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

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Title Page

<u>Sediment pollution impacts sensory ability and performance of settling coral-reef fish</u>

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28

29 **Abstract**

30   Marine organisms are under threat globally from a suite of anthropogenic

31 sources, but the current emphasis on global climate change has deflected the focus

32 from local impacts. While the effect of increased sedimentation on settlement of coral

33 species is well studied there is little known about the impact on larval fishes. Here,

34 the effect of a laterite "red soil" sediment pollutant on settlement behaviour and post-

35 settlement performance/fitness of larval reef fish was tested. In aquarium tests that

36 isolated sensory cues we found significant olfaction-based avoidance behaviour and

37 disruption of visual cue use in settlement-stage larval fish at 50mgL^{-1}, a

38 concentration regularly exceeded *in situ* during rain events. *In situ* light trap catches

39 showed a trend of lower abundance and species richness in the presence of red soil

40 but were not significantly different due to high variance in the data. Prolonged

41 exposure to red soil produced altered olfactory cue responses, whereby fish in red

42 soil made a likely maladaptive choice for dead coral compared to controls where fish

43 chose live coral. Other significant effects of prolonged exposure included decreased

44 feeding rates and body condition. These effects on fish larvae occurred in the

45 presence of a minor drop in pH and may be due to the chemical influence of the

46 sediment. Our results show that sediment pollution of coral reefs may have more

47 complex effects on the ability of larval fish to successfully locate suitable habitat than

48 previously thought, as well as impacting on their post-settlement performance and

49 ultimately recruitment success.

50

53

54 **<u>1. Introduction</u>**

2

55   Coral-reef ecosystems, which compose some of the world's species rich

56   environments, are facing threats to their survival on many fronts (Hughes et al.

57   2010). Apart from rising sea levels, surface temperatures, acidification and fishing

58   pressure, coral reefs are also highly sensitive to the effects of anthropogenic land-

59   based pollution (Bégin et al. 2014). Impacts of sediment pollution are already

60   realised in some regions (Rogers 1990), however these effects are likely to worsen,

61   as some 75% of the world's coral reefs are currently nearby human settlements and

62   because human populations in nearly all countries with coral reefs are expected to

63   double within the next 50 to 100 years (Mora et al. 2011). Sedimentation is one of

64   the biggest localized sources of reef degradation because elevated amounts of

65   sediment on coral reefs, generated by increased land development run-off and

66   dredging projects, have resulted in reduced coral cover, diversity, health and

67   productivity (Erftemeijer et al. 2012; Fabricius 2005). Sediment pollution also directly

68   impacts on the fitness of coral reef inhabitants (Wong et al. 2013), though the

69   indirect mechanisms leading to shifts in density and species richness of fishes on

70   sediment-impacted reefs are still being determined (Edinger and Risk 2013).

71   For coral reef fish populations to persist, their larvae need first to identify

72   suitable settlement habitat then establish in this habitat as juveniles and adults.

73   Coral-reef fish larvae have well-developed sensory systems capable of detecting

74   habitat-relevant cues to allow orientation towards suitable settlement habitat, as well

75   as the swimming capability to influence the direction of travel in ocean currents

76   (Kingsford et al. 2002). Visual and olfactory senses play important roles for larval fish

77   in orienting swimming direction towards suitable habitat (Lecchini et al. 2014a;

78   Lecchini et al. 2014b). Therefore sediment pollution likely influences coral reef fish

79   recruitment through impacting the capacity of these fishes to detect these cues

80   (Siebeck et al. 2014). Suspended sediment can disrupt the ability of larval fish to

81 select appropriate habitat, though our understanding of which cues are affected and

82 the potential consequences for recruitment success is limited (Wenger et al. 2011).

83   Even if coral reef fish larvae can locate and settle on suitable habitat,

84 sediment pollution may threaten the viability of reef fish populations through impacts

85 on their post-settlement fitness. Growth and behavioural development of fish larvae

86 during settlement not only determines their recruitment success (Shima and Findlay

87 2002), but also the ability of juvenile fishes to compete for resources (i.e. food,

88 shelter and living space) within coral reef environments (Thorrold and Milicich 1990).

89 Larval growth may be disrupted by increased sediment levels (Wenger et al. 2013a).

90 Even in cases where larval development is not inhibited the presence of suspended

91 sediment may still increase predator-induced mortality of post-settlement individuals

92 by disrupting sensory cues. Hence, sensory modalities are of primary importance for

93 survival and therefore ecological fitness at both pre- and post-settlement stages

94 (Wenger et al. 2013b).

95   Here we investigated the effects of red soil pollution on the settlement

96 behaviour of coral reef fish larvae and the impacts of exposure to red soil pollution

97 on post-settlement behaviour and performance. The term "red soil" refers to a laterite

98 soil that causes high levels of silt and turbidity around the coral reefs in Okinawa,

99 Japan, during run-off events exacerbated by increased land development. Although

100 this run-off has been reduced since the enforcement of the Okinawa Prefecture Red

101 Soil Erosion Ordinance in 1995 there is evidence that it is still a major contributor to

102 degradation of Okinawan coral reef communities, disrupting settlement and growth of

103 coral species, however the effect on larval fishes remains largely unknown (Omori

104 2011).

105   Here we tested the hypothesis that red soil pollution affects settlement

106 dynamics of larval fish by applying red soil treatments *in situ* to light traps catching

107 larvae as they approach the reef to settle, specifically investigating the patterns of

108 species abundance and richness. We then tested the effect of red soil on sensory

109 cue use for habitat location and selection in the laboratory by observing behavioural

110 responses of newly caught settlement-stage larvae to visual and olfactory habitat

111 cues in the presence and absence of red soil. To investigate the effects of prolonged

112 exposure of red soil on post-settlement performance we reared wild-caught fish

113 larvae under different red soil concentrations then applied similar choice experiments

114 on sensory cue response, as well as monitoring feeding behaviour and body

115 condition. This is the first study to combine *in situ* and *ex situ* techniques to

116 investigate multi-faceted effects of sediment pollution on early-stage fishes from

117 habitat selection to post-settlement fitness.

118 **2. Materials & methods**

119 **(a) Field experiments**

120 *(i) Establishing coral species important for settlement*

121 The planktivorous damselfish *Chromis viridis*, highly abundant in tropical reefs

122 and light trap catches of the area, was selected as the study species for behavioural

123 and conditioning experiments. *In situ* settlement preference of coral species for *C.*

124 *viridis* was determined using underwater visual surveys on reef habitat adjacent to

125 the light trap positions recording abundance of settlement stage individuals and the

126 coral species they were associating with. To determine whether *C. viridis* associated

127 with particular species of corals by chance, the cover of reef by each coral species

128 was quantified within the site. The most common coral that *C. viridis* associated with

129 was determined by comparing frequencies of recruits on each coral species

130 observed during surveys. To test if association of *C. viridis* recruits with a given coral

131 species occurred by chance, a Chi-squared test adapted for low values (p-values

132 evaluated by Monte Carlo simulation n=1000) was used to compare the proportion of

133 *C. viridis* that associated with a coral species to the proportion of reef it covered.

134 *(ii)* In situ *choice experiment*

135   To test the *in situ* settlement dynamics of larval fish in the presence of red

136   soil, four light traps were deployed (for more description about these light-traps, see

137   (Nakamura et al. 2009)) adjacent to fringing coral reefs surrounding the Sesoko

138   Station (Tropical Biosphere Research Center, University of the Ryukyus) on the

139   south-east side of Sesoko Jima, Okinawa (See Fig. 1A in supplementary material).

140   Traps were set at one of four locations spaced at 50m intervals and randomly

141   assigned one of four treatments upon each deployment: 1) Control: empty bottles

142   added, 2) Red soil added, 3) Live coral added and 4) Both live coral and red soil

143   added. Red soil was added by the attachment of three slow release 250mL bottles

144   filled with red soil suspended in sea water to the top, bottom and inner chamber of

145   the light trap. Live coral was added with a *Porites cylindrica* coral head ~20cm

146   diameter attached in a mesh bag suspended within the light trap. Total abundance

147   and species richness from each trap catch was recorded for a period of eleven

148   consecutive days (01 to 11 July 2013). A one-way ANOVA was used to test for

149   differences in mean abundance and species richness between treatments. Chi-

150   squared tests were used on rank-frequency data from the light trap catches to

151   investigate bias in light trap location, treatment type, and temporal effects. These

152   data were then put into a general linear model (GLM) using Poisson distribution

153   (usually adapted for abundance data) to compare the effect of each treatment on

154   larval catch patterns. Only fish caught in the control light traps were used in

155   subsequent experiments to avoid prior conditioning to coral or red soil.

156

157   **(b) Settlement-stage larval sensory experiments**

158   *(ii) Effect of sediment extract on olfactory cue use*

159   A two-channel Perspex choice flume of a similar design to that of Gerlach et

160   al. (2007) was used to test preferences between olfactory cues in water sources with

161   and without the presence of red soil. Each trial begins with a larva being placed in

162   the centre of the downstream end of the chamber to explore the chamber and

163   acclimate to the water sources for a period of two minutes. Fish that did not swim

164   actively or explore both sides of the chamber during these two minutes were

165   discarded from the trials (<5% of fish tested were discarded). After the acclimation

166   period, the position of the fish was recorded every five seconds for another two

167   minute period as being on one side of the chamber or the other. Water sources

168   entering the chamber from buckets gravity-feeding into the left and right side were

169   then switched, with one minute being allocated for the water sources to exchange

170   and flush completely, in order to control for side preference in individuals. After

171   switching water sources, another two-minute acclimation period was given, followed

172   by another two-minute observation period. In this way it is possible to tease apart

173   choice for chemical properties of the water sources and side preference of the

174   apparatus. Each individual was tested only once after which they were released back

175   to the capture site and the chamber was rinsed thoroughly with freshwater. Flow rate

176   was maintained at 200mL min^{-1} and dye tests were conducted after each replicate to

177   ensure a laminar flow on each side of the chamber without eddies or mixing of the

178   two water sources.

179   Experimental treatments for each fish consisted of an initial control treatment

180   where incoming water on both sides of the chamber was from the same source (i.e.,

181   coral-soaked water vs coral-soaked water), followed by the experimental treatment

182   testing preferences between water from two different sources, resulting in two

183   comparable data sets for each fish. To test the effect of red soil on behavioural

184   response to habitat cues, *C. viridis* larvae were given a choice between coral-soaked

185   water and the same coral-soaked water with red soil added in two different

186   concentrations. Twenty fish larvae were used per treatment. Coral-soaked water was

187   produced in a 150L tank containing approximately 6kg of live *P. cylindrica* coral

188   heads. After the flow-through system had been shut off for at least two hours water

189 was transferred to two 60L tanks. Depending on the treatment being tested 50 or

190 200mgL^{-1} of red soil was mixed into one of the tanks. The two water sources were

191 then left to sit for a further 2 - 4 hours to allow time for the water containing the

192 suspended red soil to equilibrate and become "clear" to minimise the effect of

193 turbidity, creating a red soil "extract". Olfactory preference data was analysed using

194 Wilcoxon's Signed Rank Test, a non-parametric test suited to the time proportion

195 data and accounting for the repeated measures of the same individual.

196   *(ii) Effect of suspended sediment on visual cue use*

197   Larvae were tested in a three-compartment test chamber (60 x 12 x 10 cm).

198 The side compartments were formed by two transparent plexiglass panels separated

199 by 1cm placed to create barriers at 8cm from each end, resulting in a central

200 compartment of 32cm length which was delimited into three equal parts. This

201 experimental system isolates visual cues available to the individual placed in the

202 central compartment from chemical cues present in the side compartments. Thus,

203 only visual cues from coral colonies were influencing larval movement in the central

204 compartment.

205   Before each trial, one live and one dead coral colony were placed in the side

206 compartments behind the plexiglass barriers. A single larva was introduced into the

207 middle of the central compartment for a one minute acclimation period during which

208 an opaque screen was placed between the plexiglass barriers to block visual cues

209 after which the screens were removed and the trial commenced. The position of the

210 larva in the central compartment was recorded every five seconds for a period of one

211 minute. This short test period also ensured that sediment added to the test chamber

212 remained in suspension. The aquarium was emptied and washed with freshwater

213 after each trial. To exclude a possible side bias of the fish, the order of adjacent

214 compartments containing each coral type was randomized for each trial. Moreover,

215 water samples were taken and parameters of temperature, pH, salinity and turbidity

216 were measured to ensure there was no biasing effect in these parameters.

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

218 1) Control – Habitat compartments (at each end): Live or dead coral, larval

219   chamber (centre): no red soil added

220 2) Low - 50mgL^{-1} red soil suspended in larval chamber

221 3) High - 200mgL^{-1} red soil suspended in larval chamber

222 Each individual was only tested once. Visual preference data were analysed using

223 Wilcoxon's Rank Sum Test, a non-parametric alternative to the two-sample t test.

224

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

226 *(i) Olfactory response*

227   To test the olfactory responses of settlement-stage larvae after being

228 acclimated to different pollution levels, *C. viridis* larvae caught in light traps were

229 placed into aerated non-flow-through 10 litre holding tanks and reared for five days,

230 fed twice daily *ad libitum* with newly hatched *Artemia salina*. Three different

231 treatments were applied to the rearing tanks: 1) Control (no red soil added), 2)

232 50mgL^{-1} red soil mixed into solution and 3) 200mgL^{-1} red soil mixed into solution.

233 After initial mixing red soil was not-remixed during the rearing period and allowed to

234 settle in the tank. Ten fish larvae from each treatment were then selected for

235 olfactory choice testing using the same methods previously described, but

236 implementing a test between olfactory cues from water soaked with live coral and

237 dead coral (coral rubble collected from the just below the intertidal zone of the reef

238 area sampled in this study).

239   *(ii) Visual response*

240   Individuals from the same treatments as used for the olfactory experiments

241 above were also tested in the visual cue test chamber with the same protocol as the

242 newly caught larvae. Fifteen fish per rearing treatment were tested in clear water

243 with live and dead coral colonies in the adjacent compartments at each end of the

244 test chamber without red soil added. Instead of comparing red soil concentrations in

245 the chamber, preferences of individuals swimming in clear water but from different

246 rearing concentrations of red soil were analysed.

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

248 *(i) Feeding rates and condition*

249 To test the effect of red soil on post-settlement performance of *C. viridis*

250 recruits, aquaria trials were used to compare feeding rates and physiological

251 condition. Following capture 10 individual larvae were randomly selected and

252 allocated to one of sixteen 15L plastic tanks - eight containing 50mgL^{-1} of red soil

253 and eight containing untreated seawater. Larvae were maintained for seven days,

254 fed live *Artemia salina* ad libitum twice daily. Following this rearing period, fishes

255 were filmed using HD video from above tanks for one minute following the addition of

256 a standardised quantity of pellet food. Feeding rates (bites per minute) of all

257 individuals within groups were counted whilst replaying this video footage. The wet

258 weight (WW) and standard length (SL) of each fish was measured at the end of the

259 conditioning period, allowing calculation of Fulton's condition (K) factor; K =

260 $100(WW/S_L^3)$. Tanks were lit naturally, aerated and contained a small piece of coral

261 for shelter. Water was changed daily; ensuring concentrations of red soil were

262 consistent. Feeding rates and body condition (Fulton's) were compared between red

263 soil and no red soil treatments (fixed) and groups (random; nested within red soil

264 treatment) using a two factor nested ANOVA (individual fish being the residual).

265 **(e) Post-experiment red soil pH analysis**

266 Following the analysis of the data on the sensory responses of fish reared for

267 a prolonged period under different sediment treatments further measurements of the

268 effect on pH levels were required. Measurements were done on site using a 713 pH

269 meter (Metrohm Japan Ltd.) using 6 replicates in 1L bottles for each of the three

270 treatment concentrations of red soil used for the rearing tanks (namely 0, 50 and

271 200mgL^{-1}). As with the rearing trials sediment was initially mixed and not disturbed

272 for the remainder of the experimental period. Measurements of pH were taken at

273 0hrs (turbid water) and 2 hrs (clear water after sediment had settled to bottom), then

274 at 3 days and 7 days after the initial mixing.

275 **3. Results**

276 **(a) Field experiments**

277 *(i)* C. viridis *coral association*

278 Field surveys showed *C. viridis* associated with the branching coral, *Porites*

279 *cylindrica*, significantly more frequently than expected by chance; 119 of 184

280 individuals (64.7%) observed associated with *P. cylindrica*, which composed 4% of

281 live, hard corals (χ^2 = 1160, df = 10, *P* < 0.001). This preliminary survey justified the

282 use of *P. cylindrica* as a habitat cue in settlement cue experiments.

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

284 Forty-seven species of larval fish were caught in the light traps during the field

285 experiment. No significant experimental bias of light trap position (χ^2 = 16.8, df = 3, *P*

286 > 0.05) or time of deployment (Global Chi² = 0.6894) was detected. Species

287 abundance and richness between light trap treatments were not significantly different

288 (*F* = 0.36, df = 3,36, *P* = 0.78 & *F* = 1.57, df = 3,36 *P* = 0.21 respectively) due to high

289 levels of variance in the data (Welch test for homogeneity of variance in abundance,

290 *P* = 0.49) and species richness (*P* = 0.24). Though light trap catches were highly

291 variable, a similar trend for both species richness and abundance was apparent

292 between treatments (see Fig. 2A in supplementary material). Total abundance was

293 highest in treatments without red soil, with the live coral treatment attracting the most

294 larvae (32%) followed by Control (empty light trap) (26%), red soil (24%) and live

295 coral with red soil (18%). Light traps containing live coral only also attracted the

296 highest species richness (30%) followed by control (28%), red soil and live coral with

297 red soil (21% each).

298 One of the four light trap positions showed an effect of the treatment type on

299 the number of larvae caught that approached significance (χ^2 = 18.6, df = na, P =

300 0.07) and a significant difference in species richness (χ^2 = 37.9, df = na, P= 0.01).

301 Comparing catches at this location showed a significant difference in larval

302 abundance between traps that contained live coral and those that did those that did

303 not (χ^2 = 10, df = na, P = 0.01). General Linear Modelling indicates this difference

304 was driven by an increasing trend in catch by light trap treatment, with significant

305 coefficients ordered as Control + Red soil < Live Coral + Red Soil < Control < Live

306 Coral, a similar trend seen in the non-significant mean abundance and species

307 richness between treatments (see Table 1A in supplementary material). Comparing

308 rank frequencies of larval abundance at each of the four light trap positions for each

309 day with chi-squared tests showed no difference in three of the four light traps (χ^2 =

310 6.2, df = na, P = 0.69), however the southerly light trap position showed significantly

311 higher catch over time irrespective of which treatments were being applied (χ^2 =

312 10.8, df = na, P = 0.02). It should be noted that this light trap was excluded from the

313 GLM due to its potential bias.

314 **(b) Settlement-stage larvae sensory experiments**

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

316 No bias to the chamber or experimental conditions was detected in *C. viridis*

317 larvae, with no side preference shown when presented with the same habitat cue on

318 both sides of the chamber. Larvae spent approximately equal time on each side

319 (mean ±SE percentage of time spent in the left flow 52 ± 0.63%; and in the right 48 ±

320 0.63%), justifying this comparison as a control treatment to test for experimental

321 bias. Larvae responded to the red soil infused treatment water by spending

322 significantly more time in the live coral soaked water (>70%), strongly avoiding the

323 water infused with 50mgL^{-1} of red soil (Z= -3.643, *P* < 0.001). This effect was

324 increased when the concentration of red soil was increased to 200mgL^{-1} spending

325 over 74% of time (Fig. 1) in the live coral water (Z= -3.642, *P* < 0.001). Turbidity

326 measurements did show a slight increase in turbidity with red soil concentration from

327 live coral water to 50 and 200mgL^{-1} (0.12, 0.15 and 0.19 NTU/FTU respectively),

328 however this difference was regarded as nominal.

329   *(ii) Visual cue use disrupted by suspended sediment*

330   Larvae presented with visual cues of both live coral and dead coral spent

331 significantly more time swimming near the live coral than the dead coral (Wilcoxon's

332 Rank Sum test, z-value = 3.0, p-value = 0.002), spending twice the proportion of time

333 exploring the compartment closest to the live coral chamber compared to the

334 compartment near the dead coral (56% vs. 26% respectively). When 50mgL^{-1} of red

335 soil solution was added to the central compartment with the larvae, this ability to

336 visually discriminate between the live and dead coral chambers disappeared (see

337 Fig. 2), with no significant difference in time spent at either end of the choice

338 chamber (Wilcoxon's test, Z = 0.05, *P* = 0.96). Time spent exploring the chamber

339 dropped dramatically when 200mgL^{-1} of red soil solution was added to the chamber:

340 larvae were in the central compartment 75% of the time, as opposed to only 28%

341 under the 50mgL^{-1} conditions. Again, approximately equal time was spent exploring

342 compartments close to both live and dead coral (Wilcoxon's test, Z = 0.05, *P* =

343 0.957).

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

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

346   After 5 days of being reared in test aquaria, fish under red soil conditions

347 displayed significantly different behaviour than those raised in "clean" control water

348 (see Fig. 3). Individuals reared under control conditions strongly preferred live coral

349 cues over dead coral cues (Wilcoxon's Rank Sum; Z = 2.81, *P* = 0.01), spending

350 over 70% of the time in the flow containing live coral cues. By comparison the fish

351 reared in water containing 50mgL^{-1} shifted their preference, displaying significant

352 choice towards dead coral cues over live coral cues (Wilcoxon's Rank Sum; Z =

353 2.20, P = 0.03), spending 63% the time in the flow containing dead coral cues. After

354 being reared in 200mgL^{-1} conditions there was no significant choice between the two

355 cues (Wilcoxon's Rank Sum; Z = -0.05, P = 0.96).

356   *(ii) Choice behaviour undetectable in response to visual cues*

357   Larvae reared under control conditions spent equal time near the live and

358 dead coral compartments (31% each). Responses of larvae reared with 50mgL^{-1} of

359 sediment were similar, with 32% and 29% of time spent near live and dead coral

360 compartments respectively. Under 200mgL^{-1} rearing conditions this dynamic

361 changed somewhat, with the largest proportion of time spent near the dead coral

362 compartment (40%). This change in choice behaviour is similar to what occurred in

363 the olfaction trials, though the difference was not statistically significant (Wilcoxon's

364 test, Z = 0.86, P = 0.39). Furthermore, position distributions were not significantly

365 different between the rearing treatments for time spent close to both live coral (K-S

366 Test, P = 0.99) and dead coral (K-S Test, P = 0.93).

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

368   *(i) Decreased feeding rates and body condition*

369 Feeding rates and condition were significantly impacted by the presence of red soil

370 (See Fig. 4). Feeding rates were significantly slower for red soil conditioned than

371 control fish ($F_{1, 144}$ = 7.12; P = 0.04), whereas, within the sediment treated tanks

372 there were significant differences among groups ($F_{1, 144}$ = 3.65; P = 0.02). Condition

373 was also significantly poorer in red soil than control fish ($F_{1, 99}$ = 5.52; P = 0.04),

374 though it did not significantly differ amongst groups within the sediment treatment

375 tanks ($F_{1, 99}$ = 0.15; P = 0.12). There was no difference in mortality between control

376 and red soil treated fishes; 25 and 20 individuals from 80 respectively in the

377 sediment treatment tanks (t_{14} = 0.90; *P* = 0.38).

(e) Post-experimental pH analysis

379 Average pH level declined with increasing sediment concentration.

380 Immediately after mixing mean pH of control samples, 50mgL^{-1} and 200mgL^{-1} was

381 8.1, 8.09 and 8.08 respectively. Measurements were similar at 2 hours after the

382 sediment had settled out of suspension however while the mean pH of the control

383 water remained stable over time the water treated with red soil became more acidic

384 over time, reaching 8.08 and 8.04 after 7 days for the 50 and 200mgL^{-1} treatments

385 respectively (see Table 2A in supplementary material).

4. Discussion

387 Larval fish behaviour is likely to influence their dispersal and orientation to

388 suitable settlement habitat (Cowen et al. 2006), yet the impacts of sediment pollution

389 on this process have been largely unresolved. We showed that in the presence of

390 sediment pollution, such as that caused by red soil, tropical reef fish larvae could be

391 inhibited from locating suitable coral habitats during settlement. Specifically, we

392 found that in the presence of red soil, *C. viridis* larvae failed to distinguish suitable

393 coral habitat visual cues and avoided coral habitat olfactory cues. This may lead to

394 decreased chances for recruitment in areas prone to recurrent sediment pollution,

395 such as Okinawa, Japan, compared to more pristine areas. Previous studies have

396 reported avoidance behaviour in settlement-stage fish of either degraded (by algal

397 phase shift (Lecchini et al. 2013)) or dying coral reef habitat (Feary et al. 2007), and

398 our results suggest sensory cue use may be an important factor. Evidence for

399 chemically mediated avoidance behaviour *in situ* has been shown using cues from

400 degraded seaweed-dominated reef habitats, suggesting that olfactory cues from

401 sediment-degraded reefs could affect recruitment densities and reef resilience

402 (Dixson et al. 2014).

403   Evidence of negative impacts of sediment pollution on settlement behaviour of

404 settlement-stage reef fish larvae have been found previously (Wenger and

405 McCormick 2013), yet the field relevance of these results were previously untested.

406 Here we provide the initial evidence that the presence of red soil near coral habitat,

407 even administered in small amounts, may reduce settlement success in the field.

408 Untreated light traps containing live coral with red soil absent caught a higher

409 abundance and species richness than light traps with coral in the presence of red

410 soil. The statistical significance of this result may have been affected due to a

411 positional bias of the light traps, with the southerly light trap position (the direction

412 from which the prevailing current flows) catching more larvae than the other three

413 positions. High levels of variation in catch abundance, seen in our results, is also a

414 notoriously typical effect on reliable light trap sampling (Leis et al. 2002). The non-

415 significant trend of a negative response of settling fishes to red soil treatments was

416 congruent with results obtained in the laboratory experiments.

417   Results showing either an avoidance of olfactory cues from habitat with red

418 soil present or a disruption of the ability or inclination to utilise visual habitat cues in

419 settlement stage coral reef fish larvae indicates that such pollution of coral reef

420 environments may impact larval recruitment and survival. A reluctance or inability to

421 approach potential habitats can extend the period spent by fish larvae in close

422 proximity to the reef running the "predation gauntlet", increasing the risk of predation

423 (Almany and Webster 2006). Even prolonged exposure to non-predatory fish can be

424 detrimental, with harassment of recruiting fish larvae by territorial residents observed

425 to result in elevated predation risk or avoiding the habitat altogether (Leis and

426 Yerman 2012). In addition to this, previous studies have shown an effect of sediment

427 pollution on larval development whereby the PLD is increased (Wenger et al. 2013a).

428 This effect could alter the timing and location of larval settlement, changing the

429 natural distribution and recruitment of fish larvae through the modifications of their

430 movement towards habitat can affect the spatial and community demography of fish

431 populations.

432   Even if fish larvae manage to settle on coral reef habitat degraded with

433 sediment pollution, our results show that prolonged exposure to the presence of

434 sediment could impact post-settlement behaviour and survival. Five days of

435 exposure to water containing red soil significantly altered the response to olfactory

436 cues from different habitat types, reversing the strong preference for live coral cues

437 under control conditions to instead preferring those from dead corals. At a higher

438 rearing concentration of red soil, response to olfactory cues was absent altogether.

439 As the habitat cues presented to individuals here after the rearing period contained

440 no sediment influence, it is assumed that the change in behaviour was due to

441 physiological effects of exposure on the sense organ, rather than impairment of

442 transmission of the cues themselves. The response to visual cues in individuals

443 exposed for five days to sediment differed from newly caught larvae, no longer

444 showing significant choice behaviour towards live coral over dead coral. This

445 reduced exploration of the visual cue chamber may be an artefact of rearing in

446 captivity or an ontogenetic change in behaviour, perhaps due to a reduction in the

447 inclination to use visual habitat cues to orient movement by juveniles compared to

448 settling larvae (Lecchini et al. 2007).

449   Reversal in normal behavioural responses to olfactory cues has been shown

450 in response to increased water acidification, where a normal avoidance of predator

451 cues became a strong attraction in fish larvae reared in water with higher acidity

452 (Dixson et al. 2009).   Furthermore, when the acidity of rearing conditions is

453 increased further the fish larvae no longer responded to olfactory cues (Munday et

454 al. 2009), similar to the trend in our results. The mechanism suggested for this is an

455 alteration caused by the increased acidity of anion gradients across neuronal

456 membranes which reverse normal receptor function (Nilsson et al. 2012). The

457 changes in behaviour seen in the rearing trials may be a similar response due to the

458 acidity of red soil. Behavioural avoidance of acid sulphate soils by juvenile fish has

459 been shown in juveniles of various fishes where pH levels were well within the range

460 exhibited in natural systems (Kroon 2005). Red soil in Okinawa is acidic with a pH of

461 ~5 (Mkadam et al. 2006), and analysis of rearing conditions used here showed pH

462 decreased both with increasing concentrations of red soil and with time. Reductions

463 in pH of a similar range (0.07) have been shown to elicit similar behavioural changes

464 in juvenile coral trout (*Plectropomus leopardus*) (Munday et al. 2013). This acidic soil

465 could influence the pH of water surrounding the reef on which it deposits, as has

466 been shown with organic-rich soils (Weber et al. 2012).

467 Previous studies on the effect of red soil on water chemistry showed that as

468 red soil concentration increased in a solution, availability of H^+ ions (increasing

469 acidity) and heavy metals, particularly Al^{3+} also increased (Kombo et al. 2005).

470 Exposure to elevated concentrations of heavy metals can also lead to changes in

471 behavioural responses of fish to olfactory cues (Scott and Sloman 2004). Increased

472 Al^{3+} concentrations can exacerbate the damage done to the olfactory epithelium by

473 increased acidity alone, working synergistically to disrupt olfactory abilities in

474 salmonid fish (Klaprat et al. 1988). Our results suggest that chemical properties of

475 sediment pollution may cause greater impacts on coral reef environments than

476 turbidity alone. Of course, it is not only the use of habitat cues that can affect the

477 ecology of early-stage fishes. Chemical pollution of the aquatic environment may

478 also disrupt communication, social recognition and shoaling in fish, which can have

479 drastic effects on population fitness (Fisher et al. 2006; Ward et al. 2008).

480 Exposure to sediment pollution negatively impacted the condition and

481 performance in *C. viridis.* Both feeding rates and condition reduced with increasing

482 concentration of red soil. The sensory disruption detected in other individuals of the

483 same cohort during this experiment as well as other physiological effects from

484 prolonged exposure to sediment pollution may have played a role in reduced

485 foraging success. As evidence suggests that growth is a critical variable in the

486 recruitment success and survival of post-settlement fish this is another aspect in

487 which sediment pollution can impact on affected fish populations (Bergenius et al.

488 2002). Our results concur with data on juvenile reef fish reared under similar

489 concentrations of suspended sediment (0 – 180mgL^{-1}), where reduced foraging

490 behaviour led to reduced growth, condition and survival (Wenger et al. 2012). This

491 effect has also been shown to reduce foraging success *in situ* when prey detection

492 may be limited by absolute light levels rather contrast (Fiksen et al. 2002).

493 In conclusion, this experiment used a variety of techniques and life history

494 stages to investigate the effect of sediment pollution on coral reef fish larvae around

495 the crucial settlement period. At the settlement stage we found evidence of

496 disruption of natural use of sensory cues related to locating suitable habitat by fish

497 larvae in the presence of increased sediment levels. Following settlement (after five

498 days captive rearing) metamorphosing post-settlement fish experienced reduced

499 feeding behaviour and condition under the sediment treatments. Response to

500 habitat-relevant sensory cues became confused, reversing the preference from live

501 coral to dead coral in some cases in individuals which had experienced prolonged

502 exposure to sediment concentrations. The responses to sediment pollution reported

503 here are in many aspects the similar to those reported in from elevated levels of CO_2

504 which also can simultaneously affect a range of fish behaviours (Jutfelt et al. 2013).

505 These include avoidance behaviour, reversal of behavioural responses to

506 ecologically relevant sensory cues, reduced growth and reduced feeding rates

507 (Baumann et al. 2012; Briffa et al. 2012; Cripps et al. 2011; Dixson et al. 2009;

508 Munday et al. 2009). This suggests that the interaction of red soil with the

509 surrounding water can cause chemically altered environments detrimental for fish

510 survival. In any case, these behavioural changes can be biomarkers for significant

511 biological effects of sediment pollution in marine environments (Galloway et al.

512 2004). If the impacts on sensory cue use in recruiting larval fishes influences the

513 recruitment success of coral reef fish populations this can have implications for both

514 fisheries and marine park management as import and export of larvae between

515 populations and habitats may be constrained by degraded habitat (Dixson et al.

516 2014).

517   The concentrations of sediment used in this experiment are regularly

518 encountered on coral reef ecosystems across the world, for example on the Great

519 Barrier Reef, Australia, sediment concentrations are regularly recorded near the

520 lower level used here ($50 mgL^{-1}$) and can exceed the higher level ($200 mgL^{-1}$) during

521 the wet season or as a result of dredging (Bak 1978; Wenger and McCormick 2013).

522 This indicates negative impacts on settlement behaviour and success of reef fish

523 larvae may be exacerbated if sediment load influx to coral reef environments

524 increases due to anthropogenic activities. The close proximity of acidic sediment

525 similar to Okinawa's red soil to coral reef environments (e.g. over 600,000ha of acid

526 sulfate soil resides within the Great Barrier Reef (GBR, Queensland) catchment

527 alone (Powell and Martens 2005)), means that chemical effects of sediment are

528 likely to have substantial impacts on coral reefs and their inhabitants. Future work is

529 required to better understand these effects and how they interplay with effects of

530 sediment-induced turbidity.

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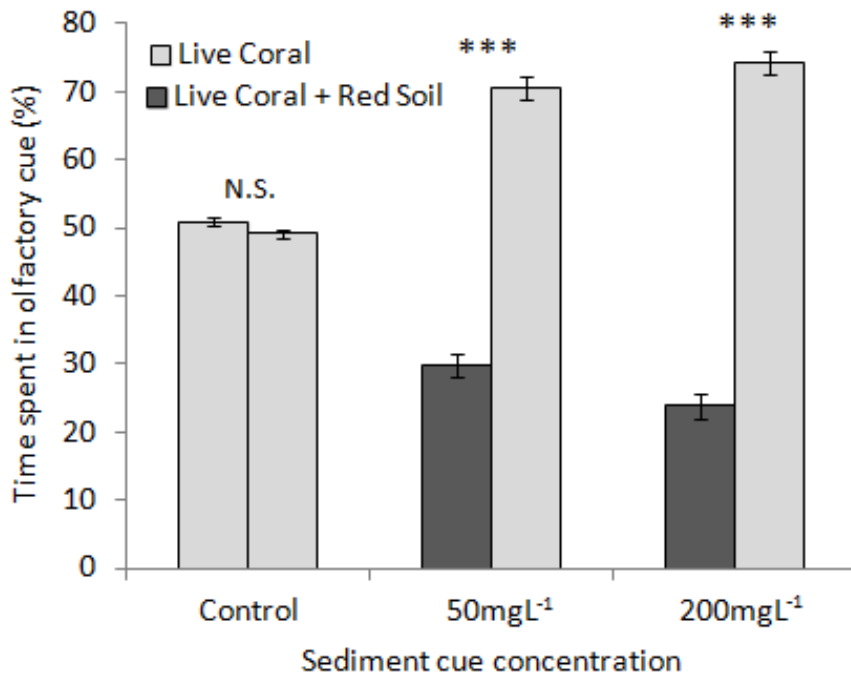717

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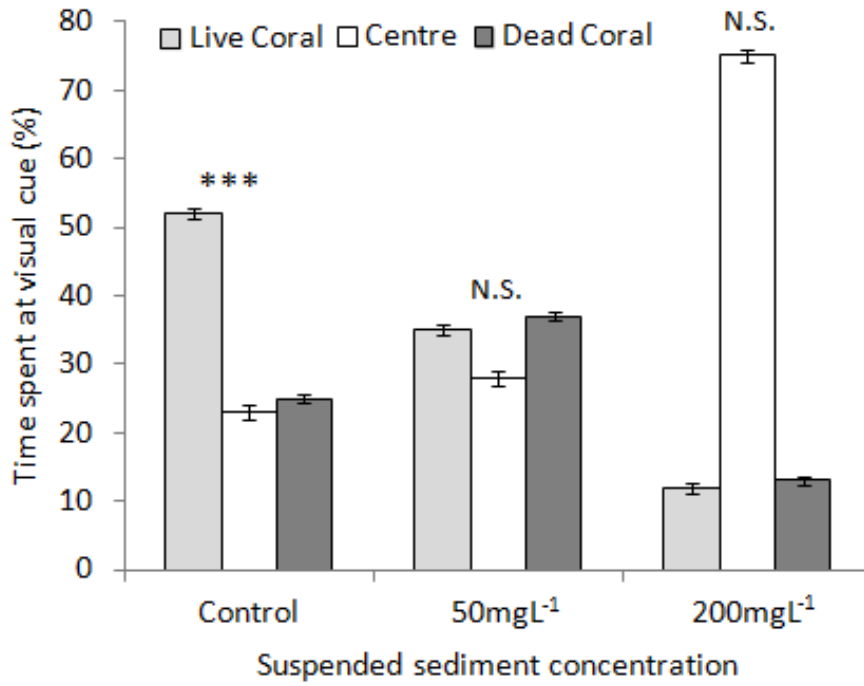732

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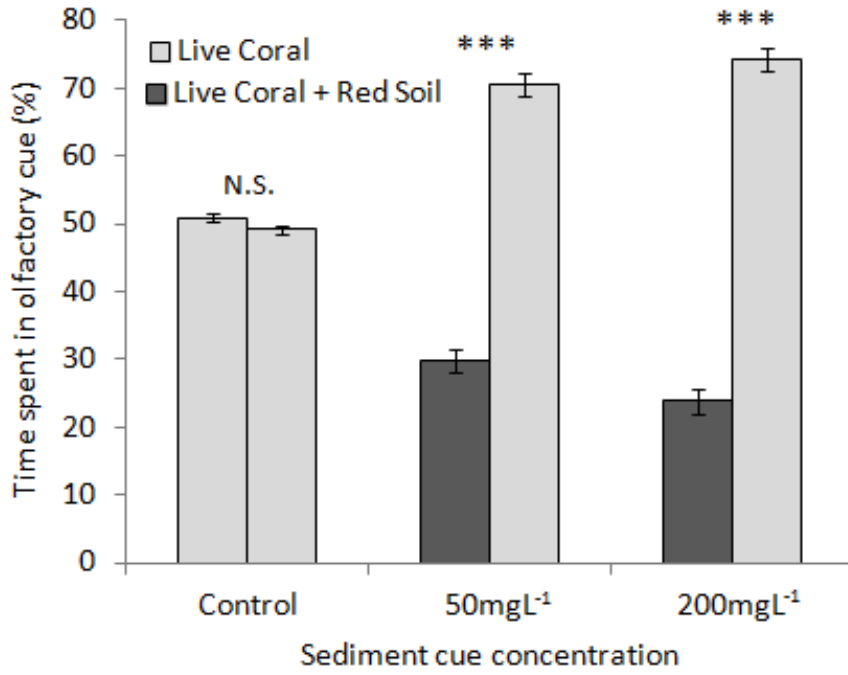753

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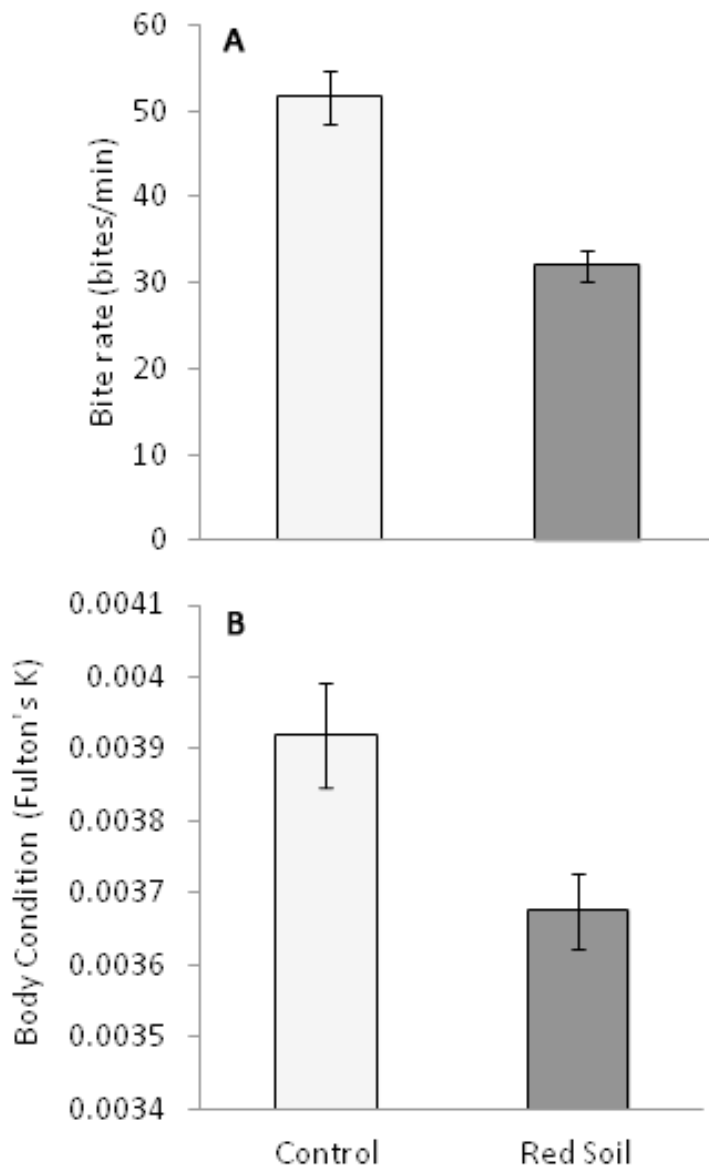
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