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Author for correspondence:
Matthew D. Venesky
e-mail: mvenesky@gmail.com

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Pathogen biology

Optimal digestion theory does not predict the effect of pathogens on intestinal plasticity

Matthew D. Venesky[1], Shane M. Hanlon[2], Kyle Lynch[2], Matthew J. Parris[2] and Jason R. Rohr[1]

[1]Department of Integrative Biology, University of South Florida, Tampa, FL 33620, USA
[2]Department of Biological Sciences, University of Memphis, Memphis TN, 38152, USA

One prediction of optimal digestion theory is that organisms will increase the relative length of their digestive tracts when food resources become limited. We used theory of optimal digestion to test whether tadpoles can adjust the relative length of their intestines when challenged with the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*). The degree of tadpole mouthpart damage, a symptom of *Bd* infections that reduces food consumption, was associated positively with the length of tadpole intestines relative to their body size, consistent with optimal digestion theory. After controlling for mouthpart damage, tadpoles exposed to *Bd* had shorter intestines relative to their body size, opposite to the predictions of optimal digestion theory. One explanation of why tadpoles with higher *Bd* loads have shorter relative intestinal lengths is that they divert energy from maintaining intestinal and overall growth towards anti-parasite defences.

1. Introduction

Defences against parasites can use a substantial amount of an organism's energy budget [1]. To meet the increased energy demands associated with parasite resistance, some host species can offset the costs of resistance by choosing diets that optimize their immune defences [2]. However, if hosts do not have access to high quantity or quality food, they might use alternative strategies to meet the high costs associated with anti-parasite defences.

One such way for an organism to improve energy intake without increasing food consumption is to increase its digestive efficiency by increasing the relative length of its intestine. Optimal digestion theory predicts that digestive tract length should be inversely correlated with food consumption (or food quality), because longer intestines can improve digestive efficiency by increasing food transit time [3]. Although optimal digestion theory has traditionally been used to predict phenotypic changes in digestive tract length as a function of changes in food quantity/quality (reviewed in [4]), there is increasing interest surrounding other ecological contexts that result in intestinal plasticity. For example, Relyea & Auld [5] combined theory on optimal digestion and phenotypic plasticity to show that tadpoles raised with a high density of competitors developed long intestines relative to their body size, whereas tadpoles raised in predator environments developed relatively short intestines.

We conducted a laboratory study using southern leopard frog (*Lithobates sphenocephalus*) tadpoles and tested whether tadpoles can adjust the relative length of their intestine when challenged with the pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*; hereafter '*Bd*'). *Bd* infects the keratinized mouthparts of tadpoles and results in tooth row loss [6], and reduced feeding efficiency [7] and growth [8]. Thus, changes in intestinal length relative to body size could be influenced by a number of mechanisms, such as reduced food intake from mouthpart damage or from trade-offs with anti-parasite

Table 1. The standardized path coefficients and the standard error of the coefficient (in parentheses) for each path in our path analyses.

paths	model 1[a]	model 2[b]	model 3[c]
Bd → damage	0.287 (0.191)	0.189 (0.193)	0.019 (0.267)
Bd → SVL	−0.602 (0.169)	−0.465 (0.176)	−0.222 (0.246)
damage → SVL	0.248 (0.167)	0.163 (0.175)	0.316 (0.246)
damage → gut length	0.321 (0.140)	0.302 (0.122)	0.131 (0.188)
Bd → gut length	−0.407 (0.166)	−0.514 (0.137)	−0.475 (0.183)
SVL → gut length	0.404 (0.158)	0.398 (0.133)	0.427 (0.193)

[a]All tadpoles with *Bd* exposure as a categorical predictor ($n = 28$).
[b]All tadpoles with *Bd* abundance (log + 1) as a continuous predictor ($n = 28$).
[c]Only tadpoles exposed to *Bd* with *Bd* abundance (log + 1) as a continuous predictor ($n = 14$).

defences. We used path analyses to test how *Bd* exposure/infection, mouthpart damage and tadpole size influence relative intestinal length. Borrowing from theory on optimal digestion, we predicted that, when controlling for mouthpart damage, tadpoles that were exposed to *Bd* or those that were infected but relatively resistant to *Bd* (i.e. those with lower *Bd* abundance) would have long intestines relative to their body size to meet the energetic demands of *Bd* resistance. We also predicted that tadpoles with more mouthpart damage, a pathology that reduces food consumption, would be positively associated with intestinal length relative to body size.

2. Material and methods

(a) *Bd* inoculation and tadpole husbandry

Bd was maintained according to standard protocols [9]. We placed 28 southern leopard frog (*L. sphenocephalus*) tadpoles of similar developmental stages in 75 ml water baths and haphazardly assigned them to receive *Bd* or not. Tadpoles in the *Bd*-exposed treatment were inoculated with 1.1×10^7 *Bd* zoospores. We then re-exposed the same tadpoles to 4.5×10^6 zoospores 72 h after the first exposure. Tadpoles from the non-exposed treatment were given a similar volume inoculate without *Bd* zoospores. Twenty-four hours after the final inoculation, we transferred the tadpoles to plastic containers filled with 1 l of aged tap water and raised in the laboratory at 19°C. Tadpoles were fed every 3 days, and we changed the water in each container once per week. Tadpoles were euthanized 46 days after the second dose of *Bd*. See the electronic supplementary material for more details on tadpole husbandry.

(b) Morphological traits

We measured the size (snout–vent length; hereafter 'SVL') of each tadpole with digital callipers (to the nearest 0.1 mm) after euthanasia. We then dissected the mouthparts and the alimentary tract (excluding the foregut and the colon; hereafter 'intestine') of each tadpole. We straightened the intestine on a dissection pan and measured the length of each intestine with digital callipers (to the nearest 0.1 mm). Last, for each tadpole, we calculated a mouthpart deformation score, which was adapted from Venesky *et al.* [6], based on the percentage of teeth missing from each side (left and right) of the five labial tooth rows. See the electronic supplementary material for more details on the deformation score.

(c) Quantitative PCR analysis

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 methods of Boyle *et al.* [10] and Hyatt *et al.* [11]. DNA extractions were diluted 1 : 100 and processed in triplicate in a step-one real-time PCR system (Applied Biosystems).

(d) Statistical analyses

We used path analysis with maximum-likelihood estimation to examine the relationship between *Bd*, mouthpart damage, tadpole size (SVL) and relative intestinal length (by controlling for differences in SVL on changes in intestinal length). We ran three separate path analyses that differed only in whether we treated *Bd* as a categorical predictor (exposed/not exposed) or as a continuous predictor (abundance; the amount of *Bd* in infected and non-infected tadpoles). When considering *Bd* abundance as a predictor, we ran two path models; the first included the *Bd* abundance of all tadpoles (exposed and non-exposed) and second included only the tadpoles exposed to *Bd*. The results from these models were consistent with each other (table 1), and we thus refer to the model using the categorical predictor *Bd* exposure in the main text.

We first tested the hypothesis that SVL and mouthpart damage were influenced by *Bd* exposure. We predicted that tadpoles exposed to *Bd* would be smaller and would have more mouthpart damage compared with non-infected tadpoles. We then tested the hypothesis that relative intestinal length is influenced by *Bd* exposure. Our model included both direct (e.g. *Bd* exposure to intestinal length) and indirect (e.g. *Bd* exposure to mouthpart damage to intestinal length) pathways by which *Bd* could influence relative intestinal length. If defences towards *Bd* infection use energy resources, then the path from *Bd* exposure to intestinal length should be significant and have a positive coefficient. Additionally, if the relative length of the intestine is influenced by mouthpart deformities [12], then the path to intestinal length from mouthpart damage should be significant and have positive coefficients.

All path analyses were conducted using the 'lavaan' package in R. We assessed the relative strength of each path by comparing their standardized coefficients, where higher absolute values indicate a more parsimonious path; indirect paths in our model were determined by multiplying the standardized coefficients [13]. We used two-tailed tests to evaluate significance in all paths with the exception of the path from *Bd* exposure to mouthpart damage (because *Bd* infections can only reduce the number of teeth, not increase them).

3. Results

Tadpoles exposed to *Bd* were smaller ($p < 0.001$) and tended to have more mouthpart damage ($p = 0.059$) compared with non-exposed tadpoles (see figure 1 and electronic supplementary material). Mouthpart damage, however, was not a significant predictor of tadpole size ($p = 0.130$). Contrary to

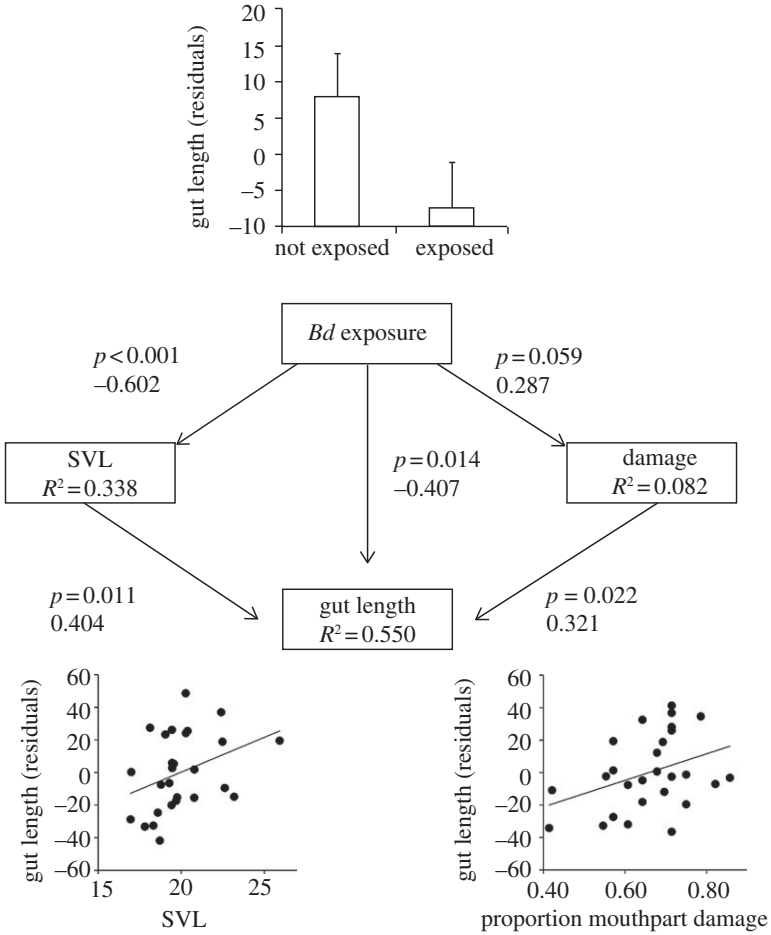

Figure 1. Results of a path analysis testing for relationships among *Bd* exposure, mouthpart damage, tadpole size (SVL) and intestine length in southern leopard frog (*Lithobates sphenocephalus*) tadpoles. Probability values, standardized coefficients and ancillary plots are provided next to each path. For intestinal length, we obtained the residuals from the regression models containing SVL + mouthpart damage, *Bd* + mouthpart damage, and *Bd* + SVL, respectively. Opposite to what we predicted, tadpoles exposed to *Bd* had relatively shorter intestines than non-exposed tadpoles.

our predictions, *Bd* exposure was a significant negative predictor of intestinal length relative to body size ($p = 0.014$; figure 1). *Bd* abundance was also a significant negative predictor of intestinal length relative to body size (see table 1 and electronic supplementary material, figures S1 and S2) and the relative intestinal length of tadpoles that were *Bd*+ was approxiamately 33 per cent shorter than those that were resistant to *Bd* (i.e. exposed but not infected). However, the relative intestinal length of resistant tadpoles did not differ from non-exposed tadpoles, suggesting that tadpoles do not increase the relative length of their intestines to meet the energetic demands of *Bd* resistance. Instead, tadpoles with high *Bd* abundance had shorter intestines relative to their body size than tadpoles with low or no *Bd* (electronic supplementary material, figure S3).

The direct path between *Bd* exposure and intestinal length was the strongest path in our model (path coefficient = −0.407), followed by the indirect paths from *Bd* abundance to SVL to intestinal length (path coefficient = −0.243), and *Bd* abundance to mouthpart damage to intestinal length (path coefficient = 0.092). Our results were consistent with each other regardless of the predictor that we used (table 1).

4. Discussion

Our results show that *Bd*-infected tadpoles of *L. sphenocephalus* were significantly smaller and tended to have more mouthpart deformities compared with non-infected tadpoles, consistent with previous findings [8,12]. Additionally, we found that tadpoles can adjust the length of their intestines relative to their body size. Even after controlling for the effects of *Bd* exposure and differences in body size, tadpoles with a higher proportion of mouthpart deformities had significantly longer intestines (figure 1). This anatomic change probably occurs as a response to reduced food intake, because tadpoles with more mouthpart deformities, even in the absence of *Bd* infection, cannot feed as effectively as tadpoles with fewer missing teeth [14]. This increase in intestinal length relative to their body size is probably a plastic response to increase nutrient absorption and maintain or increase growth rates, consistent with the predictions from optimal digestion theory [3].

Based on optimal digestion theory, one might also predict that tadpoles exposed to *Bd* should develop a longer intestine relative to their body size to increase nutrient absorption to meet the energetic costs of increased resistance. Following that logic, tadpoles that are the most resistant (i.e. tadpoles exposed to *Bd* with zero or low *Bd* abundance) should have longer relative gut length compared with non-exposed tadpoles. Contrary to our predictions, we found that, even after controlling for mouthpart damage, tadpoles exposed to *Bd* had relatively shorter intestines than non-exposed tadpoles. Moreover, tadpoles with higher *Bd* abundance (i.e. those that were less resistant) had relatively shorter intestines than tadpoles with low *Bd* abundance (see the electronic supplementary material, figures S1 and S2), and the relative gut

length of tadpoles that were most resistant did not differ from those not exposed to *Bd* (see the electronic supplementary material, figure S3). These results, coupled with the fact that the strongest path in our path analyses was always the direct path from *Bd* to intestinal length, does not support the hypothesis that optimal digestion theory predicts the effects of *Bd* on intestinal plasticity; instead, our results suggest that the phenotypic change might be pathogen-induced.

In conclusion, our results suggest that tadpoles are capable of increasing the relative length of their intestines when resources are limited, presumably to increase digestive efficiency. Opposite to the prediction from optimal digestion theory, when tadpoles are challenged with *Bd*, the relative lengths of their intestines decrease. *Bd* infections can reduce tadpole food consumption [7] and tadpoles that are resource limited have reduced defences against *Bd* [15]. Thus, one explanation for our results is that tadpoles divert energy resources from maintaining intestinal and overall growth towards deploying anti-parasite defences (e.g. antimicrobial peptide secretions; reviewed in Rollins-Smith *et al*. [16]), similar to the patterns observed by Relyea & Auld [5], who found that predator-induced tadpoles had shorter intestines relative to their body size. Alternatively, *Bd* might somehow directly reduce the relative intestinal length of tadpoles. Future research using optimal digestion theory and phenotypic plasticity are needed to fully understand the costs associated with parasite resistance.

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