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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.

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

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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 × 0.3 × 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%

and <1% of target values, respectively, which were monitored for 10 days before the experiment (Tanaka et al., 2014). The temperature of the seawater was 27.4 °C (SD = 0.2).

To test the effect of acidification on the behavioural response towards conspecifics cues, *C. viridis* or *S. hispidus* larvae were given a choice between conspecifics and heterospecific treated seawater adjusted to one of three acidification levels. Seawater was first acidified to the desired level. Cue treatments were then made by soaking either 5 conspecific or 5 heterospecific individuals in 5 L of filtered seawater for 3 h. Cue preferences were determined using the 30 *C. viridis* and 15 *S. hispidus* larvae. Trials run consisted of conspecific vs. heterospecific cue in seawater at: i) 400 ppm, ii) an acidified condition (700 or 1000 ppm), and again iii) 400 ppm. Between each consecutive test, the larva was maintained in an individual tank of untreated control seawater (400 ppm) for 60 min (Fig. 1).

A z-test with the null hypothesis that larvae would spend 50% of their time in either water source was used to determine if marine larvae were significantly i) attracted to conspecifics chemical cues in control

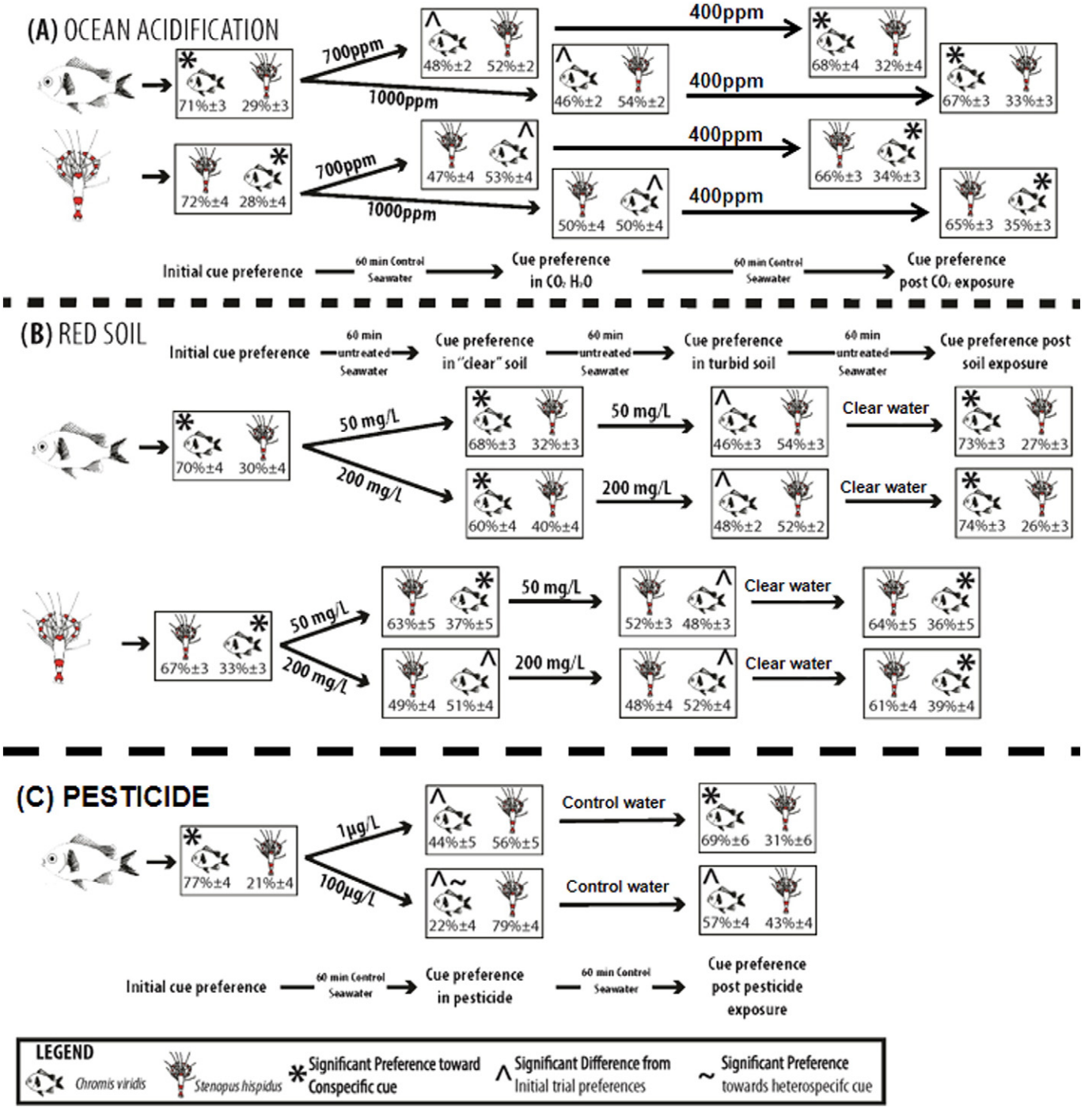

Fig. 1. Infogram of the experimental methods to test for the effect of A) ocean acidification (Sesoko, $n = 30$ *C. viridis*, $n = 15$ *S. hispidus* for each acidified seawater concentration: 700 or 1000 ppm), B) red soil pollution (Sesoko, n = 30 *C. viridis*, n = 15 *S. hispidus* for each red soil concentration: 50 or 200 mg/L) and C) pesticide chlorpyrifos (Moorea, n = 15 *C. viridis* for each pesticide concentration: 1 or 100 µg/L) on the larval ability to discriminate between conspecific and heterospecific chemical cues before, during and after exposure to the pollutants. Chemical cues from conspecifics were tested against the chemical cues of heterospecifics. Percent values indicate the mean percentage of time (±SE) larvae spent in one water source opposed to the other. * indicate a significant preference towards the conspecific chemical cue; ^ indicate a significant difference in preference compared to the initial trial; ~ indicate a significant preference towards the heterospecific chemical cue.

conditions, ii) lost the attraction towards the conspecifics cues in acidified seawater (700 or 1000 ppm), and iii) remained attracted towards conspecifics cues in control conditions after exposure to the acidified water. The pairwise comparison between all treatments was analyzed by a generalized linear model (GLM) of fish distribution, followed by post hoc pairwise comparison Dunnet test. Dunnett's test, a post-hoc multiple comparison procedure, is designed to hold the family wise error rate at or below alpha when performing multiple comparisons of treatment group with control. For the same individual repeatedly, the GLM used a repeated measures design. In this case, a correction factor like Bonferroni may be unnecessary.

2.4. Experiment 2: Red soil treatment

Two concentrations (50 and 200 mg/L) of unpolluted red soil (*Kunigami mahgi*), obtained from the Okinawa Prefectural Agricultural Research Centre, were tested. The suspended sediment concentration of <50 mg/L is regarded as low level pollution in marginal near shore reefs. While >100 mg/L is considered a high state of pollution worldwide (Omija, 2004; Fabricius et al., 2005; Wenger et al., 2012). Red soil pollution alters marine systems in two ways; first by introducing additional chemical cues emitted from the red soil into the water column and second, by increasing water column turbidity. Therefore, it was important to experimentally separate the impact of turbidity from the additional of red soil chemical cues.

To test the effect of red soil on the behavioural response towards conspecifics, *C. viridis* or *S. hispidus* larvae were given a choice between conspecifics seawater and heterospecifics seawater. The same larva was tested four consecutive times i) untreated seawater (control trial), ii) seawater treated with either 50 or 200 mg/L of settled red soil (testing chemical cues and removing turbidity), iii) seawater treated with either 50 or 200 mg/L of red soil in turbid water (testing both chemical cues and turbidity) and, iv) untreated seawater (a second control for the exposure to turbidity and red soil chemical cues). Water treatments were created by dissolving red soil into 5 L of filtered ocean seawater with either 5 conspecifics or 5 heterospecifics for 3 h. The conspecifics and heterospecifics were removed from the tank, and the water sources were left for a further two hours. This allowed the sediment to settle out of solution for the isolation of red soil chemical cues or remain in suspension by adding aeration to test turbidity. Between each test, the larva was maintained in an individual tank supplied with flow-through ocean seawater for 60 min before being re-tested. The experiment was conducted on 30 *C. viridis* larvae and 20 *S. hispidus* larvae for each red soil concentration (50 or 200 mg/L).

2.5. Experiment 3: Pesticide treatment

The effect pesticides had on conspecific recognition of *C. viridis* was tested using a seawater control, a solvent control (acetone) and two chlorpyrifos concentrations (1 and 100 μg/L). Chlorpyrifos are one of the most common pesticides found in coral reefs. The concentrations tested reflect levels previous research had identified to inflict negative effects on fish biology, ecology and behavior (Botte et al., 2012). A total of 50 mg of chlorpyrifos was first dissolved into 125 mL acetone at a final concentration of 400 mg/L (Botte et al., 2012). To mimiize potential solvent effects, stock concentrations were diluted 10 L tanks, ensuring acetone volume did not exceed 0.02% (v/v).

To test the effect of chlorpyrifos on the behavioural response towards conspecifics cues, 15*C. viridis* larvae per concentration level were given a choice between conspecifics treated seawater and heterospecifics treated seawater. The same larva was tested three consecutive times i) using untreated filtered seawater (control), ii) using filtered seawater with 1 or 100 μg/L of pesticides, and iii) using untreated filtered seawater (control for previous pesticide exposure). Between each test, the larva was maintained in an individual tank of untreated filtered seawater for 60 min. Additionally, to determine any effect exposure to acetone may have had on fish behavioural preferences, 10 *C. viridis* larvae were given the choice between untreated filtered seawater and filtered seawater containing acetone (125 mL of acetone in 10 L of sea-water; z-test).

2.6. Sample preparation and analysis of seawater chemical fingerprints

High performance liquid chromatography (HPLC) was used to acquire the profiles of seawater metabolites. This allowed the comparison of the chemical fingerprints of conspecifics between untreated seawater and the different experimental treatments (acidification, red soil, and pesticides). Water samples were prepared by holding 10 juvenile *C. viridis* for 3 h in 10 L of: i) filtered untreated seawater at each location (Moorea and Sesoko), ii) seawater treated to either 400, 700 or 1000 ppm CO_2, iii) "clear" seawater made with either 50 or 200 mg/L settled red soil, iv) turbid seawater made with either 50 or 200 mgL^{-1} of red soil and lastly, v) seawater with 1 or 100 μg/L of chlorpyrifos. Each water sample (10 L) was vacuum filtered through solid phase extraction (SPE) cartridges containing C_{18} silica-gel-based bonded phase sorbent, washed with 50 mL distilled water, and subsequently desorbed with 50 mL methanol. The organic phase of each sample was freeze-dried leaving a powdery organic residue. The organic extracts were dissolved in 1 mL methanol before analysis. The data were processed to create a max plot chromatogram that plotted the maximum spectral absorbance measured at each time point. Max plot chromatogram detects all UV-absorbing components in a sample. The absorbance of each component (computed as the area of peak and expressed in absorbance units, AU) was used to identify the molecular diversity of conspecifics odor in presence or absence of acidified seawater, red soil or pesticides.

3. Results

3.1. Experiment 1: Anthropogenic ocean acidification

Both *C. viridis* and *S. hispidus* displayed a significant attraction to the chemical cues of conspecifics when tested against the chemical cues of heterospecifics under current day conditions (400 ppm; z test, *p*-value < 0.001 for both taxa; Fig. 1A). Larvae spent ~70% of their time in the chemical cue containing the conspecific cue opposed to seawater containing heterospecific cues (mean % ± SE; *C. viridis:* 71 ± 3.2%; *S. hispidus:* 70 ± 3.5%). However, when seawater was treated to a either 700 ppm or 1000 ppm CO_2, this attraction was significantly reduced. When compared to the initial present day control trial, *C. viridis* displayed a >23% decreased attraction and *S. hispidus* decreased conspecific attraction by >25% (GLM, $p < 0.001$; Dunnet test, $p < 0.001$ for both taxa). Furthermore, at both 700 ppm or 1000 ppm CO_2, marine larvae lost their attraction towards the conspecific cue, spending equal time in water containing either the conspecific or heterospecifc signal (z test, $p > 0.19$ for both taxa).

Larvae of both species, previous exposed to either 700 ppm or 1000 ppm CO_2, regained their preference for conspecific chemical signals when tested for a third time in present day (400 ppm) conditions (*C. viridis:* 68 ± 3.5%; *S. hispidus:* 66 ± 3.0%; z test, $p < 0.003$ for both taxa; Fig. 1A). *C. viridis* and *S. hispidus* larvae displayed no significant difference between their initial 400 ppm trial and the final 400 ppm experimental trial (Dunnet test, $p = 0.97$ for *C. viridis*, $p = 0.94$ for *S. hispidus*).

3.2. Experiment 2: Red soil treatment

C. viridis and *S. hispidus* larvae displayed signficiant preferences towards the chemical cues of conspecifics opposed to heterospecifics prior to red soil exposure (*C. viridis:* 70 ± 3.6% and *S. hispidus:* 67 ± 3.1%; Fig. 1B).

Preferences for the conspecific chemical signal persisted in both species when experiment trials were run in water treated with 50 mg/L of red soil that was allowed to precipitate out of solution (Fig. 1B). The

attraction towards conspecific cues was reduced by 2% for *C. viridis* and 4% for *S. hispidus* (GLM $p < 0.001$; Dunnet test *C. viridis* $p = 0.98$; *S. hispidus* $p = 0.55$). *C. viridis* was able to maintain a significant preference for the conspecific chemical cue when the red soil concentration was increased to 200 mg/L (z test, $p = 0.03$). However, the preference for the conspecific cue decreased from 70% to 60% (Fig. 1B). In contrast, *S. hispidus* lost their preference for the conspecific chemical cue when the amount of red soil was increased to 200 mg/L (z test, $p = 0.16$) with the preference for the conspecific cue decreased from 67% to 49% (Fig. 1B).

In the turbid water treatment containing both the chemical cues and turbidity of the red soil, regardless of soil concentration 50 mg/L or 200 mg/L, the attraction towards conspecific cues was significantly reduced in both species compared to the initial trial with no red soil (Fig. 1B). The turbidity associated with 50 mg/L red soil significantly reduced the time spent in conspecific chemical cues by 24% for *C. viridis* and 19% for *S. hispidus* (Dunnet test, $p < 0.001$ for both taxa). Resulting in a loss of chemical attraction behavior in both *C. viridis* and *S. hispidus* larvae at 50 mg/L (z test, $p > 0.43$ for both taxa) and 200 mg/L red soil (z test, $p > 0.17$ for both taxa).

Larvae were retested for their chemical preferences of conspecific vs. heterospecific cues post exposure to either levels of red soil (50 or 200 mg/L) and exposure types (chemical cue only and turbid red soil water). Both species significantly regained attraction to conspecifics chemical cues ($p < 0.02$ for all tests). There were no significant differences in the strength of their cue preference between the initial control and final post exposure control trials ($p > 0.90$ for all tests).

3.3. Experiment 3: Pesticide treatment

Exposure to acetone had no effect on the choice behavior of *C. viridis* larvae (mean ± SE percentage of time spent in the control flow: 54 ± 2.1% - z test, $p = 0.85$). As expected by previous results, initial preferences demonstrated a significant attraction towards the chemical cues of conspecifics compared to heterospecifics (Fig. 1C).

Exposure to seawater treated with 1 μg/L of chlorpyrifos reduced the attraction towards conspecific cues by 33%, resulting in *C. viridis* larvae no long spending significantly more time in the conspecific cue choice (z test, $p = 0.29$). Time spent in the conspecific cue was further reduced in seawater treated with 100 μg/L of chlorpyrifos. *C. viridis* initially spent 77% of their time in the seawater containing chemical cues of the conspecific. However, 100 μg/L of chlorpyrifos reduced the amount of time spent in conspecific water to only 22%. Unlike all other anthropogenic experimental trials, exposure to 100 μg/L altered the chemical preference of *C. viridis* so greatly that fish in this treatment displayed a significant preference for heterospecific opposed to conspecific chemical cues (z test, $p < 0.001$).

In the post exposure control trial (without pesticide), larvae previously exposed to 1 μg/L chlorpyrifos were significantly attracted to the conspecific chemical cues (z test, $p = 0.01$) and no significant difference was found in the strength of their preference between the initial and post exposure control trials (Dunnet test, p-value = 0.36). Recovery of chemical preference was not shown in *C. viridis* that had been exposed to 100 μg/L, individuals did not significantly prefer the chemical cues of conspecifics, spending only 57% of their time this cue (z test, $p = 0.06$). As a result, cue preference was significantly different when comparing pre and post exposure control trials (Dunnet test, $p = 0.04$).

3.4. Analysis of seawater chemical fingerprints

HPLC determined the chemical fingerprints of *C. viridis* in presence or absence of acidified seawater, red soil and pesticides (Figs. 2,3,4). Three control seawater samples (no anthropogenic treatments or conspecifics) were characterized by very few minor peaks. Therefore, each peak found was due to the odor of conspecifics rather than the seawater itself.

In acidification treatments, the chemical fingerprint of conspecifics at 400 ppm was characterized by two major peaks (noted 1 and 2 in Fig. 2) and one peak complex (noted 3 in Fig. 2). At 700 ppm and 1000 ppm CO_2, the fingerprint still contained one major peak (noted 2) and one peak complex (noted 3). However, the absorbance of each peak decreased: from 5.37 AU at 400 ppm to 5.44 AU at 1000 ppm for the peak 2; and from 7.28 AU at 400 ppm to 6.54 AU at 1000 ppm. No significant variation was highlighted between the absorbance of each peak and acidified water (no significant regression, slope = 0.0001, $p = 0.97$ for peak 2; slope = −0.001, $p = 0.72$ for peak 3). Interestingly, the peak noted 1 at 400 ppm (0.56 AU) disappeared in seawater treated to 700 ppm and 1000 ppm; and a new minor peak appeared at 1000 ppm (0.08 AU; noted A in Fig. 2).

The chemical fingerprint of conspecific cues in the absence of red soil was characterized by three major peaks (noted 1 to 3 in Fig. 3) and one peak complex (noted 4 in Fig. 3). The fingerprint of unpolluted red soil (*Kunigami mahgi*) did not show any peaks. In presence of red soil, the chemical fingerprint of conspecific cues was still characterized by the one major peak and one peak complex (noted 2 and 4). No significant variation was found between the peak absorbance and red soil concentration. The first major peak (noted 1, 0.18 AU) disappeared when red soil was added at any concentration and the third peak (noted 3) was only present on the chromatograms of 50 mg/L red soil (0.98 AU without red soil, 0.28 AU in clear water, and 0.85 AU in turbid water). Two peaks appeared in presence of red soil: a minor peak (noted A, from 0.13 to 0.33 AU) on all chromatograms and a major peak (noted B, 2.22 AU) on the chromatogram of 50 mg/L red soil in clear water (Fig. 3).

The chemical fingerprint of conspecifics in the absence of a pesticide was characterized by seven major peaks (noted 1 to 7 in Fig. 4) and one peak complex (noted 8 in Fig. 4). The fingerprint of chlorpyrifos was characterized by one major peak at 18′31 min (4.79 AU). This peak was present on the fingerprint of conspecifics at 1 μg/L (0.28 AU) and 100 μg/L (5.10 AU). In presence of 1 μg/L pesticides, the fingerprint of conspecific chemical cues was characterized by a decrease in absorbance of all but one peak (peak 7) and the disappearance of 1 peak (noted 6) and the peak complex (noted 8). For example, the absorbance of peak 1 was reduced 10-fold (from 12.8 AU to 1.2 AU). One minor peak appeared in seawater treated with 1 μg/L pesticides (noted A in Fig. 4). In presence of 100 μg/L pesticides, the chemical fingerprint of conspecific cues was characterized by virtually no peaks, with the exception of two (noted 1 and 7), both had low absorbance reduced by 11 and 18 fold respectively (Fig. 4).

4. Discussion

Anthropogenic induced stressors, such as ocean acidification, sedimentation/turbidity, and pesticide runoff result in direct consequences for coral reef habitats. These anthropogenic alterations also impact the ability of larvae during the recruitment stage to recognize and respond to the chemical cues used for habitat identification. The use of chemical cues in habitat selection (Kingsford et al., 2002; Dixson et al. 2014; Barth et al., 2015) and the importance of conspecific chemical cues has been widely accepted (Kingsford et al., 2002; Dixson et al., 2011; Coppock et al. 2013; Lecchini and Nakamura 2013; Barth et al., 2015). Results collected here, confirm an attraction towards conspecific opposed to heterospecific chemical cues, in fish (*C. viridis*) and crustacean (*S. hispidus*) larvae. However, when chemical cues were exposed to anthropogenic induced stressors (ocean acidification, sedimentation/turbidity, and pesticide runoff), waterborne chemical information is altered. This change results in fish and crustacean larvae no longer displaying an attraction towards important conspecific chemical signatures. The reduced attraction could result in decreased recruitment to reefs experiencing said stressors.

Fish and shrimp larvae lost their chemical attraction to conspecifics cues in seawater treated to mimic conditions expected to occur in 50–100 years (Fig. 1A). The larval attraction was reduced by 23% in

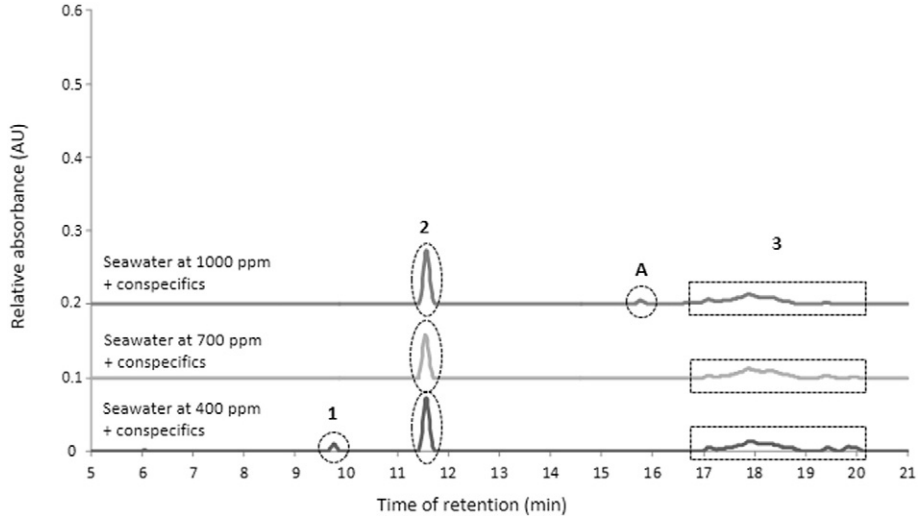

Fig. 2. High performance liquid chromatography chromatograms (HPLC) of conspecific fingerprint different levels of acidified water (400, 700, 1000 ppm). The first 5 min of chromatograms were removed, as it corresponded to dead volume in the column, and to maximize sensitivity, the data were processed to create max plot chromatograms, which plots the maximum spectral absorbance measured at each time point. The specific peaks of conspecific fingerprint in absence of stressor were noted 1 to 3. The alarm peak of conspecifics emitted in polluted environment was noted A.

either 700 ppm or 1000 ppm CO_2 seawater when compared to a present day control condition (400 ppm). Similarly, *Amphiprion percula* (clownfish) larvae reared in seawater simulating future ocean acidification conditions, resulted in the inability of larvae to discriminate between predators and non-predators (Dixson et al. 2010) and parents and non-parents (Munday et al. 2009). Our results are consistent with previous studies, demonstrating an acidification induced effect on fish behavior and the perception of chemical cues (Dixson et al. 2010, Munday et al. 2012; Heuer and Grosell, 2014).

Red soil also affected the chemical abilities of marine larvae to recognize their conspecifics (Fig. 1B). When red soil was suspended in water and allowed to precipitate from the water column, leaving red soil chemical cues but removing turbidity, the larval attraction to conspecifics was only lost for *S. hispidus* at high concentrations (200 mg/L) of red soil. Both *C. viridis* and *S. hispidus* were able to recognize and respond to conspecific cues at the lower concentration tested (50 mg/L). While the "clear" red soil treatment no longer contains the red soil particulates, it does contain the waterborne chemical components produced by each concentration level of red soil within the water column. In contrast, *C. viridis* and *S. hispidus* larvae lost their chemical attraction to conspecific cues in turbid water at both 50 mg/L and 200 mg/L of red soil. A reduction in fish abundance, biomass and species diversity has been documented at inshore sites and sites highly impacted by sediment compared to offshore or low sediment sites (Fabricius et al., 2005; Mallela et al., 2007). Our results indicate that crustacean and fish larvae are more chemically sensitive to water turbidity (i.e. sedimentation) than the chemical pollution of red soil (Fig. 1B). Similarly, an increase in turbidity (suspended sediment levels >45 mg/L) impaired habitat choice and foraging success of coral reef fish recruits due to reduced ability to distinguish visual and chemical cues (Wenger et al., 2012, 2015; O'Connor et al., 2016). Importantly, the rainy season corresponds with a high recruitment levels on the Great

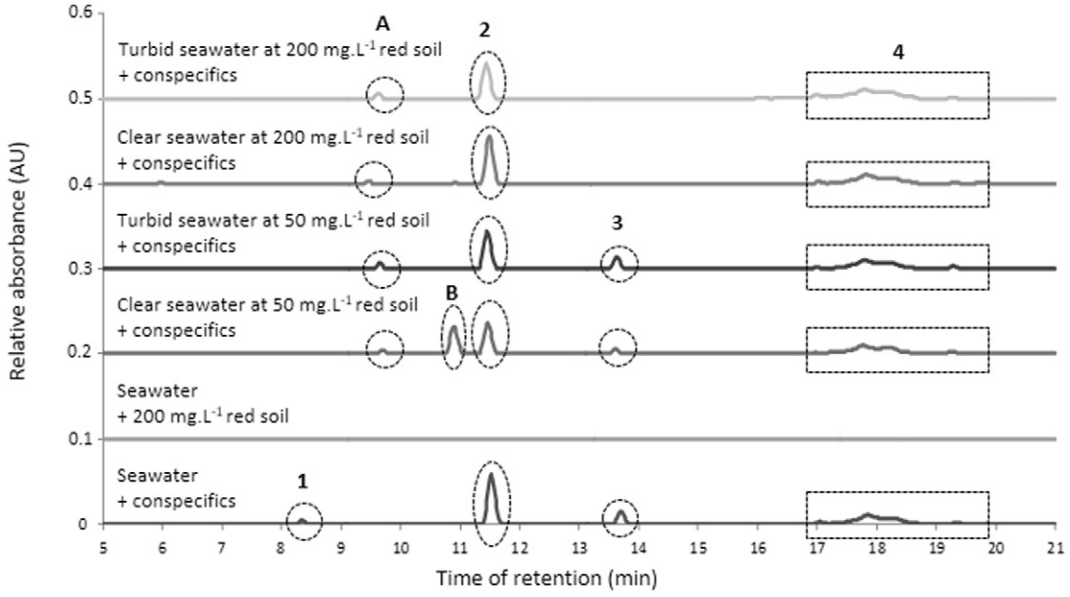

Fig. 3. HPLC of conspecific fingerprint in different levels of concentrations of red soil pollution (0, 50 or 200 mg/L of red soil in clear or turbid water) and of unpolluted red soil *Kunigami mahgi*. The specific peaks of conspecific fingerprint in absence of stressor were noted 1 to 4. The alarm peaks of conspecifics emitted in polluted environment was noted A and B.

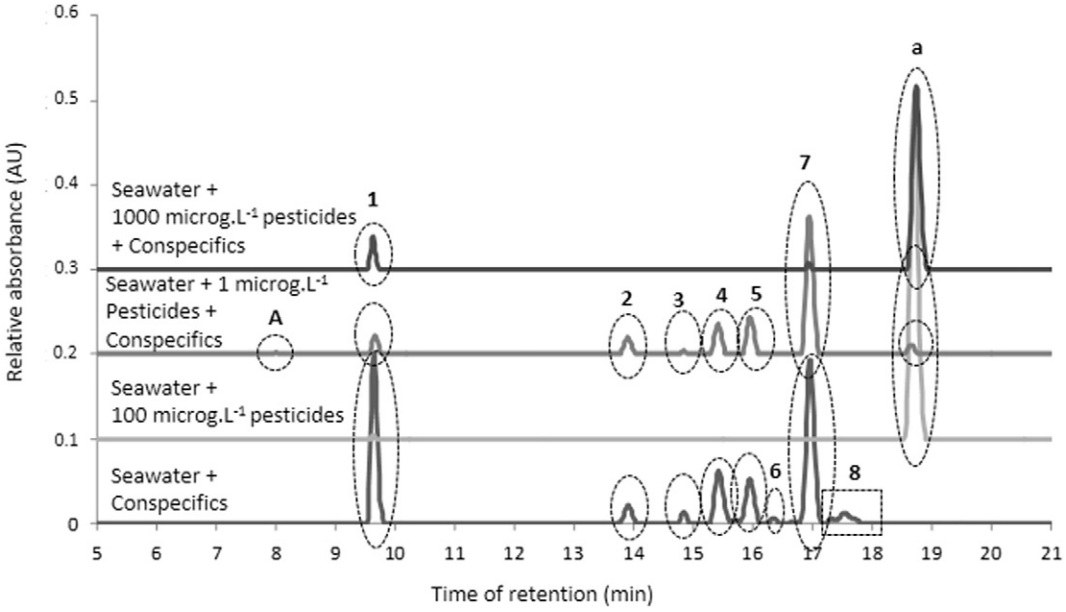

Fig. 4. HPLC of conspecific fingerprint in different concentrations of pesticide (0, 1 or 100 μg/L) and of pure chlorpyrifos. The specific peaks of conspecific fingerprint in absence of stressor were noted 1 to 8. The alarm peak of conspecifics emitted in polluted environment was noted A. The specific peak of chlorpyrifos was noted 'a'.

Barrier Reef, Ryukyu Island, and French Polynesia (Nakamura et al., 2009a; Lo-Yat et al., 2011); meaning fish and crustacean larvae experience sub-optimal conditions during recruitment as sedimentation is increased due to precipitation induced runoff.

In addition to terrestrial runoff, some pesticides, used in agriculture, are carried into coral lagoons, as in Moorea Island (Roche et al., 2011). Toxicology research in coral reefs have focused on the pesticide concentration found the tissue of different marine organisms (Roche et al., 2011). Few studies highlighted the influence of pesticides on biology, ecology and ontogenetic development of coral and fishes (Fabricius et al., 2005; Botte et al., 2012). No studies to date have tested the impact of pollutants on recruitment cues used by settlement stage larvae. *C. viridis* larvae significantly lost their ability to chemically recognize conspecifics cues at 1 μg/L and 100 μg/L chlorpyrifos (Fig. 1C). Moreover, among all anthropogenic treatments studied, exposure to the 100 μg/L chlorpyrifos pesticide had lasting effects on *C. viridis*. Fish were unable to recognize conspecific chemical cues after pesticide exposure when tested in untreated filtered seawater.

Although several studies suggest the presence of alarm cues emitted as a signal of stress (Barth et al., 2015), no studies in coral reefs have explored the effects of human pollution on social interactions (e.g., reproduction, recruitment, foraging activities – Leis et al., 2011). Coral reef fish and crustaceans are exposed to an enormous diversity of chemical cues that are mixed and dispersed by waves, currents, and tides. Therefore, extracting useful information from multiple cues is crucial to the survival of marine organisms (Barth et al., 2015). It has been shown that the chemical cues from specific reef components can impact recruitment, with coral acting as a positive cue and seaweed acting as a negative source of chemical stimuli (Dixson et al. 2014). Lecchini et al. (2013) found, during the recruitment stage, 70% of the fish species preferred water from reefs dominated by live coral compared to reefs dominated by algae. It was suggested that fish larvae could respond to the quantity of chemical cues emitted by conspecifics whose abundance changes in response to coral and algal cover, or the chemical cues varied based on the environment. Here, we demonstrate the latter, showing a modification of the chemical fingerprints of conspecifics according to the environment (Figs. 2,3,4). In acidification and red soil treatments, conspecific fingerprints were modified with a disappearance of some peaks and the appearance of some 'alarm' peaks (i.e. emission of metabolic disturbance factors). For example, at 700 ppm CO_2, there was a disappearance of peak noted 1 (Fig. 2), which suggest that conspecific behavior is altered by acidified water. At 1000 ppm CO_2, there was the appearance of one peak noted A (Fig. 2), suggesting the production of alarm cues by conspecifics. Similarly, two peaks appeared in the presence of red soil, a minor peak A (from 0.13 to 0.33 AU) on all chromatograms and a major peak B (2.22 AU) on the chromatogram of 50 mg/L in water containing the chemical cues of red soil alone (Fig. 3). The presence of alarm peaks in the red soil experiments did not inhibit the larval attraction towards conspecific cues, suggesting alarm signals are minor compared to the cue of conspecific juveniles. In the 1 μg/L pesticide treatment, the conspecific chemical fingerprint was modified with a decrease in absorbance of nearly all peaks, the disappearance of two peaks and the appearance of one alarm peak (Fig. 4). At a concentration of 100 μg/L pesticide, the conspecific chemical fingerprint contained only two peaks at low absorbance (Fig. 4). These results suggest that conspecific behavior was altered by the presence of 1 μg/L pesticides with the release of alarm cues. At 100 μg/L pesticides, conspecific cues were so strongly altered that almost no chemical signal was emitted into the seawater. Consequently, the larval attraction for conspecific chemical signals was decreased. Overall, these results highlight, for the first time, that polluted seawater modified the specific chemical signature of conspecifics. A weak stressor evoked the production of alarm cues, a strong stressor resulted in reduced cue emission.

Our results highlight that both global (ocean acidification) and local stressors (red soil and pesticides) to coral reef ecosystems could have significant effects on the sustainability of reefs by altering the behavior of fish and crustacean populations during a critical life-history transition (i.e. recruitment phase). In chemical polluted environments, marine larvae displayed a reduced or no attraction to conspecifics, thus larvae may not locate suitable settlement habitat, resulting higher predation and lower recruitment levels. Predicting how anthropogenic changes will affect connectivity and recruitment in coral reef ecosystems is important. The dispersal of larvae between reefs is a key component in population dynamics of reef organisms and reef connectivity (Munday et al., 2009). Our study investigated non-lethal behavioural effects of chemical cues in changing environments. Although the levels of ocean acidification, pesticide pollution and terrestrial runoff did not result in mortality among the exposed organisms, indirect consequences were found. Thus, if polluted seawater disrupts the recruitment process, fish larvae may misinterpret important chemical signals resulting in lower

recruitment levels to impacted reefs. Stability of marine organisms is dependent, in part, on the stability of social interactions. Therefore disruption to larvae-conspecifics relationships could have major consequences for recruitment and adult population with further repercussions for the ecosystem. Understanding the relationship between reef health and recruitment potential could allow management planning for the maintenance of coral cover and biodiversity on reefs that are increasingly degraded.

Competing interests

The authors have declared no competing interests exist.

Author contributions

D.L., Y.T., B.B. and Y.N. designed research; D.L., G.L., D.L.D., N.R., B.F., M.B. and Y.N. collected and analyzed data; D.L., G.L., D.L.D. and Y.N. wrote the paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2016.08.083.

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