Habitat selection by marine larvae in changing chemical environments

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ABSTRACT

The replenishment and persistence of marine species is contingent on dispersing larvae locating suitable habitat and surviving to a reproductive stage. Pelagic larvae rely on environmental cues to make behavioural decisions with chemical information being important for habitat selection at settlement. We explored the sensory world of crustaceans and fishes focusing on the impact anthropogenic alterations (ocean acidification, red soil, pesticide) have on conspecific chemical signals used by larvae for habitat selection. Crustacean (*Stenopus hispidus*) and fish (*Chromis viridis*) larvae recognized their conspecifics via chemical signals under control conditions. In the presence of acidified water, red soil or pesticide, the ability of larvae to chemically recognize conspecific cues was altered. Our study highlights that recruitment potential on coral reefs may decrease due to anthropogenic stressors. If so, populations of fishes and crustaceans will continue their rapid decline; larval recruitment will not replace and sustain the adult populations on degraded reefs.

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1. Introduction

Coral reefs are home to approximately 25% of the ocean's biodiversity while only accounting for 0.02% of the ocean's surface area (Spalding et al., 2001). Due to the economic and environmental importance, unique assemblage of organisms, and high species diversity found on coral reefs, protective measures are critical (Chin et al., 2011; de Groot et al. 2012). Beginning in the early 1990's, questions have been raised concerning the resilience of coral reefs to global changes (Grigg and Dollar, 1990). Today, it is estimated that 20% of coral reefs have been destroyed, 25% are under great immediate threat, and a further 25% will be under threat by 2050 (Chin et al., 2011). Several studies have shown that coral reefs exposed to a disturbance event often exhibit a decline in adult populations. This decline leads to accelerated rates of extirpation compared to "non-degraded" habitats (Hughes et al., 2003; Munday et al., 2009). For example, the decline in adult populations of coral reef fishes on degraded reefs in Papua New Guinea was a reflection of larval recruitment failure rather than adult mortality.

Results suggest the "rescue" effect of recruitment may be ineffective in degraded habitats (Jones et al. 2004). Unfortunately, the mechanisms that determine how pelagic larvae respond to different environmental stressors and the role anthropogenic induced change plays during settlement site selection by recruiting organisms remains poorly understood (Hanski and Gilpin, 1997; Lecchini et al., 2013; Dixson et al., 2014).

Most species of coral reef fishes and crustaceans have stage-structured life histories: a relatively sedentary benthic stage (juveniles and adults) produces highly dispersive pelagic larvae (Kingsford et al., 2002). The transition from the pelagic environment to a benthic reef (i.e., recruitment process) represents a key period in the ontogeny of marine organisms (Lecchini, 2005). At recruitment, fish and crustacean larvae are subjected to strong selective pressure to choose a suitable reef habitat that will promote post-recruitment survival and growth of individuals (Doherty, 2002). Up to 90% of fish larvae may be removed by predation during the first week post-recruitment if suitable habitat was not selected (Doherty et al., 2004). Thus, many fish and crustacean species show high selectivity in suitable reef habitat; basing decisions on the presence of specific substrates and/or conspecifics, as well as the absence of predators and/or competitors (Kingsford et al., 2002; Lecchini et al., 2010; Barth et al., 2015). Chemical cues are often used

by fish and crustacean larvae to locate suitable reefs (Leis et al., 2011; Lecchini et al., 2013; Dixson et al., 2014), to identify conspecifics (Dixson et al., 2011), and to avoid predators (Dixson et al., 2012). For example, settling fish larvae are capable of olfactory discrimination among reefs, preferring the water-borne odors of their home reefs compared to neighboring reef habitats (Gerlarch et al., 2007). The understanding of how animals make decisions is a fundamental question in behavioural ecology (Feely et al., 2009; Barth et al., 2015). While evidence is mounting that larval organisms are active participants in the process of dispersal and recruitment (Lecchini et al., 2010), the sensory and behavioural mechanisms by which larvae disperse and return to appropriate habitat remain unknown. Understanding the recruitment process is especially important in the context of global reef decline due to natural and/or anthropogenic stressors (Munday et al., 2009; Lecchini et al., 2013; Dixson et al., 2014).

We investigated the behavioural response towards conspecific chemical stimuli by fish and crustacean larvae used in habitat selection during recruitment at two reef locations (Moorea Island, French Polynesia; Sesoko Island, Ryukyu Archipelagos, Japan). Research was conducted in the context of anthropogenic-induced change, specifically focusing on 1) ocean acidification, 2) red soil pollution and 3) pesticide pollution.

Since the beginning of the industrial revolution, atmospheric concentrations of carbon dioxide (CO_2) have risen dramatically. As atmospheric CO_2 passively diffuses into the ocean's surface waters, seawater pH decreases, and in turn increasing water acidity (Feely et al. 2009). Several studies have shown that changes to seawater chemistry are predicted to impact the health and function of fishes (Munday et al., 2012; Leduc et al., 2013). We investigated how acidified seawater (700 and 1000 ppm) could alter the chemical cues of conspecifics available to fish and crustacean larvae.

Turbidity is one of the biggest sources of habitat degradation. The elevated amount of sediment suspended in the water column is generated by increased urbanisation and agricultural development run-off (Fabricius, 2005). We investigated the effects of red soil pollution (50 and 200 mg/L of red soil in clear or turbid water) on the chemical abilities of fish and crustaceans to recognize their conspecifics. The term "red soil" refers to a laterite soil prominent in Ryukyu Islands, Japan (Omija, 2004). The geographic features and rainfall patterns combined with surges in land development since the 1970s on Ryukyu Islands has resulted in the periodic erosive run-off and re-suspension of red soil. The increase in red soil runoff pollutes the surrounding coral reefs with high levels of silt and turbidity (Omija, 2004; O'Connor et al., 2016).

In contrast to the large numbers of studies testing the influence of pesticides on food and human health, few studies have explored its influence on reef biodiversity (Fabricius et al., 2005; Botte et al., 2012). In French Polynesia, Roche et al. (2011) illustrated the contamination of marine organisms (fish, green algae, mollusk, coral and holothurian) by several herbicides (chloroacetamide and triazine derivatives) and several insecticides (organophosphates and organochlorines). We examined the effect of the organophosphorus pesticide (1 and 100 μg/L of chlorpyrifos) on the chemical abilities of marine larvae to detect conspecific cues.

We hypothesized that conspecifics emit chemical cues that larvae are able to recognize in un-polluted seawater. However in the presence of anthropogenic pollution, the ability to chemically recognize conspecifics will be altered. Thus, if the recruitment potential of coral reefs has decreased due to these anthropogenic stressors (i.e. larvae unable to detect important chemical cues in polluted environment), the populations of fishes and crustaceans will continue to rapidly decline, as larval recruitment will not sustain adult populations on degraded ecosystems.

2. Materials and methods

2.1. Sampling locations and target species

Habitat naïve larval fish (*Chromis viridis*) and crustaceans (*Stenopus hispidus*) were collected just before settlement using light traps (Nakamura et al., 2009a,b) set 300 m off of the fringing reefs on the south-east side of Sesoko Island (26°38′08.94″N, 127°51′55.04″E). The acidification and red soil experiments were conducted at the Sesoko research station in August 2012. To conduct the pesticide experiment, habitat naive larvae were collected just before settlement using crest nets (Lecchini et al. 2004, 2006; Lo-Yat et al., 2011) set off the west coast of Moorea Island (17°30′58.85″S, 149°55′26.77″W) in March–April 2013. The capture of *S. hispidus* was low, therefore the pesticide experiment focused on *C. viridis*. All collected larvae were transferred and maintained in habitat free individual aquaria ($0.3 \times 0.3 \times 0.2$ m; water temperature: 26–27 °C) supplied with flow-through ocean seawater. Laboratory experiments were performed within 24 h of larval capture (Dixson et al., 2011; Lecchini et al., 2013). Conspecifics and heterospecifics, used as cues transmitters, were *C. viridis* and *S. hispidus* larvae reared in aquaria for 7 days post capture. *C. viridis* was used as the heterospecific cue for *S. hispidus* and vice versa. This was done to represent a post-settled juvenile stage. Larvae, conspecifics and heterospecifics were fed three times per day (live *Artemia* sp. Nauplii - *C. viridis* and dead fish - *S. hispidus*).

2.2. Behavioural experiments in choice flume

A two-channel choice flume described in Gerlach et al. (2007) was used to test larval preferences between olfactory cues in present day and acidified seawater conditions (Exp. 1), water treated with or without red soil (Exp. 2), and pesticides (Exp. 3). Briefly, a flow rate of 100 mL/min was maintained using flow meters. The low flow allowed larvae to swim without struggle against the current, ensuring movement patterns were a result of cue preference (Leis et al., 2011). Dye tests were conducted to confirm laminar flow within the chamber without eddies or areas of water mixing.

A larva was placed in the center of the downstream end of the flume during a 2 min habituation period. During this time the larva could explore the chamber and swim between the two parallel flowing water sources. Individuals that did not actively swim or explore both sides of the chamber during the habituation period were discarded (<2% of fish and <6% of crustacean). After the habituation period, the position of the larva, in either the right or left water channel, was recorded at five-second intervals for a two-minute test period. The water sources entering the chamber were then switched, with a one-minute rest period allocated to ensure the flushing of both channels (verified by dye tests). This was done to control for any side preferences individuals may display. After water sources were switched, another two-minute habituation period was given, followed by a second two-minute test period.

Preliminary experiments were conducted to 1) ensure no unanticipated biases existed for the two channel choice chamber;, 2) the use of chemical cues in the recognition of conspecific and heterospecific cues; and 3) to ensure repeated measurements did not interfere with larval responses (Supp. Mat).

2.3. Experiment 1: Anthropogenic ocean acidification

The partial pressure of CO_2 (pCO_2) in seawater was adjusted with a high-precision pCO_2 control system (Kimoto Electric). Using methods described in Tanaka et al. (2014), fresh filtered seawater (pore size, 1 μm) was treated with a gas mixture of CO_2 and ambient air in a bubbling tank. pCO_2 of the seawater flowing from the bubbling tank was directly measured and maintained at the desired level by continuously regulating pCO_2 in the gas mixture. Three types of seawater were simultaneously prepared using this system: 400 ppm (pH value = 8.11), 700 ppm (pH value = 7.96) and 1000 ppm (pH value = 7.84). These concentrations were chosen to reflect a present day control (400 ppm), and two future scenarios expected by 2050 (700 ppm) and 2100 (1000 ppm) (Feely et al., 2009; Munday et al., 2012; Leduc et al., 2013). The standard deviation of pCO_2 and pH values were <15%