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# EXTRINSIC AND INTRINSIC PREDICTORS OF VARIATION IN INFECTION BY *POSTHODIPLOSTOMUM MINIMUM* MACCALLUM, 1921 (TREMATODA) IN SUNFISHES (*LEPOMIS* RAFINESQUE, 1819) FROM EASTERN OHIO

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ABSTRACT: The trematode *Posthodiplostomum minimum* MacCallum, 1921 (white grub) is a common parasite of centrarchid fishes, frequently reaching high prevalence and intensity in local populations. However, significant variation in infection has been observed across *Lepomis* Rafinesque, 1819 sunfish species, which are common and abundant hosts for this parasite. Previous observations suggest both extrinsic factors (e.g., habitat-specific characteristics and environmental parameters) and intrinsic factors (e.g., host size, behavior, and susceptibility) as important predictors of infection in this parasite–host system. In the present study, we evaluated the prevalence and intensity of *P. minimum* in 6 sympatric species of *Lepomis* sunfish (total of 563 individuals) across 9 lakes in eastern Ohio, U.S.A., that range in surface area from 5.6 to 1,448.7 hectares, and assessed the importance of both extrinsic and intrinsic factors as predictors of infection. We found that collection site (extrinsic factor) and host body mass and species identity (intrinsic factors) were the strongest predictors of infection intensity. Specifically, infection intensity was negatively associated with lake surface area and positively associated host body mass. *Lepomis macrochirus* (bluegill sunfish) and hybrid sunfish displayed the highest infection intensities, whereas *Lepomis cyanellus* (green sunfish) and *Lepomis gulosus* (warmouth sunfish) were significantly less burdened. We were unable to conclude if the observed variation among host species was due to host ecology or susceptibility. These general findings were supported by classification and regression tree (CART) analysis, which optimally partitioned variation in individual host infection intensities by using lake size and host body mass (but not host species identity). Although infection intensity was negatively associated with lake surface area (even among host individuals of the same size and species), the causal mechanisms involved remain unresolved and should be the impetus of future work on this parasite–host system.

The trematode *Posthodiplostomum minimum* MacCallum, 1921 (white grub) is one of the most ubiquitous parasites in North America, infecting more than 100 species of centrarchid fishes (Hoffman, 1999; but see Moszczynska et al., 2009; Locke et al., 2010; Lane et al., 2015 for discussion of cryptic species and host specialization), often reaching 100% prevalence in sunfish (*Lepomis* Rafinesque, 1819) host populations (Palmieri, 1973). *Posthodiplostomum minimum* displays a typical 3-host trematode life cycle, where pulmonate snails, centrarchid fishes, and piscivorous birds act as the first intermediate, second intermediate, and definitive hosts, respectively (Hoffman, 1999), although other definitive hosts have been observed (Palmieri, 1976, 1977). Cercariae are released from the snail intermediate host, and actively penetrate host fishes, encysting as metacercariae in the viscera (most frequently the heart, liver, and kidneys), often resulting in substantial pathology, host mortality, and variation in host development (Smitherman, 1968; Spall and Summerfelt, 1969; Hoffman and Hutcheson, 1970; Lutterschmidt et al., 2007). In aquaculture ponds and fish hatcheries, *P. minimum* can reach infection levels sufficient to alter host population survivorship and growth (Smitherman, 1968; J. Wetzel, pers. comm.), making this parasite of economic importance as well (Davis, 1953). However, significant variation has been observed among hosts (e.g., differences in infection rates across host populations and among host species).

The factors involved in producing variation in infection are often grouped into extrinsic sets (properties of the host's surrounding environment) and intrinsic sets (properties of the host individual) (e.g., Kanerek and Zaleśny, 2014). With respect to extrinsic factors, habitat structure and environmental components (e.g., lentic or lotic system type, water temperature, pond or lake size) have been implicated in explaining the observed patterns of infection by *P. minimum*. Hoffman and Hutcheson (1970) observed marked differences in infection prevalence, intensity, and host mortality among study ponds, but they did not address the drivers involved in producing the observed variation. Lewis and Nickum (1964) also observed marked differences in infection intensity among lakes of differing sizes. There, individuals of *Lepomis macrochirus* (bluegill sunfish) from the 2 largest lakes displayed the highest infection loads, and differences in visitation rates by the definitive host, *Ardea herodias* (great blue heron), were speculated as a relevant driver. Given that spatial overlap among the hosts involved in the *P. minimum* life cycle is confined to shoreline or littoral habitat, it is reasonable to predict that infection rates might be higher in bodies of water displaying larger perimeter-to-area ratios (i.e., the relative amount of shoreline habitat would be higher, and cercariae could effectively saturate local environs). Indeed, using parasite richness as an indicator of fish movements and habitat preferences, Wilson et al. (1996) observed that sunfish inhabiting the littoral zone were hosts to more parasites than those using the limnetic zone. Notably, the authors observed a 5-fold increase in *P. minimum* infections in littoral-dwelling sunfish. Although such observations have been made regarding these extrinsic components, very few studies have explicitly tested them in relation to *P. minimum* infection, and the few studies that do exist have very small sample sizes for each host population (typically fewer than 30 individuals).

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Table I. Mean infection intensity, prevalence (in parentheses), and sample size for each *Lepomis* species–lake combination. Sites are listed in order of ascending lake surface area. Dash (—) indicates the absence of a host species.

| Lake (surface area, ha) | *L. macrochirus* | *L. cyanellus* | *L. megalotis* | *L. gibbosus* | *L. gulosus* | *Lepomis* hybrid |
|---|---|---|---|---|---|---|
| Barnesville (5.6) | 46.8 (1.0), n = 46 | 18.0 (0.57), n = 7 | 78.5 (1.0), n = 2 | — | — | — |
| Cambridge (6.4) | 261.4 (1.0), n = 19 | 8.3 (0.67), n = 24 | 94.6 (1.0), n = 8 | 170.1 (1.0), n = 2 | 1.3 (0.22), n = 9 | 248.7 (1.0), n = 8 |
| Caldwell (19.4) | 287.9 (1.0), n = 25 | 58.0 (0.67), n = 3 | — | 314.0 (1.0), n = 2 | — | 441.8 (1.0), n = 7 |
| Belmont (47.3) | 132.3 (1.0), n = 34 | 16.3 (0.75), n = 4 | — | 389.0 (1.0), n = 2 | — | 204.0 (1.0), n = 2 |
| Wolf Run (81.3) | 25.7 (0.97), n = 36 | 37.1 (1.0), n = 21 | 22.0 (1.0), n = 2 | 14.0 (1.0), n = 2 | — | 6.0 (1.0), n = 2 |
| Monroe (535.2) | 34.3 (0.96), n = 24 | 38.8 (0.81), n = 21 | — | — | — | 69.8 (1.0), n = 20 |
| Piedmont (918.6) | 20.9 (1.0), n = 29 | 7.5 (0.93), n = 13 | 40.7 (1.0), n = 12 | — | — | 57.1 (1.0), n = 15 |
| Salt Fork (1,238.3) | 28.1 (1.0), n = 32 | 71.0 (1.00), n = 7 | 18.6 (1.0), n = 23 | — | — | 44.0 (1.0), n = 6 |
| Seneca (1,448.7) | 27.0 (1.0), n = 58 | 14.0 (0.77), n = 13 | — | 10.2 (1.0), n = 13 | 3.0 (0.17), n = 6 | 81.0 (1.0), n = 4 |

Several intrinsic factors have also been implicated in describing *P. minimum* infection patterns. As has been shown in other parasite–host systems (e.g., Poulin and Valtonen, 2001; do Amarante et al., 2015, but see Poulin, 2000), *P. minimum* infection intensities tend to be more severe in larger fish hosts (Avault and Allison, 1965; Wilson et al., 1996). In addition, numerous studies have observed differences in infection prevalence and intensity among different host species. In surveys (Hoffman, 1958; Lewis and Nickum, 1964; Palmieri, 1976) and in experimental infection studies (e.g., Avault and Smitherman, 1965), sunfish species seem to have differing susceptibilities to *P. minimum* infection, leading to markedly different infection loads (intensities). Consistently in the literature, *L. macrochirus* has displayed higher infection prevalence and intensity values (e.g., Buck and Lutterschmidt, 2017; although Gruninger et al., 1977 did report higher prevalence in *Lepomis megalotis* [longear sunfish], whereas infections in *Lepomis cyanellus* [green sunfish] are greatly reduced [e.g., Avault and Smitherman, 1965]). Thus, there is evidence that habitat, as well as host size and species identity, may contribute to *P. minimum* infection patterns. In the present study, we examine the infection patterns of *P. minimum* in mixed-species assemblages of *Lepomis* sunfishes from lakes of differing size (surface area) and shape (shoreline perimeter), and we evaluated extrinsic and intrinsic factors as predictors of those patterns. As such, this the first study in this parasite–host system to explicitly examine infection in the context of habitat (lake) size and structure.

## MATERIALS AND METHODS

### Data collection

From 2012 to 2015, we surveyed *P. minimum* infections in *Lepomis* sunfish from 9 lakes in eastern Ohio, U.S.A. Prior pilot work with *P. minimum* from a small pond in central New Jersey, U.S.A., found very high infection loads, whereas anecdotal evidence based on fish collected from larger lakes suggested lower overall levels of infection. Thus, the lakes used in this study were chosen to test the prediction that infection would be negatively associated with lake size, and they represented a gradient of lake sizes ranging from 5.6 to 1,448.7 ha in surface area (see Table I for lake surface areas). All study lakes are nested within an area roughly 50 km in diameter (Fig. 1). Fish were captured using both hook-and-line and baited-trap (32 × 53-cm collapsible crayfish trap) methods, allowing us to target all age classes (from adult to young-of-the-year [YOY] classes). Collections made in 2012 were very small (only 83 fish were collected, and sample sizes were biased across lakes). More intense sampling was conducted in 2013 and 2015 (no collections were made in 2014). All collections were made between 1 June and 1 October of each year. Fish were humanely euthanized on site by using a technique approved by Waynesburg University's Institutional Review Board, consistent with the Animal Welfare Act (1966). Fish collected in the field were then immediately stored in iced coolers and frozen after transport for later dissection. Fish were thawed, identified to species (including hybrids), measured for length (maximum anterior–posterior length) and mass and then necropsied under dissection microscopes, where the viscera were removed and examined for infection. Metacercariae in intense infections are often found along the swim bladder or elsewhere in the body, and these metacercariae were included in count data. We attempted to precisely quantify the total number of encysted metacercariae in each host.

### Statistical analysis

Using the method described by Shapiro and Wilk (1965), we determined that our response variable (individual infection intensities, *Z*-standardized based on mean and 2 SDs) did not conform to a normal distribution (at $P < 0.05$; Fig 2). Thus, we

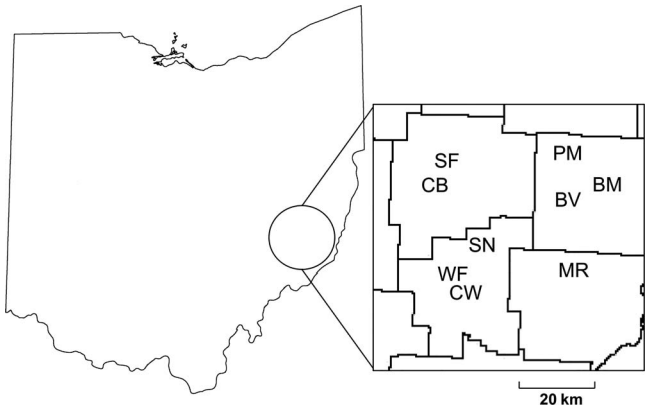


FIGURE 1. Map of Ohio, U.S.A., and the 4 counties containing the surveyed lakes. SF = Salt Fork, CB = Cambridge Reservoir, PM = Piedmont, BM = Belmont, BV = Barnesville Reservoir, SN = Seneca, WF = Wolf Run, CW = Caldwell, MR = Monroe. The maximum distance between any 2 lakes is 53 km.

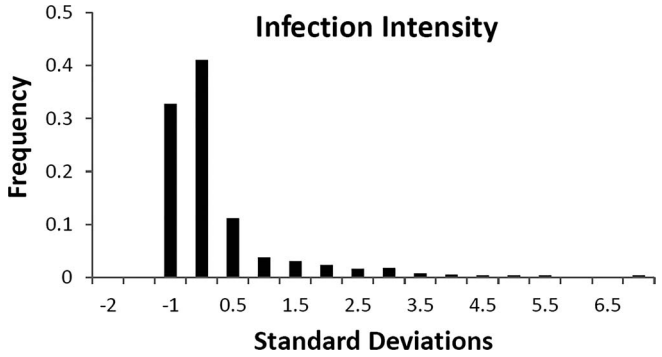


FIGURE 2. Negative binomial frequency distribution for *Z*-standardized values (based on mean and 2 SDs) of infection intensity in host fishes. Most host individuals displayed either no infection or very low infection intensities (i.e., negative values relative to mean infection intensity), whereas a small number of individuals displayed exceedingly large infection intensities (values well beyond 2 SDs). For scale, the mean infection intensity was 80.13 and the maximum infection intensity was 888. Thirty-five individuals had no infection.

opted for nonparametric methods of statistical analysis. We used a generalized linear model (GLM) to assess the importance of both extrinsic and intrinsic variables in predicting *P. minimum* infection. We began with the following predictor variables: site (lake collected from), collection year, lake surface area (ha), lake perimeter (km), ratio of lake perimeter-to-surface area, host species, host mass (g), and host length (cm). Because the distribution of infection intensities was over-dispersed (variance was 2 orders of magnitude larger than the mean), we assumed a negative binomial distribution for the response variable and designed our GLM by using the MASS package (Venables and Ripley, 2002) in R v3.4.0 (R Development Core, 2017). When using categorical variables as predictors in a GLM, one of the levels is used as a reference, and the resulting coefficients for all other levels are relative to this reference level. Thus, for host species, we assigned *L. macrochirus* as the reference group, because this species was the most abundant, occurred in all lakes, and displayed high average infection intensities. Cambridge Reservoir, which displayed high across-the-board intensity values and contained all host species, was used as the reference level for the site variable.

Several variables were either highly correlated or were perfectly co-varying. For example, values for lake surface area and perimeter would be identical for all fish collected from a given site and thus functioned as redundant categorical (nominal) variables. Still, these variables had to be considered individually because, for example, it could be that lake surface area is a weak predictor of infection intensity (in that there is no clear pattern of increase or decrease in infection intensity with increasing or decreasing lake surface area), whereas collection site could still be a strong predictor. Based on the restricted maximum likelihood procedure offered in JMP v13.0.0 (SAS Institute Inc., 2017), Spearman's rank correlation values indicated that host mass and length were also strongly correlated ($r = 0.911$, $P < 0.0001$). In searching for an optimal set of predictor variables, we examined combinations of these co-varying and highly correlated variables, and we eliminated those that were either nonsignificant ($P > 0.05$) as predictors of infection intensity, or those that did not greatly affect the Akaike information criterion (AIC) score (Akaike, 1974) for each candidate model. The contribution of each variable to the AIC score for each model was evaluated and ranked using the "step()" function in R v3.4.0. Our approach was similar to the "backwards stepwise selection" process described by James et al. (2013) in which selection begins with the full set of predictors and iteratively removes the least useful variable (this is also similar to the approach used by Lane et al., 2015).

Using the optimal set of predictor variables as determined in our GLM, we also performed a data partition using the classification and regression tree (CART) procedure in JMP v13.0.0. Whereas other multivariate methods (such as GLMs) assess the overall significance or predictive power of multiple variables in relation to a response variable, CART analyses attempt to optimally partition variation in a response variable based on some set of predictor variables. The advantage of this approach is that predictor variables can be split at cut-points that optimize partitioning of the response variable (Phelps and Merkle, 2008). For example, rather than determining that fish length is a significant predictor of infection intensity, CART analysis will find the cut-point along the distribution of fish lengths that optimally partitions the observed variance in infection intensity. Based on our CART analysis, we then estimated the main and total effect of each predictor variable by using the partial least squares method provided in JMP v13.0.0 (Wold, 1994). To avoid overfitting of the model (a concern with CART models; see Gray and Fan, 2008), we truncated the number of bifurcations (partitions) based on observed declines in LogWorth values [calculated as $-\log_{10}(P\text{-value})$], or when the model repeatedly split a predictor variable (e.g., multiple cut-points along the distribution of fish lengths). Support for this approach was demonstrated in the abrupt decline in variable importance (as indicated by a decline in effect magnitude).

## RESULTS

In total, 563 *Lepomis* sunfish, representing 6 species were collected and examined for *P. minimum* infection: 303 *L. macrochirus* (bluegill sunfish), 113 *L. cyanellus* (green sunfish), 47 *L. megalotis* (longear sunfish), 21 *Lepomis gibbosus* (pumpkinseed), 15 *Lepomis gulosus* (warmouth sunfish), and 64 *Lepomis* hybrids. We observed infection prevalence values that approached 1.0 for all species except *L. cyanellus* and *L. gulosus* (average prevalence was 0.80 and 0.19 for those species, respectively). Intensity values ranged from 0 to 888, with average intensities varying across sites and host species (a more detailed presentation of the data is provided in Table I). We also note that in 1 *L. gulosus* identified as infected by *P. minimum*, only a few cysts were observed and all were empty (lacking any larvae).

Our final GLM, which included collection site, host body mass, and host species identity, was able to account for 69.57% of the observed variation in infection intensity (Table II; residuals presented in Fig 3). With respect to the extrinsic variables we considered, the following predictor variables were included in the initial GLM: collection site, collection year, lake surface area, lake perimeter, and the perimeter-to-area ratio (along with intrinsic variables discussed below). Neither year nor perimeter-to-area ratio were significant predictors of infection intensity ($P = 0.790$ and 0.067, respectively), and they were removed from the model. Site, lake surface area, and perimeter were all highly significant predictors ($P < 0.0001$ in all cases), but because they were

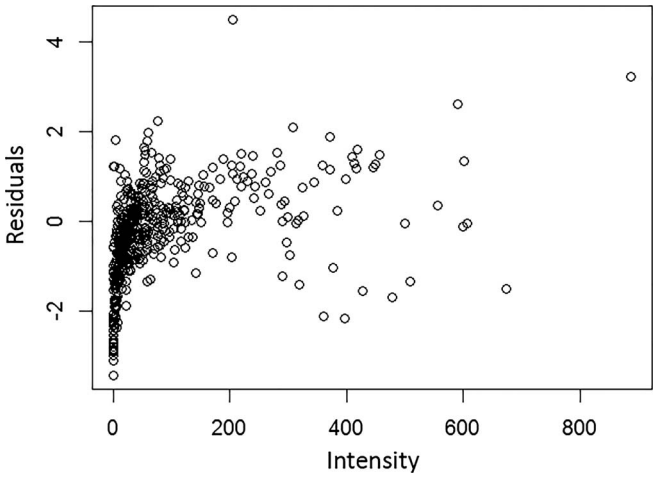


FIGURE 3. Residual variance for the optimal general linear model. We observed a broadly symmetrical distribution of residuals, with most predicted points. However, there was evidence of 0 inflation, and several outliers (beyond 2 SDs) were observed. Null deviance for the model was 1,564.39, and the residual deviance was 644.01.

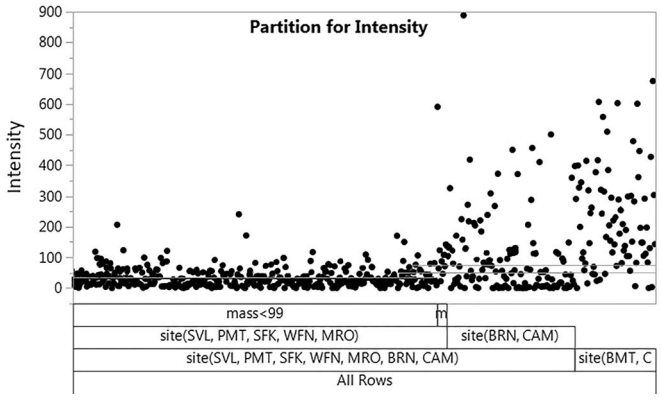


FIGURE 4. Visual output of CART analysis. Infection intensity was optimally partitioned by three cut-points along two variables; site and host mass. All infection intensities over 240 cysts could be collapsed into a group of fish hosts over 99.0 g and collected from the 4 smallest lakes (Barnesville Reservoir, Cambridge Reservoir, Caldwell Lake, and Belmont Lake).

perfectly co-varying, they could not be considered in the GLM model simultaneously (i.e., they produced singularities). Collection site was preferred over the other 2 variables because its inclusion yielded a lower relative AIC score, and its removal resulted in the largest increase in AIC score (an increase of 249.10) and residual variance (a 41.12% increase) based on our step analysis. By these same metrics, the site variable was determined to be the most significant overall predictor of infection intensity.

In comparison with the reference level for the site variable (Cambridge Reservoir), all but 1 of the sites (Belmont Lake) displayed significantly lower infection intensities. The magnitudes of these differences (negative coefficients) were particularly large among the 5 largest lakes in the data set, suggesting that lake size was the primary driver associated with site. The importance of lake size as a driver of infection intensity was further supported by our CART analysis, in which collection site was also identified as the strongest predictor of infection intensity (total effect = 0.821, $P < 0.0001$), and cut-points along this predictor variable directly separated large and small lakes. The first optimal cut-point in the partition separated Belmont Lake and Caldwell Lake from all other sites. The second partition separated Barnesville Reservoir and Cambridge Reservoir from the remaining sites. Collectively, the first 2 optimal cut-points separated the 4 smallest lakes from the 5 remaining (larger) lakes. No subsequent partitions used the site variable.

With respect to intrinsic factors, infection intensity varied with both host body size (mass and length) and species identity. Although both host mass and length were highly significant predictors of infection intensity in our GLM, they were tightly co-varying, and host mass was preferred in the final GLM because its removal had a larger effect on AIC score and residual variance (an increase of 173.5 in AIC score and a 27.21% increase in residual variance). The importance of host body mass was corroborated by our CART analysis, which identified hosts above 0.99 g as the third optimal cut-point in the partition (total effect = 0.458, $P < 0.0001$). When considered alongside the first two partitions resulting from cut-points based on the site variable, the CART analysis was able to correctly identify all hosts with infection intensities above 240 cysts, and the remaining variation was very small (Fig. 4). The 4 subsequent partitions in the CART analysis were also cut-points along the distribution of host masses (evidence of over-fitting), were substantially lower in LogWorth values, and had almost no effect on the AIC score.

Host species was also identified as a strong predictor of infection intensity in our GLM, and removal of this variable increased the AIC score by 205 and the residual variation by 33.35%. The final GLM indicated that host species *L. gulosus* and *L. cyanellus* displayed dramatically lower infection intensities than the reference host species, *L. macrochirus* (coefficients of –4.80 and –1.03, respectively; $P < 0.0001$ for both species). Both *L. gibbosus* and *L. megalotis* also displayed significantly lower infection intensities, whereas hybrid sunfish infection intensities were significantly higher (see Table II). However, host species was not identified as a strong predictor of infection intensity by our CART analysis (total effect = 0.084). Thus, only the first 3 cut-points (described above) were kept in the final partition.

## DISCUSSION

Determining the sources of variation in infection prevalence and intensity in wild populations remains an area of intense interest for parasitologists and ecologists alike (reviewed in Guégan et al., 2005; Pullan et al., 2014). Generally, both extrinsic factors (e.g., geographic, seasonal, or otherwise environmental) and intrinsic factors (e.g., taxonomic, genetic, ontogenetic, behavioral, or physiological) have been implicated in producing heterogeneity in the patterns of infection in host populations (e.g., Ondračková et al., 2004; Poulin, 2006; Kanarek and Zaleśny, 2014). The *P. minimum–Lepomis* spp. parasite–host system offers an opportunity to examine potential determinants of infection patterns because *P. minimum* displays marked variation in infection prevalence and intensity in host populations, and both the parasite and its host fishes are ubiquitous in freshwater ecosystems (making them easy to study). In the present study, we examined the patterns of *P. minimum* infection in *Lepomis* spp. from 9 freshwater lakes of differing size and shape, and we found strong evidence for both extrinsic and intrinsic factors as

Table II. Final general linear model results. Reference level for the site variable was Cambridge Reservoir. There was no statistical difference between intensity values observed in the reference level and Caldwell Lake. Estimate coefficients represent differences in *y*-intercept for each site against Cambridge Reservoir. Infection intensities for the largest 5 lakes (Wolf Run Lake to Seneca Lake) were markedly lower than Cambridge Reservoir. *Lepomis macrochirus* was used as the reference level for the Species variable. Infection intensities in hybrid sunfish and *Lepomis megalotis* did not differ significantly from *L. macrochirus*. Both *Lepomis cyanellus* and *Lepomis gulosus* displayed markedly lower infection intensities. Host mass was significantly positively associated with infection intensity.

| Variable | Estimate | SE | *Z*-value | Pr(>\|z\|) |
|---|---|---|---|---|
| Site | | | | |
| Barnesville | −0.478 | 0.169 | −2.823 | 0.0048 |
| Caldwell | −0.456 | 0.226 | −2.018 | 0.0435 |
| Belmont | 0.294 | 0.181 | 1.628 | 0.1036 |
| Wolf Run | −1.126 | 0.163 | −6.930 | <0.0001 |
| Monroe | −0.693 | 0.160 | −4.320 | <0.0001 |
| Piedmont | −1.711 | 0.161 | −10.62 | <0.0001 |
| Salt Fork | −1.578 | 0.166 | −9.528 | <0.0001 |
| Seneca | −1.295 | 0.153 | −8.473 | <0.0001 |
| Species | | | | |
| *L. cyanellus* | −1.035 | 0.104 | −9.998 | <0.0001 |
| *L. megalotis* | −0.247 | 0.147 | −1.675 | 0.0938 |
| *L. gibossus* | −0.655 | 0.208 | −3.156 | 0.0016 |
| *L. gulosus* | −4.808 | 0.440 | −10.93 | <0.0001 |
| Hybrid | 0.246 | 0.134 | 1.835 | 0.0665 |
| Host mass | 0.021 | 0.0015 | 13.709 | <0.0001 |

predictors of infection intensity. More specifically, this study is the first to show large differences in patterns of *P. minimum* infection associated with habitat (lake) size.

## Extrinsic factors

Collection location (site) was identified as the most important predictor of infection intensity in host fishes. More specifically, our GLM and CART analyses demonstrated that infection intensity was negatively associated with sites representing larger lakes. This runs counter to the findings of Lewis and Nickum (1964) in which infection by *P. minimum* was greatest in the 2 largest lakes sampled (however, those authors' sample sizes were all 25 fish or less for each lake, and lake size was not explicitly tested in the study). Although their sample sizes were confined to 30 individuals or less at each site, Zelmer and Campbell (2011) found no clear relationship between *P. minimum* infection intensity and pond size. Among 5 ponds of differing sizes, the second smallest pond presented the largest mean infection intensity, whereas the second largest pond produced the second largest infection intensity. So, although our study, which considered more sites and a larger host data set, found strong evidence of a negative relationship between infection intensity and lake surface area, the generality of this pattern remains unclear. However, it should be noted that, based on our CART analysis, it could be that infection patterns related to habitat (lake) size were simply not discernable in the Zelmer and Campbell (2011) study, because only ponds (i.e., small habitats relative to our sampled lakes) were considered. There may be a lake size threshold at which differential infection can be detected.

We also argue that the patterns observed in Belmont and Wolf Run lakes suggest lake surface area alone may not be sufficient to explain the variation in *P. minimum* infection. Although similar in size to Wolf Run Lake, Belmont Lake clearly grouped with smaller lakes in terms of infection intensity values, whereas Wolf Run Lake presented the lowest overall intensities of any lake considered (even though 4 lakes in this study are larger in surface area). We think additional extrinsic factors should be taken into consideration in trying to explain the observed patterns of infection, as has been suggested in studies dealing with infection patterns in other parasite–host systems. For example, Hall et al. (2010) demonstrated that, along with lake size (and shape), food web structure, water current, and turbulence all play into infection dynamics in *Daphnia*. Rossiter and Sukhdeo (2012) found that snail hosts confined to intertidal pannes were significantly more likely to harbor 2 species of trematodes than snails found on tidal mudflats, and they speculated that infective larvae might saturate confined areas (pannes), greatly increasing parasite–host contact rates (wave action and turbulence were also suggested as putative drivers; see also Fingerut et al., 2003). Marcogliese and Cone (1991) found that parasite communities associated with *Salmo salar* (Atlantic salmon) and *Salvelinus fontinalis* (brook trout) were markedly different in small, medium and large lakes, but also that larval trematode infections were more frequent in shallow lakes.

These studies suggest that spatial overlap among hosts and infective stage larvae might be a critical driver in infection dynamics, and consideration of these factors could add context to patterns of infection in relation to system size. With respect to the present study, it is well established that sunfish tend to prefer complex habitats (snags, downed trees, vegetation) and are bound to coastline habitats (as are the other hosts in the *P. minimum* life cycle). Sunfish are not typically considered open pelagic or deep-water species, and the role of host–host (and thus, host–parasite) overlap has already been suggested as a driver in *P. minimum* infections in centrarchid fishes (e.g., Anderson et al., 2015). Furthermore, Wilson et al. (1996) directly demonstrated that *L. macrochirus* using littoral (shoreline) habitats were significantly more burdened by *P. minimum* than those dwelling in adjacent "open water" (limnetic) habitats. Although we did not extensively evaluate the physical characteristics of each lake, we note that larger lakes tended to have greater average depths, maximum depths, and steeper slopes from shoreline to profundal zone. For example, most of the shoreline of Piedmont Lake (918.6 ha) is very steep, rapidly transitioning from shallows to more than 8 m in depth. This may reduce snail habitat, bird and mammal feeding habitat, shoreline water temperatures, and contact rates between hosts and free-living larval stages of *P. minimum*. That is, we suggest that the larger lakes in our study might display narrower littoral zones, and thus minimize zones of high infection risk. This would be consistent with the extremely low infection levels observed for the aforementioned Wolf Run Lake, which has a maximum depth of 15.5 m, is very steep along its shoreline, and is greater than 9 m in depth nearshore. Additional considerations regarding habitat structure and infection risk are discussed below in the context of shifts in host habitat use (both ontogenetic and competition driven).

Another possible explanation for the observed patterns of infection is that smaller lakes display increased infection

intensities because host densities are greater (reviewed in Patterson and Ruckstuhl, 2013). However, full surveys of host abundances were not conducted in our study, and our collection methods were not aimed at quantifying host relative abundance or density (although these have been implicated as drivers of infection in others studies, e.g., Krkošek, 2010; Clausen et al., 2012; but see Buck and Lutterschmidt, 2017). Thus, we are not able to relate host abundance or density to the observed patterns of infection in *Lepomis* spp. However, the numerical dominance of *L. macrochirus* in our study is consistent with nearly all prior studies of sunfish in ponds and lakes of the Midwest (e.g., Mittelbach, 1988), and its relative abundance cannot be excluded as a driver of the elevated infection intensities seen in this species (as has been shown for other parasite–host systems, e.g., Bagge et al., 2004).

### Intrinsic factors

As has been seen in other host–parasite studies in fishes (e.g., Zelmer and Arai, 1998; do Amarante et al., 2015; discussed in Poulin, 2011), we found that host body size (mass) was an important predictor of *P. minimum* infection intensity. Both our GLM and CART analyses identified host body mass as the secondary determinant of infection intensity, and we observed a strong positive relationship between infection intensity and host size (both mass and length). Larger hosts displayed larger infection intensities and the size distributions of fishes from smaller lakes (namely, Belmont Lake) did include larger individuals. Taken in the context of the observed negative relationship between infection intensity and lake surface area, it is important to note that infection intensities were higher in smaller lakes, even among individuals of the same size, and mass-specific infection loads (metacercariae per unit mass) were also larger. As indicated in our analyses, site (not host mass) was the primary predictor of infection intensity.

We suggest that future work on the *P. minimum–Lepomis* spp. system should also include consideration of the effects of differences in host size and species in relation to habitat preferences. Sunfish (particularly *L. macrochirus*) are known to hatch in the littoral zone and migrate into the limnetic zone to feed on zooplankton as YOY. They then return to the littoral zone for several years, before again preferentially exploiting the limnetic zone to feed on larger zooplankton (Mittelbach, 1981; Dimond and Storck, 1985; Werner and Hall, 1988). Given that Wilson et al. (1996) found evidence for differential infection dynamics in littoral and limnetic habitats, respectively, it may be that the increased infection intensities observed in larger fishes are due to changes in habitat use (i.e., small fish are less exposed while in the limnetic zone, and contact rates with infective larvae increase when older fish move back into littoral habitat). As mentioned, in lakes displaying narrowed littoral habitats (i.e., such as the larger lakes in our study), infection risks during ontogenetic shifts in host life cycles might be lower, resulting in lower infection intensities. However, it is worth noting that Avault and Allison (1965) subjected 2 size classes of *L. macrochirus* to identical artificial infection trials and found significantly larger infection intensities in the larger size class. Treating hosts as habitat for the parasite, it may be that larger hosts simply represent larger surface areas for contact with infective stage larvae. However, this would not explain the observed variation in infection intensities among similarly-sized fishes from small and large lakes.

With respect to host species identity, we observed high intensity and prevalence values in all host fishes except *L. cyanellus* and *L. gulosus*, with infections being most intense in *L. macrochirus* and in hybrids. Our findings were broadly consistent with the existing literature on *P. minimum* infection patterns in different sunfish species (e.g., Fellis and Esch, 2004). Anderson et al. (2015) found lower infection intensities in co-occurring *L. cyanellus* individuals, and they speculated that this pattern may result from differential susceptibility to the parasite. Avault and Smitherman (1965) subjected several sunfish species (along with other fish species) to artificial infection by *P. minimum* and demonstrated that although nearly all centrarchid fishes contracted infections, *L. macrochirus* and its hybrids were disproportionately burdened by the parasite. They were also unable to demonstrate infection of *L. gulosus* or *L. cyanellus* by *P. minimum*, and infection was noted in just 12.5% of *L. cyanellus* hybrids. In the present study, we find that wild populations of both *L. cyanellus* and *L. gulosus* display markedly lower infection intensities than co-occurring host species. Based on the findings of Avault and Smitherman (1965), we speculate that this pattern may be partially explained by innate differences in sunfish species' susceptibilities to *P. minimum*.

As with the role of size-based ontogenetic shifts in habitat usage, we suggest that future work might consider infection dynamics in the context of species-based competition regimes among sympatric sunfish. *Lepomis macrochirus* is known to modify its feeding preferences in the presence of *L. cyanellus*, shifting from foraging for large prey in complex habitat (vegetation associated with the littoral zone) to consuming available prey in the limnetic zone (Werner and Hall, 1976, 1979; but in a different study Werner and Hall, 1977 found no such pattern). A similar response has been observed in *L. gibbosus* in the presence of *L. cyanellus* (Werner and Hall, 1976), and *L. macrochirus* and *L. gibbosus* are also known to compete intensely (particularly early on, in small size classes; Arendt and Wilson, 1997; see also Mittelbach, 1988; Robinson et al., 1993; Osenberg et al., 1994). This is important, given that *L. cyanellus* is both a superior competitor and apparently displays reduced susceptibility to *P. minimum*. It seems likely that modification of fish behavior (e.g., changes in habitat usage and foraging behavior) in response to competition might also alter contact rates with infective stage larvae (and perhaps even physiological responses to infection).

In summary, although the present study demonstrates significant differences in *P. minimum* infections in relation to lake surface area, and it supports previous observations regarding differential infection loads in relation to host size and species identity, the roles that habitat structure, ontogenetic shifts in host behavior, and size- and species-based competition regimes play in *P. minimum* infection remain unresolved. Still, based on the results presented here, the extensive existing literature on *P. minimum* in snail, fish, and bird hosts, and the known behavioral shifts among *Lepomis* spp., this host–parasite system seems ideal for future exploration of the interactions among host species, habitat structure, community dynamics, and parasitism.

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