Effects of water acidity and recovery on the viability of both *Daphnia longispina* (O. F. Müller) and *Daphnia obtusa* (Kurz) in Lake Orta (N. Italy)

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**Introduction**

The acidity of Lake Orta caused by industrial pollution (ammonium and copper sulphate) is a well known case in the history of polluted lakes. The toxic effects of both low pH (<4), and high trace metal concentrations on different trophic levels of the lake ecosystem since 1926 have been well documented by several authors (Monti 1930, Baldi 1949, Vollenweider 1963, Bonacina 1970, Bonacina & Bonomi 1984). On the other hand, first, the construction of a treatment plant for the removal of ammonia content in the main industrial effluent (late, 1980) and, second, the liming intervention on the lake basin during the period May, 1989–June 1990, resulted in marked improvements in both water quality and biotic richness (Mosello et al. 1986, Bonacina et al. 1988a,b, Calderoni et al. 1992).

The zooplankton community structure before the pollution of the lake was described as normal, with seven species of cladocera (four of them being *Daphnia* sp.), five species of copepods and abundant presence of rotatorians (Bonacina et al. 1988a). The checklist included *Daphnia longispina* which was no longer found after the onset of pollution (Monti 1930), until the end of 1991 (i.e. one year after the liming intervention). In 1986, when the lake water was still very acidic, but with decreasing ammonia content due to the reduction of the main industrial loading, a small population of *Daphnia obtusa* (new for the lake) suddenly appeared (Bonacina 1988b). Its density increased rapidly but, after the liming, it disappeared from the samples for about one year (1990–1991). Now (1995), both *D. longispina* and *D. obtusa* are established in the lake, showing an irregular temporal distribution.

To understand the dynamics of both species in relation to pH variations and liming effects, life tables were followed at pH values of 5, 6, 7 before and after CaCO₃ addition, using filtered lake water as a reference medium.

In the present paper only results relating to animals survivorship and production of new-born individuals will be discussed. Results relating to growth rate and other measured demographic parameters will be published elsewhere.

**Materials and methods**

**Simulation of pre- and post-liming conditions**

About 100 L of lake water was sampled from the epiplankton and filtered through GF/C filters. pH adjustment of the filtered lake water (original pH = 6.3) to pH values 5 and 6 was carried out in 25 L containers, using 0.05 N HCl solution; pH value 7 was reached with a diluted solution of NaHCO₃-Na₂CO₃. The remaining filtered water was used as a reference without any addition. A standard solution of copper nitrate was added to each container in order to reach 40 g Cu L⁻¹ equivalent to the concentration in the lake before the liming (Calamari & Marchetti 1975). The containers were then left for two weeks at room temperature to stabilize. Finally, each sample was divided into two sub-samples forming two groups of pH 5, pH 6 and pH 7 media; to one group, natural limestone was added to reach a concentration of 10 mg L⁻¹ CaCO₃ simulating the liming process in the lake. During the experiment, chemical analysis were carried out on the media and on the reference lake water (APHA 1989).

**Daphnia acclimatization and experimental conditions**

Individuals of both *D. longispina* and *D. obtusa* were sampled from Lake Orta and reared separately in a 20°C indoor incubator under controlled 12:12h light and dark cycle. A food suspension of 4 × 10⁶ cells ml⁻¹ of *Selenastrum capricornutum* clone cultured in WC medium was centrifuged and resuspended in separate subsamples of the different media, and was then used to feed animals during rearing and experimental phases. The new-borns (aged 12 ± 12), obtained from the third generation of each species were isolated and distributed in beakers with different pH media and...
the reference medium (4 ind. 100 ml⁻¹). New-borns belonging to the second generation of the pH acclimatized animals were used in the study of life tables; five new-borns from each species-specific treatment were grown individually in 50 ml polycarbonate transparent vials. Both media and food suspensions were renewed 3 times per week. During the animals lifespan, observations on movement and egg development, measures of total length, counting of eggs and new-borns, and isolation of new-borns were carried out daily.

Results

Calcium carbonate addition led to a shift of the adjusted media toward neutral or alkaline levels (Table 1); a marked reduction in both N-NO₃, and Cu concentrations also took place in all media. Chemical analysis of the control medium (filtered lake water) for all elements except copper showed concentrations comparable to those measured in the pH 6 adjusted medium before CaCO₃ addition (Table 1).

No significant variations in the lifespans of both D. longispina and D. obtusa individuals were observed in control medium (Fig. 1A). However, new-born production of D. obtusa was significantly higher (av. = 92 new-borns adt⁻¹), in comparison with that obtained from D. longispina (Fig. 1B).

At all manipulated pH values D. longispina individuals died without reaching maturation (5–6 days) before CaCO₃ was added. The same result was obtained in two other attempts. However, after liming, 100% of the animals survived to maturity at pH values 5 and 6, while only 60% reached maturity at pH 7. Although the estimated 50% survivorship was higher at pH 6 (28 days), than at pH 5 and 7 (Fig. 2A), the highest number of new-borns (>40 new-borns adt⁻¹), was produced by animals grown at pH 5 (Fig. 2B). The regression analysis between pH after liming and new-born production of D. longispina was not significant (p = 0.325).

Table 1. Chemical analyses of the experimental media before and after CaCO₃ addition.

<table>
<thead>
<tr>
<th>Chemical parameters</th>
<th>Before CaCO₃ addition</th>
<th>After CaCO₃ addition</th>
<th>Control L. water</th>
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<tbody>
<tr>
<td></td>
<td>pH5</td>
<td>pH6</td>
<td>pH7</td>
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<tr>
<td>pH</td>
<td>4.97</td>
<td>6.04</td>
<td>7.19</td>
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<tr>
<td>Alk. meq L⁻¹</td>
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<td>Cond. μs L⁻¹</td>
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<td>119</td>
</tr>
<tr>
<td>N-NO₃ μg L⁻¹</td>
<td>3200</td>
<td>3200</td>
<td>3170</td>
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<tr>
<td>P-PO₄ μg L⁻¹</td>
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<td>1</td>
<td>1</td>
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<tr>
<td>Ca mg L⁻¹</td>
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<td>12.89</td>
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<tr>
<td>Cu μg L⁻¹</td>
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<tr>
<td>Al μg L⁻¹</td>
<td>28</td>
<td>27</td>
<td>23</td>
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</table>

Fig. 1. Control medium average survivorship (%) (A) and average new-born production (new-born adt⁻¹) (B) of the two species.
In *D. obtusa*, the percentage of individuals reaching maturity at different pH values was similar (80–100%) before and after CaCO₃ addition. Although the 50% survivorship at pH 5 without lime was lower (av. = 17 days) than that measured at pH 6 and 7 (av. = 27 and 32 days, respectively), no significant variations were observed in the limed media, irrespective of the different pH values (Fig. 3 A, B). The average new-born production at pH 6 before liming was higher (>100 new-borns adt.⁻¹) than at pH 5 and 7 (av. = 40 and 63 new-borns adt.⁻¹, Fig.
3 C). On the other hand, the average production values were negatively correlated ($r = -0.92, p < 0.0001$) with the experimental pH values after CaCO$_3$ addition.

Discussion

The observed viability and fertility of both species in control medium may reflect the present improvement in the quality of the lake water. The death of *D. longispina* at all pH values before lime addition can be attributed mainly to the toxic effect of the copper addition. In his review, Locke (1991) reported that pH 5 was critical for *D. longispina* with a previous history of pH 7. These two statements may help in understanding (1) the disappearance of *D. longispina* after the onset of pollution in 1926 (mean lake concentration of copper in 1954: 80 g L$^{-1}$, Picotti 1958), and (2) its reappearance after the lake was limed (mean lake pH at the end of 1991: 6, Bonacina 1992). Our results also confirm the toxic effect of copper on the viability of *D. longispina* even at pH 7. The insignificant variations in new-born numbers at different pH values after liming (Fig. 2B) may be a result of sensitivity to H$^+$ buffering and ionic balance effects (Locke 1991).

The observed low 50% survivorship of *D. obtusa* grown at pH 5 (Fig. 3A) may indicate the synergistic effect of both low pH and high copper concentration. Although *D. obtusa* is known to be resistant to low pH values (Pruter 1985), our results indicate that pH 5 may still be critical for its fertility and that pH 6 is the optimum, even in the presence of copper pollution (Fig. 3B).

The negative correlation between new-born numbers and the experimental pH values after lime addition may indicate the inability of Lake Orta's *D. obtusa* clone to live in neutralized conditions. This may also explain its disappearance after liming and its recolonization one year later when the lake returned to be only slightly acidic (Table 1). In their study on the genetic composition of various *D. obtusa* clones, Bachiiori et al. (1991) have concluded that the Lake Orta clone is more successful in colonizing an environment which is toxic for other populations of the same species. One of the authors (Rossi pers. comm.) also found that the Lake Orta clone showed a significant drop in new-born production in Lake Maggiori water (slightly alkaline).

In the light of our results, we may attribute the present variability in the densities of both *D. longispina* and *D. obtusa* populations in Lake Orta (unpubl. data) to interspecific competition resulting from species-specific sensitivity to the continuous variations in lake water chemistry. Other reasons, such as fish or invertebrate predation effects (Havens 1991), and/or food supply and its composition (Stenson & Svensson 1994) cannot be ignored as secondary reasons.

References


Calamari, D. & Marchetti, R., 1975: Predicted and observed acute toxicity of copper and ammonia to rainbow trout (Salmo gairdneri Rich.). — Prog. Wat. Tech. 7: 569–577.


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