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Interactive effects of temperature, ultraviolet radiation and food quality on zooplankton alkaline phosphatase activity☆

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ABSTRACT

Ultraviolet Radiation (UVR) is a stressor for aquatic organisms affecting enzyme activities in planktonic populations because of the increase in reactive oxygen species. In addition, UVR exposure combined with other environmental factors (i.e. temperature and food quality) could have even higher detrimental effects. In this work, we aimed to determine the effect of UVR on somatic Alkaline Phosphatase Activity (APA) and Glutathione *S*-Transferase (GST) activity on the cladoceran *Daphnia commutata* under two different temperatures (10 °C and 20 °C) and under three food qualities (carbon:phosphorus ratios: 1150, 850 and 550). APA is a biomarker that is considered as a P deficiency indicator in zooplankton. Since recovery from UVR damage under dark conditions is an ATP depending reaction we also measured APA during recovery phases. We carried out a laboratory experiment combining different temperatures and food qualities with exposition to UVR followed by luminic and dark phases for recovery. In addition, we exposed organisms to H_2O_2, to establish if the response on APA to UVR was a consequence of the reactive oxygen species produced these short wavelengths. Our results showed that somatic APA was negatively affected by UVR exposure and this effect was enhanced under high temperature and low food quality. Consistently, GST activity was higher when exposed to UVR under both temperatures. The H_2O_2 experiments showed the same trend as UVR exposure, indicating that APA is affected mainly by oxidative stress than by direct effect of UVR on the enzyme. Finally, APA was affected in the dark phase of recovery confirming the P demands. These results enlighten the importance of food quality in the interacting effect of UVR and temperature, showing that C:P food ratio could determine the success or failure of zooplanktonic populations in a context of global change.

1. Introduction

Aquatic ecosystems are being affected by increasing temperature and ultraviolet radiation (UVR) (Häder et al., 2007, 2015; Zepp et al., 2003). These changes modify light intensity and nutrient inputs to aquatic ecosystems that consequently affect the C:P ratio (carbon:phosphorus) of primary producers (Sterner et al., 1997), hence, the food quality for zooplankton (Dobberfuhl and Elser, 2000; Elser et al., 2000). In that context, organisms with high nutrient content (like *Daphnia* needs high P food) are more susceptible to low food qualities (Sterner et al., 1993), and well-nourished animals will cope with stressors more efficiently than those that are nutrient-depleted (Balseiro et al., 2008).

Herbivorous zooplankton constitutes an important link in aquatic food webs, because they transfer nutrients from primary producers to higher consumers (Carpenter et al., 2001; McQueen et al., 1989; Sterner and Elser, 2002). Nutrient uptake by zooplankton can be linked to some enzymatic activities, for example P acquisition is closely related to Alkaline Phosphatase (AP). AP is a prevalent enzyme in aquatic environments commonly found attached to cell surface or in the dissolved fraction (Tank et al., 2005). This enzyme releases phosphates from macromolecules allowing P transportation and sub-cellular uptake from digested food (Elser et al., 2010; Wojewodzic et al., 2011). AP activity (APA) may be considered as an indicator of P deficiency in zooplankton (Elser et al., 2010; McCarthy et al., 2010; Wagner and Frost, 2012) because its activity increases under P-depleted food

Abbreviations: APA, Alkaline Phosphatase Activity; GST, Glutathine *S*-Transferase; UVR, Ultraviolet Radiation; PAR, Photoenzymatic Active Radiation; PER, Photoenzymatic Recovery; NER, Nucleotide Excision Repair.

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and in the presence of other stressors such as temperature that can affect the nutritional requirements (Persson et al., 2011).

UVR was observed to affect enzyme activities in planktonic populations because of the increase in reactive oxygen species (ROS) (Barata et al., 2005a; Hessen, 1993; Souza et al., 2010). Antioxidant enzymes as Glutathione *S*-transferase (GST) represent a major defense mechanism against ROS, inactivating secondary metabolites during oxidative stress (Hayes et al., 2005). In addition, GST reduces lipid hydroperoxides generated during UVR oxidative stress (Collinson and Grant, 2003; Hurst et al., 1998) that lead to membrane destruction (Cho et al., 2000). On the other hand, important enzymes may be negatively affected by UVR, i.e. extracelular APA was observed to diminish under UVR exposure in algae (Li et al., 2013; Tank et al., 2005). It would be expected that membrane-anchored AP has a refuge from UVR inside the animal body, but non photoprotected animals, like *Daphnia commutata*, may not exert enough protection to AP from hazardous short wavelength. Cladocerans cannot take advantage from photoprotective compounds such as microsporin-like aminoacids (Siebeck et al., 1994) thus other mechanisms such as photorepair become very important for animal success in transparent UVR environments. On the other hand, if the main effect of UVR on APA is through the generation of ROS, then photoprotective compounds would have a minor effect.

To deal with hazardous effects of UVR on DNA, organisms exhibit two alternative mechanisms: Photo-Enzymatic Repair (PER) and Nucleotide Excision Repair (NER) (Essen and Klar, 2006). The main difference between both mechanisms is that PER does not demand energy from the animal while NER does, requiring ATP. PER acts under light between 380 and 400 nm (Black light), activating the enzyme photolyase that reverses DNA photoproducts (Mitchell and Karentz, 1993; Sutherland, 1981). NER acts under dark conditions and is a multi-enzymatic reaction depending on ATP supply that also reverses UV-induced DNA damage (Sancar, 1996). Because of the differences in P demand between both mechanisms it could be expected differences in APA.

Andean lakes are ultra-oligotrophic, highly transparent and with very low nutrient concentrations, in particular P (Markert et al., 1997; Modenutti et al., 2000; Morris et al., 1995). Moreover, Northern Patagonian Andean lakes exhibit high UVR penetration due to their low dissolved organic matter content (Modenutti et al., 2013; Morris et al., 1995); lack of aerial pollutants (Mladenov et al., 2011) and proximity to the ozone layer depletion area (Villafañe et al., 2001). Furthermore, this area is also likely to be affected by increasing temperature in the next century (Barros et al., 2015). In this area, P-demanding organisms, like *Daphnia* are distributed according to the C:P ratio of their phytoplanktonic food (Balseiro et al., 2007) and their enzymatic responses to UVR stress are constrained by nutrient availability (Balseiro et al., 2008).

Temperature, UVR exposure and food quality are important stressors affecting *Daphnia* success, however the combined effect of these environmental factors would interact leading to the possibility of synergic or antagonistic responses in APA. In this sense here we aimed to analyze how *D. commutata* respond to different stressor combinations using APA as a proxy of their P requirement and GST as an oxidative stress biomarker. For this purpose we carried out an experimental laboratory design in which *D. commutata* was grown under different controlled conditions of food quality (C:P ratio), temperature and UV light simulating Andean lakes. Based on Balseiro et al. (2008) and Persson et al. (2011) we hypothesize that *Daphnia* growing under high C:P ratios and high temperature will be more affected by UVR, where APA should increase under these conditions and GST decrease. But at the same time, APA may not respond linearly to P requirements.

2. Methods

We used a clonal zooplankton population of *D. commutata*, started from a single female isolated from lake Mascardi (Nahuel Huapi National Park, Patagonia, Argentina). The clone was maintained under laboratory conditions, at 15 °C at least 5 years prior the experiment, and fed with *Chlamydomonas reinhartii* culture.

2.1. Experimental design

We conducted a UVR exposure experiment (UVR + PAR, 290–700 nm hereon UVR vs. photosynthetically active radiation + black light, hereon PAR, 380–700 nm exposure) under two different temperature treatments (10 °C and 20 °C) and in three different food qualities: high, with low C:P ratio = 550; medium, with C:P ratio = 850 and low, with high C:P ratio = 1150. Previous to starting the experiment, we acclimated *D. commutata* for 6 days under the two temperatures and the three food qualities. We used six days of acclimation based on previous studies (Hessen et al., 2002). 20 *D. commutata* neonates (<24 h old) were arrayed in 250 mL experimental quartz flasks filled with 250 mL of P-free COMBO medium (Kilham et al., 1998) and *C. reinhartii* as food (0.5 mg L^{-1} of C), with the corresponding food quality (high, medium and low, see above). COMBO medium and food were replaced every 48 h. Concentrations of total particulate P and C of food were also monitored every 48 h with standard analyses before adding new food to flasks (see below "*Biochemical determinations*"). The acclimation comprised a total of 36 flaks (3 replicates for each treatment) and was conducted in a walk-in culture chamber for both temperatures and under PAR (400–700 nm wave band and intensity of 92 μmol m^{-2} s^{-1}) with 14:10 (light:darkness) photoperiod. All glassware was carefully cleaned and sterilized. During the whole experiment no *Daphnia* mortality was observed.

After the 6 days of acclimation, half of the quartz flasks (randomly selected) were exposed to UVR while the other half to PAR protected with a new sheet of cut-off filters to remove wavelengths shorter than 400 nm (Courtgard™, CPFilms (Doyle et al., 2005)) during 4 h. After the 4 h of UVR exposure, we disposed the flasks to 6 h of photorecovery radiation (PER, 380–700 nm) and afterward 6 h under dark conditions (NER).

Temperatures and the levels of food quality used in our experiments are based on Balseiro et al. (2007) and Laspoumaderes et al. (2013) in particular in the epilimnion of Lake Mascardi where *Daphnia commutata* lives. Andean deep lakes are cold environments in which is very infrequent that temperatures exceed 16 °C in the warmest seasons. For that reason, we consider that an increase in the epilimnion to 20 °C is sufficiently higher and it is in accordance with global change predictions (IPCC, 2014).

2.2. Light features

Light source was provided by two UVA340 fluorescent lamps (Q-Panel Lab Products), two daylight fluorescent lamps (Philips TLT 40 W) and two black-light fluorescent lamps (UVA340 lamp from Q-Panel Lab Products, with maximum emission at 380 nm) The UV spectrum of the UVA340 light closely resembles the solar spectrum between 280 nm and 350 nm (Shick et al., 1999). The black light was included to fill the gap between maximum emission of UVA (340 nm) and the daylight fluorescent lamps (400 nm) (Fig. 1). During the incubation, animals received 35 μW cm^{-2} nm^{-1} of 340 nm wave band, an irradiance level of the wave band that is equivalent to surface sunlight in Andean lakes during summer (Modenutti et al., 2005). The total 340-nm wave-band dose was of 5040 J m^2 s^{-1}.

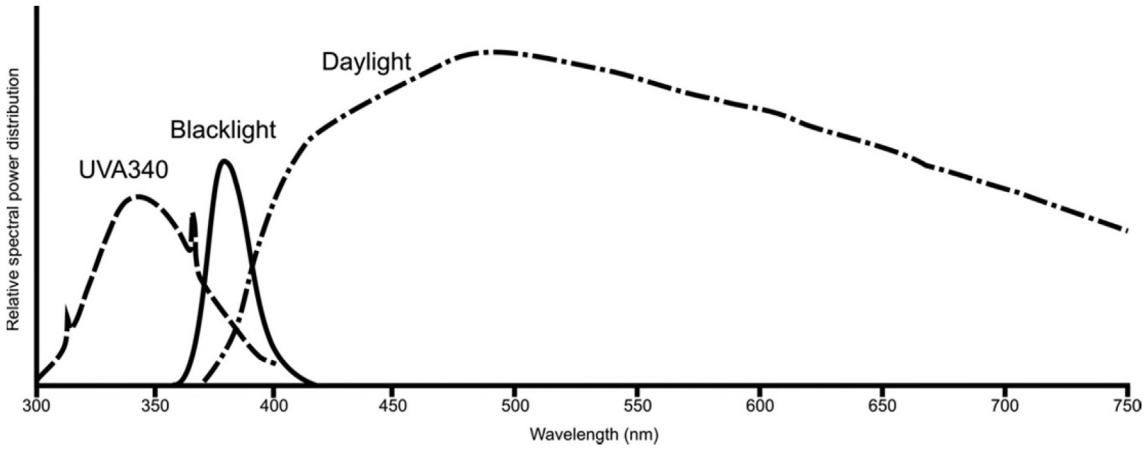

Fig. 1. Relative spectral power distribution of the experimental light panels according to their wavelength emission in nanometers and manufacture's specifications. The first curve (from 300 to 400 nm) represents UVR relative doses, the second curve (from 350 to 410 nm) represents black light relative doses and the third curve (from 400 to 750) represents daylight (PAR) relative doses.

2.3. Algal culture

Food for *Daphnia* experiments was obtained from *Chlamydomonas reinardtii* algal cultures. To obtain different C:P ratios we used low-P MBL culture medium (Guillard and Lorenzen, 1972) (10 folds less than the original P concentration). Low food quality (C:P ratio = 1150) was obtained directly from the low-P MBL medium. The other two food qualities (C:P medium and high) were obtained following Persson et al. (2011) with a P spike, adding two different amounts of K_2HPO_4 to the algal batches of P-depleted algae allowing the algae to assimilate the P during 1 h in the dark.

After this procedure (no P spike or P spike), algal cultures were centrifuged at 13,000 x g, and the supernatant was discharged. Then the pellet was re-suspend with P free COMBO and used to feed *Daphnia* and also for particulate C and P analyses. Based on C concetration we added sufficient algal culture to reach a concentration of 0.5 mg C L^{-1} of *C. reinhartii* (similar concentration to that of Lake Mascardi) to each 250 mL experimental flasks containing *D. commutata* in P-free COMBO.

2.4. Treatments sampling

After the 4 h of UVR and PAR exposure we sampled 8 individuals from each replicate of the different treatments. All sampled individuals were immediately frozen at −80 °C for further APA and GST determinations. Before biochemical determinations each individual was rinsed with Milli-Q water and measured by taking lateral images and then processing the image via Image-Pro Plus, (Media Cybernetics) software, following Acharya et al. (2004) and Balseiro et al. (2007). These measurements were converted to dry weight (dry wt) based on the length–weight regression obtained from Balseiro et al. (2008) and finally determined growth of individuals exposed to different food qualities and temperature. After each period of UVR recovery we sampled four animals from each replicate to determine APA.

2.5. Oxidative stress on APA

We conducted an experiment in which *D. commutata* was exposed to hydrogen peroxide (H_2O_2) for 4 h in order to determine the effect of oxidative stress on GST and APA. A direct effect would result from the damage on APA by the short wavelengths of UVR itself (Li et al., 2013; Tank et al., 2005), while the indirect one from the damage on APA caused by UVR generated ROS. To disentangle these two possible effects, we analyzed APA in organisms exposed to H_2O_2 in a concentration of ~1000 nM, based on Rusak et al. (2006) and Meinertz et al. (2008). This concentration was equivalent to the UVR doses of our previous experiment (see *Light features*) (Rusak et al., 2006) and was considered not lethal for *Daphnia* (Meinertz et al., 2008). The concentration of H_2O_2 at the beginning of the experiment was 975 nM and after 2 h 650 nM (in 4 h most of the H_2O_2 was reduced). Because of the decay of H_2O_2, we only showed the results for 2 h of exposition. The experiment was run in two different food qualities (medium: C:P ratio = 850 and low: C:P ratio = 1150) and in two treatments with and without H_2O_2. Previous to starting the experiment, we acclimated *D. commutata* under the two food qualities during 6 days. The experiment was run in darkness at 20 °C. 10 *D. commutata* neonates (<24 h old) were arrayed in 50 mL experimental flasks in the same condition as the UVR exposure experiment. After the 2 h of experimentation (with and without H_2O_2) we collected the *Daphnia* with the same procedure as the UVR experiment.

2.6. Biochemical determinations

Carbon from *C. reinhartii* was analyzed with a CHN elemental analyzer (Thermo Finnigan EA1112, Thermo Fisher, Milan, Italy). Total phosphorus from *C. reinhartii* was analyzed with persulfate digestion followed by molybdate reaction (APHA, 2005). All determinations were carried out in at least three replicates.

APA was determined following Wagner and Frost (2012) with 1 specimen per measure. Each *D. commutata* was homogenized with an Ultrasonic Homogenizer (Sartorius, LABSONIC M, with 0.6 Cycles, 80% of amplitude and 2 mm Ø point) in 120 μL buffer solution (Tris pH = 8) and centrifuged at 13,000 x g for 10 min at 4 °C to obtain the supernatant source of enzyme. APA was determined fluorometrically (Perkin–Elmer LS45) with the excitation 360 nm/10 nm and emission 420 nm/10 nm and for each sample 100 μL of 4-Methylumbelliferyl Phosphate (MUP) 0.05 μM as substrate. APA was expressed in μmol of Methylumbelliferone (MU) per minute per mg of animal dry wt.

GST activities were determined according to Habig et al. (1974) in 0.1 mol L^{-1} phosphate buffer (pH 6.5), with 0.1 mg mL^{-1} 1-chloro-2.4-dinitrobenzene in acetonitrile (1% v/v) and 0.75 mg mL^{-1} L-glutathione reduced as substrates recording the absorbance at 340 nm, using a Shimadzu 2450 spectrophotometer

at 23 ± 0.5 °C. GST activity was expressed in μmoles of product developed per minute per mg of animal dry wt.

2.7. Statistical analyses

Differences in APA and GST activity from *D. commutata* exposed to UVR and PAR in the different temperatures and food treatments were tested with a three way ANOVA (factor A: temperature, factor B: food quality, factor C: light). To analyze the subsequent recovery phases, we performed a two-way ANOVA test (factor A: temperature, factor B: food quality) for each recovery phase (PER and NER). When significant, after the ANOVA we run *a posteriori* multiple comparison *Tukeýs HSD* test procedure (= 0.05). In order to evaluate the effect of the oxidative stress experiment (with and without H_2O_2 exposure) we run a two-way ANOVA test.

Somatic growth was analyzed also by a two way ANOVA test (factor A: temperature, factor B: food quality) followed by a Holm-Sidak method, *a posteriori* multiple comparison procedure (= 0.05). All statistical analyses were performed in Sigma Plot 12.5 (Systat Software Inc., San Jose, CA, USA). All data were checked for normality and homocedasticity. Somatic growth was log-transformed in order to fit a normal distribution.

3. Results

3.1. Somatic growth

Somatic growth was affected by the interaction of temperature and food quality (ANOVA; $F_{2, 192} = 6.027$ $p = 0.003$; Fig. 2). *Daphnia* dry mass was higher at 20 °C (Holm-sidak method: $p < 0.001$) than at 10 °C for all food quality levels. At 20 °C we observed significant differences between food quality levels, low food quality had significantly lower biomass than medium and high ones (Holm-Sidak method: $p < 0.001$). In contrast, at 10 °C biomasses were similar for all levels of food quality (no significant differences were observed) (Fig. 2).

3.2. UVR experiment

During the 4 h of UVR exposure we observed a significant decrease in APA relative to APA in PAR treatment (control). In addition, temperature also affected APA response being higher at 20 °C (three-way ANOVA, $F_{1, 62} = 26.806$ $p < 0.001$; Fig. 3). However, food quality though significant (three-way ANOVA, $F_{2, 62} = 7.064$ $p = 0.002$; Fig. 3) did not respond monotonically to temperature. At low temperature (10 °C) APA decreased as food quality increased, at 20 °C the highest response in APA was observed in medium food quality (*a posteriori* Tukey test $p < 0.001$), while low food quality showed the lowest response (*a posteriori* Tukey test $p < 0.001$; Fig. 3b).

GST activity exhibited significant differences between light treatments only at 20 °C (two-way ANOVA $F_{1, 36} = 6.507$ $p = 0.015$; Fig. 4) being higher under UVR in food quality treatments. In addition, at 20 °C we observed a trend of higher GST activity under higher food quality, with significant differences between low quality and medium and high food qualities (Fig. 4b). Interestingly, temperature resulted with significant differences in UVR exposure but not under PAR.

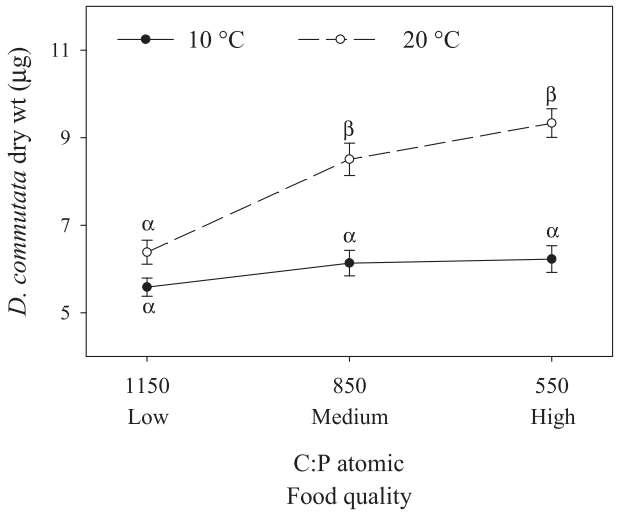

Fig. 2. Somatic growth. D. commutata somatic growth under the three food qualities (Low: C:P 1150, Medium: C:P 850, High: C:P 550) and two temperatures (10 °C and 20 °C). Error bars represent one standard error.

3.3. UVR recovery

After the first period of UVR recovery, APA did not change from the values obtained after UVR exposure (Fig. 5). In contrast, after the second period of UVR recovery we observed that the overall response of APA at 20 °C resulted higher than at 10 °C (two-way ANOVA $F_{1, 21} = 60.950$ $p < 0.001$; Fig. 5c and d). At 20 °C the treatment of medium food quality showed the highest response, and low food quality the lowest one (Fig. 5d), although the humped fit curve resulted not significant the interaction food quality X temperature was significant (two-way ANOVA $F_{2, 21} = 12.453$ $p < 0.001$).

3.4. Oxidative stress on APA

During the 2 h of exposure at 20 °C we observed that the activity of the biomarker GST increased in the two food treatments in the presence of H_2O_2 (two-way ANOVA $F_{1, 17} = 47.026$ $p < 0.001$; Fig. 6a), whereas APA was observed to decrease (two-way ANOVA $F_{1, 22} = 46.757$ $p < 0.001$; Fig. 6b). However, the response in APA resulted higher in the medium food quality treatment (C:P 850) (two-way ANOVA $F_{1, 22} = 60.068$ $p < 0.001$; Fig. 6b).

4. Discussion

In aquatic ecosystems, UVR is recognized as an important biological stressor that causes cell damage of important molecules (DNA, proteins, and lipids), apoptosis and oxidative stress (Martindale and Holbrook, 2002; Souza et al., 2012), altering reduced gluthathione levels and increasing the activity of enzymes as Catalase, Gluthathione Reductase and GST among others (Barata et al., 2005; Borgeraas and Hessen, 2000). The increase in these antioxidant defenses imply high energy demands and requires higher food quality for an adequate response (Balseiro et al., 2008; Souza et al., 2010). In addition, ROS production increases at higher temperatures (Lesser, 1996). Accordingly, our results showed that higher GST activity was observed at 20 °C and at high food quality (Fig. 4). In contrast, under low food quality (C:P 1150) GST exhibited comparatively lower values suggesting that *Daphnia* was highly P-limited. The same trend was observed in our experiment where *Daphnia* was exposed to H_2O_2 (Fig. 6) confirming that GST responded with low activity to oxidative stress under low food quality. Oxidative stress may affect other parameters as malondialdehyde or reduced gluthathione levels however our study was limited to GST activity.

APA was observed to respond negatively under both H_2O_2 (Fig. 6) and UVR exposure (Fig. 3). Although, absolute values of APA

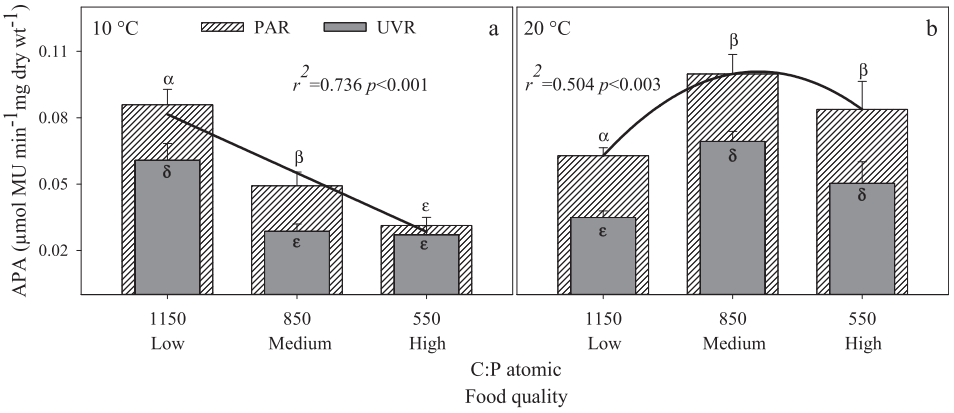

Fig. 3. APA under UVR exposure. D. commutata APA response to UVR exposure vs. PAR exposure in the three food qualities (Low: C:P 1150, Medium: C:P 850, High: C:P 550), under both temperatures at 10 °C (a) and 20 °C (b). Error bars represent one standard error.

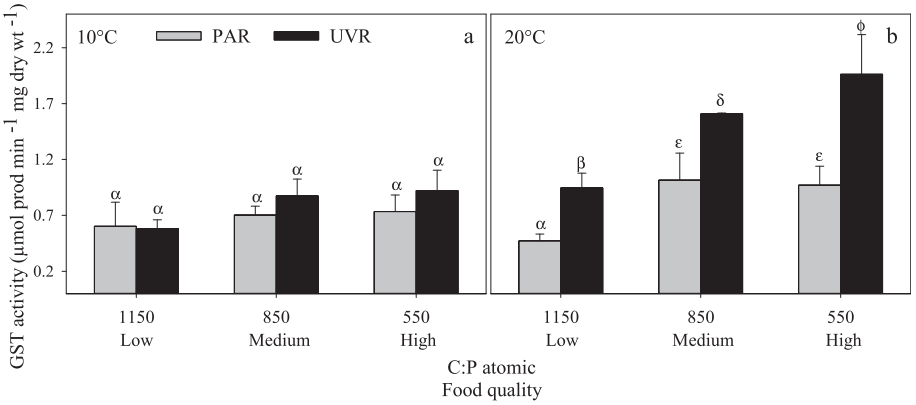

Fig. 4. GST activity under UVR exposure. GST activity from D. commutata in each food quality (Low: C:P 1150, Medium: C:P 850, High: C:P 550), after UVR and PAR exposures, under both temperatures at 10 °C (a) and at 20 °C (b). Error bars represent one standard error.

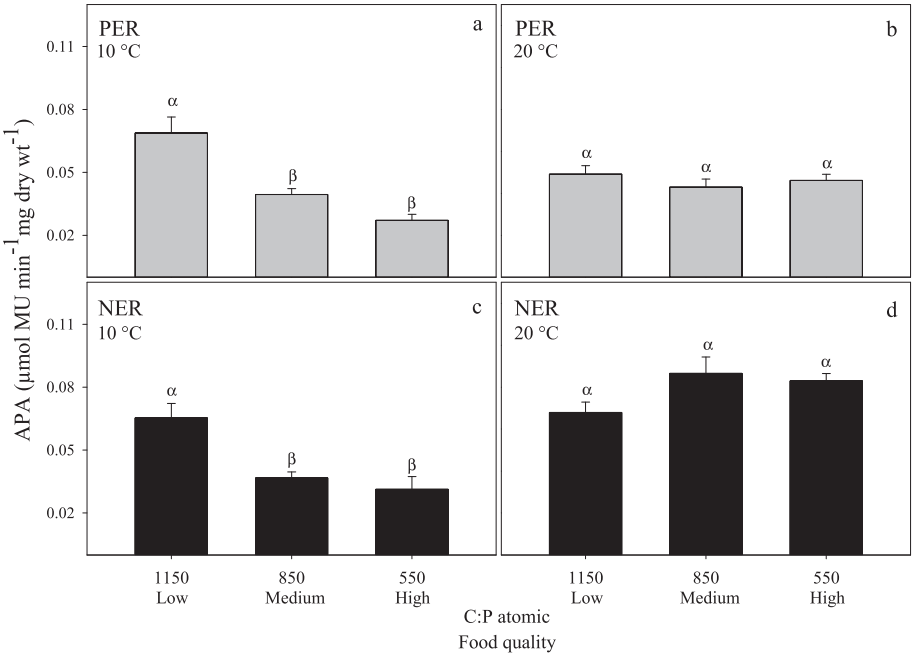

Fig. 5. APA under recovery phases. Response of D. commutata APA after recovery phases (PER and NER) in the three food qualities (Low: C:P 1150, Medium: C:P 850, High: C:P 550). PER phase at 10 °C (a) and 20 °C (b) and NER at 10 °C (c) and 20 °C (d). Error bars represent one standard error.

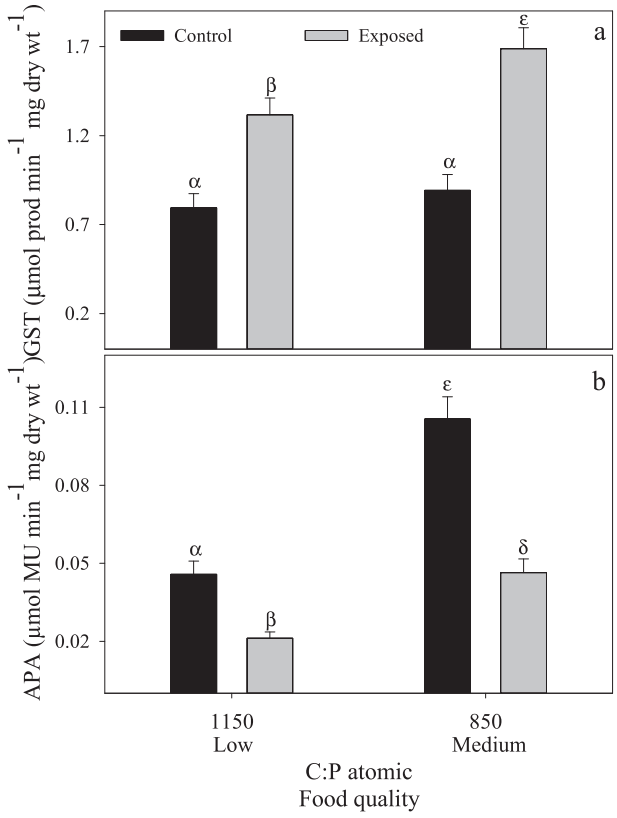

Fig. 6. GST and APA under H_2O_2 exposure. (a) GST activity after 2 h of H_2O_2 exposure vs. control without H_2O_2 in D. commutata under low and medium food quality, at 20 °C. (b) APA after 2 h of H_2O_2 exposure vs. control without H_2O_2 in D. commutata under low and medium food quality, at 20 °C. Error bars represent one standard error.

were quite different under UVR and H_2O_2, the r response to food quality in both experiments at 20 °C resulted similar. We observed that the response of *D. commutata*'s APA depended on the C:P ratio of the food with a significant increase in the 20 °C treatment compared to the 10 °C (Fig. 4). High temperature increases nutrient (P) limitation because it would imply higher nutrient requirements to maintain metabolism and growth (Persson et al., 2011) and to provide more energy for increased locomotion, respiration and life history processes (Wojtal-Frankiewicz, 2012). On the other hand, at 10 °C we observed that APA increased under low food quality, confirming that APA responds monotonically to food quality since APA decreases as food quality increases. Under low temperatures, low nutrient requirements were observed for somatic growth (Rhee and Gotham, 1981) and no changes in UVR susceptibility (Borgeraas and Hessen, 2000). Therefore, the response of APA under low food quality (C:P 1150) seemed to be sufficient for the P requirements at 10 °C with a negative relationship between APA and P concentration, as expected. However, at 20 °C APA showed a different trend with lower values at low food quality than at medium or high food qualities. In addition, we observed the same trend in our experiment of H_2O_2 exposure that was run at 20 °C (Fig. 6). In a study with *Daphnia magna*, Wojewodzic et al. (2011) indicated that APA was higher at lower temperatures, however in our study APA resulted higher at the higher temperature. Persson et al. (2011) showed that P limitation increases with temperature so, it is likely to assume that individuals at higher temperature are more P limited and would exhibit higher APA, as it was observed in this study.

Poor algal food (low P content) reduces the dietary mineral P available for *Daphnia*, thus, somatic APA can be used as a proxy of P-stress (McCarthy et al., 2010). P-nutrition also regulates the amount of AP that is released into the water and McCarthy et al. (2010) showed that reduced AP loss would be advantageous for P-limited *Daphnia*. So under P-starvation the extracellular APA is reduced and the concentration of membrane-anchored AP is higher than in well fed animals (McCarthy et al., 2010). In our experiments higher somatic APA was observed under low food quality at 10 °C, but not at 20 °C. Persson et al. (2011) showed that P limitation increases with temperature, so it could be assumed that under these conditions animals were so P-depleted that they probably couldn't even synthesize AP. In this sense, it can be suggested that under severe P limitation (low food quality and high temperature) there is a trade-off between the production of AP and other constitutive proteins, so APA did not show the expected increasing trend with decreasing food quality (Wojewodzic et al., 2011).

During the first and second phases of DNA photoproducts repair (not measured), we observed different trends in APA. The first phase (PER, 380–700 nm) was characterized by no changes in APA. During this phase the enzyme photolyase uses light photons as energy source, thereby does not require extra energy (ATP) to repair UVR damage (Essen and Klar, 2006; Park and Rho, 2002). In the following phase (darkness), we observed changes in APA indicating changes in P demands. Previous studies observed that ATP was required for DNA repair (Park and Rho, 2002), so P requirements should increase. Probably this increased P demands for repair (DNA damage was not measured in the present study) would imply higher APA. Interestingly, again at 20 °C, APA showed a similar trend to that observed after the UVR exposure, with the maximum in the medium food quality. Similarly, we can assume that at this high temperature, under low food quality the organisms were so P-limited that even the AP synthesis was affected. The fact that in the three exposures (UVR, H_2O_2 and NER) at 20 °C showed the same trend in APA reinforces that APA doesn't increase monotonically with decreasing food quality.

5. Conclusions

Our experiments highlight the relationship between variables as UVR and temperature with the nutritional status of *Daphnia* simulating predicted changes in Andean lakes. We demonstrated that somatic APA is affected by oxidative stress (both by H_2O_2 and UVR exposure). However, the response of APA to UVR is observed to be also affected by temperature and food quality. In this context we determined that at higher temperatures, UVR has a more detrimental effect on APA (Macfadyen et al., 2004) probably due to higher metabolic rates and more reactivity to UVR. At 20 °C and under low food quality (C:P = 1150) *D. commutata* was so P-depleted that they probably couldn't even synthesize AP because of the increase of P limitation due to higher temperature. *D. commutata* growing under high C:P ratios and high temperature will be more affected by UVR, therefore low food quality and transparent environments such as oligotrotrophic lakes are a real challenge for *Daphnia* fitness. In a global change context temperature, UVR and nutrients are variables that will interact differentially, thus, in *Daphnia* population response models to global change it is important to take into account all these interactive variables.

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