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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