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RESEARCH ARTICLE

Cleaner fish are potential super-spreaders

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ABSTRACT

Cleaning symbiosis is critical for maintaining healthy biological communities in tropical marine ecosystems. However, potential negative impacts of mutualism, such as the transmission of pathogens and parasites during cleaning interactions, have rarely been evaluated. Here, we investigated whether the dedicated bluestreak cleaner wrasse, *Labroides dimidiatus*, is susceptible to and can transmit generalist ectoparasites between client fish. In laboratory experiments, *L. dimidiatus* were exposed to infective stages of three generalist ectoparasite species with contrasting life histories. *Labroides dimidiatus* were susceptible to infection by the gnathiid isopod *Gnathia aureamaculosa*, but were significantly less susceptible to the ciliate protozoan *Cryptocaryon irritans* and the monogenean flatworm *Neobenedenia girellae*, compared with control host species (*Coris batuensis* or *Lates calcarifer*). The potential for parasite transmission from a client fish to the cleaner fish was simulated using experimentally transplanted mobile adult (i.e. egg-producing) monogenean flatworms on *L. dimidiatus*. Parasites remained attached to cleaners for an average of 2 days, during which parasite egg production continued, but was reduced compared with that on control fish. Over this timespan, a wild cleaner may engage in several thousand cleaning interactions, providing numerous opportunities for mobile parasites to exploit cleaners as vectors. Our study provides the first experimental evidence that *L. dimidiatus* exhibits resistance to infective stages of some parasites yet has the potential to temporarily transport adult parasites. We propose that some parasites that evade being eaten by cleaner fish could exploit cleaning interactions as a mechanism for transmission and spread.

KEY WORDS: Parasite, Transmission, Cleaning symbiosis

INTRODUCTION

Cleaning symbiosis is one of the most emblematic mutualistic relationships in marine environments. Hundreds of reef fish species have been reported as cleaners or clients, making cleaning interactions widespread in both tropical and temperate reef systems (Quimbayo et al., 2021). In cleaning interactions, cleaner fish remove and ingest parasites from the body surface of other fish, called 'clients' (Côté, 2000). Cleaner fish may clean throughout their entire lifespan, using cleaning as their primary way of acquiring food (dedicated cleaners), or engage in cleaning interactions predominantly as juveniles or as a secondary source of food (facultative cleaners; Vaughan et al., 2017). Research on cleaner fish has often highlighted the benefits of cleaners to the health of their clients, and associated biological communities (e.g. Clague et al., 2011; Waldie et al., 2011; Sun et al., 2015; Binning et al., 2018; Ros et al., 2020) through parasite removal (Grutter, 1999; Arnal and Morand, 2001; Narvaez et al., 2015), aiding the healing process of injuries and wounds (Foster, 1985; Vaughan et al., 2018a; Grutter et al., 2020a), and decreasing the stress level of fish (Soares et al., 2011).

However, costs are also associated with cleaning symbiosis. Cheating behaviour by cleaner fishes, for example, introduces costs that may negatively impact their clients (e.g. Poulin and Vickery, 1995; Soares et al., 2014; Truskanov et al., 2020). Some cleaner fish, such as the bluestreak cleaner wrasse, *Labroides dimidiatus*, prefer to eat client fish mucus than parasites, probably because of the higher nutritional value of mucus (Arnal and Morand, 2001; Eckes et al., 2015). As this mucus loss is detrimental to the client (Grutter and Bshary, 2004), cheating by the cleaners often results in the client terminating the cleaning interaction by jolting and swimming off, or actively chasing the cleaner fish (Bshary and Grutter, 2002). Cleaner fish can also be preyed upon by their clients, making engagement in the interaction a potentially risky business (e.g. Francini-Filho et al., 2000; Messias and Soares, 2015).

Another cost that has only more recently been considered is the potential for parasite transmission between cleaners and clients (Narvaez et al., 2021a). This cost has been exposed in fish farms in northern Europe, where cleaner wrasses are used as a biocontrol to remove sea lice in salmon fish farms (Erkinharju et al., 2021). Following the introduction of cleaner wrasses in salmon farming in the early 1990s, cleaners have been found to acquire parasites and other pathogens from the salmon they were intended to clean (Treasurer, 1997; Treasurer, 2012). This has exposed the possibility that cleaners could potentially act as a reservoir and vector of parasites for salmon (for review, see Erkinharju et al., 2021). Disease transmission by cleaners in the wild, however, has been poorly studied. Only one recent study has highlighted the potential for a wild cleaner fish (the cleaner goby *Elacatinus prochilos* in the Caribbean) to share potentially harmful skin bacteria with their clients and vice versa (Xavier et al., 2019). The role of cleaner fish in the transmission of parasites has yet to be evaluated and presents a critical knowledge gap on whether parasite transmission is a potential cost associated with cleaning symbiosis in the wild.

The aim of this study was to investigate whether the dedicated cleaner wrasse *L. dimidiatus* is susceptible to, and can transmit, generalist ectoparasites potentially associated with their clients. This species was selected because each individual cleans several thousand clients a day (Grutter, 1996a), their clients have a high abundance and diversity of parasites (Grutter, 1994, 1996b), and

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they may themselves host parasites (Narvaez et al., 2021b). Here, individual *L. dimidiatus* and control fish species were exposed to infective stages of three generalist ectoparasite species with contrasting life histories: the gnathiid isopod *Gnathia aureamaculosa*, the ciliate protozoan *Cryptocaryon irritans*, and the monogenean flatworm *Neobenedenia girellae*. These three ectoparasites are known for their low host specificity (Ferreira et al., 2009; Li et al., 2022; Whittington and Horton, 1996, respectively), cosmopolitan distribution (Ferreira et al., 2009; Diggles and Adlard, 1997; Brazenor et al., 2018, respectively) and pathological effects (Smit and Davies, 2004; Colorni and Burgess, 1997; Trujillo-González et al., 2015a). Potential for transmission from a client fish to the cleaner fish was then simulated using experimentally transplanted mobile adult (i.e. egg-producing) *N. girellae* on *L. dimidiatus.* Based on the parasite community of wild *L. dimidiatus* (Narvaez et al., 2021b), we hypothesised that this species would be susceptible to the three generalist parasites tested here. Furthermore, given the frequent, repeated and close contact between cleaner fish and their clients, we propose that the mobile *N. girellae*, which can crawl along the surface of their host using specialised attachment organs, may be transmitted from clients to cleaners and vice versa. We hypothesised that *N. girellae* would remain attached to cleaners for a duration that would permit transmission to a new client.

MATERIALS AND METHODS

Ethics statement

The experiments were done in accordance with the James Cook University Animal Ethics guidelines (Approval A2558 and A2457).

Fish acquisition and husbandry

The dedicated cleaner wrasse *Labroides dimidiatus* (Valenciennes 1839) was used as the study species, given it is one of the most geographically widespread, conspicuous and active marine cleaners (Côté and Cheney, 2004; Cheney and Côté, 2005; www.fishbase.org, version 08/2021). Susceptibility experiments were performed with the gnathiid isopod species *Gnathia aureamaculosa* at Lizard Island Research Station (Great Barrier Reef, Australia). Twenty *L. dimidiatus* (mean±s.d. total length, TL 73.6±1.34 mm) and 20 Batu rainbow-wrasse *Coris batuensis* (Bleeker 1856) (control species, TL 78.6±1.61 mm) were collected using SCUBA diving on reefs around Lizard Island in January 2020 with barrier- and hand-nets. For this experiment, *C. batuensis* was used as a positive control because it has been previously found to be infected with gnathiid parasites at the study location and is of similar size to *L. dimidiatus* (Muñoz and Cribb, 2006; Muñoz et al., 2007). The fish were acclimatised and quarantined for 1 week prior to experiments. Cleaner fish were held in 32 l tanks (dimensions: 39×28×30 cm L×W×H) individually or in pairs. Individuals of *C. batuensis* were held in two 300 l round plastic tanks (dimensions: 1.0 m diameter×0.4 m deep). These tanks were connected to a flow-through aquarium system, with water directly pumped from the adjacent reef via a holding tank. Varying sizes of PVC plastic tubes were introduced into the tanks as shelters and fish were fed twice daily with frozen *Mysis* sp. shrimp. The seawater temperature was 30.4±0.12°C (mean±s.d.) during the experiment (i.e. the same water temperature as the adjacent reef).

We performed the susceptibility experiments with the protozoan ciliate *Cryptocaryon irritans* and the monogenean flatworm *Neobenedenia girellae* at James Cook University (Townsville, QLD, Australia). Twenty adult *L. dimidiatus* (TL 65.55±2.19 mm) were purchased from a commercial ornamental fish supplier (Cairns Marine) in April 2019. Twenty barramundi, *Lates calcarifer* (Bloch 1790) (TL 169.35±4.04 mm) (Latidae) were also purchased from an aquaculture supplier (Spring Creek Barramundi farm) in February 2019. For these two experiments, *L. calcarifer* were used as a positive control, because this species is known to be susceptible to *N. girellae* and *C. irritans* under laboratory conditions (Skilton et al., 2020) and is routinely used as a host for *in vivo* parasite cultures in the Marine Parasitology Laboratory at James Cook University. On arrival, *L. dimidiatus* were quarantined for 2 weeks in separate tanks (dimensions: 22×14×13 cm L×W×H; 4 l) and in two recirculating systems and monitored for clinical signs of disease. Water parameters were monitored daily (salinity, pH, ammonia, nitrate, nitrite and temperature), with temperature and salinity maintained, respectively, at 26°C and 35 ppt. Each tank contained one PVC plastic tube as a shelter. Fish were fed daily to satiation with *Mysis* sp. shrimp. *Lates calcarifer* were housed in a freshwater tank (dimensions: 63×37×45 cm L×W×H; 100 l) and 2/3 of the water was renewed once a day. They were fed with commercial pellets specifically formulated for *L. calcarifer* (Ridley Aquafeed – Marine Float Range). Artificial lights were set on a 12 h day/night light regime. The two recirculating systems used were built following Vaughan et al. (2018b), where the seawater was recirculated through an algae scrubber containing *Caulerpa taxifolia* for nitrate export. The sump contained bio balls, used for the growth of beneficial bacteria, a protein skimmer and a seawater UV treatment system.

After fish collection or purchase, fish were first introduced into a dechlorinated freshwater bath for 5 min to kill and remove any potential ectoparasites.

Parasite cultures

The marine isopod gnathiid *G. aureamaculosa* was cultured continuously at Lizard Island Research Station from 2001 until 2020 (Nagel and Grutter, 2007; Grutter et al., 2020b). Gnathiids are parasitic during their three larval stages before becoming adults, when they then aggregate on the bottom to reproduce (Tanaka, 2007). In between each larval stage, gnathiids also return to the bottom to moult to the next stage (Tanaka, 2007). Therefore, suitable benthic habitats such as algal turf and dead coral were present in the culture. A small number of susceptible fish were also needed for the gnathiids to feed on to maintain the continuity of the culture (see Grutter et al., 2020b). The culture of *G. aureamaculosa* and the associated experiment were conducted outdoors, and thus fish and parasites were exposed to natural light.

The marine monogenean *N. girellae* was continuously cultured at James Cook University in the Marine Parasitology Laboratory between 2010 and 2019 (see Hutson et al., 2018, 2021) using *L. calcarifer* as the principal host. In brief, freshwater *L. calcarifer* (maintained in a 100 l aquarium) were gradually acclimatised to seawater over 3 days before being introduced into the seawater culture tank. *Neobenedenia girellae* develop on the host until reaching sexual maturity (~7 days post-infection at 26°C and 35 ppt salinity; Brazenor and Hutson, 2015) and produce eggs for the next 17 consecutive days (Hoai and Hutson, 2014). Eggs were incubated at the same temperature (26°C) in a Petri dish containing fresh, filtered seawater (35 ppt salinity). Finally, free-swimming larvae (oncomiracidia), which hatch after 4 days of incubation (see Brazenor and Hutson, 2015), were counted, and collected using a pipette and transferred to a beaker of fresh seawater for re-infection of naive acclimated *L. calcarifer*.

The marine ciliate protozoan *C. irritans* has been cultured at James Cook University in the Marine Parasitology Laboratory for

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previous studies (Vaughan et al., 2018c; Skilton et al., 2020; Vaughan and Hutson, 2021). Similar to the methodology for *N. girellae* culture, freshwater *L. calcarifer* were gradually acclimatised to seawater over 3 days before being introduced into the seawater culture tank (100 l tank). Twenty reproductive stages (tomonts) were counted and added to the culture tank (26°C, 35 ppt). Fish presented clinical signs of infection (via the infective theronts), as evidenced by white spots on the skin (trophont stage), after 3 days. Trophonts leave the host and encyst into tomonts (Diggles and Lester, 1996), which were apparent on the bottom of the culture tank with the naked eye. At the same time, barramundi were removed from the culture tank and re-acclimatised to freshwater progressively over 3 days. Parasites were then collected from the tank bottom by scraping using a microscope slide and a pipette for suction. Trophonts were incubated at 26°C in a Petri dish containing fresh seawater (35 ppt) for the release of theronts. For both experiments with *N. girellae* and *C. irritans*, fish and parasites were maintained using artificial light with a 12 h day/night light regime.

Experiment 1: susceptibility of *Labroides dimidiatus* to generalist parasites

The susceptibility of *L. dimidiatus* to generalist parasites was determined in challenge trials following the World Organisation Animal Health (OIE) criteria (OIE Aquatic Code, 2019 available at https://www.oie.int/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/?id=169&L=1&htmfile=chapitre_criteria_species.htm; Table 1). Two different aquatic anaesthetics were used for the following experiments according to the stage of anaesthesia needed: AQUI-S® was used for deeper anaesthesia, while 2-phenoxyethanol was used for light anaesthesia (Ackerman et al., 2005).

Gnathia aureamaculosa

Gnathiid isopods were cultured and used experimentally following Grutter et al. (2020b). After 1 week of acclimation, the cleaner fish *L. dimidiatus* collected at Lizard Island (n=20) and the control host species *C. batuensis* (n=20) were introduced into experimental tanks (36×21×20 cm L×W×H) with fresh seawater. No water recirculation was used for the experiment and water was aerated using air stones. The fish were first acclimatised for 30 min to reduce the stress of handling. After the acclimation period, five unfed infectious juvenile third stage gnathiid isopod *G. aureamaculosa* were added to each tank (Fig. 1A). Only third stage infectious juvenile gnathiids were used because they are larger than the two first stages (~1.05–1.45 mm; Grutter, 2003) and the most visible with the naked eye (Nagel and Grutter, 2007). The experiment started at 11:00 h and fish remained in the tank for 2 h. This time is considered ample for a gnathiid parasite to attach and feed on labrid hosts, before dropping off (Grutter, 2003). During the 2 h of experiments, we visually checked the fish every 15 min, looking for visible gnathiids on their skin. After 2 h of exposure, the cleaner fish was removed from the tank and introduced into a dechlorinated freshwater bath for 5 min to kill and remove the parasites. Then, the fish was measured (TL), transferred to a seawater bath for recovery and quarantined for 3–5 days prior to being released to the collection sites. The water was filtered from all tanks (including the experimental tank, and freshwater and seawater recovery bath) with a 60 µm sieve. Engorged and unfed third stage gnathiids were recovered from the water, counted and preserved in 70% ethanol. To control for potential predation from the fish on gnathiids and the loss of gnathiids during fish transfer, *G. aureamaculosa* were also kept in control tanks (n=20) with no fish (Fig. S1).

Cryptocaryon irritans

Cleaner fish *L. dimidiatus* (n=20) and the control host *L. calcarifer* (n=20) were transferred to individual 30 l tanks (26°C and 35 ppt salinity) and exposed to 10 *C. irritans* tomonts (reproductive stage) each (Fig. 1B). Numerous theronts (infective stage) can hatch from tomonts and mature as trophonts (parasitic stage). The fish exposed to *C. irritans* tomonts were monitored for 10 days to enable the hatching of theronts as well as the development of trophonts on the fish, which subsequently produce the second generation of tomonts. To optimise theront infection, we started to perform the daily exchange of 70–80% of the seawater, 48 h after introduction. Water changes were done in a biosecure manner to prevent contamination of equipment, which was cleaned with diluted bleach daily using equipment assigned to different treatments (Vaughan and Hutson, 2021). After the first appearance of trophont infection (~day 5; see Fig. S2), we maintained the fish for a further 5 days with daily checks for any remaining trophonts and tomonts (reproductive stage) on the bottom of the tank. After 10 days, fish were removed from their tanks, anaesthetised with AQUI-S® (~85 µl l^{-1}) in seawater and transferred to a deep Petri dish (for *L. dimidiatus*) and a tray (*L. calcarifer*). We then assessed their skin under the dissection microscope (magnification ×20 and×40; see Fig. S2 for evidence of *C. irritans* infection) for up to 2 min before fish were released into recovery tanks. The bottom of each experimental tank was scraped using a different synthetic sponge for each tank to suspend the tomonts in the water column, which was then filtered using a 60 µm mesh sieve. The contents were collected in a Petri dish and examined under a dissection microscope. The number of tomonts was counted and preserved in 70% ethanol. After the visual check, *L. dimidiatus* were placed into quarantine tanks (36×21×20 cm L×W×H; maximum four per tank, with similar size conspecifics, or isolated if aggressive toward the others – a rare occurrence). Fish were moved to new, clean tanks every day over a period of 1 week to break the *C. irritans* life cycle in the unlikely event that 100% of

Table 1. Susceptibility of *Labroides dimidiatus* to generalist parasites

OIE Aquatic Code criteria	Approach
(1) Determine whether the route of transmission is consistent with natural pathways for the infection	All experiments were conducted through exposure to the parasites' juvenile, infective life stage in the water column
(2) Determine whether the pathogenic agent has been adequately identified	All cultures used were previously identified using combined morphological and molecular approaches (*Neobenedenia girellae*: Brazenor et al., 2018; *Cryptocaryon irritans*: B. Gomes, personal communication; *Gnathia aureamaculosa*: Ferreira et al., 2009)
(3) Determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection	Defined as parasites surviving to adulthood for *N. girellae* and *C. irritans*; defined as parasites dropping off their hosts in an engorged (fed) state for *G. aureamaculosa*

World Organisation Animal Health (OIE) Aquatic Code criteria for listing species as susceptible to infection with a specific pathogen were followed for experimental challenges of cleaner wrasse (*Labroides dimidiatus*) with three parasite species: *Gnathia aureamaculosa*, *Neobenedenia girellae* and *Cryptocaryon irritans*.

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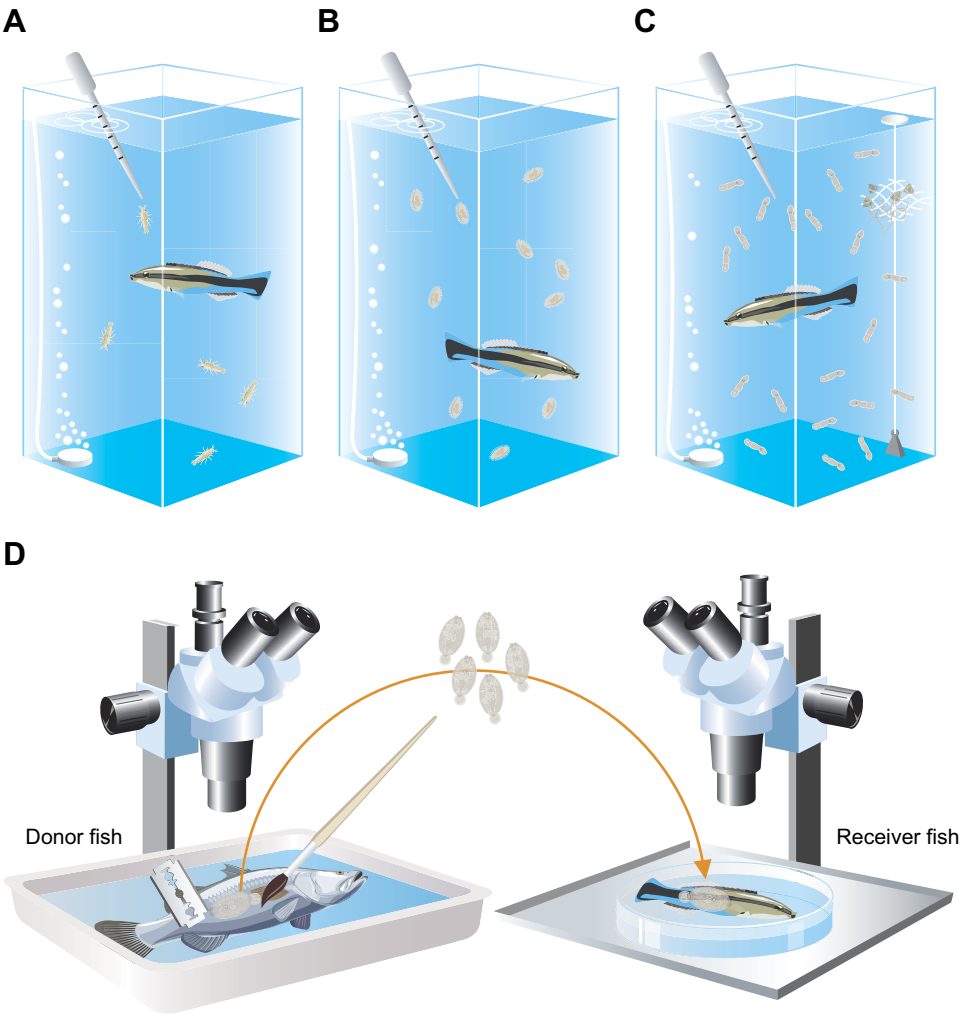

Fig. 1. Schematic diagram showing the experimental treatments conducted in this study. (A–C) First, susceptibility of the cleaner wrasse *Labroides dimidiatus* to (A) the gnathiid isopod *Gnathia aureamaculosa* third stage, (B) the ciliate protozoan *Cryptocaryon irritans* theronts and (C) the flatworm monogenean *Neobenedenia girellae* oncomiracidia was tested. (D) Then, to test transmission success of parasites from clients to cleaners, adult *N. girellae* were transferred from a susceptible control fish to the cleaner fish and their subsequent survival was determined on the cleaner fish. Illustration ©2022, Eden Cartwright, Bird Circus (with permission).

parasites were not recovered. We added the individual *L. calcarifer* to individual tanks containing half freshwater and half seawater, before transferring it to freshwater again; *C. irritans*, being a marine organism, cannot persist in freshwater.

Neobenedenia girellae

In the interest of animal ethics and the recommendations of reducing the number of animals used, fish from the *C. irritans* challenge were rested for 30 days and then subsequently re-used for the *N. girellae* challenge experiments. The *L.. dimidiatus* (n=20) and *L. calcarifer* (n=20) were transferred to individual 30 l tanks (38×28×29 cm L×W×H) with PVC pipe for shelter and air stones for air supply. Experimental fish were exposed to 20 freshly hatched free-swimming oncomiracidia larvae (infectious stage) of *N. girellae*, previously collected from the laboratory culture using a pipette and transferred to a glass beaker of fresh seawater (Fig. 1C). Fish were maintained in their experimental tanks, and the water was monitored for up to 18 days for evidence of egg production (parasites typically infect fish within 48 h at ∼25°C; Trujillo-González et al., 2015b) and sexual maturity on approximately day 7 post-infection at 26°C and 35 ppt salinity (Brazenor and Hutson, 2015). To collect monogenean eggs, a piece of 3 cm^2 clean bridal tulle cloth material was immersed in each tank for the period of the experiment. At the end of the monitoring period, fish were individually given a dechlorinated freshwater bath for 5 min with an anaesthetic concentration of 2-phenoxyethanol at 0.10–0.15 ml l^{-1} for 5 min. This process anaesthetised the fish and removed the parasites present on the skin (Vaughan et al., 2018c). After the freshwater bath, adult *N. girellae* were removed from the water using a pipette or tweezers. Freshwater was filtered using a 60 µm mesh sieve and the contents examined in a Petri dish under a dissection microscope. The number of adult *N. girellae* was counted and preserved in individual vials per fish in 70% ethanol.

Experiment 2: egg production of adult *N. girellae* on *L. dimidiatus* post-transfer

We investigated whether adult *N. girellae* remained attached, survived and produced viable eggs when transferred to the skin of live *L. dimidiatus*. To do so, individual *L. calcarifer* (n=15) were used as donors of adult parasites. To avoid potential immunity following exposure during experiment 1 (Hutson et al., 2018), 10 *L. dimidiatus* and 10 *L. calcarifer* that were not previously infected by *N. girellae* were used as receivers. Survival and egg-laying ability of adult parasites were assessed concurrently in seawater as a control (n=10). Five adult *N. girellae* (between 16 and 20 days post-hatching) were transferred to each receiving fish following Hutson et al. (2018). In brief, we separately and simultaneously anaesthetised two fish (the donor and the receiver) using AQUI-S® (85 µl l^{-1}). We placed the anaesthetised *L. calcarifer* in trays and *L. dimidiatus* in a deep Petri dish filled with seawater. Two dissecting microscopes were used simultaneously, one for the donor and one for the receiver. Adult parasites were gently removed from

the donor using a blunt-edged blade placed underneath the attachment organ (the haptor) and a paintbrush, and transferred immediately to the body of the receiver fish (flanks or tail; Fig. 1D). A transfer was considered successful when *N. girellae* reattached to its new host as observed under the microscope and did not detach in the few minutes post-transfer (see Fig. S3 for *N. girellae* successfully attached to the new host). Following successful transfer (n=5 per fish), we immediately placed the donors into a recovery tank and the receivers into individual experimental tanks (30 l tanks, 38×28×29 cm L×W×H) with PVC pipes for shelter and air stones for air supply. Receiver fish were monitored over the following 7 consecutive days. To collect monogenean eggs, a piece of 3 cm^2 tulle was immersed in each tank and replaced with a new piece each day. The tulle previously immersed in the tank was observed under the dissecting microscope and the number of eggs was counted. Because *L. dimidiatus* produces nocturnal mucous envelopes (Lenke, 1991), this mucus was removed daily and observed under the dissecting microscope for the presence of entangled eggs. Air stones were also examined for possible entangled eggs. A seawater exchange was performed daily (70–80% of the water) using a siphon and a 60 µm filter. The remaining material filtered was examined under the dissecting microscope for detached and dead *N. girellae* as well as eggs (preserved in 70% ethanol). After 7 days, we removed the fish and placed them into a seawater 2-phenoxyethanol bath (0.10–0.15 ml l^{-1}) and then a dechlorinated freshwater 2-phenoxyethanol bath (0.10–0.15 ml l^{-1}) to remove any remaining attached *N. girellae*. The water from each tank was filtered using a 60 µm filter and the tank was placed into a freshwater bath to determine whether any potential monogeneans had survived *in vitro* with no host (individuals that become detached from the host but survive and attach to the bottom of the tank, e.g. Tubbs et al., 2005; Ogawa et al., 2014; Reyes-Becerril et al., 2017). For the control experiment without a host, the same transfer procedure was done from a barramundi donor but the adult *N. girellae* were attached to the bottom of the tank. Similar daily procedures were applied to control tanks (i.e. changing the tulle every day and siphoning). Each day, the control tanks were scrutinised using a flashlight to look for dead *N. girellae* on the bottom (easily detected because they become opaque when dead), which were removed with tweezers and preserved in 70% ethanol.

Experiment 3: survival of adult *N. girellae* on *L. dimidiatus* post-transfer

In the third experiment, we tested whether *L. dimidiatus* remained infected with transposed adult *N. girellae* over 48 h. The same procedure of transfer as per the experiment 2 was performed. New *L. dimidiatus* individuals (n=10), i.e. that had not been subjected to experiment 2, were used. After transferring adult *N. girellae* to each fish (n=5), they were allocated to an experimental tank (22×14×13 cm L×W×H) and checked for parasite presence with the naked eye at 0.5, 1, 2, 4, 8, 24 and 48 h post-infection. The flatworm *N. girellae* is mostly transparent and hard to locate when attached to the host (Trujillo-González et al., 2015b). However, after transfer, it was often possible to distinguish them on the body of the new host (see Fig. S3 for *N. girellae* coloration). After each visual check, we gently guided the fish into their PVC pipe shelter (with only one side open and the other side sealed) and transferred them, immersed in seawater, to another tank with new seawater: this method avoids the need for handling fish with a net, which could dislodge the parasite. The water from the previous tank was filtered using a 60 µm filter and the contents analysed under a dissection microscope for detached and/or dead *N. girellae*. The previous tank was also immersed in freshwater and bathed to remove any potential *in vitro* parasites. After 48 h, *L. dimidiatus* were removed and introduced into a seawater bath with 2-phenoxyethanol (0.10–0.15 ml l^{-1}) and then to a dechlorinated freshwater 2-phenoxyethanol bath (0.10–0.15 ml l^{-1}) to recover any remaining parasites attached to the host.

To confirm the egg viability of the parasites used in this experiment, a subsample of eggs produced by *N. girellae* from the susceptibility experiment with *L. calcarifer* and from the transfer experiment with *L. calcarifer* and *L. dimidiatus* was maintained in filtered seawater in Petri dishes until hatching. All eggs from the subsamples developed and hatched into live and mobile larvae (infective stage oncomiracidia). Infection success was not examined.

Quantification and statistical analysis

All data analyses were performed in R (http://www.R-project.org/). A Bayesian analytical framework was used to structure generalised linear models and generalised linear mixed models to test for (1) differences in the recovery rates of gnathiids at the end of the susceptibility experiment (that could be due to potential predation on gnathiids by the fish); (2) differences in the susceptibility of *L. dimidiatus* and the control species to each parasite species; (3) differences in the egg production of *N. girellae* following the transfer experiment over 7 days between *L. dimidiatus*, the control fish and the control with no fish; and (4) survival rates of adult *N. girellae* transferred from *L. calcarifer* to *L. dimidiatus*, to the control fish and to the control with no fish over a 48 h time period. Markov Chain Monte Carlo (MCMC) chains were implemented using the No-U-Turn sampler algorithm in the Stan language with the rstanarm interface to R (https://CRAN.R-project.org/package=rstanarm, version 2.21.1). For all models, 5000 iterations, three chains, a 50% warmup and a thinning of 1 in every 3 iterations was used. Default priors were kept, which in most cases consisted of normally distributed priors on both the intercept and coefficients (mean=0, s.d.=2.5), and an exponential (rate=1) auxiliary prior. Each model was inspected using posterior predictive checking, trace plots of MCMC chains, effective sample sizes, Rhat values and autocorrelation plots for each parameter. All indicators for all models were satisfactory.

For the models in experiment 1, we tested for potential differences in the recovery rate of gnathiids between tanks with fish (both cleaner and control) and tanks without fish (control for the recovery). This model is meant to estimate potential predation rates from *L. dimidiatus* and *C. batuensis* on gnathiids. A negative binomial Bayesian generalised linear model was fitted, with the total number of gnathiids recovered from each tank as the response variable and the treatment (*L. dimidiatus*, *C. batuensis* or control) as the predictor variable. The recovery rate was lower for the fish treatments, and particularly for *C. batuensis*, compared with the no-fish treatment (*L. dimidiatus* 2.6, 95% credibility interval CI 1.8–3.5; *C. batuensis* 1.39, 95% CI: 0.9–2; and no fish treatment 4.4, 95% CI: 3.2–5.8; mean and 95% CI from the posterior distribution of the number of parasites recovered; Fig. S4). Therefore, this indicated that comparisons of the susceptibility to gnathiids between species had to account for the differential detection of parasites. Consequently, two different tests were performed using the two different variables for gnathiid detection (visual count and individuals recovered that had fed, i.e. had an engorged gut).

For the models in experiment 1, interspecies comparisons required accounting for differences in body surface area exposed

to experimental infections. Given logistical constraints that precluded surface area from being directly measured for the experimental fishes, we first devised a modelling procedure relating body length with visible external surface area (i.e. not including gill area) from photographs of different individuals of each species. We first retrieved lateral pictures with associated length measurements for a minimum of eight individuals for each of the three fish species used. We used our own photos and photos publicly available from FishBase (www.fishbase.org, version 08/2021). The visible external surface area was estimated from each photo by measuring the total lateral area of the fish using the software Fiji (Schindelin et al., 2012) and multiplying it by two. Estimated visible external surface area as a function of body length was then modelled and the obtained relationship used to predict the visible external surface area for each of the fish individuals used across the susceptibility experiments. To test for the susceptibility to *G. aureamaculosa*, two independent tests were done: (1) the number of gnathiids visually observed attached to each individual fish (*L. dimidiatus* or *C. batuensis*) during the experiment; and (2) the number of fed gnathiids recovered at the end of the experiment. For each of these two models, a binomial Bayesian generalised linear mixed-effect model was fitted, with each parasite as an observation and the outcome (success or failure) as the response variable. For the first model, each outcome represented successfully detecting versus not detecting each individual gnathiid infecting the fish by visual means, with fish species as the predictor variable and fish individual as a random effect. For the second model, each outcome represented direct evidence of successful feeding versus unsuccessful feeding for each individual gnathiid. In this case, parasites either not recovered or recovered unfed were considered as representing an unsuccessful infection event. Similar to the previous model, fish species was used as the predictor variable and fish individual as a random effect, but we additionally incorporated the estimated visible external surface area of each fish individual (mm^2) as a log-link offset term. To test for the susceptibility to *C. irritans* and *N. girellae*, two negative binomial Bayesian generalised linear models were fitted. The number of tomonts collected and the number of adults collected at the end of the experiment for *C. irritans* and *N. girellae*, respectively, was used as the response variable, and fish species as the predictor variable. In both cases, the estimated surface area of each individual fish (mm^2) was used as a log-link offset term.

For the models in experiment 2, we tested whether *N. girellae* egg production changed during the 7 day experiment involving experimental parasite transfer from a donor host to a receiver host. A negative binomial generalised linear mixed-effect model was fitted using the number of eggs produced per treatment (*L. dimidiatus*, *L. calcarifer* or control) per experimental day as the response variable, and experimental day (from 1 to 7), including an interaction with species, and parasite age (as this is known to impact fecundity; Hoai and Hutson, 2014; Brazenor et al., 2020) as the predictor variables. Parasite age had no effect on the model and was therefore removed (effect=−0.005; 95% CI: −0.199–0.179). Experimental day was included as a categorical variable, rather than a numerical variable, to account for the possibility of non-linear responses in egg production. Finally, this model also included fish individual identity as a random effect.

For the models in experiment 3, the survival rates of *N. girellae* were tested at different time points during the 48 h experiment involving experimental parasite transfer from the donor host to the receiver host. Two binomial Bayesian generalised linear models were fitted. In the first model, the number of visible *N. girellae* still attached to *L. dimidiatus* (i.e. number of successes) relative to the number unattached (i.e. failures) during the experiment was considered the response variable and the different time points assessed (i.e. 0.5, 1, 2, 4, 8, 24 and 48 h) as the categorical predictor variable. For the second model, instead of the number of visible individuals of *N. girellae*, the number of attached individuals relative to the number of dropped individuals (dead and *in vitro*) was used as the number of successes/failures for the response variable, respectively, and time as the predictor variable.

RESULTS

Susceptibility of the cleaner wrasse *L. dimidiatus* to generalist ectoparasites

The cleaner wrasse *L. dimidiatus* was more susceptible to *G. aureamaculosa* than to *C. irritans* and *N. girellae*. During the exposure to *G. aureamaculosa*, the probability of visually detecting a gnathiid attached to the cleaner was 2.24 times higher (95% CI: 1.36–3.55; *L. dimidiatus* P=0.46, 95% CI: 0.36–0.56; *C. batuensis* P=0.20, 95% CI: 0.12–0.29) than for the control species, *C. batuensis* (see Fig. S4), and after 2 h of exposure we recovered a median 2 (raw data, interquartile range IQR 0.75–4) and 1 (IQR 0–2) *G. aureamaculosa* individuals infecting the cleaners and the control, respectively (Fig. 2A). Differences in the likelihood of fish predation on *G. aureamaculosa* (see Fig. S1) were accounted for by using the number of fed parasites recovered at the end of the experiment. The rate of recovery of fed gnathiids from tanks with cleaner fish was slightly higher than that from the control tanks, although their CI overlapped (*L. dimidiatus* recovery rate 0.90, 95% CI: 0.77–0.999; *C. batuensis* recovery rate 0.73, 95% CI: 0.47–0.992) (Fig. 2B).

All individuals of *L. dimidiatus* and all individuals of the control species, *L. calcarifer*, were successfully infected by theronts (i.e. the infective stage) of the ciliate protozoan *C. irritans*. However, the median number of tomonts (i.e. the reproductive stage) produced by *C. irritans* (i.e. an indicator of life cycle progression) was considerably lower when infecting cleaners (raw data median 8; IQR 5–19.2) compared with control fish (median 438; IQR 170–707; Fig. 2C). Even when accounting for differences in body surface area between the two host fish species, the cleaner was on average 73% less susceptible to *C. irritans* infection than the control (95% CI: 54–86%; Fig. 2D).

Finally, no *N. girellae* flatworm larvae successfully infected *L. dimidiatus*. This was in stark contrast with the control *L. calcarifer*, in which all individuals were infected with a median 5.5 (raw data, IQR 5–11) parasites per fish, from an exposure of 20 (Fig. 2E). Accounting for differences in body surface area between species and conservatively assuming limitations on the capacity to detect zero infections provided a predicted susceptibility to *N. girellae* infection for the cleaner fish that was on average 97% lower than that for the control species (95% CI: 87–100%; Fig. 2F).

Despite the apparent resistance of *L. dimidiatus* to infection by *N. girellae* larvae (Fig. 2E,F), it is conceivable that adult parasites, which are mobile and can crawl along the skin surface of host fishes (Trujillo-González et al., 2015b; see movie of *Benedenia* sp. crawling: https://doi.org/10.5281/zenodo.6207357), could transfer to *L. dimidiatus* from an infected client during close physical contact while cleaning. Whether adult parasites that may become attached to cleaner wrasse during cleaning interactions survive to enable subsequent transmission from *L. dimidiatus* to new clients is unknown. To test this hypothesis, we manually transferred adult *N. girellae* between donor host individuals of *L. calcarifer* and *L. dimidiatus* and monitored survival.

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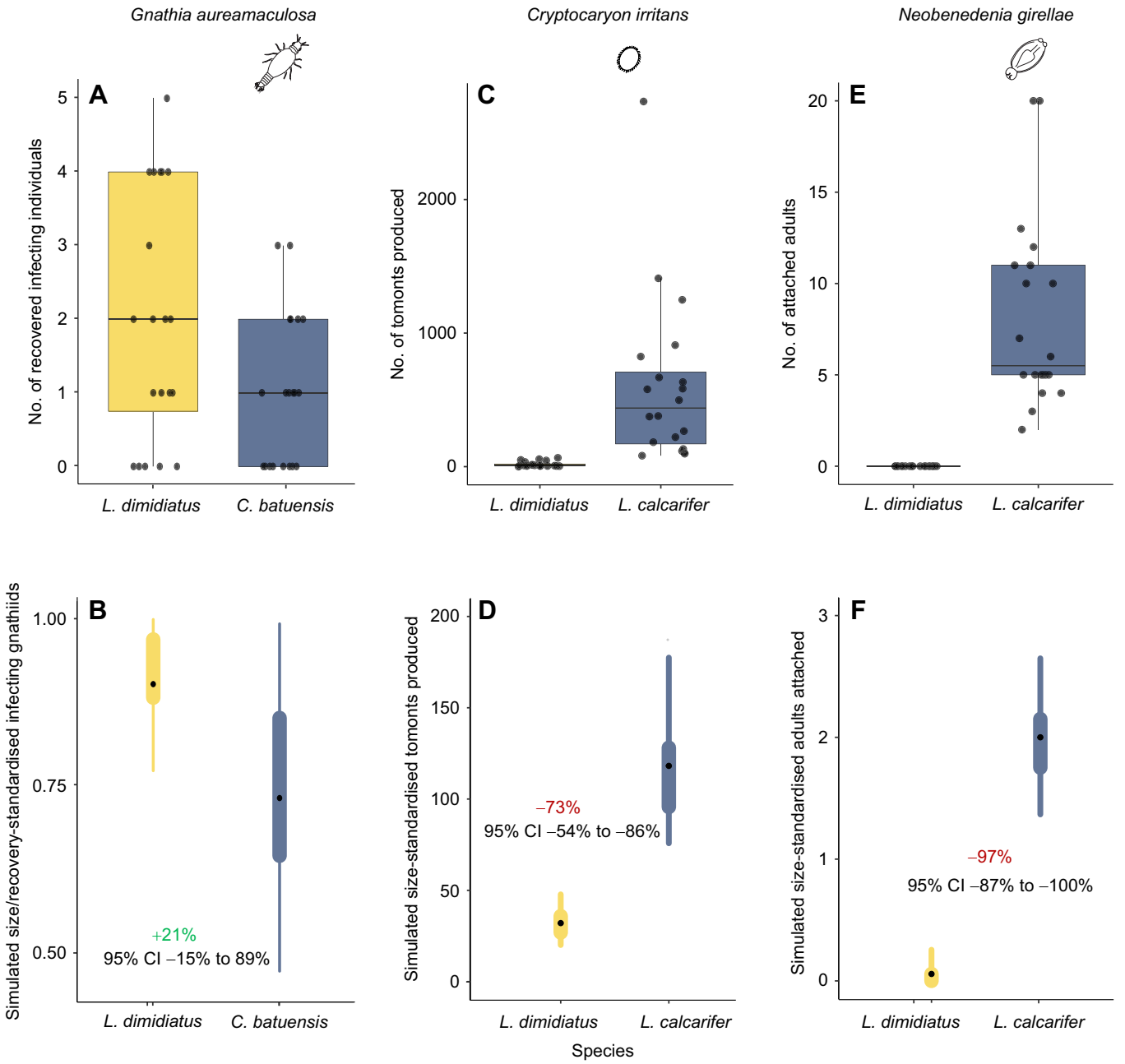

Fig. 2. Raw data and predictive models describing rates of infection of the cleaner wrasse *Labroides dimidiatus* by three types of ectoparasite compared with the control species *Lates calcarifer* and *Coris batuensis*. (A,C,E) Raw data from each susceptibility experiment. (A) Number of gnathiid isopods, *G. aureamaculosa*, recovered fed (i.e. with an engorged gut) at the end of the experiment, from a total of five originally added per trial. (C) Number of second generation tomonts produced by the protozoan ciliate *C. irritans* after initial exposure to 10 tomonts per trial. (E) Number of adult flatworms, *N. girellae*, attached to the host following exposure to 20 infective-stage larvae (oncomiracidia) per trial. Black data points were jittered on the *x*-axis. Boxplots show the median (horizontal line), the 25% and 75% interquartile intervals (box), and the maximum and minimum values (whiskers). (B,D,F) Model coefficients and simulated data for each experiment, accounting for differences in surface area between individuals from the two fish species. (B) Simulated infection by *G. aureamaculosa* at the end of the experiment (fed gnathiids), with 0 and 1 representing unsuccessful and successful infection, respectively. (D) Simulated number of tomonts produced by *C. irritans* at the end of the experiment. (F) Simulated number of adult *N. girellae* attached to the fish at the end of the experiment. The effect size of the comparison between cleaner and control (percentage ratio) in each case (with the 95% credibility interval, CI) is shown in B–F.

Egg production and viability of adult *N. girellae* flatworms experimentally transferred to *L. dimidiatus*

In the first 24 h after being transferred from the donor fish to the recipient fish, the number of eggs produced by flatworms on the cleaner fish was only 7% (95% CI: 2–15%) of that produced on the *L. calcarifer* treatment (Fig. 3A; Table S1). Between 48 and 72 h post-transfer, flatworms attached to cleaner fish achieved only 16.6% (95% CI: 0.87%–25.8%) of their initial egg production (i.e. first 24 h), and only 1.6% (95% CI: 0.3–4%) of the eggs produced by flatworms attached to *L. calcarifer* in the same period. Egg production on the cleaner treatment declined to only 6.7% of initial egg production between 72 and 96 h (Table S1). At the end of the experiment (i.e. 7 days), no adult flatworms were alive in the cleaner treatment, with 20% found dead and detached from the fish,

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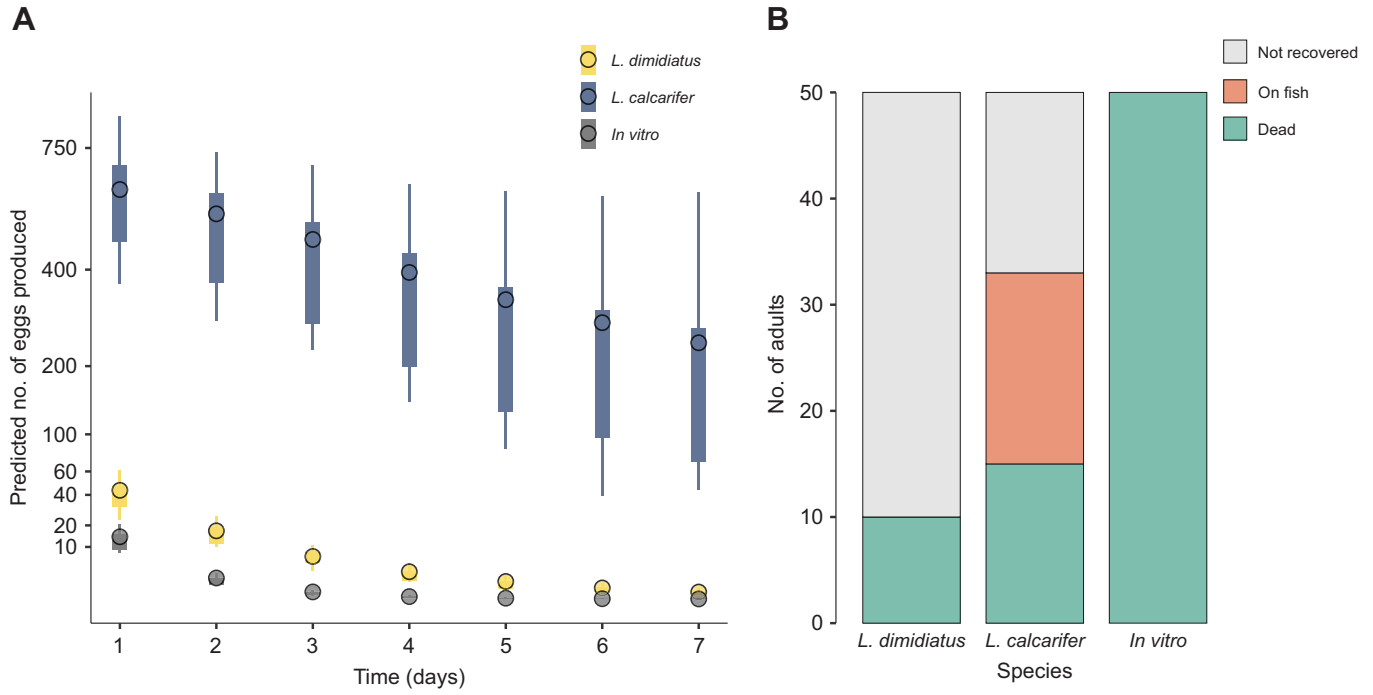

Fig. 3. Parasite egg production and survival on cleaner fish. (A) Estimated number of eggs produced at daily time intervals by adult *N. girellae* flatworms transferred from donor *L. calcarifer* to the cleaner wrasse *L. dimidiatus*, uninfected *L. calcarifer* and in an *in vitro* control. (B) Fate of adult *N. girellae* 7 days post-transfer on *L. dimidiatus*, *L. calcarifer* and in the *in vitro* control treatments. Parasite fate 'not recovered' includes *N. girellae* that dropped off during the experiment and either degraded in the water or were consumed by the fish. Parasite fate 'on fish' represents *N. girellae* recovered after dropping off the fish following a freshwater bath treatment on day 7. Parasite fate 'dead' represents parasites found dead at any point in time during the experiment. All contrasts (Time×Treatment) have a posterior probability of differences >98%.

and 80% not recovered, i.e. degraded or possibly consumed by the cleaners (Fig. 3B). *In vitro* flatworms achieved only 1.4% (95% CI: 0.2–3.9%) of their initial egg production between 48 and 72 h (Fig. 3A; Fig. S4) and, after 4 days, all *in vitro* parasites had died (Fig. 3B), with no further egg production. Flatworms transferred to *L. calcarifer* maintained high egg production throughout the experiment, with an average 35% of the initial egg production (95% CI: 2.3–111%) after 7 days. At the end of the experiment, 36% of flatworms transferred to barramundi were alive on the fish, 30% had died and detached from the fish, and 34% were not recovered (Fig. 3B).

Given that most adult flatworms (80%) experimentally transferred to the cleaners were not found at the end of the experiment (i.e. 7 days), a third experiment was performed to determine changes in the survival rate of these parasites on the cleaner *L. dimidiatus* over the course of the first 2 days following attachment. After transferring adult *N. girellae* flatworms (n=5 parasites per trial; 16–20 days post-hatching) from donor barramundi *L. calcarifer* to cleaner *L. dimidiatus* (n=10 trials), the probability of detecting parasites still visible while attached to *L. dimidiatus* was ∼68% at 0.5, 1 and 2 h (95% CI: 55–81%; Fig. 4). No parasites were found dead or living *in vitro* on the bottom of the tank for the first 8 h of the experiment. After this time, the number of detached parasites (dead or alive) increased, with a probability of 23.8% (95% CI: 13.1–36%) and 27.8% (95% CI: 16.2–40.5%; Fig. 4) of finding a detached parasite after 24 and 48 h, respectively. After 24 h, approximately half of the transferred parasites were still visible on the bodies of *L. dimidiatus*. The probability of finding a visible flatworm on fish decreased from over 48% (95% CI: 34.4–61.3%) after 24 h to 17.7% (95% CI: 8.2–28.6%) after 48 h. At the end of the second experiment (48 h post-transfer), 5 out of 10 cleaners still had *N. girellae* attached with a median of 0.5 (IQR 0–2.5) flatworms per fish.

DISCUSSION

We showed that the bluestreak cleaner wrasse *L. dimidiatus* is capable of transporting attached, egg-producing adult ectoparasites (*N. girellae*) for up to 48 h, despite showing some resistance to infection by common generalist ectoparasites (*C. irritans* and *N. girellae*). This offers strong support to the hypothesis that cleaning interactions provide parasites ample opportunity for transmission, potentially making the cleaner wrasse an unsuspected parasite 'super-spreader'.

In the wild, *L. dimidiatus* individuals interact with an average of 2300 fish per day, for an average 260 min per day (Grutter, 1996a). In a high proportion of these interactions, *L. dimidiatus* executes detailed gill inspections (48–78% of interactions; Grutter et al., 2002), which often involve the cleaner inserting its whole body inside the client's gill chamber. Moreover, nearly 50% of all these interactions also involve direct tactile stimulation (i.e. when cleaner fish touch the client's body with their pectoral and pelvic fins; Bshary and Würth, 2001). During cleaning interactions, mobile ectoparasites, such as adult *N. girellae*, could switch from infected clients to cleaner fish by crawling across the body surface of these fish (Trujillo-González et al., 2015b; see movie of *Benedenia* sp. crawling: https://doi.org/10.5281/zenodo.6207357). Indeed, other related mobile ectoparasites, such as gyrodactylid monogeneans (Bakke et al., 2007) or caligid copepods (Ritchie, 1997), can switch hosts when conditions become unsuitable (e.g. stress, host immunity, death) or when accidentally dislodged (Bakke et al., 2007). In a cleaning symbiosis context, cleaning interactions thus present a potentially impending stress for parasites because of the

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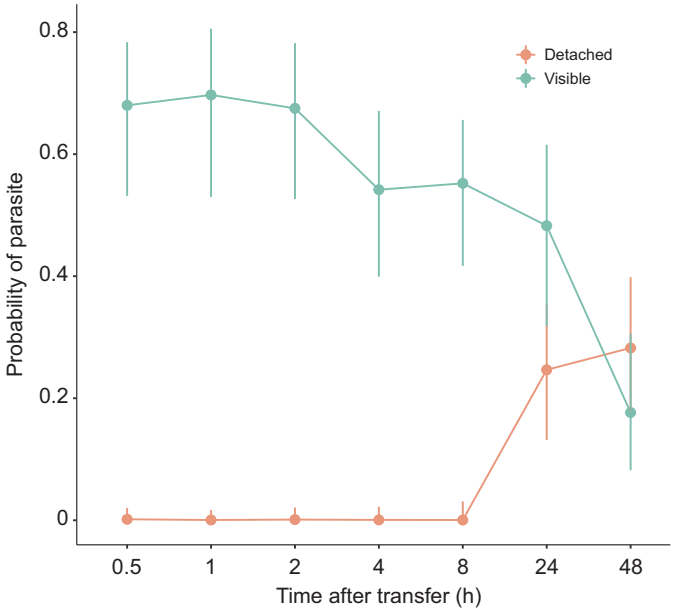

Fig. 4. Estimated probability of different outcomes of the transfer of *N. girellae* from donor fish *L. calcarifer* to cleaner fish *L. dimidiatus* at different time intervals over a 48 h experiment. *Neobenedenia girellae* were found either 'detached' inside the tank (dead or alive) or 'visible', attached to the body of *L. dimidiatus* (n=10). The circles represent the posterior probability for each outcome and the vertical lines the 95% CI.

risk of predation by cleaner organisms, suggesting detachment from the host may be advantageous (Narvaez et al., 2021a). Overall, this reinforces the potential ecological impacts of the combination of parasite mobility, cleaner's capacity to transport parasites (even without being infected, i.e. phoresy) and the prolific, transient and intimate nature of cleaning interactions.

Some parasites may have evolved to exploit cleaning symbiosis as a mechanism for transmission. Indirect evidence is provided by the bucephalid trematode *Rhipidocotyle labroidei* that infects *L. dimidiatus* in their digestive tracts. This parasite is probably acquired from cleaning interactions, when *L. dimidiatus* removes parasites from the skin of its clients, and ingests encysted bucephalid metacercariae (Jones et al., 2004). Similarly, some parasites exploit their original host's predator (Strona, 2015; Antonovics et al., 2017) and develop host-finding and infection strategies based on the interactions between predators and prey (Strona, 2015). When considering the close and intimate contact between cleaner and client fish, cleaning interactions may be compared with prey–predator interactions and could therefore facilitate parasite transmission in a similar way to prey–predator interactions (Jones et al., 2004).

Susceptibility of the cleaner wrasse *L. dimidiatus* to generalist ectoparasites

The cleaner wrasse *L. dimidiatus* is susceptible to infection by gnathiid isopods, which supports recent evidence that wild cleaners are infected by gnathiids at comparable, and sometimes even higher infection intensity than that of other wild wrasse species from the same environments (including *C. batuensis*; Narvaez et al., 2021b). Gnathiids are the main prey item of wild *L. dimidiatus*, constituting up to 95% of their diet (Grutter, 1997, 2000). However, gnathiids are not permanently attached to their hosts, normally dropping off quickly after successful feeding, or as a response to increased host stress or risk of predation (Penfold et al., 2008; Grutter et al., 2020b). Nevertheless, given the fast attack speeds of gnathiids (up to 24.5 cm s^{-1} for an average length gnathiid of 1.5 mm; Grutter, 2003; Grutter et al., 2020b), and their quick response to disturbance, the horizontal transmission from infected client fish to cleaners and vice versa is plausible (Narvaez et al., 2021a).

Individual *L. dimidiatus* were less susceptible to infection by *C. irritans* than control barramundi, *L. calcarifer*, and exhibited some level of resistance toward infection by *N. girellae*. While these species have not been previously documented from *L. dimidiatus* (Narvaez et al., 2021b), these results are surprising because *C. irritans* and *N. girellae* are geographically widespread, harmful, and have remarkably low host specificity among marine fishes (Colorni and Burgess, 1997; Luo et al., 2008; Whittington and Horton, 1996; Brazenor et al., 2018). Indeed, *C. irritans* has been reported as infecting more than 30 fish species from 17 families in the wild (Burgess and Matthews, 1995; Bunkley-Williams and Williams, 1994; Diggles and Lester, 1996) and more than 120 species in captivity (Burgess, 1992). Outbreaks of *C. irritans* in captivity can infect multiple species simultaneously and cause very high mortality (Burgess and Matthews, 1995; Colorni and Burgess, 1997; Montero et al., 2007). Similarly, *N. girellae* has been reported to infect more than 135 marine fishes from 43 families, both in the wild and in captivity, including Labridae and Latidae, among others (Brazenor et al., 2018).

Differences among fishes in host susceptibility to *C. irritans* of a similar magnitude have been reported from laboratory experiments for the rabbitfish *Siganus canaliculatus* (compared with six other marine fish species: Wang et al., 2010). This resistance was attributed to a blood protein which induces protozoan cilia detachment and cell membrane rupture (Wang et al., 2010). Furthermore, components of the fish innate immune system such as physical and physiological (e.g. antibacterial peptides, lysozyme, lectins) mucus parameters may offer effective barriers against some pathogens (Magnadóttir, 2006; Parida et al., 2018; Reverter et al., 2018). It is therefore plausible that the cleaner wrasse *L. dimidiatus* may be resistant to *C. irritans* and *N. girellae* as a result of some specific physiological mechanisms (e.g. a blood protein such as in *S. canaliculatus*) and/or some features of the mucus (e.g. chemical composition, thickness, etc.) that confer some sort of parasite protection. However, future studies are needed to understand the exact mechanism(s) behind this resistance.

The role of cleaner fish in ectoparasite transmission

Given that *C. irritans* penetrate and settle within the first and secondary lamella layers of the epithelium, where they become encysted (Yambot et al., 2003), it may be unlikely that cleaners, interacting with infected clients, engage in physical contact with this parasite. In contrast, *N. girellae* most often attach to the outer surface of the skin of their hosts and are highly mobile (Trujillo-González et al., 2015b; see movie of *Benedenia* sp. crawling: https://doi.org/10.5281/zenodo.6207357). As such, given the intimate and frequent contact between cleaner fish and clients, it is reasonable to hypothesise that *N. girellae* and other mobile ectoparasites (e.g. copepods) can come in physical contact with the cleaner wrasse and become attached to them during cleaning interactions (Narvaez et al., 2021a). Therefore, whilst not observing successful experimental infection of *L. dimidiatus* by *N. girellae* larvae, we showed that simulated attachment of adult ectoparasites can be conducted successfully. Most adult *N. girellae* experimentally transferred to *L. dimidiatus* remained attached to the fish and produced viable eggs for up to 4 days, demonstrating that it is plausible for *N. girellae* to be transported by *L. dimidiatus* for at

least 48 h (68% parasites were still attached to the fish body for at least 2 days).

Conclusions and future research

The benefits of cleaning symbiosis in marine environments appear obvious and are seemingly identifiable (e.g. parasite removal, enhanced abundance of client fish). However, there may be costs to this interaction associated with the possibility of parasite transmission from infected cleaners. Difficulties with detecting cryptic, small parasites in the field and even in captivity, as well as potential assumptions that cleaning symbiosis involves mostly positive outcomes may have contributed to the historical omission of considering this aspect of cleaning symbiosis. Given the apparent resistance of cleaner fish to some generalist ectoparasites as observed here, experiments simulating direct transmission of parasites between cleaners and clients could focus on pathogens shared between cleaners and clients. This study presents the first experimental examination of the susceptibility of a common cleaner fish to generalist parasites and reveals their potential role in the transmission of parasites during cleaning interactions. Given the prolific nature of dedicated cleaners such as *L. dimidiatus*, which may interact with thousands of clients every day, client ectoparasite infection holds the potential for significant transmission rates and, as such, ecological impacts. Thus, understanding the frequency of direct parasite transmission and downstream outcomes for parasite survival and reproduction is key to accurately assessing the potential for an underlying cost to a well-known mutualism.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: P.N., K.S.H.; Methodology: P.N., R.A.M., D.B.V., A.S.G., K.S.H.; Formal analysis: P.N., R.A.M.; Investigation: P.N.; Writing - original draft: P.N.; Writing - review & editing: R.A.M., D.B.V., A.S.G., K.S.H.; Visualization: P.N.; Supervision: K.S.H.; Funding acquisition: P.N., A.S.G., K.S.H.

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Data availability

Data from this research are available from James Cook University Research Data Repository: https://doi.org/10.25903/txvf-1216. A movie of *Benedenia seriolae* crawling is available from Zenodo: https://doi.org/10.5281/zenodo.6207357

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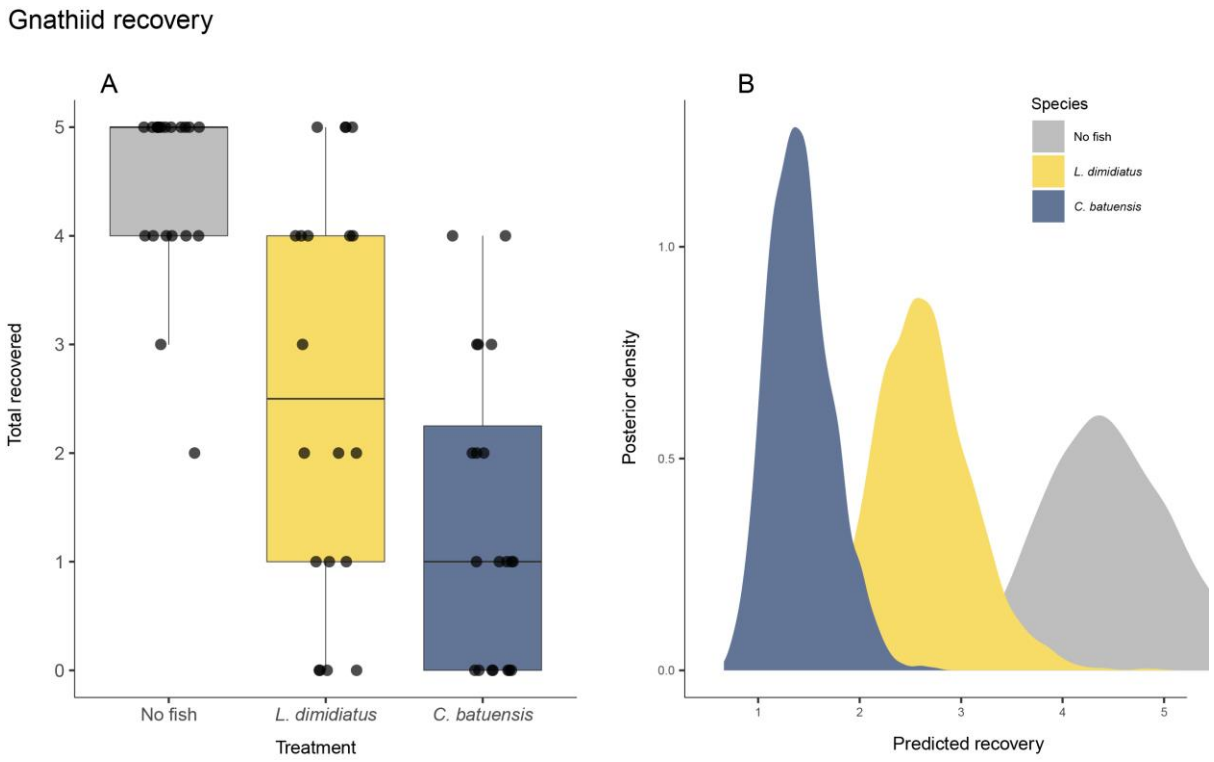

Fig. S1. (A) Number of gnathiids recovered at the end of the experiment in control tank with no fish (grey), *Labroides dimidiatus* (yellow) and control fish *Coris batuensis* (blue). Black dots show jittered data points representing each individual or tank (for control with no fish). Boxplots show the median (horizontal bar), the interquartile (rectangle) and maximum and minimum values (error bars). (B) The predicted recovery of gnathiids was higher for the control with no fish than the two fish treatments. However, the predicted recovery from the *L. dimidiatus* tanks was higher than from *C. batuensis*.

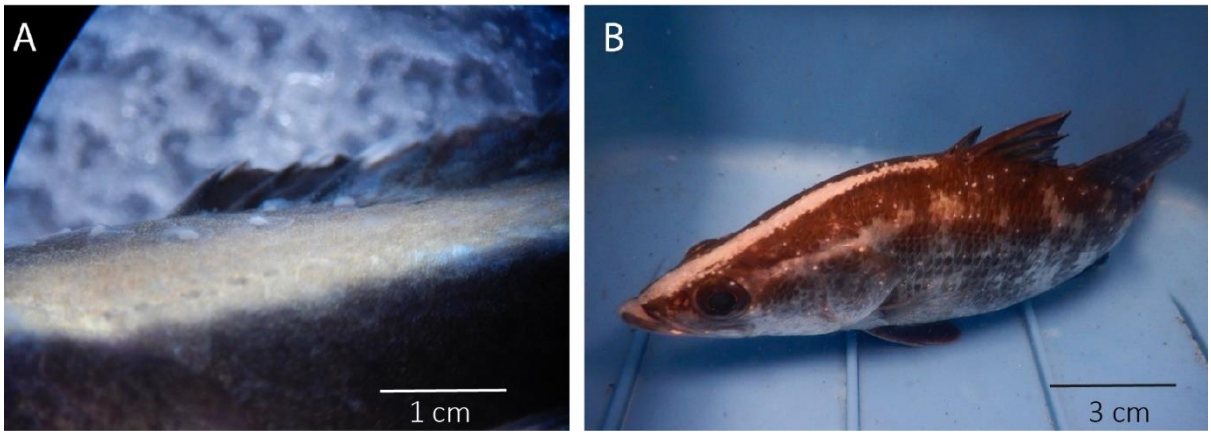

Fig. S2. Trophonts (parasitic stage) of *Cryptocaryon irritans* infecting the cleaner wrasse *Labroides dimidiatus* were rare and visible only under a dissecting microscope (magnification x 40) (A). However, trophonts of *C. irritans* infecting barramundi *Lates calcarifer* were visible with the naked eye and occurred in high infection intensities (B).

Fig. S3. Adult (parasitic stage) flatworm *Neobenedenia girellae* (inside the orange circle) on the body of an anesthetised individual cleaner wrasse *Labroides dimidiatus* after transfer from a donor fish.

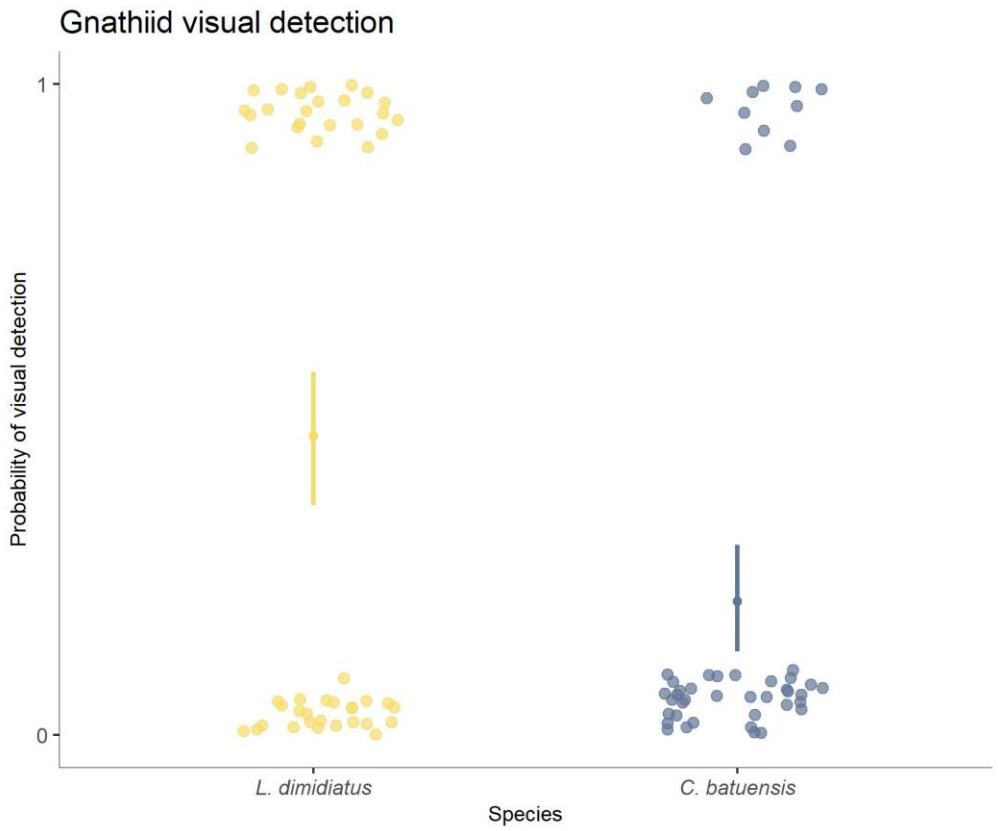

Fig. S4. Probability of visually detecting *Gnathia aureamaculosa* during the experiment with *Labroides dimidiatus* and *Coris batuensis* with 1 being the probability of detection and 0 no detection. The middle dot represents the median and the line the lower and upper 95 % Credibility Interval. Simulated data points were jittered in the x- and y- axis.

Table S1. (A) Egg production estimation based on the ratio of *Labroides dimidiatus* / *Lates calcarifer* and *L. dimidiatus* / *in vitro* using a Bayesian generalised linear models via Stan (Stan Development Team, 2018). Lower.HPD represents the lower endpoint of the highest posterior density interval and Upper.HPD represents the upper endpoint of the highest posterior density interval. (B) Total number of eggs produced per day of experiment for *Labroides dimidiatus* (n=10), *Lates calcarifer* (n=10) and *in vitro* (n=10) treatments.

A Contrast	Day	Estimate	lower.HPD	upper.HPD
Lab.dim / *Lat.cal*	1	0.07	0.021	0.15
Lab.dim / *Lat.cal*	2	0.034	0.009	0.08
Lab.dim / *Lat.cal*	3	0.016	0.003	0.04
Lab.dim / *Lat.cal*	4	0.008	0.001	0.03
Lab.dim / *Lat.cal*	5	0.004	0	0.02
Lab.dim / *Lat.cal*	6	0.002	0	0.01
Lab.dim / *Lat.cal*	7	0.001	0	0.006
Lab.dim / *in vitro*	1	3.2	0.90	8.33
Lab.dim / *in vitro*	2	10.9	2.35	29.5
Lab.dim / *in vitro*	3	36.6	4.73	156
Lab.dim / *in vitro*	4	125	7.58	870
Lab.dim / *in vitro*	5	427	8.01	5175
Lab.dim / *in vitro*	6	1461	8.47	33088
Lab.dim / *in vitro*	7	5055	8.95	195309

B Total number of eggs produced			Day
Lab. dim	*Lat. cal*	*In vitro*	
710	4,938	223	1
146	7,195	1	2
48	5,882	29	3
41	6,834	0	4
30	5,588	0	5
7	4,842	0	6
3	4,911	0	7