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Reduced attachment strength of rocky shore gastropods caused by trematode infection

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ABSTRACT

For rocky shore gastropods, attachment strength is a key determinant of survival, as getting dislodged by wave action or predators has negative consequences. Yet little is known of the factors that cause inter-individual variation in attachment strength among conspecifics. Here, we test the influence of trematode infection on the suction-mediated attachment strength of periwinkles from two New Zealand species, *Austrolittorina cincta* and *A. antipodum*. Using a standardised experimental protocol, we measured both the strength of attachment of individual snails to the substrate, and its repeatability, i.e. the consistency of measurements taken on different occasions on the same individuals. We then compared the attachment of snails infected with a philophthalmid trematode with that of their uninfected conspecifics. First, we found that for a given snail mass, infected snails were easier to detach from the substrate than the uninfected ones, although this pattern was only significant for *A. cincta*, the larger of the two snail species. Second, the repeatability of attachment strength measurements per individual snail did not differ between infected and uninfected conspecifics, for either of the two periwinkle species. Our findings show that parasitism can weaken snail attachment, and indirectly increase snail mortality, on exposed rocky shores, suggesting a new way in which parasites can affect host population dynamics.

1. Introduction

Invertebrates living on exposed rocky shores must withstand the substantial forces generated by wave action to avoid dislodgement. Tight attachment to the substrate is also a key defence mechanism against predation. To adhere to the substrate, most rocky shore gastropods, such as limpets and periwinkles, use a combination of suction and the secretion of adhesive mucus (Davies and Case, 1997; Smith, 1991, 1992). Suction can be achieved by raising the centre of the foot away from the substrate, thus creating a negative pressure (relative to ambient) under the foot (Smith, 1991). It is generally a mechanism used during locomotion or immediately after the gastropod settles in one spot. In contrast, the production of adhesive mucus is a mechanism that gastropods normally use to glue themselves to the substrate during long periods of inactivity (Davies and Case, 1997; Smith, 1992; Smith and Morin, 2002). In ecological studies of periwinkles (Littorinidae), such as mark-recapture studies, snails that fail to remain attached to the rocky substrate are generally assumed to have died because the consequences of being dislodged can only be negative (Boulding and Van Alstyne, 1993; O'Dwyer et al., 2014; Rolan-Alvarez et al., 1997). Yet, little is known of the factors that affect attachment strength, and thus determine which snails remain attached and which become detached.

Parasitism could be one such factor though this has rarely been investigated to date (but see Zardi et al., 2009). In intertidal habitats, trematodes (Phylum Platyhelminthes) are very common parasites of gastropods (Mouritsen and Poulin, 2002). These flatworms use gastropods as their first intermediate hosts, in which they multiply asexually before their infective stages (cercariae) leave the snail to seek the next host in the life cycle (Galaktionov and Dobrovolskij, 2003). Trematodes are known to impact all key aspects of snail biology, including behaviour (Curtis, 1987; Miller and Poulin, 2001), fecundity (Fredensborg et al., 2005; Mouritsen and Jensen, 1994) and survival (Lafferty, 1993; Mouritsen and Poulin, 2002). In particular, with regard to periwinkles trematode parasitism has strong effects on their behaviour, fecundity, growth and survival (e.g., Huxham et al., 1993; McCarthy et al., 2000; McDaniel, 1969; Mouritsen et al., 1999; O'Dwyer et al., 2014). Although these documented impacts represent the *direct* effects of infection on periwinkle biology, trematodes could also *indirectly* affect their snail host; for example, a subtle reduction in attachment strength caused by trematode infection could indirectly cause greater dislodgement and higher mortality in parasitised snails.

Here, we investigate the influence of trematode infection on the short-term, suction-mediated attachment strength of periwinkles from two New Zealand species, *Austrolittorina cincta* and *A. antipodum*. In both these species, the disappearance of infected snails was slightly higher than that of uninfected snails during a five-month mark–recapture study (O'Dwyer et al., 2014). There are two ways in which trematode infection could affect attachment strength. Firstly, infection may weaken a

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snail and lead to a decrease in its *average* attachment strength. Secondly, infection may cause temporal variation in a snail's ability to generate strong suction, and increase the *variance* in its attachment strength. For example, consider two snails with the same average attachment strength measured across several trials, but with one snail showing extremely consistent values every time while the other snail shows huge differences in how strongly it is attached to the substrate from one trial to the next. The snail showing variable attachment strength should be more likely to become dislodged under natural conditions, because the strength of the suction it generates is likely to frequently fall below the minimum threshold necessary to remain attached to the substrate.

Our specific objectives are to test the hypotheses that (i) trematode infection reduces the attachment strength of periwinkles, and (ii) trematode infection reduces the repeatability (i.e. increases the variance) of measurements of periwinkle attachment strength over time. By investigating the effect of the same parasite on two different snail host species with different body sizes, we aim to examine the generality of any observed effect of infection.

2. Methods

2.1. Study organisms and maintenance

A total of 384 periwinkles were collected by hand at Portobello, Otago Harbour, Dunedin, New Zealand (45.83°S, 170.64°E) on 24 November 2013, consisting of 199 *A. cincta* and 185 *Austrolittorina antipodum*. These two sympatric littorinid species are the dominant organisms on the high rocky shores of New Zealand, with *A. cincta* having generally much larger body sizes (mean mass +/− SD 0.497 g +/− 0.246 g) than *A. antipodum* (mean mass +/− SD 0.181 g +/− 0.059 g). Both species are host to the same philophthalmid trematode species, which has been studied both morphologically and genetically but is yet to be described formally (K. O'Dwyer, unpubl data). These parasites infect the gonad and digestive tissue using the snail as the first intermediate host, before leaving the snail and encysting on hard substrates in the environment and awaiting ingestion by an avian definitive host. The collection site was chosen because earlier sampling revealed a high trematode prevalence in that locality, approximately 70%. Snails were kept in the lab in 1 l containers with 1 cm deep seawater and rocks from the collection site. The shell length and aperture width of each snail were recorded to the nearest 0.1 mm using digital callipers, and the snails were weighed to the nearest 0.001 g. Each individual was labelled with a numbered bee tag (Bee Works, Orillia, Canada) and fitted with a loop of braided fishing line, approximately 20 mm in length, both applied with glue. The fishing line was used to minimise any effect of strain during the measurement of attachment strength (see below). Snails were then allowed to acclimatise in tanks containing rocks from the collection site, seawater and *Ulva* sp. for 48 h before the experiment was carried out. Prior to the experiment, snails were kept in dry conditions for approximately 12 h, to promote movement when wetted.

2.2. Attachment strength assessment

The experimental arena consisted of a 120 × 170 mm sheet of perfectly smooth perspex. The perspex platform was placed on a balance (precision: 0.0001 g) that was then positioned under a custom-made crane. The crane consisted of an electrical motor powering the crane arm, with a hook attached to the end of the arm. One at a time, snails were wetted in seawater, attached by the braid loop to the hook, and placed on the perspex plate. The time required for the snail to begin moving was recorded; at this point, the snail was considered to be attached to the platform as its foot was flush with the platform surface. The crane arm was then raised, pulling the snail upward from the plate at a constant speed of 3 mm/s. The balance readout was recorded throughout the whole process on a handheld compact camera. From the digital videos, the attachment strength of the snail, or the maximum force required to detach it from the perspex platform, was calculated as the maximum difference in mass relative to the initial reading on the balance. This initial reading included the mass of the perspex sheet and the snail; therefore, the mass of the snail was subtracted from this result. At the precise time the snail was detached, the only acceleration was due to gravity; therefore the force required to detach the snail could be calculated as the mass difference (described above) in kilograms multiplied by gravity, giving the resulting force in Newtons. Three measurements were obtained for each individual snail, at intervals of 4 days, though only two measurements could be made for a small number of snails. All measurements were recorded 'blind' to the infection status of the snails, which were only dissected at the end of the experiment to determine whether or not they were infected by trematodes. The very few snails that died during the experiment, and those that harboured a trematode parasite other than the common philophthalmid species, were discarded. The total number of snails used in the final analysis was: 125 infected *A. cincta*, 65 uninfected *A. cincta*, 120 infected *A. antipodum* and 50 uninfected *A. antipodum*.

2.3. Statistical analysis

All statistical analyses were carried out in R version 3.0.0 (R Core Team, 2013) using generalised linear mixed models with Gaussian errors with the *lmer* function from the package *lme4*. Separate models were run for each species of snail. The response variable, attachment force measured in Newtons, was log transformed. As snail mass was correlated with shell length and aperture width only mass was included in the analysis. The fixed-effect predictor variables were infection status, snail mass (after centering) and their interaction. The random-effect variables were snail identity, the identity of the container in which the snail was housed, and the dates of each measurement. To obtain an 'effect size' of the effect of trematode infection on attachment strength Cohen's *d* effect sizes were calculated (Nakagawa and Culthill, 2007).

In addition, to assess the repeatability of the estimated attachment strength across the three measurements taken for each snail (13 snails contributed only 2 measurements to analysis; 1 snail was excluded because it had only a single measurement), Bayesian posterior modes and 95% credibility intervals were calculated, as outlined in Dingemanse and Dochtermann (2013). Univariate mixed models, using a weakly informative prior (R = list(V = 1, nu = 0.002), G = list(V = 1, nu = 0.002)), were run for infected and uninfected snails in the R package *MCMCglmm* (Hadfield, 2010), ensuring that autocorrelation between successive samplings was <0.1. The posterior mode and 95% credibility interval for the random effect, snail identity, were compared between infected and uninfected individuals in order to detect any difference between the two categories of snail. Here the variance components were partitioned so that the variation accounted for by individual snails (i.e. repeatability) could be calculated for both infected and uninfected snails. Where a credibility interval crosses zero no significant difference is indicated.

3. Results

3.1. Snail attachment strength

In both snail species, we observed a trend toward lower attachment force in individuals infected by the philophthalmid trematode parasite relative to uninfected conspecifics. However, this result was only significant for *A. cincta* ($t = -2.796$, $p = 0.005$; Table 1) and not for *A. antipodum* ($t = -1.379$, $p = 0.168$; Table 2). Still, for a given snail mass, infected snails tend to be easier to detach from the substrate than uninfected ones (Figs. 1 and 2). We obtained Cohen's *d* effect sizes for these models and found that there was a small to medium negative effect of trematode infection on the attachment strength in both snail species (*A. cincta*, $d = -0.428$; *A. antipodum*, $d = -0.232$).