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Temporal changes in environmental conditions of a mid-latitude estuary (southern Chile) and its influences in the cellular response of the euryhaline anemone *Anthopleura hermaphroditica*

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A R T I C L E I N F O

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A B S T R A C T

The cellular response of the estuarine anemone *Anthopleura hermaphroditica* was assessed during a tidal cycle (low tide at noon) and compared between organisms acclimated to summer and winter environmental conditions (UV-B and PAR levels, salinity and temperature), with the aim of understanding the strategies used by this dominant invertebrate in an estuary of southern Chile. Anemones were gathered every two hours from 8:00 to 20:00 h from the low intertidal area of the Quempillén estuary and immediately snap frozen to carry out biochemical analyses (oxidative damage, antioxidant capacity, photo-protective compounds). Additionally, levels of environmental radiation (UV-B and PAR), salinity and water temperature were recorded during the field survey. Our results indicate that during summer, lipid peroxidation and protein carbonyl levels in *A. hermaphroditica* were 3.72 and 16.51 folds higher than during winter, respectively. Maximum lipid peroxidation levels in *A. hermaphroditica* during summer occurred after UV-B radiation peaked (14:00 h) when water temperatures reached 31 °C. Carbonyl protein levels increased progressively reaching maximum levels at the end of the day. On the contrary, during winter when radiation levels are low and water temperature remained almost constant in the estuary throughout the day, significant variation in salinity levels occurred due to oxidative damage levels that increased significantly at the end of the day. Surprisingly, the mean antioxidant capacity level in *A. hermaphroditica* during winter was 1.7 folds higher than in summer, a situation that was positively correlated to total phenolic compounds content. Mycosporine 2-glycine (λ_{max}: 331 nm) was the only mycosporine like amino acid (MAA) found in *A. hermaphroditica*, a photo-protective compound with constant levels throughout the year. *Anthopleura hermaphroditica*, is an estuarine anemone that are adapted to tolerate rapid fluctuations in the environmental parameters during tidal changes. Those fluctuations are highly affected by seasonal components that expose this invertebrate to different intensity and duration of stress factors between summer and winter. Thus, an efficient antioxidant strategy added to the use of secondary metabolites (phenolic compounds and MAA) may efficiently intercept and neutralize ROS in this animal that lacks of physical protection, allowing it to succeed in a very unstable environment characterized by strong fluctuations of salinity, temperature and environmental radiation through the year.

1. Introduction

It is understood that climate change can impact different marine ecosystems through an increase in environmental radiation, water temperature and acidification in the water column (Henson et al., 2017). Consequently, collateral effects include a rise in the death of symbiotic cnidarians (Downs et al., 2002; Hill et al., 2014; Pramanik, 2014), a situation that has been occurring during the last 30 years (Roberts, 1987), mainly due to the inability of these organisms to intercept and efficiently neutralize reactive oxygen species (ROS) (Asada, 2006; Dai et al., 2014). Consequently, ROS such as superoxide radical (O_2^{-}), singlet oxygen ($^1O_2$), hydroxyl radical ($HO^{\cdot}$) and hydrogen peroxide (H_2O_2), can lead to an oxidative stress affecting the functionality of macromolecules such as lipids, proteins, and DNA in the cell (Ahmad, 1995; Bergamini et al., 2004; Lesser, 2006). Particularly in cnidarians, high levels of oxidative stress are associated with the

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expulsion of microalgae symbionts from the host cell (bleaching), a process that can result in the death of the animal (Weis, 2008). Up until now, physiological studies on corals and anemones have been able to determine their tolerance levels against a range of climate change scenarios (*e.g.* temperature variation and UV-B radiation), but have only considered marine species (Castrillón-Cifuentes et al., 2017; Gardner et al., 2016; Liñán-Cabello et al., 2009). In marine ecosystems, environmental variations occur on a time scale of either months or years, which gives the possibility of physiological acclimatization of animals (Cubillos et al., 2014). On the other hand, periodic variations in the physical-chemical conditions of the water column (*e.g.* temperature and salinity) are associated with more dynamic systems such as estuaries (Vilas, 2009), which impose physiological restrictions on the organisms that develop on an hourly scale (Chaparro et al., 2008b,c; Pardo et al., 2011). Tidal changes in an estuary can generate great variations in salinity, temperature, seston, oxygen availability, and indirectly, exposure to UV-B radiation (Burritt et al., 2002; Cubillos et al., 2016; Chaparro et al., 2008b), allowing us to predict the effects of environmental stress, and determine which species approach their tolerance limits, and which physiological systems establish these limits (Somero, 2010).

Anthopleura hermaphroditica (Carlgren, 1899) is an endemic anemone to Chile that has a wide latitudinal distribution between 23°S and 41°S (Spano et al., 2013). Predominantly, this anemone has been described in bays and estuaries in densities of more than 10,000 ind m^{-2} living semi-buried in the sediment, projecting only the tentacular crown where dinoflagellates of the zooxanthellae-type symbionts are housed (Schories et al., 2011; Spano et al., 2013). In southern Chile, there is a population of *A. hermaphroditica* that lives in the Quempillén estuary, a body of water that due to its small size, has rapidly changing environmental conditions in the water column, which can impact the physiology of this intertidal cnidarian. The Quempillén estuary is located in a mid-latitude area of the southern hemisphere, a geographical area that is characterized to have almost 50% higher UV-B levels than other similar sized estuaries which have been observed during summer in the northern hemisphere (Seckmeyer et al., 2008). Additionally, seasonal variations in radiation doses at a mid-latitude places of the southern hemisphere show variations up to 40-folds the monthly doses of ultraviolet B radiation (UV-B; > 280–315 nm) and photosynthetically available radiation (PAR; > 400–700 nm) radiation between winter and summer (Cubillos et al., 2015). Strategically, benthic and sessile species that inhabit these ecosystems have developed different mechanisms in order to avoid increases in oxidative damage levels. Unlike mollusks, which use shells as physical shields, anemones may be more vulnerable to environmental fluctuations, because their soft tissues are directly exposed to periodic variations of salinity, temperature and environmental radiation, which would increase levels of cellular damage (Dahms and Lee, 2010). Particularly during low tide in summer, the symbiont anemone *A. hermaphroditica* could be confined under a thin layer of water, resulting in exposure to high levels of ambient radiation, and consequently high temperature levels, which would cause high levels of cellular damage. In this context, a previous study carried out using the sea anemone *A. elegantissima* indicated that continuous exposure to elevated PAR radiation levels (400–700 nm) can substantially increase ROS production as a by-product of photosynthesis, increasing oxidative damage (Dykens and Shick, 1984). Additionally, water temperature plays a pivotal role increasing oxidative damage in cnidarians (Lesser, 1996; Lesser, 1997; Senona et al., 2014; Weis, 2008). For example, high levels of superoxide ion (O_2^-) were observed in the symbiont sea anemone *Aiptasia pulchella* after being exposed under thermal stress (Nii and Muscatine, 1997). Consequently, ROS accumulation by thermal stress can lead to a deterioration of the D1 protein which is directly related to the water splitting process and acts as a PSII repair molecule against high levels of radiation (Pospíšil, 2009; Rögner et al., 1996), a situation that can lead to chronic photoinhibition (Weis, 2008). In addition, thermal stress can damage the thylakoid membrane which decouples the electron transport between PSII and PSI, finally generating the radical hydroxyl reactant (HO˙), that rapidly diffuses into the animal cell generating more oxidative damage to other tissues (Weis, 2008). It has been documented that thermal stress initiates the process of apoptosis through the expression of the caspase-like activity enzyme, as observed in the symbiotic anemone *Anemonia viridis* (Richier et al., 2006). Salinity variation due to tidal changes represents a determinant factor that modulates the physiological response of estuarine organisms (Bertrand et al., 2017; Gonçalves et al., 2017). Although salinity levels are mostly constant during summer in the Quempillén estuary, prolonged precipitation during winter can significantly reduce salinity to approximately 5 PPT for up to 72 h (Chaparro et al., 2008b; Chaparro et al., 2009).

The potential variation in environmental stress experienced by *A. hermaphroditica* throughout the year can impact the physiological response of this estuarine anemone. During summer, when low tide episodes coincide with the maximum radiation peak, which was found to occur at midday, a concomitant increase in water temperature may impact the response of this invertebrate. On the contrary, prolonged rainfall during winter generates important reductions in the salinity of the Quempillén estuary, which can generate osmotic problems to *A. hermaphroditica*. Thus, variations in the surrounding environmental conditions influenced by temporal and seasonal variables can impose different levels of cellular and physiological damage in *A. hermaphroditica*. Therefore, the present study was designed to: 1) examine the interaction between the set of anti-stress mechanisms (i.e., increase of the antioxidant activity, phenolic compounds and secondary metabolites, with a photo-protective role) and 2) determine the cellular responses (oxidative damage to lipids and proteins) during a tidal cycle of a typical summer and winter day in the Southern Hemisphere. Understanding the physiological and biochemical adaptations that allow *A. hermaphroditica* to tolerate and consolidate itself as a dominant species in an estuarine system will allow us to infer some metabolic requirements used to biological adapt in a scenario of climate change.

2. Material and methods

2.1. Field survey methodology

To understand how temporal variations (daily and seasonal) influence the cellular response of *Anthopleura hermaphroditica* (Fig. 1A) samples of this estuarine anemone were collected at the southern Chile (Fig. 1B) from the lower intertidal zone of the Quempillén estuary (41°520S; 73°460 W, Chiloé Island, Southern Chile (Fig. 1C)) during a clear sky day of summer (January 2016) and winter (August 2016) every 2 h from 8:00 h until 20:00 h (replicated three times). During the sampling period, tidal conditions were as follows: 8:00 am/high tide; 14:00/low tide and 20:00/high tide. During each colleting hour, 20 individuals were collected, rapidly frozen using liquid nitrogen and then stored in an ultra-freezer (−80 °C) to perform biochemical analyzes of oxidative stress to lipids and proteins, and total antioxidant capacity.

2.2. Environmental parameters

Temperature and salinity levels were continuously measured using an YSI-65 multiparametric instrument in the Quempillén estuary, during tidal changes between 8:00 AM and 8:00 PM during winter and summer. UV-B and PAR radiation levels were continuously recorded during the study period using a portable radiometer (Solarlight PM-2000/USA) placed at the surface of the water level over a floating dock. Radiation doses for PAR and UV-B were calculated for each season according to the irradiance levels of the sampling period.