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# Sublethal effects of ultraviolet radiation on crab larvae of *Cyrtograpsus altimanus*

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ABSTRACT

Ultraviolet radiation (UVR, 280–400 nm) is known to be lethal to several aquatic species; however, more subtle, 'sublethal' effects of UVR have recently received more attention. Larvae of the crab *Cyrtograpsus altimanus* are a transient component of the plankton community in the Atlantic northern Patagonia (Argentina) and thus they may be exposed to solar UVR in both open and coastal waters. The aim of this study was to determine if previous sublethal UVR exposure on larvae of *C. altimanus* affects development, body size and motility. Larvae which were pre-exposed to UVR had a delay/absence of molting from Zoea I to Zoea II, coupled to arrested body growth, but showed enhanced swimming behavior. In contrast, the control group (i.e., exposed only to visible light) molted from Zoea I to Zoea II after 6–9 days, with a significant increase in body size, and did not change their motility. Since hatching of this species occurs in summer (i.e., season with highest UVR levels) our results suggest that, by significantly affecting development, growth and motility, natural UVR may influence the plankton–benthos coupling in coastal waters.



## 1. Introduction

It is widely known that solar ultraviolet radiation (UVR 280–400 nm) can produce several harmful effects on aquatic organisms and ecosystems. Previous studies addressed the effects of increased levels of ultraviolet-B radiation (UVB, 280–315 nm), highlighting the susceptibility of marine ecosystems (de Mora et al., 2000; Helbling and Zagarese, 2003). UVR may directly damage cellular targets such as DNA, proteins and/or membranes (Sinha and Häder, 2002). Ultraviolet-A radiation (UVA, 315–400 nm) may also produce reactive oxygen species (ROS) with its concomitant negative effects (Lesser et al., 2001; Vega and Pizarro, 2000). In general terms, UVR may produce a direct and immediate decrease in survival (i.e., 'lethal exposure') or indirect, more subtle effects that do not include immediate mortality (i.e., 'sublethal exposure').

Early life stages of marine organisms, particularly eggs and larvae, are usually regarded as being more vulnerable to solar UVR radiation than older stages (Häder et al., 2011). Many planktonic organisms have mechanisms to avoid or to minimize UVR-induced damage (e.g. behavioral avoidance, bioaccumulation of UVR-absorbing compounds, efficient DNA repair systems, etc.) but they may still be affected by solar radiation even when there is no immediate or evident effects on survival. The ecological implications of this 'sublethal exposure' on natural populations have not been extensively studied. For example, UVR may affect early stages of an organism (i.e., eggs, larvae) but its effects might be observed during subsequent developmental stages (i.e., juveniles, adults) even when UVR ceased to be a stress factor. In fact, indirect effects were observed in larvae of *Rana temporaria* several weeks after metamorphosis; in this case, the duration of the larval period and developmental abnormalities increased, while the body weight decreased (Pahkala et al., 2001). Ulterior effects of early exposure to sublethal UVR have also been determined for gastropods, bivalves, echinoderms, polychaetes, crustaceans, bryozoans, urochordates, and vertebrates (Pechenik, 2006). Other effects of sublethal UVR exposure on zooplankton include those on feeding and respiratory rates (Fischer et al., 2006; Freitag et al., 1998; Ylönen et al., 2004), delayed metamorphosis or settlement (Kuffner, 2001; Pahkala et al., 2001), malformations (Adams and Shick, 2001; Lermanda et al., 2009), body lesions and reduced growth rates (Browman et al., 2000), vertical distribution (Shick et al., 1996), swimming motility (Alemanni et al., 2003; Gonçalves et al., 2007), protein synthesis (Tartarotti and Torres, 2009), among others.

In coastal zones of the Atlantic Patagonia (Argentina), the varunid crab *Cyrtograpsus altimanus* (Brachyura: Grapsoidea) (Rathbun, 1914), is a typical, highly abundant species in rocky intertidal pools and on shallow sandy bottoms (Scelzo and Lichtschein de Bastida, 1979). Its larvae, together with those of *Cyrtograpsus angulatus*, contribute with a significant share (35%) of the total amount of larvae in local coastal waters (Dellatorre, 2009). Settlement of adults occurs after the metamorphosis of the planktonic larvae, which may be exposed to solar UVR in their natural environment. However, a previous study carried out with *C. altimanus* showed that the first larval stage (Zoea I) is highly tolerant (in terms of survival) to short-term exposure under artificial UVB radiation, as compared to other crab species (Hernández Moresino and Helbling, 2010). However, and

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to the best of our knowledge, there is no information on sublethal effects after exposure to UVR in *C. altimanus*. Thus, the aim of the present study was to evaluate changes in morphology, development and swimming behavior of larvae of *C. altimanus* as may occur after an initial exposure to sublethal UVR levels. To this aim, we exposed Zoea I larvae to a sublethal UVR dose under artificial conditions and followed possible changes in their development and swimming behavior for ~2 weeks after that initial exposure.

## 2. Materials and methods

This study was carried out during austral summer (March) of 2010 and 2011 with zoea lavae of *C. altimanus*. The source of these specimens were ovigerous females collected during low tide from intertidal rocky pools at Puerto Madryn (42°46′ S, 65°02′ W), Chubut, Argentina.

The collected females were kept in an aquarium that was placed in a culture chamber (MiniCella) at 19–20 °C with bubbling and a 12:12 h photoperiod, for ca. 48 h; after this period larvae started to hatch. Three experiments were done, each one using newly hatched larvae that were exposed under a solar simulator (Hönle, Sol 1200) at 109 cm from the lamp. The irradiances output from the lamp were 84.5, 30, and 0.76 W $m^{-2}$ for PAR, UVA and UVB, respectively. These irradiance conditions, together with the exposure time (120 min) – that resulted in a UVB dose of 5.5 kJ $m^{-2}$ – were chosen based on preliminary tests and previous studies conducted with this species (Hernández Moresino and Helbling, 2010). This latter study determined that neither PAR, nor UVA had significant effects on larvae mortality, whereas mortality occurred just after the incubation period with UVB doses of >22.5 kJ $m^{-2}$ (i.e., after 495 min under UVB irradiance of 0.76 W $m^{-2}$). Considering this for the present study we chose the same irradiance level (i.e., 0.76 W $m^{-2}$), but a much shorter time of ca 25% (i.e., 120 min), giving a UVB dose of 5.5 kJ $m^{-2}$; this sublethal dose was used to carry out our experiments. It should be noted that the summer daily doses of UVB reaching the sea surface over the Patagonian region often exceed 30 kJ $m^{-2}$ (up to 45 kJ $m^{-2}$) while noon UVB irradiance can be as high as 1.8 W $m^{-2}$ (Villafañe et al., 2004).

Pools of 300 larvae were used in each experiment, with larvae of less than 16 h from hatching. They were sorted, using a wide-mouth plastic pipette, into two aquaria (17 × 17 × 4 cm; length × width × depth) containing 300 ml of sterilized seawater and exposed under two radiation treatments: 1) One aquarium – UVR treatment – was covered with a filter film (Ultraphan 290) to eliminate any UVC output from the lamp, so that the pool of larvae received full radiation (PAR + UVA + UVB); 2) One aquarium – PAR treatment or control – was covered with a film filter (Ultraphan UV Opak Digefra) so that larvae received only PAR. The transmission characteristics of these filters are published elsewhere (Villafañe et al., 2003).

After the exposure period, both aquaria were removed from the solar simulator and kept in the same culture chamber at 19–20 °C. The aquaria were gentle bubbled and larvae were fed with a mix of diatoms — *Thalassiosira weissflogii* and *Phaeodactylum tricornutum* (final concentration of 6–8 × $10^4$ cell $ml^{-1}$). The culture chamber had a photoperiod of 12:12 h and an irradiance level of 250 μmol photons $m^{-2}$ $s^{-1}$ of PAR. The water and food in both aquaria were renewed completely every 2 days (but 3 days between days 6 and 9), and dead larvae were counted, removed, and fixed with 4% formaldehyde for size measurements. The low proportion of dead individuals (<5%) that was observed in both treatments during the three experiments was most likely the result of a combination of factors such as culturing conditions, container effects, handling, etc. rather than exposure to UVR. In addition, every two days (before renewing the water and food) 20 larvae from each treatment were collected and video recorded for motility measurements as explained below. Data from the three experiments were used to determine changes in body size and larval stages, while data from only two of these were used for motility measurements. We decided to leave one experiment without manipulation (as may occur during video recording and motility measurements) to establish if it might have caused any stress that could have affected the development of larvae. However, we did not find any differences between this experiment and the other two; therefore data from the three were used to determine changes in body size and development.

### 2.1. Size measurements

Fixed larvae were examined under a stereoscope for malformations as well as to determine body size using the Micro Image Analysis Software (MIAS 2003, ver. 1.3B). Between 5 and 10 individuals per radiation treatment were measured every 2 days in each experiment. Total length, length from the rostral to the dorsal spin, length of the carapace, and carapace surface were measured in each individual. From all these variables, the length of the carapace was chosen as an estimation of body size, as it was the one that displayed less variability.

### 2.2. Larval stage determination

Larval stages were determined in fixed samples by counting the number of plumosae setae present in the exopodite of the second pair of maxillipeds; the first two larval stages, Zoea I and II, have four and six setae, respectively. Another distinguishable feature was the length of the facial spin or the size of the carapace, that were shorter and smaller in Zoea I as compared to Zoea II (Scelzo and Lichtschein de Bastida, 1979).

### 2.3. Motility measurements

Every 2 days, 20 larvae were randomly chosen (therefore representing the pool of individuals under each radiation treatment) and placed in a rectangular glass vessel (6 × 5 × 1 cm, vertical × horizontal × optical depth) with 25 ml of sterilized seawater, so that the filmed surface had an area of 25 $cm^2$ (vessel filled with a 5 cm water column). Before starting the video recordings, larvae were acclimated for ca. 1 min in the dark, which was enough time to dissipate the weak turbulence generated by introducing the individual into the vessel. After less than 1 min, the normal swimming behavior of larvae was observed. Then they were video-recorded during 1 min in darkness, using infrared light (IR) and an IR-sensitive video camera (Sony DCR SR85) at 30 fps. In this way (i.e., filming in darkness) the normal swimming behavior was not altered (e.g., due to differential phototactic behavior produced by non-regular angular distribution of light inside the vessel, etc.). It should be noted that when the individuals moved near the water surface (i.e., <0.5 cm), optical artifacts precluded larvae detection and measurements of swimming speed. Therefore, only clearly visible, defined trajectories were analyzed in this study. More than one of the detected trajectories could belong to any given individual, so the overall motility data for each video of the 20 individuals was considered as representative of their treatment (UVR or PAR) at that stage of the development. After video recording, the larvae were returned to their original container until the next measurement; all the procedure took less than 5 min. Fig. 1 shows some examples of upward swimming (hereafter "tracks") of two larvae exposed to the different radiation treatments. Non-exposed larvae (at day 0) remained near the water surface (i.e., in the top 0.5 cm of the water column) thus their movements were not analyzed; larval motility at days 2, 4, 6, 9 and 11 after exposure was measured for both radiation treatments.

Video recordings were transformed into individual images (one image per frame) and were pre-processed and binarized (i.e., leaving only the moving larvae as white objects in a dark background) using the software ImageJ (Abramoff et al., 2004). These binary images were

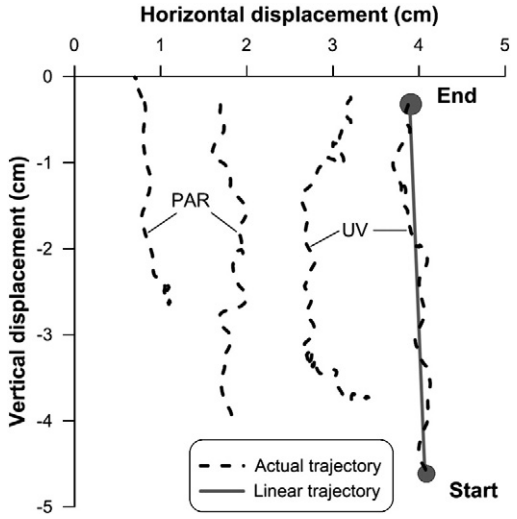


**Fig. 1.** Examples of upward swimming trajectories ('tracks') of larvae, indicating the actual (broken lines) and linear (solid line) trajectories. The −5 value of the Y axis indicates the bottom of the video-recording vessel (i.e., 0 cm is the water surface).

then analyzed to obtain the X–Y position of each detected larva within each frame, using the software CellProfiler (Lamprecht et al., 2007).

In general, these larvae have two types of displacements: (1) a vertical passive sinking, and (2) an almost vertical active upward swimming. We focused in the upward movements (i.e., tracks) as these imply a metabolic cost. From the analysis of these tracks, we obtained swimming velocity, angle displacement with respect to a vertical, linearity, X–Y position and traveled distance for each larva. Linearity of a trajectory between points A and B was calculated as the ratio of the linear distance between these two points and the actual distance traveled by the larvae (i.e., a straight trajectory has a linearity of 1).

To avoid confounding effects from comparing swimming speed of individuals with different sizes (i.e., growth during larval development), the velocity was 'normalized' with body size, thus the velocity is expressed as *body size* $s^{-1}$. As no significant body size differences were found between preserved versus live larvae (one-way ANOVA, $F_{(1,18)} = 0.45$, $P = 0.51$) we used the size measurements from the fixed samples to normalize the motility variables.

### 2.4. Statistical analysis

Due to the effective space under the solar simulator, we were unable to fit replicates for each sublethal exposure. However, replicates were obtained by conducting the same exposure experiment under equal conditions three times, each one with a fresh group of newly-hatched larvae.

There was a small but non-negligible chance of selecting the same larvae more than once for the motility measurements. In addition, all the variables (i.e., body size, modal velocity, vertical position, actual displacement and linearity) were repeated measurements throughout the time, and no homoscedasticity was observed among days. Therefore, a non-parametric (Friedman) test was used considering multiple dependent-samples comparisons. When the results of the Friedman tests were significant, pair dependent-samples comparisons were done using the non-parametric Wilcoxon test.

Linear regressions were used to fit the modal velocity, number of tracks, actual displacement, linearity versus the time (sampling days), and the significance of the slopes were determined using an ANOVA test. One-way ANOVA was used to test for differences between radiation treatments, on specific days (Zar, 1999). We used the R Software Package version 2.11 to test for significance (which was fixed at $P<0.05$).

## 3. Results

### 3.1. Molting and growth

Fig. 2 shows the differential development and body size in larvae pre-exposed to PAR-only or PAR + UVR. There were significant changes in the developmental stages (Fig. 2a) and in body size (Fig. 2b) between the two radiation treatments. The larvae that received UVR remained as Zoea I during the whole experimental period, not reaching the Zoea II stage (Fig. 2a). On the other hand, larvae that received only PAR started to molt from Zoea I to Zoea II between days 6 and 9, reaching ca 50% of the second larval stage on day 11, and 100% of Zoea II at the end of the experiment (Fig. 2a). No malformations were observed in any of the radiation treatments at any time of development. The body size of larvae (Fig. 2b) in the UVR treatment ranged from 0.041 to 0.045 cm and did not show a significant increase during the experiment (Friedman test, $X^2_{(N=2,\ df=6)} = 11.58$, $P = 0.07$). On the other hand, the body size of larvae in the PAR treatment had a significant increase (Friedman test, $X^2_{(N=2,\ df=6)} = 20.15$, $P<0.01$) from 0.041 (SD = 0.004) to 0.054 (SD = 0.003) cm after 13 days. The differential increase in body size among larvae from the two radiation treatments resulted in significantly larger larvae in the PAR treatment as compared to those in the UVR treatment, on day 11 (ANOVA, $F_{(1,\ 4)} = 8.49$, $P<0.01$) and on day13 (ANOVA, $F_{(1,\ 4)} = 14.82$, $P<0.01$).

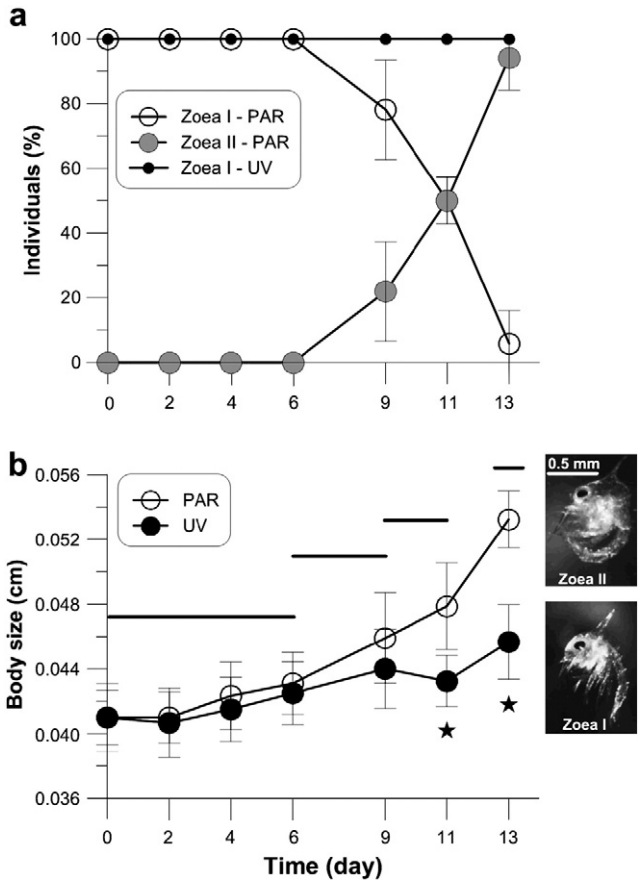


**Fig. 2.** Molt and growth of larvae after exposure to UVR (black circles) and to PAR (white and gray circles). a) Proportion (%) of Zoea I and Zoea II. b) Body size of larvae (in cm) throughout the experimental period (13 days). Symbols represent the mean of three experiments except for day 6 in which one sample was lost (n = 2). The vertical lines indicate the standard deviations, while the asterisks indicate significant differences among radiation treatments ($P<0.05$). The horizontal lines in panel b connect the PAR treatment samples that were not significantly different (Wilcoxon test).

### 3.2. Motility

A general pattern of motility was observed in every video recording: a variable amount of larvae stayed very near the water surface, while the rest performed the sink-and-swim-up motion described above. None of the individuals in any video session was observed to stay quiet at the bottom or at a particular depth (i.e., any individual which was not at the surface performed the "sink and swim up" pattern). Therefore for every track that was accounted, a previous sink was always observed. A LogNormal function was the best fit for the larvae swimming speed frequencies distribution (Fig. 3); the frequency distributions shifted towards slowest velocities in both treatments as the experiments progressed (Fig. 3a). Differences were observed between radiation treatments only at days 2 and 6 when comparing the modal velocity (i.e., the most frequent value) (Fig. 3b) (ANOVA, $F_{(1,\ 2)}=39.49$, $P=0.02$ and $F_{(1,\ 2)}=41.97$, $P=0.02$, respectively) with larvae pre-exposed to UVR having higher modal velocity than those that received only PAR (Fig. 3b). As depicted by the modal velocity throughout the experiments, there was a significant slowing down of the swimming speed at the two last days (9 and 11, as compared to days 2, 4 and 6) (Friedman test, $X^2_{(N=4,\ df=4)}=12.00$, $P=0.02$; Wilcoxon signed-ranks test, $T=2.52$, $N=8$, $P=0.01$).

As 20 larvae were filmed in each video for motility measurements, observing less than 20 tracks indicated that some larvae migrated down while others stayed at the surface and thus these latter were not accounted for motility measurements. On the other hand, when more than 20 tracks were observed this would reflect that a) at least one individual did more than one downward/upward excursion and b) higher overall motility on that treatment. The number of observed tracks did not show significant changes for the PAR treatment and remained between 10 and 15 (Fig. 4a), indicating that these were the maximum number of larvae moving down (and then up) while the rest of them stayed at the surface. In the UVR treatment, however, the number of tracks increased significantly as the experiment progressed (i.e., 10 tracks at day 2 to 26 tracks at day 9), with a significant linear increase of 1.41 per day ($r^2=0.64$, $F_{(1,\ 8)}=8.65$, $P=0.02$). The locomotion pattern was in general highly linear (Fig. 4b), and there were no differences among radiation treatments or sampling days (mean overall value of 0.71, SD = 0.09, N = 20).

In order to relate the changes in the number of tracks recorded (Fig. 4) with the actual vertical position of larvae, the time-integrated vertical distribution of larvae during the upward swimming path was obtained by considering all the occurrences of larvae at different depths (i.e., swimming up) during the one-min video, using 0.1 cm depth intervals (Fig. 5). The average larval occurrence at any specific depth was then plotted against their vertical position (i.e., depth) for each sampling day. There were no significant differences in the amount of larvae as a function of their vertical position in the PAR treatment, indicating that not only the number of tracks did not change (Fig. 4), but also that the number of "sink events" did not change during the experiment. However, larvae that received UVR had a higher number of occurrences at all depths in the water column

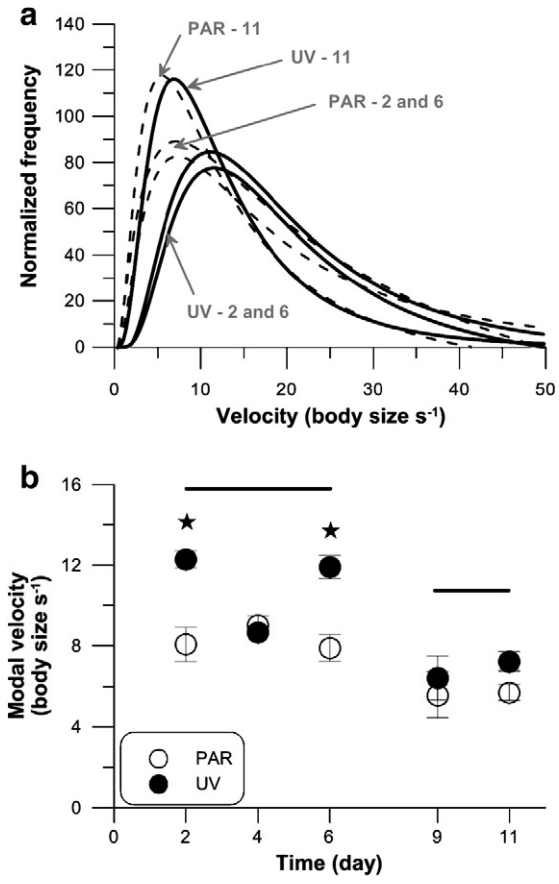


**Fig. 3.** Swimming speed during the experiments. a) Mean frequency distributions of two experiments for both radiation treatments as adjusted by LogNormal curves. b) Modal velocity (expressed as body size $s^{-1}$) determined from the LogNormal frequency distributions. The vertical lines indicate the half mean range, while the asterisks indicate significant differences among radiation treatments ($P<0.05$). The horizontal lines indicate samples that were not significantly different pooling the two radiation treatments (Wilcoxon test).

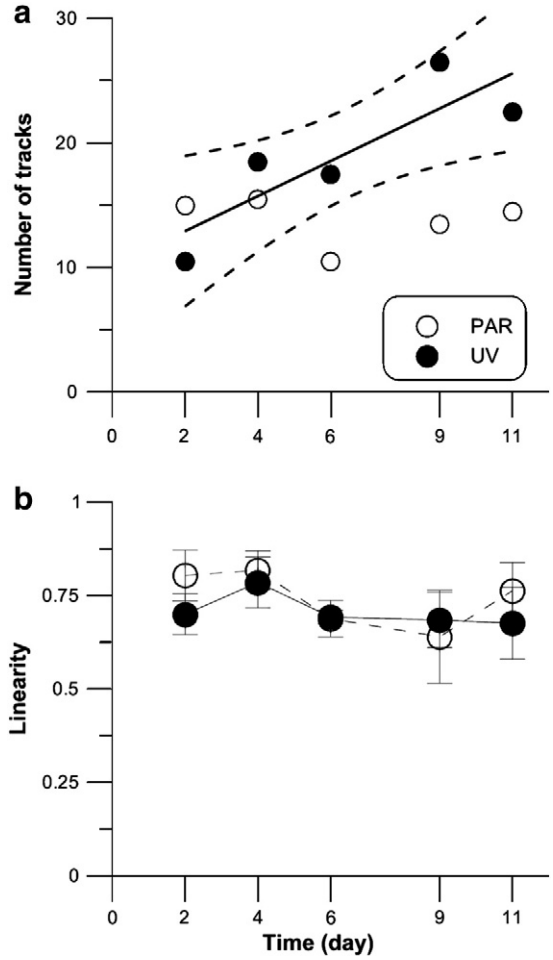


**Fig. 4.** Motility as a function of time, as determined by tracks number and linearity. a) Average number of tracks. Broken lines indicate the 95% confidence limits for the linear fit (solid line); b) Linearity of the tracks. Vertical error bars indicate the half mean range.

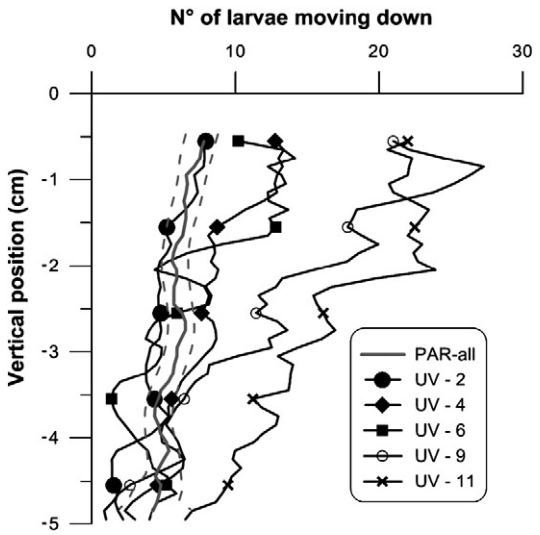


**Fig. 5.** Larval position in the water column as a function of time for the two experiments. For the UVR treatment, each black solid line indicates the number of larvae encountered at the corresponding depth for each day. The gray solid and broken lines indicate the mean distribution and 95% confidence limit, respectively, for larvae in the PAR treatment for all days. For simplicity, symbols are shown every 1 cm.

(below the top 0.5 cm), showing an increased overall activity in this treatment. As the total amount of individuals was the same in every video (i.e., 20 larvae), we can also infer from Fig. 5 that the number of individuals at the surface (i.e., excluded from our analysis –see above) decreased in the UVR treatment during the experiment: initially (days 2–6) there were between 5 and 10 occurrences at all depths, which means that more larvae stayed at the surface as compared to the last part of the experiment (days 9 and 11) when more occurrences were observed below 0.5 cm (i.e., less larvae stayed at the surface).

The overall higher activity in the UVR treatment was also confirmed by calculating the integrated vertical displacement of larvae, which resulted in a linear increase as the experiments progressed (Fig. 6). In this way we quantified the increased deeper distribution of the larvae that received UVR with a linear increase of 6.92 cm per day for the whole pool of larvae moving below the 0.5 cm depth ($r^2 = 0.65$, $F_{(1, 8)} = 14.86$, $P < 0.01$).

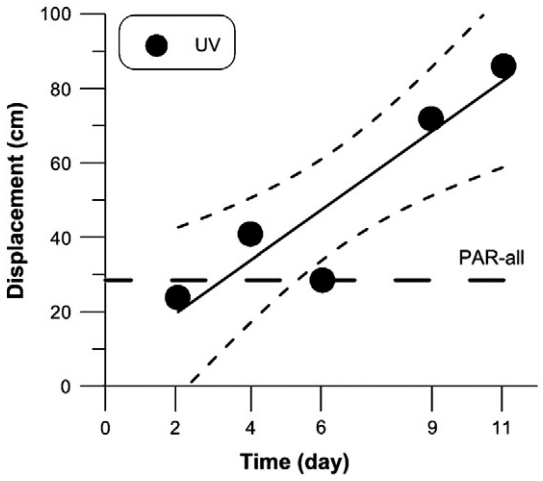


**Fig. 6.** Actual overall displacement of larvae in the UVR treatment, calculated from the occurrences versus vertical position, as a function of time. Thin, solid and broken lines indicate the linear fit and the 95% confidence limits for the UVR treatment. The thick broken line indicates the constant larvae displacement under the PAR treatment, for comparison.

## 4. Discussion

Most studies dealing with UVR upon invertebrate larvae have focused on lethal-exposure effects such as survival rate (Browman et al., 2000; Wübben and Vareschi, 2001; Hernández Moresino and Helbling, 2010). Sublethal effects, however, may be also very important, especially when early stages are the targets for UVR. Our results show that molting, body size and motility of *C. altimanus* larvae are affected by sublethal pre-exposure to UVR. In the following paragraphs we will discuss how these UVR effects might affect the behavior and reproductive success of *C. altimanus* from Patagonian waters.

It has been observed that molting of decapods larvae is usually delayed under unfavorable conditions such as changes in temperature, salinity, nutrition, and water chemistry (Anger 2003 and references therein). In our study, we further determined that a sublethal dose of UVR may also act as an additional stress factor, resulting in a delay/absence of molting, and thus extending the duration of a particular developmental stage. Since *C. altimanus* settles on the bottom when they reach the juvenile stage, this extended larval period will lead to a longer planktonic stage. This in turn, most likely would decrease its survival due to starvation, exposure to other physical stress, predators (among others) as seen in other studies (Morgan, 1995). In addition, a longer planktonic stage would expose larvae to more UVR, with the potential of higher mortality as observed in previous studies carried out with this crab species (Hernández Moresino and Helbling, 2010). A delayed metamorphosis, preceding the benthic juvenile form, may also affect post-metamorphic performance (e.g., reduction of growth, survival and development rates) as observed in juvenile decapods that had an extended time of larvae stage (Gebauer et al., 2003), this being a rather general pattern for many organisms. For example, energy reserves, metamorphosis and growth rates might be affected, as observed in fish larvae from the Lake Pyhäselkä (Pechenik, 2006; Ylönen et al., 2004), in the barnacle *Balanus amphitrite* (Thiyagarajan et al., 2007) and in several marine invertebrates — see review by Pechenik (2006). Under this scenario, the chances of a given larva to become a reproductive member of the population decreases, with the concomitant cost for the population.

Molting is also very important, as body size depends on it. For example, the body length of brachyuran crab larvae increases linearly with the consecutive larval stages (Anger, 2001). In many zooplankton organisms, size has a key importance for feeding, searching ability and predation risk, as observed for fish larvae (Miller et al., 1988). In our study, the absence of molting in the UVR-pre-exposed larvae had a direct impact on their body size as they did not grow during the experiment. On the other hand, PAR only-exposed larvae reached a size that is within the "normal" growth range (Scelzo and Lichtschein de Bastida, 1979).

It could be argued that any sublethal UVR-induced impact would decrease the swimming activity, as seen for frog larvae (Hatch and Blaustein, 2000), although it does not seem to be a general feature — e.g., swimming behavior of juvenile trout can be enhanced under UVR exposure (Alemanni et al., 2003). In terms of swimming speed, our results support the hypothesis of a velocity reduction due to UVR (Fig. 3a and b). However, in terms of overall activity the contrary can also be argued, as UVR larvae were increasingly active during their development as compared to those in the PAR treatment (Figs. 4–6). This was observed as more tracks (Fig. 4a), but as linearity was unchanged (Fig. 4b), it was ultimately translated into more effective cumulative displacement (Fig. 6). This is especially important in terms of energy budget since the displacement was obtained by measuring the active vertical movement during the upward swimming of individuals. Therefore larvae exerted the necessary upward force during these displacements (Fig. 6), and thus the active upward swimming involved a metabolic cost, unlike the passive sinking mode. As the individual

has to outperform gravity plus drag by exerting an upward momentum, Fig. 6 also suggest that more energy was needed as the displacement increased over time in the UVR treatment. Regarding the vertical distribution of these displacements, they in turn resulted in a higher number of occurrences at any depth as compared to the PAR treatment (Fig. 5). This increased activity and its concomitant energetic cost might leave less energy for molting/growth, which ultimately would result in a delay/absence of molting. The increased activity moving down in the water column might be due to (1) a natural mechanism of the larvae to "escape" from the water surface where UVR is high, and/or (2) a tendency to spend more time 'sinking' to increase the probability of encounter food particles — i.e., these crabs feed while they are sinking (Anger, 2001). This pattern was not observed in the PAR-only exposed larvae that had the same distribution in the water column at all times but, most important, more than 50% of the PAR larvae always remained near the surface. This would indicate that food might not be a limitation, but rather, the downward excursions might be more related to the need of changing their position in the water column to avoid UVR or, alternatively, it was a particular behavior related to their lack of molting.

Our laboratory results suggest that larvae of *C. altimanus* may avoid surface layers when they have been previously exposed to UVR (Fig. 5). In an ecological context, the changes in the vertical position of larvae (i.e., away from a potential UVR-exposure at the surface) may strongly affect their dispersal behavior. It has been shown that the larvae of *C. altimanus* are dispersed near the surface after hatching from the shallow coastal area towards offshore waters (Dellatorre, 2009). Thus, larvae that stay less time near the surface would have fewer chances for dispersal, and they may not be able to reach favorable areas for their next stage of development.

In their natural environment, it is expected that larvae will have one or several mechanisms to avoid or minimize UVR damage. Some of them are the diel vertical migration (Alonso et al., 2004; Queiroga and Blanton, 2005), DNA repair mechanisms (Gonçalves et al., 2002; Mitchell et al., 2009), and acquisition of protective compounds through diet (e.g., carotenoids, mycosporine-like amino acids) (Banaszak, 2003; Helbling et al., 2002; Newman et al., 2000). We have previously shown that *C. altimanus* larvae have UV-absorbing compounds and carotenoids ((Hernández Moresino and Helbling, 2010)). However, our set up of experiments did not allow for the induction of these compounds by feeding the adults or the larvae with food rich in UV-absorbing compounds. So our results were obtained with the "normal" concentration of this compounds that the larvae had in nature, and of course this would vary from one location to another, depending on the food availability.

It should be noted however, that the overall performance of *C. altimanus* larvae will be the result of the interaction of several factors that can act synergistically or antagonically (Dunne 2010). In our case, we tested one of the potential stressors (i.e., UVR) and our results indicate that sublethal UVR doses might have more consequences for the population and the ecosystem than previously thought. It remains to be explored if this negative effect – as observed in molting, body size and motility – can be counteracted by changes in other variables as seen in previous studies that observed, for example, that higher temperature can reduce UVR-induced damage in other zooplankton species (MacFadyen et al., 2004; Sanders et al., 2005; Cooke et al., 2006).

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