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Sediment pollution impacts sensory ability and performance of settling coral-reef fish

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Abstract

Marine organisms are under threat globally from a suite of anthropogenic sources, but the current emphasis on global climate change has deflected the focus from local impacts. While the effect of increased sedimentation on settlement of coral species is well studied there is little known about the impact on larval fishes. Here, the effect of a laterite "red soil" sediment pollutant on settlement behaviour and post-settlement performance of reef fish was tested. In aquarium tests that isolated sensory cues we found significant olfaction-based avoidance behaviour and disruption of visual cue use in settlement-stage larval fish at 50mgL^{-1}, a concentration regularly exceeded *in situ* during rain events. *In situ* light trap catches showed lower abundance and species richness in the presence of red soil but were not significantly different due to high variance in the data. Prolonged exposure to red soil produced altered olfactory cue responses, whereby fish in red soil made a likely maladaptive choice for dead coral compared to controls where fish chose live coral. Other significant effects of prolonged exposure included decreased feeding rates and body condition. These effects on fish larvae reared over five days occurred in the presence of a minor drop in pH and may be due to the chemical influence of the sediment. Our results show that sediment pollution of coral reefs may have more complex effects on the ability of larval fish to successfully locate suitable habitat than previously thought, as well as impacting on their post-settlement performance and ultimately recruitment success.

Keywords:

behavioural ecology; coral reefs; environmental pollution; larval settlement; olfaction;

1. Introduction

Coral-reef ecosystems, which compose some of the world's most species rich environments, are facing threats to their survival on many fronts (Hughes et al. 2010). Apart from rising sea levels, surface temperatures, acidification and fishing pressure, coral reefs are also highly sensitive to the effects of anthropogenic land-based pollution (Bégin et al. 2014). Impacts of sediment pollution are already realised in some regions (Torres and Morelock 2002), however these effects are likely to worsen, as some 75% of the world's coral reefs are currently nearby human settlements and because human populations in nearly all countries with coral reefs are expected to double within the next 50 to 100 years (Mora et al. 2011). Sedimentation is one of the biggest localized sources of reef degradation because elevated amounts of sediment on coral reefs, generated by increased land development run-off and dredging projects, have resulted in reduced coral cover, diversity, health and productivity (Erftemeijer et al. 2012; Fabricius 2005). Sediment pollution also directly impacts on the fitness of coral reef inhabitants (Wong et al. 2013), though the indirect mechanisms leading to shifts in density and species richness of fishes on sediment-impacted reefs are still being determined (Edinger and Risk 2013).

For coral reef fish populations to persist, their larvae need first to identify suitable settlement habitat then establish in this habitat as juveniles and adults. Coral-reef fish larvae have well-developed sensory systems capable of detecting habitat-relevant cues to allow orientation towards suitable settlement habitat, as well as the swimming capability to influence the direction of travel in ocean currents (Kingsford et al. 2002). Visual and olfactory senses play important roles for larval fish in orienting swimming direction towards suitable habitat (Lecchini et al. 2014a; Lecchini et al. 2014b). Therefore sediment pollution likely influences coral reef fish recruitment through impacting the capacity of these fishes to detect these cues (Siebeck et al. 2014). Suspended sediment can disrupt the ability of larval fish to select appropriate habitat, though our understanding of which cues are affected and the potential consequences for recruitment success is limited (Wenger et al. 2011).

Even if coral reef fish larvae can locate and settle on suitable habitat, sediment pollution may threaten the viability of reef fish populations through impacts on their post-settlement fitness. Growth and behavioural development of fish larvae during settlement not only determines their recruitment success (Shima and Findlay 2002), but also the ability of juvenile fishes to compete for resources (i.e. food, shelter and living space) within coral reef environments (Thorrold and Milicich 1990). Larval growth may be disrupted by increased sediment levels (Wenger et al. 2014). Even in cases where larval development is not inhibited the presence of suspended sediment may still increase predator-induced mortality of post-settlement individuals by disrupting sensory cues. Hence, sensory modalities are of primary importance for survival and therefore ecological fitness at both pre- and post-settlement stages (Wenger et al. 2013).

Here we investigated the effects of red soil pollution on the settlement behaviour of coral reef fish larvae and the impacts of exposure to red soil pollution on post-settlement behaviour and performance. The term "red soil" refers to a laterite soil common to Okinawa, Japan, that innundates coastal areas with high levels of silt and turbidity during run-off events. These run-off events co-incide with seasons of heavy rainfall, often three times that of other areas in Japan, from June – July and September – October (Higashi et al. 1985), corresponding with the seasonal recruitment of larval fishes during May – September (Nanami and Nishihira 2002). Red soil pollution is regarded to have dramatically increased along with land development of coastal sites in Okinawa. Although there is no quantative data of pollution levels before and after development, surveys show 97% of respondees from local government and business believe it emerged after intensive implementation of road and agriculture projects from the early 1970s (Okinawa Prefecture 1993). Although this run-off has been reduced since the enforcement of the Okinawa Prefecture Red Soil Erosion Ordinance in 1995 there is evidence that it is still a major contributor to degradation of Okinawan coral reef communities, disrupting settlement and growth of coral species, however the effect on larval fishes remains largely unknown (Omori 2011).

Here we tested the hypothesis that red soil pollution affects settlement dynamics of larval fish by applying red soil treatments *in situ* to light traps catching larvae as they approach the reef to settle, specifically investigating the patterns of species abundance and richness. We then tested the effect of red soil on sensory cue use for habitat location and selection in the laboratory by observing behavioural responses of newly caught settlement-stage larvae to visual and olfactory habitat cues in the presence and absence of red soil. To investigate the effects of prolonged exposure of red soil on post-settlement performance we reared wild-caught fish larvae under different red soil concentrations then applied similar choice experiments on sensory cue response, as well as monitoring feeding behaviour and body condition. This is the first study to combine *in situ* and *ex situ* techniques to investigate multi-faceted effects of sediment pollution on early-stage fishes from habitat selection to post-settlement fitness.

2. Materials & methods

(a) Field experiments

(i) Establishing coral species important for settlement

To decide which coral species to use in settlement behaviour trials of *C. viridis*, *in situ* settlement preference was determined using underwater visual census. First, roaming surveys, 20 minutes in length, were conducted daily over a period of five days on reef habitat adjacent to the light trap positions at Sesoko Island, an area of Okinawa regarded as unimpacted by red soil pollution (Arakaki et al. 2005). Abundance of settlement stage individuals (~10mm length) encountered and the coral species they were associating with were recorded. Surveys were conducted within two hours of first light, with surveys extending from the intertidal zone to a depth of 4m, encompassing all subtidal habitats within the site. Surveys were conducted across a ~16,000m^2 patch of reef with water visibility >10m and swell <0.5m. Second, to determine whether *C. viridis* associated with particular species of corals by chance, the cover of reef by each coral species was quantified within the site. The proportion of reef covered by coral species was quantified along ten haphazardly positioned 30m transects laid perpendicular from shore ~20m apart, recording coral species found directly under the transect tape at 50 cm intervals. Coral identification was conducted *post-hoc* from photographs taken at each interval. The most common coral that *C. viridis* associated with was determined by comparing frequencies of recruits on each coral species observed during surveys. To test if association of *C. viridis* recruits with a given coral species occurred by chance, a Chi-squared test adapted for low values (p-values evaluated by Monte Carlo simulation n=1000) was used to compare the proportion of *C. viridis* that associated with a coral species to the proportion of reef it covered.

(ii) In situ *choice experiment*

To test the *in situ* settlement dynamics of larval fish in the presence of red soil, four light traps were deployed (for more description about these light-traps, see (Nakamura et al. 2009)) adjacent to fringing coral reefs surrounding the Sesoko Station (Tropical Biosphere Research Center, University of the Ryukyus) on the south-east side of Sesoko Jima, Okinawa (See Fig. 1A in supplementary material). Traps were set at one of four locations spaced at 50m intervals and randomly assigned one of four treatments upon each deployment: 1) Control 2) Red soil added

3) Live coral added and 4) Live coral and red soil added. Red soil was added by the attachment of three slow release 250mL bottles filled with red soil suspended in sea water to the top, bottom and inner chamber of the light trap. The flow of red soil from the bottles was adjusted such that although water movement dispersed the red soil as it was released there would be suspended red soil present around the trap from the time of deployment until collection (~12 hours). Live coral was added with a *Porites cylindrica* coral head ~20cm diameter attached in a mesh bag suspended within the light trap (see Fig. 2A in supplementary material). Slow release bottles filled with clean sand and empty mesh bags were attached to treatments without red soil and/or live coral to account for potential visual bias on the trap. Total abundance and species richness from each trap catch was recorded for a period of eleven consecutive days (01 to 11 July 2013). A one-way ANOVA was used to test for differences in mean abundance and species richness between treatments. Chi-squared tests were used on rank-frequency data from the light trap catches to investigate bias in light trap location, treatment type, and temporal effects. These data were then put into a general linear model (GLM) using Poisson distribution (usually adapted for abundance data) to compare the effect of each treatment on larval catch patterns. Only fish caught in the control light traps were used in subsequent experiments to avoid prior conditioning to coral or red soil.

(b) Settlement-stage larval sensory experiments

(i) Effect of sediment extract on olfactory cue use

A two-channel Perspex choice flume of a similar design to that of Gerlach et al. (2007) was used to test preferences between olfactory cues in water sources with and without the presence of red soil. Each trial begins with a larva being placed in the centre of the downstream end of the chamber to explore the chamber and acclimate to the water sources for a period of two minutes. Fish that did not swim actively or explore both sides of the chamber during these two minutes were discarded from the trials (<5% of fish tested were discarded). After the acclimation period, the position of the fish was recorded every five seconds for another two minute period as being on one side of the chamber or the other. Water sources entering the chamber from buckets gravity-feeding into the left and right side were then switched, with one minute being allocated for the water sources to exchange and flush completely, in order to control for side preference in individuals. After switching water sources, another two-minute acclimation period was given, followed by another two-minute observation period. In this way it is possible to tease apart choice for chemical properties of the water sources and side preference of the apparatus. Each individual was tested only once after which they were released back to the capture site and the chamber was rinsed thoroughly with freshwater. Flow rate was maintained at 200mL min^{-1} and dye tests were conducted after each replicate to ensure a laminar flow on each side of the chamber without eddies or mixing of the two water sources.

Previous studies on olfactory preferences of *C. viridis* demonstrate, as with other coral reef species, a preference for chemical cues released from coral over blank seawater (Lecchini et al. 2014a). In this experiment treatments for each fish consisted of an initial control testing for positional bias in the apparatus where incoming water on both sides of the chamber was from the same source (i.e. coral-soaked water vs coral-soaked water), followed by the experimental treatment testing preferences between water from two different sources, resulting in two comparable data sets for each fish. To test the effect of red soil on behavioural response to habitat cues, *C. viridis* larvae were given a choice between coral-soaked water and the same coral-soaked water with red soil added in two different concentrations. Twenty fish larvae were used per treatment. Coral-soaked water was produced in a 150L tank containing approximately 6kg of live *P. cylindrica* coral heads. After the flow-through system had been shut off for at least two hours water was transferred to two 60L tanks. Depending on the treatment being tested 50 or 200mgL^{-1} of red soil was mixed into one of the tanks. The two water sources were then left to sit for a further 2 - 4 hours to allow time for the water containing the suspended red soil to equilibrate and become "clear" to minimise the effect of turbidity, creating a red soil "extract". Olfactory preference data was analysed using Wilcoxon's Signed Rank Test, a non-parametric test suited to the time proportion data and accounting for the repeated measures of the same individual.

(ii) Effect of suspended sediment on visual cue use

Larvae were tested in a three-compartment test chamber (60 x 12 x 10 cm). The side compartments were formed by two transparent plexiglass panels separated by 1cm placed to create barriers at 8cm from each end, resulting in a central compartment of 32cm length which was delimited into three equal parts (see Fig. 3A in supplementary material). This experimental system isolates visual cues available to the individual placed in the central compartment from chemical cues present in the side compartments. Thus, only visual cues from coral colonies were influencing larval movement in the central compartment.

Before each trial, one live and one dead coral colony were placed in the side compartments behind the plexiglass barriers. A single larva was introduced into the middle of the central compartment for a one minute acclimation period during which an opaque screen was placed between the plexiglass barriers to block visual cues after which the screens were removed and the trial commenced. The position of the larva in the central compartment was recorded every five seconds for a period of one minute. This short test period also ensured that sediment added to the test chamber remained in suspension. The aquarium was emptied and washed with freshwater after each trial. To exclude a possible side bias of the fish, the order of adjacent compartments containing each coral type was randomized for each trial. Moreover, water samples were taken and parameters of temperature, pH, salinity and turbidity were measured to ensure there was no biasing effect in these parameters.

Five treatments were applied as follows (*n* = 20 per treatment):

1) Control – Habitat compartments (at each end): Live or dead coral, larval chamber (centre): no red soil added
2) Low - 50mgL^{-1} red soil suspended in larval chamber
3) High - 200mgL^{-1} red soil suspended in larval chamber

Each individual was only tested once. Visual preference data were analysed using Wilcoxon's Rank Sum Test, a non-parametric alternative to the two-sample t test.

(c) Effect of prolonged exposure to sediment on sensory responses

(i) Olfactory response

To test the olfactory responses of settlement-stage larvae after being acclimated to different pollution levels, *C. viridis* larvae caught in light traps were placed into aerated non-flow-through 10 litre holding tanks and reared for five days, fed twice daily *ad libitum* with newly hatched *Artemia salina*. Three different treatments were applied to the rearing tanks: 1) Control (no red soil added), 2) 50mgL^{-1} red soil mixed into solution and 3) 200mgL^{-1} red soil mixed into solution. After initial mixing red soil was not resuspended during the rearing period and allowed to settle in the tank. The time during which red soil stayed in suspension varied with concentration however after two hours sediment had settled in all rearing tanks. Ten fish larvae from each treatment were then selected for olfactory choice testing using the same methods previously described, but implementing a test between olfactory cues from water soaked with live coral and dead coral (coral rubble collected from the just below the intertidal zone of the reef area sampled in this study, rinsed thoroughly to remove any sediment or algae present).

(ii) Visual response

Individuals from the same treatments as used for the olfactory experiments above were also tested in the visual cue test chamber with the same protocol as the newly caught larvae. Fifteen fish per rearing treatment were tested in clear water with live and dead coral colonies in the adjacent compartments at each end of the test chamber without red soil added. Instead of comparing red soil concentrations in the chamber, preferences of individuals swimming in clear water but from different rearing concentrations of red soil were analysed.

(d) Red soil acclimation effects on post-settlement performance

(i) Feeding rates and condition

To test the effect of red soil on post-settlement performance of *C. viridis* recruits, aquaria trials were used to compare feeding rates and physiological condition. Following capture 10 individual larvae were randomly selected and allocated to one of sixteen 15L plastic tanks - eight containing 50mgL^{-1} of red soil and eight containing untreated seawater. Larvae were maintained for seven days, fed live *Artemia salina* ad libitum twice daily. Following this rearing period, fishes were filmed using HD video from above tanks for one minute following the addition of a standardised quantity of pellet food. Feeding rates (bites per minute) of all individuals within groups were counted whilst replaying this video footage. The wet weight (WW) and standard length (SL) of each fish was measured at the end of the conditioning period, allowing calculation of Fulton's condition (K) factor; K = $100(WW/S_L^3)$. Tanks were lit naturally, aerated and contained a small piece of coral for shelter. Water was changed daily; ensuring concentrations of red soil were consistent. Sediment was suspended by stirring tanks twice daily (controls were stirred to the same extent). Sediment settled out of suspension after approximately two hours, with feeding trials conducted randomly across all treatments between 30 min and 1 hr after tanks were stirred. Feeding rates and body condition (Fulton's) were compared between red soil and no red soil treatments (fixed) and groups (random; nested within red soil treatment) using a two factor nested ANOVA (individual fish being the residual).

(e) Post-experiment red soil pH analysis

Following the analysis of the data on the sensory responses of fish reared for a prolonged period under different sediment treatments further measurements of the effect on pH levels were required. Measurements were done on site using a 713 pH meter (Metrohm Japan Ltd.) using 6 replicates in 1L bottles for each of the three treatment concentrations of red soil used for the rearing tanks (namely 0, 50 and 200mgL^{-1}). As with the rearing trials sediment was initially mixed and not disturbed for the remainder of the experimental period. Measurements of pH were taken at 0hrs (turbid water) and 2 hrs (clear water after sediment had settled to bottom), then at 3 days and 7 days after the initial mixing.

3. Results

(a) Field experiments

(i) C. viridis *coral association*

Field surveys showed *C. viridis* associated with the branching coral, *Porites cylindrica*, significantly more frequently than expected by chance; 119 of 184 individuals (64.7%) observed associated with *P. cylindrica*, which composed 4% of live, hard corals (χ^2 = 1160, df = 10, $P < 0.001$). This preliminary survey justified the use of *P. cylindrica* as a habitat cue in settlement cue experiments.

(ii) In situ *larval abundance and species richness trends*

Forty-seven species of larval fish were caught in the light traps during the field experiment. No significant experimental bias of light trap position (χ^2 = 16.8, df = 3, $P > 0.05$) or time of deployment (Global Chi² = 0.6894) was detected. Species abundance and richness between light trap treatments were not significantly different (F = 0.36, df = 3,36, P = 0.78 & F = 1.57, df = 3,36 P = 0.21 respectively) due to high levels of variance in the data (Welch test for homogeneity of variance in abundance, $P = 0.49$) and species richness ($P = 0.24$). Though light trap catches were highly variable, a similar trend for both species richness and abundance was apparent between treatments (see Fig. 4A in supplementary material). Total abundance was highest in treatments without red soil, with the live coral treatment attracting the most larvae (32%) followed by Control (empty light trap) (26%), red soil (24%) and live coral with red soil (18%). Light traps containing live coral only also attracted the highest species richness (30%) followed by control (28%), red soil and live coral with red soil (21% each).

One of the four light trap positions showed an effect of the treatment type on the number of larvae caught that approached significance ($\chi^2 = 18.6$, df = na, $P = 0.07$) and a significant difference in species richness ($\chi^2 = 37.9$, df = na, $P = 0.01$). Comparing catches at this location showed a significant difference in larval abundance between traps that contained live coral and those that did those that did not ($\chi^2 = 10$, df = na, $P = 0.01$). General Linear Modelling indicates this difference was driven by an increasing trend in catch by light trap treatment, predicting increasing catch abundance with treatments as follows: Control + Red soil < Live Coral + Red Soil < Control < Live Coral, a similar trend seen in the non-significant mean abundance and species richness between treatments (see Table 1A in supplementary material). Comparing rank frequencies of larval abundance at each of the four light trap positions for each day with chi-squared tests showed no difference in three of the four light traps ($\chi^2 = 6.2$, df = na, $P = 0.69$), however the southerly light trap position showed significantly higher catch over time irrespective of which treatments were being applied ($\chi^2 = 10.8$, df = na, $P = 0.02$). It should be noted that this light trap was excluded from the GLM due to its potential bias.

(b) Settlement-stage larvae sensory experiments

(i) Larvae avoid habitat chemical cues with sediment extract present

No bias to the chamber or experimental conditions was detected in *C. viridis* larvae, with no side preference shown when presented with the same habitat cue on both sides of the chamber. Larvae spent approximately equal time on each side (mean ±SE percentage of time spent in the left flow 52 ± 0.63%; and in the right 48 ± 0.63%), justifying this comparison as a control treatment to test for experimental bias. Larvae responded to the red soil infused treatment water by spending significantly more time in the live coral soaked water (>70%), strongly avoiding the water infused with $50 mgL^{-1}$ of red soil (Z= -3.643, $P < 0.001$). This effect was increased when the concentration of red soil was increased to $200 mgL^{-1}$ spending over 74% of time (Fig. 1) in the live coral water (Z= -3.642, $P < 0.001$). Turbidity measurements did show a slight increase in turbidity with red soil concentration from live coral water to 50 and $200 mgL^{-1}$ (0.12, 0.15 and 0.19 NTU/FTU respectively), however this difference was regarded as nominal.

(ii) Visual cue use disrupted by suspended sediment

Larvae presented with visual cues of both live coral and dead coral spent significantly more time swimming near the live coral than the dead coral (Wilcoxon's Rank Sum test, z-value = 3.0, p-value = 0.002), spending twice the proportion of time exploring the compartment closest to the live coral chamber compared to the compartment near the dead coral (56% vs. 26% respectively). When $50 mgL^{-1}$ of red soil solution was added to the central compartment with the larvae, this ability to visually discriminate between the live and dead coral chambers disappeared (see Fig. 2), with no significant difference in time spent at either end of the choice chamber (Wilcoxon's test, Z = 0.05, $P = 0.96$). Time spent exploring the chamber dropped dramatically when $200 mgL^{-1}$ of red soil solution was added to the chamber: larvae were in the central compartment 75% of the time, as opposed to only 28% under the $50 mgL^{-1}$ conditions. Again, approximately equal time was spent exploring compartments close to both live and dead coral (Wilcoxon's test, Z = 0.05, *P* = 0.957).

(c) Effect of prolonged exposure to sediment on sensory responses

(i) Choice behaviour in response to olfactory cues reversed or eliminated

After 5 days of being reared in test aquaria, fish under red soil conditions displayed significantly different behaviour than those raised in "clean" control water (see Fig. 3). Individuals reared under control conditions strongly preferred live coral cues over dead coral cues (Wilcoxon's Rank Sum; Z = 2.81, *P* = 0.01), spending over 70% of the time in the flow containing live coral cues. By comparison the fish reared in water containing 50mgL^{-1} shifted their preference, displaying significant choice towards dead coral cues over live coral cues (Wilcoxon's Rank Sum; Z = 2.20, *P* = 0.03), spending 63% the time in the flow containing dead coral cues. After being reared in 200mgL^{-1} conditions there was no significant choice between the two cues (Wilcoxon's Rank Sum; Z = -0.05, *P* = 0.96).

(ii) Choice behaviour undetectable in response to visual cues

Larvae reared under control conditions spent equal time near the live and dead coral compartments (31% each). Responses of larvae reared with 50mgL^{-1} of sediment were similar, with 32% and 29% of time spent near live and dead coral compartments respectively. Under 200mgL^{-1} rearing conditions this dynamic changed somewhat, with the largest proportion of time spent near the dead coral compartment (40%). This change in choice behaviour is similar to what occurred in the olfaction trials, though the difference was not statistically significant (Wilcoxon's test, Z = 0.86, *P* = 0.39). Furthermore, position distributions were not significantly different between the rearing treatments for time spent close to both live coral (K-S Test, *P* = 0.99) and dead coral (K-S Test, *P* = 0.93).

(d) Red soil acclimation effects on post-settlement performance

(i) Decreased feeding rates and body condition

Feeding rates and condition were significantly impacted by the presence of red soil (See Fig. 4). Feeding rates were significantly slower for red soil conditioned than control fish ($F_{1,\ 144}$ = 7.12; *P* = 0.04), whereas, within the sediment treated tanks there were significant differences among groups ($F_{1,\ 144}$ = 3.65; *P* = 0.02). Condition was also significantly poorer in red soil than control fish ($F_{1,\ 99}$ = 5.52; *P* = 0.04), though it did not significantly differ amongst groups within the sediment treatment tanks ($F_{1,\ 99}$ = 0.15; *P* = 0.12). There was no difference in mortality between control and red soil treated fishes; 25 and 20 individuals from 80 respectively in the sediment treatment tanks (t_{14} = 0.90; *P* = 0.38).

(e) Post-experimental pH analysis

Average pH level declined with increasing sediment concentration. Immediately after mixing mean pH of control samples, 50mgL^{-1} and 200mgL^{-1} was 8.1, 8.09 and 8.08 respectively. Measurements were similar at 2 hours after the sediment had settled out of suspension however while the mean pH of the control water remained stable over time the water treated with red soil became more acidic over time, reaching 8.08 and 8.04 after 7 days for the 50 and 200mgL^{-1} treatments respectively (see Table 2A in supplementary material).

4. Discussion

Larval fish behaviour is likely to influence their dispersal and orientation to suitable settlement habitat (Cowen et al. 2006), yet the impacts of sediment pollution on this process have been largely unresolved. We showed that in the presence of sediment pollution, such as that caused by red soil, tropical reef fish larvae could be inhibited from locating suitable coral habitats during settlement. Specifically, we found that in the presence of red soil, *C. viridis* larvae failed to distinguish suitable coral habitat visual cues and avoided coral habitat olfactory cues. This may lead to decreased chances for recruitment in areas prone to recurrent sediment pollution, such as Okinawa, Japan, compared to more pristine areas. Previous studies have reported avoidance behaviour in settlement-stage fish of either degraded (by algal phase shift (Lecchini et al. 2013)) or dying coral reef habitat (Feary et al. 2007), and our results suggest sensory cue use may be an important factor. Evidence for chemically mediated avoidance behaviour *in situ* has been shown using cues from degraded seaweed-dominated reef habitats, suggesting that olfactory cues from sediment-degraded reefs could affect recruitment densities and reef resilience (Dixson et al. 2014).

Evidence of negative impacts of sediment pollution on settlement behaviour of settlement-stage reef fish larvae have been found previously (Wenger and McCormick 2013), yet the field relevance of these results were previously untested. Here we provide the initial evidence that the presence of red soil near coral habitat, even administered in small amounts, may reduce settlement success in the field. Untreated light traps containing live coral with red soil absent caught a higher abundance and species richness than light traps with coral in the presence of red soil. The statistical significance of this result may have been affected due to a positional bias of the light traps, with the southerly light trap position (the direction from which the prevailing current flows) catching more larvae than the other three positions. High levels of variation in catch abundance, seen in our results, is also a notoriously typical effect on reliable light trap sampling (Leis et al. 2002). The non-significant trend of a negative response of settling fishes to red soil treatments was congruent with results obtained in the laboratory experiments.

Results showing either an avoidance of olfactory cues from habitat with red soil present or a disruption of the ability or inclination to utilise visual habitat cues in settlement stage coral reef fish larvae indicates that such pollution of coral reef environments may impact larval recruitment and survival. A reluctance or inability to approach potential habitats can extend the period spent by fish larvae in close proximity to the reef running the "predation gauntlet", increasing the risk of predation

(Almany and Webster 2006). Even prolonged exposure to non-predatory fish can be detrimental, with harassment of recruiting fish larvae by territorial residents observed to result in elevated predation risk or avoiding the habitat altogether (Leis and Yerman 2012). In addition to this, previous studies have shown an effect of sediment pollution on larval development whereby the PLD is increased (Wenger et al. 2014). This effect could alter the timing and location of larval settlement, changing the natural distribution and recruitment of fish larvae through the modifications of their movement towards habitat can affect the spatial and community demography of fish populations.

Even if fish larvae manage to settle on coral reef habitat degraded with sediment pollution, our results show that prolonged exposure to the presence of sediment could impact post-settlement behaviour and survival. Five days of exposure to water containing red soil significantly altered the response to olfactory cues from different habitat types, reversing the strong preference for live coral cues under control conditions to instead preferring those from dead corals. At a higher rearing concentration of red soil, response to olfactory cues was absent altogether. As the habitat cues presented to individuals here after the rearing period contained no sediment influence, it is assumed that the change in behaviour was due to physiological effects of exposure on the sense organ, rather than impairment of transmission of the cues themselves. The response to visual cues in individuals exposed for five days to sediment differed from newly caught larvae, no longer showing significant choice behaviour towards live coral over dead coral. This reduced exploration of the visual cue chamber may be an artefact of rearing in captivity or an ontogenetic change in behaviour, perhaps due to a reduction in the inclination to use visual habitat cues to orient movement by juveniles compared to settling larvae (Lecchini et al. 2007).

Reversal in normal behavioural responses to olfactory cues has been shown in response to increased water acidification, where a normal avoidance of predator cues became a strong attraction in fish larvae reared in water with higher acidity (Dixson et al. 2010). Furthermore, when the acidity of rearing conditions is increased further the fish larvae no longer responded to olfactory cues (Munday et al. 2009), similar to the trend in our results. The mechanism suggested for this is an alteration caused by the increased acidity of anion gradients across neuronal membranes which reverse normal receptor function (Nilsson et al. 2012). The changes in behaviour seen in the reared post-settlement fish may be a similar response due to the acidity of red soil. Behavioural avoidance of acid sulphate soils by juvenile fish has been shown in juveniles of various fishes where pH levels were well within the range exhibited in natural systems (Kroon 2005). Red soil in Okinawa is acidic with a pH of ~5 (Mkadam et al. 2006), and analysis of rearing conditions used here showed pH decreased both with increasing concentrations of red soil and with time. Reductions in pH of a similar range (0.07) have been shown to elicit similar behavioural changes in juvenile coral trout (*Plectropomus leopardus*) (Munday et al. 2013). This acidic soil could influence the pH of water surrounding the reef on which it deposits, as has been shown with organic-rich soils (Weber et al. 2012).

Previous studies on the effect of red soil on water chemistry showed that as red soil concentration increased in a solution, availability of H^+ ions (increasing acidity) and heavy metals, particularly Al^{3+} also increased (Kombo et al. 2005). Exposure to elevated concentrations of heavy metals can also lead to changes in behavioural responses of fish to olfactory cues (Scott and Sloman 2004). Increased Al^{3+} concentrations can exacerbate the damage done to the olfactory epithelium by increased acidity alone, working synergistically to disrupt olfactory abilities in salmonid fish (Klaprat et al. 1988). Our results suggest that chemical properties of sediment pollution may cause greater impacts on coral reef environments than turbidity alone. Of course, it is not only the use of habitat cues that can affect the ecology of early-stage fishes. Chemical pollution of the aquatic environment may also disrupt communication, social recognition and shoaling in fish, which can have drastic effects on population fitness (Fisher et al. 2006; Ward et al. 2008).

Exposure to sediment pollution negatively impacted the condition and performance in *C. viridis.* Both feeding rates and condition reduced with increasing concentration of red soil. The sensory disruption detected in other individuals of the same cohort during this experiment as well as other physiological effects from prolonged exposure to sediment pollution may have played a role in reduced foraging success. As evidence suggests that growth is a critical variable in the recruitment success and survival of post-settlement fish this is another aspect in which sediment pollution can impact on affected fish populations (Bergenius et al. 2002). Our results concur with data on juvenile reef fish reared under similar concentrations of suspended sediment (0 – 180mgL^{-1}), where reduced foraging behaviour led to reduced growth, condition and survival (Wenger et al. 2012). This effect has also been shown to reduce foraging success *in situ* when prey detection may be limited by absolute light levels rather contrast (Fiksen et al. 2002).

In conclusion, this experiment used a variety of techniques and life history stages to investigate the effect of sediment pollution on coral reef fish larvae around the crucial settlement period. At the settlement stage we found evidence of disruption of natural use of sensory cues related to locating suitable habitat by fish larvae in the presence of increased sediment levels. Following settlement (after five days captive rearing) metamorphosing post-settlement fish experienced reduced feeding behaviour and condition under the sediment treatments. Response to habitat-relevant sensory cues became confused, reversing the preference from live coral to dead coral in some cases in individuals which had experienced prolonged exposure to sediment concentrations. These results are similar to those reported for elevated levels of CO_2 which also can simultaneously affect a range of fish behaviours (Jutfelt et al. 2013). These include avoidance behaviour, reversal of behavioural responses to ecologically relevant sensory cues, reduced growth and reduced feeding rates (Baumann et al. 2012; Briffa et al. 2012; Cripps et al. 2011; Dixson et al. 2010; Munday et al. 2009). This suggests that the interaction of red soil with the surrounding water can cause chemically altered environments detrimental for fish survival.

In any case, these behavioural changes can be biomarkers for significant biological effects of sediment pollution in marine environments (Galloway et al. 2004). If the impacts on sensory cue use in recruiting larval fishes influences the recruitment success of coral reef fish populations this can have implications for both fisheries and marine park management as import and export of larvae between populations and habitats may be constrained by degraded habitat (Dixson et al. 2014). This is the first study assesing the effects of red soil soil pollution on settlement behaviour of larval fishes and further work is needed to assess how these impacts are influencing recruitment on sediment polluted reefs.

The concentrations of sediment used in this experiment are regularly encountered on coral reef ecosystems across the world, for example on the Great Barrier Reef, Australia, sediment concentrations are regularly recorded near the lower level used here ($50 mgL^{-1}$) and can exceed the higher level ($200 mgL^{-1}$) during the wet season or as a result of dredging (Bak 1978; Wenger and McCormick 2013). This indicates negative impacts on settlement behaviour and success of reef fish larvae may be exacerbated if sediment load influx to coral reef environments increases due to anthropogenic activities. The close proximity of acidic sediment similar to Okinawa's red soil to coral reef environments (e.g. over 600,000ha of acid sulfate soil resides within the Great Barrier Reef (GBR, Queensland) catchment alone (Powell and Martens 2005)), means that chemical effects of sediment are likely to have substantial impacts on coral reefs and their inhabitants. Future work is required to better understand these effects and how they interplay with effects of sediment-induced turbidity.

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List of Figures

Fig. 1 Mean percentage of time (±SE) spent by settlement-stage *C. viridis* larvae in each side of the olfactory choice chamber when presented with cues from live coral with and without red soil extract at different concentrations. *** $P < 0.001$, N.S. no significant choice behaviour (n = 20)

Fig. 2 Mean percentage of time (±SE) spent by settlement-stage *C. viridis* larvae in compartments close to live coral, dead coral, and in the centre in the presence of suspended sediment at different concentrations. *** $P < 0.01$, N.S. no significant choice behaviour between live and dead coral cues (n = 20)

Fig. 3 Mean percentage of time (±SE) spent by reared *C. viridis* when presented with live coral and dead coral soaked water after 5 days of exposure to different red soil concentrations. * $P < 0.05$, N.S. no significant choice behaviour between live and dead coral cues (n = 10)

Fig. 4 Mean percentage of time (±SE) spent by reared *C. viridis* in compartments close to live coral, dead coral and in the centre after 5 days exposure to different concentrations of red soil (n = 15)

Fig. 5 Mean feeding rates (panel a) and body condition (panel b) (±SE) for *C. viridis* after rearing in the presence of red soil compared to control conditions $P = < 0.05$ (n= 160)

Fig.1

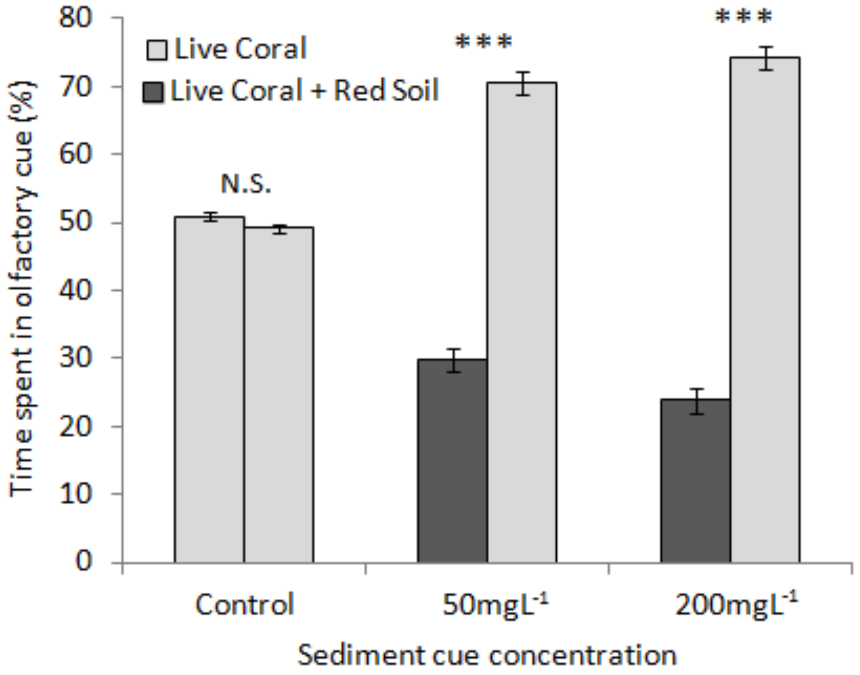

80
70
60
50
40
30
20
10
0
Time spent in olfactory cue (%)
Live Coral
Live Coral + Red Soil
N.S.
***
***
Control
50mgL-1
200mgL-1
Sediment cue concentration

Fig.2

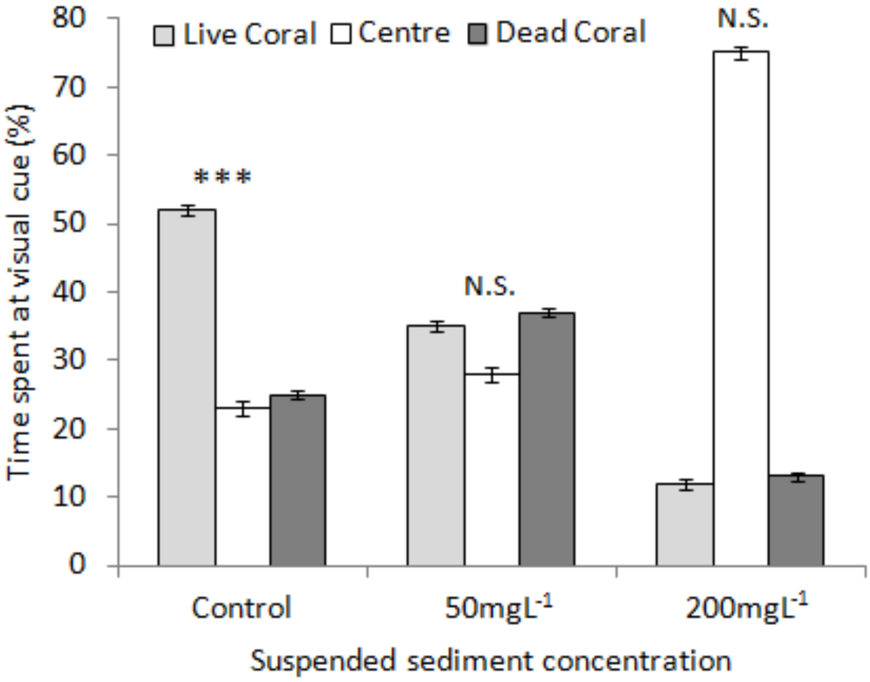

80
70
60
50
40
30
20
10
0
Time spent at visual cue (%)
Live Coral
Centre
Dead Coral
***
N.S.
N.S.
Control
50mgL-1
200mgL-1
Suspended sediment concentration

Fig.3

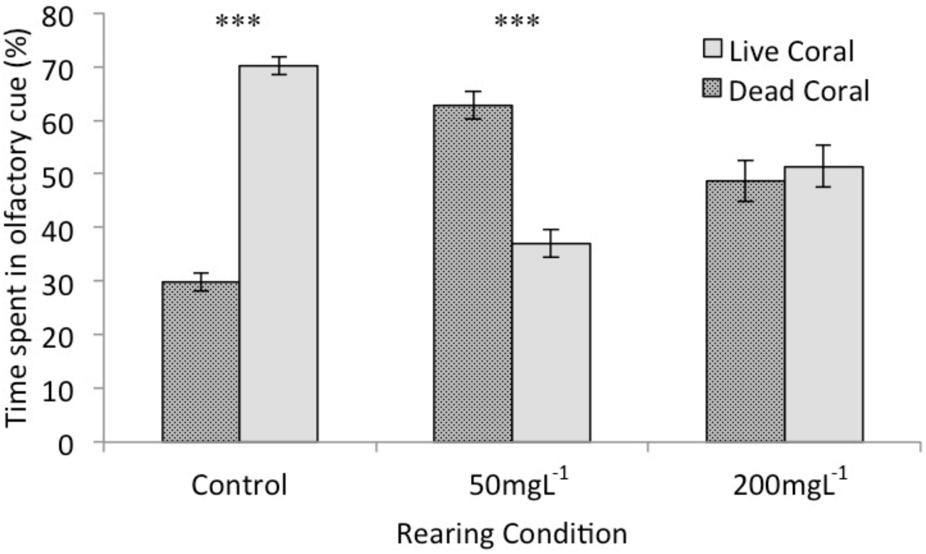

Time spent in olfactory cue (%)
80
70
60
50
40
30
20
10
0
***
***
Live Coral
Dead Coral
Control
50mgL-1
200mgL-1
Rearing Condition

Fig.4

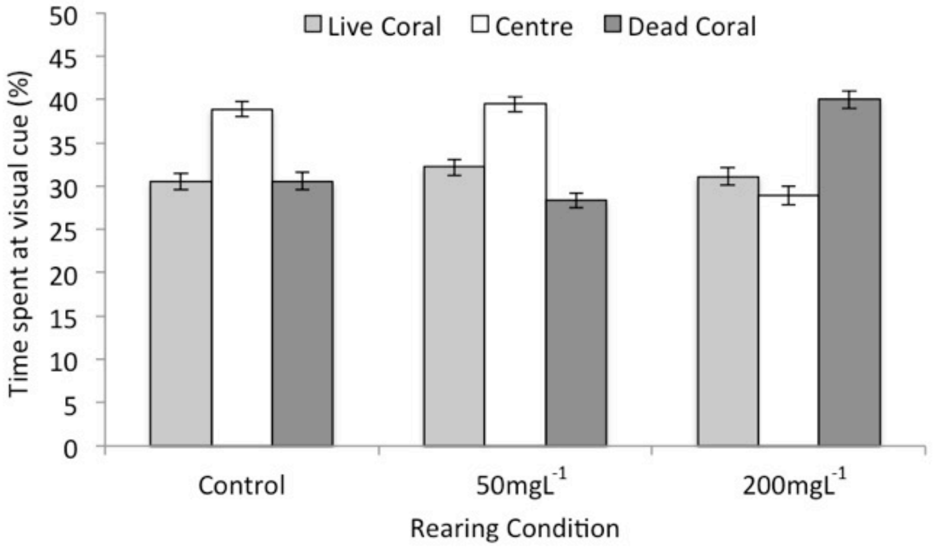
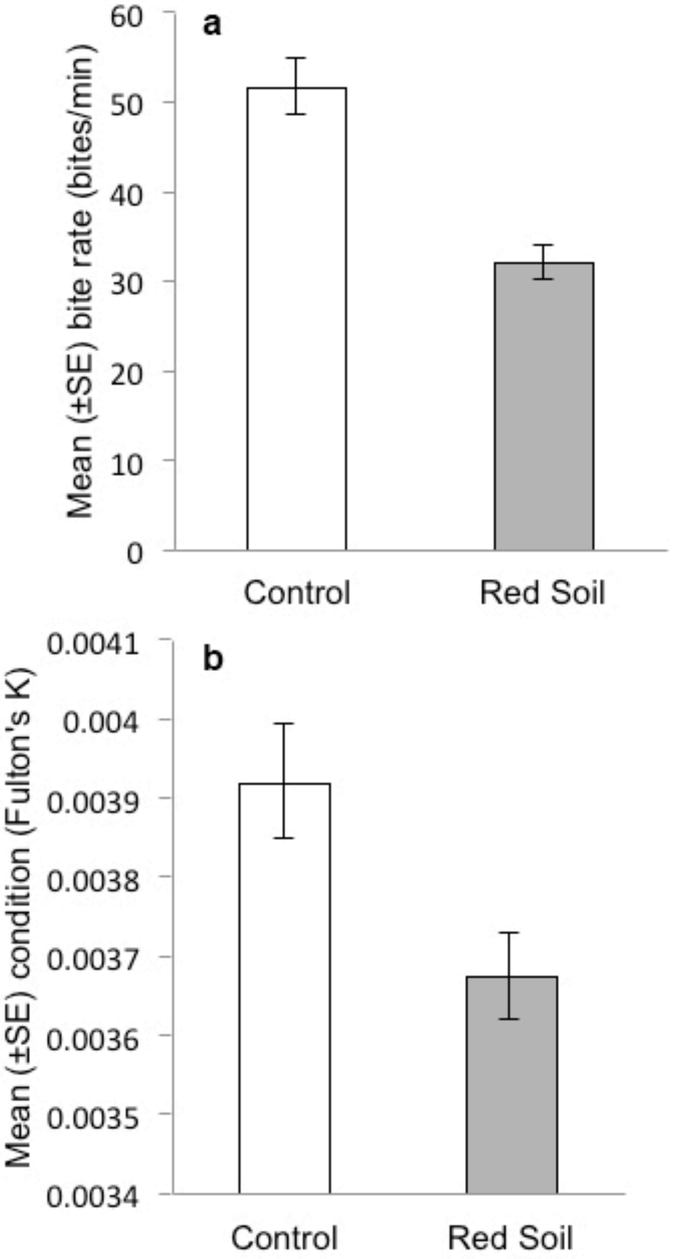

Live Coral
Centre
Dead Coral
Time spent at visual cue (%)
50
45
40
35
30
25
20
15
10
5
0
Control
50mgL-1
200mgL-1
Rearing Condition

Fig.5


a
Mean (±SE) bite rate (bites/min)
60
50
40
30
20
10
0
Control
Red Soil
b
Mean (±SE) condition (Fulton's K)
0.0041
0.004
0.0039
0.0038
0.0037
0.0036
0.0035
0.0034
Control
Red Soil