J. Limnol., 2014; 73(2): 340-349
DOI: 10.4081/jlimnol.2014.886

ORIGINAL ARTICLE

Changes in life-history parameters of *Daphnia longispina* (Cladocera, Crustacea) as a function of water chemistry

Fátima T. JESUS,*# Celso MARTINS,# António J.A. NOGUEIRA

Department of Biology & CESAM, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal
*Corresponding author: jesusfatima@gmail.com
#Both authors contributed equally to the study.

ABSTRACT

Health, distribution and life-history of aquatic crustaceans strongly depend on water hardness. However hardness is commonly correlated with alkalinity, which highlights the need to assess the joint effects of both hardness and alkalinity. This study aims to test the hypothesis that water hardness and alkalinity affect the life-history parameters (growth, reproduction and population growth rate) of D. longispina*. Following this, life table experiments were carried out in order to study the effects of high levels versus low levels of water hardness and alkalinity. Low levels of hardness and alkalinity caused a significant reduction in the growth of daphnids after a 7-days period, which augmented during the 21-day-test period reaching a 14.5% reduction compared to high hardness and alkalinity. Allied to the reduced growth, daphnids reared at low hardness and alkalinity showed delayed reproduction, increased body length at first reproduction, reduced fertility at first brood and, consequently, a 36.6% reduction in total fertility, compared to daphnids reared at high hardness and alkalinity. Accordingly, daphnids with the same size produced smaller broods at low hardness and alkalinity, reflecting a direct effect of water chemistry on daphnids reproduction. The impaired growth and reproduction at low hardness and alkalinity levels was likely a consequence of increased maintenance costs, and was not related to changes in the feeding activity. Population growth rate of daphnids reared at low hardness and alkalinity was 13.4% lower than that of daphnids reared at high hardness and alkalinity. Thus, despite* D. longispina *can survive at low hardness and alkalinity, their life-history parameters are significantly affected. This study raises concerns about the effects of decreasing hardness and alkalinity, which has been reported in Europe and North America, on populations of* D. longispina *and, thus, on the structure of aquatic ecosystems.*

Key words: Hardness, alkalinity, calcium, crustaceans, growth, reproduction.

Received: October 2013. Accepted: January 2014.

INTRODUCTION

Water chemistry exerts a strong influence on the health, distribution and life-history of aquatic organisms. For crustacean species, one of the most important water chemistry parameters is hardness. Indeed, survival and distribution of crustaceans is affected by water hardness, because of their high calcium (Ca) demand (Ashforth and Yan, 2008). Aquatic crustaceans have a calcified exoskeleton that is replaced regularly as they grow and a high proportion of the body Ca is lost with the shed moult (Alstad *et al.*, 1999). Thus, they periodically need to extract considerable amounts of Ca from the water to rebuild their exoskeleton. Among crustaceans, *Daphnia* is considered the most vulnerable genus to low Ca concentrations (Ashforth and Yan, 2008), which supported its recent use as a model to study the effects of Ca decline in aquatic ecosystems (Jeziorski *et al*., 2008). Furthermore, *Daphnia* species play an important role in most freshwater systems, not only because of their abundance (Jeziorski *et al*., 2008) but also because of their central position in aquatic food webs. Despite the effects of hardness to *Daphnia magna* Straus have been studied (Hessen *et al*., 2000), little is known about the effects to *Daphnia longispina* O.F. Muller. The latter is a small planktonic crustacean widely distributed in Europe, frequent in lentic systems, both oligo and mesotrophic. This species is found in small-scale systems (small lakes, small pools and ponds) but also in the pelagial and littoral zones of larger lakes with various extent of fish predation (Adamczuk, 2012; Seda and Petrusek, 2011). The small-scale systems are particularly exposed to variations of water chemistry since chemical variations are not buffered by the presence of a large water volume.

In aquatic ecosystems hardness is commonly correlated with alkalinity (Moiseenko *et al.*, 2013). This occurs because the main source of alkalinity is usually carbonate rocks (*e.g*., limestone) that are mostly $CaCO_3$. So, soft waters usually have low alkalinity, whereas hard waters have high alkalinity, except when the dominant anions in the water are chloride and sulfate rather than carbonate. Furthermore, water hardness and alkalinity vary both geographically and temporally. The geographic variability is mainly determined by bedrock geology, weathering, climate and land cover/use of the surrounding landscape (Moiseenko *et al.*, 2013). Concerning temporal variability, it might occur, for instance, as a result of acid

deposition. In fact, acid deposition reduces not only water pH and alkalinity, but also water hardness, as found in many regions of Europe and North America (Skjelkvåle *et al.*, 2001).

Given the trend for concurrent changes in water hardness and alkalinity it is important to assess the joint effects of these parameters on the biology of crustaceans. Keeping this in mind, this study focused on the effects of water hardness and alkalinity on the life-history changes of *D. longispina*. Our objective was to evaluate growth, reproduction and population growth of *D. longispina* reared at two different water chemistry scenarios: low hardness and low alkalinity *vs* high hardness and high alkalinity. We intended to address the following questions: i) to what extent do water hardness and alkalinity affect the life-history parameters of *D. longispina*? ii) what is the ecological relevance of such effects? Following this, life table experiments were carried out in order to study the effects of two levels of water hardness and alkalinity on the life-history parameters (growth, reproduction and population growth rate) of *D. longispina*. For high levels, hardness and alkalinity were 174.7 and 106.4 mg L^{-1} $CaCO_3$, respectively, whereas for low levels hardness and alkalinity were 46.8 and 31.4 mg L^{-1} $CaCO_3$, respectively. Ca concentration was 29.86 and 7.02 mg Ca L^{-1}, respectively for high and low levels.

METHODS

Test organisms

Daphnia longispina, clone EV20 *sensu* Antunes *et al.* (2003), were used in the experiments. Individuals originated from *ephippia* collected in Lagoa da Vela, a shallow lake located in the central region of Portugal (Figueira da Foz, Portugal) [see Antunes *et al.* (2003) for additional details]. The mean Ca concentration of the lake water is 29.4 mg L^{-1}, the alkalinity is 101.7 mg $CaCO_3$ L^{-1} and pH varies between 8.10 and 8.74 (Castilho, 2008). Cultures of daphnids were maintained in ASTM hard water (ASTM, 2004) enriched with a standard organic additive (suspension extracted from *Ascophyllum nodosum*, commercialized as Marinure seaweed extract by Glenside Organics Ltd., Stirling, UK) (Antunes *et al.*, 2003). Daphnids were fed daily the algae *Chlorella vulgaris* at a concentration of 3.5 µg dw (dry weight) mL^{-1}. Following the recommendations of Ashforth and Yan (2008), a small flake of cetyl alcohol was placed at the surface of the medium to reduce surface tension and the probability of daphnids entrapment. Culture medium was renewed every other day. The cultures were maintained under a 16:8 h light:dark cycle at a temperature of 20±1°C.

Females carrying the first brood, 7-8 days old, were arbitrarily selected and assigned to each medium. The first two broods were discarded, as these animals were not exposed to the test media during their entire developmental period (Barata *et al.*, 2007). Only neonates from the third-to-fourth broods were used in the experiments.

Life-history experiments

In this study we compared the growth, reproduction and population growth of *D. longispina* reared in two media with different hardness and alkalinity: medium L (low hardness and alkalinity) and medium H (high hardness and alkalinity). Media were selected from USEPA protocols for preparation of synthetic freshwaters (USEPA, 2002a). Media were prepared by addition of different volumes of stock solutions of $NaHCO_3$, $MgSO_4$, KCl and $CaSO_4$ to ultrapure water. The pH of medium H was adjusted to pH 7.8±0.1 (mean ±SD) with HCl. The main chemical parameters of the test media are presented in Tab. 1. Medium H corresponds to ASTM hard water (ASTM, 2004), which is commonly used for *Daphnia* culture and testing (ASTM, 2004; OECD, 2008). Note that both Ca concentration and alkalinity are very similar to those of the lake were daphnids were collected. Medium L has about 3.5-fold lower hardness and alkalinity than medium H, being classified as soft water according to USEPA (2002b). Tests were carried out following OECD guideline 211 (OECD, 2008). Tests were initiated with neonates (aged less than 24-h) originated from parental daphnids acclimated to each test medium, using 15 replicates *per medium*. Each organism was kept individually in glass beakers containing 50 ml of the respective medium; the algal and seaweed extract concentrations, as well as the photoperiod and temperature conditions were as described for cultures. The test duration was 21 days, during which media were renewed every other day.

Tab. 1. Chemical properties of the test media: mean (standard deviation), n=12.

	Medium L	Medium H
Hardness (mg $CaCO_3$ L^{-1})	46.8 (2.2)	174.7 (5.9)
Alkalinity (mg $CaCO_3$ L^{-1})	31.4 (1.7)	106.4 (6.0)
Conductivity (µS cm^{-1})	167.7 (3.9)	555.9 (34.7)
pH	7.85 (0.21)	8.10 (0.33)
Concentration of major ions (mg L^{-1})		
Ca	7.02 (0.55)	29.86 (1.49)
Mg	6.42 (0.57)	25.54 (1.65)
Na	15.35 (1.39)	57.94 (2.19)
K	4.11 (0.32)	7.30 (0.51)
Cl	3.58 (0.14)	10.68 (1.08)
SO_4	58.33 (1.92)	195.74 (7.30)

Medium L, low hardness and low alkalinity; medium H, high hardness and high alkalinity.

Organisms were checked twice a day for reproduction, moulting and mortality. Offspring as well as aborted eggs and embryos were counted and the shedded carapaces were collected for posterior determination of daphnids body length (BL). BL (from the top of the head to the base of the carapace spine) was estimated based on the length of the first exopodite of the second antennae (AL) which was measured in the carapace released at the end of each instar using a stereoscope fitted with an ocular rule (MS5, Leica Microsystems, Houston, TX, USA). The following equation was used:

$$BL = 9.116 * AL - 0.110 \quad \text{(eq. 1)}$$

(both BL and AL in mm, r^2=0.904, n=63, P<0.001). This regression model was developed previously to the start of the experiments and based on the measurement of BL and AL in daphnids from day zero up to day 21.

Following the life-history experiments, the effects of water hardness and alkalinity on growth, reproduction and population growth of *D. longispina* were determined. The effects on growth were assessed based on initial BL (BL of the daphnids at the start of the test), BL at day 7 and final BL (BL at day 21). BL at day 7 was chosen to represent the start of reproduction, since at day 7 all the daphnids were carrying the first brood in the brood chamber. Concordantly, after day 7 a reduction in growth was observed, as illustrated in Fig. 1. The effects on growth in both these periods (pre-reproductive period: 0-7 d and reproductive period: 8-21 d) were assessed by determining the growth rate using the following equation:

$$growth\ rate = \frac{\ln(lf) - \ln(li)}{\Delta t} \quad \text{(eq. 2)}$$

where growth rate is expressed in day^{-1}, l_f and l_i are the final and initial BL of daphnids, respectively (mm), and Δt is the time interval (days). Furthermore, the somatic growth of daphnids was modeled using the von Bertalanffy equation, as defined by Gurney and Nisbet (1998):

$$L = L_{max} - (L_{max} - L_0)e^{-kt} \quad \text{(eq. 3)}$$

where L represents the BL (mm) of a daphnid at age t, L_{max} and L_0 represent the theoretical maximum BL of adults and the BL of neonates, respectively, and k is the growth coefficient. The von Bertalanffy equation was fitted to the growth trajectory of each individual, giving independent parameter estimates for each individual.

The effects on reproduction were assessed based on the following endpoints: age at first reproduction (AFR, age of daphnids when the first brood is released to the external medium); size at first reproduction (SFR, length of daphnids when carrying the first brood in the brood chamber); fertility at first reproduction (FFR, number of viable juveniles produced in the first reproduction), total fertility (number of viable juveniles produced during the 21-days period), number of broods and mean brood size. Moreover, the effects of water chemistry on the relationship body length - brood size were also assessed.

The effects at the population level were assessed by determining the intrinsic rate of population increase (*r*) using the Euler-Lotka equation and the jackknife method (Meyer *et al.*, 1986), following the equation:

$$1 = \sum_{x=0}^{n} e^{-rx} . l_x m_x \quad \text{(eq. 4)}$$

where r is expressed in day^{-1}, x is the age class (1 . . . n days), l_x is the probability of surviving to age x, and m_x is the fecundity at age x.

Feeding experiments

The feeding rate of daphnids in both test media was determined to assess whether the effects in growth and reproduction were related to the feeding activity of daphnids. Feeding tests followed the procedure outlined by Agra *et al.* (2010). Tests were carried out with individuals originating from parental daphnids acclimated to each test medium. Five fourth instar daphnids (4-day-old) were transferred to 50 mL glass vials containing 20 mL of medium and algae (*C. vulgaris* at a concentration of 3.5 µg dw mL^{-1}). Each treatment consisted of three replicates (with algae and daphnids) and three blanks (with algae but without daphnids). Daphnids were allowed to feed during 24 hours, at 20°C, in the dark. At the end of tests, daphnids were carefully removed and vials were vigorously shaken before the measurement of the optical density (OD) at 440 nm in a UV-spectrophotometer (Jenway 6505 ultraviolet/Vis.). Algae concentration (expressed in µg dw mL^{-1}) was estimated from OD using a standard calibration curve based on 24 data points, with an r^2>0.98. The change in algae concentration during 24 h allowed the determination of individual feeding rates (µg dw ind^{-1} h^{-1}), using the equation by Allen *et al.* (1995), with slight adaptations, namely on the units of cell density and by incorporating the number of animals per replicate (N):

$$F = \frac{V(C_0 - C_t)}{tN} \quad \text{(eq. 5)}$$

where:

F=feeding rate of single individuals (µg dw ind^{-1} h^{-1});
V=volume of medium (mL);
C_0=cell concentration in the vials without daphnids (µg dw mL^{-1});
C_t=final cell concentration in the treatment (µg dw mL^{-1});
t=time animals were allowed to feed (hours);
N=number of animals per replicate.

Since the BL of 4th instar daphnids differed between media (L:1.27 mm; H:1.46 mm), and given that differing BL could affect the feeding rates, another feeding experiment was carried out to assess the feeding rates of daphnids as a function of BL. This experiment contributed to assess whether the observed effects in the feeding rates of daphnids reared in both media were due to water chemistry or the differing BL of the daphnids or both. The experimental procedure was similar to the described previously, except that all daphnids were reared in the reference medium ASTM hard water and three BL classes were tested (1.28, 1.41 and 1.51 mm). Individuals of different BL classes differed in age. Each treatment consisted of seven replicates (with algae and daphnids) and six blanks (with algae but without daphnids).

Chemical analyses

Conductivity and pH were measured using a WTW Cond 330i meter and a WTW pH 330 meter, respectively. The concentrations of major ions were determined in filtered samples (0.45 µm cellulose acetate membrane). Cations (Ca, Mg, Na and K) were quantified in acidified samples using inductively coupled plasma mass spectrometry (ICP-MS Thermo Scientific X-Series) following ISO 17294. Anions (chloride and sulfates) were analyzed in a Hach DR2000 spectrophotometer (Düsseldorf, Germany) using the mercuric thyocianate and the Sulfaver 4 methods, respectively. Total hardness and total alkalinity were quantified by the EDTA and the bromocresol green titrimetric procedures, respectively (American Public Health Association, 2005). All chemical measurements were performed in fresh and 48h-old media, *i.e.*, before and after media renewal.

Statistical analyses

Statistical analyses were performed using the statistical package SigmaPlot (version 11, Systat Software Inc.). Two-tailed Student's *t*-test was used to test whether each endpoint differed significantly between both media (high hardness and alkalinity *vs* low hardness and alkalinity). For non-normally distributed (Shapiro-Wilk test) or heteroscedastic data, the nonparametric Mann-Whitney U Test was used. Two-way ANOVA's (involving medium and individual) were used to assess variation in parameter estimates of the von Bertalanffy equation between these groups.

To assess the effects of water chemistry on the relationship body length–brood size for both media, analysis of covariance (ANCOVA) was performed following Zar (1999). All statistical analyses were based on a 0.05 significance level.

RESULTS

The chemical parameters of the test media are summarized in Tab. 1. As in natural waters, higher hardness and alkalinity levels are related to increased pH, conductivity and concentration of major ions. No mortality of test organisms reared in both media was observed during the 21 days period.

All the studied life-history parameters of *D. longispina* were affected by water chemistry, except the initial BL, the growth rate in the period 0-7 days and the number of broods (Tab. 2). Growth was significantly reduced in medium L, compared to medium H (Fig. 1). The initial BL of daphnids in both media was not significantly different (0.04 mm, P=0.064, Tab. 2). However, during the test period the effects of water chemistry on the growth of daphnids became more pronounced (Fig. 1). After only 7 days, the difference in the body length of daphnids reared in both media increased to 0.13 mm (equivalent to 8%), and these differences were statistically significant P≤0.001, Tab. 2). At day 21 the body length of daphnids differed in 0.35 mm, corresponding to a 14.5% reduction in medium L, which was statistically significant

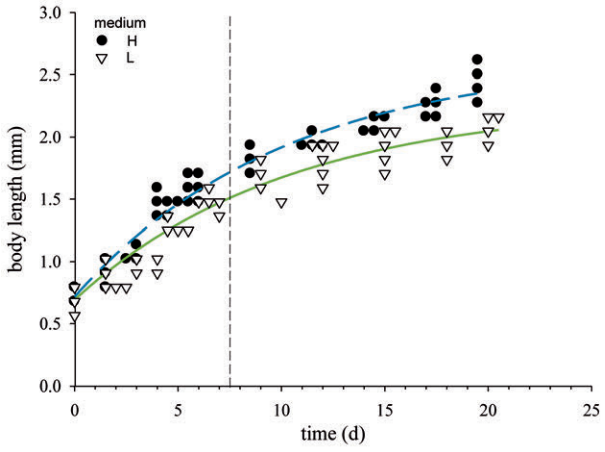

Fig. 1. Changes in body length of *D. longispina* reared in media L (triangles) and H (dots) (see Tab. 1 for the chemical properties of the media) during 21 days. The symbols represent experimental data and the lines represent the adjustment to the von Bertalanffy growth model in media L (solid line) and H (dashed line). The vertical dashed line represents the onset of maturation, separating the pre-reproductive period (0-7 d) and the reproductive period (8-21d).

(P≤0.001, Tab. 2). The growth rate in the pre-reproductive period (0-7 d) did not differ significantly between media (p=0.305, Tab. 2) but in the reproductive period (8-21 d) there were significant differences (p=0.006; Tab. 2). Nevertheless, the growth coefficient of the von Bertalanffy equation (k) did not differ significantly between both media (P=0.856); indeed, values were very similar. In addition, L_0 was not significantly affected by water chemistry (P=0.299). However, low hardness and alkalinity levels reduced L_{max} from 2.64 to 2.30 mm (P<0.001; Tab. 3). Since the parameter estimates did not differ significantly among individuals for each treatment (2-way ANOVA, P>0.05; Tab. 3) the growth trajectory for each medium was described by a single curve, as depicted in Fig. 1.

Regarding reproduction, a significant effect of water chemistry was found in all the studied endpoints, except the number of broods (Tab. 2). Briefly, daphnids reared in medium L were smaller (8.0%) but older (6.7%) at first reproduction and, therefore, released fewer juveniles at first reproduction (34.9% reduction), compared to daphnids reared in medium H (Tab. 2). The number of juveniles per brood was lower for daphnids reared in medium L, as depicted in Fig. 2a. As a consequence, the total fertility of daphnids reared in medium L was reduced in 36.6%. Accordingly, the average brood size of daphnids reared in medium L was reduced, corresponding to a 35.6% reduction.

The relationship body length - brood size was linear within the data range (Fig. 2b). Analysis of covariance showed that regression lines for the different test media differed significantly ($F_{2, 175}$=8.515, P<0.001). Despite no differences among slopes (t_{175}=0.442, P=0.659), significant differences between intercepts were detected (t_{176}=-4.124, P<0.001). This shows that daphnids with the same size released fewer juveniles when reared in medium L compared to medium H. In other words, the brood size depended not only on the size of the daphnids but also on the hardness and alkalinity of the test media.

As expected from the above mentioned results, water chemistry affected the intrinsic rate of population growth (*r*): *r* was lower for medium L than for medium H (0.27

Tab. 2. Summary of the major endpoints studied during the 21-days test with both media (L and H), expressed as mean (SD), and the appropriate statistical analysis: Student's *t*-test or Mann-Whitney U test.

Parameter	Medium L	Medium H	Statistics
Initial BL (mm)	0.73 (0.07)	0.78 (0.04)	U=65.500, n_1=13, n_2=15, P=0.064
BL at day 7 (mm)	1.48 (0.05)	1.61 (0.09)	U=30.000. n_1=n_2=15, P≤0.001
Final BL (mm)	2.06 (0.07)	2.41 (0.12)	U=0.000. n_1=n_2=15, P≤0.001
GR (0-7d) (day^{-1})	0.10 (0.01)	0.10 (0.01)	t_{27}=-1.047, P=0.305
GR (8-21d) (day^{-1})	0.02 (0.00)	0.03 (0.01)	t_{27}=-3.003, P=0.006
AFR (d)	9.07 (0.26)	8.50 (0.00)	U=0.000, n_1=n_2=15, P≤0.001
SFR (mm)	1.48 (0.05)	1.61 (0.09)	U=30.000, n_1=n_2=15, P≤0.001
FFR	4.73 (1.71)	7.27 (0.88)	U=23.500, n_1=n_2=15, P≤0.001
Total fertility	45.40 (8.24)	71.60 (5.59)	t_{28}=-10.187, P≤0.001
N. of broods	4.93 (0.26)	5.00 (0.00)	U=105.000, n_1=n_2=15, P=0.351
Avg brood size	9.22 (1.68)	14.32 (1.12)	t_{28}=-9.804, P≤0.001
r (day^{-1})	0.27 (0.03)	0.31 (0.01)	U=24.000, n_1=n_2=15, P≤0.001

Medium L, low hardness and low alkalinity; medium H, high hardness and high alkalinity; BL, body length; GR, growth rate; AFR, age at first reproduction; SFR, size at first reproduction; FFR, fertility at first reproduction.

Tab. 3. Parameter estimates of the von Bertalanffy growth equation for each medium, expressed as mean (SE) and results from the statistical analysis (2-way ANOVA for medium and individual).

Parameter	Medium L	Medium H	Statistics
L_{max} (mm)	2.30 (0.06)	2.64 (0.06)	Medium: $F_{1, 13}$=14.934, P=0.002 Individual: $F_{14, 13}$=0.450, P=0.924
L_0 (mm)	0.71 (0.01)	0.73 (0.01)	Medium: $F_{1, 13}$=1.297, P=0.275 Individual: $F_{14, 13}$=1.341, P=0.301
k	0.10 (0.01)	0.10 (0.01)	Medium: $F_{1, 13}$=0.0962, P=0.761 Individual: $F_{14, 13}$=0.480, P=0.907

Medium L, low hardness and low alkalinity; medium H, high hardness and high alkalinity; L_{max}, theoretical maximum body length; L_0, theoretical body length of neonates; k, growth coefficient.

and 0.31 day^{-1} respectively, Tab. 2), representing 13.4% reduction. The effects of water chemistry on the life-history parameters are concordant with the effects on the feeding rate of daphnids. Indeed, the feeding rate of daphnids in medium L was 42.9% lower than in medium H (L: 0.148 µg dw ind^{-1} h^{-1}; H: 0.258 µg dw ind^{-1} h^{-1}). The relationship between BL of daphnids and the feeding rate was described by a power function (Fig. 3): the feeding rate of daphnids increased with their BL. Plotting the feeding rates of daphnids reared in both media on the graph that relates BL and feeding rate shows no deviation, which suggests that BL is the only factor needed to explain the variability in the feeding rates of daphnids in both media, *i.e.*, the reduced feeding rate of daphnids in medium L can be explained solely by their smaller size.

DISCUSSION

In this study we aimed to assess the effects of water hardness and alkalinity on the life history parameters (growth, reproduction and population growth rate) of *D. longispina*. Thus, we performed life table experiments with *D. longispina* reared under two different water chemistry scenarios: low hardness and low alkalinity (medium L) *vs* high hardness and high alkalinity (medium H). The vast majority of the life-history parameters was affected by water chemistry, being the total fertility the most affected. Indeed, low levels of hardness and alkalinity caused 14.5% reduction in the final body length of daphnids, 36.6% reduction in the total fertility and 13.4% reduction in *r* compared to high levels of hardness and alkalinity.

In general, the findings of this study support previous studies concerning the effects of reduced Ca concentration on growth, reproduction and population growth of *Daphnia*. However, not only the effects of Ca have to be considered, but also the effects of alkalinity. Although the effects of decreasing alkalinity to crustaceans are less pronounced than those of decreasing hardness (Cowgill and Milazzo, 1991), decreased alkalinity might enhance the susceptibility to low Ca, *i.e.*, at low pH/alkalinity higher concentrations of Ca are needed to support *Daphnia* populations (Hooper *et al.*, 2008). The reduced somatic growth of daphnids reared in medium L is most likely a consequence of low Ca concentration, since Ca is essential for the formation of moults (Alstad *et al.*, 1999; Hessen *et al.*, 2000). It is accepted that growth of *Daphnia* is most affected by low Ca in the days immediately after hatching for two reasons. First, because moult cycles are shorter in neonates or juveniles than adults, leading to greater Ca demands (Hessen *et al.*, 2000). Second, because surface-to-volume ratios are greater in neonates and juveniles, increasing Ca demand for a given carapace thickness (Cairns and Yan, 2009). For instance, *D. magna* reared in media with Ca concentrations between 3.4 and 32.5 mg Ca/L exhibited significant differences in BL at day 7, but no significant differences at day 14 and day 21, revealing an uniformization of BL during the 21-days period (Muyssen *et al.*, 2009). However, this was not the case with *D. longispina*, since the growth of daphnids reared in media L and H became more divergent along the test period. This might be due to the following reasons. As daphnids grow, they have greater Ca demands in order

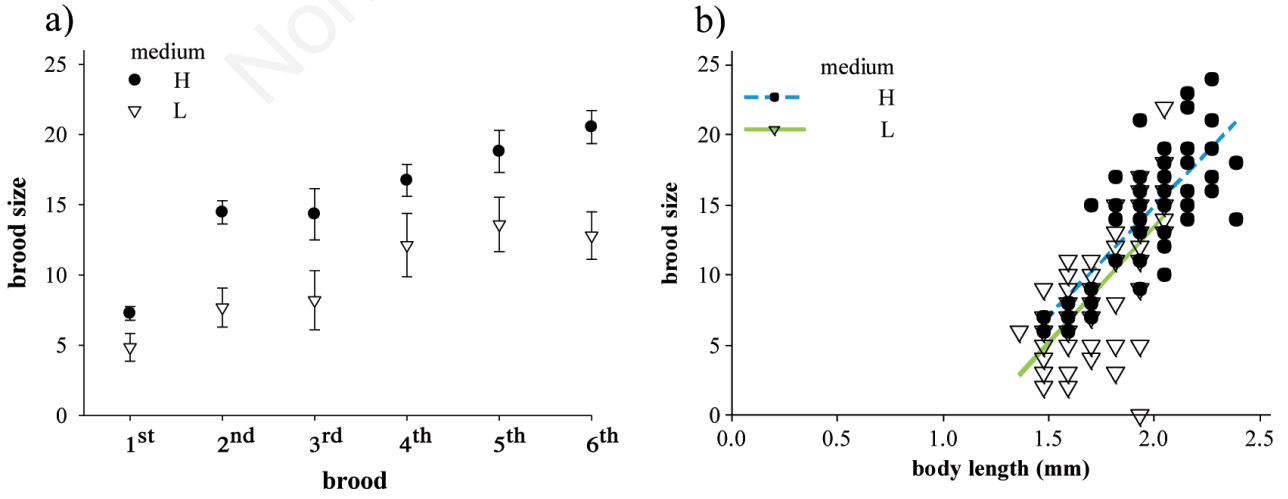

Fig. 2. Brood size (number of neonates) of *D. longispina* reared in media L (triangles) and H (dots) (see Tab. 1 for the chemical properties of the media): a) brood size in each brood (symbols represent mean values and the error bars represent 95% confidence intervals); b) relationship between body length (mm) and brood size: symbols represent experimental data and the lines represent the regressions for media L (solid line) and H (dashed line).

to accomplish the formation of a larger moult. As a consequence, daphnids reared at low hardness and alkalinity must increase their metabolism and, hence, have higher maintenance costs to maintain their ionic balance (Arnér and Koivisto, 1993) and form larger moults. Additionally, part of the energy uptake by the organisms is required for reproduction, *i.e.*, the onset of reproduction represents additional costs, leaving less energy available for growth. This agrees with the deceleration in the growth rate even for daphnids reared in medium H, which has high Ca levels (9.86 mg/L); however the deceleration was more pronounced for daphnids reared in medium L. Hence, the higher maintenance costs allied to the onset of reproduction might explain why the growth of daphnids in medium L and H became more divergent along the test period.

Although low hardness and alkalinity levels reduced the maximum body length, the von Bertalanffy growth coefficient (k) was not significantly affected. The k value is mostly determined by the initial slope of the growth curve, *i.e.*, the growth of individuals during the pre-reproductive period which, according to the growth rate of daphnids in the period 0-7 days, was not affected by low hardness and alkalinity. Moreover, the fact that low hardness and alkalinity reduced the growth rate in the period 8-21 days agrees with the significant differences in the estimated maximum body length (L_{max}) between both media. The reproduction impairment in medium L is partly explained by the reduced body length, since smaller daphnids produce smaller broods which, ultimately, results in reduced total fertility. Nevertheless, the effects of water chemistry on reproduction are not exclusively a consequence of the BL of daphnids. In fact, the relationship body length - brood size showed that water chemistry exerted significant effects on reproduction, independently of the body length: daphnids with the same body length produced smaller broods when reared in medium L compared to medium H. Besides Ca and alkalinity, the slope and the intercept of this regression are known to be affected by food availability (Nogueira *et al.*, 2004), food quality and possibly also by the size structure of the population (Hülsmann, 2001). In our experiment, these factors were constant for both treatments, thus the likely explanation for the effects on this relationship is that metabolic costs associated with Ca transport and ionic balance are higher in individuals maintained at low Ca (Arnér and Koivisto, 1993), as mentioned previously. Note that the effects on the relationship body length – brood size cannot be attributed to differences in the energy uptake since we showed that feeding activity, and thus energy uptake, was not affected by water chemistry but was only a function of the BL of daphnids. As the relationship body length – brood size is commonly used to assess the reproductive potential of *Daphnia* populations (Hülsmann, 2001), our finding highlights the importance of water hardness and alkalinity to *D. longispina* and might be particularly relevant in the assessment of the reproductive potential of populations of *D. longispina* in natural aquatic systems.

Other reproduction-related parameters, namely AFR, SFR and FFR were significantly affected as a consequence of decreased hardness and alkalinity. AFR increased with decreasing hardness, concordantly to previous studies (Cairns and Yan, 2009). Given the pronounced effects on growth, despite daphnids in medium L showed a higher AFR they were smaller (lower SFR) and, therefore, produced fewer juveniles in the first brood (lower FFR) compared to daphnids in medium H. The high reduction in total fertility of daphnids reared in medium L (36.6%) is, hence, a combined effect of their later start of reproduction, their smaller size and, thus, reduced broods, and the effects of water chemistry on the relationship body length – brood size.

The *r* value found for medium H is similar to values reported for the same clone (Antunes *et al*., 2003). As a consequence of the reduced growth and reproduction in medium L, *r* was lower in this medium. Such a decrease is concordant with a previous study reporting reduced *r* with decreasing Ca and pH in *D. magna* (Hooper *et al.*, 2008). *D. longispina* seemed to be more sensitive to low hardness and alkalinity than the large-bodied *D. magna*. Indeed, in a study carried out in our lab (Jesus *et al.*, in

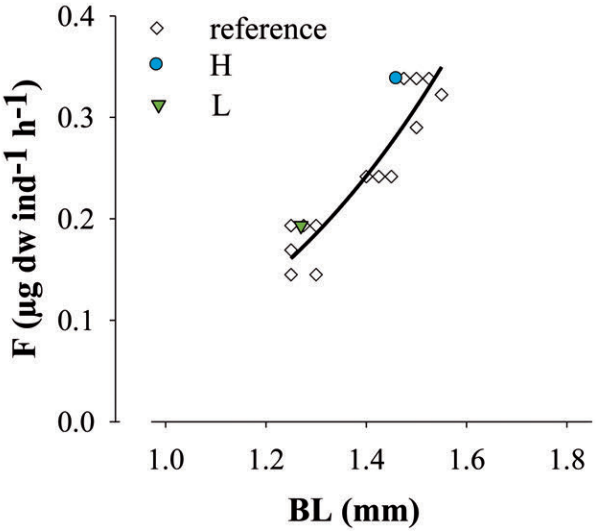

Fig. 3. Relationship between BL of *D. longispina* and the respective feeding rate (F); diamonds represent daphnids reared in the reference medium ASTM hard water; the circle represents daphnids reared in medium H; the triangle represents daphnids reared in medium L; standard deviation is 0.00 for both media (n=3). The solid line represents the regression line for the reference data: $F=0.0726 \times BL^{3.566}$ ($r^2=0.857$, n=21, $P<0.001$).

preparation) we found that *D. magna* (clone F, *sensu* Baird *et al.*, 1990) reared at low levels of hardness and alkalinity (equivalent to medium L) exhibited 1.7% reduction in the final body length, 6.4% reduction in total fertility and 2.5% reduction in *r* compared to daphnids reared at high hardness and alkalinity (equivalent to medium H). Muyssen *et al.* (2009) also found a smaller variation in the growth and reproduction of *D. magna* (clone K6) compared to our results for *D. longispina*. The authors reported that *D. magna* reared at 5.7 mg Ca L^{-1} exhibited 2.1% increase in the final body length and 4.8% reduction in total fertility compared to those reared at 32.5 mg Ca L^{-1}. Even though the variation in Ca concentration was higher in their study, *D. magna* showed a less pronounced response compared to that of *D. longispina* in this study. Concerning the body Ca content, which is lower in *D. longispina* than *D. magna* [about 1.5% dw and 4.4% dw, respectively; see Waervagen *et al.* (2002)], it would be expected that *D. longispina* was less sensitive to low Ca than *D. magna*. Therefore, other factors must be considered to explain the higher sensitivity of *D. longispina*. First, within the *Daphnia* genus the ability of a species to cope with low Ca also depends not only on its body Ca content, but also on its ability to extract and retain Ca from water and food, as suggested by Tan and Wang (2010). For instance, these authors reported that although *D. galeata* had lower Ca content than *D. carinata*, it had worse performance in low Ca conditions, which might be due to its lower ability to extract and retain Ca from the environment. Second, it is possible that the tested clone of *D. longispina* has a reduced tolerance to low Ca. This hypothesis is supported by the inter-population variation of the tolerance to low Ca found in *D. galeata* (Rukke, 2002), which might also be valid for *D. longispina* and would therefore be linked with the high variability in the body Ca content of *D. longispina* (Waervagen *et al.*, 2002). Note that the clone was obtained in a lake with high Ca (29.4 mg Ca L^{-1}). Finally, not only the sensitivity to low Ca has to be considered but also the sensitivity to low alkalinity/pH. Since *D. longispina* were obtained in a lake with high alkalinity (101.7 mg $CaCO_3$ L^{-1}) and pH (8.10-8.74) (Castilho, 2008), they might have reduced tolerance to low alkalinity and pH values.

However, the lower tolerance of *D. longispina* than *D. magna* observed in laboratorial studies is not concordant with field distributions of these species. *D. magna* is found at Ca concentrations above 5.0 mg L^{-1} and pH 6.9 (Hooper *et al.*, 2008), whereas *D. longispina* can be found at Ca concentrations above 0.6 mg L^{-1} and pH 5.5 (Hessen *et al.*, 1995; Nilssen and Waervagen, 2002). The explanation for the divergence between the results of laboratorial and field experiments is probably the genetic intra-species diversity in ecosystems, which translates into differential sensitivity to low Ca (Rukke, 2002), allied to the lower Ca content in *D. longispina* than *D. magna* (Waervagen *et al.*, 2002). Additionally, in natural ecosystems the organisms commonly are not exposed to sudden changes in water chemistry as they were in our experiment, but to gradual changes, which allows their adaption to changing conditions.

This study shows that under conditions of low levels of hardness and alkalinity, the growth, reproduction and population growth of *D. longispina* are significantly affected, in particular reproduction. These findings might be relevant for populations of *D. longispina* under the current scenario of decreased hardness and alkalinity which has been reported in many surface waters in Europe and North America over the past decades (Skjelkvåle *et al.*, 2005). Actually, such effects might, at long-term, compromise the abundance and eventually the survival of populations of *D. longispina*, in particular those with reduced tolerance to low Ca. Note that, despite medium L caused pronounced effects on the life-history parameters of *D. longispina*, the Ca concentration was 7.02 mg L^{-1}, which is higher than Ca concentrations in many aquatic systems (Hessen *et al.*, 1995; Jeziorski *et al.*, 2008). Moreover, low hardness and alkalinity not only cause direct effects on the life-history parameters of *Daphnia*, but can also reduce the stress-tolerance to other environmental factors such as temperature (Ashforth and Yan, 2008), acidity (Hooper *et al.*, 2008) and UV radiation (Hessen and Rukke, 2000) and also to low food availability (Ashforth and Yan, 2008). This raises concern about the effects of reduced hardness to the populations of *D. longispina* in natural aquatic systems, mainly because many aquatic ecosystems face not only reduced hardness but also increasing acidity (Skjelkvåle *et al.*, 2001) and temperature and reduced algal biomass (Ashforth and Yan, 2008). These factors might affect not only the persistence of populations of *D. longispina* but also the structure of aquatic ecosystems due to the abundance and key role of these crustaceans on aquatic food webs.

CONCLUSIONS

Despite *D. longispina* can survive at low hardness and alkalinity, their life-history parameters are significantly affected, mainly the total fertility. Low levels of hardness and alkalinity (46.8 and 31.4 mg $CaCO_3$ L^{-1}, respectively) caused 14.5% reduction in the final body length, 36.6% reduction in the total fertility and 13.4% reduction in the population growth rate of daphnids compared to high levels of hardness and alkalinity (174.7 and 106.4 mg $CaCO_3$ L^{-1}, respectively). Additionally, this laboratorial study shows that this clone of *D. longispina* is more susceptible to low hardness and alkalinity than some clones of *D. magna,* which does not agree with field data since *D. longispina* is commonly found at lower Ca

concentrations and pH (correlated to alkalinity) than *D. magna*. This might suggest that the tested clone of *D. longispina* used in this experiment was more sensitive to low hardness and alkalinity than other clones of this species, hence highlighting the importance of using autochthonous species to promote the ecological value and application of scientific experiments. The high sensitivity of this species to low hardness and alkalinity raises concern about the effects of decreasing hardness that has been reported in Europe and North America, as decreasing hardness increases the susceptibility of daphnids to increasing acidity and temperature and also to decreasing food availability. Ultimately, decreasing hardness and alkalinity might affect not only populations of *D. longispina* but also the structure of aquatic ecosystems.

Given the important role of water chemistry on the life-history endpoints of *D. longispina*, further studies should address the effects of a wider range of water hardness and alkalinity. Moreover, the joint effects of water chemistry and other parameters, such as temperature and food concentration, should also be studied, given their importance in aquatic ecosystems.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Bruno Castro (Dept. of Biology, University of Aveiro, Portugal) for providing the culture of *D. longispina* and Abel Ferreira (Dept. of Biology, University of Aveiro, Portugal) for culturing *C. vulgaris*. This work was supported by fellowships from Fundação para a Ciência e a Tecnologia (SFRH/BD/27637/2006) and from CESAM (BII/UI88/POCI/AMB/55801/2004/2008) and co-financed by the European programme FEDER (FCT/POCI/AMB/55801/2004).

REFERENCES

Adamczuk M, 2012. Spatial distribution of juvenile and adult stages of limnetic Cladocera in relation to selected environmental factors. J. Limnol. 71:112-118.

Agra AR, Guilhermino L, Soares AMVM, Barata C, 2010. Genetic costs of tolerance to metals in *Daphnia longispina* populations historically exposed to a copper mine drainage. Environ. Toxicol. Chem. 29:939-946.

Allen Y, Calow P, Baird DJ, 1995. A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. Environ. Toxicol. Chem. 14*:*1625-1630.

Alstad NEW, Skardal L, Hessen DO, 1999. The effect of calcium concentration on the calcification of *Daphnia magna*. Limnol. Oceanogr. 44*:*2011-2017.

American Public Health Association, 2005. Standard methods for the examination of water and wastewater, 21 ed. Published jointly by American Public Health Association, American Waters Works Association and Water Pollution Control Federation, Washington, DC, USA.

Antunes SC, Castro BB, Gonçalves F, 2003. Chronic responses of different clones of *Daphnia longispina* (field and ephippia) to different food levels. Acta Oecol. 24*:*S325-S332.

Arnér M, Koivisto S, 1993. Effects of salinity on metabolism and life history characteristics of *Daphnia magna*. Hydrobiologia 259*:*69-77.

Ashforth D, Yan ND, 2008. The interactive effects of calcium concentration and temperature on the survival and reproduction of *Daphnia pulex* at high and low food concentrations. Limnol. Oceanogr. 53*:*420-432.

ASTM, 2004. Standard guide for conducting *Daphnia magna* life-cycle toxicity tests, ASTM E1193–97. American Society for Testing and Materials, West Conshohocken, PA, USA.

Baird DJ, Barber I, Calow P, 1990. Clonal variation in general responses of *Daphnia magna* Straus to toxic stress. 1. Chronic life-history effects. Funct. Ecol. 4*:*399-407.

Barata C, Baird DJ, Nogueira AJA, Agra AR, Soares AMVM, 2007. Life-history responses of *Daphnia magna* Straus to binary mixtures of toxic substances*:*Pharmacological versus ecotoxicological modes of action. Aquat. Toxicol. 84*:*439-449.

Cairns A, Yan N, 2009. A review of the influence of low ambient calcium concentrations on freshwater daphniids, gammarids, and crayfish. Environ. Rev. 17*:*67-79.

Castilho AMA, 2008. [Quiaios lakes - Contribution to their geological and hydrogeological knowledge].[PhD thesis in Portuguese]. Faculdade de Ciências e Tecnologia-Departamento de Ciências da Terra. University of Coimbra.

Cowgill UM, Milazzo DP, 1991. Demographic effects of salinity, water hardness and carbonate alkalinity on *Daphnia magna* and *Ceriodaphnia dubia*. Arch. Hydrobiol. 122:33-56.

Gurney W, Nisbet RM, 1998. Ecological dynamics. Oxford University Press: 352 pp.

Hessen DO, Alstad NEW, Skardal L, 2000. Calcium limitation in *Daphnia magna*. J. Plankton Res. 22*:*553-568.

Hessen DO, Faafeng BA, Andersen T, 1995. Competition or niche segregation between *Holopedium* and *Daphnia* - empirical light on abiotic key parameters. Hydrobiologia 307:253-261.

Hessen DO, Rukke NA, 2000. UV radiation and low calcium as mutual stressors for *Daphnia*. Limnol. Oceanogr. 45:1834-1838.

Hooper HL, Connon R, Callaghan A, Fryer G, Yarwood-Buchanan S, Biggs J, Maund SJ, Hutchinson TH, Sibly RM, 2008. The ecological niche of *Daphnia magna* characterized using population growth rate. Ecology 89:1015-1022.

Hülsmann S, 2001. Reproductive potential of *Daphnia galeata* in relation to food conditions: implications of a changing size-structure of the population. Hydrobiologia 442:241-252.

Jeziorski A, Yan ND, Paterson AM, DeSellas AM, Turner MA, Jeffries DS, Keller B, Weeber RC, McNicol DK, Palmer ME, McIver K, Arseneau K, Ginn BK, Cumming BF, Smol JP, 2008. The widespread threat of calcium decline in fresh waters. Science 322:1374-1377.

Meyer JS, Ingersoll CG, McDonald LL, Boyce MS, 1986. Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques. Ecology:1156-1166.

Moiseenko TI, Skjelkvåle BL, Gashkina NA, Shalabodov AD, Khoroshavin VY, 2013. Water chemistry in small lakes along a transect from boreal to arid ecoregions in European

Russia: effects of air pollution and climate change. Appl. Geochem. 28:69-79.

Muyssen BTA, De Schamphelaere KAC, Janssen CR, 2009. Calcium accumulation and regulation in *Daphnia magna*: links with feeding, growth and reproduction. Comp Biochem. Physiol. A Mol. Integr. Physiol. 152:53-57.

Nilssen JP, Waervagen SB, 2002. Recent re-establishment of the key species *Daphnia longispina* and cladoceran community changes following chemical recovery in a strongly acid-stressed region in southern Norway. Arch. Hydrobiol. 153*:*557-580.

Nogueira AJA, Baird DJ, Soares AMVM, 2004. Testing physiologically-based resource allocation rules in laboratory experiments with *Daphnia magna* Straus. Ann. Limnol - Int. J. Lim. 40:257-267.

OECD, 2008. OECD Guidelines for the testing of chemicals. Test No. 211: *Daphnia magna* reproduction test. Organisation for Economic Co-operation and Development, Paris, pp. 23.

Rukke NA, 2002. Tolerance to low ambient calcium shows inter-population differences in *Daphnia galeata*. J. Plankton Res. 24*:*527-531.

Seda J, Petrusek A, 2011. *Daphnia* as a model organism in limnology and aquatic biology: introductory remarks. J. Limnol. 70*:*337-344.

Skjelkvåle BL, Stoddard JL, Andersen T, 2001. Trends in surface water acidification in Europe and North America (1989-1998). Water Air Soil Poll. 130:787-792.

Tan QG, Wang WX, 2010. Interspecies differences in calcium content and requirement in four freshwater cladocerans explained by biokinetic parameters. Limnol. Oceanogr. 55:1426-1434.

USEPA, 2002a. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, EPA-821-R-02-012, 5th ed. United States Environmental Protection Agency-Office of Water (4303T), Washington, DC, USA.

USEPA, 2002b. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, EPA-821-R-02-013, 4th ed. United States Environmental Protection Agency-Office of Water (4303T), Washington, DC, USA.

Waervagen SB, Rukke NA, Hessen DO, 2002. Calcium content of crustacean zooplankton and its potential role in species distribution. Freshwater Biol. 47:1866-1878.