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2 **Density, parasitism, and sexual reproduction are**  
3 **strongly correlated in lake *Daphnia* populations**  
4

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21  
22 RUNNING HEAD: Variation in sex in *Daphnia* populations

23  
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27

28 ABSTRACT

29 Many organisms can reproduce both asexually and sexually. For cyclical parthenogens, periods  
30 of asexual reproduction are punctuated by bouts of sexual reproduction, and the shift from  
31 asexual to sexual reproduction has large impacts on fitness and population dynamics. We studied  
32 populations of *Daphnia dentifera* to determine the amount of investment in sexual reproduction  
33 as well as the factors associated with variation in investment in sex. To do so, we tracked host  
34 density, infections by nine different parasites, and sexual reproduction in 15 lake populations of  
35 *D. dentifera* for three years. Sexual reproduction was seasonal, with male and ephippial female  
36 production beginning as early as late September and generally increasing through November.  
37 However, there was substantial variation in the prevalence of sexual individuals across  
38 populations, with some populations remaining entirely asexual throughout the study period and  
39 others shifting almost entirely to sexual females and males. We found strong relationships  
40 between density, prevalence of infection, parasite species richness, and sexual reproduction in  
41 these populations. However, strong collinearity between density, parasitism, and sexual  
42 reproduction means that further work will be required to disentangle the causal mechanisms  
43 underlying these relationships.

44

45 INTRODUCTION

46 A major challenge in evolutionary biology is explaining variation in reproductive strategies —  
47 especially why so many organisms reproduce sexually (Otto 2009; Lively and Morran 2014;  
48 Neiman et al. 2017). Sexual reproduction has several potential drawbacks, including the  
49 “twofold cost” of sex (Otto 2009; Stelzer 2011; Neiman et al. 2017), challenges in finding a  
50 mate, acquisition of sexually transmitted infections, and shuffling of alleles that worked well in a  
51 parent (Otto 2009; McLeod and Day 2014; Kokko 2020). At the same time, sexual reproduction  
52 also has advantages, including providing an opportunity to purge deleterious mutations and  
53 producing novel genotypes that can avoid infection by parasites (Muller 1964; Jaenike 1978;  
54 Kondrashov 1984; Lively 2010). However, framing reproduction as a dichotomy between  
55 (entirely) sexual or (entirely) asexual ignores the abundance of organisms that combine the two  
56 (Gerber et al. 2018; Kokko 2020). By being able to shift between sexual and asexual  
57 reproduction, cyclical parthenogens are often described as experiencing the “best of both worlds”

58 (Kokko 2020), gaining the benefits of sexual reproduction while also avoiding its costs.  
59 However, this ability to shift between these two modes of reproduction raises a new question:  
60 how much to invest in asexual vs. sexual reproduction?

61 When considering investment in sexual reproduction, it is important to consider that  
62 sexual reproduction in cyclical parthenogens is often associated with dormancy (Walsh 2013;  
63 Gerber et al. 2018; Gerber and Kokko 2018; Kokko 2020). Sexual reproduction thus not only  
64 affords the benefits of creating novel genotypes and purging mutational load (Cáceres et al.  
65 2009), but also can allow a lineage to escape through time, potentially waiting out harsh  
66 conditions. Given the strong spatial and temporal variation in biotic and abiotic conditions that  
67 exists in nature, it is perhaps not surprising that populations of cyclical parthenogens can vary  
68 substantially in the degree to which they reproduce sexually (Walsh 2013) — as seen, for  
69 example, in studies of *Daphnia* populations (e.g., Tessier and Cáceres 2004; Johnson et al. 2009;  
70 Walsh and Post 2012; Gerber et al. 2018).

71 Prior research on *Daphnia*, a dominant member of pond and lake food webs, has  
72 identified a variety of factors that contribute to asexual vs. sexual reproduction, including  
73 predation, parasitism, crowding, resource limitation, and changing abiotic conditions (Stross and  
74 Hill 1965; Walsh 2013; Gerber et al. 2018; Haltiner et al. 2020). A potential role of parasitism in  
75 sexual reproduction in *Daphnia* has received particular attention in recent years. Sexually  
76 produced *Daphnia* offspring are more fit against contemporaneous parasites (Ebert et al. 2007;  
77 Auld et al. 2016), and more susceptible genotypes are more likely to shift to sexual reproduction  
78 (Mitchell et al. 2004; Duncan et al. 2006). Moreover, studies on two different *Daphnia*-parasite  
79 systems found the production of males was more likely in the presence of parasites (Roth et al.  
80 2008; Hite et al. 2017) and, in a third, sexual reproduction was higher in years with more  
81 infection by a chytrid parasite (Johnson et al. 2009).

82 A potential role of parasites in driving sexual reproduction has also been studied in other  
83 systems, including plants (Busch et al. 2004), *C. elegans* (Morran et al. 2011; Slowinski et al.  
84 2016; Lynch et al. 2018), and snails (Schrag et al. 1994; Ben-Ami and Heller 2005; Dagan et al.  
85 2013), most notably the New Zealand freshwater snail *Potamopyrgus antipodarum* (e.g., Lively  
86 1987; Lively and Dybdahl 2000; Gibson et al. 2018). Asexual *P. antipodarum* are most common  
87 in habitats with no or low levels of infection by virulent parasites (King et al. 2009; McKone et  
88 al. 2016). Moreover, male snails were more common when a virulent parasite was common

89 (Vergara et al. 2013) and asexual snails tended to have higher levels of infection (Vergara et al.  
90 2014), though a more recent study found the opposite pattern (asexual snails having lower levels  
91 of infection, perhaps because they have become rare (Gibson and Lively 2019)). This prior work  
92 on *P. antipodarum* demonstrates the value of studies comparing levels of parasitism and sexual  
93 reproduction in natural populations.

94 In this study, we explored the prevalence of sexual reproduction in lake populations of  
95 *Daphnia dentifera* (Fig. 1) and whether particular lakes have consistently high levels of sexual  
96 reproduction across years. We then asked what factors are associated with the amount of sexual  
97 reproduction. We were particularly interested in the degree to which the prevalence of sexual  
98 reproduction in a population is related to the level of parasitism and/or to overall population  
99 density. We explored this by tracking sexual reproduction, density, and infections by multiple  
100 parasites in 15 *D. dentifera* populations over three years to better understand variation in sexual  
101 reproduction in this dominant member of lake food webs. We found that parasitism and density  
102 were both associated with sexual reproduction, but strong correlations between parasitism,  
103 density, and sexual reproduction highlight the need for additional work to uncover the  
104 mechanisms driving these patterns.

105

## 106 MATERIALS

### 107 *Study system*

108 *Daphnia dentifera* are a dominant zooplankton species in lakes in the Midwestern US, feeding  
109 on phytoplankton and serving as prey to small fish and invertebrate predators (Tessier and  
110 Woodruff 2002). *Daphnia* often switch to sexual reproduction at particular times of the year,  
111 when it becomes less costly (Gerber et al. 2018); the species we focused on, *D. dentifera*, shifts  
112 to sexual reproduction in autumn (Duffy et al. 2008). During sexual reproduction, female  
113 *Daphnia* create clones that are males and haploid resting eggs, which the males then fertilize  
114 (Ebert 2005). The resting eggs (encased in a chitinous envelope called an ephippium) are  
115 released by the sexually reproducing females and remain dormant before later hatching, ideally  
116 when environmental conditions have improved (Hairston 1996).

117

118 *D. dentifera* occurs at varying densities across our 15 study lakes in Southeast Michigan, US and  
119 is infected by a suite of parasites (Duffy et al. 2010). We tracked *D. dentifera* population sizes

120 through time, as well as infections of nine microparasites (Green 1974; Wolinska et al. 2008;  
121 Duffy et al. 2010, 2015; Lu et al. 2020): *Metschnikowia bicuspidata* (fungus), *Pasteuria ramosa*  
122 (bacterium), *Spirobacillus cienkowskii* (bacterium), *Blastulidium paedophthorum* (oomycete),  
123 *Gurleya vayrai* (microsporidian), *Larssonia obtusa* (microsporidian), *Caullerya mesnili*  
124 (ichthyosporean), an undescribed microsporidian gut parasite (“MicG”), and an unknown  
125 *Saprolegnia*-like oomycete (“spider”).

126

### 127 *Field sampling*

128 We studied host and parasite communities in 15 lakes in Southeast Michigan, USA (Table S1)  
129 over three years (2014-2016). We sampled lakes roughly once every two weeks from mid-July to  
130 mid-November each year (usually 9 sampling events per year). In addition, we intensively  
131 sampled four of the study sites (Gosling, North, Pickerel, and Sullivan Lakes) every three days  
132 during 2016 for a study focused on population dynamics. For each lake, on each sampling date,  
133 we collected three replicate vertical tows from the bottom of the lake with a 153  $\mu\text{m}$  Wisconsin  
134 plankton net and sampled from three different locations in each lake. This yielded three replicate  
135 samples per lake per sampling day, each of which contained one tow from each of the three  
136 locations within the lake. We used one of these samples to quantify infection prevalence and  
137 investment in sex. To quantify infection prevalence, we visually diagnosed parasite infections in  
138 live hosts under a dissection microscope at 20-50x magnification using dark field microscopy (or  
139 under a compound microscope at 200 to 400x magnification for early-stage infections). As  
140 *Daphnia* are mostly transparent, many parasite infections are visibly detectable with this method.  
141 We also identified individuals as juvenile females, asexually reproducing females, sexually  
142 reproducing females, or males based on morphological differences (Brooks 1957). For this  
143 sample where we quantified infection prevalence and investment in sex, we randomly  
144 subsampled the collected hosts, surveying at least 200 *D. dentifera* individuals for possible  
145 parasite infections or surveying all individuals when fewer than 200 individuals were present.  
146 We preserved the other two replicate samples in 90% ethanol. Later, we estimated the density of  
147 each host species by randomly subsampling and counting one of these samples (which combined  
148 one tow from each of the three locations in the lake) to estimate the density of each host species.  
149 We counted at least two subsamples from each lake-date; if the total density of the two  
150 subsamples were not within 80% of each other, additional subsamples were counted. The

151 subsamples were averaged yielding a single density estimate per lake-date, with density  
152 calculated as the number of hosts throughout the water column for a given surface area of the  
153 lake (number of hosts per m<sup>2</sup> of lake surface).

154  
155 *Statistical analysis*

156 We explored relationships between density, parasitism, and investment in sex. For density, we  
157 integrated the total density of *D. dentifera* for each lake in a year over all sampling dates (i.e., we  
158 calculated the area under the curve with day on the x-axis and host density on the y-axis), then  
159 took the log of that value. We analyzed two metrics related to parasitism: 1) integrated  
160 prevalence, determined by integrating the proportion of hosts infected with any parasite across  
161 sampling events within a lake and year, and 2) parasite species richness, calculated by tallying  
162 the number of parasite species observed infecting *D. dentifera* in a particular lake in a given year.  
163 Analyses with mean host density and parasitism yielded qualitatively similar results (Fig. S1).

164  
165 We also analyzed two metrics related to investment in sex: 1) the maximum investment in sex in  
166 the population as either the percent sexual ((males + ephippial females)/(total population)) or the  
167 percent sexual adults ((males + ephippial females)/(males + adult females)); 2) integrated  
168 investment in sex, which, similar to the above metrics, was determined by integrating the  
169 proportion of hosts that were sexually reproducing (ephippial females or males) across sampling  
170 events within a lake and year. When determining the maxima, we only used samples that  
171 included at least 15 *D. dentifera* so that we could have greater confidence in the estimate of the  
172 investment in sex.

173  
174 We plotted and analyzed data in R version 4.0.5. We analyzed whether lakes varied in  
175 investment in sex using a generalized linear model. The response variable was the number of  
176 sexual and number of asexual individuals observed on the day with the maximum percent sexual  
177 for that lake and year; because of overdispersion of the data, we used a quasibinomial error  
178 distribution. Because of limitations on mixed models and quasi- distributions, our model  
179 included lake and year as fixed effects.

180

181 In addition to determining whether populations differed in the degree to which they reproduced  
182 sexually, we were also interested in assessing whether variation in investment in sex was  
183 associated with density or parasitism. We did not use a time series approach for this, because,  
184 based on our prior work on this system, we knew that investment in sex is strongly seasonal.  
185 Moreover, because sexual reproduction is associated with dormancy in this system, density  
186 would be expected to decrease as a result of sexual reproduction, even if high density had  
187 initially triggered investment in sex. Finally, we do not have any information on potential time  
188 lags that might occur between parasitism and investment in sex, especially given the presence of  
189 maternal and grandmaternal effects in *Daphnia* (e.g., Lynch and Ennis 1983; Little et al. 2003;  
190 Poulsen et al. 2021) and the ability of parasite spores to persist outside the host (King et al. 2013;  
191 Duffy and Hunsberger 2018). As a result, our analyses focused on integrated metrics of density,  
192 parasitism, and sexual reproduction, as well as parasite species richness across the entire  
193 sampling season. We calculated correlations between sexual reproduction (measured as the  
194 integrated investment in sexual reproduction) and 1) integrated *D. dentifera* density, 2) parasite  
195 species richness, and 3) integrated prevalence of infection. In order to check for collinearity, we  
196 also calculated correlations between integrated density, parasite species richness, and integrated  
197 prevalence of infection. Finally, we used a model selection approach to compare different  
198 possible models for investment in sexual reproduction. For all of these models, integrated  
199 investment in sexual reproduction was the response variable. These models included different  
200 combinations of integrated *D. dentifera* density, parasite species richness, integrated prevalence  
201 of infection, and year as independent variables. We created various sub-models and then used  
202 model selection and Akaike information criteria (AIC) to compare 15 different models (as  
203 detailed in Table 1 in the Results section, below).

204

## 205 RESULTS

206 There was substantial variation in investment in sex, density, and parasite prevalence in the study  
207 populations of *D. dentifera* (Fig. 2). Sexual reproduction was seasonal, with male and ephippial  
208 female production beginning as early as late September and generally increasing through  
209 November (black lines in Fig. 2). In some lakes and years, we never observed any males or  
210 ephippial females, whereas in others, populations shifted to nearly all sexual. Lakes that had  
211 higher investment in sex in one year tended to also have high investment in sex in the other two

212 years (Fig. 3a&c; maximum investment in sex in the total population: lake:  $F = 4.02$ ,  $p =$   
213 0.0008).

214

215 There was also substantial variation in the prevalence of parasites (ocean blue lines in Fig. 2)  
216 across lakes. In some lakes and years, there was very little parasitism; in other lakes and years  
217 infection prevalence exceeded 50% at the peak of infections. Density was generally fairly  
218 consistent within lakes over time (purple lines in Fig. 2), but populations crashed to near or  
219 below detection limits in some lakes and years.

220

221 Investment in sexual reproduction by *D. dentifera* was strongly associated with the log of  
222 integrated *D. dentifera* density (Fig. 4a;  $r = 0.637$ ,  $p < 0.0001$ ) and parasite species richness (Fig.  
223 4b;  $r = 0.602$ ,  $p < 0.0001$ ); it was also associated with the integrated prevalence of infection (Fig.  
224 4d;  $r = 0.350$ ,  $p = 0.019$ ). The log of integrated *D. dentifera* density, parasite species richness,  
225 and integrated prevalence of infection were also correlated with one another (density & parasite  
226 species richness: Fig. 4c;  $r = 0.791$ ,  $p < 0.0001$ ; prevalence of infection & density: Fig. 4e;  $r =$   
227 0.359,  $p = 0.015$ ; prevalence of infection & parasite species richness: Fig. 4f;  $r = 0.371$ ,  $p =$   
228 0.012). Comparing the AICs of models incorporating different possible drivers of variation in  
229 investment in sex suggests the importance of density and/or parasitism: all top models ( $\Delta AIC <$   
230 4.0) included one or more of log of integrated density, parasite species richness, and integrated  
231 prevalence of infection as a predictor of sexual reproduction (Table 1).

232

233 The strength of the relationship between the integrated prevalence of individual parasites and the  
234 integrated prevalence of sexual reproduction varied across parasites (Fig. 5, Table 2). The  
235 correlation between *Blastulidium paedophthorum*, an oomycete that attacks developing embryos,  
236 was the strongest and similar to the correlation between total parasitism and sex ( $r = 0.350$ ,  $p =$   
237 0.0186). The relationship between the most common parasite, the parasitic castrator *Pasteuria*  
238 *ramosa*, and sexual reproduction was less strong (Fig. 5, Table 2).

239

## 240 DISCUSSION

241 We found substantial variation in investment in sexual reproduction in natural populations of  
242 *Daphnia dentifera*, with some populations remaining entirely asexual and others becoming



243 almost entirely sexual in autumn. That variation was fairly consistent across years, with lakes  
244 that had high investment in sex one year also tending to have high investment in sex in the other  
245 two years. We found strong relationships between density, parasitism, and sexual reproduction in  
246 this system, suggesting that density and/or parasitism might be linked with investment in sex in  
247 these populations. However, strong collinearity in the underlying data means that further work  
248 will be required to disentangle the drivers of these relationships.

249  
250 Our findings are consistent with earlier studies that found density to be an important factor  
251 influencing the shift from asexual to sexual reproduction in cyclical parthenogens like *Daphnia*  
252 and rotifers (Stross and Hill 1965; Larsson 1991; Berg et al. 2001; Stelzer and Snell 2003;  
253 Haltiner et al. 2020; Gilbert 2020). One possible explanation for this association is that, in many  
254 cyclical parthenogens, sexual reproduction is associated with the production of long-lasting  
255 resting stages, meaning sexual reproduction may serve as a means of temporal dispersal when  
256 faced with strong competition in dense populations (Gerber et al. 2018; Gilbert 2020). High  
257 densities also reduce the relative costs of sexual reproduction; as populations approach carrying  
258 capacity, asexual reproduction is less beneficial, reducing the opportunity costs of sexual  
259 reproduction (Burt 2000; Gerber et al. 2018).

260  
261 We also found that parasitism was positively correlated with sexual reproduction in *D. dentifera*.  
262 Prior work has especially focused on the bacterial parasite *Pasteuria ramosa* and investment in  
263 sex. *Pasteuria* is highly virulent (Ebert et al. 2000; Auld et al. 2012) and can reach quite high  
264 prevalence (Duncan and Little 2007). It also shows very strong host-parasite genotype  
265 specificity, with parasite infectivity (and host susceptibility) being determined by host (and  
266 parasite) genotype (Carius et al. 2001; Ebert et al. 2016). One would expect this matching  
267 mechanism to favor genetic recombination (and it does in Auld et al. 2016), which could, in turn,  
268 drive Red Queen dynamics, where reciprocal evolutionary dynamics arise from selection of two  
269 antagonists on one another. Indeed, one of the best examples of Red Queen dynamics comes  
270 from the *Daphnia-Pasteuria* system (Decaestecker et al. 2007). In our present study, *Pasteuria*  
271 was the second most common of the nine parasites that we tracked (after “gut” parasites; Table  
272 2). The overall relationship between *Pasteuria* infection levels and investment in sex in *D.*  
273 *dentifera* was consistent with that of the combined infection levels and investment in sex (Fig.

274 5), but was not significant. Instead, the strongest correlation was between the integrated  
275 prevalence of an oomycete that attacks developing embryos, *Blastulidium paedophthorum*.  
276 Overall, prior work in *Daphnia* suggested that parasites might favor sexual reproduction in hosts;  
277 our work expands this by showing that the prevalence of sexually reproducing individuals in  
278 natural lake populations is associated with parasitism (as well as density).

279  
280 Intriguingly, there was a strong positive relationship between parasite species richness (the  
281 number of parasite taxa observed over the summer and fall in a particular lake) and the amount  
282 of sexual reproduction (Fig. 4b). An earlier study on hermaphroditic snails found that male  
283 outcrossing ability correlated with an index that combined trematode prevalence and species  
284 richness (Schrag et al. 1994); similar to our study, that study found a correlation between species  
285 richness and prevalence (in the snail study, the prevalence of one particular trematode) that made  
286 it hard to disentangle their relative effects. Looking at a much larger scale, a study on plants  
287 found that species that are attacked by more fungal pathogens have higher outcrossing rates, as  
288 compared to species that are attacked by fewer pathogens (Busch et al. 2004). Collectively, these  
289 results suggest additional research on parasite species richness and sexual reproduction is  
290 warranted.

291  
292 We focused on the influences of parasitism and density on investment in sex. An interesting  
293 avenue for future research would be to consider, in addition to density and parasitism, the  
294 impacts of resources and predators, which have also been shown to influence shifts to sexual  
295 reproduction in *Daphnia* (Walsh 2013). However, doing so becomes logistically challenging.  
296 While it is relatively straightforward to quantify the abundance of invertebrate predators such as  
297 *Chaoborus* larvae, directly quantifying the rate of fish predation is challenging, though body size  
298 can be used as a proxy (Brooks and Dodson 1965; Kitchell and Kitchell 1980). Similarly,  
299 directly quantifying resource quality can be challenging, since chlorophyll levels in a lake do not  
300 strongly correlate with the resources experienced by *Daphnia* (Tessier and Woodruff 2002).  
301 However, the average clutch size (known as the “egg ratio”) of uninfected hosts can be used as  
302 an indicator of resource levels as experienced by *Daphnia* (Threlkeld 1979; Kerfoot et al. 1988)  
303 so, similar to predation, it is possible to use proxies to assess resource levels. Thus, future studies  
304 that measure invertebrate predators, *Daphnia* body size, and *Daphnia* egg ratio in addition to the

305 factors we measured in this study would give greater insight into the factors driving variation in  
306 investment in sex.

307

308 It would also be interesting for future research to consider the potential impacts of abiotic factors  
309 on sexual reproduction in lake *Daphnia* populations. In particular, temperature and light are  
310 known cues for *Daphnia* reproductive cycles (Stross and Hill 1965). This work should consider  
311 not only direct impacts of those abiotic factors on sexual reproduction, but also the potential for  
312 indirect effects. Prior studies in this system have shown that habitat structure (including light and  
313 thermal structure) can have a range of direct and indirect effects on parasitism (Penczykowski et  
314 al. 2014; Strauss et al. 2016; Shaw et al. 2020), and it is possible (perhaps even likely) that the  
315 same is true for investment in sex.

316

317 Shifts from asexual to sexual reproduction in cyclical parthenogens have large impacts on fitness  
318 (Gerber et al. 2018) and population dynamics. We found that wild *Daphnia dentifera* populations  
319 varied greatly in the degree to which they invested in sexual reproduction, with some remaining  
320 entirely asexual and others shifting almost entirely to sexual reproduction. Host density and  
321 parasitism were strongly predictive of the frequency of sexual females and males in these  
322 populations, providing evidence in support of links between parasitism, density, and sexual  
323 reproduction.

324

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330

#### 331 AUTHORS' CONTRIBUTIONS

332 MAD initiated the project and CDG helped shape the project; CDG, MAR, CLS, and KKH  
333 collected and analyzed samples; CDG and MAD analyzed the data; CDG and MAD wrote the  
334 manuscript, and MAR, CLS, and KKH also contributed to the writing.

335

336 CONFLICT OF INTEREST

337 The authors declare no conflicts of interest.

338

339 DATA ACCESSIBILITY STATEMENT

340 Data and associated code are available at Dryad: <https://doi.org/10.5061/dryad.pzgmsbcm6>

341

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511

512 TABLES

513 **Table 1.** Model selection results from linear models with total integrated sexual reproduction as  
 514 the response variable. Models are arranged by AIC score. “Int. density” indicates integrated *D.*  
 515 *dentifera* density, “int. inf. prev.” indicates integrated infection prevalence, and “parasite SR”  
 516 indicates parasite species richness.

	<i>Model</i>	<i>AIC</i>	$\Delta AIC$	<i>AIC weight</i>
1	sex ~ log(int. density) + parasite SR	253.10	0.00	0.264
2	sex ~ log(int. density)	253.12	0.01	0.262
3	sex ~ log(int. density) + int. inf. prev.	253.83	0.73	0.183
4	sex ~ log(int. density) * int. inf. prev.	255.81	2.71	0.068
5	sex ~ parasite SR	256.26	3.15	0.055
6	sex ~ log(int. density) + year	256.68	3.58	0.044
7	sex ~ parasite SR + int. inf. prev.	256.94	3.83	0.039
8	sex ~ log(int. density) + int. inf. prev. + year	257.72	4.61	0.026
9	sex ~ log(int. density) + int. inf. prev. + parasite SR + year	257.78	4.67	0.026
10	sex ~ log(int. density) + int. inf. prev. * year	258.01	4.91	0.023
11	sex ~ log(int. density) * int. inf. prev. + year	259.69	6.59	0.010
12	sex ~ int. inf. prev.	270.72	17.62	3.95E-05
13	sex ~ int. inf. prev. + year	270.77	17.67	3.85E-05

14	sex ~ int. inf. prev. * year	270.89	17.79	3.62E-05
15	sex ~ year	278.04	24.93	1.02E-06

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519 **Table 2.** Summary of the virulent effects and prevalence of the five most common parasites in  
520 this study, as well as the correlation between the integrated prevalence of each parasite and the  
521 integrated prevalence of sexual reproduction (as shown in Fig. 5). Information on virulence in *D.*  
522 *dentifera* comes from prior studies (Duffy and Hall 2008; Auld et al. 2012; Duffy et al. 2015;  
523 Wale et al. 2019; Rogalski et al. unpubl.). “Gut” parasites are the ichthyosporean *Caullerya*  
524 *mesnili* and a microsporidian currently known as “MicG” (Rogalski et al. unpubl.; Genbank  
525 accession MH635259). Parasite prevalences come from the 15 lake populations and three years  
526 that were the focus of the present study. The correlation was calculated between the integrated  
527 prevalence of each particular parasite and the integrated prevalence of sexual individuals (Fig.  
528 5).

Parasite	Parasite virulence		Parasite prevalence			Correlation between integrated prevalence and sexual reproduction	
	Impact on reproduction	Impact on lifespan	Median	Mean	Max	r	p
<i>Pasteuria ramosa</i>	Castrating	Low	1.9%	4.9%	36.5%	0.218	0.150
<i>Metschnikowia bicuspidata</i>	Moderate	High	0.0%	0.9%	14.0%	0.018	0.908
<i>Spirobacillus cienkowskii</i>	Castrating	Very high	0.5%	1.7%	20.1%	0.256	0.090
<i>Blastulidium paedophthorum</i>	Castrating	None detected	0.9%	2.2%	11.2%	0.369	0.013
Gut	Variable	High for <i>C. mesnili</i> , none	7.3%	13.5%	57.8%	0.231	0.127

		detected for MicG					
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533 **Figure S1.** Analyses with mean host density and parasitism yielded qualitatively similar results.

534 **(a)** Populations of *D. dentifera* with higher densities had higher sexual reproduction ( $r = 0.649$ ,  $p$

535  $< 0.0001$ ). **(b)** *D. dentifera* populations with more total parasitism tended to have more sexual

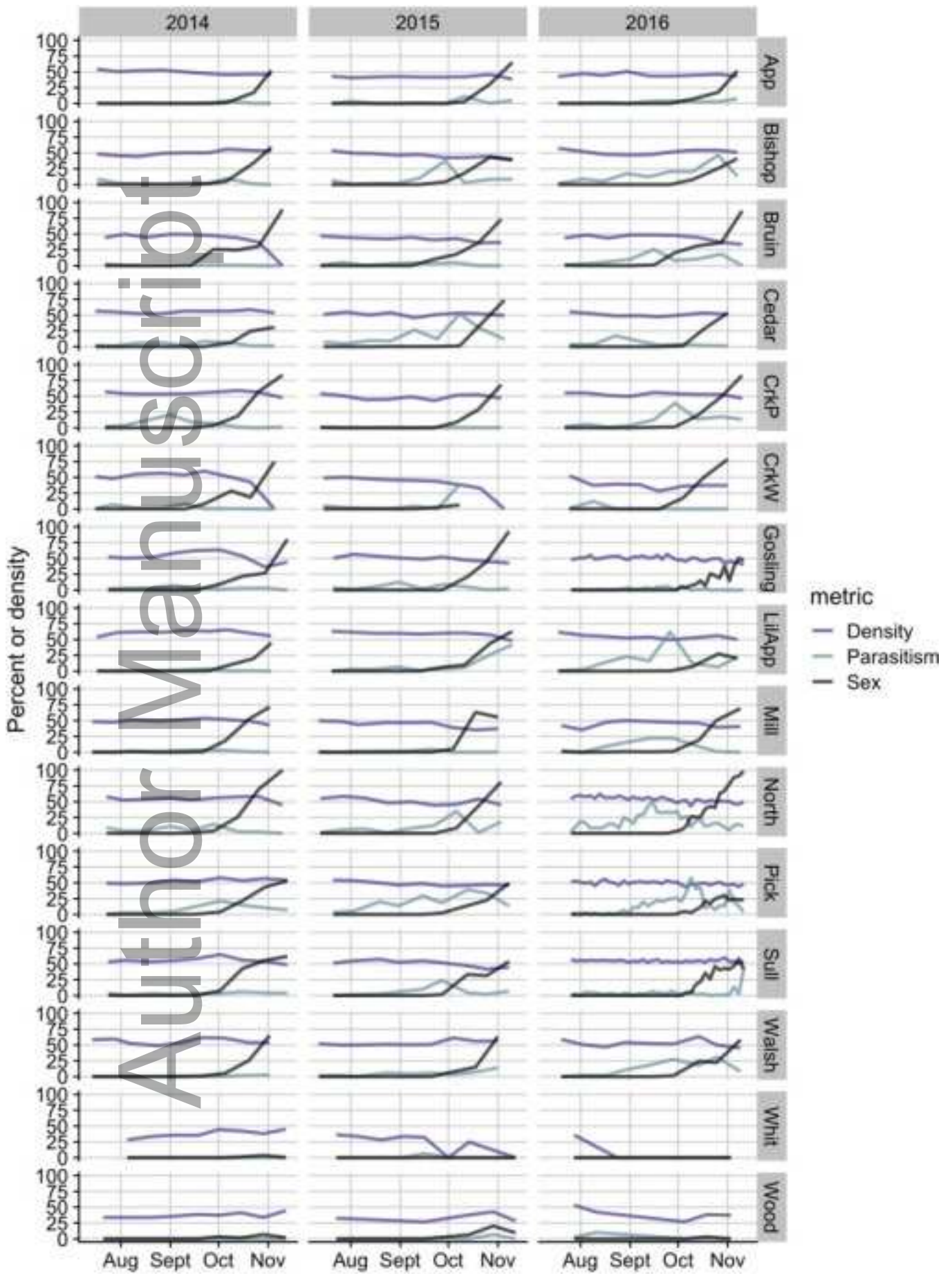
536 reproduction ( $r = 0.316$ ,  $p = 0.034$ ). **(c)** Populations with greater *D. dentifera* density higher

537 parasitism ( $r = 0.372$ ,  $p = 0.012$ ).

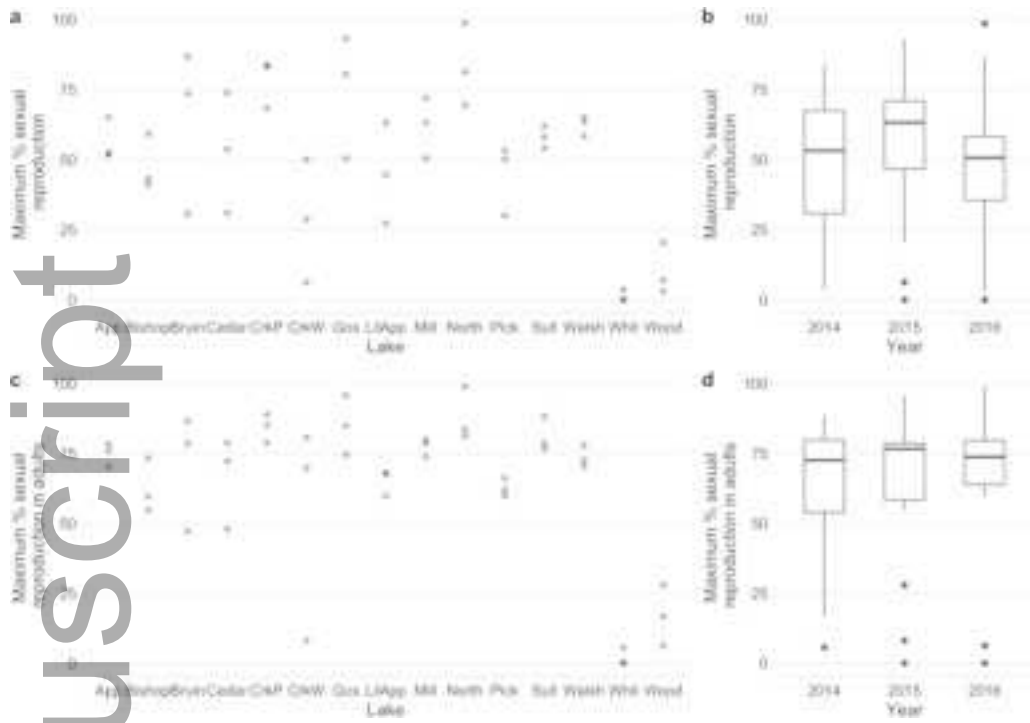
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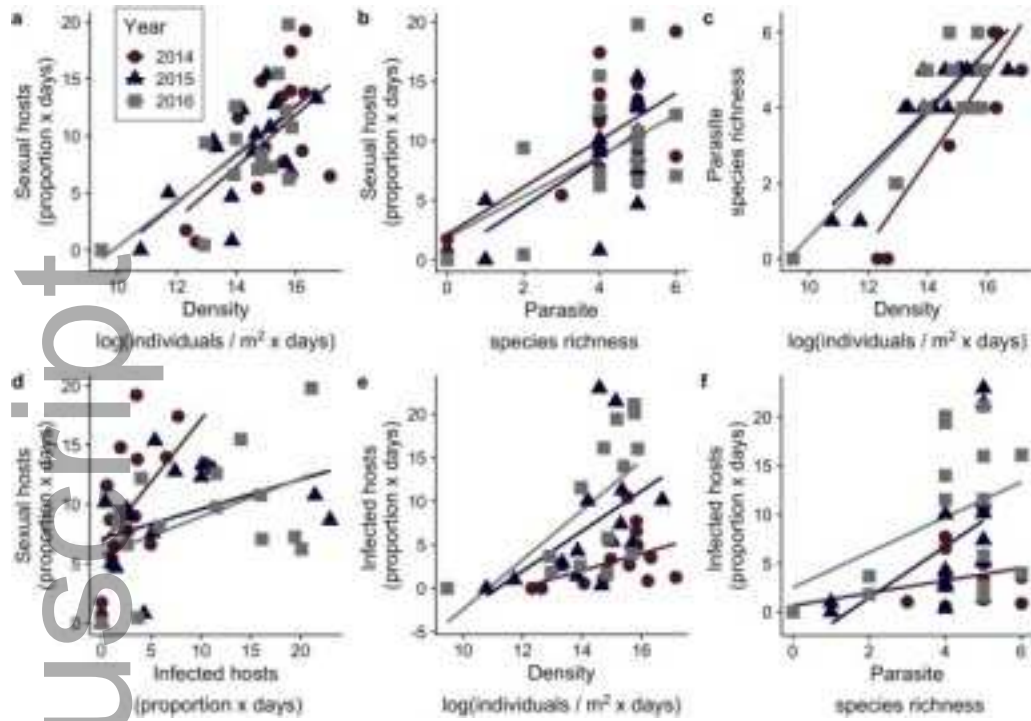
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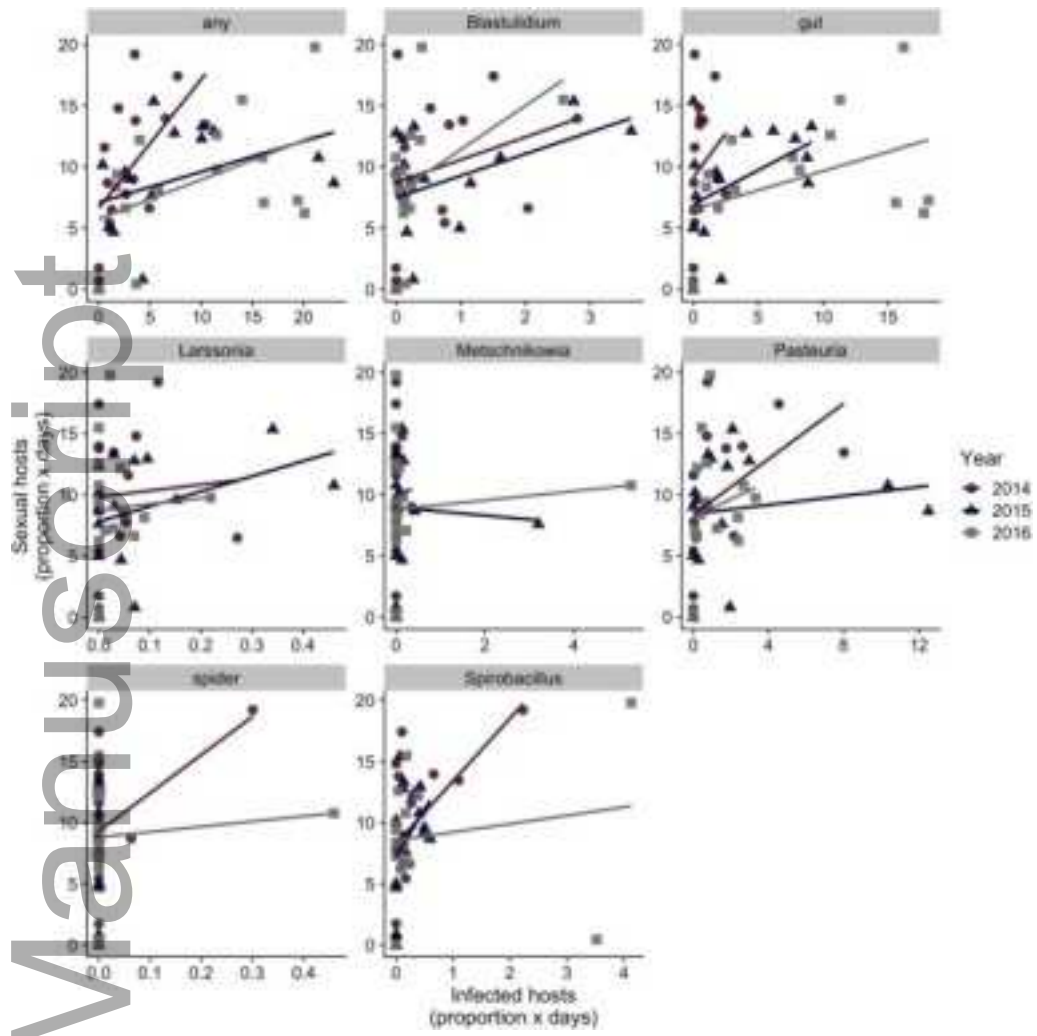
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