Population-level effects of parasitism on a freshwater ecosystem engineer, the unionid mussel *Anodonta anatina*

Joshua I. Brian | Sebastian E. Dunne | Christine L. Ellis | David C. Aldridge

Aquatic Ecology Group, Department of Zoology, University of Cambridge, Cambridge, U.K.

**Correspondence**
Joshua I. Brian, Aquatic Ecology Group, Department of Zoology, The David Attenborough Building, University of Cambridge, Cambridge CB2 3QZ, U.K.
Email: jib33@cam.ac.uk

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**Abstract**

1. Parasites can negatively affect hosts at individual, population, and species-level scales. However, the link between individual- and population-level impacts is often poorly understood. In particular, the population-level response to parasitism may alter wider ecosystem dynamics if animals with ecosystem engineering capabilities are infected.

2. Here, we examine the effects of parasitism on a freshwater ecosystem engineer, the unionid mussel *Anodonta anatina*, at two different sites. We study three common parasites: the digenean trematode *Rhipidocotyle campanula*; the unionicolid mite *Unionicola intermedia*; and the ectoparasitic invasive zebra mussel *Dreissena polymorpha*. As well as demonstrating the individual-level effects of parasitism on the native host mussel, we construct a simple model to estimate the reduction in population-level reproductive output caused by parasites.

3. We show that both infection prevalence and intensity were population-specific, with one site having more than three times as many native mussels infected by trematodes and mites than the other, but more than four times fewer mussels afflicted by invasive zebra mussels. Negative reproductive consequences for individual host mussels were documented as a result of parasitism, with trematodes causing castration at both sites. Mites were also correlated with a reduction in the viability of larval offspring (glochidia) by more than 25%, but only at one site, suggesting some potential impacts of parasitism may be population specific. The population-level model shows that parasitism alone reduces larval output of the two populations by 10% and 13%, respectively.

4. Our study takes the important step of scaling individual-level effects of parasitism to population-level processes, and highlights the influence that parasites may have in the population dynamics of unionid mussels. Given the ecosystem engineering capabilities of *A. anatina*, such effects may have important impacts on the wider biota.

5. Even at relatively low prevalences, the observed effects of parasites on native mussel populations suggests that parasitism must be considered in the conservation of freshwater mussels, one of the world’s most globally imperilled faunal
Parasites are ubiquitous feature of ecosystems (Lafferty et al., 2008), and host-parasite interactions show an extensive evolutionary history (Zhang et al., 2020). While parasites are crucial for the functioning of ecosystems (Hudson et al., 2006), they may affect individuals in a range of negative ways, such as reducing fecundity (Auld et al., 2012) and lowering body condition (Sánchez et al., 2018). The degree of this effect depends on the virulence of the parasite (Figure S1). In turn, these individual-level effects can scale to the population level (Figure S1), and may significantly impact the success of populations. There is evidence of this link from freshwater ecosystems; for example, the invasion success of the non-native amphipod Dikerogammarus haemobaphes is predicted to be limited by microsporidian parasites (Bojko et al., 2019), and there is a correlational link between fungal parasites and freshwater fish and amphibian declines (Rowley et al., 2013). In extreme cases, the impacts of parasitism on populations are observed as localized outbreaks with often devastating consequences (e.g. Katsanevakis et al., 2019). It is likely that parasite-induced population fitness differences are common to a less observable degree, but in general the link between individual impacts and population-level effects is poorly understood and requires further characterisation (Wood & Johnson, 2015).

The impacts of parasitism are particularly pertinent for populations of ecosystem engineers, as parasites could affect their influence on the environment (Dunn & Hatcher, 2015). Freshwater mussels (Unionoida) are one such ecosystem engineering group. Through their burrowing, they increase oxygenation (Vaughn & Hakenkamp, 2001), and their extensive filtering enhances water clarity and facilitates nutrient deposition (Howard & Cuffey, 2006). They are associated with increased biodiversity in the rivers and lakes they inhabit (Aldridge et al., 2007; Chowdhury et al., 2016), and play a significant role in the healthy functioning of freshwater ecosystems. Unionid mussels are affected by a broad range of parasites (Brian & Aldridge, 2019); however, few studies of parasitism in this group examine the impact on the hosts. Of the 237 studies reviewed in Brian and Aldridge (2019), which assessed all published host-parasite records for North American and European freshwater mussels, just 20% looked at the effects of parasitism, and these studies are largely focused at the level of the individual mussel. However, the negative fitness consequences of parasites on individual mussels may scale to population-level metrics of success, with subsequent impacts on the wider ecosystem.

The most commonly studied parasites of freshwater mussels are digenean trematodes, unionicolid mites (both endoparasites), and zebra mussels (an ectoparasite). The effects of trematodes are generally well-understood, causing significant reductions in reproductive output and potentially castrating their hosts (Jokela et al., 1993; Taskinen et al., 1994). However, the effects are generally reported at an individual level (e.g. is the mussel producing offspring or not), with no quantification of population-level effects (but see Taskinen & Valtonen, 1995). The effects of unionicolid mites are less clear, with some studies reporting parasitic behaviour affecting the health of the host (e.g. Fisher et al., 2000) and others providing evidence for commensalism (e.g. McElwain et al., 2016). Previously reported parasitic behaviours of mites include consumption of gill tissue (Fisher et al., 2000; Walker, 2017), thus interfering with larval brooding by females that use the gills as marsupia for their developing glochidia. However, similarly to trematodes, to our knowledge no studies have examined the population-level impacts of mite parasitism on mussels. Zebra mussels are invasive dreissenids that have spread from the Ponto-Caspian region of eastern Europe throughout North America and Europe (Aldridge et al., 2004). These attach to the shell of native mussels and intercept food particles drawn in by the underlying native mussel, thus providing a physical and physiological stress that can lower body condition (Sousa et al., 2011).
2 | METHODS

2.1 | Study species and sampling

The non-endangered unionid duck mussel Anodonta anatina (Linnaeus 1758) was chosen as our study species as it is common throughout Europe (Lopes-Lima et al., 2017), and is infected with a broad range of parasites (Brian & Aldridge, 2019, 2021). We collected 60 mussels from each of two sites that are hydrologically connected but separated by approximately 20 km: the River Great Ouse at Brandon Creek (52.5002°N, 0.3650°E; henceforth BC) and the Old West River at Stretham, a tributary of the Great Ouse (52.3343°N, 0.2243°E; henceforth OW).

We sampled mussels by hand from the river margin, retaining mussels of all sizes until 60 had been collected at each site. Sampling of both sites took place on a single day (7th November 2019). We transported mussels back to the laboratory in the river water they were collected in, and held them in aerated water at ambient temperature (8°C) until subsequent dissection (maximum of 10 days until dissection). Transfer of parasites between individuals while being held is highly improbable, as all parasites considered were either encysted (mite larvae and eggs, zebra mussels) or require multiple hosts to complete their life cycle (trematodes) and so cannot be directly infected by conspecifics.

2.2 | Dissection and parasite quantification

Prior to dissecting each native mussel, we removed and counted any invasive zebra mussels (Dreissena polymorpha) present on their shells. We then measured the maximum length of each native mussel to the nearest 0.1 mm with Vernier callipers, before sacrificing mussels by slicing the posterior and anterior adductor muscles. The mantle of each A. anatina was inspected under a dissecting microscope to identify the presence of encysted larvae and eggs of the parasitic mite Unionicola intermedia (henceforth referred to as mites), and scored as a binary presence/absence. We then inspected the gonad of the mussel to identify and quantify infection with the digenean Rhipidocotyle campanula (henceforth referred to as trematodes), following Brian and Aldridge (2020). Briefly, we removed the visceral mass and made an incision c. 1 cm from the posterior end, then used forceps to remove samples of gonad tissue which we squashed between two glass microscope slides. These gonad squashes were inspected under 40x magnification to identify trematode infection. If trematodes were present, we photographed these gonad squashes (12 replicate photographs in total) and used the program ImageJ to estimate the percentage of each mussel gonad filled with trematode tissue. In total, we therefore obtained presence/absence data for zebra mussels, mites, and trematodes, in addition to a quantitative level of infection for zebra mussels and trematodes. While zebra mussels are themselves affected by parasites, a comprehensive review of invasive and native mussels from Europe and North America demonstrated that zebra mussels do not host U. intermedia or R. campanula (Brian & Aldridge, 2019), and so zebra mussel parasites were not considered further in the present study.

Following identification of parasites and processing of gravid mussels (see below), we dried all mussel tissue to constant mass for 48 hr and then weighed it to the nearest 0.001 g; we also weighed the dried shells for each mussel to the nearest 0.001 g.

2.3 | Processing gravid mussels

For all mussels, we removed the right outer demibranch to determine the sex of the mussel and quantify gravidity if applicable. First, demibranchs were weighed to the nearest 0.001 g. Gravid mussels were immediately apparent by the swollen appearance of the demibranch, indicating the marsupia were filled with glochidia (larval mussels). For these mussels, we quantified glochidia viability. This involved breaking open the centre of the removed right demibranch using fine forceps and taking a sample of glochidia. These glochidia were gently mixed into water on a petri dish, and table salt (NaCl) was added to determine glochidial viability (following Bringolf et al., 2007). We examined the sample of glochidia under a microscope and the first 100 were counted. Glochidia that were snapping or had closed were counted as viable, and any that remained open were counted as non-viable, with overall viability being expressed as a percentage.

For mussels that were not gravid, we gently teased apart the removed demibranch under a dissecting microscope to search for marsupia, the interlamellar tubes that bear glochidia. Mussels lacking marsupia were classed as male, while mussels possessing marsupia (in addition to those that were gravid) were classed as female/hermaphroditic.

2.4 | Statistical analysis

All statistical analyses were executed in R v.3.6.3 (R Core Team, 2020). We utilised logistic regression (using a generalised linear model with a logit link) to explore significant factors explaining the presence or absence of parasites. We modelled the presence of each of the three parasites (zebra mussels, mites, trematodes) as the response variable in turn, and included site, mussel weight, mussel length, gravid status, and the other two parasites as possible explanatory variables. Further, for zebra mussels and trematodes (which had quantitative data), we explored the influence of site and mussel length on the intensity of infection. For the site comparisons we used a non-parametric Kruskal–Wallis test, as assumptions of parametric tests could not be met using either raw or transformed data. For the trematode length analysis, we used standard linear regression. For the zebra mussel length analysis, we utilised standard linear regression on log-transformed data using site BC only, as there was a maximum of two zebra mussels on any given native mussel at OW (median = 0).

Next, we examined the impact of factors on native mussel weight, using three general linear models with a Gaussian link,
which had tissue weight, shell weight, and total weight as the response variable, respectively. Explanatory variables included were site, gravid status, and the three parasites. Assumptions were checked and verified to have been met using normal Q-Q and residuals versus fitted values plots. In addition, for these and the above analyses that included multiple explanatory variables, we verified an absence of multicollinearity by confirming the variance inflation factors were all <5.

Finally, we examined the effect of parasites on the reproductive capacity of native mussels. First, we utilised logistic regression with gravidity (yes/no) as the response variable, and site, mussel length and the three parasite types as possible explanatory variables. Next, we used non-parametric Kruskal–Wallis tests to look at the effect of zebra mussel and mite presence on glochidia viability. This was not attempted for trematodes, as no trematode-infected mussels were gravid (see section 3).

To estimate parasite influence on the reproductive output of mussel populations, we incorporated results of all the above analyses into a model to predict glochidial output in the absence of parasitism, and in the presence of parasitism (i.e. the actual scenario). We chose to express reproductive output of the population in terms of glochidial production (in g) per 100 g of shell mass, as this measure does not require assumptions about total population size. The model described below was run four times, each with 1,000 replicates: for OW with and without parasites, and for BC with and without parasites. For each site, we used the binomial 95% confidence intervals for both the proportion of gravid mussels and the proportion of mussels infected by trematodes to estimate the minimum and maximum number of mussels expected to be gravid at each site in the presence or absence of trematodes. Then, we sampled a random number of mussels from the population within those intervals (representing the pool of gravid mussels for that particular replicate), and summed their glochidial mass. The probability of any one mussel being selected in a given replicate was weighted according to their probability of being gravid, as larger mussels were more likely to be gravid. For mussels that were not gravid originally, their glochidial mass was estimated using their length using site-specific linear equations, as glochidial mass was strongly correlated with length. The summed glochidial mass was multiplied by the average glochidial viability for that site, according to whether the run included the presence of parasites or not (as mites had a site-specific influence on glochidial viability, see section 3). This yielded the total mass of viable glochidia, which we then divided by the total shell weight of all 60 mussels (of either BC or OW mussels depending on the run). This produced an estimate for each replicate of the mass of viable glochidia per 100 g of shell weight. While this exercise used the sample size of 60 mussels at each site, assuming that our sample is representative of the overall mussel populations, this estimate is generalisable to the population as a whole, as it is independent of the actual number of mussels sampled. The overall results of these models were four means (averaged over the 1,000 replicates) with associated 95% confidence intervals: viable glochidial mass per 100 g of shell weight for BC in the absence of parasitism; for BC in the presence of parasitism; for OW in the absence of parasitism; and for OW in the presence of parasitism. We also calculated the actual value of viable glochidial mass per 100 g shell mass for BC and OW in the study, and compared those values to the model results in the presence of parasitism, to confirm that our model gave realistic predictions. For full details of model specification, see Supplementary Methods.

## 3 RESULTS

### 3.1 Native mussel parameters

In total, 120 A. anatina were dissected across both sites. Using the presence or absence of marsupia in the outer demibranchs, three were identified as male (two at OW, one at BC) and 117 as either female or hermaphroditic (i.e. possessing the female trait of marsupia). There were no differences between the two sites in terms of length (overall length 69.7 ± 11.6 mm, mean ± SD, \( t_{118} = -1.02, p = 0.310 \)), total dried weight (8.4 ± 4.0 g, \( t_{118} = -1.04, p = 0.194 \)), or the proportion of mussels that were gravid (45% of all mussels, \( z = 0.27, p = 0.788 \)).

### 3.2 Factors influencing parasite distribution and abundance

Factors correlated with the presence or absence of parasites were investigated with logistic regression. Modelling showed that for both mites and zebra mussels, site had a significant relationship with the proportion of native mussels infected: there were on average 5.8 times more mussels infected with mites at OW (\( z = 3.60, p < 0.001 \)), and 4.5 times more native mussels with attached zebra mussels at BC (\( z = -4.93, p < 0.001 \); Figure 1a). There were also 3 times as many native mussels infected by trematodes at OW than BC, although the lower total prevalences meant that the sites were not statistically different (\( z = 1.67, p = 0.095 \), Figure 1a). For the two parasites that had a quantitative measure taken (zebra mussels and trematodes), site was also a significant predictor when considering intensity of infection—the abundance of zebra mussels per native mussel was on average 40 times higher at BC (\( \chi^2 = 87.85, p < 0.001 \), Figure 1b), and the infection intensity of trematodes was 2.2 times higher at BC (\( \chi^2 = 6.23, p = 0.013 \), Figure 1c).

Native mussel length was not associated with the probability of infection with zebra mussels or trematodes, or the infection intensity of trematodes (\( p > 0.05 \), all cases). However, at BC, native mussel length was significantly correlated with zebra mussel abundance (\( t_{58} = 2.26, p = 0.028 \), Figure 2a), and was also correlated with the probability of infection with mites (\( z = 2.42, p = 0.016 \), Figure 2b). For a given parasite, no other factors, including the presence of the other two parasites, were significantly related to patterns of distribution or abundance among sites or mussels.
3.3 Relationship between parasites and host population reproductive capacity

General linear models were used to explore the relationship between parasites and the weight of host mussels. After accounting for the effects of site and mussel gravidity (which increases tissue weight), parasites had no correlation with the weight of native mussels, regardless of whether tissue weight, shell weight or combined weight was considered ($p > 0.05$, all cases).

Next, a logistic model was used to explore the factors determining whether or not a mussel was gravid. Larger mussels were significantly more likely to be gravid ($z = 3.49$, $p < 0.001$), and mussels infected with trematodes were significantly less likely to be gravid ($z = -0.02$, $p < 0.001$); indeed, no mussels with trematodes were observed to be gravid in either site (Figure 3a). Mites and zebra mussels had no relationship with the likelihood of a native mussel being gravid ($p > 0.05$ in both cases).

For gravid native mussels, the viability of glochidia was compared between mussels infected and not infected with zebra mussels, and between mussels infected and not infected with mites. This was not carried out for trematodes, as no mussels infected with trematodes were gravid. Zebra mussels were not associated with the viability of glochidia produced by native mussels, regardless of site ($p > 0.05$ in both cases). However, mite presence was correlated with a site-specific effect on glochidia viability; glochidia viability from mite-infected mussels at BC was significantly lower than non-infected mussels at BC (Kruskal–Wallis, $X^2 = 4.45$, $p = 0.035$), and glochidial viability was also lower than mite-infected mussels at OW ($X^2 = 5.14$, $p = 0.023$; Figure 3b). In contrast, there was no difference in glochidial viability between non-infected mussels at BC and OW ($X^2 = 0.17$, $p = 0.681$) or between infected and non-infected mussels at OW ($X^2 = 0.055$, $p = 0.815$; Figure 3b).

Finally, our model that incorporates the individual-level effects of trematodes (Figure 3a) and mites (Figure 3b) to predict...
population-level glochidial output revealed a significant reduction of 13.0% at OW and 9.6% at BC caused purely by the combined presence of these parasites (Figure 3c). The actual values observed in the data aligned very closely with the predicted model values (Figure 3c), suggesting that the model constructed is applicable and reliable.

4 | DISCUSSION

In this paper, we have explored factors explaining the rate of parasitism and its consequences in a common unionid mussel, at both individual and population scales, showing significant negative consequences for native mussel reproduction. In this discussion, we first consider our individual-level results in light of previous research, before considering the implications of our population-level findings for mussel conservation and ecosystem services.

4.1 | The effect of parasitism on individuals

At an individual level, we found mussel length to be a significant predictor for the presence of mites, and for the number of zebra mussels at the site with high intensity (BC). Both these results are intuitive and support previously documented patterns. As hosts constitute a resource base, larger hosts represent a larger resource supply and are therefore preferentially infected by parasites with the ability to select hosts. The ability of mites to select hosts is well-established (e.g. Downes, 1986, 1991), and previous studies found that mites were more likely to infect larger mussel hosts (Dimock, 1985; Wen et al., 2006), something borne out by our results. Similarly, larger native mussels also have a greater exposed shell area for attachment by zebra mussels (Sousa et al., 2011), in addition to the long lifespan of A. anatina (Aldridge, 1999) meaning that larger mussels will be likely to accumulate greater numbers of zebra mussels over time. As zebra mussels can settle on a range of substrates, at low abundance any effect of native mussel shell length may reflect this lifetime chance of infection rather than zebra mussels actively choosing larger mussels, which may explain why native mussel size was not correlated with the presence of zebra mussels, only their intensity. Given these patterns, we show that larger mussels are therefore at greater risk of parasitism: sites with low incidence of recent recruitment will have predominantly larger individuals in the population and so may be disproportionately vulnerable to parasitism, which could further limit the production and recruitment of juvenile mussels.

Host mussel length was not a significant predictor for trematode presence or intensity, in contrast to the above. This is also different to previous studies, which reported an increased prevalence among larger mussels (e.g. Müller et al., 2015; Taskinen & Valtonen, 1995). These studies hypothesised that trematodes preferentially infected larger mussels, or that larger mussels were older, and therefore had
a greater lifetime chance of being infected. We did not detect this pattern, possibly because our sample size was too small to detect a significant effect. However, our results did align with previous research in showing a significant negative effect of *R. campanula* on the reproductive potential of their hosts, with no mussels observed as gravid at even low levels of infection. The eventual castrating effects of digenean trematodes are well-documented (e.g. Jokela et al., 1993; Walker, 2017), particularly for the virulent *R. campanula* (Müller et al., 2015; Taskinen et al., 1994), but these studies still record native mussels being able to reproduce at low infection intensities. We have also previously recorded gravid mussels at low infection intensities of *R. campanula* (Brian & Aldridge, 2021), suggesting that understanding the degree of infection is as important as understanding its presence (Brian & Aldridge, 2020). Nevertheless, our results highlight that even intermediate infection intensities are correlated with mussel castration, showing the need to understand the presence and distribution of this highly virulent parasite across individuals and populations.

The mite *U. intermedia* also showed a possible site-specific effect on the reproductive potential of their hosts, as this parasite was correlated with a 28% reduction in glochidial viability at BC relative to uninfected mussels. We acknowledge that these data are correlative only; it is possible that mussels inherently producing low-viability glochidia were also more susceptible to mite infection. Further, the mechanism for this reduction is not immediately apparent, given that mite presence was observed as eggs and larval mites encysted in the mantle and therefore separated in space from the gills where glochidia are brooded. However, eggs and larvae signify...
the presence of adult mites, which are transient and return to mussels to produce offspring (Baker, 1988) and may have previously imposed stress or damage on the native mussels. Adult *U. intermedia* have been observed feeding on gill tissue (Baker, 1976, 1977), and, in general, mites of multiple species have been shown to damage gill tissue (Fisher et al., 2000; Walker, 2017). This provides a plausible mechanism for reduced viability of host glochidia in the presence of mites, a trend that has also been previously reported (Gangloff et al., 2008).

4.2 | The population-level implications of parasitism

We combined the negative reproductive effects of trematodes and mites in our model to show that parasitism correlates with a 9%–13% reduction in population glochidial output of native mussels relative to predictions in the absence of parasitism. To our knowledge, this is the first study that demonstrates a reduction in population-level reproductive capacity caused by parasitism in freshwater mussel populations. While Taskinen and Valtonen (1995) did estimate the proportion of glochidia-bearing mussels in the population reduced by trematodes, this did not explicitly predict glochidial output, which may be influenced by other factors such as adult mussel size and glochidia viability patterns. We note that our predicted reduction in glochidial output does not necessarily translate to population impact: if recruitment is not glochidia-limited, a reduction in glochidial production may not matter to overall population success. However, glochidia are highly sensitive and suffer massive mortality (Jansen et al., 2001); in vulnerable or endangered populations, any significant reduction in the glochidial pool could have serious consequences. Even with the relatively low trematode and mite prevalence in the current study, especially at BC, we found a minimum reduction of 9.6% in glochidial production by the population. Other studies have reported much higher prevalences (e.g. Taskinen and Valtonen [1995] reported 32.3% of all studied *A. anatina* infected with *R. fennica*, which eventually castrates its host), which may translate to dramatic reductions in reproductive potential. Recent years have seen a high number of enigmatic declines in freshwater mussels, many of which are characterised by a cessation of recruitment rather than by immediate death of all individuals (Haag, 2019). This pattern of recruitment cessation is consistent with parasite-driven reductions in reproductive potential, something acknowledged to be an under-studied impact (Brian & Aldridge, 2019; Ferreira-Rodríguez et al., 2019). Our demonstration that parasites are correlated with significant reductions in glochidial productivity, even at low prevalence, shows that parasites should be carefully considered in the conservation of threatened or endangered mussels.

The population-level impacts of parasitism may not just be limited directly to reproductive output; parasites also have the potential to influence the broader reproductive strategy of the population. For example, populations of the freshwater snail *Pisidium amnicum* become semelparous breeders under high levels of trematode parasitism (Rantanen et al., 1998). We found evidence for a similar influence in our study, with just 2.5% of the 120 mussels identified as male, in contrast to other reports for *A. anatina* of 47% (Zieritz & Aldridge, 2011) and between 20% and 66% (Hinzmann et al., 2013). The high number of marsupia-bearing individuals means that an extraordinary female bias was present, or that there was a high number of hermaphrodites. While females and hermaphrodites were not separated in our study, hermaphroditism has previously been linked with trematode infection (Kat, 1983; Walker, 2017). As hermaphroditism increases the number of phenotypic females, this may help compensate for the loss of other larval-bearing mussels, especially as females may be targeted to a greater extent than males by trematodes (Taskinen & Valtonen, 1995). Hermaphroditism is common in the genus *Anodonta* (Heard, 1975), although in the U.K. it is mainly observed in *A. cygnea* (Chase et al., 2018; Zieritz & Aldridge, 2011). However, the reproductive plasticity in this genus makes it possible that parasitism induces an alteration of population-level reproductive strategy, something consistent with the observed sexual bias in our study.

Our analysis of two sites also demonstrates that different populations may be affected differently, in terms of the prevalence and intensity of parasites, as well as their effects. For example, BC had a lower prevalence of mites and trematodes (by 5.8 and 3 times, respectively) but a higher intensity of trematode infection (by 2.2 times) and a significant negative correlation between mite presence and larval viability. A potential explanation is that parasite populations only recently invaded BC mussels, and host mussels at BC are therefore more evolutionarily naïve than OW mussels (i.e. naïve host syndrome, see Lymbery et al., 2014; Mastitsky et al., 2010). These results also agree with previous studies that individuals in different native mussel populations may respond differently to parasitism by trematodes (Jokela et al., 2005) and bitterling (*Rhodeus amarus*) fish (Reichard et al., 2015), although these studies do not consider the population-level outcomes of these differences. The differential effects of parasitism across populations, even across small spatial scales in interconnected waterways as in the current study, suggests that considerable caution is required when mussels are transported between populations, even across short distances within the same river (Brian et al., 2021).

While we did not detect a relationship between parasites at the individual host level, it is possible that they may interact at a host population level, further highlighting the need to link individual and population scales. Specifically, while zebra mussels did not alter the likelihood of a specific mussel hosting trematodes, there was a clear site-wide inverse relationship between the two, with BC mussels hosting high numbers of zebra mussels but few trematodes, and OW mussels hosting few zebra mussels but a higher number of trematodes. As we only sampled two sites, we were not able to statistically assess this trend, but it aligns closely with the results of Müller et al. (2015), Müller et al. (2021), who also showed, using multiple Polish lakes, that zebra mussels were not correlated with trematodes at the individual level but that their prevalences were inversely related at the population level. Given that zebra mussels do not host *R. campanula* (Brian & Aldridge, 2019), it may be that...
attempted trematode infections in D. polymorpha are wasted (see Rigaud et al., 2010), which reduces the site-wide prevalence of trematodes. We recommend that further consideration is given to parasite interactions at the population level, as well as within hosts.

In our study, we have focused on how the effects of parasites on individual native mussels may influence population-level reproductive output. Such an effect may also cascade to the community and ecosystem level. Native mussels perform multiple ecosystem engineering roles, such as water filtration, bioturbation, and nutrient deposition (Vaughn, 2018). Therefore, any parasite-induced alteration in population outcomes will also affect the services provided by that population, thus changing the dynamics of whole ecosystems. Further, in our sites, A. anatina lives sympatrically with other unionid mussels such as Unio pictorum and Unio tumidus, which are not affected to the same degree by trematodes in particular (Brian & Aldridge, 2019). The reproductive suppression and potential alteration in strategy (hermaphroditism) experienced largely by A. anatina may also significantly alter community structure: for example, the composition of crustacean communities in freshwater systems can be altered by parasites differentially affecting its constituent species (Friesen et al., 2020). This will also shift ecosystem dynamics if the native mussel species differ in their contribution to ecosystem engineering processes.

To conclude, we have demonstrated a relationship between parasitism and reduced reproductive performance of individuals and populations of an important ecosystem engineer. We suggest that further research is required into the impacts of parasitism on freshwater mussels, especially at a population level. Documenting effects on individual mussels is valuable but may not scale to demographic impacts, while general statements on the effects of parasites may miss differences between populations. In general, the effects of parasites and pathogens on unionid mussel communities are poorly understood (Brian & Aldridge, 2019; Ferreira-Rodriguez et al., 2019). While this paper takes a first step to exploring the implications of parasitism at a population level, future work is required to both establish clear causality for the effects of multiple parasites, and further demonstrate how parasites may influence host population dynamics. From there, the consequent impacts on ecosystem services deserves consideration.

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CONFLICT OF INTEREST
All authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
D.C.A. and J.I.B. conceived the study; S.E.D., C.L.E., J.I.B. and D.C.A. performed sampling; S.E.D., C.L.E. and J.I.B. carried out all laboratory work and analysed the data; J.I.B. and D.C.A. wrote the manuscript; all authors approved the final version.

DATA AVAILABILITY STATEMENT
All data and code supporting the conclusions of this paper are available from the corresponding author upon reasonable request.

ORCID
Joshua I. Brian https://orcid.org/0000-0001-9338-4151
David C. Aldridge https://orcid.org/0000-0001-9067-8592

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