was conducted with 5 replicates. Larval swimming behavior and the number of larvae in either the upper or lower layer of the water column was noted at twenty minute intervals for 1 h upon exposure to the extract.

2.5. Larval settlement assay

Ten actively swimming larvae were introduced into polystyrene 6-well plates with each well containing 12 ml of 0.2 μm filtered seawater. Freeze-dried extracts from the three coral species were dissolved in dichloromethane (DCM) to obtain 1 or 1000 μg/ml solutions. Rubble chips approximately 6 mm^2 in size were cut from *Acropora* coral rubble. Chips were oven dried and cooled before use. Chips were soaked in the DCM solutions for 1 h to allow adsorption of the extracts, then air dried to allow solvent evaporation. Extract-adsorbed coral rubble chips were randomly selected and added to each well of the test plate. Chips of similar size cut from coral rubble covered with crustose coralline algae (CCA) served as positive controls and rubble chips soaked in DCM served as negative controls. The experiment was conducted with 5 replicates per treatment. After 24 h, the number of coral larvae that had settled on the rubble chips was enumerated under a dissecting microscope.

2.6. Statistical analyses

For all experiments, the percent of larvae responding to different concentrations of each crude coral extract was compared with the appropriate controls using Kruskal-Wallis one-way analysis of variance (ANOVA) by ranks followed by pairwise Mann-Whitney *U* tests. For the swimming behavior experiment, changes in response through time for each of the different treatments was analyzed using Friedman repeated measures ANOVA by ranks. All statistical analyses were conducted using Statistica software.

3. Results

Crude ethanolic extracts from *P. cylindrica* and *S. glaucum* exhibited some toxicity on *P. damicornis* larvae. While concentrations of 1 μg/ml and 10 μg/ml of the extracts from other coral species had no visible effect on larval survivorship (Fig. 1), larval mortality began to be apparent at 100 μg/ml of the *P. cylindrica* extract, which decreased survivorship to 86%. At 1000 μg/ml of the extract, survivorship decreased to 22%