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Joint additive effects of temperature and UVB radiation on zoeae of the crab *Taliepus dentatus*

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ABSTRACT: Warming and enhanced ultraviolet B (UVB) radiation are 2 global stressors acting across the ocean. We tested their effects on the survival and performance (consumption rates and activity) on the zoea I stage of the Chilean kelp crab *Taliepus dentatus*. Our goal was to resolve whether these stressors, when acting concurrently, had additive or interactive effects, either synergistic or antagonistic. A multifactorial experiment of 4 temperatures and 3 UVB irradiance levels was run. The larvae showed a significant increase in mortality with increasing temperature. Exposure to UVB reduced the thermal tolerance of the larvae by a significant increase of their mortality rate. Oxygen consumption increased as temperature increased. When exposed to UVB radiation, larval oxygen consumption increased significantly for all the temperatures tested. Two statistical models of joint effects confirmed that the combined effect of both stressors was additive, with no interaction, either synergistic or antagonistic. One of them, the independent action (IA) model, also revealed that concurrent effects on mortality remained additive when doubling the UVB dose. Additivity of the stressors improved the predictability of their effects on larval mortality. Exposure to UVB radiation increased mortality rates by 1.5 times at any temperature tested, independently of the dose.

KEY WORDS: UVB · Temperature · Larval survival · Oxygen consumption · Multiple stressors

INTRODUCTION

Ozone is continuously produced, destroyed and circulated in the stratosphere by a variety of natural factors (Crutzen 1971, Wofsy et al. 1975). During recent decades the emission of chlorofluorocarbon compounds (CFCs) has led to a reduction of ozone concentration in the atmosphere (Weatherhead & Andersen 2006), resulting in an increase of ultraviolet B (UVB) radiation reaching Earth (Munakata et al. 2009). The largest zonal average increase of UVB irradiance since the 1970s has been recorded in the Southern Hemisphere, where the stratospheric ozone decline has been greatest (Herman 2010, Agustí et al. 2015). UVB radiation penetrates to considerable depths into the water column in clear oceanic waters (Tedetti & Sempéré 2006), damaging marine organisms especially in the Southern Hemisphere, where incident UVB radiation is higher (Dütsch 1974, Llabrés et al. 2013). Marine organisms show mechanisms of photoprotection to avoid deleterious effects of ultraviolet radiation. These mechanisms include production of antioxidant enzymes (e.g. Hylander & Hansson 2010), pigmentation (e.g. Hansson et al. 2007), or ingestion of particles that can aid in protection such as mycosporine-like amino acids (MAAs;

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Helbling et al. 2002, Rocco et al. 2002, Tartarotti & Sommaruga 2006). Avoidance by migration is another response (Hansson et al. 2007, Hylander & Hansson 2010, Zengling et al. 2010). Despite these mechanisms, experimental studies have shown that elevated UVB radiation has significant impacts on marine biota (Häder et al. 2011).

In a large meta-analysis, Llabrés et al. (2013) found that the impacts of increased UVB radiation resulted in a significant increase in the mortality of marine biota, with early stages such as eggs and larvae being the most sensitive to UVB (Llabrés et al. 2013). For instance, it is known that UVB radiation causes mortality to encapsulated molluskan embryos (Carefoot et al. 1998, Przeslawski 2004). Marine organisms from the Southern Hemisphere seem to be less vulnerable to UVB radiation than those from the Northern Hemisphere (Llabrés et al. 2013, Agustí et al. 2015), but they are still affected, possibly because of strong selective pressure under the recent elevated UVB regimes in the Southern Hemisphere (Agustí et al. 2015).

In addition to changes in the stratospheric ozone layer, Earth is also experiencing substantial changes in temperature. Earth's temperature is increasing due to anthropogenic greenhouse gas emissions (Trenberth et al. 2007). These changes have been observed in the atmosphere and the ocean, but not all of the regions are warming. As an example, a decrease in temperature of both air and water has been reported along the coast of Chile, at a rate of 0.2°C per decade (Falvey & Garreaud 2009). Changes in temperature regimes affect marine ecosystems by influencing reproductive success (Pörtner & Knust 2007), recruitment (Jordà et al. 2012), development (O'Connor et al. 2007), mortality (Coma et al. 2009, Jordà et al. 2012) and changing the geographical distribution of organisms (Hawkins et al. 2008). Ectothermic animals are extremely dependent on the temperature window of their natural environment (Pörtner 2001).

Thermal tolerance windows differ among species depending on the range of environmental temperatures in their habitat (Pörtner 2001, Somero 2010), and vary greatly across developmental stages (Anger et al. 2003, Parker et al. 2009, Weiss et al. 2010, Storch et al. 2011). While adult organisms are often protected from physiological extremes (e.g. by shells and operculi of mollusks), their pelagic eggs or larvae may be highly vulnerable because they may experience strong short and long-term temperature fluctuations in the water column (Anger et al. 2003). Moreover, temperature may also influence dispersal since larval development time is affected (O'Connor et al. 2007). Temperature changes therefore affect marine organisms, particularly larval stages, across a broad range of processes and scales (Duarte 2007).

A stressor is any environmental factor that reduces survival or performance of optimum conditions (Folt et al.1999). Increasingly stressful conditions such as shifts in temperature might decrease the ability of organisms to cope with other concurrent stressors, such as ultraviolet radiation. Thus, understanding the complex responses of organisms to environmental changes requires addressing the interaction between concurrent stresses (Duarte 2014). The multiple stresses concept, developed originally in the toxicology literature (Heugens et al. 2001), is now used more broadly to refer to the presence of accumulated stressors, not necessarily including pollutants, which have been identified as responsible for population declines and community changes in aquatic systems (e.g. Schisler et al. 2000). Multifactorial studies can help to analyze deviations from linear responses and test for additive effects of joint stressors (Echeveste et al. 2011, Sett et al. 2014). Models such as concentration addition and independent action (IA) have been developed to estimate the toxicity of chemical mixtures in organisms (Payne et al. 2001). IA is widely used in toxicology and has been successfully applied to test the interaction between stressors other than toxic chemicals in zooplankton (Coors & De Meester 2008). When using IA the stressors are assumed to act on different systems in the organism. UVB radiation affects cells by damaging their DNA and increasing oxidative stress (Sinha & Häder 2002, Häder et al. 2011), while temperature affects the organismal metabolic rates of processes. Multi-stressor studies have already shown that the prevailing type of stressor interaction is synergistic over additive effects and that early life stages are more sensitive; in these studies, temperature and ocean acidification have received most attention (Byrne & Przeslawski 2013, Kroeker et al. 2013, Ban et al. 2014, Przeslawski et al. 2015). The influence of the concurrent stress of temperature and UVB has mostly focused on coral reefs (Ferrier-Pagès et al. 2007, Ban et al. 2014). Improved understanding the concurrent effects of temperature and UVB on marine organisms in general, and particularly on their most vulnerable stages, is needed to predict responses to global change.

In this study, we tested the sensitivity of marine zoea I larvae of the crab *Taliepus dentatus* to joint effects of temperature and UVB radiation, with the ultimate goal of elucidating whether the interaction

between increasing temperature and UVB radiation leads to multiplicative synergistic or antagonistic effects that deviate from those expected under the assumption of additivity of single-stressor effects. We chose *T. dentatus* as our model species because it is a common kelp crab inhabiting the near shore rocky habitats of the Chilean and Peruvian coast. Moreover, larval stages of this species are neustonic (Palma et al. 2006, Pardo et al. 2007, Mujica & Nava 2010), and can therefore be affected by UVB radiation, and exhibit a clear thermal tolerance window (Storch et al. 2009). Crustacean and fish larvae are the most sensitive to increased UVB radiation (Llabrés et al. 2013). Southern South America receives elevated UV radiation due to reduced stratospheric ozone levels, which may particularly impact larvae inhabiting surface waters (Mujica & Nava 2010). Previous studies have already shown the vulnerability of these larval stages to temperature changes (Storch et al. 2009). Departures from the narrow optimal temperature range of zoea larvae can affect both metabolism and behaviour of the larvae (Storch et al. 2009). For example, the influence of upwelling, frequent in this region, generates periods where water temperature is below the optimum reported for the larval stage of these species (Storch et al. 2011). Predicted long-term changes in seawater temperatures in this geographic region associated with climate change highlight the relevance of evaluating the interplay between these 2 critical environmental factors, i.e. temperature and UVB radiation, on larval development (Falvey & Garreaud 2009).

MATERIALS AND METHODS

To meet the goal of evaluating the influence of temperature and UVB radiation on first life stage (zoea I) of the Chilean kelp crab *Taliepus dentatus,* we conducted laboratory experiments exposing larvae to simultaneous variations in both factors, testing the effects on survival, metabolism and larval activity. The experiments were conducted at Estación Costera de Investigaciones Marinas (ECIM), in Las Cruces (33° 29′ S, 71° 38′ W), in Central Chile. This region is characterized by a narrow continental shelf, and nearshore hydrography is dominated by wind-induced upwelling during austral spring and early summer (Strub et al.1998). There is large thermal oscillation in sea surface temperature over the year, with a minimal temperature of 11°C and a maximum temperature of 21°C during the warmest months (Kaplan et al. 2003).

Experimental design

Brooding females of *Taliepus dentatus* were collected from Las Cruces area by local professional divers between October and December 2011, and transported to the laboratory of ECIM. Females were maintained individually in 50 l aquaria with running seawater at the experimental temperatures (see below), and approximately 34 ppt salinity. Females were kept at experimental temperatures (see below) for at least 5 d to allow embryo acclimation. They were fed with algae (*Lessonia trabeculata*) and monitored daily until they were ready to spawn. Most often larvae hatched during the night or early morning. Newly hatched larvae from each female were collected and transferred to labeled 0.5 l culture vessels with a constant density of 40 individuals per vessel (all replicates contained the same number of larvae). Larvae were maintained in the same temperature controlled conditions as adult females. Both adults and larvae were exposed to a light:dark photoperiod (12:12 h) using fluorescent lamps (photosynthetically active radiation [PAR]). Approximately 75% of the seawater of the culture vessels was changed daily with UV-treated, filtered and aerated seawater. Freshly hatched *Artemia* were added daily to feed the larvae.

In order to test the sensitivity of marine zoea I larvae of *Taliepus dentatus* to joint effects of temperature and UVB, we carried out laboratory experiments under indoor conditions, implementing a factorial design to test of range of combinations of temperature and UVB radiation. We used 4 experimental temperatures covering the full range between the lowest and highest temperature observed in the study area (11, 15, 19 and 23°C) using thermoregulated baths. These temperatures are within the natural thermal oscillation of sea surface temperature in the area in austral spring and summer (Kaplan et al. 2003, Vargas et al. 2004, Pulgar et al. 2006), including the optimum temperature of 15°C for zoea I larvae (Storch et al. 2011). The upper thermal limit is reported to occur between 19 and 23°C (Storch et al. 2011) and for this reason we set in 23°C as our upper experimental temperature. Temperature was monitored every minute in all experiments using Onset TidbiT temperature loggers. This information allowed us to confirm that our experimental temperatures did not vary over the course of the experiment.

Besides temperature treatments described above, larvae were exposed to 2 levels of underwater UVB radiation for 6 h each day (between 10:00 and

16:00 h): 0.7 (low) and 1.5 (high) W m^{-2}. These UVB levels represented daily doses of 15.2 and 32.4 kJ m^{-2} respectively. Larvae were also exposed to a control treatment without UVB radiation (PAR only). Radiation intensities were selected taking into account natural radiation and the attenuation between water surface and 50 cm deep on a sunny day in ECIM coast. We provided UVB radiation under laboratory conditions using UVB-313 EL lamps (Q-LAB), emitting in the range of 280 to 360 nm, with a peak at 313 nm. The timing of the treatment (10:00 to 16:00 h) corresponded to the time of day when the highest UVB levels are recorded daily in Las Cruces area (B. Carreja pers. obs.). We measured radiation intensities with a PMA 2100 radiometer and a UVB sensor (Model 6, Serial 14400, Solar Light Company) submerged in the incubation tank.

Each of the vessels containing larvae was randomly assigned to one experimental condition of UVB and temperature. We did not use larvae from the same female in the same experimental condition, and larvae from the same female were not replicated in the whole range of experimental condition. The experimental runs lasted 4 d. Between 5 and 13 replicates were run in most cases for the 2 temperatures closer to the optimum (15 and 19°C) and between 3 and 4 replicates for the extreme temperatures, due to low hatching and survival rates (Table 1).

We measured larval mortality and performance during the first 4 d after hatching. The duration of the zoea I stage is approximately 10 d (Fagetti & Campodónico 1971). Larval performance was assessed by measuring their oxygen consumption at the middle of the experimental period (second day).

Table 1. Number of replicates for each combination of UVB radiation daily dose (kJ m^{-2}) and temperature for the experiments conducted using Zoea I larvae of *Taliepus dentatus*

Temperature (°C)	UVB (kJ m^{-2})	Survival	O_2 consumption
11	0	4	4
11	15.12	4	4
15	0	12	11
15	15.12	7	6
15	32.4	5	5
19	0	13	9
19	15.12	7	5
19	32.4	6	4
23	0	3	3
23	15.12	4	3

Survival

To measure larval survival, we counted the number of larvae that were alive and dead immediately after turning off the UVB lamps (in all treatments, whether exposed or not to UVB radiation). Larvae were considered dead when they could not swim and remained motionless on the bottom of the flasks. Mortality was monitored daily, after UVB experimental exposition. We calculate mortality rate as the slope of the linear regression between natural logarithm of larval abundance and time (days).

We used linear regression to analyze the relationships between mortality rate and temperature. To be able to test the combined effect of UVB radiation and temperature and to evaluate if the multiple stress resulted in synergistic, additive or antagonistic effect on the mortality rate of the larvae, we applied a statistical model, analyzing the variance of the linear relationships between mortality rate and temperature in absence and presence of UVB radiation, following Echeveste et al. (2011):

$$\text{Larval mortality} = a1 + b1 \cdot T + a2\,(\text{UV}) + b2 \cdot (\text{UV} \cdot T) \quad (1)$$

where T is the temperature, $a1$ is the intercept on the y-axis of the linear regression, $b1$ is the slope of that linear regression, $a2$ is the variation of the intercept in the presence of UVB and $b2$ is the variation of the slope in the presence of UVB. When UVB was not present, the model applies the equation: larval mortality = $a1 + T \cdot b1$, that shows the effect of increasing temperature on the different parameters studied. When UVB radiation was present, the model applies the equation: larval mortality = $(a1 + a2) + T(b1 + b2)$, that shows the joint action of increasing temperature and presence of UVB on the different parameters that are being studied.

In order to evaluate whether doubling UVB doses, from 15.2 to 32.4 kJ m^{-2}, could have a synergetic effect with temperature increase on larvae mortality, we used a joint effect model applied to test interaction by multiple stressors in other planktonic organisms (Coors & DeMeester 2008). As temperature and UVB radiation may operate at different molecular levels in larvae, we used an IA model of joint effects that assumes additivity and denotes synergy and antagonism by positive and negative deviations of the observed relative to the predicted effect, respectively, as described in the analysis of multiple effects by toxic chemicals (Payne et al. 2001). The IA model allowed us to estimate the expected joint effects of both stressors.

To develop the IA model, we chose as a control the temperature treatment showing the lowest mortality rate (15°C). The IA model calculates the predicted effects e_{mix} of a mixture of known composition by using the expression (Payne et al. 2001):

$$e_{mix} = 1 - \Pi[1 - E_i] \quad (2)$$

where E_i is the effect of each single stressor *i*. IA is a probabilistic model, i.e. E_i is a fraction of a maximal possible effect that cannot exceed 1. When this model is applied to proliferative effects (products of effects), a maximal effect e_{max} must be defined. For this purpose, the maximal mortality rate observed on the zoea I larvae during the experiment was used as a reference point, and the effects of tested temperature and UVB radiation were expressed relative to the maximal effect (Payne et al. 2001):

$$E_i = e_i / e_{max} \quad (3)$$

where e_i is the effect of each single stressor *i*. We identified as synergistic effect treatments when the observed mortality rate was significantly higher than the predicted mortality rate e_{mix}. Student's *t*-test was used to test the significance of the differences between predicted and observed effects.

The models and statistic tests were analyzed using the program JMP, Version 9.0. The significance level was 5%.

Oxygen consumption

We measured oxygen consumption after exposing the larvae to UVB treatment during the second day of experiment, between 17:00 and 20:00 h. Two larvae were randomly selected and placed in Hamilton microliter precision syringes (volume 500 µl), used as closed respiration chambers. Larvae were at the same temperature as in the experimental temperature treatment when we measured their respiration. They were not exposed to UVB radiation while oxygen was monitored in the respiration chambers. Blanks were run to monitor oxygen consumption in the seawater without the presence of larvae. Oxygen partial pressures in the respiration chamber were recorded by oxygen micro-optodes (needle-type fiber-optic micro sensor with flat broken tip, diameter: 140 µm) connected to a Microx TX2 (PreSens). Optodes were calibrated using an oxygen-free solution (1 g Na_2SO_3 per 100 ml distilled water) to calibrate 0% of oxygen and aerated seawater to calibrate 100% of oxygen. After calibration, 2 larvae were carefully introduced into the precision syringes, leaving a 40 µl volume. Next, the optode was inserted in the syringe and the sensitive tip was positioned in the middle of the respiration chamber, leaving enough space for the larvae to swim freely. Measurements lasted until the oxygen tension in the chamber decreased by 20%. At the end of the oxygen measurement, larvae were removed from the chambers, carefully dried on paper and weighed on a Sartorius BP 211 D high-precision balance. Oxygen consumption rates were expressed as µg O_2 mg^{-1} larva h^{-1}. Due to high larval mortality at the extreme conditions, it was not possible to replicate all combinations of temperatures and UVB levels (Table 1). Thus, for extreme temperatures we could only perform oxygen uptake measurements under control and the lower dose of UVB radiation. For this reason, we conducted 2 different 2-way ANOVA testing on (1) the influence of UVB across the wide range of temperatures that included extreme temperature conditions, and (2) the influence of the full range of UVB levels using only the 2 temperatures closer to the optimum temperature reported for this larval stage (Storch et al. 2009). Tukey tests were applied to determine differences between levels.

RESULTS

Larval survival declined with time and was strongly dependent on the different treatments, increasing with temperature and UVB (Fig. 1). Larval mortality rate varied highly from 0.036 ± 0.007 d^{-1} (mean ± SE) observed at 11°C to 2.2 ± 0.4 d^{-1} at 23°C for larvae exposed to UVB. Mortality rate increased with increasing temperature both in the absence and presence of UVB radiation. Precisely, in the absence of UVB radiation larval mortality was $-1.95 + (0.165 \times T)$ d^{-1} ($r^2 = 0.61$, $p < 0.0001$), while in the presence of UVB radiation mortality was $-1.37 + (0.158 \times T)$ d^{-1} ($r^2 = 0.59$, $p < 0.0001$). Similarly, larval mortality was significantly higher, for all temperatures tested, when exposed to UVB radiation ($p < 0.0001$). These effects were best described by the statistical model, which showed that both temperature and UVB radiation had significant effects on larval mortality rate (Table 2). Although the mortality rate was higher at the highest temperature and in the presence of UVB radiation, the interaction term between these parameters (*b*2 in Eq. 1) was not significant (Table 2), indicating that the effects of temperature and UVB radiation on larvae mortality rate were additive (Table 2).

The IA model was applied in temperature treatments where larvae were exposed to 2 levels of UVB

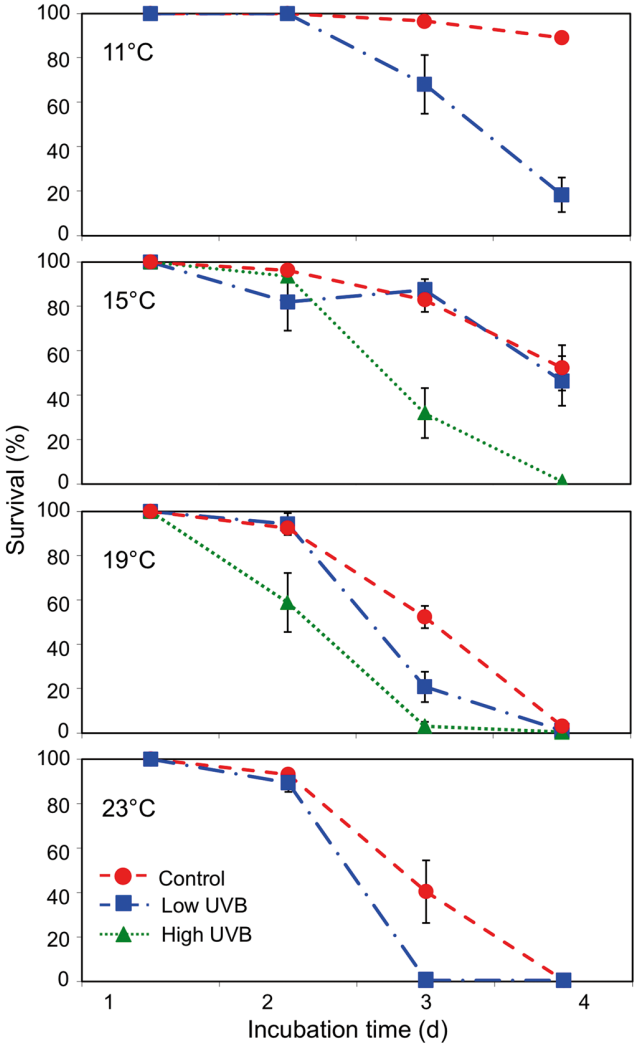

Fig. 1. Changes in the percent survival of *Taliepus dentatus* zoea I larvae over time observed at combinations of experimental temperature (11, 15, 19 and 23°C) and UVB radiation treatments (control, low and high). The low and high UVB treatments were 15.12 and 32.4 kJ m^{-2} daily doses, respectively

(low and high), to check for possible stressor interactions associated with the level of UVB irradiance. Larval mortality increased relative to that in the control treatment (15°C) when larvae were exposed to either UVB radiation or 19°C temperature (Fig. 2, single stressors). The increase in mortality rate in response to UVB at 15°C was higher at the highest UVB dose tested (Fig. 2, single stressor). Larval mortality also increased when exposed to multiple stressors (i.e. to both increase in temperature to 19°C and exposure to UVB radiation) but the observed mortality rate was similar to that predicted under additive effects by the IA model (t = 0.45, df = 6, p = 0.33, and t = 0.02, df = 5, p = 0.40, for low and high UVB,

Table 2. Results of the statistical model (Eq. 1) testing the relationship between larval mortality of zoea I larvae of *Taliepus dentatus* and temperature and exposure to UVB radiation. *p < 0.05

Parameters	Estimate	p
*a*1	−1.90 ± 0.32	0.0001*
*b*1 (Temperature)	0.16 ± 0.02	0.0001*
*a*2 (UVB)	0.47 ± 0.12	0.0003*
*b*2 (Temperature+UVB)	0.007 ± 0.03	0.852

respectively), confirming, consistent with previous analysis (Table 2), the lack of synergistic or antagonistic interaction between these 2 stressors when the UVB dose was doubled (Fig. 2).

Oxygen consumption

Zoea I larvae exposed to UVB radiation showed the highest oxygen consumption rates, and oxygen consumption increased as temperatures departed from

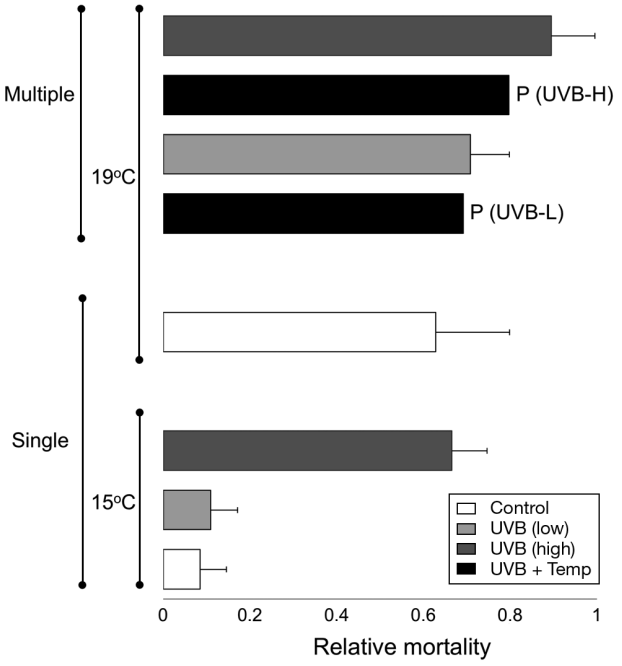

Fig. 2. Observed concurrent effects of temperature and UVB radiation on larval mortality of *Taliepus dentatus* and additive effects of combined stressors predicted by the independent action (IA) model. White bars show larvae not exposed to UVB radiation, light grey bars larvae exposed to low UVB radiation (UVB-L), and dark grey bars larvae exposed to high UVB radiation (UVB-H), in each case at the control temperature (15°C) and 19°C. Black bars show the joint effects of UVB radiation and temperature predicted (P) by the IA model. 'Single' indicates treatments with 1 stressor, and 'multiple' indicates treatments with 2 stressors

Table 3. Results of the 2-way ANOVA conducted to assess the influence of UVB and temperature on oxygen consumption (response variable) by zoea I larvae of *Taliepus dentatus*: across the full range of experimental temperatures in combination with the control (no UVB radiation) and low UVB treatment; and for 2 temperature treatments in combination with the control and 2 UVB levels (low and high). High mortality prevented analysis of oxygen consumption across the full range of temperature treatments with high UVB levels. *p < 0.05

Analysis	Factor	*F*	df	p
Four temperatures and low UVB	Temperature	10.02	1,97	0.002*
	UVB	3.45	3,97	0.019*
	Interaction	0.33	1,97	0.8
Two temperatures and 2 UVB levels	Temperature	6.24	1,86	0.002*
	UVB	5.4	1,86	0.02*
	Interaction	2.6	2,86	0.08

the optimum (15°C; Fig. 3). Moreover, oxygen consumption increased with exposure to UVB radiation with respect to the control in the same rates across the experimental temperature range (the interaction term was not significant: $F = 0.33$, df = 1,97, $p = 0.8$; Table 3). Oxygen consumption was significantly higher at 11 and 23°C than at 15 and 19°C ($p < 0.05$). The same pattern was observed when we compared UVB levels for 2 experimental temperatures (15 and 19°C; Fig. 3). Oxygen consumption increased under UVB radiation ($F = 5.4$, df = 1,86, $p = 0.02$; Table 3) and when temperature increased ($F = 6.24$, df = 1,86, $p = 0.002$; Table 2), but there was no significant interaction between UVB and temperature (interaction term: $F = 2,6$, df = 2,86, $p = 0.08$; Fig. 3, Table 3).

DISCUSSION

Our results showed that survival and oxygen consumption rate of the zoea I larvae stage of the Chilean kelp crab *Taliepus dentatus* were significantly affected by warming. UVB radiation also had a significant effect, increasing larvae mortality and oxygen consumption. However, our results show that these 2 stressors operate on larval mortality through additive, not synergistic, effects.

Temperature is a key driver of biological processes (Duarte 2007) and changes in seawater temperature can cause biological stress to early larvae stages of marine organisms affecting their development, behaviour and survival (Pörtner 2002, Anger et al. 2003, Storch et al. 2011). Zoea I larvae were highly sensitive to temperature, as shown by the high increase in mortality rate to the highest temperature tested (23°C).

UVB radiation also stressed *T. dentatus* larvae, causing an increase in mortality as the UVB dose increased. This observation also agrees with previous studies conducted with early-hatched crab larvae and other crustacean larvae showing increased mortality under UVB radiation (Hovel & Morgan 1999, MacFadyen et al. 2004, Hernández Moresino & Helbling 2010, Llabrés et al. 2013). Larval mortality could be related to the failure of the photo-repair systems when the dose exceeds the system tolerance limits (Damkaer et al. 1981, Damkaer & Dey 1983). Indeed, increased mortality has been identified as the main effect of UVB radiation on marine biota (Llabrés et al. 2013), with early stages of marine organisms identified as the most sensitive to UVB radiation (Häder et al. 2011, Llabrés et al. 2013). Our high experimental UVB dose mimicked the upper range of natural UVB radiation in central Chile in springtime, where the specimens were collected. Therefore, the current natural UVB conditions in the study area seem to influence larval survival, considering that larvae of this species have been reported in the neuston (Palma et al. 2006, Pardo et al. 2007, Mujica & Nava 2010) and the peak of reproduction of brachyuran crabs match the highest UVB levels in the southeast Pacific. Moreover, in southern Chile, where larvae of *T. dentatus* are more sensible to high temperatures (Storch et al. 2009), UVB radiation can reach twice our maximum experimental levels (Huovinen et al. 2006). It is also important to remark that our study was conducted with the larval stage of *T. dentatus* that shows the greatest tolerance to temperature variation (Storch et al. 2011). Therefore, we can predict a stronger effect of temperature and UVB on larval mortality at later stages of larval develop-

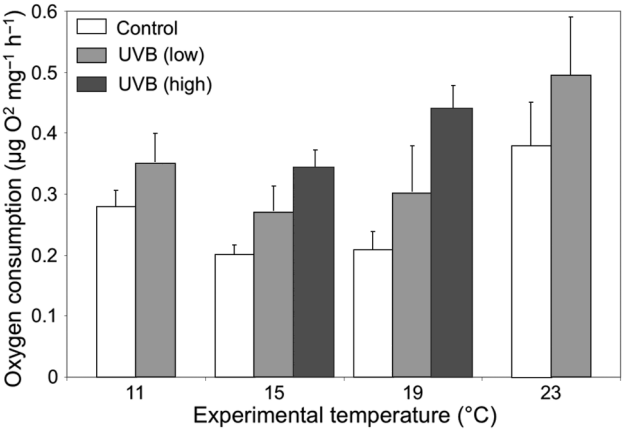

Fig. 3. Average (+SE) oxygen consumption of *Taliepus dentatus* zoea I larvae under a range of combined temperature and UVB radiation treatments

ment in southern Chile, and also on physiological indicators.

We showed that the oxygen consumption of zoea I larval stage increased as temperature departed from the optimum (15°C), showing a Q_{10} of 1.5. Our results agree with previous studies that described a non-linear exponential increase in oxygen consumption with temperature in *T. dentatus* larvae (Storch et al. 2009). Oxygen consumption comprises the energy demand for both maintenance and swimming activity and thus includes elements of performance or aerobic scope that are crucial in setting the thermal tolerance of zoea I (Pörtner & Knust 2007, Pörtner & Farrell 2008). We also observed an increase in oxygen consumption following exposure to UVB, as previously described in other organisms, where it was related to increasing stress (Wieland & Kühl 2000, Alemanni et al. 2003). Oxygen consumption increase was also related to increasing larval activity (Alemanni et al. 2003), although this hypothesis could not be tested properly in our study due to the high larval mortality observed under UVB. Warming produces a mismatch between oxygen consumption and demand (Frederich & Pörtner 2000), due to a limited capacity of ventilation at extreme temperatures (Frederich & Pörtner 2000). This mismatch appears above 19°C, as described by Storch et al. (2009), helping to explain the high mortality rate that we observed in our study. This suggests that UVB radiation increased zoea I oxygen demand, reducing their capacity to support thermal stress.

Increased UVB radiation and warming can affect physiological processes in different ways, so that organisms exposed to their joint effect may experience impacts across a broader suite of physiological traits than when these stresses act in isolation, amplifying the consequences of environmental stress (Folt et al. 1999). We found that both stressors have significant additive effects on larval mortality, and exposure to UVB increased *T. dentatus* mortality rate by 1.5 times (as the ratio of the linear regression's intercepts in presence and absence of UVB). Additivity of the joint effects of temperature and UVB radiation on larval mortality rate benefits larval survival, in contrast to a synergistic effect, as additive implies a sum of each individual effect and synergism implies that the combined effect is higher than the sum of individual effects (Folt et al. 1999). Other studies addressing temperature effects with concurrent stressors (e.g. acidification, light, UVB) found synergistic effects (Hernández Moresino & Helbling 2010, Kroeker et al. 2013, Ban et al. 2014, Przeslawski et al. 2015). The additivity found in this study might be related to some degree of adaptation of zoea I larvae to local changes in environmental conditions. Heritable variation in organisms' responses to environmental changes has already been reported in other marine organisms (Foo et al. 2012). Moreover, Storch et al. (2009) were able to identify differences in the thermal tolerance between *T. dentatus* zoea I larvae sampled from South and Central Chile coastal locations, with larvae from the southern site being less tolerant to temperature increase. Selection for most resistant organisms to UVB could have occurred in the area in response to a 7 to 14% decrease in stratospheric ozone over southern Chile between 1970 and 2012 (Agustí et al. 2015). The significant tolerance to UVB radiation in early stages and organisms from the Southern Hemisphere (Agustí et al. 2015) has been attributed to prolonged exposure to increased UVB, forcing adaptive responses and the selection of the more resistant genotypes.

The impact of global change on natural systems results from a range of concurrent stressors (Duarte 2014). The cumulative effects of multiple stressors on marine organisms, biogeochemistry and ecosystems remain largely unknown (Crain et al. 2008, Achterberg 2014, Duarte 2014). There are still few studies assessing the magnitude and mode of interacting effects (additive vs. interactive), but different works have already demonstrated that inferences made from single-factor experiments are not valid for prediction of joint effects (Hoffman et al. 2003, Gianguzza et al. 2014). For instance, Przeslawski et al. (2005) showed for mollusk larvae that the interactions could be complex, as increasing the degree of stress due to concurrent stressors, like UV radiation that increased its effect on larval mortality (up to 12 times) when it was combined with other stressors.

Our analysis identified additive effects of UVB radiation and temperature on a species that inhabits a region exposed to high UVB radiation (Herman 2010, Agustí et al. 2015) and to wide diurnal and seasonal temperature variations (Kaplan et al. 2003). Our results predict that zoea I larvae mortality is expected to increase by 1.5 times when exposed to ambient levels of UVB radiation in surface waters and temperatures that deviate from the optimum. This effect is higher than the average effect of UVB on mortality of marine organisms (Llabrés et al. 2013). Additive effects allow a more reliable prediction of responses to multiple stresses than is possible when interactions between temperature and UVB radiation occur, and also allow cumulative impacts to be resolved (Duarte 2014). Synergistic interactions, in contrast, are considered extremely complex and

challenging to predict (Przeslawski et al. 2015). So, even if this species is living in an area with a slight drop in temperature (Falvey & Garreaud 2009), we can predict a joint effect of UVB radiation and temperature when the high temperature peak during the day coincides with high UVB conditions. Whether the results presented here are specific to the model species tested or whether warming and UVB radiation act, when concurrent, in an additive manner across taxa needs to be resolved by extending the approach presented here to other species.

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