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Localization of multiple opsins in ocular and non-ocular tissues of deep-sea shrimps and the first evidence of co-localization in a rhabdomeric R8 cell (Caridea: Oplophoroidea)

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ABSTRACT

Bioluminescence is a prevalent phenomenon throughout the marine realm and is often the dominant source of light in mesophotic and aphotic depth horizons. Shrimp belonging to the superfamily Oplophoroidea are mesopelagic, perform diel vertical migration, and secrete a bright burst of bioluminescent mucous when threatened. Species in the family Oplophoridae also possess cuticular light-emitting photophores presumably for camouflage via counter-illumination. Many species within the superfamily express a single visual pigment in the retina, consistent with most other large-bodied mesopelagic crustaceans studied to date. Photophore-bearing species have an expanded visual opsin repertoire and dual-sensitivity visual systems, as evidenced by transcriptomes and electroretinograms. In this study, we used immunohistochemistry to describe opsin protein localization in the retinas of four species of Oplophoroidea and non-ocular tissues of *Janicella spinicauda*. Our results show that *Acanthephyra purpurea* (Acanthephyridae) retinas possess LWS-only photoreceptors, consistent with the singular peak sensitivity previously reported. Oplophoridae retinas contain two opsin clades (LWS and MWS) consistent with dual-sensitivity. *Oplophorus gracilirostris* and *Systellaspis debilis* have LWS in the proximal rhabdom (R1-7 cells) and MWS2 localized in the distal rhabdom (R8 cell). Surprisingly, *Janicella spinicauda* has LWS in the proximal rhabdom (R1-7) and co-localized MWS1 and MWS2 opsin paralogs in the distal rhabdom, providing the first evidence of co-localization of opsins in a crustacean rhabdomeric R8 cell. Furthermore, opsins were found in multiple non-ocular tissues of *J. spinicauda*, including nerve, tendon, and photophore. These combined data demonstrate evolutionary novelty and opsin duplication within Oplophoridae, with implications for visual ecology, evolution in mesophotic environments, and a mechanistic understanding of adaptive counter-illumination using photophore bioluminescence.

1. Introduction

The marine realm represents the largest habitat on Earth and is home to virtually every light environment. Light penetrates the ocean depths in a density dependent manner, with the composition of suspended particles determining which wavelengths of light are deepest reaching. In epi- (0–200 m) and mesopelagic (200–1000 m) depths, ambient light is comprised of downwelling atmospheric light that varies in spectra based on its origin (e.g., sunlight, moonlight, stars, aurora, and anthropogenic light) (Johnsen, Widder and Mobley, 2004), and depending on depth may be dominated by bioluminescence (Cronin et al., 2016). At these depths light offers pelagic animals a high fidelity signal over long distances for visually guided behaviors that range from following a visual isolume for diel vertical migration (Cohen and Forward, 2016), foraging, congener recognition, prey capture, and being lured or deterred by bioluminescence (see review: Haddock, Moline, & Case, 2010).

Among the many different sources of light throughout the ocean, bioluminescence is remarkably prevalent, with upwards of 75% of pelagic animals exhibiting some form of light emission (Martini and Haddock, 2017). Bioluminescence comes in many forms, having independently evolved at least 94 times (Lau and Oakley, 2021), with

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functions ranging from offense and defense to communication (Haddock, Moline, & Case, 2010). At the molecular level, bioluminescence is produced by the oxidation of a molecule, luciferin, by an enzyme, luciferase, that is either encoded by the genome or a bacterial symbiont. A dynamic range of depths called the bioluminescence compensation depth exists throughout the ocean, and is based on *in situ* abundance of bioluminescent species and prevailing atmospheric light (Cronin et al., 2016). Below the bioluminescence compensation depth, incident bioluminescent light exceeds atmospheric light and dominates visual ecological dynamics. Ambient light is only half the equation, and to understand the visual ecology of a habitat, we must also understand the optical properties of its inhabitants.

Animals detect light using a wide variety of different eye-types with molecular and optical adaptations corresponding to their life history (Cronin, 2014). The light-sensitive molecules in animal eyes that facilitate vision are *opsins,* G-protein coupled receptors that absorb light when bound to a vitamin-A chromophore (Ovchinnikov, 1982). Decapods possess a wide range of opsin diversity from some anomurid crabs with no opsins at all to prawns with upwards of 19 opsins (Palecanda et al., 2022). The putative visual opsins in decapods form three groups broadly defined by the wavelength of absorption, i.e., long-wavelength (LWS), middle-wavelength (MWS), and short-wavelength (SWS/UVS) sensitive (Palecanda et al., 2022). A typical decapod crustacean retina is comprised of a complex array of ommatidia each with a cornea and crystalline cone that guide light onto eight rhadbomeric cells that contain opsin proteins. Many deep-sea decapods have reflecting superposition optics that concentrate light from multiple angles onto large rhabdoms (see review: Nilsson, 1989). By both concentrating light and increasing the surface area of rhabdoms, photons are more likely absorbed by an opsin protein with a resultant increase in photosensitivity. The rhabdoms are comprised of seven proximal cells (R1-7) that usually express middle- or long-wavelength sensitive opsins, and an eighth distal cell (R8) expressing UV- or short-wavelength sensitive opsins (Cronin and Hariyama, 2002), although the size or presence of the R8 cell varies among species (Gaten, Shelton and Nowel, 2003).

The decapod shrimp superfamily Oplophoroidea (Fig. 1), composed of the two families Oplophoridae (Fig. 1A–C) and Acanthephyridae (Fig. 1D), use bioluminescence primarily for defense in the form of a secreted spew used to startle or deter predators (Herring, 1976). Many species are cosmopolitan and strong vertical migrators with depth ranges that span epi- and bathypelagic zones depending on time of day. Examples include species such as *Acanthephyra purpurea* migrating within the meso- and bathypelagic depths (300–3292 m) and *Janicella spinicauda, Systellaspis debilis,* and *Oplophorus gracilirostris* all migrating into the epipelagic at night (Burdett et al., 2017). The family Oplophoridae, including species from the genera *Janicella, Oplophorus,* and *Systellaspis*, also possess cuticular photophores that emit ventrally directed light presumably for counter-illumination throughout diel vertical migration (Wong et al., 2015). Counter-illumination is achieved across those depths by precisely matching the intensity of

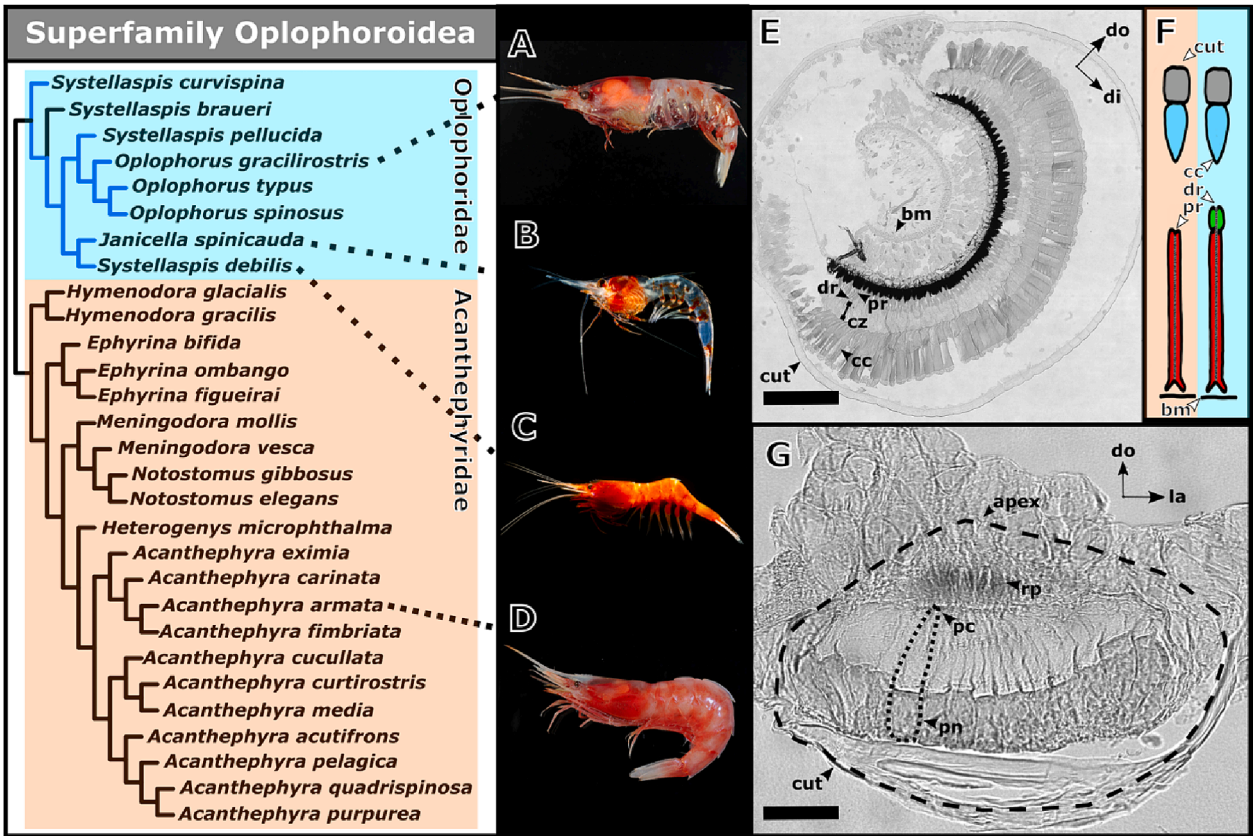

Fig. 1. Phylogeny of the superfamily Oplophoroidea, including families Oplophoridae (blue shaded) and Acanthephyridae (red shaded) recreated from Wong et al. (2016). Blue branches indicate photophore-bearing species. A–D. Images of select species (with dotted line corresponding to placement on the phylogeny) reproduced under open access and creative commons license. A. *Oplophorus gracilirostris* (Corbari, L., MNHN-IU-2013-2565); B. *Janicella spinicauda* (Bracken-Grissom et al., 2020); C. *Systellaspis debilis* (DeLeo & Bracken-Grissom 2022); D. *Acanthephyra armata* (Corbari, L. MNHN-IU-2013-2693) is shown to represent the genus. E,F,G. Schematics of focal tissues for the present study. E. Transmitted light micrograph of *J. spinicauda* retina. F. Graphic depicting key features of photoreceptors from Acanthephyridae (red shaded) and Oplophoridae (blue shaded); note the presence and location of the distal rhabdom (dr) in Oplophoridae. G. Transmitted light micrograph of *J. spinicauda* pleopod photophore (bounded by thick dashed line) with an individual photocyte shown (bounded by short dashed line). Abbreviations: bm = basement membrane, cc = crystalline cone, cut = cuticle, cz = clear zone, di = distal, do = dorsal, dr = distral rhabdom, la = lateral, pc = paracrystalline bodies, pn = photocyte nuclei, pr = proximal rhabdom, rp = reflecting pigment. Scalebars, E: 250 μm; G: 50 μm.

bioluminescence to that of downwelling light, and can involve tilting the photophores to maintain a downward orientation in some oplophorids (Nowel, Shelton and Herring, 1998) and other crustaceans (Land, 1980; Grinnell et al., 1988). Many species in the superfamily have a single peak spectral sensitivity in the retina, consistent with most other large-bodied mesopelagic crustaceans studied to date (Marshall et al., 1999; Warrant and Adam Locket, 2004). In the family Acanthephyridae, all tested species have a single peak visual sensitivity with some variation among them, namely *Notostomus gibbosus* and *Notostomus elegans* ($\lambda max = 490$ nm) and *Acanthephyra smithi* and *Acanthephyra curtirostris* ($\lambda max = 510$ nm) (Frank and Case, 1988). In Oplophoridae, species that possess cuticular photophores have an expanded opsin repertoire compared to Acanthephyridae (Wong et al., 2015) and dual visual sensitivity (Frank and Case, 1988; Cronin and Frank, 1996). Oplophorids: *Oplophorus gracilirostris, Oplophorus spinosus, Systellaspis debilis,* and *Janicella spinicauda*, possess cuticular photophores and visual sensitivity with peak absorbance at approximately 400 nm and 500 nm (Frank and Widder, 1994a).

Oplophoridae dual sensitivity was confirmed behaviorally, with *Janicella, Systellaspis,* and *Oplorphorus* species responding equally to blue-green and near-UV light compared to *Acanthephyra* species that were significantly less reactive to the near-UV light (Frank and Widder, 1994b, 1994a). Interestingly, electroretinogram data from *Janicella spinicauda* may suggest either an additional pigment or a broad short-wavelength sensitivity relative to *Oplophorus* and *Systellaspis*, with peak absorbance ranging from 350 nm to 420 nm (Frank and Case, 1988) (Frank *personal communication*). Dual sensitivity in mesopelagic light environments may facilitate color opponency for congener recognition of photophore bioluminescence, for discriminating between photophore and spew, or enhance foraging success by broadening the range of light absorbance for increased sensitivity to visual stimuli (see review: Nilsson, 2013). Additionally, many oplophorid shrimp are strong migrators and dual sensitivity may also aid in behaviorally tracking a preferred light environment, or isolume, during diel vertical migration particularly for UV light, which is detectable at daytime depths to 600 m (Frank and Widder, 1996). It is also possible dual sensitivity operates in parallel channels for independent functions, with one photoreceptor type for motion detection during navigation and the other for prey detection (Baden, 2024).

The broad depth horizon of oplophorids almost certainly overlaps with the predicted bioluminescence compensation depth. Under mesophotic conditions, counterillumination in shrimp is achieved by precisely tuning the amount of light emitted from photophores to match downwelling irradiance, and the regulation involves a combination of internal control within photophores integrated with the visual system (Warner, Latz and Case, 1979). Previous work has found a whole complement of phototransduction genes expressed in photophore transcriptomes (Wong et al., 2015), and opsin mRNA and G-q protein have been identified within the photophores through fluorescent *in situ* hybridization and immunohistochemistry (Bracken-Grissom et al., 2020). Furthermore, photophores exposed to light experienced similar tissue damage pathology as observed in the retina (Bracken-Grissom et al., 2020). In the present study we have developed antibodies for Oplophoroidea LWS, MWS1, and MWS2 opsins and demonstrated protein localization both in the retina and in non-ocular tissues. This work confirms the molecular basis for dual sensitivity seen in oplophorids and provides additional evidence that shrimp photophores and nervous tissue possess proteins fundamental for light sensitivity and may be involved in regulating emissions for counterillumination.

2. Methods

2.1. Animals

The study of visual sensitivity and photophore photosensitivity in deep-sea species requires that the animals be protected from damaging surface light (Lawry, 1974; Bracken-Grissom et al., 2020) and warm water temperatures (Frank and Case, 1988). In this study, live specimens of *Acanthephyra purpurea* (A. Milne-Edwards, 1881), *Oplophorus gracilirostris* (A. Milne-Edwards, 1881), *Systellaspis debilis* (A. Milne-Edwards, 1881), and *Janicella spinicauda* (A. Milne-Edwards, 1883), were collected aboard the R/V Walton Smith and R/V Points Sur as part of DEEPEND | RESTORE cruises into the Gulf of Mexico using a light-tight, thermally insulated cod-end that could be closed at depth (Bracken-Grissom et al., 2020), sorted using dim red light, and paraformaldehyde-fixed (4 % PFA in 0.2 μm filtered sea water) in dark conditions. Fixed whole specimens were dehydrated in an ethanol series (ethanol in filtered-seawater: 25 %, 50 %, 75 %, 90 %, 100 %) and stored in 100 % ethanol at −20 °C until rehydration, paraffin embedding and sectioning.

2.2. Immunohistochemistry

PFA-fixed tissues were paraffin embedded. Eyes from all four species were serially sectioned at 7 μm thick across four slides (three opsins and one no-primary control) and the abdomen of *Janicella spinicauda*, including pleopod photophores, was sectioned at 10 μm. Serial sections of eyes allowed us to label the same photoreceptors with multiple opsin antibodies across several slides. Custom anti-Opsin antibodies (Pacific Immunology Corporation) were designed against *J. spinicauda* LWS, MWS1, and MWS2 coding sequences confirmed from previously published transcriptome data (Bracken-Grissom et al., 2020). The amino acid peptides (LWS: SHPKYRAALEKKLPC (15 aa); MWS1: CSYDYISQDWNTKSYNL (17 aa); MWS2: RNHEKALRDQAKRMGVESLRSN-Cys (23 aa)) were selected for homology across the Oplophoroidea superfamily including sequences, where available, from *J. spinicauda, O. gracilirostris, O. spinosus, S. debilis,* and *A. purpurea*. The host for the antibodies were New Zealand White Rabbit.

Sectioned tissues were deparaffinized in xylene, rehydrated in an ethanol series, and rinsed three times in Phosphate Buffered Saline (PBS) for 30 min each. Unspecific binding sites were blocked using 10 % Normal Goat Serum in PBS (NGS) for 2 h at room temperature. The NGS solution was replaced with 0.1 % Triton x-100 in 1x PBS (PBSTx). Primary antibody was diluted in PBSTx (1:200) and applied to sections at 4 °C overnight. Sections were rinsed in 10 % NGS three times for 10 min each then replaced with PBSTx. Secondary antibody (DyLight™ 633; Invitrogen) was diluted in PBSTx (1:200) and applied to sections at 4 °C overnight. Sections were rinsed three times in PBS for 10 min each and mounted in ProMount Pro Long Gold with DAPI (Invitrogen). No-primary controls were created identically for sectioned tissues, replacing primary antibody with PBS.

2.3. Imaging

Labelled and mounted sections were imaged on a Leica SP8 X Confocal Laser Scanning Microscope with white light (470–670 nm) and UV lasers on an upright DM6 microscope at the Biological Electron Microscope Facility, University of Hawai'i at Mānoa. Fluorophores used were DAPI and Dylight 633 with gating and sequential detection. Laser power remained consistent across all samples for each magnification. Tile scan z-stacks were collected for focal images of each opsin label in the eye and a no-primary control cross section of the retina and pleopod for context. All opsin antibody labelled images were gathered as z-stacks with maximum projection and LIGHTNING deconvolution.

3. Results

The salient features of superfamily Oplophoroidea eye and photophore morphology are depicted with transmitted light microscopy and graphically in Fig. 1E–G. Anti-LWS opsin antibodies labeled the proximal rhabdom (R1-7 cells) in all Oplophoroidea investigated, including species from the family Acanthephyridae: *Acanthephyra purpurea* (Fig. 2A, C), and the family Oplophoridae: *Oplophorus gracilirostris*

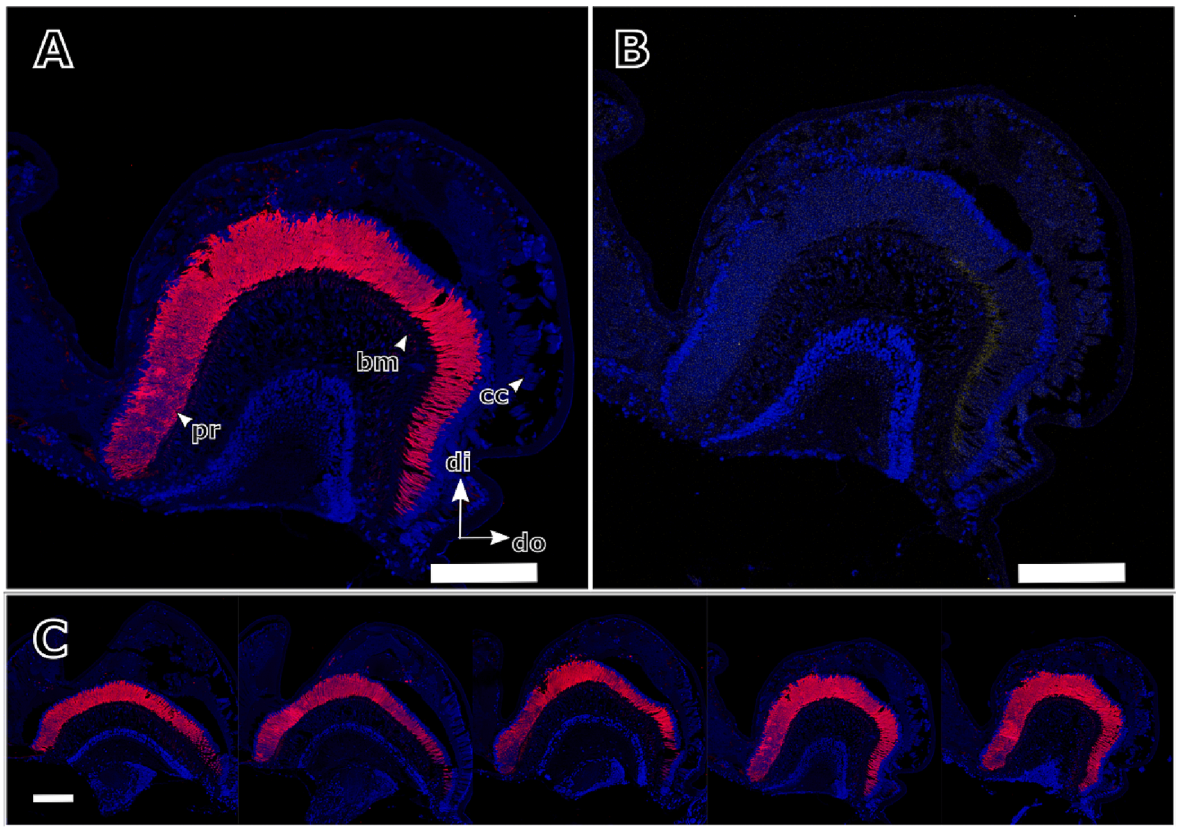

Fig. 2. A: Tile scan maximum projection of *Acanthephyra purpurea* retina with LWS opsin labeling in the rhabdoms. B: No primary control demonstrating minimal autofluorescence and non-specific secondary labeling (yellow and non-punctate blue). C: Serial section of retina demonstrating ubiquitous localization of LWS opsin proteins with no apparent intraretinal variation. Red: LWS (panels A,C), blue: DAPI (panels A-C). Abbreviations: bm = basement membrane, cc = crystalline cone, di = distal, do = dorsal, pr = proximal rhabdom. Scalebars = 250 μm.

(Fig. 3A, C), *Systellaspis debilis* (Fig. 4A, C), and *Janicella spinicauda* (Fig. 5A, D). MWS localization was not observed in *A. purpurea* (Fig. 2B). MWS2 was localized in the distal rhabdoms (R8 cell) of all Oplophoridae species investigated (*O. gracilirostris,* Fig. 3B, D; *S. debilis,* Fig. 4B, D; *J. spinicauda,* Fig. 5B, E). Unexpectedly, both MWS1 and MWS2 were found co-localized in *J. spinicauda* R8 cells (Fig. 5B, C, E, F). MWS1 was not found in retinas of *A. purpurea* as predicted, nor *O. gracilirostris* and *S. debilis* retinas despite being present in eye transcriptomes.

Serial sections of the eyes in all species revealed that opsin localization was uniform in both the distal (LWS) and proximal (MWS1, MWS2) rhabdoms with no clear pattern of differential expression across the retina (Figs. 2–5). For all antibodies tested, autofluorescence and background labeling were minimal throughout the retina in the 633 channel, with only some autofluorescence in the basement membrane of *A. purpurea, O. gracilirostris,* and *S. debilis* (e.g., Fig. 2B) and abdomen (Fig. 6A) when using a no-primary control (see Supplementary Figures for a complete set of no-primary control images). Secondary labeling was observed in optical structures, notably MWS2 punctate at the base of *S. debilis* crystalline cones and all three visual opsins surrounding *J. spinicauda* crystalline cones (Fig. 5).

Opsin labeling was also observed in multiple non-ocular tissues of *J. spinicauda*. Minimal autofluorescence was observed when using a no-primary control and a tile scan of the whole abdominal cross section is shown for context (Fig. 6A). In photophores, LWS was localized in a punctate pattern along the tendon attached to the apex of the photophore (Fig. 6B) while MWS1 labelled the apex of pleopod photophores in proximity to the paracrystalline bodies (Fig. 6C), the structures presumed to be the source of bioluminescence (Nowel, Shelton and Herring, 1998). Opsin antibodies also labelled putative nervous tissue, namely LWS in tissue projecting through dorso-lateral white muscle tissue with features consistent with a crustacean ensheathed nerve (Young, 1936; Villegas and Sánchez, 1991) (Fig. 6D) and MWS1 in the ventral nerve ganglia (Fig. 6E).

4. Discussion

Here we provide molecular evidence of opsin protein localization in ocular tissues of select species from the superfamily Oplophoroidea, the discovery of both LWS and MWS opsins in non-ocular tissues, and the first evidence of opsin co-localization in R8 retinular cells of *Janicella spinicauda.* As previously reported, oplophorid species express visual opsins in their eyes from four clades: one long-wavelength sensitive clade (LWS), two middle-wavelength sensitive clades (MWS1 and MWS2), and one short-wavelength sensitive clade (SWS/UVS) (Wong et al., 2015; Bracken-Grissom et al., 2020; DeLeo and Bracken-Grissom, 2020). Our opsin localization data support the previous studies demonstrating that LWS and MWS clades are not only expressed in the retinas, but translated into proteins in rhabdomeric cells. Further, we found molecular evidence for dual-sensitivity in oplophorid shrimp (Frank and Case, 1988) by confirming that LWS was localized in the proximal rhabdom (R1-7 cells), and MWS was localized in the distal rhabdom (R8 cells).

Our finding of MWS co-localization in the R8 cell is novel in decapod crustaceans and provides additional data for the mounting evidence against the one cell, one opsin orthodoxy. The novel co-localization found in *J. spinicauda* is supported by previous work that found opsin co-expression in fish (Hofmann et al., 2010), butterfly (Arikawa, 2003), decapod crabs (Sakamoto et al., 1996; Rajkumar et al., 2010), red flour beetle (Jackowska et al., 2007), and horseshoe crab (Katti et al., 2010). In decapods, the co-expression was restricted to R1-7 cells (Sakamoto

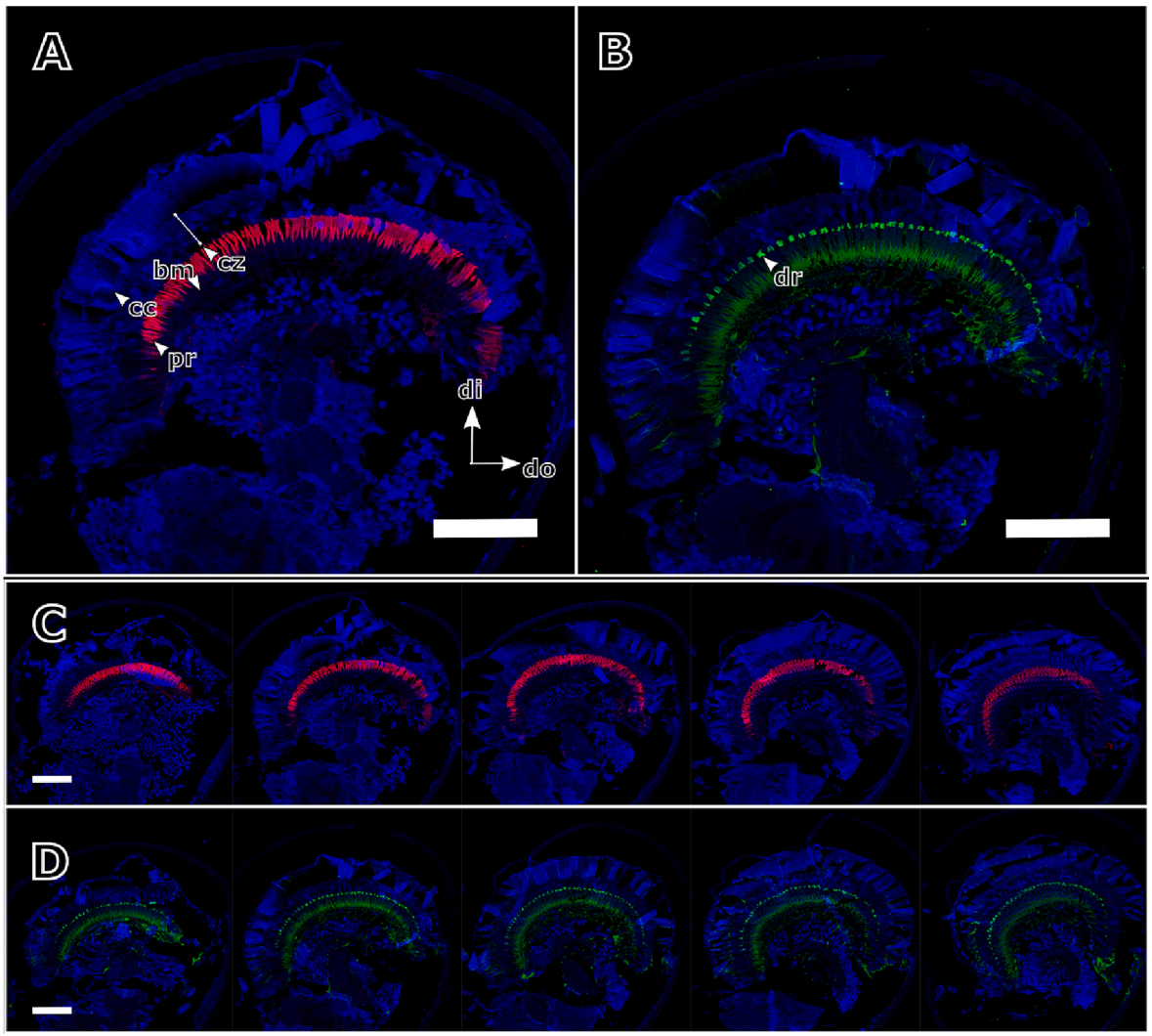

Fig. 3. A, B: Tile scan maximum projection of *Oplophorus gracilirostris* retina with opsin labeling in the rhabdoms. C, D: Serial sections of retina demonstrating ubiquitous localization of LWS and MWS2 opsin proteins with no apparent intraretinal variation. LWS is localized in the proximal rhabdoms (R1-7) and MWS2 is localized in the distal rhabdoms (R8). Red: LWS (panels A,C); green: MWS2 (panels B,D); blue: DAPI (panels A-D). Abbreviations: bm = basement membrane, cc = crystalline cone, cz = clear zone, di = distal, do = dorsal, dr = distal rhabdom, pr = proximal rhabdom. Scalebars = 250 μm.

et al., 1996; Rajkumar et al., 2010) and evidence for protein localization is absent. Interestingly, co-expression in the red flour beetle involved both UV- and long-wavelength sensitive opsins (Jackowska et al., 2007). Co-localization presumably functions to broaden the peak sensitivity of co-expressing cells. Previous electroretinogram data suggest *J. spinicauda* possess broader peak sensitivity of 350–420 nm compared to sister genera, *Oplophorus* and *Systellaspis* (Frank and Case, 1988). Furthermore, *J. spinicauda* have increased temporal resolution after light adaptation similar to euphausiid shrimp, a phenomenon not normally observed in deep-sea species (Frank, 2003), and a broader absorption may account for greater photon capture contributing to a quicker response. Although an SWS/UVS opsin was found in *J. spinicauda* eye transcriptomes (Bracken-Grissom et al., 2020), the transcript recovered was a fragment from which we were unable to develop an antibody, and it was not detected in any other members of the superfamily. When short-wavelength sensitivity is present in crustaceans, it is typically confined to the R8 cells and absorbs light before it is scattered by deeper tissues including MWS/LWS-sensitive R1-7 retinular cells. Based on the co-localization of MWS1 and MWS2 in the R8 combined with the fact that transcriptomes contained only fragments of SWS/UVS opsin with comparatively low expression, we therefore consider it unlikely the SWS/UVS opsin contributes to the short-wavelength peak absorbance previously reported in adults. Rather, we predict earlier developmental stages of oplophorid shrimp use the SWS/UVS opsin instead of the adults. Investigation into *J. spinicauda* larvae could reveal visual adaptions throughout their life history, including the expression of a different complement of opsins in the larval retina.

Oplophorids have several modifications for increased sensitivity in mesophotic environments, including superposition optics, reflective tapetum, and intraretinal variation in rhabdom morphology (Shelton, Gaten and Herring, 1992; Gaten, Shelton and Nowel, 2003, 2004). Variation in eye morphology has been described in members of both families with considerable differences found in the proportion of distal (R8) and proximal (R1-7) rhabdoms; the distal rhabdom may account for up to 25 % of the total rhabdom volume for *Systellaspis debilis*, whereas in *Acanthephyra pelagica* distal rhabdoms are reduced to <1 % of the total volume and only visible using electron microscopy (Gaten, Shelton and Nowel, 2003). In Oplophoridae, the relative length of the distal to proximal rhabdoms is greater in the ventral retina presumably to detect short-wavelength scattered light and bioluminescence (Gaten, Shelton and Nowel, 2004). Violet upwelling light from scattered atmospheric sources is present to depths of 200 m and orders of magnitude dimmer than downwelling light (Warrant and Adam Locket, 2004). Broadening the peak absorption of the distal rhabdom by co-localizing two MWS

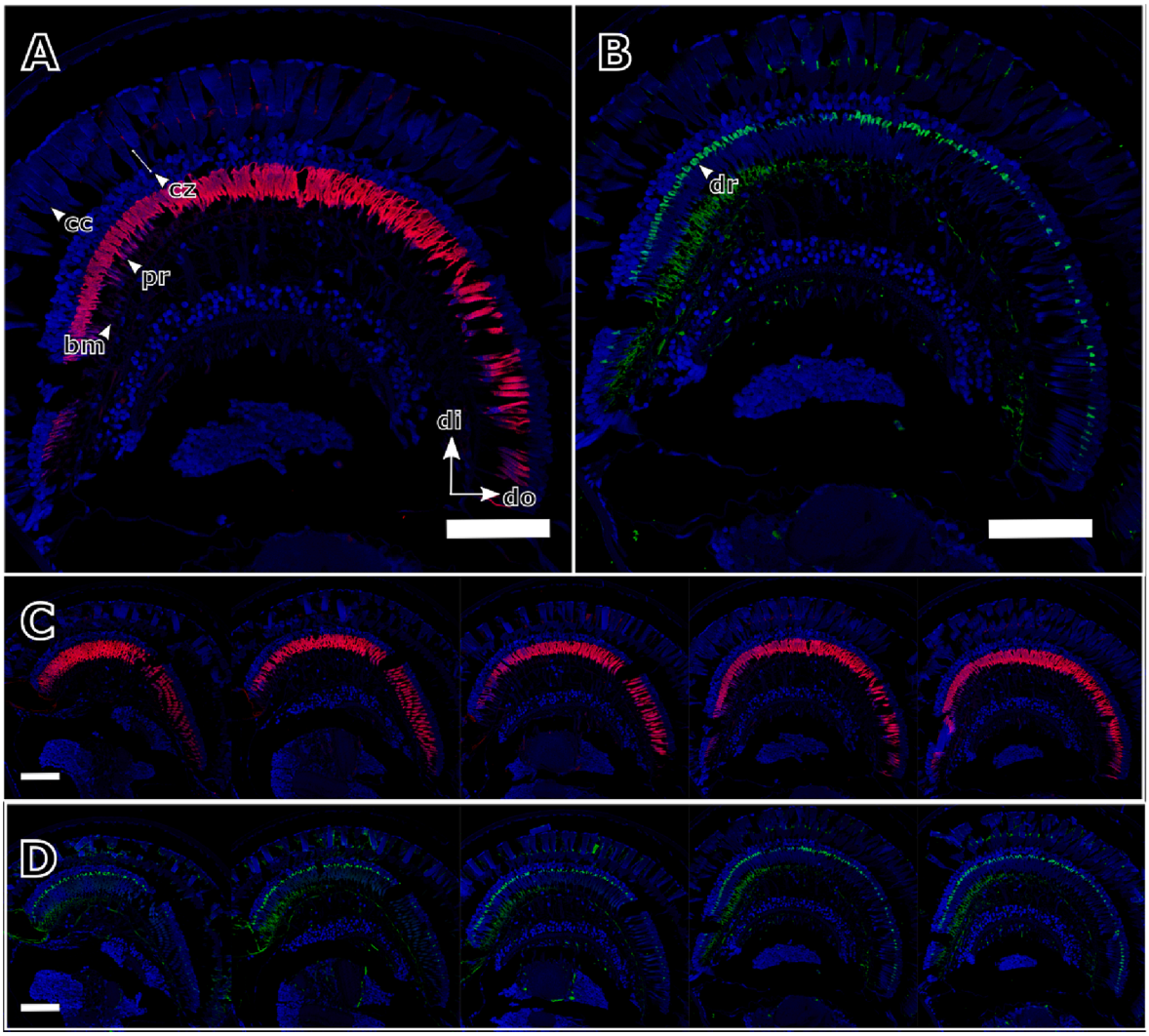

Fig. 4. A, B: Tile scan maximum projection of *Systellaspis debilis* retina with opsin labeling in the rhabdoms. C, D: Serial section of retina demonstrating ubiquitous expression of LWS and MWS2 opsin proteins with no apparent intraretinal variation. LWS is localized in the proximal rhabdoms (R1-7) and MWS2 is localized in the distal rhabdoms (R8). Red: LWS (panels A,C); green: MWS2 (panels B,D); blue: DAPI (panels A–D). Abbreviations: bm = basement membrane, cc = crystalline cone, cz = clear zone, di = distal, do = dorsal, dr = distal rhabdom, pr = proximal rhabdom. Scalebars = 250 μm.

opsins may also serve to increase overall sensitivity of the photoreceptor in *J. spinicauda.* Opsin labeling was observed between the crystalline cones of *J. spinicauda* and *S. debilis* and may act as filters to reduce off-axis light. This function has only recently been posited in salticid jumping spiders (Steck et al., 2024), though opsin transcript expression has been observed between crystalline cones of crickets (Henze et al., 2012), within crystalline cones of bumblebees (Spaethe and Briscoe, 2005), in the clear zones of butterflies (Briscoe et al., 2003), and surrounding amphipod crystalline cones (Ramos et al., 2019). While this type of labeling is often categorized as autofluorescence or background, our negative controls present no evidence of autofluorescence matching the observed patterns and likely represent a biological phenomenon.

Species with dual-sensitivity also possess cuticular photophores, presumably for counterillumination. Although oplophorids match the irradiance of downwelling light, color vision could enable oplophorids to detect the color contrast between photophores as compared to downwelling light for congener recognition. The two modes of bioluminescence, spew and photophore, differ spectrally both within and among species (Herring, 1983), and photophores vary in size, number, and pigmentation (Collins & Bracken-Grissom in press). Color discrimination may be useful in detecting different types of bioluminescence, as well as for species recognition. The color produced by photophore bioluminescence may not intrisically match downwelling light but counterillumination may be achieved by matching light intensity or by filtering emitted light. For example, in lantern fish the photophores possess filtering properties to tune spectral characteristics of bioluminescent light to match ambient light (Paitio et al., 2020). Photophores tuned to match the intensity of downwelling light, but differ from it spectrally, may offer a “hidden” signal for dual-sensitive species surrounded by monochromats. Several species of bioluminescent shrimp, including euphausiids and sergestids, have exhibited swarming behavior in multi-species assemblages (Vereshchaka, 2009; Vereshchaka, Kulagin and Lunina, 2019) and although swarming has not been reported in Oplophoroidea, multiple visual pigments may facilitate color vision and discrimination among complex, heterogenous light signals originating from downwelling light, shrimp, and other bioluminescent species. In d eep sea fish multiple distinct rhodopsins are thought to be adapted for bioluminescence detection under mesophotic and aphotic atmospheric light conditions (Musilova, 2018; Partridge, Archer, & Lythgoe, 1988). In shrimp, however, given the constraints for vision under scotopic conditions, and the trade-off between visual actuity and sensitivity, individuals may be limited in their ability to detect conspecifics across meaningful distances (Schweikert, 2022).

Oplophorids migrate hundreds of vertical meters daily and the

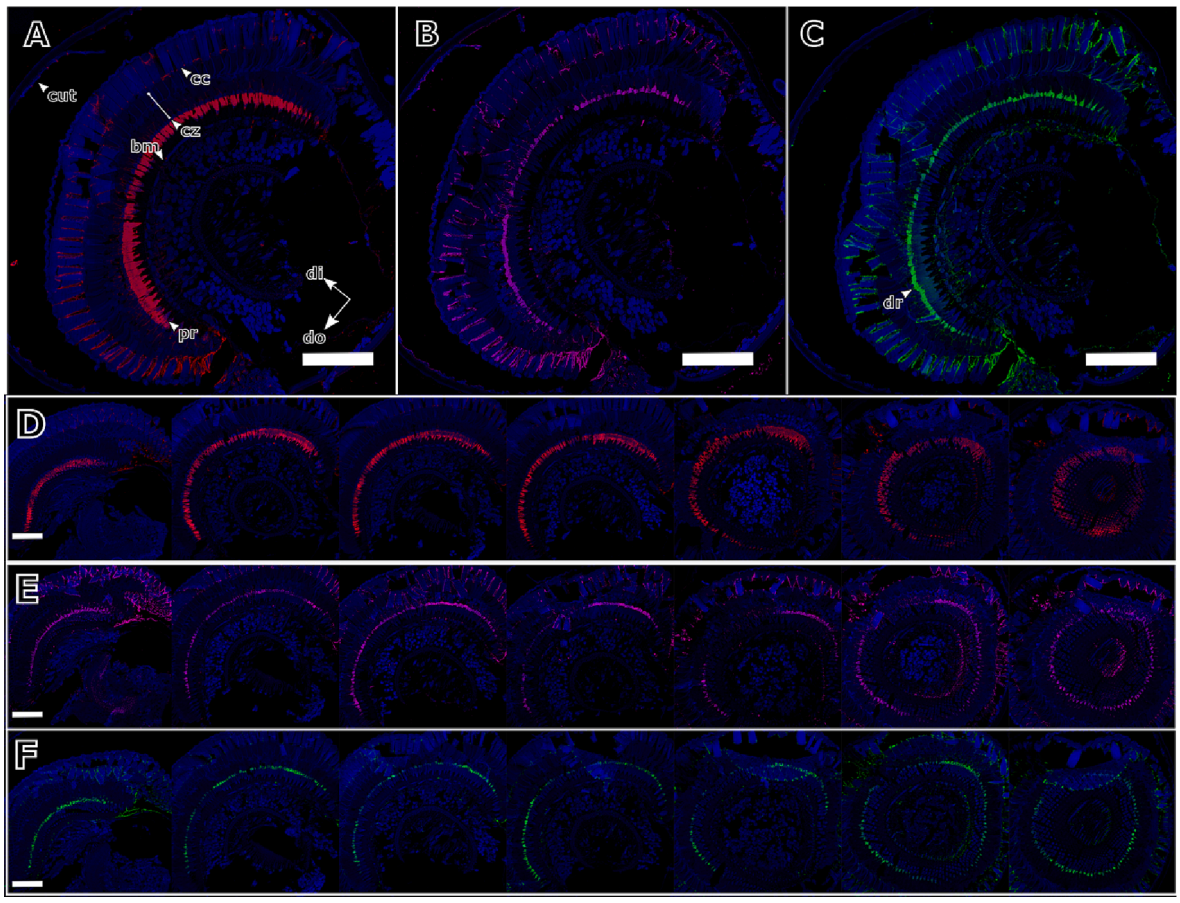

Fig. 5. A, B, C: Tile scan maximum projection of *Janicella spinicauda* retina with opsin labeling in the rhabdoms. D, E, F: Serial section of retina demonstrating ubiquitous localization patterns for each opsin with no apparent intraretinal variation. Red: LWS (panels A, D); magenta: MWS1 (panels B,E); green: MWS2 (panels C, F); and blue: DAPI (panels A–F). MWS1 and MWS2 were co-localized in the distal rhabdom (R8), while LWS was localized in the proximal rhabdom (R1-7). Abbreviations: bm = basement membrane, cc = crystalline cone, cut = cuticle, cz = clear zone, di = distal, do = dorsal, dr = distal rhabdom, pr = proximal rhabdom. Scalebars = 250 μm.

downwelling irradiance changes with depth, time of day, and weather, and the mechanism enabling finely tuned photophore irradiance for counterillumination is unknown. Here we provide evidence for phototranduction proteins localized within bioluminescent organs and nervous tissue that may be used to detect and regulate the amount of bioluminescence emitted. Sergestid shrimp are capable of adjusting bioluminescent intensity in a matter of seconds, and due to the rapidity of change and that retinal information is required for successful tuning, some degree of neural control is expected (Warner, Latz and Case, 1979). Although rapid changes in bioluminescent output to match downwelling light has only been experimentally demonstrated in the sergestid, *Sergestes similis*, given the preponderance of ventrally facing photophores the function is presumed to be ubiquitous throughout the ocean and across taxa. Cuticular photophores in Oplophoridae are comprised of multiple photocytes, each with a distally positioned photocyte nuclei, cytoplasm, and a clear area with paracrystalline bodies at the proximal apex of the cell (Nowel, Shelton and Herring, 1998) (See: Fig. 1G). The aggregate of these photocytes forms the photophore, which has several muscles and tendons attached to the apex that may be used to pivot the organ and direct the emission (Nowel, Shelton and Herring, 1998). Throughout the day shrimp swim in the water column orienting their whole body up, down, and sideways, depending on foraging needs or for predator avoidance. Photophores likely move internally to maintain a downward facing position to ensure the directionality of counter-illumination as observed in the sergestid Organ of Pesta (Latz and Case, 1982). Nerve tissues labeling with opsin were previously reported in other crustaceans (Kingston and Cronin, 2015), and the punctate LWS expression on the tendon and LWS and MWS expression in nervous tissues may serve to detect diffuse downwelling light and initiate the mechanism to tilt emitting photophores vertically down. The intensity of light is also important for counter-illumination, and MWS may be used to detect the amount of light produced internally by the photophore. That information may then be integrated through the nervous system with downwelling visual cues captured in the retina. This is not unprecedented, as lantern fish are able to visually integrate photophore emissions with downwelling light (Lawry, 1974) and *Sergestes* bioluminescence was reduced to zero when eye shields were placed on the shrimp (Warner, Latz and Case, 1979). Based on the kinetics of induction, sergestid shrimp use both neural and hormonal inputs to regulate bioluminescence from the Organ of Pesta based on the visual environment (Latz, 1995), however more work is needed to explore this system. In other systems, light sensitivity can contribute to the regulation of bacterial photophore emissions, as seen in *Euprymna scolopes* (Tong et al., 2009) and even non-bioluminescent color change in vertebrates (Schweikert, Fitak and Johnsen, 2018; Schweikert et al., 2023). Opsin localization both internal to photophores, in the cytoplasm and near the paracrystalline bodies, and external to photophores, in nervous tissues and tendons, from two opsin clades provides additional evidence that oplophorid photophore bioluminescence is in part regulated by non-ocular photosensitivity.

Here we describe the localization of opsin genes in the retinas of four species from the superfamily Oplophoroidea and non-ocular tissues of *Janicella spinicauda.* In ocular tissues, LWS opsins were found in the proximal rhabdom (R1-7 cells) and MWS opsins were found in the distal

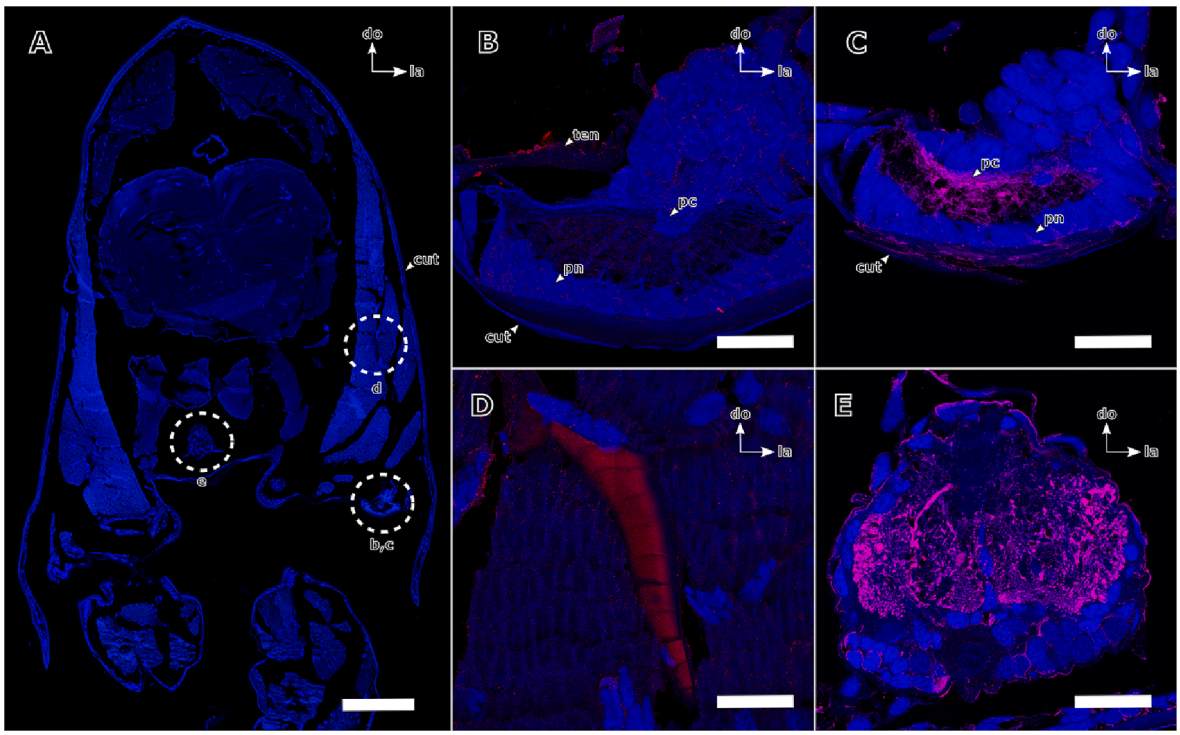

Fig. 6. Immunohistochemistry of *Janicella spinicauda* abdomen cross-section, including pleopod photophores. A: Tilescan of the no-primary control is provided for context with relative regions of images in B, C, D, and E highlighted. B: Punctate LWS labeling (red) on the tendon connected to the photophore. C: MWS1 labeling (magenta) in the photophore near where paracrystalline bodies are located. D: Dorso-lateral muscle with an oblique section of nerve labelled with LWS opsin (red). E: Ventral nerve ganglia with MWS1 opsin label (magenta). Scale bars, A: 250 um; B, C, D: 50 um; E: 10 um. Abbreviations: cut = cuticle, do = dorsal, la = lateral, pc = paracrystalline bodies, pn = photocyte nuclei, ten = tendon.

rhabdom (R8). Further, we provide the first evidence for co-localization of middle-wavelength sensitive opsin proteins (MWS1 and MWS2) in the distal rhabdom (R8 cell) of a decapod shrimp. These data confirm at the protein level the opsins contributing to dual-sensitivity visual systems in Oplophoridae (LWS and MWS2). Opsin localization is not limited to the retina, and we found opsins in the bioluminescent photophores and nervous tissue, which may contribute to fine-tuning the counter-illumination of bioluminescence during diel vertical migration. This research provides a basis for future studies that will advance the field, including studies of MWS co-localization in other Oplophoridae species as well as opsin expression across life history stages to test whether the UV opsin fragment found in adult transcriptomes is functional in larval retinas. Additionally, future studies should take a comparative approach to map non-ocular opsin localization among other photophore bearing species and an experimental approach to test if photosensitivity is directly involved in bioluminescent control for counter-illumination.

CRediT authorship contribution statement

Tom Iwanicki: Conceptualization, Methodology, Investigation, Writing – review & editing, Writing – original draft, Data curation, Visualization. **Mireille Steck:** Writing – review & editing, Writing – original draft, Investigation. **Heather Bracken-Grissom:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Conceptualization. **Megan L. Porter:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Conceptualization.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.visres.2024.108403.

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