

# The interactive effects of calcium concentration and temperature on the survival and reproduction of *Daphnia pulex* at high and low food concentrations

Dawn Ashforth

York University, Department of Biology, 4700 Keele Street, Toronto, Ontario M3J 1P3, Canada

Norman D. Yan<sup>1</sup>

York University, Department of Biology, 4700 Keele Street, Toronto, Ontario M3J 1P3, Canada; Dorset Environmental Science Centre, 1026 Bellwood Acres Road, Dorset, Ontario P0A 1E0, Canada

## Abstract

We reared *Daphnia pulex* in a fully defined medium at seven calcium (Ca) concentrations (0.1, 0.5, 1, 1.5, 2, 5, and 10 mg Ca L<sup>-1</sup>), two mixed algal densities (high food: 30 µg chlorophyll *a* L<sup>-1</sup> and low food: 3 µg Chl *a* L<sup>-1</sup>), and four temperatures (20°C, 24°C, 28°C, and 32°C) in a fully factorial design. The minimum Ca concentration required for the survival and reproduction of *D. pulex* was between 0.1 and 0.5 mg Ca L<sup>-1</sup>. Daphniids reared at 0.1 mg Ca L<sup>-1</sup> lived no longer than 10 d and did not reproduce. Although offspring were produced at 0.5 mg Ca L<sup>-1</sup> and above, reproduction was reduced below 1.5 mg Ca L<sup>-1</sup> due to delays in maturity and reductions in both the brood size and number of broods produced within the 15 d of the experiment. As a result, the intrinsic rate of natural increase, *r*, decreased dramatically between 1.5 and 1 mg Ca L<sup>-1</sup>, and was undefined at 0.1 mg Ca L<sup>-1</sup>. Higher temperatures and reduced algal biomass enhanced the susceptibility of *D. pulex* to low Ca concentrations by raising the reproductive threshold to 1.5 mg Ca L<sup>-1</sup>. Thermal stress at 32°C was so great that daphniids lived no longer than 5 d and did not reproduce. Hence, *r* was undefined at 32°C. In order to sustain *D. pulex* populations and potentially other Ca-sensitive daphniids, fresh waters must maintain a Ca concentration of at least 0.5 mg Ca L<sup>-1</sup>, although concentrations as high as 1.5 mg Ca L<sup>-1</sup> may be required for daphniids to withstand the concurrent reductions in algal biomass and rising water temperatures that are now commonplace on the south-central Canadian Shield.

Sustained acid-loading since the onset of industrialization and large clearances of vegetation have depleted the calcium (Ca) content of watershed soils (Federer et al. 1989; Watmough and Dillon 2003), decreasing inputs to fresh waters. As a result, Ca concentrations in the soft-water lakes of the south-central Canadian Shield have dropped by over 45% on average since preindustrial times (Keller et al. 2001), with individual lakes experiencing losses of 10–60% since just the 1980s (Keller et al. 2003; Yan et al. in press). In 1990, the median Ca concentration of the majority of Canadian Shield lakes was near 3 mg Ca L<sup>-1</sup> (Neary et al. 1990) but concentrations less than 1.5 mg Ca L<sup>-1</sup> are now common in small lakes and ponds (D. McNicoll unpubl. data).

The effects of the current widespread Ca decline on aquatic organisms are not well-understood. Low ambient Ca concentrations can inhibit the ability of heavily calcified

organisms to grow (Rukke 2002a) or maintain their protective shells or carapaces (Orr et al. 2005). Among the crustacean zooplankton, daphniids are likely the most vulnerable taxa to low ambient Ca concentrations since their carapace is predominantly comprised of calcium carbonates (Compère et al. 2004). Daphniids moult frequently (Ebert 1992) and lack the ability to store Ca while moulting (Alstad et al. 1999); hence, their Ca demand is very high (Jeziorski and Yan 2006) and the majority of their Ca must be extracted from the external medium immediately after moulting (Hessen et al. 2000; Rukke 2002a). An inadequate supply of Ca could threaten daphniid persistence.

Our knowledge of the external Ca concentrations required by daphniids is quite limited. Thresholds for survival lie between 0 and 2 mg Ca L<sup>-1</sup> for *Daphnia galeata* (Rukke 2002b) and between 0.1 and 0.5 mg Ca L<sup>-1</sup> for *D. magna* (Hessen et al. 2000). However, since the average Ca concentration of Canadian Shield lakes is approaching 2 mg Ca L<sup>-1</sup>, we must determine whether the threshold for daphniid survival lies closer to 0 or to 2 mg Ca L<sup>-1</sup>. Our first objective was to address this knowledge gap for *D. pulex*, a taxon with a large Ca requirement (Jeziorski and Yan 2006) that commonly inhabits soft-water Canadian Shield lakes and ponds (Yan et al. 1988), unlike *D. magna*. Ultimately, we must determine if these thresholds are representative of all daphniids.

By themselves, lab-derived estimates of mortality are inadequate to assess the effects of stressors on natural

<sup>1</sup> Corresponding author (nyan@yorku.ca).

## Acknowledgments

We thank Alison Croft and Allegra Cairns for their assistance in the laboratory, Don Evans, Peter Sutey, and Vince Ferraro for their technical assistance, Celia Chen, Winfried Lampert, and Kathleen Keating for their advice with culturing techniques, Howard Riessen for the *Daphnia pulex* culture, and Derek Taylor for the molecular analyses that confirmed the identity of our *Daphnia*. We also thank Edward McCauley and two anonymous reviewers for their helpful comments on the manuscript.

Support was provided by the Natural Sciences and Engineering Research Council (NSERC) and a government of Ontario Premier's Research Excellence Award (PREA) to N. D. Yan.

populations (Walthall and Stark 1997; Raimondo and McKenney 2006). Small reductions in reproductive rate or delays in maturity can lead to population declines or extinction even when death rates are stable (Walthall and Stark 1997). Hessen et al. (2000) reported delays in primiparity, fewer total broods, and reduced numbers of gravid female *D. magna* when Ca concentrations were reduced, even though survival rates were similar across all Ca concentrations. Our second objective was to determine the effects of low Ca concentrations on the maturation and reproductive rates of *D. pulex* using the cohort generation time, basic reproductive rate, and intrinsic rate of natural increase as integrative metrics. The cohort generation time,  $T_c$ , is the average length of time between the birth of an individual and the birth of its offspring. The basic reproductive rate,  $R_o$ , is the mean number of neonates produced by an individual in its lifetime. For species with discrete generations,  $R_o$  can be used to describe the overall changes in population size. However, for species with overlapping generations, such as *Daphnia*, the intrinsic rate of natural increase must be used to describe changes in population size since it takes into account the simultaneous contributions made by various cohorts. The intrinsic rate of natural increase,  $r$ , measures the potential growth of a population, or in our case, the viability of a clone, in a nonlimiting environment. Since daphniids reproduce parthenogenetically, the magnitude of  $r$  is indicative of the potential success of a particular genotype (Stearns 1992). Low values of  $r$  indicate low relative fitness or a strong likelihood that a species or clone will be replaced by those with higher  $r$  values (Wolf and Weider 1991), assuming they are in competition with one another. Any decline in  $r$  is indicative of a loss in competitive status within a community, possibly increasing the susceptibility of a population to local extinction. Thus, a reduction in  $r$  can have dramatic and rapid effects on a population.

Ca concentration is not the only potential zooplankton stressor that is changing in Canadian Shield lakes, and stressors can interact to affect biota in ways that cannot be predicted from the sum of their individual effects (Folt et al. 1999; Christensen et al. 2006). Our third objective was to determine the effects of low Ca concentrations on *D. pulex* in the context of declining algal biomass and a warming climate. Over the last 30 yr, total phosphorus and chlorophyll concentrations have dropped by 25–30% in south-central Canadian Shield lakes (A. Paterson, pers. comm.), which may reduce longevity, growth, and reproduction of daphniids (Tessier et al. 1983; Lampert 1987; Peters 1987) and increase their susceptibility to low ambient Ca concentrations (Hessen et al. 2000). Temperatures  $>25^\circ\text{C}$  increase the susceptibility of daphniids to stress (Moore et al. 1996; Folt et al. 1999) and will increase their demand for Ca since all physiological processes, including those involved in moulting, are accelerated at higher temperatures (Hessen et al. 2000). This is especially troubling since the temperature of many ponds and shallow lakes in temperate North America is presently  $\geq 25^\circ\text{C}$  in the summer (Moore et al. 1996) and the frequency and duration of such events are expected to increase (Cubasch et al. 2001). Should elevated temperatures and reduced

algal biomass influence sensitivity to low Ca concentrations, the minimum Ca concentrations required for daphniid survival and reproduction will likely rise.

Here we determine the following three items: (1) what the minimum Ca concentration required for the survival and reproduction of *D. pulex* is; (2) whether low ambient Ca concentrations delay the maturation of *D. pulex* or affect neonate production rates; and (3) whether warmer temperatures or reduced algal biomass influence these Ca effects.

## Materials and methods

**Daphniid culture**—In the spring of 2004, a culture of *Daphnia pulex* was obtained from H. Riessen (Buffalo State College, Buffalo, New York, USA). The culture was identified as a member of the common *D. pulex* panarctic clade via ND5 and ND2 gene assessments (Colbourne et al. 1998; D. Taylor pers. comm.). The founding stock for this culture was collected from a natural roadside pond south of the Dorset Environmental Science Centre (DESC) in Dorset, Ontario, Canada. The road adjacent to the pond is used only during the summer and has little traffic.

At York University's Field Laboratory for the Assessment of Multiple Ecological Stressors located at the DESC, *D. pulex* was reared in a medium based on Lynch et al. (1986), with supplemented trace elements (Table 1). The *D. pulex* culture was maintained in the laboratory for 8 months prior to its use in experimental trials.

**Algal cultures**—Pure cultures of *Pseudokirchneriella subcapitata* and *Chlorella kesslerii* obtained from the phytoplankton culture collection of the University of Toronto (Toronto, Ontario, Canada) were grown in a modified Bristol's medium using deionised (DI) water and stored prior to use at  $4^\circ\text{C}$  in clear polyethylene terephthalate (PET) bottles in 100-mL and 150-mL quantities, for *P. subcapitata* and *C. kesslerii*, respectively. For the experiment, mixed algal suspensions containing an equal concentration of each species were prepared daily at two densities: 65 and 6  $\mu\text{g}$  chlorophyll *a*  $\text{L}^{-1}$ . The algal suspensions contained minimal amounts of Ca, 0.027 mg Ca  $\text{L}^{-1}$  (0.000–0.060) and 0.003 mg Ca  $\text{L}^{-1}$  (0.000–0.010) on average, in the high and low food suspensions, respectively. This was achieved by siphoning off most of the Bristol's medium from the settled stock algal solutions and replacing it with an equal amount of Ca-free Bristol's medium.

Prior to each daphniid feeding, we measured several water-quality parameters of each algal suspension. Oxygen concentration, conductivity, and temperature were measured with a YSI Model 85 Handheld Oxygen, Conductivity, Salinity, and Temperature System. We measured pH with a Thermo Orion Benchtop 520 Plus pH meter (Fisher Scientific) after calibration with pH 4, 7, and 10 buffers. Ca concentration was measured with a Varian SpectraAA 300plus flame atomic absorption spectrophotometer following the protocol of Evans (2001). Chl *a* was determined with a Beckman DU Series 600 spectrophotometer using 90% acetone after filtering the samples through 1.2- $\mu\text{m}$

Table 1. Composition of the zooplankton medium used in this study. Deionised water was used throughout. Zooplankton medium and vitamin solution recipes provided by Lynch et al. (1986). ANIMATE recipe provided by Kilham et al. (1998). Selenium ( $\text{SeO}_2$ ) and vanadium ( $\text{NH}_4\text{VO}_3$ ) were added to the ANIMATE solution based on recommendations from K. I. Keating (pers. comm.) and Keating (1985).

Zooplankton medium recipe			
KCl	50 mg L <sup>-1</sup>	$\text{NaSiO}_3 \cdot 9\text{H}_2\text{O}$	20 mg L <sup>-1</sup>
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	40 mg L <sup>-1</sup>	$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	1.1 mg L <sup>-1</sup>
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	42 mg L <sup>-1</sup>	Biotin	0.1 mg L <sup>-1</sup>
$\text{K}_2\text{HPO}_4$	6 mg L <sup>-1</sup>	Vitamin solution	0.3 ml L <sup>-1</sup>
$\text{KH}_2\text{PO}_4$	6 mg L <sup>-1</sup>	ANIMATE	1 ml L <sup>-1</sup>
$\text{NaNO}_3$	50 mg L <sup>-1</sup>		

Final concentrations in the prepared zooplankton medium.

To obtain the concentrations in the test jars containing *Daphnia pulex*, halve these values (due to dilution by the addition of the algal suspension).

Vitamin solution			
Calcium pantothenate ( $\text{B}_5$ )	7 g L <sup>-1</sup>	Nicotinamide	1.3 g L <sup>-1</sup>
Cyanocobalamin ( $\text{B}_{12}$ )	0.0003 g L <sup>-1</sup>	Folic acid	3.3 g L <sup>-1</sup>
Thiamin ( $\text{B}_1$ )	0.6 g L <sup>-1</sup>	Putrescine	0.3 g L <sup>-1</sup>
Riboflavin ( $\text{B}_2$ )	0.4 g L <sup>-1</sup>	Choline	5 g L <sup>-1</sup>
		Inositol	11 g L <sup>-1</sup>

Concentrations given are for a vitamin stock solution.

Animal trace elements (ANIMATE) solution			
LiCl	500 mg L <sup>-1</sup>	KI	22 mg L <sup>-1</sup>
RbCl	50 mg L <sup>-1</sup>	$\text{SeO}_2$	1 mg L <sup>-1</sup>
$\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$	300 mg L <sup>-1</sup>	$\text{NH}_4\text{VO}_3$	0.5 mg L <sup>-1</sup>
NaBr	64 mg L <sup>-1</sup>		

Concentrations given are for an ANIMATE stock solution.

nylon filters and fixing with 0.5 mL of 1%  $\text{MgCO}_3$  for each litre of sample following the protocol of Wright and Ferraro (2004).

**Media preparation**—The zooplankton medium was prepared 2 d prior to the start of the experiment in Apr 2005. Seven separate 20-liter batches were prepared at Ca concentrations of 0, 0.5, 1, 1.5, 2, 5, and 10 mg Ca L<sup>-1</sup>. The Ca gradient was established by varying the amount of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  added to each batch. The pH of the media was lowered to 7.08–7.24 by adding several drops of 0.1 mol L<sup>-1</sup> HCl. Each batch was stored in a separate 20-liter carboy at room temperature (19–23°C); media can be stored at this temperature for as long as one month before the build-up of bacteria becomes a concern (Lynch et al. 1986). Finally, we recorded the oxygen concentration, conductivity, and temperature of each batch prior to the start of the experiment, and weekly thereafter, along with pH and Ca concentration. Pilot studies showed the oxygen concentration of the media within the test jars was between 8 and 9 mg L<sup>-1</sup>, which remained constant at 20°C or moderately increased at 24°C, 28°C, and 32°C, over a 2-d period with daphniids present (D. Ashforth unpubl. data). Thus, sufficient oxygen was available for all test daphniids at all times.

At the start of the experiment, the measured Ca content of each batch of media was within 0.1 mg Ca L<sup>-1</sup> of its

nominal value, once added to the test jars and diluted by the algal suspensions. Over the course of the experiment, the Ca content of the  $\leq 2$  mg Ca L<sup>-1</sup> media remained within 0.1 mg Ca L<sup>-1</sup> of the nominal concentrations. The Ca content of the 5- and 10-mg Ca L<sup>-1</sup> media varied by up to 1 mg Ca L<sup>-1</sup> from nominal concentrations by the end of the experiment; however, as the daphniids suffered no ill-effects at any of these high Ca concentrations, our results were not affected by these few departures from planned exposures.

**Experimental set-up**—We used <24-h-old, F2-generation females from the third brood of our clone of *D. pulex*. Our use of <24-h-old neonates ensured that the test animals would be exposed to the treatments for the majority of their juvenile period, while the third brood source from F1 generation individuals of identical age controlled for maternal effects (Sakwińska 2004) and minimized variance among test animals (Dodson et al. 1997). We ran 10 replicates of each treatment ( $N = 560$ ) with daphniids placed individually in 250-mL glass jars. Each jar contained 40 mL of algal suspension and 40 mL of zooplankton medium at the appropriate Ca concentration. The initial chlorophyll concentration in each test jar was approximately 30 and 3  $\mu\text{g Chl } a \text{ L}^{-1}$  for the high and low food treatments, respectively. Pilot studies indicated little, if any, change in Chl *a* concentrations over a 2-d period (D.

Ashforth, unpubl. data). The algal density of the low food treatment resembled lake concentrations: algal standing stocks are typically low in Canadian Shield lakes, averaging 2–3  $\mu\text{g Chl } a \text{ L}^{-1}$  (Marshall and Peters 1989; Ontario Ministry of the Environment, unpubl. data).

A small flake, 0.4–1.1 mg, of cetyl alcohol [ $\text{CH}_3(\text{CH}_2)_{14}\text{CH}_2\text{OH}$ ] was placed at the surface–air interface of the medium to reduce surface tension and the likelihood of daphniid entrapment. This tiny flake size ensured no toxic effect (Desmarais 1997), and Ashforth (2006) demonstrated that the remaining infrequent incidence of entrapment did not affect results. Of the 560 daphniids in this experiment, only 154 ever became trapped in the surface film, and only 14% of these individuals were trapped more than once. Most daphniids continued to live and reproduce for days after entrapment (Ashforth 2006); only 15% of daphniids reared at 20°C, 13% at 24°C, and 8% at 28°C died shortly after entrapment. At 32°C, 40% of entrapped daphniids died shortly afterward; however, elevated temperature likely accounts for this mortality.

The test jars were covered with Parafilm® M laboratory sealing film (Picheney Plastic Packaging) to avoid evaporation and contamination and were positioned in a fully randomized way within CONVIRON Cmp4030 controlled environment chambers. The temperature within all chambers was initially 20°C. One chamber was maintained at this temperature for the duration of the experiment. The temperature in the remaining chambers was increased from 20°C at a rate of 0.5°C h<sup>-1</sup> until one of three experimental temperatures (24°C, 28°C, or 32°C) was reached. These higher temperatures were maintained thereafter. The initial gradual ramping rate of temperature was selected to minimize impacts on the daphniids (Chen 1994; C. Y. Chen pers. comm.). The chambers were programmed for 14:10-h light:dark cycles with 30-min dawn:dusk ramping periods.

The experiment was run for 15 d, as this time period was sufficient for derived population parameters to stabilize (Riessen and Sprules 1990). There were 56 treatments altogether in a fully factorial design of seven Ca concentrations, four temperatures, and two algal densities.

*Media changes*—Every other day, daphniids were transferred via pipette to a clean jar containing fresh zooplankton medium and algal suspension that were preheated to the appropriate experimental temperature and maintained at that temperature ( $\pm 0.5^\circ\text{C}$ ) during the transfer using water baths. The jars were always covered with Parafilm® M laboratory sealing film to prevent evaporation and contamination. Once the daphniids were transferred, we added a fresh flake of cetyl alcohol to the surface of the media and replaced the Parafilm® before returning the jar to its position within the chamber. All jars were machine-washed with a non-residue-forming cleanser and rinsed several times with reverse-osmosis water prior to transfers.

Ashforth (2006) determined that the intrinsic rate of natural increase,  $r$ , not the juvenile growth rate, was the most reliable integrative metric to assess daphniid performance in Ca-based assays. Hence, at each transfer, the survivorship and offspring production of each daphniid

was scored to generate the life tables needed for the calculation of  $r$  (Stearns 1992). Individuals were considered dead if they were motionless, even when probed. Neonates were counted and then discarded.

*Intrinsic rate of natural increase, reproductive rate, and generation time*—Employing the PopTools version 2.6.9 add-in for Microsoft Excel (<http://www.cse.csiro.au/pop-tools>), we used the survivorship and reproductive data from abbreviated life tables to calculate three population metrics: the intrinsic rate of natural increase,  $r$ , the basic reproductive rate,  $R_o$ , and the cohort generation time,  $T_c$ . We calculated  $r$  using the equation  $\sum e^{-rx} l_x m_x = 1$ , where  $e$  is the base of natural logarithms,  $l_x$  is the proportion of individuals surviving to time  $x$ , and  $m_x$  is the mean number of eggs produced per surviving individual at time  $x$  (Stearns 1992; Begon et al. 1996). The basic reproductive rate,  $R_o$ , was calculated using the equation  $R_o = \sum l_x m_x$ . The cohort generation time,  $T_c$ , was calculated using the equation  $T_c = \ln R_o \times r^{-1}$ . Standard errors for each metric were determined using a Jackknife technique (Meyer et al. 1986) with Microsoft Excel.

*Statistical analyses*—As survivorship curves violated assumptions of normality due to censorship in the data, parametric tests could not be used. Data are said to be censored when the event of interest (e.g., death) does not occur before the end of the observation period. Typically, the Wilcoxon–Gehan test is employed for survivorship curve-by-curve comparisons; however, due to the number of comparisons required, it was more appropriate to describe the overall relationship between survival and temperature, Ca concentration, and food concentration using Proportional Hazards or Cox Regression (Cox 1972). Cox Regression describes the effect of several variables on the time required for a specified event (i.e., death) to occur, given that the event has not yet happened. Cox Regression generates cumulative survival and hazard ratio data. Cumulative survival is the probability of surviving to time  $t$ . Cumulative survival to day 15 was determined separately for each Ca concentration ( $n = 80$ ), temperature ( $n = 140$ ), and food concentration ( $n = 280$ ;  $N = 560$ ). While Kaplan–Meier curves are often used to describe survivorship, cumulative survival rates determined by Cox Regression are more accurate, if the model is sound. The hazard ratio indicates the likelihood of death for each point of increase in a predictor and is calculated by raising the log odds parameter estimate ( $\beta$ ) to the base of natural logarithms ( $e$ ; Peng et al. 2002). The hazard ratio may also be thought of as a relative death rate. Hazard ratios were determined separately for each predictor and were used to calculate the likelihood of death occurring for each point of increase in all predictors.

Since the data for  $r$ ,  $R_o$ , and  $T_c$  violated the assumption of normality for the analysis of variance (ANOVA), the nonparametric equivalent Kruskal–Wallis H test with Scheirer–Ray–Hare extension (Scheirer et al. 1976; as described in Sokal and Rohlf 1995 and Dytham 2003) was used to determine the main and interactive effects of each factor. For each of the 56 treatments, 10 values for

each dependent variable were calculated using a Jackknife technique (Meyer et al. 1986) with Microsoft Excel. Each dependent variable was then ranked and a three-way ANOVA was performed on these ranks using temperature, Ca concentration, and food concentration as factors. The sum of squares data from ANOVA were used to calculate the Kruskal–Wallis  $H$  test statistic, which was used to calculate  $p$ -values for each factor.

All tests were performed with the Statistical Package for the Social Sciences version 12.0 (SPSS).

## Results

**Survival**—The minimum Ca concentration required for our clone of *Daphnia pulex* to survive at least 15 d was between 0.1 and 0.5 mg Ca L<sup>-1</sup>, and this range was not influenced by temperature or food concentration (Fig. 1). At 0.1 mg Ca L<sup>-1</sup>, all daphniids died within 10 d regardless of temperature or food concentration, whereas the daphniids reared at  $\geq 0.5$  mg Ca L<sup>-1</sup> lived for at least 15 d (Fig. 2a). However, temperature and food concentration individually exerted strong effects on survival. As temperature increased, survivorship declined, and at 32°C all daphniids died within 5 d, regardless of Ca or food concentration (Fig. 2b). At low food, all daphniids died within 12 d, regardless of temperature or Ca concentration, whereas survival among the daphniids reared at high food was much greater (Fig. 2c).

In general, raising temperature or lowering Ca or food concentration reduced survival. The Cox Regression revealed highly significant effects ( $p < 0.0001$ ) of temperature, Ca concentration, and food concentration on the survival of *D. pulex*. The overall fit of the model was sound ( $\chi^2 = 399.57$ ,  $df = 3$ ,  $p < 0.001$ ) and the addition of each factor improved the model (temperature: Wald's  $\chi^2 = 195.54$ ,  $df = 1$ ,  $p < 0.001$ ; Ca concentration: Wald's  $\chi^2 = 53.52$ ,  $df = 1$ ,  $p < 0.001$ ; food concentration: Wald's  $\chi^2 = 157.11$ ,  $df = 1$ ,  $p < 0.001$ ). In addition, we quantified the likelihood of death occurring as temperature, Ca concentration, and food concentration varied using hazard ratios. The hazard ratios for temperature, Ca concentration, and food concentration were 2.155, 0.824, and 0.244, respectively. A hazard ratio of 1 indicates the independent variable has no effect on the dependent variable. If the hazard ratio is  $>1$ , then an increase in the independent variable (e.g., temperature) will increase the likelihood that the event of interest (e.g., death) will occur. If the hazard ratio is  $<1$ , then an increase in the independent variable (e.g., Ca or food concentration) will decrease the likelihood that the event of interest (e.g., death) will occur. For variables with two conditions, or in other words, one point of increase, the interpretation of the hazard ratio is straightforward. For example, the likelihood of *D. pulex* dying decreased from 1.0 to 0.244 ( $=e^{-1.411}$ ) when food concentration was raised from low to high. For variables with more than two conditions, or more than one point of increase, the interpretation of the hazard ratio is more complex. Since the hazard ratio indicates the likelihood of death for a single point of increase, the hazard ratio must be modified to determine the likelihood of death for

predictors with multiple points of increase. This is done by multiplying  $\beta$  by the appropriate number of points of increase. For example, to determine the effects of temperature on the likelihood of death, hazard ratios must be calculated for each of the three points of 4°C temperature increase. The likelihood of *D. pulex* dying increased from 1.0 to 2.155 ( $=e^{0.768 \times 1}$ ) as temperature increased one point from 20°C to 24°C. An increase of two points, from 20°C to 28°C, increased the likelihood of death from 1.0 to 4.646 ( $=e^{0.768 \times 2}$ ). An increase of three points, from 20°C to 32°C, increased the likelihood of death from 1.0 to 10.01 ( $=e^{0.768 \times 3}$ ), and so *D. pulex* was 10 times more likely to die at 32°C than at 20°C. When this procedure was repeated for each of the six points of increase in Ca concentration, the likelihood of *D. pulex* dying decreased from 1.0 to 0.824 (0.5 mg Ca L<sup>-1</sup>), 0.680 (1.0 mg Ca L<sup>-1</sup>), 0.560 (1.5 mg Ca L<sup>-1</sup>), 0.462 (2 mg Ca L<sup>-1</sup>), 0.381 (5 mg Ca L<sup>-1</sup>), and 0.314 (10 mg Ca L<sup>-1</sup>).

**Reproduction**—The minimum Ca concentration required for *D. pulex* to reproduce was between 0.1 and 0.5 mg Ca L<sup>-1</sup>. No neonates were produced at 0.1 mg Ca L<sup>-1</sup>, regardless of temperature or food concentration (Fig. 3). Daphniids reared at 0.5 or 1 mg Ca L<sup>-1</sup> could only reproduce at 20°C, whereas the daphniids reared at  $\geq 1.5$  mg Ca L<sup>-1</sup> were able to produce at least one brood at both food concentrations and every temperature, with the exception of 32°C (Fig. 4). No neonates were produced in any of the 32°C treatments.

Daphniids reared at 0.5 or 1 mg Ca L<sup>-1</sup> seldom produced more neonates than those reared at  $\geq 1.5$  mg Ca L<sup>-1</sup>, indicating a reproductive disadvantage of low Ca concentrations. This was particularly apparent at higher temperatures and low food (Fig. 3). However, temperature and food concentration also affected the average number of neonates produced in the lifetime of an individual,  $R_o$  (Table 2). An increase in temperature from 20°C or 24°C to 28°C reduced  $R_o$  by 40–45% at high food and by 30–90% at low food (Fig. 3). A reduction from high to low food dropped  $R_o$  in most treatments by more than 90% (Fig. 3). However, the strong influence of food concentration on  $R_o$  obscured the relationship between temperature and Ca concentration on  $R_o$ , and so we assessed the effect of each food concentration on  $R_o$  separately to determine the strength of this interaction. The interaction between temperature and Ca concentration on  $R_o$  was significant only at low food (Table 2).

The proportion of adults that reproduced at 0.5 or 1 mg Ca L<sup>-1</sup> was almost always lower than those reared at  $\geq 1.5$  mg Ca L<sup>-1</sup> (Fig. 4). As temperature rose, increasingly fewer daphniids reproduced at 0.5 or 1 mg Ca L<sup>-1</sup>. At 24°C, 10–20% fewer adults reproduced at 0.5 or 1 mg Ca L<sup>-1</sup> than at  $\geq 1.5$  mg Ca L<sup>-1</sup> and at 28°C this percentage increased to 20–50% (Fig. 4). At low food, fewer adults reproduced than at high food at all Ca concentrations and for all broods, and the daphniids reared at 0.5 or 1 mg Ca L<sup>-1</sup> were still the least likely to reproduce (Fig. 4).

Furthermore, the onset of brood production was delayed below 1.5 mg Ca L<sup>-1</sup>. Daphniids reared at 0.5 or

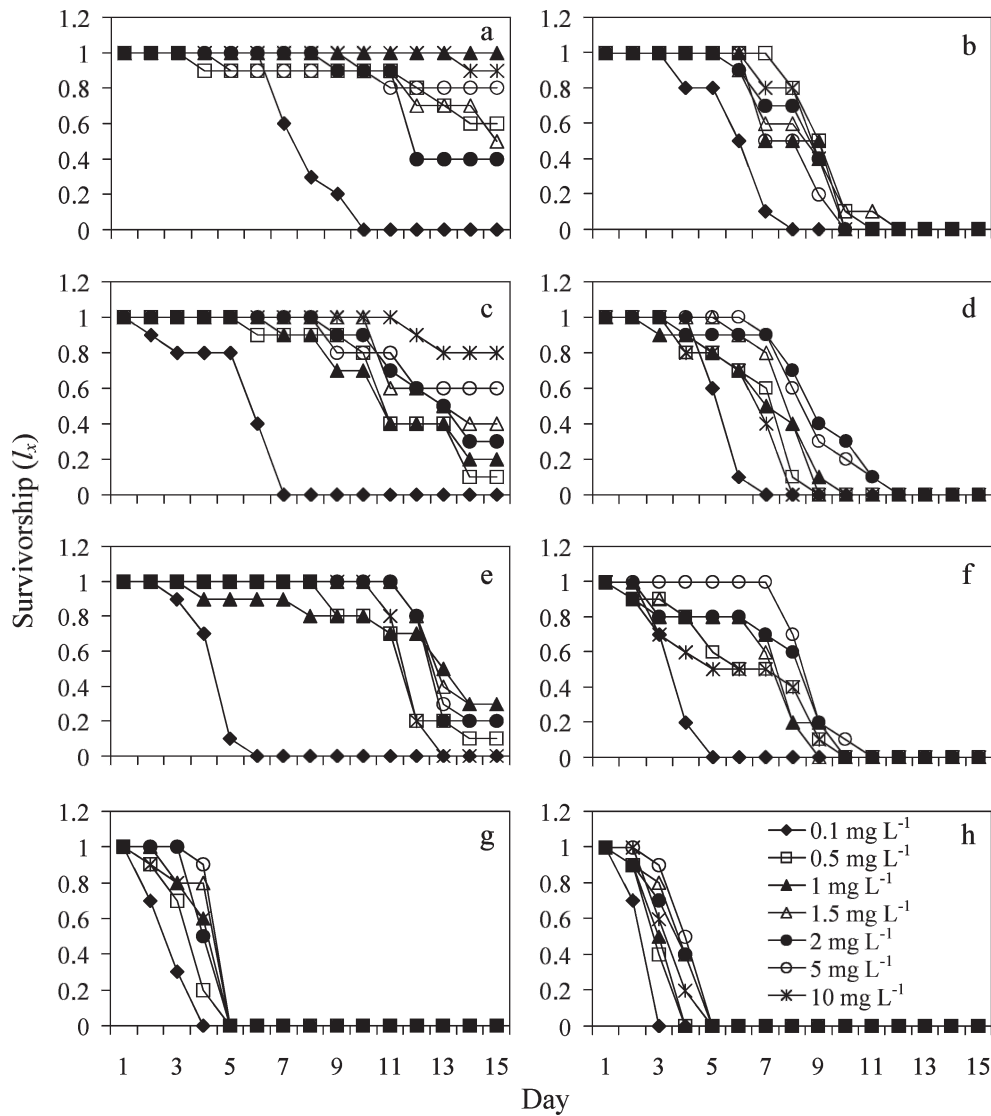


Fig. 1. The age-specific survivorship ( $l_x$ ) of *Daphnia pulex* when reared across a calcium gradient at four temperatures (20°C, 24°C, 28°C, and 32°C) and two algal densities (HF [high food] and LF [low food]) over a period of 15 d: (a) 20°C HF; (b) 20°C LF; (c) 24°C HF; (d) 24°C LF; (e) 28°C HF; (f) 28°C LF; (g) 32°C HF; and (h) 32°C LF.

1 mg Ca L<sup>-1</sup> produced broods a few days later than those reared at higher Ca concentrations. The first few broods were particularly delayed at low Ca concentrations. This significantly increased the cohort generation time,  $T_c$  (Fig. 5; Table 3). As expected, a temperature effect was detected whereby increases in temperature decreased the time between generations. Food concentration did not significantly affect  $T_c$ .

At 1.5 mg Ca L<sup>-1</sup> and above, a greater proportion of adults generally produced more neonates and reached primiparity faster than those reared at  $\leq 1$  mg Ca L<sup>-1</sup>. This suggests a second reproductive threshold exists between 1 and 1.5 mg Ca L<sup>-1</sup> at warmer temperatures and low algal density.

*Population-level response*—Integrating the survival and reproduction data gave an overall indication of the

response of *D. pulex* to low Ca concentrations at the population level. Food concentration exerted the strongest effect on the intrinsic rate of natural increase,  $r$ , reducing the median value from 0.396 (range = 0.232–0.495) neonates female<sup>-1</sup> d<sup>-1</sup> at high food to 0.0457 (range = -0.153–0.300) neonates female<sup>-1</sup> d<sup>-1</sup> at low food (Fig. 6). However, temperature and Ca concentration also exerted strong effects on  $r$ , whether analysed in conjunction with food or as separate food treatments (Table 4). The 24°C treatments produced the highest  $r$  values, and whether  $r$  was higher in the 20°C or 28°C treatments was dependent upon Ca and food concentration (Fig. 6). Ca concentration mainly affected  $r$  below 1.5 mg Ca L<sup>-1</sup>. At high food, we observed a significant decrease in  $r$  at all temperatures as Ca concentration dropped from 1.5 to 1 mg Ca L<sup>-1</sup> and again from 0.5 and 0.1 mg Ca L<sup>-1</sup> (Fig. 6). At low food, the effect of Ca on  $r$  was more variable:  $r$  rose and fell around 2 mg Ca L<sup>-1</sup>

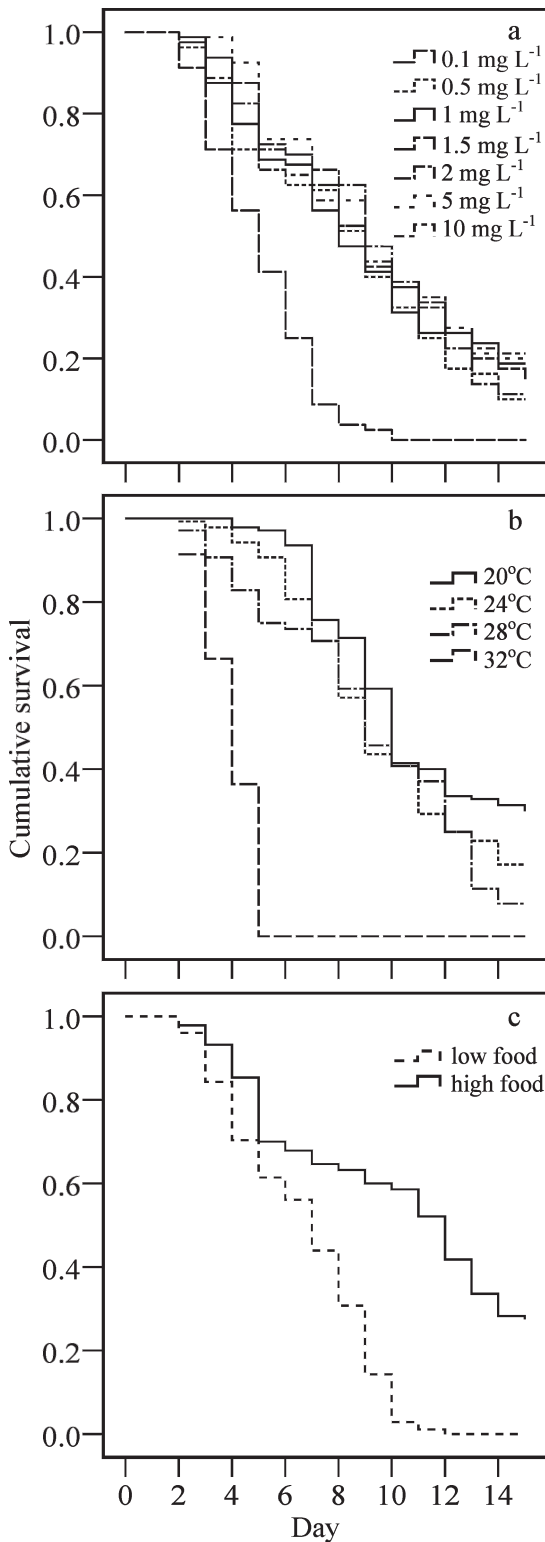


Fig. 2. Cumulative survival functions for *Daphnia pulex* reared across (a) a calcium gradient, (b) at four temperatures, and (c) at two algal densities, for a period of 15 d.

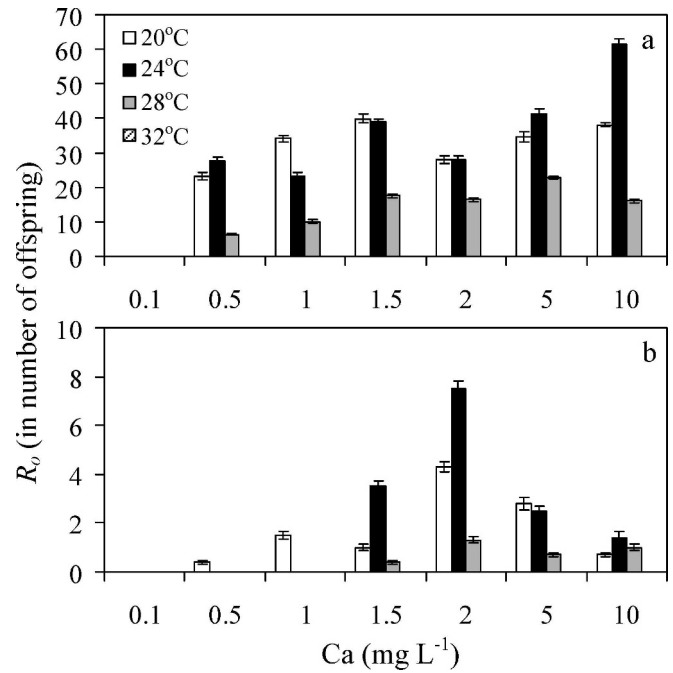


Fig. 3. The average number of offspring produced by *Daphnia pulex* over a period of 15 d,  $R_0$ , when reared across a calcium gradient at four temperatures and (a) high or (b) low algal density. Error bars indicate  $\pm 1.96$  SE. At 32°C, no offspring were produced; hence,  $R_0$  was undefined.

(Fig. 6). In all 32°C and 0.1 mg Ca L<sup>-1</sup> treatments,  $r$  was undefined since no offspring were produced under these conditions.

### Discussion

**Survival**—As daphniids have a continuous need for Ca, which they acquire via active uptake (Compère et al. 2004), the metabolic demands of Ca acquisition may rise to levels that threaten survival when Ca availability is low. In a low-Ca environment, daphniids may also be unable to build a fully functional exoskeleton, which is necessary for mobility, homeostasis, and protection against predation and other stressors (Alstad et al. 1999; Orr et al. 2005). The minimum Ca concentration required for the survival of our clone of *Daphnia pulex* was between 0.1 and 0.5 mg Ca L<sup>-1</sup> and is in agreement with the Ca threshold concentrations proposed by Hessen et al. (2000) and Rukke (2002b) for other *Daphnia* species.

Our study is the first to examine the effects of low Ca concentrations on daphniids at temperatures  $>18^\circ\text{C}$  ( $\pm 1^\circ\text{C}$ ). Higher temperatures reduced daphniid survival even at higher Ca concentrations (Fig. 1). Temperature influences the metabolic rate of daphniids, accelerating moulting (Hessen et al. 2000), which raises Ca demand to compensate for the accelerated rate of Ca loss and increases the energy required to acquire Ca. High temperatures can also denature and destabilize cell proteins, affecting their function. Organisms protect themselves from the potentially lethal effects of misfolded proteins by increasing the expression of genes that code for stress proteins (e.g., heat

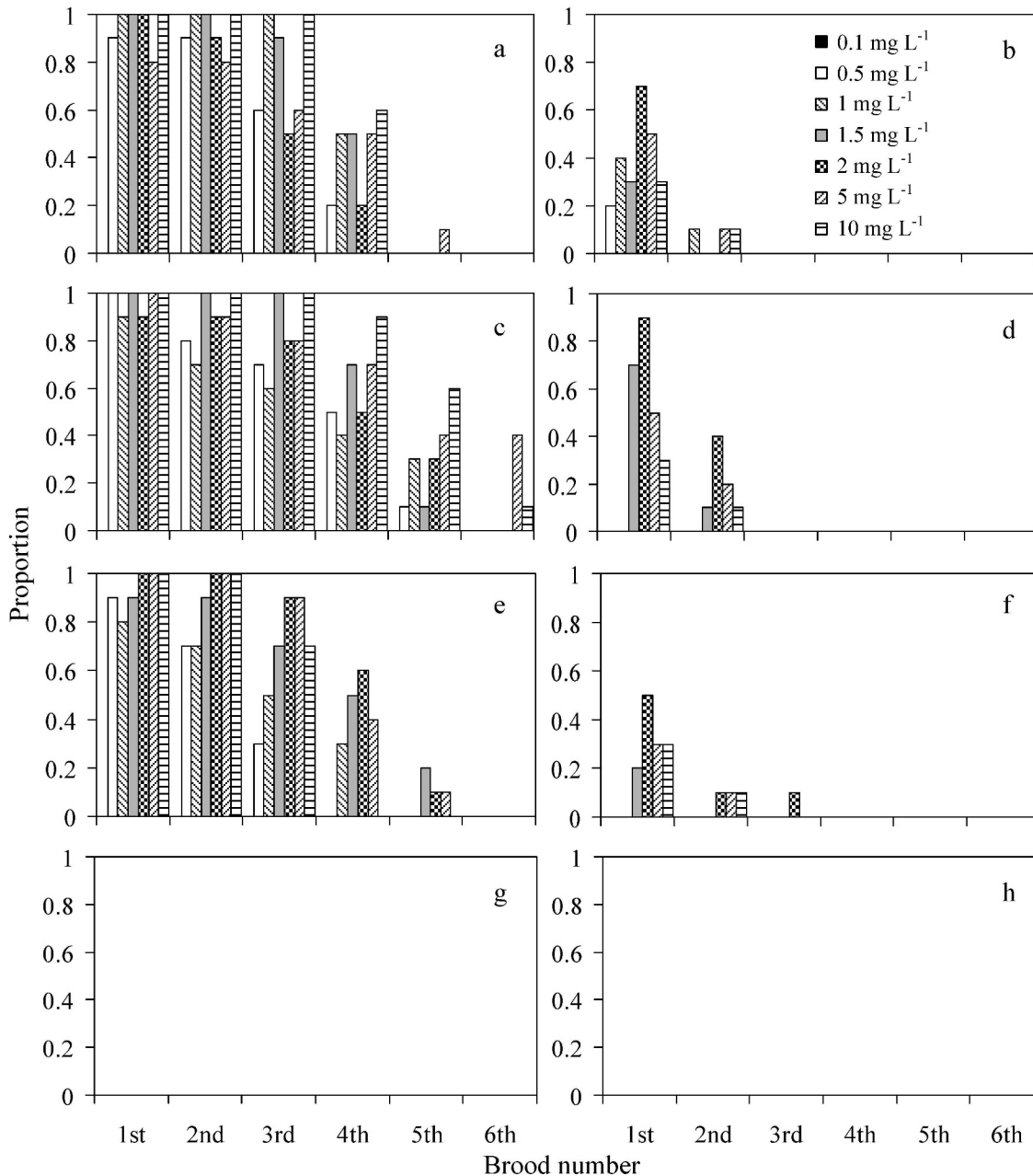


Fig. 4. The proportion of adult *Daphnia pulex* that produced broods when reared across a calcium gradient at four temperatures (20°C, 24°C, 28°C, and 32°C) and two algal densities (HF [high food] and LF [low food]) over a period of 15 d: (a) 20°C HF; (b) 20°C LF; (c) 24°C HF; (d) 24°C LF; (e) 28°C HF; (f) 28°C LF; (g) 32°C HF; and (h) 32°C LF. At 0.1 mg L<sup>-1</sup> of Ca, and 32°C, no brood were produced.

shock proteins). Stress proteins help stabilize and repair partly denatured proteins to restore their proper functioning. As stresses increase, the synthesis of stress proteins also increases (Feder and Hofmann 1999), which requires additional energy and may raise maintenance costs. Although high temperatures are a well-documented trigger, other factors are known to induce stress protein synthesis in *Daphnia*, including predation (e.g., Pauwels et al. 2005) and metal toxicity (e.g., Chen et al. 1999). Perhaps low Ca concentrations also invoke this defensive response in

*Daphnia*, in which case low available Ca would be especially energetically draining for daphniids.

Thermal stress was so overwhelming at 32°C that it is unclear whether a Ca effect at  $\geq 0.5$  mg Ca L<sup>-1</sup> exists at this temperature. Whereas the daphniids reared at 0.1 mg Ca L<sup>-1</sup> were again the first group to succumb, 32°C did not change this Ca effect. Few studies have explored the effects of higher temperatures ( $\geq 25^\circ\text{C}$ ) on daphniids and although our animals were acclimatized to 20°C for multiple generations, perhaps reducing high-

Table 2. The effects of temperature, calcium concentration, and food concentration on the basic reproductive rate,  $R_o$ , for *Daphnia pulex* using three-way and two-way nonparametric ANOVA (Kruskal–Wallis  $H$  test with Scheirer–Ray–Hare extension). For the two-way analysis, the effects of temperature and calcium concentration on  $R_o$  were determined for each food concentration separately. Significant values are in bold.

Factor	df	SS	$H$	$P$
Temp	2	321972.461	42.17274	<b>&lt;&lt;0.0001</b>
Ca	5	100973.356	13.22574	<b>0.0214</b>
Food	1	1782205.303	233.4376	<b>&lt;&lt;0.0001</b>
Temp $\times$ Ca	10	33652.794	4.407925	0.9271
Temp $\times$ Food	2	24822.131	3.251263	0.1968
Ca $\times$ Food	5	97198.816	12.73134	<b>0.0260</b>
Temp $\times$ Ca $\times$ Food	6	48571.660	6.362034	0.3839
Error	288	26044.550		
Total MS		7634.611508		
High food				
Temp	2	310619.425	114.4139	<b>&lt;&lt;0.0001</b>
Ca	5	114320.733	42.10903	<b>&lt;&lt;0.0001</b>
Temp $\times$ Ca	10	48360.192	17.81305	0.0582
Error	162	12662.150		
Total MS		2714.874302		
Low food				
Temp	2	83261.713	50.97494	<b>&lt;&lt;0.0001</b>
Ca	5	95107.927	58.22750	<b>&lt;&lt;0.0001</b>
Temp $\times$ Ca	6	35390.437	21.66693	<b>0.0014</b>
Error	126	13280.450		
Total MS		1633.385086		

temperature tolerance (Goss and Bunting 1980), death after only a few days of exposure to temperatures  $>25^\circ\text{C}$  is common (Chen 1994; Folt et al. 1999). An upper limit of  $30^\circ\text{C}$  to  $35^\circ\text{C}$  has been proposed as the critical thermal maximum for *D. pulex* (Goss and Bunting 1976) and is consistent with our observations at  $32^\circ\text{C}$ .

Low food concentrations also reduced daphniid survival despite high Ca concentrations. None of the daphniids in our low food treatment lived longer than 12 d, whereas many daphniids lived to the end of the experiment in our high food treatment (Fig. 1). Thus, the availability of food is an integral determinant of survival, regardless of whether or not Ca needs are met.

**Reproduction**—Since low available Ca may strongly influence daphniid energy budgets, reproduction may also be limited as daphniids divert more energy to maintenance in low-Ca environments. The minimum Ca concentration required for *D. pulex* to reproduce was between 0.1 and 0.5 mg Ca  $\text{L}^{-1}$ , since neonates were only produced by daphniids reared at  $\geq 0.5$  mg Ca  $\text{L}^{-1}$ . This is a novel result since Hessen et al. (2000) did not design their methods to pinpoint a specific Ca threshold limiting reproduction. However, Hessen et al. (2000) did discover that reproduction in the hard-water species *D. magna* is greatly limited between 0.5 and 1 mg Ca  $\text{L}^{-1}$  at high food concentrations and between 1 and 5 mg Ca  $\text{L}^{-1}$  at low food, indicating (as expected) that *D. magna* is less tolerant of low Ca

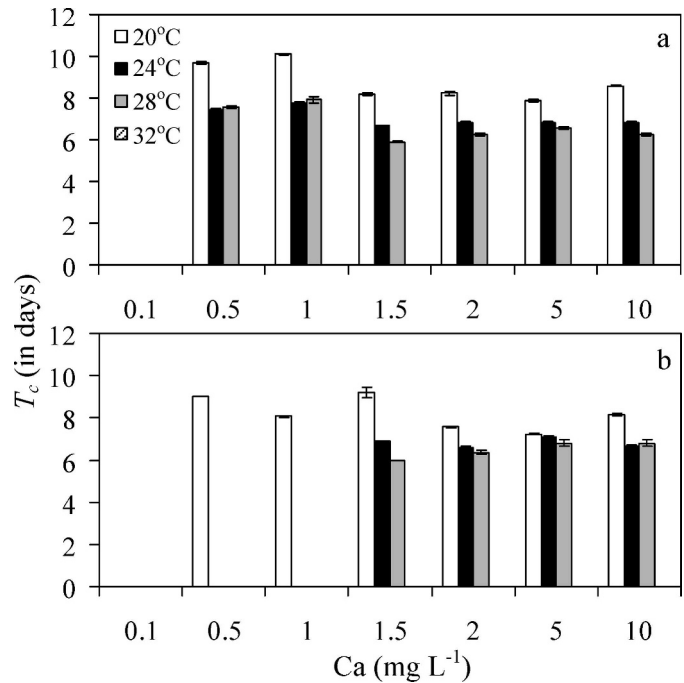


Fig. 5. The cohort generation time,  $T_c$ , defined as the average number of days between the birth of a parent and its offspring, for *Daphnia pulex* reared across a calcium gradient at four temperatures and (a) high or (b) low algal density. Error bars indicate  $\pm 1.96$  SE.  $T_c$  was undefined at  $32^\circ\text{C}$  as no brood were produced.

concentrations than *D. pulex*. We agree with Hessen and colleagues that daphniid reproduction will cease at low ambient Ca. At some point between 0.1 and 0.5 mg Ca  $\text{L}^{-1}$ , the energetic cost of survival became too great to permit energy allocation to reproduction in our clone, which we suspect may apply to other *Daphnia* species as well.

While offspring were produced at Ca concentrations as low as 0.5 mg Ca  $\text{L}^{-1}$ , reproduction was reduced in all treatments below 1.5 mg Ca  $\text{L}^{-1}$ . Fewer neonates were produced (Fig. 3) by fewer adults (Fig. 4) at 0.5 or 1 mg Ca  $\text{L}^{-1}$  than at  $\geq 1.5$  mg Ca  $\text{L}^{-1}$ , especially at higher temperatures and low food. However, the most striking observation was that maturity was delayed below 1.5 mg Ca  $\text{L}^{-1}$  (Fig. 5). This indicates a second possible reproductive threshold between 1 and 1.5 mg Ca  $\text{L}^{-1}$ .

In daphniids, maturation is size-dependent (Ebert 1992, 1994) and body length and weight decrease as Ca concentration decreases (Hessen et al. 2000). Thus, a low-Ca environment would delay the onset of brood production by reducing growth rate. At  $\geq 1.5$  mg Ca  $\text{L}^{-1}$ , sufficient Ca was present to enable steady growth toward the size maturity threshold without detriment to survival, and so these daphniids reached maturity sooner (Fig. 5). Hence, daphniids reared at  $\geq 1.5$  mg Ca  $\text{L}^{-1}$  had a reproductive advantage over the others, especially at higher temperatures and low food, where Ca concentrations needed to be at least 1.5 mg Ca  $\text{L}^{-1}$  in order for reproduction to occur (Figs. 3, 5). Higher temperatures and low food concentrations further reduced energy allocation toward reproduc-

Table 3. Three-way nonparametric ANOVA (Kruskal–Wallis  $H$  test with Scheirer–Ray–Hare extension) with the cohort generation time,  $T_c$ , as the dependent variable and temperature, calcium concentration, and food concentration as factors for *Daphnia pulex*. Significant values are in bold.

Factor	df	SS	$H$	$P$
Temp	2	1295043.137	194.1967	<<0.0001
Ca	5	381124.931	57.15116	<<0.0001
Food	1	848.224	0.127194	0.7214
Temp $\times$ Ca	10	247156.575	37.06209	0.0001
Temp $\times$ Food	2	38581.258	5.785409	0.0554
Ca $\times$ Food	5	48648.367	7.295011	0.1996
Temp $\times$ Ca $\times$ Food	6	56116.208	8.414843	0.2093
Error	288	59802.100		
Total MS		6668.717241		

tion by raising the energetic costs of survival and forcing trade-offs.

Since individual growth rates are accelerated at higher temperatures and the size maturity threshold decreases as temperature increases (McKee and Ebert 1996), it is not surprising that the time to maturity decreased as temperature increased (Fig. 5). However, the daphniids reared at 28°C produced fewer neonates than those reared at lower temperatures. Up to the thermal optimum, that lies between 24°C and 28°C for the daphniids in our study and is in agreement with Goss and Bunting (1983) and Chen (1994), daphniids become increasingly more efficient at assimilating energy for both respiration and reproduction (Lampert 1987; Peters 1987). Hence, more daphniids reproduced at 24°C than at any other temperature, and the most neonates and broods were also produced at 24°C (Figs. 3, 4). Above the thermal optimum assimilation efficiency falls, reducing the amount of energy available for neonate production. Thus, temperatures of 28°C and higher, are detrimental to daphniid reproduction even though daphniids reach maturity sooner at these temperatures. Low Ca enhanced this temperature effect: with each subsequent brood the number of reproducers at 0.5 and 1 mg Ca L<sup>-1</sup> dropped more than those reared at higher Ca concentrations, especially at 28°C (Fig. 4). We expect that the thermal optimum for reproduction in our clone lies closer to 24°C than to 28°C. This is distressing since lakes and ponds in temperate North America commonly maintain temperatures >25°C for prolonged periods during the summer (Moore et al. 1996), and epilimnetic temperatures >28°C are now also common (A. Paterson unpubl. data). While to our knowledge no studies have examined the effects of >30°C on daphniid reproduction, Chen (1994) did observe minimal reproduction at 30°C.

Algal quantity also typically affects the timing of broods (McCauley et al. 1990); however, algal concentrations need to be one or two orders of magnitude lower than our low food concentration to generate this effect (Ebert 1992). Our daphniids in the high and low food treatments likely reached the threshold size for egg production at approximately the same time since food concentration did not affect time to maturity (Fig. 5; Table 3). Thus, even in the low food treatments, the supply of food was adequate for

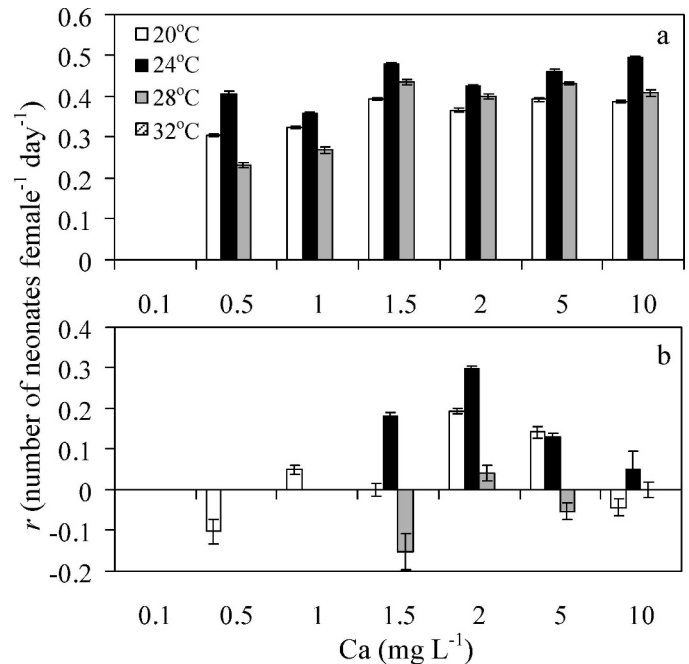


Fig. 6. The intrinsic rate of natural increase,  $r$ , for *Daphnia pulex* reared across a calcium gradient at four temperatures and (a) high or (b) low algal density over a period of 15 d. Error bars indicate  $\pm 1.96$  SE. At 32°C no brood were produced; hence,  $r$  was undefined.

the daphniids to steadily grow without the need for additional instars.

The trade-offs made at low food, therefore, pertained to the number of neonates and broods produced. While daphniids still allocate resources toward egg production when starving (Tessier et al. 1983), the number of offspring (Fig. 3) and broods (Fig. 4) produced at low food was far below that produced at high food. Folt et al. (1999) have shown similar changes in neonate production under high and low food diets. Although more neonates were produced in our high food treatments than are typically generated by natural populations (Lampert 1978), the high food conditions were designed to stimulate neonate production. The number of neonates generated at the more realistic low food concentration reflected natural production levels in south-central Canadian Shield lakes (e.g., Ramcharan et al. 2001).

**Population-level response**—The results of the trade-offs between survival and reproduction are reflected in the intrinsic rate of natural increase,  $r$ . At high food, our population of *D. pulex* would grow at all Ca concentrations, except 0.1 mg Ca L<sup>-1</sup>, and at all temperatures, except 32°C (Fig. 6). At 0.1 mg Ca L<sup>-1</sup> and 32°C  $r$  was undefined, indicating population collapse since reproduction did not occur. However, the success of *D. pulex* in nature will depend on the  $r$  values of competitors. Apart from the change in  $r$  between 0.1 and 0.5 mg Ca L<sup>-1</sup>, we found that the largest change in  $r$  at high food, an average increase of 25%, occurred between 1 and 1.5 mg Ca L<sup>-1</sup> (Fig. 6). This suggests that *D. pulex* populations have the

Table 4. The effects of temperature, calcium concentration, and food concentration on the intrinsic rate of natural increase,  $r$ , for *Daphnia pulex* using three-way and two-way nonparametric ANOVA (Kruskal–Wallis H test with Scheirer–Ray–Hare extension). For the two-way analysis, the effects of temperature and calcium concentration on  $r$  were determined for each food concentration separately. Significant values are in bold.

Factor	df	SS	H	P
Temp	2	216778.967	26.36274	<b>&lt;&lt;0.0001</b>
Ca	5	233832.994	28.43670	<b>&lt;&lt;0.0001</b>
Food	1	1881283.128	228.7850	<b>&lt;&lt;0.0001</b>
Temp × Ca	10	68515.481	8.332247	0.5964
Temp × Food	2	72297.658	8.792202	<b>0.0123</b>
Ca × Food	5	91739.146	11.15650	<b>0.0484</b>
Temp × Ca × Food	6	31346.542	3.812089	0.7021
Error	288	27320.700		
Total MS		8222.929831		
High food				
Temp	2	157385.733	57.96908	<b>&lt;&lt;0.0001</b>
Ca	5	261974.933	96.49189	<b>&lt;&lt;0.0001</b>
Temp × Ca	10	53541.933	19.72083	<b>0.0320</b>
Error	162	13081.400		
Total MS		2714.994408		
Low food				
Temp	2	91500.467	55.86889	<b>&lt;&lt;0.0001</b>
Ca	5	84847.500	51.80669	<b>&lt;&lt;0.0001</b>
Temp × Ca	6	37498.133	22.89583	<b>0.0008</b>
Error	126	13804.100		
Total MS		1637.771223		

potential to grow at Ca concentrations between 0.5 and 1 mg Ca L<sup>-1</sup>, but they would be more prolific at 1.5 mg Ca L<sup>-1</sup> and above. They also risk being outcompeted at Ca concentrations of 0.5 or 1 mg Ca L<sup>-1</sup> by species that are more tolerant of low Ca concentrations. At low food, the ability of our *D. pulex* population to proliferate was unclear due to the highly varied response in  $r$  (Fig. 6). Although, since  $r$  was negative or undefined below 1 mg Ca L<sup>-1</sup> at 20°C, 1.5 mg Ca L<sup>-1</sup> at 24°C, and 2 mg Ca L<sup>-1</sup> at 28°C, this suggests the threshold Ca concentration varies with temperature at low food. *D. pulex* is more susceptible, therefore, to low Ca concentrations when temperatures are high and food concentrations are low. Daphniids that inhabit shallow lakes and ponds may be unable to find refuge in cooler strata and, thus, would be exposed to this potentially lethal combination of stressors for the duration of their lives.

The energetic costs of survival and reproduction are higher at low Ca concentrations, higher temperatures, and low food. To cope with this stress, *D. pulex* produced fewer neonates and broods and/or delayed maturity, if they reproduced at all. These trade-offs had different effects on  $r$ . Assuming individuals survive to maturity, the timing of offspring production tends to be the main determinant of  $r$ , exerting more of an influence than the number of neonates per brood or the number of broods produced. When food

and Ca concentrations were nonlimiting, we found this to be the case. For example, at high food and  $\geq 1.5$  mg Ca L<sup>-1</sup>, even though fewer neonates were produced at 28°C than at 20°C,  $r$  was greater at 28°C (Fig. 6) since these daphniids reached maturity faster. Near the thermal optimum at 24°C, faster maturation resulted in the highest  $r$  values of nearly all treatments even though neonate production was similar to that of 20°C (Figs. 3, 6). However, fecundity is also an important component of  $r$ , and on occasion high neonate production can offset delays in maturity. The more neonates produced, the larger the  $r$  value, but only at low food, or at low Ca concentrations and high food (Figs. 3, 6). Faster maturation at 28°C did not result in greater  $r$  values than the treatments with slower maturation rates, except at Ca concentrations of  $\geq 1.5$  mg Ca L<sup>-1</sup> at high food, and  $r$  was only higher at 28°C than at 20°C (Figs. 5, 6). Below a certain level of neonate production, or food or Ca concentration, the importance of timing and fecundity on  $r$  is reversed. When each daphniid in a treatment produced only one offspring,  $r$  equalled zero (Figs. 3, 6), maintaining the size of the population. Thus, populations can in theory persist under Ca-related stress if individuals produce at least one offspring. Although populations in decline are not necessarily headed for catastrophe, any loss in competitive status in the community is likely unfavourable.

A loss of Ca-rich daphniids could significantly alter the structure and function of aquatic food webs since daphniids are both important algal grazers and sources of food for fish and predatory invertebrates. The effects could be far-reaching since Ca-rich daphniids are some of the most abundant zooplankton in Canadian Shield lakes (Yan et al. 1988) and Shield lakes are among the most common lakes in the world.

We have presented results for only one clone of one species of *Daphnia* in the absence of competition and predation. More work is clearly needed. Nevertheless, we have shown that ambient Ca concentration does affect daphniid fitness and that warmer temperatures and reduced algal biomass interact with this Ca effect. Thus, the definition of a suitable habitat for *D. pulex*, and potentially other daphniids, in Canadian Shield lakes should be expanded to include a minimum Ca concentration that will permit persistence of healthy populations or the recovery of populations from historical damage, such as that caused by lake acidification (e.g., Holt and Yan 2003). Fresh waters should have a Ca concentration of at least 0.5 mg Ca L<sup>-1</sup> to sustain *D. pulex* populations and potentially other Ca-sensitive daphniids. However, to increase the likelihood that daphniids will withstand concurrent reductions in total phosphorus and rising water temperatures, concentrations of 1.5 mg Ca L<sup>-1</sup> or higher are likely required.

## References

- ALSTAD, N. E. W., L. SKARDAL, AND D. O. HESSEN. 1999. The effect of calcium concentration on the calcification of *Daphnia magna*. *Limnol. Oceanogr.* **44**: 2011–2017.

- ASHFORTH, D. 2006. Potential interactive impacts of declining ambient calcium levels, reduced algal biomass, and rising summer water temperatures on *Daphnia pulex*: A laboratory study of multiple stressors. M.Sc. thesis, York Univ.
- BEGON, M., J. L. HARPER, AND C. R. TOWNSEND. 1996. Ecology: Individuals, populations, and communities, 3rd ed. Blackwell.
- CHEN, C. Y. 1994. Demographic consequences of seasonal variation in environmental stress. Ph.D. thesis, Dartmouth College.
- , K. B. SILLETT, C. L. FOLT, S. L. WHITTEMORE, AND A. BARCHOWSKY. 1999. Molecular and demographic measures of arsenic stress in *Daphnia pulex*. *Hydrobiologia* **401**: 229–238.
- CHRISTENSEN, M. R., M. D. GRAHAM, R. D. VINEBROOKE, D. L. FINDLAY, M. J. PATERSON, AND M. A. TURNER. 2006. Multiple anthropogenic stressors cause ecological surprises in boreal lakes. *Glob. Change Biol.* **12**: 2316–2322.
- COLBOURNE, J. K., T. J. CREASE, L. J. WEIDER, P. D. N. HEBERT, F. DUFRESNE, AND A. HOBÆK. 1998. Phylogenetics and evolution of a circumarctic species complex (Cladocera: *Daphnia pulex*). *Biol. J. Linn. Soc.* **65**: 347–365.
- COMPÈRE, P., C. JEUNIAUX, AND G. GOFFINET. 2004. The integument: Morphology and biochemistry, p. 59–144. *In* J. Forest, J. C. von Vaupel Klein and F. R. Schram [eds.], *The Crustacea: Revised and updated from the Traité de Zoologie*, v. 1. Brill Academic.
- COX, D. R. 1972. Regression models and life-tables. *J. Royal Stat. Soc. Series B (Methodological)* **34**: 187–220.
- CUBASCH, U., AND OTHERS. 2001. Projections of future climate change, p. 527–582. *In* J.-W. Kim and J. Stone [eds.], *Climate change 2001: The scientific basis. Contributions of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge Univ. Press.
- DESMARIS, K. H. 1997. Keeping *Daphnia* out of the surface film with cetyl alcohol. *J. Plankton Res.* **19**: 149–154.
- DODSON, S. I., S. RYAN, R. TOLLRIAN, AND W. LAMPERT. 1997. Individual swimming behaviour of *Daphnia*: Effects of food, light, and container size in four clones. *J. Plankton Res.* **19**: 1537–1552.
- DYTHAM, C. 2003. Choosing and using statistics: A biologist's guide, 2nd ed. Blackwell.
- EBERT, D. 1992. A food-independent maturation threshold and size at maturity in *Daphnia magna*. *Limnol. Oceanogr.* **37**: 878–881.
- . 1994. A maturation size threshold and phenotypic plasticity of age and size at maturity in *Daphnia magna*. *Oikos* **69**: 309–317.
- EVANS, D. 2001. The determination of cations in Precambrian Shield waters by atomic absorption spectrophotometry. Ontario Ministry of the Environment.
- FEDER, M. E., AND G. E. HOFMANN. 1999. Heat-shock proteins, molecular chaperones, and the stress response: Evolutionary and ecological physiology. *Annu. Rev. Physiol.* **61**: 243–282.
- FEDERER, C. A., J. W. HORNBECK, L. M. TRITTON, C. W. MARTIN, R. S. PIERCE, AND C. T. SMITH. 1989. Long-term depletion of calcium and other nutrients in eastern US forests. *Environ. Manage.* **13**: 593–601.
- FOLT, C. L., C. Y. CHEN, M. V. MOORE, AND J. BURNAFORD. 1999. Synergism and antagonism among multiple stressors. *Limnol. Oceanogr.* **44**: 864–877.
- GOSS, L. B., AND D. L. BUNTING. 1976. Thermal tolerance of zooplankton. *Water Res.* **10**: 387–398.
- , AND ———. 1980. Temperature effects on zooplankton respiration. *Comp. Biochem. Physiol.* **66A**: 651–658.
- , AND ———. 1983. *Daphnia* development and reproduction: Responses to temperature. *J. Thermal Biol.* **8**: 375–380.
- HESSEN, D. O., N. E. W. ALSTAD, AND L. SKARDAL. 2000. Calcium limitation in *Daphnia magna*. *J. Plankton Res.* **22**: 553–568.
- HOLT, C., AND N. D. YAN. 2003. Recovery of crustacean zooplankton communities from acidification in Killarney Park, Ontario, 1971–2000: pH 6 as a recovery goal. *Ambio* **32**: 203–207.
- JEZIORSKI, A., AND N. D. YAN. 2006. Species identity and aqueous calcium concentrations as determinants of calcium concentrations of freshwater crustacean zooplankton. *Can. J. Fish. Aquat. Sci.* **63**: 1007–1013.
- KEATING, K. I. 1985. A system of defined (*Sensu stricto*) media for daphnid (Cladocera) culture. *Water Res.* **19**: 73–78.
- KELLER, W., S. S. DIXIT, AND J. HENEBERRY. 2001. Calcium declines in northeastern Ontario lakes. *Can. J. Fish. Aquat. Sci.* **58**: 2011–2020.
- , J. H. HENEBERRY, AND S. S. DIXIT. 2003. Decreased acid deposition and the chemical recovery of Killarney, Ontario, lakes. *Ambio* **32**: 183–189.
- KILHAM, S., D. KREEGER, S. LYNN, C. GOULDEN, AND L. HERRERA. 1998. COMBO: A defined freshwater culture medium for algae and zooplankton. *Hydrobiologia* **377**: 147–159.
- LAMPERT, W. 1978. A field study on the dependence of the fecundity of *Daphnia* species on food concentration. *Oecologia* **36**: 363–369.
- . 1987. Feeding and nutrition in *Daphnia*. *Mem. Ist. Ital. Idrobiol.* **45**: 143–192.
- LYNCH, M., L. WEIDER, AND W. LAMPERT. 1986. Measurement of the carbon balance in *Daphnia*. *Limnol. Oceanogr.* **31**: 17–33.
- MARSHALL, C. T., AND R. H. PETERS. 1989. General patterns in the seasonal development of chlorophyll a for temperate lakes. *Limnol. Oceanogr.* **34**: 856–867.
- MCCAULEY, E., W. W. MURDOCH, AND R. M. NISBET. 1990. Growth, reproduction, and mortality of *Daphnia pulex* Leydig: Life at low food. *Funct. Ecol.* **4**: 505–514.
- McKEE, D., AND D. EBERT. 1996. The effect of temperature on maturation threshold body-length in *Daphnia magna*. *Oecologia* **108**: 627–630.
- MEYER, J. S., C. G. INGERSOLL, L. L. McDONALD, AND M. S. BOYCE. 1986. Estimating uncertainty in population growth rates: Jackknife vs. bootstrap techniques. *Ecology* **67**: 1156–1166.
- MOORE, M. V., C. L. FOLT, AND R. S. STEMBERGER. 1996. Consequences of elevated temperatures for zooplankton assemblages in temperate lakes. *Arch. Hydrobiol.* **135**: 289–319.
- NEARY, B. P., P. J. DILLON, J. R. MUNRO, AND B. J. CLARK. 1990. The acidification of Ontario lakes: An assessment of their sensitivity and current status with respect to biological damage. Ontario Ministry of the Environment.
- ORR, J. C., AND OTHERS. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**: 681–686.
- PAUWELS, K., R. STOKS, AND L. DEMEESTER. 2005. Coping with predator stress: Interclonal differences in induction of heat-shock proteins in the water flea *Daphnia magna*. *J. Evol. Biol.* **18**: 867–872.
- PENG, C.-Y. J., K. L. LEE, AND G. M. INGERSOLL. 2002. An introduction to logistic regression analysis and reporting. *J. Educ. Res.* **96**: 3–14.
- PETERS, R. H. 1987. Metabolism in *Daphnia*. *Mem. Ist. Ital. Idrobiol.* **45**: 193–243.

- RAIMONDO, S., AND C. L. MCKENNEY, JR. 2006. From organisms to populations: Modeling aquatic toxicity data across two levels of biological organization. *Environ. Toxicol. Chem.* **25**: 589–596.
- RAMCHARAN, C. W., A. PEREZ-FUENTETAJA, D. M. MCQUEEN, N. D. YAN, E. DEMERS, AND J. RUSAK. 2001. Dynamics of zooplankton productivity under two different predatory regimes: The Dorset food web piscivore manipulation project. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* **56**: 151–169.
- RIESSEN, H. P., AND W. G. SPRULES. 1990. Demographic costs of antipredator defenses in *Daphnia pulex*. *Ecology* **71**: 1536–1546.
- RUKKE, N. A. 2002a. Effects of low calcium concentrations on two common freshwater crustaceans, *Gammarus lacustris* and *Astacus astacus*. *Funct. Ecol.* **16**: 357–366.
- . 2002b. Tolerance to low ambient calcium shows inter-population differences in *Daphnia galeata*. *J. Plankton Res.* **24**: 527–531.
- SAKWIŃSKA, O. 2004. Persistent maternal identity effects on life history traits in *Daphnia*. *Oecologia* **138**: 379–386.
- SCHEIRER, C. J., W. S. RAY, AND N. HARE. 1976. The analysis of ranked data derived from completely randomized factorial designs. *Biometrics* **32**: 429–434.
- SOKAL, R. R., AND F. J. ROHLF. 1995. *Biometry*, 3rd ed. W. H. Freeman.
- STEARNS, S. C. 1992. *The evolution of life histories*. Oxford Univ. Press.
- TESSIER, A. J., L. L. HENRY, AND C. E. GOULDEN. 1983. Starvation in *Daphnia*: Energy reserves and reproductive allocation. *Limnol. Oceanogr.* **28**: 667–676.
- WALTHALL, W. K., AND J. D. STARK. 1997. A comparison of acute mortality and population growth rate as endpoints of toxicological effect. *Ecotoxicol. Environ. Safety* **37**: 45–52.
- WATMOUGH, S. A., AND P. J. DILLON. 2003. Calcium losses from a forested catchment in south-central Ontario, Canada. *Environ. Sci. Technol.* **37**: 3085–3089.
- WOLF, H. G., AND L. J. WEIDER. 1991. Do life-history parameters of *Daphnia* as determined in the laboratory correctly predict species successions in the field? *Verh. Internat. Verein. Limnol.* **24**: 2799–2801.
- WRIGHT, B., AND V. FERRARO. 2004. The determination of chlorophyll in river and lake samples by spectrophotometry. Ontario Ministry of the Environment.
- YAN, N. D., W. KELLER, J. R. PITBLADO, AND G. L. MACKIE. 1988. *Daphnia-Holopedium* relationships in Canadian Shield lakes ranging in acidity. *Verh. Internat. Verein. Limnol.* **23**: 252–257.
- , AND OTHERS. In press. Long-term changes in crustacean zooplankton communities of Dorset, Ontario lakes: The probable interactive effects of changes in pH, TP, dissolved organic carbon, and predators. *Can. J. Fish. Aquat. Sci.*

Received: 17 April 2007

Accepted: 18 November 2007

Amended: 20 November 2007