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Shelter selection by intertidal amphipods: the role of UVR and photoprotective compounds

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Abstract Shelters are crucial for intertidal organisms as a way to protect from environmental stress. The present study examines whether the shelter selection of the amphipod *Ampithoe valida* is influenced by ultraviolet radiation (UVR) and whether this selection depends on the amount of photoprotective compounds (UV-absorbing compounds, UVAC) that the amphipods acquire through the diet. Amphipods were exposed to radiation (photosynthetically active radiation and UVR) and offered a choice of UVR-transparent versus UVR-shielded shelters. Experiments were carried out (1) in the short term (i.e., hours) and (2) in the middle term (i.e., days), with individuals feeding on diets with different amounts of UVAC either prior to or during the exposure, respectively. In the short term, both sexes preferentially selected UVR-shielded shelters. In the middle term, the preference for this shelter type was only observed after 5 days of exposure in females and in males just partially; however, no shelter preferences could be detected during the first two days of exposure in either sex. In the case of the experiment with females, this latter was possibly related to lower irradiances due to cloudy conditions, whereas males may have traded shelter for higher mobility. UVAC acquired through the diet did not alter *A. valida's* shelter selection, indicating that seeking shelter remains an important strategy to protect from deleterious levels of UVR even when other mechanisms of photoprotection are available.

Keywords *Ampithoe valida* · Patagonia · Shelter · UV-absorbing compounds · Ultraviolet radiation · UVR avoidance

Abbreviations

UVR	Ultraviolet radiation
PAR	Photosynthetically active radiation only (> 400 nm)
UVAC	UV-absorbing compounds
FCR	Food consumption rates

Introduction

Cryptic behavior such as hiding beneath rocks, within crevices and between macrophytes is a common strategy displayed by several intertidal species as a way to obtain refuge against predators (Duffy and Hay 1991; Boström and Mattila 1999; Machado et al. 2019), but also to decrease the impact of physical stresses of the environment (Jones and Boulding 1999; Sotka 2007; Pulgar et al. 2017). One of the stressors against which

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shelters may offer protection is solar ultraviolet radiation (UVR). UVR, mainly UV-B (280–315 nm), is a serious threat for aquatic organisms with effects ranging from increased respiration rates and changed feeding behavior to reductions in the individual´s survival and growth rates (Williamson et al. 1994; Fischer et al. 2006; Leech et al. 2009). The detrimental effects of UVR have been intensively studied for almost 50 years and raised special concern since the discovery of the Antarctic ozone hole in the 1980s (Farman et al. 1985) which led to dramatic increases in the solar UVR reaching the Earth's surface (Madronich et al. 1998). Despite a partial recovery of the stratospheric ozone layer reported during the last years as a result of the successful implementation of the Montreal Protocol (Barnes et al. 2021), there is now growing concern about other factors such as climate change-related variations in cloud cover, aerosols and surface reflectivity which may lead to variations in incident solar UVR levels (Bais et al. 2019). In intertidal areas, where organisms experience regularly acute short-term-exposures to maximum irradiances during low tide (Häder et al. 2011), seeking shelter has been reported in some species as an important behavioral strategy to avoid or at least ameliorate the exposure to this stressor (Adams 2001; Pulgar et al. 2017).

Besides behavioral avoidance, there are other protective strategies to cope with UVR, like photoprotective compounds (UV-absorbing compounds, hereafter: UVAC, Häder et al. 2011). These are secondary plant metabolites synthetized by some species of macroalgae, phytoplankton and fungi, which absorb in the UVR range and thus are able to screen off harmful radiation before it reaches important targets within the cells (Shick and Dunlap 2002). The synthesis of these compounds is highly variable among primary producers. For example, red macroalgae from shallow waters commonly contain high amounts of UVAC, whereas green and brown ones do not synthesize them or exhibit only trace concentrations (Karsten et al. 1998). Animals can acquire these compounds by feeding on diets rich in UVAC and accumulate them in their bodies. The photoprotective role of UVAC has been shown for numerous consumer species, with effects including reductions in oxidative stress, respiration rates and mortality in organisms exposed to UVR (Adams 2001; Moeller et al. 2005; Obermüller et al. 2005; Valiñas and Helbling 2016). In some species, the accumulation of these compounds may allow the exposure to higher levels of UVR and thus weaken the avoidance reaction of animals in response to this stressor (Hansson et al. 2007). However, in others, these compounds did not play a significant role regarding protection against UVR.

In the present study, we experimentally tested whether shelter selection of the epibenthic amphipod *Ampithoe valida*—a common species from coastal intertidal areas of the Southern Atlantic Ocean which lives closely associated with macroalgae (Rumbold 2019)—is influenced by UVR, and whether UVAC acquired by the amphipods from the diet alter this selection. If UVR is a stressor in this species and UVAC provide protection, we expect that amphipods feeding on diets with low amounts of UVAC will preferentially select UVR-shielded shelters. However, there would be no preference of these shelters when amphipods feed on diets containing high amounts of UVAC.

Material and methods

To achieve the aim of this study, two types of experiments were carried out, hereafter called: Refuge experiments and Refuge with food experiments, respectively. In the Refuge experiments, we evaluated the shelter selection of *A. valida* individuals containing high and low amounts of UVAC in their bodies, by direct observation of amphipods exposed to artificial radiation during the short term (i.e., 6 h). In the Refuge with food experiments, we tested the shelter selection under solar radiation conditions in the middle term (i.e., 6 days) by estimating the food consumption rates beneath each shelter when amphipods were feeding on different diets.

The experiments were conducted in November 2015 and February 2016 at the Estación de Fotobiología Playa Unión (EFPU, Chubut, Argentina, 43° 18′ S–65° 02′ W) with amphipods and macroalgae collected from a nearby rocky beach located in the Patagonian coast (Playa Bonita, 43° 22′ S–65° 03′ W). In the laboratory, adult amphipods were separated by sex and maintained at 20 °C in plastic pools filled with aerated seawater inside a temperature controlled chamber (Minicella, Argentina) under a 12:12 h light:dark photoperiod until the beginning of the experiments. The water used for the experiments was collected from the same area as the amphipods and macroalgae to provide a similar background as in the field. No unusual behavior of the animals under laboratory conditions was noted; therefore, it was assumed that the period between field sampling and start of the experiments was sufficient for them to recover from the sampling procedure and to acclimatize to the laboratory conditions.

Refuge experiments

Individuals of *A. valida* were fed for 4 days with either *Pyropia columbina* (Rhodophyceae, formerly referred to as *Porphyra columbina*) or *Ulva rigida* (Chlorophyceae), representing a diet with high and low amounts of UVAC, respectively (Valiñas and Helbling 2015). Both macroalgae and water were replaced after 48 h. At the end of the 4-day feeding period and before starting the experiments, determinations of UVAC content in the amphipods were performed to confirm that the individuals fed with *P. columbina* had bioaccumulated significant amounts of these compounds in contrast with those fed with *U. rigida* (see below). For the experiments, individuals of *A. valida* from the high- or low-UVAC treatments described above were placed in experimental containers (32 cm × 23 cm × 8 cm, L × W × H, Fig. 1a) and exposed to artificial radiation of 112.6; 43; and 1.08 W m^{-2}, for PAR (400–700 nm), UV-A (315–400 nm) and UV-B (280–315 nm), respectively, under a solar simulator (SOL 1200 W, Dr Hönle AG, Gräfelfing/München, Germany; for details to the experimental setup see Table 1). Thirty minutes after the radiation exposure started, the four corners of the experimental containers were provided with shelters

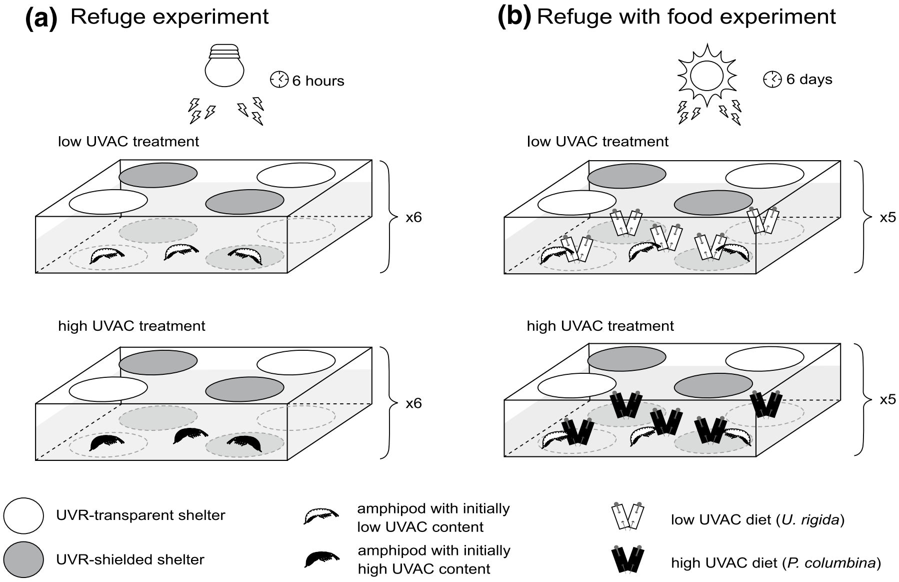

Fig. 1 Experimental set up for the **a** Refuge and **b** Refuge with food experiments (not at scale) to test shelter selection of the amphipod *A. valida*. Experiments were carried out separately for males and females. Numbers of individuals were **a** Refuge experiments: 6 for both sexes; **b** Refuge with food experiments: 8 for males; 10 for females

Table 1 Details of the experimental setup for the Refuge and Refuge with food experiments

	Refuge experiments	Refuge with food experiments
Dimensions of experimental containers (L × W × H)	32 cm × 23 cm × 8 cm	25 cm × 18 cm × 5 cm
Nr. of replicates	6	5
Nr. of individuals per replicate	Males: 6 Females: 6	Males: 8 Females: 10
Source of radiation	Artificial	Natural
Temperature during exposure	Constant (20 °C)	Fluctuating (13–21 °C)
Time of exposure	6 h	6 days

made of circular plastic films (diameter: 9 cm); two of these films blocked UVR (transmission of PAR only, > 400 nm, hereafter: UV-shielded shelters), while the other two allowed all the radiation to pass through (transmission of the full solar spectrum, hereafter: UV-transparent shelters). The four shelters were placed randomly in each corner of the experimental containers in order to avoid any site effect. The transition zone between the shelters remained uncovered (hereafter "bare area") and occupied ca. 60% of the exposed area. Within the subsequent 6 h after adding the shelters to the containers, the location of the individuals was registered by visual observation; every 15 min during the first hour and every 30 min during the subsequent 5 h, resulting in a total of 14 observations per replicate. Individuals were considered beneath a particular shelter when they were situated at least with half of the body beneath the respective film at the moment of the first sight. Individuals that were situated within the bare area, or those with less than half of the body beneath one film or above a film, respectively, were not counted. Due to size limitation of the effective area of the solar simulator, and in order to ensure that the individuals received similar irradiances, 2 experimental containers (1 containing males and the other 1 containing females, coming from low- or high-UVAC treatment) were exposed at a time. We ensured that the individuals had the same feeding times on the different macroalgae diets by starting the feeding periods shifted in time.

Refuge with food experiments

One day after their collection, amphipods were placed in experimental containers (25 cm × 18 cm × 5 cm, L × W × H, Fig. 1b, Table 1) provided in each corner with the same shelters as described above, and exposed during 6 days to natural solar radiation. Each container was randomly assigned to one of two diet treatments: low-UVAC content (i.e., *U. rigida*) and high-UVAC content (i.e., *P. columbina*). Macroalgae used as food were taken directly from the field, cut in strips (~ of 0.5 × 2 cm), dried from excess water, weighted and fixed beneath each shelter in an upright position in order to minimize the shading effects (Fig. 1b). As *A. valida* may show territoriality and aggressive behavior against conspecifics, we put two pieces of food beneath each shelter, in order to give the individuals the possibility to maintain distance to each other while feeding. Additionally, to confirm that there were no effects of the shelters other than the spectral properties, food was also placed in the center of the bare area. The food was put every day at 8 a.m. and taken out at 7 p.m., so during the night the individuals were left without food. In this way, the food consumption beneath each shelter was measured only under the period of radiation exposure. The food was replaced completely every 48 h, so the individuals fed on the same strips of macroalgae for 2 days, after which the leftovers were dried from excess water and weighted again. Dead amphipods were removed every day, but not replaced; thus at the end of the experimental period, all individuals in the containers received the same doses of radiation. The food consumption rates (FCR) were calculated for the first two days (hereafter, $t_{1\text{-}2}$) and for the fifth and sixth day of solar radiation exposure (hereafter, $t_{5\text{-}6}$) as the difference between the macroalgal wet weight before and after 48 h of feeding, divided by the number of alive amphipods. No controls for autogenic changes of macroalgal wet weight were done based on the findings of previous studies that growth changes macroalgal wet weight just after 3 days of incubation (Valiñas and Helbling 2015). Experiments were run outdoors of the EFPU; experimental containers were maintained in a 200 L water baths at temperatures between 13 and 21 °C during this period, which is within the natural thermal range that amphipods experience in the study area during the summer season (Valiñas and Helbling 2015). For more details to the experimental setup see Table 1. For logistic reasons, the experiments were carried out at different times for males and females, with the experiment using males starting one day later than that for females.

Determination of UVAC

UVAC content determinations were performed in amphipods collected from their natural environment and from those that were fed during 4 days with the different macroalgae ($n = 3$, with 3 individuals per sample). Individuals were starved for 24 h to empty their gut contents prior the determinations and stored frozen until further analysis. Subsequently, samples were dried from excess water, weighted, and put in 15 ml centrifuge tubes, where 5 ml of absolute methanol were added. After breaking the tissues with a glass rot, the samples were sonicated for 20 min at

25 °C, left for 1 h for extraction and subsequently centrifuged for 15 min at 1500 rpm. Finally, the spectral characteristics of the supernatant were measured between 250 and 750 nm using a scanning spectrophotometer (Hewlett Packard model HP-8453E). UVAC content was measured by peak analysis between 310 and 360 nm and expressed as peak area per mg wet weight of tissue.

Radiation data

The irradiance output of the solar simulator used during the Refuge experiments was measured using an Ocean Optics spectroradiometer (UV–Vis-IR HR 2000). The spectroradiometer was calibrated using a solar calibration procedure. For this calibration, the irradiance data during a clear sky condition were compared with the output of radiation transfer models such as STAR (Ruggaber et al. 1994) and Daylight (Björn and Murphy 1985).

During the Refuge with food experiments, solar radiation was continuously monitored (every minute) using a broadband filter radiometer European Light dosimeter Network (ELDONET; Real Time Computers Inc., Möhrendorf Germany), which has three channels for PAR (400–700 nm), UV-A (315–400 nm) and UV-B (280–315 nm). This radiometer is permanently installed on the roof of the Estación de Fotobiología Playa Unión (EFPU, Häder et al. 2007).

Data treatment and statistical analysis

All analyses were done separated for males and females using the statistical software R (R Core Team 2013). Differences in UVAC content among individuals taken from the field (t_0), and those fed during 4 days on *U. rigida* or *P. columbina,* respectively, were evaluated using one-way ANOVA with a posteriori Fisher's LSD tests (Zar 1999). To test for differences in shelter selection across the time of radiation exposure, the numbers of individuals registered beneath each shelter in the Refuge experiments were compared among the 14 observations both graphically and statistically using Friedman-tests. Since both analyses did not reveal differences in shelter selection across time (Friedman $p > 0.05$), the data of the 14 observations were pooled and the mean percentages of individuals beneath each shelter type (i.e., as sum of individuals beneath the two UVR-transparent or UVR-shielded shelters, respectively) were compared by means of a mixed-effects ANOVA using the package nlme (Pinheiro et al. 2020). Diet (high- vs. low-UVAC content in *A. valida*) was set as between-subjects factor and shelter type (UVR-transparent vs. UVR-shielded) was regarded as within subject's factors in order to account for the lack of independence among shelters.

In the Refuge with food experiments, differences in FCR between the average of the UVR-transparent shelters and the transition zone (bare area) were tested using *t* tests (Zar 1999), to discard an artifact effect. As in more than 80% of the cases there were no significant differences, the bare area was excluded from the statistical analysis. Differences in FCR among shelter types (i.e., total amounts of food consumed beneath the two UVR-transparent and UVR-shielded shelters, respectively) and diet were evaluated separately for $t_{1\text{-}2}$ and $t_{5\text{-}6}$ using a mixed-effects ANOVA as described above. Significant interaction terms were a posteriori analyzed using pairwise comparisons with Bonferroni correction from the package emmeans (Lenth et al. 2020).

In all cases, model residuals were tested for normality and homoscedasticity using Shapiro and Levene´s tests, respectively, and data were arcsine-square-root or log transformed if necessary (Zar 1999). In the very few cases where data transformation failed to achieve normality and homoscedasticity, we proceeded with parametric tests since ANOVA is considered very robust against deviations from these assumptions (Underwood 1997).

Results

Refuge experiments

Amphipods collected in the field (t_0) had low amounts of UVAC in their bodies, as inferred from the small "shoulders" between 310 and 360 nm in the absorption spectra of both sexes (Fig. 2a, b). There were no significant differences in the amount of UVAC in the amphipod bodies at t_0 as compared to that after feeding on *U. rigida* for 4 days (Fig. 2c). However, when feeding on *P. columbina*, individuals from both sexes accumulated around 15 times higher amounts of UVAC than those individuals measured immediately

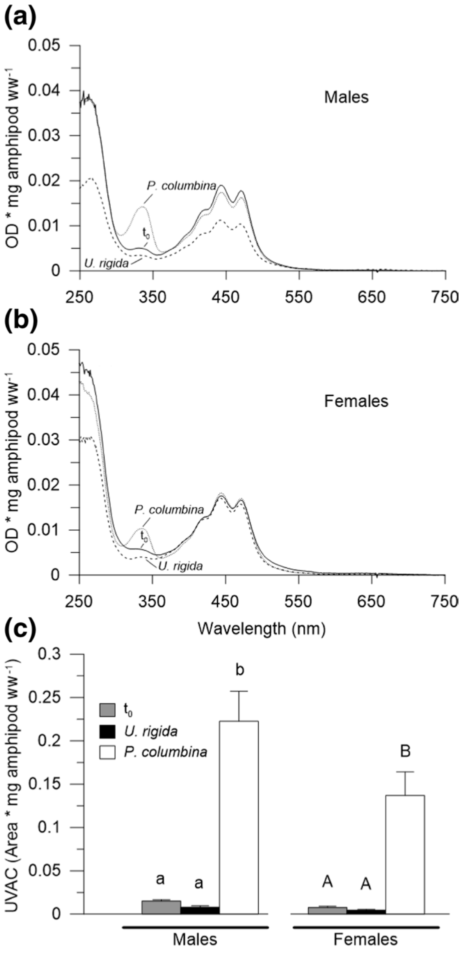

Fig. 2 Mean optical density (OD) per mg wet weight of amphipod´s tissue as a function of the wavelength (250–750 nm) for males (**a**) and females (**b**) of *A. valida* collected from their natural environment (t_0) and after 4 days of feeding on diets with low (*U. rigida*) or high amounts of UVAC (*P. columbina*), respectively. **c** Mean UVAC (±SD, as peak area at 336 nm) per mg wet weight of tissue in amphipod males and females, before (t_0) and after the feeding period. The statistical analysis was performed separately for males and females. Different letters indicate significant differences in the UVAC content between the amphipods at the moment of sampling and of those fed on *U. rigida* or *P. columbina*, respectively

after their collection in the field and up to 30 times higher amounts than those feeding on *U. rigida* (ANOVA, $p < 0.001$ in all cases), respectively.

Shelter type, but not diet, had a significant effect on shelter selection in both males and females of *A. valida* (Table 2, Fig. 3), as in both diet treatments more than 70% of individuals selected UVR-shielded shelters.

Table 2 Results from the mixed-effects ANOVA of the effects of diet (in terms of UVAC- content accumulated by *A. valida,* i.e., high- vs. low-UVAC content) and shelter type (i.e., UVR-transparent vs. UVR-shielded shelters) on individual numbers beneath the shelters

	Males		Females	
	$F_{1,10}$	*p*	$F_{1,10}$	*p*
Diet	0.00	1.00	0.00	1.00
Shelter type	55.98	< 0.0001*	32.68	< 0.001*
Diet × shelter type	1.95	0.19	3.32	0.10

*Represents significance at the alpha = 0.05 level

Refuge with food experiments

The daily irradiances received by the amphipods during the experiments are shown in Fig. 4. Despite

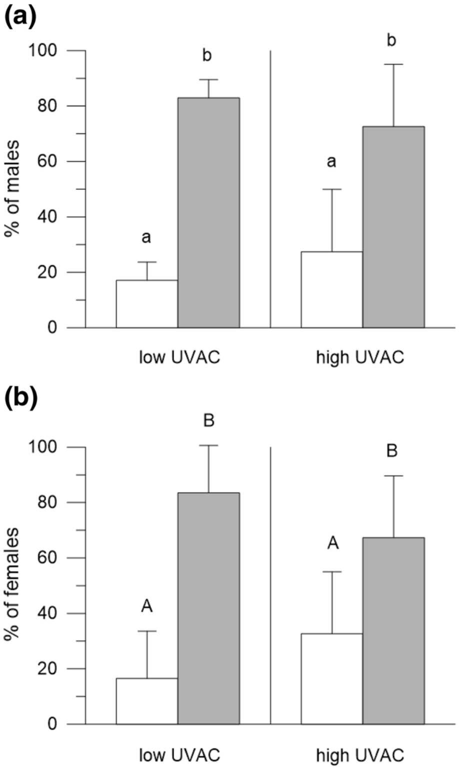

Fig. 3 Mean percent (±SD) of *A. valida* males (**a**) and females (**b**) registered beneath shelters during the Refuge experiments as a function of the diet (i.e., high- vs. low-UVAC content in amphipods) and shelter type (i.e., UVR-transparent [white bars] vs. UVR-shielded [gray bars]). Total number of individuals per replicate was six. Different letters indicate significant differences between shelter types

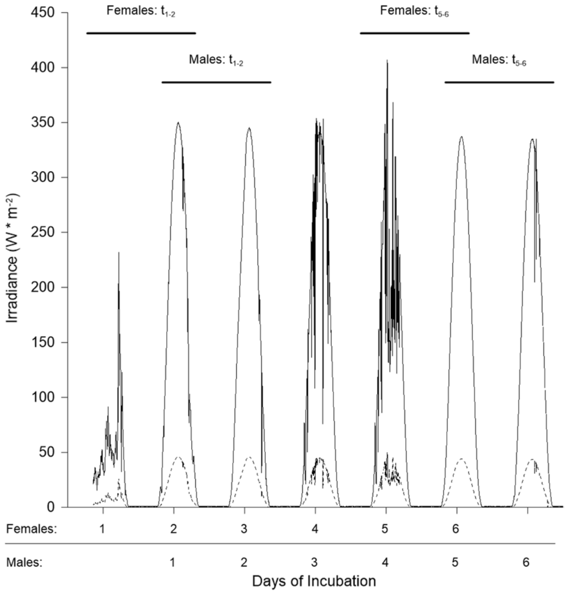

Fig. 4 Solar radiation conditions in terms of PAR (solid lines) and UVR (dashed lines) during the Refuge with food experiments. Note that the experiments for males and females were carried out time shifted

some differences in cloudiness among the days of exposure, mean daily irradiances were similar during most time of the experimental period (i.e., PAR: 208.4 ± 15.5 W m^{-2}, UV-A: 25.9 ± 1.6 W m^{-2}, UV-B: 0.7 ± 0.05 W m^{-2}), with the exception of the first day of females' exposure, where cloudy conditions led to almost 70% lower irradiances than the rest of the days.

During the first two days of exposure to solar radiation ($t_{1\text{-}2}$), there was a general trend of higher FCR beneath UVR-shielded shelters in both male and female amphipods (Figs. 5a and 6a); however, these differences were not significant between shelter types (UVR-transparent versus UVR-shielded) or between diets (low- and high-UVAC content; Table 3). After 5 days of exposure ($t_{5\text{-}6}$), males feeding on diets with high-UVAC content showed higher FCR beneath UVR-shielded shelters, while FCR of males feeding on diets with low-UVAC content did not significantly differ between shelters (Table 3, Fig. 5b). In the case of females, they showed higher FCR beneath UVR-shielded shelters regardless of the diet they were feeding on (Table 3, Fig. 6b).

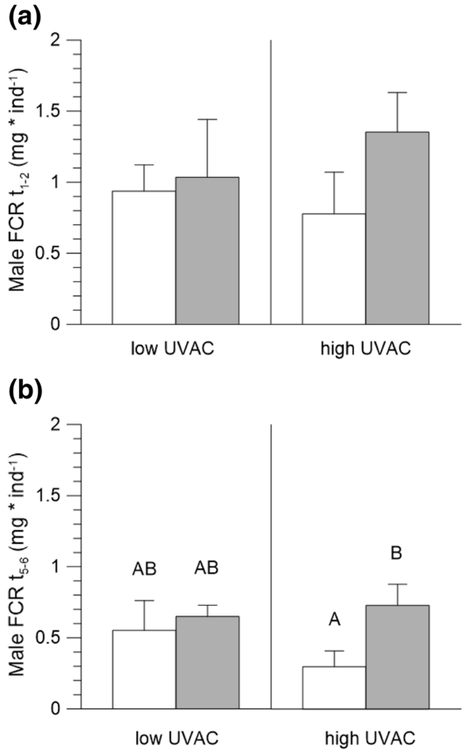

Fig. 5 Mean (± SD) food consumption rates (FCR) in *A. valida* males during the first two days ($t_{1\text{-}2}$, **a**) and after 5 days ($t_{5\text{-}6}$, **b**) of solar radiation exposure as a function of shelter types and diets offered. White bars: UVR-transparent shelters, gray bars: UVR-shielded shelters. Different letters indicate significant differences for the interaction of diet and shelter type

Discussion

UVR can alter the behavior of aquatic animals as these may use and select shelters differently under exposure to this stressor (Pulgar et al. 2017). Previous studies found that, under a short-term exposure to radiation (i.e., hours), several taxa including amphibian tadpoles (Connolly et al. 2011), sea urchins (Adams 2001) and fish (Kelly and Bothwell 2002; Pulgar et al. 2015) preferred UVR-shielded sites. This is in accordance with the results obtained in the Refuge experiments where amphipods clearly preferred UVR-shielded shelters (Fig. 3). Under naturally fluctuating levels of UVR, avoidance and escape reactions are especially important to cope with short-term peaks of this stressor (Rautio et al. 2003). For example, zooplankton have been shown to respond quickly (i.e., within minutes) to fluctuating UVR threats by alternated up- and downward swimming

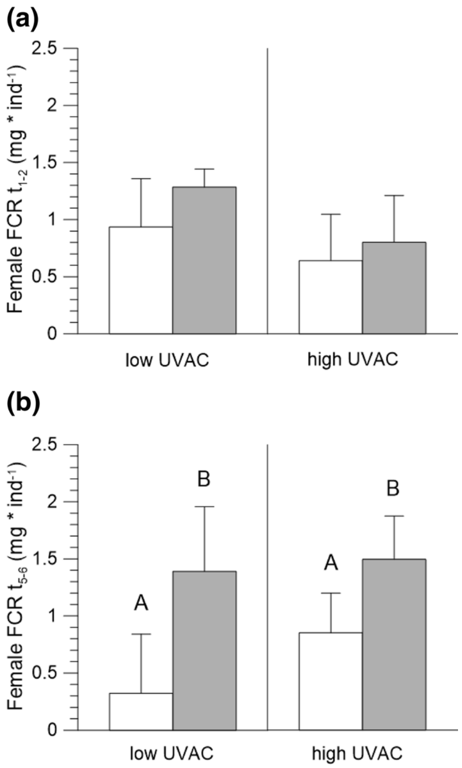

Fig. 6 Mean (±SD) food consumption rates (FCR) in *A. valida* females after the first two days ($t_{1\text{-}2}$, **a**) and after 5 days ($t_{5\text{-}6}$, **b**) of solar radiation exposure as a function of shelter types and diets offered. White bars: UVR-transparent shelters, gray bars: UVR-shielded shelters. Different letters indicate significant differences between shelter types

Table 3 Results from the mixed-effects ANOVA of the effects of diet (i.e., high- vs. low-UVAC content) and shelter type (i.e., UVR-transparent vs. UVR-shielded shelters) on food consumption rates (FCR) of *A. valida* beneath the shelters. The analyses were carried out separately for the first two days ($t_{1\text{-}2}$) and the fifth and sixth day of exposure ($t_{5\text{-}6}$)

		Males		Females	
		$F_{1,7}$	*p*	$F_{1,8}$	*p*
$t_{1\text{-}2}$	Diet	0.20	0.67	3.65	0.09
	Shelter type	4.48	0.07	2.55	0.15
	Diet × shelter type	2.65	0.15	0.35	0.57
$t_{5\text{-}6}$	Diet	1.04	0.34	1.88	0.21
	Shelter type	18.36	< 0.01*	13.71	< 0.01*
	Diet × shelter type	6.35	0.04*	0.85	0.38

*Represents significance at the alpha = 0.05 level

in response to the instantaneous level of UVR, which may have evolved as a response to short-term pulses resulting from changing cloudiness (Hansson et al. 2016). In the study area, amphipods are exposed to semidiurnal tides, and they naturally experience large fluctuations of UVR during the tidal cycle, reaching daily maxima when low tide matches with the solar noon. The exposure time and irradiances used during the Refuge experiments (i.e., UV-A: 43.00 W m^{-2}, UV-B: 1.08 W m^{-2}) represent an acute UVR-stress, as individuals would experience in the study area during midday low tides in spring–summer seasons. For instance, in November, when experiments were carried out, maximum irradiances at noon reach mean values of 48.8 ± 8.0 (SD) W m^{-2} for UV-A and 1.6 ± 0.24 (SD) W m^{-2} for UV-B (mean values of daily maxima between 2015 and 2020, data provided from EFPU database; www.efpu.org.ar). By seeking shelter to avoid peak irradiances, amphipods may lower the UVR damage in the short term, what might improve their energy balance (Pulgar et al. 2017; Vargas et al. 2018), and consequently enhance their fitness, growth, survival and reproductive output in the longer term (Belden and Blaustein 2002; Fischer et al. 2006).

In some species, feeding behavior is affected by UVR exposure (Ruelas et al. 2006; Leech et al. 2009; Fernández and Rejas 2017); however, in the case of *A. valida*, a previous study carried out in the study area found no difference in FCR when amphipods were exposed either to PAR only or to PAR + UVR (Valiñas and Helbling 2015). Based on these previous findings, and taken into account that in the Refuge with food experiments amphipods had the opportunity to choose among feeding sites, we therefore assume that a higher FCR beneath a certain shelter would be related with a longer time the individuals spent under that shelter rather than with changes in their rates of consumption due to UVR. This was the case for females at $t_{5\text{-}6}$, where individuals had the highest FCR beneath UVR-shielded shelters, independently of the diet they were offered (Fig. 6b), meaning that the individuals preferred these shelters as feeding sites. However, at $t_{1\text{-}2}$, despite a general trend of higher FCR beneath UVR-shielded shelters, no significant differences in FCR between shelter types were detected. This could be related to the lower irradiances received by the females during the first day of exposure due to cloudy conditions

(Fig. 4). The impact of UVR depends strongly on the strength of the stressor, with some species being tolerant at lower irradiances, but experiencing increasingly negative effects when intensities exceed a certain threshold (Dey et al. 1988). Consequently, UVR-avoidance behavior becomes more pronounced under increasing levels of this stressor (Barcelo and Calkins 1980; Rautio et al. 2003), but there might be a threshold for triggering such a behavior. Although we did not determine the lethal dose 50 (LD50) in our experiments, a previous study carried out in the study area found an LD50 of 82.5 kJ m^2 of UV-B and a significant decrease in survival of *A. valida* when UV-B doses were higher than 40 kJ m^2 (Helbling et al. 2002). In the Refuge with food experiments, the UV-B dose received by the females after the first two days of exposure was 37.2 kJ m^2 (data not shown), which might be below the critical dose for inducing a behavioral avoidance reaction. It is therefore possible that the irradiances received by the females during the first two days of exposure were too low to trigger shelter selection, which may have led to the non-significant outcome of the experiment at $t_{1\text{-}2}$.

In the case of males, despite sunny conditions during both $t_{1\text{-}2}$ and $t_{5\text{-}6}$ (Fig. 4), no significant preference for UVR-shielded-shelters could be observed when measuring food consumption at $t_{1\text{-}2}$, while at $t_{5\text{-}6}$ individuals consumed more beneath UVR-shielded shelters just when they were feeding on *P. columbina* (Fig. 5). Males therefore showed in general less preference for UVR-shielded shelters than females. Males of *A. valida* are generally more active than females, leaving their shelters more often and swimming around in search for mates (Borowsky 1983). Males therefore have to make a trade-off between protecting themselves by sheltering and exposing themselves to environmental threats in order to increase their reproductive success (Vesakoski et al. 2008). In our experiment, such a higher mobility could have led to a more scattered feeding “by chance” on the algae offered beneath the different shelters and therefore have accounted for the less pronounced shelter selection of this sex. Furthermore, males are naturally more exposed to UVR and therefore may be better adapted to support exposure to this stressor, while for females, sheltering to avoid UVR exposure would be more important, at least at the levels of irradiance used in this study. This is consistent with previous findings which report that females of this species have a higher vulnerability to UVR than their male counterparts (Valiñas and Helbling 2015). We therefore conclude that over a longer time period, sheltering to protect from elevated levels of UVR may be more crucial for females than for males, for whom other factors such as reproductive behavior may become more important.

Regarding the accumulation of UVAC, our findings indicate that *A. valida* can accumulate UVAC in their bodies by feeding on diets with high amounts of these compounds, as it was already shown in previous studies (Helbling et al. 2002; Valiñas and Helbling 2015, 2016). Moreover, we infer that in both the Refuge experiments and in the end of the Refuge with food experiments, amphipods had the opportunity to accumulate the maximum amounts of UVAC in their bodies, for at least two reasons: First, *P. columbina*, which was used as source of UVAC in our experiments, is among the rhodophyte species with the highest concentration of these compounds in the study area (Helbling et al. 2004; Huovinen et al. 2004). In this species, the synthesis of UVAC increases with increasing irradiance and nitrogen availability (Peinado et al. 2004); thus, UVAC contents would be highest during spring–summer months when irradiances peak and nutrient loads increase by the input of the Chubut river (Helbling 1989), coinciding with the time when experiments were carried out. Second, an additional experiment we performed showed that *A. valida* can accumulate significant amounts of UVAC after one day of feeding exclusively on *P. columbina*, with the peak of accumulation occurring after 3 days (Blum, unpub. data). Thus, we assume that under natural conditions, amphipods would not be able to accumulate higher amounts of UVAC than in our experiments. Therefore, the individuals from the low- and high-UVAC treatments in both the Refuge experiments and in the end of the Refuge with food experiments would represent two contrasting conditions, while the UVAC concentrations of individuals in the field would lie between these two extremes.

UVAC accumulation can increase the survival rates of *A. valida* exposed to UVR (Helbling et al. 2002; Valiñas and Helbling 2015), evidencing the photoprotective role of these compounds in this species (Adams and Shick 1996; Obermüller et al. 2005). Such an accumulation of UVR-screening substances can influence the behavioral reaction of aquatic invertebrates in response to UVR, as it was shown for pelagic zooplankton that escape to significantly deeper depth when they contain just low amounts of UVAC, whereas the accumulation of these compounds allows them to stay at better feeding sites closer to the surface (Rhode et al. 2001; Hansson et al. 2007). In the present study, UVAC did not play a significant role in shelter selection. In the Refuge experiments, even when individuals had UVAC accumulated, more than 70% of them selected UVR-shielded shelters, meaning that seeking shelter remains an important strategy to protect from high levels of UVR even when other photoprotective mechanisms are available. It is possible that the accumulation of UVAC provides just partial protection from UVR damage (Lesser 1996; Shick and Dunlap 2002). For example, a study on crab larvae from Patagonia showed that the species with the highest UVAC content had the lowest mortality rates when exposed to UVR, indicating the protective value of these compounds. However, a temperature increase further decreased mortality rates in that species due to the enhancement of enzymatic repair mechanisms, suggesting that UVAC accumulation alone did not provide full protection for the larvae, which rather may rely on a combination of both protective mechanisms (Moresino and Helbling 2010). In the case of *A. valida*, previous studies showed that UVAC accumulation could significantly lower, though not fully prevent, UVR-induced mortality (Valiñas and Helbling 2015). For this species, sheltering, or a combination of both sheltering and UVAC accumulation, may therefore be a more effective way of protection.

In the Refuge with food experiments, we observed a different impact of the diet on shelter selection in males and females at $t_{5\text{-}6}$. While females in the two diet treatments showed no differences in shelter selection (Fig. 6b), males preferred UVR-shielded shelters just when they were feeding on *P. columbina* (Fig. 5b). In the latter case, if UVAC played a role in shelter selection, one would have expected that the higher amount of UVAC provided by *P. columbina* would lead to a decrease in the selection of UVR-shielded shelters. It is possible, though, that the observed pattern in males is related to other factors such as a higher nutritional quality of *P. columbina* compared to *U. rigida* (Valiñas and Helbling 2015). However, these findings clearly contradict our initial hypothesis that *A. valida* would select UVR-shielded shelters only when chemical photoprotection through UVAC accumulation is not provided. Therefore, the search for shelter may have evolved in this amphipod species as an effective way to protect from UVR (Fernández et al. 2020).

Intertidal animals may be able to separate among microhabitats which offer different degree of protection against UVR (Pulgar et al. 2017). As *A. valida* lives closely related to macrophytes, which may offer different degrees of protection against UVR, those macrophytes that offer the best protection may be preferentially selected as shelter, what would indirectly determine the feeding sites of the amphipods (Duffy and Hay 1991; Boström and Mattila 1999). Studies on macrophyte-herbivore interactions traditionally focused on the role of the former as feeding areas (Nicotri 1980; Buschmann 1990), as well as shelter against predators (Duffy and Hay 1991; Boström and Mattila 1999; Vesakoski et al. 2008) and to a lesser extent against physical stressors such as wave action and desiccation (Jones and Boulding 1999; Sotka 2007). Based on the findings of the present study, we therefore suggest that future research should address the role and relative importance of macrophytes to offer UVR-protection in order to fully understand their selection by associated herbivores in the field.

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Data Availability The authors are pleasured to share the data after formal request.

Declarations

Conflict of interest The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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