

THE BEHAVIOR RESPONSE OF AMPHIPODS INFECTED BY *HEDRURIS SUTTONAE* (NEMATODA) AND *PSEUDOCORYNOSOMA* SP. (ACANTHOCEPHALA)

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21 ABSTRACT. The manipulation of intermediate host behavior may increase chances of parasite transmission to the definitive host. In freshwater environments of the Neotropical Region, studies on behavioral manipulations by parasites are rare, and the majority of these consider only a single parasite species and/or 1 life stage of a particular parasite species. In Andean Patagonian lakes of Argentina, the amphipod *Hyaella patagonica* is infected by larvae of the fish nematode *Hedruris suttonae* and by the bird acanthocephalan *Pseudocorynosoma* sp. The 3 objectives of the present study were to determine whether *H. suttonae* and *Pseudocorynosoma* sp. differ in their effects on behavior of *H. patagonica*, whether such modification is associated with parasite development, and to assess the associations between behavioral traits. From naturally parasitized amphipods, activity (swimming levels) and phototaxis (light preference) was measured. Only in phototaxis trials did larvae of *H. suttonae* induce significantly higher levels of photophilia, suggesting that they are manipulative. Scores of activity and phototaxis were positive and significantly related for non-parasitized female amphipods and for amphipods parasitized by larvae of *Pseudocorynosoma* sp. but were not associated in amphipods parasitized with larvae of *H. suttonae* (infective and non-infective), suggesting that infection separated the relationship between these variables.

Adaptive manipulation of intermediate host behavior should increase the chances of parasite transmission to a suitable definitive host (Moore, 2002; Cézilly and Perrot-Minnot, 2005). Considerable research on this topic has focused on larvae of acanthocephalans infecting crustaceans. Those investigations found that a number of host traits could be manipulated: e.g., infected specimens are more active (Dezfuli et al., 2003), more photophilic (Bethel and Holmes, 1973; Rauque et al., 2011), swim at the water surface (Cézilly et al., 2000), or show different responses depending on the wavelength of light (Benesh et al., 2005). *Skrjabinoclava morrisoni*, probably the only manipulative nematode reported in aquatic environments, infects the marine amphipod *Corophium volutator* and increases surface activity in the host during the day only. Such activity was not recorded at night, when the definitive bird host *Calidris pusilla* does not feed (McCurdy et al., 1999). In theory, a host could be parasitized by a developing or a mature larva, although only the latter one would be immediately ready to be transmitted to a definitive host. Thus, larvae can be grouped as non-infective or infective to the final host (Weinreich et al., 2012). To be most successful, manipulation should only be induced by an infective larva. Sparkes et al. (2004) found that cystacanths (the infective stage) of the acanthocephalan *Acanthocephalus dirus* strongly altered the pigmentation of the isopod *Caecidotea intermedius*, but only minor color changes occurred when isopods were infected by acanthellae (a non-infective stage). Recently researchers have highlighted another facet of manipulation, wherein parasites alter the magnitude and the directions of the associations among host behavioral traits. For instance, an infected host may show a strengthening, reversing, or breaking up of an association between traits, presumably induced by a parasite to increase the probability of transmission from its intermediate to its final host (Coats et al., 2010; Poulin, 2010, 2013; Thomas et al., 2010). Only a few studies have focused on such compounded alterations (Benesh et al., 2008; Coats et al., 2010; Hammond-Tooke et al., 2012). Most of these studies of complex manipulations have considered only a single parasite species and/or 1 life stage of a particular parasite

species. However, in nature, hosts are challenged by many parasite species and/or different life stages of the same species, so multiple infections can be frequent. Thus, host-parasite interactions are complex, and studies that involve multiple parasite species and several host traits can give us better clues to understanding the infection patterns of the hosts in nature. Although the phenomenon of multiple behavioral manipulations induced by parasites has gradually gained more worldwide recognition in the last decade, no such studies are reported in freshwater environments of the Neotropical Region.

In Andean Patagonian lakes of Argentina, the freshwater nematode *Hedruris suttonae* and the acanthocephalan *Pseudocorynosoma* sp. have a 2-host life cycle using the amphipod *Hyaella patagonica* as the intermediate host. The amphipods are infected by eating parasite eggs. Afterwards, the larval stages (L1 to L5 for the nematode and acanthellae to cystacanths for the acanthocephalan) develop in the amphipod's hemocoel. Native species of fishes and aquatic birds acquire parasites when they ingest infected amphipods, *H. suttonae* in the case of fishes and *Pseudocorynosoma* sp. in birds (Rauque and Semenas, 2007; Brugni and Viozzi, 2010). The objectives of the present study were to determine whether *H. suttonae* and *Pseudocorynosoma* sp. differ in their effects on behavior of *H. patagonica* and whether this modification is associated with parasite development and to assess associations between any such behavioral traits.

MATERIALS AND METHODS

Sample collection

Specimens of *H. patagonica* were collected on 1 occasion in December 2010 (austral spring) from Lake Moreno (41°03'S, 71°31'W), Argentina, in a vegetated zone along the coast using a sieve (1 mm mesh size). Crustaceans were transported alive in water from the lake to the laboratory within 2 hr of capture. They were maintained in aquaria with aquatic plants for food, supplementary aeration, and under the same temperature that the lake (14 C ± 1 C). All amphipods were tested within 72 hr post-capture.

Behavioral experiments

To assess behavior of amphipods, activity (through the measure of swimming levels) and phototaxis (light preference) were recorded using trials adapted from Cézilly et al. (2000) and Rauque et al. (2011). Each

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amphipod was placed in a 22.5 ml glass tube (1.5 cm diameter by 18 cm long) filled with water at room temperature, sealed with a film, and positioned on its side 45 cm beneath a fluorescent 40 watt cold lamp. The specimen was acclimatized for 5 min before beginning the tests of activity and phototaxis. In both experiments, measurements were done at 30 sec intervals during a 5 min period. To measure activity, each glass tube was placed above a grid that divided it into 4 equal zones. The initial location (i.e., in zone 1, 2, 3, or 4) was scored for each amphipod at the start of the trial. Amphipod movement was scored in the following way: 0 if the animal stayed in the same zone during a 30-sec scoring interval, or a value between 1 and 4 for a move between 1 to 4 of the zones within the tube during an interval. Thus, an inactive amphipod would have a 0 amount of activity. The most active animal had a total score of 27. To measure phototaxis, half of the glass tube with the same amphipod inside was then covered with a paper tube to produce zones of light and dark. Location, with respect to position in the light or dark parts of the tube, was recorded for each amphipod (1 for light and 0 for dark) at 30 sec intervals during this 5 min test. Total scores ranged from 0 for extremely photophobic animals to 10 for extremely photophilic ones. After the trials were completed, the animals were fixed in 5% formalin and later necropsied under a stereoscopic microscope to determine sex and to determine their parasite(s) and their stage(s) of development. We divided the 4 larval stages (L2–L5) of *H. suttonae* into 2 groups: Group A (stages L2–L3) were non-infective for the definitive host, and Group B (stages L4–L5) were infective. No categories were needed for *Pseudocorynosoma* sp. parasites because only the cystacanth stage was found, which is infective to the definitive host (Crompton and Nickol, 1985).

Statistics

All tests were conducted using a significance level of 95%. Due to non-normality of data, a non-parametric test was used to evaluate differences. The influence of sex (female vs. male) on behavior of amphipods (measured as activity and phototaxis) for each category was analyzed with a Mann-Whitney Mean Rank test. The influence of parasite infection (uninfected and infected by *H. suttonae*, and *Pseudocorynosoma* sp.) on behavior of amphipods was analyzed with a Kruskal-Wallis Mean Rank test, using Dunn's method to isolate the group or groups that differ from the others. To analyze the relation between scores of phototaxis and activity a Spearman Correlation Test was used within the groups of amphipods.

RESULTS

Among 619 amphipods used for behavioral trials, 401 were females and 211 were males, and 7 showed no external or internal sexual characters that allowed determination of sex. For the total number of amphipods analyzed, 214 (34.6%) were parasitized and 405 (65.4%) were non-parasitized with a relation male:female of 1:0.7 for parasitized and 1:3.4 for non-parasitized. Among those infected, 18 (2.9%) were parasitized with non-infective larvae of *H. suttonae*, 107 (17.3%) with infective larvae of *H. suttonae*, and 72 (11.6%) with infective larvae of *Pseudocorynosoma* sp., 17 amphipods with multiple infections (10 with non-infective and infective larvae of *H. suttonae*, and 7 with *H. suttonae* and *Pseudocorynosoma* sp.) were excluded from statistical analysis because of small sample size.

For activity trials: Because scores of amphipods parasitized by non-infective *H. suttonae* and infective *H. suttonae* showed no differences ($T = 0.808$; $n = 122$; $P = 0.421$) they were pooled to increase power to detect effects of the parasite on host behavior. Activity scores were not significantly associated with sex (female vs. male) in the 3 amphipod categories (uninfected $T = 16,135$; $n = 395$; $P = 0.3$; parasitized by *H. suttonae*: $T = 0.82$; $n = 122$; $P = 0.22$, or by *Pseudocorynosoma* sp.: $T = 1,115$; $n = 69$; $P = 0.87$). Activity scores were also not significantly different among the 3 amphipod categories ($X^2_{(2)} = 0.168$; $P = 0.919$).

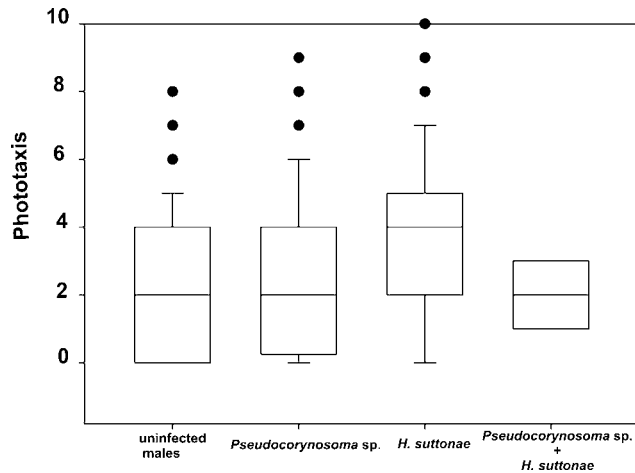


FIGURE 1. Phototaxis scores of parasitized and non-parasitized amphipods *Hyalella patagonica*. Median, quartiles, and data outside 10th and 90th percentiles are indicated.

For phototaxis trials: Scores were significantly associated with sex in uninfected amphipods ($T = 14,880$; $n = 395$; $P = 0.02$), with females having the higher values (female mean score 3.4, male mean score: 2.4). No associations with sex were recorded in the other groups (parasitized by *H. suttonae*: $T = 2,967$; $n = 122$; $P = 0.68$; or by *Pseudocorynosoma* sp.: $T = 1,039$; $n = 69$; $P = 0.47$). Therefore, uninfected males and uninfected females were considered as separated groups. Because phototaxis scores of amphipods parasitized by non-infective *H. suttonae* and infective *H. suttonae* did not differ ($T = 898$; $n = 122$; $P = 0.273$) they were pooled. Phototaxis scores showed significant differences between groups ($H_{(3)} = 20.86$, $P < 0.001$) (Fig. 1), with parasitized amphipods by *H. suttonae* differing from both uninfected male amphipods ($Q = 3.91$) and those parasitized with larvae of *Pseudocorynosoma* sp. ($Q = 3.58$). However, scores of amphipods parasitized by *H. suttonae* and those of uninfected females were not significantly different ($Q = 2.54$).

Relation between activity and phototaxis: Activity and phototaxis scores were positive and significantly correlated for non-parasitized female amphipods ($R_s = 0.15$, $P = 0.009$, $n = 309$) and for amphipods parasitized by larvae of *Pseudocorynosoma* sp. ($R_s = 0.24$, $P = 0.042$, $n = 72$). However, phototaxis and activity scores showed no association in non-parasitized male amphipods ($R_s = 0.19$, $P = 0.08$, $n = 86$) or in amphipods parasitized by *H. suttonae* ($R_s = -0.01$, $P = 0.88$, $n = 122$).

DISCUSSION

Manipulation induced by parasites is a strategy that may involve positive (cooperation) or negative (conflict) interactions with other parasite species (Cézilly et al., 2000; Moore, 2002). Thus, the kind of life cycle and the availability of resources and space within the host can determine the direction of the relationship among co-infecting parasites (Moore, 2002). In fact, 2 opposite strategies have been found based on ontogenetic development: Young parasite larvae are able to induce a protective behavior against predators, whereas older stages induced a diminishing antipredator behavior (Koella et al., 2002; Dianne et al., 2011). In the present study, only amphipods parasitized by larvae of *H. suttonae* (non-infective and infective)

showed an altered behavior, with higher levels of photophilia. Therefore, we infer that larvae of *H. suttonae* manipulate the behavior of amphipods and could cause them to be active in lighted areas and so become more vulnerable to predation by definitive hosts. Non-infective larvae of *H. suttonae* were uncommon, so comparisons between infective and non-infective should be made with caution. In general non-infective parasites should avoid predation by definitive host in a way to permit the immature stages of parasites develop in the intermediate host, favoring the survival of this intermediate host until the parasite can development to an infective stage (Weinreich et al., 2012). This pattern was not found in our study.

Unfortunately, only 7 amphipods were simultaneously parasitized by *H. suttonae* and *Pseudocorynosoma* sp. However these data might inform future studies, because the jointly infected amphipods did not express the altered phototaxis seen when infected only by *H. suttonae*.

Uninfected amphipods had differences in phototaxis scores between the sexes (females showing higher levels than males), while no such differences were detected in parasitized amphipods. The differences in levels of photophilia between sexes in normal amphipods (uninfected) indicate differences in the behavior and location of both sexes and makes males more likely to be exposed to getting infection.

The modification of multiple traits of host could increase efficiency of transmission to the next host (Poulin, 2010; Thomas et al., 2010). For instance, a particular parasite can enhance its chances of transmission to the definitive host by strengthening, reversing, or breaking up relationships between behavioral traits of the intermediate host that act to protect it against predators (Poulin, 2010). Coats et al. (2010) recorded that amphipods infected with the trematode *Microphallus* sp. showed not only an altered behavior but also a strengthening of association between horizontal movement, phototaxis, and vertical distribution. However, Rauque et al. (2011) found no correlation between activity and phototaxis in amphipods infected with digeneans, cestodes, and acanthocephalans. In the present study, the 2 traits evaluated (activity and phototaxis) were positively related for non-parasitized female amphipods and for amphipods parasitized by larvae of *Pseudocorynosoma* sp. However, this correlation was abrogated when amphipods were parasitized by *H. suttonae*, suggesting that the larvae broke the relationship between these traits. The implications of this pattern to the transmission of *H. suttonae* are uncertain to us, and we cannot discard the possibility that this is only a side effect of becoming infected, per se.

In summary, we found that only larvae of *H. suttonae* induce a photophilic behavior in parasitized amphipods, that this modification seems not to be associated with parasite development, and that the natural relation between activity and phototaxis is broken by infection. Interpretation of these results must be limited because a manipulated trait does not necessarily lead to an increase in transmission and also because an increased transmission rate may be produced by a mixture of several traits (Perrot-Minnot et al., 2012). Alterations in the transmission rate to the definitive host still remains unproved in our system, so future experimental studies should be focused on this topic. Additionally, because we did not use experimental infection, we cannot assess if the alterations in behavior that we detected are a cause or a consequence of infection by nematodes. Although other acanthocephalans are known as manipulators, in our

experiment we found no such evidence for *Pseudocorynosoma* sp. However, we have to be careful and consider that this species could be manipulating traits other than those evaluated in the present study.

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