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Author(s): Gerber, Nina; Kokko, Hanna; Ebert, Dieter; Booksmythe, Isobel

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1 ***Daphnia* invest in sexual reproduction when its relative costs are reduced**

2

3 Nina Gerber^{1,2,3}, Hanna Kokko¹, Dieter Ebert^{3,4}, Isobel Booksmythe^{1,3}

4 1) Department of Evolutionary Biology and Environmental Studies, University of Zurich,
5 Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

6 2) Centre of Excellence in Biological Interactions, Department of Biological and Environmental
7 Science, University of Jyväskylä, Jyväskylä, Finland

8 3) Tvärminne Zoological Station, J.A. Palmenintie 260, 10900 Hanko, Finland

9 4) University of Basel, Department of Environmental Sciences, Zoological Institute, Vesalgasse 1,
10 4051 Basel, Switzerland

11

12 **Abstract**

13 The timing of sex in facultatively sexual organisms is critical to fitness, due to the differing
14 demographic consequences of sexual vs. asexual reproduction. In addition to the costs of sex itself,
15 an association of sex with the production of dormant life stages also influences the optimal use of
16 sex, especially in environments where resting eggs are essential to survive unfavourable conditions.
17 Here we document population dynamics and the occurrence of sexual reproduction in natural
18 populations of *Daphnia magna* across their growing season. The frequency of sexually reproducing
19 females and males increased with population density and with decreasing asexual clutch sizes. The
20 frequency of sexually reproducing females additionally increased as population growth rates
21 decreased. Consistent with population dynamic models showing that the opportunity cost of sexual
22 reproduction (foregoing contribution to current population growth) diminishes as populations
23 approach carrying capacity, we found that investment in sexual reproduction was highest when
24 asexual population growth was low or negative. Our results support the idea that the timing of sex is
25 linked with periods when the relative cost of sex is reduced due to low potential asexual growth at
26 high population densities. Thus, a combination of ecological and demographic factors select on the
27 optimal timing of sexual reproduction, allowing *D. magna* to balance the necessity of sex against its
28 costs.

29

30 **Keywords:** Timing of sex, *Daphnia*, population density, cost of sex

31 Many treatments of the evolution of sex consider sexual and asexual forms as discrete lineages
32 (reviewed in [1,2]). In nature, however, competition between these types is often more subtle.
33 Organisms that use sex facultatively potentially gain the ‘best of both worlds’, as they avoid paying
34 costs of sexual reproduction much of the time, while maintaining access to the benefits of genetic
35 recombination [3-5]. This flexibility brings about a suite of life history consequences: the ability to
36 reproduce either sexually or asexually, depending on current conditions, means that the frequency
37 and timing of the sexual life cycle is an evolvable trait [6].

38 The timing of sex in facultative sexual organisms is critical to fitness for several reasons stemming
39 from the differing demographic consequences of sexual vs. asexual reproduction. Asexual
40 reproduction is usually the more efficient strategy in terms of converting resources into offspring, as
41 it avoids the ‘twofold cost’ of male production [7,8]. A genotype’s asexual and sexual success are
42 not independent, and strongly traded off against each other, because a female can only do one at a
43 time. Switching from asex to sex too early also entails potentially large opportunity costs: foregoing
44 the opportunity to contribute to asexual generations (i.e. current population growth) can, in an
45 exponentially growing population, drastically reduce the representation of a clonal genotype in the
46 mating pool, and hence in the sexually produced offspring generation. This opportunity cost (birth
47 rate disadvantage) of sexual reproduction may be particularly pronounced when offspring gained
48 through sex are not equivalent to offspring produced asexually, as often observed in nature. For
49 example, in facultative sexual organisms sexual offspring are often dormant or dispersing life stages
50 (e.g. Cladocera: [9]; rotifers [10,11]; aphids [12]) that do not contribute to current local population
51 growth.

52 In populations that undergo periods of inhospitable conditions, an association between sexual
53 reproduction and dormancy results in potentially complex selection on the timing of sex. Sexually
54 produced, diapausing offspring are vital for the persistence of a lineage across favourable
55 (‘growing’) seasons, and must be produced before the intervening periods when the habitat is
56 unsuitable. In such a system, the measure of fitness that is expected to be maximized is the total
57 count of sexually produced dormant stages at the end of the favourable season [13,14]. In contrast,
58 during the growing season, selection in the short term favours asexual reproduction due to its
59 efficiency [15]. If individuals can precisely predict the duration of each favourable season, we
60 expect a simple switch from asexual to sexual reproduction towards the end of the growth season
61 (Gerber, Booksmythe & Kokko, unpublished) [16,17]. However, in unpredictable environments
62 individuals might hedge their bets with regards to reproductive mode, while in more predictable
63 environments the use of environmental cues could allow this plasticity [18]. Indeed, in various
64 facultatively sexual species, changes in temperature [12], population density or crowding [19,20],

65 food quality [21,22], photoperiod [22] and predation [23] contribute to the induction of sexual
66 reproduction. All of these cues potentially inform females about seasonal changes and/or
67 deteriorating conditions, under which sex may be advantageous [24]. The reproductive response to
68 such cues can be complicated by the need to produce both males and sexually reproductive females
69 so that they coincide at sexual maturity during the period optimal for the production of sexual
70 offspring [25,26].

71 The cost of sex is not a fixed parameter, but may depend itself on current conditions. Investment in
72 each reproductive mode is expected to be influenced by the relative costs of sexual and asexual
73 reproduction. For example, mate-finding costs may be higher at low densities, when encounter rates
74 between individuals are low [27,28]. However, this cost may be diminished if individuals can
75 flexibly switch to asexuality should a mate not be found. Similarly, the cost of sex may depend on
76 the current scope for asexual population growth [29-31]. Assuming that adults are better able than
77 offspring to survive at high densities, when a population approaches carrying capacity the
78 recruitment rate declines.

79 A handful of population dynamic models highlight the consequences of these dynamics for the
80 demographic advantage of asexual lineages over sexuals [32-34]. They show that asexuality cannot
81 always realize its demographic advantage: if high densities prevent immediate population growth,
82 the opportunity cost of sex diminishes for populations nearing carrying capacity. Although this is
83 not always sufficient to modify the cost if sex and asex occur simultaneously [8], the prediction for
84 facultative sexuals with dormant sexual eggs is clear: they should switch to sex as resources become
85 limiting and the opportunity cost of sexual reproduction is reduced. This important prediction has,
86 to date, been largely overlooked by empirical studies of the costs and benefits of sex.

87 In facultative sexual *Daphnia* (Cladocera: Daphniidae) we expect strong selection on the timing of
88 sex. *Daphnia* sex appears costly relative to asexual reproduction in the short term due to its
89 demographic effects. The largest asexual clutches recorded for *D. magna* contain ~110 eggs [35],
90 whereas sexual clutches contain at most two eggs. This clutch size difference represents a
91 potentially extreme opportunity cost of reproducing sexually, which necessarily entails foregoing an
92 asexual reproductive bout. Non-equivalence of sexually and asexually produced offspring holds for
93 this system: asexually produced eggs develop immediately in the maternal brood chamber into free-
94 swimming plankton, whereas fertilized sexual eggs must undergo a period of dormancy, encased in
95 a hardy capsule known as an ephippium [9]. Additionally, sexual reproduction requires the
96 (asexual) production of males, which reduces the asexual growth rate of a lineage. However, in
97 terms of resource allocation, the extent to which investment in sex trades off with other life history

98 traits, including asexual investment, is not clear. While production times for sexual and asexual
99 clutches are equal, the large number of eggs in an asexual clutch could translate into higher resource
100 requirements compared to the two eggs per sexual clutch. Alternatively, melanisation of the
101 ephippium and provisioning for dormancy might require additional resources when producing a
102 sexual clutch.

103 Ultimately, only sexual, dormant eggs are able to withstand harsh conditions, including freezing
104 and desiccation, so sexual reproduction is vital for the long-term persistence of a lineage over
105 inhospitable periods. At the start of each growing season, when environmental conditions become
106 suitable, dormant eggs hatch into females that found the planktonic population anew. Male
107 production and the female switch to sexual reproduction may occur in response to different cues, or
108 with different sensitivity to the same cues: in *D. magna*, production of males and of sexual clutches
109 responded differently to manipulations of photoperiod [36], and male production has been observed
110 to occur more stochastically throughout the growing season compared to ephippia production [25].
111 The relative roles of environment and genotype in determining the likelihood of male and ephippia
112 production also vary: for example, in *Daphnia pulex* inhabiting temporary ponds over a short
113 growing season, substantial male production occurred very early while population densities were
114 still low, and was also not linked to other environmental factors such as pond temperature [37].

115 Most studies on the timing of sex and male production in *Daphnia* have focused on cues that allow
116 dormant eggs to be produced before environmental deterioration or the end of the season, and have
117 been conducted under laboratory conditions (e.g. [22,26,36,38,39], but see [37]). We aim to add the
118 costs and consequences of sex itself to this picture, and focus on population density as a variable
119 connecting the ecological and demographic influences on the timing of sex. We highlight the
120 hypothesis of demographically varying costs of sex [32-34] as an important alternative to the
121 prevailing emphasis on sex as a response to deteriorating conditions. This prevailing view sees sex
122 either providing a direct escape route (e.g. dormancy [26,36]) or generating diversified offspring
123 through recombination, to explain why particularly stressful conditions induce sex [40].

124 Of these three options, we focus on the first two (the demographic cost hypothesis, and the habitat
125 deterioration hypothesis). The third hypothesis appears unlikely to explain the precise scheduling of
126 sex in the current context. While high density (and its correlates, e.g. increased resource limitation
127 or disease risk) may constitute a stressful environment, it is difficult to envisage a benefit of
128 producing diverse offspring genotypes in response to this transient stress. Offspring hatch in
129 subsequent seasons under benign density conditions; the range of densities a lineage may later
130 encounter is independent of the density when the lineage-founding ephippia were produced.

131 Returning to the two focal hypotheses, previous work has shown that crowding promotes sex
132 induction and reduces asexual fecundity in laboratory populations of *Daphnia* [41]. Observing these
133 patterns in natural populations would support the habitat deterioration hypothesis, with support
134 strengthening if populations do not persist after reaching high density. The demographic cost
135 hypothesis, in contrast, predicts that density directly modifies the relative costs of sex and asexual
136 reproduction through its relationship with the population's capacity for growth [32-34]. In this case,
137 we would expect sex induction to be related to population growth rates in addition to density.

138 We used an intensive longitudinal sampling regime to document population dynamics and the
139 occurrence of sexual reproduction over the main part of the growing season in natural populations
140 of cyclically parthenogenetic *Daphnia magna*. We investigated the interacting effects of population
141 density, asexual reproductive investment, and growth rates on the frequency of sexually
142 reproducing individuals. Additionally, in the laboratory we estimated resource allocation trade-offs
143 between the production of ephippia and asexual fecundity over the lifespan of individual females, to
144 clarify whether investment in sex imposes costs beyond its immediate demographic disadvantage.

145 **Methods**

146 *Population sampling*

147 We sampled 11 natural *D. magna* populations every three-to-four days for 60 days (May 30 – July
148 28, 2015). Populations inhabited separate rock pools distributed over 6 islands (FU1, HA, K, LON,
149 N, and SMF) in the Finnish archipelago near Tvärminne Zoological Station (59.8420° N, 23.2018°
150 E). We recorded density and demographic structure ('stage-structure') of the populations at each
151 sampling point. To assess population density, 350 ml water samples were collected at 15
152 haphazardly chosen locations spanning the pool area and depth. These were combined in a bucket
153 and stirred to distribute individuals evenly, and a 350-ml subsample was taken as the final density
154 sample. The remaining animals were returned to the rock pool. After collecting the density sample a
155 small hand net was swept through the pond to take a representative population sample.

156 Live samples were brought back to the lab and analysed the same day. All *D. magna* individuals in
157 the 350-ml density sample were counted under a dissecting microscope and converted to an
158 estimate of individuals/L. The stage-structure samples were variable in size; to make larger samples
159 manageable (< 1000 individuals) they were split using a Folsom plankton sample divider. The
160 sample was then sieved through 0.6 mm nylon mesh to separate the smallest individuals.

161 Individuals that remained in the sieve were counted and classified into the following categories
162 under a dissecting microscope: females with asexual eggs or embryos in the brood pouch, females

163 with an empty brood pouch but filled ovaries, females with ephippia, adult females without eggs,
164 embryos or filled ovaries, juvenile females (indicated by short 1st abdominal process [9]); adult
165 males (prolonged first antenna, copulatory hook on the first thoracic leg [9, 42]) and juvenile males.
166 After assessing stage-structure, up to ten females (where possible; median = 10, mean \pm SE = 8.72
167 \pm 0.17) with asexual eggs in the brood pouch were isolated from the sample and maintained in
168 individual 35 ml jars until they released their clutch. The number and sex of offspring was
169 determined under a dissecting microscope. This paper's focus is the timing of investment in sexual
170 reproduction, and does not present the data on offspring sex allocation, which is addressed in a
171 second study using the population density and stage-structure data collected here (Booksmythe,
172 Gerber, Ebert & Kokko, unpublished).

173 *Reproductive life history trade-offs*

174 We collected large population samples from five additional rock pool populations and isolated 60
175 females (F0) carrying asexual clutches. Females were kept individually in 50 ml falcon tubes filled
176 with artificial *Daphnia* medium (ADaM [43]) and fed daily with *Scenedesmus* algae (~5 million
177 cells per individual per day) until they released their first clutch. We isolated four F1 daughters per
178 F0 female and housed them in pairs in 50 ml falcon tubes until they produced their first clutch.
179 Twenty-two of these groups of four sister F1 females (henceforth 'clones') synchronously produced
180 enough daughters that we could isolate ten F2 females per clone, half of which were assigned to a
181 long day length treatment (18:6 hours light:dark) and the other half to a short day length treatment
182 (6:18 hours light:dark) on the day of their release from the maternal brood pouch. We used extreme
183 day lengths (naturally occurring at midsummer and midwinter at the study site) to induce
184 propensities for sex that were as different as possible between treatment groups. Over the 35-day
185 experimental period, females experienced these photoperiod treatments under otherwise
186 standardized conditions in climate chambers (20°C, with *Daphnia* placed ~20 cm below the
187 fluorescent light source). Individual F2 females were fed and checked daily for the release of
188 asexual clutches or sexually produced ephippia. When an asexual clutch was released, the date,
189 number and sex of offspring were recorded, the offspring removed and the water changed. When an
190 ephippium was produced, the date was recorded, the ephippium removed and the water changed.
191 We recorded the date of any deaths. Females that did not reproduce were excluded from the
192 analysis. We also excluded 7 females that produced an ephippium in the very first clutch, which
193 needed twice the time to produce their first clutch compared to other females, indicating very
194 unusual behaviour.

196 We were interested in how the frequency of sex relates to population density, population growth,
197 and asexual reproductive effort. We ran separate models to predict the frequencies of sexual
198 females and males, as they could respond differently to these predictors. Models for the frequency
199 of sexual females in a sample used population density from the previous sampling point ('lag
200 density') as a predictor, because these prior conditions (3-4 days before) coincide with the point at
201 which female reproductive mode would have been determined [9]. However, models for the
202 frequency of adult males used current density, as conditions at the previous sampling point do not
203 coincide with the production of these males. The appropriate lag period (the amount of time males
204 need to mature) is at least 10 days/3 sampling points, and a predictor variable using this lag would
205 have unacceptably reduced our sample size. Using current density in the analysis instead allows us
206 to examine whether males are produced so as to coincide with periods of high density in adulthood.
207 As density varied by orders of magnitude across populations, and within populations over time, we
208 used log-transformed density in all analyses. We calculated the intrinsic rate of per capita
209 population growth per time step as $r = \frac{\ln(N_{t2}) - \ln(N_{t1})}{t2 - t1}$ [44].

210 Asexual reproductive effort was estimated from the clutch size of ~10 females, carrying asexual
211 eggs, per population sample. The clutch size among sampled females overestimates the mean
212 population asexual reproductive effort, as a small but variable proportion of mature females in each
213 population sample showed no current reproductive investment (with neither eggs in the brood
214 pouch nor filled ovaries). We weighted the mean clutch size of sampled females by the proportion
215 of currently reproductive females among all mature, non- sexually reproducing females to estimate
216 the mean asexual reproductive effort in the population. We used log-transformed clutch size and
217 reproductive effort in our analyses to normalize their distribution. Because asexual reproductive
218 effort and growth are related (collinearity) we included them separately in models with density as
219 the only other predictor. To determine the threshold asexual reproductive effort at which
220 populations switch to sexual reproduction, we created a binary dependent variable for whether a
221 population sample contained females investing in sexual reproduction or not. We fitted a logistic
222 regression of this variable over asexual reproductive effort and determined its inflection point.

223 Statistical analyses were performed in R (v. 3.2.2) [45]. We used linear mixed-effects models in the
224 package lme4 [46] for analyses of density, clutch size, and growth rates in the natural populations,
225 and of asexual clutch size and mean interval between clutches in the laboratory experiment. For
226 analyses of proportions of males and sexual females we used generalized linear mixed-effects
227 models (GLMMs) with binomial error and logit link in lme4. To account for repeated

228 measurements the population ID (natural populations) or family ID (laboratory experiment) was
229 included as random factor. If binomial models were overdispersed an observation-level random
230 factor was included [47]. Predictor variables in binomial GLMMs were standardized to aid in
231 interpretation of parameter estimates, reported on the log odds scale; as an indication of effect sizes,
232 we also present the odds ratio for each parameter, and marginal and conditional R^2 [50] for each
233 GLMM. Summary statistics are presented as mean \pm 1 standard error (SE), unless otherwise
234 specified.

235

236 **Results**

237 *Sex is associated with high density and low asexual reproductive effort*

238 The frequency of sexual females (adult females carrying sexual eggs) was larger following high
239 population densities, and when mean reproductive effort among asexual females was low (Fig. 1,
240 Table 1). The value for asexual reproductive effort at which the majority of populations contained
241 females investing in sexual eggs was 11.47 offspring (Figure S1). The frequency of sexual females
242 also increased with decreasing growth rate r (Fig. 1, Table 1). The frequency of males in the adult
243 population was larger when density was high, and when asexual effort was low, but was not
244 significantly related to population growth rate (Fig. 1, Table 1). Odds ratios in Table 1 show the
245 predicted change in odds with each standard deviation increase in the predictor, for a constant
246 (mean) value of the covariate. For example, the odds of a female carrying an ephippium were 0.035
247 at the intercept (i.e. for mean values of density and asexual effort). For each standard deviation
248 increase in density, keeping asexual effort constant, these odds increased by a factor of 4.75.

249 The association of higher frequencies of sexual females with high population density and low
250 growth rates can also be seen when looking at patterns across the growing season (Fig. S2). Across
251 all populations, there were two main peaks in density during our sampling period. These peaks are
252 followed by periods of reduced growth rate (Fig. S2) reflecting the negative relationship between
253 population growth and population density (LMM: Slope = -0.053 ± 0.014 , $\chi = 13.83$, $p < 0.001$).
254 Lag population density was also negatively related to asexual clutch size (LMM: Slope = $-0.26 \pm$
255 0.047 , $\chi = 31.02$, $p < 0.001$) (Fig. S2).

256 *Ephippia production trades off with asexual clutch size*

257 The number of females that reproduced in the long-day and short-day experimental treatments did
258 not differ (83 of 110 and 88 of 110, respectively; $z = 0.81$, $p = 0.42$). The short-day treatment

259 successfully induced ephippia production, with 41 of 88 females producing at least one ephippium,
260 compared to 1 of 83 females in the long-day treatment (GLMM: Slope = -4.700 ± 1.199 , $z = -3.92$,
261 $p < 0.001$; Fig. 2). Total asexual reproductive success (number of offspring) was higher in the long-
262 day treatment (LMM: Slope = 7.507 ± 2.475 , $\chi^2 = 9.20$, $p = 0.002$; Fig. 2). Day length did not affect
263 the total number of reproductive bouts, the latency to first reproduction, or the mean interval
264 between clutches (all $p > 0.05$). Nor did day length affect the mean asexual clutch size when
265 calculated across all of a female's asexual clutches (LMM: Slope = 0.178 ± 0.420 , $\chi^2 = 0.18$, $p =$
266 0.67). Within the short-day treatment, we could compare individuals that reproduced only asexually
267 with those that produced at least one sexual clutch. The latter had fewer total asexual offspring,
268 losing on average 9 offspring compared to their clone mates that reproduced only asexually
269 (asexual, 54.1 ± 1.9 , sexual, 45.4 ± 3.2 ; LMM: Slope = -9.167 ± 3.856 , $\chi^2 = 5.65$, $p = 0.02$), but
270 whether or not females reproduced sexually did not affect their mean asexual clutch size ($-0.974 \pm$
271 0.801 , $\chi^2 = 1.48$, $p = 0.22$). The absolute number of sexual clutches (0, 1 or 2) was not related to
272 mean asexual clutch size (-0.523 ± 0.588 , $\chi^2 = 0.79$, $p = 0.37$). However, the proportion of a
273 female's reproductive events that were sexual was negatively related to her mean asexual clutch
274 size (LMM: Slope = -5.958 ± 2.535 , $\chi^2 = 5.52$, $p = 0.019$); if half of a female's clutches were
275 sexual, the mean size of her asexual clutches was reduced by around 3 eggs compared to females
276 producing only asexual clutches (Fig. 2c). Females that produced relatively many ephippia had
277 smaller clutches when they reproduced asexually.

278

279 **Discussion**

280 *Daphnia magna* in the rock pool habitat studied here experience a short growing season followed
281 by completely inhospitable conditions in which only dormant, sexually produced eggs persist.
282 Besides winter freezing, pools often experience summer droughts [49]. The resulting strong
283 selection for the production of dormant eggs is therefore expected to shape the timing of sexual and
284 asexual reproduction during the growing season. While approaching inhospitable conditions do
285 influence investment in sexual reproduction [36], here we argue that in line with theoretical
286 predictions, the reduced cost of sexual relative to asexual reproduction at high densities may
287 plausibly favour density as a cue for the switch to sexual reproduction in *D. magna*. Our results
288 suggest that an understanding of how population dynamics affect the costs of sex can inform
289 predictions of when sex should occur, and highlight the importance of considering variation in the
290 costs of sex when seeking explanations for the maintenance of sexual reproduction.

291 Investment in sexual reproduction in our sampled populations was strongly predicted by population
292 density: when density was high at the previous sampling point, a greater proportion of females in
293 the current sample carried sexual eggs. The frequency of males in the adult population also
294 increased with density. These relationships were consistent over the sampled time period, where we
295 observed two density peaks (Fig. S2). However, neither of these peaks in density and sexual
296 reproduction was followed by extinction of the planktonic population, indicating that sex did not
297 immediately precede inhospitable periods where the dormant stage would be vital. This conflicts
298 with the habitat deterioration hypothesis, suggesting that the approach of inhospitable conditions
299 alone cannot explain the marked increase in investment in sex at these points.

300 We suggest, in accordance with the demographic cost hypothesis, that the association of sexual
301 reproduction and population density results from declining efficiency of asexual reproduction as
302 populations approach carrying capacity — newborn offspring may then have difficulty recruiting
303 into the current population, which reduces the returns on asexual offspring. Consequently, the
304 relative profitability of sexual reproduction (which does not contribute to current population
305 growth) increases with population density. Furthermore, sexually produced offspring are not
306 affected by current conditions (e.g. competition), as they do not hatch until subsequent growing
307 seasons. We could not measure juvenile survival, which is predicted to decrease with population
308 density under this scenario. However, the frequency of sexual females increased with decreasing (or
309 negative) population growth, and decreasing asexual reproductive effort. Thus, for a given
310 population density, females increased their investment in sexual reproduction when potential
311 growth was low or populations were declining. This supports the demographic cost hypothesis that
312 sex in ephemeral *D. magna* populations is timed to coincide with periods when the opportunity cost
313 of sexual reproduction is reduced [32-34] (in addition to the likelihood of sex increasing with
314 ecological cues, such as changes in day length, ensuring that it occurs before the season end [36]).
315 Our data on the clutches of females taken from natural populations suggest that this cost is balanced
316 when the mean asexual reproductive effort in a population is around 11.5 eggs. Below this value,
317 sexually reproducing females could be found in the majority of populations (Fig. S1).

318 Interestingly, in other facultative sexual systems showing an association between population density
319 and sex induction (e.g. rotifers [14,20,50]), density-dependent induction of sex has been shown to
320 contribute to regulating population density in a controlled laboratory setting [51]. Because of the
321 production of males and dormant stages, which require resource investment and do not contribute to
322 current population growth, increasing rates of sex can feed back negatively on growth rates [16,17].
323 In rotifers the density threshold for sex induction is low, and sex is directly related to population
324 density, rather than indirectly through resource depletion [20], suggesting that the induction of sex

325 influences the growth potential of the population [50]. This effect on population growth has also
326 been demonstrated in laboratory *Daphnia* populations [52]. Such negative feedback is also possible
327 in our dataset, but is much more difficult to detect in natural populations due to the many other
328 uncontrolled variables (e.g. pool volume, algal productivity) likely to affect rates of sex, density,
329 and their relationship.

330 In contrast to the frequency of sexual females, the frequency of adult males was not related to
331 population growth. High male frequencies coincided with periods of high density and low asexual
332 reproductive effort, but generally male occurrence appears to be timed less precisely than ephippia
333 production. This is perhaps not surprising when considering that males require time (~10 days at
334 20°C) to mature, which reduces the likelihood that cues available when males are produced will
335 reliably predict population dynamics at their maturity. Furthermore, as male lifespan is substantially
336 longer than the moult cycle over which a female bears an ephippium, male frequency increases
337 cumulatively over time while ephippia frequency reflects much more closely the current conditions.
338 Previous studies of *Daphnia* species in temporary habitats have found similar patterns of male
339 appearance in a population preceding the first production of ephippia [37,53].

340 High population density leads to increased investment in both males and ephippia in laboratory
341 populations of *Daphnia* [19,54-56] and to smaller asexual clutch sizes [41]. A negative relationship
342 between density and asexual reproduction was also apparent in our dataset. This could reflect
343 increased competition at high densities, resulting in reduced reproductive condition that restricts
344 female fecundity. However, we observed increased sexual reproduction at high densities, when
345 resources are limited. This finding is difficult to reconcile with the suggestion that the sexual
346 ephippia have a high resource cost [57]. If resources limit the production of large asexual clutches
347 (as shown in many experiments, e.g. [40,58,59]), these conditions should also constrain production
348 of costly ephippia. Our laboratory results on reproductive trade-offs suggest that producing a sexual
349 clutch is costly: individual females producing a greater proportion of sexual clutches over their
350 lifespan produced, on average, smaller asexual clutches. The cost imposed on asexual reproductive
351 potential by a sexual event is thus greater than the loss of one asexual clutch. However, quantifying
352 the absolute cost of producing a sexual clutch requires experiments manipulating asexual clutch
353 sizes by altering resource availability.

354 The major cost of sex in our experiment appeared to be the immediate trade-off arising from the
355 inability to produce a sexual and asexual clutch simultaneously: females that produced more
356 ephippia had a lower total number of asexual offspring. If a female producing a sexual clutch has
357 fewer opportunities and/or resources left available for asexual reproduction, there are clear

358 consequences for the competitiveness of clonal lineages with different propensities for sexual
359 reproduction in terms of their numerical representation in the population. *D. magna* clones vary in
360 their propensity to produce males and, independently, ephippia in response to environmental cues
361 [26,36]. Sexually produced, dormant offspring are the measure of long-term fitness in *Daphnia* and
362 many facultative sexual organisms, but total sexual output depends both on sexual and asexual
363 fecundity. The timing of sexual reproduction is thus expected to optimise investment in the two
364 reproductive modes.

365 **Conclusions**

366 In wild populations of facultative sexual *D. magna*, females invest in sexual reproduction following
367 high population densities and when the population growth rate and asexual reproductive effort are
368 low, conditions that reduce the relative cost of sexual reproduction. We provide empirical support
369 for the idea that a facultative sexual population will show increased rates of sex as it approaches
370 carrying capacity and the cost of sex declines. Combining our new finding with previous results we
371 suggest that three underlying rules determine the induction of sexual reproduction in *D. magna* on a
372 large biogeographic scale: First, ephemeral, seasonal populations that frequently experience
373 inhospitable periods should generally invest more in sexual reproduction compared to populations
374 in permanent, less seasonal habitats [32]. Second, we have found that within a season, sex induction
375 co-occurs with conditions that are theoretically predicted to reduce its costs relative to asexual
376 reproduction [32-34]. This is the case at high population densities when asexual clutch size is small
377 and the cost of foregoing asexual reproduction is low. Third, previous studies have shown that this
378 pattern can be modified by the timing and predictability of onset of inhospitable conditions, such
379 that investment in sexual reproduction increases towards the anticipated end of the growing season
380 [36]. We conclude that timing of sex in cyclical parthenogens is not only shaped by the approach of
381 inhospitable conditions, but appears to respond to effects of density and population growth on the
382 relative costs of sexual and asexual reproduction.

383

384 **Competing interests**

385 We have no competing interests.

386 **Data accessibility**

387 All data are archived in the DRYAD digital repository (DOI provided on manuscript acceptance)

388 **Authors' contributions**

389 NG, IB, DE and HK conceived the study; NG, IB and DE designed the study; NG and IB carried
390 out data collection, analysed the data, and drafted the manuscript; all authors revised the manuscript
391 and gave final approval for publication.

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529

530 **Figure 1.** The relationship of the proportion of sexual females (a-c) and males (d-f) with lag (a) or
531 current (d) population density (*Daphnia*/L), asexual reproductive effort (b, e), and growth rate (c, f).
532 Bold lines show the global logistic regression with 95% confidence intervals, with significant
533 relationships in colour and non-significant in grey. Thin black lines show regressions for each
534 population, and light grey points show raw data.

535 **Figure 2.** The effect of day length treatment (short-day, 6:18 hours light:dark; long-day, 18:6 hours
536 light:dark) on (a) the mean proportion of a female's clutches that were sexual and (b) the mean size
537 of asexual clutches; and (c) the relationship between the proportion of a female's clutches that were
538 sexual and her mean asexual clutch size, for the short-day treatment only. Asterisks indicate
539 significance (n.s., $p > 0.05$; *, $p < 0.05$; ***, $p < 0.001$).

540 **Table 1.** Effects of population density, mean asexual reproductive effort (RE), and growth rate on
541 the proportions of sexual females and adult males in a population; estimated by binomial GLMM
542 with logit link. Parameter estimates are presented as the log odds ratio (β) and its standard error
543 (SE); we additionally present the odds ratio (OR) as a measure of effect size. $R^2_{\text{GLMM}(m)}$, marginal R^2
544 (variance explained by fixed effects); $R^2_{\text{GLMM}(c)}$, conditional R^2 (variance explained by fixed + random
545 effects) [50].

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Model: Density and asexual reproductive effort

Proportion sexual females (N = 174)						Proportion adult males (N = 185)					
Fixed effects:	β	SE	z	p	OR	Fixed effects:	β	SE	z	p	OR
(Intercept)	-3.344	0.333	-10.04	< 0.001	0.035	(Intercept)	-1.878	0.172	-10.91	< 0.001	0.153
ln(lag density)	1.558	0.221	7.04	< 0.001	4.751	ln(density)	0.976	0.129	7.56	< 0.001	2.654
ln(mean asexual RE)	-0.914	0.155	-5.91	< 0.001	0.401	ln(mean asexual RE)	-0.470	0.108	-4.36	< 0.001	0.625
Random effects:	SD					Random effects:	SD				
Population ID	0.983					Population ID	0.466				
Observation ID	1.412					Observation ID	1.206				
R²:	R²_{GLMM(m)}	R²_{GLMM(c)}				R²:	R²_{GLMM(m)}	R²_{GLMM(c)}			
	0.426	0.514					0.224	0.258			

Model: Density and growth rate

Proportion sexual females (N = 166)						Proportion adult males (N = 177)					
Fixed effects:	β	SE	z	p	OR	Fixed effects:	β	SE	z	p	OR
(Intercept)	-3.299	0.274	-12.05	< 0.001	0.037	(Intercept)	-1.910	0.171	-11.15	< 0.001	0.148
ln(lag density)	1.879	0.240	7.83	< 0.001	6.544	ln(density)	1.177	0.144	8.19	< 0.001	3.243
growth rate r	-0.389	0.155	-2.52	0.012	0.678	growth rate r	0.161	0.114	1.41	0.16	1.175
Random effects:	SD					Random effects:	SD				
Population ID	0.721					Population ID	0.443				
Observation ID	1.586					Observation ID	1.277				
R²:	R²_{GLMM(m)}	R²_{GLMM(c)}				R²:	R²_{GLMM(m)}	R²_{GLMM(c)}			
	0.394	0.444					0.191	0.222			
