

Ultraviolet vision in larval *Neogonodactylus oerstedii*

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Summary statement

This paper provides evidence for ultraviolet vision in the larval stomatopod crustacean, *Neogonodactylus oerstedii* using electrophysiological and molecular techniques.

Abstract

Stomatopod crustaceans have among the most complex eyes in the animal kingdom, with up to twelve different color detection channels. The capabilities of these unique eyes include photoreception of ultraviolet (UV) wavelengths (<400 nm). UV vision has been well characterized in adult stomatopods but has not been previously demonstrated in the comparatively simpler larval eye. Larval stomatopod eyes are developmentally distinct from their adult counterpart and have been described as lacking the visual pigment diversity and morphological specializations found in adult eyes. However, recent studies have provided evidence that larval stomatopod eyes are more complex than previously thought and warrant

closer investigation. Using electroretinogram recordings in live animals we found physiological evidence of blue and UV sensitive photoreceptors in larvae of the Caribbean stomatopod species *Neogonodactylus oerstedii*. Transcriptomes of individual larvae were used to identify the expression of three distinct UV opsins transcripts, which may indicate the presence of multiple UV spectral channels. This is the first paper to document UV vision in any larval stomatopod, expanding our understanding of the importance of UV sensitivity in plankton. Similar to adults, larval stomatopod eyes are more complex than expected and contain previously uncharacterized molecular diversity and physiological functions.

Introduction

Once believed to be relatively rare, ultraviolet (UV) vision is wide spread across animal phyla, particularly in the marine realm (Cronin and Bok, 2016; Tovee 1995). UV vision is hypothesized to be critical to the behavior and ecology of animals in pelagic habitats, including but not limited to controlling vertical migration (Frank and Widder, 1996; Lampert, 1989; Williamson et al., 2011), increasing feeding efficiencies (Browman et al., 1994; Siebeck and Marshall, 2007), and protecting from photodamage (Morgan and Christy, 1996; Rhode et al., 2001). Adult stomatopods have one of the most complex visual systems currently described, including multiple photoreceptors sensitive to different wavelengths of UV light. At the most complex, adult stomatopods have up to 16 photoreceptor types (Marshall, 1988; Marshall et al., 1991a), which allow for specialized linear and circular polarization vision as well as twelve different color detection channels (Cronin et al., 2017a; How et al., 2014; Marshall, 1988; Marshall et al., 1991b), including UV vision (Bok et al., 2015; Cronin et al., 1994; Kleinlogel and Marshall, 2009; Kleinlogel et al., 2003). In the well-studied species *Neogonodactylus oerstedii* (Fig. 1) (Cronin and Marshall 1989; Donohue et al. 2018; Porter et al. 2020), the complex adult eyes contain six spectrally distinct types of UV photoreceptors, achieved by tuning two visual pigments with one of four distinct optical filters (Bok et al., 2014). Due to this photoreceptor diversity, UV vision is believed to be ecologically important to the adult lifestyle (Bok et al., 2018; Franklin et al., 2018).

Despite the adult *N. oerstedii* visual system being well characterized, the larval visual system is still poorly understood because larval and adult stomatopod eyes are physiologically and morphologically distinct (Fig. 1) (Cronin and Jinks, 2001; Cronin et al., 1995; Feller et al., 2015). During metamorphosis, the adult retina develops adjacent to the larval retina and forms new neural connections before the larval eye degenerates back into the body (Feller et al., 2015; Marshall et al., 1991b). Unlike the complex photoreceptor arrangement of adult stomatopod eyes (Marshall et al., 2007), larval stomatopods have a simple transparent apposition compound eye where, similar to other larval crustaceans, ommatidial units form a uniform array. However, recent studies have found that some species of larval stomatopods have unexpected eye complexity that has not yet been fully explored (Feller et al., 2019).

Like many crustaceans, stomatopod larvae are pelagic. During this planktonic phase, larval eyes are tuned to the light environment of the open ocean and the ecological demands of the larva. Most crustacean larvae, including stomatopods, have a single spectral channel peaking in the blue/green portion of the spectrum (Cronin and Jinks, 2001; Cronin et al., 2017b; Feller and Cronin, 2016; Nilsson, 1983). However, UV vision has been hypothesized to exist in some decapod larvae based on behavioral trials (Forward and Cronin, 1979) and ultrastructural studies (Douglass and Forward, 1989; Meyer-Rochow, 1975), suggesting UV vision may occur more generally in larval crustaceans. However, despite being a long-standing hypothesis, few studies have directly measured larval crustacean UV vision using either physiological or molecular approaches. The behavioral and anatomical evidence for UV sensitivity in crustacean larvae, as well as the diversity of UV receptors in adult stomatopods, suggests the possibility that larval stomatopods may also possess UV sensitivity.

In this study we tested the hypothesis that UV vision is present in larval *N. oerstedii* through a combination of electrophysiological and molecular techniques and found strong evidence for UV photoreception. This study is the first to demonstrate UV vision in larval stomatopods and adds to the growing evidence that UV vision is important to larval crustaceans more broadly.

Methods

Specimen Collection

Samples were collected between May–July 2019 at the Keys Marine Laboratory (Long Key, FL, USA). Larvae (~3 mm in length) were collected nearshore at night using underwater dive lights and dipnets, and then sorted by hand. After collection, larvae were kept in room temperature (~25 °C) finger bowls in the laboratory with daily water changes for up to 3 days in ambient lighting. As the larvae used were wild caught, we cannot definitively determine their age, as the larval stages of *N. oerstedii* have not been previously characterized. However, all larvae were positively phototactic and free swimming with intact raptorial appendages, indicating that they were in one of the pelagic feeding stages (Harrison et al., 2021). As well, all larvae used were the same size, which means that they all were likely the same age and stage (Hamano et al., 1995).

Electroretinogram Recordings

To physiologically test larval stomatopod vision, electroretinogram (ERG) recordings were used. ERG recordings measure the summed potential of a group of photoreceptors to a stimulus, in this case brief flashes of light. Over the course of the study, larvae were presented with stimuli at two different colors (blue, UV). Using these measurements, the magnitude of electrical responses over a series of four intensity levels for each color were compared to determine photosensitivity, an approach that can help determine relative sensitivity among wavelengths when organism sensitivity is insufficient for generating a full spectral sensitivity curve (Mason and Cohen, 2012). ERGs were measured under two different light adaptation conditions: (1) full dark, referred to as ‘dark adaptation’; and (2) a constant >570 nm orange light, referred to as ‘chromatic adaptation.’ ERGs measured under dark adaptation were used to test the dominant response of the eye, with the expectation that sensitivity in the blue spectral region would dominate. ERGs under chromatic adaptation are an effective method for uncovering secondary sensitivity peaks at shorter wavelengths even if there are low levels of expression of the secondary visual pigment (Goldsmith, 1986). Studies on larval stomatopod visual systems are limited, and there has been no published ERG recordings or characterization of opsins in any larval stomatopods prior to this study. However, the spectral absorption of retinal photoreceptors in eight larval stomatopod were completed using microspectrophotometry (MSP), and all species tested had a peak sensitivity between 450–500 nm (Feller and Cronin, 2016). While *N. oerstedii*

was not included in the previous study, we hypothesized that it would follow a similar trend with a peak sensitivity in the blue portion of the spectrum. Therefore, we chose to use an orange >570 nm light for chromatic adaptations in order to decrease the physiological response to the blue light stimulus, without impacting the response to the UV light stimulus.

During experimentation, animals were attached to an acrylic support by the dorsal carapace with cyanoacrylate gel adhesive (Loctite, Rocky Hill, CT, USA) and suspended in a seawater chamber that was maintained at 22 °C. The chamber was placed in a light-tight Faraday cage and grounded to the cage with an AgCl coated wire. An epoxy-insulated tungsten microelectrode (FHC 75 µm standard fine, Bowdoin, ME, USA) was placed subcorneally in one eye with a micromanipulator under dim red light supplied by the adapting light with a filter (RG630, Schott, Elmsford, NY, USA). The primary light stimulus for ERGs was provided by a xenon arc lamp (Spectral Products ASB-XE-175, Putnam, CT, USA). Light was supplied to the animal via one branch of a bifurcated fused-silica fiber optic light guide (P806, EXFO, Richardson, TX USA) positioned directly on the eye, allowing the entire eye to be bathed in light. The other branch of the light guide was connected to an accessory light for specimen preparation and chromatic adaptation (Ocean Optics HL-2000 lamp, Dunedin, FL, USA; OG570 filter, Schott, Elmsford, NY, USA). Initial trials determined that the larval eye did not have a sufficiently high response to determine photosensitivity to discrete wavelengths, therefore recordings made to broadband flashes of light were used (Mason and Cohen, 2012). For experimental responses, light was filtered through placement of broad bandpass colored glass filters placed directly in the light path to obtain either blue light (FG39, Schott Elmsford, NY, USA) or UV light (FGUV5, Schott, Elmsford, NY, USA). The intensity of the blue and UV was adjusted by adding fused silica neutral density filters to the light path from 0 OD to 4.0 OD at 0.1 OD steps (Melles Griot, Rochester, NY, USA). The intensity of both colors was measured with each neutral density filter using a radiometrically calibrated spectrometer (Fig. S1) (QEPro, 1000 µm diameter fiber with a cosine corrector, Ocean Insight, Orlando FL, USA). For each trial, four light intensities of blue light (14.8–16.04 log photons cm⁻² s⁻¹) and four light intensities of UV light (14.0–14.7 log photons cm⁻² s⁻¹) were used in order to compare responses across a stimulus intensity gradient. Because we were interested in understanding how the UV response changed relative to blue, all responses were normalized to a bright blue flash of light (16.14 log photons cm⁻² s⁻¹) by dividing both blue and UV responses by the response to this blue flash for a given

preparation. Using this method, we are able to directly test how the UV response is affected by chromatic adaptation relative to the blue response. Our goal with the chromatic adaptation is to visualize any secondary photoreceptor, in this case UV, by knocking down the dominant photoreceptor response without affecting the secondary photoreceptor. If there is a UV photoreceptor, we would expect the blue normalized UV response to increase relative to the blue response under chromatic adaptation with orange light.

During experimentation the animal was left to adapt for a minimum of 30 minutes until a consistent response to a dim test flash of UV light was achieved. For any given preparation, the test flash was used periodically throughout experimentation to make sure the animal remained at its initial state of adaptation throughout the testing period. Each test and stimulus flash lasted for 75 ms and was supplied through a computer-controlled shutter (Uniblitz model VS25, Rochester, NY, USA). For each trial, regardless of the adaptation, animals were supplied with all four intensity levels of both blue and UV light. Stimulus response recordings were viewed in real time and digitized for post-processing (AD Instruments Powerlab and Labchart 7, Colorado Springs, CO, USA). Typically, animals were tested under only one adapting condition, either dark (n=5) or chromatic adaptation (n=4), as an individual preparation typically did not stay stable long enough to complete both adaptations on the same animal. Upon completion of the trials, a series of Welch's t-tests with a Bonferroni correction were used for both the blue and UV responses, to compare differences at each irradiance level between dark and chromatic adaptation within each color (R Core Team 2021).

Species Identification

Animals used in electrophysiology experiments were preserved in 100% ethanol and shipped back to the University of Hawai'i at Mānoa to confirm species identity post hoc using DNA barcoding (Barber and Boyce, 2006; Palecanda et al., 2020; Tang et al., 2010). For each individual, DNA was extracted using a DNeasy kit (Qiagen, Hilden, DE, USA) following manufacturer protocols, and the cytochrome oxidase I (COI) mitochondrial gene was amplified via polymerase chain reaction (PCR) using degenerate primers designed to target stomatopod COI (Palecanda et al. 2020). PCR was conducted using MyTaq Mix (Bioline, London, UK) following manufacturer protocols with 20 µl reactions containing 0.2 mM forward and reverse primer and 2.5–4 ng of DNA. The cycling parameters of each PCR were a single 30 second incubation at 94 °C; 20 cycles of 20 sec. 94 °C denaturing, 15 sec. 46 °C annealing, and 1 min 72

°C elongation; 20 cycles of 20 sec. 94 °C denaturing, 15 sec. 51 °C annealing, and 1 min 72 °C elongation; final elongation of 1 min at 72 °C. PCR amplicons were cleaned using EXO-SAP-IT (Thermo Fisher, Waltham, MA, USA) and sequenced at the Advance Studies in Genomics, Proteomics, and Bioinformatics facility at the University of Hawai'i at Mānoa (Honolulu, HI, USA). The identity of animals used for transcriptomes was confirmed by extracting COI sequences from individual assemblies and using NCBI's Basic Local Alignment Tool (BLAST) to determine species.

Ultraviolet Opsin mRNA Transcript Identification

Samples preserved in RNAlater were transported to the University of Hawai'i for processing. RNA was extracted from whole larvae using a RNeasy Mini Kit (Qiagen) following manufacturer protocols. An on-column DNase digestion was performed to remove residual DNA. RNA was quantified using a Qubit fluorometer and sent to Novogene (Sacramento, CA, USA) for further quality assessment, cDNA library preparation, and sequencing. Three larval individuals were sequenced using Illumina NovaSeq 6000 technology.

After sequencing, read quality was assessed using FastQC (Andrews, 2010). Adapter sequences were trimmed and sequences with an average quality score of less than 25 averaged over 4 bases were removed using Trimmomatic v0.36 (Bolger et al., 2014). Subsequently, reads with a length less than 25 base pairs (bp) were also discarded. Trimmed reads were then assembled *de novo* with Trinity v2.6.6 (Grabherr et al., 2011; Haas et al., 2013) using in silico read normalization and default minimum contig length and kmer sizes of 200 bp and 25 bp respectively (Grabherr et al., 2011; Henschel et al., 2012). Single larval datasets were assembled first individually and then a combined assembly was produced including all three datasets to streamline downstream gene identification. Assembly statistics were calculated (Haas et al., 2013) and assessments of transcriptome completeness using BUSCO v3.0.2 (Simão et al., 2015) were calculated for individual and combined assemblies. BUSCO evaluations were done using a reference set of orthologous groups (n=255) found across Eukaryotes.

The combined larval transcriptome was analyzed using the Phylogenetically Informed Annotation (PIA) tool (Speiser et al., 2014) which is designed to identify putative phototransduction genes in a phylogenetic context. PIA analysis consisted of three steps. First, all open reading frames (ORFs) were extracted using a minimum ORF length of 150 amino acids

to remove small gene fragments. Putative rhabdomeric opsin transcripts were then identified via BLAST searches (e-value threshold = $1e-5$) against a database of known opsins. Finally, significant hits were aligned using MAFFT and placed into a pre-existing phylogeny to further differentiate between visual opsins transcripts and closely related proteins.

Transcripts that clustered with non-visual opsins or outgroups were removed and the remaining transcripts were used to generate a final opsin tree using a comprehensive opsin alignment curated by Porter et al. (2011). Translated opsin transcripts and published protein sequences were compiled into a MAFFT alignment using the software Geneious R10 (Kato et al., 2002; Kearse et al., 2012). Maximum likelihood trees were constructed using RAxML (Stamatakis, 2014) on the CIPRES platform (Miller et al., 2010) using default input parameters. Trees were visualized in FigTree (v.1.4.3) (Rambaut, 2007). Identified visual opsin transcripts clustered with crustacean long (>500 nm) and middle (~400–500 nm) wavelength sensitive opsin clades as well as the UV-sensitive opsin clade (Cronin and Porter, 2014; Porter et al., 2007). Only putative UV-sensitive opsin transcripts were included in this study. Gene expression data was generated by mapping reads from each individual back to the combined assembly using RSEM (Li and Dewey, 2011) and putative opsin transcripts with an average expression (TPM) of less than one across all three replicate samples were discarded (Hart et al., 2013; Suvorov et al., 2017). Additional BLASTp searches were performed on putative UV-sensitive opsin transcripts. Once a final set of opsin transcripts from the combined assembly was compiled, sequences were checked against individual larval assemblies to verify similarity. The expression values for each mRNA transcript identified, as well as their mean and standard deviation across replicates, were calculated.

Results

Electrophysiology

Our results show that *N. oerstedii* larvae display negative (downward) ERG waveforms (Fig. 2A) and the magnitude of responses increased as the stimulus intensity increased for both blue and UV stimuli (Fig. 2B). In the raw data, at any given irradiance, the chromatic adapted blue response decreased compared to dark adapted blue responses, while the UV response minimally between adaptations (Fig. 2A). This was expected, as chromatic adaptation was used to knock down the blue response while the UV remained essentially unchanged.

In order to statistically test the effect of adaptation at each color and irradiance level, both the blue and UV responses were normalized to a bright blue flash. When the responses were normalized, we expected to see the chromatic adapted UV response increase relative to the dark adapted response given the decrease in sensitivity to the blue normalizing response, while the normalized blue responses should remain unchanged. Our results support this, as we saw no significant difference in the normalized blue response adaptations at any of the intensity levels measured (Fig. 2B). For the normalized UV response, the chromatic adapted UV responses were significantly higher than dark adapted UV response at all four irradiance levels tested (Fig. 2B). These results demonstrate that relative to the dominant blue response, the UV response increased under chromatic adaptation, indicating there is a physiologically responsive UV photoreceptor in the larval *N. oerstedii* retina.

Transcriptomes

An average of 63 million paired-end reads were generated per larval sample. These data are available on NCBI's Sequence Read Archive (SRA) database under BioProject: PRJNA790459. The combined larval assembly contained 236,330 contigs with a mean contig length of 759 bp and an N50 of 1,367 bp indicating fairly high contiguity (Cahais et al., 2012) (Table 1). The BUSCO score for the combined larval assembly was 91.8% indicating that the assembly is relatively complete.

Three UV opsin transcripts, hereby referred to as NoUV1, NoUV2, and NoUV3, (GenBank accession numbers OK432515–OK432517) were identified from the combined larval assembly (Fig. 3). These three UV opsin transcripts correspond with previously published adult *N. oerstedii* UV opsins (Bok et al., 2014) with similarity of at least 97% at both the nucleotide and amino acid levels. At the amino acid level NoUV1, NoUV2, and NoUV3 appear unique from each other with the highest similarity between NoUV1 and NoUV3 at 58%. Similarity between NoUV1 and NoUV2 was 39% while NoUV2 and NoUV3 were 35% similar. All identified UV opsin transcripts contained a lysine residue at the amino acid position corresponding with bovine rhodopsin position 90, a site which has been shown to be important in arthropod UV spectral tuning (Salcedo et al., 2003).

Although expression levels varied between samples (Table 1), average expression for each opsin transcript was high at 532 TPM with standard deviation of 239 TPM for NoUV1, 574 TPM with standard deviation of 266 TPM for NoUV2, and 486 TPM with standard deviation of 416 TPM for NoUV3.

Discussion

Despite UV vision being relatively widespread in the visual systems of pelagic organisms, there have been few studies that have provided evidence of UV vision in larval crustaceans (Forward and Cronin, 1979; Mishra et al., 2006). Using electrophysiology and molecular approaches, this study is the first to document UV photoreception in larval stomatopod crustaceans. Previous studies have reported that larval stomatopods have a dominant blue/green sensitivity (Feller and Cronin 2016). Using electroretinogram recordings, we tested the hypothesis that larval stomatopods also have UV vision though broadband comparisons of blue and UV light. We utilized chromatic adaptation to continually photoactivate the dominant blue photoreceptor cells (Feller and Cronin, 2016), which allowed us to visualize the secondary UV photoreceptor trace (Frank et al., 2009; Goldsmith and Fernandez, 1968). We found that under chromatic adaptation and after normalizing to a blue test flash, UV responses were significantly higher than under dark adaptation at all four irradiance levels tested, while the blue response remained unchanged between adaptations (Fig. 2B). Based on these results, we propose that larval *N. oerstedii* retinas have at least one physiologically active spectral class of UV cells.

Based on the expression of three UV opsins transcripts, there may be even more diversity in UV photoreception than suggested by the ERG data, although verification would require antibody labeling. The sequences of the UV opsin transcripts found in larval *N. oerstedii* are different enough from each other to unambiguously represent three unique mRNAs. The presence of a lysine residue at bovine rhodopsin amino acid position 90 provides evidence that if translated into a functional protein these opsins would be UV sensitive, as a shift from asparagine or glutamate to lysine at this position has been shown to be largely responsible for shifting wavelength sensitivity from blue to UV in invertebrate opsins (Salcedo et al., 2003). Transcripts corresponding with published middle and long wavelength sensitive opsins are the subject of ongoing investigation and will be discussed in future studies. As has been shown in

previous studies, the existence of an opsin mRNA transcript does not necessarily signify the synchronous existence of a functional opsin protein and antibody labeling is necessary to verify the existence of such proteins (Arikawa et al., 2017; McCulloch et al., 2017). However, we feel that the high expression levels of UV-sensitive opsin transcripts that were observed in combination with physiological markers of UV sensitivity provide strong evidence for UV vision in larval *N. oerstedii*.

Bok et al. (2014) defined two well-supported clades of crustacean UV opsins-cUV1 which contains adult *N. oerstedii* opsin transcripts NoUV1 (λ_{max} of 334 nm) and NoUV3 (λ_{max} unknown), and cUV2 which contains adult *N. oerstedii* opsin transcripts NoUV2 (λ_{max} of 383 nm). Based on *in situ* hybridization studies, the mRNA transcripts of opsins NoUV1 and NoUV2 were expressed in different R8 photoreceptor cells in adult eyes and MSP measurements were used to determine the different absorbance peaks of each visual pigment (Bok et al. 2014). Our results demonstrate that larval *N. oerstedii* express the same three UV opsin mRNAs found in adults, indicating that not only do these larvae have the same molecular components of UV vision found in adults, but they may also have multiple UV photoreceptor classes. Adult *N. oerstedii* use unique UV optical filters to further tune their visual system to include 6 distinct UV spectral classes (Bok et al. 2014; Cronin et al. 2014). There is no documented evidence of UV filters in stomatopod larval retinas, although only one species has been tested at this time (Bok et al., 2015). Because of this putative lack of larval UV filters, we do not expect larval *N. oerstedii* to possess an equivalent number of UV spectral channels to adults despite having comparable opsin transcript diversity. Future studies should determine if the multiple expressed UV opsins correspond to multiple UV spectral channels in *N. oerstedii* larval visual systems.

Because of the small size of stomatopod larvae (~3 mm), whole body RNA extractions were used to generate transcriptomes. This leaves open the possibility that the characterized UV opsins may be expressed outside of the eye. For example, Bok et al. (2014) determined expression patterns for opsin transcripts NoUV1 and NoUV2 in adult *N. oerstedii* eyes but an expression pattern for NoUV3 was not found in the retina, making NoUV3 a potential candidate for extraocular expression. Further protein localization studies will be necessary to determine where these opsins are found in larvae. Expression levels for all three UV opsins mRNAs identified were consistent with use in vision across all samples (Chauhan et al., 2014; Lowe et al., 2018), however variation did exist between replicates. Sample 3 (Noer lar 3) had

significantly higher expression for all 3 opsin mRNAs, with expression of NoUV3 being particularly high (Table 1). NoUV3 was still highly expressed in all samples, lending support to its existence as a functional protein.

With current knowledge of stomatopod larval ecology being very limited, largely due to the difficulty involved in studying these tiny transparent animals in their natural habitat, the discovery of UV sensitivity may shine light on this transitory but important phase of stomatopod life history. There are several hypotheses for the presence of UV vision in planktonic marine animals. For pelagic crustaceans, UV light has been suggested to play a role in diel vertical migration behavior (Frank and Widder, 1996; Leech and Johnsen, 2002), where many zooplankton species move into deeper waters during the day and up to the surface at night (Lampert, 1989) possibly following daily shifts in light intensities. UV vision has also been hypothesized to be beneficial for pelagic planktivores, particularly larval reef fish, by increasing transparent prey contrast with the open water background and aiding in prey capture (Browman et al, 1994; Siebeck and Marshall, 2007). Stomatopods are capable swimmers and feed in the larval phase, although their diet is not currently well documented outside of lab settings. It is possible that stomatopod larvae may be utilizing UV sensitivity in similar ways to larval fish, to aid in the perception of smaller, transparent prey. UV sensitivity may be particularly useful at crepuscular periods, which are the transition points for diel vertical migration and are considered an “optical foraging niche” for predators with UV vision. This is due to the increased proportion of UV sky light and enhanced target background contrast found at dawn and dusk (Leech and Johnsen, 2002).

Summary and Future Directions

This study provides the first evidence of UV vision in larval stomatopods, and is among only a few to demonstrate UV sensitivity electrophysiologically in any larval crustacean (e. g., Ziegler et al. 2010). This study is novel in its use of an integrative physiological and molecular approach to clearly demonstrate the presence of UV photosensitivity in larval *N. oerstedii*. While at this time UV vision has only been identified in larvae of a single species, future studies will expand our current dataset to determine if UV vision is a widespread trait across larval stomatopods. There are also few studies, mostly from decapods, demonstrating behavioral evidence of UV vision in larval crustaceans (e.g., Forward and Cronin, 1979; Ziegler et al., 2010). Behavioral

studies in stomatopods are necessary to determine the ecological role of UV sensitivity in these larvae.

Abbreviations:

UV	Ultraviolet
ERG	Electroretinogram
COI	Cytochrome oxidase I
PCR	Polymerase Chain Reaction
BLAST	Basic Local Alignment Tool
bp	Base pairs
PIA	Phylogenetically Informed Annotation
ORFs	Open Reading Frames

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Figures:

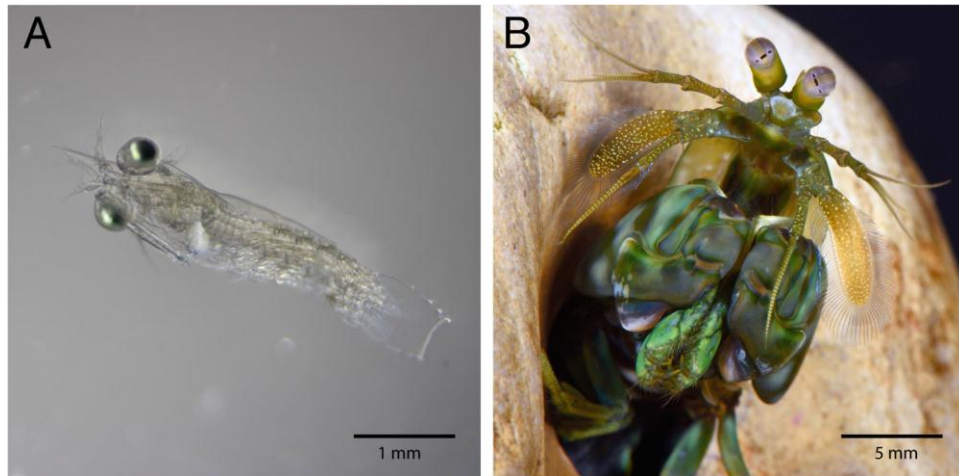


Figure 1: Representative photos of (A) larval (M. McDonald) and (B) adult (R. Caldwell) *Neogonodactylus oerstedii*. Larvae used in this study were 3–4 mm in size while adults have a maximum size of 68–76 mm (Schram and Muller, 2004).

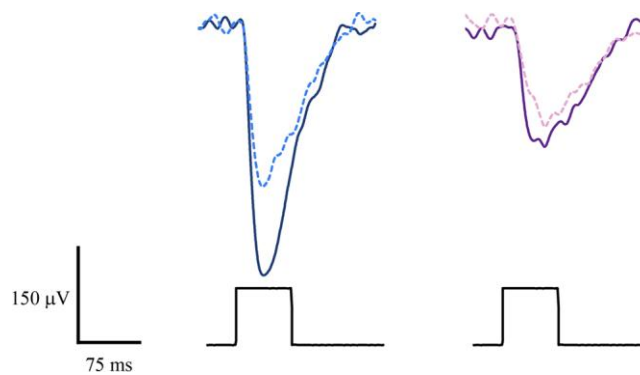


Figure 2A: Representative ERG responses to a 75 ms flash of blue (16.04 log photons $\text{cm}^{-2}\text{s}^{-1}$, blue lines) and UV (14.76 log photons $\text{cm}^{-2}\text{s}^{-1}$, purple lines) light under dark (solid line) and chromatic (dashed line) adaptations.

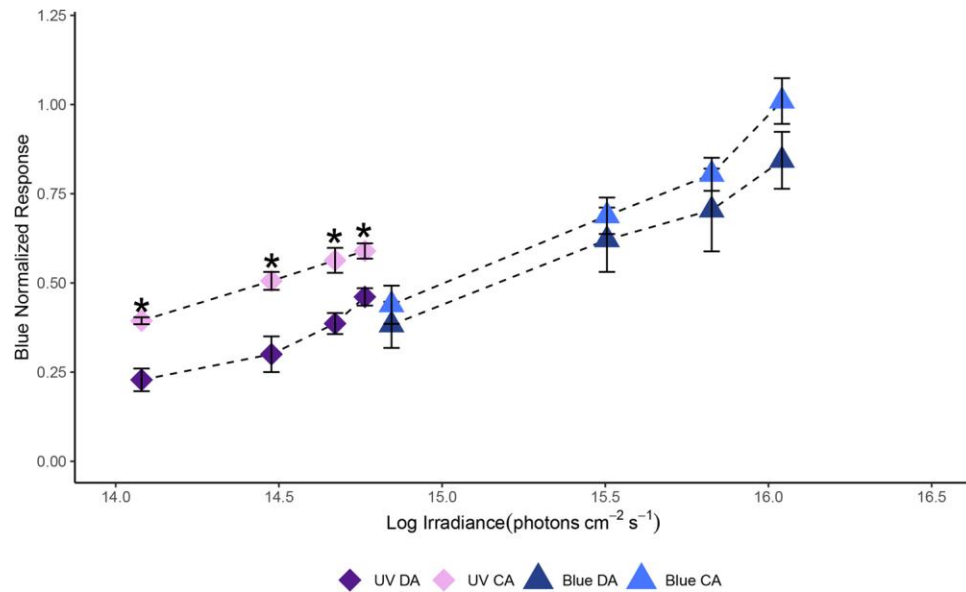


Figure 2B: Graded response to blue and UV light in dark (n=5) and chromatic (n=4) adapted states at four quantal intensities for each color. Replicates are individual animals. Responses were recorded as μV before being normalized to a bright blue flash of light ($16.14 \log \text{photons cm}^{-2} \text{s}^{-1}$) for each replicate. Normalized responses are plotted as mean $\pm$ s.e.m and compared at each irradiance level using Welch two sample t-tests with a Bonferroni correction (* $p < 0.05$).

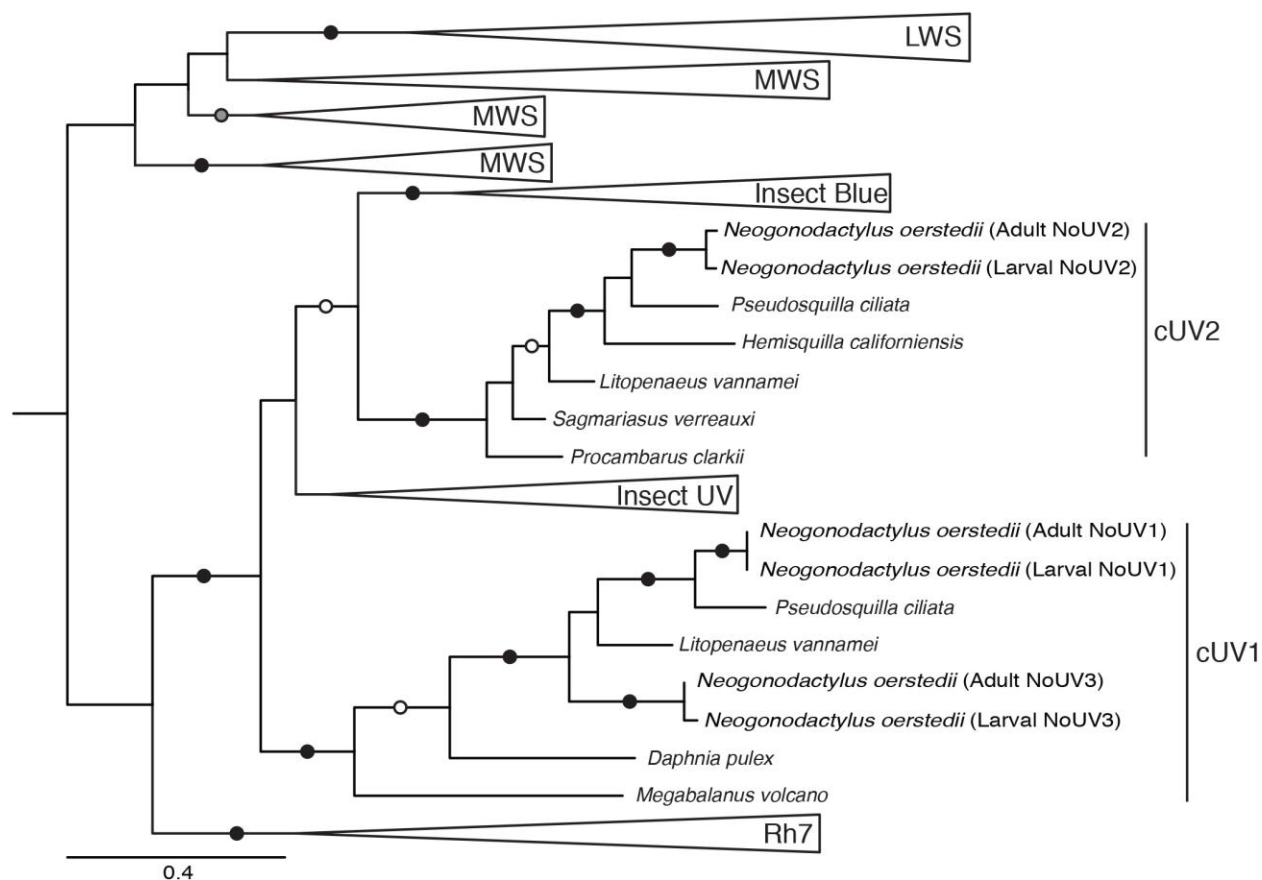


Figure 3: Maximum likelihood phylogeny of arthropod short wavelength sensitive (SWS) opsin sequences. Collapsed clades show middle wavelength sensitive (MWS) and long wavelength sensitive (LWS) opsins as well as insect SWS and Rh7 opsins. The outgroup is not shown but consisted of Placozoa opsin-like sequences. Crustacean UV opsin clades (cUV) are labeled. Bootstrap values for nodes are represented by white circles (70-79%), grey circles (80-89%), and black circles (90-100%).

Table 1: De novo transcriptome assembly statistics of individual and combined larval *Neogonodactylus oerstedii* assemblies

Assembly	Number of transcripts	Mean transcript length (bp)	N50 (bp)	Complete BUSCOs (%)	NoUV1 mRNA expression (TPM)	NoUV2 mRNA expression (TPM)	NoUV3 mRNA expression (TPM)
Noer Lar 1	138,683	740	1,301	82.4	539	512	250
Noer Lar 2	129,686	803	1,484	85.5	290	345	242
Noer Lar 3	145,073	847	1,651	89.9	768	866	965
Combined	236,330	759	1,367	91.8			

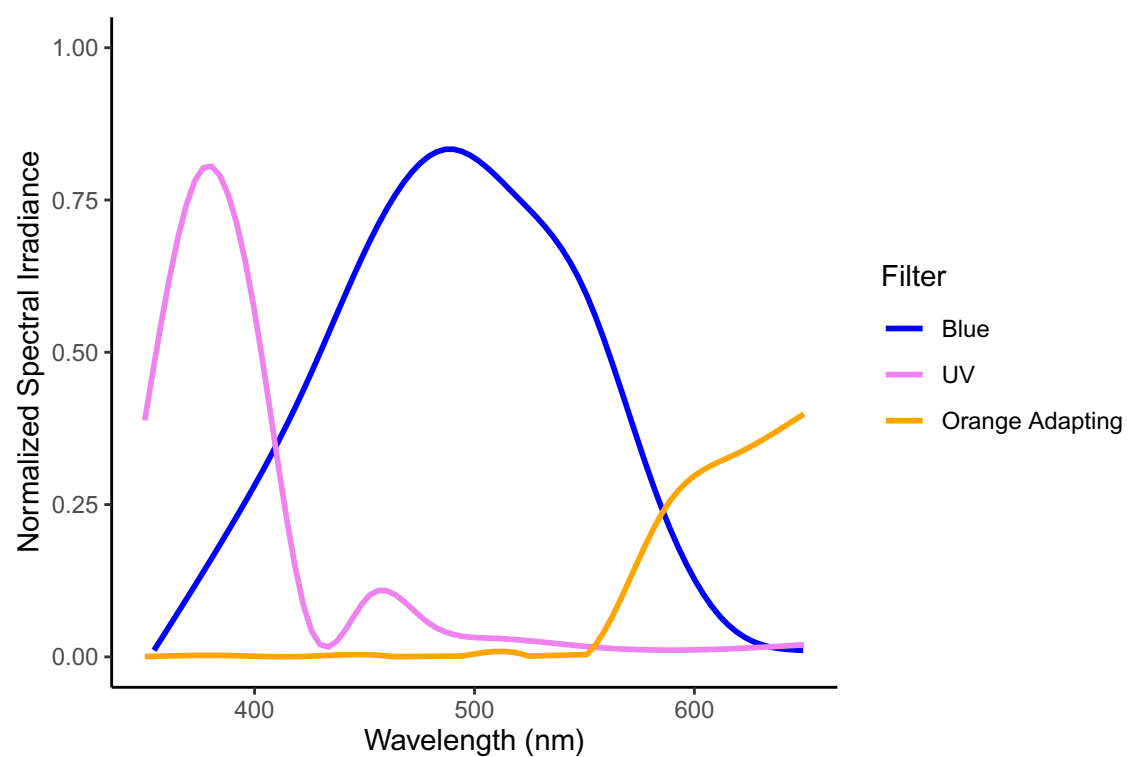


Fig. S1. Filter spectra used in the study measured using a radiometrically calibrated spectrometer (QEPro, 1000 micron diameter fiber with a cosine corrector, Ocean Insight, Orlando FL, USA), displaying representative blue (FG39, 1.2 ND), UV (FGUV5, no ND), and orange (OG570, 0 ND) lights used in this study.

Table S1. Sequence information used to generate the maximum likelihood phylogeny shown in Figure 4.

ID	species	spectral sensitivity	NCBI accession	Alt. source	Notes
LMS.triCa	<i>Tribolium castaneum</i>	LWS	ABA00706		
LWS.Catbo	<i>Cataglyphis bombycinus</i>	LWS	AF042787		
LMS.meoOe	<i>Neogonodactylus oerstedii</i>	LWS	DQ646869		
LMS1.droM	<i>Drosophila melanogaster</i>	LWS	NP_524407		
LWS.Megvi	<i>Megoura viciae</i>	LWS	AF189714		
CRUST.Par	<i>Paramysis intermedia</i>	LWS	EU233609		
Crust.Eup	<i>Euphasia superba</i>	LWS	DQ852580		
CRUST.Hom	<i>Homarus gammarus</i>	LWS	DQ852590		
LMS2.Limp	<i>Limulus polyphemus</i>	LWS	P35361		
LMS.lucCr	<i>Luciola cruciata</i>	LWS	BAH56227		
Dapu.77472	<i>Daphnia pulex</i>	MWS	EFX77472		
BCR1.triG	<i>Triops granarius</i>	MWS	BAG80979		
CRUST.Bra	<i>Branchinella kugenumaensis</i>	MWS	AB298789		
BCR1.triL	<i>Triops longicaudatus</i>	MWS	BAG80982		
Dapu.63277	<i>Daphnia pulex</i>	MWS	EFX63277		
BCRb.hemS	<i>Hemigrapsus sanguineus</i>	MWS	D50584		
MWS.Tical	<i>Tigriopus californicus</i>	MWS	ADZ45237		
MWS.Vatsu	<i>Vargula tsujii</i>	MWS	ADZ45236		
BCR2.braK	<i>Branchinella kugenumaensis</i>	MWS	BAG80986		
BCR3.braK	<i>Branchinella kugenumaensis</i>	MWS	BAG80985		
Dapu.77471	<i>Daphnia pulex</i>	MWS	EFX77471		
Dapu.72329	<i>Daphnia pulex</i>	MWS	EFX72329		
Dapu.63569	<i>Daphnia pulex</i>	MWS	EFX63569		
BCR.triGr	<i>Triops granarius</i>	MWS	BAG80976		
Dapu.72331	<i>Daphnia pulex</i>	MWS	EFX72331		
MWS.Gevom	<i>Gelasimus vomeris</i>	MWS	ACT31580		
SWS.anoGa	<i>Anopheles gambiae</i>	SWS	XP_319247		
SWS.Nyman	<i>Nymphalis antiopa</i>	SWS	AAY16526		
SWS.diaNi	<i>Dianemobius nigrofasciatus</i>	SWS	BAF45422		
SWS.Papxu	<i>Papilio xuthus</i>	SWS	BAA93469		
SWS.Piera	<i>Pieris rapae</i>	SWS	BAE19945		
UV7.droMe	<i>Drosophila melanogaster</i>	Rh7	NP_524035		
UV7.anoGa	<i>Anopheles gambiae</i>	Rh7	XP_308329		
UV7a.acyP	<i>Acyrtosiphon pisum</i>	Rh7	XP_001950416		

UV7.droYa	<i>Drosophila yakuba</i>	Rh7	XP_002094554		
UV7.culQu	<i>Culex quinquefasciatus</i>	Rh7	XM_001861603		
UV5.pedHu	<i>Pediculus humanus</i>	UV	XP_002422743		
UV.Bomim	<i>Bombus impatiens</i>	UV	AAV67326		
UV.Camab	<i>Camponotus abdominalis</i>	UV	AAC05092		
UV5.diaNi	<i>Dianemobius nigrofasciatus</i>	UV	BAG71429		
UV5.papXu	<i>Papilio xuthus</i>	UV	BAA93470		
UV5.manSe	<i>Manduca sexta</i>	UV	AAD11965		
UV.Hearm	<i>Helicoverpa armigera</i>	UV	ADW20311		
UV.Grbim	<i>Gryllus bimaculatus</i>	UV	AEG78686		
UV.Hasal	<i>Harpegnathos saltator</i>	UV	EFN88089		
UV4.droMe	<i>Drosophila melanogaster</i>	UV	NP_476701		
Adult NoUV1	<i>Neogonodactylus oerstedii</i>	cUV1	AIF73507		
Larval NoUV1	<i>Neogonodactylus oerstedii</i>	cUV1	OK432515		Present study
PsciIUV1	<i>Pseudosquilla ciliata</i>	cUV1		Porter et al., 2013	contig02135
UV_Pvan	<i>Litopenaeus vannamei</i>	cUV1	XP_027206668		
Adult NoUV3	<i>Neogonodactylus oerstedii</i>	cUV1	AIF73509		
Larval NoUV3	<i>Neogonodactylus oerstedii</i>	cUV1	OK432517		Present study
Dapu.81332	<i>Daphnia pulex</i>	cUV1	EFX81332		
UV.Mevol	<i>Megabalanus volcano</i>	cUV1	ATQ64330		
Adult NoUV2	<i>Neogonodactylus oerstedii</i>	cUV2	AIF73508		
Larval NoUV2	<i>Neogonodactylus oerstedii</i>	cUV2	OK432516		Present study
PsciIUV2	<i>Pseudosquilla ciliata</i>	cUV2		Porter et al., 2013	contig03244
HecalUV2	<i>Hemsquilla californiensis</i>	cUV2		Porter et al., 2013	contig0257
UV2_Pvan	<i>Litopenaeus vannamei</i>	cUV2	XP_027215370		
UV_Saver	<i>Sagmariasus verreauxi</i>	cUV2		Buckley et al., 2016	Sv_GPCR_A53
UV_Prcla	<i>Procambarus clarkii</i>	cUV2	KT304797		
					opsin-like but lacking the lysine in retinal-binding site
TRIADDRAFT_53608	<i>Trichoplax adhaerens</i>	Outgroup	XP_002109502		
					opsin-like but lacking the lysine in retinal-binding site
TRIADDRAFT_58557	<i>Trichoplax adhaerens</i>	Outgroup	XP_002114578		
					opsin-like but lacking the lysine in retinal-binding site
TRIADDRAFT_58590	<i>Trichoplax adhaerens</i>	Outgroup	XP_002114592		
					opsin-like but lacking the lysine in retinal-binding site
TRIADDRAFT_28157	<i>Trichoplax adhaerens</i>	Outgroup	XP_002114763		
					opsin-like but lacking the lysine in retinal-binding site