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

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Running head: Low O₂ alters *Daphnia* fitness over multiple broods

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Summary

1. Periods of hypoxia lasting up to weeks are now anticipated in freshwaters, owing to anthropogenic influences. However, the cumulative effects of hypoxia on *Daphnia*, over multiple broods, have received virtually no attention, and to establish and evaluate such responses there is a need to make measurements over a wide range of oxygen concentrations, potentially revealing non-linear patterns. We predict that the effect of hypoxia on growth, survival, and fecundity (i.e., production of new individuals) of *Daphnia* will increase over multiple broods, and with increasing oxygen these responses will approach asymptotic maxima, following a rectangular-hyperbolic response.

2. *Daphnia similoides* were exposed to 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 mg oxygen L⁻¹. To determine effects on the first brood we examined: number of offspring; time to the first eggs; time to the first brood; and size of the female at the first eggs and the first brood. To determine cumulative effects of oxygen over multiple broods (up to 8 broods over 21 days) we measured: total number of offspring produced by a female; survival time; and total number of moults and broods. To investigate how the cumulative effects arose over the multiple broods, we examined the number of offspring per brood in each brood over 8 broods. To assess treatment effects and indicate responses, functions were fit to data using the most parsimonious function that reflected trends in the data.

3. Measurements associated with a single brood responded linearly, or not at all, with changing oxygen concentration, while measurements made over the 21 days followed a rectangular hyperbolic response, increasing to an asymptote as oxygen increased. For the first brood, as oxygen concentration was raised from 1 to 8 mg L⁻¹: the number of offspring produced and the time required to produce the brood were not affected; the time required to produce eggs decreased ~0.3 fold; and the size of individuals at the time when the eggs and the brood were produced increased ~0.1 fold. Over the 21 days, between 1 and 8 mg L⁻¹: the total number of
offspring produced increased ~3.4 fold; individual survival and the number of moults increased ~2 fold; and the number of broods increased ~1 fold. For single brood responses, there was no effect from decreasing oxygen levels on the number of offspring in the first brood, and there were negative effects on the second to fourth broods; the number of offspring in the remaining broods were not significantly related to oxygen levels, as there were fewer data at low levels due to poor survival.

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

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

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

It has long been recognized that reduced oxygen levels, for periods of hours to days, can be deleterious to Daphnia (e.g., Green, 1956), although more recently it has been noted that low levels can also benefit Daphnia, as they seek refuge from predators in deep hypoxic layers (Larsson & Lampert, 2011, 2012). Consequently, a range of studies has examined the impact of varying oxygen concentration on the behavior and physiology of Daphnia (e.g., Kring & O'Brien, 1976; Kobayashi &
Likewise, other studies have explored growth and reproductive responses under hypoxic conditions (e.g., Green, 1956; Homer & Waller, 1983; Nebeker et al., 1992; Hanazato, 1996). However, these past studies tend to examine responses at only a few oxygen levels, which do not provide sufficient data to assess trends in growth and reproductive responses over the full range of oxygen levels that Daphnia may experience in nature (i.e., ~0 to 8 mg L\(^{-1}\)). Furthermore, most measurements have been made over periods encompassing no more than a single brood, and thus will not assess cumulative effects. At present we, therefore, lack sufficient information to evaluate the relative impacts of depleted oxygen on multiple broods. Given the increase in reliance on ecosystem models as predictive tools to evaluate our changing environment, it is timely to assess and parameterize the potential impacts of reduced oxygen on fecundity and survival over multiple broods.

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

### Methods

#### Test organism

We used a clone of Daphnia similoides (from Lake Taihu, China) that has been used as a model for assessing life-history parameters (Xiang et al., 2010; Xiang et al., 2011). This species was also considered useful to assess oxygen effects, as in Lake Taihu, D. similoides experiences oxygen ranging from hypoxic to saturating levels;
e.g., annually, due to algal blooms, dissolved oxygen levels decline to 2 mg L\(^{-1}\) for several weeks (Cao et al., 2006; Zhang et al., 2011). Furthermore, *D. similoides* is morphologically similar to other important species such as *D. magna* (Lampert, 2006) and is widely distributed in freshwater systems (e.g., Australia, New Zealand, South Africa, East Asia) (Hudec, 1991; Korovchinsky, 2013).

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

**Experimental design**

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

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

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

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

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

Here, we mainly consider this rectangular hyperbolic function as phenomenological, to illustrate trends. However, the function may also be applied to describe, mechanistically, a rate-reaction where a required substance (e.g., oxygen) is rate-limiting when it is at low concentrations, and an increase in the substance increases the rate. Then at high levels the reaction is no longer substance-limited, and the response becomes asymptotic, reaching a maximum. Such responses have been applied to enzyme reactions (Michaelis–Menten kinetics), nutrient-dependent microbial growth (the Monod equation), prey-dependent ingestion rate (the Type II functional response), and nutrient-dependent growth of autotrophs (see Berges *et al.*, 1994). The function can be further modified to include a positive $y$-intercept (e.g., $O'$), reflecting that at low (but positive) substance-levels rates becomes zero; e.g., this has been applied to show that organism growth is zero when food is still present (Montagnes & Berges, 2004). We have, therefore, applied a function that allows us to examine trends and may provide initial insights into mechanisms associated with how
oxygen becomes rate limiting. Non-linear regression analysis was performed using the iterative curve-fitting function of SigmaPlot 11.0 (Systat Software Inc).

**Results**

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

Over eight broods (21 days), there were, relatively, greater effects of oxygen level on the cumulative reproductive and survival parameters (Fig. 2). Between 1.0 and 8.0 mg L\(^{-1}\), the total number of offspring produced increased by 3.4 fold. These data followed a non-linear (rectangular hyperbolic) response, increasing from ~40 at 1.0 mg L\(^{-1}\) to ~160 at saturating oxygen levels (Fig. 2a; Table 2). Further measurements assessed demographic changes associated with this response.

Individual survival time exhibited a similar asymptotic response, increasing ~2 fold over the range (Fig. 2b; Table 2): there was no mortality at 8.0 mg L\(^{-1}\), but the survival time rapidly declined with decreasing oxygen to ~7 days at 1.0 mg L\(^{-1}\) oxygen (with only one of four replicates surviving >14 days). Likewise, the number of moults and the number of broods responded non-linearly (Fig. 2c, d; Table 2), with, respectively, 2- and 1-fold declines with depleted oxygen levels.

To assess how oxygen depletion affected *Daphnia* fecundity of each individual brood over the eight broods, we focused efforts on measuring the number of offspring per brood. There was no effect of depleted oxygen on the number of offspring produced in the first brood (Fig. 3a, Table 1). However, as time progressed decreasing oxygen levels had a negative effect on the second to fourth broods, as illustrated by the significant linear regression through the data (Fig. 3b-d, Table 1).
The number of offspring in the fifth and sixth broods was not significantly related to oxygen levels (there were insufficient data to perform tests on the seventh or eighth broods, Fig. 3 e-h, Table 1); these results arose from fewer data at low oxygen levels due to poor survival under these conditions (Fig. 2b).

Discussion

To date, research on the effects of hypoxia on *Daphnia* fecundity (production of new individuals), growth, and survival has focused on events associated with periods over the first brood. *Daphnia*, however, continue to reproduce and typically increase their reproductive output over multiple broods. Here, we indicate that in the first brood fecundity and survival were not affected by a reduction in dissolved oxygen. Likewise, in the first brood, there were only small (< 1 fold) but significant impacts on the time to produce eggs (but not the time to produce the brood) and on the growth of the animals, as reflected by their size when producing the first eggs and first brood.

Our results suggest that there were some effects of low oxygen on the first brood. Correspondingly, previous work has revealed that exposure to hypoxia, during less than a single brood-period, down-regulated functional genes involved with repairing cellular proteins and antioxidation (Lyu *et al*., 2015). We can, therefore, surmise that prior to the onset of multi-brood effects on fecundity, survival, and growth, hypoxia-exposed *Daphnia* will be impacted at the cellular level.

We are still faced with why phenotypic responses of the first brood were small or absent. It is possible that as parental females were raised at saturating oxygen levels (8.0 mg L⁻¹), their offspring (<24 h old), which were subjected to hypoxia, were initially influenced by the conditioning of the mothers. In contrast, a striking finding was that over subsequent broods, cumulative effects on these individuals resulted in substantial shifts in the measured parameters. Such longer term impacts undoubtedly arise from a range of factors, including, energy allocated to up-regulating proteins (including hemoglobin and enzymes associated with degrading stored carbohydrates), increased responses of ventilatory and circulatory systems, reallocation of energy
away from somatic growth and reproduction, and the accumulation of toxic by-products arising from anaerobic metabolism (Seidl et al. 2005; Zeis et al. 2009; Ekau et al. 2010). If such periods of hypoxia last for up to three weeks (i.e., a period that produces up to 8 broods), between highly depleted levels and oxygen saturation the accumulated effect was a 3-fold increase in total offspring per female, a 2-fold increase in survival time and total number of moults, and a 1-fold increase in the total number of broods. Thus it appears that, over multiple broods, demographic effects can be fully appreciated only if the response to low oxygen over the life span of Daphnia is considered. Furthermore, associated impacts will undoubtedly extend to future generations. For example, although not directly measured in this study, we can expect that smaller offspring will be produced by the smaller adults arising from low oxygen levels in the first brood (Seidl, Paul & Pirow 2005). Multi-generational response may now be an appropriate focus for evaluating prolonged impacts of hypoxia.

To explore trends over this multi-brood period, we have applied a rectangular hyperbolic function, which we treat primarily as predictive but has a mechanistic basis (see Methods). For instance, we indicate clear threshold levels of oxygen ($O'$) where responses reach zero-levels, and we illustrate the rate at which these responses approach asymptotic maxima near saturating oxygen concentrations. Such trends will impact on predictions associated with population dynamics of Daphnia when oxygen is limiting. They also reflect physiological limits that now may be investigated, through gene expression methods (e.g. Lyu et al., 2013). Consequently, our responses provide useful information, allowing modelers to incorporate hypoxia into food web simulations and explore mechanisms associated the observed phenomena. Moreover, even without complex modeling efforts, it is clear that if oxygen levels drop for extended periods there will be significant consequences for Daphnia populations. Given that such extended reductions are likely to be more common and more pronounced in the future (Moran et al., 2010; Foley et al., 2012; Wojtal-Frankiewic,
From our data it is also possible to begin to assess how reduced oxygen may directly affect *Daphnia*. The rapid reduction in individual survival as oxygen decreases suggests that this may be the main factor contributing to depressed fecundity. Reduced oxygen may also influence the total number of moults and of broods, both of which require energy that may be diverted to cope with the stress of hypoxia (Bergman Filho & Loureiro, 2011). These data build on our work on *D. similis* that indicated exposure to dissolved oxygen of 2.0 mg L$^{-1}$ decreased the number of broods by 50% over two weeks (Lyu et al., 2013) and agree with work by Homer & Waller (1983) who indicated that the production of *D. magna* offspring is reduced by 50% at 1.8 mg L$^{-1}$. Our results may, therefore, be robustly applicable to *Daphnia*, although there can be species and clonal differences in oxygen responses within *Daphnia* (Heisey & Porter, 1977; Weider & Lampert 1985), and even acclimation of clones to reduced oxygen may occur (Weider & Lampert, 1985).

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

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Reference


Kring R. L. & O'brien W. J. (1976) Effect of varying oxygen concentrations on the


Daphnia magna to warm temperatures is dependent on haemoglobin expression. *Journal of Thermal Biology, 30*, 532-544.


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

<table>
<thead>
<tr>
<th>Life history trait</th>
<th>Slope ± SE, p, F (n)</th>
<th>Intercept (or average) ± SE, p</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring in first brood (Fig. 1a, 3a)</td>
<td>0*, 0.39, 0.746 (21)</td>
<td>15.9 ± 0.6, --</td>
<td>--</td>
</tr>
<tr>
<td>Time (d) to first egg (Fig. 1b)</td>
<td>-0.19 ± 0.03, &lt;0.0001, 29.9 (21)</td>
<td>5.2 ± 0.17, &lt;0.0001</td>
<td>0.6</td>
</tr>
<tr>
<td>Time (d) to first brood (Fig. 1c)</td>
<td>0*, 0.17, 2.0 (21)</td>
<td>5.7 ± 0.53, --</td>
<td>--</td>
</tr>
<tr>
<td>Size (mm) at first egg (Fig. 1d)</td>
<td>0.015 ± 0.003, &lt;0.0001, 26.2 (21)</td>
<td>0.94 ± 0.01, &lt;0.0001</td>
<td>0.6</td>
</tr>
<tr>
<td>Size (mm) at first brood (Fig. 1e)</td>
<td>0.022 ± 0.005, 0.0002, 21.5 (21)</td>
<td>1.0 ± 0.02, &lt;0.0001</td>
<td>0.5</td>
</tr>
<tr>
<td>Offspring in second brood (Fig. 3b)</td>
<td>3.6 ± 0.6, 0.0001, 34.6 (19)</td>
<td>0*, &lt;0.089</td>
<td>0.7</td>
</tr>
<tr>
<td>Offspring in third brood (Fig. 3c)</td>
<td>2.2 ± 0.9, 0.02, 6.0 (18)</td>
<td>12.0 ± 4.8, &lt;0.02</td>
<td>0.3</td>
</tr>
<tr>
<td>Offspring in fourth brood (Fig. 3d)</td>
<td>4.9 ± 0.7, &lt;0.0001, 43.9 (18)</td>
<td>0*, &lt;0.52</td>
<td>0.7</td>
</tr>
<tr>
<td>Offspring in fifth brood (Fig. 3e)</td>
<td>0*, 0.46, 0.6 (17)</td>
<td>16.2 ± 10, --</td>
<td>--</td>
</tr>
<tr>
<td>Offspring in sixth brood (Fig. 3f)</td>
<td>0*, 0.43, 0.7 (9)</td>
<td>21.7 ± 8.6, --</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 2. Equations and associated parameters for responses fit to data in Fig. 2 (see text for details); $Y$ is the response of life-history traits of *D. similoides* and $O$ is dissolved oxygen (mg L$^{-1}$). The adjusted $R^2$ is provided as an indication of goodness of fit.

<table>
<thead>
<tr>
<th>Life history trait</th>
<th>Equation</th>
<th>Adjusted $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring per female (Fig. 2a)</td>
<td>$Y = 200(O-0.91)/[(2.5+( O-0.91)]$</td>
<td>0.59</td>
</tr>
<tr>
<td>Survival (Fig. 2b)</td>
<td>$Y = 21(O-0.67)/[(0.6+( O-0.67)]$</td>
<td>0.63</td>
</tr>
<tr>
<td>Moult (Fig. 2c)</td>
<td>$Y = 14(O-0.04)/[(2.1+( O-0.04)]$</td>
<td>0.66</td>
</tr>
<tr>
<td>Broods (Fig. 2d)</td>
<td>$Y = 8.2(O-0.36)/[(2.1+( O-0.36)]$</td>
<td>0.31</td>
</tr>
</tbody>
</table>
**Figure caption**

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

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

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

- Offspring in first brood
- Time to first eggs (d)
- Time to first brood (d)
- Size at first eggs (mm)
- Size at first brood (mm) vs. Dissolved oxygen (mg L⁻¹)
Fig. 2

(A) Total offspring vs. dissolved oxygen (mg L\(^{-1}\)).

(B) Survival time (d) vs. dissolved oxygen (mg L\(^{-1}\)).

(C) Number of molts vs. dissolved oxygen (mg L\(^{-1}\)).

(D) Number of broods vs. dissolved oxygen (mg L\(^{-1}\)).
Fig. 3

Offspring per brood vs. Dissolved oxygen (mg L$^{-1}$)

a, 1$^{st}$

b, 2$^{nd}$

c, 3$^{rd}$

d, 4$^{th}$

e, 5$^{th}$

f, 6$^{th}$

g, 7$^{th}$
h, 8$^{th}$