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ORIGINAL PAPER

Seeing double: visual physiology of double-retina eye ontogeny in stomatopod crustaceans

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Abstract Stomatopod eye development is unusual among crustaceans. Just prior to metamorphosis, an adult retina and associated neuro-processing structures emerge adjacent to the existing material in the larval compound eye. Depending on the species, the duration of this double-retina eye can range from a few hours to several days. Although this developmental process occurs in all stomatopod species observed to date, the retinal physiology and extent to which each retina contributes to the animal's visual sensitivity during this transition phase is unknown. We investigated the visual physiology of stomatopod double retinas using microspectrophotometry and electroretinogram recordings from different developmental stages of the Western Atlantic species *Squilla empusa.* Though microspectrophotometry data were inconclusive, we found robust ERG responses in both larval and adult retinas at all sampled time points indicating that the adult retina responds to light from the very onset of its emergence. We also found evidence of an increase in the response dynamics with ontogeny as well as an increase in sensitivity of retinal tissue during the double-retina phase relative to single retinas. These data provide an initial investigation into the ontogeny of vision during stomatopod double-retina eye development.

Keywords *Squilla empusa* · Vision · Ontogeny · Electroretinogram · Microspectrophotometry

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Introduction

Many marine crustacean larvae have compound eyes that are distinct in structure and sensitivity from the adult compound eyes. Regardless of the adult eye type, many larvae (including those of euphausiids, decapods, and stomatopods) possess similar compound eye structures specialized for life in the pelagic environment, often including transparent apposition optics (Nilsson 1983) and a single spectral class of photoreceptors (Cronin and Jinks 2001). Transparent apposition eyes are specialized to minimize contrast of the retina by reducing the necessarily opaque pigments to a tiny ball at the center of the eye. This reduction is thought to reduce visual predation, which likely selects for a similar eye design among pelagic larvae from diverse crustacean taxa (Nilsson 1983).

When a crustacean larva undergoes metamorphosis, the animal must develop eyes that are specialized to meet the visual demands of the adult phase. Most crustacean eye metamorphoses result from a direct modification of the existing larval eye tissue into the adult structures, as evidenced by ontogenetic studies of euphausiids (Nilsson 1983; Nilsson et al. 1986), mysids (Nilsson et al. 1986), and decapods (Meyer-Rochow 1975; Nilsson 1983; Fincham 1984; Douglass and Forward 1989; Cronin et al. 1995; Gaten 1998; Jinks et al. 2002). Stomatopods, however, display a more unusual method of eye development, such that in a single stomatopod larval eye just prior to metamorphosis the adult retina and its associated optics emerge adjacent to, but separate from, the existing larval tissue (Fig. 1b, e; Williams et al. 1985; Cronin et al. 1995; Cronin and Jinks 2001; Schiff et al. 2007). Thus, a "double-retina" type eye persists through metamorphosis into the postlarval phase. Over time, the adult tissue will

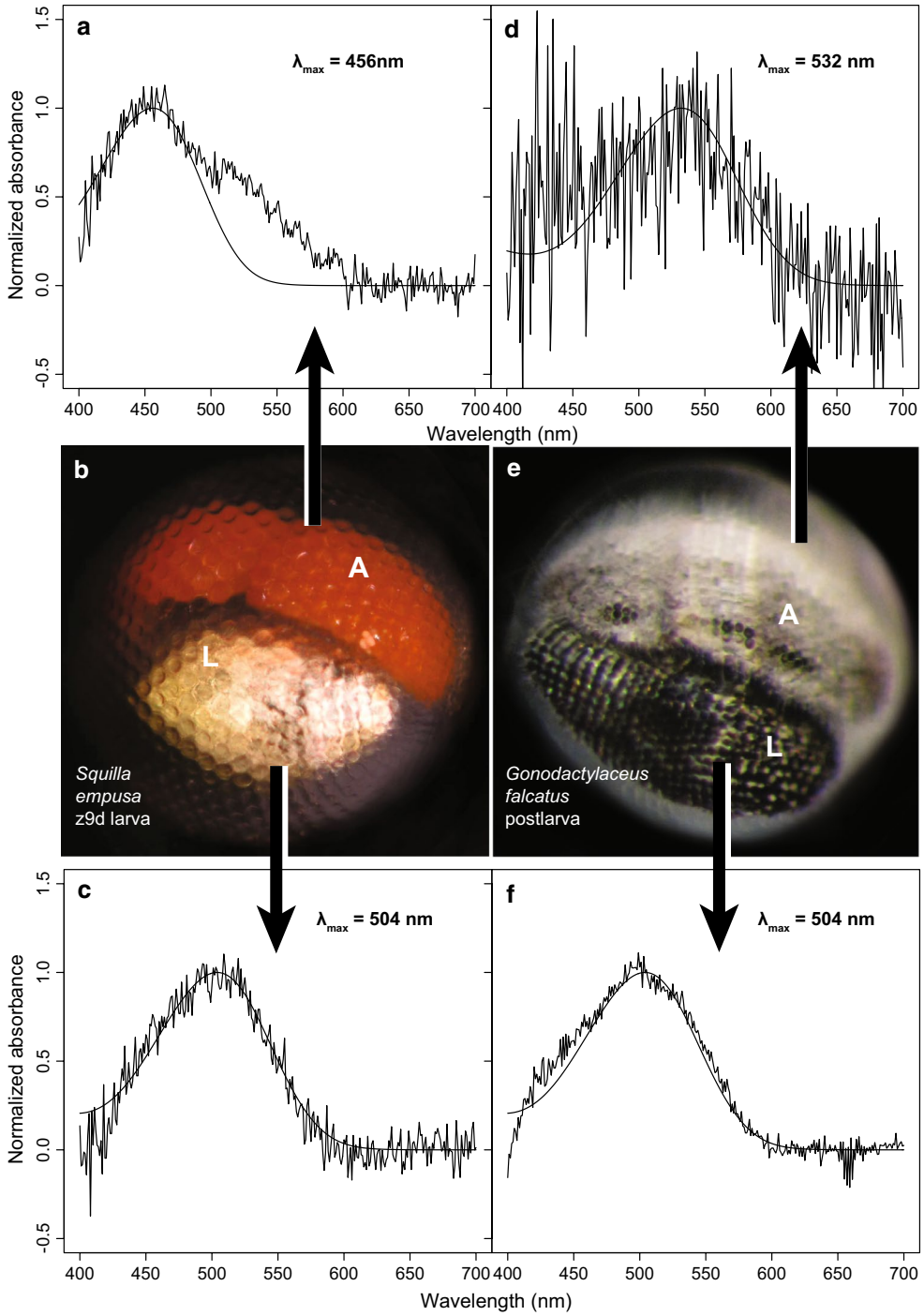

Fig. 1 Microspectrophotometric (MSP) data from double-retina eyes of *Squilla empusa* (**a**–**c**) and *Gonodactylaceus falcatus* (**d**–**f**). The data from each average spectral absorption curve (*jagged line*) are normalized and fit to a template for A1-visual pigments (Govardovskii et al. 2000) (*smooth line*). **a** Average adult retina absorption curve ($n = 3$). **b** Photograph of double-retina eye of *S. empusa* late z9d larval stage. **c** Average larval retina absorption curve ($n = 8$). **d** Average adult retina absorption curve ($n = 3$). **e** Photograph of double-retina eye of *G. falcatus* postlarva. **f** Average larval retina absorption curve ($n = 12$). *A* adult retina, *L* larval retina. *Arrows* link specific retinas in each photograph to their respective spectral absorption curve

continue to increase in size until it completely fills the volume of the eye, while the larval tissue simultaneously degenerates into obscurity.

The adult stomatopod retina itself is very unusual among crustaceans, which may play a part in its unique developmental process. Unlike larval eyes, which contain only a

single known class of photoreceptors, adult stomatopod eyes can possess up to 16 photoreceptor classes (for review see Marshall et al. 2007). A single adult compound eye is divided into a dorsal and ventral hemisphere by 2–6 rows of enlarged ommatidia along the equator of each eye. In the most elaborate 6-midband-row eyes, the specialized ommatidia of the midband detect a wide range of visible wavelengths (Marshall et al. 1991b), provide polychromatic ultraviolet vision (Marshall and Oberwinkler 1999; Bok et al. 2014), and also enable both linear (Marshall et al. 1991a) and circular (Chiou et al. 2008) polarization sensitivity. The complexity of an adult retina is so great that the existing larval structures may be an insufficient scaffold on which to build such a visual system, thus necessitating the evolution of the double-retina eye developmental scheme. Interestingly, this unique system of eye development is present in species with the two-midband row adult eye type, despite the presence of simpler ommatidia more similar to those of the larval eye. Two-midband-row eyes are likely derived from six-midband-row ancestors, which may explain retention of the double-retina eye development process in these species (Porter et al. 2010).

Aside from gross morphological descriptions of the phenomenon, very little is known about stomatopod double-retina eye development (Williams et al. 1985; Cronin et al. 1995; Jutte et al. 1998; Cronin and Jinks 2001; Schiff et al. 2007). The double-retina eye developmental process itself is highly varied among species in its onset and duration. *Heterosquilla tricarinata* (Superfamily: Lysiosquilloidea), a stomatopod from the temperate waters of the Otago region of New Zealand, does not present a pigmented adult retina until the fourth or fifth day of the first postlarval stage, with the entire double-retina period lasting more than 10 days (Williams et al. 1985). Lysiosquilloid species from the tropical waters of Lizard Island (Australia), including *Pullosquilla thomassini, Alachosquilla vicinia*, and *Lysiosquillina maculata,* present a small, pigmented adult retina in the terminal larval stage a few hours before their metamorphic molt, with the double-retina period persisting approximately five to seven days in the first postlarval stage (KD Feller pers. observ.). By contrast, larvae of the Squilloid species *Alima pacifica*, which are also found at Lizard Island, present a much more developed adult retina in the terminal larval stage, such that the existing larval and emerging adult retinas are of roughly equal size prior to metamorphosis. Thus, the double-retina phase in *A. pacifica* postlarvae is completed in a shorter period than other Lizard Island species, lasting approximately one to two days post metamorphosis. The full diversity and trends in the length of stomatopod double-retina development time periods are currently unknown, though eye development appears to be accelerated in the superfamily Squillidae (Ahyong et al. 2014).

While little is known about the morphological ontogeny of visual organs in stomatopods, even less is understood regarding the physiology of each of these two retinas in developing eyes. Based on our understanding of the anatomical organization of these eyes from previous work (Cronin et al. 1995), we hypothesized that the emerging adult retina does not become physiologically responsive to light until a critical point in the postlarval stage when the larval retina visibly begins to deteriorate. We tested this hypothesis using two methods: microspectrophotometry (MSP) and electroretinogram (ERG) recording. This paper presents an ontogenetic investigation into the visual physiology of the stomatopod eye development system.

Materials and methods

Animals

The primary target species for both MSP and ERG experiments was the Western Atlantic Ocean Squilloid, *Squilla empusa*, which was selected for several reasons. First, *S. empusa* larvae are abundant along the Eastern coast of the United States, and a range of stages is readily accessible at a field experimentation site, Duke University Marine Laboratory (Beaufort, NC, USA), during the summer months. Second, the complete description of the larval stages is published for *S. empusa* (Morgan and Provenzano 1979), which allows for visual identification and staging of captured animals in the field. Third and finally, adults of this species only contain a single class of photoreceptor (Trevino and Larimer 1969), similar in peak absorption (λ_{max}) to the larval retina (adult $\lambda_{max} = 507$ nm; Cronin 1985; larval $\lambda_{max} = 509$ nm; Cronin and Jinks 2001). Thus, for ERG experiments, a single light source will stimulate both larval and adult photoreceptors equally.

Squilla empusa larvae were captured during maximum current, nighttime flood tides using a stationary plankton net (500-μm-mesh, 0.75-m diameter) set near Beaufort Inlet, NC, USA (34°4′N; 76°4′W) in June 2013. Larval catches were brought to the laboratory and sorted by stage according to the morphological characters unique to each of the nine (I–IX) *S. empusa* larval stages (Morgan and Provenzano 1979). Individuals were contained in natural light/dark conditions in fingerbowls of ambient seawater (35 ppt salinity) that was changed daily. Larvae were fed *Artemia* nauplii once daily and monitored for eye development prior to experimentation. To examine ontogenetic changes in light intensity sensitivity in *S. empusa*, we measured retinal responses from a range of eye developmental stages: single-retina stage VIII (z8), single-retina early-stage IX (z9s), double-retina late stage IX (z9d; specified as either larval or adult retina), and double-retina postlarvae (PL).

A second species, *Gonodactylaceus falcatus* was included only in MSP experiments. Previous spectral sensitivity data are also available for separately measured *G. falcatus* larval and adult retinas, providing data with which to compare our double-retina measurements (Cronin et al. 1995). The animals used in this study were captured using a handheld underwater light source and dip-nets at Lizard Island Research Station (LIRS; Queensland, Australia) and identified using DNA barcoding of the cytochrome oxidase I gene from non-ocular tissue (KM982433; Feller et al. 2013). Due to its unavailability throughout the course of this study and general limits on developmental stages able to be captured and identified at Lizard Island, *G. falcatus* was not able to be included for investigation via ERG.

Microspectrophotometry (MSP)

MSP was used to characterize visual pigments expressed in individual rhabdoms, or photoreceptive units, located in either the larval or adult retinal regions of double-retina eyes. All tissue preparation and measurements were carried out either in the dark or under dim red room lighting. Each individual MSP preparation contained rhabdomeric cross-sections of both larval and adult retinas. MSP data from *S. empusa* z9d larvae were collected in 1996, using published methods (Cronin and Marshall 1989). MSP of *G. falcatus* postlarva was performed at LIRS in June, 2012 via similar methods and a portable MSP system (Loew 1982).

Electroretinogram (ERG) Recording

We measured the extracellular ERG response as a function of stimulus irradiance from the following *S. empusa* eye development stages: z8 ($n = 5$), z9s ($n = 6$), z9d larval retina ($n = 3$), z9d adult retina ($n = 3$), and PL adult retinas ($n = 2$). Recordings from late stage IX individuals with robust double-retina eyes (z9d) permitted us to collect and compute simultaneous, but separate, irradiance sensitivities for each larval or adult retina. While the PL eyes did contain the remnants of a larval retina, the area of the remaining larval tissue was too small for ERG recording, thus only adult retina recordings are reported for this stage.

The dorsal carapace and eyestalks of a single *S. empusa* larva were attached to a stationary pin mounted on an acrylic support using a cyanoacrylate gel adhesive. The animal was then suspended just below the surface of a seawater bath held at a constant temperature of 20 °C. The recording microelectrode was an epoxy-insulated and electrolytically sharpened tungsten wire (125 µm, 5–7 MΩ impedance; FHC, Bowdoinham, ME, USA) inserted subcorneally into the target retina. The tip of a second electrode was lowered into the seawater bath to serve as the differential reference. For recordings of double-retina eyes, a recording microelectrode was inserted subcorneally into the larval retina of one eye while a second recording microelectrode was inserted into the emerging adult retina of the other eye. A third microelectrode was lowered into the seawater bath near the animal to serve as the differential reference for both recording channels. The differential AC signals from all recordings were amplified and filtered (EXT-02B; NPI, Tamm, Germany), then digitized and stored for later analysis (PowerLab 25T, LabChart v.7 software; AD Instruments, Colorado Springs, CO, USA).

Specimen eyes were stimulated with a DC-regulated 150-W QTH lamp (Dolan Jenner DC-950; Boxborough, MA, USA) that was filtered to remove heat (#32-765 Edmund Optics, Barrington, NJ, USA) and restrict spectral composition of the stimulus light to blue wavelengths matching the visual sensitivity of *S. empusa* (Fig. 2a; Corning Glass Works 9-46, Corning, NY, USA). Irradiance was controlled by fused silica neutral density filters (Melles Griot, Rochester, NY, USA). The filtered light was coupled to a liquid light guide that emitted a circle of light large enough to simultaneously stimulate both eyes of the specimen. Details of stimulus delivery to the eye, irradiance control and calibration are provided elsewhere (Cohen et al. 2010). Animals were dark adapted in the experimental apparatus and given dim test flashes of low intensity light at five-minute intervals until a consistent response above background noise was established (approximately 50 µV) for a minimum of three consecutive flashes. A series of 75-ms experimental flashes at half-log irradiance steps was then delivered, from which a voltage log-intensity (*V*log*I*) curve was determined and modeled as described in Cohen et al. (2010; see Fig. 2b for example). Curves were accepted only from retinas that reached a minimum of 60 % of the calculated maximum value (V_{max}) during experimentation. Based on these criteria, the final data set included a proportion of curves from five individuals at 60 %, eight at 75 %, and seven at 90 % V_{max} recordings. To examine irradiance sensitivity in retinas at each developmental stage, the *V*log*I* data were averaged from all individuals of a given stage and used to calculate an average fitted curve (Fig. 3a).

To compare sensitivity among developmental stages, the log irradiance required to elicit 50 % of the maximum response (Log *K*), as well as the slope, was calculated from *V*log*I* curves modeled from each individually retina sampled for each stage (Fig. 3b, c). After establishing normal distribution of the data using a Shapiro–Wilk normality test, multiple comparisons of Log *K* and slope parameters at developmental time points were achieved using one-factor ANOVAs with Holm–Sidak post hoc testing ($\alpha = 0.05$;

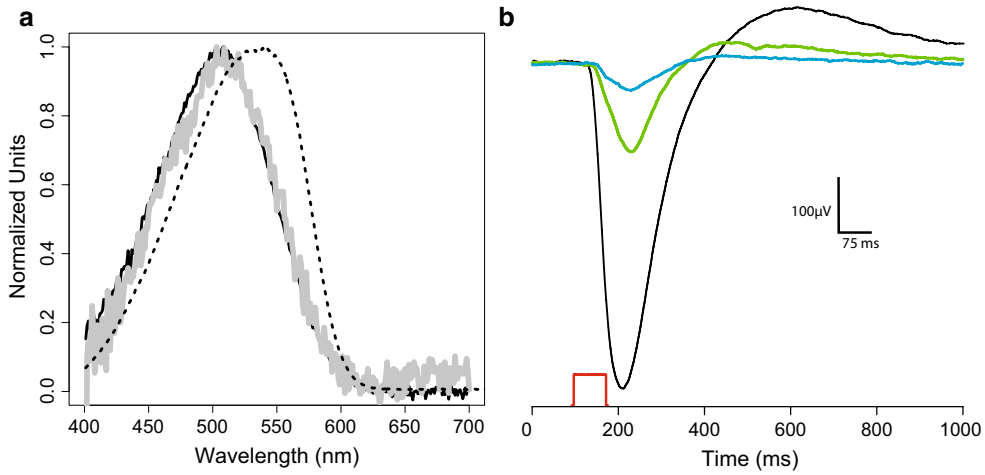

Fig. 2 Electroretinogram (ERG) responses for stomatopod larvae. **a** Normalized plot of light stimulus spectrum (*dotted trace*) with published *S. empusa* larval (*gray*) and adult (*black*) visual pigment absorption curves (Cronin and Jinks 2001). **b** Representative ERG traces in response to irradiance stimuli of different log intensities: *blue trace* 11.9 log photons cm^{-2} s^{-1}; *green trace* 12.8 log photons cm^{-2} s^{-1}, and *black trace* 13.8 log photons cm^{-2} s^{-1}. The *red line* indicates the 75 ms period of stimulus light exposure

SigmaPlot version 12.0). To test for differences in sensitivity of retinas measured during the day versus night, Log *K* values from z9s retinas were separated into day and night collected sample sets and tested using a two-tailed Student's *t* test ($\alpha = 0.05$).

Results

Microspectrophotometry

A single visual pigment with a peak absorption (λ_{max}) of 504 nm was found in the larval retinal tissue of both the last larval stage of *Squilla empusa* and the postlarval stage of *Gonodactylaceus falcatus* when double retinas were present (Fig. 1c, f). The data from *S. empusa* larval double-retina tissue are similar to previously published data from larvae with single retinas ($\lambda_{max} = 507$ nm; Cronin and Jinks 2001). Additionally, the data from *G. falcatus* larval double-retina tissue are similar to previously published spectral absorption values ($\lambda_{max} = 499$ nm; formerly *Gonodactylus aloha,* Cronin et al. 1995). While absorption measurements were robust from larval rhabdoms in the double-retina eye preparations of each species, the emerging adult retinal tissue yielded poor visual pigment absorption. Only a small number of the adult scans in both species could be fit to a visual pigment template (Fig. 1a, d), despite similar sampling of adult and larval rhabdoms in each preparation. From these data, a single visual pigment was identified in the emerging photoreceptors of both *S. empusa* ($\lambda_{max} = 456$ nm) and *G. falcatus* ($\lambda_{max} = 532$ nm).

Electroretinogram recording

Recordings from larval and adult retinas had corneal positive waveforms that were dependent on stimulus irradiance (e.g. Fig. 2b). We observed a general change in sensitivity from *V*log*I* models fit to these ERGs, as determined by the Log *K* value, during the z9 double-retina period relative to that of the early-stage single-retina period and the postlarval stage (Fig. 3a, b) ($p = 0.026$, one-way ANOVA). Recordings from the adult structure of z9 double retina (z9d) indicated a greater sensitivity (had lower Log *K* values) than those from single retinas in early-stage z9s ($p = 0.043$, Holm–Sidak post hoc test; Fig. 3b). All other post hoc pairwise comparisons indicated similar sensitivities, including the Log *K* values of z9 larval and adult retinas recorded simultaneously from the opposite eye ($p = 0.916$, Holm–Sidak post hoc test). Interestingly, Log *K* values from the adult PL retinas show an unexpected similarity in sensitivity in both early-stage single-retina stages (z8 $p = 0.856$, z9s $p = 0.684$).

The slopes of *V*log*I* curves increased with ontogeny ($p = 0.0024$, ANOVA with Holm–Sidak post hoc analysis) (Fig. 3c). Slopes for postlarval adult retinas were significantly greater than for single-retina eyes (z8 $p = 0.032$; z9s $p = 0.027$) (Fig. 3c). Both larval and adult *V*log*I* slopes from double-retina eyes were not significantly different from either single-retina larval or adult retina PL data (Fig. 3c),

Like previously described squilloids (Feller et al. 2013), *S. empusa* metamorphosis into postlarvae appears to coincide with the last quarter phase of the moon. The complete *S. empusa* double-retina eye development

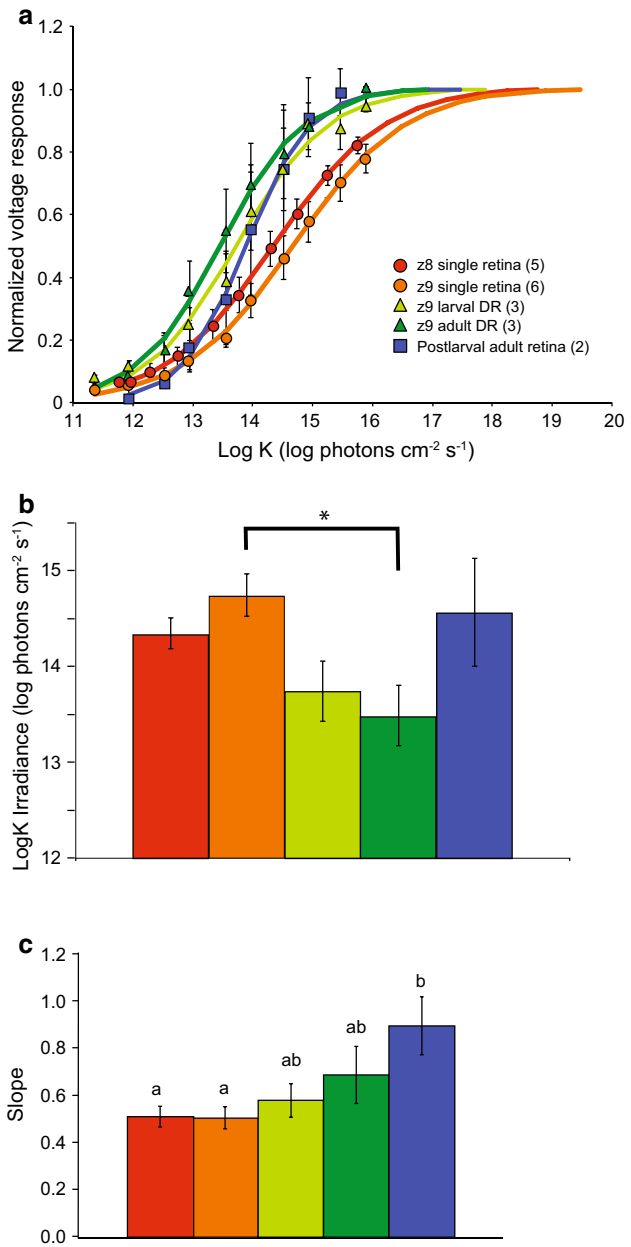

Fig. 3 *VlogI* curves for *S. empusa* developmental stages. **a** Mean normalized ERG response (±SE) for each stage as a function of stimulus intensity; sample sizes in *parentheses*. *Solid lines* represent best-fit Naka–Rushton curves to the respective *VlogI* data averaged from all individuals of a given developmental stage. **b** Boxplot of Log *K* values determined from *VlogI* curves for each individual at a given developmental stage. Each developmental stage is represented by *color code* and sample sizes in (**a**). *Asterisk* denotes statistically different sensitivities (ANOVA with Holm–Sidak post hoc, z9s vs. z9d adult, $p = 0.043$). **c** Mean slope (±SE) *VlogI* curve slope. Each developmental stage is colored as in (**a**). *Letters* denote significance groups among the five developmental stages, determined from an ANOVA with Holm–Sidak post hoc analysis. Only PL and single-retina slopes demonstrated significant differences from one another (PL vs. z8, $p = 0.032$; PL vs. z9s, $p = 0.027$)

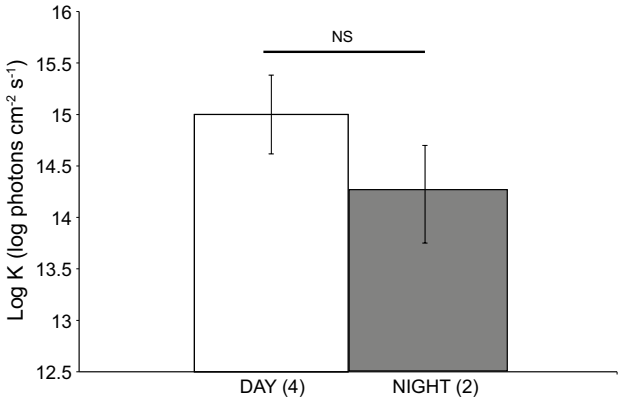

Fig. 4 Differences in "day" and "night" Log *K* values from all z9s recordings. Sample sizes for each group given in *parentheses*. Though retinas measured at night trend towards more sensitive responses (lower Log *K* values), the difference in the two groups is not significant (two-tailed Student *T* test, $p = 0.094$)

associated with this metamorphosis occurs within a 12-h period during the nighttime hours. On average, individual z9 double-retina larvae present an initial deposit of adult retina pigmentation after sunset (2020 h, June 2013), with the most robust expression of a double retina by 0100 hour. Molting occurred prior to sunrise (0550 h, June 2013) and by 0800 h only a small remnant of the larval retina remained laterally adjacent to the enlarged adult structure in the PL eye. This unexpectedly rapid eye development necessitated the acquisition of ERG measurements throughout the diel cycle, thus we were unable to control for putative circadian cycles in eye sensitivity. We compared available day vs. night z9s Log *K* values to test for diel changes in retinal sensitivity. While there is a trend towards increased sensitivity of z9s retinas at night, a pairwise comparison of the day vs. night Log *K* values from this developmental stage reveals that the change is not significant ($p = 0.094$ two-tailed Student *T* Test; Fig. 4).

Discussion

The primary goal of this research was to investigate stomatopod visual physiology during the double-retina eye developmental process. Using MSP, we found a single visual pigment in larval rhabdoms of double-retina eyes that is similar to previous MSP findings in single-retina larval eyes for both *S. empusa* (Cronin and Jinks 2001) and *G. falcatus* (Cronin et al. 1995). The adult tissue in the same double-retina preparations, however, did not yield results that were robust from either species or, in the case of *S. empusa,* in agreement to previous

findings from adult photoreceptors. Despite similar sample numbers of larval and adult rhabdoms, the majority of the scans from the adult retinas of both species could not be fit to a visual pigment template. In *S. empusa,* the best approximation of an adult visual pigment was an average spectrum from three rhabdoms fit to a 456 nm template, which is much shorter than the 507 nm λ_{max} known for adult retinas of this species (Fig. 1a; Cronin 1985; Cronin and Jinks 2001). The adult retinal tissue of *G. falcatus* double retinas produced a 532-nm λ_{max} template fit to a noisy average of three rhabdomeric scans (Fig. 1d). Unlike the situation observed with the *S. empusa* adult data, a 532-nm visual pigment in *G. falcatus* agrees with previous adult measurements that identify a long-wavelength sensitive pigment with a 531 nm λ_{max} value expressed in the distal tier of midband row 3 (Cronin et al. 1995). Taken together, the larval and adult data raised the question as to whether the emerging adult retina must achieve a critical period in its development before becoming physiologically active. We refuted this hypothesis by collecting ERG recordings from an ontogenetic series of *S. empusa* larvae and postlarvae. These ERG data reveal a robust electrophysiological response to light from adult retinal tissue at its earliest formation in late stage z9 double-retina eyes (Fig. 3). This was evidenced through simultaneous ERG recordings from both the larval and adult retinas of the same individual as it began its double-retina eye development. Despite the incomplete formation of the adult retina at this time point, simultaneous recordings produced similar responses from both retinas in double-retina eyes (Fig. 3a).

The ERG retinal responses recorded from *S. empusa* z9d larval and adult retinas both appear more sensitive to lower levels of light stimuli than single-retina larval eyes, though only z9d adult recordings were significantly different from the single-retina larval state (Fig. 3a, b). Given that ERGs record the extracellular potential from an undefined population of photoreceptor cells, and both larval and adult retinal tissue are present in the double-retina stage, this apparent sensitivity increase could be resulted from the added contribution of cells from the non-target retina to the ERG response in z9 double-retina eyes. The increased slope in the postlarval *V*log*I* curve is consistent with a shift in spectral sensitivity between larval and adult retinas (e.g. Chapman and Lall 1967; Figs. 1, 2). Slopes for double-retina eyes are intermediate between those of PL and single-retina eyes, suggesting the putative contribution from both larval and adult receptor classes. Intracellular recordings are needed to confirm these findings and to resolve whether double-retina photoreceptors are actually more photosensitive than those in single-retina stomatopod eyes (particularly those of the z8/z9 larvae).

MSP is a powerful tool that directly measures the spectral absorption and photochemical response of the visual pigments expressed photoreceptor membranes. Though MSP is an established method for analyzing spectral sensitivity, this method was ultimately inadequate for investigating ontogenetic changes in spectral sensitivity during stomatopod double-retina eye development. Several MSP-specific factors may be responsible for the good larval, but poor adult visual pigment absorption results generated from double-retina preparations. MSP measures the light absorbed from a small, focused beam of light as it passes through the cross-section of an individual rhabdom. Light absorption in such a minute measurement system can be affected by low concentrations of visual pigment in the rhabdomeric membrane, leeching of pigment from the sectioned tissue, and/or simple error from non-visual pigments in the surrounding cells. These MSP results may have additionally been influenced by dark adaptation during the experimental procedure. Specifically, before collecting MSP data, animals were housed in the dark for up to 12 h before experimentation to optimize visual pigment absorption and minimize the quantity of screening pigments between receptors. In the absence of light, proper formation of the developing rhabdoms may have been affected (Deruntz et al. 1994). Since the larval retina was completely formed long before the prolonged dark adaptation, it would not be affected by this procedure and would (and did) perform as expected during the MSP experiments (Fig. 1c, f). Though *S. empusa* eye development happens during the nocturnal hours, light may still be required for proper eye formation. Further work is needed to understand the effects of sensory deprivation on stomatopod visual pigment expression and photoreceptor development. Molecular analyses of developmental patterns of opsin expression would also serve to address the observed MSP results in double-retina eyes.

Though the difference in sensitivity between day- and nighttime recordings was not significant in z9 single retinas, given the observed trend in the data in the context of previous findings, we cannot completely rule out a potential for circadian cycling in sensitivity in *S. empusa eyes* (Fig. 4). Previous work demonstrates a robust circadian cycle in the pupillary light response in the eyes of adult *S. empusa* (Cronin 1992). The pupillary light response occurs as a function of the radial migration of pigment granules in response to light and infers a circadian cycle in sensitivity in adult *S. empusa*, whereby the eyes are less sensitive during the day. Visual rhythms are well documented in many non-stomatopod crustaceans and can be derived from changes in daily membrane shedding (Chamberlain and Barlow 2003), variations in mRNA expression (Arikawa et al. 1987, 1988), changes to the shape of the ommatidial components (Meyer-Rochow and Keskinen 2003; Schiff

et al. 2007) or, as previously mentioned, via migration of accessory pigment granules (Stowe 1980; Cronin 1992). Given the ubiquity of circadian visual rhythms among crustaceans, it is likely that one of these mechanisms produces a circadian cycle in sensitivity in the eyes of *S. empusa* adults and larvae, which may be weakly reflected in our data (Fig. 4). An increased sample size is needed to establish whether or not these variations are statistically significant. Studies that specifically measure circadian cycles in ERG responses from *S. empusa* larval and adult eyes are needed to properly address this question.

This study characterizes the ontogeny of irradiance sensitivity during the double-retina transition period in stomatopod crustaceans. Our data suggest a shift in sensitivity during the double-retina phase as well as an ontogenetic change in the response dynamics of stomatopod retinas. It remains unclear whether or not the measured responses from early developing adult retinas are transmitted to the brain. We plan to perform ontogenetic studies of neuroanatomical projections from the retina to the optic neuropils and other downstream structures to address this question. The data presented here will serve as a foundation for future investigations of stomatopod double-retina eyes. The authors hope to use ERG to measure the ontogeny of spectral sensitivity and flicker fusion thresholds during double-retina eye development. Due to the limited availability of animals, we were not able to pursue these questions in the present study. Additionally, in the context of existing literature, this study provides preliminary evidence that further investigation of circadian visual rhythms in *S. empusa* adults and larvae is warranted.

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Conflict of interest There are no conflicts of interest to declare by the authors regarding this work.

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