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Photoprotective benefits of pigmentation in the transparent plankton community: a comparative species experimental test

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Abstract. Plankton live under the countervailing selective pressures of predation and ultraviolet radiation (UVR). In lakes, zooplankton are transparent reducing visibility to predatory fishes but are pigmented in the absence of fishes, hypothetically reducing UVR damage. In the sea, planktivorous fishes are widespread, so plankton typically are transparent and ascend to productive surface waters at night to forage and descend during the day to reduce visibility to predators. However, larvae of some species face the unique constraint of traveling in surface currents in the daytime during migrations between adult and larval habitats. We would expect these larvae to be transparent since companion studies demonstrated increased predation risk of pigmented larvae under strong sunlight. Paradoxically, larvae range from being darkly to lightly pigmented. We hypothesize that some larvae are more heavily pigmented to reduce UVR damage, while other species travelling in subsurface currents with low UVR might be more transparent. Linking larval morphology to depth-dependent selective pressures would add a key element to help improve predictions of larval vertical distributions, which are important for simulating larval transport trajectories. We quantitatively tested the hypothesis that selection may have favored photoprotective pigmentation for larvae in the predominantly transparent plankton community while testing the differential effects of UVA and UVB radiation. We measured larval pigmentation of 12 species of crabs and exposed them to visible light only, visible + UVA, or visible + UVA + UVB in the tropics. Controlling for phylogeny, more pigmented species survived UVR better than less pigmented species, especially on sunnier days, though intraspecific comparisons for four species were equivocal. Most species died even from UVA exposure, which has long been regarded as relatively harmless despite penetrating deeper underwater than UVB. Thus, we demonstrate with a phylogenetically controlled analysis that crab larvae are pigmented in the predominantly transparent planktonic community to protect from UVR, improving our understanding of the selective forces acting on animal coloration and the factors determining planktonic distributions, survival, and dispersal. This linkage of morphology with susceptibility will be important for developing mechanistic models of environmental stress responses to better predict larval dispersal in current and future climates.

Key words: *Brachyura; coloration; dispersal; larvae; marine; plankton; transparency; ultraviolet radiation.*

Introduction

On land, almost all animals have some coloration, whether brilliant or drab. This coloration is often thought to be driven by selection for camouflage or inter- or intraspecific signaling. Some moths are colored and patterned to match tree bark when in the correct orientation (Webster et al. 2009), butterflies are brightly colored to warn potential predators of accumulated toxins or to mimic the warning colorations of other species to trick predators into avoiding them (Bates 1862), and male peafowl display dazzlingly colored and patterned rump feathers to attract females, even though these feathers increase predation risk (Zahavi 1975).

But in the open water of the pelagic zones of seas and lakes, the absence of color (transparency) is thought to provide the best protection (McFall-Ngai 1990, Johnsen 2001). Thus, many animals inhabiting this realm are mostly transparent, ranging from huge cephalopods and jellyfish to microscopic copepods and free-floating planktonic larval stages of many animals (McFall-Ngai 1990, Johnsen 2001, 2014).

Yet, not all pelagic animals are transparent and few are fully transparent; most have at least some pigmentation, even though this pigmentation often increases the risk from visual predators (Hairston 1979a, Luecke and

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O'Brien 1981, Utne-Palm 1999). Thus, transparency must impose some cost or pigmentation some benefit for pelagic animals. The advantage of pigmentation could be related to the inadequacy of transparency as camouflage (Johnsen 2003) or inter- or intraspecific signaling. Most marine animals have pigmented pelagic larval stages that feed and grow in the plankton community for days to months before metamorphosing to juveniles. The colors of these reproductively immature life stages are unaffected by sexual selection, and aposematism (warning coloration) does not apply to most species that lack chemical defense (such as our study species; Bashevkin and Morgan 2019). Thus, neither inter- nor intraspecific signaling are likely explanations for the pigmentation of otherwise transparent larvae. Protection from ultraviolet radiation (UVR) is another less considered alternative that could apply to all zooplankton. Pigments often absorb in the ultraviolet as well as the visible range (Bandaranayake 2006), so the ubiquitous transparency of planktonic animals may increase their risk of damage from UVR.

In freshwater habitats, more pigmented individuals were shown to be better protected from photodamage than less pigmented individuals of the same species of copepods (Hairston 1976, 1979b) or *Daphnia* (Luecke and O'Brien 1983, Herbert and Emery 1990, Hessen 1996, Hessen et al. 1999). More pigmented morphs occur in clear ponds and lakes at high altitudes and latitudes where the risk of photodamage is high (Herbert and Emery 1990, Hansson et al. 2007) and visual predators are absent (Hairston 1976, Hylander et al. 2012).

In the sea, visual predators are widespread, so zooplankton typically are transparent and undertake diel vertical migrations, ascending to productive surface waters at night to forage and descending during the day to reduce visibility to predators (Williamson et al. 2011). However, larvae of some species face the unique constraint of migrating between adult and larval habitats in surface currents during the daytime where they are more susceptible to UVR and predation (Morgan and Christy 1996, Hovel and Morgan 1999, Morgan and Anastasia 2008). Larvae can be colorful and darkly pigmented, with shades of red, orange, green, yellow, blue, and brown, and have been proposed to be more heavily pigmented to reduce the UVR damage they encounter in dangerous surface waters (Morgan and Christy 1996, Hovel and Morgan 1999).

Few studies have been conducted on the costs and benefits of visible pigmentation in larvae and other marine zooplankton. Crab larvae occupy surface waters exposed to UVR (Morgan and Anastasia 2008) and face strong visual predation pressure from fish (Bashevkin and Morgan 2019) driving the evolution of defensive traits (Morgan 1989). In companion studies, we examined the cost of pigmentation in increasing fish predation on crab larvae. We found that more pigmented species of tropical crab larvae are more susceptible to a planktivorous fish in the tropics on sunnier days (S. M. Bashevkin, J. H. Christy, and S. G. Morgan, *unpublished manuscript*) and that experimentally reduced pigmentation reduces the susceptibility of temperate crab larvae to planktivorous fishes under natural sunlight (S. M. Bashevkin, et al., *unpublished manuscript*). In this study, we focus on the potential benefits of pigmentation in protecting from UVR. Previous studies on UVR and pigmentation in crab larvae have found conflicting results. Morgan and Christy (1996) studying four species of tropical crab larvae found that species with the darkest coloration were most resistant to UVR damage, whereas Hovel and Morgan (1999) studying three species of temperate crab larvae found that the darkest species tested were among the most susceptible to UVR damage. These contrasting findings have left as an open question the efficacy of larval pigmentation in reducing UVR damage. Furthermore, no study in any aquatic system has yet established a formal link between quantified visible pigmentation level and UVR protection. There is no phylogenetic signal in crab larval pigmentation (S. M. Bashevkin, *unpublished data*) so our phylogenetically controlled approach will enable us to accurately test the evolutionary correlation between larval pigmentation and UVR susceptibility while controlling for other species traits that confer similar UVR susceptibility to related species.

Animals can also use invisible means to defend against damage from UVR. Mycosporine-like amino acids (MAAs) absorb UVR but not visible light, antioxidants can neutralize the damaging free radicals produced by UVR exposure, and DNA damage can be repaired with enzymes fueled by UVA and blue light energy or light-independent enzymes (Banaszak 2003). If these invisible protections are much more effective than visible pigments in protecting from UVR, then we would expect no relationship between visible pigmentation and UVR survival. However, if visible pigmentation is strong enough to predict UVR survival alone, our quick cost-effective technique to quantify pigmentation could vastly improve our ability to predict understudied zooplankton species' sensitivity to UVR.

We exposed crab larvae of 12 species to UVR in a quantitative and phylogenetically controlled test of the hypothesis that zooplankton visible pigmentation protects from UVR. Alternatively, if zooplankton rely more on invisible protections from UVR, we would expect no relationship between visible pigmentation and UVR susceptibility. To test the hypothesis that visible pigmentation alone can predict larval survival under UVR, we quantified the pigmentation of 12 tropical species of crab larvae and compared their mortality when exposed to natural levels of UVR, including different wavelengths, while controlling for phylogeny. Furthermore, intraspecific differences in pigmentation were observed in four of these species enabling us to test whether the same pattern held within as well as among species.

Methods

Study system

This study was conducted at the Smithsonian Tropical Research Institute's Galeta Marine Laboratory on the Caribbean coast of Panama (9°24′10.35″ N, 79°51′39.26″ W) in July–October 2015 and June–July 2016. Gravid females of 12 species of crabs were collected by hand (Fig. 1, Table 1) and identified to species aided by Rathbun (1918, 1925, 1930), Klompmaker et al. (2015), Abele (1976, 1992), and Crane (1975). Majoids and xanthoids were collected during the day, while all other species were primarily collected at night with a flashlight. Gravid females were then held until they released larvae in individual 1-L plastic containers partially submerged in a table with flowing seawater to maintain ambient temperatures. Each container was checked for freshly hatched larvae and the water was changed every morning.

Experiments were conducted on stage 1 zoeae since they are released into surface waters and swim upward to occupy surface waters (Epifanio and Cohen 2016) with the highest UVR levels. Our study species develop through two to six or more zoeal stages depending on the species for a total zoeal duration of 3–30 or more days before metamorphosing into the terminal megalopal stage. The pigmented chromatophores change little over larval development and can be used to identify larvae to species (Aikawa 1929, Webber and Wear 1981).

UVR survival experiments

To assess the protection afforded by pigmentation, we determined the susceptibility of larvae of each species to UVR damage. Experiments were conducted opportunistically with newly hatched larvae of all species available at the time (Table 1). Larvae were exposed outside to natural surface levels of either full sunlight (visible + UVA + UVB), visible + UVA, or visible (no UVR) light only. Freshly hatched larvae (stage 1 zoeae) were placed into compartmentalized trays (3.2 × 3.2 × 3.1 cm compartments filled halfway with 15 mL seawater), with four larvae of a single species per compartment, 18 compartments per tray, and three trays per UVR treatment. The unusually large larvae of one species, *Pitho laevigata,* were placed individually in compartments. This species was thus excluded from interspecific analyses (see Statistical analyses). The trays were floated in three polystyrene foam coolers (26 × 32 × 19 cm) filled with newly collected seawater to maintain ambient temperatures under intense sunlight. Water temperature was higher in the coolers on sunnier days, ranging from 22°C to 32°C, but more commonly between 24°C and 30°C. However, temperature was the same in all treatments and never exceeded natural water temperatures measured in the adjacent lagoon (S. Paton, *unpublished data*).

The three UVR treatments were haphazardly assigned to different coolers, and they were fabricated by covering the coolers with plastic filters with known transmission properties. In 2015, the coolers assigned to the visible + UVA + UVB treatment were left open, exposing larvae in trays directly to natural sunlight, while in 2016, these coolers were covered with visible and UV-transparent acrylic glass. In both years, coolers assigned to the other two UVR treatments were covered with a polyethylene terephthalate film filter that selectively blocks UVB transmitting visible + UVA, or a polycarbonate filter that blocks all UVR transmitting only visible light (Appendix S2: Fig. S1). Plastic filters were partially open at the sides to permit air circulation and avoid warming by the greenhouse effect. In 2015, when larvae in the visible + UVA + UVB treatment were exposed directly to the sun, the experiment was covered nightly and within 5 min of rain showers. Water in the coolers was replaced daily for the duration of the experiments. Experiments ran for 2–3 d. Mortality was recorded daily by examining the trays under a dissecting microscope.

This experimental design exposed larvae to intense UVR since they were covered in just 1.5 cm seawater. However, crab larvae are often found very close to the surface in the neuston (Roff et al. 1986, Epifanio et al. 1988) and UVR penetrates deeply, decaying slowly with depth, in the clear tropical waters where our study was conducted. For example, at a Jamaican reef, 10% surface irradiance persisted to 25 m and attenuation averaged 0.33 m^{-1} (Fleischmann 1989).

We occasionally noticed intraspecific differences in coloration among larvae of the same species hatched on the same day. In these cases, larvae were separated into two groups (lighter vs. darker color) and their UVR tolerance was tested as described above (Table 1).

Larvae were not fed during experiments to eliminate potential confounding effects of UVR on prey or larval foraging, because starvation was unlikely after 2–3 d. An additional experiment was performed to determine if fed *Armases ricordi* larvae survived UVR better than unfed larvae. This experiment was performed as above except that half the larvae were fed for 2 h on newly hatched *Artemia* nauplii before the experiment began (Guerao et al. 2007). There was no difference in UVR susceptibility or survival between fed and unfed larvae.

To account for the mortality of "control" larvae in the visible light treatment, relative survival was calculated for larvae in the visible + UVA and visible + UVA + UVB treatments by dividing the percent survival of each compartment by the average percent survival across compartments in the visible light treatment. Hereafter, this will be referred to as "relative survival," whereas "survival" will refer to the raw percent survival of larvae.

Concurrent with these experiments, light intensity was measured 100 m away at a monitoring station maintained by the Smithsonian Tropical Research Institute Physical Monitoring Program (S. Paton, *unpublished data*). Solar radiation was measured with two LiCor Model Li200x Pyranometers (Li-COR Lincoln, Lincoln, Nebraska, USA) every minute, and the data averaged every 15 min.

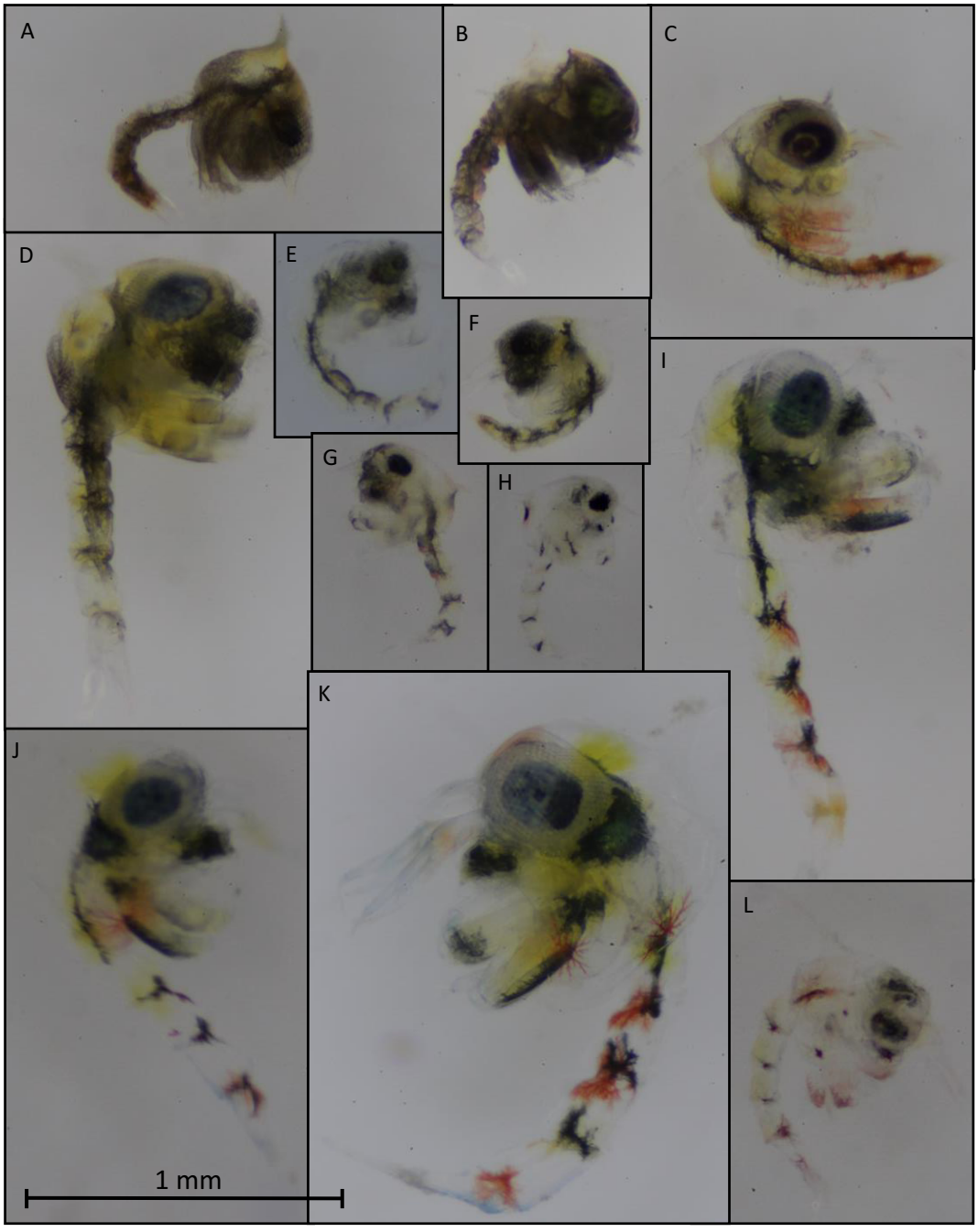

FIG. 1. Characteristic photos of study species against white backgrounds, similar to those used to calculate percent pigment cover. All photos are to the same scale. Photos may not exactly reflect average pigmentation for each species. (A) *Cyclograpsus integer*, (B) *Armases ricordi*, (C) *Grapsus grapsus*, (D) *Gecarcinus ruricola*, (E) *Cardisoma guanhumi*, (F) *Pachygrapsus transversus*, (G) *Minuca mordax*, (H) *Minuca rapax*, (I) *Mithraculus sculptus*, (J) *Omalacantha bicornuta*, (K) *Pitho laevigata*, (L) *Cataleptodius floridanus*.

Pigment cover

To determine the percent cover by pigment of larvae for each species, larvae were photographed under a dissecting microscope at 45× with a Canon EOS Rebel T3 Digital SLR Camera (Canon, Ōta, Tokyo, Japan) fitted with a microscope adapter. A single live larva was pipetted onto a depression slide, isolated in a few drops of seawater, and photographed while illuminated from above with white LED lights, against a white background. Larvae were photographed alive from the lateral view when still (Fig. 1). Since some larvae expand their chromatophores (pigment

Table 1. Species studied, number of intraspecific and total trials conducted on each species, and total number of larvae tested from each species. Most trials tested the survival of multiple species simultaneously.

Superfamily and species	Abbreviation	Intraspecific trials	Total trials	Larvae tested
Grapsoidea				
Cyclograpsus integer	CI		3	752
Armases ricordi	AR	1	14	4,316
Grapsus grapsus	GG		4	968
Pachygrapsus transversus	PT		2	536
Gecarcinus ruricola	GR		1	324
Cardisoma guanhumi	CG		1	216
Ocypodoidea				
Minuca mordax	MM		3	648
Minuca rapax	MR		4	1,048
Majoidea				
Mithraculus sculptus	MS	1	3	704
Omalacantha bicornuta	OB	3	7	2,292
Pitho laevigata	PL		1	108
Xanthoidea				
Cataleptodius floridanus	CF	1	2	628
Total		6	27	12,576

cells) in response to light (Pautsch 1967, Miner et al. 2000), larvae were left in the light for at least 30 min before being photographed. Sample sizes depended on larval availability, but usually five hatches of larvae from different females were photographed per species and 20 larvae were photographed from each hatch.

The proportion of pigment cover was quantified from the larval photographs with the image analysis program imageJ through the Fiji platform (Schindelin et al. 2012). Images were first converted to binary format, which transformed all pigmentation to black and all transparent segments to white, and the black (pigment) surface area was measured in this binary image. Then, the total exposed surface area of the larva was measured by tracing the larva in the original color photo. The proportion of pigment cover was calculated by dividing the pigment surface area by the total surface area of the larva. A pilot experiment with *Pachygrapsus crassipes* larvae from California demonstrated no effect of different lighting conditions on percent cover.

Our approach of photographing larvae and calculating percent pigment cover to quantifying pigmentation is very similar to the approach described by Siegenthaler et al. (2017) to study background matching in shrimp (Siegenthaler et al. 2018). This approach was found superior to traditional methods ranking chromatophore size with an index from 1 to 5 in speed, accuracy, and precision (Siegenthaler et al. 2017). Moreover, our approach was the best available option due to the small size of crab larvae and the limited equipment available at the remote Galeta Marine Laboratory, which provided unparalleled access to extraordinary crab diversity needed to conduct our comparative study.

Statistical analyses

Statistical models were fit in a Bayesian framework with Stan and the R package Rstan (Stan Development Team 2017). A Bayesian framework was chosen because the Stan Bayesian modeling framework provided the flexibility required to analyze repeated measures experimental data with continuous predictors and a phylogenetic correction. In addition, a Bayesian framework was the best approach for our complex random effects structure and unbalanced design due to its high flexibility and accuracy (Harville and Carriquiry 1992, Browne and Draper 2006, Bolker et al. 2009, Cressie et al. 2009, Gelman et al. 2013, McElreath 2015). Our priors were weakly informative as recommended by the package authors, using the Stan language manual (Stan Development Team 2016), Gelman and Hill (2006) and McElreath (2015) as references. Model results were robust to prior specifications. For each model, the diagnostics and posterior predictive checks were thoroughly inspected before proceeding.

We conducted a total of three binomial generalized linear mixed models (GLMMs). (1) To test the relationship between larval pigmentation and survival among species, we fit a model with a phylogenetically constrained random intercept (Appendix S1; Garamszegi 2014) and a random intercept for each replicate compartment. We included fixed effects for UVR treatment coded as an ordinal variable, average percent cover of pigment for each species, cumulative light exposure, days of exposure, and all interactions up to three-way. (2) To test the intraspecific relationship between pigmentation and UVR survival, we fit a model separately on each species with a random intercept for replicate and fixed effects for pigmentation (dark or light), cumulative light exposure (only for *Omalacantha bicornuta*), days of exposure, and all interactions. (3) To determine whether each species was sensitive to UVA or UVA + UVB, we fit separate models on each species identical to the former models except without a fixed effect for pigmentation. More details on the statistical and phylogenetic methods are available in Appendix S1 and the full Stan models are in Data S1 and Data S2.

To statistically test our hypotheses, each of the models was used to simulate the fitted values of survival over the range of treatment levels. These simulated data were then manipulated in the same way as the actual data to calculate relative survival values. The mean and 95% confidence intervals were then calculated from the resulting posterior distribution of relative survival at each parameter combination.

Results

Larval survival

Survival was usually high (>75%) after the first day of exposure in all treatments, and it often plummeted while becoming much more variable on the second day. Consistently, fewer larvae survived during sunnier than cloudier days (Appendix S2: Fig. S3) and UVR decreased survival more on sunnier days (Fig. 2).

Seven out of the 12 species tested (*A. ricordi, Grapsus grapsus, Pachygrapsus transversus, Minuca rapax, Mithraculus sculptus, O. bicornuta, Cataleptodius floridanus*) were significantly sensitive to UVA, and survival of these larvae exposed to visible + UVA was often intermediate between larvae exposed to visible and those exposed to visible + UVA + UVB (Fig. 2A; Appendix S2: Fig. S4, Table S2). Five species (*Cyclograpsus integer, Gecarcinus ruricola*, *Cardisoma guanhumi*, *Minuca mordax,* and *P. laevigata*) did not exhibit any more mortality in the visible + UVA treatment than in the visible control treatment. These species were also relatively resilient to UVB, even after 2 d of exposure (Fig. 2A; Appendix S2: Fig. S4, Table S2). These two groups of species with differing UVA sensitivities have similar average pigmentations and include both darkly and lightly pigmented species.

Interspecific pigmentation and survival

Species with more pigmented larvae were significantly better protected from UVR than species with more transparent larvae, as evidenced by the higher relative survival (survival of UVR exposed larvae divided by survival of visible light exposed larvae) of more pigmented species (Fig. 2B, C; Appendix S2: Table S3). However, the effect varied with the duration of exposure and cloud cover; the effect of pigmentation in protecting larvae from UVR was strongest on sunnier days after 2 d of exposure (Fig. 2C). Pigmentation had protective benefits against UVA and UVA + UVB (Fig. 2C). Although excluded from this analysis, *P. laevigata* exhibited higher relative survival (Fig. 2A) and higher pigmentation than the other majoid species (*O. bicornuta* and *M. sculptus*; Appendix S2: Fig. S2).

Intraspecific pigmentation and survival

Intraspecific differences in survival and relative survival became most apparent after 2 d of exposure (Fig. 3; Appendix S2: Fig. S5, Table S4). More pigmented individuals of *A. ricordi* larvae survived better under all light treatments, and the effect was greater after 2 d. In contrast, more pigmented larvae of *M. sculptus* survived slightly worse, but only in the Visible + UVA treatment after 2 d of exposure (Fig. 3B; Appendix S2: Fig. S5, Table S4).

Greater pigmentation significantly decreased relative survival for *O. bicornuta,* but the effect was only apparent after 2 d of exposure on sunny days (Fig. 3; Appendix S2: Table S4). In contrast, more pigmented *A. ricordi* and *M. sculptus* larvae trended nonsignificantly toward higher relative survival than less pigmented larvae of the same species. There was no impact of individual pigmentation on larval survival or relative survival only for *C. floridanus* (Fig. 3; Appendix S2: Fig. S5, Table S4). This species was generally very sensitive to UVR exposure and had little pigmentation (Fig. 2A; Appendix S2: Fig. S2).

Discussion

This study provides a rigorous test of the hypothesis that pigmentation protects from UVR in planktonic crab larvae. Previous studies provided some evidence for the protective power of pigmentation in zooplankton by qualitatively describing pigmentation difference and either comparing the UVR susceptibility of two to four species (Morgan and Christy 1996, Hovel and Morgan 1999, Kessler et al. 2008) or different phenotypes of one species (Hairston 1976, 1979*b*, Luecke and O'Brien 1983, Herbert and Emery 1990, Hansson et al. 2007, Hylander et al. 2012). Our study is the first to use a quantitative phylogenetically controlled approach to test the hypothesis that the transparency of zooplankton across species is directly related to their susceptibility to UVR. This finding suggests that pigmented larvae are free to utilize the entire water column while transparent larvae may be forced to descend to subsurface waters during the daytime to avoid damaging UVR. While invisible photoprotections, such as MAAs, may play a role in protecting crab larvae from UVR (Moresino and Helbling 2010), we found that visible pigmentation alone can predict larval susceptibility to UVR. Thus, knowledge of larval pigmentation may add a key element to help improve predictions of larval vertical distributions, which are important for simulating larval transport trajectories.

Ultraviolet radiation can induce significant mortality in zooplankton. For example, Hunter et al. (1982) estimated that 13% of all northern anchovy larvae die annually from UVR exposure in the temperate Southern California Bight, where UVR is much weaker than the tropics. A number of studies have documented lethal and sublethal impacts of UVR on marine larvae (Damkaer et al. 1980, Gleason and Wellington 1995, Morgan and Christy 1996, Hovel and Morgan 1999, Chiang et al. 2007, Moresino and Helbling 2010, Moresino et al. 2014) and even more have documented these impacts on freshwater and marine holoplankton (Bancroft et al. 2007, Häder et al. 2007).

Our study provides definitive evidence of the damage UVR causes underwater by demonstrating its lethal impact on the larvae of eight crab species. In addition, UVA killed seven species of crab larvae. UVA is generally regarded as less damaging than UVB and sometimes even beneficial since it can activate defensive pathways

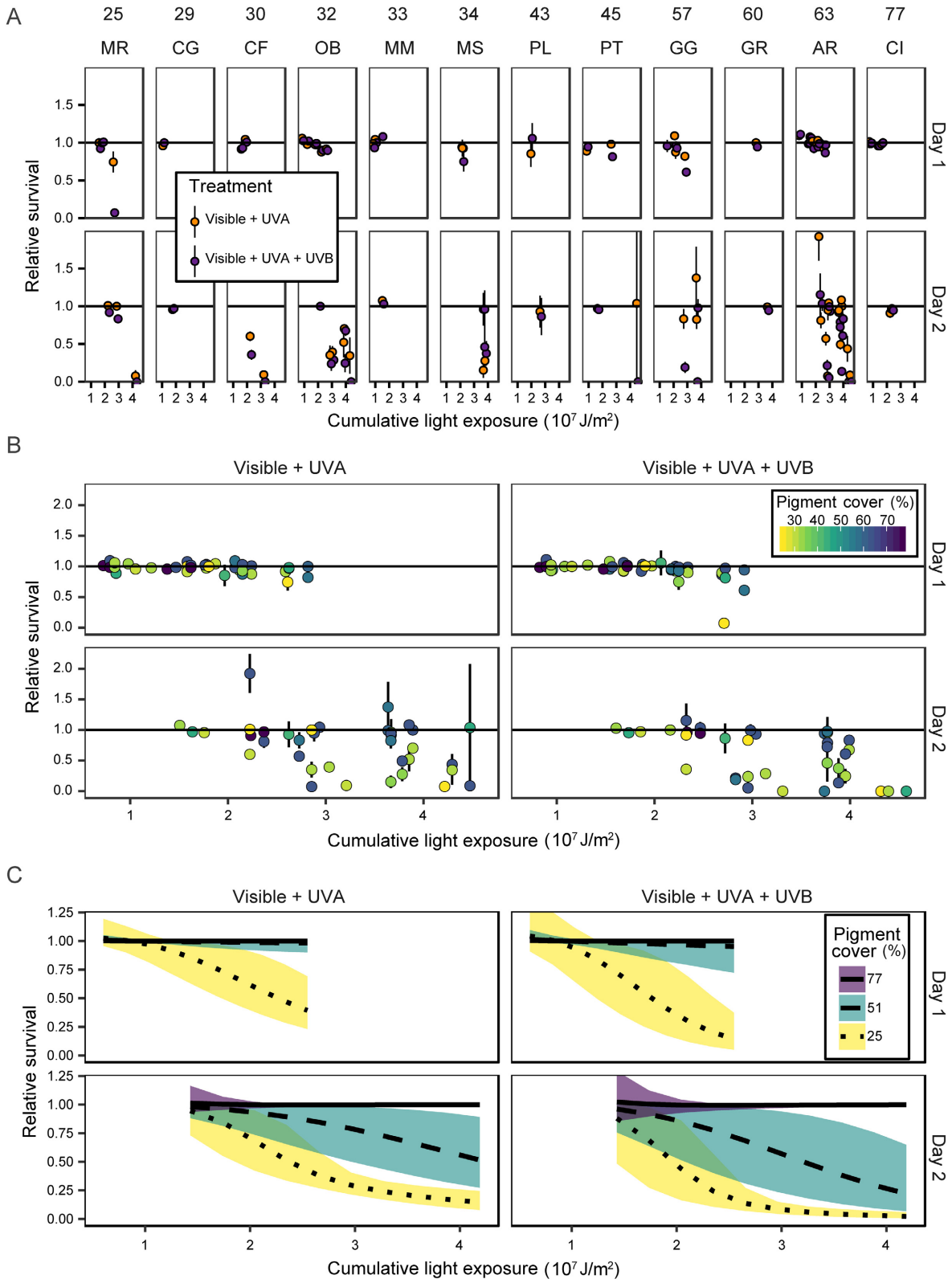

FIG. 2. Crab larval pigmentation and relative survival after exposure to either visible + UVA or visible + UVA + UVB. Relative survival was calculated by dividing the percent survival of each compartment by the average percent survival across

(Fig. 2. *Continued*)
compartments in the visible light treatment. Larvae were exposed to natural UVR in outdoor experiments. Relative survival (mean ± SE) arranged by (A) species ordered by pigment cover (numbers above plots) or (B) pigmentation. See Table 1 for species abbreviations. The horizontal line indicates relative survival = 1, where there is no difference in survival between the visible light exposed controls and the UVR exposed treatment larvae. (C) Relative survival of three simulated species of crabs with differing pigment coverage from a Bayesian generalized linear mixed model. Shading indicates 95% confidence intervals.

to repair UVR damage (Banaszak 2003, Häder et al. 2007). This is especially important because UVA penetrates much deeper than UVB. In clear tropical waters, UVA can penetrate as deep as green light to about 70 m while UVB only reaches about 25 m depth, the same as red light (Johnsen 2014). While UVB is stronger and induced higher mortality in our study and others (Häder et al. 2007), the much greater penetration of UVA and its lethal effects should discourage future studies from neglecting the physiological and ecological impacts of UVA.

The damaging effects of UVR on larvae combined with their reduced ability to avoid UVR via vertical migrations due to transport constraints may result in a strong selective pressure for protection from UVR, especially in the tropics where underwater UVR is strongest. Our study demonstrates a quantitative and phylogenetically controlled link between crab larval pigmentation and protection from UVR, thereby providing important evidence that the coloration of larvae evolved in response to mortality caused by exposure to UVR. The lack of a phylogenetic signal in crab larval pigmentation (S. M. Bashevkin, *unpublished data*) indicates that species are not limited in their pigmentation by their evolutionary history; rather, as selective forces shift (e.g., larvae are exposed to more UVR) pigmentation can change in tandem (e.g., larvae can become more darkly pigmented). Furthermore, crab larval chromatophores are generally located over important organs such as the nervous and digestive systems (Morgan and Christy 1996, Spitzner et al. 2018), supporting a photoprotective role.

In comparing larval pigmentation and survival within species, however, we did not find clear evidence that darker larvae were better protected from UVR, although there was a slight trend in this direction for *M. sculptus* and *A. ricordi*. In contrast, less pigmented *O. bicornuta* larvae were better protected from UVR than more pigmented individuals (Fig. 3B), indicating that other factors may be influencing differences in UVR susceptibility within species. In terms of overall survival, more pigmented *A. ricordi* larvae always had higher survival while more pigmented *M. sculptus* had lower survival, but only in the visible + UVA treatment at day 2. This may just be an issue of statistical power since *O. bicornuta* was the only species for which we were able to conduct multiple experiments over multiple days with differing weather conditions. However, the effect sizes for these intraspecific differences were also much lower than those from the interspecific comparisons (Figs. 2C, 3B). Further research is needed to determine the extent to which intraspecific differences in pigmentation affect susceptibility to UVR. This would have important implications for understanding trade-offs in maternal investment since pigments of freshly released larvae must be maternally derived (e.g., carotenoids that animals must obtain from food) or rely on maternally provisioned energy.

If pigments protect from UVR, how do transparent larvae survive in the field and why are not all larvae pigmented? The lack of phylogenetic signal in pigmentation among our study species (S. M. Bashevkin, *unpublished data*) indicates that the minimal pigmentation of some species is not related to species evolutionary history since pigmentation is equally likely to evolve anywhere on the phylogenetic tree. To survive, less pigmented larvae likely spend less time dispersing at the surface and more time at depth during the daytime. To address this question, the vertical distributions of crab larvae with differing pigmentations should be investigated in the field to determine whether more pigmented larvae spend more time at the surface during the daytime while more transparent larvae hide in deeper waters. If some species can successfully migrate without inhabiting surface waters during the daytime, then pigmentation may be unnecessary for those species and transparency could be favored by selection if pigments are energetically costly or increase visibility to predators (Hairston 1979*a*, 1981, Kerfoot 1982, Morgan and Christy 1996, Gorokhova et al. 2013). In our companion study, reef silversides selectively preyed on darkly pigmented larvae over lightly pigmented larvae only in the absence of UVR, which would give lightly pigmented larvae an increasing advantage with increasing depth. When UVR was present, fish showed no preference between darkly pigmented and lightly pigmented larvae (S. M. Bashevkin, J. H. Christy, and S. G. Morgan, *unpublished manuscript*), indicating that the lightly pigmented larvae may appear more pigmented in the presence of UVR since some planktivorous fish can see UVR (Losey et al. 1999). Thus, the combination of selection for camouflage and reduced energetic cost may favor transparency among crab larvae that spend the majority of their dispersal in deeper waters.

Another possibility is that larvae may be utilizing UVR-absorbing compounds that are invisible under visible light such as mycosporine-like amino acids (Moresino and Helbling 2010), but this may not be an effective strategy to avoid predation by some fish predators that can utilize UV-vision (Losey et al. 1999). Larvae may also use other invisible defenses such as antioxidants or repair enzymes (Hansson and Hylander 2009, Reef et al.

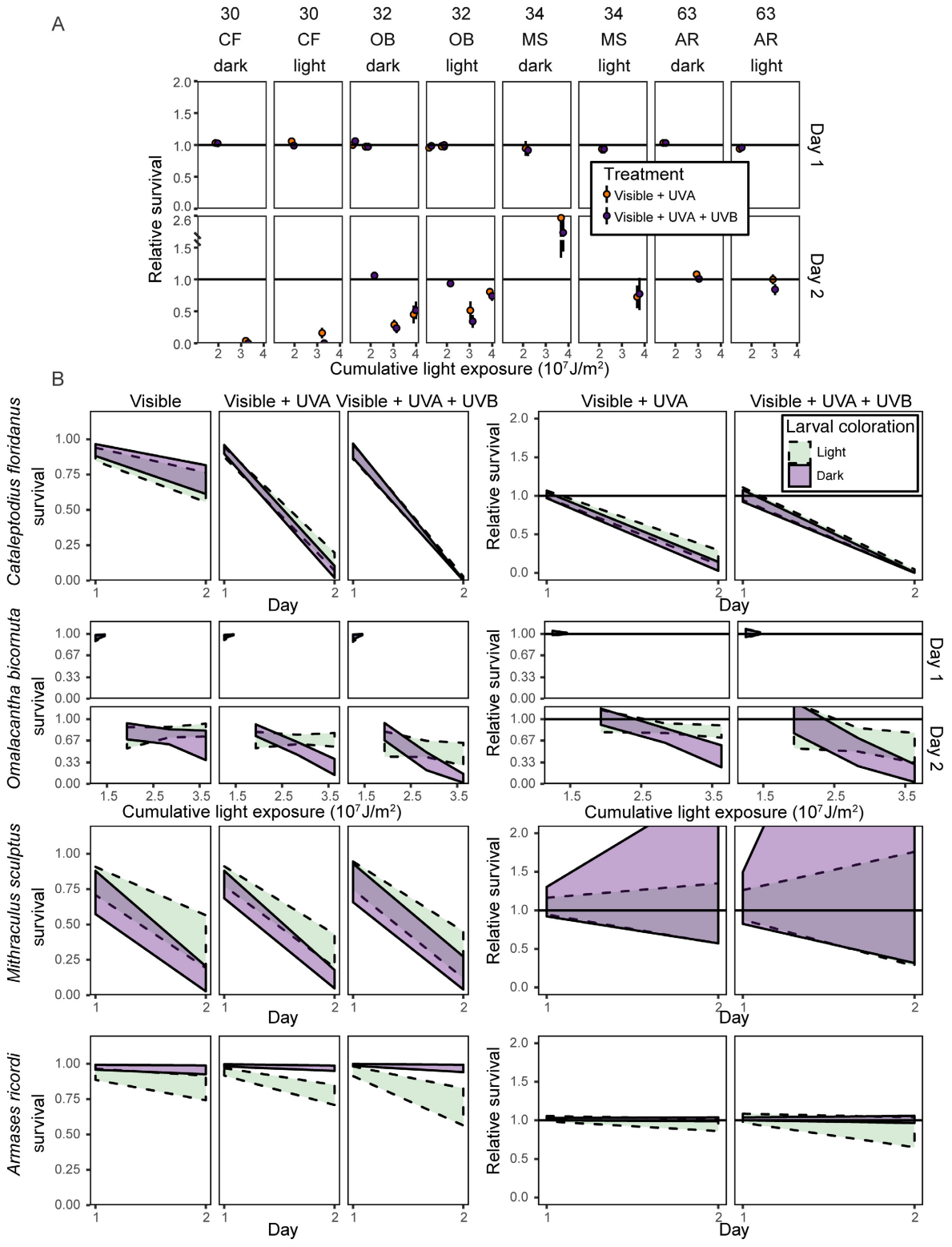

FIG. 3. Effects of intraspecific pigmentation differences on larval survival under UVR. (A) Relative survival (mean ± SE) of four species of crabs. See Fig. 2 for methods. (B) Survival (left column) and relative survival (right column) (±95% confidence intervals) of simulated larvae with intraspecific differences in pigmentation from each of the four species. Results were simulated from a Bayesian generalized linear mixed model. In the *Mithraculus sculptus* graph in the right column of panel B, the trend line for lightly pigmented larvae is entirely surrounded by the line for darkly pigmented larvae.

2009). These defenses may explain why some larval susceptibilities to UVR differed from expectations based on pigmentation (e.g., some *A. ricordi* hatches; Fig. 2A). Nevertheless, we were able to model UVR susceptibility based on visible pigmentation alone, potentially enabling us to utilize this more easily measured trait to predict UVR susceptibility. Furthermore, by controlling for phylogeny, we eliminated the influence of unmeasured and phylogenetically constrained species traits, further bolstering our evidence for the importance of pigmentation.

We demonstrated the advantage of pigmentation in planktonic crab larvae as protection from UVR. Larvae of seven of 12 species of crabs were vulnerable to mortality from UVA in addition to UVB with important implications for the depths these larvae would need to descend to avoid deadly UVR and the ecological importance of UVA, which has historically been dismissed. This study also advanced our understanding of the selective forces acting on animal coloration and vertical distributions of zooplankton. With a better understanding of the links between species traits and their resilience to stressors, we can construct mechanistic models of species responses to environmental stressors, which will be increasingly important in a changing ocean as organisms are exposed to increasing UVR (Ball et al. 2018) and novel environments.

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