

## **Temporal Variation in Cleanerfish and Client Behaviour: Does It Reflect Ectoparasite Availability?**

Isabelle M. Côté & Philip P. Molloy

*School of Biological Sciences, University of East Anglia, Norwich, UK*

### **Abstract**

We tested the importance of ectoparasites as the proximate cause of cleaning interactions by comparing the activity of Caribbean cleaning gobies (*Elacatinus evelynae*) and of their clients during three daily periods (early morning, midday, and late afternoon) in which ectoparasite availability varied naturally. Emergence from the benthos of gnathiid isopod larvae, the main target of cleaning goby predation, was higher at night, when cleaners were inactive, than during the day. As a result, overall ectoparasite loads on client fish tended to be higher in the morning. Inspection bouts by cleaning gobies were longest in the morning, but also at midday when ectoparasite availability on clients was lower. Client fish were observed at cleaning stations most often in the afternoon, when they harboured few ectoparasites, but they were more likely to adopt incitation poses, which increase the likelihood of being cleaned, in the morning than later in the day. Most cleaner and client behaviours therefore did not change predictably in response to natural diurnal variation in ectoparasite availability. Our study suggests that the ultimate and proximate causes of cleaning behaviour need not necessarily coincide.

Corresponding author: Isabelle M. Côté, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK. E-mail: [i.cote@uea.ac.uk](mailto:i.cote@uea.ac.uk)

### **Introduction**

Cleaning symbioses among fish are ubiquitous and conspicuous interactions, particularly on coral reefs. The adaptive significance of cleaning symbioses has long been debated (reviewed in Poulin & Grutter 1996; Côté 2000). However, recent empirical studies have shown significant reductions in client ectoparasite loads as a result of cleaner activity (Grutter 1999a; Cheney & Côté 2001; Grutter & Lester 2002), supporting the notion that cleaning symbioses are mutualisms based on ectoparasite removal. By contrast, controversy remains over the proximate causes of cleaning behaviour, particularly for clients.

From the cleaner's perspective, cleaning is related to food acquisition and as such, hunger should influence willingness to clean. Lenke (1982) tested this prediction experimentally but found no effect of cleaner satiation state on the cleaning activity of cleaner wrasses *Labroides dimidiatus* on dummy clients. However, cleanerfish's propensity to clean, which may reflect degree of hunger, has been linked to client ectoparasite loads, with cleaner wrasses and cleaning gobies preferring larger fish and those with more ectoparasites (Gorlick 1984; Grutter 1995a; Arnal et al. 2000).

From the client's perspective, two main factors have been proposed as motivation to seek cleaners. The first is related to relieving the irritation ectoparasites cause to their host's skin (Limbaugh 1961; Feder 1966). Assuming that more ectoparasites cause increased discomfort, there should be a link between client ectoparasite load and client propensity to visit cleaners. However, Losey (1971, 1979) found no such link under experimental conditions. Moreover, captive clients will perform cleaning solicitation poses to a variety of inanimate objects (Fricke 1966; Losey 1979). These observations prompted an alternative explanation, namely that clients primarily seek tactile stimulation from cleaners, rather than relief from ectoparasite infestation (Losey 1972, 1979). The two proximate hypotheses thus differ in the importance of ectoparasites for eliciting cleaner-seeking behaviour in clients.

The main targets of cleanerfish predation are usually gnathiid isopod larvae (Grutter 1996; Arnal & Côté 2000; Arnal & Morand 2001). These small (1–2-mm long) crustaceans attach temporarily to fish hosts to feed on body fluids and then return to the substratum to moult (Kabata 1984). The dynamics of benthic emergence of gnathiids, and their pattern of colonization of hosts, are now becoming clearer. Gnathiids emerge more often at night (including dawn and dusk) than during the day (Hobson & Chess 1976; Jacoby & Greenwood 1988; Chambers & Sikkell 2002; but see Grutter et al. 2000), particularly during the full moon (Jacoby & Greenwood 1988). Host colonization, though occurring through the day, also peaks at night when the rate of predation is low because of cleanerfish's nocturnal inactivity (Grutter 1999b; Grutter & Hendrikz 1999). Ectoparasite availability on clients should therefore be highest at the end of each night and decrease through the day as a result of cleaning activity (Grutter & Hendrikz 1999; Chambers & Sikkell 2002).

Any temporal variation in client ectoparasite load should affect both the foraging patterns of cleanerfish and the pattern of daily use of cleaning stations by fish clients if ectoparasites are the proximate cause of cleaning. Cleaner hunger levels should be higher in the morning, after nocturnal fasting, and clients with more ectoparasites visit cleaning stations more often (Arnal et al. 2001; Grutter 2001). In addition, clients that adopt stereotyped, immobile poses at cleaning stations are more likely to become inspected by cleaners (Côté et al. 1998). An early morning peak in ectoparasite availability should therefore be associated with longer inspection by cleaners and higher rates of visits and poses by clients.

In this paper, we examine empirically the relationships between the activity of cleaning gobies (*Elacatinus evelynae*), client use of cleaning stations, and

concurrently measured client ectoparasite loads. We first document the daily pattern of emergence of gnathiid isopod larvae on our Barbadian study reef to confirm the pattern of nocturnal emergence described elsewhere in the Caribbean (Chambers & Sikkell 2002). We then test the predictions that peak ectoparasite availability on clients should result in (1) high cleaning goby foraging activity, as reflected by long inspection bouts and/or rapid biting rate, and (2) high rates of use by clients to cleaning stations and/or high rate of adoption of incitation poses.

## Methods

### Study Site and Cleanerfish Species

The study was carried out on the North Bellairs fringing reef within the Barbados Marine Reserve on the west coast of Barbados, West Indies, in July and August 2000, and June 2002. All benthic emergence trapping, collection of fish clients for ectoparasite enumeration and observations of cleaning stations were carried out in the crest and spur-and-groove zones of the reef, 60–110 m from shore and at depths of 2.5–4.75 m. The North Bellairs reef is largely degraded, with turf algae cover ranging from 45 to 75% in the spur-and-groove and reef crest zones, respectively (I.M.C., unpublished data).

*E. evelynae*, the sharknose cleaning goby, is one of two species of cleaning gobies on Barbadian reefs. It is small (maximum total length: 4 cm) but conspicuously coloured, with a bright yellow lateral stripe running from the tip of the snout to the tail. The social and cleaning behaviour of *E. evelynae* on Barbadian reefs has been described in detail elsewhere (Whiteman & Côté 2002).

### Emergence of Benthic Ectoparasites

To establish the pattern of benthic emergence of gnathiid isopod larvae, we used four emergence traps, consisting of a 1-m<sup>2</sup> base of PVC piping and a 1-m high pyramid-shaped net made of 200- $\mu$ m nylon mesh. A sampling jar (500-ml plastic bottle with 5-cm-wide opening), made buoyant with a bubble of air, was screwed to the top of each trap. Two of the traps had inverted funnels (1-cm-wide at narrow end) extending 5 cm into the sampling jars. We found no difference in gnathiid numbers between traps with and without funnels (Wilcoxon signed ranks test,  $Z = 0.82$ ,  $N = 4$ ,  $P = 0.41$ ). The traps were secured to the reef using lead weights on each corner.

Traps were set on a variety of habitats (including live coral, coralline rock, sand and rubble) over 4 d around full moon, which usually corresponds to peak gnathiid emergence (Jacoby & Greenwood 1988). Each trap was moved to a new location every 24 h, with sampling jars being changed at 06.00 and 18.00 h every day. There were thus 16 night trap-periods and 16 day trap-periods which were carried out in June 2002.

The contents of the sampling jars were filtered through a 200- $\mu$ m mesh filter, preserved in 70% alcohol, and examined under a dissecting microscope.

### Assessment of Ectoparasite Load on Client Fish

Five species of client fish (brown chromis *Chromis multilineata*, smallmouth grunt *Haemulon chrysargyreum*, French grunt *H. flavolineatum*, longfin damselfish *Stegastes diencaeus* and yellow goatfish *Mulloidichthys martinicus*) were selected for ectoparasite assessment because they are abundant on the reef but they exhibit different frequencies of visits to cleaning stations (Arnal & Côté 1998; this study). Individual fish were selectively herded into a barrier net (4-mm mesh size), subsequently caught in a hand net and quickly transferred into a sealable plastic bag. A small amount of clove oil, a natural anaesthetic, was squirted into the bag and fish died quickly (less than 20 s) with little evidence of distress.

Ectoparasites were recovered using the method of Grutter (1995b). Fish were placed in a 4% chlorobutanol bath for 60 min. The body, fins and gills of each fish were then gently scraped with the nozzle of a squirt bottle. All liquids, including the contents of the plastic bag, were filtered. Filtrates were preserved in 70% alcohol for later identification under a binocular microscope.

Finally, the surface area of each fish was estimated by drawing an outline of the body with fins spread and of one pectoral fin. The area of each outline was obtained using the software WinDias v 2.0 (Delta-T Devices Ltd, Cambridge, UK). Body area was doubled and pectoral fin area quadrupled before summing these values to estimate total fish area.

### Behavioural Observations

Eleven cleaning stations occupied by sharknose cleaning gobies were selected haphazardly on the study reef. Detailed behavioural observations were carried out at each focal cleaning station to quantify goby cleaning activity and use of cleaning stations by fish clients. When cleaning stations were occupied by a pair of cleaning gobies, a single goby was selected for observations. This individual was recognized by clear size difference or, in the absence of natural distinguishing features, by injection of a small subcutaneous elastomer marker (VIE, Northwest Marine Technology, Seattle, USA) on the caudal peduncle. Previous experiments have shown that the cleaning behaviour of *E. evelynae* is not affected by elastomer tagging (Whiteman & Côté 2002). No mortality was recorded following tagging in this study.

Behavioural observations were carried out during three different time periods: morning (06.00–08.00 h), midday (11.00–13.00 h) and evening (16.00–18.00 h). Each station was observed for seven 15-min sessions in each of the three time periods, with each session of a given station occurring on a different day. The order in which cleaning stations were observed each day was randomized. The total observation time was 57 h.

Observations were made by a SCUBA diver sitting 2–2.5 m away from a cleaning station and began after a 5-min delay to allow fish to become accustomed to diver presence. For each cleaning interaction, we recorded: client species, total inspection time (including interruptions in cleaning of less than 10 s if the cleaning

go by did not inspect another client during this time), whether the client posed or not, and the number of flinches made by the client. We assumed that a flinch indicated the removal of an ectoparasite and thus used flinch number as an index of biting rate.

### Client Fish Censuses

Client visits to cleaning stations are often related to client abundance on the reef (Kuwamura 1976; Grutter & Poulin 1998; Arnal et al. 2000). To quantify this relationship and remove any effect of client abundance, three 15-m long transects were placed haphazardly on the reef crest and spur-and-groove zones of the reef. The three reef crest transects were located parallel to shore whereas, owing to the narrowness of the spurs, the three transects in the spur-and-groove zone were placed perpendicular to shore. Individuals of all client fish species within 1 m on either side of the transects were recorded. Juvenile grunts, *Haemulon* spp., were combined for ease of recording because they typically occurred in mixed-species shoals and their identification may be difficult. A list of potential client species of *E. evelynae* at this site was obtained from Arnal & Côté (1998).

To investigate temporal patterns of abundance of clients on the study reef, all transects were run three times in a single day, coinciding with the behavioural observation periods described above. This initial survey revealed no significant effect of time of day on client species diversity (repeated-measures (RM)-ANOVA;  $F_{2,10} = 1.24$ ,  $p = 0.58$ ), total number of individual clients (RM-ANOVA;  $F_{2,10} = 0.45$ ,  $p = 0.65$ ), or number of individuals of a given species (RM-ANOVA with Greenhouse–Geisser adjustment for heterogeneity of covariance;  $F_{1,29,42,24} = 1.36$ ,  $p = 0.26$ ). All transects were therefore repeated four times, 4–7 d apart, at high tide, and their data were averaged to provide an index of client species abundance on the reef.

### Statistical Analyses

Ectoparasite availability derived from the benthic emergence traps was compared between day and night using a Wilcoxon signed rank test, matching diurnal and nocturnal catches of each trap at each sampling location. Temporal variation in ectoparasite availability derived from gnathiid abundance on fish clients was first examined by averaging the number of gnathiids recovered on each client species within each time period, and using a RM-ANOVA, with client species as subjects and time period as the within-subjects factor. We also investigated temporal patterns within each client species with non-parametric analyses of variance since raw ectoparasite numbers showed significantly skewed distributions.

Cleaning goby activity in relation to time of day was examined using RM-ANOVAS, in which individual cleaning gobies were used as subjects and time period as the within-subjects factor. Data from all client species were included, regardless of the number of interactions recorded per species, to obtain an accurate reflection of cleaning activity from the cleaners' perspective. For each

cleaning goby, mean length of inspection and mean biting rate (number of client finches per second of inspection) were calculated by pooling the seven observation sessions for each time period separately.

Finally, to examine the temporal pattern of client use of cleaning stations, data from all cleaning stations were pooled, and the total number of cleaning interactions for each client species was obtained for each time period. In this case, pooling across cleaning stations was justified because, although variation in clientele existed among cleaning stations owing to their proximity to specific clients, it was the rate of use of cleaners by clients rather than the specific identity of cleaning stations visited which was relevant in this analysis. A nominal abundance of 0.01 individuals  $m^{-2}$  was ascribed to client species using cleaning stations which were not recorded on the transects ( $n = 2$  cases). The numbers of cleaning interactions and proportions of interactions with incitation poses by clients were analyzed with RM-ANOVAS, with client species used as subjects and time period as the within-subjects factor. Clients which were seen fewer than five times at cleaning stations were omitted from the analysis of proportions of clients posing.

Throughout, when the data violated the assumption of homogeneity of covariance implicit in RM-ANOVAS (Zar 1999), the Greenhouse–Geisser adjustment of degrees of freedom was used in the calculation of the within-subjects effects.

## Results

### Ectoparasite Availability

Eight of the 32 trap-periods yielded gnathiids. Gnathiids were caught significantly more often in night than day trap-periods ( $\chi^2 = 4.5$ ,  $df = 1$ ,  $p = 0.03$ ; Fig. 1a). Moreover, at a given location, night trapping yielded significantly more gnathiid larvae than day trapping (Wilcoxon signed ranks test,  $Z = 2.11$ ,  $p = 0.035$ ; Fig. 1b).

Overall ectoparasite load on the five species of clients examined was low. The total number of parasites, including gnathiid isopod larvae, caligid copepods and monogeneans, varied from 0 to 4 per individual client. Gnathiid isopod larvae comprised 45% of all ectoparasites recovered, and gnathiid load varied from 0 to 3 per client. Overall, there was no relationship between gnathiid load and client body area ( $r_s = 0.05$ ,  $n = 71$ ,  $p = 0.66$ ) or total length ( $r_s = -0.17$ ,  $n = 71$ ,  $p = 0.17$ ). Neither measure of client size was therefore included as a covariate in subsequent analyses.

There were no differences in gnathiid numbers among the three time periods ( $\bar{x} \pm 1$  SD, morning:  $0.51 \pm 0.36$  gnathiids client $^{-1}$ , midday:  $0.23 \pm 0.25$ , afternoon:  $0.35 \pm 0.31$ ; RM-ANOVA with Greenhouse–Geisser adjustment,  $F_{1,06,4,25} = 1.63$ ,  $p = 0.27$ ). There was, however, a trend for higher total ectoparasite loads on clients in the morning than later in the day (morning:  $1.19 \pm 0.72$  ectoparasites client $^{-1}$ , midday:  $0.52 \pm 0.22$ , afternoon:  $0.84 \pm 0.47$ ; RM-ANOVA,  $F_{2,8} = 3.65$ ,  $p = 0.07$ ). When client species were considered individually, four of the five species harboured more ectoparasites in the morning

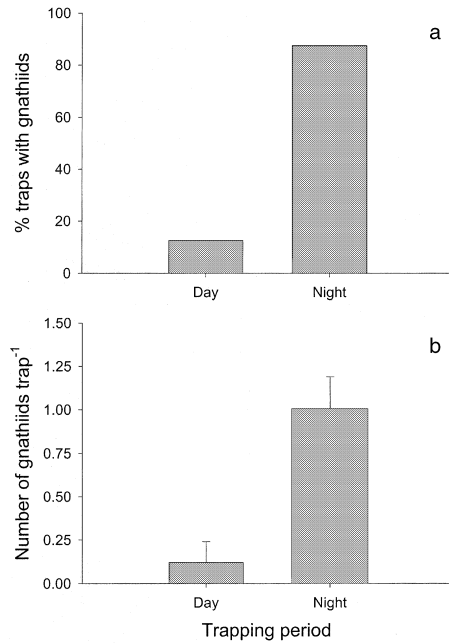


Fig. 1: (a) Percentage of emergence traps yielding gnathiids and (b) number of gnathiids per trap during daytime and night-time sampling on the study reef

(Table 1), although only smallmouth grunt showed a near-significant trend of temporal variability, with more gnathiids in the morning (Kruskal–Wallis test,  $\chi^2 = 4.88$ ,  $df = 2$ ,  $p = 0.09$ ).

### Cleaning Goby Activity

Cleaning goby cleaning activity was assessed by examining the length of inspection bouts and biting rate. The length of inspection bouts tended to vary through the day (RM-ANOVA,  $F_{2,20} = 3.87$ ,  $p = 0.06$ ). Inspection bouts were significantly longer in the morning than in the afternoon (RM-ANOVA, post hoc

Table 1: Total ectoparasite numbers on five client species in each of three daily time periods. Means are shown  $\pm 1$  SD. Sample sizes are given in parentheses

Client species	Morning	Noon	Afternoon
Brown chromis	0.80 $\pm$ 0.84 (5)	0.40 $\pm$ 0.55 (5)	0.40 $\pm$ 0.89 (5)
French grunt	0.40 $\pm$ 0.55 (5)	0.33 $\pm$ 0.82 (6)	1.00 $\pm$ 1.15 (4)
Smallmouth grunt	2.25 $\pm$ 0.96 (4)	0.75 $\pm$ 0.96 (4)	1.60 $\pm$ 0.89 (5)
Longfin damselfish	1.00 $\pm$ 1.22 (5)	0.80 $\pm$ 0.84 (5)	0.60 $\pm$ 0.89 (5)
Yellow goatfish	1.50 $\pm$ 1.64 (5)	0.33 $\pm$ 0.58 (3)	0.60 $\pm$ 0.89 (5)

test,  $p = 0.03$ ; Fig. 2), although there was no difference between morning and midday inspection bout length (RM-ANOVA, post hoc test,  $p = 0.16$ ; Fig. 2). By contrast, cleaning goby biting rate, as reflected by client finches, did not vary among the three time periods ( $\bar{x} \pm 1$  SD, morning:  $0.14 \pm 0.07$  finches per second of inspection, midday:  $0.15 \pm 0.10\%$ , afternoon:  $0.17 \pm 0.10\%$ ; RM-ANOVA,  $F_{2,20} = 0.43$ ,  $p = 0.65$ ).

### Client Use of Cleaning Stations

A total of 35 fish species were involved in 1428 cleaning interactions at the 11 focal cleaning stations. The commonest clients were yellow goatfish ( $n = 491$  visits, 34.4% of all visits) and brown chromis ( $n = 393$ , 27.5% of all visits). The overall number of cleaning interactions (combined across all cleaning stations and time periods) was significantly related to client abundance on the reef derived from transect data ( $r^2 = 0.30$ ,  $F_{1,33} = 14.4$ ,  $p < 0.001$ ), and this relationship also held for each time period separately ( $p < 0.05$  in all cases). Client abundance was therefore included as a covariate in the RM-ANOVA of interaction rates.

Nine of the 21 client species were recorded at cleaning stations most often in the morning, while 10 interacted with cleaners most often in the late afternoon. Only two species were recorded most frequently at midday ( $\chi^2 = 5.42$ ,  $df = 2$ ,  $p = 0.06$ ). This temporal pattern of client diversity at cleaning stations was largely mirrored by the pattern of client abundance recorded at cleaning stations. There were significant differences among time periods in the number of clients using cleaning stations (RM-ANOVA with Greenhouse–Geisser adjustment,  $F_{1,44,47.62} = 3.55$ ,  $p = 0.05$ ). Significantly higher rates of cleaning interactions were recorded in the afternoon than at midday (RM-ANOVA, post hoc test,  $p = 0.004$ ; Fig. 3a). Although the morning rate of interaction was also higher

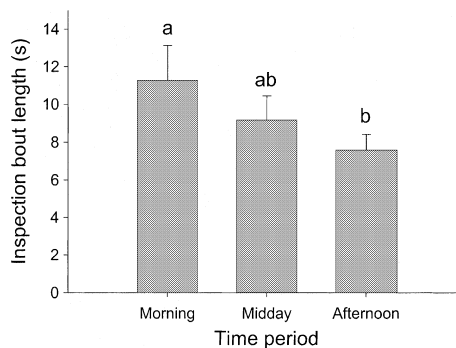


Fig. 2: Length of inspection bouts by cleaning gobies in each of three daily time periods. Estimated marginal means derived from a RM analysis of variance are presented  $+1$  SE. Bars with different superscripts are significantly different from each other ( $p < 0.05$ ).  $n = 11$  cleaning gobies in all cases

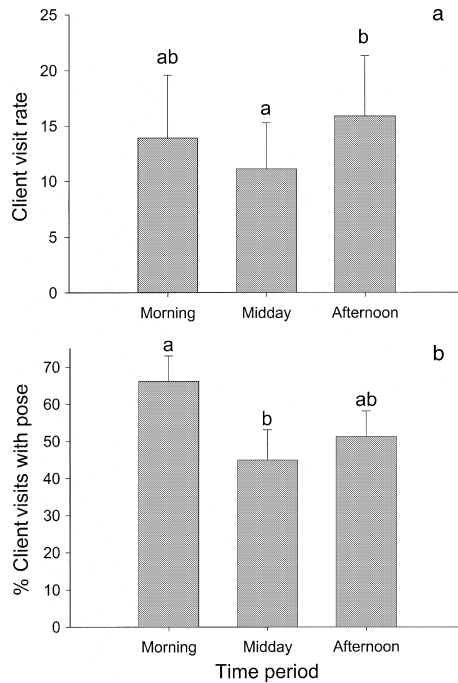


Fig. 3: (a) Rate of client visits (number of visits 15 min<sup>-1</sup>), combined for 11 focal cleaning stations (n = 35 client species), and (b) percentage of clients adopting an incitation pose during a visit in each of three daily time periods (n = 21 client species). Estimated marginal means derived from a RM analysis of variance are presented +1 SE. Bars with different superscripts are significantly different from each other (p < 0.05)

than at midday, this difference was not significant (RM-ANOVA, post hoc test, p = 0.33; Fig. 3a).

The proportion of clients exhibiting incitation poses in the morning tended to be greater than at midday or in the afternoon (RM-ANOVA,  $F_{2,36} = 2.80$ , p = 0.07; Fig. 3b). Fourteen client species were omitted from this analysis because they were recorded at cleaning stations fewer than five times.

### Discussion

In this study, we attempted to link temporal variability in behaviour of cleaning gobies and of their clients to concurrently measured ectoparasite availability. On our study reef, natural variation existed through the day in the total numbers of ectoparasites on clients, although little temporal variability was observed in numbers of gnathiids, the main prey of most cleanerfish. Most cleaning goby and client behaviours did not change as predicted with ectoparasite

availability, casting doubt on the role of ectoparasites as sole proximate cause of cleaning behaviour.

### **Patterns of Ectoparasite Availability**

We found a nocturnal bias in benthic emergence of gnathiids, which is consistent with patterns recorded elsewhere in the Caribbean (Chambers & Sikkel 2002), Australia (Jacoby & Greenwood 1988; but see Grutter et al. 2000), and California (Hobson & Chess 1976). Although our trap rates were low, they were not unusually so. Grutter et al. (2000), for example, usually collected between 0 and 6 gnathiids  $\text{m}^{-2} \text{12 h}^{-1}$  at Lizard Island on the Great Barrier Reef. Note, however, that night-time trapping in our study (from 18.00 to 06.00 h) encompassed dawn and dusk hours. Shorter sampling periods could have pinpointed more precisely the timing of gnathiid emergence. Chambers & Sikkel (2002), for example, have shown with 6-h sampling periods a peak in gnathiid emergence at dusk and dawn, rather than at night, in Puerto Rico and St. John, US Virgin Islands. Nevertheless, gnathiid emergence occurring primarily between dusk and dawn, combined with cleaning goby inactivity during this period, should lead to higher ectoparasite loads on client fish in the morning.

Although none of the five client species examined supported this prediction when considered individually, the overall mean number of gnathiids on clients was 50–120% higher in the morning than at midday or in the afternoon. These differences were not significant, probably owing to the small number of client species tested and thus limited statistical power. However, there was an overall trend ( $p = 0.07$ ) for higher total ectoparasite loads on clients in the morning than later in the day. Our results thus support previous studies of diel variability in fish ectoparasite infestation which showed significant morning peaks in Australia (Grutter 1999b; Grutter & Hendrikz 1999) and in the northern Caribbean (Sikkel et al. in press).

### **Temporal Variation in Cleaner and Client Behaviour**

Changes in both cleaning goby and client behaviour through the day were generally not correlated as predicted with variation in ectoparasite availability. The biting rate of cleaning gobies, measured as the number of perceptible flinches by clients, did not vary in relation to ectoparasites. Inspection bouts were significantly longer in the morning than in the afternoon, thus mirroring ectoparasite availability; however, equally long inspection times were also observed at midday, at a time of lower ectoparasite numbers on clients. Moreover, clients used cleaning stations at highest frequency in the afternoon, again at times of low ectoparasite infestation.

These results are consistent with the idea of hunger as a proximate cue for cleaner behaviour, as longer foraging bouts occurred in the morning after nocturnal fasting. If ectoparasite ingestion rates are low, owing to the generally low ectoparasite intensity at our study site (Sikkel et al. 2000; Arnal et al. 2001),

satiation could perhaps be expected to be reached as late as noon. In wild *L. dimidiatus*, satiation (as measured by fullness of gut) is reached approx. 3.5 h after the onset of cleaning in the morning, with inspection activity being much greater than in cleaning gobies (Grutter 1996).

Biting rate may also have been expected to increase in response to hunger (e.g. Grutter 1996). It is possible that the constant rate of biting observed through the day conceals a variable energetic intake which peaks in the morning if, for example, larger gnathiids are available in the morning (Grutter 1999b; Chambers & Sikkell 2002). It is also possible that the number of client finches we used as a proxy for cleaner biting rate may not represent the removal of ectoparasites. Client finches have been interpreted as resulting from dishonest foraging by *L. dimidiatus* that remove scales and tissue rather than ectoparasites (Bansemmer et al. 2002; Bshary & Grutter 2002). 'Cheating' on the part of cleaning gobies is possible, although their small size limits the scope for taking scales from most clients. Moreover, in *L. dimidiatus*, the incidence of cheating has been linked to low availability of ectoparasites (Bansemmer et al. 2002). If this holds for cleaning gobies, cheating should occur more frequently at midday and in the afternoon, when ectoparasite availability is lower, but this was not the case. Finally, if client finches represent cheating, cleaning bouts in which clients finch should be shorter than those during which finches were not observed, because cheating often leads to the termination of cleaning interactions (Bshary & Grutter 2002). In fact, there was no difference in cleaning bout length for interactions with and without finches, either when pairing cleaning bouts by cleaning station or by client species (paired t-tests,  $p > 0.10$  in all cases). Client finches in our study may therefore not represent dishonest cleaning.

The high rate of client use of cleaning stations we observed in the afternoon is not consistent with the idea of ectoparasites driving clients to seek cleaners, as ectoparasite loads were lower in the afternoon than in the morning. An alternative proximate cause for temporal variation in use of cleaning stations may be related to biases in the activity patterns of reef fish. Herbivores such as parrotfish, for example, often forage more actively from midday onward (Choat & Clements 1993) and could thus encounter cleaning stations more frequently later in the day. Many species providing parental care of eggs, such as damselfish, spawn early in the morning and thus decrease their morning use of cleaning stations during spawning periods (Cheney & Côté, in press). In addition, if predatory fish are more active in the morning, clients could shift their use of cleaning stations to later in the day according to risk of predation. Note, however, that other studies conducted on shallow tropical reefs have found morning or afternoon peaks in cleaning activity that coincide with peaks in ectoparasite availability. For example, the frequency of inspections by *L. dimidiatus*, which reflects rate of client visits, is highest in the morning (Grutter 1996) when gnathiid infestation rates are highest on clients (Grutter 1999b). Similarly, more clients were recorded at cleaning stations at dawn and dusk in Puerto Rico (Chambers & Sikkell 2002), which corresponds to peaks in client ectoparasite load (Sikkell et al. in press).

The temporal pattern of incitation poses by clients is more obviously linked to ectoparasites. Côté et al. (1998) have shown that the stereotyped incitation poses exhibited by some clients led to a significantly greater likelihood of being cleaned. Such poses should therefore be expected of clients with high ectoparasite loads. Indeed, we found that clients tended to adopt incitation poses more often in the morning than later in the day.

In conclusion, few cleaner and client behaviours changed as predicted in response to natural diurnal variation in ectoparasite availability. Although cleaning symbioses are generally becoming well established as mutualisms based on ectoparasite removal (Grutter 1999a; Cheney & Côté 2001; Grutter & Lester 2002), the proximate and ultimate causes of behaviour need not always coincide. Our study suggests that this may be the case for interactions between cleaning gobies and their clients in Barbados.

### Acknowledgements

We thank the director and staff of the Bellairs Research Institute for facilitating this study, Karen Cheney and Liz Whiteman for invaluable help in the field, Lexa Grutter for advice and encouragement, and Graham Kearns for help in identifying ectoparasites. We are grateful to John Reynolds for comments on the manuscript. PPM was supported by a Nuffield Undergraduate Summer Bursary.

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Received: July 3, 2002

Initial acceptance: October 10, 2002

Final acceptance: February 21, 2003 (M. Taborsky)

