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3 Cumulative effects matter: multi-brood responses of *Daphnia* to hypoxia

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15 Running head: Low O<sub>2</sub> alters *Daphnia* fitness over multiple broods

16

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18 fecundity.

19

20 **Summary**

- 21 1. Periods of hypoxia lasting up to weeks are now anticipated in freshwaters, owing  
22 to anthropogenic influences. However, the cumulative effects of hypoxia on  
23 *Daphnia*, over multiple broods, have received virtually no attention, and to  
24 establish and evaluate such responses there is a need to make measurements over a  
25 wide range of oxygen concentrations, potentially revealing non-linear patterns. We  
26 predict that the effect of hypoxia on growth, survival, and fecundity (i.e.,  
27 production of new individuals) of *Daphnia* will increase over multiple broods, and  
28 with increasing oxygen these responses will approach asymptotic maxima,  
29 following a rectangular-hyperbolic response.
- 30 2. *Daphnia similoides* were exposed to 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 mg oxygen L<sup>-1</sup>.  
31 To determine effects on the first brood we examined: number of offspring; time to  
32 the first eggs; time to the first brood; and size of the female at the first eggs and the  
33 first brood. To determine cumulative effects of oxygen over multiple broods (up to  
34 8 broods over 21 days) we measured: total number of offspring produced by a  
35 female; survival time; and total number of moults and broods. To investigate how  
36 the cumulative effects arose over the multiple broods, we examined the number of  
37 offspring per brood in each brood over 8 broods. To assess treatment effects and  
38 indicate responses, functions were fit to data using the most parsimonious function  
39 that reflected trends in the data.
- 40 3. Measurements associated with a single brood responded linearly, or not at all, with  
41 changing oxygen concentration, while measurements made over the 21 days  
42 followed a rectangular hyperbolic response, increasing to an asymptote as oxygen  
43 increased. For the first brood, as oxygen concentration was raised from 1 to 8 mg  
44 L<sup>-1</sup>: the number of offspring produced and the time required to produce the brood  
45 were not affected; the time required to produce eggs decreased ~0.3 fold; and the  
46 size of individuals at the time when the eggs and the brood were produced  
47 increased ~0.1 fold. Over the 21 days, between 1 and 8 mg L<sup>-1</sup>: the total number of

48 offspring produced increased ~3.4 fold; individual survival and the number of  
49 moults increased ~2 fold; and the number of broods increased ~1 fold. For single  
50 brood responses, there was no effect from decreasing oxygen levels on the number  
51 of offspring in the first brood, and there were negative effects on the second to  
52 fourth broods; the number of offspring in the remaining broods were not  
53 significantly related to oxygen levels, as there were fewer data at low levels due to  
54 poor survival.

55 4. We conclude that assessments of *Daphnia* demographics should not rely on  
56 estimates of the effect of oxygen concentration on single-broods. Studies should  
57 consider cumulative changes over multiple broods. Following our approach,  
58 studies may now explore the impacts on congeners and other zooplankton and  
59 assess the mechanisms associated with multi-brood responses.

60

61

62 **Introduction**

63 In virtually all freshwater systems, levels of dissolved oxygen are important in  
64 structuring the distribution, survival, and fecundity (i.e., production of new  
65 individuals) of animals (Moss, 1988), and there is now a growing need to appreciate  
66 the impacts of oxygen, as hypoxic conditions are predicted to intensify in magnitude  
67 and frequency due to the combined impacts of global warming and anthropogenic  
68 eutrophication (Moran *et al.*, 2010; Foley *et al.*, 2012; Wojtal-Frankiewicz, 2012;  
69 Jenny *et al.* 2015). In large bodies of water, where oxygen levels are depressed below  
70 the thermocline, animals may escape hypoxia by migrating to oxygen rich zones  
71 (Moss, 1988; Larson & Lampert, 2011, 2012). In contrast, in the shallow waters and  
72 wetlands that are ecologically important and can spatially dominate freshwater  
73 ecosystems (Downing *et al.*, 2006; Feuchtmayr *et al.* 2009), oxygen may become  
74 fully depleted or reduced for extended periods (weeks), except for a few centimeters  
75 at the interface (e.g., Luo, 2002; Moran *et al.*, 2010; Vad *et al.*, 2013).

76 In these shallow waters *Daphnia* is a keystone zooplankter, being the primary  
77 consumer of phytoplankton and transferring energy and material to higher trophic  
78 levels (Moss, 1988; Sarnelle, 2005; Downing *et al.*, 2006; Lampert, 2006; Persson *et al.*  
79 *et al.*, 2007; Wojtal-Frankiewicz, 2012). Therefore, if *Daphnia* is inhibited by hypoxia,  
80 food web structure and productivity in shallow waters may be adversely affected.  
81 Furthermore, *Daphnia* is routinely used as a micro-crustacean model organism  
82 (Lampert, 2006); observations on it may then also inform our understanding of how  
83 other freshwater zooplankton respond to hypoxia.

84 It has long been recognized that reduced oxygen levels, for periods of hours to  
85 days, can be deleterious to *Daphnia* (e.g., Green, 1956), although more recently it has  
86 been noted that low levels can also benefit *Daphnia*, as they seek refuge from  
87 predators in deep hypoxic layers (Larsson & Lampert, 2011, 2012). Consequently, a  
88 range of studies has examined the impact of varying oxygen concentration on the  
89 behavior and physiology of *Daphnia* (e.g., Kring & O'brien, 1976; Kobayashi &

90 Tanaka, 1991; Lamkemeyer, Zeis & Paul, 2003; Seidl, Pirow & Paul, 2005; Becker *et*  
91 *al.*, 2011; Gerke *et al.*, 2011; Lyu *et al.*, 2015). Likewise, other studies have explored  
92 growth and reproductive responses under hypoxic conditions (e.g., Green, 1956;  
93 Homer & Waller, 1983; Nebeker *et al.*, 1992; Hanazato, 1996). However, these past  
94 studies tend to examine responses at only a few oxygen levels, which do not provide  
95 sufficient data to assess trends in growth and reproductive responses over the full  
96 range of oxygen levels that *Daphnia* may experience in nature (i.e., ~0 to 8 mg L<sup>-1</sup>).  
97 Furthermore, most measurements have been made over periods encompassing no  
98 more than a single brood, and thus will not assess cumulative effects. At present we,  
99 therefore, lack sufficient information to evaluate the relative impacts of depleted  
100 oxygen on multiple broods. Given the increase in reliance on ecosystem models as  
101 predictive tools to evaluate our changing environment, it is timely to assess and  
102 parameterize the potential impacts of reduced oxygen on fecundity and survival over  
103 multiple broods.

104 In this study we, therefore, examined a suite of traits associated with population  
105 growth models (Gurney *et al.*, 1990). Specifically we contrast the responses of the  
106 first brood with that of multiple broods at seven oxygen levels ranging from near  
107 anoxia to saturation. We examined the hypothesis that the effect of hypoxia on  
108 fecundity and survival of *Daphnia* will increase over multiple broods. The results  
109 will provide responses that may be applied to further evaluate the impact of oxygen  
110 on *Daphnia*.

111

## 112 **Methods**

### 113 *Test organism*

114 We used a clone of *Daphnia similoides* (from Lake Taihu, China) that has been  
115 used as a model for assessing life-history parameters (Xiang *et al.*, 2010; Xiang *et al.*,  
116 2011). This species was also considered useful to assess oxygen effects, as in Lake  
117 Taihu, *D. similoides* experiences oxygen ranging from hypoxic to saturating levels;

118 e.g., annually, due to algal blooms, dissolved oxygen levels decline to 2 mg L<sup>-1</sup> for  
119 several weeks (Cao *et al.*, 2006; Zhang *et al.*, 2011). Furthermore, *D. similoides* is  
120 morphologically similar to other important species such as *D. magna* (Lampert, 2006)  
121 and is widely distributed in freshwater systems (e.g., Australia, New Zealand, South  
122 Africa, East Asia) (Hudec, 1991; Korovchinsky, 2013).

123 *Daphnia similoides* was cultured in 200-mL flasks containing dechlorinated water  
124 (Mirza & Pyle, 2009) and fed the chlorophyte *Scenedesmus obliquus*. The  
125 chlorophyte was cultured in sterile BG-11 medium (Lürling & Beekman, 2006), at 25  
126 °C, under fluorescent light (at 40 μmol photons m<sup>-2</sup> s<sup>-1</sup>) with a light-dark cycle of  
127 12:12 h, and at 8.0 mg L<sup>-1</sup> oxygen. Except for the oxygen levels, all experiments were  
128 conducted under the above conditions, which are typical of those where *D. similoides*  
129 may experience reduced oxygen concentrations. To ensure change in prey abundance  
130 was not a confounding factor *Daphnia similoides* were maintained at 5.0 × 10<sup>5</sup> *S.*  
131 *obliquus* mL<sup>-1</sup> by replacing media and prey daily. Thus, waste materials did not  
132 accumulate and pH remained at 8.0±0.5.

### 133 *Experimental design*

134 Clonal lines were acclimated to the above conditions for multiple generations (>  
135 3, following methods of Yang *et al.*, 2012) before subjecting them to oxygen  
136 treatments. The isolates were then exposed to seven oxygen treatments, ranging from  
137 near anoxia to saturating levels. Individuals (<24 h old) were randomly allocated to  
138 50-mL containers (i.e., one per container), to avoid density effects (Martínez-  
139 Jerónimo, Espinosa-Chávez & Villaseñor, 2000). Each treatment was replicated (*n* =  
140 4, although at low oxygen levels, some replicates died over time; see Results). To  
141 obtain dissolved oxygen levels of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 8.0 mg L<sup>-1</sup>, the media  
142 was bubbled with compressed nitrogen gas and clean air (Hanazato & Dodson, 1995);  
143 to ensure constant levels the media was monitored every 3 h, and it was regulated  
144 when required (Bante A820, Bante Instrument, China).

145 To assess effects of oxygen on the first brood of *Daphnia*, the following

146 parameters were measured daily (every 2 h between 6:00 and 24:00) until the first  
147 brood occurred: the number of offspring in the first brood; the time (days) required to  
148 produce the first eggs in the brood chamber (henceforth, time to first eggs); the time  
149 (days) required for the first brood of offspring to be released (henceforth, time to first  
150 brood); the size (mm) of the female (top of head to base of tail spine) at the time to  
151 first eggs (henceforth, size at first eggs); and the size (mm) of female at the release of  
152 the first brood (henceforth, size at first brood). Note that although most growth of  
153 *Daphnia* occurs between moults, intra-moult growth also occurs, and small changes in  
154 the size at various stages (e.g., between when the first eggs occur and first brood is  
155 released) may be indicative of stress.

156 To measure the effect of oxygen on multiple broods, each day (at 8:00) the  
157 number of offspring produced by a female was determined over 21 days; once  
158 counted, offspring were removed. To examine further demographic processes that  
159 resulted from impacts of hypoxia, changes in survival time, the total number of  
160 moults, and total number of broods over the lifespan were also recorded. Dead  
161 individuals were identified if they lacked a heartbeat. Moults were determined by  
162 counting shed carapaces. The number of moults was considered a useful parameter to  
163 measure when considering the *Daphnia* life-history. For mature *Daphnia* a single  
164 moult is associated with a single brood (Green, 1956), but moults may also occur (i.e.,  
165 individual growth) without the production of broods. Given that moults are  
166 equivalent to 15-20% of an individual's energy budget, and this may decrease when  
167 animals are stressed (Wissing & Hasler, 1968), presenting both number of broods and  
168 moults provides potentially useful information to interpret energy use under stress.

169 Finally, we assessed how hypoxic conditions affect broods over the observed  
170 eight broods. To do this we focused on determining the number of offspring per  
171 brood; for these measurements, animals were examined daily, at 8:00. Note that not  
172 all individuals survived to produce eight broods, especially at low oxygen levels.

173 *Data analysis*

174 To assess treatment effects and indicate trends, functions were fit to data, using  
175 the most parsimonious function that reflected trends in the data. Traits measured over  
176 a single brood varied linearly, or were invariant, over the observed range of oxygen  
177 levels (see Results); these were assessed by fitting a linear function, where a slope  
178 significantly differing from zero ( $\alpha = 0.05$ ) indicated an effect of oxygen on the trait.  
179 In contrast to single-brood responses, responses examined over multiple broods  
180 exhibited cumulative effects that were non-linear, suggesting an asymptotic response  
181 at higher oxygen levels (see Results). To assess these, the following rectangular  
182 hyperbolic function was fit to the data:  $Y = Y_{\max} (O - O') / [k + (O - O')]$ , where  $Y$  is the  
183 predicted value of the trait variable,  $O$  is dissolved oxygen ( $\text{mg L}^{-1}$ ),  $Y_{\max}$  is the  
184 theoretic asymptotic maximum value of the trait variable,  $O'$  is the threshold  
185 concentration of oxygen at which  $Y = 0$ , and  $k$  is a constant, reflecting the rate at  
186 which  $Y_{\max}$  is reached.

187 Here, we mainly consider this rectangular hyperbolic function as  
188 phenomenological, to illustrate trends. However, the function may also be applied to  
189 describe, mechanistically, a rate-reaction where a required substance (e.g., oxygen) is  
190 rate-limiting when it is at low concentrations, and an increase in the substance  
191 increases the rate. Then at high levels the reaction is no longer substance-limited, and  
192 the response becomes asymptotic, reaching a maximum. Such responses have been  
193 applied to enzyme reactions (Michaelis–Menten kinetics), nutrient-dependent  
194 microbial growth (the Monod equation), prey-dependent ingestion rate (the Type II  
195 functional response), and nutrient-dependent growth of autotrophs (see Berges *et al.*,  
196 1994). The function can be further modified to include a positive y-intercept (e.g.,  
197  $O'$ ), reflecting that at low (but positive) substance-levels rates becomes zero; e.g., this  
198 has been applied to show that organism growth is zero when food is still present  
199 (Montagnes & Berges, 2004). We have, therefore, applied a function that allows us to  
200 examine trends and may provide initial insights into mechanisms associated with how



201 oxygen becomes rate limiting. Non-linear regression analysis was performed using  
202 the iterative curve-fitting function of SigmaPlot 11.0 (Systat Software Inc).

### 203 **Results**

204 The effects of reduced oxygen on individuals over the first brood period were  
205 minimal or absent (Fig. 1, Table 1). There was no effect of oxygen on the number of  
206 offspring produced or the time to produce the brood, but over the observed range there  
207 was a significant reduction of ~1 day (~0.3 fold decrease) in the time required to  
208 produce the first eggs (Fig. 1a-c, Table 1). Over the observed range there were also  
209 significant, ~0.1 fold, increases in the size of individuals at both the time to produce  
210 the first eggs and produce the first brood (Fig. 1d,e, Table 1).

211 Over eight broods (21 days), there were, relatively, greater effects of oxygen  
212 level on the cumulative reproductive and survival parameters (Fig. 2). Between 1.0  
213 and 8.0 mg L<sup>-1</sup>, the total number of offspring produced increased by 3.4 fold. These  
214 data followed a non-linear (rectangular hyperbolic) response, increasing from ~40 at  
215 1.0 mg L<sup>-1</sup> to ~160 at saturating oxygen levels (Fig. 2a; Table 2). Further  
216 measurements assessed demographic changes associated with this response.  
217 Individual survival time exhibited a similar asymptotic response, increasing ~2 fold  
218 over the range (Fig. 2b; Table 2): there was no mortality at 8.0 mg L<sup>-1</sup>, but the survival  
219 time rapidly declined with decreasing oxygen to ~7 days at 1.0 mg L<sup>-1</sup> oxygen (with  
220 only one of four replicates surviving >14 days). Likewise, the number of moults and  
221 the number of broods responded non-linearly (Fig. 2c, d; Table 2), with, respectively,  
222 2- and 1-fold declines with depleted oxygen levels.

223 To assess how oxygen depletion affected *Daphnia* fecundity of each individual  
224 brood over the eight broods, we focused efforts on measuring the number of offspring  
225 per brood. There was no effect of depleted oxygen on the number of offspring  
226 produced in the first brood (Fig. 3a, Table 1). However, as time progressed  
227 decreasing oxygen levels had a negative effect on the second to fourth broods, as  
228 illustrated by the significant linear regression through the data (Fig. 3b-d, Table 1).

229 The number of offspring in the fifth and sixth broods was not significantly related to  
230 oxygen levels (there were insufficient data to perform tests on the seventh or eighth  
231 broods, Fig. 3 e-h, Table 1); these results arose from fewer data at low oxygen levels  
232 due to poor survival under these conditions (Fig. 2b).

### 233 **Discussion**

234 To date, research on the effects of hypoxia on *Daphnia* fecundity (production of  
235 new individuals), growth, and survival has focused on events associated with periods  
236 over the first brood. *Daphnia*, however, continue to reproduce and typically increase  
237 their reproductive output over multiple broods. Here, we indicate that in the first  
238 brood fecundity and survival were not affected by a reduction in dissolved oxygen.  
239 Likewise, in the first brood, there were only small (< 1 fold) but significant impacts  
240 on the time to produce eggs (but not the time to produce the brood) and on the growth  
241 of the animals, as reflected by their size when producing the first eggs and first brood.  
242 Our results suggest that there were some effects of low oxygen on the first brood.  
243 Correspondingly, previous work has revealed that exposure to hypoxia, during less  
244 than a single brood-period, down-regulated functional genes involved with repairing  
245 cellular proteins and antioxidation (Lyu *et al.*, 2015). We can, therefore, surmise that  
246 prior to the onset of multi-brood effects on fecundity, survival, and growth, hypoxia-  
247 exposed *Daphnia* will be impacted at the cellular level.

248 We are still faced with why phenotypic responses of the first brood were small or  
249 absent. It is possible that as parental females were raised at saturating oxygen levels  
250 (8.0 mg L<sup>-1</sup>), their offspring (<24 h old), which were subjected to hypoxia, were  
251 initially influenced by the conditioning of the mothers. In contrast, a striking finding  
252 was that over subsequent broods, cumulative effects on these individuals resulted in  
253 substantial shifts in the measured parameters. Such longer term impacts undoubtedly  
254 arise from a range of factors, including, energy allocated to up-regulating proteins  
255 (including hemoglobin and enzymes associated with degrading stored carbohydrates),  
256 increased responses of ventilatory and circulatory systems, reallocation of energy

257 away from somatic growth and reproduction, and the accumulation of toxic by-  
258 products arising from anaerobic metabolism (Seidl *et al.* 2005; Zeis *et al.* 2009; Ekau  
259 *et al.* 2010). If such periods of hypoxia last for up to three weeks (i.e., a period that  
260 produces up to 8 broods), between highly depleted levels and oxygen saturation the  
261 accumulated effect was a 3-fold increase in total offspring per female, a 2-fold  
262 increase in survival time and total number of moults, and a 1-fold increase in the total  
263 number of broods. Thus it appears that, over multiple broods, demographic effects  
264 can be fully appreciated only if the response to low oxygen over the life span of  
265 *Daphnia* is considered. Furthermore, associated impacts will undoubtedly extend to  
266 future generations. For example, although not directly measured in this study, we can  
267 expect that smaller offspring will be produced by the smaller adults arising from low  
268 oxygen levels in the first brood (Seidl, Paul & Pirow 2005). Multi-generational  
269 response may now be an appropriate focus for evaluating prolonged impacts of  
270 hypoxia.

271 To explore trends over this multi-brood period, we have applied a rectangular  
272 hyperbolic function, which we treat primarily as predictive but has a mechanistic basis  
273 (see Methods). For instance, we indicate clear threshold levels of oxygen ( $O'$ ) where  
274 responses reach zero-levels, and we illustrate the rate at which these responses  
275 approach asymptotic maxima near saturating oxygen concentrations. Such trends will  
276 impact on predictions associated with population dynamics of *Daphnia* when oxygen  
277 is limiting. They also reflect physiological limits that now may be investigated,  
278 through gene expression methods (e.g. Lyu *et al.*, 2013). Consequently, our responses  
279 provide useful information, allowing modelers to incorporate hypoxia into food web  
280 simulations and explore mechanisms associated the observed phenomena. Moreover,  
281 even without complex modeling efforts, it is clear that if oxygen levels drop for  
282 extended periods there will be significant consequences for *Daphnia* populations.  
283 Given that such extended reductions are likely to be more common and more  
284 pronounced in the future (Moran *et al.*, 2010; Foley *et al.*, 2012; Wojtal-Frankiewicz,

285 2012; Jenny *et al.* 2015), our results have considerable implications and applicability.

286 From our data it is also possible to begin to assess how reduced oxygen may  
287 directly affect *Daphnia*. The rapid reduction in individual survival as oxygen  
288 decreases suggests that this may be the main factor contributing to depressed  
289 fecundity. Reduced oxygen may also influence the total number of moults and of  
290 broods, both of which require energy that may be diverted to cope with the stress of  
291 hypoxia (Bergman Filho & Loureiro, 2011). These data build on our work on *D.*  
292 *similis* that indicated exposure to dissolved oxygen of 2.0 mg L<sup>-1</sup> decreased the  
293 number of broods by 50% over two weeks (Lyu *et al.*, 2013) and agree with work by  
294 Homer & Waller (1983) who indicated that the production of *D. magna* offspring is  
295 reduced by 50% at 1.8 mg L<sup>-1</sup>. Our results may, therefore, be robustly applicable to  
296 *Daphnia*, although there can be species and clonal differences in oxygen responses  
297 within *Daphnia* (Heisey & Porter, 1977; Weider & Lampert 1985), and even  
298 acclimation of clones to reduced oxygen may occur (Weider & Lampert, 1985).

299 In conclusion, we strongly suggest that freshwater ecologists interested in  
300 *Daphnia* demographics must be concerned with the impacts of hypoxia over multiple  
301 broods. In contrast, it seems that low oxygen events impacting the first-brood, even  
302 extreme ones, may be of less concern. Now that we have revealed this distinction, it  
303 will be appropriate to expend effort exploring the impacts on congeners and other  
304 zooplankton and assessing the mechanisms associated with these multi-brood  
305 responses. For instance, there is now great potential to develop links between  
306 individual changes in *Daphnia* and the molecular mechanisms underlying the  
307 response to hypoxia, following multi-gene expression methods (Lyu *et al.* 2015).

308

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315

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458 and C-N imbalance in submersed and floating-leaved aquatic plants in Lake  
459 Taihu, China. *Chemosphere*, **82**, 329-339  
460

461 Table 1. Equations and associated parameters for the linear responses fit to data in  
 462 Fig. 1 and 3 (see text for details), where the y-axis is the life-history traits of *D.*  
 463 *similoides* and the x-axis is dissolved oxygen ( $\text{mg L}^{-1}$ ). When a value was not  
 464 significantly different from zero ( $\alpha = 0.05$ ), it was presented as “0\*”. When the  
 465 slope was not significantly different from zero (or in the case of Fig. 2g,h, where  
 466 there were too few data to determine a slope), the average response was  
 467 determined. Statistics for the responses are presented: standard error (SE), p-  
 468 values for the regression and intercept (p), F-value for regression (F), sample size  
 469 (n), goodness of fit ( $R^2$ ).

470

Life history trait	Slope $\pm$ SE, p, F (n)	Intercept (or average) $\pm$ SE, p	$R^2$
Offspring in first brood (Fig. 1a, 3a)	0*, 0.39, 0.746 (21)	15.9 $\pm$ 0.6, --	--
Time (d) to first egg (Fig. 1b)	-0.19 $\pm$ 0.03, <0.0001, 29.9 (21)	5.2 $\pm$ 0.17, <0.0001	0.6
Time (d) to first brood (Fig. 1c)	0*, 0.17, 2.0 (21)	5.7 $\pm$ 0.53, --	--
Size (mm) at first egg (Fig. 1d)	0.015 $\pm$ 0.003, <0.0001, 26.2 (21)	0.94 $\pm$ 0.01, <0.0001	0.6
Size (mm) at first brood (Fig. 1e)	0.022 $\pm$ 0.005, 0.0002, 21.5 (21)	1.0 $\pm$ 0.02, <0.0001	0.5
Offspring in second brood (Fig. 3b)	3.6 $\pm$ 0.6, 0.0001, 34.6 (19)	0*, <0.089	0.7
Offspring in third brood (Fig. 3c)	2.2 $\pm$ 0.9, 0.02, 6.0 (18)	12.0 $\pm$ 4.8, <0.02	0.3
Offspring in fourth brood (Fig. 3d)	4.9 $\pm$ 0.7, <0.0001, 43.9 (18)	0*, <0.52	0.7
Offspring in fifth brood (Fig. 3e)	0*, 0.46, 0.6 (17)	16.2 $\pm$ 10, --	--
Offspring in sixth brood (Fig. 3f)	0*, 0.43, 0.7 (9)	21.7 $\pm$ 8.6, --	--

471

472 Table 2. Equations and associated parameters for responses fit to data in Fig. 2 (see  
 473 text for details);  $Y$  is the response of life-history traits of *D. similoides* and  $O$  is  
 474 dissolved oxygen ( $\text{mg L}^{-1}$ ). The adjusted  $R^2$  is provided as an indication of  
 475 goodness of fit.

Life history trait	Equation	Adjusted $R^2$
Offspring per female (Fig. 2a)	$Y = 200(O-0.91)/[(2.5+(O-0.91))]$	0.59
Survival (Fig. 2b)	$Y = 21(O-0.67)/[(0.6+(O-0.67))]$	0.63
Moults (Fig. 2c)	$Y = 14(O-0.04)/[(2.1+(O-0.04))]$	0.66
Broods (Fig. 2d)	$Y = 8.2(O-0.36)/[(2.1+(O-0.36))]$	0.31

476

477

478 **Figure caption**

479 Fig. 1. The effect of ambient dissolved oxygen concentration on a range of  
480 parameters measured on the first brood: the number of offspring produced by a  
481 mother in the first brood (a); the time (day) taken to produce the first eggs (b) the time  
482 (day) taken to produce the first brood (c); the size (mm) of mothers when the first  
483 eggs appeared (d); and the size (mm) of mothers when the first brood occurred (e).  
484 Solid dots are replicate measurements, and lines represent linear regressions where  
485 there were significant trends (Table 1).

486

487 Fig. 2. The effect of ambient dissolved oxygen concentration on: the total number of  
488 offspring produced by a single mother over the 21 day period which encompassed up  
489 to 8 broods (a); the number of days that the mother survived (b); the number of moults  
490 over this lifespan (c); and the number of broods the mother produced over this time  
491 (d). Solid dots are mean measurements, error bars are one standard error, and the  
492 lines are the best fit of a rectangular hyperbolic response through the data (see Table 2  
493 for parameters and Methods for details of the equation).

494

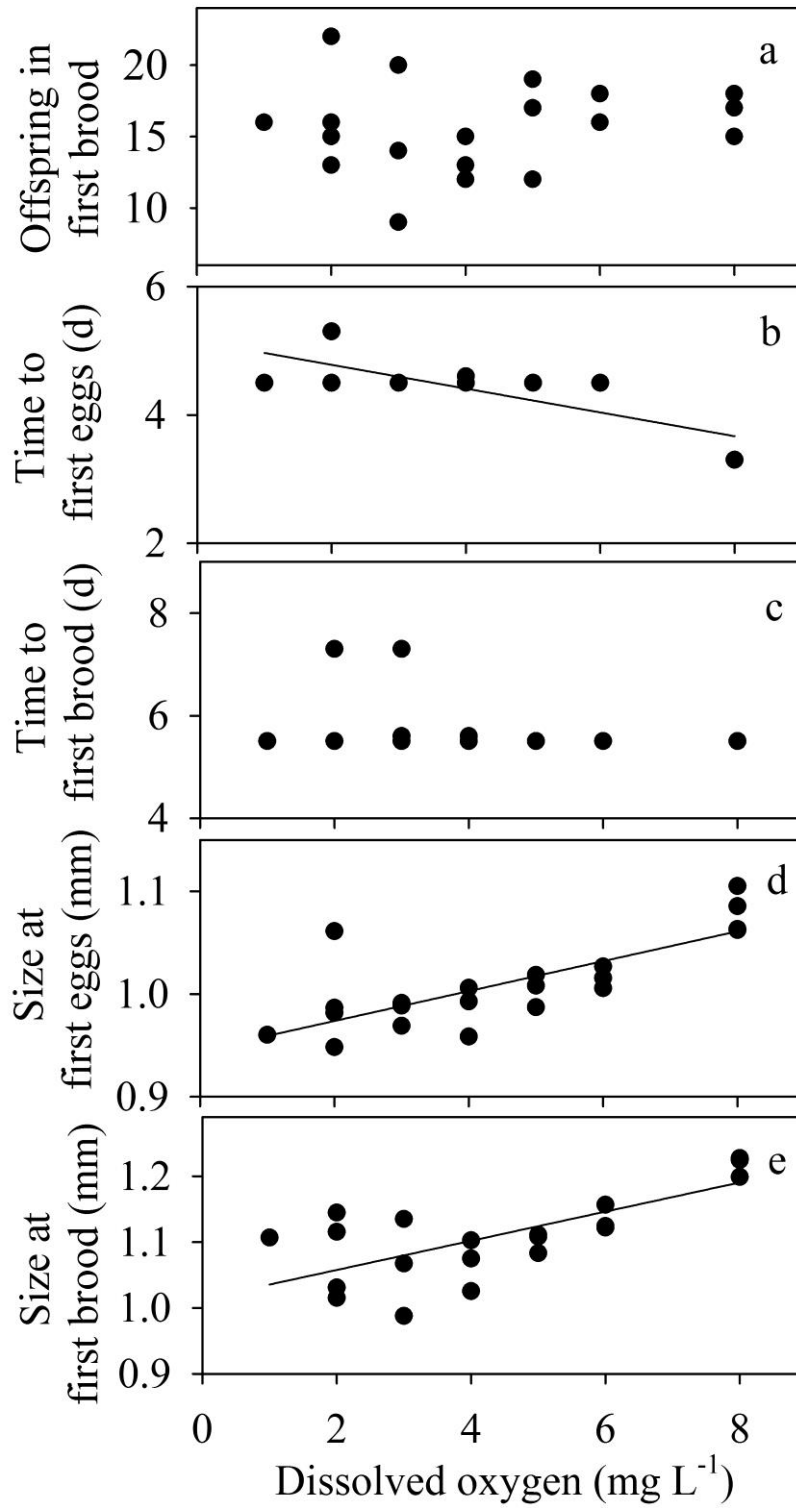
495 Fig. 3. The effect of ambient dissolved oxygen concentration on the number of  
496 offspring per brood, over multiple broods; a to h represent the 1<sup>st</sup> to 8<sup>th</sup> broods,  
497 respectively. Solid dots are replicate measurements, and lines represent linear  
498 regressions where there were significant trends (Table 1). Note that fewer  
499 measurements at low oxygen levels, on later dates, reflect mortality of individuals at  
500 these levels (see Fig. 2b).

501

502 Fig. 1

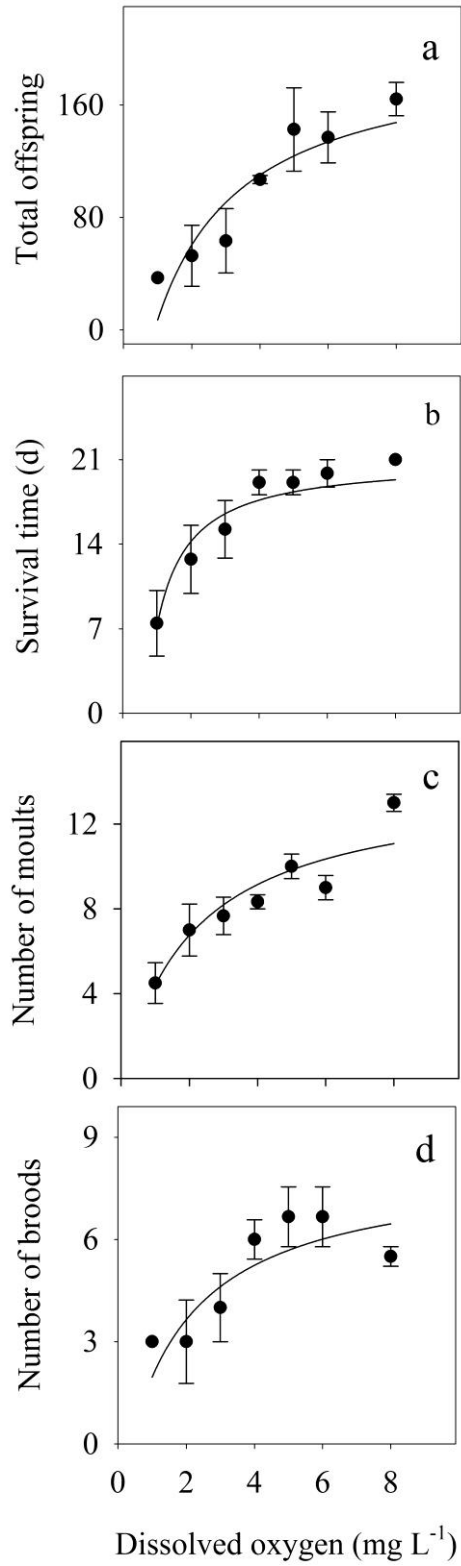
503

504



505 Fig. 2

506





507 Fig. 3

