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Author(s)	Joseph A. DeMarchi, Jordan R. Gaston, Amanda N. Spadaro, Claire A. Porterfield, Matthew D. Venesky
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Tadpole Food Consumption Decreases with Increasing *Batrachochytrium dendrobatidis* Infection Intensity

JOSEPH A. DEMARCHI, JORDAN R. GASTON, AMANDA N. SPADARO, CLAIRE A. PORTERFIELD, AND MATTHEW D. VENESKY*

Allegheny College, Department of Biology, 520 North Main Street, Meadville, Pennsylvania, USA

*Corresponding author email: mvenesky@gmail.com

LRH: J. DeMarchi et al.

RRH: *Bd* infection and tadpole feeding efficiency

ABSTRACT.—Parasitized hosts frequently experience behavioral side effects associated with pathology. Although this phenomenon is fairly well documented, we do not fully understand the relationship between changes in host behavior and parasite infection intensity. Building upon previous research on *Batrachochytrium dendrobatidis* (hereafter "*Bd*") and feeding efficiency in anuran tadpoles, we conducted a laboratory experiment using *Bd*-infected tadpoles of the Green Frog (*Lithobates clamitans*). We allowed tadpoles to feed during a 5-hour trial and tested whether *Bd* infection intensity was a significant predictor of the amount of food that the tadpoles consumed during the trial. As predicted, tadpole feeding efficiency decreased linearly with *Bd* infection intensity. Our results indicate that *Bd*-infected tadpoles suffer a reduced potential to obtain food and that feeding performance is correlated with *Bd* infection intensity. Our result indicates that if tadpoles cannot clear low-level *Bd* infections, their health might decline at a rapid rate which could further reduce their ability to defend themselves from other micro- and macro-parasites.

Key words: Amphibian; Chytridiomycosis; Disease; Feeding efficiency; Intestine; *Lithobates clamitans*

Parasite-induced modifications in host behavior are often categorized as responses that increase transmission rates and ultimately parasite fitness (Poulin, 2010) or as adaptive host responses to clear and/or eliminate their infection burden (often referred to as "sickness behaviors"; reviewed in Adelman and Martin, 2009). However, the behavior of parasitized animals is not always adaptive in respect to the host or parasite (Poulin, 1995). Host behavior can

change in response to physiological, immunological, and/or anatomic changes associated with pathology from the infection. For example, amphibian larvae infected with *Ranavirus* have behavioral deficits that include erratic swimming and lethargy (reviewed in Gray et al., 2009), which can impair their ability to perform as well as non-infected amphibian, which doesn't appear to increase host or parasite fitness. Behavioral side effects of infection, such as lethargy, can thus reduce host fitness by decreasing a host's ability to acquire food and escape predators.

All hosts, however, are not equal and there is individual variation in how hosts respond to parasitism. Relatively few individuals in a population typically carry the heaviest infection burdens whereas the majority of hosts are not infected (Woolhouse et al., 1997). Moreover, of those infected, individuals vary in their tolerance to infection and the healthiest individuals (e.g., those in the best body condition) do not always carry the lowest infection intensity (Raberg et al., 2007). This variation in host species resistance to parasites, coupled with variation in host responses when they are infected, has generated considerable interest in the relationship between host performance and/or fitness change with increasing infection intensity. For example, Rohr et al. (2010) demonstrated that the relationship between change in host fitness and parasite infection intensity was not always negative. In other words, the most heavily infected tadpoles of *Anaxyrus* [= *Bufo*] *americanus* and *Lithobates* [= *Rana*] *clamitans* did not necessarily die earlier than tadpoles with lower infection burdens. This result emphasizes the need to move beyond categorizing animals as "infected" or "non-infected" to better understand the relationship between infection burden and host performance.

Larval anurans are an excellent model system to study parasite-mediated changes in host behavior because they are infected with a diversity of micro- and macro-parasites (Sutherland, 2005) and exhibit many different parasite-induced behavioral changes (e.g., Taylor et al., 2004;

Venesky et al., 2009; Koprivnikar et al., 2014). The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (hereafter "*Bd*"), is of particular interest to ecologists because it is associated with amphibian declines and extinctions on each continent on which it occurs (Fisher et al., 2009). Many biotic and abiotic factors interact to affect the outcome and severity of *Bd* infections, such as temperature (Raffel et al., 2013), amphibian biodiversity (Searle et al., 2011; Venesky et al., 2014), and immune responsiveness (Fites et al., 2013; McMahon et al., 2014). Once infected with *Bd*, the most common behavioral changes in tadpoles include reduced activity (Parris et al., 2006) and reduced feeding activity and food consumption (Venesky et al., 2009).

Previous work on *Bd*-induced behavioral changes, however, did not quantify infection burden and considered infection status as a categorical variable (Parris et al., 2006; Venesky et al., 2009). In the present study, we examined the relationship between parasite infection and feeding performance in Green Frog (*L. clamitans*) tadpoles. Our aim was to build upon the findings of Venesky et al. (2009) and test for a relationship between *Bd* infection intensity and food consumption. If foraging performance correlates with *Bd* infection intensity, we predicted that there would a negative relationship between *Bd* infection intensity and food consumption.

MATERIALS AND METHODS

Animal Collection and Husbandry.—We collected tadpoles of *L. clamitans* (n = 13) from Crawford County, Pennsylvania and brought them back to the laboratory at Allegheny College. The tadpoles were all at relatively similar developmental stages (34-37; Gosner, 1960). Throughout the duration of the experiment, the tadpoles were maintained individually in 650 mL plastic containers (55 mm radius x 68 mm depth) filled with approximately 300 mL of aged tap water. The tadpoles were maintained on a 12 h light: 12 h dark photoperiod at 18 C (±1 C) and

were fed a mixture of Sera Micron® (Sera, Germany) and freshwater fish flakes *ad libitum* twice per week.

We changed approximately 75% of the water in each container on a weekly basis by pouring water from each container into an 18.9L plastic bucket. We avoided the potential for unintentional transfer of *Bd* zoospores or DNA between replicates by not touching any of the tadpoles during this process. Upon completing each water change (and also at the end of the experiment) we thoroughly disinfected all containers, equipment, and water soaking the equipment in a 10% bleach solution to kill *Bd* (Johnson et al., 2003).

Laboratory Experiment.—Bd (JEL 660, isolated from an amphibian in OH, USA) was grown in the laboratory on 1% tryptone agar plates according to standard protocol (Longcore et al., 1999). We harvested *Bd* zoospores by adding 10.0 mL of sterile reagent water to the cultures and collected the zoospores that emerged from the zoosporangia after 45 min. We then exposed the tadpoles to water baths containing infectious concentrations of *Bd* zoospores (approximately $1x10^6$ total zoospores in 150 mL of aged tap water) for 48 hours. After the exposure period, the tadpoles were placed back into the containers described above (Day 0).

On Day 7, we fasted all of the tadpoles for two days to empty their intestines. In addition, we checked the focal containers periodically during the two days to remove any fecal matter, which could be a food source for tadpoles. Previous research has demonstrated that this is an effective method of emptying the intestine (Venesky et al., 2009). On Day 9, we placed two microscope slides covered in dried Sera Micron® along the bottom of each tadpole's container. The microscope slides were prepared the previous day by dissolving Sera Micron® into aged tap water, which created a saturated solution of food (Venesky et al., 2011a). We then uniformly brushed the solution along one side of each microscope slide and allowed them to air dry for 24

hours. We removed all of the tadpoles after 5 hours and euthanized them with an overdose of MS-222, which does not kill *Bd* and is safe for using in studies on chytridiomycosis (Webb et al., 2005).

Prior to swabbing the mouthparts of each tadpole for *Bd*, we visually inspected the oral apparatus of each tadpole under a dissecting microscope and noted any damage/deformations to the keratinized feeding structures, as these can compromise tadpole feeding efficiency (Venesky et al., 2010). We then swabbed the mouthparts of each tadpole using sterile fine-tipped swabs (Advantage Bundling, Durham, North Carolina, USA; product # MW113). Using sterile equipment, we then dissected and straightened each intestine and photographed it next to a scale bar for future analysis. We haphazardly selected and photographed 4 of the microscope slides (two of which were from the only tadpoles that consumed all of the food from the slides and two of which did not consumed a negligible amount of food).

To quantify the amount of food ingested during the experimental trial, we analyzed each digital photograph using imageJ (Abramoff et al., 2004). We first measured the entire length of each intestine (in mm), excluding the foregut and the colon, and then the width of the intestine in three locations: the anterior, midpoint, and posterior ends (Venesky et al., 2009). We took the mean of the three width measurements and then estimated the total volume of the intestine. Sera Micron® is green and provides a sharp contrast to an empty intestine, allowing us to calculate the percentage of each intestine filled with food that was consumed during the 5-hour trial. This measure of food intake assumes that tadpoles have equal digestion rates and/or that variation in digestion rates is incorporated into the error term in the statistical model.

We used quantitative PCR (qPCR) to quantify the amount of *Bd* on the mouthparts of each tadpole. Our DNA extractions and qPCR analysis followed the protocols outline in Boyle et

al. (2004) and Hyatt et al. (2007). DNA extractions were diluted 1:100 and processed in duplicate in a Step-One Real-Time PCR system (Applied Biosystems, Foster City, California, USA). We used the average of the two qPCR values in our statistical analyses.

Statistical Analyses.—We used linear regression to examine the relationship between *Bd* infection intensity (using log(Bd+1) as a continuous predictor variable) and food consumption during the 5-hour trial. We ran two separate statistical models that differed only in the response variable. In our first statistical model, we arc sine transformed our response variable (percentage of volume of the intestine with food). Our second statistical model tested the relationship between *Bd* infection intensity and the absolute volume of food consumed (i.e., we did not control for individual differences in the volume of the intestinal tract). Both analyses were conducted using Statistica (StatSoft, Tulsa, Oklahoma, USA; version 6.1).

RESULTS

Upon examination of the data, we noticed that the two tadpoles which consumed all of the food from the microscope slides also cleared the majority of the food from their intestines and thus were not representative of their low food consumption score. However, because we only haphazardly selected four microscope slides to photograph, we could not use the amount of food removed from each slide as a response variable. Thus, we removed these two individuals from the statistical analyses.

As predicted, tadpoles with a greater *Bd* infection consumed significantly less food than tadpoles with less *Bd*, but only after controlling for individual size differences in the volume of each tadpole's intestinal tract ($F_{1,9} = 9.31$; $P = 0.014$; $R^2 = 0.509$; Figure 1A). The absolute volume of food consumed was not predictable based on *Bd* infection intensity ($F_{1,9} = 3.18$; $P =$

0.108; $R^2 = 0.260$; Figure 1B). None of the tadpoles had any noticeable mouthpart deformities (e.g., missing teeth or gaps in the keratinized jaw sheaths).

DISCUSSION

Our result corroborates and builds upon the findings of previous research by demonstrating that *Bd* infection reduces tadpole feeding efficiency and that feeding performance is negatively correlated with *Bd* infection intensity (Venesky et al., 2009). In addition, we now have evidence that *Bd* infection reduces tadpole feeding efficiency in species of three different genera (*Anaxyrus*, *Hyla*, and *Lithobates*), suggesting that this is a generalized pathology associated with *Bd* infection. Whether these generalities indicate a similar mechanism of reduced food consumption is unknown and future studies are needed to elucidate this.

Our finding provides evidence that *Bd*-infected tadpoles suffer a reduced potential to obtain food that is independent of *Bd*-induced changes to the mouthparts of tadpoles. None of the tadpoles used in our experiment had any visible signs of mouthpart damage, which is consistent with previous work which found that *Bd*-induced mouthpart deformations occur 3–4 weeks post infection in another ranid tadpole (Rachowicz and Vredenburg, 2004). Our result, however, is not consistent with previous work showing that reduced food consumption in tadpoles with chytridiomycosis is associated with *Bd*-induced deformations to the keratinized mouthparts (Venesky et al., 2009; Venesky et al., 2010). Generalized pond-type tadpoles all have 2 anterior and 3 posterior tooth rows which function in concert to lift food from a substrate (Venesky et al., 2011a). The fact that tadpoles with the highest *Bd* infections consumed less food than tadpoles with lower infections despite having a full complement of teeth suggests that another

mechanism, such as changes in tadpole behavior, might also contribute to reduced feeding efficiency observed in tadpoles with chytridiomycosis.

Our result that food intake decreases as a function of increasing *Bd* infection intensity has important implications for amphibian disease ecology. Hosts in poor nutritional condition often have dampened immune responses and are more susceptible to parasite infection (Peck et al., 1992; Feder et al., 1997; Houdijk et al., 2000). If food intake is positively correlated with immunocompetence (as it is with dietary protein intake; Venesky et al., 2011b), our result suggests that tadpoles of *L. clamitans* with higher *Bd* infections could suffer a reduced potential to resist other species of parasites. Concurrent infections by multiple species of parasites have been documented in amphibian host species (Sutherland, 2005); yet, it is unclear how co-infections affect host pathology (Romansic et al., 2011; Johnson and Hoverman, 2012). Future research is needed to understand the net effects of reduced food consumption on tadpole health and physiology, and how this relates to resistance to multiple species of parasites.

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FIG. 1.—The relationship between the amount of food consumed by tadpoles of *Lithobates* [= *Rana*] *clamitans* and *Batrachochytrium dendrobatidis* (*Bd*) infection intensity. Each datum point is an individual tadpole. Panel A shows the arcsine transformed percentage of food consumed, relativized by the total length of each tadpole's intestine ($Y = -0.553x + 2.14$; $R^2 = 0.509$). Panel B shows the absolute volume of food consumed by each tadpole ($Y = -77.929x + 297.30$; $R^2 = 0.260$).