

**Multiple factors likely explain variation in investment in
sexual reproduction by lake *Daphnia* populations**

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RUNNING HEAD: Variation in sex in *Daphnia* populations

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CDG, MAR, CLS, and KKH collected and analyzed samples; CDG analyzed the data, with input
from MAD; CDG and MAD wrote the manuscript, and MAR, CLS, and KKH also contributed to
the writing.

ABSTRACT

Many organisms can reproduce both asexually and sexually. For cyclical parthenogens, periods of asexual reproduction are punctuated by bouts of sexual reproduction, and the timing of the shift from asexual to sexual reproduction has large impacts on fitness and population dynamics. We studied populations of *Daphnia dentifera* to determine the amount of investment in sexual reproduction as well as the factors associated with variation in investment in sex. To do so, we tracked host density, parasite infections, sexual reproduction, temperature, and light attenuation in 15 lake populations of *D. dentifera* for three years. We monitored infections by nine common parasites; this is notable since most prior studies on investment in sex and parasitism have focused on a single parasite, even though multiparasite communities are the norm in nature. We found substantial variation in investment in sex, with some populations reproducing entirely asexually throughout the study period and others shifting almost entirely to sexual reproduction by late autumn. We found that higher host density and parasitism were associated with greater investment in sex. Temperature and light attenuation were not as predictive of investment in sex, but received some statistical support. While correlational, our results leverage a large time series dataset and suggest multiple factors likely drive variation in sexual reproduction in this dominant member of lake food webs.

INTRODUCTION

A major challenge in evolutionary biology is explaining variation in reproductive strategies — often framed as a question of why some species reproduce asexually and others sexually. Asexual reproduction has many benefits, including avoiding the “twofold cost” of sex (Otto 2009; Stelzer 2011), as well as other potential drawbacks of sexual reproduction (e.g., challenges in finding a mate, acquisition of sexually transmitted infections, shuffling of alleles that worked well in a parent (Otto 2009; McLeod and Day 2014; Kokko 2020)). At the same time, sexual reproduction also has advantages, including providing an opportunity to purge deleterious mutations and producing novel genotypes that can avoid infection by parasites (Muller 1964; Jaenike 1978; Kondrashov 1984; Lively 2010). However, framing reproduction as a dichotomy between (entirely) sexual or (entirely) asexual ignores the abundance of organisms that combine the two (Gerber et al. 2018; Kokko 2020). By being able to shift between sexual and asexual reproduction, cyclical parthenogens are often described as experiencing the “best of both worlds” (Kokko 2020), gaining the benefits of sexual reproduction while also avoiding its costs. However, this ability to shift between these two modes of reproduction raises a new question: when to shift from asexual to sexual reproduction?

When considering the optimal timing of a shift from asexual to sexual reproduction, it is important to consider that sexual reproduction in cyclical parthenogens is often associated with dormancy (Walsh 2013; Gerber et al. 2018; Gerber and Kokko 2018; Kokko 2020). Sexual reproduction thus not only affords the benefits of creating novel genotypes and purging mutational load (Cáceres et al. 2009), but also can allow a lineage to escape through time, potentially waiting out harsh conditions. Given the strong spatial and temporal variation in biotic and abiotic conditions that exist in nature, it is perhaps not surprising that populations of cyclical

parthenogens can vary substantially in the degree to which they invest in sexual reproduction (Walsh 2013) — as seen, for example, in studies of *Daphnia* populations (e.g., Tessier and Cáceres 2004; Johnson et al. 2009; Walsh and Post 2012; Gerber et al. 2018). Prior research on *Daphnia spp.*, a dominant member of pond and lake food webs, has identified a variety of factors that contribute to a shift from asexual to sexual reproduction, including predation, parasitism, crowding, resource limitation, and changing abiotic conditions (Stross and Hill 1965; Walsh 2013; Gerber et al. 2018; Haltiner et al. 2020).

A potential role of parasitism in sexual reproduction in *Daphnia* has received particular attention in recent years. Sexually produced *Daphnia* offspring are more fit against contemporaneous parasites (Ebert et al. 2007; Auld et al. 2016), and more susceptible genotypes are more likely to shift to sexual reproduction (Mitchell et al. 2004; Duncan et al. 2006). Moreover, studies on two different *Daphnia*-parasite systems found the production of males was more likely in the presence of parasites (Roth et al. 2008; Hite et al. 2017) and, in a third system, sexual reproduction was higher in years with more infection by a chytrid parasite (Johnson et al. 2009). One notable feature of prior studies on the role of parasitism in investment in sexual reproduction in *Daphnia* (and other species: (Lively 1987; Lively and Dybdahl 2000; Morran et al. 2011; Slowinski et al. 2016)) has been a focus on a single parasite (but see (Wolinska and Spaak 2009), which looked for Red Queen dynamics in a *Daphnia*-multiparasite system). This likely leads to an incomplete picture of the role of parasites in investment in sex, since *Daphnia*, like all hosts, are infected by an abundance of parasites (Green 1974; Ebert 2005), including in the same population (Stirnadel and Ebert 1997; Wolinska et al. 2009; Duffy et al. 2010).

In addition to biotic factors, abiotic factors like photoperiod and temperature can be associated with sexual reproduction in *Daphnia* (Stross and Hill 1965), and factors can interact

(e.g., decreasing photoperiod in conjunction with lower temperature or higher density triggers sexual reproduction (Stross and Hill 1965)). Moreover, abiotic factors can influence biotic factors. As one example, because temperature and light levels influence phytoplankton growth, with different taxa thriving at different temperatures and light levels (Prescott 1962; Singh and Singh 2015), differences in temperature and light attenuation might alter phytoplankton communities and, in turn, alter competition, density, and parasitism in grazers such as *Daphnia*. If those factors are important for investment in sex, abiotic differences among populations might indirectly influence investment in sex.

In this study, we take advantage of a large multi-year, multi-population dataset to assess multiple possible drivers of investment in sexual reproduction in *Daphnia*. We were particularly interested in exploring associations between parasitism by multiple pathogens and investment in sex. Because there is also substantial evidence for a role of density as a cue for sexual reproduction in *Daphnia* (Stross and Hill 1965; Larsson 1991; Berg et al. 2001; Gerber et al. 2018; Haltiner et al. 2020), likely due to increased competition for resources, we also considered density as a possible driver of investment in sex. We also explored whether two key features of the abiotic environment, temperature and light attenuation, were associated with investment in sex. Overall, our study leverages an unusually comprehensive dataset to better understand the factors influencing investment in sex in natural populations of a dominant member of lake food webs.

117 MATERIALS

118 *Study system*

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

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129 *D. dentifera* occurs at varying densities across our 15 study lakes in Southeast Michigan, US and
130 is infected by a suite of parasites (Duffy et al. 2010). We tracked *D. dentifera* population sizes
131 through time, as well as infections of nine microparasites (Green 1974; Wolinska et al. 2008;
132 Duffy et al. 2010, 2015; Lu et al. 2020): *Metschnikowia bicuspidata* (fungus), *Pasteuria ramosa*
133 (bacterium), *Spirobacillus cienkowskii* (bacterium), *Blastulidium paedophthorum* (oomycete),
134 *Gurleya vavrai* (microsporidian), *Larssonia obtusa* (microsporidian), *Caullerya mesnili*
135 (ichthyosporean), an undescribed microsporidian gut parasite (“MicG”), and an unknown
136 *Saprolegnia*-like oomycete (“spider”).

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Field sampling

We studied host and parasite communities in 15 lakes in Southeast Michigan, USA (Table S1) over three years (2014-2016). We sampled lakes roughly once every two weeks from mid-July to mid-November each year (usually 9 sampling events per year). In addition to our normal sampling efforts, we intensively sampled four of the study sites (Gosling, North, Pickerel, and Sullivan Lakes) every three days during 2016 for a study focused on population dynamics. For each lake, on each sampling date, we collected three replicate vertical tows from the bottom of the lake with a 153 μm Wisconsin plankton net and sampled from three different locations in each lake. This yielded three replicate samples per lake per sampling day, each of which contained one tow from each of the three locations within the lake. For one replicate sample, we visually diagnosed parasite infections in live hosts under a dissection microscope at 20-50x magnification using dark field microscopy (or under a compound microscope at 200 to 400x magnification for early-stage infections). As *Daphnia* are mostly transparent, many parasite infections are visibly detectable with this method. We also counted males and sexually reproducing females based on morphological differences (Brooks 1957). We randomly subsampled the collected hosts, surveying at least 200 individuals of each host species for possible parasite infections or surveying all individuals when fewer than 200 individuals of a host species were present. We preserved the other two replicate samples in 90% ethanol, and, at a later date, we randomly subsampled and counted one of the samples to estimate the density of each host species. Density was calculated as the number of hosts throughout the water column for a given surface area of the lake (number of hosts per m^2 of lake surface).

In addition to the two potential biotic cues for shifts to sexual reproduction in *D. dentifera* (parasitism and total host density), we also measured two abiotic factors: temperature and light attenuation. Lake water temperature was recorded with a sonde (Hydrolab MS5 multiprobe, Hach Hydromet (now OTT Hydromet)) during nearly every sampling event (the exceptions resulted from equipment malfunctions). In our analyses, we used the temperature at 2 meters below the surface of the lake and averaged the temperature values within each lake for each year. Due to an equipment malfunction that limited temperature data for October 2016, we averaged the temperature for mid-July through the end of September; results were qualitatively the same if we use data for just August or just September. For light measurements, we calculated metrics for visible light attenuation (ad440) for each lake in both 2014 and 2016; because we did not have measurements from 2015, we averaged the values from a given lake in 2014 and 2016 and used that as the 2015 value. This metric is based on absorbance of filtered lake water in a spectrophotometer (Kirk 1994).

Statistical analysis

In our analysis, sexual reproduction in *D. dentifera* could depend on the effects of host density, parasitism, temperature, or light attenuation. For host density, we integrated the total host density of *D. dentifera* for each lake in a year over all sampling dates (i.e., we calculated the area under the curve with day on the x-axis and host density on the y-axis). Parasitism, calculated as the proportion of hosts infected with any parasite, was also integrated across sampling events within a lake and year. Similarly, we integrated the proportion of hosts that were sexually reproducing (ephippial) females or males. These integrated measurements are common for this type of data;

using mean and maximum values of density, infection prevalence, and sexually reproducing animals gave similar results (Fig. S3, Fig. S4).

In R (version 3.5), we used a generalized linear model that included host density, parasitism, their interaction (density*parasitism), visible light attenuation (ad440), and temperature (mean at 2m depth) as predictors with sexual reproduction as the dependent variable, with a Gaussian error distribution. We created various sub-models and then used model selection and Akaike information criteria (AIC) to compare 13 different sub-models (as detailed in Table 1 in the Results section, below).

RESULTS

There was substantial variation in investment in sex and parasite prevalence in the study populations of *D. dentifera* (Fig. 1). Sexual reproduction was seasonal, with male and ephippial female production beginning as early as late September and generally increasing through November. In some lakes and years, we never observed any males or ephippial females, whereas in others, the population shifted to nearly all (98%) being sexual. Maximum parasite prevalence was also quite variable, ranging from 0% in some lake-years to 62% in others.

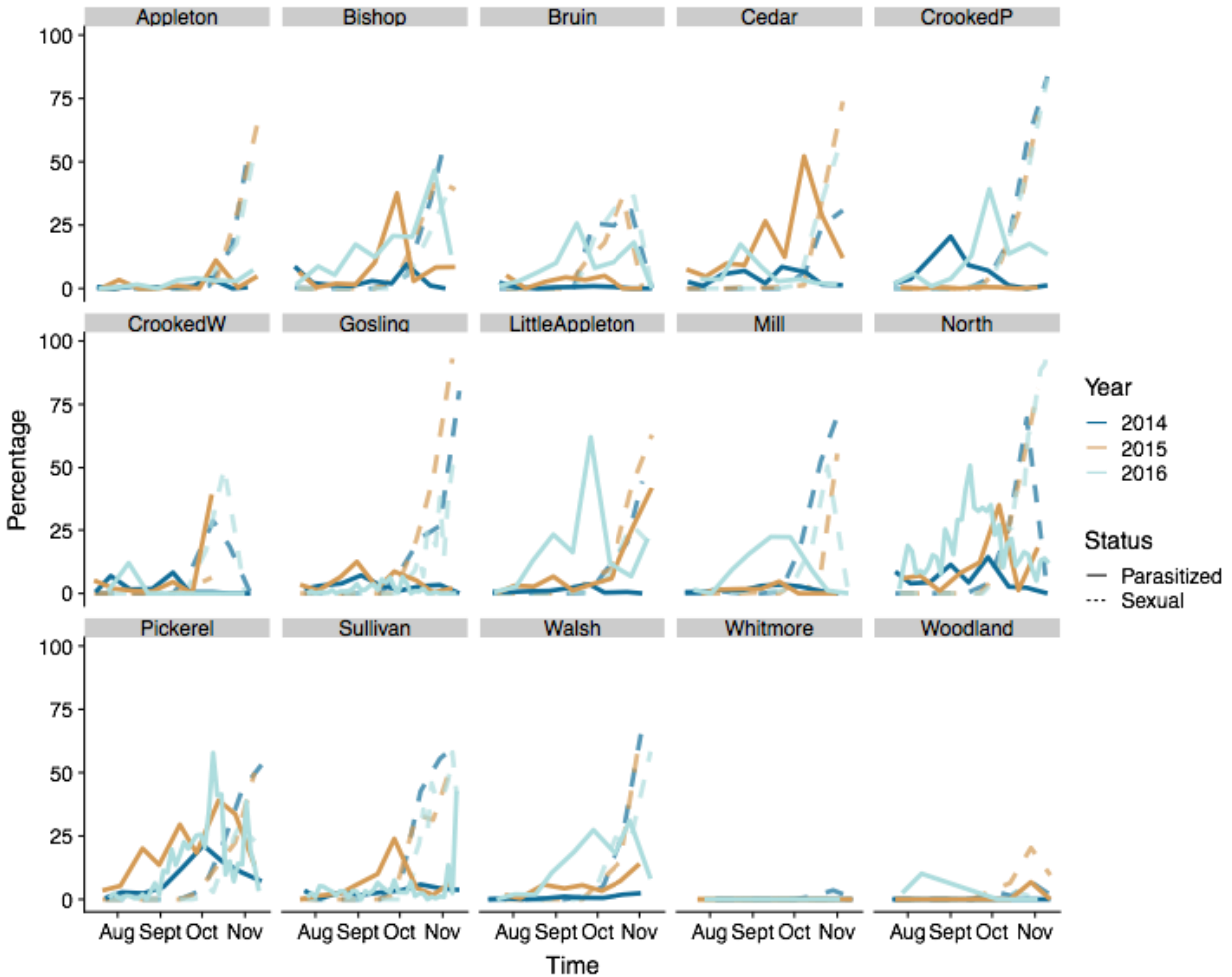


Figure 1. Across lakes, years, and calendar day, there was considerable variation in the percentage of *D. dentifera* reproducing sexually, as well as in the percent infected with at least one parasite. The percent sexual was derived from the ratio of males and ephippial females out of the total population counted. Percent infected was calculated as the percent of *D. dentifera* with any parasitic infection, including coinfections. Solid lines represent percent of population parasitized and dashed lines represent the percentage reproducing sexually. Colors denote different sampling years. Figure S6 shows percent sexual and percent infected in separate panels for each year, with individual lakes overlain.

Integrated host density strongly correlated with sexual reproduction of *D. dentifera* (Fig. 2a, $F = 29.44$, $p < 0.001$, Table 1). As the density of hosts increased, we observed more sexual reproduction (Fig. 2a and Fig. S1a). Interestingly, both the density of conspecific hosts and the density of all *Daphnia* species correlated with sexual reproduction (Fig. S2a, $\text{sex} \sim \log(\text{combined}$

host density), $F = 6.27$, $p = 0.02$). A model selection approach suggests the importance of density: all top models included $\log(\text{density})$ as a predictor of sexual reproduction (Table 1).

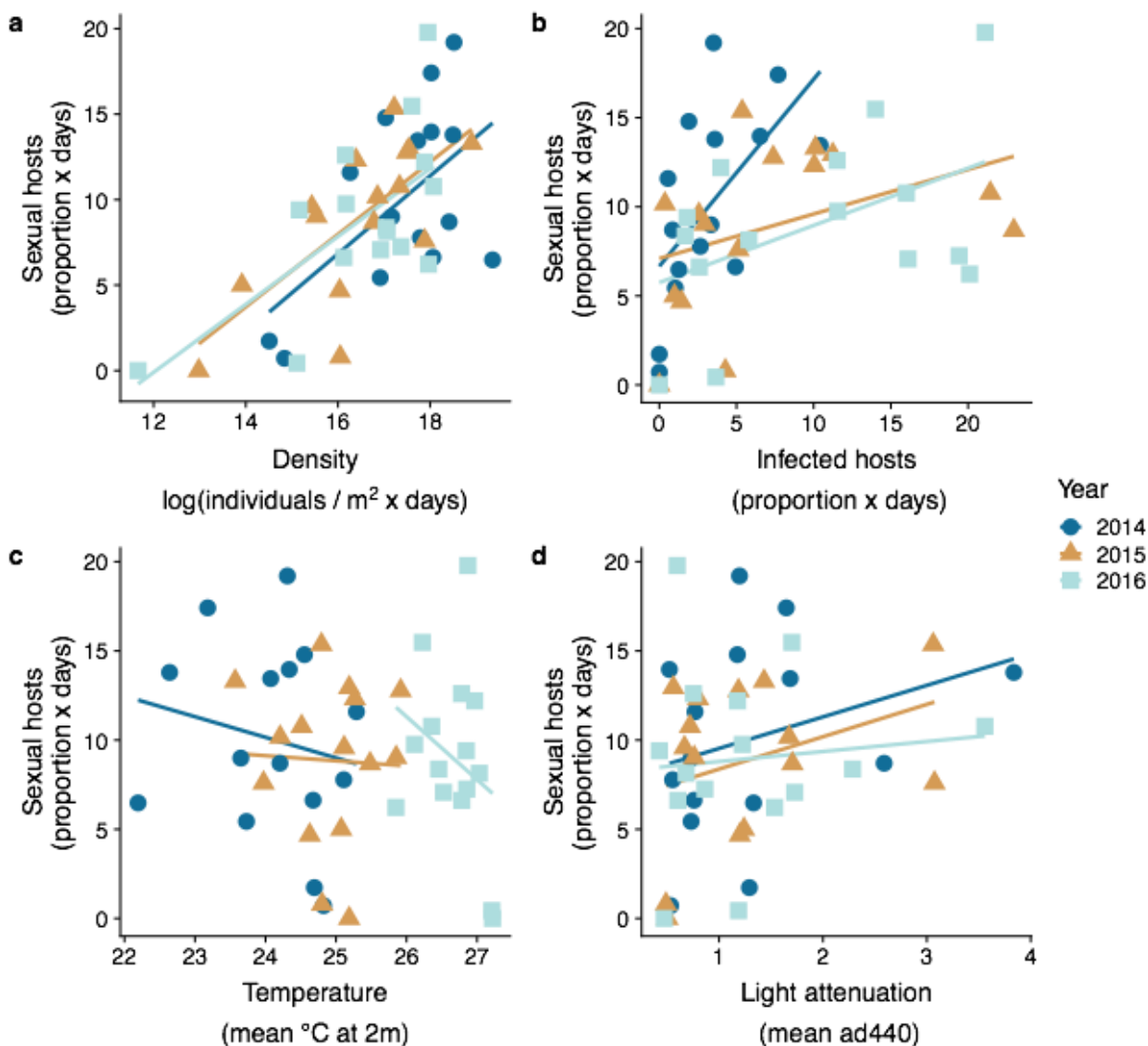


Figure 2. *D. dentifera* density and levels of parasitism were strongly associated with the amount of sexual reproduction in *D. dentifera* populations. (a) shows integrated total *D. dentifera* density on a log scale, and (b) shows integrated area of the proportion of *D. dentifera* infected with any parasite. Two abiotic factors, (c) mean temperature at 2m below the surface, and (d) light attenuation, as measured by mean ad440, were less predictive but modestly improved model fit (Table 1). The areal density of *D. dentifera*, the proportion of infected *D. dentifera*, and the proportion of male and sexual female *D. dentifera* values were each separately integrated across sampling events to obtain a single value (each point represents a single lake in a given year); analyses with mean and maximum *D. dentifera* density and parasitism generally yielded qualitatively similar results (Fig. S3, Fig. S4).

Table 1. Model selection results from linear models with total integrated sexual reproduction as the response variable. Models are arranged by AIC score.

	<i>Model</i>	<i>AIC</i>	ΔAIC	<i>AIC weight</i>
1	sex ~ log(density)	253.12	0.00	0.346
2	sex ~ log(density) + parasitism	253.83	0.72	0.242
3	sex ~ log(density) + parasitism + temperature	255.55	2.43	0.103
4	sex ~ log(density) * parasitism	255.81	2.70	0.090
5	sex ~ log(density) + parasitism + light	255.82	2.70	0.090
6	sex ~ log(density) + light + temperature	256.10	2.99	0.078
7	sex ~ log(density) + parasitism + light + temperature	257.55	4.43	0.038
8	sex ~ log(density) * parasitism + light + temperature	259.51	6.39	0.014
9	sex ~ parasitism + light + temperature	270.39	17.28	6.14E-05
10	sex ~ parasitism	270.72	17.61	5.21E-05
11	sex ~ light	273.95	20.83	1.04E-05
12	sex ~ light + temperature	275.59	22.47	4.57E-06
13	sex ~ temperature	275.60	22.49	4.53E-06

Besides host density, parasitism was most strongly associated with the degree of sexual reproduction in *D. dentifera* (sex ~ parasitism, *gaussian*, $F = 5.99$, $p = 0.019$). We used a model comparison approach to allow us to jointly test the effects of density, parasitism, light, and temperature. Using this approach, the model with density and parasitism as predictors was the second best model and performed similarly to the model with just density ($\Delta AIC = 0.72$). Moreover, the next three models ($\Delta AIC = 2.43$ - 2.70) all incorporated parasitism in addition to density. Thus, host density and parasitism were both positively associated with sexual reproduction in *D. dentifera*.

We also explored the possibility that temperature and light attenuation might be important predictors of the amount of sexual reproduction, and they moderately improved the model fit.

While the two best performing models did not incorporate abiotic factors (Table 1), three of the next four models ($\Delta AIC = 2.43$ -2.99) included temperature (mean °C at 2m below the surface) and/or visible light attenuation (ad440). However, in linear models with a single predictor, temperature did not significantly affect sexual reproduction (sex ~ temperature, $F = 0.94$, $p = 0.34$) nor did light attenuation (sex ~ light, $F = 2.60$, $p = 0.11$). Overall, temperature and light attenuation may be associated with the amount of sexual reproduction in *D. dentifera*, but to a lesser degree than density and parasitism.

DISCUSSION

We found substantial variation in investment in sexual reproduction in natural populations of *Daphnia dentifera*, with some populations remaining entirely asexual and others becoming almost entirely sexual in autumn. Host density and parasitism were most predictive of this variation in investment in sex (Fig. 2, Table 1). However, model selection approaches revealed that temperature and light attenuation might also be associated with the amount of sexual reproduction. Taken together, our results suggest multiple factors likely impact investment in sexual reproduction in populations of *D. dentifera*.

Our findings are consistent with earlier studies that found density to be an important factor influencing the shift from asexual to sexual reproduction in cyclical parthenogens like *Daphnia* and rotifers (Stross and Hill 1965; Larsson 1991; Berg et al. 2001; Stelzer and Snell 2003; Haltiner et al. 2020; Gilbert 2020). One possible explanation for this association is that, in many cyclical parthenogens, sexual reproduction is associated with the production of long-lasting resting stages, meaning sexual reproduction may serve as a means of temporal dispersal when

faced with strong competition in dense populations (Gerber et al. 2018; Gilbert 2020). High densities also reduce the relative costs of sexual reproduction; as populations approach carrying capacity, asexual reproduction is less beneficial, reducing the opportunity costs of sexual reproduction (Gerber et al. 2018).

We also found that parasitism was positively correlated with sexual reproduction in *D. dentifera*, which is also consistent with prior work. However, contrary to this study, prior studies have generally focused on relationships between the prevalence of a single parasite and investment in sex. There has been a particular focus on the bacterial parasite *Pasteuria ramosa* and investment in sex. *Pasteuria* is highly virulent (Ebert et al. 2000; Auld et al. 2012) and can reach quite high prevalence (Duncan and Little 2007). It also shows very strong host-parasite genotype specificity, with parasite infectivity (and host susceptibility) being determined by host (and parasite) genotype (Carius et al. 2001; Ebert et al. 2016). One would expect this matching mechanism to favor genetic recombination (and it does in Auld et al. 2016), which could, in turn, drive Red Queen dynamics, where reciprocal evolutionary dynamics arise from selection of two antagonists on one another. Indeed, one of the best examples of Red Queen dynamics comes from the *Daphnia-Pasteuria* system (Decaestecker et al. 2007). In our present study, *Pasteuria* was one of nine parasites that we tracked. The overall relationship between *Pasteuria* infection levels and investment in sex in *D. dentifera* was consistent with that of the combined infection levels and investment in sex (Figure S5). However, we can also see positive relationships between other parasites and sexual reproduction in at least some years (Figure S5). Given that we know that most hosts are infected by multiple parasites (Rigaud et al. 2010), if we wish to fully

understand the relationship between parasitism and investment in sex, we should consider the multiparasite environment experienced by hosts.

While we were most interested in the influences of parasitism and density on investment in sex, we also considered the potential for temperature and light, which are known cues for *Daphnia* reproductive cycles (Stross and Hill 1965), to influence sexual reproduction. Our results suggest that temperature and light attenuation may also influence the variation in sexual reproduction in these populations. Drawing broader conclusions from the impact of abiotic forces is more difficult, including because there are a myriad of ways in which they can influence parasitism, host density, and sexual reproduction. Given the metrics of temperature and light available to us (seasonal averages), the effects of light and temperature are likely occurring via a different mechanism than those occurring in the study by Stross and Hill (1965). In particular, our study is not measuring whether a change in temperature or photoperiod triggered a shift to sexual reproduction. Instead, it is asking whether light attenuation in a lake and/or (epilimnion) temperatures are associated with overall levels of investment in sex. Prior studies in this system have shown that habitat structure (including light and thermal structure) can have a range of direct and indirect effects on parasitism (Penczykowski et al. 2014; Strauss et al. 2016; Shaw et al. 2020), and it is possible (perhaps even likely) that the same is true for investment in sex.

An interesting avenue for future research would be to consider the impacts of resources and predators, which have also been shown to influence shifts to sexual reproduction in *Daphnia* (Walsh 2013), in addition to all the factors we considered in this study. However, doing so becomes logistically challenging. While it is relatively straightforward to quantify the abundance

of invertebrate predators such as *Chaoborus* larvae, directly quantifying the rate of fish predation is challenging, though body size can be used as a proxy (Brooks and Dodson 1965; Kitchell and Kitchell 1980). Similarly, directly quantifying resource quality can be challenging, since chlorophyll levels in a lake do not strongly correlate with the resources experienced by *Daphnia* (Tessier and Woodruff 2002). However, the average clutch size (known as the “egg ratio”) of uninfected hosts can be used as an indicator of resource levels as experienced by *Daphnia* (Threlkeld 1979; Kerfoot et al. 1988) so, similar to predation, it is possible to use proxies to assess resource levels. Thus, future studies that measure invertebrate predators, *Daphnia* body size, and *Daphnia* egg ratio in addition to the factors we measured in this study would give greater insight into the factors driving variation in investment in sex.

Shifts from asexual to sexual reproduction in cyclical parthenogens have large impacts on fitness (Gerber et al. 2018) and population dynamics. We found that wild *Daphnia dentifera* populations varied greatly in the degree to which they shift from asexual to sexual reproduction, with some remaining entirely asexual and others shifting almost entirely to sexual reproduction. Our results suggest that multiple factors, especially host density and parasitism, can explain this variation in investment in sex. While there is important prior work on investment in sexual reproduction by cyclical parthenogens, including *Daphnia*, our study is notable for the number of populations (15), the number of years of study (3), and for using a multiparasite framework. Future work that builds on this to additionally consider the impacts of predation and resource levels would further untangle the multiple factors that influence investment in sex in the wild.

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DATA ACCESSIBILITY STATEMENT

Data and associated code will be deposited to Dryad upon acceptance of this manuscript.

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478 APPENDIX

479 **Table S1.** Lakes in Michigan, USA sampled as part of this study.

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Lake Name	Site	County	Latitude & Longitude	Max depth (m)
Appleton	Brighton	Livingston	42°30'37"N, 83°50'03"W	11.5
Bishop	Brighton	Livingston	42°30'04"N, 83°50'24"W	16.5
Bruin	Pinckney	Washtenaw	42°25'07"N, 84°02'23"W	14.5
Cedar	Waterloo	Washtenaw	42°18'52"N, 84°04'45"W	7
Crooked P	Pinckney	Washtenaw	42°25'11"N, 83°58'57"W	12
Crooked W	Waterloo	Washtenaw	42°19'32"N, 84°06'43"W	6
Gosling	Pinckney	Livingston	42°26'22"N, 84°00'12"W	6
Little Appleton	Brighton	Livingston	42°30'24"N, 83°50'19"W	6
Mill	Waterloo	Washtenaw	42°19'46"N, 84°05'27"W	6
North	Pinckney	Washtenaw	42°23'35"N, 84°00'23"W	17
Pickerel	Pinckney	Washtenaw	42°24'37"N, 83°58'58"W	16
Sullivan	Pinckney	Washtenaw	42°23'55"N, 84°03'25"W	6.5
Walsh	Waterloo	Washtenaw	42°20'15"N, 84°04'4"W	6
Whitmore	Brighton	Livingston & Washtenaw	42°25'42"N, 83°45'08"W	19.5
Woodland	Brighton	Livingston	42°33'12"N, 83°46'29"W	10.5

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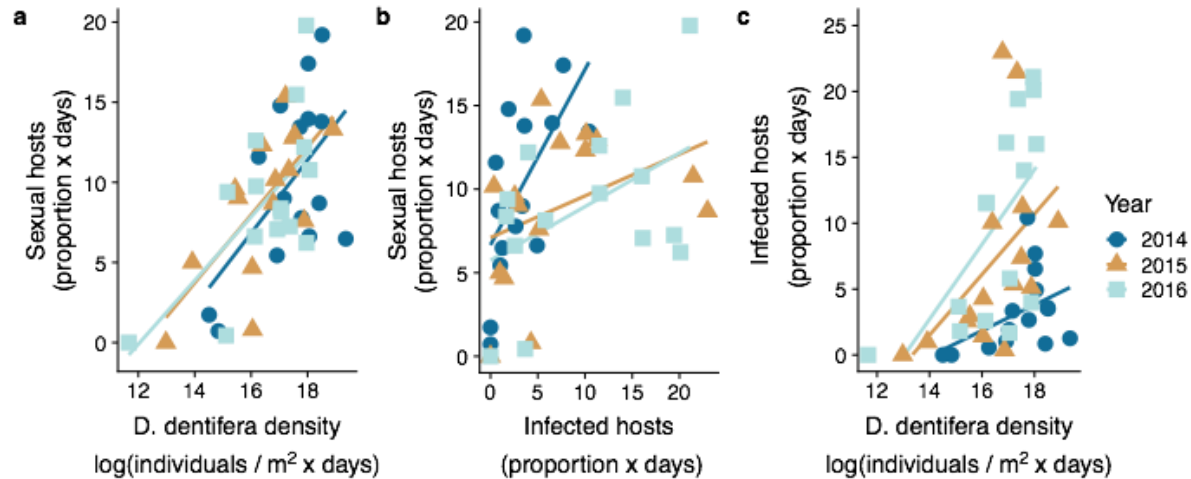


Figure S1. Variation in investment in sexual reproduction is best predicted by host density and parasitism. **(a)** Populations with higher *D. dentifera* densities had higher sexual reproduction (sex $\sim \log(\text{density})$, *gaussian*, $F = 29.44$, $p < 0.001$). **(b)** Populations with more total parasitism tended to have more sexual reproduction (sex \sim parasitism, *gaussian*, $F = 5.99$, $p = 0.019$). Additionally, **(c)** lakes where *D. dentifera* was at high densities typically had more total parasite infections (parasitism $\sim \log(\text{density})$, *negative binomial*, $z = 4.37$, $p < 0.001$). In a model selection approach, *D. dentifera* density consistently came out as a main predictor, followed by parasitism (see Table 1). The areal density of hosts, the proportion of infected hosts, and the proportion of male and sexual female hosts values were each separately integrated across sampling events to obtain a single value (each point represents a single lake in a given year).

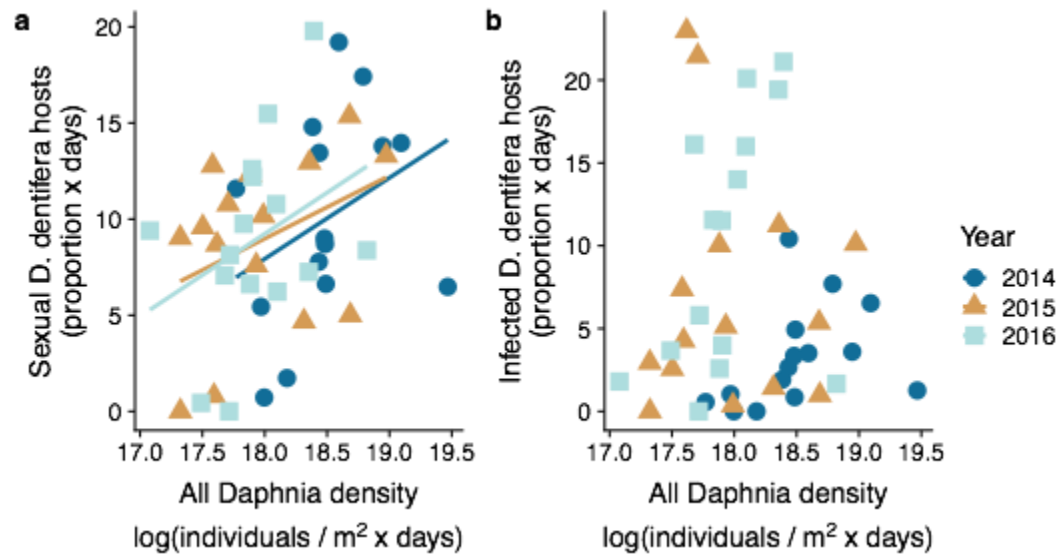
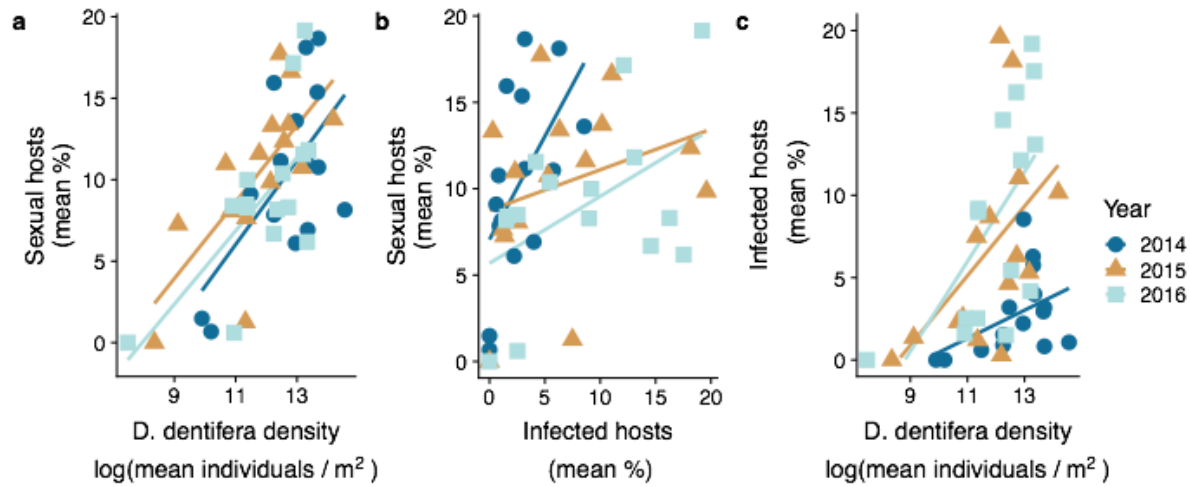


Figure S2. Variation in *D. dentifera* sexual reproduction also correlated with the density of all *Daphnia spp.* hosts (**a**) (sex $\sim \log(\text{combined host density})$, *gaussian*, $F = 6.27$, $p = 0.016$), but (**b**) variation in infection in *D. dentifera* did not correlate with the density of all *Daphnia* hosts (parasitism $\sim \log(\text{combined host density})$, *negative binomial*, $z = -0.48$, $p = 0.64$).

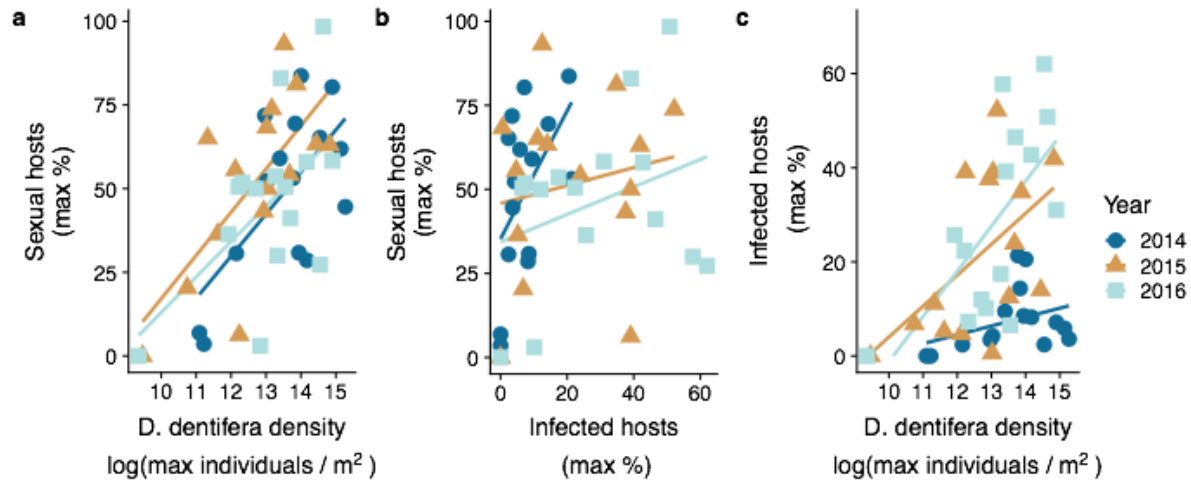
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506 **Figure S3.** Analyses with mean host density and parasitism yielded qualitatively similar results,
 507 except for the relationship between *D. dentifera* density and infected *D. dentifera*. **(a)**
 508 Populations of *D. dentifera* with higher densities had higher sexual reproduction (sex ~
 509 log(density), *gaussian*, $F = 31.35$, $p < 0.001$). **(b)** *D. dentifera* populations with more total
 510 parasitism tended to have more sexual reproduction (sex ~ parasitism, *quasipoisson*, $F = 4.34$, p
 511 = 0.043). However, **(c)** there was no correlation between *D. dentifera* density and total parasite
 512 infections (parasitism ~ log(density), *negative binomial*, $z = 0.58$, $p = 0.53$).
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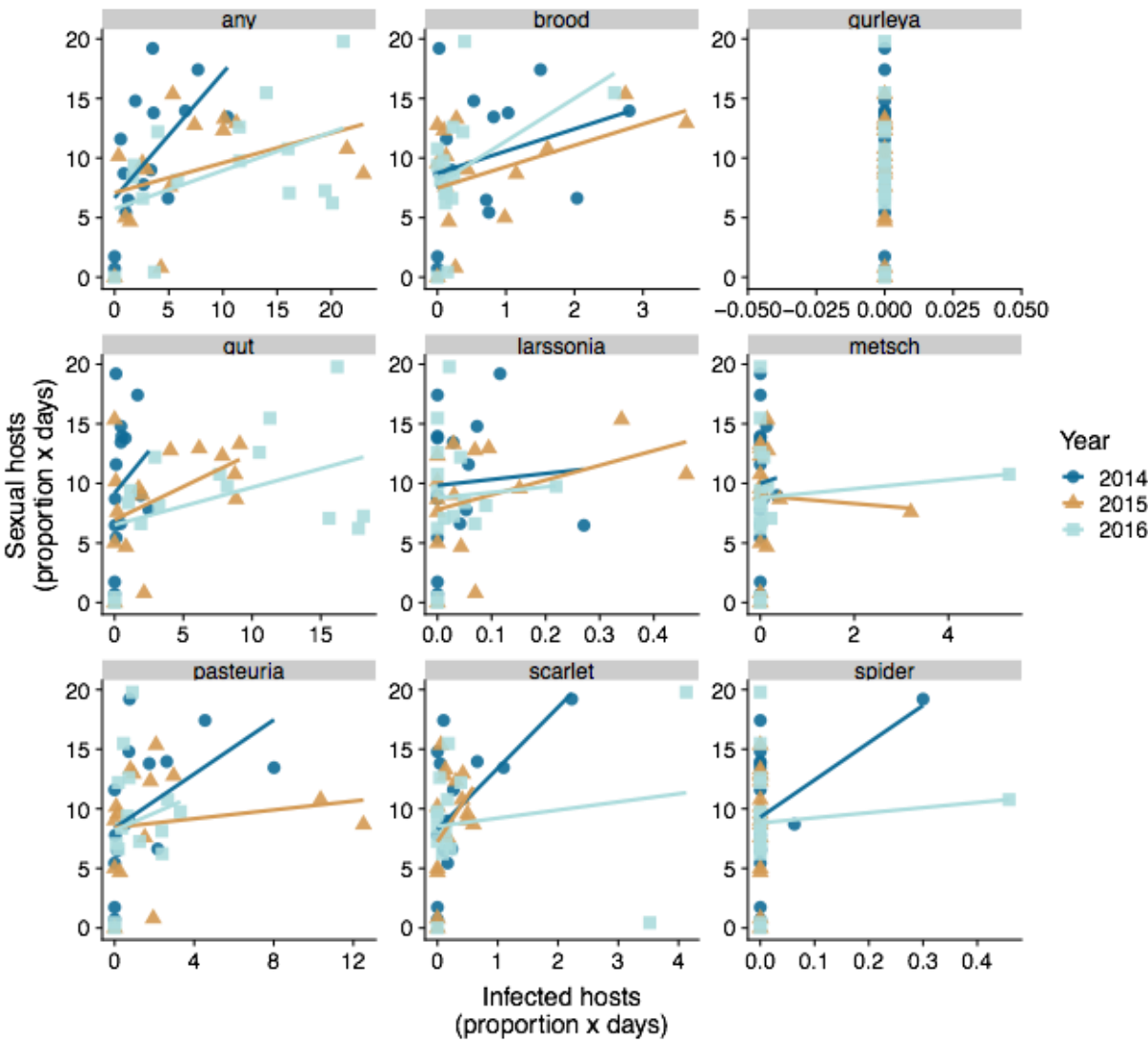
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516 **Figure S4.** Analyses with maximum host density and parasitism yielded qualitatively similar
 517 results for the correlation between *D. dentifera* density and sexual reproduction **(a)** (sex ~
 518 log(density), *gaussian*, $F = 25.03$, $p < 0.001$). On the other hand, **(b)** there was no longer a
 519 correlation between sex and parasitism (sex ~ parasitism, *gaussian*, $F = 2.06$, $p = 0.16$), **(c)** nor
 520 was there was a correlation between density and total parasite infections (parasitism ~
 521 log(density), *negative binomial*, $z = 0.98$, $p = 0.30$).
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525 **Figure S5.** Total integrated sexual hosts compared to integrated infected hosts with different
526 parasite species. “Gut” parasites are a combination of two parasite species (which were not
527 initially distinguished): *Caullerya mesnili* (ichthyosporcan), an undescribed microsporidian gut
528 parasite (“MicG”).
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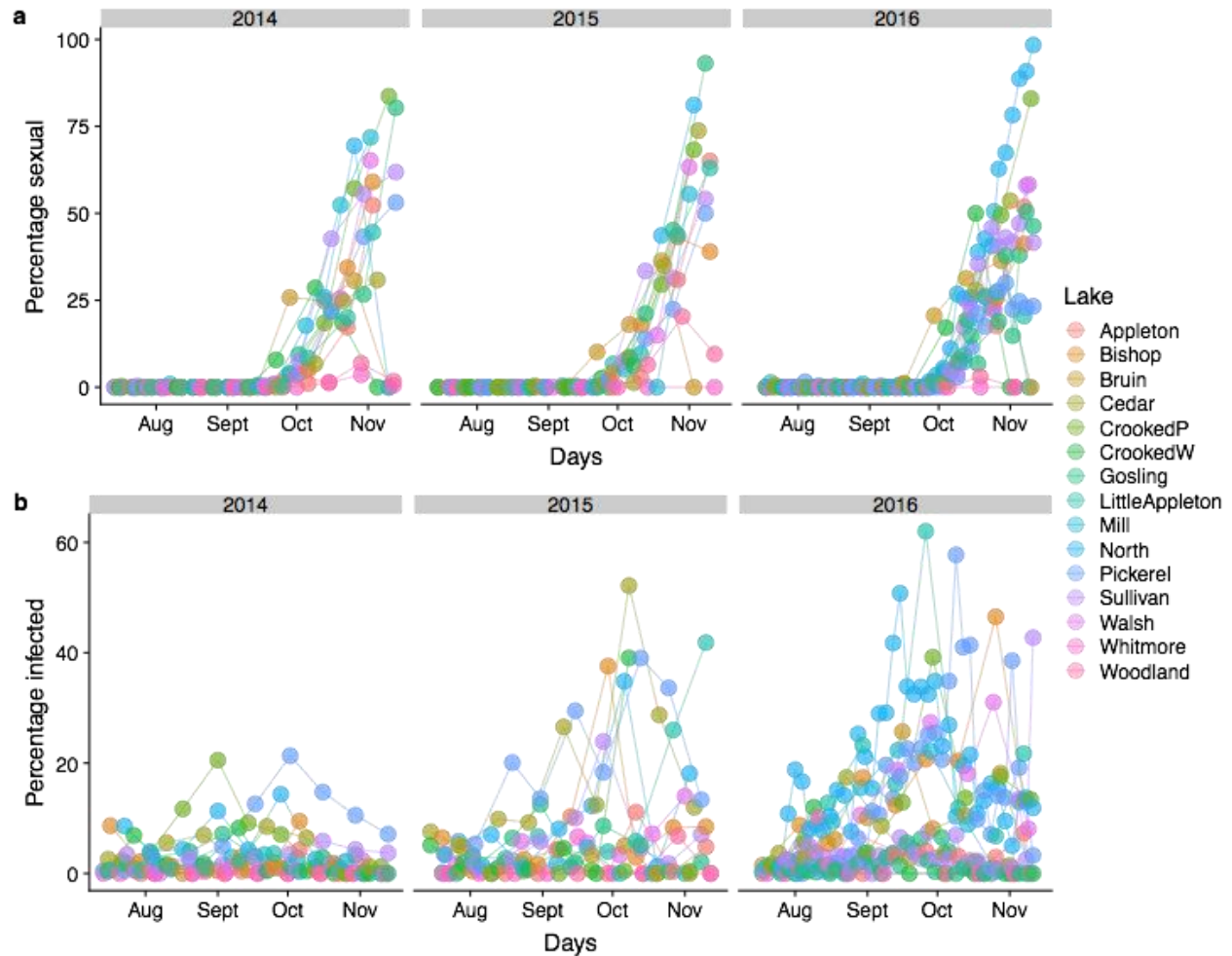


Figure S6. Across lakes, years, and calendar day, there was considerable variation in the percentage of *Daphnia dentifera* (a) reproducing sexually and (b) infected with at least one parasite. The percentage sexual was derived from the ratio of males and ephippial females out of the total population counted and percentage infected was calculated as the percentage of *Daphnia* with any parasitic infection, including coinfections. Each point represents a single sampling event, and colors denote different study populations.