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# Mutual visual signalling between the cleaner shrimp *Ancylomenes pedersoni* and its client fish

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Cleaner shrimp and their reef fish clients are an interspecific mutualistic interaction that is thought to be mediated by signals, and a useful system for studying the dynamics of interspecific signalling. To demonstrate signalling, one must show that purported signals at minimum (a) result in a consistent state change in the receiver and (b) contain reliable information about the sender's intrinsic state or future behaviour. Additionally, signals must be perceptible by receivers. Here, we document fundamental attributes of the signalling system between the cleaner shrimp *Ancylomenes pedersoni* and its clients. First, we use sequential analysis of *in situ* behavioural interactions to show that cleaner antenna whipping reliably predicts subsequent cleaning. If shrimp do not signal via antenna whipping, clients triple their likelihood of being cleaned by adopting darker coloration over a matter of seconds, consistent with dark colour change signalling that clients want cleaning. Using experimental manipulations, we found that visual stimuli are sufficient to elicit antenna whipping, and that shrimp are more likely to 'clean' dark than light visual stimuli. Lastly, we show that antenna whipping and colour change are perceptible when accounting for the intended receiver's visual acuity and spectral sensitivity, which differ markedly between cleaners and clients. Our results show that signalling by both cleaners and clients can initiate and mediate their mutualistic interaction.

## 1. Introduction

For a signalling system to arise and persist, the signal must, on average, increase the fitness of both the sender and the receiver, and be reliable [1–3]. A signal is reliable if some aspect of the signal (including presence/absence) correlates consistently with some attribute of the signaller (including future behaviour) [4]. Additionally, receiving the signal, or having information about the signaller's state, should result in a state change in the receiver that benefits the receiver [4,5]. In large part, work exploring signal reliability has focused on intraspecific signals, showing that signal reliability is maintained by genetic relatedness between signaller and receiver or by costs associated with signalling [4,6,7]. However, signals also mediate interspecific interactions, where genetic relatedness cannot explain signal reliability and where signallers and receivers may have vastly different sensory systems. Studying interspecific signalling systems offers an opportunity to test whether signal function and reliability arise in the absence of genetic relatedness, as well as to examine how senders and receivers with very different sensory capabilities may perceive and place selection pressure on signalling traits. Here, we use the interspecific mutualistic interactions between cleaner shrimp and their client fish to determine whether certain candidate signals reliably predict signaller intent.

The cleaner shrimp *Ancylomenes pedersoni* (Palaemonidae) and their reef fish clients are an example of a mutualistic system in which signalling probably occurs. *Ancylomenes pedersoni* live at 'cleaning stations' on tropical coral reefs from which they provide cleaning services to reef fish clients, removing ectoparasites and dead skin from their mouths, gills and scales [8]. The cleaner receives

a meal [8,9] and the client benefits from parasite removal [10]. Cleaning mutualisms provide a particularly promising system for studying interspecific signalling. First, signals that result in the mutualistic behaviour by definition have an average benefit for both sender and receiver [11]. Second, selection on signals that identify cleaners as beneficial partners rather than as food should be particularly intense, because misidentification by clients could result in the death of the cleaner [12]. Selection also may act on clients to identify themselves as seeking cleaning services, rather than a meal, because cleaners can choose not to clean clients.

Several behaviours observed in interactions between *A. pedersoni* and their clients have been proposed as signals. In particular, *A. pedersoni* have long white antennae that they often whip vigorously in the presence of clients. Numerous reports refer to this behaviour as a signal of intent to clean (e.g. [13–18]), but no studies have tested this interpretation. Many crustaceans also have chemosensory sensilla on the antennules (see [19]); so, alternatively, or in addition to being a signal, cleaners could whip their antenna to assess their olfactory environment. Additionally, client fish often rapidly change to darker coloration at cleaning stations [20–22]. Although some have suggested that colour change is a signal to cleaners (e.g. [23–25]), others have suggested it may be a physiological response to the act of being cleaned [26,27] or that it may serve to make parasites more visible to cleaners [23,28]. As with antenna whipping by cleaners, the signalling function of client colour change has not been rigorously investigated.

Here, we use several approaches to test whether antenna whipping and client colour change are reliable signals. First, we recorded *in situ* cleaner–client interactions and annotated the sequence of behaviours of both parties. We then used sequential analysis of the component behaviours to test whether the occurrence of one behaviour depends on a previous behaviour, as well as the probability of observing any particular sequence of behaviours [29]. We also used behavioural experiments in the laboratory to test whether shrimp whip their antennae in response to various visual stimuli in the absence of olfactory cues. We also observed whether shrimp that had antenna whipped then approached the visual stimulus, tapped it with their antennae, or climbed on it as if to clean.

Lastly, it is often implicitly assumed that a receiver can perceive a given signal. However, given the diversity of sensory capabilities across animals [30], it is important to account for the sensory abilities of a given receiver to test that the signal is perceptible. Here, we use information about colour vision and acuity in both cleaners and clients to modify still images and movies. These images show whether the spatial information available in the purported signals is perceptible by the relevant receiver visual system.

## 2. Material and methods

### (a) Field recordings

To record interactions between cleaner shrimp and client fish, we deployed GoPro cameras (Hero3+, GoPro Corporation, San Mateo CA, USA) at cleaning stations. Two cameras were deployed at each station: one attached to a dive weight rested on the substrate 17–30 cm from the station, and a second attached to a PVC pole that provided a top-down view from between 37 and 65 cm away. All videos were recorded on the

House Reef, CARMABI field station in Curaçao, the Netherlands Antilles (12°7' N, 68°5' W) during August 2015. Deployments occurred twice per day and lasted for 3–4 h, depending on camera battery life. The first deployment began at 07.00 and the other at 16.00, though *A. pedersoni* clean consistently throughout the day [17]. After deployments, divers left the area so that the cleaners and clients were undisturbed during filming, because diver presence can alter cleaner–client interactions [31]. In total, we recorded 133 h of footage at 10 cleaning stations ( $10.4 \pm 1.8$  h per station). Cleaning stations were between 5 and 8.5 m deep.

### (b) Video annotation

Two observers who had been trained to identify cleaner and client behaviours, but were naive to our research question, watched and annotated all footage using the annotation program BORIS [32]. This resulted in an ethogram of nine focal behaviours (table 1). The observers also noted all interactions, defined as when a client was visible in the camera's field of view for more than five seconds, and which began when clients entered and ended when clients exited the field of view of the camera. A third observer then annotated all interaction footage at half speed to create a final dataset of behavioural interactions. Clients were identified to species during each interaction (see electronic supplementary material, table S1). Interactions were initially identified using the top-down footage, and final annotations were created using the side view footage, which provided a closer view and thus allowed for more detailed observation. These final annotations were checked against the order of behaviours noted by the first two annotators. Differences were resolved by majority rule, though in no instances did all three observers disagree about the identity or order of behaviours. Repeat instances of whipping, posing, dark colour change and jolting were often seen, and so were allowed in our sequences.




All cleaning stations where footage was annotated housed 3–5 individual cleaners. Although we annotated the behaviours of all shrimp, we analysed data from only the first shrimp to either antenna whip or clean (whichever came first) during each interaction. This allowed us to isolate the influence of single pairwise cleaner–client interactions, without the secondary influence of additional cleaners. Additionally, we restricted our analyses to the subset of client species in which we observed colour change or for which we found published evidence of colour change ability (electronic supplementary material, table S2).

### (c) Sequential analysis

We analysed behavioural sequences from 199 interactions at eight cleaning stations (11–41 interactions per cleaning station); in total, we observed 18 individual shrimp and 10 species of client fish (electronic supplementary material, table S1). Sequences were analysed in two ways. First, we identified common strings of behaviours across the dataset and used the R [33] package *TraMineR* [34] to calculate the likelihood that a given series of behaviours occurred in a certain order. This allowed us to examine all behavioural sequences that lead to cleaning, as well as the probability that a shrimp that antenna whipped would clean later in the interaction, even if it was not the immediate next behaviour in a sequence.

Second, we examined sequences by focusing only on transitions between pairs of behaviours. We rearranged the data into a two-column format where the left column represented a behaviour and the right column represented the behaviour immediately following, reducing the behavioural sequences to their component transitions. Transitions were then summarized using an adjacency matrix [29], in which each row corresponds to an antecedent behaviour and each column to a consequent behaviour, with each cell representing the number of times each

**Table 1.** Focal client and cleaner behaviours. Icons correspond to those used in network figures.

party	behaviour	description
client	interaction begin	client fish enters the camera's field of view
	pose	client fish stops forward motion near a cleaning station, either resting on, or remaining still just above, the bottom; often accompanied by a flaring of the opercula and/or fins
	colour change (dark)	a rapid change to darker coloration, including barring/striping or full body colour change
		
	colour change (light)	a rapid change to lighter coloration
		
	jolt	a sudden twitching movement
	interaction end	client fish exits the camera's field of view
cleaner	antenna whipping	whipping of the antennae; usually accompanied by a body rocking motion
		
	clean begin	begin physical contact between cleaner and client
	clean end	end physical contact between cleaner and client

transition occurred across the entire dataset. We first created adjacency matrices for each cleaning station, to examine how the frequency of each behavioural transition varied across stations. Stations were highly consistent in which transition was the most common for each antecedent behaviour (see Results and electronic supplementary material, table S3); thus, data for all stations were combined for further analysis.

We analysed these transitions using the package *igraph* [35] in R. To test whether behaviours were followed by other behaviours more often than would be expected by chance, we created a permuted null distribution showing the frequency of each transition expected by chance and compared that distribution to our observed data (as in [36,37]). To do this, we permuted the second column of our two-column database of observed behavioural transitions 10 000 times, maintaining the relative frequency of each behaviour while randomizing the transitions between behaviours. For each of the 10 000 resampling iterations, we generated an adjacency matrix.

When permuting the transitions in our dataset, several temporally impossible transitions were created, including 'clean begin → clean begin', 'clean end → clean end', transitions where any behaviour comes before 'interaction begin' and transitions where any behaviour comes after 'interaction end'. Additionally, we disallowed 'colour change to light → colour change to light' because once an animal adopted light coloration, it was never observed to get even lighter immediately. By contrast, we did observe individuals transitioning to a darker mottled morph, and then later adopting fully dark coloration, meaning that 'colour change to dark → colour change to dark' was possible. This resulted in a set of 20 disallowed transitions. To account for this, after generating a resampled adjacency matrix, we replaced the disallowed transitions with 0 and divided the entire matrix by the total number of allowed transitions. Thus, each cell in the adjacency matrix represented the frequency of a given transition, normalized to the number of allowed transitions in the permuted dataset.

We then calculated a null distribution for each behavioural transition using the adjacency matrices from all resampling iterations; this represented a distribution of expected transition frequencies for each pair of behaviours if behavioural transitions occurred randomly, but with the relative frequency of each behaviour remaining constant. From the null distribution, we extracted the 99.92% quantile (the Bonferroni-corrected significance given that 61 allowed transitions were tested in parallel, although our results were qualitatively similar without the Bonferroni correction) for each transition and compared these quantiles to our observed values. Transitions in our observed dataset that occurred more frequently than their respective 99.92% null quantile were considered to have occurred significantly more often than expected by chance. We then used *igraph* to visualize the significant transitions as networks.

#### (d) Experiments with visual 'synthetic clients'

To test whether antenna whipping may be an olfactory sensing behaviour, we examined whether shrimp antenna whip in the absence of client olfactory cues. We presented shrimp with purely visual 'synthetic clients' in the laboratory. Shrimp were housed individually at Duke University (Durham, NC) on a 12 L:12 D cycle, in artificial seawater (23–24°C; salinity 28–31ppt) made from Instant Ocean (United PetGroup, Blacksburg, VA, USA). Animals were fed one stick of Crab Cuisine (Hikari, CA, USA) on the day before a trial.

We used an iPad mini 2 (Apple, Inc., Cupertino, CA, USA), which rested against the glass outside of the tank, to display synthetic clients to cleaners ( $n = 9$  individuals). At the beginning of the majority of trials, shrimp were resting on plastic perches 25 cm from the iPad. Synthetic clients were images that varied in shape (rectangles, circles or triangles), colour (black or white) and motion ('moving' stimuli, which moved across without stopping, and 'still' stimuli, which moved to the centre of the screen and stopped). Each stimulus covered the same total area,

was present on the screen for 20 s, and was displayed on the same grey background. In between each 20 s stimulus presentation, 2 min of grey background served as a control. Stimuli were presented to the shrimp in random order, and over the course of the experiment, each shrimp saw each stimulus between four and ten times, for a total of 1210 trials.

To test whether cleaners were responding to the spatial aspects of the stimulus or the change in ambient light that occurred when stimuli entered the screen, we designed stimuli where the entire image was either lighter or darker grey. This eliminated the spatial stimulus while maintaining the same change in ambient light. We then performed a second set of behavioural trials ( $n = 5$  individuals) where from the control grey screen, the screen would change to either the darker or the lighter shade for 20 s.

During trials, the tank was lit from overhead by an LED white light panel (Fotodiox Pro LED 312-DS, Fotodiox Inc., IL, USA) held at constant brightness and a correlated colour temperature of 5600 K. Trials were recorded using a GoPro camera that was placed at the side of the tank so that the observer was blind to stimulus identity. All footage was annotated using BORIS, and all instances of antenna whipping, moving towards the stimulus, tapping the stimulus with antennae or climbing on the stimulus were noted. To compare equal amounts of time for stimulus present and stimulus absent (control), we randomly extracted 20 s from each 2 min control clip for use in data analysis.

To statistically examine frequency of antenna whipping, we used the statistical software JMP Pro 13 (SAS Institute, Inc.). Shapiro–Wilk tests showed non-normality, and both Brown & Forsythe [38] and Levene [39] tests showed unequal variances, so we used an unequal variances (Welch's)  $t$ -test on ranked data to compare the mean tendencies of two groups [40], and a Welch's ANOVA to compare between more than two groups. Significance level was Bonferroni-corrected to 0.01 ( $n = 5$  comparisons).

### (e) Accounting for receiver vision

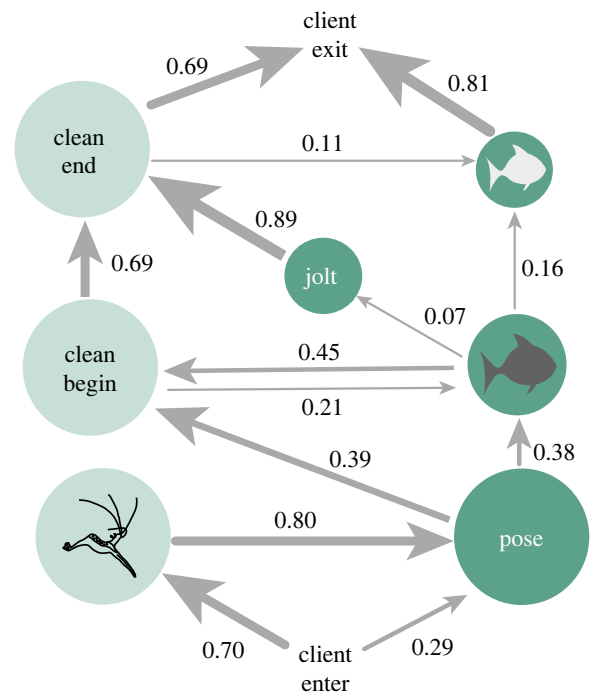
To examine whether spatial and colour information from signalling traits is available to the relevant receivers when accounting for receiver visual capabilities, we created still frames and videos of putative signals that were modified based on the receiver's acuity and colour vision. First, we used the R package *AcuityView* [41] to modify individual frames to display only the spatial information that is resolvable by a viewer, given the viewer's acuity and the distance to the object being viewed. We then modified the images based on receiver colour vision, where possible, and recompiled those frames into a video to examine whether signals are perceptible by signal receivers.

*Ancylomenes pedersoni* has low acuity ( $0.12$  cycles degree $^{-1}$ ) and monochromatic vision ( $\lambda_{\max} = 518$  nm [42]). Therefore, we approximated *A. pedersoni* colour vision by displaying only the green channel from the image. For client vision, we selected clients with both the lowest and highest known acuities for reef-dwelling fish reported in a recent review of fish acuity [43]. Most reef fish, especially those in the shallow waters where cleaners are found, have either tri- or tetra-chromatic colour vision [30,44]; therefore, we display images as the colours appear to humans, under the assumption that many client fish could see at least all of the colour information available to humans.

## 3. Results

### (a) Cleaning interactions are initiated by antenna whipping and/or colour change

A network of our complete dataset showed that, following client arrival at a cleaning station, there were only two



**Figure 1.** Network diagram of the significant behavioural transitions across 199 interactions between cleaner shrimp (light) and client fish (dark). Icons represent antennae whipping by shrimp, and colour change to dark and light by clients (table 1 caption). Network vertices (circles) represent behaviours and are scaled to represent the number of times that behaviour was observed. Transitional probabilities—the probability of progressing to a subsequent behaviour from a given current behaviour—between each behaviour are shown by numbers and the thickness of the network edges (arrows; thicker edges represent more common transitions). (Online version in colour.)

significant transitions away from client arrival. Thus, after a client arrived at a cleaning station, the immediate next behaviour was either antenna whipping by cleaners (probability = 0.70) or posing by clients (0.29) (figure 1; see electronic supplementary material, figure S1 for a complete network that includes transitions that were observed significantly less often than predicted by chance). Cleaning was observed 137 times, but only seven behavioural sequences lead to the initiation of cleaning (table 2). Of those, 95% of sequences pass through either antenna whipping, client colour change, or both.

### (b) Antenna whipping signals cleaner intent to clean

#### (i) Sequential analysis

Visualizing the behavioural sequences where antennae whipping did occur ( $n = 144$ ; figure 2a) and those where it did not ( $n = 55$ ; figure 2b) showed that, when antennae whipping occurred, the only statistically significant transition from antenna whipping was to client posing. This indicates that antenna whipping is followed by a consistent state change by the receiver. When antenna whipping occurred, it was immediately followed by client posing in 80% ( $n = 115$ ) of interactions. Additionally, in the 169 interactions where posing occurred, it was immediately preceded by antenna whipping in 73% ( $n = 119$ ) of interactions. In an additional four interactions, antenna whipping preceded posing, but not immediately, as cleaning began before the fish had

**Table 2.** Behavioural sequences that led to cleaning ( $n = 137$  interactions), and the probability of that sequence occurring. If a given behaviour occurred twice in immediate succession, it was collapsed to a single instance for clarity in the table.

sequence	$n$	probability
antenna whip → pose → clean begin	67	0.49
antenna whip → pose → colour change dark → clean begin	32	0.23
pose → colour change dark → clean begin	18	0.13
antenna whip → clean begin	9	0.07
pose → clean begin	7	0.05
pose → antenna whip → clean begin	3	0.02
antenna whip → colour change dark → pose → clean begin	1	0.01

adopted a completely still pose. Examining each station individually showed that the most common behaviour after antenna whipping was posing at 6 of 8 stations; at the remaining two, antenna whipping was most often followed by further antenna whipping, and then by posing (electronic supplementary material, table S3).

Antenna whipping also predicted cleaning behaviour by shrimp (i.e. the future behaviour of the signaller). In 82% of interactions ( $n = 112$ ) where cleaning occurred, the previous behaviour by that same shrimp was antenna whipping. Additionally, of 144 interactions in which we observed antenna whipping, it was ultimately followed by cleaning in 80% of interactions ( $n = 115$ ).

### (ii) Synthetic clients

Experiments using synthetic clients showed that a visual stimulus is sufficient to elicit antenna whipping behaviour; shrimp antenna whipped in a significantly higher proportion of trials when stimuli were present than when stimuli were absent ( $t_{11.3} = 4.0$ ,  $p = 0.002$ ; figure 3). However, not all visual stimuli elicit antenna whipping. Shrimp antenna whipped significantly more frequently in response to black than white stimuli ( $t_{12} = 4.5$ ,  $p = 0.0007$ ), but equally to moving and still stimuli ( $t_{14} = 0.49$ ,  $p = 0.63$ ), as well as to circles, rectangles and triangles ( $F_{16} = 0.60$ ,  $p = 0.56$ ) (figure 3a). Antenna whipping towards dark stimuli was not explained simply by the change in ambient light associated with black stimuli: shrimp antenna whipped in response to black stimuli significantly more often than to the screen darkening ( $t_{9.7} = 5.6$ ,  $p = 0.0003$ ) (figure 3).

If antenna whipping was only a way to sample the olfactory environment and assess whether clients were present or not, one would expect no further behaviour after antenna whipping, because the lack of olfactory cues should indicate the stimulus is not a client fish. However, we found that after antenna whipping, shrimp interacted further with visual stimuli in more than half of trials. Moving towards the stimulus occurred in the highest proportion of trials, with a median (interquartile range) of 0.41 (0.33–0.47), followed by climbing on the stimulus as

if to clean it (0.15, 0.05–0.30) and tapping on the stimulus with antennae (0.03, 0–0.05). Additionally, climbing on the stimulus was preceded by antenna whipping by the shrimp in a high proportion of trials, with a median (interquartile range) of 0.67 (0.63–0.74).

### (iii) Accounting for reef fish vision

Both static images (figure 4) and videos (electronic supplementary material, videos S1–S3) suggest that antenna whipping is visible to clients with low acuity from 10 cm (a distance previously cited as a standard interaction distance [45,46], and which aligns with our observations of cleaner–client interactions), and to fish with high acuity from at least 30 cm.

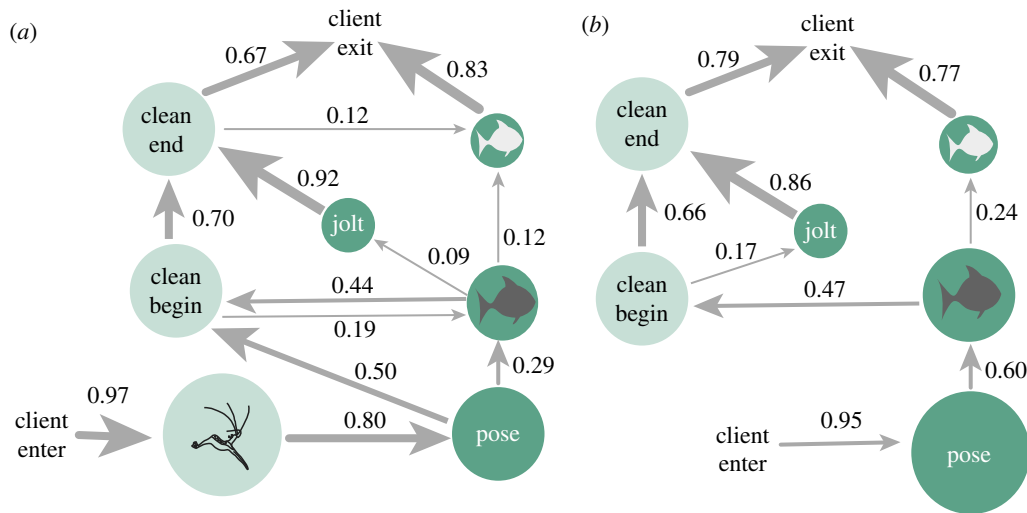
### (b) Colour change may signal client desire to be cleaned in the absence of antenna whipping

Given that antenna whipping by cleaners appears to indicate intent to clean, we examined whether clients can still induce cleaning in the absence of antenna whipping. Examining a network for the 55 interactions in which antenna whipping did not occur (figure 2b) shows that once clients pose, the only statistically significant path to cleaning is via colour change to dark. In these interactions, change to dark was followed by cleaning in 47% of interactions ( $n = 25$ ), and cleaning was preceded by a change to dark in 50% of interactions ( $n = 28$ ). In the absence of antenna whipping, clients that posed but did not colour change to dark had a 15% chance of being cleaned. Thus, changing to a dark morph more than tripled the likelihood of cleaning. Further, in our experiments with synthetic clients we found that shrimp climbed onto dark stimuli in a significantly higher proportion of trials (median, interquartile range; 0.94, 0.67–0.95) than light stimuli (0.06, 0.00–0.33;  $t_{15.7} = 10.5$ ,  $p < 0.0001$ ).

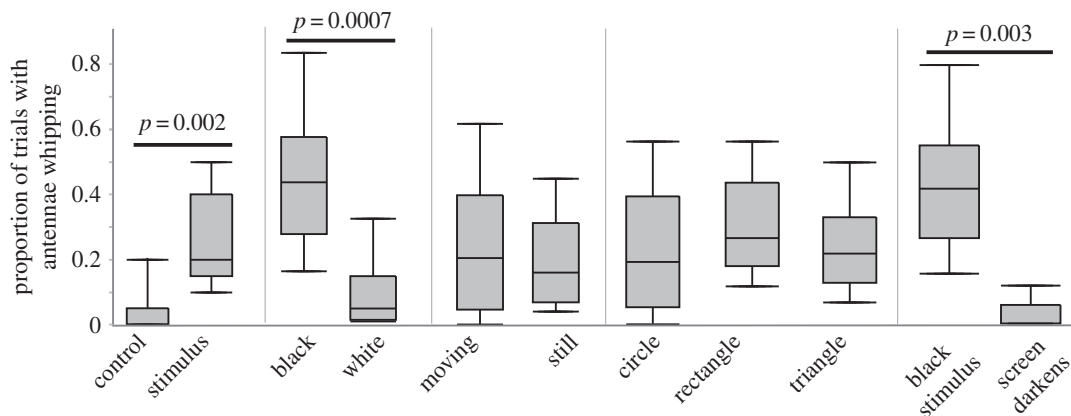
The order of colour change and cleaning that we observed suggests that colour change to a dark morph is not a response to the act of being cleaned. As can be seen in figures 1 and 2, and in electronic supplementary material, table S1, changing to a dark morph occurred before, or entirely without, physical contact by a cleaner shrimp in 74 of 110 cases (67%). Additionally, in 102 out of 137 cases, cleaning occurred without any colour change by the client, indicating that physical contact by cleaners is not sufficient to induce colour change. Thus, colour change to a dark morph does not appear to be an automatic response to presence at a cleaning station or to physical contact by shrimp. Lastly, despite having coarse, monochromatic vision, visual information about changes from light to dark is available to *A. pedersoni* viewers, especially given the short distances over which cleaners and clients interact (figure 5; electronic supplementary material, video S4).

## 4. Discussion

Taken together, the data presented here support the hypothesis that signalling mediates cleaning interactions. This study (1) is the first study of signalling in cleaner shrimp to use *in situ* behavioural observations in the absence of the confounding presence of human observers, (2) suggests that antenna whipping by *A. pedersoni* is a signal and (3) is the first



**Figure 2.** Network diagram of the significant transitions between behaviours across (a) 144 interactions in which antenna whipping by cleaner shrimp occurred and (b) 55 interactions in which antenna whipping by cleaner shrimp did not occur. Network parameters are described in figure 1. (Online version in colour.)



**Figure 3.** The frequency with which the cleaner shrimp *A. pedersoni* antenna whips in response to visual stimuli on an iPad screen. Boxes are quantile plots, showing the median (line), 25th and 75th quantiles (box) and minimum and maximum (whiskers). Significant differences are shown by bars with  $p$ -values; note that the Bonferroni-corrected significance level for five comparisons was 0.01. Statistics are from a Welch's  $t$ -test (or ANOVA in the case of three groups) of unequal variances. Sample size for all comparisons is nine individuals, except for the black stimulus versus screen darkens, where the sample size was five individuals.

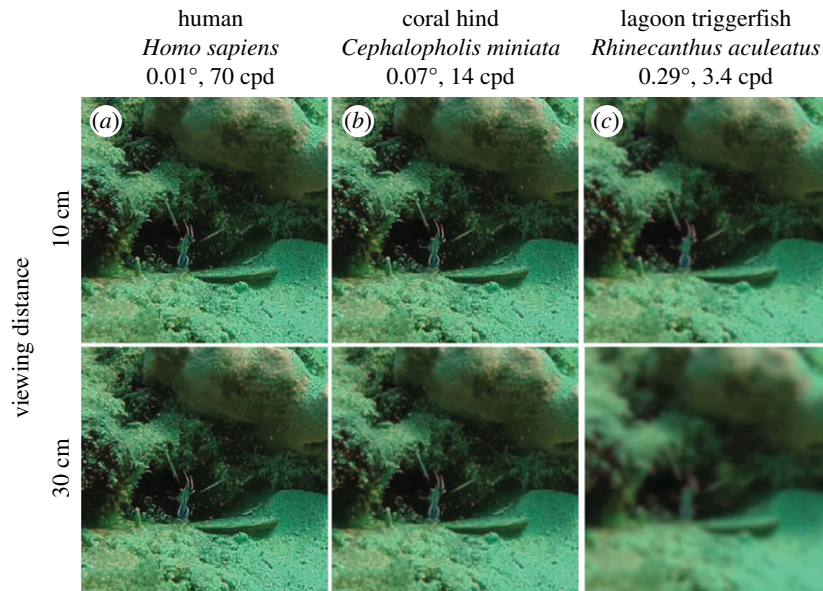
study of which we are aware to demonstrate a signalling role for client colour change.

### (a) Antenna whipping is a reliable signal of a cleaner's intent to clean

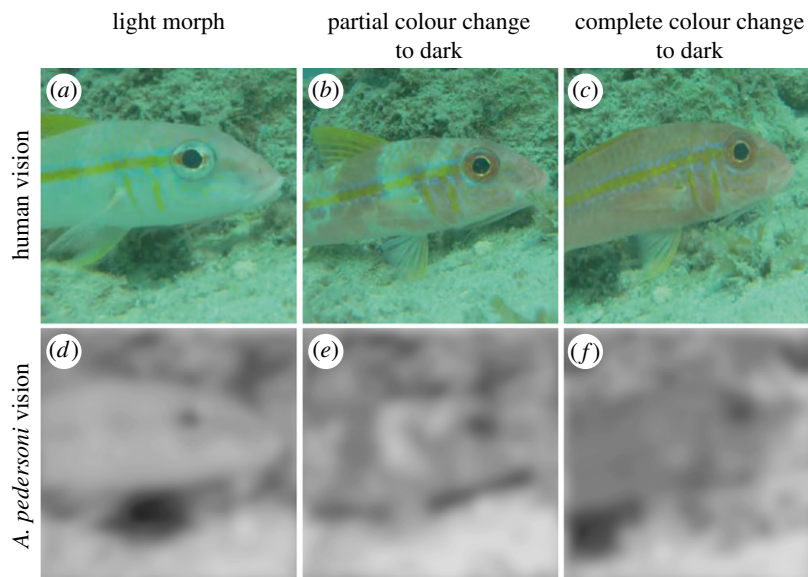
Antenna whipping was consistently associated with the outcome of the interaction (i.e. subsequent cleaning behaviour by that shrimp), indicating that a receiver could extract reliable information about a cleaner's intent to clean from the signal. Additionally, antenna whipping is consistently followed by a state change (adopting a cleaning solicitation pose) by the receiver, a behaviour which allows cleaning to begin and thus benefits the receiver. Currently, the balance of evidence from two other empirical studies of signalling in cleaner shrimp [45,46] aligns with the claim that cleaner shrimp signal to identify themselves as willing to clean, rather than, for example, to attract clients from long distances to cleaning stations. However, it has been shown that clients will pose for anemones where *A. pedersoni* is not visible [13], and we viewed some interactions where clients posed without any

signalling; thus, antenna whipping is not a necessary precursor to posing. Whether signalling modifies client posing behaviour, perhaps causing them to pose for longer or reinforcing that a given cleaning station is still active and thus resulting in repeated visits by the same client, has not yet been studied.

Several lines of evidence support the hypothesis that cleaner signalling traits have been evolutionarily modified for signalling. First, it has been noted in scientific and hobby literature that the antennae of *A. pedersoni* are longer and whiter than those of related non-cleaning species [14,15,47], suggesting some morphological modification of antennae in *A. pedersoni* for signalling. Second, studies of the congener *A. longicarpus* have shown that this species signals with slender white chelae [46], a signal similar in form to *A. pedersoni*, but using a different body part. Third, the cleaner shrimps *A. longicarpus* and *Urocaridella sp. C* both modify their signalling (chelae clapping and body rocking, respectively) based on hunger level [9,45,46], and when given a choice between a hungry and a satiated cleaner, client fish more often choose to be cleaned by the hungry cleaner [45], showing that clients respond to the modified signal.



**Figure 4.** Images of *A. pedersoni*, for humans (a) and two reef fish at the high (b) and low (c) ends of known reef fish acuity. Based on the size of the shrimp, the image was assumed to be 5 cm across. Column labels show minimum resolvable angle in degrees and acuity in cycles per degree (cpd).



**Figure 5.** As a representative example, the yellow goatfish *Mulloidichthys martinicus* in light (a,d), partial dark (b,e) or complete dark coloration (c,f), as seen by a human (a–c) or *A. pedersoni* (d–f). We assumed the client was 10 cm from the cleaner, a standard interaction distance [45,46], and that the portion of the fish being viewed is 7 cm long.

### (b) Colour change as a signal by client fish

Our results also suggest that client colour change to a darker morph may signal that a client wants to be cleaned. Clients who changed colour to a darker morph roughly tripled their chances of being cleaned when cleaners had not already signalled intent to clean, colour change is perceptible by cleaner shrimp, and in laboratory manipulations cleaners preferentially tried to clean dark stimuli over light stimuli. As further evidence, when we observed clients change back to a light morph ( $n = 41$ ), it was followed either by departing the cleaning station (probability of 0.83) or by cleaning ending (0.17). Thus, clients may adopt a dark coloration as a signal before or during cleaning and switch back to light coloration only as cleaning ends. Ours is the first study, to our knowledge, to demonstrate any purported signalling function for client colour change by quantifying how it affects cleaner behaviour.

Our study focused on species of client fish with observed or previously recorded colour changing abilities. There are, however, client species that we never observed changing colour and for which we could find no evidence of colour change ability in the literature (electronic supplementary material, table S2), but these species also received cleaning services. Although it has been shown that cleaner fish prefer client species with higher average ectoparasite loads [48], the role that client signalling might play in whether some clients are preferred has not been examined.

Whether colour change has been modified in some way for a signalling function has not been studied. Numerous fishes colour change for mating, aggression or for camouflage [49], so a colour change signal may have been exapted from colour change in a different behavioural context. Lastly, behavioural sequences showed that colour change to dark is



not a response to cleaning or posing, and that not all clients change colour in the presence of cleaners; this is in line with the hypothesis that colour change is a signal to solicit cleaning. The conditions under which individuals choose to colour change deserve further study.

## 5. Conclusion

Our study suggests that signalling, primarily by the cleaner but secondarily by the client, is important to initiate cleaning. In only 4 of 199 interactions did cleaning result when neither antenna whipping nor colour change occurred. These findings have implications for understanding how cleaning mutualisms are mediated and maintained. Cleaners and clients are an intriguing and accessible system for studying interspecific, communication-style exchanges of signals, in which reliability appears to have arisen as a result of shared interests between parties and not genetic relatedness between sender and receiver. Finally, mutualisms are a promising area for exploring the selection pressures that receivers can place on senders when the two parties have very different

sensory capabilities. As shown here, differences in sensory systems between species can sometimes result in signals, such as antenna whipping, that the producer probably cannot resolve, but which are directed solely at partner species.

**Data accessibility.** Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.4b4q7nq> [50].

**Authors' contributions.** E.M.C. carried out fieldwork, annotated behavioural videos, performed sequential and network analysis and drafted the manuscript; P.A.G. assisted with fieldwork and the statistical aspects of sequential and network analysis, and gave feedback on the manuscript; S.J. participated in the design of the study and gave feedback on the manuscript.

**Competing interests.** We declare we have no competing interests.

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## References

- Kokko H. 1997 Evolutionarily stable strategies of age-dependent sexual advertisement. *Behav. Ecol. Sociobiol.* **41**, 99–107. (doi:10.1007/s002650050369)
- Otte D. 1974 Effects and functions in the evolution of signaling systems. *Annu. Rev. Ecol. Syst.* **5**, 385–417. (doi:10.1146/annurev.es.05.110174.002125)
- Johnstone RA, Grafen A. 1993 Dishonesty and the handicap principle. *Anim. Behav.* **46**, 759–764. (doi:10.1006/anbe.1993.1253)
- Searcy WA, Nowicki S. 2005 *The evolution of animal communication*. Princeton, NJ: Princeton University Press.
- Vehrencamp SL, Hall ML, Bohman ER, Depeine CD, Dalziel AH. 2007 Song matching, overlapping, and switching in the banded wren: the sender's perspective. *Behav. Ecol.* **18**, 849–859. (doi:10.1093/beheco/arm054)
- Guilford T, Dawkins MS. 1995 What are conventional signals? *Anim. Behav.* **49**, 1689–1695. (doi:10.1016/0003-3472(95)90090-X)
- Vehrencamp SL. 2000 Handicap, index, and conventional signal elements of bird song. In *Animal signals: signalling and signal design in animal communication* (eds Y Espmark, T Amundsen, G Rosenqvist), pp. 277–300. Trondheim, Norway: Tapir Academic Press.
- Becker JHA, Grutter AS. 2004 Cleaner shrimp do clean. *Coral Reefs* **23**, 515–520. (doi:10.1007/s00338-004-0429-3)
- Becker JHA, Grutter AS. 2005 Client fish ectoparasite loads and cleaner shrimp *Urocaridella* sp. C hunger levels affect cleaning behaviour. *Anim. Behav.* **70**, 991–996. (doi:10.1016/j.anbehav.2005.01.004)
- Bshary R, Oliveira RF, Oliveira TS, Canário AV. 2007 Do cleaning organisms reduce the stress response of client reef fish? *Front. Zool.* **4**, 21. (doi:10.1186/1742-9994-4-21)
- Boucher DH, James S, Keeler KH. 1982 The ecology of mutualism. *Annu. Rev. Ecol. Syst.* **13**, 315–347. (doi:10.1146/annurev.es.13.110182.001531)
- Hasson O. 1994 Cheating signals. *J. Theor. Biol.* **167**, 223–238. (doi:10.1006/jtbi.1994.1065)
- Huebner LK, Chadwick NE. 2012 Reef fishes use sea anemones as visual cues for cleaning interactions with shrimp. *J. Exp. Mar. Bio. Ecol.* **416–417**, 237–242. (doi:10.1016/j.jembe.2012.01.004)
- Limbaugh C, Pederson H, Chace FA. 1961 Shrimps that clean fishes. *Bull. Mar. Sci. Gulf Caribb.* **11**, 237–257.
- Ellis GR. 1985 Cleaner Shrimp. *Freshw. Mar. Aquarium* **8**, 83–86.
- Mahnken C. 1972 Observations on cleaner shrimps of the genus *Periclimenes*. *Bull. Nat. Hist. Museum Los Angeles Cty. Sci.* **71**, 71–83.
- Titus BM, Daly M, Exton DA. 2015 Temporal patterns of Pederson shrimp (*Ancylomenes pedersoni* Chace 1958) cleaning interactions on Caribbean coral reefs. *Mar. Biol.* **162**, 1651–1664. (doi:10.1007/s00227-015-2703-z)
- Johnsson M. 1987 Fish cleaning behaviour of shrimp. *J. Zool. Lond.* **213**, 117–131. (doi:10.1111/j.1469-7998.1987.tb03682.x)
- Mead K, Koehl M. 2000 Stomatopod antennule design: the asymmetry, sampling efficiency and ontogeny of olfactory flicking. *J. Exp. Biol.* **203**, 3795–3808.
- Côté IM. 2000 Evolution and ecology of cleaning symbioses in the sea. *Oceanogr. Mar. Biol.* **38**, 311–355.
- Côté IM, Arnal C, Reynolds JD. 1998 Variation in posing behaviour among fish species visiting cleaning stations. *J. Fish Biol.* **53**, 256–266. (doi:10.1111/j.1095-8649.1998.tb01031.x)
- Losey GS. 1972 Behavioural ecology of the 'cleaning fish'. *Aust. Nat. Hist.* **17**, 232–238.
- Trivers RL. 1971 The evolution of reciprocal altruism. *Q. Rev. Biol.* **46**, 35–57. (doi:10.1086/406755)
- Feder HM. 1966 Cleaning symbiosis in the marine environment. In *Symbiosis I: associations of microorganisms, plants, and other organisms* (ed. SM Henry), pp. 327–380. New York, NY: Academic Press.
- Randall JE. 1962 Fish service stations. *Sea Front.* **8**, 40–47.
- Hobson ES. 1965 Diurnal-nocturnal activity of some inshore fishes in the Gulf of California. *Copeia* **3**, 291–302. (doi:10.2307/1440790)
- Hobson ES. 1971 Cleaning symbiosis among California inshore fishes. *Fish. Bull.* **69**, 491–523.
- Vaughan DB, Grutter AS, Costello MJ, Hutson KS. 2017 Cleaner fishes and shrimp diversity and a re-evaluation of cleaning symbioses. *Fish. Fish.* **18**, 698–716. (doi:10.1111/faf.12198)
- Castellan NJ. 1979 The analysis of behavior sequences. In *The analysis of social interactions: methods, issues, and illustrations* (ed. RB Cairns), pp. 81–118. Hillsdale, NJ: Lawrence Erlbaum Associates.
- Cronin TW, Johnsen S, Marshall NJ, Warrant EJ. 2014 *Visual ecology*. Princeton, NJ: Princeton University Press.
- Titus BM, Daly M, Exton DA. 2015 Do reef fish habituate to diver presence? Evidence from two reef sites with contrasting historical levels of SCUBA intensity in the Bay Islands, Honduras. *PLoS ONE* **10**, e0119645. (doi:10.1371/journal.pone.0119645)

32. Friard O, Gamba M. 2016 BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* **7**, 1325–1330. (doi:10.1111/2041-210X.12584)
33. R Development Core Team. 2016 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See <https://www.R-project.org/>.
34. Gabadinho A, Ritschard G, Müller NS, Studer M. 2011 Analyzing and visualizing state sequences in R with TraMineR. *J. Stat. Softw.* **40**, 1–37. (doi:10.18637/jss.v040.i04)
35. Csardi G, Nepusz T. 2006 The igraph software package for complex network research. *InterJournal Complex Syst.* **1695**.
36. Green PA, Patek SN. 2018 Mutual assessment during ritualized fighting in mantis shrimp (Stomatopoda). *Proc. R. Soc. B* **285**, 20172542. (doi:10.1098/rspb.2017.2542)
37. Bakeman R, Robinson BF, Quera V. 1996 Testing sequential association: estimating exact p values using sampled permutations. *Psychol. Methods* **1**, 4–15. (doi:10.1037/1082-989X.1.1.4)
38. Brown MB, Forsythe AB. 1974 Robust tests for the equality of variances. *J. Am. Stat. Assoc.* **69**, 364–367. (doi:10.1080/01621459.1974.10482955)
39. Levene H. 1960 Robust tests for the equality of variances. In *Contributions to probability and statistics* (ed. I Olkin), pp. 278–292. Palo Alto, CA: Stanford University Press.
40. Ruxton GD. 2006 The unequal variance t-test is an underused alternative to Student's t-test and the Mann-Whitney U test. *Behav. Ecol.* **17**, 688–690. (doi:10.1093/beheco/ark016)
41. Caves EM, Johnsen S. 2017 AcuityView: an R package for portraying the effects of visual acuity on scenes observed by an animal. *Methods Ecol. Evol.* **9**, 793–797. (doi:10.1111/2041-210X.12911)
42. Caves EM, Frank TM, Johnsen S. 2016 Spectral sensitivity, spatial resolution, and temporal resolution and their implications for conspecific signalling in cleaner shrimp. *J. Exp. Biol.* **219**, 597–608. (doi:10.1242/jeb.122275)
43. Caves EM, Sutton TT, Johnsen S. 2017 Visual acuity in ray-finned fishes correlates with eye size and habitat. *J. Exp. Biol.* **220**, 1586–1596. (doi:10.1242/jeb.151183)
44. Marshall J, Carleton KL, Cronin T. 2015 Colour vision in marine organisms. *Curr. Opin. Neurobiol.* **34**, 86–94. (doi:10.1016/j.conb.2015.02.002)
45. Becker JHA, Curtis LM, Grutter AS. 2005 Cleaner shrimp use a rocking dance to advertise cleaning service to clients. *Curr. Biol.* **15**, 760–764. (doi:10.1016/j.cub.2005.02.067)
46. Chapuis L, Bshary R. 2010 Signalling by the cleaner shrimp *Periclimenes longicarpus*. *Anim. Behav.* **79**, 645–647. (doi:10.1016/j.anbehav.2009.12.012)
47. Wicksten MK. 1995 Within-species variation in *Periclimenes yucatanicus* (Ives), with taxonomic remarks on *P. pedersoni* Chace (Crustacea: Decapoda: Caridea: Palaemonidae). *Proc. Biol. Soc. Washingt.* **108**, 458–464.
48. Arnal C, Côté IM, Sasal P, Morand S. 2000 Cleaner-client interactions on a Caribbean reef: influence of correlates of parasitism. *Behav. Ecol. Sociobiol.* **47**, 353–358. (doi:10.1007/s002650050676)
49. Townsend C. 1929 Records of changes in color among fishes. *Zoologica* **IX**, 321–378.
50. Caves EM, Green PA, Johnsen S. 2018 Data from: Mutual visual signalling between the cleaner shrimp *Ancylomenes pedersoni* and its client fish. Dryad Digital Repository. (doi:10.5061/dryad.4b4q7nq)