


# Water transparency affects the survival of the medusa stage of the invasive freshwater jellyfish *Craspedacusta sowerbii*

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**Abstract** *Craspedacusta sowerbii* is a global invader of inland waters, but little is known on what environmental factors control its colonization success. We hypothesized that water transparency and thus, exposure to UV radiation are relevant for the colonization success of this relatively transparent invasive hydroid. Here we used field and laboratory experiments to assess the effect of natural solar radiation and artificial UV radiation on the medusa stage of *C. sowerbii* across natural and simulated water

transparency gradients in three Chilean Patagonian lakes. Short-term exposure of the jellyfish to artificial UV radiation under low Dissolved Organic Carbon (DOC) treatments induced mortality and caused sublethal effects such as swimming anomalies and production of reactive oxygen species. Outdoor exposure of jellyfish to full solar radiation in the most transparent water (Lake Ranco; DOC = 0.6 mg l<sup>-1</sup>) resulted in almost complete mortality. However, higher DOC contents provided partial or almost complete protection against the adverse impact of UV radiation. Overall, our findings provide evidence of the role of underwater light conditions in the underlying mechanisms that may favor the invasion of this gelatinous alien species in inland waters. The results imply that under the current “brownification” of lakes, an increase in water color might provide more

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favorable conditions for the invasion process of this hydroid.

**Keywords** Bio-invasions · UV radiation · DOC · CDOM · Patagonian lakes · Brownification

## Introduction

*Craspedacusta sowerbii* Lankester 1880 (Hydrozoa, Limnomedusae) is a freshwater jellyfish, native to the Yangtze valley in China (Kramp, 1961) with a complex life cycle alternating between benthic, asexually reproducing polyps, and the pelagic sexually reproducing medusa stage (De Vries, 1992). Sexual reproduction on *Craspedacusta* is restricted to the encounter of both sexes in the pelagic habitat of lakes. Therefore, the medusa stage is probably crucial for the invasion process of this exotic hydroid at micro- and macroevolutionary scale (Lundberg et al., 2005), which can be related to increases on the invasive potential due to natural selection on new genotypes locally adapted to environmental conditions outside its native range.

*Craspedacusta sowerbii* is the most successful invasive freshwater Hydrozoa and it is globally reported to colonize both natural and artificial freshwater ecosystems across diverse climatic regions, except for Antarctica (Dumont, 1994). The historical expansion dynamics of *C. sowerbii* outside its native range of distribution (i.e., China) represents a well-documented case of passive global dispersal of an aquatic organism during the Anthropocene, with hundreds of new reports in the last three decades, mostly records of free-living medusae stages colonizing sub-tropical and temperate mesotrophic lacustrine ecosystems (Moreno-Leon & Ortega-Rubio, 2009). In these environments, medusae feed on medium-sized zooplankters, preferentially micro-crustaceans (Spadinger & Maier, 1999) and can reach high abundances during summer and autumn (Stefani et al., 2010; Minchin et al., 2016). Although in the new colonized habitats, *Craspedacusta* commonly acts as a top planktonic predator (Spadinger & Maier, 1999) in the absence of top-down control or competitors, little is known about its impact on the invaded ecosystem (Costello et al., 2008; Oscoz et al., 2010).

The earliest historical record of *C. sowerbii* in the Central- and South American region dates back to 1925 in the artificial Lake Gatún, adjacent to the Panama channel (Ringuet, 1950). New localities infested by jellyfish were later reported in Venezuela, Brazil, Argentina, Uruguay, and Chile suggesting a progressive expansion of *Craspedacusta* from the tropical zone towards southern regions (Fuentes, 2015), apparently mediated by aquatic birds that use the Andean corridor as part of their migration routes (Caputo et al., 2013). Currently, the known distribution of this invasive hydroid in Chile includes oligomesotrophic coastal lagoons, Mediterranean reservoirs, and Andean lakes distributed between 32° and 40°S (Caputo et al., 2013). The southernmost lacustrine environment invaded by jellyfish is the Lake Illahuapi (Caputo et al., 2013), a north Patagonian brown-colored lake located very close to the large and deep (> 200 m) oligotrophic Lake Ranco. A recent molecular study on the invasion process of freshwater jellyfish (Fuentes, 2015) confirms the establishment of clonal populations of *C. sowerbii* (Lankester, 1880) distributed in the lake ecoregions in Chile.

Temperature, food availability, and water quality have been recognized as the most important environmental factors that globally determine the geographical distribution of *C. sowerbii* (Folino-Rorem et al., 2016). In fact, optimal temperatures for growth under laboratory conditions are between 21 and 30°C (Folino-Rorem et al., 2016), which coincides with the optimum range of growth temperatures reported for sexually mature jellyfish in thermally stratified lakes during summer and early autumn (Pérez-Bote et al., 2006; Stefani et al., 2010; Lewis et al., 2012). This jellyfish exhibits a swimming behavior (e.g., diel vertical migration) apparently influenced by solar radiation, which suggests a strategy of sunlight evasion or predation avoidance during different times of the day (Dumont, 1994; Spadinger & Maier, 1999). Moreover, daylength and temperature are important environmental signals triggering ontogenetic transition from the polyp to medusa stage in *C. sowerbii* (Folino-Rorem et al., 2016). On the contrary, a temperature decrease and an increase in turbidity related to lake mixing have been associated with the disappearance of *Craspedacusta* from the water column (De Vries, 1992).

In general, high solar radiation appears to cause detrimental effects on different biological processes of

various hydrozoans. For example, the freshwater medusa *Limnocoñida tanganyicae* Böhm, 1883 (Hydrozoa, Limnomedusae) is highly sensitive to UV radiation and dies after 1 h exposure to short wavelengths (Salonen et al., 2012). Similarly, sessile zooxanthellate polyps of *Cassiopea* sp. are sensitive to high levels of UV-B radiation, with a decrease in asexual reproduction and survival rates (Salonen et al., 2012). Therefore, it is plausible to hypothesize that sunlight and the optical properties of the water column can be relevant to understand the colonization and further expansion success of *Craspedacusta* populations outside of its native range, especially when factors such as water temperature or food availability are not limiting.

Here we studied short-term effects of natural solar radiation (outdoor exposures) and artificial UV radiation (laboratory exposures) on the free-living medusa stage of *C. sowerbii* across natural and simulated water transparency gradients in three Chilean Patagonian lakes. We tested the hypothesis that low lake water transparency caused by turbidity and Dissolved Organic Carbon (DOC) reduces the negative effects of solar radiation on the jellyfish using lethal and sublethal endpoints, such as mortality, swimming behavior, and physiological stress (i.e., production of reactive oxygen species, ROS).

## Materials and methods

### Study area

The study area is located in the pre-Andean zone of the Rio Bueno Basin (15,367 km<sup>2</sup>), which is representative of North Patagonian ecosystems at 40°S (Fig. 1A, B). High number and diversity of lentic and lotic freshwater ecosystems is a distinctive feature of the landscape at these latitudes. These water bodies share similar limnological characteristics such as low mineralized waters, circumneutral pH, and low phytoplankton biomass (Soto & Campos, 1995), and are frequently co-limited by nitrogen and phosphorus (Steinhart et al., 2002). Usually, two distinctive types of zooplankton communities with low species diversity (Soto & Zúñiga, 1991) characterize Patagonian lakes (Woelfl, 2007), namely, a first type with predominance, in terms of biomass, of large mixotrophic ciliates and calanoid copepods, and a second

one dominated by calanoid copepods, followed by cladocerans, and the absence of mixotrophic ciliates. Three lakes were selected for the present study to represent a gradient of water transparency: Lake Illahuapi (maximum depth: 15 m, area: 0.14 km<sup>2</sup>), Lake Verde (maximum depth: 32 m, area: 0.15 km<sup>2</sup>), and Lake Ranco (maximum depth: 200 m, area: 4426 km<sup>2</sup>). The humic Lake Illahuapi is the only system infested with *C. sowerbii* in the Rio Bueno Basin.

### Water characteristics

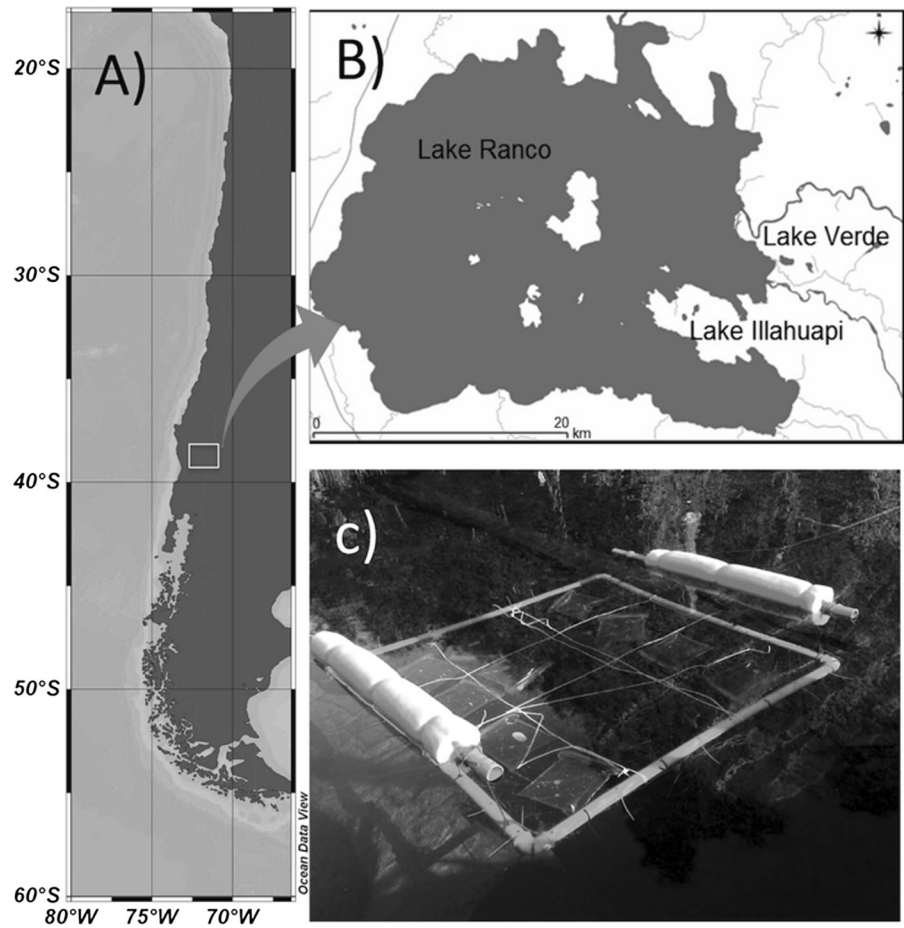
Samples of surface water (0–50 cm) were collected using a 5-l bottle (Uwitec, Austria). Samples for the analysis of DOC were filtered through 0.2 µm pore size filters (Sartorius, Minisart®) and acidified to pH 2 with HCl (2 M) in the field. The analysis was run using a total organic carbon analyzer (Shimadzu TOC-Vc series, Japan). Chlorophyll-*a* content was analyzed according to standard methods (APHA, 2005). Water temperature was recorded with a multiprobe (YSI V2 model 6920, USA) and the water transparency was measured with a 20 cm diameter Secchi disk (Table 1).

The absorbance of the chromophoric dissolved organic matter (CDOM) (200–800 nm) was measured in the same filtrate as for DOC with an Optizen scanning spectrophotometer (Mecasys Co., Ltd.; Daejeon, Korea) using a 5 cm quartz cuvette and 0.2 µm filtered Milli-Q water as a reference. The absorbance at 680–700 nm was subtracted for scattering correction. The absorption coefficient ( $a_d$ ) was calculated as  $a_d = 2.303 \times D/r$ , where  $D$  is the absorbance and  $r$  the pathlength (in meters) (Kirk, 1994). Furthermore, underwater light attenuation ( $K_d$ ) of Photosynthetically Active Radiation (PAR), ultraviolet B (UV-B) and A (UV-A) were measured in Lake Ranco and Lake Illahuapi using a PUV-2500 radiometer (Biospherical Inc., San Diego, CA) and calculated using the following equation:

$$E_d(z) = E_d(0)e^{-K_d z},$$

where  $K_d$  is the vertical diffuse attenuation coefficient for irradiance,  $E_d(z)$  is the irradiance at depth  $z$ , and  $E_d(0)$  is the irradiance just below the surface (Kirk, 1994). The slope of the linear regression between the ln of  $E_d(z)$  and depth  $z$  was used to estimate the  $K_d$  (Table 2).

**Fig. 1** A, B Geographic location of the studied lakes Ranco, Verde, and Illahuapi in the North Patagonia Chile; C the experimental setup used in the outdoor experiments



**Table 1** Background parameters for the surface water during the experiments with jellyfish in the three studied lakes

	Lake Illahuapi	Lake Verde	Lake Ranco
Temperature (°C)	20.3/20.4	20.8/21.2	20.2/20.2
Secchi depth (m)	6.5/6.0	9.0/10.0	14.0/15.0
DOC (mg l <sup>-1</sup> )	2.6/2.6	1.5/–	0.64/–
Chlorophyll- <i>a</i> (µg l <sup>-1</sup> )	2.1/–	1.2/–	1.4/–

Values for the experimental conditions in March and April 2016 are given. No data = (–)

### Outdoor experiments

In Lake Illahuapi, the jellyfish were collected from multiple integrated vertical tows in the top 5 m (ca. the thickness of the epilimnion) using an 80 µm mesh size plankton net. They were kept in 4-l glass containers overnight (12 h) before the experiments and fed ad libitum with zooplankton that was also collected from the lake. In both cases, individuals between 4 and

20 mm diameter were randomly sorted. Two levels of transparency were used in the experiments in the three lakes: one consisting of the original lake water (OW) from the lake where the outdoor exposure was done and another with water from the humic Lake Illahuapi (IW). UV transparent polyethylene bags (20 × 18 × 5 cm; Bitran Inmark, Inc., USA) and non-transparent dark bags (Virutex, Ilko, Chile) were filled with 1 L of screened (55 µm) lake water (OW or

**Table 2** Absorption coefficients ( $a_d$ ) of UV-B radiation, vertical diffuse attenuation coefficients ( $K_d$ ), and 1% depth limits ( $Z_{1\%}$ ) for UV-B, UV-A, and Photosynthetically Active Radiation (PAR) for Lake Ranco (January) and Lake Illahuapi

(March), according to the field measurements with the multichannel radiometer PUV-2500 (Biospherical Instruments Inc., USA)

Lake	$a_d$ ( $m^{-1}$ )				$K_d$ ( $m^{-1}$ )			$Z_{1\%}$ (m)
	UV-B <sub>(315 nm)</sub>	UV-B <sub>(313 nm)</sub>	UV-A <sub>(395 nm)</sub>	PAR <sub>(400–700 nm)</sub>	UV-B <sub>(313 nm)</sub>	UV-A <sub>(395 nm)</sub>	PAR <sub>(400–700 nm)</sub>	
Ranco		0.52	0.17	0.12	8.8	26	39	
Verde	1.2	1.4 <sup>a</sup>	–	–	3.3 <sup>a</sup>	–	–	
Illahuapi	9.8	11.2	3.7	0.68	0.41	1.2	6.8	

In Lake Verde,  $K_d$  was estimated from the absorption coefficient of a filtered water sample

<sup>a</sup>Estimation based on absorption coefficient ( $a_d$ )

IW), six jellyfish individuals, and concentrated zooplankton (3–4 ml) as food source. Each treatment was five times replicated for full sunlight exposure and twice for dark controls. Bags were incubated at the lake surface using PVC frames (1 × 1 m) whose inner area was meshed with transparent nylon nets to keep and secure the bags (Fig. 1C). Experiments were done either under cloudy or clear sky conditions (Table 3), estimated using the tropospheric ultraviolet–visible (TUV) model (version 4.2; Madronich, 1993). The bags used in the field exposures attenuate approximately 20% of FULL (PAR), 26% of UV-A, and 32% of UV-B radiation. Total ozone concentrations ranged between 260 and 265 Dobson Unit (DU), respectively (Ozone data used in the model were extracted from Ozone Monitoring Instrument (OMI), <https://ozoneaq.gsfc.nasa.gov/tools/ozonemap/>). No special

permission was required for conducting sampling or collecting jellyfish in these lakes.

*Laboratory experiments*

Sexually mature jellyfish (17 and 22 mm umbrella diameter) and concentrated zooplankton samples were collected from Lake Illahuapi (as described above) and transported to the laboratory in 60-l plastic containers. The jellyfish were distributed into 12-l aquaria, fed with zooplankton and acclimatized at 20°C during four days before the experiments. Lake water used in the experiments was previously screened (55 μm) and stored under dark conditions at 18°C. A gradient of water transparency was set using dilutions with deionized Milli-Q water (conductivity: 50 μS cm<sup>-1</sup> and pH: 7.1) resulting in DOC concentrations of

**Table 3** Estimations of solar radiation maximum daily irradiance and dose during the outdoor experiments (3 March and 5 April 2016) in the studied lakes based on the tropospheric ultraviolet–visible (TUV) model (version 4.2; Madronich, 1993)

Type of exposure	Maximum daily irradiance			Dose			Exposure time (h)
	UV-B (W m <sup>-2</sup> )	UV-A	PAR (μmol m <sup>-2</sup> s <sup>-1</sup> )	UV-B (kJ m <sup>-2</sup> )	UV-A	PAR	
Outdoor (March)							
Clear sky	1.7	50	1700	35	1260	9840	
Cloudy <sup>a</sup>	0.8	25	870	15	630	4920	24
Outdoor (April)							
Clear sky	0.9	38	1330	16	800	6370	8
Laboratory exposure							
Fluorescent tubes	2.3	8.4	120	66	240	750	8

Maximum daily levels and total doses considering the duration of the exposure are given. For comparison, irradiance levels and doses for the laboratory experiments are also given

<sup>a</sup>At least 50% decrease in irradiance levels is estimated under cloudy conditions prevailing in March (based on Kirk, 2011)

2.6, 2.0, 1.2, and 0.7 mg l<sup>-1</sup>. Three plastic flasks (Ø = 30 mm mouth) containing three jellyfish individuals and 50 mL of lake water were placed in a thermoregulated chamber (20°C) 30 min before the experiment. In order to set PAR and PAR + UV irradiation treatments, cut-off filters Ultraphan 395 nm (Digefra; Munich, Germany) and Ultraphan 295 nm, respectively, were used to cover the flasks, resulting in 2.3 W m<sup>-2</sup> UV-B and 8.4 W m<sup>-2</sup> UV-A and 85 µmol m<sup>-2</sup> s<sup>-1</sup> PAR levels. For the dark control, an opaque plastic foil was used. The UV-B levels correspond to the maximum values recorded in the study area during summer (Huovinen et al., 2006). After the exposure, the jellyfish were kept in darkness for 12 h at 20°C to evaluate their recovery capacity. Seven to ten replicates were used for each transparency level, PAR and PAR + UV treatments and four flasks were used as dark controls.

#### *Determination of sublethal and lethal effects after inspection of swimming capacity*

The responses of the jellyfish to UV radiation were categorized as follows: (1) Normal swimming: coordinated and synchronous movements between umbrella pulsations with the tentacle impulses (bull-whip type) that produce ballistic movements; (2) Erroneous swimming: asynchronous movements between umbrella pulsations with tentacle pumping, as a rule, observed in specimens at the bottom of the flasks with the bell upside down and with spiral motion of tentacles; (3) Eversion syndrome: morphological deformation when animals turn their bell inside out, but preserving their natatorium capacity (Freeman et al., 2009); (4) Lethal effect (mortality): the organisms neither show pulsations of umbrella nor movement of tentacles. This was tested by applying pulses of hydraulic perturbation against the medusa without movement using a plastic pipette to induce responses (tentacles and/or umbrella). If motion was induced by hydraulic stimulus, the jellyfish reaction was categorized as “erroneous swimming.” These stress responses were recorded by visual inspection of animals (for 1 min) after exposure to UV radiation, as well as after the recovery period.

#### *Oxidative stress assay*

Temporal changes in the level of oxidative stress in jellyfish incubated in the most transparent water treatment (DOC 0.7 mg l<sup>-1</sup>) and exposed to artificial PAR + UV radiation in the laboratory (as described above) were assessed. The method described by Collén & Davison (1997) using 2',7'-dichlorofluorescein-diacetate (DCFH-DA) to detect and quantify intracellular production of H<sub>2</sub>O<sub>2</sub> was adapted. In the presence of intracellular ROS and other peroxides, DCFH-DA is switched to highly fluorescent dichlorofluorescein (DCF). Briefly, 3 ml of the dye (5 mM DCF-DA in distilled water) was added to three flasks containing three jellyfish in 50 ml of lake water (20%). The flasks were kept in the dark for 20 min to allow the absorption of DCF-DA into the animal tissue. Additionally, a group of three flasks containing a similar number of jellyfish as in treatment PAR + UV were incubated under dark condition to assess the variation in DCFH-DA in the absence of UV stress. A solution of water containing 3 ml of DCF-DA, without jellyfish, was prepared and used as chemical control. To assess the production of ROS with time, replicate vials were removed randomly at different time intervals (1, 3, 5, 8, and 12 h). From each vial, two jellyfish were selected, gently washed with distilled water, and immediately frozen at -80°C. After 24 h, frozen organisms were cold homogenized with 1250 µl Tris-HCL in a sonicator. DCF-based fluorescence of the extracts was measured with excitation at 498 nm and emission at 522 nm in a Varioskan Flash microplate reader (Thermo Fisher Scientific, Waltham, MA). Production of ROS in the samples exposed to PAR + UV and dark condition was estimated as percentage to the basal fluorescence of the chemical control.

#### *UV-absorbing substances in jellyfish*

To evaluate the presence of UV-absorbing substances, 20 jellyfish were kept alive and starved for 24 h to allow for evacuation of the gut content. Later, the animals were separated into two tubes and frozen in liquid nitrogen for the extraction of UV-absorbing compounds using aqueous methanol 25% (v/v) (Tartarotti & Sommaruga, 2002) and also pure water as solvents. The extracts were scanned in 1 cm quartz



cuvette using an Optizen scanning spectrophotometer (Mecasys Co., Ltd.; Daejeon, Korea).

### Statistical analyses

Differences in mortality of jellyfish between Lake Illahuapi, Lake Ranco, and Lake Verde, with two transparency levels (IW and OW), and under cloudy and clear sky conditions were tested using three-way ANOVA. In the case of mortality and sublethal (erroneous swimming) measured in the laboratory, responses to PAR and UV treatments across a transparency gradient (four DOC levels) were compared using two-way ANOVA. When statistically significant differences among DOC levels were detected ( $P < 0.05$ ) a post hoc analysis (Tukey's HSD) was applied. Differences in ROS production by medusae incubated in the higher transparency level were tested using two-way ANOVA with UV treatment and incubation time as factors. In all cases normal distribution and homogeneity of variances were examined using Shapiro–Wilk's and Levene's tests, respectively. All analyses were done using the Statistica 7 software (StatSoft, Inc., USA).

## Results

### Mortality of jellyfish under field exposure to solar radiation

Animals exposed in the most humic system, Lake Illahuapi, were not affected by solar radiation. Mortality of *C. sowerbii* transplanted from Lake Illahuapi to Lake Verde and Lake Ranco increased with increasing transparency, both under cloudy and clear sky conditions (Fig. 2A, B). Using the water from Lake Illahuapi inside the incubation bags (IW treatment) instead of the original lake water (OW) decreased significantly the mortality of jellyfish in Lake Verde ( $P < 0.01$ ; Tukey HSD) and even more in Lake Ranco ( $P < 0.01$ ; Tukey HSD). In Lake Verde, mortality in OW treatments averaged 20%, whereas in IW treatment no mortality was observed under cloudy condition (Fig. 2A), but reached 10% under cloudless sky (Fig. 2B). In Lake Ranco, exposure under cloudless conditions resulted in 100% mortality of the individuals in OW treatment, whereas 77% survived in IW treatment. Mortality of the jellyfish was

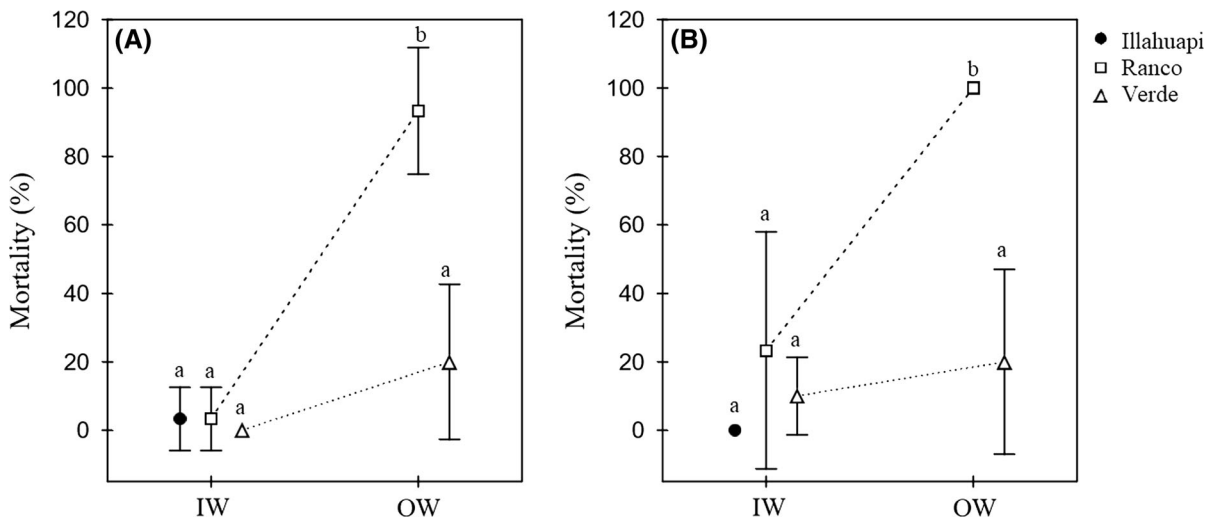
significantly affected by the lake in which experiments were done (i.e., factor 'lake') and the origin of the lake water (i.e., factor 'water') used in incubations (three-way ANOVA,  $P < 0.001$ , Supplementary Table 1). In addition, there was also a significant interaction effect between the factors, 'lake' and 'water' on jellyfish mortality (three-way ANOVA,  $P < 0.001$ ). In contrast, the factor 'sky condition' did not significantly affect the mortality (three-way ANOVA,  $P = 0.163$ ).

### Mortality and swimming capacity of jellyfish under laboratory UV exposure

Jellyfish were negatively affected by PAR and PAR + UV radiation in the 0.7 and 1.2 mg l<sup>-1</sup> DOC treatments and < 0.7 mg l<sup>-1</sup> DOC swimming capacity was reduced by 80% in the PAR and 39% in the PAR + UV treatment. When jellyfish were incubated at 1.2 mg l<sup>-1</sup> DOC and illuminated with PAR, swimming was reduced by 60%, whereas in treatment PAR + UV, swimming capacity was close to 30% (Fig. 3A, B). However, this effect was only temporary in the PAR treatment as revealed by partial recovery of animals within 12 h, particularly noticeable in DOC level of 1.2 mg l<sup>-1</sup> (Fig. 3A). The highest mortality (35%) was observed in PAR + UV treatments with 0.7 mg l<sup>-1</sup> DOC (equivalent to the transparency of Lake Ranco) (Fig. 3C, D). During dark recovery, the mortality increased up to 54% (Fig. 3D). The erroneous swimming after dark recovery became chronic in 75% of the jellyfish ( $n = 6$ ) which developed morphological deformations (eversion) with lethal effect after 36 h. The two-way ANOVA indicated that although UV treatment and DOC levels had an effect on erroneous swimming and mortality of jellyfish, the analysis demonstrated, consistently, a significant interaction between both factors, after exposure and during dark recovery ( $P < 0.001$ ; Supplementary Table S2).

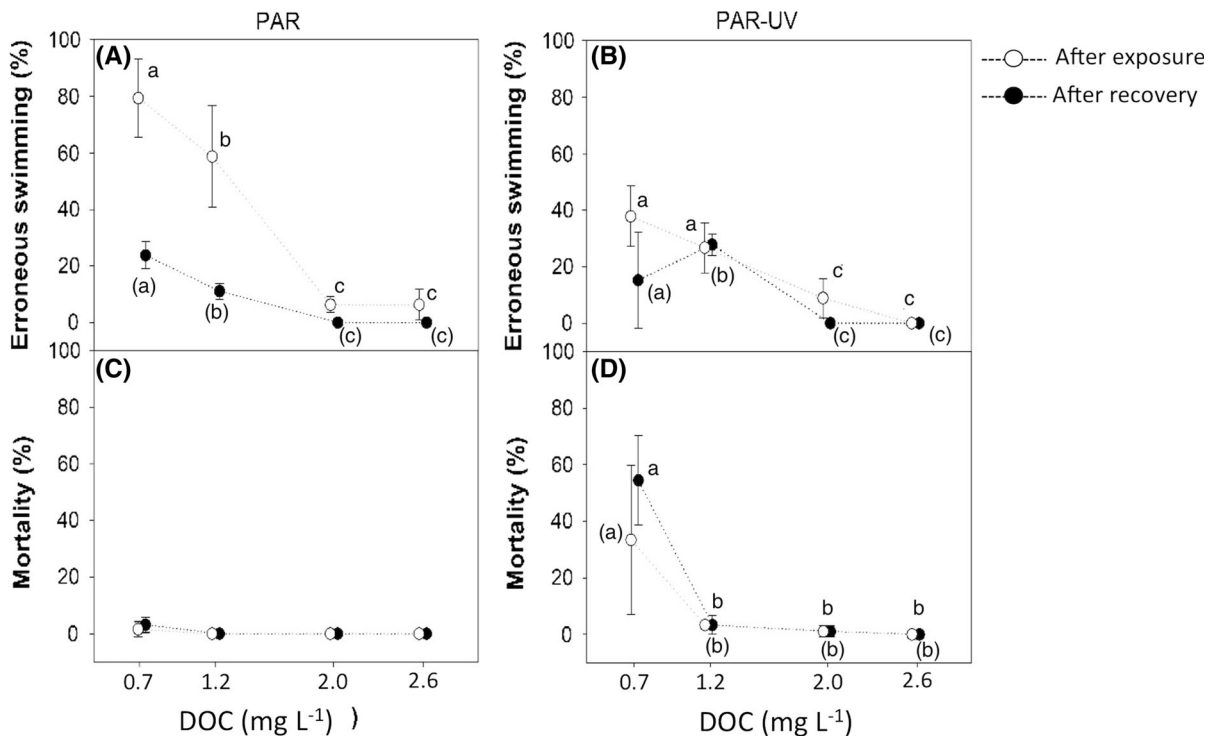
### Oxidative stress

The production of reactive oxygen species (ROS) was evident after five hours of exposure to UV radiation in the low DOC (0.7 mg l<sup>-1</sup>) treatment. Fluorescence signatures, indicative of formation of oxidized DCF, were close to 228% of chemical control in jellyfish exposed for 12 h to UV radiation ( $P < 0.05$ ; Tukey test; Fig. 4). In contrast, samples kept in the dark did



**Fig. 2** Jellyfish mortality after 24 h exposure to solar radiation under cloudy conditions (A) and after 8 h under clear sky condition (B) at the surface of the lakes Illahuapi (black circles), Ranco (squares), and Verde (triangles) in suspended bags

containing transparent water from Lake Illahuapi (IW) and the original lake water (OW). Mean values  $\pm$  SE ( $n = 5$ ). Different letters denote significant statistical differences after ANOVA and Tukey test ( $P > 0.05$ )

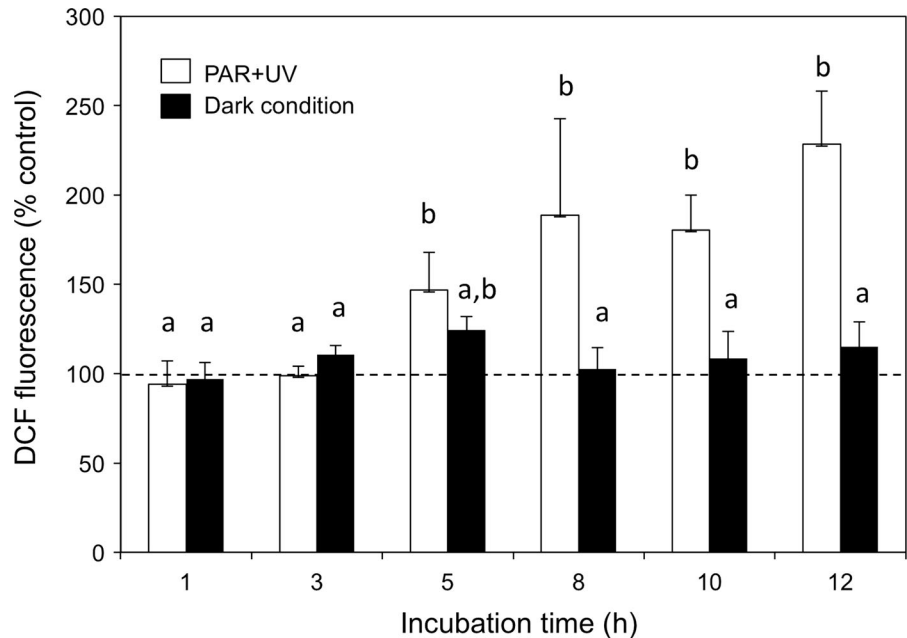


**Fig. 3** Effects of 8 h exposure to artificial Photosynthetically Active Radiation (PAR) + UV radiation on swimming (A, B) and mortality (C, D) of *C. sowerbii* along a Dissolved Organic Carbon (DOC) gradient and after 12 h recovery in darkness. Jellyfish responses to irradiance treatments are

expressed as mean values  $\pm$  SE ( $n = 10$  PAR + UV;  $n = 7$  PAR). Different letters denote significant statistical differences after ANOVA and Tukey test ( $P > 0.05$ ). Differences for recovery in darkness are indicated by letters in brackets



**Fig. 4** Changes in the percentage of the production of reactive oxygen species (ROS) quantified as the relative fluorescence of intracellular oxidized DCF in *C. sowerbii* exposed to Photosynthetically Active Radiation (PAR) + UV radiation treatment and dark condition. Mean  $\pm$  SE ( $n = 6$ ) are relative to a DCF-DA control. Different letters denote significant statistical differences after two-way ANOVA and Tukey test ( $P > 0.05$ ) for PAR + UV and dark condition



not increase significantly in relation to the basal control ranging between 84 and 108% ( $P > 0.05$ , Tukey test; Fig. 4). The two-way ANOVA analysis indicated a strong interactive effect of UV radiation treatments and the time of exposure ( $P < 0.001$ ; Supplementary Table S3). Extracts from visually transparent jellyfish showed no absorption in the range of UV-B and UV-A radiation. Absorption maxima of water and MeOH extracts were detected between 230 and 275 nm (data not shown).

## Discussion

### Impact on mortality and behavioral responses

Our results show that short-term exposure to UV radiation affected the survival and swimming responses, as well as induced the production of reactive oxygen species (ROS) in *C. sowerbii*. Increase in mortality in the outdoor solar exposure differed according to the gradient of water transparency. Thus, while the most humic Lake Illhuapi (DOC:  $2.6 \text{ mg l}^{-1}$ ) provided protection from UV damage, detrimental effects of solar radiation increased in the more transparent lakes. Mortality decreased markedly in the lakes Verde and Ranco when water from Lake Illhuapi was used inside the

experimental bags instead of the more transparent waters from the experimental lakes. Results from the laboratory UV exposure confirmed that  $\text{DOC} \geq 2.0 \text{ mg l}^{-1}$  protect *C. sowerbii* from detrimental effects of solar radiation such as mortality, erroneous swimming, and production of ROS. Photosynthetically Active Radiation (PAR) and UV treatments with DOC of  $1.2 \text{ mg l}^{-1}$  significantly resulted in reduced swimming capacity of *C. sowerbii*, which however was reversible, while exposure in the DOC treatment of  $0.7 \text{ mg l}^{-1}$  caused more acute effects on swimming behavior, increased the production of ROS (200% higher compared to control), and increased jellyfish mortality under UV radiation exposure.

Although our data indicated that PAR induces some degree of stress to organisms, most of the effects measured in the laboratory were sublethal and temporary, as reflected by the noticeable recovery capacity in the dark after exposure. Under highly transparent condition, PAR induced erroneous swimming with a noteworthy directional change in natatorium motion towards the bottom of the flask. This behavioral response confirmed previous observations reported by Dumont (1994), who suggested that high PAR levels effectively trigger vertical migration in the medusa stage of *Craspedacusta* sp. Similarly, this behavior has been reported for other marine species such as the deep-water scyphomedusae *Chrysaora quinquecirrha*

Desor, 1848 (Schuyler & Sullivan, 1997) and *Periphylla periphylla* Peron & Lesueur, 1809 (Dupont et al., 2009), as well as for the hydromedusae *Limnocoñida tanganyicae* whose range of vertical migration can reach depths around 20 m (Salonen et al., 2012).

Little is known about the effects of solar radiation on the physiology of freshwater hydromedusae and how these organisms counteract UV damage. Nonetheless, our results agree with studies done with the hydromedusae *Limnocoñida tanganyicae* from Lake Tanganyika, a relatively transparent lake ( $Z_{1\%}$  UV-B; 290–300 nm: 5 m), where jellyfish exposed to full solar radiation reached 100% mortality after 70 min and a UV-B dose of  $6 \text{ kJ m}^{-2}$  (Salonen et al. 2012). In our experiment conducted in April in Lake Ranco ( $Z_{1\%}$  UV-B: 8.8 m), mortality of *C. sowerbii* reached 100% under full solar exposure (clear sky) after 8 h, which was equivalent to a UV-B dose of  $16 \text{ kJ m}^{-2}$ . Despite the considerable differences in the levels of incident solar UV-B radiation between both studies, *C. sowerbii* seems to be more tolerant to UV radiation under natural conditions compared to *Limnocoñida*. This would outline the apparently invasive character of the former species, whereas *Limnocoñida*—as far as we know—is geographically restricted to African lakes. Nevertheless, *C. sowerbii* was less UV tolerant when transferred to more transparent lakes. These findings may be explained by the protecting function of Chromophoric Dissolved Organic Material (CDOM) in humic waters that reduced the negative effects of UV radiation on medusa. Based on our observations, *C. sowerbii* does not accumulate UV-absorbing compounds, such as mycosporine-like amino acids (MAAs), in its body as a first line of defense against harmful UV radiation, contrasting with other cnidarian groups, such as marine corals and hydrozoans, where internal photoprotective substances (e.g., MAAs from algal symbionts) can minimize UV damage (Reynolds et al., 2008). In other organisms, such as the cladoceran *Daphnia*, CDOM has been reported to shield harmful UV wavelengths efficiently (Hessen & Færøvig, 2001); however, they can also accumulate UV-blocking substances such as melanin (Rautio & Korhola, 2002) or antioxidants such as specific vitamins (Connelly et al., 2015).

The exposure to enhanced UV radiation for 12 h caused oxidative damage in *C. sowerbii*, as indicated

by the oxidized fluorescent DCF (2',7'-dichlorofluorescein). This suggests that the sublethal effects of UV radiation determined on a behavioral basis (e.g., tentacle movement) were accompanied by physiological damage. UV radiation results in the formation of ROS (Shiu & Lee, 2005), which causes damage to different cellular components such as lipid membranes, DNA, and proteins (Shiu & Lee, 2005; Kazerouni et al., 2016). These detrimental effects of ROS result not only in impaired metabolism, but also can damage tissues, increase muscle fatigue, and consequently, decrease movement performance (Bogdanis, 2012; Kazerouni et al., 2016). In natural ecosystems, however, diel vertical migration (DVM) by planktonic organisms can be an effective mechanism to minimize those negative effects (Dodson, 1990). Although we did not evaluate the DVM of *C. sowerbii*, its existence has been reported for the freshwater *Limnocoñida tanganyicae* (Salonen et al., 2012), as well as for other marine jellyfish (Schuyler & Sullivan, 1997; Dupont et al., 2009). DVM patterns in these organisms were characterized by a migration to the deep zone during the onset of solar radiation, while at sunset, the medusae could be found at the surface (Schuyler & Sullivan, 1997). In our experiments, high levels of PAR induced “erroneous swimming” in medusa. Thus, an active behavioral response for searching refuge in deeper water layers and, so avoiding UV damage is possible. Although we do not have evidence for UV effects on the polyp stage of this species, our results on the free-living phase of the *C. sowerbii* allow inferring some environmental constraints that this species could experience. In fact, sexual reproduction is restricted to adult individuals of the medusa stage and thus, selection processes as well as the genetic and physiological mechanisms to endure physical factors probably occur in this phase and in the sexually formed planula larvae (Holstein & Laudet, 2014). In terms of their natural exposure to UV radiation, the polyps of *Craspedacusta* remains attached to the bottom and so, they could be protected from exposure to high levels of solar radiation. At present, however, it is unknown how the benthic phase of Cnidarians (polyps) cope with environmental factors (Bosch et al., 2014). Therefore, to achieve a more complete picture on the invasive process of *Craspedacusta*, it will be crucial to evaluate the impact of UV radiation not only in the medusa stage, but also on the attached polyp phase. Thus, further

approaches are required to gain new insights into the adaptive strategies that invasive organisms with complex life cycles can display to disperse, establish, and finally become dominant (Leclère et al., 2016).

#### The light environment and implications for expansion of *C. sowerbii*

The negative effects of natural solar radiation and artificial UV radiation increased significantly in lakes characterized by high UV transparency, which could be related to the low CDOM and DOC concentrations (Morris et al., 1995; Huovinen et al., 2003). The increased mortality of *C. sowerbii* in more transparent water bodies could explain why there is a large number of reports on the invasion progression of *C. sowerbii* at global scale in mesotrophic or humic environments, but few in transparent ones (Fritz et al., 2007; Duggan & Eastwood, 2012).

The first evidence of the present study indicates that lakes with low UV transparency and high DOC effectively provide environmental suitability for UV-sensitive aquatic organisms (Kessler et al., 2008). Only small increases in CDOM are necessary to exponentially change light climate in aquatic ecosystems (Williamson et al., 1996; Sommaruga & Garcia-Pichel, 1999; Cooke et al., 2006). Therefore “browning” and/or eutrophication processes that decrease UV penetration (Huovinen et al., 2000; Huovinen & Goldman, 2000) could lead to favorable environmental conditions for the establishment and further expansion of *Craspedacusta*, especially if sexual reproduction occurs and new phenotypes, locally adapted to environmental conditions, are exposed to natural selection.

Our laboratory essays suggest, at least at a mechanistic level, that UV radiation affect swimming, mortality, and ROS production, which confirmed the importance of water transparency in the survival of *C. sowerbii* observed in the outdoor conditions. Probably these responses observed at organismal scale reveal environmental limits to the distribution of *C. sowerbii*, which could have implications for the ecology of natural populations in these ecosystems. Although few studies are available about UV penetration in inland water ecosystems in Chile, diverse studies conducted in temperate lakes, indicate that UV has important consequences for the species composition and dynamics in these ecosystems (Scully & Lean, 1994;

Helbling et al., 2001; Marinone et al., 2006). For example, in highly UV-exposed ecosystems, such as polar and high mountain lakes, organisms can be exposed to deleterious levels of UV-B in the water column (Vincent et al., 1998; Sommaruga, 2001; Rose et al., 2009). In fact, measurements done in some Antarctic lakes in the McMurdo Dry Valleys revealed that small changes in CDOM affect more significantly the UV penetration and UV inhibition of phytoplankton than changes in incidence UV-B radiation (e.g., due to changes in stratospheric ozone) (Vincent et al., 1998). In marine ecosystems the effects of eutrophication coupled with ocean warming have been related with an increase in the frequency of blooms of invasive gelatinous organisms (jellyfish and ctenophore), with relevant adverse consequences for local and regional biodiversity (Purcell, 2005; Brotz et al., 2012; Duarte et al., 2013). Similarly, brownification of lakes or their increase in water color (higher CDOM) could increase the invasion success of *C. sowerbii*.

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