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Daphnids adaptive strategies to UV radiation

Carla E. Fernández

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Abstract

This thesis focuses on the adaptive strategies of daphnids that allows them to maintain stable populations while deal with UV radiation, highlighting the differences in the responses that populations with different evolutionary histories may have.

Although exposition to lethal doses in nature is unlikely, sub-lethal UV-B radiation doses may have strong impacts on zooplankton. This work initiates with a study aimed at determining the effect of sub-lethal doses UV-B radiation on filtration rates of two cladoceran species (Daphnia pulicaria and Ceriodaphnia dubia) and showed that filtration rates of D. pulicaria and C. dubia decreased by 50% and more than 80%, respectively, enhancing the importance of avoidance behaviour and recovery strategies for freshwater zooplankton species.

This study was followed by a second one that assessed the ability of Daphnia pulex to develop tolerance to UV radiation, while disentangling, at the same time, the relative importance of local adaptations and ontogenetic processes behind such tolerance. Two populations from environments strongly differing in UV radiation conditions (Bolivian Andes and southern of Sweden) were UV-induced to produce photo-protective compounds and changes in behavioural responses that were monitored by 3D Nano-tracking. Although changes in tolerance capacity of both populations were evident in the results, differences between populations were more related to local evolution than to short-time ontogenetic processes, showing that tolerance to UV radiation is dependent on the evolutionary history of each species population.

Such evolutionary differences should be reflected in the life-histories of both populations so that the next study, through structural equation modelling, shows clear differences in the life-history structures of both populations of Daphnia pulex. Main differences involve indirect effects of UV radiation on offspring production as well as the age at first reproduction. In addition to known tolerance strategies to UV radiation, a population strategy that includes early reproduction and high fertility to compensate for the fitness loss imposed by UV radiation stress, may be developed by populations from high-UV environments. A further study was conducted to explore how these high fertility populations may maintain stable populations in a gradient of resource availability and different sources of light. The results suggest that Daphnia may use resting eggs as self-regulating strategy, which can work as a dissipative structure which slows entropy production in the system, or as a mechanism of population-density control that allows re-population on the long term..

Key words: Cladocera, zooplankton, Andean lakes, ultraviolet radiation, migration strategy, photo-protection, *Daphnia*.

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To everyone who wants to read this work……

Contents

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Thank you!

Yes!!! It is finished! ლ(´￣∇￣`)ლ

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Popular summary

The depletion of the ozone layer has led to greater exposure to ultraviolet (UV) radiation, increasing susceptibility to the negative impacts of over-exposure for terrestrial and aquatic organisms. Nevertheless, side effects of climate change and water extraction for human consumption may lead to even more pronounced increases in ultraviolet radiation that freshwater organisms receive. Especially in high mountain lakes where organisms are naturally exposed to high levels of UV radiation due to a thinner atmosphere as an effect of altitude. In high-Andean lakes this effect is further enhanced due their location at tropical latitudes, thus the species inhabit these ecosystems should be expected to have developed strategies to cope with high levels of UV radiation.

Zooplankton is one of the most vulnerable groups of freshwater organisms to UV radiation. In this work my results show that sub-lethal effects of UV radiation may have strong negative impacts on the grazing activity of key species such as *Daphnia* and *Ceriodaphnia*. Therefore, increased doses of UV radiation received by the organisms due to natural changes or human influence may strongly disturb the structure and functioning of high-Andean lakes.

During the last decades, a considerable amount of research has been done regarding the resistance processes and strategies that zooplankton have adopted to avoid UV damage. Among them, the importance of photo-protective substances has captured most attention, mainly due to their efficiency but also because of the trade-offs involved. The development of photo-protection may involve energetic costs and risk of predation, but also several benefits that may improve the fitness of organisms. Performing vertical migration downward in the water column is the counterbalance strategy, thus an inverse relationship between vertical migration and the content of photo-protective substances is expected. I examined two populations of *Daphnia pulex* which differed in the UV radiation levels they historically received at their place of origin, from extremely high UV radiation stress at high-altitude Bolivian lakes to low UV radiation stress near sea level in southern Sweden. My results indicate that tolerance capacity, in terms of photo-protective substances plus swimming behaviour, may be induced in relatively short times, but that the expression of this tolerance depends on the evolutionary history of each population.

I also examined the life-history traits of both populations and identified the involved trade-offs. Main differences between populations involved UV radiation effects that affected offspring production as well as the age at first reproduction. In addition to the well-known strategies against UV radiation, including avoidance, prevention, or repairing of damages, I propose here an evolutionary population strategy that

includes the selection of early reproduction and high fertility lines, as response to UV radiation.

Finally, my work explores the resting eggs production and how it is influenced by resources availability and solar radiation. Cladocerans are able to produce resting eggs inside a protective capsule that may resist harsh conditions and maintain viable even for decades. My results show that solar radiation increases the production of resting eggs, principally at high food availability, but also suggest that it might be used as a strategy to maintain the stability of populations, preventing high density and competition.

Abstract

This thesis focuses on the adaptive strategies of daphnids that allows them to maintain stable populations while deal with UV radiation, highlighting the differences in the responses that populations with different evolutionary histories may have.

Although exposition to lethal doses in nature is unlikely, sub-lethal UV-B radiation doses may have strong impacts on zooplankton. This work initiates with a study aimed at determining the effect of sub-lethal doses UV-B radiation on filtration rates of two cladoceran species (*Daphnia pulicaria* and *Ceriodaphnia dubia*) and showed that filtration rates of *D. pulicaria* and *C. dubia* decreased by 50% and more than 80%, respectively, enhancing the importance of avoidance behaviour and recovery strategies for freshwater zooplankton species.

This study was followed by a second one that assessed the ability of *Daphnia pulex* to develop tolerance to UV radiation, while disentangling, at the same time, the relative importance of local adaptations and ontogenetic processes behind such tolerance. Two populations from environments strongly differing in UV radiation conditions (Bolivian Andes and southern of Sweden) were UV-induced to produce photo-protective compounds and changes in behavioural responses that were monitored by 3D Nano-tracking. Although changes in tolerance capacity of both populations were evident in the results, differences between populations were more related to local evolution than to short-time ontogenetic processes, showing that tolerance to UV radiation is dependent on the evolutionary history of each species population.

Such evolutionary differences should be reflected in the life-histories of both populations so that the next study, through structural equation modelling, shows clear differences in the life-history structures of both populations of *Daphnia pulex*. Main differences involve indirect effects of UV radiation on offspring production as well as the age at first reproduction. In addition to known tolerance strategies to UV radiation, a population strategy that includes early reproduction and high fertility to compensate for the fitness loss imposed by UV radiation stress, may be developed by populations from high-UV environments. A further study was conducted to explore how these high fertility populations may maintain stable populations in a gradient of resource availability and different sources of light. The results suggest that *Daphnia* may use resting eggs as self-regulating strategy, which can work as a dissipative structure which slows entropy production in the system, or as a mechanism of population-density control that allows re-population on the long term.

Papers

Appended papers

I. **Fernández, C.E**. & D. Rejas. 2017. Effects of UVB radiation on grazing of two cladocerans from high-altitude Andean lakes. *PLOS ONE* 12: e0174334. https://doi.org/10.1371/journal.pone.0174334

II. **Fernández, C.E.**, M. Campero, G. Bianco, M. Ekvall, D. Rejas, C. Uvo, and L-A. Hansson. UV radiation tolerance on *Daphnia pulex*: a behavioural and physiological approach. *(Manuscript).*

III. **Fernández, C.E.**, M. Campero, C. Uvo, and L-A. Hansson. 2018. Disentangling population strategies of two cladocerans adapted to different ultraviolet regimes. *Ecology and Evolution* 8: 1995-2005. https://doi.org/10.1002/ece3.3792

IV. Campero, M., **Fernández, C.E.** & F. Acosta. On entropy, game theory and *Daphnia*. *(Submitted manuscript).*

Author's contributions

The co-authorship of the papers reflects the collaborative nature of the underlying research.

Paper I. DR conceived the original idea. CF and DR designed and performed the experiment. CF analysed the data. CF and DR wrote the paper.

Paper II. CF, LAH and MC conceived and designed the experiment. CF and LAH conducted the experiment with assistance of ME. CF and GB analysed the tracking data. CF made the pigments analysis. GB, CF, MC and CU decided the statistic approach. CF wrote the manuscript. DR contributed substantially in the revision of the manuscript. All authors contributed to the final version.

Paper III. CF, MC, and L-AH conceived the idea and designed the experiment. CF and L-AH collected the data. CU led the revision of the methods. CF and MC led the analysis of data. CF wrote the manuscript and all authors contributed to the final version for publication.

Paper IV. MC conceived the idea and designed the experiment. CF performed the experiment and collected the data. MC analysed the data with the assistance of CF and FA. MC ran the simulation model. MC wrote the manuscript and all authors contributed to the final version.

Authors: Carla Fernández (CF), Cintia Uvo (CU), Melina Campero (MC), Lars-Anders Hansson (LAH), Giuseppe Bianco (GB), Danny Rejas (DR), Mikael Ekvall (ME), Francisca Acosta (FA).

Other related work

Cossio, C.X., **Fernández, C.E.**, Gonzales, A., Cossio, V., Helgegren, I., Rauch, S. Critical issues in water and wastewater management in small towns. Impacts of increasing population in Tiraque, Bolivia. *12th Urban Environment Symposium, Oslo, Norway 1–3, June 2015.* http://hues.se/assets/Abstract_final_2015-05-27-final1.pdf

M. Campero, **Fernández, C.E**. & D. Rejas. 2015. Lagunas altoandinas: Adaptaciones de los organismos a sistemas extremos y posibles efectos del cambio climático. In: Navarro, G., L. Aguirre & M. Maldonado Eds. Biodiversidad, ecología y conservación del Valle Central de Cochabamba. Centro de Biodiversidad y Genética CBG. Universidad Mayor de San Simón. Cochabamba-Bolivia. 300 p. ISBN: 978-99974-53-83-9.

1. Introduction

Even the most primitive civilizations known to man, recognized the sun as a life-giving force that control many aspects of life. From crop growth to our mood, the sun regulates most of the processes on Earth. Solar radiation allowed photosynthesis to evolve and increased the atmospheric oxygen concentration, leading to a complex multicellular life. The oxygen helped to create the ozone layer, which formed a shield against the harmful portion of solar light, the ultraviolet (UV) radiation.

After the 1970s, a significant reduction in the protective ozone layer was detected (Farman et al., 1985), causing an increment of studies on the effects of UV radiation on ecosystems. Since then, actions to revert ozone layer depletion and mitigate climate change, have received worldwide attention. The Montreal protocol, agreed on 1987 and entered in full force in 1989, has been successful controlling ozone-depleting substances. Nowadays, the stratospheric ozone concentrations are recovering, especially at low and mid-latitudes (McKenzie et al., 2011, Wu et al., 2013). Nevertheless, climate change can still interact with UV radiation, and influence terrestrial and aquatic ecosystems (Häder et al., 2011). The intensity of solar UV radiation that reaches Earth's surface depends not only on the ozone, but factors such as clouds and aerosols, coupled with geographic characteristics as altitude and latitude, also play an important role on the high variability of UV radiation levels. Further, surface levels of UV radiation do not define the UV levels under water, which are highly dependent of the physical and chemical characteristics of each ecosystem (Kirk, 2007).

High-altitude mountain lakes are considered sentinels of climate change due their extreme environmental conditions and their relatively simple trophic structure (Catalan et al., 2006, Barry, 1992, Blumthaler et al., 1992, Williamson et al., 2009). In lakes of the Andean Cordillera, zooplankters are commonly top predators, since those lakes are usually fishless ecosystems. Thus, any factor that affects zooplankton will affect the entire ecosystem (Cottenie et al., 2003, Stoks et al., 2014). Several studies have been done on how UV radiation affects zooplankton, from acute to long term studies (Cabrera et al., 1997, Hansson et al., 2007, Leech and Williamson, 2001, Williamson et al., 1994). Most of those studies have reported increased mortality (Williamson et al., 1994, Zagarese et al., 2003), decreased reproduction (Grad et al., 2001), reduction on growth rates (De Lange et al., 1999) and we recently showed detrimental effect in grazing activity (Fernández and Rejas, 2017), between many others. However, zooplankters have evolved strategies that allow them to survive and succeed in diverse types of UV radiation environments. Behavioural responses, photo-repair systems, and accumulation of photo-protective compounds are the most remarkable strategies to avoid or diminish the deleterious effects of UV radiation. In this sense, effects of UV radiation will widely vary depending on the species, the environment it inhabits, and on its own capacity of photo-protection and recovery (Rautio and Tartarotti, 2010).

Determining the ability of key grazers, as *Daphnia*, to express tolerance to natural selection pressures as UV radiation, is fundamental to understand if they may quickly respond to environmental changes. Many studies have demonstrated that species from high-altitude and high-latitude lakes are more resistant when dealing with UV radiation (Tartarotti et al., 2001, Sommaruga, 2001, Tartarotti et al., 1999, Sommaruga, 2010), due to constant or cyclic exposure to high levels of UV radiation. The central Andes, located within the tropical band, has many lakes of glacial origin that naturally receive high levels of UV radiation at more than 3700 m a.s.l. (Campero et al., 2011). Thus, organisms from high mountain Andean lakes are particularly valuable to study biological adaptations to UV radiation.

My research has aimed at investigating the adaptive strategies of zooplankton (cladocerans) that allows them to maintain stable populations under high UV radiation. The research in my thesis is based on sub-lethal effects that UV radiation has on cladocerans from high altitude Andean lakes (**Paper I**), the different strategies between them and populations from an environment with low-UV radiation levels (**Papers II, III**) and the energy allocation they use to maintain stable populations (**Paper IV**). The specific objectives of this work were:

- To evaluate sub-lethal effects of UV radiation on grazing activity of common Andean daphnids (*Daphnia pulicaria* and *Ceriodaphnia dubia*).
- To assess the relative importance of local adaptations and short-term ontogenetic processes on the ability of *Daphnia pulex* to express tolerance to UV radiation.
- To address causal relationships in life-history traits by which two non-pigmented lineages of *Daphnia pulex*, from different UV radiation environments, endure UV radiation exposure.
- To explore the relationship between energetically expensive resting eggs and the stability in time of *Daphnia* populations.

This thesis is composed of two parts. The first is a general summary that includes a brief introduction, the theoretical background behind the research, an overview of the methodology, a discussion of main results and concluding remarks and perspectives. The second part is a compilation of the four articles that address the above objectives.

2. Theoretical background

2.1. Climate change and ultraviolet radiation

Since the beginning, our planet has been in permanent change, from profound geological transformations to the evolution and expansion of life. Natural changes occur all the time at diverse time scales, influencing climatic parameters as temperature, precipitation, cloudiness, etc., just to mention a few of them. Both short-term climatic variability as well as long-term climate change, have been features ever present on the planet Earth, although, the speed in which climate is changing nowadays, is unusual. The continued use of fossil fuels, particularly oil and coal for industrial and domestic purposes, have peaked carbon dioxide (CO_2) concentrations in the atmosphere, increasing global warming through the greenhouse effect. Agricultural and industrial activities have increased the emission of other greenhouse gases (GHG), such as methane (CH_4) or nitrous oxide (N_2O). Between those, fluorinated gases (e.g., chlorofluorocarbons, hydrochlorofluorocarbons) stand out as they also have a stratospheric ozone-depleting effect. Each of these gases can remain in the atmosphere for different amounts of time, ranging from a few to thousands of years. That means that even if the emissions are regulated now, the effects of passed emissions will still be present for long time.

The ozone layer has been in a dynamic equilibrium for millions of years, since this gas is continuously produced and destroyed, keeping its proportion cyclically constant in the atmosphere. This balance has been altered by reactions between ozone and chlorine, nitrogen or bromide molecules, been chlorofluorocarbons (CFCs) the most harmful, due to their permanence time in the atmosphere. Ozone plays a critical role in the ecological balance of the earth due to its strong absorption of highly damaging ultraviolet (UV) radiation, which is a central factor in this study.

The UV radiation composes 8.03% of the incoming solar radiation at the top of the atmosphere, covers wavelengths between 100 and 400 nm (Iqbal, 1983) and contains more energetic wavelengths than the photosynthetically active radiation (PAR: 400-700 nm). It is usually divided into three bands based on its interaction with the atmosphere and biota (UNEP et al., 2010): UV-C radiation (100-280 nm) that is the highest energetic and highest harmful fraction for organisms, but that does not reach the surface of the Earth, since it is completely absorbed by ozone and other gases in the atmosphere. UV-B radiation (280-320 nm) that is the medium energetic fraction and is greatly removed by stratospheric ozone. However, the filtration is not total, and the portion that reaches the surface turns out to be the most harmful radiation for life. Some authors set the division between UV-B and UV-C at 290 nm since shorter wavelengths are unlikely to be present at Earth's surface (Diffey, 1991), but there is a marked exception at high altitudes were short wavelengths may be present (Henderson, 1977). Finally, the last fraction is the UV-A radiation (320-400 nm) that is not appreciably absorbed by the atmosphere. This fraction of radiation is also harmful, but thousand times less energetic than UV-B radiation (Diffey, 1991).

When solar radiation enters into the Earth's atmosphere, it is modified by several phenomena, not only by the ozone content. UV radiation levels are basically conditioned by the solar zenith angle, the cloudiness, the aerosols and other particles like pollutants, the albedo and the altitude above sea level (Frederick et al., 1989). For instance, it is known that surface UV radiation levels are dependent on the position of the sun (Iqbal, 1983), in Tropical regions, where the sun is close to a vertical angle, summer levels of UV radiation are significantly high higher than elsewhere. Contrarily, in Polar Regions, the elevation of the sun even in summer is low, which will promote low or moderate levels of radiation on these areas.

The solar zenith angle (SZA) represents the angle between the local zenith and the line of sight to the sun, both drawn from a specific location on the surface. The value of the SZA depends on the day of the year, the latitude of the location and the time of day, reaching the lowest values in the central hours of the day. The SZA is the main factor that controls the variation of the radiation on a horizontal surface during the day. When the SZA is lower, solar rays will arrive more perpendicular on the horizontal surface of the Earth. As the SZA grows, the flow of radiation that reaches the horizontal plane is lower, until reaching the SZA limit at 90º, when the sun disappears over the horizon and the received solar radiation is equal to zero (Iqbal, 1983). The extra-terrestrial solar radiation arrives in a direction marked by the SZA, however, when crossing the Earth's atmosphere, radiation is able to be absorbed or dispersed to other directions. The radiation that reaches Earth's surface can be decomposed in two: direct radiation, which reaches the surface in the direction marked by the SZA, and diffuse radiation, which is the radiation dispersed by the atmosphere that reaches the surface from any other direction than the direct radiation (Allaart et al., 2004).

The importance of cloudiness over surface UV radiation is also well recognized. After ozone, clouds and aerosols are the most important factors affecting it (Coakley Jr et al., 1983, Li, 1998). The cloudiness has a horizontal effect on UV radiation without wavelength distinction (Bais et al., 1993, Riordan et al., 1990). This cloudiness attenuates the spectrum to the same extent for the entire range, apparently without modifying the spectral structure. The amount of UV radiation attenuated by the cloud will be a function of the type of cloud and its development (Li, 1998). Denser and darker clouds will block UV radiation more efficiently, while less developed vapours and white clouds will be less efficient (Calbó et al., 2005). There are many aspects regarding cloudiness to be taken into account. The thickness of the cloud, its height, the distribution of the size of the drops and the albedo of the surfaces influence the transmissivity of the UV radiation (Calbó et al., 2005). In this way, clouds are generally difficult to represent realistically in a model (Madronich, 1993). That is why most models of solar radiation are restricted to clear skies (McKenzie, 1991). However, the net cloudiness effect does not always result in a reduction in UV radiation. Sometimes the clouds cover the sky, but they do not hide the direct radiation. In those circumstances, UV radiation reflected upwards by the Earth's surface is in turn reflected by the clouds, thereby being trapped between clouds and the surface and, as a consequence, increasing the level of UV radiation on the surface (Madronich, 1993, Madronich and Flocke, 1997, Calbó et al., 2005).

Atmospheric aerosols (particles suspended in the atmosphere) may also modify the incident UV radiation. Air molecules disperse radiation, while aerosols disperse, but also absorb solar radiation. The optical thickness and the albedo of the particles are directly linked to their chemical composition and will determine the wavelength of absorption. The albedo of a surface is defined as the ratio of the reflected irradiance to the incident irradiance, being a function of the type and surface texture, and of the wavelength (Madronich, 1993) and depends on the angle of incidence and the SZA (Blumthaler et al., 1993). Different types of surfaces reflect UV radiation in different degrees. Reflections from water, vegetation or other surfaces can increase the received UV radiation, either directly or indirectly.

Finally, between the main factors that affect the UV radiation level at Earth’s surface, the altitude or elevation above the sea level is extremely important. The higher the elevation, the lower the attenuation through the atmosphere. This effect is a consequence of the thinner air mass traversed at higher altitudes, and is highly dependent on the UV radiation wavelength, the SZA, air composition, surface cover and season of each specific place. For instance, increases of 11% km^{-1} was reported in the Alps (Blumthaler et al., 1992), while 8 to 10% km^{-1} were reported in the Chilean Andes (Piazena, 1996) for the UV-B band. On the same line, measures of erythemal radiation (fraction of UV that cause skin erythema) have reported increases of 16 to 31% km^{-1} at the highest pics of Bolivian Andes (Pfeifer et al. (2006).

2.2. High-mountain lakes, sentinels of climate change

High-mountain lakes by definition are those who are above the treeline (Pechlaner, 1971). However, as vegetation is so dependent on temperature, the treeline position will be more affected by temperature and latitude, than by altitude (Legros, 1992). High mountain lakes altogether have a global importance since they are considered as sentinels of climate change (Laurion et al., 2000, Williamson et al., 2009), because their simple biotic communities are very sensitive to environmental perturbations (Straškrabová et al., 1999, Catalan et al., 2006).

Mountain systems are the sources of the major rivers of the world (Viviroli et al., 2011), host 915 million people (90% in developing countries) and cover 22% of the surface of the Earth (Romeo et al., 2015). These systems are widely distributed from tropical to arctic latitudes, supporting varied biota and human population densities. Nevertheless, most of the studies on high-mountain lakes are done in temperate lakes of the northern hemisphere (high-Alpine lakes). While considerable research have been done in the Andean Titicaca Lake (Lazzaro and Gamarra Peralta, 2014, Villafañe et al., 1999), it cannot be used for general hypotheses due to its vast surface area (8448 km^2) and its depth (~280 m), which is unusual for lakes in the Andean Cordillera.

Most of high-Andean lakes have a glacial origin (Hutchinson, 1957) and provide water for consumption, industrial and agricultural proposes to surrounding towns (Bradley et al., 2006). High-Andean lakes are ecosystems with natural extreme conditions such as high UV radiation (Campero et al., 2011), oligotrophic (waters with low nutrient contents) to mesotrophic conditions (Modenutti et al., 1998) and nutrient limitations (Rejas et al., 2012). In this sense, all system processes are greatly influenced by solar radiation, which do not change significantly throughout the year. The seasonal climate variation is not very notorious and is mainly determined by the dry and wet season. On the other hand, high-Alpine lakes have also glacial origin and low nutrients availability (Pechlaner, 1984), but they experience short growing seasons (3 – 5 months) and extreme seasonal changes in light conditions and high solar radiation periods. The onset ice/melt cycle greatly influences high-Alpine lakes, by minimizing radiation transmitted to water under ice, by reflection (Vincent and Roy, 1993, Vincent et al., 2007) or by white ice (ice with air inclusions), which has a strong attenuation effect on visible light and an even greater effect on UV radiation (Vincent and Belzile, 2003). This type of attenuation is not observed in the high-altitude Andean lakes, since they rarely get to freeze and when they do, they thaw after a few sunlight hours. Thus, in general chemical and physical properties as well as biological processes of tropical lakes (high-Andean lakes) greatly differ from temperate lakes (Lewis, 2000, Aguilera et al., 2013).

Further, high-Andean lakes are polymictic, characterized by frequent periods of mixing and circulation, which determines low mineralization and low availability of nutrients (Barbosa, 2009, Vila and Muhlhauser, 1987). All these harsh conditions, allow only for simple trophic structures in these lakes. Naturally fishless, these systems host rich phytoplankton diversity, which support zooplankton communities that usually are the main predators of the system. However, rainbow trout (*Oncorhynchus mykiss*) have been introduced in many lakes, most of the time with infructuous farming but affecting the ecosystems (Aguilera et al., 2006, Buria et al., 2007).

Due to their remote location and reduced accessibility high-Andean lakes are, in general, much less influenced by pollution compared to lowland lakes. However, climatic conditions in the tropical Andes have changed considerably during the last century. Regional climatic patterns of the Southern Hemisphere are highly influenced by the Andean mountain range, principally because it harbours the second highest and most extensive plateau in the world, which constitutes a barrier of air circulation (Gregory-Wodzicki, 2000). The average temperature has increased about 0.7 °C between 1939 and 2006 (Vuille et al., 2008), depending on the elevation and slope (Urritia and Vuille, 2009, Vuille et al., 2003). According to future scenarios of climate change, the high mountain ecosystems of the Andes will become warmer and dryer (IPCC, 2008), and particularly, locations at higher elevations will be more affected (Urritia and Vuille, 2009, Vuille, 2013, Vuille et al., 2003). In this sense, Bolivia is expected to be one of the most affected countries by continued reductions in water supply (Winters, 2012), since many of their glaciers have already retreated, and the main water supply for several cities relies on precipitation. Nowadays, the increasing water demand for agricultural purposes, obliges to supply the water from many high altitude lakes in the Andes (Chevallier et al., 2011, Rangecroft et al., 2013), making their biota more exposed to UV radiation by diminishing water depth.

However, when talking about aquatic systems, many factors come into play to define the levels of UV radiation that reaches the system (Häder et al., 2007). To assess the impact of UV radiation on aquatic ecosystems, it is necessary to consider more than only incident radiation, since the effective UV radiation levels received by aquatic organisms become much lower than surface levels due to water attenuation processes (Rae and Vincent, 1998, Kirk, 2007). Once UV radiation has penetrated the water column, its attenuation mainly depends on the concentration of chromophoric dissolved organic matter (CDOM, Fig. 1), which is the light absorbing fraction of dissolved organic carbon (DOC) and the concentration of microorganisms present in the water (Zagarese et al., 1998).

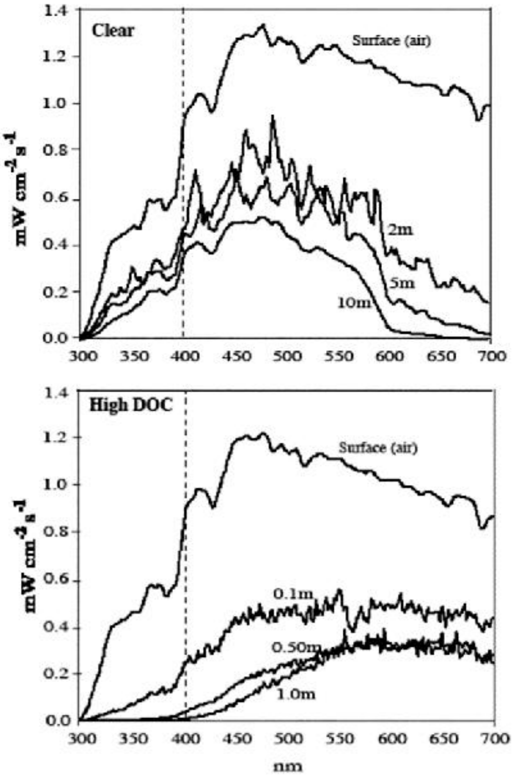

Figure 1. Spectral profiles of a) a clear alpine lake (Bessvatn, Norway) and b) a forest lake with higher levels of chromophoric dissolved organic matter (CDOM) (Skjervatjern, Norway). Irradiation at various depths indicated. UV radiation to the left of the broken vertical line. Mid-summer measurements at noon for both localities. In Hessen (2008), the author enfazices that the intensities of UV radiation is far higher at 10 m depth in the clear lake compared with 10 cm in the DOC-rich lake. Extracted from Hessen (2008) with permission of the author.

CDOM occurs principally as a result of tannin-stained waters released from decaying detritus and is considered the main attenuator of UV radiation in the water column (Morris et al., 1995, Rautio and Korhola, 2002a), selectively absorbing the UV wavelengths (Williamson and Rose, 2010) and decreasing its transmittance in the water column (Schindler and Curtis, 1997).

CDOM is composed by fulvic materials and humic acids, which are extracted from the soil or incorporated into the water by vegetation and microbial activities (Morris et al., 1995). Within freshwater ecosystems, variations in CDOM might be more important on UV radiation levels than stratospheric ozone depletion (Rautio and Korhola, 2002a), since it acts as a screen for most sensitive organisms such as plankton (Häder et al., 2007, Sinha and Häder, 2002) and amphibians (Morris et al., 1995, Crump et al., 2000). The UV radiation penetration may vary from a few centimetres in highly humic lakes (Kirk, 1994, Hessen, 2008), to tens of meters in the oceans (Smith et al., 1992). In this sense, attenuation within the water will depend on specific characteristics of each ecosystem (Fig. 1). Any factor that decreases the CDOM concentrations will affect the water transparency, causing a greater exposure of organisms to UV radiation. Increased acidity or temperature, can decrease the concentration of CDOM i.e., being both variables susceptible to be affected by climate change (Shindell et al., 1998). High water acidity causes organic matter precipitation (Häder et al., 2011, Häder et al., 2007). On the other hand, the increase in temperature may cause decreased rainfall, resulting in less runoff and less water saturation in the soils, entailing a reduced transport of organic matter to the lakes (Williamson and Zagarese, 2003, Häder et al., 2007) and hampering UV refuge for the organisms.

We also should consider the effects of large-scale climatic fluctuations on freshwater communities. El Nino–Southern Oscillation (ENSO) is a large-scale climatic oscillation of atmospheric mass and the main driver of Earth's interannual climate variability. It affects the periodic climate variation worldwide, but particularly tropical eastern Pacific Ocean, changing winds and sea surface temperatures affecting tropical and subtropical climate, rain patterns and consequently freshwater ecosystems (Arthington et al., 2016). The North Atlantic Oscillation (NAO) is a similar phenomenon that affects climate variability of large part of the northern hemisphere, and the Pacific Decadal Oscillation (PDO) affecting the variation in the North Pacific climate. Zooplankton are especially sensitive to all those changes affecting the de density, structure and succession of their assemblages (Fig. 2; Aronés et al., 2009, Ji et al., 2017, Vadadi-Fülöp et al., 2012).

2.3. The trophic cascade and the ecological relevance of zooplankton

The ‘*Trophic Cascade Hypothesis*’ (Carpenter and Kitchell, 1996, Carpenter et al., 1985) outlines that the control flows downward in the trophic pyramid. The potential production at a given trophic level is determined by the availability of nutrients and food (*bottom-up* control; Odum, 1966), whereas the real production at each trophic level is regulated by the immediately higher trophic level (*top-down* control; Hairston et al., 1960). In this system, keystone predators can strongly affect the community structure at each trophic level (Shapiro and Wright, 1984, Carpenter and Kitchell, 1996, Start and Gilbert, 2017). For instance, the introduction of planktivorous fish may result in an increased phytoplankton biomass (Shapiro and

Wright, 1984). Fish are visual size-selective predators that prefer to prey upon large zooplankton (Li et al., 2017, Drenner, 1980), changing zooplankton structure to a dominance of smaller species and individuals, which are less efficient grazers (‘Size efficiency hypothesis’; Brooks and Dodson, 1965). Within the dynamics of pelagic ecosystems, grazing can be considered as the main energy regulatory factor of phytoplankton biomass (Lampert & Sommer, 2007), thus a structure dominated by small zooplankters may lead to increased phytoplankton biomass.

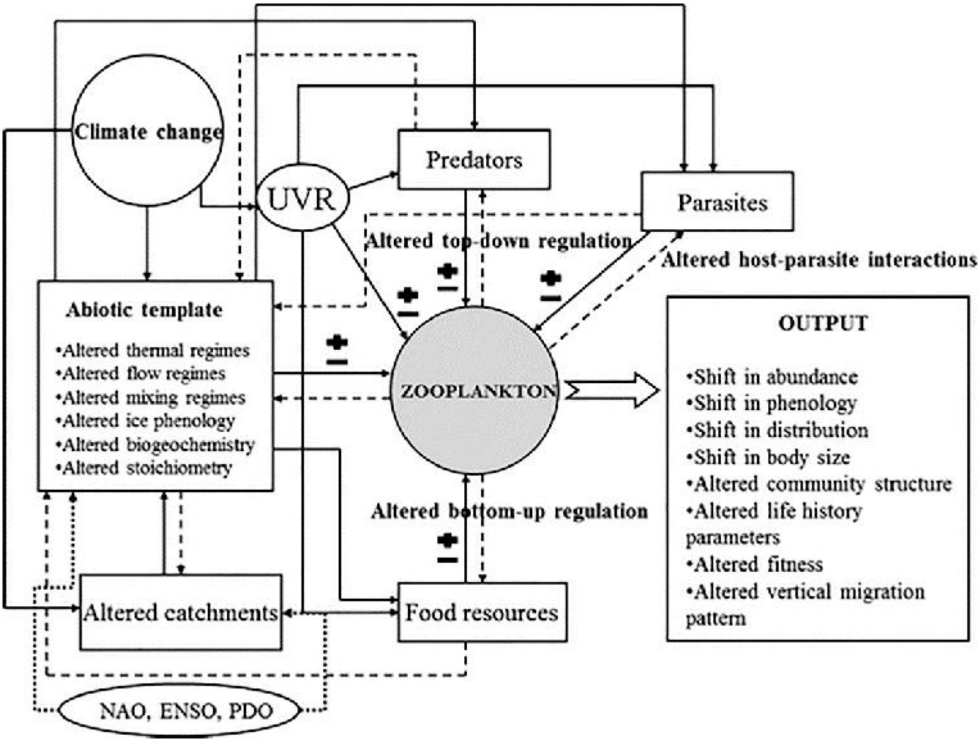

Figure 2. Conceptual model of the possible direct and indirect effects of climate change on freshwater zooplankton. Solid arrows indicate linkage between components while dashed arrows indicate feedbacks. Dotted lines indicate synchronizing effects of NAO, ENSO, and PDO (for more details, see the text). Plus and minus signs indicate potential positive and negative effects on zooplankton. Extracted from Vadadi-Fülöp et al. (2012) with permission of Springer.

The structure of zooplankton assemblage is able to modify both the productive state of the lakes (Lampert and Sommer, 2007), as well as the matter and energy that is transferred to the higher trophic levels, serving as prey for vertebrate and invertebrate planktivores (Gliwicz, 2002). Additionally, another important function of zooplankton is the recycling of nutrients, mainly when these are limiting for phytoplankton and bacterioplankton (Rejas et al., 2005, Rejas et al., 2012, Attayde and Hansson, 2001). For instance, in oligotrophic lakes, which are more dependent on internal recycling, zooplankton regulates the nutrient availability in the environment.

In most freshwater ecosystems, zooplankton are mainly composed by protozoans, rotifers, cladocerans and copepods. Bolivian high-Andean lakes are mainly regulated by cladocerans (genus *Daphnia*, *Simocephalus* and *Ceriodaphnia*) and copepods (mostly genus *Boeckella*). Cladocerans (Order Anomopoda, formerly Cladocera) sequester more nutrients than copepods, leaving less available nutrients for phytoplankton growth (Sommer and Sommer, 2006). And, unlike copepods, cladocerans are non-selective filter feeders (Martinez, 2000). The superiority of cladocerans (especially of the *Daphnia* genus) as phytoplankton regulator has been the basis of several bio-manipulation studies (Navarro and Rejas, 2009, Wissel et al., 2000, Matveev et al., 1994). They are responsible for approximately 80 % of phytoplankton consumption in lentic ecosystems (Brito et al., 2006), and therefore essential for water clarification of these systems (Brendelberger, 1985, Muylaert et al., 2006).

This group of organisms have two different reproduction strategies. The most frequent is the asexual cycle (Fig. 3-I) by parthenogenesis. This type of reproduction occurs under favourable environmental conditions and low population density (Green, 1956, Boikova, 2017). Parthenogenetic females produce diploid eggs that are incubated in the brood chamber of the mother and generate morphologically and genetically identical individuals (clones) to the mother. The sexual cycle (Fig. 3-II) mostly occurs under environmental stress. Certain factors, such as water temperature, photoperiod or food availability changes, may induce the appearance of male offspring (parthenogenetic sons) and parthenogenetic daughters with haploid eggs (Toyota et al., 2016, Wolff and Gerberding, 2015). After mating and fertilisation the female produces sexual resting eggs, which are enclosed in a protective capsule called *ephippium* or ephippia. After a diapause, sexually produced daughters hatch from the ephippia and start to reproduce asexually again. Resting eggs are crucial to maintain genetic diversity in *Daphnia* populations (Brendonck et al., 1998, De Meester et al., 2006), although there are obligatory parthenogenetic linages which may produce ephippia asexually.

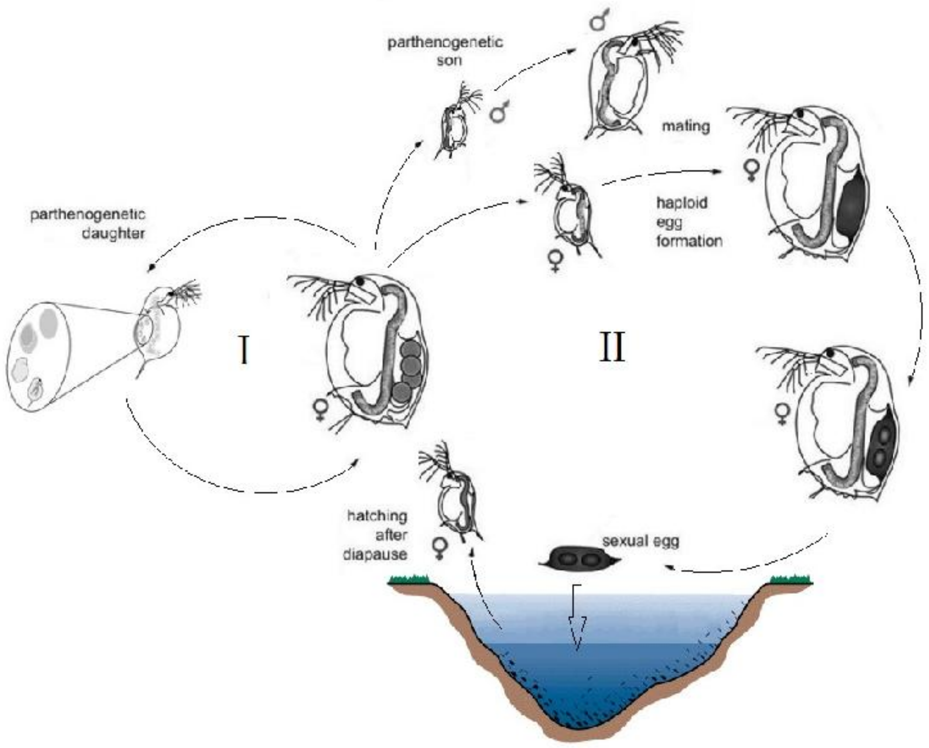

Figure 3. Reproduction strategies of daphnids: I. asexual cycle. II. sexual cycle.

2.4. Effects of UV radiation on aquatic organisms

UV radiation acts from molecular level, denaturing numerous organic compounds, such as DNA, proteins and pigments (Vincent and Neale, 2000, Roy et al., 2000) altering ecosystem processes (Solomon, 2008). In aquatic ecosystems the effects at organisms and populations level may have great impact on the entire ecosystem. Sensitive species to UV radiation would reduce their populations or even disappear from the most exposed habitats. This could lead to a loss of species richness and biodiversity (Solomon, 2008) but also to changes in the food web. However, the indirect effects of UV radiation may cause more damage than the UV radiation itself. UV radiation damage induces formation and accumulation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hydroxyl radicals (HO), superoxides (O_2^-), etc., inside the cells, thus the structure and physiology of cells are altered, decreasing their growing rate and therefore the density of the species (Häder et al., 2007).

UV radiation can prevent or diminish the process of photosynthesis (photo-inhibition), destroying photosynthetic pigments (chlorophyll and phycobilins; Singh et al., 2010) and key enzymes for the incorporation of carbon and nitrogen (Viñegla, 2000). Phytoplankton is the basis of aquatic food chains, supporting the production of higher trophic levels. UV radiation may alter the internal composition (proteins, lipids and carbohydrates) of algae, thereby affecting the nutrition of grazers and the secondary production (Leech & Williamson, 2001). Nevertheless, phytoplankton may produce photoprotective substances; cyanobacteria synthesize scytonemin (Rastogi et al., 2013) and many phytoplankton and macroalgae species produce several mycosporine-like amino acids (MAAs) to diminish the impact of UV radiation (Llewellyn et al., 2012, Laurion et al., 2002).

Heterotrophic microorganisms (bacteria and flagellates) are also affected by UV radiation, which may limit their density in high mountain systems. A mesocosm experiment showed increased heterotrophic microorganism biomass when UV radiation was excluded, even in phosphorus limitation conditions (Medina-Sánchez et al., 2013).

Some species of zooplankton are especially sensitive to UV radiation (Williamson et al., 1994) presenting DNA damage (Malloy et al., 1997) and high mortality rates (Zagarese et al., 2003). But sub-lethal effects were also reported, including reduction in growth (de Lange et al., 1999) and fecundity rates (Williamson et al., 1994; Zellmer, 1996 & 1998; Huebner et al., 2006). Negative effects of feeding on algae pre-exposed to UV radiation were also reported (Zellmer et al., 2004), as well as increased susceptibility when UV radiation interacted with other environmental stressors (Hessen & Alstad Rukke, 2000; Cooke et al., 2006). Therefore, variations in the intensity of UV radiation could have a strong effect on the functioning of lentic aquatic ecosystems (Williamson et al., 2001), but the effect will greatly depend on the environmental conditions and the species specific abilities to avoid and recovery from UV damage.

Strategies to cope with UV radiation

In the water column, and depending of the particular characteristics of each system, only the superficial layer is exposed to UV radiation, therefore, deep lakes may have dark areas that constitute a safe refuge for organisms (Zagarese et al., 1994). Thus, one of the best protection mechanisms adopted by some species to avoid UV radiation is vertical migration to the deeper layers of the lake. For instance, several zooplankters makes large migrations to the deep, dark zone of the water column during daylight, and migrates back to the surface to feed at night (De Meester et al., 1999, Hylander and Hansson, 2010). These migrations are so common and widespread that numerous associated predation behaviours have developed. Many aquatic species are equipped with receptors to detect UV radiation, with negative or positive phototactic behaviour (Hessen, 1994). Numerous organisms (including fish) have vision in the UV-A and UV-B bands (Williamson and Rose, 2010). UV radiation photoreceptors were found in the compound eye of *Daphnia magna* with peak sensitivity within UV and PAR bands (348, 434, 525, and 608 nm; Smith and Macagno, 1990). This is useful for navigation, identification and avoidance of depths where UV radiation still penetrates (Leech and Johnsen, 2003).

However, not all organisms have enough mobility or the ability to choose their position in the water column, or simply, the water column is not deep enough to allow migration to safer layers. For instance, phytoplankton needs to absorb PAR to photosynthesize, thus is forced to remain in the photic zone, where is exposed to UV radiation (Roy et al., 2000). Some algae developed protective cellular structures, these mechanisms are physical in nature and involve the formation of different structures, such as mucilage covers, whose function is to unite the cells in colonies and at the same time reflect UV radiation, avoiding its penetration in the cells (Banaszak et al., 2003) or the creation of crystals walls (holococcoliths) that may reflect UV radiation (Quintero-Torres et al., 2006). But one of the most important mechanisms of protection against UV radiation is based on the production of chemical substances, capable to act as photo-protective or photo-repairer compounds. Photo-protective substances absorb UV radiation wavelengths acting like sunscreens and protecting the cells from photo-damage (i.e melanin, scytonemin, MAAs; Moeller et al., 2005, Garcia-Pichel and Castenholz, 1991). On the other hand, photo-repairer substances (i.e. carotenoids) act neutralizing toxic photo-products of UV radiation exposure, such as free radicals, by antioxidant activity (Banaszak et al., 2003, Roy et al., 2000).

Carotenoids, scytonemin and MAAs are produced by algae and incorporated to zooplankton by feeding (Hansson, 2000, Korbee et al., 2006) or associated bacteria (García et al., 2010). In zooplankton, melanin is the most representative of synthesized pigments, and is mainly present in cladocerans of high-latitude or high-altitude zones (Rautio et al., 2009, Rautio and Korhola, 2002b, Hessen, 1996, Rautio and Tartarotti, 2010).

There are striking differences between the energetic costs of synthetized and accumulated photo-protectors. For example, melanin in the carapace, has to be synthetized every time cladocerans moult (Herbert and Emery, 1990). Concentration of melanin is commonly reduced if UV radiation is removed (Hansson et al., 2007, Hessen, 1996) or shows seasonal variations (Rautio and Korhola, 2002, Hansson, 2004). Under such evidence, it is assumed that melanin production has a metabolic cost. However, some species could be able to maintain melanisation without UV radiation stress. Hessen et al. (2002) experiments suggest that visible light may also induce melanin synthesis, which agrees with Connelly et al. (2009) observations that maintained *Daphnia middendorffiana* pigmented for six months in laboratory conditions without UV radiation. Nevertheless, a metabolic cost of melanin synthesis is evident, as Hessen (1996) found that melanic morphs of *Daphnia pulex* showed lower growth rates than non-melanic ones, suggesting a trade-off between growth rate and protection from UV radiation, while Weider (1987) observed delayed age at first reproduction and smaller clutch size in melanic *Daphnia*.

Carotenoids accumulation like melanin synthesis may decrease in absence of UV radiation (Hansson et al., 2007), suggesting metabolic costs. However, further studies have not revealed any metabolic detriments. Recently, Gorokhova et al. (2013) reported higher growth, egg production and antioxidant capacity in pigmented *Eurytemora affinnis* (copepod) than unpigmented pairs, suggesting reduced fitness in the last ones but also reduced predation pressure. However, avoiding predation risk at the expense of diminished antioxidant reserves, may strongly affect copepods' immune system, as Van der Veen (2005) experimentally found higher parasitic infection by a cestode (*Schistocephalus solidus*) in the copepod *Macrocyclops albidus*, when copepods reduced their carotenoid reserves. Although carotenoids have a well-known photo-protective role, carotene-pigmented copepods were observed under ice in conditions of complete darkness (Hairston, 1976, Hairston, 1981), and were also found in ovaries and eggs of cladocerans exposed and non-exposed to UV radiation (Green, 1957, Siebeck, 1978). All these evidences suggest that carotenoids have other ecological functions than solely photo-protection.

As colourless substances, MAAs (mycosporin-like amino acids) give zooplankton an invaluable protection against UV radiation, because they do not increase predation risk (Hylander et al., 2014, Moeller et al., 2005). These amino acids are water soluble and only bacteria, fungi and algae have the ability to synthesize them, but they can be incorporated into the organisms that feed on them, accumulating in the predators which will benefit from their photoprotective function transmitting it to the food web (Sommaruga & García-Pichel, 1999; Tartarotti et al., 2001). In a recent study Hylander et al. (2014) showed that marine copepod *Acartia tonsa* can maintain its fitness and even increase it, feeding on a diet rich in MAAs, even if it is exposed to UV radiation. MAAs concentration in copepods, as melanin in cladocerans, have shown a seasonal variability with higher quantities in summer, which apparently was related to seasonal production of MAAs. Further, developing eggs, nauplii, and young copepodites were adapted to this MAAs availability, showing an important ecological role of MAAs (Tartarotti and Sommaruga, 2006). In addition, copepods apparently can facultatively chose their photo-protective substances, increasing levels of MAAs when carotenoids are low and vice versa (Hylander et al., 2009a)

But, not all the damages resulting from exposure to radiation can be avoided, thus organisms have also developed important systems to repair the damage caused by the UV radiation in their cells (Vincent & Neale, 2000; Banaszak, 2003). One of them is the acceleration of cell renewal by eliminating damaged cells and replacing them with new cells (Buma et al., 2003). But, the most important photo-repair systems involve DNA repair (Vincent & Neale, 2000). The alteration of DNA bases and formation of adjacent pyrimidine dimers (CPDs, cyclobutane pyrimirine dimers) are the most important damage derived from UV exposure, because they block the replication activity of the cells (Buma et al., 2003).

There are two main forms of repair systems: i) photo-reactivation, which is stimulated by blue light and UV-A light, and ii) dark repair, which is independent of light (Banaszak, 2003). In both systems enzymes that operate on damage are synthesized. They identify the affected area, make an incision in the chain, synthesize the correct sequence and stick it in the injured area (Vincent & Neale, 2000). These repair systems are found in all types of cells, both eukaryotic and prokaryotic (Banaszak, 2003) and have evolved into indispensable systems for organisms. The photo-reparation process can compensate to a certain extent the phytoplankton photo-inhibition when temperatures are moderate, however, at low temperatures photo-reparation process is limited (Häder et al., 2007).


a.
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3. Materials and methods

3.1. The origin of zooplankton-cultures

Zooplankton samples from high-altitude lakes were taken from different lakes of Pucara basin, from Tiraque, Bolivia (Fig.4a). Pucara basin (17.31°- 17.6°S and 65.37°- 65.88°W), is located at 60 km Northeast from the city of Cochabamba. It covers a big part of Tiraque province, and small areas of Punata, Carrasco, Arani and Chapare provinces (Lazo Carpio, 2009). The watershed has an approximate area of 440.9 km2, and it is divided into five sub-basins: Ch'aki Mayu (82.3 km2), headers of Millu Mayu (77.5 km2), Pucara Mayu (106.0 km2), Toralapa (74.7km2) and Ch'aqo Millu Mayu (100.4 km2; Cruz, 2009, Rojas et al., 2007).

High-altitude zooplankton came from Pucara basin, from lakes with varying depths between 1.8 and 21 m. Attenuation depths (UVA, 1% of surface radiation) range from 0.40 to 2.98 m in in the lakes of the area (Aguilera et al., 2013), depending on the CDOM content. Low-altitude zooplankton came from Dalby Quarry, a small lake at southern Sweden (55.67°N, 13.5°E). This lake is about 10 m deep, with an absorption coefficient of approximately 2.0 and an attenuation depth of 1.6 m (320 nm, 1% of surface radiation).

3.2. Field work

To collect the zooplankters, we took vertical and horizontal samples using a 75 µm mesh size plankton net (Fig. 4b) in several lakes of the basin. Captured individuals were gathered and transported alive to the Limnology and Aquatic Resources Unit (ULRA), in Cochabamba, in 2 L plastic bottles filled with lake water.

Figure 4 (left). Materials and methods used in this study. a) Andean lake (innominate) from Pucara Basin in Bolivia. b) Plankton net. c) Microscopic view of *Daphnia pulicaria* gut with fluorescent microspheres (see 3.4 section). d) Sampling mission. e) and g) *Daphnia pulex* labelled with quantum dots under blue light. f) Tubes with a suspention of quantum dots fluorescent in red (655nm) and yellow (585nm) under blue light. h) Video film system: cameras and aquaria illuminated by led blue light.

Sediment samples were collected at tree random points in the limnetic zone with plastic samplers (bottle 15 cm depth and 8 cm diameter) in 5 lakes and ponds of the basin. The first 10 centimetres of sediment were sampled and placed in dark at 4 °C until ephippia separation for incubation.

3.3. Cultures

The parthenogenetic mode of reproduction (segregating genetic variability) and short life cycle (egg to adult in ~10 days) make daphnids (Fig. 5) ideal organisms for studying environmental stress responses (Lampert and Kinne, 2011).

For this research I maintained different types of daphnids cultures. Monoclonal (**Paper I**) and monospecific (**Paper IV**) cultures developed from live samples transferred directly from field to laboratory to be acclimatized, however, also monospecific populations were hatched from ephippia isolated from sediments (**Papers II** and **III**).

For **Paper I**, high-altitude monoclonal lines of *Daphnia pulicaria* and *Ceriodaphnia dubia* (coming from Totorakhocha Lake (3730 m a.s.l.; 17.46° S, 65.63° W) were used for the experiments. Monoclonal cultures were started with a single female. Five neonates (<24 h old) from the female were used as parental culture. Subsequent cultures were initiated with 15 neonates (<24 h old) per flask, chosen from a pool of offspring produced by age-synchronised adults from the parental culture.

For **Papers II** and **III**, the High-altitude monospecific cultures were started from sediments of Totorakhocha Lake. For this, sediment samples were mixed with sucrose solution (1:1) and the ephippia were pipetted from the supernatant. Then ephippia with similar morphology were separated and maintained in dark at 4 °C for one week. After that time, resting eggs were incubated in dechlorinated tap water under continuous PAR light. New hatched individuals were feed *ad libitum* by an algae culture mainly composed by *Scenedesmus* sp. Species identification was done 3-5 days after hatching. With individuals of *Daphnia pulex*, a monospecific culture was started in 250 ml flasks. Parental cultures were initiated with 15 (<24 h old) neonates per flask, coming from different females. Subsequent cultures were initiated with 15 (<24 h old) neonates coming from the pool of offspring produced by the parental cultures.

Figure 5 (right). Microphotographies of daphnids. Lateral view of (a) *Ceriodaphnia sp.* and (b) *Daphnia pulex*. Frontal view of (c, d) *Ceriodaphnia sp.* and (e, f) *Daphnia pulex*. Photos: Copyright © Christian Englbrecht, permition required for reproduction.

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When parental cultures had about 50 individuals, they were transferred into a 10 L tank with dechlorinated water, where the experiments were carried out. As Low-altitude culture, I used a Swedish monospecific culture of *Daphnia pulex* coming from Dalby Quarry Lake, which was kept for several years at the Aquatic Ecology laboratory from Lund University. A subsample of the main culture (approx. 100 individuals) was transferred to a 10 L tank and left to grow.

For **Paper IV**, a monospecific culture (started and kept as stated above) of *Daphnia pulex*-complex from an innominate lake of Pucara Basin (3680 m a.s.l., 17°23’23” S, 65°36’30” W) was used.

3.4. Experimental techniques and tools

Sub-lethal effects on grazing activity

The potential effects of sub-lethal doses of UV-B radiation on filtration rates of *Daphnia pulicaria* and *Ceriodaphnia dubia*, two cladoceran species that are common and widely distributed in high Andean lakes were studied in **Paper I**. To properly find the filtration rate and the effect of UV radiation on it, I made pilot experiments to find the gut passage time (GPT) and the incipient limiting concentration (ILC, Fig. 6). Both parameters are essential to avoid over/underestimation of filtration rate.

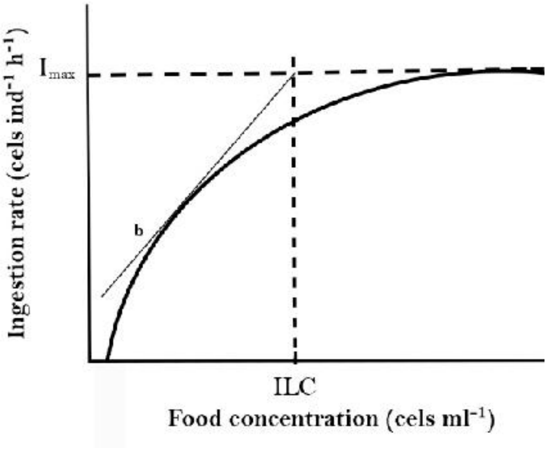


Figure 6. Functional response of feeding activity. Parameters: Imax = Maximum ingestion rate; b = Consumption efficiency and ILC = Incipient Limiting Concentration. The functional response curve is depicted by the equation Ic = Imax ($1-e^{-bc}$), were c = Resource concentration (Ivlev model, figure inspired by Crawley (1992) and Martinez (2000). For further details see Paper I.

Food (algae) concentration is known to influence the per capita-ingestion rate of cladocerans. In general, the effect of food concentration follows a functional response model under short-term conditions (Fig 6; Crawley 1992). This model reflects active feeding behaviour and is consistent with energy optimization models, which predict food concentration thresholds for ingestion rates and changes in consumption efficiency in response to resource availability differences and physical restrictions of the food particles. The ingestion rate increases to a maximum level (I_{max}), which occurs at a threshold resource concentration called Incipient Limiting Concentration (ILC, (McMahon and Rigler, 1965). At food concentrations higher than ILC, the ingestion rate is kept constant by decreasing filtration activity and by rejecting excess of food particles.

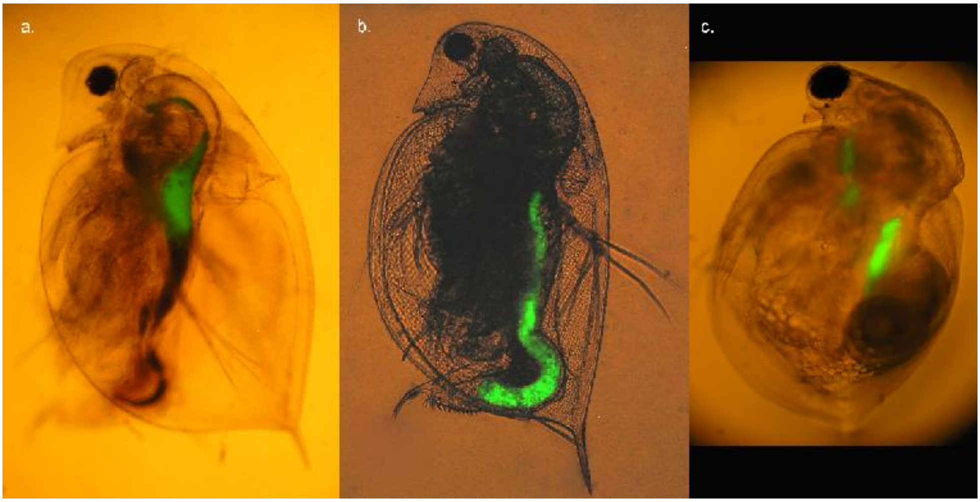
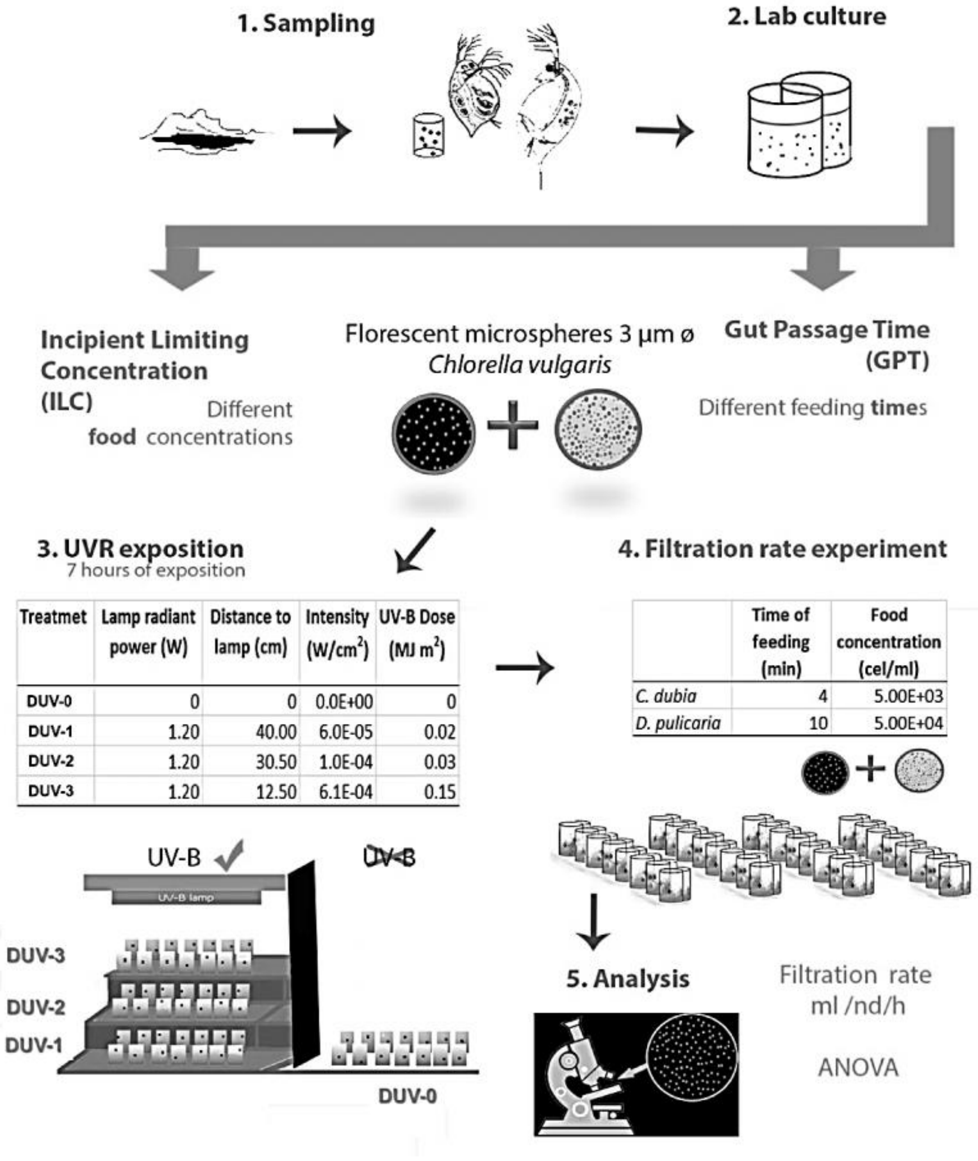


Figure 7. ***Daphnia pulicaria* (a,b) and *Ceriodaphnia dubia* (c)** at minutes a) 5, b) 60 and c) 5 feeding on a mix of *Chlorella vulgaris* and fluorescent microspheres (gut passage time experiment, Paper I).

The GPT, ILC as well as the filtration rate, were obtained using fluorescent microspheres (3 µm ø Fluoresbrite® YG – Polysciences, Germany) mixed in an algal suspension to fed the cladocerans (Fig. 4c and 7). This technique is widely used to determine grazing activity of zooplankters (Agasild, 2005, Pace and Bailiff, 1987, Scherer et al., 2017).

Based on the results of the ILC and GPT assays, food concentrations lower than the ILC (to prevent inhibition of ingestion rates) and feeding times shorter than the GPT (to prevent excretion of particles during the experiments) were selected for the filtration rate experiment. To determine the effect of sub-lethal doses of UV radiation on filtration rates of both species, I exposed clones of each of them to four increasing UV-B treatments: i) DUV-0 (Control), ii) DUV-1 (0.02 MJ m^2), iii) DUV-2 (0.03 MJ m^2) and iv) DUV-3 (0.15 MJ m^2) and estimated their filtration rates after a single exposure. The flow diagram for the grazing experiment is presented in figure 8, see methods section of **Paper I** for further details.



Treatmet	Lamp radiant power (W)	Distance to lamp (cm)	Intensity (W/cm²)	UV-B Dose (MJ m²)
DUV-0	0	0	0.0E+00	0
DUV-1	1.20	40.00	6.0E-05	0.02
DUV-2	1.20	30.50	1.0E-04	0.03
DUV-3	1.20	12.50	6.1E-04	0.15

	Time of feeding (min)	Food concentration (cel/ml)
C. dubia	4	5.00E+03
D. pulicaria	10	5.00E+04

Figure 8. Flow diagram of the experiment to determine the effect of sub-lethal doses of UV-B radiation on the grazing activity of *Daphnia pulicaria* and *Ceriodaphnia dubia* (Paper I).

Photo-protective compounds and 3D Nano-tracking

In order to evaluate the regional influence on the induction of *Daphnia pulex* tolerance to UV radiation and the relative importance of short-term and long-term acclimation, I made an experiment to induce the production of photo-protective compounds in this species. Two contrasting populations (from a high-UV region in Bolivian high-Andes and from a low-UV region in Sweden, **Paper II**) were compared on the basis of its production of photo-protective compounds, after an inductive exposure to UV-A radiation (70 days). Melanin, carotenoids and mycosporine-like amino acids (MAAs) contents per gram of dry mass (DM) were measured and compared between the resulting treatments. See methods in **Paper II** for further details about photo-protective compounds extraction.

In terms of migration behaviour, I measured the “refuge demand” imposed by each species' life-history and the level of UV radiation. This refuge demand was estimated as the integrated vertical position of the organisms over time in a Non UV-UV-Non UV routine (Hansson et al., 2016). To measure this, I used nano-labelling (**Paper II**). This method was proposed to track individual zooplankters by Ekvall et al. (2013) and it has demonstrated high efficiency showing individual responses (Hansson et al., 2016, Heuschele et al., 2017, Hylander et al., 2014). Fluorescent quantum dots (Qdots; Fig. 4f) were conjugated with poly-L-lysine in order for them be able to attach to *Daphnia*'s carapaces. Then, a system of cameras controlled by a computer, filmed the movements of two individual *Daphnia* labelled with different colours of fluorescent Qdots (red and yellow) and tracked by video-cameras. The camera system (Fig. 4h) calibration is explained in detail by Bianco et al. (2013) and (Palmér et al., 2016). The data from the four synchronized cameras were triangulated to obtain the 3D position of each individual, which was then translated to position in the water column over time.

During the data analyses (see **Paper II** for further details) some *Daphnia* with abnormal behaviour that suggested disability or the presence of bubbles in the carapace were found (Fig. 9a-b). All these tracks were discarded from the analysis, maintaining only animals who were able to move (Fig. 9c).

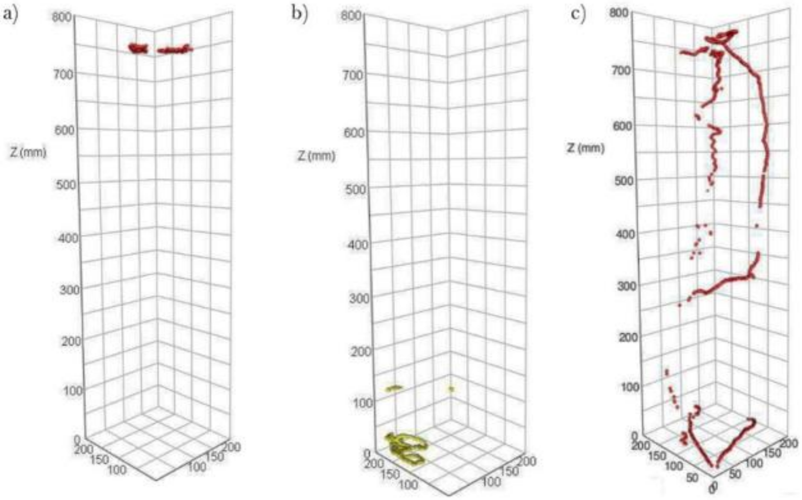


Figure 9. Examples of discarded and used tracks. Tracks were excluded from the analysis when *Daphnia* remained swimming at surface (a) or at the bottom (b). Kept tracks were those with active movement behaviour (c).

Life history and structured equation modelling

Life table methods were used in experiments of **Papers III** and **IV**. This is a fundamental technique based on "time to event" principle (Cox, 2018). Recorded life-history variables were: age at first reproduction (FR), size and number of neonates at FR, number of clutches, total number of born males, total offspring, total ephippia (resting eggs) per female and longevity. All of these variables were used in **Paper III** to get a descriptive model of Swedish and Bolivian *Daphnia pulex* strategies to cope with UV radiation stress (see methods section of **Paper III** for further details). In **Paper IV,** only longevity, number of resting eggs and population stability (in terms of number of days before population collapse) were analysed and used to model population dynamics influenced by a set of different factors (see **Paper IV** for further details).

In **Paper III** I also used structural equations modelling (SEM) in order to analyze causal relationships between life history variables of both populations (Bolivian, Swedish) and the effect of UV radiation. This technique is a combination between factorial and multiple regression analysis, which allows for estimation of multiple direct and indirect dependence relationships between variables in a single analysis. Specifically, I used Path Analysis, which is the application of structural equation modelling without latent (non-quantifiable) variables. The initial path model is a theoretical model based on literature, and is stated by a flow diagram (**Paper III**, Fig. 2), showing the independent, intermediate, and dependent variables in the dataset. In the diagram, single-headed arrows show causality, while double-headed arrows show covariance between two variables. Significance and goodness of fit of the model were tested by the Lavaan package (Rosseel, 2012) for R (R Core Team, 2016).

4. General results and discussion

Sub-lethal effects of UV radiation

It is known that *Ceriodaphnia* and *Daphnia* species can feed on bacterial food as efficiently as on unicellular algae (Brendelberger, 1985, Brendelberger, 1991, Hayashi-Martins et al., 2017), and their active filtration activity confers them an important role in freshwater ecosystems. Allometry also plays an important role, as large species are more efficient grazers (DeMott, 1982, Navarro and Rejas, 2009), and their impact on the energy flux in aquatic ecosystems is extremely high compared to that of small species (Gianuca et al., 2016). For instance, the effectivity of top-down control on phytoplankton is (co)determined by the proportional relationship between large and small zooplankton species. As it was expected, my results showed a positive correlation between cladoceran size and their filtration rates. Further, in my experiments, although both species were influenced by UV radiation, *D. pulicaria* was less affected than *C. dubia*. Nevertheless, due to their different sizes, a reduction of 50% in filtration rates of *D. pulicaria* may have greater impact on the overall community than a reduction of 80% in filtration rates of *C. dubia* (Fig. 10). Additionally, malnutrition of individuals could enhance individuals' mortality, decreasing zooplankton grazing pressure and the zooplankton/phytoplankton biomass ratio, which could eventually lead to a break up in the energy flux of the system as final outcome. Despite the fact that zooplankton can avoid, protect themselves or repair damage produced by UV radiation up to certain limits, sub-lethal UV radiation effects may cause important perturbation, especially in clear, shallow lakes.

Different sub-lethal effects of UV radiation have been profusely reported in literature. These may include changes in respiration rates (Fischer et al., 2006), lower reproductive rates (Huebner et al., 2006), etc. Effects related to grazing have also been reported, as Zellmer et al. (2004) observed damage on the intestine of some UV-exposed individuals of *Daphnia pulex*, and this damage was increased when individuals were fed on algae also UV-exposed. In my case, no ruptures were observed in the gut of the animals of the experiment (**Paper I**) and indirect effects on grazers via changes in the biochemistry of the phytoplankton (De Lange and Van Donk, 1997) were discarded because the animals were fed with non-irradiated algae.

Thus all reductions observed on the filtration rate of both species were directly caused by the exposure to UV-B radiation.

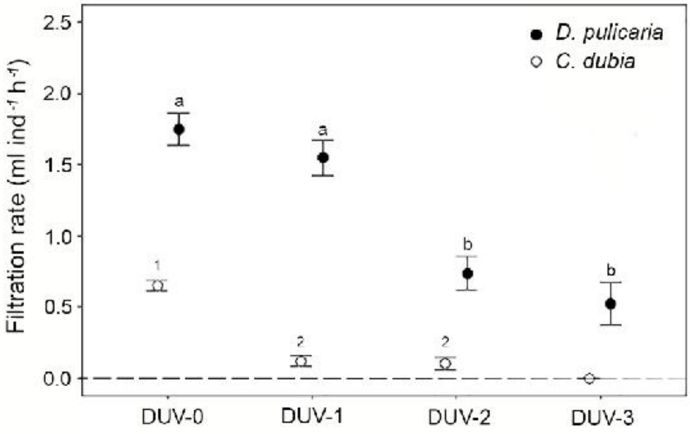


Figure 10. Filtration rates of *D. pulicaria* (black dots) and *C. dubia* (open dots) exposed to sublethal UV-B doses. DUV-0: no exposition, DUV-1: 0.02 MJ m^2, DUV-2: 0.03 MJ m^2 and DUV-3: 0.15 MJ m^2. Error bars correspond to the standard deviation of the mean. Different letters indicate statistically significant differences between treatments (Contrasts's post-hoc test). Figure from paper I.

Photo-protective substances and Migration behaviour

Synthesizing photo-protectants involve increased energetic costs (Vincent and Neale, 2000) and risk of predation (Hansson, 2004, Hansson, 2000). To synthetizing melanin is costlier than accumulate other pigments, since has to be synthetized in every moult (about 3-4 days, Hebert and Emery, 1990). On the other hand, some studies showed that accumulating photo-protective compounds may have other benefits that could improve fitness of those who possess these substances (Gorokhova et al., 2013). In this study, I was able to induce melanisation in the high-altitude population (H-A in **Paper II**). The reverse process has also been observed when UV radiation is removed (Hansson et al., 2007, Hessen, 1996) or shows seasonal variations (Rautio and Korhola, 2002, Hansson, 2004), suggesting metabolic costs of its production, and enhancing the importance of behavioural avoidance in cladocerans, principally in the presence of predators. Even the heavily melanised *Daphnia melanica* are able to modify its melanin level in each moult, showing high and rapid plasticity in presence of predators (Scoville and Pfrender, 2010). In this experiment, the high-altitude population came from fishless lakes and the low-altitude population (L-A in **Paper II**) spent years without contact with predators, therefore, melanisation should be easier for these populations. Regarding the other photo-protective compounds, unlike copepods, the presence of carotenoids and MAAs is not common in cladocerans, but the induction of both substances was evident even at low levels in our experiment (Fig. 11).

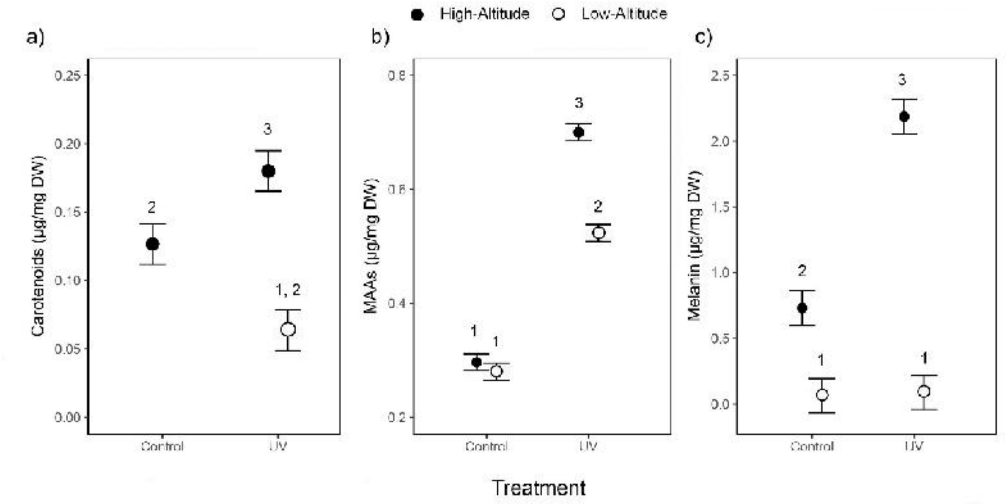


Figure 11. Concentrations of photo-protective compounds for high-altitude (Bolivian) and low-altitude (Swedish) *Daphnia pulex* populations. a) Carotenoids, b) Mycosporine-like amino acids and c) Melanin. n = 3 samples per treatment per pigment, each composed by 40 individuals. Different numbers indicate statistically significant differences between treatments (Contrasts's post-hoc test). From Paper II.

Due to the energetic cost involved with photo-protection, behavioural responses of daphnids to UV radiation are usually inversely related to their pigmentation or photo-protective content (Rhode et al., 2001). This inverse relation has been reported before when comparing different cladoceran species (Hansson et al. 2016), and smaller migrated distance was observed in UV-induced *D. magna* when re-exposed to UV radiation (Hylander et al. 2014). In my experiment (**Paper II**), the behaviour of the low-altitude (Swedish) *Daphnia* population followed these patterns. The content of carotenes, as well as mycosporine-like amino acids (MAAs), increased somewhat, but significantly, after 70 days in the induced animals compared to their control relatives (Fig. 11a,b), but the melanin content remained near to cero in this population. Despite the small increase in quantity of the photo-protective compounds, smaller migrated distances were observed in the low-altitude population (Fig. 12a). Therefore, ontogenetic processes may change the expression of tolerance in just few generations.

On the other hand, induced high-altitude (Bolivian) *Daphnia* population displayed greater amounts of pigments (i.e. melanin induction from 0.73 to 2.19 μg·mg^{-1}· DM, Fig. 11c), but did not migrated less than their non-induced, control siblings when re-exposed to UV radiation (Figs.12a). Thus, here I postulate that populations that come from high-UV radiation environments, may have an evolutionary memory of photoreception that induces them to make more pronounced migrations even at low UV levels and regardless of the increment on photo-protective substances. This may signify that their natural environment may usually require cumulative strategies to deal with UV radiation.

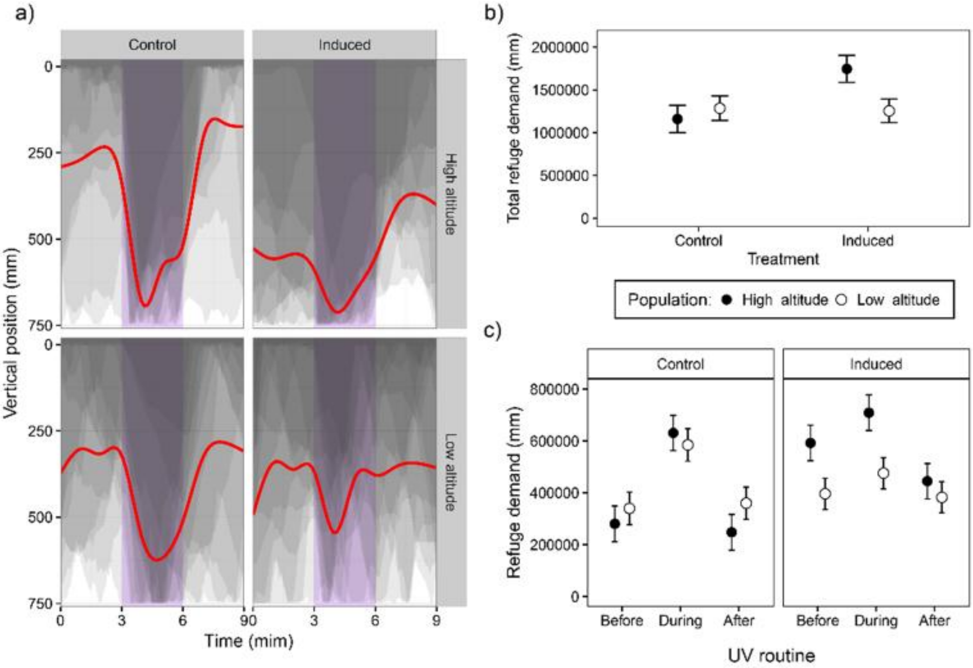


Figure 12. Vertical position and refuge demand of Control (only PAR) and Induced (70 days with UVA+PAR) *Daphnia pulex* from High and Low altitude populations in a non-UVR:UVR:non-UVR recording routine. a) Average (red lines) and individual (overlapping gray areas) vertical position. Purple areas represent the UV radiation phase of the routine. b) Total refuge demand (mean ±SE) in a 9 minutes routine. c) Specific refuge demand (mean ±SE) before, during and after the UV radiation exposure. Closed and open circles represent high and low-altitude populations respectively. Figure from paper II.

The ability of *Daphnia* to perceive UV radiation through photoreceptors should result in appropriate depth selection when exposed to UV radiation (Smith and Macagno 1990). My results show discrepancy in the depth selection and the refuge demand that *Daphnia pulex* may require to "feel" safe (Fig. 12c). It is known that evolutionary processes influences the expression of the critical genes for photoreception (opsins; Brandon et al., 2017); therefore it is difficult to disentangle whether the different responses are due to differences in tolerance or photoreception.

Differences in swimming speed again suggests evolutionary imprinted memory (Fig. 13). The high-altitude non-induced *Daphnia* reacted and swam faster than the other treatments when UV radiation was turned on (Mixed models $F_{2,1733} = 8.89$, $P = 0.0001$, see **Paper II**). This leads us to the hypothesis that populations from high-UV radiation environments may compensate the absence of physiological photo-protection with a fast reaction, even without previous experience with UV radiation.

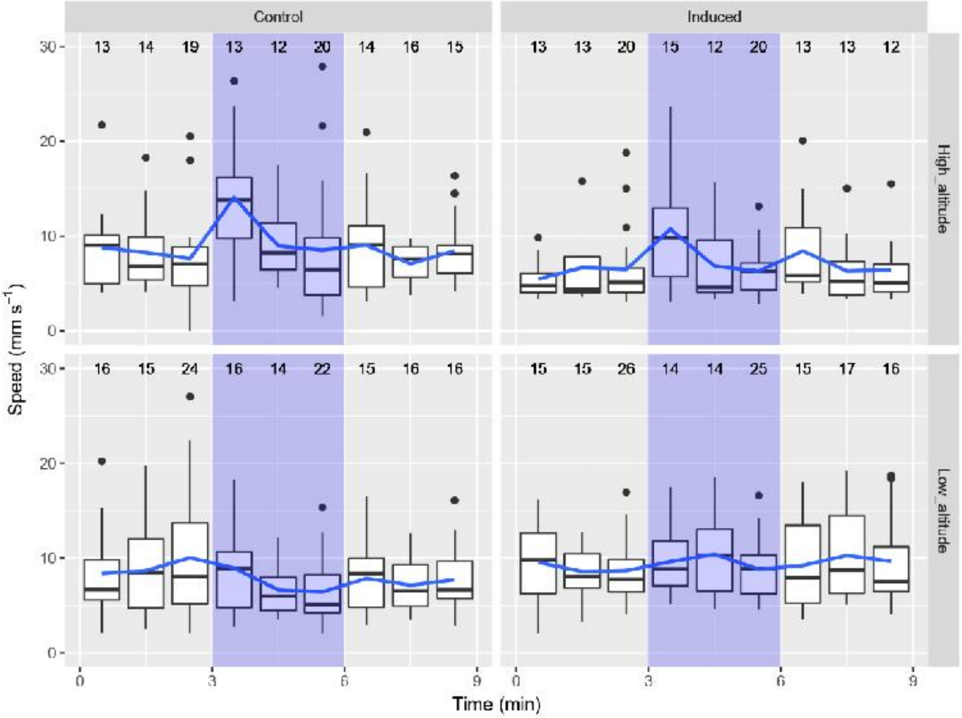


Figure 13. Swimming speed responses of high-altitude and low-altitude *Daphnia pulex* grown under UV radiation (Induced) or regular PAR-light (Control) in a non-UVR:UVR:non-UVR routine. Purple bands represent UV radiation exposure for 3 min (minutes 4 to 6). Top numbers = number of tracks used in each minute analysis; black lines within the boxes = median of the minute; blue line = mean; dots = outliners.

Responses to the presence or absence of UV radiation were immediate, indicating rapid and active reaction of *Daphnia* to changes in UV radiation. Therefore, migration behaviour might vary during the day in function to real time changes on UV radiation incidence. Local adaptation and ontogenetic processes can lead to different responses to UV radiation within a single species, hence, my results show that tolerance may vary depending on the environmental context, but at the end, evolutionary history will control its expression.

Life history strategies and population stability

It may be expected that populations exposed to higher doses of UV radiation are adapted, or at least have a higher capacity to acclimatize to it, as the distribution of the species is determined by specific environmental conditions (Siebeck and Böhm, 1994). As avoidance mechanisms, protective and repair systems may not be completely effective under certain conditions, and require a considerable energy budget, organisms may modify life history traits in order to compensate the energy required to deal with UV radiation. My results showed that UV radiation was detrimental for almost all life-history traits of both *D. pulex* populations (**Paper III**). Total effects were higher for the high-altitude population (HighUV in Table 2 of **Paper III**), except for the number of offspring at first reproduction. The principal outcome comparing both populations was that the ability to exhibit different traits to counteract UVR damage is not only species-specific, but also differs between populations (lineages) of the same species with different evolutionary histories. This means that the involved trade-offs to deal with UV radiation depend on the environment in which the organisms live and the evolutionary history of the specific place.

Generation time is highly connected to age at first reproduction and may play an important evolutionary role (Kawecki & Ebert, 2004). In my experiment low-altitude population (LowUV lineage in Paper III) had its first reproduction (FR) at a younger age under UV radiation exposure. This is in line with previous observations that refer early reproduction as a typical phenotypic plasticity response to new environmental conditions (Barata, Baird, & Soares, 2001; Fischer & Fiedler, 2002), strong stressors (Spitze, 1991), or seasonal triggers (Nylin, 1992; Nylin & Gotthard, 1998). Unexpectedly, the high-altitude population (HighUV lineage in **Paper III**) started to reproduce earlier even without UVR exposure, suggesting that this population has adopted early reproduction as an evolutionary adaptation. Hence, whereas the low-altitude animals were forced to respond with phenotypic plasticity to the elevated experimental UVR conditions, the high-altitude population required no warning trigger to start reproducing earlier.

The other unexpected finding was the elevated number of offspring of high-altitude population when compared with low-altitude population (Fig. 14). This high fecundity easily offset the reduction in offspring production due to UV exposure, equalling and even overtaking the offspring production of the low-altitude population unexposed to UV radiation. High fecundity and early age at first reproduction was suggested before as a strategy of Antarctic tardigrades to cope with extreme and stochastic environmental conditions (Altiero et al., 2015). In this sense, my results show an alternative strategy that does not prevent, limit or repair the UV radiation damage, but handle UV radiation effects over the population by maintaining fitness and a stable population size through high fecundity and early reproduction.

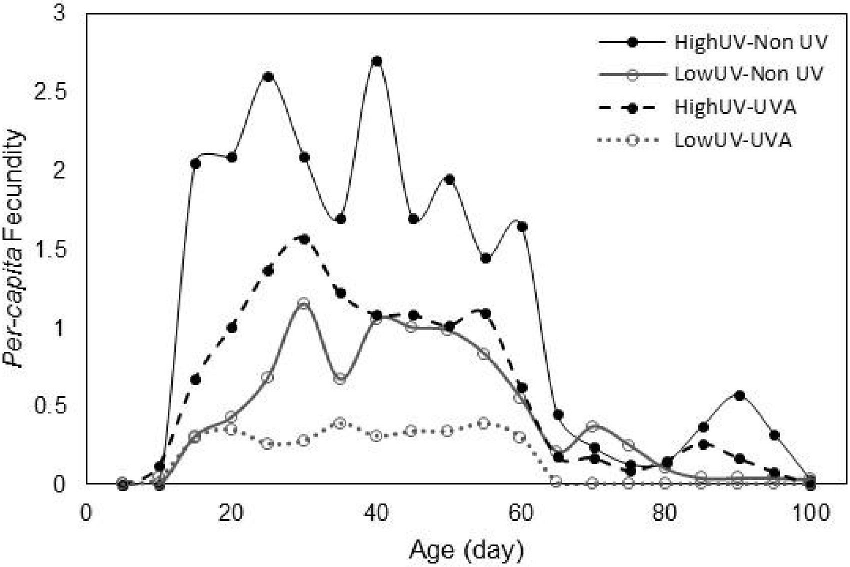


Figure 14. Five-day average life-time fecundity of high-altitude (HighUV) and low-altitude (LowUV) population of *D. pulex*. non-UVR treatments are represented by solid lines, while dashed lines represent UV radiation treatments. Closed and open circles represent high and low-altitude populations, respectively. From Paper III.

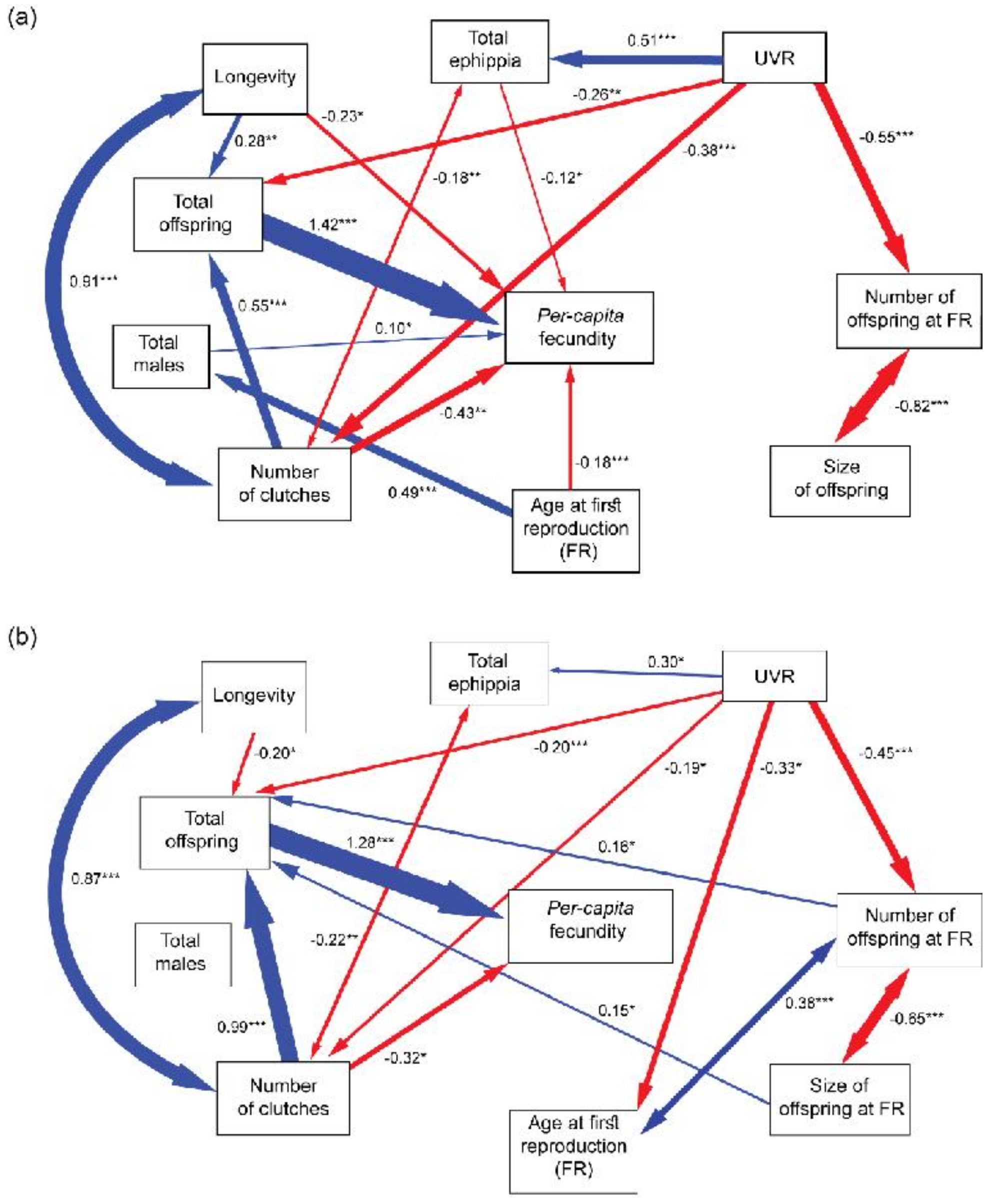


Figure 15. Structural equation model of the influence of UV radiation on life-history traits of *Daphnia pulex*. Resulting path models for: (a) HighUV and (b) LowUV lineage. Arrow widths are proportional to path coefficients. One-headed arrows depict causal relationships whereas two-headed arrows depict correlations. The sign of coefficients denotes positive or negative effects. The level of statistical significance is indicated by asterisks (**p* < .05; ***p* < .01; ****p* < .001). Figure from paper III.

Other differences between life history traits of both populations were exposed by the path model (Fig. 15, see results of **Paper III**). These differences may be caused by the seasonality of the UV radiation regimen at the place of origin of both populations; zooplankton in Andean lakes suffer high UV stress year around, whereas animals in Swedish lakes are exposed from moderate to low UV radiation stress in spring-summer, and fall-winter, respectively. Additionally, the UV radiation treatment reduced the number of clutches and increased number of ephippia in both, Bolivian and Swedish *Daphnia* populations, reflecting a trade-off between investing energy in the present or in the future generations. But longevity was not significantly or directly affected by the UV treatment for any population.

On the other hand, in the experiment of **Paper IV**, longevity was negatively affected by solar spectrum radiation (Fig. 16c), possibly due to differences in the UV lamps between experiments. In this last experiment, a trade-off between longevity and the number of resting eggs was found, due to the inherently high energetic cost of the later. Various factors can trigger the production of resting eggs, the most common ones (besides photoperiod) are high population density and/or low-resource availability (Carvalho and Hughes, 1983, Dinh et al., 2018). Food limitation has been reported as a transmittable stimulus for producing ephippia, female *Daphnia* transmit information about food and photoperiod to their offspring inducing the production of resting eggs in the next generation (Alekseev and Lampert, 2001). Conversely, my results show that food limitation is not an obligate requirement for ephippia production in this population, furthermore, the ephippia production was greater at higher feeding frequencies but only under solar radiation ($F_{2;96} = 30.42$; $P < 0.001$, Fig 17b).

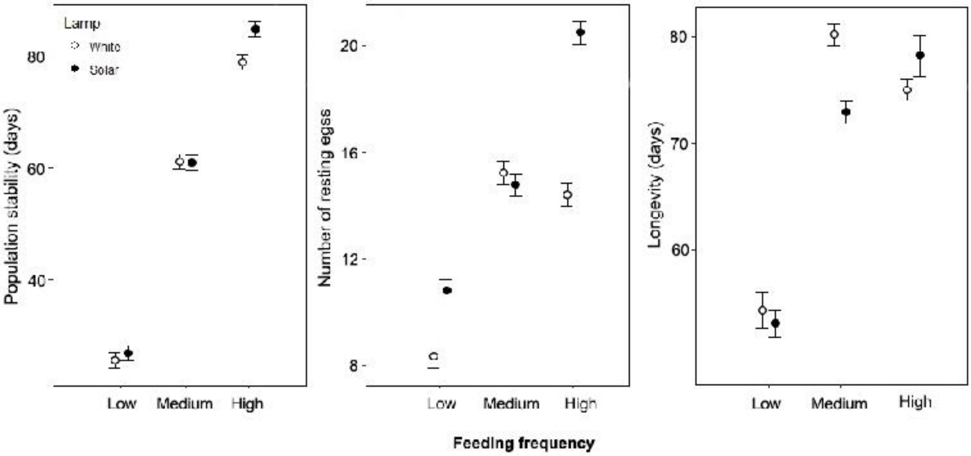


Figure 17. Effects of feeding frequency and artificial solar radiation on a) population stability, b) number of resting eggs and c) individual longevity. Mean ± standard error are shown.

The stability of this population was higher at higher feeding frequencies and enhanced by the solar treatment (Fig.17a), suggesting that this population is highly adapted to solar radiation. Conversely to temperate High-Alpine lakes that may have ultra-oligotrophic conditions, High-Andean lakes usually have oligotrophic to mesotrophic conditions (Modenutti et al., 1998). This can be translated on higher feeding frequencies and hence more available energy that may represent better opportunities to reproduce for zooplankters. Nevertheless, high densities may become inconvenient for the population at some point. Ephippia production is energetically more expensive than a clutch of parthenogenetic offspring, thus ephippia might have an ecological function as a dissipative structure of energy in the system in addition to its well-known function as a structure of resistance and repopulation, as it is shown in **Paper IV**.

5. Conclusions and perspectives

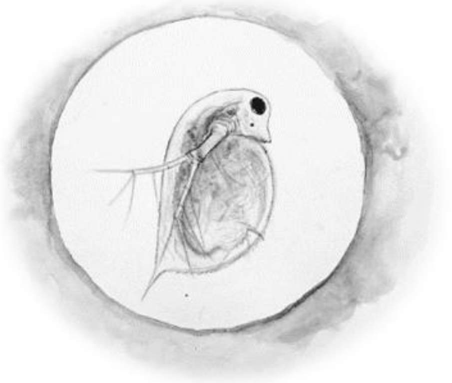
In this thesis I have investigated the adaptive strategies of zooplankton (mainly *Daphnia*) that allows them to maintain stable populations while dealing with UV radiation, highlighting the differences in the responses that may have populations with different evolutionary histories.

Results from **Paper I** indicate that UV radiation is a strong selective force in high-Andean lakes, which may limit grazing activity of daphnids in surface waters during the day. Sub-lethal doses of UV radiation, at low water levels, may strongly reduce zooplankton grazing and ultimately the energy flux in these ecosystems, consequently decreasing the water quality for human uses.

To prevent this effects, *Daphnia* can induce vertical migration or photo-protection (**Paper II**), which may both be modified by ontogenetic processes in relatively short time but determined by local adaptation that lead to different responses to UV radiation within a single species.

UV radiation influences almost every aspect of the life cycle of *Daphnia* populations, either directly or indirectly. However, different populations have adopted different strategies to deal with UV radiation. Results of **Paper III** proposes UV radiation as a selective force, favouring increased fecundity and earlier age at first reproduction as a major evolutionary strategy to compensate the losses caused by UV radiation in populations from high-UV environments. However, this could produce intra-specific competition (for either space or resources) under some conditions, which may require another strategy to maintain through time stable populations. Findings in **Paper IV** strongly suggest that production of resting eggs may have another ecological role, besides resistance and repopulation, acting as an immediate population control mechanism by dissipating the energy, regulating the species and therefore, stabilizing the system.

More research is needed to address how UV radiation would affect population dynamics of key organisms, such as zooplankton, especially in high vulnerable and poorly studied areas such as high-Andean lakes as all research contained in this thesis was done under laboratory conditions. Thus, environmental factors were standardized, including water chemistry, temperature, photoperiod, food quality/quantity, seasonality and predation pressure. This approach makes it possible to identify important processes and assess mechanisms, although in the future it is imperative to couple laboratory with field studies to improve our understanding of these systems, and how multiple environmental factors have shaped *Daphnia* life-history strategies to succeed in different habitats.



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