Title: **Symbiont virulence is a poor predictor of impacts on host population dynamics**

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Abstract: Symbionts are classified as parasites or mutualists based on their impacts on host individuals, but timescale, ecological context, and level of biological organization can all influence symbiosis outcomes, and hence whether they are deemed harmful or beneficial. We designed experiments exposing the key freshwater grazer *Daphnia dentifera* to two symbionts varying in virulence, to test how well individual-level effects translate into population-level patterns. We found that a seemingly mildly virulent microsporidian strongly reduced host population size, with consequences for primary production, while a yeast that was highly virulent to individuals had no

31 detectable negative effects on host population size. Our experiments show that studies that fail to
32 consider the impacts of symbionts at multiple scales risk focusing mitigation and control measures
33 on the wrong symbionts.
34
35 **Main text:**
36 Scientists have long recognized that symbionts can have major effects on host fitness (*1*),
37 classifying symbionts with net positive impacts as mutualists and those with net negative impacts
38 as parasites (*2*). This classification is typically based on individual-level measurements of changes
39 in fitness-related traits of the host, like symbiont-induced changes in host survival (*3–5*) or
40 reproductive success (e.g., (*5–7*)). A common measure of the impact of symbionts on hosts is
41 virulence, a term that has a variety of definitions, but in a broad sense is defined as damage inflicted
42 on the host by the symbiont (e.g., (*5*)), and in a narrow sense as symbiont-induced increases in
43 mortality (*3–5*, *8*). Here, we use the broad sense definition, but emphasize that both definitions of
44 virulence refer to an individual-level measure of symbiosis outcome. Measuring virulent effects
45 of parasites on life-history traits helps assess the impact of a given symbiont on its host, and is
46 commonly used in human and veterinary medicine, conservation, and organismal biology (*4*, *9*,
47 *10*). However, besides the individual-level impacts, it is also important to identify how symbionts
48 affect host population trajectories; after all, a common reason for measuring individual-level
49 effects is to predict the population-level patterns. Accurate prediction of the large-scale, long-term
50 consequences of symbiosis outcomes is crucial to identify risks and benefits of symbiont spread
51 through host populations, and can be used in improving management practices in agriculture and
52 conservation biology (*11*, *12*). Yet, there is growing evidence that effects at the individual-level
53 can poorly predict effects at the population-level (*12*), and that symbionts that seem benign at the

54 individual-level can have harmful population-level effects and vice versa (e.g., (*13*, *14*)). These
55 contradictory patterns stem from scale-dependent impacts like density-dependent effects, trans-
56 and multigenerational effects or environmental context (*13*, *15–18*), and studies of symbionts at
57 the individual level cannot detect these impacts. Hence the individual-level studies of virulence
58 are burdened with a risk of incorrectly classifying a symbiont as harmful, neutral or beneficial for
59 the population. Such incorrect labeling of a symbiont can lead us astray when we attempt to assess
60 the food web- and ecosystem-level impacts of symbionts, or when we try to decide which
61 symbionts should be combated or propagated. To improve our understanding of the role symbionts
62 play in host populations, food webs and ecosystems, we need to recognize that the outcomes of
63 symbiosis can vary depending on the time scale, level of biological organization, and ecological
64 context in which the outcome is measured (e.g., (*12–14*)).
65
66 In this study, we explored how the net impact of symbiosis may change with scale and context,
67 using the freshwater zooplankton *Daphnia dentifera* as a model organism. *Daphnia*, like all
68 organisms, are subject to and dependent on symbiotic interactions; some of these interactions are
69 vital for their survival (e.g., *Daphnia* without gut microbiomes die (*19*)), while others are
70 detrimental (e.g., highly virulent parasites, (*20*)). A well-studied symbiont of *Daphnia* spp. is
71 *Metschnikowia bicuspidata*, a yeast pathogen considered highly virulent mostly due to its large
72 reduction in host lifespan (*21–23*). A recently discovered and seemingly much less virulent
73 microsporidian symbiont of *Daphnia* species, *Ordospora pajunii* (*24*, *25*), shows potential to
74 become a mutualist when *M. bicuspidata* is present in the environment (*26*). *Ordospora pajunii*
75 infection reduces the penetrability of *Daphnia* guts to attacking *M. bicuspidata* spores (*26*, *27*).
76 Additionally, an experimental investigation of potential drivers of context-dependent mutualism

77 revealed that *O. pajunii* causes *Daphnia* to become a dead-end host for *M. bicuspidata* upon
78 sequential co-infection due to increased host mortality (*27*). However, *O. pajunii* has also been
79 found to greatly reduce offspring quality through transgenerational effects, which should decrease
80 host population size over longer time scales (*28*). Therefore, our expectation was that the net
81 impact of *O. pajunii* might change depending on whether the impacts were measured at the
82 individual-level over short timescales versus at the population-level over longer time scales, as
83 well as depending on whether the ecological context included the highly virulent *M. bicuspidata*.
84 We first investigated the short-term, individual-scale effects of each symbiont on the host with a
85 life-table experiment, then tested for potential shifts in outcomes based on ecological context and
86 longer timescales (spanning generations) using a population-level experiment.
87
88 **Individual-level effects**
89 At the individual-level, infections affected host reproduction (parasite:clutch interaction F=43.52,
90 p<0.001) and lifespan (parasite effect F=29.35, p<0.001), but the effects of each symbiont strongly
91 differed. Specifically, the yeast *M. bicuspidata* was highly virulent to the host, reducing its
92 reproduction by 28-46% in clutches 2-4 (t-ratio>5.4 and p<0.001 for each comparison, Fig. 1A)
93 and lifespan by 63.5% (t-ratio=6.22, p<0.001, Fig. 1B), the latter resulting in the host producing
94 no more than four clutches. *Ordospora pajunii* infection, on the other hand, was less harmful to
95 host individuals. The infections of *O. pajunii* had no effect on the number of offspring in the first
96 three clutches, but this microsporidian had a negative effect on later reproduction, with the size of
97 clutches 4-8 reduced by 8-29% (t-ratio>3.2 and p<0.003 for each comparison, Fig. 1A) and
98 clutches 9-14 reduced by 31-54% (t-ratio>11.2 and p<0.001 for each comparison). *O. pajunii* did
99 not significantly reduce host lifespan (t-ratio=1.74, p<0.27, Fig. 1B). Parasite treatment had a

100 significant effect on reproduction time of *Daphnia* in clutches 1-4 (parasite effect F=5.00,
101 p=0.007) (Fig. S1). While neither *M. bicuspidata* nor *O. pajunii* exposed *Daphnia* differed from
102 the control (C-MB t-ratio=-0.74 and p=1.000; C-OP t-ratio=1.43 and p=0.466), the *O. pajunii*
103 exposed *Daphnia* reproduced faster than those exposed to *M. bicuspidata* (MB-OP t-ratio=3.15
104 and p=0.005) (Fig. S1). Overall, *M. bicuspidata* had larger negative effects on the reproduction
105 and survival of individuals than *O. pajunii*.
106
107 **Scaling up**
108 Based on the life-table experiment results and previous studies of these symbionts, we predicted
109 that the highly virulent yeast *M. bicuspidata* would strongly reduce the population density of
110 *Daphnia*. The life-table experiment suggested that *O. pajunii* should have negligible effects on
111 *Daphnia* populations. However, we know that *O. pajunii* induces negative transgenerational
112 effects (i.e., transgenerational virulence, (*28*)), and therefore, we expected that the microsporidian
113 could negatively impact *Daphnia* population density over multiple generations. We also
114 hypothesized that *O. pajunii* would prevent, or at least reduce, *M. bicuspidata* outbreaks,
115 benefiting the host. Hence, we exposed *Daphnia* populations to *O. pajunii*, *M. bicuspidata*, both,
116 or neither as a control. Because *O. pajunii* outbreaks in natural lakes occur before those of *M.*
117 *bicuspidata* (*27*), and the hypothesized protection mechanism against *M. bicuspidata* requires
118 established infection of *O. pajunii* (*26*, *27*), we added *O. pajunii* spores at the start of the
119 experiment and waited until day 31 for its epidemics to be established at ~15% prevalence to add
120 *M. bicuspidata*. We analyzed the growth rates and densities of host populations, as well as
121 prevalences of each parasite. We also investigated differences in host demographics, expecting
122 that transgenerational virulence of *O. pajunii* would reduce the proportion of juveniles in the

123 populations. Additionally, we quantified the standing stock of algae in the microcosms to
124 determine how the symbionts altered host-resource interactions.
125
126 For the population-level experiment, the effects of single- and co-exposure to the symbionts were
127 assessed during two time intervals: the initial growth phase (until day 23) and the post-peak phase
128 (days 30-93). The separation was determined by both population dynamics and the experimental
129 design: *M. bicuspidata* spores were added on day 31, when *O. pajunii* epidemics were established
130 (~15% prevalence) and the host populations had peaked in abundance. Thus, we used the time
131 before the *M. bicuspidata* exposure to quantify how *O. pajunii* affects initial host population
132 growth rate, and we combined the two groups exposed to *O. pajunii* into one group (OP and
133 OPMB, labeled 'OP' in Fig. 2A), and the two unexposed groups into a separate group (C and MB,
134 labeled 'C' in Fig. 2A). The post-peak phase was used to analyze the impact of both symbionts on
135 host population trajectories, with the four treatments analyzed separately.
136
137 **Population-level effects**
138 Contrary to what the individual-level measurements of virulence predicted, we found strong
139 negative effects of *O. pajunii* on the host populations and no negative effects of *M. bicuspidata*.
140 In the initial growth phase of the experiment, *O. pajunii* reduced the population growth rate by
141 13% (χ^2 = 17.37, p<0.001, Fig. 2A). This negative impact of *O. pajunii* on hosts continued after
142 the peak in densities. In populations with only *O. pajunii*, host population size on days 30-61 was
143 reduced by, on average, 47% (t-ratio>2.7 and p<0.034 for each comparison between OP and C on
144 each day); in the co-exposure treatment, host population size on days 30-64 was reduced by, on
145 average, 42% (t-ratio>2.8 and p<0.026 for each comparison; Fig. 2B). Conversely, *M. bicuspidata*

146 had no observable negative effects on the host populations; instead, later in the experiment (days
147 71-83), the *Daphnia* population size was larger in comparison to the control populations by, on
148 average, 41% (t-ratio<-2.84 and p<0.028 for comparisons on each day; Fig. 2B).
149
150 The most likely explanation for the decimation of *Daphnia* populations by *O. pajunii* is
151 transgenerational virulence (i.e., negative effects of maternal exposure on offspring fitness, even
152 when those offspring are not themselves exposed (*28*)). A mathematical model estimating the
153 population-level impacts of transgenerational virulence predicted an approximately 30% reduction
154 in population size (*28*), which is similar to the effect we observed in the population experiment.
155 Additionally, we found a lower proportion of juveniles in the *O. pajunii*-exposed populations
156 (treatment effect F=9.66, p<0.001; Fig. 3), which is consistent with high juvenile mortality caused
157 by transgenerational virulence. Adverse transgenerational effects of maternal exposure to stressors
158 have been observed before, via, for example, altered embryo nutrition or epigenetic inheritance
159 (*29*, *30*) or, for some parasites, vertical transmission (*31*). We do not know yet the process
160 responsible for the transgenerational virulence of *O. pajunii*, but we do know that the
161 microsporidian is not vertically transmitted and that the embryos produced by exposed mothers
162 are more fragile (*28*).
163
164 **Resource availability**
165 We also hypothesized that some of the discrepancy between the individual-level effects on host
166 fitness and population-level patterns could be explained by host-resource interactions. Some
167 models indicate that intraspecific host competition for resources can amplify the virulence of
168 seemingly benign parasites (*13*). Specifically, strong negative effects of *O. pajunii* could be the

169 result of an infection-driven increase in resource demand that led to starvation and smaller
170 population sizes. Contrary to this, *O. pajunii* exposed populations had leftover algae on days 29-
171 56 in single exposure and on days 25-64 in co-exposure (C-OP t-ratio<-2.88 and p<0.025 ; C-
172 OPMB t-ratio<-2.68 and p<0.045 for the given time spans) (Fig. S2), when the host populations
173 were undergoing the strongest reductions in density. This indicates that the decrease in population
174 size of *Daphnia* exposed to the microsporidian was not driven by resource depletion and starvation.
175 Conversely, it is possible that the exposed *Daphnia* were undergoing illness-mediated anorexia
176 (*32*, *33*), which would lower the overall food consumption. In a different study, we found that *O.*
177 *pajunii* can depress host feeding rates (*34*). Hence, the unexpected harmfulness of *O. pajunii* is not
178 likely to be a result of resource shortage. We acknowledge that *O. pajunii* infection might hamper
179 host nutrition through processes independent of food density in the environment, although
180 assimilation efficiency of hosts seems to be unaffected by the microsporidian (*34*).
181
182 In contrast, *M. bicuspidata*-exposed *Daphnia* populations consumed all the available food (Fig.
183 S2). Previous work has suggested that infection-driven decreases in foraging rates in *M.*
184 *bicuspidata*-infected hosts (*32*, *35*) can allow for increased resource uptake by other (susceptible,
185 uninfected) hosts, driving a phenomenon known as a disease-induced hydra effect (*36*). The lack
186 of a standing stock of algae in the *M. bicuspidata*-exposed treatment suggests that the very high
187 densities of hosts consumed all the available food; if infected hosts consumed less food, that would
188 leave additional food for the uninfected hosts, fueling a hydra effect, which is consistent with the
189 periodically higher population size of the yeast-exposed population in comparison to the control
190 (Fig. 2B). Additionally, during *M. bicuspidata* epidemics, the older and larger *Daphnia* experience
191 the greatest infection-driven mortality. Old and large adults are effective grazers (*37*), but

192 contribute very little to the population growth rate (*38*). Therefore, their increased mortality would
193 release a fraction of resources from their control, and nourish the juveniles, increasing their
194 survival, and stabilizing the whole population. Hence, we suspect that a joint effect of increased
195 resource availability due to foraging depression, and age-dependent mortality eliminating large
196 grazers, could be responsible for the lack of negative effects of *M. bicuspidata* at the population-
197 level despite its high individual-level virulence.
198
199 **Antagonistic relationship of the symbionts**
200 This study was motivated by the hypothesis that *O. pajunii* is a context-dependent mutualist whose
201 impact switches from mildly negative to positive in the presence of the more harmful yeast
202 symbiont. Consistent with prior work on *O. pajunii* (*26*, *27*) and on its close relative (*39*), *M.*
203 *bicuspidata* prevalence was reduced in our experiment in the presence of *O. pajunii*, and *O. pajunii*
204 prevalence was lower during *M. bicuspidata* outbreaks (Fig. 4A). Specifically, the prevalence of
205 *M. bicuspidata* was lower in co-exposure from day 56 of the experiment onward, and reached a
206 66% reduction by the end of the experiment (treatment:day interaction z-value=-2.33, p=0.020;
207 Fig. 4A). *Ordospora pajunii* was also less prevalent in the co-exposure treatment from day 44
208 onward, reaching a 57% reduction at the end of the experiment (treatment:day interaction F=15.39,
209 p<0.001; Fig. 4B). Despite the latter, co-exposed populations had similar host population dynamics
210 as the populations exposed just to *O. pajunii* (Fig. 2B). All in all, our assumption that *O. pajunii*
211 could be beneficial for *Daphnia* is not supported because *O. pajunii* had a strong negative
212 population-level effect whereas *M. bicuspidata* did not. Citing Francis Bacon "*the remedy is worse*
213 *than the disease*".
214

215 **Beyond individual-level virulence measurements**

216 Awareness of how ecological context affects the outcome of symbiosis is crucial for epidemiology,
217 environmental management and conservation, medicine, and agriculture (*14*). While there is
218 growing consideration of context-dependent changes in symbiosis outcomes, few studies consider
219 the role that time span and level of biological organization play in the functioning of symbiosis.
220 Switching from individual-level analysis to population-level studies done over multiple
221 generations can dramatically change the perspective on the outcome or even nature of the focal
222 symbiosis. For example, anthelmintic treatment that was predicted to help the buffalo population
223 fight off tuberculosis infection actually increased the spread of bovine tuberculosis (*12*).
224 Furthermore, *Wolbachia* is known to have complex transgenerational effects (*40*); for example, in
225 *Drosophila melanogaster,* grandmother age determines the strength of the cytoplasmic
226 incompatibility impact on fitness of the offspring of *Wolbachia*-infected males (*41*). These
227 examples, and many other studies (e.g., (*23*, *42–44*)), show that individual-level, single generation
228 studies are burdened with a risk of overlooking important patterns and interactions, such as
229 transgenerational inheritance, selection of host and symbiont genotypes, or shifts in symbiosis
230 outcomes stemming from population/community level interactions. These effects have the
231 potential to change our classification of a focal symbiosis from mutualistic to parasitic or vice
232 versa. Here, we present how transgenerational virulence inflicted by a microsporidian that does
233 minimal harm to the infected host can decimate the host population, and how a highly virulent
234 yeast parasite that was expected to be extremely harmful to the host population turned out to be
235 benign. We would not have been able to predict such strong population-level consequences if we
236 had relied solely on individual-level measurements of virulence in a single generation.
237

238 Understanding the large-scale and long-term consequences of symbiosis is crucial for ecology,
239 evolutionary biology, and epidemiology. The strong population-level effects of *O. pajunii* detected
240 in this study indicate that long-lasting epidemics of this widespread microsporidian (as have been
241 seen in nature; (*26*, *45*, *46*)) have the potential to decrease the abundance of *Daphnia*; given their
242 central role in lake food webs, this likely has consequences for primary producers and ecosystem
243 functioning. Indeed, our finding that there was excess algae in populations exposed to *O. pajunii*
244 but not in control populations or those exposed to only *M. bicuspidata* suggest cascading effects
245 on the food web. More generally, we found that switching between individual- and population-
246 level studies revealed patterns that change our understanding of how a focal symbiosis functions
247 and how it should be classified. Our study emphasizes that individual-based methods of virulence
248 estimation are not enough to make accurate predictions about the impact of a symbiont on a host
249 population, and hence parametrizing models using only within-generation, individual-level
250 measurements may not be sufficient to predict the population- and food web-level effects of
251 epidemics. To combat the spread and harmful effects of infectious diseases in humans, wildlife
252 and agriculture (e.g., (*47–49*)), and to use symbiosis in disease biocontrol (e.g., (*50*, *51*)), we
253 require a deep understanding of the outcomes of symbioses at levels beyond individuals. Such
254 understanding comes from determining the impacts of symbionts at the population scale over
255 longer time scales, as well as the role of ecological context in shaping those interactions.
256 Incorporating these factors into our investigations of infectious diseases and symbioses will be
257 crucial not only for furthering our knowledge of the ecology and evolution of symbionts but also
258 for decision making in agriculture, conservation, and management.
259
260

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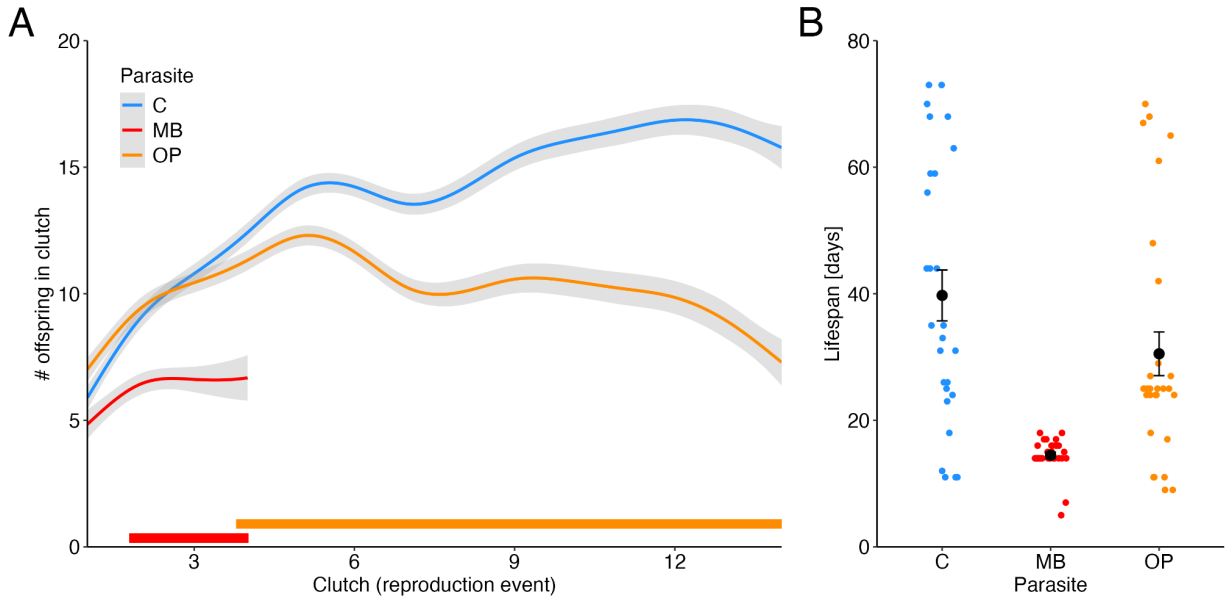
433 **Figures**



434

435 Fig. 1. **The number of offspring per clutch (A) and the lifespan (B) of *Daphnia* were strongly**
436 **reduced by *M. bicuspidata* (red), while *O. pajunii* (orange) reduced clutch size only in late life**
437 **stages (after clutch 3) and had no effect on host lifespan.** Panel A shows fitted values±SE of
438 clutch size (number of offspring per reproduction event) in consecutive clutches. Thick lines on
439 the bottom of the figure indicate clutches at which the difference in clutch size between control
440 and the treatment of corresponding color is significant. Panel B shows mean±SE lifespan (black
441 points and error bars) of *Daphnia* in each treatment, with individual data points color-coded by
442 treatment.
443

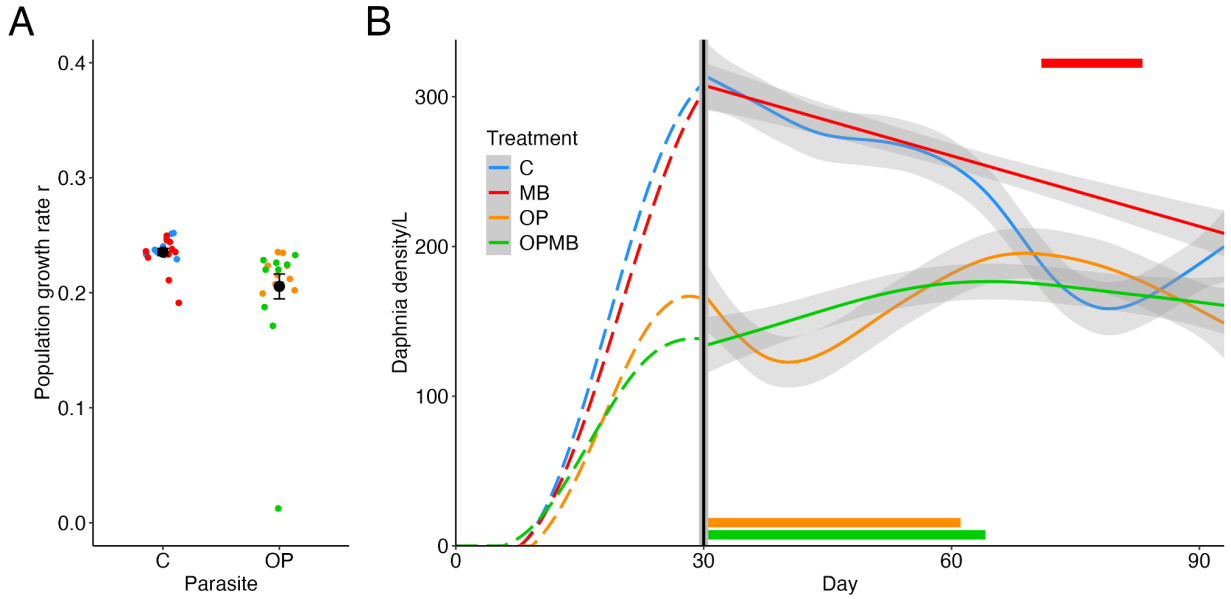


Fig. 2. ***Daphnia* initial population growth rate (A) and population size (B) were reduced by *O. pajunii* whereas *M. bicuspidata* increased population size.** Panel A shows population growth rate during the initial growth phase (through day 23) in groups unexposed (C and MB) and exposed to *O. pajunii* (OP and OPBM), grouped into parasite treatment C or OP, respectively, because the population growth rates were measured before *M. bicuspidata* dosing. The black points and error bars indicate mean±SE, the data points are plotted underneath with colors corresponding to treatments. Panel B shows GAM modeled population densities in each treatment, the vertical gray line with black stripe on day 30 separates the time before the populations peaked (dashed lines) and after they peaked (solid lines); *M. bicuspidata* was dosed to the system on day 31, around when densities peaked. Only the modeled population sizes after day 30 were analyzed with the GAM. The population size in the single infection (OP) treatment was reduced on days 30-61, while, in the co-infection (OPMB) treatment, it was reduced on days 30-64. *M. bicuspidata* (MB) had a positive effect on the host population on days 71-83. The solid lines and gray ribbons indicate fitted values±SE, thick lines on top and bottom of the figure indicate the time span at which the treatment of the corresponding color had significantly larger or smaller host population size, respectively, in comparison to the control.

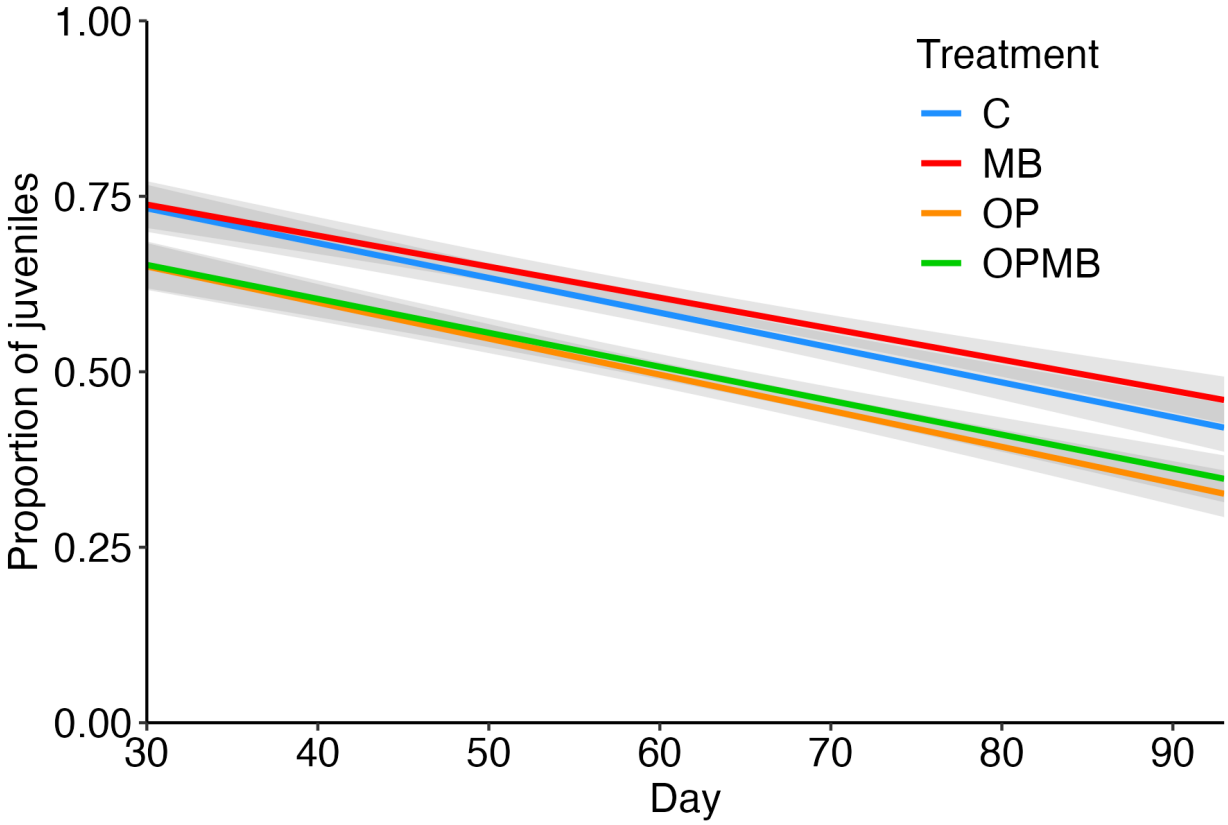


Fig. 3. **Proportion of juveniles decreased over time in all treatments, but populations with *O. pajunii* outbreaks consistently had a lower proportion of juveniles than either control populations or *M. bicuspidata* exposed populations.** Fitted values of linear regression±SE are presented.

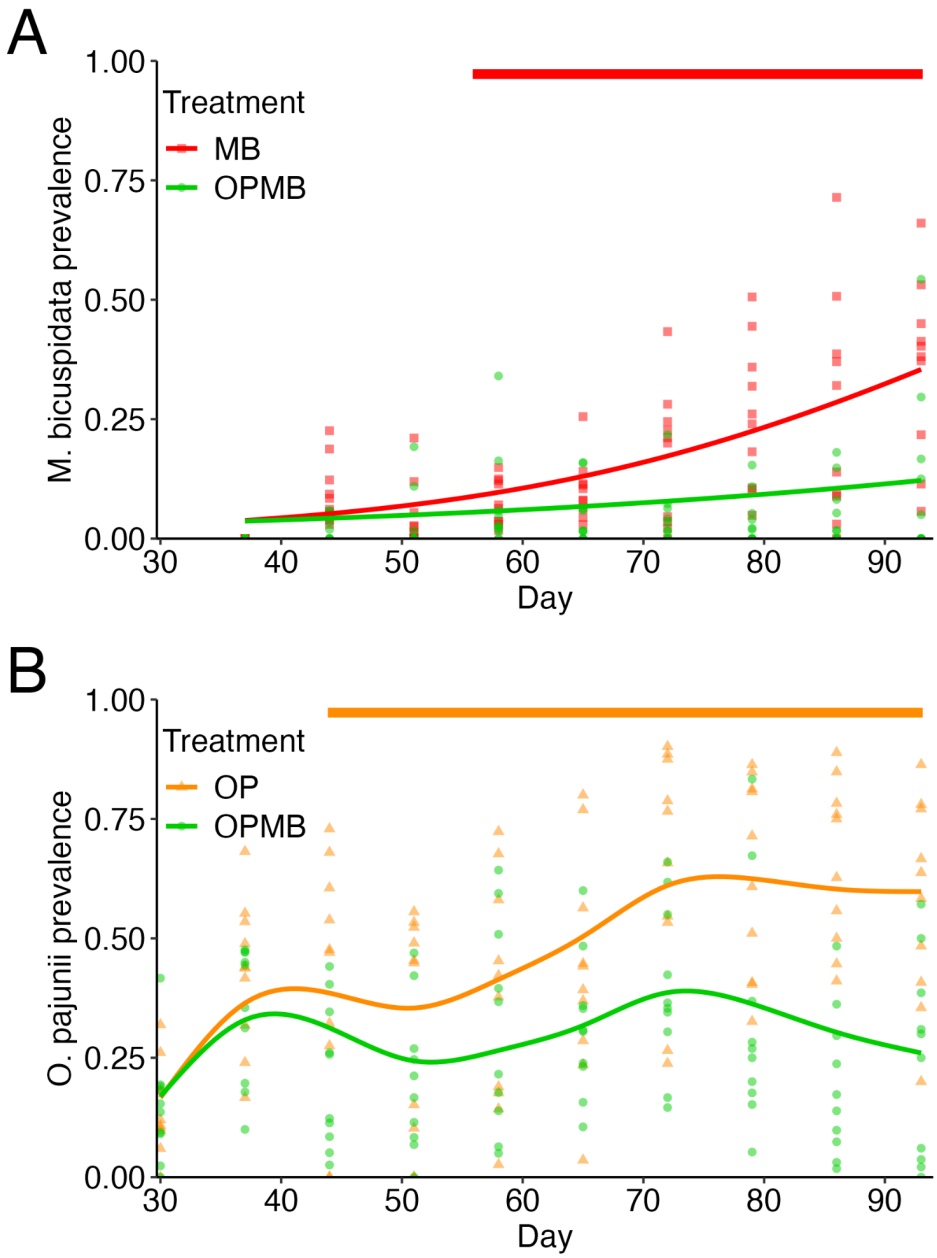


Fig. 4. **Prevalence of *M. bicuspidata* (A) and *O. pajunii* (B) were lower in the co-infection treatment in comparison to the single infection treatments.** The plots show fitted curves of beta regression for *M. bicuspidata* (A) and GAM for *O. pajunii* (B), with data points plotted underneath; the thick lines above the figures show the time span when the prevalence of the respective parasite was higher in the single infection treatment than in the co-infection treatment.