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Contrasting patterns of MAAs accumulation in two populations of the copepod *Boeckella gracilipes*

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The bio-accumulation of mycosporine-like amino acids (MAAs) is common in planktonic copepods that inhabit environments exposed to high levels of solar radiation. MAAs accumulation in copepods can be affected both by extrinsic (environmental) and intrinsic factors (local adaptation, genotype, *etc.*). Laboratory experiments were performed to study the bio-accumulation of MAAs in two geographically-isolated populations of *Boeckella gracilipes* from a mountain and a piedmont lake of North Patagonia. We performed two series of 10-day incubations of *B. gracilipes* from the different lakes applying two radiation conditions (PAR + UVR and darkness), at five different temperatures (5 to 20 °C) and providing a MAA-free flagellate as food. We assumed that differences in final MAAs concentrations between copepod populations should be exclusively due to environmental factors, and that any difference in the patterns of MAAs accumulation should exclusively arise from differences in MAAs concentration at the time of collection. MAAs concentration was three fold higher in *B. gracilipes* from Lake Verde than in copepods from the Lake Morenito. The MAAs suite was dominated (~90%) by a combination of porphyra-334 and mycosporine-glycine in copepods from Lake Verde, and porphyra-334 and MAA-332 in those from Lake Morenito. Two exclusive MAA compounds were identified, mycosporine-glycine in copepods from Lake Verde and shinorine in the copepod population from Lake Morenito. Laboratory experiments showed that: (i) exposure to PAR + UVR stimulated the accumulation of MAAs in both copepod populations; (ii) temperature affected the response of MAAs and, remarkably, low temperatures stimulated MAAs accumulation even in dark incubations, (iii) the response to radiation and temperature in MAAs accumulation was more pronounced in the population with low initial MAAs than in the population with high initial MAAs concentrations. The differences in intrinsic factors between *B. gracilipes* populations, such as local adaptation to contrasting UV and temperature scenarios, among others, appear to play an important role in determining levels and patterns of MAAs accumulation in *B. gracilipes*.

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Introduction

Bio-accumulation of mycosporine like amino acids (MAAs) is a widespread response of planktonic copepods to ultraviolet radiation exposure (UVR, 290–400 nm).[1–5] MAAs are a family of water-soluble molecules with low molecular weight and high molar extinction coefficients (absorption maxima between 309–362 nm). It is widely accepted that the primary role of MAAs is to serve as sunscreens, which confer protection against harmful exposure to UVR.[6,7] In addition, they are increasingly being regarded as multi-purpose secondary metabolites that may be involved in nitrogen storage, osmotic regulation and anti-oxidant activity.[8,9] MAAs are synthesized by fungi, bacteria and algae. In contrast, (virtually all) animals acquire MAAs either from their diet[10] or through symbiotic flora (*i.e.* algal and/or bacterial endo-symbionts).[7,11]

The presence of MAAs is ubiquitous in freshwater copepods.[2,4,12–14] The suite of MAAs in copepods typically includes several compounds with different absorbance maxima, which may confer an extended range of photo-protection over the UV region. The accumulation of MAAs is known to be affected by a number of extrinsic (*i.e.*, environmental) and intrinsic factors. Among the extrinsic factors, the stimulating role of visible and UV radiation exposure has been extensively studied,[4,10,14,15] and remarkable differences in MAAs composition and concentrations have been reported for copepod populations inhabiting natural or experimental environments with contrasting exposure to solar radiation.[13,15,16] In addition, several other environmental factors (*i.e.*, nitrogen availability, osmotic strength, and temperature) are recognized to influence the accumulation of MAAs.[3,4,7,16,17]

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Different intrinsic factors are known to affect the accumulation of MAAs in copepods. The concentration of MAAs has been found to vary among different species,[2,18] as well as among populations of the same species.[12,15,16] Moreover, even within a single copepod population, the MAA concentration often differs between developmental stages, with eggs and younger copepodids bearing comparatively higher MAAs levels than the adults.[19] Furthermore, MAA concentration has been found to vary among different body structures, with higher concentrations found in the egg-sacs followed by the thorax/abdomen region and the cephalothorax.[20]

Boeckella gracilipes is a freshwater calanoid copepod that has a broad distribution in South America. In North Patagonia, *B. gracilipes* is the dominant copepod in piedmont lakes, but it has also been recorded in several mountain lakes.[2,21–26] Interestingly, individuals from mountain lakes usually bear much higher MAA concentrations than those from piedmont lakes.[2] The reason for these differences in MAA concentration remains unknown. It could be that intra-specific variability is low, and that environmental differences (extrinsic factors) account for much (or all) of the observed differences in MAAs concentration. Alternatively, it could be that low dispersal (of copepods and/or their associated microorganisms), due to lack of hydrological connectivity between some mountain and piedmont lakes, have resulted in intraspecific differences (intrinsic factors) in the accumulation of MAAs.

In this paper, we compare the accumulation of MAAs in individuals of the calanoid copepod *Boeckella gracilipes* from Lake Verde (mountain lake, higher MAAs) and Lake Morenito (piedmont lake, lower MAAs). Individuals from both populations were exposed to a prescribed suite of radiation and temperature, under controlled experimental conditions. As a working hypothesis, we assumed that differences in MAA concentration between lakes should be exclusively due to the environmental peculiarities of each particular habitat (*i.e.*, we assumed negligible effects due to intrinsic factors). Under such assumption, any difference in the patterns of MAAs accumulation should exclusively arise from differences in MAAs concentration at the time of collection. More specifically, we predict that (i) individuals with low initial MAA concentration (Lake Morenito) will display a stronger tendency to acquire these compounds when exposed to PAR + UVR; (ii) individuals with high initial MAA concentration (Lake Verde) will be more prone to lose MAAs when maintained in darkness, and (iii) the individual MAA compounds that are acquired or lost, under different combinations of radiation and temperature, should be the same, regardless of the copepods' origin.

Material and methods

Sampling sites

Boeckella gracilipes was collected from two Andean lakes, Lake Morenito (41° 03′S, 71° 30′W) and Lake Verde (41° 16′S, 71° 18′W) within the Nahuel Huapi National Park (Northwestern Patagonia, Argentina). These lakes occur at different elevations; the piedmont lake Morenito (758 m.a.s.l) is surrounded by a mixed evergreen forest characterized by the presence of *Nothofagus dombeyii*, while the mountain lake Verde (1525 m.a.s.l) is a small, fishless system located near the tree line and surrounded by a deciduous forest of *N. pumilio*. These lakes differ in several environmental features, such as depth, thermal structure and light penetration (Table 1). Morenito is a warm monomictic piedmont lake, with indigenous (galaxid) and introduced (salmonid) fish populations. Lake Verde freezes every year from mid fall until late spring. Light penetration is higher in Lake Verde than in Lake Morenito; the PAR fraction reaches the bottom in Lake Verde while in Lake Morenito penetrates up to 8.4 m. The UV wavelength 320 nm is attenuated at 0.84 m in Lake Morenito while in Lake Verde penetrates up to 2.07 m; reflecting their contrasting DOC concentrations (Table 1). Despite the above differences, their plankton communities are similar.[26] In particular, they both share the presence of the calanoid copepod *Boeckella gracilipes* which is often found as the dominant zooplanktonic species.

Table 1 Limnological features of Lakes Morenito and Lake Verde. K_d = extinction coefficient; Z = depth

Variable	Lake Morenito	Lake Verde
Altitude (m.a.s.l.)	758	1525
Maximum depth (m)	12	5
K_{d320} (m^{-1})	5.5	2.2
K_{dPAR} (m^{-1})	0.55	0.5
$Z_{320\ 1\%}$ (m^{-1})	0.84	2.07
$Z_{PAR\ 1\%}$ (m^{-1})	8.4	9.2
Chlorophyll a ($\mu g\ l^{-1}$)	1.2	9.7
DOC ($mg\ l^{-1}$)	2.4	1.2
Conductivity ($\mu S\ cm^{-1}$)	70	29

In this investigation we performed an experimental study with individuals of *B. gracilipes* collected from Lake Morenito between June and December 2009, and from Lake Verde between January and November 2009 (excluding the ice-covered period from May to October). The copepods were sampled by performing vertical and horizontal tows, using a 55 μm plankton net. The plankton samples were placed into polycarbonate carboys and transported to the laboratory within 2–3 hours. Adult *B. gracilipes* individuals were manually sorted from the sample using a Pasteur pipette. In order to assess the initial MAAs concentration in copepods from each lake and sampling date, one group of copepods was rinsed with distilled water and allowed to void the digestive tracks for a period of two hours. Subsequently, they were sorted into 2 ml vials, and stored frozen (−20 °C) for the analyses of MAAs. Experiments were performed with a second group of copepods, maintained in filtered (GF/F, 0.7 μm) lake water, plus additions of *Chlamydomonas reinhardtii* from cultures (Marine Biological laboratory medium without NaCl) during 24 h before the experiments.

Experimental procedures

The following general conditions and procedures were applied to all the experiments. *Boeckella gracilipes* individuals were collected from either Lake Morenito or Lake Verde the day before to the beginning of the experiments as explained above. Once in the laboratory, the copepods were acclimated in dark at the prescribed temperature for each assay (see below). An attempt was made to set the experimental temperatures as closer as possible to the lake temperature at the time of collection to minimize thermal stress. All assays consisted of ten day incubations in batch cultures in 2 l, UV transparent flasks (UVT, Plasmatic, Spain). Two radiation treatments were assayed: PAR + UVR (PAR: 110 μmol quanta m^{-2} s^{-1}, UVA: 3.5 W m^{-2}, and UVB: 0.0095 W m^{-2}) and dark (wrapped with aluminum foil). Individuals under the PAR + UVR treatment received the output radiation from 10 Sanyo 40-W PAR and 2 Q-panel 340 lamps placed vertically on the sides of the test chamber, in a 12 h light:12 h dark photoperiod. Daily fluences, measured inside the test chamber (USB 2000 spectroradiometer, Ocean Optics) were 10.8 E m^{-2}, 166.5 kJ m^{-2} and 5.5 kJ m^{-2} for PAR, UVA and UVB, respectively.

During the course of the assays, the copepods were fed a suspension of *Chlamydomonas reinhardtii* (1×10^4 cell ml^{-1}) in GF/F filtered spring water. The culture medium was fully renewed every other day. The experimental units were cleaned by pouring the content of each vessel through a 220 μm mesh. The collected adult individuals were placed into clean flasks with fresh culture media. All assays were performed in an environmental test chamber (Sanyo MLR-5). For all the experiments the initial MAA concentration in the copepods and their dry weights (DW) were determined in two replicated samples of 50 individuals each.

A series of ten laboratory experimental runs were performed to analyze the accumulation of MAAs in the copepod *Boeckella gracilipes* from lakes Morenito and Verde. These experiments consisted of 10 independent assays (two populations of *B. gracilipes*: L. Morenito and L. Verde × 5 temperatures: 5, 8, 12, 16 and 20 °C). For each assay, approximately 300 adult copepods were incubated exposed to PAR + UVR or in dark. Each of these treatments was run with 6 replicates. At the end of each assay, 50 individuals per replicate were sorted into 2 ml vials and frozen (−20 °C) until MAAs analysis by High Performance Liquid Chromatography (HPLC).

Analytical procedures

MAAs determination. The initial and final MAA concentrations and composition of *B. gracilipes* in all the experiments were assessed using the HPLC technique. The HPLC analyses were performed following Tartarotti *et al.*[2] and Garcia *et al.*[4]. The freeze-dried copepods' samples from the different experiments were treated with 25% aqueous methanol and then sonicated (1 minute at 0.5 cycles and 20% amplitude; Sonic Vibra cell) to extract the MAAs. The extracts obtained were filtered through 0.22 μm Durapore membranes (Millipore). Aliquots of 300 μl were injected in a chromatographer (Äktabasic; Amersham©) equipped with a Phenosphere of 5 mm pore size C-8 column (250 × 4.6 mm internal diameter; Phenomenex©), protected with a RP-8 guard column (Brownlee©). The samples were run with a mobile phase of 0.1% acetic acid in 25% aqueous methanol (vol : vol) and a flow rate of 0.79 ml min^{-1}. In the absence of certified standards, MAAs were identified by relative retention time, absorption peaks, co-chromatography with *Porphyra* extracts and similarly to the extracts from *B. antiqua.*[11] The total MAA concentration in each sample was calculated from the HPLC peak areas at 310, 332 and 334 nm, using published extinction coefficients.[15,27,28] We used an average molar extinction coefficient (n = 40 000) for the unknown MAA332 (hereinafter MAA-332), previously recorded in *B. antiqua.*[11,29] The MAA concentration was normalized to the dry weight of each sample and expressed as μg mg^{-1} DW.

Data analysis

The total and individual concentration of MAAs compounds present in *B. gracilipes* collected in different sampling dates in lakes Morenito and Verde were compared applying the non-parametric Kruskal–Wallis test to determine the existence of intra-population variability among sampling dates. The concentrations of total individual MAAs compounds between populations were compared applying the non-parametric Mann–Whitney test.

The final MAAs concentrations of the copepods from each assay were normalized using the corresponding initial concentration to avoid the noise produced by the differences in the initial concentration. The resulting variable (hereinafter referred as ΔMAAs) is defined as:

$$\Delta\text{MAAs} = [\text{MAAs}_{\text{final}}] - [\text{MAAs}_{\text{initial}}]$$

where; $[\text{MAAs}_{\text{final}}]$ = concentration of MAAs in *B. gracilipes* after incubation in PAR + UVR or dark, $[\text{MAAs}_{\text{initial}}]$ = initial concentration of MAAs in *B. gracilipes* at the beginning of the assay.

Subsequently, the 10 independent assays were analyzed using three-way ANOVA considering the lake of origin, radiation treatment, and temperature as main factors. In order to assess inter-population differences, the analysis was completed by performing the corresponding contrasts, as required. The effect of temperature and radiation on *B. gracilipes* of the two populations was analyzed by means of *t*-tests.

Results

MAAs composition and concentration in *Boeckella gracilipes*

The mean total MAAs concentration was ~3-fold higher in *B. gracilipes* from Lake Verde (8.76 ± 3.6 μg mg^{-1} DW) than in the copepods from Lake Morenito (3.07 ± 1.1 μg mg^{-1} DW) (Mann–Whitney, U = 64, p = 0.002; Fig. 1). Total MAAs concentration in *B. gracilipes* from field samples displayed some variability between sampling dates within lakes Morenito and Verde respectively, but the differences between dates were not statistically significant (Kruskal–Wallis, H = 7 and H = 7.3, $p > 0.05$, respectively).

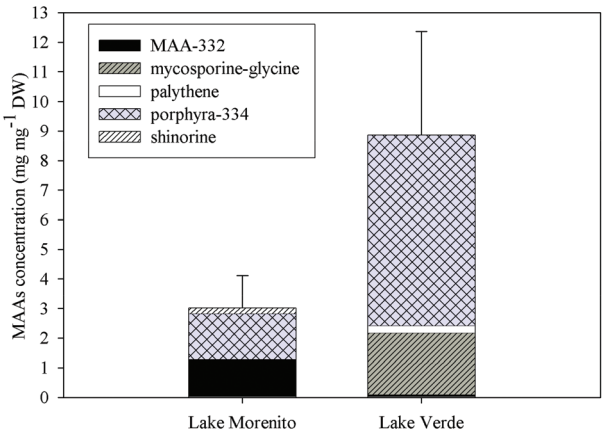

Fig. 1 MAA concentration and composition in the copepod *Boeckella gracilipes* from populations of Lake Morenito and Lake Verde (Patagonia).

The MAAs suites present in *B. gracilipes* from field samples of the different lakes were slightly different. The population from Lake Morenito presented the compounds porphyra-334 (50.91% of total MAAs), an unknown compound named MAA-332 (41.97%), shinorine (6.63%) and palythene (0.48%). In the population of *B. gracilipes* from Lake Verde the compounds found were porphyra-334 (73.03%), mycosporine-glycine (23.56%), palythene (2.64%) and MAA-332 (0.75%). Shinorine was found only in the population of Lake Morenito while mycosporine-glycine was recorded just in copepods from field samples of Lake Verde. The comparison between the concentrations of individual MAA compounds among different copepod populations, showed significantly higher concentrations of porphyra-334 and palythene in copepods from Lake Verde compared to those from Lake Morenito (Mann–Whitney, $U = 129$, $p = 0.002$ and $t = -7.1$, $p < 0.001$, respectively). In contrast, the concentration of MAA-332 was significantly higher in copepods from Lake Morenito compared to those of Lake Verde ($t = -7.4$, $p < 0.001$). Notably, the two prevailing MAA compounds in copepods from both populations accounted for more than 90% of the total MAA concentration (Fig. 1).

MAAs bio-accumulation in *B. gracilipes*

The three-way ANOVA revealed significant differences in the MAAs accumulation among populations of *B. gracilipes* from lakes Morenito and Verde ($F = 157.95$, $p < 0.001$). Also, significant differences were found in bio-accumulation at different temperatures ($F = 33.67$, $p < 0.001$) and in exposures to PAR + UVR and dark ($F = 318.31$, $p < 0.001$). The interactions between these main factors were also significant ($F = 5.09$, $p < 0.001$).

In order to compare the bio-accumulation of MAAs (Total MAAs and individual MAA compounds) of *B. gracilipes* from Lake Morenito (Low MAAs) and Lake Verde (High MAAs), we focused on their response to the radiation treatments and compared them using *t*-tests at each of the five temperature levels applied in the experiments.

The accumulation of total MAAs in PAR + UVR was higher in copepods from Lake Morenito than in those from Lake Verde (Table 2). This pattern was found at four out of the five temperature levels assayed. Only at 16 °C, total MAAs were accumulated similarly by the two *B. gracilipes* populations (Fig. 2a; Table 2). In the incubations in darkness, the copepods from Lake Morenito showed higher accumulation of total MAAs at 8, 12 and 20 °C (Table 2). The copepods from Lake Verde decrease MAAs at 12 and 20 °C. Interestingly, at 5 °C the copepods from both lakes increased their MAAs even in the absence of radiation stimuli (Fig. 2b).

In copepods from lakes Morenito and Verde, the prevailing MAAs compound porphyra-334 increased in the PAR + UVR treatment, although the increment was significantly higher in the copepods from Lake Morenito. This pattern was observed at all the temperatures assayed (Fig. 3a, Table 2). In the dark treatment the accumulation of porphyra-334 in copepods from Lake Morenito was higher than in copepods from Lake Verde at 8 and 12 °C (Table 2, Fig. 3b).

MAA-332, the second compound in importance, increased when exposed to PAR + UVR at 5, 8 and 12 °C in copepods from Lake Morenito, while in those from Lake Verde the increase was higher at 16 and 20 °C (Table 2, Fig. 3c). In the dark treatment, the MAA-332 significantly increased in Lake

Table 2 Contrasts between the responses of individual MAAs to the different light treatments applied in laboratory assays to *B. gracilipes* copepods from Lake Morenito and Lake Verde

	B. gracilipes Lake Verde *vs.* *B. gracilipes* Lake Morenito							
	PAR + UVR				Dark			
Temperature (°C)	Total MAAs	Porphyra-334	MAA-332	Palythene	Total MAAs	Porphyra-334	MAA-332	Palythene
5	$t = 8.96$ $p < 0.05$	$p > 0.05$	$t = 8.21$ $p < 0.05$	—	$p > 0.05$	$p > 0.05$	$p > 0.05$	—
8	$t = 6.21$ $p < 0.05$	$t = 3.08$ $p < 0.05$	$p > 0.05$	—	$t = 5.60$ $p < 0.05$	$t = 16.06$ $p < 0.05$	$t = 14.28$ $p < 0.05$	—
12	$t = 3.43$ $p < 0.05$	$t = 8.53$ $p < 0.05$	$t = 3.62$ $p < 0.05$	—	$t = 9.28$ $p < 0.05$	$t = 3.45$ $p < 0.05$	$p > 0.05$	—
16	$p > 0.05$	$p > 0.05$	$p > 0.05$	—	$p > 0.05$	$p > 0.05$	$p > 0.05$	—
20	$t = 6.96$ $p < 0.05$	$p > 0.05$	$p > 0.05$	$t = 15.51$ $p < 0.05$	$t = 6.06$ $p < 0.05$	$p > 0.05$	$p > 0.05$	$t = 4.69$ $p < 0.05$

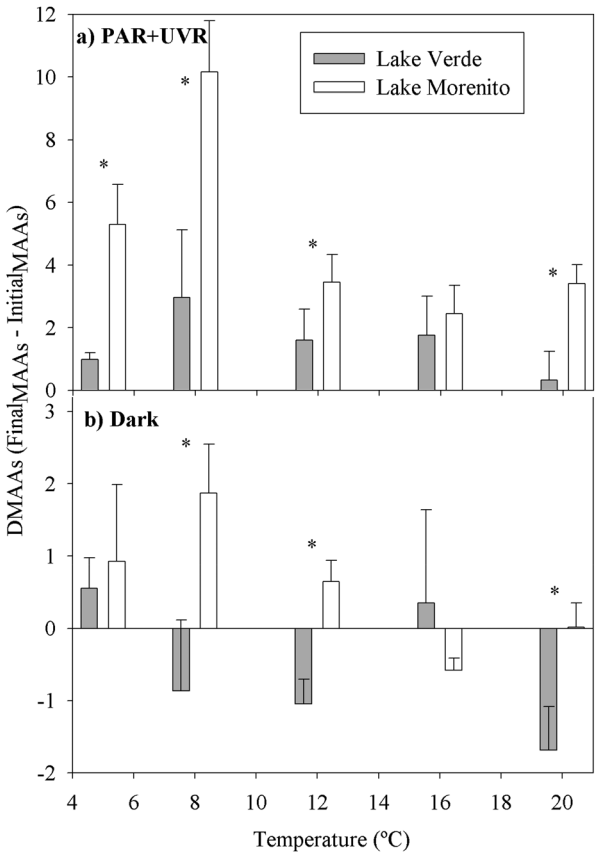

Fig. 2 Changes in Total MAAs concentrations (ΔMAAs) in *Boeckella gracilipes* observed in experimental incubations in a temperature gradient (5–20 °C): (a) in PAR + UVR and, (b) in dark. White bars represent the changes in MAAs in the population of *B. gracilipes* from Lake Morenito and grey bars from Lake Verde. Asterisks (*) indicate significant differences between populations of *B. gracilipes* (*t*-test).

Morenito compared to copepods from Lake Verde at 8 and 12 °C while at 16 °C the increment of this compound was higher in copepods from Lake Verde (Fig. 3d).

The compound palythene was found at concentrations at the limit of the HPLC detection level and only in some initial copepod samples (Fig. 1) with higher occurrence in copepods from Lake Verde (Table 2). Therefore, palythene was excluded of the comparative analysis between copepod populations.

In the case of MAA compounds exclusive from each population of *B. gracilipes*, the behavior in their response to the different radiation treatments was compared within each population of *B. gracilipes*. The compound shinorine, found only in copepods from Lake Morenito, increased significantly in PAR + UVR as compared to the dark treatment at most temperatures, except 5 °C (Table 3). Mycosporine-glycine, present only in copepods from Lake Verde, had a significant increase in the PAR + UVR treatment at 8, 12 and 16 °C, as compared to the dark treatment. At 20 °C, there was a significant decrease of this compound in the dark treatment (Table 3).

Discussion and conclusions

The composition of MAAs of *Boeckella gracilipes* from Lake Verde and Lake Morenito populations differed not only in total concentration, but also in the composition of individual compounds. As could be expected, the average MAA concentration in *B. gracilipes* was higher (~3 fold) in the population from the mountain Lake Verde than in the population from the piedmont Lake Morenito. This pattern in MAAs concentration has been observed also in other freshwater copepods around the world.[13,16,18] Particularly, in *B. gracilipes* previous investigations have recorded higher levels of photo-protective compounds (including MAAs) in mountain as compared to piedmont lakes populations.[2,22,23] As regards to the qualitative composition, it is interesting to mention that two compounds were exclusive of either one of the two populations: shinorine was only present in copepods from Lake Morenito, while mycosporine-glycine occurred only in copepods from Lake Verde. In both populations, the two more abundant compounds accounted for over 90% of the total MAA concentration, but the two main compounds differed between populations: porphyra-334 and MAA-332 in Lake Morenito, and porphyra-334 and mycosporine-glycine in Lake Verde. Despite some variability in copepods' total MAA concentration over time, the qualitative composition remained fairly stable during the period of the study in both lakes.

The concentration and composition of MAAs in copepods are the result of dynamic processes, which are known to be affected by light, temperature[4,11,29] and biotic interactions.[10,11,30] Lake Morenito is a warm monomictic, piedmont lake that does not stratify in summer and that only exceptionally freezes in extremely harsh winters.[31] On the other hand, Lake Verde, as most mountain lakes, freezes every year for several months and displays either inverse stratification or homogeneous temperature profiles in the water column.[23,32] Although the actual radiation doses experienced by the copepod populations would be difficult to quantify, it is likely that they do differ between the two lakes, being likely higher in the mountain lake Verde, due to differences in altitude, light penetration and the lack of a depth refuge.[2,23]

In addition to differences in the physical environment, the two lakes have different trophic structures: most notably, fish (*i.e.*, visual planktivores) are only present in Lake Morenito. Biotic interactions, such as visual predation[30] and food source[10,11] might influence the accumulation of MAAs in copepods. Given that MAAs are colorless, it has been suggested that these compounds would be accumulated preferentially in the presence of fish.[30] However in this study, the copepods from the fishless lake Verde had much higher MAA concentrations than those from Lake Morenito that support strong predation by indigenous and exotic fish yearlings.[33,34]

Food resources for *B. gracilipes* also differ between lakes and it is well known that food may be a significant source of MAAs in freshwater copepods.[10,14,15,35] *B. gracilipes* has an omnivorous diet, preferentially feeding on motile prey, likely nanoplanktonic flagellates and small (3.9–33 μm) ciliates.[25] In

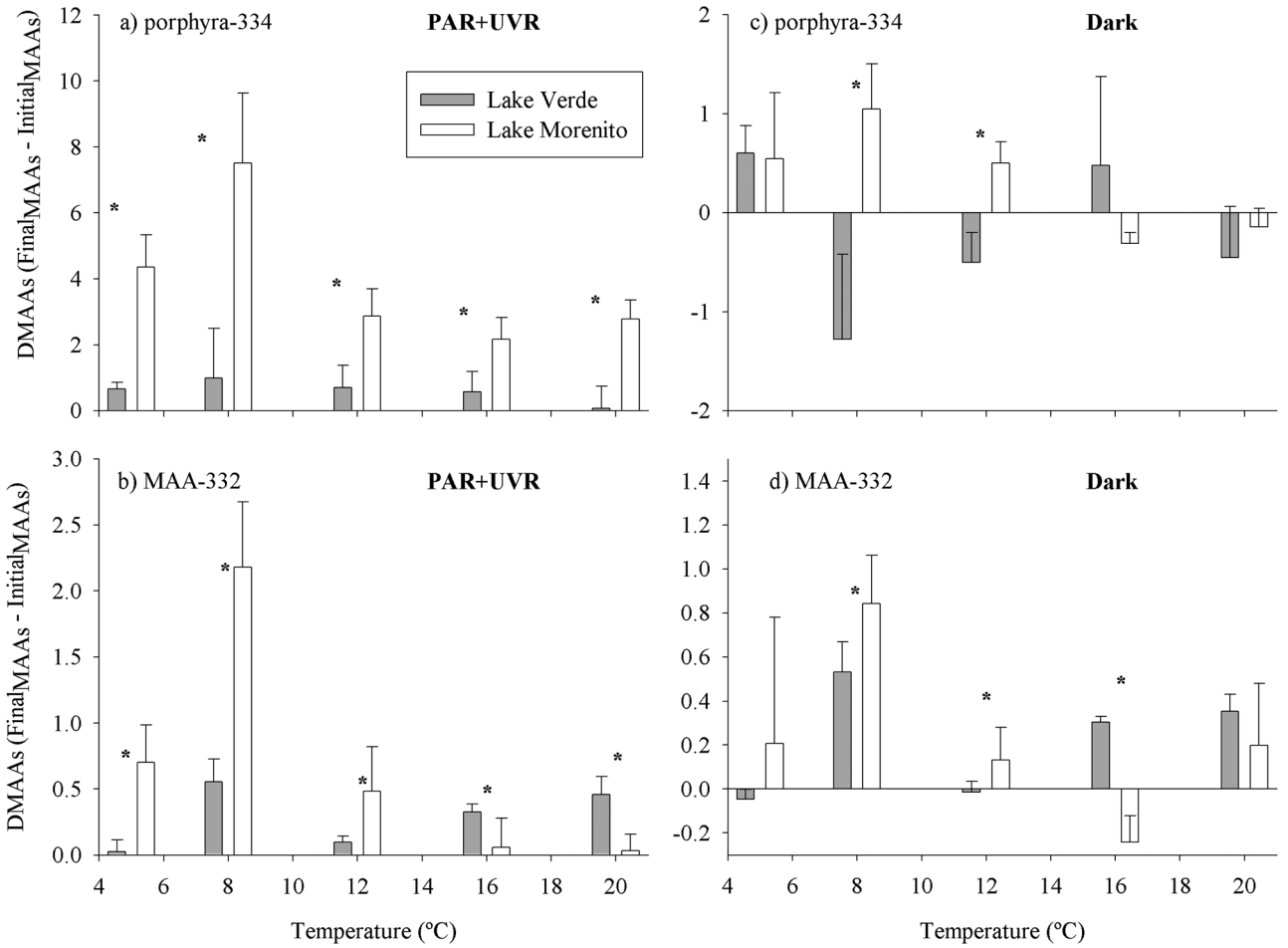

Fig. 3 Comparison of the changes in individual MAAs concentrations (ΔMAAs) in *B. gracilipes* in experimental incubations in a temperature gradient (5–20 °C): (a) porphyra-334 under PAR + UVR, (b) porphyra-334 in dark, (c) MAA-332 PAR + UVR and (d) MAA-332 in dark. White bars represent the changes in MAAs in the population of *B. gracilipes* from Lake Morenito and grey bars from Lake Verde. Asterisks (*) indicate significant differences between populations of *B. gracilipes* (*t*-test).

Table 3 Contrasts between the response of exclusive MAAs (shinorine in copepods from Lake Morenito and mycosporine-glycine in copepods from Lake Verde) to the radiation treatments assayed (PAR + UVR and dark). (*t*-values are shown for $p < 0.05$)

	PAR + UVR *vs.* dark	
Temperature (°C)	Shinorine	Mycosporine-glycine
5	$p > 0.05$	$t = 4.907$ $p < 0.05$
8	$t = 3.245$ $p < 0.05$	$t = 6.555$ $p < 0.05$
12	$t = 3.376$ $p < 0.05$	$t = 11.52$ $p < 0.05$
16	$t = 5.767$ $p < 0.05$	$t = 3.968$ $p < 0.05$
20	$t = 7.564$ $p < 0.05$	$t = 11.71$ $p < 0.05$

Lake Morenito, the nanoplanktonic flagellates dominate the phytoplankton, with *Chrysochromulina parva*, *Rhodomonas lacustris* and *Cryptomonas* spp. as the most common species.[36] In Lake Verde, the phytoplankton is dominated by non-motile algae such as *Staurastrum minutissimum*, and to a lesser extent by nanoplanktonic flagellates.[23] Despite of the differences in phytoplankton species composition, MAAs have never been detected in seston samples from any of these two lakes, suggesting that phytoplanktonic algae bear very low MAA concentrations.[2]

Additionally, the presence of particular microbiota in copepods has been related with their potential to accumulate MAAs.[4,11] Hosts may provide refuge for associated bacteria, also benefiting from these prokaryotes. Microscopy studies and bioassays have shown that copepod's gut is heavily colonized by microbes. The acquisition of novel functions from bacterial endosymbionts is increasingly being investigated. The activity of prokaryotes may augment that of the copepod in transforming materials that pass through the gut.[37] Perez *et al.*[11] described the microbial community retrieved from the copepod *Boeckella antiqua* and linked the presence of the beta-proteobacteria *Limnobacter*, which is known to have genes encoding for the shikimic acid pathway, as a source of MAAs

in an experiment providing both a MAA free-diet (*Chlamydomonas reinhardtii*) and a MAA-rich diet (*Chlamydomonas reinhardtii* + *Peridinium inconspicuum*). Ongoing studies of the gut microbiota of *B. gracilipes* found three prokaryotic entities exclusive of the copepods from Lake Morenito, and one entity exclusive of copepods from Lake Verde (unpublished data). Although not conclusive, this evidence indicates differences among the prokaryotic microbiota in *B. gracilipes* populations from the mountain and piedmont lake.

All the above factors, alone or in combination, plus potential genotypic differences between the two *B. gracilipes* populations may be responsible for the observed differences in total MAA concentration and qualitative MAA composition. Given these initial differences, our major interest in this study was to elucidate which factors influence the accumulation of MAAs when individuals of the two populations were experimentally exposed to identical external conditions (*i.e.*, extrinsic factors: radiation levels, temperature and food source). As a working hypothesis, we assumed negligible effects due to intrinsic factors. Our experimental approach provided the same extrinsic factors: two radiation conditions (PAR + UVR and dark), a temperature range (5 °C to 20 °C) and the MAA-free flagellate *Chlamydomonas reinhardtii* as food source. We assumed that these conditions would affect in the same way the two populations of *B. gracilipes*, although we expected the magnitude of the response to be different. Under these assumptions, we predicted that (i) individuals with the lowest initial MAA concentration (Lake Morenito) will display a stronger tendency to acquire MAA compounds when exposed to PAR + UVR, while (ii) individuals with high initial MAA concentration (Lake Verde) will be more prone to lose MAAs when maintained in darkness. In addition, we predicted that (iii) the qualitative MAA composition after experienced a period of similar environmental conditions would tend to become more similar, *i.e.*, a compound that was initially high in one population and low in the other would tend to be less accumulated in the first population and more in the second, and *vice versa*.

Our experimental results revealed that radiation (PAR + UVR) and temperature affected the accumulation of MAAs in both populations of *Boeckella gracilipes*. However, the magnitude of the response was different in the two populations; copepods from Lake Morenito (Low MAAs) adjusted more rapidly their MAAs concentrations than those from Lake Verde (High MAAs) when exposed to PAR + UVR or dark.

Interestingly, both populations of *B. gracilipes* increased their MAA concentrations in the dark, particularly at low temperatures. The fact that low temperatures alone can stimulate MAA accumulation may indirectly suggest that besides their photo-protective role these compounds may provide further protection. The sunscreening and antioxidant properties of photoprotective compounds are not always distinct. Nevertheless, it has been demonstrated that some MAAs have strong antioxidant capacity during photooxidative stress by scavenging reactive oxygen species (ROS) and suppressing singlet oxygen-induced damage.[38,39] Several MAAs are oxidatively robust, having concentration-dependent antioxidant activity.[7,39,40] Dunlap and Yamamoto[41] linked the presence of the MAA mycosporine-Gly (or mycosporine-taurine) present in the hyperoxic tissues of invertebrate-algal symbioses, suggesting an antioxidative function. At low temperature and high dissolved oxygen concentrations, the increase in MAA, such as recorded in *Boeckella* species, may respond to the comparatively higher oxidative condition prevailing in the environment. In this line, García *et al.*[4] reported the negative elimination rates of MAAs at 5 °C in *Boeckella gracilis* in experimental incubations which led to an accumulation pattern similar to what we observed in *B. gracilipes* populations.

In addition, the experimental results were consistent with our first prediction: the increase in total MAA concentration was greater in the population from Lake Morenito (Low initial MAAs) than in population from Lake Verde (High initial MAAs). Our second prediction however, was only partially supported by the experimental results. When maintained in darkness the copepods from Lake Verde (High initial MAAs) decreased their total MAAs concentration at 8, 12 and 20 °C, while those from Lake Morenito (Low initial MAAs) increased their MAAs at most temperatures assayed except at 16 °C.

Finally, our third prediction was also unsupported: rather than tending to equalize their relative MAAs composition, both populations accumulated more strongly those MAA compounds that were initially in the highest amount in their respective wild populations, *i.e.*, porphyra-334 and MAA-332 in *B. gracilipes* from Lake Morenito, and porphyra-334 and mycosporine-glycine in the copepods from Lake Verde.

We therefore conclude that differences in intrinsic factors favoured by the isolation of the populations play an important role in determining the levels and the patterns of accumulation of MAAs in *B. gracilipes*. Intrinsic factors, including the copepods' local adaptation to contrasting UV and temperature scenarios, their genotype, the composition of their associated microbiota, among others, likely modulate the pattern of acquisition of MAAs in *B. gracilipes*.

Further studies are needed to elucidate the strength genotypic differences among mountain and piedmont lakes populations of *B. gracilipes.* Such differences could account to explain their adaptation to local conditions present in these lakes, including their particular UV tolerance and thermal preference, among others. In addition, future investigations should address the potential of the microbiota associated to different populations of *B. gracilipes* to influence the photoprotective response. We suspect that the particular prokaryotic microbiota present in *B. gracilipes* populations may be a source of MAAs and also enhance the acquisition of these compounds from the diet as has been observed in the related species, *B. antiqua*.

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References

1 R. Sommaruga and F. Garcia-Pichel, UV-absorbing mycosporine-like compounds in planktonic and benthic organisms from a high-mountain lake, *Arch. Hydrobiol.*, 1999, **144**, 255–269.

2 B. Tartarotti, G. Baffico, P. Temporetti and H. E. Zagarese, Mycosporine-like amino acids in planktonic organisms living under different UV exposure conditions in Patagonian lakes, *J. Plankton Res.*, 2004, **26**, 753–762.

3 P. E. Garcia, A. P. Perez, M. C. Dieguez, M. A. Ferraro and H. E. Zagarese, Dual control of the levels of photoprotective compounds by ultraviolet radiation and temperature in the freshwater copepod *Boeckella antiqua*, *J. Plankton Res.*, 2008, **30**, 817–827.

4 P. E. Garcia, M. C. Dieguez, M. A. Ferraro, H. E. Zagarese and A. P. Perez, Mycosporine-like amino acids in freshwater copepods: potential sources and some factors that affect their bioaccumulation, *Photochem. Photobiol.*, 2010, **86**, 353–359.

5 L. A. Hansson and S. Hylander, Effects of ultraviolet radiation on pigmentation, photoenzymatic repair, behavior, and community ecology of zooplankton, *Photochem. Photobiol. Sci.*, 2009, **8**, 1266–1275.

6 J. Favre-Bonvin, N. Arpin and C. Brevard, Biosynthesis of micosporine: mycosporine-glutaminol in *Trichothecium roseum*, *Phytochemistry*, 1987, **26**, 2509–2514.

7 J. M. Shick and W. C. Dunlap, Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms, *Annu. Rev. Physiol.*, 2002, **64**, 223–262.

8 A. Oren and N. Gunde-Cimerman, Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites?, *FEMS Microbiol. Lett.*, 2007, **269**, 1–10.

9 J. I. Carreto and M. O. Carignan, Mycosporine-Like amino acids: relevant secondary metabolites. chemical and ecological aspects, *Mar. Drugs*, 2011, **9**, 387–446.

10 R. E. Moeller, S. Gilroy, C. E. Williamson, G. Grad and R. Sommaruga, Dietary acquisition of photoprotective compounds (mycosporine-like amino acids, carotenoids) and acclimation to ultraviolet radiation in a freshwater copepod, *Limnol. Oceanogr.*, 2005, **50**, 427–439.

11 A. P. Perez, M. A. Ferraro and H. E. Zagarese, The relative contributions of diet and associated microbiota to the accumulation of UV-absorbing mycosporine-like amino acids in the freshwater copepod *Boeckella antiqua*, *Freshwater Biology*, 2012, **57**, 993–1004.

12 V. E. Rocco, O. Oppezzo, R. Pizarro, R. Sommaruga, M. Ferraro and H. E. Zagarese, Ultraviolet damage and counteracting mechanisms in the freshwater copepod *Boeckella poppei* from the Antarctic Peninsula, *Limnol. Oceanogr.*, 2002, **47**, 829–836.

13 B. Tartarotti, I. Laurion and R. Sommaruga, Large variability in the concentration of mycosporine-like amino acids among zooplankton from lakes located across an altitude gradient, *Limnol. Oceanogr.*, 2001, **46**, 1546–1552.

14 L.-A. Hansson, S. Hylander and R. Sommaruga, Escape from UV threats in zooplankton: A cocktail of behavior and protective pigmentation, *Ecology*, 2007, **88**, 1932–1939.

15 E. W. Helbling, F. Zaratti, L. O. Sala, E. R. Palenque, F. Menchi and V. E. Villafañe, Mycosporine-like amino acids protect the copepod *Boeckella titicacae* (Harding) against high levels of solar UVR, *J. Plankton Res.*, 2002, **24**, 225–234.

16 R. Sommaruga, Preferential accumulation of carotenoids rather than of mycosporine-like amino acids in copepods from high altitude Himalayan lakes, *Hydrobiologia*, 2010, **648**, 143–156.

17 E. Litchman, J. P. Neale and A. T. Banaszak, Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: photoprotection and repair, *Limnol. Oceanogr.*, 2002, **47**, 86–94.

18 A. D. Persaud, R. E. Moeller, C. E. Williamson and C. W. Burns, Photoprotective compounds in weakly and strongly pigmented copepods and co-occurring cladocerans, *Freshwater Biol.*, 2007, **52**, 2121–2133.

19 B. Tartarotti and R. Sommaruga, Seasonal and ontogenetic changes of mycosporine-like amino acids in planktonic organisms from an alpine lake, *Limnol.Oceanogr.*, 2006, **51**, 1530–1541.

20 M. Orfeo, M. Ventura, B. Tartarotti and R. Sommaruga, Body distribution and source of mycosporine-like amino acids in the cyclopoid copepod *Cyclops abyssorum tatricus*, *J. Plankton Res.*, 2011, **33**, 1430–1444.

21 L. Villalobos and L. R. Zuñiga, Latitudinal gradient and morphological variability of copepods in Chile: *Boeckella gracilipes* Daday, *Verh.–Int. Ver. Theor. Angew. Limnol.*, 1991, **24**, 2834–2838.

22 B. Tartarotti, S. Cabrera, R. Psenner and R. Sommaruga, Survivorship of *Cyclops abyssorum tatricus* (Cyclopoidea, Copepoda) and *Boeckella gracilipes* (Calanoidea, Copepoda) under ambient levels of solar UVB radiation in two high-mountain lakes, *J. Plankton Res.*, 1999, **21**, 549–560.

23 H. E. Zagarese, M. Diaz, F. Pedrozo and C. Ubeda, Mountain Lakes in northwestern Patagonia, *Verh.–Int. Ver. Theor. Angew. Limnol.*, 2000, **27**, 533–538.

24 S. Menu-Marque, J. J. Morrone and C. L. de Mitrovich, Distributional patterns of the south American species of *Boeckella* (Copepoda: Centropagidae): a track analysis, *J. Crustacean Biol.*, 2000, **20**, 262–272.

25 E. G. Balseiro, B. E. Modenutti and C. P. Queimaliños, Feeding *Boeckella gracilipes* (Copepoda, Calanoidea) on ciliates and phytoflagellates in an ultraoligotrophic Andean lake, *J. Plankton Res.*, 2001, **23**, 849–857.

26 M. C. Marinone, S. Menu-Marque, D. Añón Suárez, M. C. Dieguez, P. Perez, P. De Los Ríos, D. Soto and H. E. Zagarese, Symposium-in-Print: UV effects on aquatic and coastal ecosystems UV radiation as a potential driving force for zooplankton community structure in patagonian lakes, *Photochem. Photobiol.*, 2006, **82**, 962–971.
27 D. Karentz, Chemical defenses of marine organisms against solar radiation exposure: UV absorbing mycosporine-like amino acids and scytonemin, in *Marine Chemical Ecology*, ed. J. B. Mcclintock and B. J. Baker, CRC Press, Boca Raton, FL, USA, 2001, pp. 481–520. Marine Science Series.
28 F. R. Conde, M. O. Carignan, M. S. Churio and J. I. Carreto, *In vitro cis–trans* photoisomerization of palythene and usujirene. Implications on the *In vivo* transformation of mycosporine-like amino acids, *Photochem. Photobiol.*, 2003, **77**, 146–150.
29 P. A. Pérez, D. Libkind, M. C. Dieguez, M. Summerer, B. Sonntag, R. Sommaruga, M. van Broock and H. E. Zagarese, Mycosporines from freshwater yeasts: a trophic cul-de-sac?, *Photochem. Photobiol. Sci.*, 2006, **5**, 25–30.
30 L.-A. Hansson, Plasticity pigmentation induced by conflicting threats from predation and UV radiation, *Ecology*, 2004, **85**, 1005–1016.
31 G. L. Perez, C. P. Queimaliños and B. E. Modenutti, Light Climate and plankton in the deep chlorophyll maxima in North Patagonian Andean lakes, *J. Plankton Res.*, 2002, **24**, 591–599.
32 C. Ubeda, H. E. Zagarese, M. Diaz and F. Pedrozo, First steps towards the conservation of the microendemic Patagonian frog *Atelognathus nitoi*, *Oryx*, 1999, **33**, 59–66.
33 C. Alonso, V. Rocco, J. P. Barriga, M. A. Battini and H. Zagarese, Surface avoidance by freshwater zooplankton: Field evidence on the role of ultraviolet radiation, *Limnol. Oceanogr.*, 2004, **49**, 225–232.
34 L. Buria, S. J. Walde, M. Battini, P. J. Macchi, M. Alonso, D. E. Ruzzante and V. E. Cussac, Movement of a South American perch *Percichthys trucha* in a mountain Patagonian lake during spawning and prespawning periods, *J. Fish Biol.*, 2007, **70**, 215–230.
35 S. Hylander and T. Jephson, UV protective compounds transferred from a marine dinoflagellate to its copepod predator, *J. Exp. Mar. Biol. Ecol.*, 2010, **389**, 38–44.
36 C. P. Queimaliños, The role of phytoplanktonic size fractions in the microbial food webs in two north Patagonian lakes (Argentina), *Verh.–Int. Ver. Theor. Angew. Limnol.*, 2002, **28**, 1236–1240.
37 K. W. Tang, V. Turk and H. P. Grossart, Linkage between crustacean zooplankton and aquatic bacteria, *Aquat. Microb. Ecol.*, 2010, **61**, 261–277.
38 N. N. Rosic and S. Dove, Mycosporine-Like Amino Acids from coral dinoflagellates, *Appl. Environ. Microbiol.*, 2011, **77**, 8478–8486.
39 F. de la Coba, J. Aguilera, F. L. Figueroa, M. V. de Gálvez and E. Herrera, Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen, *J. Appl. Phycol.*, 2009, **21**, 161–169.
40 D. Andreguetti, E. M. Stein, C. M. P. Pereira, E. Pinto and P. Colepicolo, Antioxidant properties and UV absorbance pattern of mycosporine-like amino acids analogs synthesized in an environmentally friendly manner, *J. Biochem. Mol. Toxicol.*, 2013, **27**(6), 305–312.
41 W. C. Dunlap and Y. Yamamoto, Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine, *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.*, 1995, **112**, 105–114.

