

Does pigmentation provide protection to bdelloid rotifers in a high ultraviolet B environment?

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Abstract

Aquatic species found in habitats with limited shade and little dissolved organic carbon (DOC) have increased vulnerability to ultraviolet radiation (UVR) damage. Pigmentation is a common mechanism used by animals for protection from UVR. A pigmented bdelloid rotifer, *Philodina*, occurs in high densities in shallow rock pools in El Paso Co., TX, and is subject to repeated desiccation and high UVR. To understand the roles of DOC, pigmentation, and dormancy in reducing the effects of UVR exposure in these rotifers: (1) DOC levels in rock pools were measured before and after the summer monsoon season and (2) hydrated or dormant bdelloids (desiccated for 0, 1, 7, or 32 d) that differed in degree of pigmentation (highly, moderately, lightly, and none) were exposed to three intensities of UVB radiation (low, mid, or high) and monitored for survival after 48 h. Pigmented bdelloids were found in rock pools with lower DOC concentrations. Logistic regression analysis indicated that pigmentation level, desiccation time, and UVB intensity all affected survival. Bdelloids in the dormant form for 1 d were more resistant to UVB exposure at all pigmentation levels. However, as desiccation time increased, the odds of surviving decreased. Hydrated highly pigmented bdelloids were three times more likely to survive desiccation, UVB radiation, and their combined effects. Prolonged periods of drought due to the changing climate will alter DOC concentrations, causing photoprotection to become an increasingly important survival strategy for aquatic invertebrates, especially those inhabiting shallow waters.

Desert, ephemeral aquatic communities are highly susceptible to high temperatures, extended periods of drought, and high levels of ultraviolet radiation (UVR) (Wallace et al. 2005; Walsh et al. 2014; Kadad et al. 2020). Current climate models predict a 4°C increase in ambient temperatures by the year 2070 (Hoffmann and Beierkuhnlein 2020). Increasing temperatures will, no doubt, lead to warming of water bodies and increased evaporation, as well as fluctuations in dissolved organic carbon (DOC) concentrations (Zhou et al. 2018; Hoffmann and Beierkuhnlein 2020). Warmer temperatures may also result in shallower mixing of water columns resulting in higher intensities of UVR reaching planktonic communities (Williamson et al. 2001; Boeing et al. 2004; Watanabe et al. 2011). UVR has a wide range of damaging effects on aquatic organisms, including tissue damage and DNA mutation, both of which can reduce lifespan (Fischer et al. 2013; Mojib et al. 2014; Ulbing

et al. 2019). Synergistic effects of increasing temperatures and UVR levels on aquatic species are unknown, but are emerging as a research imperative as the climate continues to change (Watanabe et al. 2011; Bais et al. 2018; Pinceel et al. 2018).

The intensity of UVR that reaches aquatic inhabitants depends on a range of factors including angle of the sun, elevation, latitude, calmness, albedo of water, and concentration of DOC (Leech and Williamson 2001; Watanabe et al. 2011). For most aquatic species, DOC acts as a protectant against UVR; its sources include both living and decaying organic matter (Erickson et al. 2015). Components of UVR that can cause damage to aquatic species are UVA and UVB wavelengths, both of which can break down DOC (Leech and Williamson 2000; Erickson et al. 2015). If DOC is not replenished periodically, UVA/UVB wavelengths are able to penetrate up to 30 m below the water surface (Williamson et al. 2001; de Los Rios 2005), making the inhabitants of shallow, freshwater environments vulnerable to UVR damage (Boeing et al. 2004; de Los Rios 2005).

The Chihuahuan Desert of the southwestern USA and northern Mexico is recognized for its high biodiversity and numerous ephemeral waterbodies, including rock pools and shallow playas (Wallace et al. 2005; Walsh et al. 2014; Brown et al. 2022). Rock pools are typically filled by precipitation. As the water begins to evaporate in the rock

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Additional Supporting Information may be found in the online version of this article.

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pools, changes in conductivity, pH, and temperature can occur rapidly; these environmental fluctuations limit the type of species that occur in these types of habitats (Brendonck et al. 2010; Jo  que et al. 2010). Another physical challenge for species occurring in these habitats is increased exposure to UVR (Martin 2017); this is because shallow waters bodies (< 1 m) with low concentrations of DOC (< 10 mg L⁻¹) offer little to no protection from UVR damage (de Los R  os 2005; Tapia-Torres et al. 2015). It is unknown whether DOC concentrations fluctuate seasonally in Chihuahuan Desert rock pools.

To avoid or reduce damage, many aquatic invertebrates use photoprotective chemicals that may take the form of conspicuous pigmentation (Hansson et al. 2007; Hylander et al. 2009, 2014; Rautio and Tartarotti 2010). Aquatic species that use photoprotective pigments include cladocerans (Connelly et al. 2015; Leach et al. 2015; Alcocer et al. 2020), copepods (Hairston Jr. 1976, 1979; Garcia et al. 2008), and tardigrades (Bonifacio et al. 2012; Suma et al. 2020). These can be synthesized by the animal (Marcoval et al. 2020) or derived from carotenoids and/or mycosporine-like amino acids found in phytoplankton, algae, bacteria, or other food sources (Bonifacio et al. 2012; Schneider et al. 2016). Carotenoids are the primary constituents of red photoprotectants in invertebrates and they possess potent antioxidant activity that enables them to counteract the detrimental effects of oxidative stress (Br  sin et al. 2016; Bashevkin et al. 2020; Suma et al. 2020). Aquatic species that are not able to avoid UVR or obtain a source of pigmentation must repair UVR damage or lose fitness (Tartarotti et al. 2013).

Bdelloid rotifers are microinvertebrates found in diverse aquatic and limnoterrestrial habitats, including extreme environments such as cryoconitic holes in Antarctica (Cakil et al. 2021) and ephemeral rock pools in deserts (Wallace et al. 2005; Walsh et al. 2014). Their ability to withstand these extreme environments is attributed to their ability to undergo anhydrobiosis at any life stage (Ricci et al. 2003; Ricci and Caprioli 2005) by forming xerosomes (Wallace et al. 2008). When emerging from dormancy, various DNA repair mechanisms, along with antioxidants, work to mitigate oxidative stress by repairing single- and double-strand DNA breaks (Ricci et al. 2003; Ricci and Fontaneto 2009; Hespeels et al. 2023).

The combined effects of desiccation and UVR exposure increased mortality and caused genome instability through DNA strand breaks in xerosomes of the bdelloid *Adineta vaga* (Davis, 1873) (Hespeels et al. 2014, 2023). The number of breaks increased with increasing doses of UVR. The same study found that in the dormant form, neither proton radiation (positively charged particles), nor gamma rays (high energy photons) had a significant effect on survival or DNA damage (Hespeels et al. 2014). Martin (2017) found that pigmented bdelloids from desert rock pools exhibited greater resistance to UVB radiation compared to unpigmented species collected from permanent waterbodies. This implies that pigmented bdelloids have a higher resistance to UVB and

making them valuable models for investigating the protective role of pigmentation against UVR exposure in active and dormant populations.

Many bdelloid rotifers exhibit a distinct red coloration (Supporting Information Table S1), including several species of *Philodina* commonly found in rock pools in El Paso, TX, USA (Walsh et al. 2014; Martin 2017). As noted above, pigmentation can increase resistance to UVR damage in copepods, daphnids, and tardigrades. However, the photoprotective qualities of red coloration have not yet been investigated in rotifers. In addition, there is little information on concentrations of DOC in rock pools. The goal of this study was to investigate potential mechanisms that provide photoprotection in aquatic invertebrates that inhabit shallow waters. Our objectives were to (1) evaluate the presence of pigmented bdelloids in rock pools with varying DOC concentrations in situ during monsoon and dry seasons and (2) use laboratory experiments to determine whether pigmentation provides protection against regional levels of UVB exposure when bdelloids are in either the active or anhydrobiotic state.

Methods

Site characterization, rotifer collection, and culture

Hueco Tanks State Park and Historic Site (hereafter Hueco Tanks) is in El Paso Co., TX, USA (Supporting Information Fig. S1). The average summer temperature is 36  C and an average precipitation of 22 cm yr⁻¹, most of which falls during the summer monsoon season (US National Weather Service 2024). The park is named for rock pools (huecos) that are found on the rocky outcrops (Schr  der et al. 2007; Walsh et al. 2014). These shallow rock depressions provide a habitat for brightly pigmented micrometazoans including an undescribed species of *Philodina*, a bdelloid rotifer. This area is subject to high intensities of UV radiation, where the rocky outcrops reach elevations of up to ~1350 m (Supporting Information Fig. S2). Bdelloids used for this study were collected 24–72 h after a significant rainfall event (   0.5 cm) from June 2017 to February 2020. The presence or absence of highly pigmented (HP) *Philodina* was recorded at the time of collection. In the laboratory, rotifers were washed free of sediment and fed a mixture of algae consisting of *Chlamydomonas reinhardtii* Dangeard, 1888 (Culture Collection of Algae at the University of Texas at Austin [UTEX] strain 90) and *Chlorella vulgaris* Beijerinck, 1890 (UTEX strain 30) in synthetic hardwater media (modified MBL media; Stemberger 1981).

Dissolved organic carbon

Water samples for DOC analysis were collected to establish whether there was a relationship between DOC concentration and the presence of pigmented bdelloids. When sufficient water was present, rock pools were sampled at least twice during the monsoon season (mid-June to September) and dry season (October to early-June) (US National Weather

Service 2024). The distribution of pigmented bdelloids had been observed previously (EJW, pers. obs.); we sampled 12 rock pools, eight where pigmented bdelloids had been reported and from four rock pools where they were absent (Supporting Information Fig. S1; Supporting Information Table S2). Water samples were collected following the protocol of Wetzel and Likens (1991) with the following modifications. Briefly, 50 mL vials were soaked in 2% HCl overnight, rinsed with DI water, and then ashed (heated to 500°C for 2 h; plastic lids and GF/F glass fiber filters [Whatman, 0.45 µm] were heated to 100°C for 1 h). In the field, water samples were filtered, transported on ice, and stored in the dark at 4°C until examined using a Shimadzu TOC (Total Organic Carbon) L series analyzer.

Degree of pigmentation

We noted that the intense pigmentation observed in field collected *Philodina* gradually dissipated during laboratory culture. This allowed us to obtain rotifers with varying degrees of pigmentation. To categorize pigmentation level, bdelloids were induced to form xerosomes by adding 10 drops of 1X Dulbecco's phosphate-buffered saline (DPBS; Hecox-Lea and Mark Welch 2018). Images were captured using a SPOT Imaging Insight CMOS camera and SPOT software version 5.6, then analyzed using ImageJ version 1.33 and the RGB plug-in downloaded from the NIH website (<https://imagej.nih.gov/ij/plugins/>; Rasband 2009). The RGB plug-in converts images to digital numbers (DN) corresponding to red, green, or blue channels. The color spectrum for each channel ranges from dark to light, high DN numbers indicate a lighter color. The percentage of red DN in bdelloid xerosomes was calculated using the following formula (Vrekoussis et al. 2009):

$$\% \text{Red DN} = \frac{\text{Red DN}}{(\text{Blue DN} + \text{Green DN} + \text{Red DN})} \times 100 \quad (1)$$

Before each experiment, 50 xerosomes were analyzed to determine pigmentation levels and assigned into one of the following categories: highly pigmented (HP), moderately (MP), lightly (LP), or non-pigmented (NP) based on number of days since field collection.

Experimental procedure: Pigmentation, desiccation, and UVB exposure

All treatments that involved desiccation and/or UVR exposure consisted of 16 replicates comprised of 50 adult bdelloids. Rotifers ($n = 10$) were placed on five pieces of approximately 1 × 1 cm GF/F glass fiber filter paper (0.45 µm) in a 60 × 15 mm glass Petri dish. Desiccations times and UVB intensities followed Martin (2017) and Caprioli and Ricci (2001). Briefly, dishes containing bdelloids were kept in a humidity chamber at 97% ± 2% relative humidity for 48 h, after which the relative humidity was dropped to between 45% and 35% for 1, 7, or 32 d. After desiccation, rotifers were exposed to UVB radiation (280–315 nm) and UVA radiation

(315–400 nm) that was emitted from a Spectroline® XX-15B lamp (120 V, 60 Hz, 0.7 A), equipped with 2 UVB bulbs yielding a spectral output of 280–400 nm, with peak output at 306 nm wavelength (Ushio G15T8E Hammond, IN). The lamp was suspended above the dishes in a low temperature, diurnal illumination incubator (VWR). All sources of white light were removed by lining surfaces with black plastic. Incubation temperature was maintained at 25 ± 1°C.

UVB intensities used for 2 h exposures were: 1.3 ± 0.05 (low), 3.7 ± 0.05 (mid), or 5.0 ± 0.05 (high) W m⁻². The low and mid intensities are based on average UVB intensities for the winter and summer in the El Paso region, respectively (McKenzie et al. 2004; Martin 2017; Sengupta et al. 2018). For low UVR treatment, the lamp was 18.5 cm above the dishes which were covered by Edmund Optics long pass 305 nm glass filters (SKU 45061; 5.08 cm²). These filters reflect wavelengths below 305 nm. For the mid treatment, a Prism Research Glass quartz disc cover (PQ-2062D-02; 5.08 cm²) was used to allow the entire spectrum of UVR emitted from the lamp (280 to 400 nm) to reach bdelloids. The space between dishes and UVB lamp was reduced to 16 cm to increase UVB intensity required for the high treatments. A UVA/B light meter (Sper Scientific Model 850009) was used to verify intensities, the meter reads wavelengths from 280 to 400 nm, with a range of 0.01 to 400 W m⁻² and is 96% ± 4% accurate at 25°C. Negative controls consisted of rotifers prepared in the same manner as those in exposure treatments but placed in a Styrofoam box covered in black plastic during exposures. Humidity was not controlled during the 2 h exposures. After UVR exposure, desiccated bdelloids were rehydrated using 5 mL of MBL medium and cultured under ambient light and temperature. In addition, hydrated bdelloids from each of the four pigmentation levels were also exposed to UVB intensities, with the addition of 5 mL of MBL during the exposure and an additional 5 mL was added after exposure. Recovery was based on visible movement of the trophi (jaws) 48 h after UVR exposure; otherwise, they were considered unviable.

Statistical analysis

Dissolved organic carbon

To determine whether DOC was predictive of the occurrence of pigmented bdelloids and if the monsoon season affected DOC concentration, a general linear regression model (Supporting Information Eq. S1) was implemented in R version 3.4.3 (R Core Team 2020) and RStudio version 1.0.136 (RStudio Team 2020).

Degree of pigmentation

A two-way ANOVA (Supporting Information Eq. S2) was used to evaluate whether there were differences in pigmentation levels among bdelloids cultured for varying lengths of time. The difference in pigmentation levels was based on the total DN and the red channel DN values of images of xerosomes. Following the ANOVA, Av Tukey honestly

significant difference (HSD) comparison tests were conducted to confirm differences in total DN and red DN among pigmentation categories. Statistical analyses were conducted using R version 3.4.3 (R Core Team 2020) and RStudio version 1.0.1366 (RStudio Team 2020).

Effects of pigmentation, desiccation, and UVB exposure

To determine the contribution of each main factors to survival we followed methods in Agresti (2019), using the logistic regression model where percent survival was the response variable, and pigmentation level and desiccation time were predictor variables (Supporting Information Eq. S3). Briefly, (1) the accuracy and predictive power of the logistic regression model was confirmed using receiver operating characteristic (ROC) and calibration curves, (2) Wald χ^2 was used to assess the significance of each treatment, (3) maximum likelihood estimates were used to establish the confidence intervals of each explanatory variable for use in the odds ratio test, and (4) multiple pairwise comparisons with the Bonferroni adjustment were conducted on odds ratios. These analyses were conducted using SAS/STAT version 14.3 for Windows 10 (2017, Cary, NC, USA. SAS®, UT Site license 70080468).

Results

Dissolved organic carbon

Rock pools with pigmented bdelloids had 7–11 mg L⁻¹ lower DOC concentrations than rock pools where pigmented bdelloids were not found (glm, $z = -3.59$, $p = 0.0003$; Table 2).

DOC concentrations in rock pools with pigmented bdelloids were 6.2 ± 3.4 mg L⁻¹ (mean $\pm$ standard deviation [SD]) during the monsoon season and 3.7 ± 1.8 mg L⁻¹ in the dry season (Table 1a) while those containing no pigmented bdelloids had 17.3 ± 8.7 and 11.0 ± 9.9 mg L⁻¹ DOC, respectively (Table 1b). Though DOC concentrations were dependent on season, this did not affect where pigmented bdelloids occurred (glm, $z = 2.00$, $p = 0.0453$; Table 2).

Degree of pigmentation

Immediately after collection, bdelloids were classified as highly pigmented (HP) and had the highest %red DN (mean $\pm$ SD: $46.0\% \pm 0.40\%$; Fig. 1a; Table 3; Supporting Information Table S3). After 2 weeks in the laboratory, rotifers that were considered as moderately pigmented (MP) had a %red DN of $39.3\% \pm 0.02\%$ (Fig. 1b; Table 3; Supporting Information Table S3). This coloration persisted for up to one additional week when rotifers lost most pigmentation, except for a red–orange tint that remained in the lining of their gut which was seen when cultured for up to 4 weeks; these rotifers were classified as lightly pigmented (LP) and had a %red DN of $37.2\% \pm 0.22\%$ (Fig. 1c; Table 3; Supporting Information Table S3). After more than 20 weeks in the lab, bdelloids retained a light orange tint in the lining of their gut and were classified as non-pigmented (NP) and the %red DN was

Table 1. Dissolved organic carbon (DOC) concentrations of rock pools with highly pigmented bdelloids at Hueco Tanks State Park and Historic Site, El Paso, Co., TX. DOC samples were taken during the monsoon season (MS; mid-June–September) and dry season (DS; October–early-June). Mean $\pm$ SD, reported for rock pools with (a) and without (b) pigmented bdelloids.

Rock Pool	MS DOC	<i>n</i>	DS DOC	<i>n</i>
(a)				
Al	14 $\pm$ 0.6	3	3 $\pm$ 0.3	3
Edge	4 $\pm$ 0.1	3	4 $\pm$ 0.0	2
Enrique	4 $\pm$ 0.0	2	3 $\pm$ 1.7	2
Jamie	6 $\pm$ 0.4	2	6 $\pm$ 0.2	2
Luisa	7 $\pm$ 2.0	3	5 $\pm$ 2.2	3
Paw	5 $\pm$ 2.2	5	4 $\pm$ 1.4	3
Sergio	4 $\pm$ 0.1	3	3 $\pm$ 1.8	3
Walsh	5 $\pm$ 1.4	4	4 $\pm$ 0.9	2
Mean	6 $\pm$ 3.4	26	4 $\pm$ 1.3	21
(b)				
Heart	11 $\pm$ 0.9	4	6 $\pm$ 3.2	3
North Temp	9 $\pm$ 0.1	2	4 $\pm$ 0.6	2
South Temp	30 $\pm$ 3.7	4	6 $\pm$ 0.3	3
Mean	17 $\pm$ 8.7	24	11 $\pm$ 9.9	13

$31.9\% \pm 0.04\%$ (Fig. 1d; Table 3; Supporting Information Table S3). The HP treatment contained 6.5%, 8.7%, and 9.7% more red pixels when compared to other pigmentation levels (MP, LP, NP, respectively) (Table 3; Supporting Information Table S3). A statistical difference in %red DN was seen among all four pigment levels (ANOVA: $F = 30.6$, $df = 6$, $p < 0.0001$; Tukey HSD pairwise comparisons tests are given in Supporting Information Table S5).

Pigmentation, desiccation, and UVB exposure

As expected, *Philodina* that were not desiccated or exposed to UVB radiation had the highest survival ($97\% \pm 3\%$). However, recovery after desiccation and UVB exposure showed a downward trend in survival as degree of pigmentation decreased and as desiccation time and UVB intensity increased (Fig. 2). The most variation in survival was observed in LP bdelloids when subjected to desiccation for either 1 or 7 d, including samples that were not exposed to UVB (Fig. 2). When

Table 2. Seasonal (monsoon vs. dry) concentration of dissolved organic carbon (DOC) in rock pools and its relation to occurrence of pigmented bdelloids at Hueco Tanks State Park and Historic Site, El Paso, Co., TX. est, estimate; SE, standard error.

Treatment	est $\pm$ SE	<i>z</i> value	Pr ($> z $)
Intercept	2.64 $\pm$ 0.65	4.04	< 0.0001
DOC	−0.40 $\pm$ 0.11	−3.59	0.0003
Monsoon	1.58 $\pm$ 0.79	2.00	0.0453

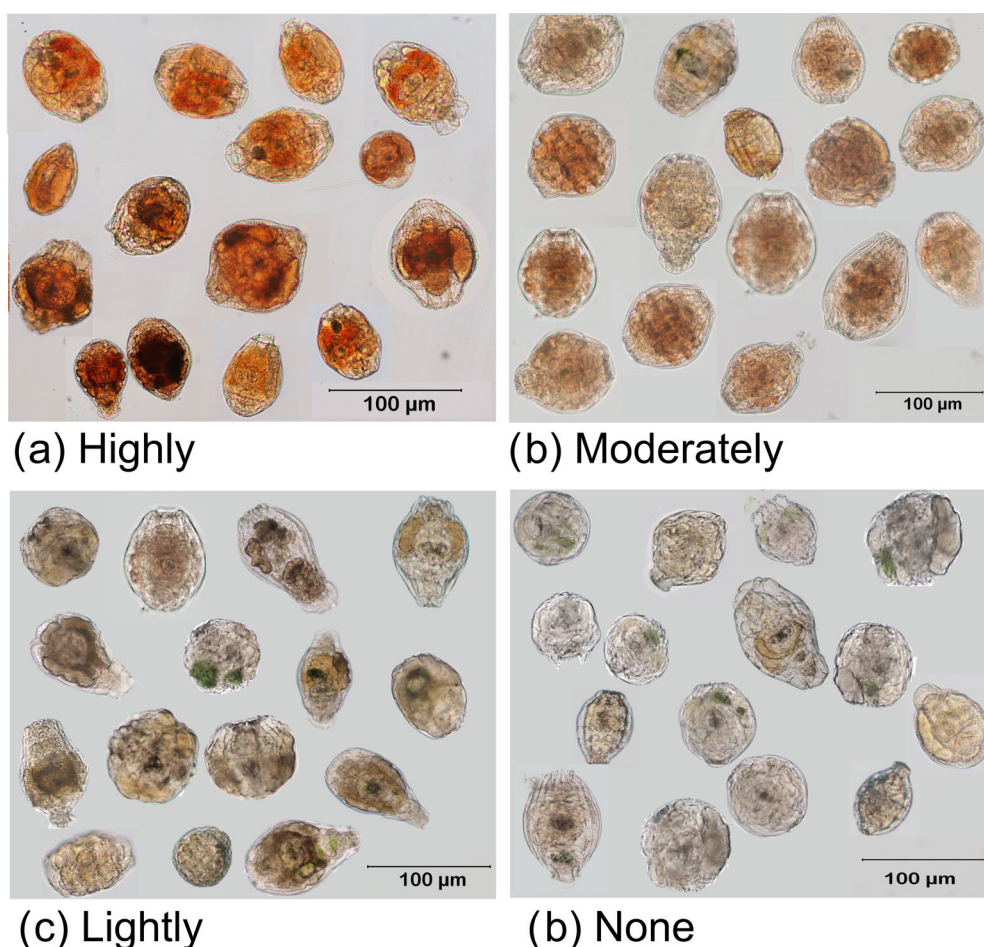


Fig. 1. Levels of pigmentation in *Philodina* xerosomes from field collections to > 20 weeks of laboratory culture. Pigment levels (a) field collected, highly pigmented (HP), (b) moderately pigmented (MP); cultured in lab for 2 weeks, (c) lightly pigmented (LP); 4 weeks in culture, or (d) non-pigmented (NP); > 20 weeks in culture.

desiccated for 32 d, almost no bdelloids recovered ($0.01\% \pm 0.01\%$). Including this treatment in the regression model resulted in increasing the type I error (e.g., introduced skew), so

Table 3. Digital numbers (DN) for the red channels were compared among the four pigmentation levels (highly, moderately, lightly, and non-pigmented) in *Philodina* from Hueco Tanks State Park and Historic Site, El Paso, Co., TX. Higher DN number indicates more white in the image. SD, standard deviation. Letter indicates significant difference at $p < 0.0001$. ANOVA Supporting Information Table S4, post hoc Tukey Supporting Information Table S5.

Pigmentation level	red DN $\pm$ SD	Total DN mean $\pm$ SD	% red DN $\pm$ SD
Highly	8041.6 $\pm$ 23 ^a	17,907.4 $\pm$ 51 ^a	46.0 $\pm$ 0.40
Moderately	9373.0 $\pm$ 19 ^b	24,308.1 $\pm$ 38 ^b	39.3 $\pm$ 0.02
Lightly	9823.2 $\pm$ 15 ^c	26,996.3 $\pm$ 49 ^c	37.2 $\pm$ 0.22
None	10,925.1 $\pm$ 13 ^d	32,570.3 $\pm$ 29 ^d	31.9 $\pm$ 0.04

it was omitted from odds ratio analysis (for results including this treatment see Supporting Information Table S6).

The logistic regression model was validated using both ROC (0.86) and calibration (slope = 0.997) curves (Supporting Information Fig. S3). The Wald's χ^2 test determined that all treatments and their interactions significantly affected bdelloid survival (Table 4). We used odds ratio analysis with confidence levels established using maximum likelihood estimates (Supporting Information Table S7) to determine and visualize the relative contribution of pairs of treatments on bdelloid survival (Figs. 3–5).

Multiple comparisons of the mean effects of each treatment on survival showed that all treatments influenced bdelloids survival (Table 5). Odds ratio analysis showed how each treatment affected survival (Figs. 3–5). Instances where the confidence interval crosses the null effect line (odds ratio = 1.0) indicate that neither treatment had an influence on bdelloid survival. First, to interpret the effect that pigmentation had on survival, the odds ratio analysis was performed with other two factors (desiccation times and UVB intensities) controlled

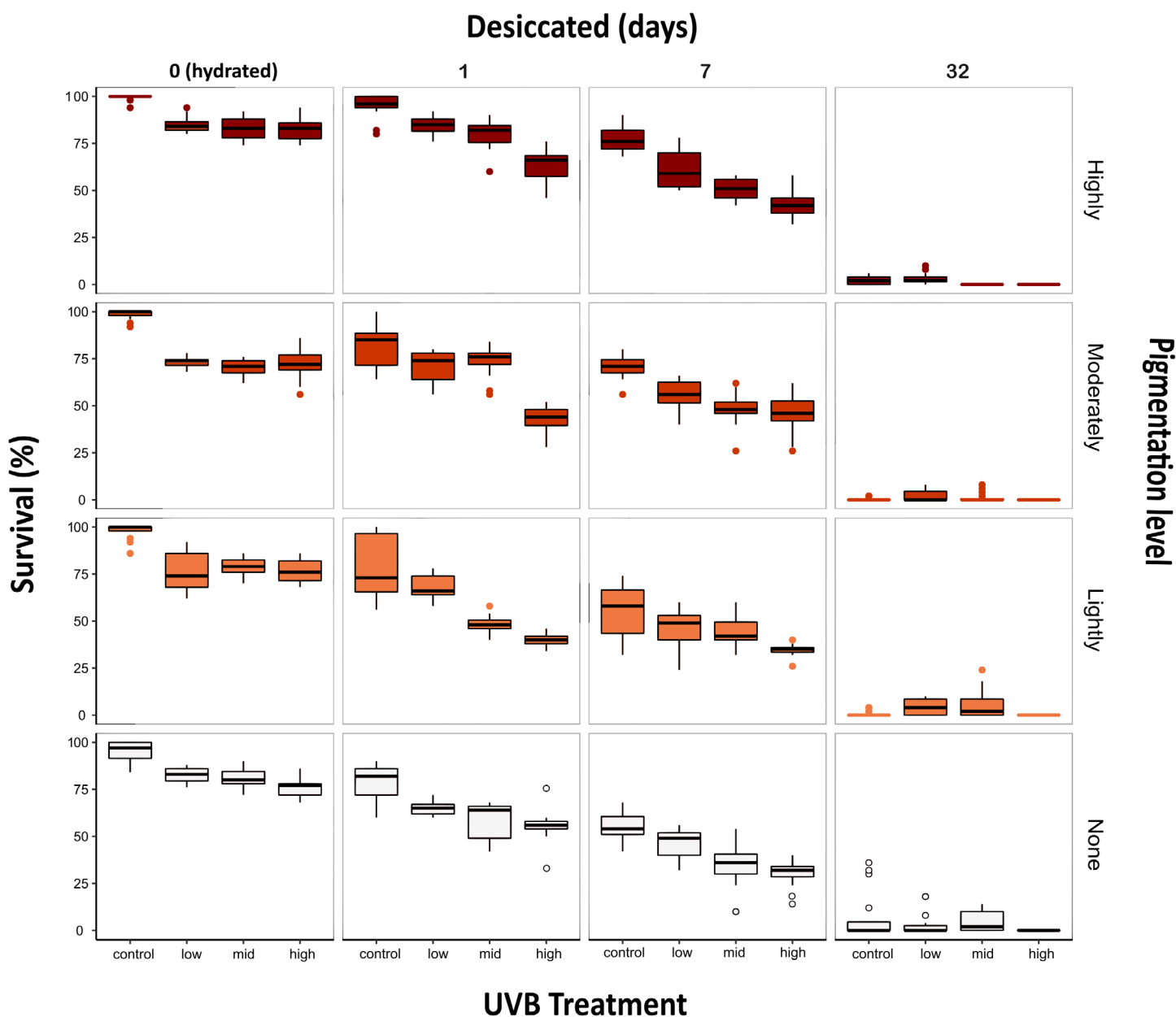


Fig. 2. Survival of *Philodina* after being desiccated for 0, 1, 7, or 32 d and then exposed to UVB intensities of 1.3 (low), 3.7 (mid) or 5.0 (high) $\pm 0.05 \text{ W m}^{-2}$. Vertical panels correspond to desiccation time; the horizontal row shows pigmentation levels (highly, moderately, lightly, and none). In boxplots, the horizontal bar is the median, the box shows the interquartile range, vertical lines represent values within 1.5 times the interquartile, and dots represent outliers.

(Fig. 3; Table 5a). For hydrated bdelloids, MP rotifers were not more likely to survive UVB exposure than LP (Fig. 3a) or NP (Fig. 3b). However, after being desiccated for 7 d, the MP treatment showed a 1.5-fold higher likelihood of survival compared to LP (Fig. 3a) or NP treatments (Fig. 3b). No significant difference in survival was observed between LP and NP bdelloids when they were hydrated or desiccated for 7 d (Fig. 3c). Highly pigmented (HP), hydrated bdelloids were at least twice as likely to survive UVB exposure than those with

LP (Fig. 3d), NP (Fig. 3e), and MP (Fig. 3f) pigmentation levels. Notably, the highest pigmentation level did not provide additional protection when the desiccation time was 7 d followed by UVB radiation exposure. Overall, HP treatments exhibited the highest odds of surviving desiccation and/or UVB exposure, except in the case when bdelloids were desiccated for 7 d where no significant difference was observed between survival of HP and MP bdelloids (odds ratio $\cong 1$; Fig. 3; Supporting Information Table S7).

Table 4. Wald chi-square analysis of *Philodina* survival following desiccation and UVB exposure. Degrees of freedom (df), probability (Pr).

	df	Wald χ^2	Pr > χ^2
Pigmentation level	3	99.0	< 0.0001
Desiccation time	3	1618.4	< 0.0001
UVB intensity	3	331.5	< 0.0001
Pigmentation $\times$ Desiccation	9	343.4	< 0.0001
Pigmentation $\times$ UVB	9	66.3	< 0.0001
Desiccation $\times$ UVB	9	361.1	< 0.0001

Next to investigate the impact of desiccation time on *Philodina* survival, an odds ratio analysis was performed with pigmentation level and UVB intensity controlled (Fig. 4; Table 5b).

The odds of survival for hydrated bdelloids and those desiccated for 1 d under UVB exposure were almost identical, as the odd ratio values ranged from 0 to 1.2 (Fig. 4a). Neither the hydrated nor the 1 d desiccation treatment had an impact on survival when exposed to low UVB radiation (Fig. 4a) for HP or MP bdelloids. Figure 4b compares the effects of 1 d versus 7 d desiccation, the odds ratio values ranged from 1.0 to 5.0 for each pigmentation level, and survival was highest in the control treatments and decreased with increasing UVR. *Philodina* that were desiccated for 1 d were 2.5 times more likely to survive UVB exposure when compared to those that were desiccated for 7 d (Fig. 4b), with odds ratio values ranging from 1.0 to 5.0. When comparing hydrated to bdelloids desiccated for 7 d, survival was negatively affected (Fig. 4c).

Finally, UVB intensity was set as the explanatory variable. The results showed that as the intensity of UVB radiation

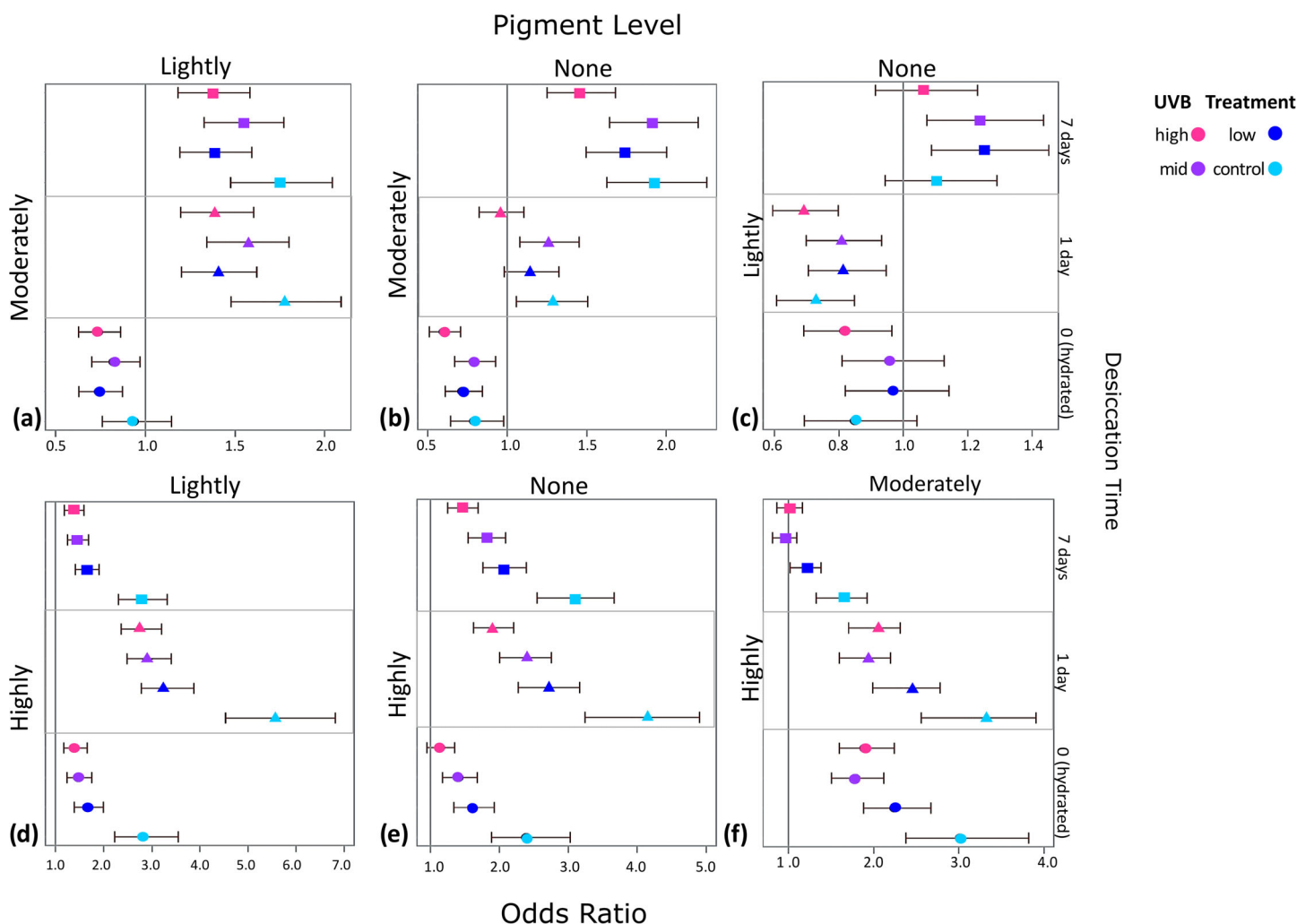


Fig. 3. Odds ratio analyses comparing survival of bdelloid rotifers with four different pigmentation levels following desiccation (0, 1, 7 d) and UVB exposure (low, mid, high). The solid-colored symbols represent the ratio estimate, and the horizontal lines denote the corresponding 95% confidence intervals. Data points located on the null line (odds ratio = 1) indicate no statistically significant effects on survival. When data points appear to the left of the null line (> 1.0), it signifies that the variable on the y-axis has a more pronounced effect on survival. Conversely, data points to the right of the null line (< 1.0) indicate a greater influence than the variable on the x-axis. Scales differ between comparisons.

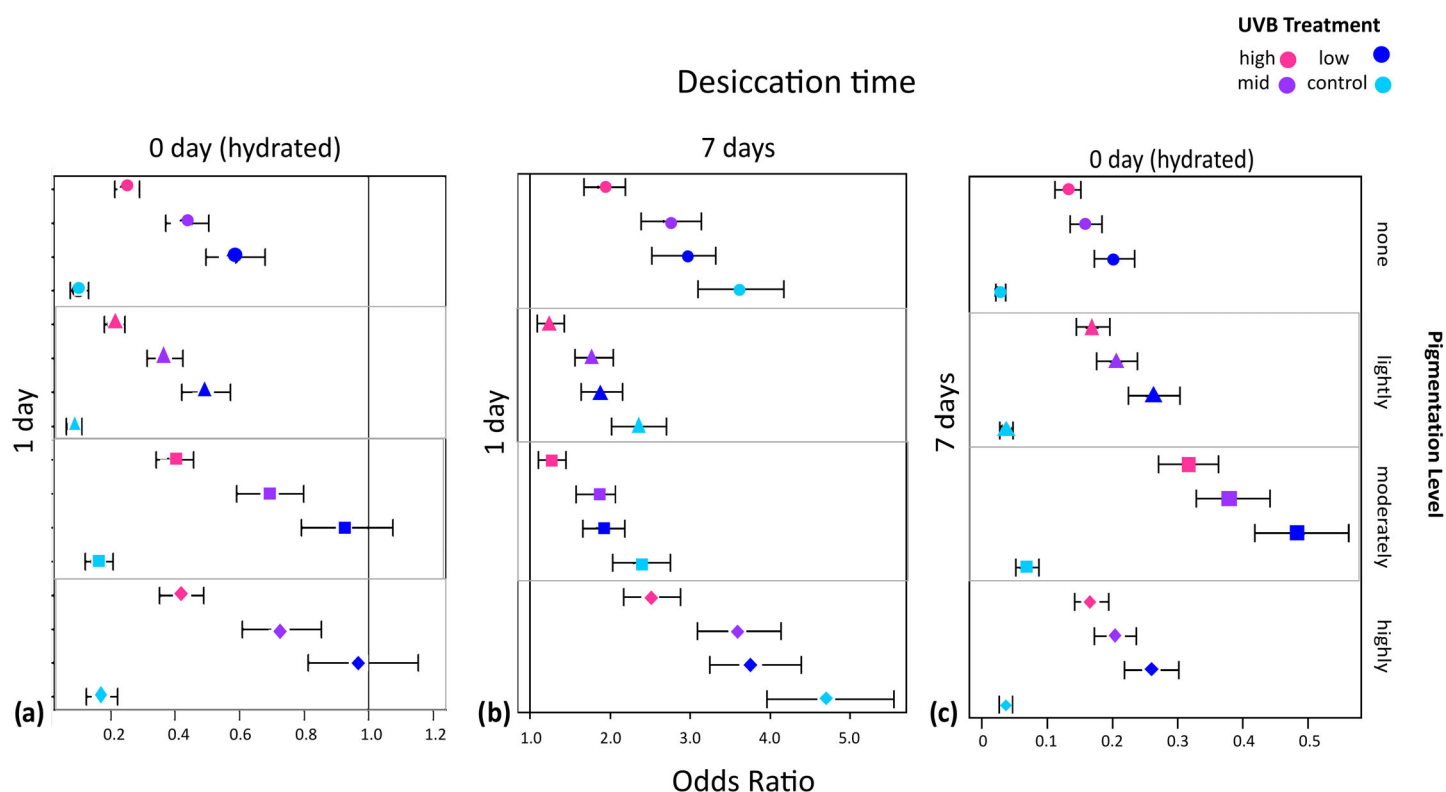


Fig. 4. Odds ratio analysis examining the impact of desiccation on bdelloid survival across various UVB intensity levels. The solid-colored symbols represent the ratio estimate, and the horizontal lines denote the corresponding 95% confidence intervals. Data points located on the null line (odds ratio = 1) indicate no statistically significant effects on survival. When data points appear to the left of the null line (> 1.0), it signifies that the variable on the y-axis has a more pronounced effect on survival. Conversely, data points to the right of the null line (< 1.0) indicate a greater influence than the variable on the x-axis. Scales differ between comparisons; in panel (c), the null line is not shown.

increased, the survival of bdelloids decreased (Fig. 5; Table 5c). This relationship is evident when bdelloids were exposed to various levels of UVB intensity (i.e., low—Fig. 5a, mid—Fig. 5b, or high—Fig. 5c) and subjected to desiccation for either 1- or 7 d. In all cases, the bdelloids were more likely to succumb to the effects of UVB radiation as compared to those not exposed to UVB. Bdelloids exposed to low UVB radiation (Fig. 5a,d,e) had a 1.2-fold higher likelihood of survival than those exposed to mid or high UVB intensity. Furthermore, as pigmentation levels decreased and desiccation time increased (Fig. 5d–f), the odds of survival decreased.

Discussion

Many studies have documented the benefits of pigmentation in cladocerans (Connelly et al. 2015; Leach et al. 2015; Alcocer et al. 2020) and copepods (Brüsin et al. 2016; Alcocer et al. 2020); however, no studies have yet explored these benefits in rotifers. This is surprising since many bdelloid species are brightly pigmented. Here we used a natural rock pool system to show that pools with low DOC concentrations and thus little absorption of UVB have pigmented bdelloids while those with higher DOC levels did not. In laboratory

experiments we found that pigmentation provides a survival advantage to bdelloids. Under laboratory conditions hydrated, highly pigmented bdelloids had double the survival odds compared to their non-pigmented counterparts when exposed to regional UV intensities. This survival advantage increased when bdelloids were desiccated for 1 day. These results lend merit to the possibility that pigments may enhance the ability of bdelloids to avoid damage caused by desiccation and UVB radiation exposure.

Many aquatic animals rely on DOC and/or diel movements for protection from UVR damage (Boeing et al. 2004; Leach et al. 2015). Prolonged periods of low DOC in an aquatic habitat will leave inhabitants vulnerable. In arid environments, where rainfall is < 50 cm a year, DOC concentrations are often much lower than in other regions (Tapia-Torres et al. 2015). In our study, DOC levels ranged from 3 to 30 mg L^{-1} , with rock pools at the low end of the range containing pigmented bdelloids while those at the high end did not. During the monsoon season, DOC levels were higher in rock pools, however, this did not affect the occurrence of pigmented bdelloids. Our hypothesis that pigmented bdelloids would be found in rock pools with lower DOC concentrations was supported by the negative relationship between DOC concentration and

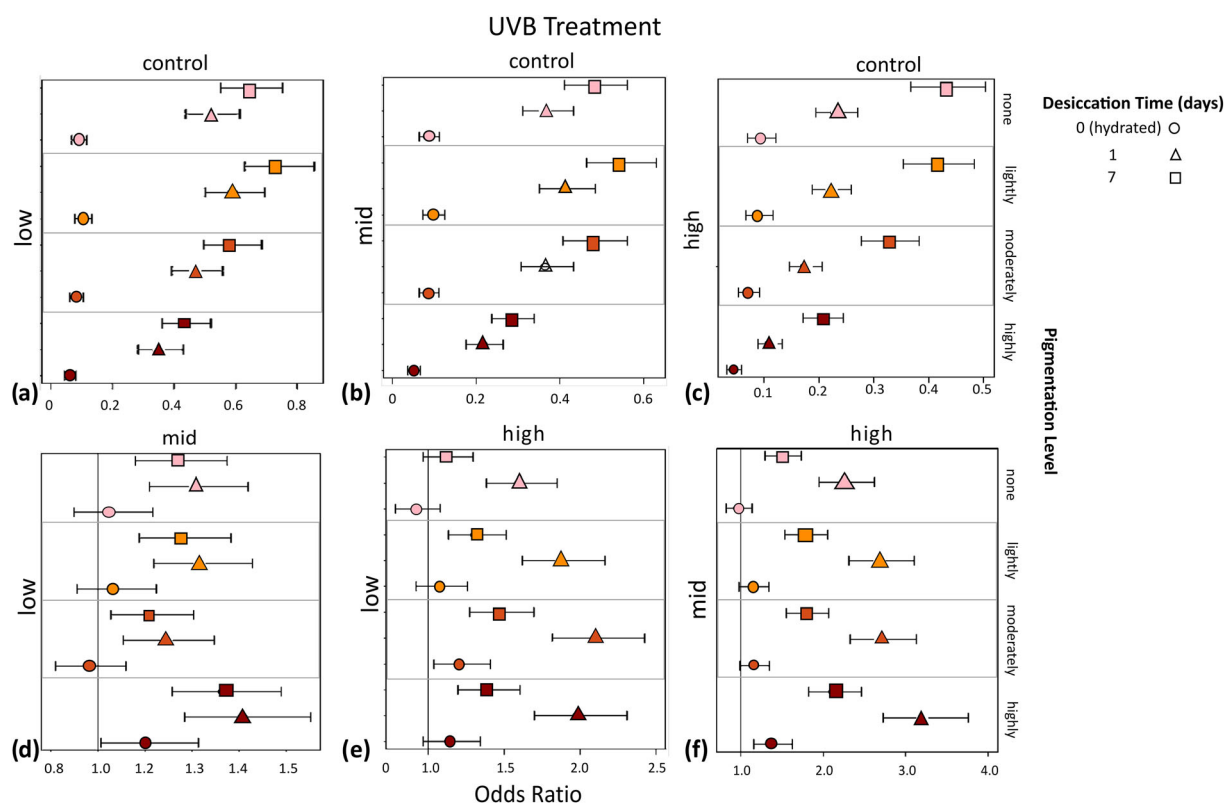


Fig. 5. Odds ratio analysis examining the impact of varying UVB radiation intensities on bdelloid survival, considering different pigmentation levels and desiccation times. The solid-colored symbols represent the ratio estimate, and the horizontal lines denote the corresponding 95% confidence intervals. Data points located on the null line (odds ratio = 1) indicate no statistically significant effects on survival. When data points appear to the left of the null line (> 1.0), it signifies that the variable on the y-axis has a more pronounced effect on survival. Conversely, data points to the right of the null line (< 1.0) indicate a greater influence than the variable on the x-axis. Scales differ between comparisons; in panels (a)–(c), the null line is not shown because it is shifted to the right far beyond the data points.

presence of highly pigmented bdelloids. Unpigmented bdelloids were found in rock pools with high DOC concentration or those that were mostly in shaded areas, which would limit exposure to UVR. These factors may negate the need for photoprotection by bdelloids inhabiting these rock pools.

In our study the low and mid UVB intensities used in exposure experiments were designed to simulate the typical UVB levels experienced during the winter and summer in west Texas. The high UVB treatment was deliberately set to an extreme UVB scenario, subjecting *Philodina* to intensities 1.4 times greater than the mean UVB levels observed during summer months. Extreme UVR events, often greater intensity than our high UVB exposure treatment, have been documented for the Atacama Desert for the past 5 yr (Cordero et al. 2023). Researchers in this area have documented some of the highest UVR intensities recorded on Earth (17.8–21.8 W m^{-2}). These exceptionally high UVB events have been attributed to several factors, including reduced ozone, high elevation (> 5900 m), and albedo enhancement. The high intensity treatment used in our study provides valuable insights into the potential resilience and adaptability of

Philodina under increasingly variable and intense UVR conditions that may occur as the climate changes and extreme events become more common.

Aquatic invertebrates with higher pigmentation levels are likely to have a survival advantage in regions with high UVB intensity (Brüsin et al. 2016; Ulbing et al. 2019). Many studies have shown that pigmentation provides photoprotection that can reduce damage from UVR exposure, but variation in degree of pigmentation can affect its efficiency (Brüsin et al. 2016; Bashevkin et al. 2020; Suma et al. 2020). Loss of pigmentation led to DNA damage after UVB exposure in the cladoceran *Daphnia melanica* Hebert, 1995 (Ulbing et al. 2019) and the copepods *Diaptomus castor* (Jurine, 1820) and *Eudiaptomus gracilis* (Sars G.O., 1863) (Brüsin et al. 2016). In these studies, animals with more melanin showed greater resistance to ecologically relevant levels of UVR damage. Similarly, the brownish-red pigmented tardigrade *Paramacrobrotus* sp. demonstrated greater resistance to UVR both in desiccated and hydrated states as compared to their non-pigmented counterparts (Altiero et al. 2011). Suma et al. (2020) further demonstrated that coating unpigmented

Table 5. Pairwise comparisons of odds ratio marginal main effects of each factor (pigmentation level, desiccation time, and UVB intensity). The effect of other two factors on bdelloid survival was averaged (see Figs. 3–5 for individual odds ratio values and confidence intervals). Adj, adjusted using Bonferroni corrections; OR, odds ratio.

		OR	Adj lower OR	Adj upper OR	Adj p value
(a) Pigmentation level					
HP	MP	1.79	1.62	1.97	< 0.0001
HP	LP	2.18	1.98	2.41	< 0.0001
HP	NP	2.04	1.85	2.25	< 0.0001
MP	LP	1.22	1.12	1.33	< 0.0001
MP	NP	1.14	1.04	1.24	0.0009
LP	NP	0.93	0.86	1.02	0.1914
(b) Desiccation time					
1 d	7 d	2.35	2.20	2.50	< 0.0001
1 d	0 d	0.34	0.31	0.38	< 0.0001
7 d	0 d	0.15	0.13	0.16	< 0.0001
(c) UVB intensity					
Low UVB	Mid UVB	1.27	1.17	1.38	< 0.0001
Low UVB	High UVB	1.77	1.63	1.92	< 0.0001
Low UVB	Control UVB	0.28	0.25	0.32	< 0.0001
Mid UVB	High UVB	1.39	1.28	1.51	< 0.0001
Mid UVB	Control UVB	0.22	0.20	0.25	< 0.0001
High UVB	Control UVB	0.16	0.14	0.18	< 0.0001

tardigrades with pigment extracts doubled their survival after UVR exposure. They also noted that pigmented tardigrades fluoresced under UV light, indicating that the pigments could be transforming damaging UVR to unharmed rays. Pigmented bdelloids from Hueco Tanks also show fluorescence (MJB, pers. obs.) so their pigments may also be capable of absorbing UVR.

Enhanced levels of antioxidants and anti-mitotic activities found in the carotenoid extracts may reduce the damage incurred by UVR exposure (Snare et al. 2013; Suma et al. 2020). For example, when the monogonont *Brachionus manjavacas* Fontaneto, Giordani, Melone & Serra, 2007 was fed red algal extracts and then exposed to low doses of UVR, their lifespan was extended (Snare et al. 2013). Researchers have hypothesized that resistance to stressors by bdelloids is a by-product of the evolutionary adaptation of anhydrobiosis, though extended desiccation time results in DNA damage (Gladyshev and Meselson 2008; Hespeels et al. 2014, 2023). Bdelloid xerosomes are resistant to UVR exposure (Fischer et al. 2013; Zhu et al. 2021), gamma radiation (Gladyshev and Meselson 2008; Krisko et al. 2012), and x-rays (Hespeels et al. 2020). Evidence of DNA strand breaks have been reported in *A. vaga* when desiccated for 7 d, while no damage or increased mortality was seen in those desiccated for 1 day (Ricci and Caprioli 2005; Hespeels et al. 2014). These results were mirrored in our study where fewer *Philodina* recovered

from desiccation and UVR exposure when desiccated for 7 d versus 1 d.

Fischer et al. (2013) found that hydrated rotifers were more susceptible to UVR exposure than those desiccated for 3.5 d in a laboratory culture of the red pigmented *Philodina roseola* (Ehrenberg, 1832). The authors speculated that *P. roseola* was able to repair DNA damage as it emerged from the xerosome, leaving hydrated samples more vulnerable to UVR. However, the level of pigmentation of *P. roseola* and the length of culture before UVR exposure were reported. In our study hydrated bdelloids had greater odds of surviving mid and high UVR intensities at all pigmentation levels. The differences between these results may be explained by (1) duration of laboratory culture before testing, (2) differences in levels of pigmentation between the species, or (3) *P. roseola* responds differently to desiccation and/or UVR than the *Philodina* species used in our study.

In contrast to our laboratory experiments, bdelloids occurring in rock pools at Hueco Tanks often undergo desiccation for periods > 32 d and can form active populations after rainfall events. This discrepancy in survival may be explained by factors such as non-optimal diet before entering desiccation, humidity regime while entering and exiting desiccation, as well as a host of other abiotic and biotic conditions that vary between the laboratory and field. However, Caprioli and Ricci 2001 also found low survival in laboratory populations of *Macrotrachela quadricornifera* (Milne, 1886) which were desiccated for over an extended time (40 d). Interestingly Hespeels et al. (2014) found that rehydration after long term desiccation was more successful in bdelloids that aggregated. We observed a similar behavior in *Philodina* during pigmentation analysis as water was evaporating. However, when bdelloids were placed on filter paper for UVR exposure, aggregations were not observed. It is unknown whether *Philodina* aggregate during drying in nature.

Inherently, small, temporary habitats like desert rock pools are highly dynamic systems where water levels and DOC concentrations are dependent on rainfall events and intensities. Precipitation events and lengths of droughts in the arid southwestern US are becoming more unpredictable in duration and intensity as the climate changes (Zhang et al. 2021). These changes will impact DOC levels in temporary habitats and increase UVR exposure to aquatic invertebrates. Our results illustrate the complex interactions of degree of pigmentation, desiccation, and UVR exposure on bdelloid survival and the need for a fuller understanding of the combined effects of climate stressors on aquatic communities.

Future directions

Red pigmented bdelloids are commonly observed in nature and have been identified in all four bdelloid families and 13 genera (Supporting Information Table S1). Many pigmented bdelloids are found in temporary habitats, such as rock pools, that are exposed to high intensities of UVR. Like

copepods and tardigrades, red pigmentation in bdelloids is likely derived from their food sources. In our study this was supported by the observation that red coloration was not retained after *Philodina* was removed from their environment and fed a mixture of green algae. However, after 20 weeks in culture *Philodina* retained some red pigmentation around the lining of the gut. From these observations, we can make three inferences: (1) bdelloids themselves can produce at least low levels of pigment independent of their diet, (2) laboratory diet contained very low levels of carotenoids or their precursors, or (3) the laboratory environment did not elicit production of the photoprotective pigments. In addition, we did not see an increase in pigmentation following UV exposure. Further research is required to confirm that bdelloids acquire pigmentation through their food sources. In our study humidity was adjusted as bdelloids entered the dormant form, but not as they exited. To optimize the number of bdelloids that successfully emerge from their xerosomes, future studies could gradually adjust nutrient availability and temperature along with humidity as bdelloids enter and exit the dormant stage. Finally, in addition to *Philodina*, many inhabitants of rock pools are brightly pigmented including flatworms, nauplii of fairy shrimp, clam shrimp, tadpole shrimp, and ostracods. It would be interesting to compare whether the findings here hold across this broad array of phylogenetically diverse taxa.

Data availability statement

All data is available upon request.

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Conflict of Interest

None declared.

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