

Parasites and personality in periwinkles (*Littorina littorea*): Infection status is associated with mean-level boldness but not repeatability

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Abstract

We demonstrate the presence of animal personality in an inter-tidal gastropod, *Littorina littorea*, both in a sample of individuals infected by the trematode *Cryptocotyle lingua* and in an uninfected sample. On average infected individuals behaved more cautiously than individuals free of infection, but the parasite did not affect repeatability. Although the parasite is not associated with greater diversity of behaviour among infected individuals, infection might be associated with state-dependent personality differences between infected and non-infected individuals.

Key words: Personality, Boldness, Parasite, Gastropod, Trematode

1. Introduction

Parasitic infection can alter the host behaviour in ways that might be adaptive for the parasite, with parasite induced behavioural changes apparent at the sample mean level (see Adamo 2013 for examples). Recently there has been much interest in longitudinal data on animal behaviour, where multiple observations are collected from each individual in the sample. Such studies can reveal mean level differences between groups (e.g. infected versus uninfected) as well as deeper patterns of behavioural variation among- and within-individuals. This approach has been stimulated by the possibility of adaptive consistent among-individual variation in behaviour, referred to as animal personality (Dall et al., 2004). Although parasites might impact behaviour at the level of animal personalities (Barber and Dingemanse, 2010; Poulin, 2013) as well as at the sample mean, only a few studies (see Hammond-Tooke et al., 2012; Kekäläinen et al., 2014; Nakagawa et al., 2010) have investigated this possibility empirically.

Animal personality can be quantified by estimating repeatability and these estimates can be compared across samples. Recent studies show that repeatability can differ between infected and uninfected samples. In the goby *Gobiomorphus cotidianus* Hammond-Tooke et al. (2012) found differences in the repeatability of boldness between individuals infected and uninfected by trematodes. A closely related concept to animal personalities is the 'behavioural syndrome', defined as an association among different behavioural traits. Evidence from a freshwater amphipod (*Austriodotea annectens*) infected with a trematode shows how parasites can alter the associations between different behaviours. In infected individuals phototaxis was positively correlated with swimming but this association was absent in uninfected individuals (Nakagawa et al., 2010).

The examples above are from freshwater habitats but as yet there are no studies of how parasites might alter animal personalities in marine systems, where similar parasites, including trematodes, are also present. *Littorina littorea*, the common periwinkle, is an intertidal gastropod, highly abundant on the rocky shores of the north Atlantic. *Littorina littorina* is an intermediate host for the trematode *Cryptocotyle lingua*. Free swimming cercariae released from infected snails in turn infect a range of fish species in which they cause ‘black-spot’ disease. Infected fish are then eaten by piscivorous birds, such as the gull *Larus argentatus*. The life-cycle is completed when *L. littorina* come into contact with the gull’s guano, which some individuals appear to avoid (Davies and Knowles, 2001). Infected snails show reduced growth, fecundity (in females) and survival (Huxham et al., 1993). They also show reduced winter migration rates (Lambert and Farley, 1968) but little else is known about the effects of *C. lingua* on *L. littorea* behaviour. Like most gastropods, *L. littorea* possess an operculum that is used to close off the shell aperture on disturbance, analogous to the hiding responses and withdrawal responses seen in other animals (e.g. Briffa, 2013). Latency to recover from these responses is commonly used as an index of boldness. Our primary aim is to determine whether there is temporally consistent among-individual variation in behaviour (i.e. animal personality) in *L. littorea*. Second, we ask whether *C. lingua* infection influences mean level latency to re-emerge (i.e. boldness). Third, we ask whether the repeatability of boldness differs between infected and uninfected samples.

2. Methods

Snails ($N = 140$; 21-30mm shell height) were collected at Mount Batten, Plymouth, UK, during September to November 2013. During collection we visually estimated infection status by the presence of orange foot colouration in infected individuals, caused by a release

of pigment from damaged tissue (Huxham et al., 1993). Snails were transferred directly to the laboratory and held individually in containers with 300ml of constantly aerated seawater at 15°C and allowed to feed *ad libitum* on *Ulva lactuca*. Following 48h acclimation, retraction times in response to a physical stimulus were measured. The snail was lifted out of its container by hand and the foot was touched with a blunt mounted needle. All observations were carried out by a single observer who had practised touching snail's feet with a consistent level of pressure. This causes the snail to retract its foot into the shell and close off the aperture with the operculum. The snail was then replaced in its container. The time from replacement of the snail in its container to the point at which the operculum re-opened, making the foot visible, was recorded as the latency to re-open (henceforth 're-opening time'). To avoid problems with right-censored data our measures of re-opening times were not cut-off by a maximum observation time. Similar to another recent study (Kekäläinen et al., 2014) on parasites and personality, we conducted three observations per animal in each infection state, over three consecutive days. We then measured the shell height of each snail before freezing them, prior to dissection. This was necessary to establish sex and infection status by the presence of *C. lingua* cercariae and rediae. To standardise for parasite species, individuals that were found to be infected with species other than *C. lingua* were excluded from the analysis, reducing the sample size to $N=120$ snails.

Statistical methods

We used a generalised linear model with binomial errors to test the effects of size and sex on infection status. To test the effect of infection status on re-opening time we used a general linear mixed-effects model, with random intercepts for individuals. Sex, size and observation number were fixed factors, as well as the sex x infection status interaction, since *a priori* we might expect males and females to show different responses to infection (Huxham et al., 1993). Data were square root transformed, which provided acceptable levels of normality and

homogeneity of residual variance. We used the Kenward-Roger method of estimating degrees of freedom. To test the effect of infection status on the repeatability of re-emergence times we calculated linear mixed model-based repeatability, R_M , and its 95% confidence intervals for infected and non-infected snails. A significant difference in repeatability would be present if the 95% CIs of the two estimates do not overlap. Analyses were conducted using the R packages lme4, lmerTest and rptR.

3. Results

There was no effect of sex ($\chi^2_1 = 0.25$, $P = 0.61$) (Table 1) or size ($\chi^2_1 = 1.46$, $P = 0.23$) on infection status and males and females did not differ in size ($t_{118} = 0.5$, $P = 0.6$). Infected snails showed longer re-opening times than uninfected snails ($F_{1,114.88} = 10.2$, $P < 0.002$) (Figure 1a) but there was no effect of sex ($F_{1,115.02} = 0.002$, $P = 0.97$), size ($F_{1,115.12} = 0.91$, $P = 0.34$) or observation number ($F_{1,237.03} = 0.74$, $P = 0.39$) and there was no interaction between infection status and sex ($F_{1,114.99} = 0.74$, $P = 0.39$). There was a significant random intercept ($\chi^2_1 = 60.4$, $P < 0.0001$) indicating the presence of consistent among-individual variation in behaviour. There was significant repeatability for both infected ($R_M = 0.5$, $P < 0.0001$) and uninfected ($R_M = 0.35$, $P < 0.0001$) snails but overlap of 95% CIs indicates no significant difference in repeatability for the two groups (Figure 1b). Similarly, there was no significant difference in variance of re-opening times between the two groups (Levene's test: $F_{1,356} = 0.7$, $P = 0.4$; coefficients of variation infected = 0.26, uninfected = 0.28).

4. Discussion

Littorina littorea show animal personalities in terms of consistent among-individual variation in boldness, similar to other marine molluscs (Sinn and Moltshaniwskyj, 2005; Pruitt et al., 2012). Infected snails had longer re-opening times on average than uninfected individuals, a result that fits with a previous study showing that infected individuals had slower migration rates (Lambert and Farley, 1968). One potential explanation for this result is that variation in re-opening time is driven by variation in physiological state, since gas-exchange and excretion are restricted when the operculum is closed off. However, parasite-induced changes to metabolism are unlikely to explain the longer re-opening times in infected snails, as the parasite does not alter respiration rates (Huxham et al. 2001). On the other hand, increased caution in the host could benefit the parasite. This might reduce the chance that the host is trapped by a predator before the parasite has completed the current phase of its life-cycle.

As in other studies comparing infected and uninfected individuals from the field (Hammond-Tooke et al., 2012; Nakagawa et al., 2010) we do not know whether infection is a cause or consequence of these behavioural differences, or in the latter case whether it represents a ‘sickness’ response (Dantzer, 2001) rather than behavioural manipulation. Thus, pre- to post-infection studies are clearly warranted. Indeed behavioural variation is present in non-infected individuals, not only in the assay of boldness used here but also in the propensity to avoid gull guano, the potential vector of infection (Davies and Knowles, 2001). Thus it would be interesting to know whether the chemoreceptive responses involved in guano avoidance are also repeatable and whether they form a behavioural syndrome with boldness.

There was no effect of the infection on the repeatability of boldness. Thus, in this example the parasite is unlikely to drive behavioural diversification in the infected portion of

the host population. Perhaps, then responses to infection are relatively homogeneous across the host population. Conversely, due to decreased survivorship (Huxham et al. 1993) *Cryptocotyle lingua* infection could drive the evolution of among-population personality differences, if the guano avoiding behaviour of some individuals makes them more susceptible to infection than others and if this behaviour is heritable. Furthermore this parasite might promote state-dependent personality differences between infected and uninfected individuals within a population (Barber and Dingemanse, 2010). *L. littorina* are easy to obtain, amenable to study in the lab and their ecological roles are well studied. Therefore, they represent ideal study subjects for further investigations of links between parasites and personalities. Our present data add to the evidence for links between parasite infection and boldness.

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Table 1: Number of infected individuals by sex.

	Females	Males	Total
Uninfected	33	20	53
Infected	45	22	67
Total	78	42	120

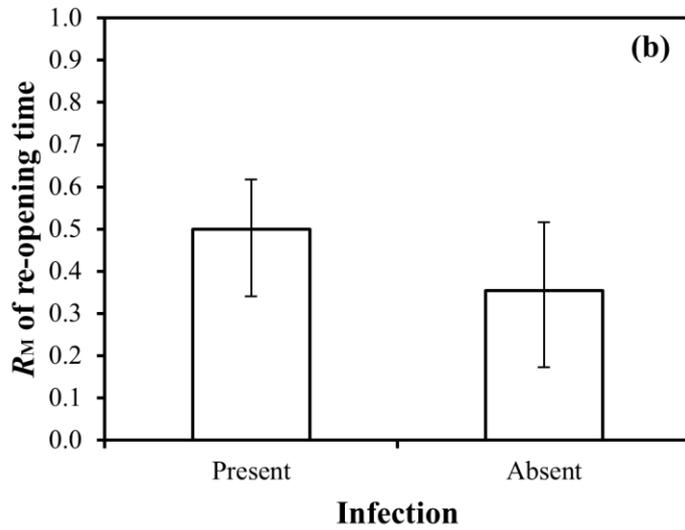
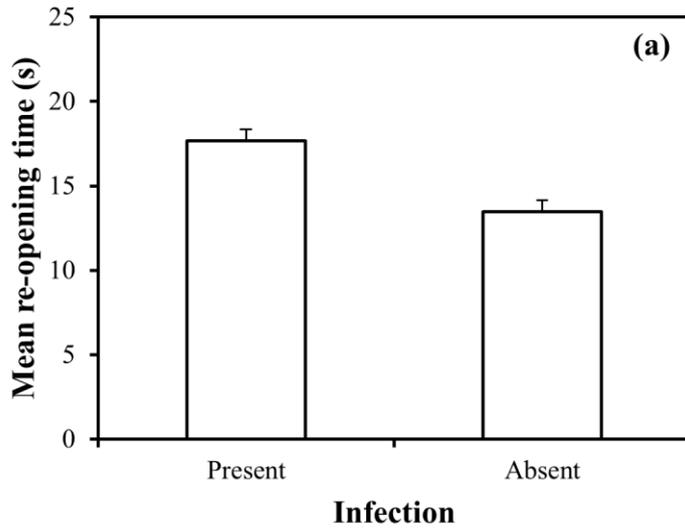


Figure 1: Mean (a) and repeatability (b) of reopening times in infected and non-infected snails. Error bars represent (a) standard errors and (b) 95% confidence intervals.