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When eating a prey is risky: Implications for predator diel vertical migration

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Abstract

Diel vertical migration (DVM) is a common behavior in zooplankton to avoid visual predation as well as potentially hazardous light wavelengths. In deep transparent lakes of Patagonia, the dark pigmented mixotrophic ciliate *Stentor araucanus* inhabits the upper layers of the epilimnion and is resistant to ultraviolet radiation (UVR). Here, we investigated if the ciliate pigment called stentorin increases oxidative stress in its predators. We studied the DVM behavior of *Mesocyclops araucanus* and the presence of stentorin in field-collected copepods and evaluated in the laboratory the rate at which the copepod releases stentorin. *S. araucanus* has a C : P ratio ~ 170 (atomic), which is one half of that of the bulk seston of the lake resulting in a very good food source in a system with very low food quality. Compared to an alternative prey without stentorin, when feeding on *Stentor*, the copepod suffered high oxidative stress (increased glutathione S-transferase activity) and the reduced glutathion levels increased from dark to visible and ultraviolet radiation. However, we also determined that exposure to only visible light was sufficient to cause oxidative stress. In the field, we observed that *M. araucanus* displays a larger amplitude DVM than other crustaceans, while the ciliate *Stentor* remained in the upper levels of the epilimnion. The DVM protects the copepods from stentorin-induced oxidative stress during daytime. Our findings are the first to show that a compound of a zooplankton prey item can influence the vertical behavior of predators in order to minimize the negative effect.

Diel vertical migration (DVM) is a common strategy in pelagic zooplankton to get access to food (Kessler and Lampert 2004; Winder and Schindler 2004), to avoid predators (Oda and Hanazato 2008; Leech et al. 2009), and to avoid harmful solar wavelengths (i.e., ultraviolet radiation, UVR) that can penetrate the upper layers of the water column (Boeing et al. 2004; Williamson et al. 2011). During the night, zooplankton generally migrate to the epilimnion and at dawn move to deeper water layers, staying there during day time. Zooplankton migrations range from less than a meter to several tens of meters in lakes and the intensity of migration is often related to light intensity and predation risk (Kessler et al. 2008; Ekvall et al. 2015). The Transparency Regulator Hypothesis (TRH), developed by Williamson et al. (2011), combines biotic (food resources and visual predation) and abiotic (temperature and ultraviolet radiation) factors regulating DVM of zooplankton. In the TRH, water transparency determines the relative importance of visual predation vs. UVR in driving zooplankton to deeper waters during the day (Williamson et al. 2011). Variation in water transparency also influences both structural drivers of DVM, such as temperature and food, as well as dynamic ones, such as light (UVR and PAR) and visual predation risk (Fischer et al. 2015).

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In addition to DVM, plankton have developed other strategies to deal with UVR, such as the accumulation of UV-protective compounds (Hairston 1979; Sommaruga and Garcia-Pichel 1999) as well as cell repair systems and enzymes that mitigate oxidative stress (Borgeraas and Hessen 2000, 2002; Macfadyen et al. 2004; Balseiro et al. 2008). For example, photoprotective compounds, such as mycosporine-like aminoacids, are obtained by consumers from their food (algae and other protists) and then used as light shields (Sommaruga and Garcia-Pichel 1999). In contrast, certain compounds present in ciliates, such as dark pigments, could act in the opposite way having a detrimental effect by increasing visibility to predators (Terazima and Harumoto 2004) by photosensitizing cells to damage (Moller 1962; Briggs and Spudich 2005). In particular, heterotrich ciliates from the genera *Stentor* and *Maristentor* have pigmented cortical granules with dark pigments named stentorin and maristentorin that are chemically very similar to hypericin (Moller 1962; Lobban et al. 2007). These pigmented ciliates are present in many aquatic environments and can achieve dense populations in transparent lakes from South America, Australia, Africa, and Europe (Foissner and Woelfl 1994). It has been suggested that stentorin and hypericin have a similar in vivo photodynamic action in the visible light spectrum (Duráan and Song 1986) and that the blue-green

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pigmented stentorin molecule can also act as a light-signaling compound (Song and Tapley 1979; Walker et al. 1979; Song et al. 1980, 1981). Recently, it has been shown that stentorin (from *Stentor coeruleus*) bioaccumulates in the blue catfish (*Ictalurus furcatus*), producing discolored tissues (Gale et al. 2015). Blue catfish is an apex predator and there is no direct evidence that it directly ingests ciliates. Thus, the potential deleterious effects of stentorin bioaccumulation by direct or indirect ciliate ingestion remain to be determined (Gale et al. 2015). Larval medaka (*Orizias latipes*) readily consume *S. coeruleus* in the laboratory, while Patagonian native and introduced fish species may also ingest it but in a very low proportion (Kamjunke et al. 2012). Direct predation on *Stentor* spp. was observed mainly for cyclopoid copepods (Lair 1990; Kamjunke et al. 2012), although there are no reports on deleterious effects after ingesting these dark-colored ciliates. Hypericin photosensitization is a reaction that causes Reactive Oxygen Species (ROS) that interact with organic molecules in the cellular membranes (Spikes 1989). Because of the similarity of stentorin with hypericin we postulate that stentorin-bearing prey could increase oxidative stress in their predators. If stentorin is bioaccumulated in the predator body, any potential negative effect (i.e., increase in oxidative stress) would last for a long period after being ingestion.

Large mixotrophic protists (i.e., ciliates) that combine phagotrophy and phototrophy in the same organism seem to possess important ecological advantages as this combined nutritional approach provides greater flexibility in the planktonic environment (Stoecker et al. 2009). Also, the combination of these nutritional modes implies that they likely have a more balanced stoichiometric composition (Flynn and Mitra 2009). Indeed, the influence of mixotrophy may modulate stoichiometric food quality for zooplankton. Purely phototrophic organisms of the epilimnion tend to increase in C content and C : nutrient ratio as light increases (Sterner et al. 1997); however, mixotrophs compensate for increased C fixation by phagotrophy to obtain N and P (Jones 2000; Modenutti and Balseiro 2002). Moreover, black ciliates such as *Stentor* may be able to regulate internal light intensity (Modenutti et al. 2005) and, consequently, their stoichiometric balance may not be the pure result of light-nutrient ratio of the lake. Thus, the overall seston C : P ratio in lake with strong influence of such mixotrophs would be lower than that of purely phototrophic organisms. Given that macrozooplankton predators are constrained by the elemental content of their prey (Laspoumaderes et al. 2015), the stoichiometric balance of mixotrophs may be a key factor affecting the stoichiometry of predator–prey relationships.

Deep large lakes in the northern Patagonia Andes are ultraoligotrophic and highly transparent, with very low nutrient and dissolved organic matter concentrations (Morris et al. 1995; Markert et al. 1997; Modenutti et al. 2000). Because of these features the lack of aerial pollutants (Mladenov et al. 2011) and their proximity to the ozone layer depletion regions (Villafañe et al. 2001), these lakes experience high UVR irradiance deep in the water column. Crustacean zooplankton have a strategy to avoid the upper, high-UV layers (Alonso et al. 2004) and are constrained in their vertical distribution by low food quality and high visual predation (Balseiro et al. 2007). In contrast, the mixotrophic ciliate *Stentor araucanus* appears to be well-adapted to high light levels, achieving high population abundances in epilimnetic depths with sufficient light energy (Im $\sim$ 600 μmol photons m^{-2} s^{-1}) to allow photosynthetic primary production (i.e., C fixation) of their endosymbiotic algae (Modenutti et al. 2005). In Andean lakes, *S. araucanus* coexists, although in the same lakes at different depths, with another mixotrophic ciliate (*Ophrydium naumanni*) that prefers metalimnetic layers and low light irradiances (Modenutti et al. 2004, 2008). Recently, it was observed that the predator cyclopoid *Mesocyclops araucanus* preys upon *S. araucanus* (Kamjunke et al. 2012). Ciliates are important prey for cyclopoid copepods (Wiackowski et al. 1994), which detect them with the help of mechanoreceptors (Rabette et al. 1998). However, since *Stentor* has the stentorin pigment, we hypothesized that stentorin consumption has negative effects on *Mesocyclops* by exposing them to a higher detrimental effects of light due to an increase in oxidative stress. As a consequence, during the day, *Mesocyclops* would need to migrate to lower depths to avoid the effects of ingesting the pigment. This hypothesis suggests that the light threshold for DVM is modified by food, providing strong support for the TRH of Williamson et al. (2011) and adding a new aspect to it. That is, we suggest that food may interact with light in which the ingestion of a particular food item (i.e., stentorin-bearing prey) modifies the predator's visible light threshold, becoming a new and primary dynamic driver for DVM.

To test this hypothesis, we assessed the relationship of UV transparency, visible (PAR) transparency and the two mixotrophic ciliates species as prey, to the vertical distribution of crustacean zooplankton. In order to analyze if stentorin-bearing prey are effectively eaten by *Mesocyclops*, we estimated its feeding rates upon two ciliate species (*S. araucanus* and *O. naumanni* with and without stentorin, respectively). We also determined the C : P ratio of *S. araucanus* and of bulk seston in order to assess if the mixotrophic prey are a better food for *Mesocyclops*. In laboratory experiments, we determined the effect of visible light and UV radiation on *Mesocyclops* fed with the two prey types, measuring the antioxidant status (activity of the enzyme glutathione S-transferase, GST) and levels of oxidative stress (as indexed by glutathione concentration, GSH) of the copepods. Finally, in the laboratory we assessed the accumulation of stentorin in the copepod body and the rate at which stentorin is metabolized in order to establish how long this pigment remains in the copepod's body. In addition, we measured stentorin concentrations in copepods collected in the field to determine not only that the copepods feed on *Stentor* but also that, during the day, copepods still retain the pigment in their bodies.

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Materials and methods

Field study – vertical distribution of ciliates and zooplankton DVM

Lake Guillelmo (41°23′ S and 71°27′ W) is an ultra-oligotrophic, highly transparent Andean North-Patagonian lake with a surface area of 5.4 km^2 and a maximum depth of 100 m. This lake was sampled between February and March of 2016, December 2016 and January 2017 (austral summer). During this period, we sampled the lake for ciliates (*S. araucanus* and *O. naumanni*) and cyclopoids (*M. araucanus*) for the various laboratory experiments and determinations. Live ciliates were collected by vertical tows from 20 m to 0 m with a plankton net of 50-μm mesh size. Live copepods were collected from 60 m to 30 m with a closing net of 100-μm mesh size. On 06 March 2016, 19 December 2016, and 25 January 2017 we carried out a complete sampling schedule analyzing light vertical distribution, ciliates, and zooplankton DVM. Temperature and light vertical profiles (photosynthetically active radiation, PAR, 400–700 nm and UVR, 320 nm and 340 nm) were measured from 0 m to 50 m with a PUV 500B submersible radiometer (Biospherical Instruments). Water samples for ciliate quantification were collected from 0 m to 50 m at five depths (10 m, 20 m, 30 m, 40 m, and 50 m) at noon and midnight with a Schindler–Patalas trap. Samples (in triplicates) were treated with acid Lugol's solution and ciliates were identified and counted with an inverted microscope using 50-mL Utermöhl chambers. Zooplankton diel vertical migration was studied by sampling carried out in four periods of 1 d: after sunrise, noon, after sunset, and midnight. Sampling at noon was carried out at 13:00 h, corresponding with solar noon in this region with midnight sampling at 0:00 h. However, as sunrise and sunset vary with season, sampling times for these periods were adjusted accordingly. On all occasions, vertical tows from 0 m to 10 m, 10 m to 20 m, 20 m to 30 m, 30 m to 40 m, and 40 m to 50 m depth were performed with two closing conical plankton nets (100-μm mesh size). These samples were used for crustacean identification (copepods, both calanoids and cyclopoids, and cladocerans) and quantification under a stereomicroscope in 5-mL Bogorov chambers. We confirmed that, as in many Andean lakes (Balseiro and Modenutti 1998), the community was dominated by the calanoid *Boeckella gracilipes*, followed by cladocerans (*Bosmina longirostris*, *Ceriodaphnia dubia*, and *Daphnia commutata*), and the cyclopoid *M. araucanus*.

Carbon : phosphorus (C : P) ratio in the seston and in *S. araucanus*

Seston elemental composition was analyzed by filtering a volume of 500 mL to 1000 mL of lake water from 10-m depth through HCl-washed and pre-combusted GF/F Whatman filters (450°C for 1.5 h). *S. araucanus* cells were sampled directly from 0 m to 10 m depth, separated in the laboratory under a stereomicroscope, carefully rinsed twice with 0.2-μm filtered lake water, and transferred on to HCl-washed and pre-combusted GF/F filters. There were 10 filters (five replicates for C and five for P) with 430 ± 23 *Stentor* per filter. Total sestonic carbon (TSC) and the C content of *Stentor* were analyzed using a Thermo Finnigan EA1112 (Thermo Finnigan) elemental analyzer. Total phosphorus (TP) and total particulate phosphorus (TPP) of both types of samples were analyzed by persulfate digestion followed by molybdate reaction (APHA 2005).

Feeding rate and stentorin content

We carried out a feeding experiment in order to determine if *Mesocyclops* effectively consumes both ciliate species (*S. araucanus* and *O. naumanni*). We collected both ciliates and *Mesocyclops* from Lake Guillelmo and transported them to the laboratory. Ciliate samples were incubated separately in Guillelmo lake water filtered through GF/C filters with (Treatments) and without (Controls) the presence of adults *Mesocyclops*. The water used in all the experiments was freshly collected from 10-m depth on the same day of experimentation. The experiments lasted for 18 h and were conducted in five replicates in a growth chamber at 14°C in darkness. 200 *S. araucanus* and 3000 *O. naumanni* were exposed separately to three *Mesocyclops* specimens in 50-mL Erlenmeyer flasks. These abundances were chosen because *Stentor* biovolume is 13 times higher than *O. naumanni* (Balseiro et al. 2001). After 18 h of exposure, copepods were removed and the remaining ciliates were quantified with an inverted microscope using 50 mL Utermöhl chambers to determine the number of prey eaten. Controls were used to confirm that the change in abundance was due only to predation by *Mesocyclops*. Results are given in prey predator^{-1} h^{-1}.

To determine if stentorin is bioaccumulated in the *Mesocyclops* body, we collected *S. araucanus* and *Mesocyclops* from Lake Guillelmo and immediately transported them to the laboratory. We then separated 50 *Stentor* cells and three *Mesocyclops* in Eppendorf tubes (at least 10 replicates) in order to determine stentorin concentration in both organisms. Copepods from the field presented a black-colored gut, indicating that *Stentor* was ingested. Stentorin content was extracted in acetone, hexane, and methanol following Gale et al. (2015) and the extract emission was determined fluorometrically with a Perkin-Elmer LS45 with an excitation wavelength of 342 nm (Gale et al. 2015). According to Walker et al. (1979) and Gale et al. (2015), fluorometric emission of stentorin is between 590 nm and 610 nm; our peak emission was at 602 nm.

Each *M. araucanus* used for stentorin determination was carefully rinsed with Milli-Q water and measured by taking images which were then processed via Image-Pro Plus (Media Cybernetics) software. In all cases, average copepod size was 1 ± 0.1 mm total length excluding furcal setae.

We carried out another laboratory experiment to determine how long stentorin remained bioaccumulated in the *M. araucanus* body. Prior to the experiment, we fed *M. araucanus* (adults) with *O. naumanni* for 20 d in order to be sure that there were no traces of stentorin in their bodies. After this time, we confirmed,

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in a subsample of copepods, the absence of stentorin (no fluorescent peak at 602 nm). The remaining copepods were transferred to 500-mL experimental flasks (four replicates) with freshly collected GF/F filtered lake water and *S. araucanus* ($\sim$ 10 ind. mL^{-1}, to feed ad libitum) as the only food source and held for 24 h in darkness. Afterwards, we again transferred the animals to another set of flaks with fresh filtered lake water with stentorin-free food (*O. naumanni*). At this moment, and every 2 d, we sampled four animals of each replicate in order to fluorometrically determine stentorin as described above. The experiment lasted for 10 d and was carried out in a growth chamber at 14°C with a 12 : 12 L : D photoperiod (only PAR). In addition, every 2 d the remaining copepods were transferred to fresh medium (lake water filtered through GF/F filters) and *O. naumanni* as food. Finally, the stentorin decay rate was estimated based on the surface area below the curve (peak at 602 nm) vs. time, applying an exponential decay model.

Light exposure experiment

To assess if *Mesocyclops* experiences increased oxidative stress when fed on *Stentor*, we performed another laboratory experiment. *M. araucanus* used for this experiment were fed with *O. naumanni* for 3 weeks prior to experimentation in order to eliminate any stentorin traces. The absence of stentorin was checked by spectrofluorometry (see above). Subsequently, *M. araucanus* were separated into two treatments, each one fed on one of the two ciliate prey species (*O. naumanni* or *S. araucanus*), in the same concentrations and conditions of the feeding experiments (see above) for 12 h. Afterwards, the copepods of each treatment were exposed to three different light levels in 50-mL quartz flasks for 4 h: Dark, PAR, and PAR + UVR (*see* "Light features" section below) with five replicates. After the 4-h exposure, we separated the copepods and kept them in a −80°C ultra-freezer until later determination of Glutathione S-Transferase (GST) activity and reduced Glutathione (GSH) concentration (*see* "Enzymatic determinations" section below).

Light features

In the laboratory UVR exposure experiments, light 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 (maximum emission at 380 nm). The UV spectrum of the UVA 340 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). During incubation, animals received 35 μW cm^{-2} nm^{-1} of 340 nm wave band, an irradiance level equivalent to surface sunlight in Andean lakes during summer (in Lake Guillermo, the irradiance of 340 nm at 1.5 m depth was 37 μW cm^{-2} nm^{-1}). The total 340-nm wave band dose was of 5040 J m^2 and PAR intensity was 40 μmol photons m^{-2} s^{-1}. In the PAR treatment and in the bioaccumulation experiment, only the two daylight fluorescent lamps (Philips TLT 40 W) were used.

Enzymatic determinations

Enzymes were determined in the *M. araucanus* exposed to the different light treatments with five replicates (each consisting of three copepods pooled). For estimation of the GST activity, each sample was homogenized with an Ultrasonic Homogenizer (Sartorius, LABSONIC M, with one Cycle, 40% of amplitude, and 2 mm Ø point) in 50 μL of buffer solution (Tris-saccharose pH = 7.4) and then incubated on ice for 10 min with an addition of 50 μL lysis buffer. Afterwards, samples were centrifuged at 13,000 $\times$ *g* for 5 min at 4°C to obtain the supernatant (enzyme source). The GST activity was determined fluorometrically (Perkin-Elmer LS45) with excitation 390 nm (10 nm band width) and emission 478 nm (10 nm band width) and for each sample, 50 μL of buffer assay, 4 μL of GSH, and 1 μL of Staurosporine as substrate were added (fluorometric glutathione assay kit, (CS1020) Sigma–Aldrich, St. Louis, Missouri, following manufacturer instructions). The GST was expressed as μmol of product per minute per individual.

Extraction of GSH was performed with the same procedures as for GST. Each sample was incubated for 1 h at 37°C with 50 μL of buffer assay, 5 μL of GST, and 1 μL of staurosporine as substrate. The GSH concentration was measured fluorometrically with excitation 360 nm (10 nm band width) and emission 460 nm (10 nm band width) and expressed in nMol of molecule per individual. The decrease of GSH in the experiment was used as a measure of the oxidative stress of the animals (Parmentier et al. 1999; Souza et al. 2010).

Statistical analysis

Differences in GST activity and GSH concentration from *M. araucanus* exposed to Dark, PAR, and UVR + PAR Light with two offered prey species (*O. naumanni* or *Stentor*) were tested with a two-way ANOVA (factor A: offered food, factor B: light). When significant, after the ANOVA, we ran an a posteriori multiple comparison Tukey's HSD test procedure (< 0.05). All statistical analyses were performed in Sigma Plot 12.5 (Systat Software, San Jose, California).

Results

Diel vertical migration

During summer, Lake Guillelmo was very transparent to the different wavelengths. The average 1% PAR depth (i.e., the euphotic zone) over all of the sampling dates was 31.79 m (standard deviation, SD = 1 m), while the 1% depths for 320 nm and 340 nm UVR were 7 m (SD = 0.25 m) and 9 m (SD = 0.19 m), respectively (Fig. 1a). The two ciliate species were differentially distributed along the water column. *O. naumanni* was present between 0 m and 50 m depths with a maximum abundance at 30 m at which PAR was close to the 1% level and far from UVR levels likely to have any effects (Fig. 1b). In contrast, most *S. araucanus* individuals remained between 0 m and 10 m depths (> 60% in the upper 10 m), where the incidence of potentially hazardous

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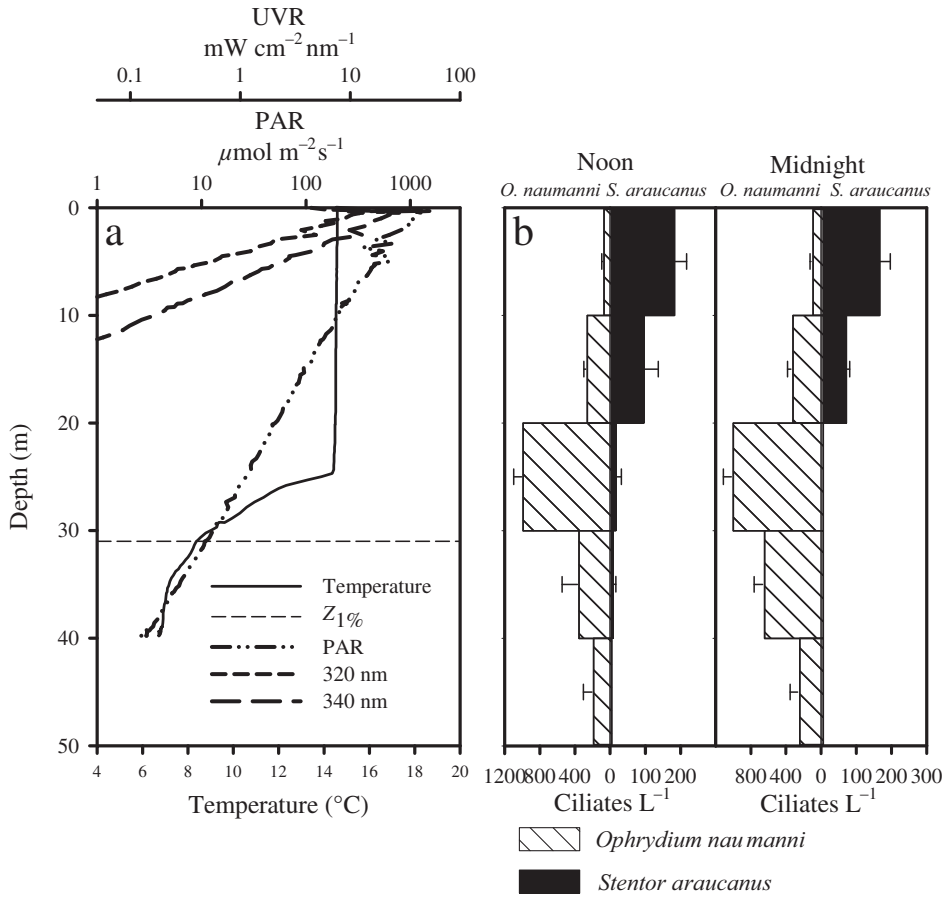

Fig. 1. (**a**) Vertical profiles of light and temperature of lake Guillelmo. Horizontal dashed line indicates the 1% surface PAR. (**b**) Vertical profiles of the abundances of the two ciliate species at midnight and noon. Left facing bars: O. naumanni and right facing bars S. araucanus. Note the different scales for each species. Error bars indicate 1 standard error (three sampling dates).

wavelengths (320 nm and 340 nm) is high (Fig. 1b). The two species did not show changes in their distribution between noon and midnight (Fig. 1b).

Distributions of the crustacean zooplankters differed sharply between day and night, showing a DVM behavior. In particular, during sunrise and noon, the *M. araucanus* population showed a marked surface avoidance, remaining between 40 m and 50 m (65–90% of total *M. araucanus* abundance) (Fig. 2). Between sunset and midnight, the population migrated to upper layers, resulting in an abundance peak between 0 m and 10 m. Interestingly, the depth of 1% surface PAR ($\sim$ 30 m) also represents *Mesocyclops*' lower and upper depth distribution during night and daytime (Fig. 2, see horizontal dashed line). Considering the whole zooplankton relative distribution, we observed that *M. araucanus* exhibited larger amplitude DVM than the other taxa. At noon, 98% of the cyclopoid population was between 30 m and 50 m (and $>$ 60% below 40 m) and the remaining 2% between 20 m and 30 m, while more than the 70% of the cladocerans and of the calanoid *B. gracilipes* population were distributed between 20 m and 40 m. After sunset, 90% of *M. araucanus* individuals were between 0 m and 10 m, while for *B. gracilipes*, only 50% of the population was present in that layer. For cladocerans, more than 70% of the population could be observed between 10 m and 20 m (Fig. 2).

The sestonic C : P ratio (atomic) of Lake Guillelmo was of 340 ± 14, while the C : P ratio of field-collected *S. araucanus* was significantly lower (170 ± 27), representing a twofold increase in P content relative to the bulk seston.

Feeding on both mixotrophic ciliates and stentorin content

In our feeding experiment, *M. araucanus* effectively ate both prey, consuming 1.1 ($\pm$ 0.2) *Stentor* predator^{-1} h^{-1} and 9.3 ($\pm$ 0.9) *O. naumanni* predator^{-1} h^{-1}.

In the laboratory, we were able to identify, by spectrofluometry, the presence of stentorin in both the ciliate (*S. araucanus*) and the copepod (*M. araucanus*), with a clear emission peak at 602 nm (Fig. 3a,b) that was the same as that registered by Gale et al. (2015). *M. araucanus* from the field showed variable emission intensity at 602 nm; however, the maximum emission observed per copepod was very similar to that of 50 *Stentor* (Fig. 3).

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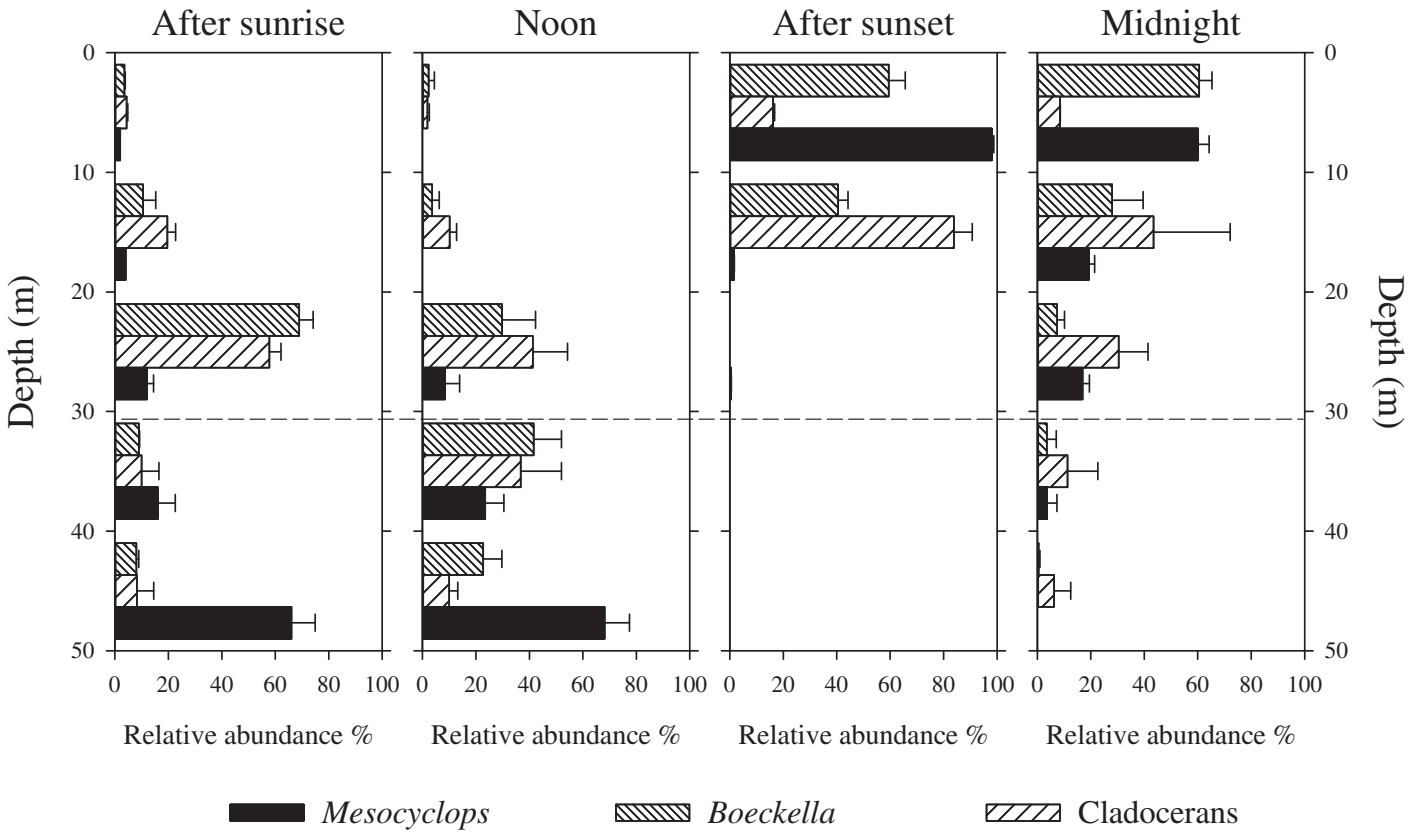

Fig. 2. Vertical relative distributions of crustacean zooplankton in lake Guillelmo after sunrise, noon, after sunset, and at midnight. Left facing bars: *M. araucanus* and right facing bars: *B. gracilipes* (Calanoid copepod) and *D. commutata* (Cladocera). Error bars indicate 1 standard error (three sampling dates). Horizontal dashed line indicates the 1% surface PAR.

In the bioaccumulation experiment, we observed a continuous, exponential decline in stentorin concentration (Fig. 4a,b) during the 9 d (decay rate = 0.129 d^{-1}).

Light exposure experiment

In the light exposure experiment, we obtained different results according to prey treatment. GST activity in *M. araucaunus* exhibited a significant light × prey (*Stentor* vs. *Ophrydium*) interaction within the PAR treatment (a posteriori Tukey's test $p = 0.006$), with GST significantly higher for *Stentor* as prey (Fig. 5a,b). Dark and UVR + PAR exposure did not result in differences within prey treatments. In contrast, light treatments with *O. naumanni* showed significantly higher GST activity in the UVR treatment than in the PAR and Dark treatments (a posteriori Tukey's test with Dark $p = 0.014$ and with PAR $p = 0.038$), while light exposure with *Stentor* as prey showed significantly higher GST activity in both PAR and UVR + PAR treatments than under Dark exposure (a posteriori Tukey's test with PAR $p = 0.005$ and with UVR $p = 0.002$).

As expected, GSH concentration showed results opposite to those for GST in *O. naumanni* (Fig. 5d) while, in *S. araucanus*, we observed decrease GSH from Dark to UVR + PAR treatments (Fig. 5c). The prey × light treatment interaction was significant (two-way ANOVA $F_{2,\ 23} = 12.0$, $p < 0.001$; Fig. 5c,d) and post-hoc tests showed prey-type differences only for the PAR treatment (a posteriori Tukey test $p < 0.001$). In the PAR treatment, higher GSH concentration occurred in copepods fed *O. naumanni* rather than *Stentor*, indicating higher oxidative damage with *Stentor* as food resource. Additionally, in the *Stentor* treatment, GSH concentration showed significant differences among the three light treatments (a posteriori Tukey test $p < 0.001$), with highest concentration in the Dark and lowest in UVR + PAR. These results suggest that PAR exposure for *Stentor*-fed copepods became stressful due to the interaction of stentorin with PAR light. However, UVR + PAR was still more hazardous than PAR only, as shown by the reduction of GSH concentration (Fig. 5c).

Discussion

We found that the copepod *M. araucanus* suffered from oxidative stress when it fed on *S. araucanus* (with stentorin). In our experiments in the PAR treatment, when *Mesocyclops* fed on *Stentor*, it exhibited an enhanced antioxidant defense response as indicated by increased activity of GST. GST activity was the same under PAR and UVR + PAR light, indicating that the presence of PAR itself was enough to cause oxidative

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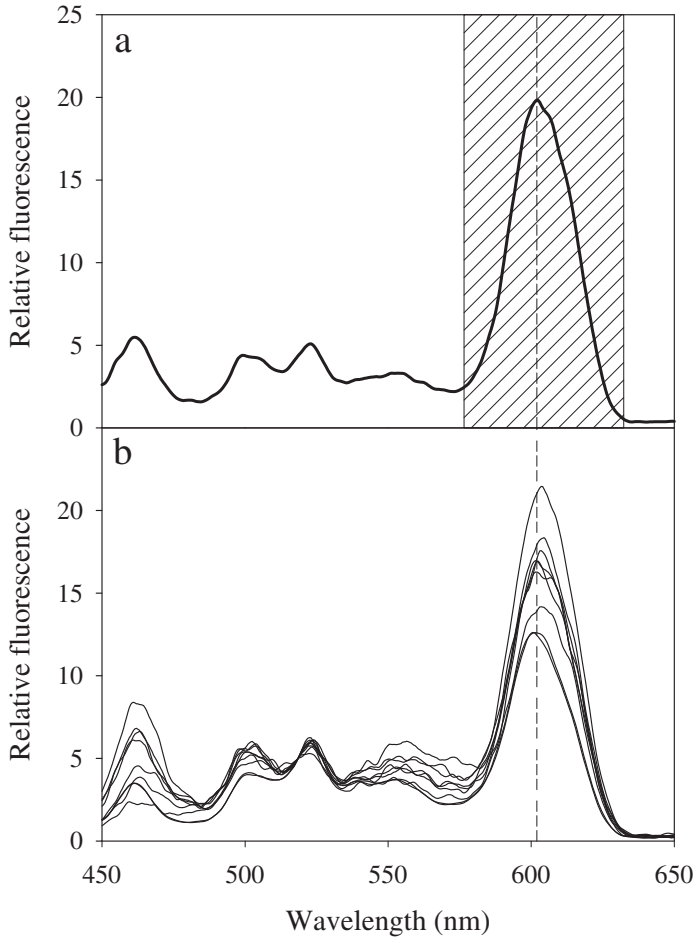

Fig. 3. a. Fluorescence spectra of stentorin extracted from 50 field collected *S. araucanus* (mean of five samples) and **b.** from one adult field collected *M. araucanus* (different sampling dates), at an excitation wavelength of 342 nm. The shadow area shows the specific peak of stentorin, and the vertical dashed line the 602 nm emission.

stress. As expected, GSH concentration showed an opposite trend relative to GST (Fig. 5), further supporting our inference of increased oxidative stress in the PAR and PAR + UVR treatments. GSH in animals fed on *Stentor* was lower under PAR than in the Dark, suggesting a stronger detrimental oxidative effect of the PAR alone than when feeding on non-bearing stentorin prey such as *O. naumanni*. Nevertheless, copepods fed on *Stentor* showed even lower GSH when exposed to UVR + PAR, indicating that the antioxidant response of GST (the same activity in both treatments) was insufficient. This may imply that the GST activity had reached its maximum and was not higher in the UVR + PAR treatment not because it was not necessary but because it was not possible. The consequence is a further decrease in GSH.

We also were able to show that stentorin bioaccumulates in copepods and remains measurable for at least a week (one third of the stentorin was still present after 9 d), suggesting that stentorin-induced oxidative stress might last for days. Stentorin has been described as a molecule similar to hypericin, a molecule known to have long-lasting photo-sensitizing effects in animals. For example, when cattle feed on *Hypericum perforatum* (a source of hypericin), they show a light sensitivity which remains for a week or more, suggesting that the pigment forms tightly bound complexes with cellular components (Duráan and Song 1986). Hypericin photosensitization is a reaction mediated by a molecule that absorbs visible light and transfers the electromagnetic radiation to adjacent molecules, generating direct damage or Reactive Oxygen Species (ROS) that interact with organic molecules in the cellular membranes (Spikes 1989). However, under dark conditions, the same molecule will not react (Song 1983); this is similar to what we observed in our Dark treatment with copepods fed on stentorin-containing food.

M. araucanus fed on both large ciliate species (*O. naumanni* and *S. araucanus*), regardless of whether stentorin was present or absent. In the field, we indirectly estimated (via stentorin accumulation in the copepod body) that *Mesocyclops* accumulate an amount of stentorin equivalent to $\sim$ 50 *Stentor* individuals per copepod. If stentorin remains for several days with a decay rate of 0.129 d^{-1}, as was shown in our experiment, the stentorin equivalent to 50 *Stentor* individuals would be reached after 5 d of feeding during the night. Thus, the amount of stentorin we observed in some copepods is within an expected range. However, most of our stentorin determinations were lower, indicating that copepods are metabolizing this compound; nevertheless, it is still present and can affect them if they are exposed to light.

Among heterotrophic protists, ciliates show rather strict stoichiometric homeostasis, with C : P ratios less than 200 (Golz et al. 2015). For *S. araucanus*, we observed a C : P ratio $\sim$ 170, which is one half of that for the bulk seston of Lake Guillelmo. This means that, from a stoichiometric perspective, *Stentor* would be a good food source in a system with low food quality. In contrast, some mixotrophs have toxic compounds to discourage predators e.g., red-tide bloom species (Burkholder et al. 2008; Flynn 2008), and therefore, reduced consumption of *Stentor* might be expected. However, our laboratory experiments showed that *S. araucanus* was eaten without any evidence of avoidance of feeding upon this stentorin-rich prey. In contrast, fishes and invertebrates avoid eating the sessile pigmented ciliate *Maristentor* that bears a chemically similar pigment (Lobban et al. 2014). The fact that *Mesocyclops* can move along the water column may help explain its feeding on *S. araucanus*, since the long range DVM displayed by *Mesocyclops* likely protects the copepods from stentorin's light effect. During the day, *M. araucanus* and *S. araucanus* are distributed far away from each other in the lake water column; one is at a depth of 50 m and the other between 5 m and 10 m. However, during the night, *Mesocyclops* migrates to surface layers to feed intensively on *Stentor* (as indicated by accumulation of stentorin in the copepod's body). Because cyclopoid copepods are predators that select on

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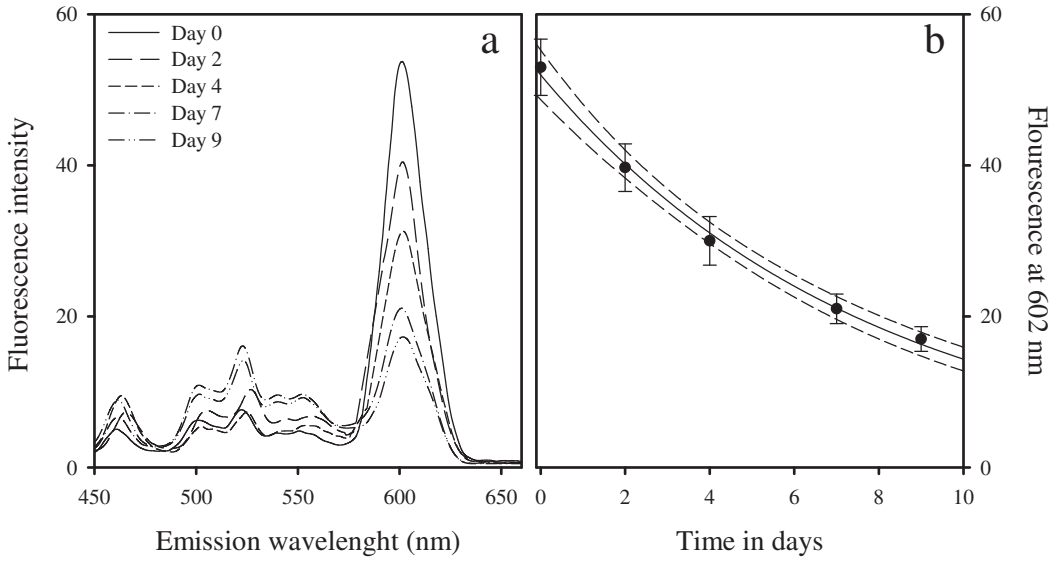

Fig. 4. Results of the laboratory experiment on the decay of stentorin in *M. araucanus*: (**a**) fluorescence spectra of stentorin (602 nm peak) extracted from 1 *M. araucanus* at increasing days after fed with *S. araucanus*. (**b**) Decay rate estimated from the maximum fluorescence at 602 nm for each day. Error bars represent standard deviation, dashed lines 95% confidence interval of adjusted curve.

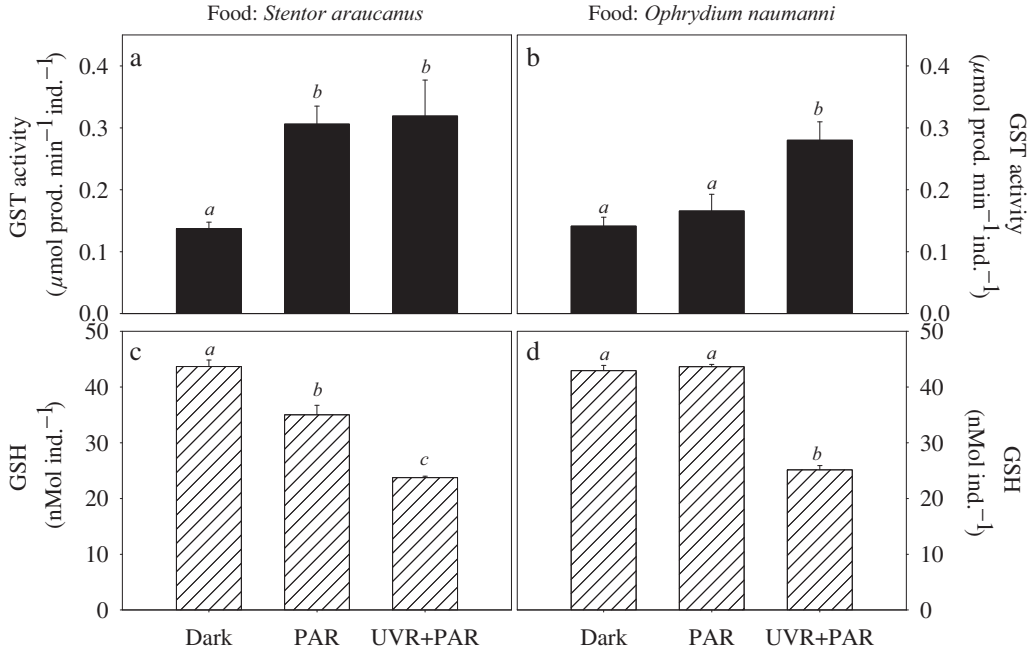

Fig. 5. Results from the exposure experiments of *M. araucanus* fed on *S. araucanus* (left graphs, **a**, **c**) and *O. naumanni* (right graphs, **b**, **d**). (**a**, **b**) Glutathione S-Transferase (GST) activity and (**c**, **d**) reduced Glutathione concentration (GSH). Letters in *italics* above bars indicate homogeneous groups determined by ANOVA.

the basis of size, hardness, behavior (Kerfoot 1978), and the abundance of alternate prey (Wickham 1995), *Mesocyclop*s probably detects *Stentor* more easily than *O. naumanni* because its larger size and its increased abundance in the upper 10 m. On the contrary, *O. naumanni* has increased abundance at a depth of 30 m during day and night, so that *Mesocyclops* would not need to migrate to surface layers to feed on them. Our data suggest that copepods are searching for a prey such as *Stentor* or, alternatively, for the higher epilimnetic temperatures that can enhance growth and reproduction (Williamson et al. 2011). In

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any case, the encounter of predator and prey is achieved in epilimnetic layers. However, since the pigment stentorin of this prey would have a hazardous effect in the presence of visible light, copepods should migrate down far away from the euphotic zone, deeper than other migrating plankton. Notably, the PAR intensity during the experiments (40 μmol photons m^{-2} s^{-1}, *see* Fig. 1a) was equivalent to the light intensity at a depth of 23 m on a summer day. On the one hand, there is no UVR at this depth (*see* Fig. 1a), so our experimental treatment very closely resembled natural conditions with low PAR and no UVR. *Mesocyclops* still showed oxidative stress (high GST with lowered GSH) (Fig. 5). This means that *Mesocyclop*s needs to migrate even deeper to avoid this low PAR intensity and cannot remain at the same depth of other crustaceans during the day (*see* Fig. 2).

Fish predation pressure is a key factor driving the DVM of planktonic organisms (Williamson et al. 2011). In Lake Guillelmo, fish predation cannot be neglected, since native (*Galaxias maculatus*) and exotic salmonid fishes are present and prey upon planktonic crustaceans (Balseiro et al. 2007). However, as fish predation is size dependent, larger zooplankton should migrate deeper to avoid fishes. *M. araucanus* is $\sim$ 1 mm in length, smaller than the mean adult size of *D. commutata* (2.5 mm length). The latter migrates to $\sim$ 25–35 m depth (around 1% surface PAR) while *Mesocyclops* migrates to $\sim$ 45 m (around 0.1% surface PAR). This substantial difference in migration depth cannot be explained by visual predation pressure, since it should be just the inverse, with the larger species migrating to deeper layers. Interestingly, our results showed that *M. araucanu*s populations remain in a deeper position during the day than the other zooplankton species of Lake Guillelmo. These taxa (which include cladocerans such as *Daphnia* and the calanoid copepod *B. gracilipes*) are not able to feed on *Stentor*, mainly due to prey large size (Balseiro et al. 2001). We can assume that these species are not at risk of consuming stentorin and therefore do not suffer from oxidative stress under low light as we observed for *Mesocyclops*. Indeed, we could not detect stentorin in the body of *Boeckella* nor *Daphnia*. Thus, the detrimental effect (i.e., increase in oxidative stress) of eating *Stentor* may explain the larger amplitude range of DVM in *M. araucanus*.

Our findings are the first to show that a compound in a prey item can change vertical behavior of predators in order to minimize negative biochemical effects, providing novel insight into of zooplankton DVM and highlighting the role of phototoxic compounds in some ciliates. Previously, the theory of zooplankton DVM was focused largely on food availability and predation (Bollens and Frost *a*,*b*). However, recent evidence has indicated that abiotic factors, such as hazardous UVR and temperature, also play important roles (Kessler et al. 2008). This adds further support to the TRH (Williamson et al. 2011), which pointed out the important role of water transparency in regulating DVM. In previous work in transparent mountain systems of the Northern Hemisphere, DVM of copepods was found to be mainly related to UVR supporting the TRH and suggesting UVR as the major driver of DVM (Fischer et al. 2015). However, our study demonstrates that the interaction of photosensitizing food and visible light can also be a primary driver of DVM. Our hypothesis that visible light is the main driving factor of DVM in our study systems is supported by the observations that oxidative stress is increased when *Mesocyclops* feeds on *Stentor* and that the DVM of *Mesocyclops* is of larger amplitude than that of crustaceans of larger size. In transparent systems such as north Patagonian Andean lakes, both sestonic food quality and visual predator abundance are often low but damaging UVR is usually high in the surface layers (Balseiro et al. 2007). In this scenario, a potentially highly nutritious prey (C : P = 170) such as *S. araucanus* developed resistance to the high UVR levels in surface waters (Modenutti et al. 2005). This high quality prey was therefore effectively consumed by cyclopoids, which consequently must exhibit strong DVM to prevent photodamage caused by the UV-protective pigment carried by the ciliate. This finding may represent a new pathway of ecological interactions that could help explain behavior, energy transfer, and differential species distributions in the pelagic community of inland waters and oceans.

References

Alonso, C., V. Rocco, J. P. Barriga, M. A. Battini, and H. Zagarese. 2004. Surface avoidance by freshwater zooplankton: Field evidence on the role of ultraviolet radiation. Limnol. Oceanogr. **49**: 225–232. doi:10.4319/lo.2004.49.1.0225

APHA. 2005. Standard methods for the examination of water and wastewater. American Public Health Association, AWWA.

Balseiro, E. G., and B. E. Modenutti. 1998. Pelagic communities and ecosystem processes in Andean Patagonian Lakes. Ecol. Austral **8**: 183–189.

Balseiro, E. G., B. E. Modenutti, and C. P. Queimaliños. 2001. Feeding of *Boeckella gracilipes* (Copepoda, Calanoida) on ciliates and phytoflagellates in an ultraoligotrophic Andean lake. J. Plankton Res. **23**: 849–857. doi:10.1093/plankt/23.8.849

Balseiro, E. G., B. E. Modenutti, C. Queimaliños, and M. Reissig. 2007. *Daphnia* distribution in Andean Patagonian lakes: Effect of low food quality and fish predation. Aquat. Ecol. **41**: 599–609. doi:10.1007/s10452-007-9113-3

Balseiro, E. G., M. S. Souza, B. E. Modenutti, and M. Reissig. 2008. Living in transparent lakes: Low food P:C ratio decreases antioxidant response to ultraviolet radiation in *Daphnia*. Limnol. Oceanogr. **53**: 2383–2390. doi:10.4319/lo.2008.53.6.2383

Boeing, W. J., D. M. Leech, C. E. Williamson, S. Cooke, and L. Torres. 2004. Damaging UV radiation and invertebrate predation: Conflicting selective pressures for zooplankton vertical distribution in the water column of low DOC lakes. Oecologia **138**: 603–612. doi:10.1007/s00442-003-1468-0

Bollens, S. M., and B. W. Frost. 1989*a*. Predator-induced diel vertical migration in a planktonic copepod. J. Plankton Res. **11**: 1047–1065. doi:10.1093/plankt/11.5.1047

Bollens, S. M., and B. W. Frost. 1989*b*. Zooplanktivorous fish and variable diel vertical migration in the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. **34**: 1072–1083. doi:10.4319/lo.1989.34.6.1072

Borgeraas, J., and D. O. Hessen. 2000. UV-B induced mortality and antioxidant enzyme activities in *Daphnia magna* at different oxygen concentrations and temperatures. J. Plankton Res. **22**: 1167–1183. doi:10.1093/plankt/22.6.1167

Borgeraas, J., and D. O. Hessen. 2002. Variations of antioxidant enzymes in Daphnia species and populations as related to ambient UV exposure. Hydrobiologia **477**: 15–30. doi:10.1023/A:1021056409446

Briggs, W. R., and J. L. Spudich. 2005. Handbook of photosensory receptors. John Wiley & Sons.

Burkholder, J. M., P. M. Glibert, and H. M. Skelton. 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae **8**: 77–93. doi:10.1016/j.hal.2008.08.010

Duráan, N., and P. S. Song. 1986. Hypericin and its photodynamic action. Photochem. Photobiol. **43**: 677–680. doi:10.1111/j.1751-1097.1986.tb05646.x

Ekvall, M. T., S. Hylander, T. Walles, X. Yang, and L.-A. Hansson. 2015. Diel vertical migration, size distribution and photoprotection in zooplankton as response to UV-A radiation. Limnol. Oceanogr. **60**: 2048–2058. doi:10.1002/lno.10151

Fischer, J. M., M. H. Olson, N. Theodore, C. E. Williamson, K. C. Rose, and J. Hwang. 2015. Diel vertical migration of copepods in mountain lakes: The changing role of ultraviolet radiation across a transparency gradient. Limnol. Oceanogr. **60**: 252–262. doi:10.1002/lno.10019

Flynn, K. J. 2008. Attack is not the best form of defense: Lessons from harmful algal bloom dynamics. Harmful Algae **8**: 129–139. doi:10.1016/j.hal.2008.08.007

Flynn, K. J., and A. Mitra. 2009. Building the "perfect beast": Modelling mixotrophic plankton. J. Plankton Res. **31**: 965–992. doi:10.1093/plankt/fbp044

Foissner, W., and S. Woelfl. 1994. Revision of the genus Stentor Oken (Protozoa, Ciliophora) and description of S. araucanus nov. spec. from South American lakes. J. Plankton Res. **16**: 255–289. doi:10.1093/plankt/16.3.255

Gale, R. W., D. M. Papoulias, and C. J. Schmitt. 2015. Bioaccumulation of stentorin, the probable causative agent for discolored ("Purple") eggs and ovaries in Blue Catfish (*Ictalurus furcatus*) from Eufaula Lake, Oklahoma, USA. Environ. Sci. Technol. **49**: 9639–9647. doi:10.1021/acs.est.5b02273

Golz, A.-L., A. Burian, and M. Winder. 2015. Stoichiometric regulation in micro- and mesozooplankton. J. Plankton Res. **37**: 293–305. doi:10.1093/plankt/fbu109

Hairston, N. G. 1979. The relationship between pigmentation and reproduction in two species of *Diaptomus* (Copepoda). Limnol. Oceanogr. **24**: 38–44. doi:10.4319/lo.1979.24.1.0038

Jones, R. I. 2000. Mixotrophy in planktonic protists: An overview. Freshw. Biol. **45**: 219–226. doi:10.1046/j.1365-2427.2000.00672.x

Kamjunke, N., M. Kramps, S. Chavez, and S. Woelfl. 2012. Consumption of large, Chlorella-bearing ciliates (*Stentor*) by *Mesocyclops araucanus* in North Patagonian lakes. J. Plankton Res. **34**: 922–927. doi:10.1093/plankt/fbs051

Kerfoot, W. C. 1978. Combat between predatory copepods and their prey: *Cyclops*, *Epischura* and *Bosmina*. Limnol. Oceanogr. **23**: 1089–1102. doi:10.4319/lo.1978.23.6.1089

Kessler, K., and W. Lampert. 2004. Depth distribution of *Daphnia* in response to a deep-water algal maximum: The effect of body size and temperature gradient. Freshw. Biol. **49**: 392–401. doi:10.1111/j.1365-2427.2004.01190.x

Kessler, K., R. S. Lockwood, C. E. Williamson, and J. E. Saros. 2008. Vertical distribution of zooplankton in subalpine and alpine lakes: Ultraviolet radiation, fish predation, and the transparency-gradient hypothesis. Limnol. Oceanogr. **53**: 2374–2382. doi:10.4319/lo.2008.53.6.2374

Lair, N. 1990. Effects of invertebrate predation on the seasonal succession of a zooplankton community: A two year study in Lake Aydat, France. Hydrobiologia **198**: 1–12. doi:10.1007/BF00048618

Laspoumaderes, C., B. Modenutti, J. Elser, and E. Balseiro. 2015. Does the stoichiometric carbon:phosphorus knife edge apply for predaceous copepods? Oecologia **178**: 557–569. doi:10.1007/s00442-014-3155-8

Leech, D. M., W. J. Boeing, S. L. Cooke, C. E. Williamson, and L. Torres. 2009. UV-enhanced fish predation and the differential migration of zooplankton in response to UV radiation and fish. Limnol. Oceanogr. **54**: 1152–1161. doi:10.4319/lo.2009.54.4.1152

Lobban, C. S., S. J. Hallam, P. Mukherjee, and J. W. Petrich. 2007. Photophysics and multifunctionality of hypericin-like pigments in heterotrich ciliates: A phylogenetic perspective. Photochem. Photobiol. **83**: 1074–1094. doi:10.1111/j.1751-1097.2007.00191.x

Lobban, C. S., M. Schefter, and T. J. Donaldson. 2014. Cluster dynamics in *Maristentor dinoferus*, a gregarious benthic ciliate with zooxanthellae and a hypericin-like pigment, in relation to biofilm grazing by the fish *Ctenochaetus striatus*. Symbiosis **63**: 137–147. doi:10.1007/s13199-014-0295-x

Macfadyen, E. J., C. E. Williamson, G. Grad, M. Lowery, W. H. Jeffrey, and D. L. Mitchell. 2004. Molecular response to climate change: Temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean *Daphnia pulicaria*. Glob. Chang. Biol. **10**: 408–416. doi:10.1111/j.1529-8817.2003.00750.x

Markert, B., F. Pedrozo, W. Geller, K. Friese, S. Korhammer, G. Baffico, M. Diaz, and S. Wolfl. 1997. A contribution to the study of the heavy-metal and nutritional element

J_ID: LNO Customer A_ID: LNO10681 Cadmus Art: LNO10681 Ed. Ref. No.: LO-17-0062.R1 Date: 8-September-17 Stage: Page: 11

status of some lakes in the southern Andes of Patagonia (Argentina). Sci. Total Environ. **206**: 1–15. doi:10.1016/S0048-9697(97)00218-0

Mladenov, N., and others. 2011. Dust inputs and bacteria influence dissolved organic matter in clear alpine lakes. Nat. Commun. **2**: 405. doi:10.1038/ncomms1411

Modenutti, B. E., G. L. Perez, E. G. Balseiro, and C. P. Queimaliños. 2000. Relationship between light availability, chlorophyll a and total suspended solid in a glacial lake of South Andes. Verh. Int. Ver. Limnol. **27**: 2648–2651.

Modenutti, B. E., and E. G. Balseiro. 2002. Mixotrophic ciliates in an Andean lake: Dependence on light and prey of an *Ophrydium naumanni* population. Freshw. Biol. **47**: 121–128. doi:10.1046/j.1365-2427.2002.00783.x

Modenutti, B. E., E. G. Balseiro, C. Callieri, C. Queimaliños, and R. Bertoni. 2004. Increase in photosynthetic efficiency as a strategy of planktonic organisms exploiting deep lake layers. Freshw. Biol. **49**: 160–169. doi:10.1046/j.1365-2427.2003.01169.x

Modenutti, B. E., E. G. Balseiro, C. Callieri, R. Bertoni, and C. P. Queimaliños. 2005. Effect of UV-B and different PAR intensities on the primary production of the mixotrophic planktonic ciliate *Stentor araucanus*. Limnol. Oceanogr. **50**: 864–871. doi:10.4319/lo.2005.50.3.0864

Modenutti, B. E., E. G. Balseiro, C. Callieri, and R. Bertoni. 2008. Light versus food supply as factors modulating niche partitioning in two pelagic mixotrophic ciliates. Limnol. Oceanogr. **53**: 446–455. doi:10.4319/lo.2008.53.2.0446

Moller, K. M. 1962. On the nature of stentorine. C. R. Trav. Lab Carlsberg **32**: 471–498.

Morris, D. P., H. Zagarese, C. E. Williamson, E. G. Balseiro, B. R. Hargreaves, B. E. Modenutti, R. Moeller, and C. Queimaliños. 1995. The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. Limnol. Oceanogr. **40**: 1381–1391. doi:10.4319/lo.1995.40.8.1381

Oda, S., and T. Hanazato. 2008. Diel vertical migration patterns in two populations of *Chaoborus flavicans* larvae (Diptera: Chaoboridae) in response to fish kairomones. J. Limnol. **67**: 93–99. doi:10.4081/jlimnol.2008.93

Parmentier, C., M. Wellman, A. Nicolas, G. Siest, and P. Leroy. 1999. Simultaneous measurement of reactive oxygen species and reduced glutathione using capillary electrophoresis and laser-induced fluorescence detection in cultured cell lines. Electrophoresis **20**: 2938–2944. doi:10.1002/(SICI)1522-2683(19991001)20:14<2938::AID-ELPS2938>3.0.CO;2-M

Rabette, C., A. Thouvenot, and N. Lair. 1998. Laboratory experiments on trophic relationships and remote detection between two ciliates and *Cyclops vicinus vicinus*. Hydrobiologia **374**: 157–167.

Shick, J. M., S. Romaine-Lioud, C. Ferrier-Pagés, and J.-P. Gattuso. 1999. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates. Limnol. Oceanogr. **44**: 1667–1682. doi:10.4319/lo.1999.44.7.1667

Sommaruga, R., and F. Garcia-Pichel. 1999. UV-absorbing mycosporine-like compounds in planktonic and benthic organisms from a high-mountain lake. Arch. Hydrobiol. **144**: 255–269. doi:10.1127/archiv-hydrobiol/144/1999/255

Song, P.-S. 1983. Protozoan and related photoreceptors: Molecular aspects. Annu. Rev. Biophys. Bioeng. **12**: 35–68. doi:10.1146/annurev.bb.12.060183.000343

Song, P. S., and K. J. Tapley. 1979. Photochemistry and photobiology of psoralens. Photochem. Photobiol. **29**: 1177–1197. doi:10.1111/j.1751-1097.1979.tb07838.x

Song, P. S., D. P. Häder, and K. L. Poff. 1980. Phototactic orientation by the ciliate, Stentor coeruleus. Photochem. Photobiol. **32**: 781–786. doi:10.1111/j.1751-1097.1980.tb04055.x

Song, P. S., E. B. Walker, R. A. Auerbach, and G. W. Robinson. 1981. Proton release from Stentor photoreceptors in the excited states. Biophys. J. **35**: 551–555. doi:10.1016/S0006-3495(81)84811-4

Souza, M. S., E. G. Balseiro, C. Laspoumaderes, and B. E. Modenutti. 2010. Effect of ultraviolet radiation on Acetylcholinesterase activity in freshwater copepods. Photochem. Photobiol. **86**: 367–373. doi:10.1111/j.1751-1097.2009.00675.x

Spikes, J. D. 1989. Photosensitization, p. 79–110. *In* K. C. Smith [ed.], The science of photobiology. Springer.

Sterner, R. W., J. J. Elser, E. J. Fee, S. J. Guildford, and T. H. Chrzanowski. 1997. The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. Am. Nat. **150**: 663–684. doi:10.1086/286088

Stoecker, D. K., M. D. Johnson, C. De Vargas, and F. Not. 2009. Acquired phototrophy in aquatic protists. Aquat. Microb. Ecol. **57**: 279–310. doi:10.3354/ame01340

Terazima, M. N., and T. Harumoto. 2004. Defense function of pigment granules in the ciliate *Blepharisma japonicum* against two predatory protists, *Amoeba proteus* (Rhizopodea) and *Climacostomum virens* (Ciliata). Zool. Sci. **21**: 823–828. doi:10.2108/zsj.21.823

Villafañe, V., E. W. Helbling, and H. E. Zagarese. 2001. Solar ultraviolet radiation and its impact on aquatic systems of Patagonia, South America. Ambio **30**: 112–117. doi:10.1579/0044-7447-30.2.112

Walker, E. B., L. Tae Young, and S. Pill-Soon. 1979. Spectroscopic characterization of the stentor photoreceptor. Biochim. Biophys. Acta **587**: 129–144. doi:10.1016/0304-4165(79)90227-7

Wiackowski, K., M. T. Brett, and C. R. Goldman. 1994. Differential effects of zooplankton species on ciliate community

ID: jwweb3b2server Time: 20:15 I Path: //chenas03/Cenpro/ApplicationFiles/Journals/Wiley/LNO#/Vol00000/170167/Comp/APPFile/JW-LNO#170167

structure. Limnol. Oceanogr. **39**: 486–492. doi:10.4319/lo.1994.39.3.0486

Wickham, S. A. 1995. *Cyclops* predation on ciliates: Species-specific differences and functional responses. J. Plankton Res. **17**: 1633–1646. doi:10.1093/plankt/17.8.1633

Williamson, C. E., J. M. Fischer, S. M. Bollens, E. P. Overholt, and J. K. Breckenridge. 2011. Towards a more comprehensive theory of zooplankton diel vertical migration: Integrating ultraviolet radiation and water transparency into the biotic paradigm. Limnol. Oceanogr. **56**: 1603–1623. doi:10.4319/lo.2011.56.5.1603

Winder, M., and D. E. Schindler. 2004. Climate change uncouples trophic interation in an aquatic ecosystem. Ecology **85**: 2100–2106. doi:10.1890/04-0151

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Conflict of Interest

None declared.

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