Cleaner wrasse indirectly affect the cognitive performance of a damselfish through ectoparasite removal

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Runing title: Parasites affect host fish cognition

Keywords: coral reef fish, Labridae, learning and memory, mutualism, pathogen infection, Pomacentridae

Abstract

Cleaning organisms play a fundamental ecological role by removing ectoparasites and infected tissue from client surfaces. We used the well-studied cleaning mutualisms involving the cleaner wrasse, *Labroides dimidiatus,* to test how client cognition is affected by ectoparasites and whether these effects are mitigated by cleaners. Ambon damselfish (*Pomacentrus amboinensis)* collected from experimental reef patches without cleaner wrasse performed worse in a visual discrimination test than conspecifics from patches with cleaners. Endoparasite abundance also negatively influenced success in this test. Visual discrimination performance was also impaired in damselfish experimentally infected with gnathiid (Crustacea: Isopoda) ectoparasites. Neither cleaner absence nor gnathiid infection affected performance in spatial recognition or reversal learning tests. Injection with immune-stimulating lipopolysaccharide did not affect visual discrimination performance relative to saline-injected controls suggesting that cognitive impairments are not due to an innate immune response. Our results highlight the complex, indirect role of cleaning organisms in promoting the health of their clients via ectoparasite removal and emphasize the negative impact of parasites on host's cognitive abilities.

Introduction

Parasite infection is costly to hosts. Infection leads to altered behavior and physiological states due to direct consumption of host tissues or resources and/or stimulation of the host's immune system [1]. As a result, infection should compromise activities requiring energy expenditure [2]. Since the brain is an energetically demanding organ, cognitive processes like learning, memory and problem solving should, therefore, be adversely affected by parasites. Research in insects, birds and mammals has found cognitive impairments in individuals following experimental infection as well as in populations with natural variation in infection intensity [3-14]. In some cases, this impairment has been linked to stimulation of the hosts' immune system [15-19], which can impinge on the normal functioning of the central nervous system through the release of pro-inflammatory cytokines [reviewed in 20]. Parasite infection has also been linked to increases in circulating levels of stress hormones such as cortisol [21, 22], which may also affect cognitive performance [23]. Yet, not all studies demonstrate a clear negative relationship between pathogen infection and cognitive abilities [24-30]. Babin et al. [29] found that aversive learning is enhanced in *Drosophila melanogaster* infected with bacterial pathogens, and suggest that immune stress can sometimes help boost cognitive abilities as part of an adaptive fight-flight response. In fact, wild populations may display positive relationships between cognitive ability and infection intensity given that learning speed can be positively correlated with boldness, activity, and/or the rapid exploration of novel environments [i.e. 31, 32], which also makes individuals more likely to encounter and acquire parasites [33-35].

Testing the direct effects of parasites on host cognitive performance can be difficult, impeding our ability to draw general conclusions regarding infection and host cognition. First, research correlating natural levels of infection with cognitive performance cannot establish causation [discussed in 3]. Second, manipulative experiments often must use short infection periods [i.e. days-weeks; 8-10, 24, 30], which does not allow for the possibility for changes in behaviour/physiology that may mitigate the effects of infection on cognition. Third, studies on infected subjects tested before and after treatment with anti-parasite medication cannot infer the amount of time each individual was infected prior to testing [e.g. 5, 12]. Fourth, many experiments use social insects (i.e. hymenoptera), as model systems [i.e. 3, 4, 15, 16, 26, 27], which may not accurately represent the trade-offs imposed by infection experienced by reproducing individuals [highlighted in 29]. Finally, studies of host-parasite relationships often fail to consider how these interactions fit within a larger ecological context. In nature, parasitized individuals continue to interact with hetero-and conspecifics. Such interactions may propagate parasite infection or reduce ectoparasite abundance, for example through allogrooming (social grooming between conspecifics) [36] or through seeking interactions with cleaning organisms specialized in removing such pests [37, 38].

Cleaning mutualisms exist in marine, freshwater and terrestrial systems [37, 39-42]. On coral reefs, obligate cleaners such as the blue-streaked cleaner wrasse, *Labroides dimidiatus,* provide a service by eating ectoparasites off the surfaces of heterospecific “client” fishes while benefiting from an easy meal [43]. In the Indo-Pacific Ocean, *L. dimidiatus*, is the most prolific cleaner, engaging in up to 2000 interactions with client species and is capable of ingesting more than 1000 ectoparasites per day [43]. Stomach content analyses indicate that *L. dimidiatus* mostly consume blood and body-fluid feeding gnathiids (Crustacea: Isopoda) during interactions with clients, which reduces client ectoparasite infection intensity [43, 44]. This represents a substantial advantage to clients with access to a cleaning station: the presence of *L. dimidiatus* is associated with increased client growth, body size and condition [45-47] and is a strong predictor of reef fish abundance, biodiversity and juvenile recruitment [48-51]. Access to cleaners may also affect endoparasite abundance, since reduced ectoparasite pressure may free-up energetic resources needed to combat other types of infection. For example, endoparasite abundance predicts ectoparasite abundance in willow ptarmigan (*Lagopus lagopus*), possibly mediated by endoparasite infections reducing host energy available for preening [52]. Cleaner presence may, therefore, help mitigate negative effects of both endo- and ecto-parasites on host cognitive performance by reducing the overall parasite burden experienced by host fish.

We first used the small, resident Ambon damselfish, *Pomacentrus amboinensis*, as a model host to first explore whether access to the cleaner wrasse, *L. dimidatus*, affects overall ecto- and endo-parasite abundance in wild-caught fish. Second, we investigated the long-term effects of parasite exposure on host cognitive performance by taking advantage of a unique experimental setup. Since 2000, a series of 16 isolated patch reefs around Lizard Island on the Great Barrier Reef, Australia have been experimentally manipulated such that seven of these patches have had all juvenile and adult *L. dimidiatus* removed every three months. This creates habitats where resident fish have reduced access to cleaning organisms, and presumably higher exposure to ectoparasites, than adjacent unmanipulated control reefs [i.e. 45, 46, 50, 51, 53]. These patches are separated by at least 5 m of sand, and small, territorial resident fishes do not cross between patches once they have settled. As this experiment has been running longer than the lifespan of most resident fishes (*P. amboinensis* live approximately 6.5 years [54]), individuals on removal reefs have experienced reduced cleaner access and presumably higher ectoparasite pressure for their entire lives. Third, the effects of short-term parasite exposure were tested by exposing wild-caught fish to gnathiid ectoparasites from an experimental culture maintained at LIRS. Finally, we examined whether non-pathogenic immune stimulation affects cognitive performance in damselfish. Bacterial lipopolysaccharides (LPS) are major components of gram-negative bacteria cell walls and are strong stimulators of the innate immune response in a wide range of taxa [55]. We used LPS injections to trigger an innate immune response in fish to assess possible proximate mechanisms underlying observed changes in performance.

Materials and methods

Long-term cleaner removal experiment

We collected adult *P. amboinensis* from 10 experimental patch reefs (3-7 m depth, 61-285 m^2 in surface area, Table S1) located in the southern lagoon habitat around Lizard Island, [see map in 51] and where this species was abundant. We chose 4 patches which had adult and juvenile *L. dimidiatus* systematically removed using hand nets, barrier nets and clove-oil every 3-4 months and 6 reefs which had been similarly disturbed by diver presence, but without cleaner manipulation [for details on cleaner removal and patch reefs, see 50, 51].

We collected four adult *P. amboinensis* per cleaner-present patch and six adult *P. amboinensis* per cleaner-removed patch (cleaner-present patches, n=24; standard length *L*s = 57.2 ± 5.8 mm; cleaner-removed patches n=24; *L*s = 57.0 ± 5.7 mm; mean ± s.d. Table S1). Individuals were housed separately and each aquarium was provided with opaque cylindrical PVC shelters to minimize stress (see *Fish Collection* in ESM). Training for the cognitive experiments commenced within three days of capture (see *Plate Training* in ESM). Fish were tested for side-bias using a double-T maze [56, 57] (see *Lateralization test* in ESM). Of the 48 fish collected, 7 were highly lateralized (4 from cleaner removed reefs, 3 from cleaner present reefs), and were excluded from the analyses. Fish were tested in three cognitive tests in the following order: spatial recognition, reversal learning and visual discrimination (see **Cognition tests** section below).

Following completion of the cognition tests, all 48 fish were sacrificed with an overdose of Aqui-S® solution. In addition, 78 individuals from the same 10 patch reefs were collected in the morning (08:30-11:30 hrs) and also sacrificed to assess overall ecto- and endoparasite abundance from freshly-caught fish (Table S1). In all 126 fish, we recorded the presence of endoparasites including trematodes, nematodes, encysted metacercaria and other cysts, as well as ectoparasites such as monogeneans, copepods and gnathiid isopods (see *Parasite screening* in ESM).

Short-term parasite exposure experiment

We collected 24 adult *P. amboinensis* (standard length *L*s= 53.2 ± 0.9 mm; here and elsewhere: mean ± SEM) on an unmanipulated continuous reef around Lizard Island. Upon arrival at LIRS, fish were treated with praziquantel (50 mg per 20 L seawater, dissolved in 3ml ethanol) for 90 minutes followed by a 60 second immersion in a freshwater bath. These treatments were intended to remove unencysted endo- and ectoparasites from all fish to ensure that individuals were relatively unparasitized prior to gnathiid exposure. Individual standard length and mass were measured, and fish were transferred to holding tanks with shelters as in the long-term cleaner removal experiment. The following day, fish were tested for side-bias in a double-T maze (see *Lateralization test* in ESM).

All 24 fish used in this experiment were non-lateralized. These fish were randomly assigned to either an experimental parasite exposure (n= 12; *L*s= 52.8 ± 1.4 mm) or control (n = 12; *L*s= 53.7 ± 1.2 mm) treatment. Fish were trained to feed on training plates twice daily (morning and early afternoon) for six days before exposure to ectoparasites, and habituated to the experimental set-up for two days prior to testing (see *Plate training* in ESM).

Two outdoor elliptical experimental tanks (220L × 120W × 40H cm) were set up side by side and supplied with water from the flow-through system at LIRS. One tank houses a culture of gnathiid ectoparasites (Crustacea: Isopoda), the other tank was identical in size and set-up, but without parasites. Fish were exposed to either the experimental infection tank or control tank for 1 h per day over 8 consecutive days before testing, and every other day once cognition tests began for a total of 16 exposures (see *Experimental parasite exposure* in ESM).

Immune-stimulation experiment

Fish were collected, transported and treated as in the short-term parasite exposure experiment. Fish were trained to feed on plates as above for seven days prior to being administered an immune stimulation treatment. In total, 31 non-lateralized fish (*L*s = 53.9 ± 3.4 mm, M = 6.6 ± 1.4 g) were assigned to either an immune stimulation treatment (n = 15, *L*s= 52.7 ± 3.3 mm; M = 6.0 ± 1.2 g) or control (n = 16, *L*s= 55.0 ± 3.2 mm, M = 7.0 ± 1.4 g) group.

To stimulate an innate immune response, fish were injected intraperitoneally (i.e. in the abdominal cavity) using heparinized 29 gauge insulin syringes (heparin concentration: 28 mg/ml) through a plastic bag partially filled with seawater to avoid stress caused by air exposure and to protect the fish from surface abrasions during handling. Treatment fish were injected with an LPS (lipopolysaccharide) dose of 50 mg/kg (Sigma-Aldrich L2880, serotype 055:B5), and control fish were injected with a 0.9 % saline solution. To keep the LPS and saline concentration constant, the volume of the injection was mass-adjusted for each fish. Testing in the visual discrimination cognitive test (see details below) began the following day and lasted seven days.

Fish were sacrificed following the last day of testing (i.e. seven days following injection) with an overdose of Aqui-S solution. Fish spleens were dissected out, weighed, preserved in RNALater (R0901 Sigma-Aldrich) stored at -20 C$^{\circ}$, and shipped to the University of Greenwich for gene expression analysis.

Spleen size is often used as an indicator of immune activation in fish. We calculated spleen-somatic index (SSI) as spleen mass (g) / fish wet weight (g) × 100 [58]. In addition, the gene *polydom/svep1* was used to verify fish immunological response to LPS injection (Table S2). This gene has been previously shown to be upregulated in response to LPS and bacterial infection in cnidarians and cell culture [59-61]. See *Immune gene analysis* section in ESM for details on RNA extraction and analysis.

Cognition tests

Cognition experiments followed protocols as described in Gingins and Bshary [62]. Fish from the long-term cleaner removal and short-term parasite exposure experiments were tested in three 2-alternative forced choice tests (spatial recognition, reversal learning, visual discrimination; Fig. 1). Fish from the immune stimulation experiment were only tested in the visual discrimination test due to the time needed to complete the cognitive tasks and the short duration period of immune activation following injection. In all tests, fish were simultaneously presented with both a reward and a detractor food plate (5.0 × 5.0 cm; Fig. S1), and we scored the number of sessions (1 session = 10 food plate trials) each fish needed to learn the location or image associated with the rewarding plate. Fish were tested in 1 to 2 sessions per day. The test was considered solved when an individual correctly chose the reward plate in 10/10 or 9/10 trials within one session, 8/10 trials in two consecutive sessions, or at least 7/10 trials in three consecutive sessions (see *Cognition tests* in ESM for more details).

Spatial recognition test

This test examined the ability of individuals to correctly associate a location (left/right of tank) with a food resource based on a learned location rather than smell [62]. The location (right or left) of the food was taken as the opposite of the choice made during the last feeding of the training phase to ensure that successful performance was based on learning and not a side bias.

Reversal learning test

Reversal learning tests the flexibility of individual learning. For individuals who successfully completed the spatial recognition test, we continued with new plates showing a slightly different pattern (Fig. S1). The location of the available and detractor food plates were reversed, and trials proceeded as above.

Visual discrimination test

In the visual discrimination test, individuals needed to classify information based on visual cues, namely distinct 2D images affixed to the front of the plate. Two different abstract images of the same colour were chosen to maximize the differences between the object shape as well as the amount of coloured area on each image (Fig. S1C). In one 10 trial session, the number of left versus right sides of available food were evenly split (five times each). The order of sides varied such that the available food plate was not presented on the same side more than three times in a row

Statistical analysis

All analyses were done in R 3.1.2 [63]. We report *P* values from type III sum of squares using the Anova function in the R package 'car'.

Parasite abundance and cleaner presence

We used a generalized lineal mixed-effects model (GLMM; glmer function in the R package 'lme4') with a negative binomial error distribution to test whether cleaner wrasse presence (fixed factor) affected parasite abundance (i.e. the total number of ecto- and endoparasites on a host [64]). Patch reef ID was specified as a random factor to account for spatial autocorrelation among fish collected from the same patch. Fish used in cognition experiments spent approximately one month in the lab between collection and being sacrificed, and may have lost parasites during this time in comparison to the freshly-caught fish. Therefore, we also included whether individuals were lab fish or freshly- caught fish as a fixed factor in the model. Fish size (*Ls*) was Z-standardized using the "scale" function in the R package 'base' [65] and included as a covariate. Normality of residuals, homogeneity of variances and overdispersion were verified using qqplots, plots of residuals vs. fitted values and the ratio of residual deviance to degrees of freedom ('overdisp.glmer' function in the R package 'RVAideMemoire'). We performed model simplification by removing the non-significant three-way interaction.

Parasite exposure and immune stimulation experiments

We used survival analysis to test the effect of 1) cleaner wrasse presence/absence (i.e. long-term exposure to ectoparasites), 2) experimental exposure to ectoparasites (i.e. short-term parasite exposure) and 3) non-pathogenic immune response (i.e. LPS injection) on the ability of *P. amboinensis* to complete different cognitive tasks. Survival analysis can handle the type of censored data generated by our experiments [62]. We present inverted survival curves since we considered success rather than death or failure as the endpoint in the tasks. We used Cox proportional hazards models ('coxph' function in the R package 'survival') and verified that model assumptions were met using the 'cox.zph' function. Fish size (*Ls*) and endoparasite abundance were included as covariates in the three models for the long-term cleaner removal experiment; fish were also clustered by reef patch using 'cluster()' in 'cox.zph' to account for spatial autocorrelation. We visualized the effect of the covariate 'endoparasite abundance' on the ratio of instantaneous success rate (analogous to the hazard ratio) using the functions 'coxsimLinear' and 'simGG' in the R package 'simPH'. The package simPH allows simulations and plots of effects estimated from Cox PH models. Fish size (*Ls*) was included as a covariate in the three models for the short-term parasite exposure experiment because ectoparasite abundance is correlated with body size [66]. Fish mass was included as a covariate in the model for the immune stimulation experiment as injection volume was a function of fish mass. Note that in the short-term parasite exposure and immune stimulation experiments, fish were not clustered since they were not collected on distinct patches as in the long-term cleaner removal experiment.

We used linear models (LMs) to test for differences in SSI and the expression of the immune gene *polydom/svep1* between control and treatment groups one week following LPS injection. We did not control for size differences in these models because SSI accounts for differences in body size and mRNA concentration had been standardized across all samples (see *Immune gene analysis* section in ESM). The effect of organ size on gene expression was therefore compensated for and could be neglected in the statistical analysis. Model assumptions were verified using standard diagnostic plots.

Results

Parasite abundance and cleaner presence

The three-way interaction among cleaner presence, fish size (*Ls*) and fish status (lab or wild-caught) was non-significant ($\chi^2 = 2.82$, df = 1, $P = 0.093$). The relationship between fish size and parasite abundance (number of endo- and ectoparasites per host) depended on whether cleaners were present or not ($\chi^2 = 4.10$, df = 1, $P = 0.043$): parasite abundance increased with increasing host body size, but the slope of this relationship was slightly greater for hosts without access to cleaner wrasse (Fig. S2). No other two-way interactions were significant (all *P*s > 0.48). There was no significant effect of whether fish had been kept in the lab or were wild caught on parasite abundance ($\chi^2 = 0.74$, df = 1, $P = 0.39$).

Long-term cleaner removal experiment

Fish with or without access to cleaner wrasse were equally able to solve both the initial (z = -1.68, $P = 0.093$, $R^2 = 0.078$) and the reverse spatial discrimination task (z = -1.38, $P = 0.166$, $R^2 = 0.094$) (Fig. S3). There was no effect of fish size (*Ls*; both *P*s > 0.40) or endoparasite abundance (initial task $P > 0.72$; reversal task $P > 0.09$) on spatial and reversal learning ability. However, fish with access to cleaners solved the visual discrimination task faster and in greater numbers than fish without access to cleaners (z = -3.27, $P = 0.0011$, $R^2 = 0.25$; Fig. 2A). Endoparasite abundance negatively affected a fish's ability to solve this task (z = -2.28, $P = 0.023$; Fig. 3), irrespective of access to cleaner wrasse (treatment*endoparasite interaction: z = 0.14, $P = 0.888$). There was no effect of fish size (*Ls*) on learning ability in this third (visual discrimination) task (z = 0.12, $P = 0.904$).

Short-term parasite exposure experiment

Over the course of the experimental parasite exposure (16 days), we found on average 4.5 gnathiids per treatment exposure in the collection buckets (4.9 after 8 treatment exposures, i.e. when experiments started; range = 0-21 per exposure, see *Experimental parasite exposure* section in EMS). No ectoparasites were ever found on control fish. Fish from both treatments were equally able to solve the original ($z = -0.78$, $P = 0.436$, $R^2 = 0.15$; Fig. S4a) and the reverse spatial discrimination task ($z = -1.18$, $P = 0.240$, $R^2 = 0.09$; Fig. S4b). However, fish experimentally infected with gnathiids had a lower probability of solving the visual discrimination task than control uninfected fish ($z = -2.00$, $P = 0.0455$, $R^2 = 0.18$; Fig. 2B). There was no effect of fish length on learning ability in any of the tasks (all *P*s > 0.15).

Immune stimulation experiment

LPS and saline treated fish were equally capable of solving the visual discrimination task ($z = 0.11$, $P = 0.913$, $R^2 = 0.002$; Fig. 2C) and there was no effect of fish size (mass) on learning ability ($z = -1.85$, $P = 0.853$).

Immune gene analysis

Spleen somatic index differed significantly between injection treatments ($F_{1,29} = 6.26$, $P = 0.018$, $R^2 = 0.15$; Fig. S5), with LPS-injected fish having a higher SSI, indicative of an enlarged spleen, compared to saline-injected fish.

We successfully established the expression of an immunity- and endotoxicity-related gene in 14 saline injected and 15 LPS injected fish from the immune stimulation experiment. LPS injection significantly altered the expression levels of the immune gene *polydom/svep1* compared to saline injected fish ($F_{1,28} = 13.85$, $P = 0.0009$, $R^2 = 0.31$; Fig. S6), indicating successful induction of an immune response.

Discussion

Cleaner wrasse perform two key ecological roles: they remove ectoparasites from client surfaces [41, 43], and reduce infection rates on hosts by lowering local ectoparasite densities [53, 67]. We hypothesized that cleaner presence could similarily affect endoparasite abundance indirectly through the removal of ectoparasites. In line with this hypothesis and previous results [41, 46, 67] we found that Ambon damselfish (*P. amboinensis*) from reef patches with access to *L. dimidiatus* had a slower increase in overall ecto- and endo-parasite abundance with increasing body size compared to fish from cleaner removal patches (Fig. S2).

Parasite infection and host cognition

The costs of even low parasite prevalence can be large, especially for small hosts like damselfish. Gnathiid ectoparasites can kill settlement-stage larval, and impose significant sub-lethal effects on juvenile damselfish including reduced swimming speeds and increased oxygen consumption rates [68, 69]. Our results highlight another benefit of cleaner wrasse to client fish: through the removal of ectoparasites, *L. dimidiatus* indirectly affects the cognitive performance of Ambon damselfish, *P. amboinensis*. Results from our long-term cleaner removal experiment show that natural access to cleaner wrasse is linked to higher cognitive performance in a visual discrimination test compared to fish denied access to cleaning services (Fig. 2A). Furthermore, damselfish endoparasite abundance was negatively associated with individual success in the visual discrimination test (Fig. 3). Thus, parasite exposure generally appeared to negatively affect the visual learning abilities of host fish. In addition, damselfish experimentally infected with gnathiids experienced similar cognitive impairments in the visual discrimination test as fish from patches without cleaners (Fig. 2B), providing evidence for a causal link between parasite infection and reduced cognitive performance. A putative mechanistic explanation for this link may be due to parasite-induced increases in circulating plasma cortisol levels [21, 22], although this hypothesis remains to be tested.

Spatial recognition tasks can be solved by applying a basic learning rule: choose the same side as in the previous interaction if food was available. The reversal learning task requires individuals to first ignore the previous association made between tank location and food and then re-learn the task. Ambon damsels are planktivorous as juveniles, but eat a variety of benthic invertebrates as adults [70]. Thus, associating a food location with spatial information and being flexible in terms of how information is relied upon over time are important cognitive skills for wild damselfish. Neither long-term or short-term parasite exposure resulted in impaired performance in these tasks. Similar studies in rodents have demonstrated that spatial learning is not always impaired as a result of ectoparasite infection [24, 30] despite being affected by immune stimulation [71] and infection with nematodes [7, 8]. Daniels et al. [24] even found improved reversal learning in rats infected with *Toxoplasma gondii*. Taken together, these studies and our results suggest that impaired cognitive performance in spatial tasks is not a ubiquitous consequence of parasite infection. In contrast, we found consistent effects of parasites on the performance in the visual discrimination test (Fig. 2A, B; 3). Damselfishes are highly visual animals that use colour, shape and/or patterns as a basis for classifying reef inhabitants and individual recognition [72]. This discrimination ability is generalizable and can be applied to arbitrary colours and abstract images when reinforced with a positive stimulus such as a food reward [73, 74]. The fitness consequences associated with an impaired ability to discriminate between visual cues in parasitized fish are potentially high. Damselfishes do not have innate predator-recognition, but rather must learn to rapidly assess the potential threat posed by a variety of reef inhabitants on the basis of visual cues such as colour and shape [75]. It is possible that parasitized fish are less able to learn and remember such categorizations or respond to a novel threat, which may lead to increased predation risk. This hypotheses remains to be tested.

Most studies of the effects of infection on host cognition use microparasites or neurotropic parasites such as *Toxoplasma gondii*, which directly affects cells of the central nervous system [i.e. 24, 25]. Cognitive impairments due to these infections seem likely given the location of infection and the structures directly impacted. Our experiments show that ectoparasites, even those only transiently associated with their hosts, can also reduce host fish performance in a learning task. This result is remarkable given the modest exposure to parasites that our fish experienced. Infection rates in our study amounted to approximately 4.5 gnathiids per exposure. Although it is difficult to estimate exact infection rates experienced by wild fishes, studies in the Caribbean on similar hosts and parasites suggest that our study represents the lower end of infestations experienced in nature [76, 77]. As gnathiid isopods do not depend on host survival to maximize their own fitness, it is conceivable that host performance is impaired even after limited exposure.

Previous research investigating the effect of parasites on host cognition have yielded conflicting results. Whereas some studies suggest that natural and experimental infection leads to decreased host cognitive performance in a range of hosts, others studies have noted boosts in cognitive performance, or no cognitive impairment as a result of infection (see references in introduction). Our results suggest that the experimental paradigm used to test performance is a potentially confounding variable explaining this discrepancy. In our fish host, we found that both long- and short-term parasite exposure negatively impacts visual discrimination abilities, while spatial and reversal learning was not affected. Daniels et al. [24] similarly found that spatial reference learning in rats remained unaffected by infection with *Toxoplasma gondii,* whereas spatial memory recall was impaired, highlighting the complexity and difficulty of assessing the overall effect of parasites on host cognitive processes.

Immune response and host cognition

LPS treated fish had higher SSI than control individuals suggesting that an immune response was triggered by endotoxin injection (Fig S5). LPS injection also resulted in modulation of the *polydom/svep1* gene in our damselfish (Fig S6), which is further indicative of an immune response. Despite this stimulation, LPS-injection did not reduce fish performance in our visual discrimination test compared with saline-injected controls. Studies of immune challenges in insects and mammals suggest that host immune stimulation can cause cognitive impairments [15-19], and, in some cases, may be a mechanism underlying cognitive impairments observed in parasite-infected individuals [78]. The energetic cost of immune activation can be high: bumblebees challenged with LPS and micro-latex beads suffer increased mortality during food limitation compared to controls [79]. Our fish were not nutritionally stressed during our experiments. Hence, the potential for complex interactive effects among infection, energetics and cognitive performance remains to be explored in this system.

Many fish species are remarkably tolerant to endotoxins [80, 81]. Our chosen dose of endotoxin injection (50 mg/kg) was based on a seperate study which examined the dosage at which this species shows behavioural signs of sickness in the days following injection (Binning et al. unpublished data). Even though the spleen was enlarged and the gene expression pattern showed that a key immune gene was upregulated seven days following LPS injection, it is possible that this response was only triggered after subjects had already completed the visual discrimination task. Alternatively, our LPS injections did not appear to lead to a reallocation of energy but, instead, to the use of endogenous reserves. In a future study, it would be interesting to test the extent to which long-term immune activation through repeated LPS injections may impact cognitive performance in fish hosts.

Conclusion

Cognitive performance is intricately linked to growth and survival, and thus individual fitness [82]. Few, if any, studies have systematically evaluated whether lifetime exposure to high parasite abundance leads to cognitive impairments, and compared results obtained from long-term infection studies with short-term experimental infections using the same species and experimental paradigm. Furthermore, to our knowledge, no studies have addressed whether cognitive abilities are affected by ectoparasites, or addressed these questions using fish hosts [35]. Our results suggest that performance in visual discrimination tasks is impaired by long- and short-term exposure to ectoparasites as well as endoparasite abundance in a fish host. Although the exact mechanism underlying this impairment remains unknown, it is unlikely to occur because of an activated host innate immune response. Using cleaning mutualisms as a model system, we experimentally demonstrated that increased access to cleaner wrasse can mitigate the negative effects of parasites on host fish cognitive performance. Thus, cleaning organisms can indirectly influence the cognitive abilities of their clients via ectoparasite removal.

Ethics

Field collections and experiments were conducted under permits from the Great Barrier Reef Marine Park Authority (G14/37048.1) with approval from the Queensland Government (DAFF) Animal Ethics Committee (CA 2015/07/878) and the University of Queensland Animal Ethics Committee (AE05703).

Data, code and materials

The data and script for this study are archived in the repository figshare and were made available to editors and reviewers upon initial submission: https://doi.org/10.6084/m9.figshare.5039713

Author Contributions

SAB, RB, DGR and ASG designed the study; SAB, DGR and SC carried out the cognition experiments; SAB and SC performed the fish dissections and tissue preservation; SAB, DGR and DS screened fish for endoparasites; JM performed the analysis of immune genes, DGR and SAB analyzed the data; SAB wrote the manuscript with input from all authors.

Acknowledgements

We thank S. Wismer, O. Rey, A. Pinto, S. Gingins, F. Quattrini, C. Demaire and staff at the Lizard Island Research Station for field support, numerous volunteers for maintaining experimental reefs, and R. Slobodeanu for statistical advice.

Funding

This study was supported by grants from the Fonds de Recherche du Québec Nature et Technologies (SAB, DGR), Subvention Egalité from the University of Neuchâtel (SAB), The Australian Research Council (ASG), The University of Queensland internal funds (ASG), and from the Swiss National Science Foundation (RB).

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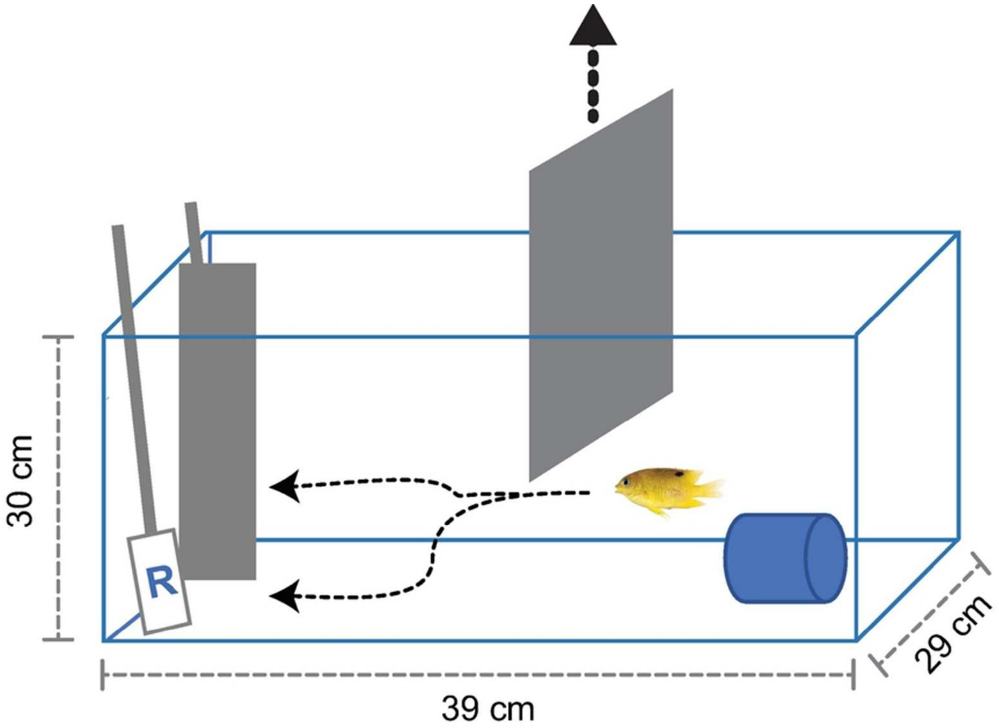
Figure Captions

Fig. 1: Diagram of the visual discrimination cognition test protocol viewed laterally. At the beginning of each trial, an opaque barrier separating the holding and the experimental compartments was lifted. In all treatments, the fish was presented with two identical plates separated by a small partition placed lengthwise at the back of the tank. One of the plates consistently had a food reward located at the back of the plate (i.e. invisible from the front).

Fig. 2: The ability of Ambon damsel (*P. amboinensis*) to successfully complete a visual discrimination task in three different experiments: **A)** long-term exposure to ectoparasites in the wild elicited by long-term removal of cleaner wrasse (cleaner present in blue, n = 21; cleaner absent in red, n = 20); **B)** short-term exposure to ectoparasites in the laboratory (uninfected in blue, n = 12; infected in red, n = 12); **C)** immune stimulation by LPS injection (saline control in blue, n = 16; LPS in red, n = 14). **Left panels** show the cumulative percent completion per trial (inverted survival curve): solid lines are Kaplan-Meier curves (i.e. raw data) and dashed lines are coxph model predictions. **Right panels** show the number of trials that fish (individual dots) needed to complete the task; dots above the dashed line represent fish that failed to complete the task in the allotted number of trials.

Fig. 3: The relationship between endoparasite abundance (controlling for fish size) and the ratio of instantaneous success rate for *P. amboinensis* completing a visual discrimination task. The model predictions indicate that fish with only four endoparasites were twice as likely to complete the task as fish with the mean parasite abundance (21.44, indicated by the red dashed line). The

dark and light blue areas represent the 95% and 50% probability interval of the simulations from the Cox PH model (see methods for details).



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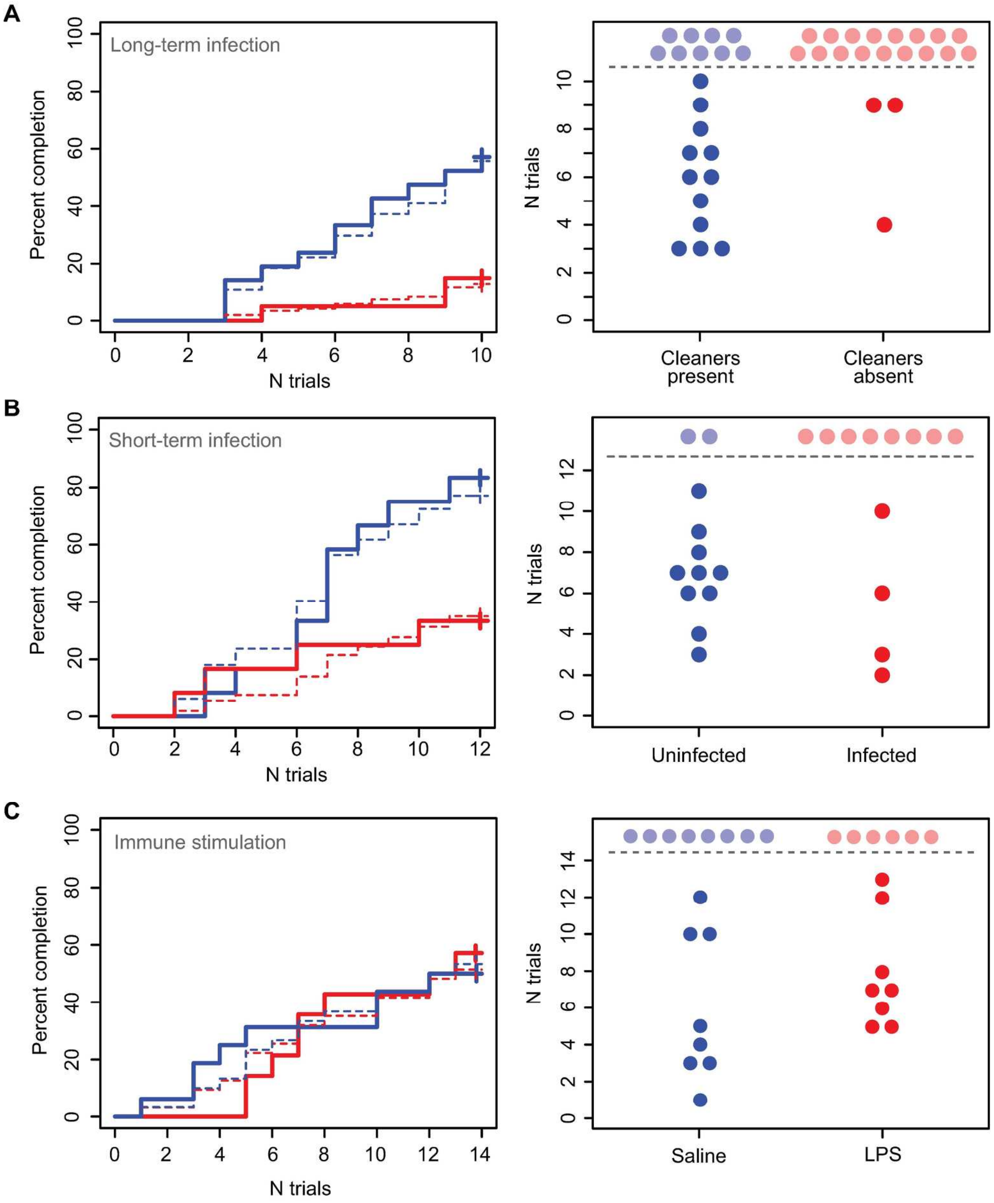

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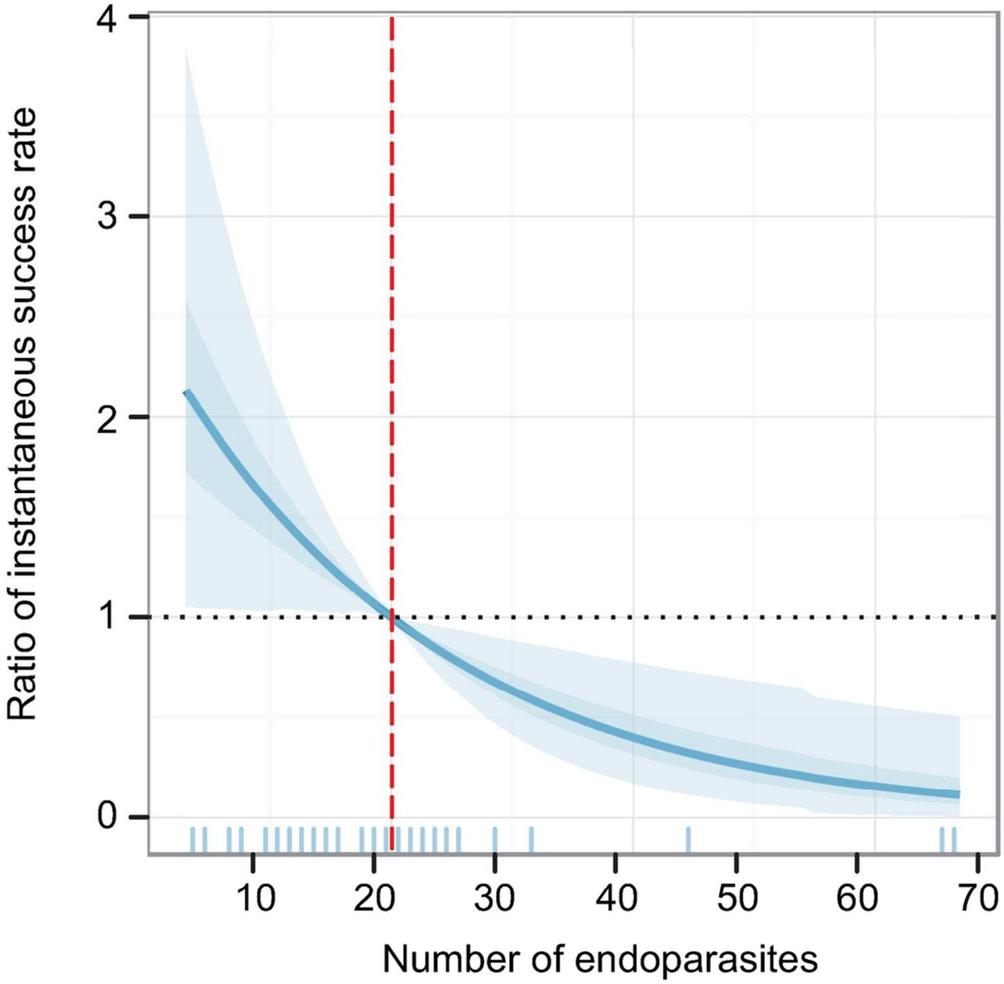

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