

1 **Cleaner wrasse influence habitat selection of young damselfish**

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22 **Abstract**

23 The presence of bluestreak cleaner wrasse, *Labroides dimidiatus*, on coral reefs increases
24 total abundance and biodiversity of reef fishes. The mechanism(s) that cause such shifts in
25 population structure are unclear, but it is possible that young fish preferentially settle into
26 microhabitats where cleaner wrasse are present. As a first step to investigate this possibility,
27 we conducted aquarium experiments to examine whether settlement-stage and young
28 juveniles of ambon damselfish, *Pomacentrus amboinensis*, selected a microhabitat near a
29 cleaner wrasse (adult or juvenile). Both settlement-stage (0 d post-settlement) and juvenile (~
30 5 weeks post-settlement) fish spent a greater proportion of time in a microhabitat adjacent to
31 *L. dimidiatus* than in one next to a control fish (a non-cleaner wrasse, *Halichoeres*
32 *melanurus*) or one where no fish was present. This suggests that cleaner wrasse may serve as
33 a positive cue during microhabitat selection. We also conducted focal observations of cleaner
34 wrasse and counts of nearby damselfishes (1 m radius) to examine whether newly-settled fish
35 obtained direct benefits, in the form of cleaning services, from being near a cleaner wrasse.
36 Although abundant, newly-settled recruits (<20 mm total length) were rarely (2%) observed
37 being cleaned per 20 min observations compared with larger damselfishes (58%). Individual
38 damselfish that were cleaned were significantly larger than the median size of the
39 surrounding nearby non-cleaned conspecifics; this was consistent across four species. The
40 selection by settlement-stage fish of a microhabitat adjacent to cleaner wrasse in the
41 laboratory, despite only being rarely cleaned in the natural environment, suggests that even
42 rare cleaning events and/or indirect benefits may drive their settlement choices. This
43 behaviour may also explain the decreased abundance of young fishes on reefs from which
44 cleaner wrasse had been experimentally removed. This study reinforces the potentially
45 important role of mutualism during the processes of settlement and recruitment of young reef
46 fishes.

47 **Introduction**

48 On coral reefs there are many mutualistic cleaners that remove parasites from other
49 organisms, including coral reef fishes. One of the most well-known of these is the bluestreak
50 cleaner wrasse, *Labroides dimidiatus* (Labridae), a cleaner wrasse common throughout the
51 Indo-Pacific (Randall et al. 1997). *Labroides dimidiatus* has been shown to reduce the
52 ectoparasite load of individual client fishes (Gorlick et al. 1987; Grutter 1999; Grutter and
53 Lester 2002; Clague et al. 2011), aggression by predators (Cheney et al. 2008), stress
54 (measured in cortisol levels) via physical contact (Bshary et al. 2007; Soares et al. 2011) and
55 increase growth and size of client fishes (Clague et al. 2011; Waldie et al. 2011). In addition,
56 these cleaner wrasse affect local populations of reef fishes, so that in their absence there are
57 reductions in the diversity and abundance of site-attached adult residents, visitors (fishes that
58 move between patch reefs) and juvenile visitor fishes (Bshary 2003; Grutter et al. 2003;
59 Waldie et al. 2011). Although the positive effects of the presence of *L. dimidiatus* on
60 individuals and populations have been demonstrated in experimental manipulations of entire
61 patch reefs ranging in size from ~61 to 285 m² (Waldie et al. 2011), the mechanisms
62 involved, particularly at small spatial scales (1 m radius, i.e., home range of juvenile cleaner
63 wrasse), remain largely unstudied.

64 One of the most critical periods that affects the population dynamics of coral reef
65 fishes occurs during the settlement of young fish from the plankton to the reef and the few
66 days or weeks of life in benthic habitats that immediately follow (post-settlement). Processes
67 at this time ultimately determine which individuals are recruited to the population of
68 juveniles. Young fish can actively choose the habitat into which they will settle and the
69 processes that underlie these choices are complex (Hoey and McCormick 2004; Heinlein et
70 al. 2010). Additionally, the mortality rate at this time is high; indeed, it has been estimated
71 that 57% of individuals die within the first 1–2 d of settlement (Almany and Webster 2006).

72 Thus, the choice of a suitable microhabitat at settlement is critical for successful recruitment.
73 Selecting the optimum microhabitat depends on numerous factors, which may include the
74 structure of the microhabitat (Tolimieri 1995), and the presence of potential predators (Vail
75 and McCormick 2011) and conspecifics (Sweatman 1985). Indeed, it was recently shown that
76 the experimental removal of *L. dimidiatus* was associated with a decrease in the abundance of
77 recent recruits on patch reefs relative to control reefs where cleaner wrasse were present (Sun
78 et al. 2015). However, it is unknown whether the presence of *L. dimidiatus* can serve as a
79 positive cue for microhabitat choice at settlement.

80 Our study used experimental and observational approaches to determine whether the
81 presence of juvenile or adult cleaner wrasse influenced the choice of microhabitat by settling
82 juveniles of the ambon damselfish, *Pomacentrus amboinensis*. In the laboratory, we
83 examined whether the presence of cleaner wrasse influences habitat choice of *P.*
84 *amboinensis*. To determine the extent to which recently settled damselfishes were cleaned by
85 *L. dimidiatus* juveniles, and thus whether the benefits of settling near a cleaner wrasse could
86 be related to the cleaning services they provide or to other indirect benefits, focal
87 observations of the cleaning interactions between *L. dimidiatus* and damselfishes were
88 conducted in the natural environment. Lastly, to determine whether juvenile cleaner wrasse
89 are size-selective in their choice of clients, we compared the size of individuals of the four
90 most abundant damselfishes that were cleaned by the wrasse with the median size of non-
91 cleaned conspecifics.

92

93 **Methods**

94 ***Laboratory experiment: does the presence of *L. dimidiatus* affect microhabitat choice in***
95 ***young damselfish?***

96 We conducted an aquarium experiment at Lizard Island Research Station, Great Barrier Reef,
97 Australia, to investigate whether the presence of *L. dimidiatus* influenced microhabitat choice
98 of *P. amboinensis*, a species chosen because of its high abundance at the study site (Sun et al.
99 2015). As much is known about its ecology, including habitat preference, recruitment, and
100 feeding habits (Kerrigan 1996; McCormick et al. 2010), this species has been extensively
101 used as a model species in many field and laboratory experiments. *Pomacentrus amboinensis*
102 were collected between November 2011 and January 2012 using light traps (see Meekan et
103 al. 2001 for design) moored overnight approximately 100 m from the reef. These fish were
104 juveniles caught just prior to settlement from the plankton and thus had no previous exposure
105 to *L. dimidiatus*. Fish were held in groups of ~20 in clear plastic holding aquaria (29 × 17 ×
106 12 cm) with a constant flow of seawater. Fish collected in November were held for around 5
107 weeks (at which point they were classified as juveniles, i.e. ~ 5 weeks post-settlement),
108 whereas fish collected in January were tested within a day of capture (classified as
109 settlement-stage, i.e. 0 d post-settlement). Fish were fed ad libitum twice a day with live
110 nauplii of brine shrimp (*Artemia* sp.).

111 Both adult and juvenile cleaner wrasse (*L. dimidiatus*) and a non-cleaner control fish
112 (pinstripe wrasse, *Halichoeres melanurus*), were collected from the Lizard Island lagoon
113 using hand-nets, barrier nets and anaesthetic clove oil mixed with alcohol and seawater (10%
114 clove oil; 40% ethanol; 50% seawater). *Halichoeres melanurus* was chosen as a control due
115 to its similar body shape to *L. dimidiatus* (Grutter 2004; Cheney et al. 2008, 2009) and
116 because it is not a predator of small recruits (Mitchell et al. 2013). All wrasses were
117 maintained in aquaria (43 × 32 × 30 cm) with running unfiltered seawater.

118 Clear perspex sheets were placed 10 cm from each end of a glass experimental
119 aquarium (35 × 36 × 65 cm), to create two end compartments with flow-through seawater
120 (Fig. 1). The middle section of the aquarium was divided into three 15 cm subsections, using

121 vertical black lines drawn on the front and back of the aquarium. Three polyvinyl chloride
122 (PVC) tube shelters (6×5 cm diameter) were placed in the centre of each subsection.
123 Aquaria were covered with black plastic on three sides to isolate the fish from external
124 activity, and a green plastic shade cloth was hung in front of the tanks to create an
125 observation blind.

126 In each end compartment a *L. dimidiatus*, a control fish or no fish was placed,
127 depending on the treatment: 1) *L. dimidiatus* and *H. melanurus*, 2) *L. dimidiatus* and no fish,
128 or 3) *H. melanurus* and no fish. These combinations were presented to a randomly-selected *P.*
129 *amboinensis* 'client' and the side in which a cleaner or control (or no fish) was placed was
130 randomised, but balanced. The 'no fish' treatment at one end compartment of the aquarium
131 was used to control for the 'client' potentially avoiding any heterospecifics, irrespective of
132 identity.

133 A *P. amboinensis* was placed in the middle section of the aquarium in a bottomless
134 clear plastic container ($8 \times 8 \times 8$ cm) with numerous small (5 mm) ventilation holes for 20
135 min prior to commencing the trial, allowing the fish to acclimate to the new surroundings.
136 The bottomless container was removed using an attached monofilament string by the
137 observer, who was positioned behind the observation blind. Each trial was conducted for 20
138 min, and the position of *P. amboinensis* within the aquarium (left, middle, or right section)
139 was recorded every 15 s. Although it is inferred that its position represented its choice of
140 habitat, it is possible that the position of the damselfish may also be the result of it being
141 curious or wanting to be cleaned.

142 Experiment 1 was divided into three parts (A, B, C) with different ontogenetic stages
143 of cleaner, control and *P. amboinensis* per treatment (see Table 1 for a summary of the fish
144 stage and species). For each part, we used eight cleaner and eight control fish that were
145 randomly selected, and 75 *P. amboinensis* ($n = 25$ individuals per treatment). At the end of

146 each trial, cleaner and control fish were replaced. Individual *P. amboinensis* were only used
147 once so that their behaviour was not affected by previous exposure.

148 Part A used adult *L. dimidiatus* and *H. melanurus*, and part B used juveniles of both
149 species. These experiments tested whether the ontogenetic stage/size of *L. dimidiatus* affected
150 habitat choice of juvenile *P. amboinensis*. Part C was conducted when settlement-stage fish
151 became available in the light traps; juvenile *L. dimidiatus* and *H. melanurus* were used for
152 this component. The aim of this experiment, relative to part B, was to determine whether *P.*
153 *amboinensis*' ontogenetic stage/size affected its habitat choice.

154

155 ***Field observations: do juvenile L. dimidiatus clean recently settled damselfish recruits and***
156 ***are they selective in their choice of clients?***

157 To assess whether recently settled damselfish recruits are cleaned by *L. dimidiatus*, and thus
158 gain direct fitness benefits from settling near them, we conducted 20 min behavioural
159 observations on juvenile *L. dimidiatus* (n = 79) on a section of continuous reef (~ 200 m) at
160 Coconut Beach (14°40'S, 145°28'E), Lizard Island. We observed juvenile *L. dimidiatus*,
161 rather than adults, due to the higher frequency of juvenile *L. dimidiatus* interacting with
162 young reef fish (Robertson 1974, D. Sun, unpublished data). Before observations
163 commenced, observers learned to accurately estimate total length (TL) of fish underwater by
164 using printed and laminated outlines of model fish (12–74 mm TL). The fish models were
165 placed on the reef at varying distances from the observer, and sizes were estimated and
166 compared with the actual length of the model until observers were more than 80% accurate at
167 estimating length. Rulers printed onto dive slates were also used as a guide for estimating fish
168 length.

169 For each observation, a juvenile *L. dimidiatus* was located and its estimated TL was
170 recorded. A 1 m radius was measured around the area where the *L. dimidiatus* was first

171 sighted using a 1 m long string and marked using five lead sinkers (7 mm diameter) attached
172 to floats (25 mm). Surrounding fish were allowed to acclimate (~ 5 min) to the presence of
173 the markers and observers, before the commencement of the fish counts and cleaning
174 interactions within this area. This acclimation period allowed adult fish that may have been
175 disturbed from the initial deployment of the markers to move back inside the observation
176 area. Fish counts were recorded in a systematic way (by size class) to ensure that an
177 individual was counted only once. All fish present were recorded to account for the
178 availability of other sizes of fish as potential clients that could influence the cleaners' choice
179 of client. Cleaning interactions were recorded as any physical contact between cleaner and
180 client, or significant inspection by the cleaner (>1 s). For each interaction, we recorded the
181 client species (where possible), cleaning duration, and estimated client size (mm TL). To
182 compare client size and the median size of nearby (non-cleaned) conspecifics, the size of all
183 other fish present within the marked observation site ($10 \text{ mm} \leq \text{TL} < 80 \text{ mm}$), but not
184 involved in any cleaning interaction, was also estimated. All other fish were identified to
185 species where possible. To ensure that each *L. dimidiatus* was observed only once, observers
186 began at one end of the reef and continued without backtracking. In contrast to adults,
187 juvenile *L. dimidiatus* stay within a highly restricted home range of around 4 m^2 (Robertson
188 1974), further reducing the possibility of observing an individual twice. Although every
189 individual within the observation area was identified to species, ultimately, for adequate
190 sample sizes for statistical analyses, only the most abundant damselfish species (both cleaned
191 and non-cleaned conspecifics) from the observations were used. These four common species
192 were *P. amboinensis*, *P. moluccensis*, *P. nagasakiensis* and *P. wardi*.

193

194 **Statistical analyses**

195 All statistical analyses used R version 3.0.1 (R Development Core Team 2013).

196 ***Laboratory experiment***

197 A full linear mixed-effects (LMER) model with a restricted maximum likelihood (REML)
198 procedure was used to examine whether *P. amboinensis* spent more time near a *L. dimidiatus*
199 than near a control fish or no fish compartment. Two analyses were conducted; the first
200 model used data from parts A and B (Table 1) to determine whether ontogenetic stage of the
201 cleaner had an effect on juvenile *P. amboinensis*' habitat choice, while the second model used
202 data from parts B and C, and included ontogenetic stage (settlement-stage and juvenile *P.*
203 *amboinensis*) of the client as a factor. In the first model, cleaner ontogenetic stage (adult or
204 juvenile), settlement stimulus type to which the microhabitat was adjacent (cleaner, control,
205 no fish), aquarium side the cleaner was placed (left or right) and fish stimulus treatment
206 combination (cleaner/control, cleaner/no fish or control/no fish) were used as fixed factors.
207 Replicate trial identity was added as a random factor, to account for an order effect. In both
208 models, proportion of time spent in each section of the aquarium (right, left and centre) was
209 transformed by taking the arcsine of the square root of the proportion to meet the assumptions
210 of normality.

211

212 ***Field observations***

213 A LMER model with a REML procedure was used to test whether there was a significant
214 difference between the median size of client fish and nearby conspecifics. The terms
215 treatment (client or nearby conspecifics), nearby conspecific size and species (four
216 damselfish species: *P. amboinensis*, *P. moluccensis*, *P. nagasakiensis* and *P. wardi*) were
217 fixed factors, and the identity of the individual cleaner fish and the cleaning event identity
218 (specifying which client and nearby conspecifics corresponded with one another) were
219 random factors. To determine whether client size varied with cleaner size or the size of all
220 other nearby species, separate (LMER) analyses were run with cleaner size and other nearby

221 fish size as covariates and cleaner identity as a random factor. A generalized linear model
222 with a binomial distribution was used to determine whether there was a significant difference
223 between the number of cleaned recruits, against the abundance of other fish species (>20 mm
224 TL) and recruits (<20 mm TL) within the 1 m radius observation area. To calculate the
225 probability that a recruit would be cleaned by a cleaner wrasse over a 12 h day, we used the
226 following equation (Eq. 1), where p_t is the probability of being cleaned over a time period, t
227 (t in units of 20 min), and p is the probability of being cleaned in a 20 min period. This
228 formula assumes that cleaning events are independent and that p is constant over the time
229 period.

230

$$231 \quad p_t = 1 - (1 - p)^t \text{ (Eq. 1)}$$

232

233 To determine the best model in all analyses, we compared the full model with models
234 in which one of the explanatory terms was dropped using the ‘drop1’ function (Chambers
235 1992). The term was dropped if an analysis of variance found that a dropped term had no
236 significant effect on the model using a Chi-square distribution. A Tukey–Kramer HSD test
237 (TK-HSD) using the glht function in the ‘multcomp’ package (Hothorn et al. 2008) identified
238 where differences occurred and a summary output table was reconstructed using the results
239 from the ‘drop1’ function. Prior to all analyses, the assumptions of normality and
240 homogeneity of variance were assessed using histograms, residuals and quantile-quantile
241 plots.

242

243 **Results**

244 *Laboratory experiment: does the presence of *L. dimidiatus* affect microhabitat choice in*
245 *young damselfish?*

246 In all three parts (A–C) of the experiment, *P. amboinensis* spent significantly more time in
247 the habitat adjacent to a *L. dimidiatus* than next to a control fish or no fish compartment (part
248 A: LRT = 9.48, $df = 2$, $P = 0.008$; part B: LRT = 64.76, $df = 2$, $P < 0.0001$, and part C:
249 LRT = 56.77, $df = 2$, $P = 0.0009$; Fig. 2a–c). The first statistical analysis, which used data
250 from parts A and B to examine whether cleaner ontogenetic stage had an effect, showed that
251 the proportion of time that juvenile *P. amboinensis* spent next to the stimulus fish (cleaner,
252 control or no fish) differed according to an interaction between fish stimulus and cleaner
253 stage (LRT = 19.98, $df = 2$, $P < 0.0001$; Fig. 2a, b); a TK-HSD test showed that juvenile *P.*
254 *amboinensis* spent significantly more time near a juvenile than an adult *L. dimidiatus* ($P <$
255 0.05). There was no significant effect of treatment combination (LRT = 0.474, $df = 2$, $P =$
256 0.788) or aquarium side (LRT = - 4.18, $df = 1$, $P = 0.772$).

257 For the second statistical analysis, which used data from parts B and C to examine
258 whether ontogenetic stage of the client affected habitat choice, the proportion of time that *P.*
259 *amboinensis* spent next to a habitat did not differ with stage (LRT = - 4.40, $df = 1$, $P = 0.749$)
260 but did differ according to fish stimulus (LRT = 144.22, $df = 2$, $P < 0.0001$; Fig. 2a, c); a TK-
261 HSD test showed that *P. amboinensis* spent significantly more time near *L. dimidiatus* ($P <$
262 0.05). The proportion of time spent in a habitat differed according to treatment combination
263 (LRT = 37.11, $df = 2$, $P < 0.0001$). A TK-HSD test showed that *P. amboinensis* spent more
264 time in both chosen microhabitats (i.e., spent less time in the middle section) in the cleaner/no
265 fish and control/no fish stimulus treatment combinations than in the cleaner/control treatment.
266 The effect of aquarium side was not significant (LRT = -1.44, $df = 1$, $P = 0.182$).

267

268 ***Field observation: do juvenile *L. dimidiatus* clean recently settled damselfish recruits and***
269 ***are they selective in their choice of clients?***

270 A total of 1107 cleaning interactions were recorded during 79 observations of individual *L.*
271 *dimidiatus* juveniles over a period of 1580 min. Cleaner wrasse cleaned 104 species of fish
272 from 19 families, including 32 species of damselfishes of which the following were cleaned
273 most often (listed from most to fewest): *Acanthochromis polyacanthus*, *Stegastes apicalis*,
274 *Amblyglyphidodon curacao*, *P. moluccensis*, *Plectroglyphidodon lacrymatus*, *Pomacentrus*
275 *amboinensis*, *P. nagasakiensis*, *P. wardi* and *Chromis lepidolepis* (see Electronic
276 Supplementary Material, ESM, Table S1 for a full list of client species). These cleaning
277 interaction frequencies were not adjusted for relative abundance of each client species. The
278 mean number (\pm SE) of clients cleaned and the duration spent cleaning per 20 min
279 observation was 14 ± 1 fish and 83.8 ± 7.3 s, respectively. Overall, only 14% of cleaning
280 interactions were between juvenile *L. dimidiatus* and the four common damselfishes (*P.*
281 *amboinensis*, *P. moluccensis*, *P. nagasakiensis* and *P. wardi*) that had the greatest
282 abundances within the 1 m radius of a cleaner wrasse. A total of 46% of interactions occurred
283 with other damselfishes (excluding the four listed species), while the remaining 40% of
284 interactions occurred between non-damselfishes and juvenile *L. dimidiatus*. Overall, 60% of
285 the clients of juvenile *L. dimidiatus* were damselfishes. The range and median size (mm TL)
286 of the common damselfishes that were cleaned were: *P. amboinensis* (20–80 and 50 mm), *P.*
287 *moluccensis* (25–80 and 50 mm), *P. nagasakiensis* (15–90 and 40 mm), and *P. wardi* (50–
288 120 and 90 mm); the duration (seconds, mean \pm SE) of time spent cleaning per 20 min
289 observation for the common species was 14 ± 2.5 s. Client size differed among the four
290 species (LRT = 68.01, $df = 3$, $P < 0.0001$; Fig. 3a); a TK-HSD test showed that *P. wardi*
291 clients were significantly larger than the other common client damselfishes. Damselfishes
292 that were cleaned were significantly larger than nearby non-cleaned conspecifics occurring
293 within a 1 m radius; this effect was consistent for all four species (LRT = 173.72, $df = 1$, $P <$
294 0.0001; Fig. 3a). Separate analyses failed to detect any correlation between the size of the

295 damselfish client with the size of the cleaner wrasse (LRT = 0.17, $df = 1$, $P = 0.675$) nor was
296 size of damselfish client correlated with the median size of all other fish species combined
297 (excluding common damselfishes) within the 1 m radius of the cleaner (LRT = 2.55, $df = 1$, P
298 = 0.110).

299 Despite the presence of numerous recently settled damselfishes (size range 15–20
300 mm) within the 1 m radius observation areas (Fig. 3b), only 2% (22 of 1107 cleaning
301 interactions) of fishes that were cleaned were <20 mm TL. There was a significant negative
302 relationship between the number of recruits that were cleaned and the abundance of both
303 other fish species (>20 mm TL) (LRT = 7.97, $df = 1$, $P = 0.004$, Fig. 4a) and recruits (<20
304 mm TL) (LRT = 16.95, $df = 1$, $P = < 0.0001$, Fig. 4b) within the 1 m radius observation area.
305 The highest probability of a recruit (<20 mm TL) being cleaned by a juvenile cleaner wrasse
306 within the observation area is 0.002/20 min (95% CI = 3.22×10^{-6} –0.22) when there were
307 eight (the lowest value encountered) other fishes (>20 mm TL); and a probability of 0.003/20
308 min (95% CI = 6.77×10^{-5} –0.07) when there were three (the lowest value encountered)
309 recruits (<20 mm TL) (Fig. 4a, b). The probability of a recruit being cleaned rapidly declines
310 to zero as the abundance of other fish and recruits increased. An extrapolation of the highest
311 probability of an individual recruit being cleaned during a 12 h day is 0.069 and 0.102.

312

313 **Discussion**

314 Our study provides some of the first evidence that the presence of cleaners could influence
315 fish settlement and potentially migration decisions. Our experimental evidence suggests that
316 the presence of cleaner wrasse, *L. dimidiatus*, could directly influence the choice of
317 microhabitats by young coral reef damselfish. In the laboratory, both settlement-stage and
318 juvenile *P. amboinensis* preferentially selected microhabitats that were in close proximity to
319 *L. dimidiatus*. This may be one explanation for the observed patterns of increased abundance

320 of fishes in the presence of cleaner wrasse on the reef. However, our observations in the
321 natural environment revealed that young damselfish were rarely cleaned by *L. dimidiatus*.
322 Thus, it is possible that even rare cleaning events and/or indirect benefits resulting from the
323 presence of cleaner wrasse may drive the selective behaviour of settling damselfish (see Sun
324 et al. 2015).

325 We assessed microhabitat choice by settlement-stage (0 d post-settlement) and
326 slightly older juvenile (~5 weeks post-settlement) *P. amboinensis* and found that individuals
327 preferentially selected a microhabitat adjacent to *L. dimidiatus*. This indicates that the
328 ‘attraction’ towards cleaner wrasse occurs at both stages. Habitat selectivity at settlement
329 appears common in young damselfishes, with some species attracted to particular
330 microhabitats such as coral heads that harbour conspecifics (Lecchini et al. 2007; Coker et al.
331 2012), while others are attracted to coral heads associated with heterospecifics (Green 1998;
332 Almany 2003). Our study adds cleaner wrasse to the list of organisms to which settlers are
333 attracted; the presence of cleaner wrasse could act as an indicator of microhabitat quality to
334 settlement-stage fish. Furthermore, these results suggest that if juveniles move between
335 microhabitats (Simpson et al. 2008), site selection may also be influenced by the presence of
336 *L. dimidiatus*. Our results indicate that damselfish are attracted to cleaners at a within-reef-
337 patch scale, however, further investigation is needed to determine whether this attraction
338 translates to decisions made during settlement.

339 The apparent attraction to *L. dimidiatus* may explain the greater number of recently
340 settled recruits (Sun et al. 2015), juvenile visiting fishes and even adult damselfishes, found
341 on patch reefs where *L. dimidiatus* are present relative to reefs without cleaner wrasse
342 (Bshary 2003; Waldie et al. 2011). There are two ways in which these patterns may arise,
343 using the presence of *L. dimidiatus* as a cue. Settlement-stage fish may either choose to settle
344 on certain patch reefs, or engage in post-settlement movement between patch reefs as

345 juveniles (Simpson et al. 2008). Both of these processes may additively result in damselfishes
346 being more abundant on reefs with cleaner wrasse than on those without. Ideally, determining
347 whether cleaners influence settlement decisions on reefs would involve a reef experiment
348 where cleaner presence/absence is manipulated and settlement is quantified. The closest
349 attempt to date involved counts of recently settled damselfish recruits (<20 mm TL) (Sun et
350 al. 2015), as few newly-settled fish could be distinguished from older recruits due to the rapid
351 change in pigmentation of damselfishes after settling onto the reef (McCormick et al. 2002).
352 Sun et al. (2015) did indeed show that the abundance of recently settled damselfish was
353 higher on reefs with cleaner wrasse. However, since fish experience high levels of predation
354 at settlement (Almany and Webster 2006), the study measured new recruits to the population
355 rather than settlement.

356 The fish used in the laboratory experiment were naïve with respect to the shape or
357 odour of *L. dimidiatus*, since they were collected by light traps while still in their planktonic
358 phase. Despite this naïveté, young fish were able to differentiate between *L. dimidiatus* and
359 another species that was similar in size and shape but differed in colour, pattern and
360 behaviour. The attraction to cleaner wrasse thus appears to be an innate behaviour. Slightly
361 older recruits displayed the same abilities. However, we did not test whether *P. amboinensis*
362 recognised *L. dimidiatus* as a cleaner per se. On some occasions, the fish did behave like a
363 client by remaining in the same position and adopting a pose with spread fins when next to
364 the compartment with a cleaner wrasse. This behaviour did not occur when they were
365 adjacent to the control fish or an empty compartment. Prospective clients of cleaner fish often
366 assume this posture when they attempt to attract a cleaner to inspect them (Côté et al. 1998).
367 Because the cleaner wrasse was behind a perspex divider, the cleaner and client were unable
368 to physically interact; such physical contact likely acts as a positive feedback (Bshary and
369 Würth 2001). Losey et al. (1995) previously showed that newly metamorphosed laboratory-

370 reared, and hence ‘cleaner-naïve’, Hawaiian humbug damselfish, *Dascyllus albisella*,
371 recognised the Hawaiian cleaner wrasse, *L. phthirophagus* by adopting the cleaning pose.
372 Our study thus confirms the hypothesis that client fish have an innate ability to recognise
373 cleaners.

374 It seems likely that *P. amboinensis* used visual cues to differentiate between the two
375 stimulus fish that were presented. The combination of black and blue and/or yellow body
376 patterns displayed by cleaner fish, including *L. dimidiatus*, allows clients to visually
377 recognise cleaners (Cheney et al. 2009). Indeed, the particular colouration and patterns
378 displayed by cleaner fish attract higher numbers of client fish than other colour and pattern
379 combinations. Client fish are also known to recognise cleaner fish based on their small body
380 size and the presence of lateral stripes (Stummer et al. 2004).

381 *Pomacentrus amboinensis* chose microhabitats adjacent to adult and juvenile *L.*
382 *dimidiatus* over the other stimulus options. This occurred despite the different colouration of
383 the two cleaner stages; while both stages have a black stripe contrasted by blue, the adults
384 also have yellow and white colours. However, juvenile *P. amboinensis* spent more time near
385 juvenile *L. dimidiatus* than near adults, implying the possibility of ontogenetic variation in
386 choice of cleaner fish. It should be noted that the preference of juvenile *P. amboinensis* for
387 juvenile cleaners shown in this study is an indirect measure, and that an experiment that
388 measures the direct preference (i.e., juvenile or adult cleaner on each side) is required.

389 Alternatively, this preference for juveniles over adult *L. dimidiatus* could simply be
390 due to the difference in relative size between the client and cleaner at different ontogenetic
391 stages. A similar choice test was not possible for settlement-stage fish due to their limited
392 numbers in the light traps. Previous studies have shown that fish larvae have good visual
393 senses, which enables them to detect and recognise conspecifics, heterospecifics and predator
394 fishes (Lecchini et al. 2005, 2014).

395 Many damselfishes settle at night (Dufour and Galzin 1993), including *P.*
396 *amboinensis* (Meekan et al. 1993) and *L. dimidiatus* sleep in the reef matrix at night
397 (Robertson 1974). Thus, cleaner wrasse could only be used as a visual cue for settlement
398 during daylight hours. There is some evidence, though, that fish “sample” the benthos during
399 the day prior to settling (Leis and Carson-Ewart 2002; Leis 2006), which could be influenced
400 by cleaner wrasse presence. However, it is likely that other cues such as odour could be used
401 by settling damselfishes at night to detect reefs where cleaner wrasse are present. More
402 information is needed to determine whether settlers can detect the presence of cleaner wrasse
403 through olfaction.

404 Focal observations of juvenile *L. dimidiatus* on reefs revealed that only 2% of their
405 clients were <20 mm TL. This suggests that small individuals are rarely cleaned by *L.*
406 *dimidiatus* and so contribute little to the cleaner wrasse’s diet. Small-bodied individuals, such
407 as recently settled damselfishes, are infected with very few ectoparasites (Grutter et al. 2010;
408 Sun et al. 2012) and the abundance of gnathiid isopods (the main food source of *L.*
409 *dimidiatus*) is low in juvenile fishes (only 3.5% of recruit *P. amboinensis* are infected)
410 (Grutter et al. 2011). From the cleaner wrasse’s perspective, these factors likely make settlers
411 an unsuitable source of ectoparasites. It was therefore unsurprising that we found that the
412 damselfish that were cleaned by juvenile *L. dimidiatus* were larger than the median size of
413 non-cleaned conspecifics within a 1 m radius. This result was consistent with previous studies
414 that show that *L. dimidiatus* selectively clean larger fish (Grutter et al. 2005; Clague et al.
415 2011), probably due to the close correlation between client size and parasite load (Grutter
416 1994,1995; Grutter and Poulin 1998); *L. dimidiatus* may thus use size as an indicator of food
417 availability.

418 From the client’s perspective, the odds of an individual new recruit (<20 mm TL)
419 being cleaned per 20 min, were low, with only two or three out of 1000 fish predicted to be

420 cleaned when there were few other fishes or recruits within a 1 m radius; this rate rapidly
421 decreased towards zero with increasing abundance of nearby other fish species and recruits.
422 Over a day, however, the extrapolated odds could be much higher, estimated to increase to 7
423 or 10 out of 100 fish when there were few other fishes or recruits within a 1 m radius. Such
424 rare events could have disproportionately significant benefits as some parasites can have
425 especially harmful effects on small individuals. While gnathiid isopods are generally
426 considered micropredators, taking small amounts of blood from their significantly larger host
427 (Lafferty and Kuris 2002), they are capable of consuming up to 85% of the total blood
428 volume of a settlement-stage *P. amboinensis* (Grutter et al. 2011). Indeed, infection by a
429 single gnathiid isopod decreases the oxygen consumption and swimming performance of a
430 recruit damselfish and reduces the likelihood of successful settlement on the reef (Grutter et
431 al. 2011). Removal of a gnathiid before it feeds on an infected fish's blood could provide a
432 major benefit for survival. Since observed cleaning interactions were brief (~1 s), it is
433 unlikely that recruits obtained other direct benefits of cleaning, such as tactile stimulation
434 leading to a reduction in client stress (cortisol) levels (Soares et al. 2011).

435 As direct cleaning interactions between cleaners and recently settled recruits were
436 relatively uncommon, it is possible that the benefits of preferentially selecting a microhabitat
437 near cleaners may be at least partly indirect. For example, cleaner wrasse may reduce
438 infection rate of parasites in the vicinity of a cleaning station. Each *L. dimidiatus* eats around
439 1200 gnathiids per day (Grutter 1996), which could significantly reduce the number of
440 ectoparasites in the immediate vicinity of a *L. dimidiatus* and hence lower the infection rate
441 of nearby settlers (Gorlick et al. 1987; Grutter 1999; Clague et al. 2011). Other indirect
442 benefits of the presence of cleaner wrasses may also exist for settling fish. 'Safe havens' from
443 predators may be created by *L. dimidiatus* (Cheney et al. 2008) in that, in the presence of
444 cleaners, some predators behave with notably reduced aggression. Settling reef fish may

445 therefore prefer to settle in a habitat with *L. dimidiatus* present, due to reduced risk of
446 predator aggression. Alternatively, or in addition, selection by young damselfish of
447 microhabitats near cleaners may simply be an investment where the return occurs only when
448 recruits have grown significantly.

449 In summary, our study provides evidence that settlement-stage reef fish may use the
450 presence of cleaner wrasse as a cue when deciding what microhabitat to settle on. As the
451 presence of this mutualistic interaction creates favourable environments, this may ultimately
452 enhance the survival and abundance of these species. This study demonstrates that mutualism
453 contributes to the already complex settlement processes of reef fishes.

454

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464

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587

588 **Figure captions**

589 **Fig. 1** Front view of the laboratory experiment tank design. A: clear perspex sheet placed on
590 either side to create compartments for stimuli, B: vertical dashed lines on front and back of
591 aquarium to create three subsections, and C: PVC tube shelters placed in each subsection. Not
592 drawn to scale

593 **Fig. 2** The percentage of time spent on microhabitat adjacent to fish treatment by damselfish
594 client *Pomacentrus amboinensis*. a) Part A: juvenile *P. amboinensis* with adult
595 cleaner/control fish, b) Part B: juvenile *P. amboinensis* with juvenile cleaner/control fish, and
596 c) Part C: settlement-stage *P. amboinensis* with juvenile cleaner/control fish

597 **Fig. 3** Cleaning interactions of juvenile cleaner wrasse, *Labroides dimidiatus* and
598 damselfishes. a) Size (total length) of damselfish clients and nearby non-cleaned
599 conspecifics. Bars represent median; error bars represent 25th and 75th quantiles, b) size
600 distribution of nearby non-cleaned damselfish per 1 m radius; bars indicate mean, error bars
601 represent SE

602 **Fig. 4** Individual recruit (<20 mm total length) being successfully (represented by a 1) or
603 unsuccessfully (represented by a 0) cleaned by a juvenile cleaner wrasse, *Labroides*

604 *dimidiatus*, in relation to the abundance of a) other fish species and b) recruits per 20 min
605 observations. The probability of a recruit successfully being cleaned decreases as the
606 abundances of other fish species and recruits increase, as determined from a logistic
607 regression model.