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Do gill parasites influence the foraging and antipredator behaviour of rainbow darters, *Etheostoma caeruleum*?

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Parasites are known to affect an array of characteristics of their hosts, including morphology, physiology and behaviour. We examined the foraging and antipredator behaviour of rainbow darters, *Etheostoma caeruleum*, that were parasitized by glochidia larvae of freshwater mussels (*Ptychobranchus occidentalis* and *Venustaconcha pleasii*: Unionidae). Glochidia attach to the gills of the host and become encapsulated in host tissue. Over a period of days or weeks the larvae develop into free-living juveniles, which then leave the host. Parasitized darters increased ventilation rates (either early in the infestation or at the height of the infestation), were less active during foraging trials, lost more body size than nonparasitized darters and showed significantly weaker responses to predation risk (signalled by the presence of a chemical alarm cue). Therefore, even for a relatively short-term infection, parasitized darters may pay a cost in terms of decreased growth and decreased probability of survival.

Parasites are defined partly by their production of negative impacts on their hosts (Begon et al. 1990), although the magnitude of these impacts can be highly variable. Increased predation of intermediate hosts due to manipulation by parasites can increase parasite fitness by facilitating their transfer to the next host (e.g. Lafferty & Morris 1996; Bakker et al. 1997; Loot et al. 2002). In contrast, single-host parasites are not expected to manipulate their hosts to increase predation risk, because death of the host would also result in death of the parasite (Smith Trail 1980). Nevertheless, single-host parasites can harm the host by co-opting host resources (overviews: Barber et al. 2000; Bush et al. 2001), and can lead to death of the host when infestations are particularly heavy (e.g. Brown et al. 1995; Northcott et al. 2003).

Negative effects of single-host parasites can include anatomical and physiological effects (Bush et al. 2001) and/or changes in behaviour that reduce success of activities such as foraging (Maksimowich & Mathis 2000; Finley & Forrester 2003), aggressive competition (Fox & Hudson 2001), courtship (Pélabon et al. 2005) and parental care (Sasal 2006). Of the studies that have examined the influence of single-host parasites on antipredator behaviour, the results have not been consistent, ranging from no effects on predation avoidance (Vaughan & Coble 1975) to increased levels of antipredator responses (Milinski 1985; Parris et al. 2006) to decreased levels of antipredator responses (LaMunyon & Eisner 1990; Krkosek et al. 2011).

The life cycle of freshwater mussels (Unionidae) includes a parasitic life stage where large numbers of larvae (i.e. glochidia) attach to fish hosts, usually on the gills (Barnhart et al. 2008). These single-host parasites have evolved specificity to their fish hosts, which are used for dispersal and development (Waller & Mitchell 1989). During the infestation process, glochidia become encapsulated in the hosts' tissues and then metamorphose over a period of days into free-living juveniles (Rogers-Lowery & Dimock 2006; Barnhart et al. 2008). Little is known of the physiological effects of glochidial infestation on host fish. However, Kaiser (2005) found that respiratory gas exchange of largemouth bass, *Micropterus salmoides*, was impaired by an infestation of glochidia from the broken ray mussels, *Lampsilis reeveiana*, resulting in elevated resting ventilation rates, reduced oxygen consumption and lower tolerance of low oxygen conditions. These effects were dependent on intensity of infestation (number of attached glochidia). In extreme cases glochidial infestations can cause mortality (Howerth & Keller 2006); however, the more typical effects that have been observed include the energetic costs of immune responses (Dodd et al. 2005) and/or increased ventilation rates, which appear to be due to reduced surface area for gas exchange over the gills (Kaiser 2005).

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The objective in this study was to examine the effects of glochidia on the behaviour and growth of a host fish, the rainbow darter, *Etheostoma caeruleum*. Our study involved two experiments that occurred over the course of approximately 5 months. We used two species of mussels in two experiments (experiment 1: kidneyshell mussel, *Ptychobranchus occidentalis*; experiment 2: Pleas' mussel, *Venustaconcha pleasii*) based on species availability. Both mussels are common at sites in the smaller tributaries of the White River system in the Missouri Ozarks (Oesch 1984) and both use darters and sculpin as the primary hosts for their glochidia (Barnhart & Roberts 1997; Riusech & Barnhart 1998). For both species of mussels, glochidia are produced in the autumn and released in the spring, and females are barren of glochidia during June–September (M. C. Barnhart, personal observations). At sites where mussels are abundant, glochidial infestations of darters are common. For example, over 60% of rainbow darters examined from a site in the James River in May were parasitized with Pleas' mussel glochidia (infestation intensity: mean $\pm$ SD $=$ 16 $\pm$ 16.7, range 1–73; Riusech & Barnhart 1998).

In our first experiment (kidneyshell mussels), we made four predictions. (1) Because of damage to the host's gills or reduction in gill surface area available for respiration (Kaiser 2005), ventilation rates should be higher for parasitized darters. (2) Because of respiratory stress, activity levels of parasitized individuals should decrease. (3) Reduced activity of darters should result in lower foraging success in behavioural trials. (4) Reduced foraging success should lead to decreased growth.

In the second experiment (Pleas' mussels), we made two predictions. (1) Increased ventilation rates should also increase in response to infestation by this species. (2) If parasitism has energetic costs, then parasitized darters should be less sensitive to predation risk while foraging (Lima 1998) than nonparasitized darters, which have been observed to decrease foraging activity under conditions of high predation risk (Woods 2008); thus, we predicted that parasitized darters would not decrease foraging behaviour under conditions of high predation risk.

METHODS

Experiment 1: Parasitism by Kidneyshell Mussels

Effects on activity, ventilation, foraging and body size of rainbow darters

In March 2009, we collected 40 rainbow darters and one adult kidneyshell mussel from the James River in Greene County, Missouri, U.S.A. We did not check whether fish were previously parasitized with glochidia; however, no exogenous glochidia were recovered from the darters during monitoring (details below), indicating that they did not carry glochidia when they were collected.

To produce infestation levels realistic for wild fishes, we used standard methods (Riusech & Barnhart 1998). This mussel species releases its glochidia in membranous packets (i.e. conglutinates). We separated glochidia from the conglutinates under a dissecting microscope. We counted the viable glochidia in 10 200 µl subsamples of this volume and extrapolated the numbers in these samples to determine the total number of glochidia. Viable glochidia were identified by their closing response to salt (Lefever & Curtis 1912). We then parasitized 20 darters by placing them in a 4-litre container with approximately 4000 viable glochidia per liter for 15 min. During this time we used a rubber-bulb 25 ml syringe (turkey baster) to gently agitate the water and keep the glochidia uniformly suspended. An additional 20 darters served as a control group; they experienced the stress of a sham infestation that followed the same methods, but no glochidia were added.

Immediately following the exposure period, darters from both treatment groups (parasitized and nonparasitized) were moved to individual 1.5-litre monitoring containers (Aquatic Habitats, Inc., Apopka, FL, U.S.A.) that received continuously flowing water (ca. 26 °C). Water from each container flowed into a common holding chamber, and a sump pump was used to pump the water through two filters (UV sterilizer and a 5 µm particulate filter) before it was recirculated. Although some chemical stimuli from individual darters may have remained in the recirculating water, these chemicals should have been distributed equally to darters in each container.

To capture adult glochidia that did not remain attached to gills and to capture juveniles that detached after transformation, the outflow from each container entered a corresponding filter cup (125 µm mesh); our methods were similar to those of Dodd et al. (2005). The minimum size of glochidia for the species that we used in this study was 200 µm (Barnhart et al. 2008), so it is unlikely that any glochidia would pass through the filter. However, any glochidia or metamorphosed juveniles that escaped would be removed by the filters attached to the common holding chamber before the water was recirculated.

At 8 days postinoculation (DPI) we began examining the filter cups of the parasitized darters by temporarily stopping the water flow, removing each filter cup, and rinsing its contents into a Bogorov tray placed under a microscope. We then counted the unmetamorphosed glochidia and metamorphosed juveniles. After 8 days, we checked the filters and counted the recovered mussels at 2-day intervals until no more were recovered (ca. 3 weeks). Because behavioural and physiological effects can be dependent on infestation intensity (Kaiser 2005), we calculated the intensity of the infestation that each fish experienced (i.e. total number of attached glochidia) by the total numbers of glochidia and metamorphosed juveniles that we recovered.

Immediately following exposure to glochidia, and also at the end of the experiment, we measured the volume of darters as the amount of water that each darter displaced in a 5 ml graduated cylinder (0.1 ml precision). We used volumetric displacement in water as a measure of body size because it is likely to be less stressful to fish than other measures that require a longer removal from the water. Beginning on 2 DPI, we fed darters with brine shrimp, *Artemia salina* (1 ml of brine shrimp solution, ca. 10 total brine shrimp), every other day between 1500 and 1700 hours.

We conducted behavioural trials on 5 different days that included both during (DPI: 2, 8, 14, 20) and after (DPI: 28) the parasitic period. Before the trials began, we lined the outer front of the tanks with dark window tinting and placed lights behind the tanks to allow us to see the darters easily while minimizing our visibility to the darters. We also placed cardboard barriers between the tanks to eliminate visual cues between adjacent fish. Each behavioural trial first consisted of recording the ventilation rate for each darter prior to feeding. At the beginning of the observation period, we stood quietly approximately 0.25 m in front of tanks. Most individuals were relatively stationary in the tanks. Once we were confident that we could clearly see at least one operculum (within 0–2 min), we counted ventilation rates for 60 s. Then, we immediately fed each darter and observed it for 120 s while recording the number of moves and the total number of brine shrimp consumed. These feeding trials were done in accordance with the darters' routine feeding schedule. After the trials on 28 DPI, we measured the volumetric displacement for each darter.

Experiment 2: Parasitism by Pleas' Mussels

Effects on activity, ventilation and antipredator behaviour of rainbow darters

During December of 2008, we collected 103 rainbow darters from Bull Creek in Taney County, Missouri, U.S.A. After 2 days in