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Metabolic and behavioral responses of the reef fish *Patagonotothen cornucola* to ultraviolet radiation: Influence of the diet

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ABSTRACT

Most of the studies devoted to assess the effects of ultraviolet radiation (UVR) on fishes have been done with early life stages (i.e., eggs and larvae) as they are the most vulnerable to these wavelengths; however, the effects of UVR on juveniles or adults are less clear. This study evaluated the effects of UVR and diet (i.e., rich and poor in ultraviolet-absorbing compounds; UVAC) on the metabolic rates (respiratory frequency) and behavior (prey capture time) of juveniles of the reef fish *Patagonotothen cornucola*, a common species inhabiting the intertidal areas of the Patagonian coast. UVR was a significant stress factor for *P. cornucola* by increasing its respiratory frequency, which could be related to the costs of repairing any cellular components damaged during the exposure. Fish exposed to UVR took more time to detect and capture their prey, which could lead to a reduction in the prey capture rates, with the concomitant effects on growth. In addition, juveniles feeding on rich-UVAC diet had a significant lower respiratory frequency than those feeding on poor-UVAC diet. This differential response was not evident in the prey capture time. In their natural environment, *P. cornucola* could feed on a varied diet and could also partially avoid the exposure to UVR by hiding under the rocks or macroalgae. Future studies should consider the broad variety of diets that could be found in rocky intertidal areas, and how their qualities (in terms of UVAC content) could help to counteract the UVR effects on juvenile fish.

1. Introduction

Solar ultraviolet radiation (UVR; 280–400 nm) has deleterious effects on aquatic organisms occupying all trophic levels (Cullen et al., 1992; Williamson, 1995; Häder et al., 2011). Some of the direct UVR effects on organisms are DNA, cellular and tissue damage, and even death (Dattilo et al., 2005; Fischer et al., 2006; Ban et al., 2007). The indirect effects include the use of sub-optimal habitats where UVR does not affect the organisms but also other abiotic factors that are critical for them, such as low food availability, and/or high predation risk (Häder et al., 2011). Since the tolerance to UVR is species-specific, some species are more likely to better cope with UVR damaging wavelengths than others (Siebeck and Bohm, 1994; Leech and Williamson, 2000). Despite these species-specific differences, there is consensus that the early life stages are the most susceptible to UVR damage (see review by Zagarese and Williamson, 2001; Bonaventura et al., 2006).

During evolution, aquatic organisms have developed different protective strategies to cope with UVR. These strategies include: (1) avoidance, such as moving away from the radiation source (i.e., vertical migration) or hiding under shaded places (Leech and Williamson, 2001; Holtby and Bothwell, 2008), (2) photoprotection, including chemical defenses such as the presence of UV-absorbing compounds (UVAC) to prevent damage (Helbling et al., 2002; Zamzow, 2007), and (3) repair of damage, either photorepair or dark enzymatic repair, after any damage has been done (Zagarese and Williamson, 2000). UVAC are the most common UV-sunscreens in marine and freshwater organisms (Shick and Dunlap, 2002; Sommaruga et al., 2006). As UVAC can be only synthesized by photosynthetic organisms, animals obtain these compounds through the diet (Carroll and Shick, 1996; Carefoot et al., 1998; Riemer et al., 2007). Most of the studies about the role of UVAC in aquatic organisms have focused on their role in reducing mortality rates when organisms were exposed to UVR (Helbling et al., 2002; Moeller et al., 2005); however, much less is known about the role of these compounds to counteract the effects of UVR on behavior and feeding.

Intertidal rocky shores are important ecotones between terrestrial and aquatic systems, playing a key role as feeding and nursery areas for several fishes (Faria and Almada, 2006; Aburto-Oropeza et al., 2007) that use them during their first life stages, before migrating to open waters. As these environments are exposed to tidal regimes,

Abbreviations: UVR, ultraviolet radiation; UVAC, ultraviolet-absorbing compounds; RF, respiratory frequency; RRF, relative respiratory frequency; PCT, prey capture time.

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several fishes use these areas only during high tide, but some others remain during low tide in tidal pools. In this latter case, the exposure to UVR, mainly during high irradiance periods (noon time at spring– summer) could be critical for the organisms.

Nototheniidae is an important family of reef fishes, playing a key role as predators, as they occupy most of the trophic niches available in the environment, feeding both on benthos and on plankton (Eastman, 1991; Eastman and McCune, 2000). Within this family, the genus *Patagonotothen* is one of the most important living in Patagonian coasts of Argentina (Galván et al., 2009). Most of the studies about this genus are descriptive (e.g., distribution and feeding habitats, Collins et al., 2007; reproduction, Brickle et al., 2006; Rae and Calvo, 1995), and much less information is available about their trophic behavior and metabolism (but see Agnisola et al., 1997; Fernández et al., 2002). Even more, the effect of variables associated to global change such as UVR on *Patagonotothen* spp. is, so far, virtually unknown. To fill this gap, the aim of this study was to evaluate the combined effects of UVR and diet on the metabolic rates (respiratory frequency) and behavior (prey capture time) in juveniles of the reef fish *Patagonotothen cornucola* (Richardson, 1844) from Patagonian coasts.

2. Methods

2.1. Study area, collection and maintenance of specimens

Juvenile specimens of *P. cornucola* were collected during the Austral Summer of 2013 from Playa Bonita (43° 21′ 40″ S, 65° 02′ 55″ W; Argentina) a rocky beach located 3 km away from the mouth of the Chubut River. Fish were caught from tide pools using a small hand net (1 cm mesh), stored in buckets and immediately transported to the laboratory at Estación de Fotobiología Playa Unión (EFPU, 20 min away from the sampling site). In the laboratory, individuals were placed in aquaria filled with seawater at 20 °C, with continuous aeration. Fish were maintained in the aquaria during three days before experimentation. During this period, individuals were fed on the amphipod *Ampithoe valida* (Smith, 1873) collected also from Playa Bonita.

2.2. Experimental procedure

To assess the combined effects of UVR and diet on fish respiratory frequency and prey capture time, individuals of *P. cornucola* were placed in 20 l plastic aquaria (1 fish per aquarium, $n = 12$), fed on 2 different diets and exposed to 2 artificial radiation conditions in an experimental set-up consisting of a 2×2 matrix, with radiation and diet as factors. The experimental conditions were as follows: a) Radiation: (1) PAB treatment, specimens receiving full radiation (PAR + UV-A + UV-B, >280 nm): 2 Phillips daylight fluorescence tubes for PAR and 2 tubes Q-Panel-UVA-340 for UVR, and (2) P treatment (control), specimens receiving only Photosynthetic Active Radiation (PAR: >400 nm): 2 Phillips daylight fluorescence tubes, and b) Diets: (1) poor in UVAC, amphipods fed on the green macroalgae *Ulva rigida* (C. Agardh, 1823), and (2) rich in UVAC, amphipods fed on the red macroalgae *Porphyra columbina* (Montagne, 1842). Three replicates per treatment were used. The duration of the experiment was of 9 days, with a 12 L:12D (light/dark) photoperiod. The irradiances received by the fish were 36.7 W m^{-2} in the P treatment, while in the PAB treatment organisms additionally received 9.87 W m^{-2} of UVR (UV-A + UV-B).

The amphipod *Ampithoe valida* was used as prey for the fish during the experiment. One week before the beginning of the experiment, individuals of *A. valida* were brought from the field, separated in 2 groups, and fed on 2 different diets: a) *U. rigida* (poor in UVAC) and, b) *P. columbina* (rich in UVAC). Previous experiments confirmed that *P. columbina* synthetized high amounts of UVAC (Helbling et al., 2004) while in *U. rigida* they were almost null. Amphipods accumulate these compounds in their bodies differentially (Valiñas and Helbling, 2015) thus it was possible to obtain amphipods rich and poor in UVAC as food source for fish. Fish were provided with food ad libitum throughout the experiment by adding amphipods in all aquaria twice a day, in the morning and in the afternoon.

2.3. Analyses and measurements

2.3.1. UV-absorbing compounds analysis

The content of UVAC in both macroalgae and in the amphipods used as food for fish was measured to verify that the food provided to the amphipods had different UVAC content, and that the amphipods used as food for fish accumulated different amounts of UVAC. The UVAC measurements in the amphipods were done at the initial time (t_0), to determine the amount of compounds that individuals brought from their natural environment, and after the 7-days feeding period. Previous to the analysis of UVAC, the amphipods were left for 24 h without any food to empty their gut content. As a general procedure (for both, amphipods and macroalgae), the tissues were weighted (wet weight), placed in 15 mL centrifuge tubes, broken with a glass rot, and extracted in absolute methanol (Holm-Hansen and Riemann, 1978). After the extraction period, a scan between 250 and 750 nm was done using a spectrophotometer (Hewlett Packard, model HP 8453E). The amount of UVAC was estimated by peak analysis in the range 310–360 nm (Helbling et al., 1996). For comparisons, the UVAC content was expressed as the peak area per wet weight of amphipod or macroalgae.

2.3.2. Respiratory frequency (RF)

The number of times that individuals opened and closed their operculum was registered during 1 min, and this was used as an indirect estimation of their degree of stress. The observations started after the individuals stayed at least 2 min motionless, so that the RF was not affected by any recent fish swimming activity. Measurements were performed at t_0 and then once a day from the third to the ninth day of the experiment. The mean RF obtained at t_0 was subtracted to the RF values obtained for the different treatments, thus the relative respiratory frequency (RRF) was used for statistical comparisons. All measurements were performed 2 h before the end of the light period. The observations of the individuals were done behind a dark curtain to avoid disturbing the fish.

2.3.3. Prey capture time (PCT)

This term refers to the time (in sec), measured with a chronometer, since the prey was thrown into the aquarium chamber until the fish caught it. To standardize the procedure, the measurements started when the fish were located in the left corner of the aquarium, throwing the prey at the opposite corner. Thus, in all measurements, the distance that fish swam to capture their prey and the visual angle from which prey appeared was the same. The PCT was measured on the last day of the experiment, 2 h before the end of the light period. Three amphipods, one at a time, were thrown to each aquarium, and the mean PCT for each replicate was calculated. A high PCT means that fish took longer to capture their prey than those with low PCT. As for RF, all the measurements were done behind a dark curtain.

3. Statistical and data analysis

To evaluate differences in the abundance of UVAC of both macroalgae species used as food for fish, a t-test was performed (Zar, 1999). Differences between the initial content of UVAC in the amphipod' bodies (UVAC that individuals accumulated in the field), and the content of these compounds after individuals were fed on *U. rigida* or *P. columbina* were analyzed using a one-way ANOVA (Zar, 1999). For RRF, and because no statistical differences were detected between days, the data from the different days were pooled and the mean RRF was compared between radiation and diet treatments using a two-way ANOVA (Zar, 1999). The same statistical analysis was used to determine differences in PCT. In all cases, Tukey's tests were performed if the