

# Cleaner wrasse influence habitat selection of young damselfish

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**Abstract** The presence of bluestreak cleaner wrasse, *Labroides dimidiatus*, on coral reefs increases total abundance and biodiversity of reef fishes. The mechanism(s) that cause such shifts in population structure are unclear, but it is possible that young fish preferentially settle into microhabitats where cleaner wrasse are present. As a first step to investigate this possibility, we conducted aquarium experiments to examine whether settlement-stage and young juveniles of ambon damselfish, *Pomacentrus amboinensis*, selected a microhabitat near a cleaner wrasse (adult or juvenile). Both settlement-stage (0 d post-settlement) and juvenile (~5 weeks post-settlement) fish spent a greater proportion of time in a microhabitat adjacent to *L. dimidiatus* than in one next to a control fish (a non-cleaner wrasse, *Halichoeres melanurus*) or one where no fish was present. This suggests that cleaner wrasse may serve as a positive cue during microhabitat selection. We also conducted focal observations of cleaner wrasse and counts of nearby damselfishes (1 m radius) to examine whether newly settled fish obtained direct benefits, in the form of

cleaning services, from being near a cleaner wrasse. Although abundant, newly settled recruits (<20 mm total length) were rarely (2 %) observed being cleaned in 20 min observations compared with larger damselfishes (58 %). Individual damselfish that were cleaned were significantly larger than the median size of the surrounding nearby non-cleaned conspecifics; this was consistent across four species. The selection by settlement-stage fish of a microhabitat adjacent to cleaner wrasse in the laboratory, despite only being rarely cleaned in the natural environment, suggests that even rare cleaning events and/or indirect benefits may drive their settlement choices. This behaviour may also explain the decreased abundance of young fishes on reefs from which cleaner wrasse had been experimentally removed. This study reinforces the potentially important role of mutualism during the processes of settlement and recruitment of young reef fishes.

**Keywords** Recruitment · Ectoparasites · Cleaning behaviour · Damselfish · Mutualism

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## Introduction

On coral reefs, there are many mutualistic cleaners that remove parasites from other organisms, including coral reef fishes. One of the most well known of these is the bluestreak cleaner wrasse, *Labroides dimidiatus* (Labridae), a cleaner wrasse common throughout the Indo-Pacific (Randall et al. 1997). *Labroides dimidiatus* has been shown to reduce the ectoparasite load of individual client fishes (Gorlick et al. 1987; Grutter 1999; Grutter and Lester 2002; Clague et al. 2011), aggression by predators (Cheney et al. 2008), stress (measured in cortisol levels) via physical contact (Bshary et al. 2007; Soares et al. 2011) and increase

growth and size of client fishes (Clague et al. 2011; Waldie et al. 2011). In addition, these cleaner wrasse affect local populations of reef fishes, so that in their absence there are reductions in the diversity and abundance of site-attached adult residents, visitors (fishes that move between patch reefs) and juvenile visitor fishes (Bshary 2003; Grutter et al. 2003; Waldie et al. 2011). Although the positive effects of the presence of *L. dimidiatus* on individuals and populations have been demonstrated in experimental manipulations of entire patch reefs ranging in size from ~61 to 285 m<sup>2</sup> (Waldie et al. 2011), the mechanisms involved, particularly at small spatial scales (1 m radius, i.e., home range of juvenile cleaner wrasse), remain largely unstudied.

One of the most critical periods that affect the population dynamics of coral reef fishes occurs during the settlement of young fish from the plankton to the reef and the few days or weeks of life in benthic habitats that immediately follow (post-settlement). Processes at this time ultimately determine which individuals are recruited to the population of juveniles. Young fish can actively choose the habitat into which they will settle, and the processes that underlie these choices are complex (Hoey and McCormick 2004; Heinlein et al. 2010). Additionally, the mortality rate at this time is high; indeed, it has been estimated that 57 % of individuals die within the first 1–2 d of settlement (Almany and Webster 2006). Thus, the choice of a suitable microhabitat at settlement is critical for successful recruitment. Selecting the optimum microhabitat depends on numerous factors, which may include the structure of the microhabitat (Tolimieri 1995), and the presence of potential predators (Vail and McCormick 2011) and conspecifics (Sweatman 1985). Indeed, it was recently shown that the experimental removal of *L. dimidiatus* was associated with a decrease in the abundance of recent recruits on patch reefs relative to control reefs where cleaner wrasse were present (Sun et al. 2015). However, it is unknown whether the presence of *L. dimidiatus* can serve as a positive cue for microhabitat choice at settlement.

Our study used experimental and observational approaches to determine whether the presence of juvenile or adult cleaner wrasse influenced the choice of microhabitat by settling juveniles of the ambon damselfish, *Pomacentrus amboinensis*. In the laboratory, we examined whether the presence of cleaner wrasse influences habitat choice of *P. amboinensis*. To determine the extent to which recently settled damselfishes were cleaned by *L. dimidiatus* juveniles, and thus whether the benefits of settling near a cleaner wrasse could be related to the cleaning services they provide or to other indirect benefits, focal observations of the cleaning interactions between *L. dimidiatus* and damselfishes were conducted in the natural environment. Lastly, to determine whether juvenile cleaner wrasse are

size-selective in their choice of clients, we compared the size of individuals of the four most abundant damselfishes that were cleaned by the wrasse with the median size of non-cleaned conspecifics.

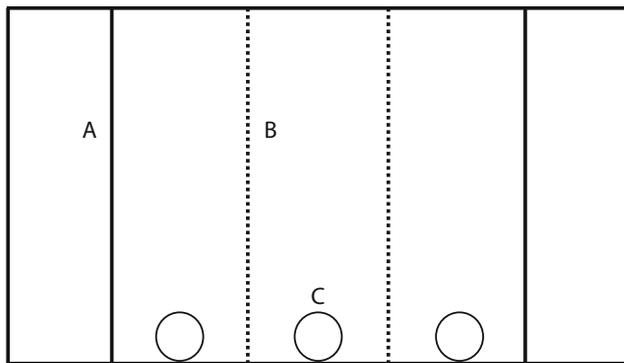
## Methods

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

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

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

Clear perspex sheets were placed 10 cm from each end of a glass experimental aquarium (35 × 36 × 65 cm), to create two end compartments with flow-through seawater (Fig. 1). The middle section of the aquarium was divided into three 15 cm subsections, using vertical black lines drawn on the front and back of the aquarium. Three



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

polyvinyl chloride (PVC) tube shelters ( $6 \times 5$  cm diameter) were placed in the centre of each subsection. Aquaria were covered with black plastic on three sides to isolate the fish from external activity, and a green plastic shade cloth was hung in front of the tanks to create an observation blind.

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

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

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

*P. amboinensis* ( $n = 25$  individuals per treatment). At the end of each trial, cleaner and control fish were replaced. Individual *P. amboinensis* were only used once so that their behaviour was not affected by previous exposure.

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

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

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

For each observation, a juvenile *L. dimidiatus* was located and its estimated TL was recorded. A 1-m radius was measured around the area where the *L. dimidiatus* was first sighted using a 1-m-long string and marked using five lead sinkers (7 mm diameter) attached to floats (25 mm). Surrounding fish were allowed to acclimate ( $\sim 5$  min) to the presence of the markers and observers, before the commencement of the fish counts and cleaning interactions within this area. This acclimation period allowed adult fish that may have been disturbed from the initial deployment of the markers to move back inside the observation area. Fish counts were recorded in a systematic way (by size class) to ensure that an individual was counted only once. All fish present were recorded to account for the availability of other sizes of fish as potential clients that could influence the cleaners’ choice of client. Cleaning

**Table 1** Ontogenetic stage and size (total length) of fish used in each part of laboratory experiment. Size is mean  $\pm$  SE

| Part | Date          | <i>Labroides dimidiatus</i> (cleaner) | <i>Halichoeres melanurus</i> (control) | <i>Pomacentrus amboinensis</i> (client) |
|------|---------------|---------------------------------------|----------------------------------------|-----------------------------------------|
| A    | December 2011 | Adult (79.1 $\pm$ 4.0 mm)             | Adult (86.1 $\pm$ 0.8 mm)              | Juvenile (19.3 $\pm$ 0.3 mm)            |
| B    | December 2011 | Juvenile (24.2 $\pm$ 0.5 mm)          | Juvenile (24.8 $\pm$ 0.4 mm)           | Juvenile (18.0 $\pm$ 0.3 mm)            |
| C    | January 2012  | Juvenile (23.1 $\pm$ 0.7 mm)          | Juvenile (24.3 $\pm$ 0.8 mm)           | Settlement-stage (15.2 $\pm$ 0.1 mm)    |

interactions were recorded as any physical contact between cleaner and client, or significant inspection by the cleaner ( $>1$  s). For each interaction, we recorded the client species (where possible), cleaning duration, and estimated client size (mm TL). To compare client size and the median size of nearby (non-cleaned) conspecifics, the size of all other fish present within the marked observation site ( $10 \text{ mm} \leq \text{TL} < 80 \text{ mm}$ ), but not involved in any cleaning interaction, was also estimated. All other fish were identified to species where possible. To ensure that each *L. dimidiatus* was observed only once, observers began at one end of the reef and continued without backtracking. In contrast to adults, juvenile *L. dimidiatus* stay within a highly restricted home range of around  $4 \text{ m}^2$  (Robertson 1974), further reducing the possibility of observing an individual twice. Although every individual within the observation area was identified to species, ultimately, for adequate sample sizes for statistical analyses, only the most abundant damselfish species (both cleaned and non-cleaned conspecifics) from the observations were used. These four common species were *P. amboinensis*, *P. moluccensis*, *P. nagasakiensis*, and *P. wardi*.

### Statistical analyses

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

### Laboratory experiment

A full linear mixed-effects (LMER) model with a restricted maximum likelihood (REML) procedure was used to examine whether *P. amboinensis* spent more time near a *L. dimidiatus* than near a control fish or no fish compartment. Two analyses were conducted; the first model used data from parts A and B (Table 1) to determine whether ontogenetic stage of the cleaner had an effect on juvenile *P. amboinensis*' habitat choice, while the second model used data from parts B and C, and included ontogenetic stage (settlement-stage and juvenile *P. amboinensis*) of the client as a factor. In the first model, cleaner ontogenetic stage (adult or juvenile), settlement stimulus type to which the microhabitat was adjacent (cleaner, control, no fish), aquarium side the cleaner was placed (left or right), and

fish stimulus treatment combination (cleaner/control, cleaner/no fish or control/no fish) were used as fixed factors. Replicate trial identity was added as a random factor, to account for an order effect. In both models, proportion of time spent in each section of the aquarium (right, left and centre) was transformed by taking the arcsine of the square root of the proportion to meet the assumptions of normality.

### Field observations

A LMER model with a REML procedure was used to test whether there was a significant difference between the median size of client fish and nearby conspecifics. The terms treatment (client or nearby conspecifics), nearby conspecific size, and species (four damselfish species: *P. amboinensis*, *P. moluccensis*, *P. nagasakiensis* and *P. wardi*) were fixed factors, and the identity of the individual cleaner fish and the cleaning event identity (specifying which client and nearby conspecifics corresponded with one another) were random factors. To determine whether client size varied with cleaner size or the size of all other nearby species, separate (LMER) analyses were run with cleaner size and other nearby fish size as covariates and cleaner identity as a random factor. A generalised linear model with a binomial distribution was used to determine whether there was a significant difference between the number of cleaned recruits, against the abundance of other fish species ( $>20 \text{ mm TL}$ ) and recruits ( $<20 \text{ mm TL}$ ) within the 1-m-radius observation area. To calculate the probability that a recruit would be cleaned by a cleaner wrasse over a 12-h day, we used the following equation (Eq. 1), where  $p_{-t}$  is the probability of being cleaned over a time period,  $t$  ( $t$  in units of 20 min), and  $p$  is the probability of being cleaned in a 20 min period. This formula assumes that cleaning events are independent and that  $p$  is constant over the time period.

$$p_{-t} = 1 - (1 - p)^t \quad (1)$$

To determine the best model in all analyses, we compared the full model with models in which one of the explanatory terms was dropped using the 'drop1' function (Chambers 1992). The term was dropped if an analysis of variance found that a dropped term had no

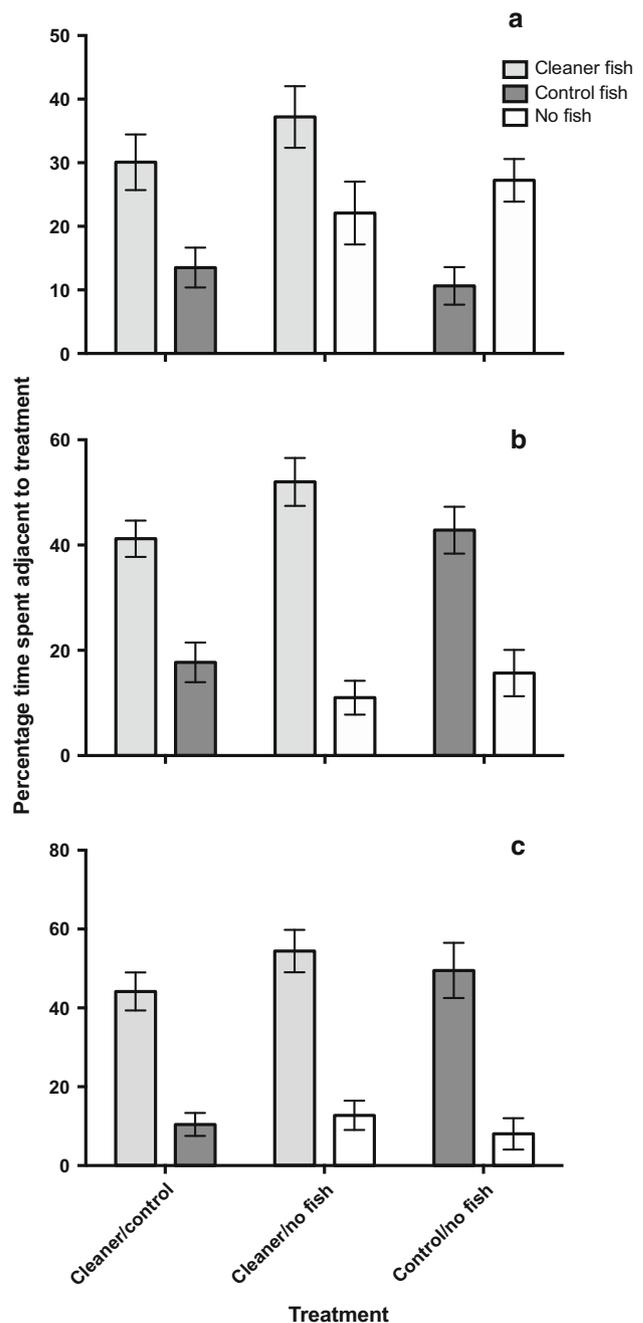
significant effect on the model using a Chi-square distribution. A Tukey–Kramer HSD test (TK-HSD) using the `glht` function in the ‘multcomp’ package (Hothorn et al. 2008) identified where differences occurred, and a summary output table was reconstructed using the results from the ‘drop1’ function. Prior to all analyses, the assumptions of normality and homogeneity of variance were assessed using histograms, residuals, and quantile–quantile plots.

## Results

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

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

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



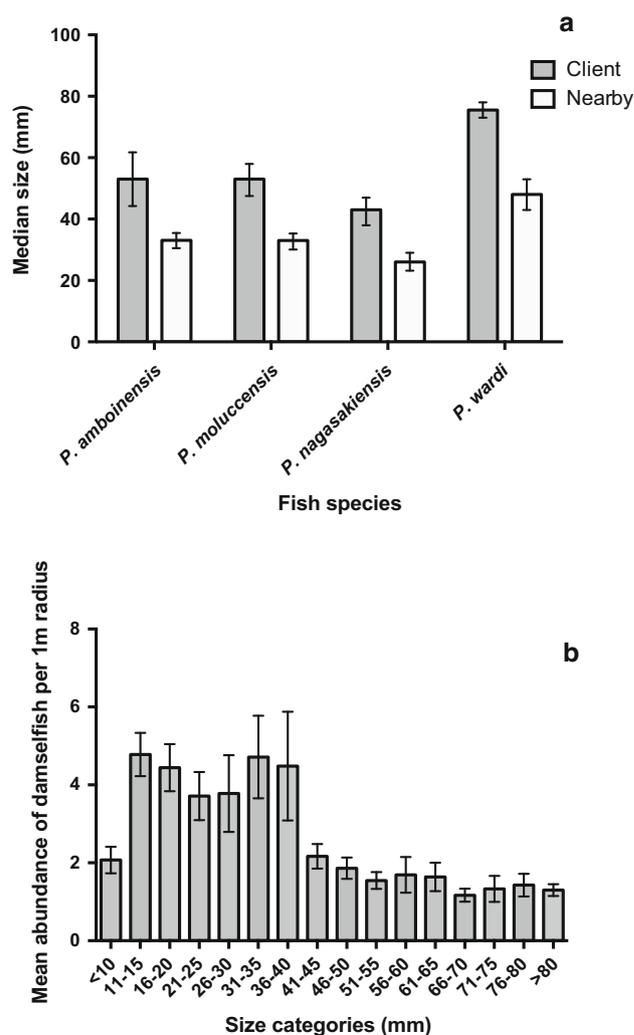
**Fig. 2** The percentage of time spent on microhabitat adjacent to fish treatment by damselfish client *Pomacentrus amboinensis*. **a** Part A: juvenile *P. amboinensis* with adult cleaner/control fish, **b** Part B: juvenile *P. amboinensis* with juvenile cleaner/control fish, and **c** Part C: settlement-stage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

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

A total of 1107 cleaning interactions were recorded during 79 observations of individual *L. dimidiatus* juveniles over a

period of 1580 min. Cleaner wrasse cleaned 104 species of fish from 19 families, including 32 species of damselfishes of which the following were cleaned most often (listed from most to fewest): *Acanthochromis polyacanthus*, *Stegastes apicalis*, *Amblyglyphidodon curacao*, *P. moluccensis*, *Plectroglyphidodon lacrymatus*, *Pomacentrus amboinensis*, *P. nagasakiensis*, *P. wardi*, and *Chromis lepidolepis* (see Electronic Supplementary Material, ESM, Table S1 for a full list of client species). These cleaning interaction frequencies were not adjusted for relative abundance of each client species. The mean number ( $\pm$  SE) of clients cleaned and the duration spent cleaning per 20 min observation was  $14 \pm 1$  fish and  $83.8 \pm 7.3$  s, respectively. Overall, only 14 % of cleaning interactions were between juvenile *L. dimidiatus* and the four common damselfishes (*P. amboinensis*, *P. moluccensis*, *P. nagasakiensis* and *P. wardi*) that had the greatest abundances within the 1-m radius of a cleaner wrasse. A total of 46 % of interactions occurred with other damselfishes (excluding the four listed species), while the remaining 40 % of interactions occurred between non-damselfishes and juvenile *L. dimidiatus*. Overall, 60 % of the clients of juvenile *L. dimidiatus* were damselfishes. The range and median size (mm TL) of the common damselfishes that were cleaned were: *P. amboinensis* (20–80 and 50 mm), *P. moluccensis* (25–80 and 50 mm), *P. nagasakiensis* (15–90 and 40 mm), and *P. wardi* (50–120 and 90 mm); the duration (seconds, mean  $\pm$  SE) of time spent cleaning per 20 min observation for the common species was  $14 \pm 2.5$  s. Client size differed among the four species (LRT = 68.01,  $df = 3$ ,  $P < 0.0001$ ; Fig. 3a); a TK-HSD test showed that *P. wardi* clients were significantly larger than the other common client damselfishes. Damselfishes that were cleaned were significantly larger than nearby non-cleaned conspecifics occurring within a 1-m radius; this effect was consistent for all four species (LRT = 173.72,  $df = 1$ ,  $P < 0.0001$ ; Fig. 3a). Separate analyses failed to detect any correlation between the size of the damselfish client with the size of the cleaner wrasse (LRT = 0.17,  $df = 1$ ,  $P = 0.675$ ) nor was size of damselfish client correlated with the median size of all other fish species combined (excluding common damselfishes) within the 1-m radius of the cleaner (LRT = 2.55,  $df = 1$ ,  $P = 0.110$ ).

Despite the presence of numerous recently settled damselfishes (size range 15–20 mm) within the 1-m-radius observation areas (Fig. 3b), only 2 % (22 of 1107 cleaning interactions) of fishes that were cleaned were  $< 20$  mm TL. There was a significant negative relationship between the number of recruits that were cleaned and the abundance of both other fish species ( $> 20$  mm TL) (LRT = 7.97,  $df = 1$ ,  $P = 0.004$ , Fig. 4a) and recruits ( $< 20$  mm TL) (LRT = 16.95,  $df = 1$ ,  $P < 0.0001$ , Fig. 4b) within the 1-m-radius observation area. The highest probability of a recruit ( $< 20$  mm TL) being cleaned by a juvenile cleaner wrasse within the observation area was 0.002/20 min

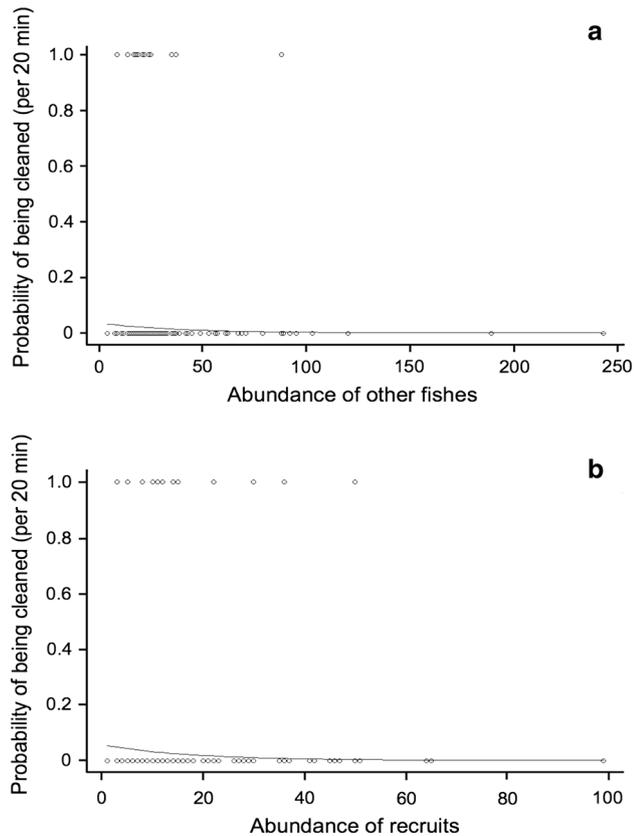


**Fig. 3** Cleaning interactions of juvenile cleaner wrasse, *Labroides dimidiatus* and damselfishes. **a** Size (total length) of damselfish clients and nearby non-cleaned conspecifics. Bars represent median; error bars represent 25th and 75th quantiles, **b** size distribution of nearby non-cleaned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

(95 % CI  $3.22 \times 10^{-6}$ –0.22) when there were eight (the lowest value encountered) other fishes ( $> 20$  mm TL), and it was 0.003/20 min (95 % CI  $6.77 \times 10^{-5}$ –0.07) when there were three (the lowest value encountered) recruits ( $< 20$  mm TL) (Fig. 4a, b). The probability of a recruit being cleaned rapidly declined to zero as the abundance of other fish and recruits increased. An extrapolation of the highest probability of an individual recruit being cleaned during a 12-h day is 0.069 and 0.102.

## Discussion

Our study provides some of the first evidence that the presence of cleaners could influence fish settlement and potentially migration decisions. Our experimental evidence



**Fig. 4** Individual recruit (<20 mm total length) being successfully (represented by a 1) or unsuccessfully (represented by a 0) cleaned by a juvenile cleaner wrasse, *Labroides dimidiatus*, in relation to the abundance of **a** other fish species and **b** recruits per 20 min observations. The probability of a recruit successfully being cleaned decreases as the abundances of other fish species and recruits increase, as determined from a logistic regression model

suggests that the presence of cleaner wrasse, *L. dimidiatus*, could directly influence the choice of microhabitats by young coral reef damselfish. In the laboratory, both settlement-stage and juvenile *P. amboinensis* preferentially selected microhabitats that were in close proximity to *L. dimidiatus*. This may be one explanation for the observed patterns of increased abundance of fishes in the presence of cleaner wrasse on the reef. However, our observations in the natural environment revealed that young damselfish were rarely cleaned by *L. dimidiatus*. Thus, it is possible that even rare cleaning events and/or indirect benefits resulting from the presence of cleaner wrasse may drive the selective behaviour of settling damselfish (see Sun et al. 2015).

We assessed microhabitat choice by settlement-stage (0 d post-settlement) and slightly older juvenile (~5 weeks post-settlement) *P. amboinensis* and found that individuals preferentially selected a microhabitat adjacent to *L. dimidiatus*. This indicates that the 'attraction' towards cleaner wrasse

occurs at both stages. Habitat selectivity at settlement appears common in young damselfishes, with some species attracted to particular microhabitats such as coral heads that harbour conspecifics (Lecchini et al. 2007; Coker et al. 2012), while others are attracted to coral heads associated with heterospecifics (Green 1998; Almany 2003). Our study adds cleaner wrasse to the list of organisms to which settlers are attracted; the presence of cleaner wrasse could act as an indicator of microhabitat quality to settlement-stage fish. Furthermore, these results suggest that if juveniles move between microhabitats (Simpson et al. 2008), site selection may also be influenced by the presence of *L. dimidiatus*. Our results indicate that damselfish are attracted to cleaners at a within-reef-patch scale; however, further investigation is needed to determine whether this attraction translates to decisions made during settlement.

The apparent attraction to *L. dimidiatus* may explain the greater number of recently settled recruits (Sun et al. 2015), juvenile visiting fishes and even adult damselfishes, found on patch reefs where *L. dimidiatus* are present relative to reefs without cleaner wrasse (Bshary 2003; Waldie et al. 2011). There are two ways in which these patterns may arise, using the presence of *L. dimidiatus* as a cue. Settlement-stage fish may either choose to settle on certain patch reefs, or engage in post-settlement movement between patch reefs as juveniles (Simpson et al. 2008). Both of these processes may additively result in damselfishes being more abundant on reefs with cleaner wrasse than on those without. Ideally, determining whether cleaners influence settlement decisions on reefs would involve a reef experiment where cleaner presence/absence is manipulated and settlement is quantified. The closest attempt to date involved counts of recently settled damselfish recruits (<20 mm TL) (Sun et al. 2015), as few newly settled fish could be distinguished from older recruits due to the rapid change in pigmentation of damselfishes after settling onto the reef (McCormick et al. 2002). Sun et al. (2015) did indeed show that the abundance of recently settled damselfish was higher on reefs with cleaner wrasse. However, since fish experience high levels of predation at settlement (Almany and Webster 2006), the study measured new recruits to the population rather than settlement.

The fish used in the laboratory experiment were naïve with respect to the shape or odour of *L. dimidiatus*, since they were collected by light traps while still in their planktonic phase. Despite this naïveté, young fish were able to differentiate between *L. dimidiatus* and another species that was similar in size and shape but differed in colour, pattern, and behaviour. The attraction to cleaner wrasse thus appears to be an innate behaviour. Slightly older recruits displayed the same abilities. However, we did not test whether *P. amboinensis* recognised *L. dimidiatus* as a cleaner per se. On some occasions, the fish

did behave like a client by remaining in the same position and adopting a pose with spread fins when next to the compartment with a cleaner wrasse. This behaviour did not occur when they were adjacent to the control fish or an empty compartment. Prospective clients of cleaner fish often assume this posture when they attempt to attract a cleaner to inspect them (Côté et al. 1998). Because the cleaner wrasse was behind a perspex divider, the cleaner and client were unable to physically interact; such physical contact likely acts as a positive feedback (Bshary and Würth 2001). Losey et al. (1995) previously showed that newly metamorphosed laboratory-reared, and hence ‘cleaner-naïve’, Hawaiian humbug damselfish, *Dascyllus albisella*, recognised the Hawaiian cleaner wrasse, *L. phthirophagus*, by adopting the cleaning pose. Our study thus confirms the hypothesis that client fish have an innate ability to recognise cleaners.

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

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

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

Many damselfishes settle at night (Dufour and Galzin 1993), including *P. amboinensis* (Meekan et al. 1993), and *L. dimidiatus* sleep in the reef matrix at night (Robertson

1974). Thus, cleaner wrasse could only be used as a visual cue for settlement during daylight hours. There is some evidence, though, that fish ‘sample’ the benthos during the day prior to settling (Leis and Carson-Ewart 2002; Leis 2006), which could be influenced by cleaner wrasse presence. However, it is likely that other cues such as odour could be used by settling damselfishes at night to detect reefs where cleaner wrasse are present. More information is needed to determine whether settlers can detect the presence of cleaner wrasse through olfaction.

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

From the client’s perspective, the odds of an individual new recruit (<20 mm TL) being cleaned per 20 min were low, with only two or three out of 1000 fish predicted to be cleaned when there were few other fishes or recruits within a 1-m radius; this rate rapidly decreased towards zero with increasing abundance of nearby other fish species and recruits. Over a day, however, the extrapolated odds could be much higher, estimated to increase to 7 or 10 out of 100 fish when there were few other fishes or recruits within a 1-m radius. Such rare events could have disproportionately significant benefits as some parasites can have especially harmful effects on small individuals. While gnathiid isopods are generally considered micropredators, taking small amounts of blood from their significantly larger host (Lafferty and Kuris 2002), they are capable of consuming up to 85 % of the total blood volume of a settlement-stage *P. amboinensis* (Grutter et al. 2011). Indeed, infection by a single gnathiid isopod decreases the oxygen consumption and swimming performance of a recruit damselfish and reduces the likelihood of successful settlement on the reef (Grutter et al. 2011). Removal of a gnathiid before it feeds on an infected fish’s blood could provide a major benefit

for survival. Since observed cleaning interactions were brief ( $\sim 1$  s), it is unlikely that recruits obtained other direct benefits of cleaning, such as tactile stimulation leading to a reduction in client stress (cortisol) levels (Soares et al. 2011).

As direct cleaning interactions between cleaners and recently settled recruits were relatively uncommon, it is possible that the benefits of preferentially selecting a microhabitat near cleaners may be at least partly indirect. For example, cleaner wrasse may reduce infection rate of parasites in the vicinity of a cleaning station. Each *L. dimidiatus* eats around 1200 gnathiids per day (Grutter 1996), which could significantly reduce the number of ectoparasites in the immediate vicinity of a *L. dimidiatus* and hence lower the infection rate of nearby settlers (Gorlick et al. 1987; Grutter 1999; Clague et al. 2011). Other indirect benefits of the presence of cleaner wrasses may also exist for settling fish. 'Safe havens' from predators may be created by *L. dimidiatus* (Cheney et al. 2008) in that, in the presence of cleaners, some predators behave with notably reduced aggression. Settling reef fish may therefore prefer to settle in a habitat with *L. dimidiatus* present, due to reduced risk of predator aggression. Alternatively, or in addition, selection by young damselfish of microhabitats near cleaners may simply be an investment where the return occurs only when recruits have grown significantly.

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

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