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Short research contribution

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SURVIVAL OF PIGMENTED FRESHWATER ZOOPLANKTON, EXPOSED TO ARTIFICIAL ULTRAVIOLET RADIATION AND TWO LEVELS OF DISSOLVED ORGANIC CARBON

ABSTRACT: The increase in penetration of ultraviolet radiation has been reported currently over southern South America. It would affect the photoprotective responses in biotic elements of freshwater ecosystems. An experiment was designed using pigmented adult individuals of the species Daphnia dadayana (Paggi 1999), Boeckella antiqua (Menu-Marque & Balseiro 2000), and Parabroteas sarsi (Mrázek 1901), characteristic for shallow fishless ponds from South American plains (located between 41 and 53°S latitude). The artificial ultraviolet radiation was involved in the experiment, with two levels of dissolved organic carbon (DOC), which would exert a protective effect against ultraviolet radiation. The results of this experiment showed that under conditions of low DOC concentrations, D. dadayana recorded a higher mortality, while under low DOC concentrations, B. antiqua and P. sarsi were not affected by ultraviolet radiation. Daphnids would be less tolerant to the exposure to ultraviolet radiation than pigmented calanoid copepods.

KEY WORDS: ultraviolet radiation, dissolved organic carbon, zooplankton, daphnids, calanoids

Zooplankton assemblages in southern South America lacustrine ecosystems are characterized by a marked predominance and broad distribution of calanoid copepods, mainly of the Boeckella genus, over daphnid cladocerans; this situation is opposed to that recorded for North America, where daphnids tend to dominate (Soto and Zúñiga 1991, Gillooly and Dodson 2000). Also, at the same time, a singificant increase in the incidence of ultraviolet radiation as an apparent consequence of a decrease in the ozone layer has been reported recently, like in the Patagonia in the 1990s (Villafañe et al. 2001). A natural photoprotective resource against ultraviolet radiation, is dissolved organic carbon, which can absorb different wavelengths of the ultraviolet spectrum (Morris et al. 1995). The existence of fishless shallow ponds, with relatively moderate and high concentrations of dissolved organic carbon, has been reported mainly for the Patagonia in Argentina (Morris et al. 1995) and southern Chile (De los Ríos 2003). Zooplanktonic assemblages in these ponds, are characterized by a marked predominance of calanoids over daphnids, both groups tend to be larger in size, and strongly pigmented with carotenoids or micosporine like aminoacids for calanoids, or melanine for daphnids (Zagarese et al. 1997, Villafañe et al. 2001, De los Ríos 2003). All the previous arguments suggest that daphnids are less

abundant due to their lower tolerance to UV radiation in comparison to calanoids.

The present study used the species Boeckella antiqua (Menu-Marque & Balseiro 2000), Daphnia dadayana (Paggi 1999), and Parabroteas sarsi (Mrázek 1901), characteristic of fishless shallow ponds in the Southern Patagonia in Chile and Argentina, whith variable contents of dissolved organic carbon (Morris et al. 1995). Zooplankton samples were collected from a temporary pond located in Rio Negro province, Argentina (Juncos pond, 41º04'S, 71º00'W, Menu-Marque and Balseiro 2000). The zooplankton collected was acclimatized for 48 hours at 5°C under 12:12 hrs light:dark photoperiod, by means of two 120 cm fluorescent tubes (Philips daylight TLT 40W/54RS); these organisms were acclimated in the original water source.

The experiment was performed at the Photobiology Laboratory, Universidad Nacional del Comahue, Centro Regional Universitario de Bariloche (Río Negro Province, Argentina). Water with different levels of dissolved organic carbon (DOC) was collected from two lakes: Escondido and Gutiérrez (Table 1, Morris *et al.* 1995, Zagarese unpublished data). Except DOC concentrations, both sites have similar chemical composition (Pedrozo *et al.* 1993). The water collected was filtered through an Apstein mesh, 60 µm pore size, to eliminate the larger zooplanktonic individuals.

The experiment was set up in triplicate (n = 18), in Petri dishes (50 mm diameter and 20 mm high), filled with 20 ml of water and exposed to ultraviolet light from a fluorescent lamp "Spectroline XX15-B"(Spectronic corporation). The lamp was covered with a diacetate cellulose plate that removed wavelengths below 295 nm, and was kept 40 cm away from the experimental dishes. The light included within the visible spectrum, came from two 120 cm fluorescent tubes (Philips daylight TLT 40W/54RS). The exposure to these radiation fractions would generate photo-protective responses against ultraviolet radiation (Zagarese et al. 1997). Photoperiod conditions were: 24 h light of visible radiation and 10 h of ultraviolet exposure at 8°C constant temperature. Petri dishes were filled with different solutions of dissolved organic carbon, and inoculated with 20 adult individuals. According to other similar experiments (Zagarese et al. 1997), this inoculation density was found to prevent densodependent effects on organisms. The experiment was set up at 18:00 hrs. and finished 24 hours later when dead individuals per treatment were counted. All individuals used were pigmented, the kind of pigmentation in daphnids is located in their caparace (Hebert and Emery 1990), whereas the pigmented area in copepods is distributed along the whole body (Rocco et al. 2002).

Due to the lack of normality (Kolmogorov-Smirnov test) and homocedasticity (Bartlet test) conditions, non-parametric tests were applied 1) to detect the effect of the two levels of dissolved organic carbon on the mortality of each zooplanktonic species under ultraviolet radiation exposure using Mann-Witney's test; 2) to check the mortality of each of the three species under high and low concentration of dissolved organic carbon using Kruskall-Wallis's test. All statistical analyses mentioned above were done with Statistica version 5.0 software. In the presence of statistical significant differences among the three species, Tukey's non parametric test of multiple comparisons described by Zar (1999), was used.

According to the first statistic analysis, considering each one of the three species kept in two levels of dissolved organic carbon,

Table 1. Dissolved organic carbon concentration and absorption coefficients for wavelengths within the ultraviolet spectrum observed for lakes Escondido and Gutiérrez (according to Morris *et al.* 1995)

Site	DOC (mg l ⁻¹)	A ₃₀₅	A ₃₂₀	A ₃₄₀	A ₃₈₀	A ₄₄₀
Lake Escondido	2.66	7.32	6.47	4.51	1.91	0.67
Lake Gutiérrez	0.32	0.32	0.71	0.60	0.46	0.11

mortality values were significantly different only for *D. dadayana* and *B. antiqua* (Table 2). Once the second analysis was applied, the significant differences were found in the mortality values of the three species under conditions of low ($\chi^2 = 7.623$, P = 0.022) and high levels of dissolved organic carbon (χ^2 = 6.230, P = 0.044). Results for Tukey's multiple comparisons test, for the treatment with low concentration of dissolved organic carbon, showed that mortality among D. dadayana was significantly higher than for two other species (Tables 2 and 3). Finally, in the treatment with high level of dissolved organic carbon, although *B. antiqua* had higher mortality in comparison to the other two species, the mortality was lower in general for all studied species (Tables 2 and 3).

The results obtained in the experiment (Tables 2 and 3), denoted clearly that the dissolved organic carbon is a zooplanktonic protective measure, against ultraviolet radiation, specially in case of daphnid cladocerans. The species used in the experiment belong to shallow ponds, with a wide range of dissolved organic carbon concentration (Morris *et al.* 1995). Facing this scenario,

where ultraviolet radiation can virtually penetrate the entire water column, zooplankton groups tend to develop photoprotective pigments as an adaptative response (Villafañe et al. 2001). Nevertheless, D. dadayana specimens used in this study, presented a higher mortality under conditions of low concentration of dissolved organic carbon (Table 3). These results would most likely indicate that this group has a lower tolerance to high levels of ultraviolet radiation than calanoid copepods. A possible cause is that daphnid cladocerans would need more energetic resources under the exposition to UV radiation (Hessen et al. 1999); during the experiment the food was not supplied, according to similar procedures described by Zagarese et al. (1997) and De los Ríos (2004). The literature, described that daphnids have low tolerance to starvation in comparison to calanoids (Sterner and Hessen 1994) Then in conditions of low food quantity and exposition to ultraviolet radiation it is very probable that the survival of daphnids would be low, whereas the calanoids have high survival (De los Ríos 2003). In spite of the the differences observed in the treatment of

Table 2. Mean number of dead animals (n = 6) after 24 hours of the experiment. 20 individuals were introduced at the start of the experiment; DOC concentrations see Table 1.

Species	Р	Low DOC	High DOC
Daphnia dadayana	< 0.01	7.33 ± 2.40	0.33 ± 0.33
Boeckella antiqua	< 0.05	0.00 ± 0.00	1.67 ± 0.33
Parabroteas sarsi		0.00 ± 0.00	0.00 ± 0.00

Table 3. Results of Tukey's multiple comparisons test for the pair of species ("Q" values higher than 4.473 indicate significant differences).

DOC concentration ¹	Species	"Q" observed
High	D. dadayana > P. sarsi	8.033
High	D. dadayana – B. antiqua	2.556
High	B. antiqua > P. sarsi	10.589
Low	D. dadayana > P. sarsi	9.859
Low	P. sarsi – B. antiqua	0.000
Low	D. dadayana > B. antiqua	9.859

¹See Table 1

high dissolved organic carbon concentration (Table 3), the mortality was relatively low and similar for all species (Table 2). This result supported the hypothesis of protective role of dissolved organic carbon against ultraviolet radiation described in the literature (Morris et al. 1995, De los Ríos 2004). Then, the notorious predominance of calanoids in zooplankton in freshwater ecosystems in southern Chile (Soto and Zuñiga 1991, Gillooly and Dodson 2000), is enhanced also because this group is most tolerant to exposition to ultraviolet radiation in comparison to daphnid cladocerans (De los Ríos 2003) These results would support the results obtained in the present study.

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