The Scientific Temper
VOL-VIII, NO.1&2; JANUARY-JULY, 2017
ISSN 0976 8653, E ISSN 2231 6396
UGC SR NO 2535; JR NO. 47226
e-mail:letmepublish@rediffmail.com
Web: www.scientifictemper.com

WATER QUALITY AND LIFE-HISTORY PARAMETERS OF *DAPHNIA CARINATA* (DAPHNIDAE : CLADOCERA) UNDER LABORATORY CONDITIONS

Chandra Bhushan Tiwary and Ashok Kumar Singh
Assistant Professor, SMD College MN Jalalpur, Gopalganj (Bihar)
Professor, Department of Zoology, Jai Prakash University, Chapra (Bihar)
Contact: 9852372068, Email ID: tiwary_cb@rediffmail.com

ABSTRACT

The life-history and population of aquatic crustaceans mainly depend on water quality. This study is aimed to test the hypothesis that water hardness and alkalinity affect the life-history parameters of *Daphnia carinata*. Experiments were carried out accordingly in laboratory conditions. There was a significant reduction in the growth of daphnids reaching up to a 14.5% as compared to high hardness and alkalinity. There were also delayed reproduction, increased body length during first reproduction and reduced number in first brood and, consequently, a 36.6% reduction in total fertility, compared to daphnids reared at high hardness and alkalinity. The impaired growth and reproduction at hardness and alkalinity levels was likely a consequence of increased salt concentrations, and not due to changes in the feeding activity.

This study raises concerns about the effects of environmental degradation and increasing acidity in the surrounding medium which is evident in the form of acid rtain etc due to increasing pollution. The decrease in populations of *D. carinata* and similar other planktons will affect the structure of aquatic ecosystems.

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

INTRODUCTION

The water quality has very strong influence on the biology and distribution of aquatic organisms. Like other crustaceans, the biological parameters of cladocerans are affected also by water hardness. For replacing the calcified cuticle during each molting, they require profuse amount of Ca++ (Ashforth and Yan, 2008) which they obtain from water. The *Daphnia* has recently been used as a model to study the effects of Ca decline in aquatic ecosystems

(Jeziorski *et al.*, 2008). In most freshwater systems, daphnid species play an important role due to their abundance and central position in aquatic food-webs. The effects of hardness on *Daphnia carinata* as small-sized cladoceran is not very significant in most small-size systems (small lakes, small pools and ponds) and also in the larger lakes with various extent of fish predation (Adamczuk, 2012). The smaller water bodies are particularly prone to variations in their water chemistry as are not buffered due to lack of a large water volume.

In aquatic ecosystems hardness is generally related with alkalinity (Moiseenko *et al.*, 2013). The main source of alkalinity is usually carbonate rocks, and, thus 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. The temporal variation is caused by acid deposition in lesser hard water and lesser alkalinity.

It is important to assess the combined effects of water hardness and alkalinity on the biology of crustaceans. Therefore, the present study has been focused upon the effects of these parameters on the life processes of *D. carinata*. The parameters taken up to study are to evaluate growth, reproduction and population growth of *D. carinata* with different water chemistry. This study would be useful to assess tolerance limit of crustaceans and ecological relevance of such effects in aquatic ecosystem.

MATERIALS AND METHODS

The daphnids cultures were maintained in hard water with a concentration of 3.5 μg dw (dry weight) mL–1 with a prepared feed material. A small flake of acetyl alcohol was placed at the surface of the medium to reduce surface tension and the probability of daphnids entrapment. Culture medium was renewed every alternate 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 were randomly selected and assigned to each medium. The first two broods were discarded and only neonates from the third-to-fourth broods were used in the experiments.

We compared the physical growth, rate of reproduction and the population growth of *D. carinata* reared in two media with different hardness and alkalinity of two ponds in this study. The main chemical parameters of the test media are presented in Tab. 1.

Table 1. Chemical parameters (mean) of the test water media.

Parameters	Medium L	Medium H
Hardness (mg CaCO3L-1)	38.4 (2.0)	21.4 (1.5)
Alkalinity (mg CaCO3L-1)	157.4 (3.6)	6.80 (0.16)
Conductivity (μScm-1)	6.56 (0.52)	6.12 (0.46)
Ph	15.12 (1.25)	3.84 (0.12)
Concentrations of major ions		
Ca	3.22 (0.10)	48.32 (1.72)
Mg	164.3 (4.7)	96.4 (5.0)
Na	545.7 (33.4)	8.10 (0.26)
K	24.76 (1.26)	20.44 (1.36)
Cl	52.54 (2.10)	6.20 (0.42)
SO4	9.48 (0.92)	182.54 (6.40)

Medium L had about 3.5-fold lower hardness and alkalinity than medium H, being classified as soft water according to USEPA (2002). 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, feed organisms and suitable conditions of the photoperiod and temperature were maintained as described for cultures. The test duration was 21 days, during which media were renewed every other day.

Organisms were checked twice a day for life-cycle parameters like reproduction, molting and mortality. The offspring, aborted eggs and embryos were counted and the shedded carapaces were collected for determination of daphnids body length (BL), the measurement was estimated based upon the length of the first exopodite of the second antennae (AL) till the end of carapace released during each molting using a stereomicroscope fitted with an ocular rule. The calculation was made with the help of regression model through equation as:-

$$\mathbf{BL = 9.11 * AL - 0.110} \qquad \text{(eq. 1)}$$

There, the unit for both BL and AL is mm, $r^2=0.904$, $n=54$, $P<0.001$.

The effects on growth in pre-reproductive and reproductive phase were assessed by determining the growth rate using equation as:-

$$\text{Growth rate} = \ln(l_f)-\Delta\ln(l_i)/\ t \qquad \text{(eq. 2)}$$

This rate is expressed in day^{-1} with l_f and l_i as the final and initial BL of daphnids. The Δ_t is the time interval (days).

The growth of daphnids was determined using the Von Bertalanffy equation, as defined by Gurney and Nisbet (1998):

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

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 endpoints for assessment of effect on reproduction were AFR (age of first reproduction), SFR (length of daphnids when carrying the first brood in the brood chamber), 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 between body length and 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:

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

Where r is expressed in day^{-1}, x is the age class (1 . . . n days), l_x is the probability of survival 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). 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):

$$F=V(C_0-C_t)/\ t\,N \qquad \text{(eq. 5)}$$

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.

Chemical analyses: Conductivity and pH were measured using a conductivity meter and p^{H} meter, respectively. The concentrations of major ions were determined in filtered samples. 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: Student's *t*-test was used to test whether each endpoint differed significantly between both media and the non-parametric Mann-Whitney U-test was used for impaired data of study. All statistical analyses were based on a 0.05 significance level.

RESULTS AND OBSERVATIONS

The chemistry of both test water media are presented in Table 1. As in aquatic systems, higher hardness and alkalinity levels are related to increased p^{H}, conductivity and concentration of major ions. The mortality of test organisms reared in both media was not observed during the 21 days period.

The water chemistry affected all life-history characteristic except the initial body length, the growth rate in initial phase (0-7days) of development and the number of broods (Table 2). The growth is more pronounced in medium H in comparison of medium L (Figure 1).

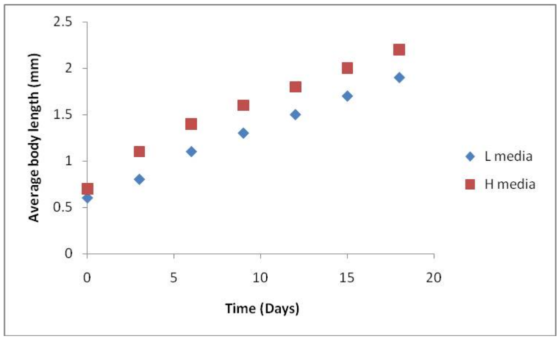

Figure 1. Changes in body length of *D. carinata* reared in different water media during study period.

on Lo were non-significant with 0.856 and 0.299 as P value between both media. Thus, only low hardness and alkalinity level reduced Lmax from 2.64 to 2.30 mm (Pd”0.001, Table 3). There was also slight difference among individuals for each treatment.

A significant effect of water chemistry was found in all the studied endpoints in regard to reproduction, except the number of broods (Table 2). The daphnids reared in medium L released fewer juveniles (34.9% reduction) at first reproduction compared to H medium due to attainment of smaller size (8.0% but older (6.7%) in the laboratory. The number of juveniles per brood was lower for daphnids reared in low medium (Figure 2).

Table 2. Summary of the major endpoints studied during the 21-days test with both media expressed as mean (SD) and the appropriate statistical analysis.

Parameter	Medium L	Medium H	Statistics
Initial BL (mm)	0.71 (0.006)	1.42 (0.03)	$U=62.400, n_1=12, n_2=15, P=0.062$
BL at day 7 (mm)	1.94 (0.03)	0.08 (0.01)	$U=28.000, n_1=n_2=15, P\leq 0.001$
Final BL (mm)	0.02 (0.00)	8.04 (0.18)	$U=0.000, n_1=n_2=15, P\leq 0.001$
Growth rate (0-7 d,day-1)	1.36 (0.03)	4.34 (1.52)	$t_{27}=-1.047, P=0.305$
Growth rate (8-21 d,day-1)	42.40 (7.14)	4.53 (0.18)	$t_{27}=-3.003, P=0.004$
AFR (day)	8.76 (1.52)	0.26 ((0.02)	$U=0.000, n_1=n_2=15, P\leq 0.001$
SFR (mm)	0.76 (0.002)	1.52 (0.06)	$U=28.000, n_1=n_2=15, P\leq 0.001$
FFR	2.32 (0.08)	0.08 (0.01)	$U=22.500, n_1=n_2=15, P\leq 0.001$
Total fertility	0.03 (0.01)	7.54 (0.00)	$t_{28}=-09.183, P\leq 0.305$
No. of Broods	1.53 (0.06)	6.87 (0.75)	$U=102.000, n_1=n_2=15, P\leq 0.001$
Average brood size	64.40 (4.47)	4.86 (0.00)	$t_{28}=-08.602, P\leq 0.001$
Population growth rate(day^1)	12.32 (1.08)	0.27 (0.01)	$U=24.000, n_1=n_2=15, P\leq 0.001$

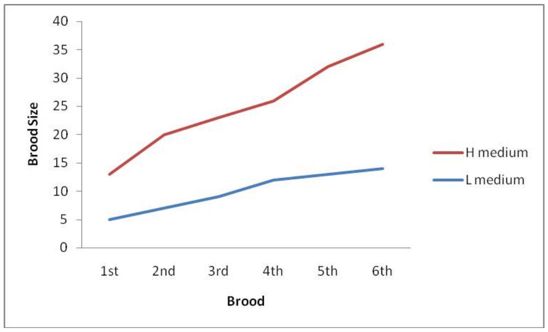

Figure 2. Brood size (Number of neonates) in different water media during study period.

The initial body length was not significantly different in both media (Table 2), however, strong effect of water chemistry on the growth of daphnids was observed during study period (Figure 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%) after only 7 days and the body length of daphnids differed in 0.35 mm at day 21 corresponding to a 14.5% reduction in low hard water media, which was statistically significant (Pd”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).

The growth coefficient value k in Von Bertalanffy equation and effect of water chemistry

Table 3. Parameter estimates of the Von Bertalanffy equation for each medium expressed as mean (se) and results from the 2-way ANOVA for medium and individual.

Parameter	Medium L	Medium H	Statistics
L_{max} (mm)	2.28 (0.04)	0.69 (0.01)	Medium $F_{1,13}$=14..632, P=0.002 Individual $_{F14,13}$=0.440, P=0.914
L_0 (mm)	0.10 (0.01)	2.622 (0.04)	Medium $F_{1,13}$=1.267, P=0.002 Individual $F_{14,13}$=1.320, P=0.99114
Growth coefficient ((k)	0.71 (0.01)	0.10 (0.01)	Medium $F_{1,13}$=0.0862, P=0.741 Individual $F_{14,13}$=0.460, P=0.807

As a consequence, the total fertility and average brood size both reduced to 36.6% and 35.6% for daphnids reared in L medium. Therefore, it might be expected that the brood size was dependent not only on the size of daphnids, but also on the hardness and alkalinity of the used media for culture.

As expected from the all other results, water chemistry affected the intrinsic rate of population growth (*r*) and its value was lower for medium L than for medium H (0.27 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. Here, 42.9% lower feeding rate was observed in L medium in comparison of H medium.

DISCUSSIONS

In present findings, most of the life-cycle parameters were affected 36.6% reduction was there in the total fertility, 14.5% reduction was found in the final body length of daphnids and 13.4% reduction in r with low levels in comparison to high level of hardness and alkalinity during the study period.

This study was concerned with both reduced calcium level and alkalinity in freshwater ecosystem. Although decreased alkalinity to crustaceans is less pronounced than those of decreasing hardness (Cowgill and Milazzo, 1991), it might enhance calcium susceptibility. The calcium is essential for moults and thus reduced growth of daphnids observed in L medium is related to low calcium level than H medium (Hessen et al., 2000). It is accepted that growth of Daphnia is most affected by low Ca in neonates than adults due to shorter moult cycles (Hessen et al., 2000) and greater surface-to-volume ratio increasing Ca demand for carapace thickness (Cairns and Yan, 2009).

The results of study were somewhat different from *D. magna* reared in media with 3.4-32.5 mg Ca/L exhibited significant differences in BL at day 7, but no significant differences at day 14 and day 21 (Muyssen et al., 2009) due to growth of daphnids in L and H media that is more divergent along the test period. This might be due to the higher requirement of the mollt, larger energy demand and ionic balance during reproduction in the circumstances.

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 growth of individuals during the pre-reproductive period, which was not affected by low hardness and alkalinity. However, reduced growth during post-reproductive period 8-21 days have significant difference in the Lmax value. It may be explained by the resulting small broods and ultimately reduced total fertility with low hardness and alkalinity. The relation between body length and brood size may be determined with considerations on water chemistry, food quality and possibly also through the size structure of population (Hülsmann, 2001). In this study, these factors were constant for both treatments, thus only the metabolic costs associated with calcium transport and ionic balance is responsible for the body length and brood size relationship.

The reproductive parameters as AFR, SFR and FFR were significantly affected as a consequence of decreased hardness and alkalinity. AFR increased with decreasing hardness, is in agreement with previous study (Cairns and Yan, 2009). However, daphnids in L medium showed lower SFR and 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 late start of reproduction, their smaller size and reduced broods.The effects of water chemistry on the relationship of body length and brood size is evident. The r value lower in L medium is in consonance with a previous study reporting reduced r with decreasing Ca and pH in *D. magna* (Hooper et al., 2008). The smaller cladoceran seemed to be more sensitive to low hardness and alkalinity than the large-bodied species. Also, the Ca concentration is lower in body of *D. carinata* as reported by Waervagen *et al.* (2002) might be associated with its ability to extract and retain Ca from water and food, as suggested by Tan and Wang (2010) and its low tolerance for low Ca level.

CONCLUSIONS

This study shows that under conditions of low levels of hardness and alkalinity, the growth, reproduction and population growth of *D. carinata* are significantly affected. 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, acidity and also to low food availability. These factors might affect not only the persistence of populations of *D. carinata*, but also the structure of aquatic ecosystems due to the abundance and key role of these crustaceans on aquatic food webs. The high sensitivity of this species to low hardness and alkalinity raises concern about the effects that has been reported in tropical freshwater systems. The decreasing hardness increases the susceptibility of daphnids to increasing acidity and temperature and also to decreasing food availability.

REFERENCES

1. Adamczuk M (2012). Spatial distribution of juvenile and adult stages of limnetic Cladocera in relation to selected environmental factors. J. Limnol. 71:112-118.
2. 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.
3. Allen Y, Calow P, Baird DJ (1995). A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. Environ. Toxicol. Chem. 14:1625-1630.
4. 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.
5. 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.
6. Gurney W, Nisbet RM (1998). Ecological dynamics. Oxford University Press: 352 pp.
7. Hessen DO, Alstad NEW, Skardal L (2000). Calcium limitation in *Daphnia magna*. J. Plankton Res. 22:553-568.
8. 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.
9. 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.
10. 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.
11. Meyer JS, Ingersoll CG, McDonald LL, Boyce M (1986). Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques. Ecology: 1156-1166.
12. 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.
13. 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.
14. USEPA (2002). 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.
15. Waervagen SB, Rukke NA, Hessen DO (2002). Calcium content of crustacean zooplankton and its potential role in species distribution. Freshwater Biol. 47:1866-1878.