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NOTE

Patterns of resource-use and competition for mutualistic partners between two species of obligate cleaner fish

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Abstract Cleaner mutualisms on coral reefs, where specialized fish remove parasites from many species of client fishes, have greatly increased our understanding of mutualism, yet we know little about important interspecific interactions between cleaners. Here, we explore the potential for competition between the cleaners *Labroides dimidiatus* and *Labroides bicolor* during two distinct life stages. Previous work has demonstrated that in contrast to *L. dimidiatus*, which establish cleaning stations, adult *L. bicolor* rove over large areas, searching for clients. We show that site-attached juvenile *L. bicolor* associate with different microhabitat than juvenile *L. dimidiatus* and that *L. bicolor* specialize on a narrower range of species than *L. dimidiatus* as both juveniles and adults. Further, we present evidence suggesting that differences in resource-use are influenced by competitive interactions between the two species. Finally, we discuss the implications of these results for understanding the ecology and evolution of the mutualism.

Keywords Cleaner fish · Mutualism · *Labroides bicolor* · *Labroides dimidiatus*

Introduction

One of the best known mutualisms on coral reefs is between cleaner fish and the client fishes that visit cleaners for the removal of ectoparasites. Recent work on cleaners has greatly increased the understanding of the maintenance of cooperation in mutualisms when one or more partners has the ability to cheat (e.g., Bshary and Grutter 2002, 2006; Raihani et al. 2010; Oates et al. 2010a). In the case of cleaner mutualisms, cheating involves cleaners feeding on client mucus or tissue instead of parasites (Bshary and Grutter 2002). While several mechanisms have been identified that can prevent cheating, a central mechanism hinges on the ability of clients to choose the cleaners with whom they interact (e.g., Bshary and Schäffer 2002; Adam 2010). This observation leads to the prediction that competition among cleaners for access to clients can affect the level of cooperation that evolves in the mutualism. Nonetheless, few studies on cleaner mutualisms have considered the potential for competition between different species of cleaners despite the observation that multiple species frequently co-occur.

Strong competition for space and food has been observed in other guilds of coral reef fishes, including herbivorous surgeonfishes (Robertson and Gaines 1986), planktivorous damselfishes (Schmitt and Holbrook 2003), and coral-dwelling gobies (Munday et al. 2001). In these well-studied systems, competitive dominance is mediated largely by interference competition, with the result that larger or more aggressive species gain access to preferred resources, thereby causing their competitors to shift to less-profitable resources. Importantly, we expect competition between cleaners to operate differently because it involves choices made by their resources (i.e., client fishes) in addition to any direct interactions between cleaners.

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Nonetheless, competition between cleaners for access to clients could lead to similar shifts in resource-use where competitively inferior species are forced to use resources not utilized by competitively superior species.

Here, we investigate patterns of resource-use during the juvenile and adult stages of two species of closely related cleaners, the bluestreak cleaner wrasse, *Labroides dimidiatus*, and the bicolor cleaner wrasse, *Labroides bicolor*. Previous work has shown that both cleaners feed on parasitic gnathiid isopods and that they clean many of the same client species (Randall 1958, see also Electronic Supplemental Material, ESM). In addition, a recent study has demonstrated experimentally that *L. dimidiatus* competes intraspecifically for access to food resources (Adam 2011). Together, these observations suggest strong potential for interspecific competition. Importantly, recent studies have also revealed that *L. bicolor* consistently cheats its clients more often than *L. dimidiatus* as both juveniles and adults (Mills and Côté 2010; Oates et al. 2010b). Given that clients often preferentially interact with cooperative cleaners rather than with cheaters (e.g., Bshary and Schäffer 2002), we hypothesize that *L. dimidiatus* will have a competitive advantage in gaining access to clients compared to *L. bicolor*. Specifically, we test the predictions that *L. dimidiatus* spends more time cleaning client fishes and cleans a greater diversity of clients than *L. bicolor*. We also predict that competition for access to clients will cause *L. bicolor* to shift their diet away from common shared resources when in close proximity to *L. dimidiatus*, forcing them to focus instead on clients rarely cleaned by *L. dimidiatus*.

Methods

Study site

The study was conducted on the east end of the Cook's Bay reef pass on the north shore of Moorea, French Polynesia (17°33′S, 149°37′W), at depths ranging from 1 to 9 m. The site had nearly continuous cover of *Porites rus*, a structurally complex coral that forms large hollow cavities and provides habitat for at least 120 species of fish (Brooks et al. 2007).

Cleaner behavioral observations

Observations of *L. bicolor* and *L. dimidiatus* were made during August 2007 between 0800 and 1730 h by a SCUBA diver or snorkeler. During each ~10-min observation, all species cleaned by a focal fish were recorded and the duration of each cleaning event was noted. Additionally, the microhabitat occupied by the focal fish was assigned to one of three possible categories: 'internal cavity' (within the recess of the reef), 'reef edge' (<10 cm from the surface of a reef), and 'water column' (>10 cm from the surface of a reef). Cleaners often occupied more than one microhabitat type, and thus, focal fish were assigned to one of six habitat categories (internal cavity only; reef edge and internal cavity; internal cavity, reef edge, and water column; reef edge only; reef edge and water column; and water column only). In total, 71 *L. bicolor* (40 juveniles and 31 adults/sub-adults) and 27 *L. dimidiatus* (12 juveniles and 15 adults/sub-adults) were observed. Juveniles (~1–5 cm TL) of each species were defined based on their color patterns, which are distinct from adults and sub-adults (~5–10 cm TL) (see Randall 2005). While fish could not be individually recognized, locations of each observation were marked, and care was taken to avoid resampling the same individuals.

Questions and data analysis

To test whether time spent cleaning differed between species and/or life stages, we conducted factorial ANOVA (effects = species, stage, and species × stage). Data were transformed using the Box–Cox transformation to meet the assumption of normality. In addition, to determine whether the different species and/or life stages cleaned different taxa, we conducted permutational MANOVA (Anderson 2001) on family-level data using the binomial deviance as our dissimilarity index (Anderson and Millar 2004). To visualize these multivariate patterns, we used canonical analysis of principal coordinates (CAP) (Anderson and

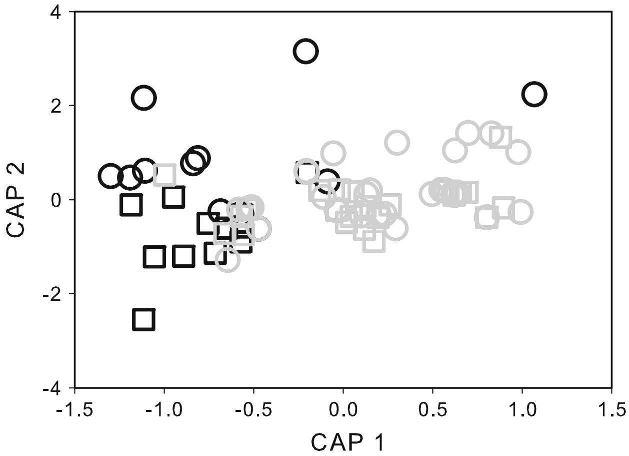

Fig. 1 Ordination plot showing separation in the composition of client fish cleaned by *L. dimidiatus* (*black symbols*) and *L. bicolor* (*gray symbols*). Plot also illustrates that juvenile *L. dimidiatus* (*black circles*) cleaned a distinct assemblage of clients compared to adult *L. dimidiatus* (*black squares*); there was no difference in the client assemblages cleaned by juvenile *L. bicolor* (*gray circles*) and adult *L. bicolor* (*gray squares*)

Willis 2003), an ordination procedure. Finally, to compare the diversity of the client species cleaned by the two species of cleaners, we constructed individual-based rarefaction curves (Gotelli and Colwell 2001). These curves reveal the rate of client species accumulation as more clients are sampled and represent a robust measurement of the diversity of clients cleaned by the two species over the range of sampling effort.

To test the prediction that access to clients by *L. bicolor* is influenced by their proximity to *L. dimidiatus*, we conducted Wilcoxon's tests on the amount of time juvenile *L. bicolor* spent cleaning different client types when in close proximity to juvenile *L. dimidiatus* and when >~3 m from *L. dimidiatus* (based on visual estimates recorded during behavioral observations). We predicted that *L. bicolor* would have access to fewer clients and, as a consequence, would have lower cleaning rates when close to *L. dimidiatus*. We also predicted *L. bicolor* would compensate for decreased access to shared clients by increasing cleaning rates of species rarely cleaned by *L. dimidiatus*.

Finally, to gain insight about the proximate mechanisms influencing the use of different client species, we determined whether microhabitat associations were species specific and/or stage specific using a series of 2 × 6 permutation-based contingency tests (equivalent to Fisher's exact test). We conducted four separate tests to compare life stages within a species as well as between species for the same life stage. All tests were two-tailed. Univariate statistics were conducted in JMP statistical software (SAS Institute). Multivariate statistics, ordination, and rarefaction were conducted in R (R Development Core Team) using the Vegan package (Oksanen et al. 2010).

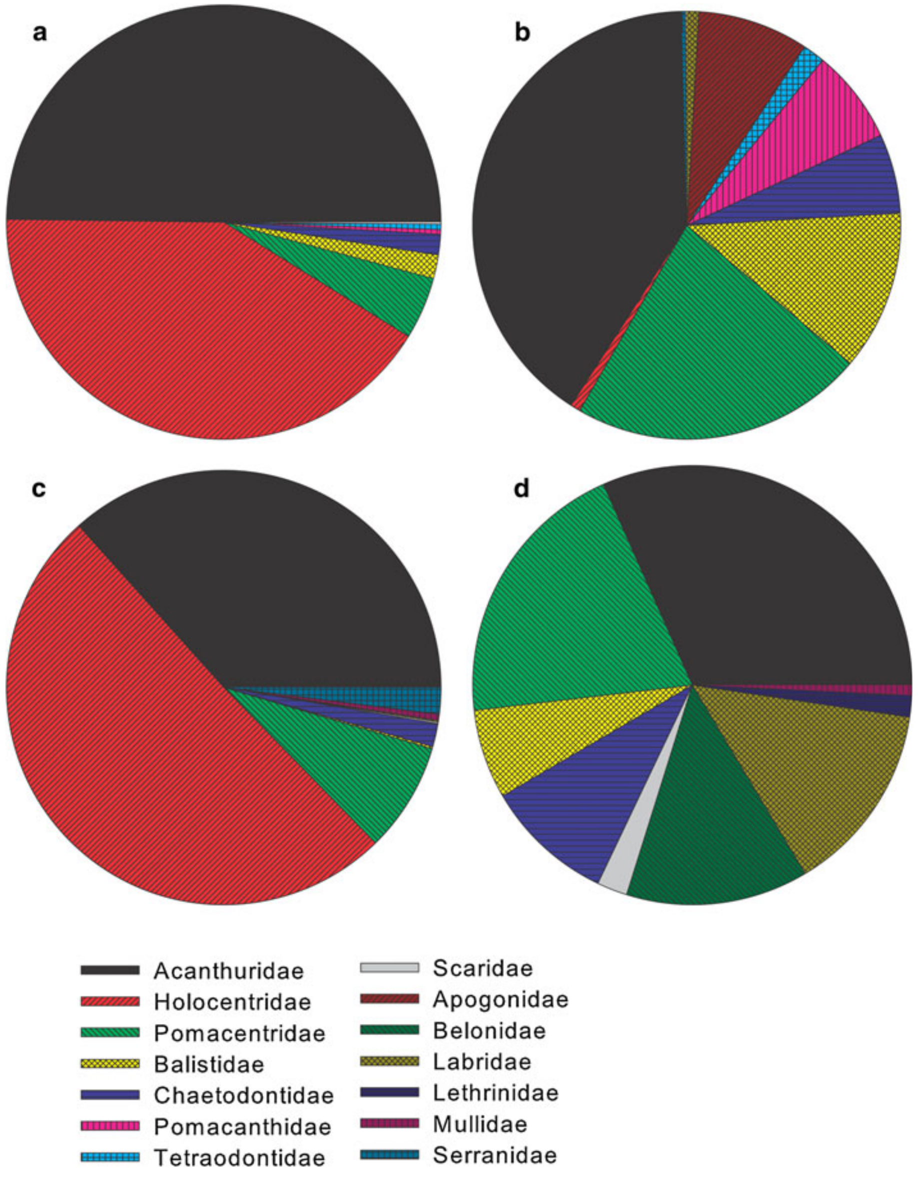

Fig. 2 Pie charts showing the client assemblages (at the family level) of **a** juvenile *L. bicolor*, **b** juvenile *L. dimidiatus*, **c** adult *L. bicolor*, and **d** adult *L. dimidiatus*. *Charts* are based on 1,000 cleaning events (*L. bicolor* juvenile = 355, *L. dimidiatus* juvenile = 133, *L. bicolor* adult = 357, *L. dimidiatus* adult = 155) lasting a total of 14,067 s (*L. bicolor* juvenile = 5,448, *L. dimidiatus* juvenile = 1,601, *L. bicolor* adult = 5,178, *L. dimidiatus* adult = 1,840)

Results and discussion

Contrary to our expectations, cleaners spent approximately one-third of their time cleaning client fish (mean = 32 %, SD = 24 %), and this did not differ between species and/or stages (ANOVA, $F_{3,94} = 1.67$ $P = 0.18$). However, *L. bicolor* cleaned a different client assemblage than *L. dimidiatus* as both juveniles (pseudo-$F_{1,50} = 8.17$, $P < 0.0001$) and adults (pseudo-$F_{1,44} = 7.52$, $P < 0.0001$) (Fig. 1). These differences resulted primarily from the tendency of *L. bicolor* to specialize on soldierfish and squirrelfish (Holocentridae), while *L. dimidiatus* cleaned a more diverse assemblage of clients (Fig. 2). Nonetheless, both species spent approximately 40 % of their time cleaning surgeonfish (Acanthuridae) (Fig. 2). While there were no stage-specific differences in the species of clients cleaned by *L. bicolor* (pseudo-$F_{1,69} = 0.11$, $P = 0.91$), juvenile *L. dimidiatus* tended to spend proportionally more time cleaning triggerfish (Balistidae), angelfish (Pomacanthidae), and cardinalfish (Apogonidae), while adults spent more time cleaning wrasses (Labridae) and needlefish (Belonidae) (pseudo-$F_{1,25} = 2.19$, $P = 0.049$) (Figs. 1,2).

Rarefaction curves indicated that *L. dimidiatus* cleaned a more diverse assemblage of species than *L. bicolor* as juveniles but not as adults (Fig. 3). The lack of consistent results among the two stages was surprising and prompted further investigation. We hypothesized that adult *L. bicolor* failed to conform to our expectations because they rove over large areas and are capable of tracking down mobile clients (Mills and Côté 2010; Oates et al. 2010b), which enables them to initiate many opportunistic interactions. Because it is likely that these interactions would be terminated quickly by clients, we tested whether cleaning interactions with species other than holocentrids and acanthurids were shorter for *L. bicolor* adults compared to *L. dimidiatus* adults. Indeed, interactions between these client species and *L. bicolor* were usually terminated very quickly (median = 3 s)—likely by the client—and were significantly shorter than interactions with *L. dimidiatus* (median = 7 s, Wilcoxon's test, $P = 0.027$). This suggests that adult *L. bicolor* have limited access to many of these species despite the fact they commonly interact with them.

As expected, *L. bicolor* spent significantly less time cleaning acanthurids when in close proximity to *L. dimidiatus* (Wilcoxon's test, $P = 0.002$) (Fig. 4a). However, there was no significant difference in the amount of time they spent cleaning all clients ($P = 0.94$), suggesting that loss of access to acanthurids was compensated by cleaning other species including holocentrids (Fig. 4b). Nonetheless, the amount of time *L. bicolor* spent cleaning holocentrids was not significantly different in the presence of *L. dimidiatus* ($P = 0.38$), and thus, patterns of resource-use only partially matched our expectations.

While it is difficult to determine whether species compete without conducting experimental manipulations, the fact that *L. bicolor* and *L. dimidiatus* clean many of the same species indicates potential for competition. In addition, the observation that *L. bicolor* apparently shifts its diet away from a common shared resource when in close proximity to *L. dimidiatus* is suggestive of an asymmetric competitive effect of *L. dimidiatus* on *L. bicolor*. Future studies should make use of manipulative field experiments to test directly the fitness consequences of each species on the other and should also focus on the quality of the clients as food resources in addition to client diversity. Still, the observation that *L. bicolor* specializes on a narrower range of clients than *L. dimidiatus* has important implications for understanding the mutualism.

For example, traits of *L. bicolor*, including habitat selection and coloration, may have evolved to facilitate specialization on particular client species. We found that as juveniles, *L. bicolor* was more closely associated with the 'internal cavity' than *L. dimidiatus* ($P = 0.003$, Fig. 5),

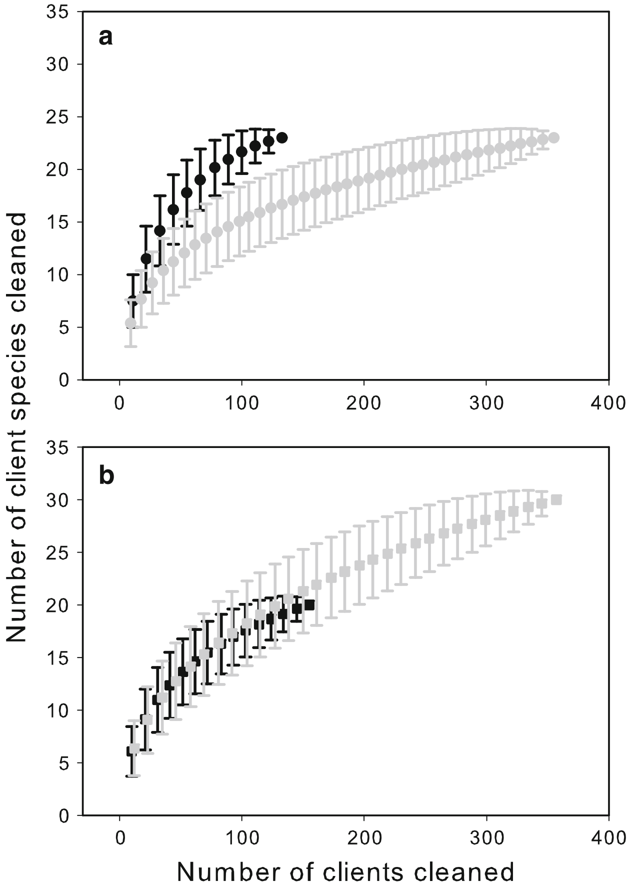

Fig. 3 Rarefaction curves showing the number of species cleaned with increasing sampling effort for **a** juvenile *L. dimidiatus* (*black symbols*) and juvenile *L. bicolor* (*gray symbols*), and **b** adult *L. dimidiatus* (*black symbols*) and adult *L. bicolor* (*gray symbols*). *Error bars* are 95 % confidence intervals

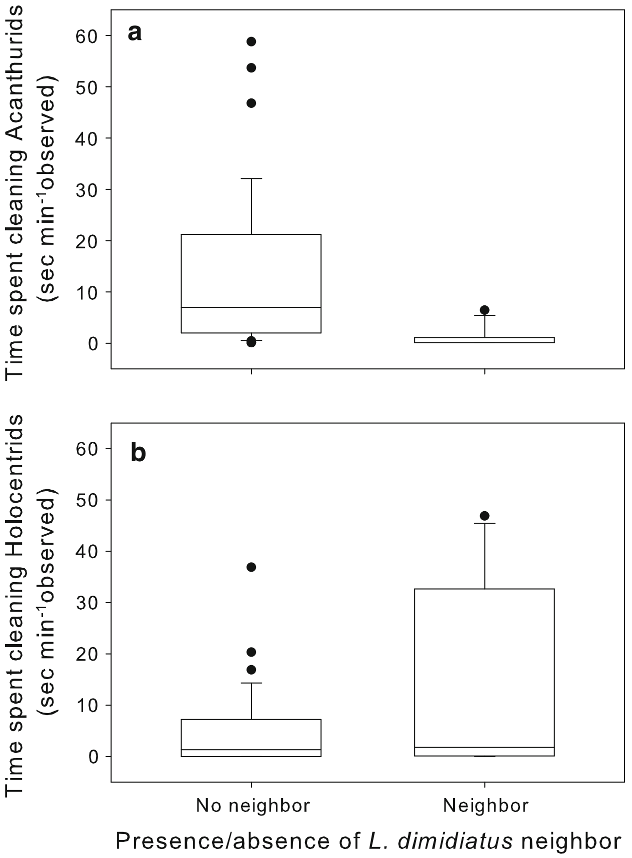

Fig. 4 Box and Whisker plot of the time juvenile *L. bicolor* spent cleaning **a** Acanthurids and **b** Holocentrids when in the absence ($N = 33$) and the presence ($N = 7$) of juvenile *L. dimidiatus*. *Boxes* are medians with 25th and 75th quartiles. Whiskers are 10th and 90th percentiles, and *dots* are data points that fall outside the 10th and 90th percentiles (determined using the Cleveland method in Sigma Plot 10.0)

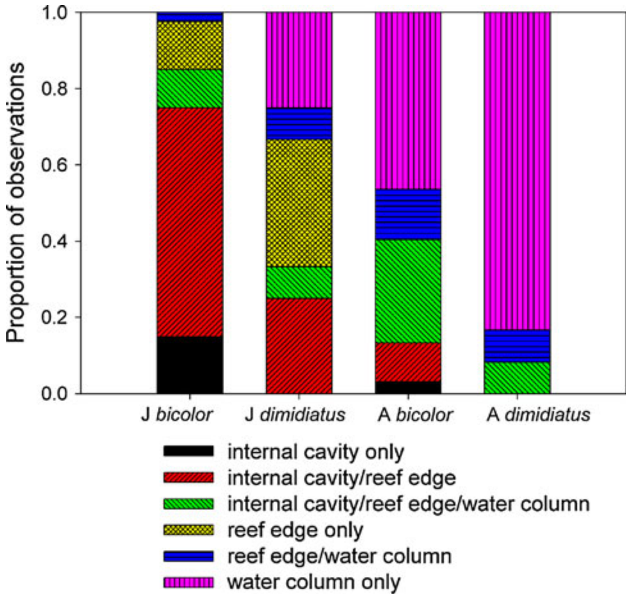

Fig. 5 Proportion of observations juvenile (J) and adult (A) *L. bicolor* ($N = 40$, $N = 30$, respectively) and *L. dimidiatus* ($N = 12$, $N = 12$, respectively) utilized combinations of three distinct habitat types, 'internal cavity' (within the recess of the reef), 'reef edge' (<10 cm of the surface of a reef), and 'water column' (>10 cm from the surface of the reef)

which is also frequently inhabited by holocentrids (Brooks et al. 2007). In addition, theoretical vision models suggest the blue stripe of juvenile *L. dimidiatus* is a more effective signal for many species of reef fishes than the yellow stripe of juvenile *L. bicolor* (Cheney et al. 2009; Lettieri et al. 2009), and hence, the yellow stripe may play a unique role in signaling to certain types of fish or signaling in specific spectral environments (e.g., within caves). Importantly, it is also possible that specialization on holocentrids arose in response to habitat-use or coloration. However, the large ontogenetic shifts in habitat-use observed in both species (Fisher's exact test; *L. dimidiatus* $P = 0.003$, *L. bicolor* $P < 0.0001$), in addition to the lack of habitat differences between their adult stages ($P = 0.34$) (Fig. 5), indicate that interspecific differences in client assemblages are not simply a by-product of habitat-use.

In conclusion, previous studies have shown that *L. bicolor* cheat their clients more often than *L. dimidiatus* (Mills and Côté 2010; Oates et al. 2010b), but these studies have not investigated the potential for interspecific competition between cleaners. Our results indicate that there is potential for competition between them. Further, the observations that *L. bicolor* apparently have access to fewer client species than *L. dimidiatus* and that they shift their diet away from common shared resources when in close proximity to *L. dimidiatus* suggest that *L. dimidiatus* may have a competitive advantage in gaining access to clients. Interspecific competition between cleaners has important implications for the ecology and evolution of the mutualism and should be considered in future studies.

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