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Illuminating the impact of diel vertical migration on visual gene expression in deep-sea shrimp

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Abstract

Diel vertical migration (DVM) of marine animals represents one of the largest migrations on our planet. Migrating fauna are subjected to a variety of light fields and environmental conditions that can have notable impacts on sensory mechanisms, including an organism's visual capabilities. Among deep-sea migrators are oplophorid shrimp that vertically migrate hundreds of metres to feed in shallow waters at night. These species also have bioluminescent light organs that emit light during migrations to aid in camouflage. The organs have recently been shown to contain visual proteins (opsins) and genes that infer light sensitivity. Knowledge regarding the impacts of vertical migratory behaviour, and fluctuating environmental conditions, on sensory system evolution is unknown. In this study, the oplophorid *Systellaspis debilis* was either collected during the day from deep waters or at night from relatively shallow waters to ensure sampling across the vertical distributional range. De novo transcriptomes of light-sensitive tissues (eyes/photophores) from the *day/night* specimens were sequenced and analysed to characterize opsin diversity and visual/light interaction genes. Gene expression analyses were also conducted to quantify expression differences associated with DVM. Our results revealed an expanded opsin repertoire among the shrimp and differential opsin expression that may be linked to spectral tuning during the migratory process. This study sheds light on the sensory systems of a bioluminescent invertebrate and provides additional evidence for extraocular light sensitivity. Our findings further suggest opsin co-expression and subsequent fluctuations in opsin expression may play an important role in diversifying the visual responses of vertical migrators.

KEYWORDS

bioluminescence, gene expression, invertebrates, opsins, transcriptomics

1 | INTRODUCTION

Diel vertical migration of marine fauna represents the largest daily migration on Earth, in terms of animal biomass (Hays, 2003). This common migratory pattern can be found among all aquatic environments—where animals swim to shallower waters at night to feed and then return to deeper, darker waters (ranging from inches in shallow ponds to hundreds of metres in the ocean) during the day to hide from visual predators (Marshall, 1979). The presence of this phenomenon among a vast array of taxa suggests this behaviour has some adaptive value (reviewed in Lampert, 1989). As ocean optics can vary greatly with depth (i.e. Frank & Widder, 1996) along these routes, fauna migrating between depth zones (e.g. epipelagic and mesopelagic) are subjected to a variety of light fields among

other variable environmental conditions (i.e. temperature and pressure); this can have notable impacts on sensory mechanisms (e.g. Schweikert, Caves, Solie, Sutton, & Johnsen, 2019). However, due to limitations in accessing these ecosystems, little is known with regard to how the sensory systems of deep-sea fauna have responded to the selective pressures associated with a diel migratory lifestyle from both an evolutionary and genomic perspective.

Among the deep-sea fauna participating in long, diel vertical migrations (up to hundreds of metres) are shrimp belonging to the family Oplophoridae. This family consists of 10 genera and approximately 70 species of predatory shrimp with a cosmopolitan distribution (Chan, Lei, Li, & Chu, 2010; Lunina, Kulagin, & Vereshchaka, 2018; Wong, Pérez-Moreno, Chan, Frank, & Bracken-Grissom, 2015). These deep-water migrations may be adaptive as oplophorids can more readily find potential mates as they aggregate in the water column during mass migrations, and/or take advantage of vertically migrating prey (Hays, 2003), similar to observations for deep-sea fishes (Afonso et al., 2014). While all species within Oplophoridae have the ability to emit a bioluminescent oral secretion (see Figure 1a) that is used as a defense mechanism to avoid predation (Herring, 1976), only a few genera (*Systellaspis*, *Janicella* and *Oplophorus*) possess a secondary light-producing mechanism in the form of cuticular photophores (Chan et al., 2010; Nowel, Shelton, & Herring, 1998). Photophores are bioluminescent light organs primarily thought to function in

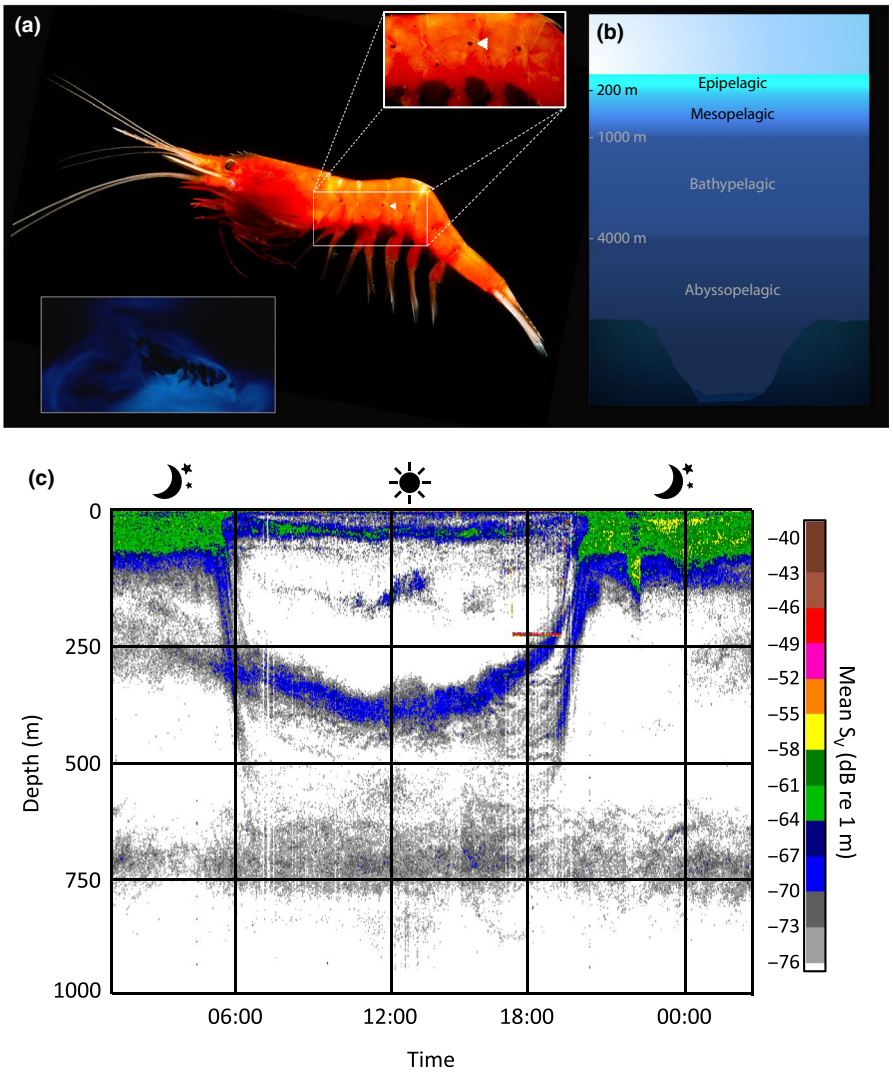

FIGURE 1 (a) The vertically migrating oplophorid *Systellapis debilis*, which possesses dual modes of bioluminescence in the form of an oral secretion (lower left image, *Oplophorus gracilirostris*) and cuticular light organs called photophores across the length of their body (enlarged, upper right—arrow labelling one organ for reference). *S. debilis* has been observed migrating between daytime depths of ~650–900 m and night-time depths of ~150–300 m (min/max 150–4,600 m), which spans several depth zones (b) and corresponding photic environments; This diel vertical migratory behaviour is illustrated by the composite echogram (c) that displays the mean depth-discrete acoustic backscatter over time (~24 hr period). This illustrates the sound-scattering layers (SSL), measured using volume backscattering strength or mean S_v, that are organized by depth and ascend at night and descend during daylight hours. This shows how the SSL (observed as greater S_v) move vertically (*y*-axis, depth[m]) through the day/night (*x*-axis, time[hr]). Data were collected from the Gulf of Mexico in May 2016, and processed and complied to generate mean S_v (log scale of backscatter reported in decibels [dB]) backscatter profiles according to the methods described in D'elia et al. (2016). Photograph credit: ©DantéFenolio

counterillumination (Herring, 1976), a form of dynamic camouflage, though recent investigations suggest they are also photosensitive (Bracken-Grissom et al., 2020). Interestingly, all photophore-bearing oplophorids participate in diel vertical migrations, where camouflage via counterillumination is particularly useful as they migrate into shallower waters at night to feed. In particular, *Systellaspis debilis* bears many photophores (Figure 1a) and has been observed migrating between daytime depths of ~650–900 m and night-time depths of ~150–300 m (Ziemann, 1975) (with min/max depths ranging from 150 to 4,600 m) (Felder, Álvarez, Goy, & Lemaitre, 2009). Their ecological range therefore spans several primary depth zones and corresponding photic habitats (Figure 1b), including the epipelagic or "sunlight" zone (0–200 m), the light-limited mesopelagic or "twilight" zone (200–1,000 m) and the dark bathypelagic or "midnight" zone (1,000–4,000 m) (Costello & Breyer, 2017). Though daily spectral changes in light, ultraviolet (UV) and visible, are considered a significant factor cueing shallow water vertical migrations (Frank & Widder, 1996; Jerlov, 1976; McFarland & Munz, 1975a, 1975b), these spectral shifts do not appear to be detectable in deep waters beyond 150 m (Frank & Widder, 1996). More recently, biochemical rhythms (i.e. circadian clocks) were also hypothesized as a possible mechanism triggering vertical migration in zooplankton inhabiting depths greater than 1,000 m (van Haren & Compton, 2013), which is well within the distributional range of *S. debilis*. Therefore, while light may be a factor cueing shallow water descents, oplophorids most likely use some other nonvisual cue to trigger their upward migrations at greater depths.

In the deep sea (>200 m), the primary sources of light are downwelling blue light (λ_{max} ~ 475 nm) (Cronin, 1986; Dartnall, 1975), that is absent by approximately 1,000 m, and bioluminescence that commonly peaks in the blue spectrum (between 460 and 490 nm) (Herring, 1983; Latz, Frank, & Case, 1988; Widder, Latz, & Case, 1983). According to the visual sensitivity hypothesis, in light-limited environments, the visual pigments of animals will be spectrally matched to that of available light (Crescitelli, McFall-Ngai, & Horwitz, 1985; Goldsmith, 1972). Correspondingly, the visual sensitivities of many deep-sea organisms appear to be limited to blue light wavelengths (rev. Douglas, Hunt, & Bowmaker, 2003; Marshall, Cronin, & Frank, 2003), though there are exceptions. Past physiological, spectrophotometric and behavioural studies investigating the visual sensitivities of oplophorid shrimp revealed that the eyes of some photophore-bearing species, including *S. debilis*, possess a near-ultraviolet (UV) photopigment (λ_{max} ~ 390–410 nm), in addition to the blue–green photopigment common among many deep-sea species (Cronin & Frank, 1996; Frank & Case, 1988; Frank & Widder, 1994a, 1994b; Gaten, Shelton, & Nowel, 2004; Kent, 1997). This "dual sensitivity" system is thought to consist of at least two photopigments with distinct spectral sensitivities—a near UV, short-wavelength-sensitive (SWS) pigment and a longer blue/green wavelength-sensitive pigment. It was proposed that this "dual-sensitivity system" may enable the photophore-bearing oplophorids to discriminate between different bioluminescent emissions (Cronin & Frank, 1996; Frank & Case, 1988; Frank & Widder, 1994b) as photophores emit light at a longer wavelength (λ_{max} ~ 475 nm) and narrower spectral bandwidth (half bandwidth ~ 55 nm) than that of the secretory luminescence (λ_{max} ~ 460 nm, half bandwidth ~ 70 nm) (Herring, 1983; Latz et al., 1988). Further, Frank and Widder (1996) presented in situ irradiance measurements that indicate the presence of UV light in the deep sea and that the intensity of this light at 600 m is within the sensitivity threshold of UV-sensitive crustaceans. This study proposed that this system may therefore function as a type of depth gauge to allow these animals to monitor changes in the spectral bandwidth of downwelling light with increasing depth (Frank & Widder, 1996), similar to what was proposed for deep-sea polychaetes (Wald & Rayport, 1977). The presence of multiple photopigments with differing spectral sensitivities among *S. debilis* and other photophore-bearing oplophorids may therefore be linked to their migratory lifestyle, as they navigate through the water column and encounter variable biotic and abiotic factors.

Underlying visual system sensitivities are the absorbance properties of photopigments, which are fundamental to light detection. They consist of visual opsins bound to a chromophore (Porter et al., 2012; Wald, 1967). These opsins are responsible for tuning spectral sensitivity, and it has been shown that altering the amino acid sequence of an opsin can alter the wavelengths to which visual pigments are sensitive (Carleton & Kocher, 2001). Photoreceptors were historically thought to express a single opsin, though there are various exceptions to this rule across metazoans (e.g. Rohlich, van Veen, & Szel, 1994; Su et al., 2006). Among Crustacea, which have rhabdomeric opsins (r-opsins) typical of invertebrates, more opsins appear to be expressed in the retina than predicted based on photoreceptor sensitivities (e.g. stomatopods, Porter, Bok, Robinson, & Cronin, 2009; Porter et al., 2013; crabs, Sakamoto, Hisatomi, Tokunaga, & Eguchi, 1996; Rajkumar, Rollmann, Cook, & Layne, 2010; mysids, Frank, Porter, & Cronin, 2009; oplophorid shrimp, Wong et al., 2015; Bracken-Grissom et al., 2020). This suggests opsins are also co-expressed among the rhabdomeral photoreceptors of some crustaceans. Multiple lines of evidence from a recent study by Bracken-Grissom et al. (2020) also revealed that opsins corresponding to a range of putative spectral sensitivities, and other phototransduction genes, are expressed in the photophores of the oplophorid *J. spinicauda*; these bioluminescent light organs are thought to function as extraocular photoreceptors.

Co-expressing opsins with different spectral sensitivities can broaden a photoreceptor spectral sensitivity (i.e. cichlids, Hofmann & Carleton, 2009). Further, opsin expression was shown to fluctuate daily in the ocular photoreceptors (compound eyes) of crabs (Arikawa, Kawamata, Suzuki, & Eguchi, 1987; Arikawa, Morikawa, Suzuki, & Eguchi, 1988), with selective expression of different opsin subsets thought to lead to different visual responses (i.e. cichlid fish; Carleton & Kocher, 2001). Since lighting environment is known to influence opsin expression (e.g. killifish, Fuller & Claricoates, 2011), it is therefore probable that similar processes are occurring in the photoreceptors (ocular and/or extraocular) of *S. debilis* during diel vertical migration. Both opsin co-expression and changes to relative opsin expression during vertical migrations may result in changes

to photoreceptor spectral tuning and allow for a diversified visual response.

Here, we present the first transcriptomic investigation into the influence of large-scale diel vertical migrations (see Figure 1c as an example, D'elia et al., 2016) on the visual sensory system (ocular and extraocular) of oplophorid shrimp. We used an integrative approach, including high-throughput sequencing and phylotranscriptomic methods, to first characterize the visual opsins and genes expressed among the ocular and extraocular photoreceptors of *S. debilis*. Gene expression analyses were subsequently conducted on shrimp collected at different phases of this diel vertical migration in order to investigate changes in opsin and gene expression linked to this migratory behaviour. Based on recent evidence from the oplophorid *J. spinicauda*, we expected to recover a higher diversity of opsins in the photoreceptors than suggested by spectral measurements. As opsin expression is known to fluctuate daily among the compound eyes of crustaceans, we also anticipated changes in relative opsin expression that may be associated with photoreceptor spectral tuning during the migratory process. Though the functional role of photophore photosensitivity is primarily speculation due to the realistic obstacles in conducting behavioural studies on cryptic deep-sea fauna, we believe corresponding shifts in extraocular opsin expression will provide insight into the functional and adaptive roles of these light organs in vertically migrating fauna. Lastly, differential gene expression analyses revealed additional cellular processes and pathways that are impacted by this migratory behaviour.

2 | METHODS

2.1 | Sample collections and processing

In this study, live specimens of *S. debilis* were collected at different stages of their diel vertical migration (day versus night) from the Florida Straits aboard the *RV Walton Smith* (July 2017). Collections were done via a 9-metre^2 Tucker Trawl fitted with a light-tight, thermally insulated cod-end that could be opened and closed at depth (Frank & Widder, 1999). This method enabled specimen collection from specific depth intervals and maintenance at in situ temperatures prior to preservation. At the surface, species were identified under dim red light to avoid any damage to photosensitive tissues. Samples were preserved in RNAlater following an abdominal incision to facilitate tissue preservation. After 24 hr, tissues were frozen at −20°C before being transported to Florida International University and stored at −80°C. Eye and photophore tissues were carefully dissected under a dissecting scope while submerged in RNAlater from five biological replicates corresponding to each sampling condition, day (n = 5) and night (n = 5), and discretely homogenized in TRIzol® reagent (Thermo Fisher Scientific). Due to their small size, photophores were collected and pooled in RNAlater from across the entire body of the shrimp, including the scaphocerite (antennae), carapace, abdomen, legs, pleopods and telson. Day and night sample replicates were caught around the same time and depth range. Day samples were collected in the morning/afternoon (presunset) from ~450 to 750 m, and night samples were collected around midnight (predawn) from ~150 to 330 m (see Figure 1b). Voucher specimens (HBG 8,390–91, 8,395–98, 8,465, 8,467–69) were ultimately curated in the Florida International Crustacean Collection (FICC).

Total RNA was discretely extracted from tissues using TRIzol/chloroform reagents and rDNase (Macherey-Nagel) treated following the protocol described in DeLeo, Pérez-Moreno, Vázquez-Miranda, and Bracken-Grissom (2018). RNA quantity was assessed via a Qubit 2.0 fluorometer (Life Technologies, USA). RNA integrity was assessed via gel electrophoresis and an Agilent Bioanalyzer. RNA sequencing (RNA-seq) libraries were constructed from high-quality RNA using the TruSeq Stranded mRNA protocol from Illumina at the GENEWIZ® Core Facility (South Plainfield, NJ). Libraries were sequenced on an Illumina HiSeq 4000 to obtain 150-bp paired-end reads.

2.2 | Transcriptome assembly and assessment

Raw sequencing data were quality assessed using FastQC (Andrews, 2010) to inform quality and adaptor trimming. Reads were trimmed using Trimmomatic v0.36 (Bolger, Lohse, & Usadel, 2014) using the newer, adaptive "maximum information" trimming strategy recommended by the Trimmomatic authors (parameters: *adapter.clip* 2:30:10:1:true, *crop* 135, *headcrop* 15, *trim.leading* 3, *trim.trailing* 3, *max.info* 40:0.999, *min.read.length* 36). Reads were then error-corrected using Rcorrector (Song & Florea, 2015) prior to assembly. Tissue-specific (eye and photophore) reference transcriptomes were assembled de novo with Trinity v2.8.4 (Grabherr et al., 2011; Haas et al., 2013) using in silico read normalization, a minimum contig length of 200 bp and a k-mer size of 23, which has proven to be the optimal k-mer size for these crustacean RNA-seq data sets (i.e. Bracken-Grissom et al., 2020; Pérez-Moreno, DeLeo, Palero, & Bracken-Grissom, 2018). A comprehensive reference assembly containing both tissue types was also assembled for downstream gene expression comparisons between tissue types. Contamination was subsequently removed from each assembly using Kraken v1.0 (Wood & Salzberg, 2014) with default parameters and NCBI's (Refseq) bacteria, archaea and viral databases. Contaminate-free assemblies were then passed through BBduk and dedupe (BBTools suite, available at: http://sourceforge.net/projects/bbmap) to remove duplicate transcripts and rRNA. Transcriptome quality and completeness for each tissue-specific reference assembly was assessed using Transrate v1.0.3 and BUSCO v3.0.2 (Benchmarking Universal Single-Copy Orthologs, Simao, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015; Smith-Unna, Boursnell, Patro, Hibberd, & Kelly, 2016). BUSCO evaluations were done in an evolutionary context using a reference data set of orthologous groups (n = 1,066) found across Arthropods (OrthoDB, Waterhouse, Tegenfeldt, Li, Zdobnov, & Kriventseva, 2013). Assembly statistics were obtained using Trinity support scripts (Haas et al., 2013).

2.3 | Characterization of opsins and light interaction genes

Each tissue-specific transcriptome was analysed using the Phylogenetically-Informed Annotation (PIA) tool (Speiser et al., 2014), modified for command-line use (Pérez-Moreno et al., 2018), which characterizes putative visual opsins and phototransduction pathway genes in a phylogenetic context. Assemblies were run through PIA using a pipeline previously described in Pérez-Moreno et al. (2018). In brief, these tools extract all open-reading frames (ORFs), identify light interaction (LIT) genes via BLAST searches against a database of known visual genes, and align and subsequently place significant hits into precomputed gene phylogenies to differentiate between false positives and genes of interest. Emphasis was placed on the rhabdomeric phototransduction pathway (rtrans), though PIA was used to characterize additional LIT genes involved in light detection (e.g. cryptochromes, Friedrich et al., 2011), absorption (e.g. pigment synthesis enzymes, Takeuchi, Satou, Yamamoto, & Satoh, 2005) and refraction (e.g. lens crystallins, Tomarev & Piatigorsky, 1996) in invertebrates.

Opsin diversity was further characterized for each tissue-specific assembly. Putative opsin sequences were aligned with PROMALS3D (Pei & Grishin, 2014) using a curated reference opsin data set ($n = 996$, Henze & Oakley, 2015; Porter et al., 2012) that comprises visual opsins across a range of spectral sensitivities as well as nonvisual opsins and related G protein-coupled receptors (GPCR). Opsin phylogenetic tree reconstruction was done with IQ-TREE (Nguyen, Schmidt, von Haeseler, & Minh, 2015) using an LG general amino acid replacement matrix, under a FreeRate model with 8 rate categories, and empirical base frequencies (LG+R8+F, Le & Gascuel, 2008; Soubrier et al., 2012) as recommended by ModelFinder (Kalyaanamoorthy, Minh, Wong, von Haeseler, & Jermiin, 2017). Support was assessed in triplicate by 1) a Shimodaira–Hasegawa-like approximate likelihood ratio test (SH-aLRT; 10,000 replicates), 2) an approximate Bayes test and 3) an Ultra-fast bootstrap approximation (UFBoot; 10,000 replicates) (Anisimova, Gil, Dufayard, Dessimoz, & Gascuel, 2011; Guindon et al., 2010; Hoang, Chernomor, von Haeseler, Minh, & Vinh, 2018; Minh, Nguyen, & von Haeseler, 2013). False positives aligning with nonvisual opsins or outgroups were removed before generating a final (invertebrate-only) opsin gene tree (see Table S4 for more details). Visual opsin identity was further confirmed via structural alignments (PROMALS3D) to bovine rhodopsin (2.8 Å) template (IF88.pdb) (Palczewski et al., 2000) and the subsequent identification of conserved domains, motifs and residues characteristic of invertebrate r-opsins (as described in Katti et al., 2010).

2.4 | Gene expression analyses

For each analysis, trimmed error-corrected reads of the sample replicates were mapped back to the reference assembly using the Trinity supported, alignment-free transcript quantification tool Salmon (Patro, Duggal, Love, Irizarry, & Kingsford, 2017). Salmon was run in trinity mode with default parameters for strand-specific libraries (--SS_lib_type RF). Transcript and gene-level count and expression matrices were generated following Trinity's transcript quantification protocol for alignment-free abundance estimation methods (Haas et al., 2013). Gene count matrices were further analysed using Trinity support scripts and the PtR package to explore relationships among sample replicates and conditions. Normalized gene-level read counts were generated via Trinity using the Trimmed Mean of *M-values* (TMM) normalization method, which uses a weighted trimmed mean of the log expression ratios to estimate scaling factors between samples (Robinson & Oshlack, 2010). The normalized TMM gene counts were generated from transcript per million (TPM) abundance values and used to compare absolute abundance measures between samples during the different stages of the vertical migration. For each tissue type, mean opsin expression for each putative opsin clade was compared between the day and night sampling conditions, using Welch's two-sample *t* test.

The software package DESeq2 (v1.22.2) (Love, Anders, & Huber, 2014) was used to test for differential gene expression due to the effects of vertical migration or the sampling "condition"—day versus night. This was done discretely for the eye and photophore data sets (design = ~ condition) using the raw gene count matrices generated from each respective transcriptome. For example, we might expect samples collected in relatively shallow waters at night to be exposed to higher environmental light levels (i.e. lunar light) than samples collected in the day from deep waters. We might also expect the eyes to be more sensitive to differences in light levels as they are the primary photoreceptors and therefore influenced by the sampling condition to a greater degree. Default functions in DESeq2 for estimating size factors, dispersion and negative binomial Wald's tests were used. False discovery rate (FDR) was controlled at 5% for each gene (alpha = 0.05), and only log2fold changes (log2FC) in gene expression levels ≥ 1, or 2-fold, were considered significant (lfcThreshold = 1).

2.5 | Gene annotation and functional enrichment

Putative annotations were broadly assigned to the differentially expressed genes (DEGs) using Trinotate, a functional annotation program designed for de novo transcriptome assemblies (Bryant et al., 2017). This program conducts a series of sequence similarity searches against preformatted Swiss-Prot and Pfam databases in addition to custom data sets. Custom BLAST databases were built from (a) UniProt Reference Clusters (Uniref90) of protein sequences with similar functional annotations across taxa (Suzek, Wang, Huang, McGarvey, & Wu, 2015), (b) the euGenes/EvidentialGene ARP7 (v2014.08) curated data set comprising arthropod orthologous gene groups (291,357 protein sequences, http://arthropods.eugenes.org/arthropods/orthologs/ARP7), (c) the newly sequenced genome of the Pacific white shrimp *Litopenaeus vannamei* (25,527 protein sequences, BioProject PRJNA438564) (Zhang et al., 2019) and (d) the National Center for Biotechnology Information (NCBI)'s

nonredundant (nr) protein database. Sequence similarity searches were performed with DIAMOND (v0.9.24.125), which is optimized for high performance and speed relative to BLAST (Buchfink, Xie, & Huson, 2015), in sensitive mode (-- more-sensitive) using default parameters. Putative genes without significant matches to any of the databases were considered orphan genes (with no detectable homologs in other lineages) for the remainder of the analyses.

DEGs were further analysed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) MAPPER v3.1 (Kanehisa, Sato, Kawashima, Furumichi, & Tanabe, 2016) to decipher molecular interaction networks (KEGG pathway mapping; *reconstruct*), based on the KEGG Orthology (KO) assignments from Trinotate. Gene Ontology (GO) functional annotations and enrichment analyses were run for each gene subset using Blast2GO (Conesa et al., 2005). GO information was obtained for the nucleotide sequences via DIAMOND searches (blastx) against NCBI's nr database and InterProScan v5.33 (Zdobnov & Apweiler, 2001) using default parameters, custom scripts and a significance threshold ≤ 1e-3.

Two independent tests were run to test for functional enrichment among the GO terms assigned to the differentially expressed genes. First, Fisher's exact test was run in Blast2GO using the subset of genes determined to have significant differential expression by DESeq2 (FDR ≤ 0.05, log2FC ≥ 1). This was done discretely for each tissue type (eye and photophore) and each set of genes, either (a) overexpressed in the day or (b) those overexpressed in night samples. Resulting significance values (*p*-values) for the enrichment analyses were adjusted in Blast2GO to control for false discovery rate (FDR), yielding *q*-values, via the Benjamini–Hochberg method (Benjamini & Yekutieli, 2001). Lastly, the program GO_MWU (Wright, Aglyamova, Meyer, & Matz, 2015) was used to perform a rank-based GO analysis, with adaptive clustering, via a Mann–Whitney U (MWU) test. This was done using a global-ranked list of *all* genes assembled in the tissue-specific transcriptomes (eye or photophore) to identify GO categories that were significantly enriched. Enriched GO categories were identified using a continuous significance measure, −log(*p*-value), calculated during the differential gene expression analyses with DESeq2. Hierarchical clustering of GO categories was based on the number of shared genes (*clusterCutHeight* = 0.25; *GO_category_min* = 5 genes; *GO_category_max* = 10% total genes).

3 | RESULTS

3.1 | Tissue-specific transcriptome assemblies

An average of 36M paired-end reads were generated per sample for a total average of approximately 1.1 billion bases per tissue type with a mean quality score of 37.7. These data are available on the NCBI's Sequence Read Archive (SRA) database under BioProject: PRJNA605562. The tissue-specific de novo transcriptome assemblies contained 176,202 and 154,153 contigs with a mean length of approximately 507 and 545 base pairs (bp) for the eye and photophore assemblies, respectively (Table 1). The corresponding contig Ex90N50 statistics (contig N50 value based on the set of transcripts representing 90% of the expression data), recommended for evaluating contiguity of transcriptomic data, were 829 bp (eye) and 966 bp (photophore). For the eye reference assembly, 72.2% of universal single-copy arthropod orthologs were identified (complete [C]:58.8% [single [S]:20.9%, duplicated [D]:37.9%], fragmented [F]:13.4%, missing [M]:27.8%, *n*:1,066), compared to 72.8% identified in the photophore assembly (C:61.0% [S:18.6%, D:42.4%], F:11.8%, M:27.2%, *n*:1,066). These BUSCO scores reflect the specificity of the targeted, tissue-specific de novo transcriptomes. It is also possible a portion of missing BUSCOs are a result of divergent or complex gene structures (Waterhouse et al., 2013) and/or technical limitations (i.e. gene prediction), which were shown to inflate proportions of fragmented or missing BUSCOS for large genomes. Given the large estimated genome size for crustaceans, and oplophorid shrimp specifically (i.e. ~38 Gb for *Hymenodora* sp., Dixon, Dixon, Pascoe, & Wilson, 2001), this may also be a contributing factor.

TABLE 1 De novo transcriptome assembly statistics for *Systellaspis debilis*

Metric	Eyes	Photophores
Number of transcripts	176,202	154,143
Mean transcript length (bp)	507.4	545.2
Reconstruction size (bases)	89,411,090	84,036,316
Transcripts over 1K bp	16,565	17,907
Transcripts over 10K bp	6	16
Number of contigs with ORFs	18,941	20,523
Mean ORF per cent	54.4	55.2
GC content (%)	38.0	38.0
N50	614	718
Ex90N50[a]	829	966

[a]Ex90N50 represents the N50 value based on the set of transcripts representing 90% of the total expression data.

3.2 | Characterization of opsins and light interaction genes

Phylogenetically-Informed Annotation (PIA) analyses of the *S. debilis* eye transcriptome revealed visual r-opsins belonging to two medium-wavelength-sensitive clades (MWS1 and MWS2) and one long-wavelength-sensitive clade (LWS2) (Figure 2, Figure S1). R-opsins corresponding to the two distinct MWS clades were highly divergent, with 169 amino acid differences between them, relative to the LWS2 r-opsins, which had 21 amino acid differences between them (Figure S2). Near-identical (98%–100% amino acid similarity) opsins were recovered from the photophore transcriptome belonging to the MWS2 and LWS2 clades (see Figure S1 for more details). Structural amino acid alignments revealed conserved elements characteristic of r-opsins, including the 7 transmembrane domains, two conserved Cys residues, a conserved Lys residue critical for Schiff base formation with the chromophore, an amino acid triplet similar to the one known

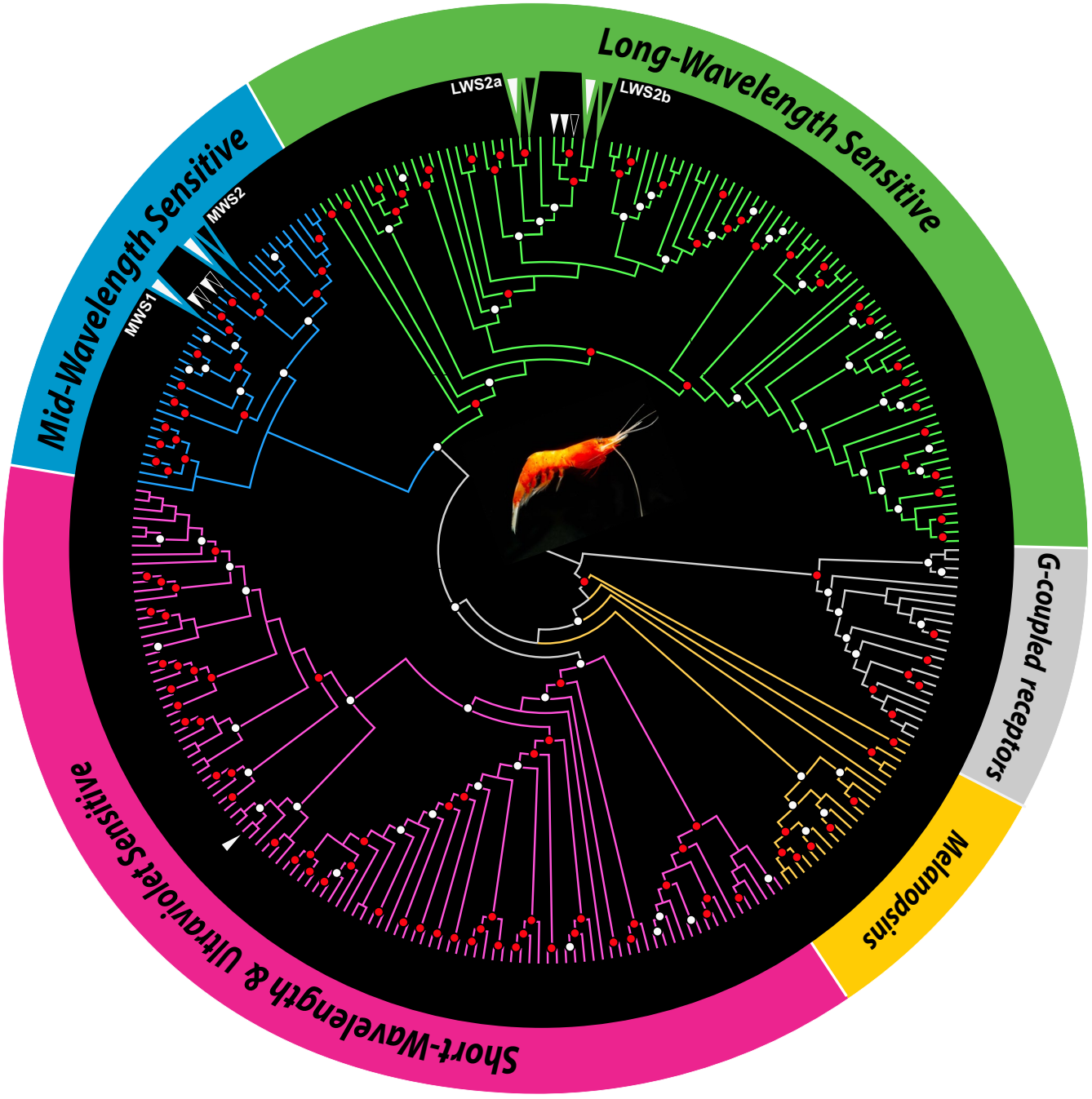

FIGURE 2 Phylogenetic opsin tree comprising 281 visual rhodopsins (r-opsins) and closely related melanopsins. Newly curated r-opsins from *S. debilis* (large blue/green triangles), eye (white center) and photophore (black center) transcriptomes were aligned with a reference opsin data set (Henze & Oakley, 2015; Porter et al., 2012) which included visual opsins of known spectral sensitivities, nonvisual opsins and related G protein-coupled receptors (GPCR) as outgroups. Also included are the recently recovered r-opsins from the eye (white triangles) and photophore (black triangles) transcriptomes of the oplophorid *J. spinicauda* (Bracken-Grissom et al., 2020) for reference. The putative spectral sensitivities of the r-opsins clades (short-wavelength-, mid-wavelength (MWS)- and long-wavelength-sensitive (LWS)) were inferred from these data sets. Significant triplicate bootstrap support is indicated by red circles (SH-aLRT > 80, aBayes > 0.95 and UFBoot > 95), and significant duplicate bootstrap support is indicated by white circles (SH-aLRT > 80 or UFBoot > 95, and aBayes > 0.95). Photograph credit: ©DantéFenolio

to couple to G-alpha$_q$ (HP(R/K)) and the "R(E/D)QAKKMN" sequence conserved among arthropod opsins (Figure S2). Phototransduction pathway analyses of the eye assembly also identified major pathway components including the opsins and the calcium ion (Ca^{2+}) channel-transient receptor potential (trp) which initiates the signalling cascade, pathway regulators such as Gq proteins, and the cascade terminators—retinal degeneration (rdg) and arrestin (Arr) (Table 2). These same genes were recovered from the photophore assembly, though the photophores contained trp-like (trpl) and trp-gamma gene isoforms instead of the trp isoform found among the eyes.

TABLE 2 *Systellaspis debilis*[a] phototransduction pathway components[b]

Gene	Eyes	Photophores
Arrestin (Arr)	◆	◆
Diacylglycerol kinase (DAGK)	◆	◆
G protein-coupled receptor kinase 1 (GPRK1)		◆
G protein-coupled receptor kinase 2 (GPRK2)	◆	◆
G-alpha$_q$ ($G_q\alpha$)	◆	◆
G-beta$_q$ ($G_q\beta$)	◆	◆
G-gamma$_q$ ($G_q\gamma$)	◆	◆
Rhabdomeric opsin (r-opsin)	◆	◆
Protein kinase C (PKC)	◆	◆
Phospholipase C (PLC)	◆	
Retinal degeneration B (rdgB)	◆	
Retinal degeneration C (rdgC)	◆	◆
Transient receptor potential (trp)	◆	◆

[a]Annotated phototransduction pathway genes for the tissue-specific, eye and photophore transcriptomes.

[b]Genes are as follows: Arr = Arr 1 & 2; DAGK = rdgA; r-opsin = Rh6; PKC = inaC; PLC = norpA; trp = trpl, trp-gamma (photophores) and trp (eyes).

Additional light interaction (LIT) genes were recovered from the tissue-specific assemblies using PIA (Table S1). For eyes, this includes the genes sine oculis (six and so isoforms) and dachshund (dac), which are essential to eye development, shaven (sv) which is involved in sensory organ development and the transcriptional regulator ovo. The eye transcriptome also contained genes involved in chromophore (visual pigment component) synthesis (ninaB and ninaG), S- and Ω-crystallins (lens crystallins), a retinol dehydrogenase (RDH8), the circadian (clock) rhythm genes, cryptochrome 2 (cry2), lark, timeless, vrille, PAR-domain protein 1 (pdp1) and tango, and genes involved in heme and melanin biosynthesis. A majority of the same genes were found in the photophore-only transcriptome with the exception of six and dac. However, when gene expression was cross-referenced between the photophore replicates using a comprehensive all-tissue (eye + photophore) transcriptome assembly, dac and six were actually expressed in at least two of the photophore replicates. This indicates the genes are likely present in the photophore transcriptomes, with low/variable expression in the specimens sampled in this study. The photophores also contained other (visual) developmental genes related to paired-box gene C (PaxC) and eyegone (eyg) that were not recovered in the eye assembly.

3.3 | Diel fluctuations in opsin expression

Approximately 70%–80% of replicate reads were mapped to the de novo reference assemblies. Absolute abundance estimates (TMM-normalized) revealed differences in relative expression among the opsin clades (Figure 3). The putative LWS opsin was the most highly expressed r-opsin in both the eye and photophore tissues, regardless of the diel sampling condition, followed by the MWS2 opsin. Although

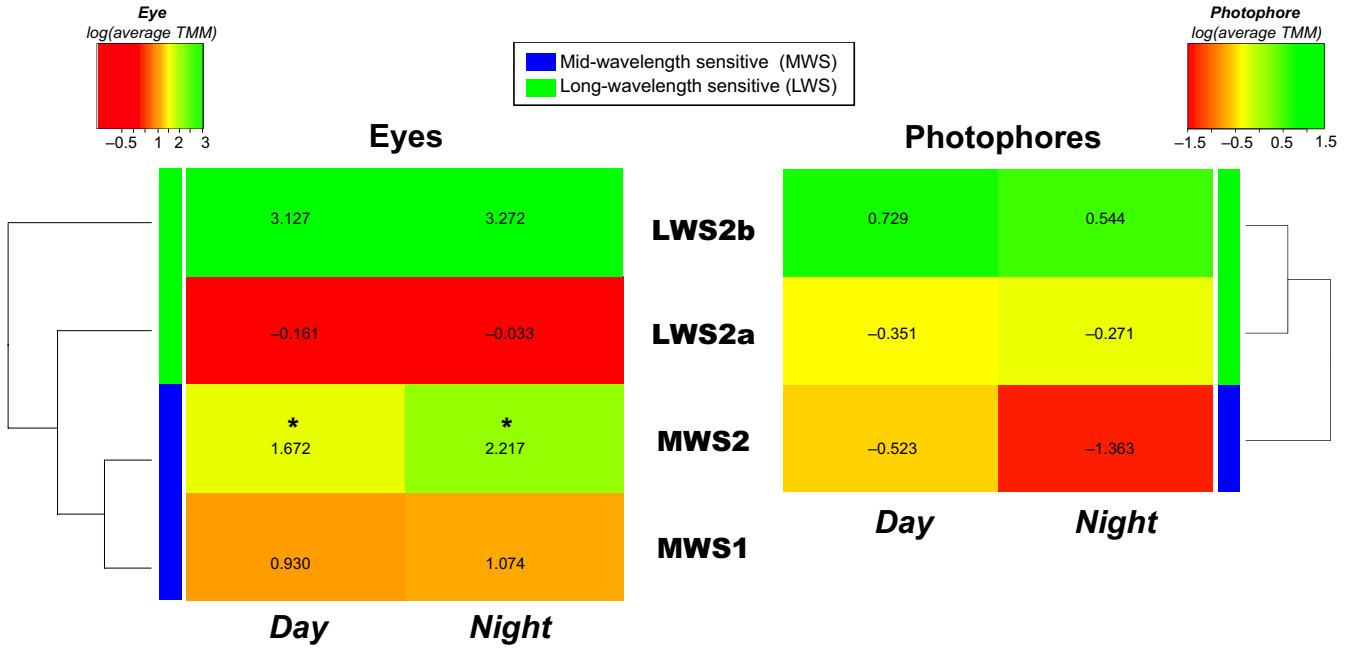

FIGURE 3 Absolute opsin abundance estimates in the eye and photophore tissues corresponding to the putative mid-wavelength (MWS1 and 2) and long-wavelength (LWS2) spectral clades. Absolute abundances are represented by tissue-specific TMM-normalized read counts, averaged across replicates for each diel sampling condition (day or night). Values represent log(average TMM) expression values. The asterisk (*) signifies significant differential expression between the day and night comparisons (FDR < 0.05). Opsin hierarchical clustering is based on similarities in expression patterns within each tissue type

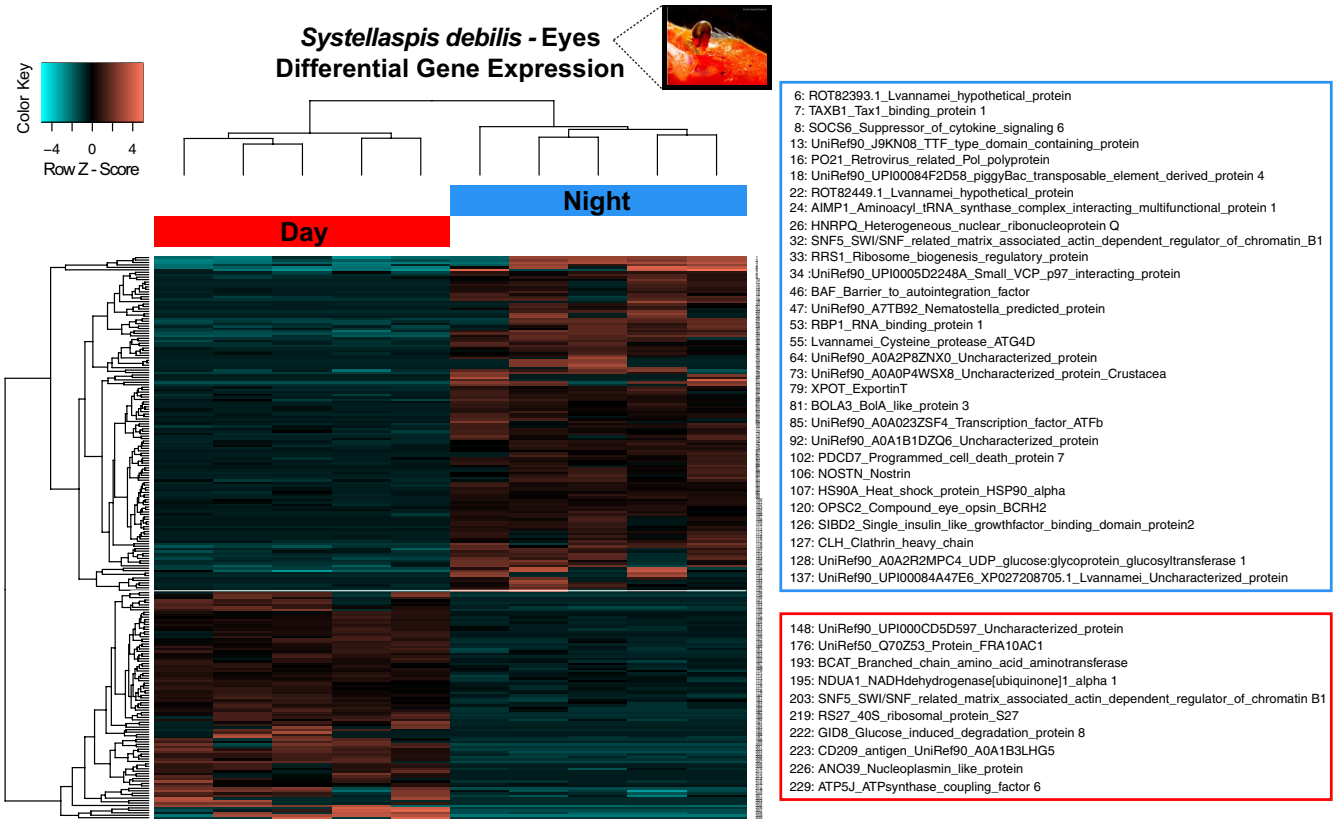

FIGURE 4 Heatmap depicting the 230 genes (numbered rows) differentially expressed among the eyes of vertically migrating *S. debilis* (FDR ≤ 0.05, log2FC ≥ 1 or 2-fold change). Each column represents a biological replicate collected during the day (red), when samples were in deeper waters (~450–750 m), or at night (blue) when the migrating shrimp were in relatively shallower waters (~150–330 m). 93 putative genes were overexpressed (coral) during the day (bottom left cluster; putative annotations in red box), while the remaining 137 genes were overexpressed (teal) at night (top right cluster; putative annotations in blue box). All available gene annotations are shown in the boxes to the right. Number prefacing annotation indicates row number for reference. Photograph credit: ©DantéFenolio

MWS expression was variable across replicates, average MWS2 expression was significantly higher in the eyes of *S. debilis* collected at night, in relatively shallow waters compared to deeper, day samples (Welsh two-sample *t* test, *p*-value = 0.03). The opposite appears to be the case in the photophores, as average MWS2 expression appears highest in deeper samples collected during the day, though this difference does not appear to be significant. The average expression of the putative LWS r-opsin appears consistent across photophore replicates (Table S2). It is important to note that while gene expression is not necessarily indicative of functionality, it has been used to infer function in other systems. Raw gene expression counts (i.e. FPKM) > 0.3–1 are thought to be suggestive of active expression rather than biological or experimental noise (Hart, Komori, LaMere, Podshivalova, & Salomon, 2013). All curated opsins had raw expression values within or exceeding this threshold.

3.4 | Differential gene expression (eyes) associated with diel vertical migration

Between the eyes of the vertically migrating shrimp *S. debilis*, there were 230 putative genes differentially expressed between the day and night samples (FDR ≤ 0.05, log2FC ≥ 1 or 2-fold change) (Figure 4). Of those, 93 genes were overexpressed during the day when samples were relatively deeper (~450–750 m) with the remaining 137 genes overexpressed at night when the migrating shrimp were in shallower waters (~150–330 m). The three top-hit BLAST species include the arthropod *Drosophila melanogaster*, *Homo sapiens* and *Mus musculus*. Annotations were obtained for approximately 22% of the differentially expressed genes with the remainder designated as orphan genes. Corresponding KEGG pathway analyses (Table S3) revealed that various genes were linked to energy metabolism and environmental adaptation (thermogenesis). Additional details regarding the KEGG and Gene Ontology (GO) analyses for this study can be found in Supplemental Results.

Among the genes significantly overexpressed in the deeper, day samples were ribosomal genes, genes associated with immune responses (CD209), muscle activity (ATP5PF), and cell proliferation and differentiation (SMARCB1). GO analyses revealed additional biological associations with energy metabolism (GO:0,006,550, 51, 73) (*p*-value < 0.01), cellular response to UV/light stimulus (GO:0,034,644) (*p*-value < 0.05), lateral inhibition (GO:0,046,331) and DNA damage/repair (GO:0,031,571) (Figure S3). The results

of the global ranked-based GO enrichment analysis (GO_MWU) further revealed significant enrichment (q-value < 0.05) among various biological processes including proteolysis and DNA modification (Figure S4).

Among the genes significantly overexpressed in the shallower, night samples were the visual r-opsin MWS2 (with similarity to the compound eye opsin BCRH2 that produces blue-/green-sensitive visual pigments), heat-shock proteins (HSP90A) that can alter transcription in response to environmental change or physiological cues, as well as genes involved in apoptosis and cytokine signalling. GO analyses revealed biological associations with endothelial cell proliferation (GO:0,001,937) (p-value < 0.01), clathrin (protein) coat assembly/clathrin light-chain binding (GO:0,048,268, GO:0,032,051) (i.e. coated vesicle formation), protein-chromophore linkage/photoreceptor activity (GO:0,018,298, GO:0,009,881) (p-value < 0.05) and defence and immune responses (GO:0,051,607, 0,050,900) (Figure S3). GO enrichment analysis with GO_MWU further revealed significant enrichment (q-value < 0.05) associated with protein-chromophore linkage, cell cycle processes including regulation, organelle organization, chromosome condensation and cellular responses to (stress) stimulus (Figure S4).

3.5 | Differential gene expression (photophores) associated with diel vertical migration

In the photophores of the vertically migrating shrimp, there were 156 genes differentially expressed (FDR ≤ 0.05, log2FC ≥ 1) between the day and night samples (Figure 5). Of those, 71 putative genes were overexpressed during the day with the remaining 85 genes overexpressed at night. Annotations were obtained for approximately 18% of the differentially expressed genes with the remainder comprised of orphan genes. The three top-hit BLAST species were the crustaceans *L. vannamei* (Pacific white shrimp), *Hyalella azteca* (aquatic amphipod) and *Armadillidium vulgare* (terrestrial isopod). Corresponding KEGG pathway analyses (Table S3) revealed genes linked to energy metabolism, protein processing, RNA degradation, cellular signalling, growth and death, as well as thermogenesis.

Among the genes significantly overexpressed in the photophores of the deeper, day samples were ribosomal genes, genes associated with the complement (immune) system (C1QL4), as well as genes associated with oxidative stress (NDUFB4). GO analyses revealed additional associations with biosynthetic processes (GO:0,009,058) (q-value < 0.05) (Figure S5). Enrichment analysis

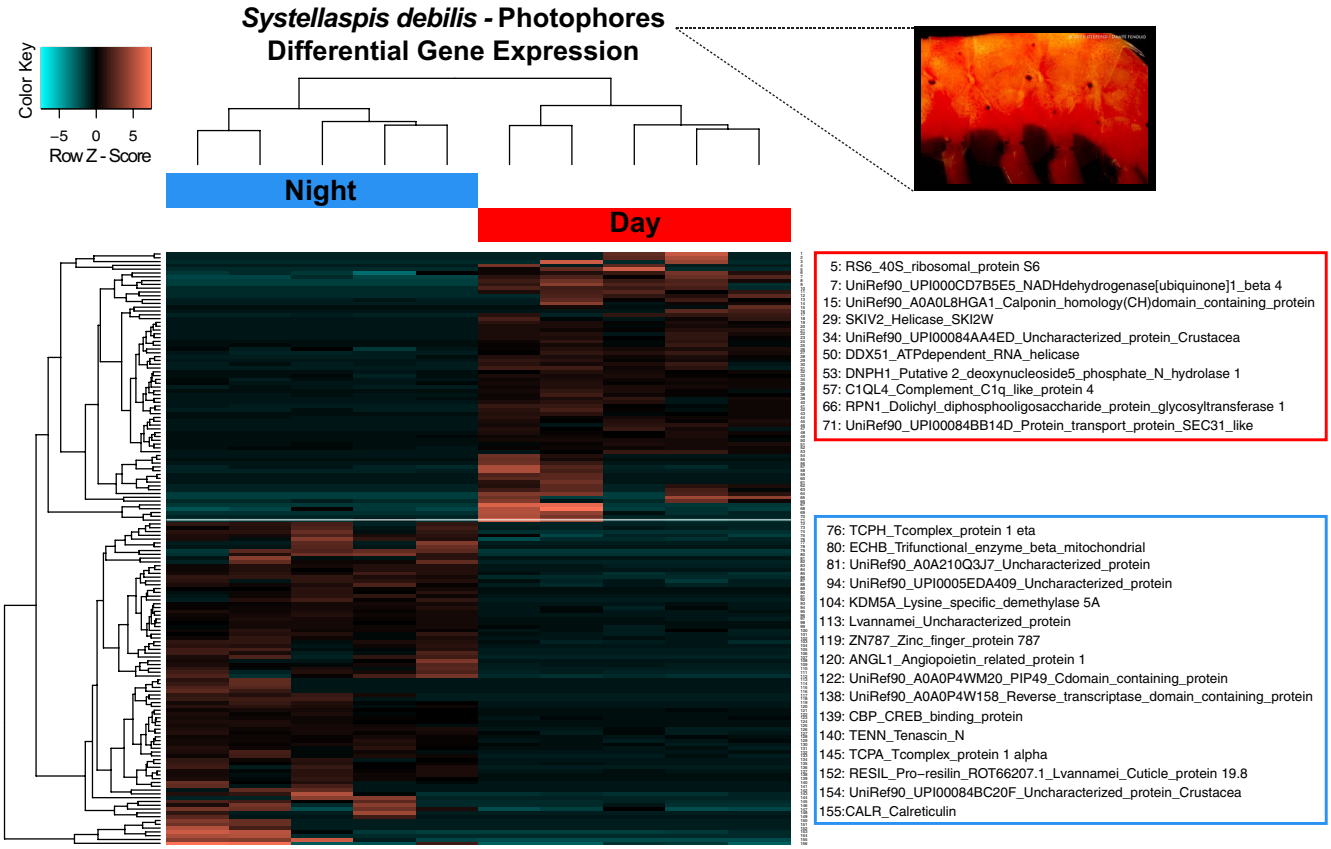

FIGURE 5 Heatmap depicting the 156 genes (numbered rows) differentially expressed among the photophores of vertically migrating *S. debilis* (FDR ≤ 0.05, log2FC ≥ 1 or 2-fold change). Each column represents a biological replicate collected during the day (red), when samples were in deeper waters (~450 – 750 m), or at night (blue) when the migrating shrimp were in relatively shallower waters (~150–330 m). 71 putative genes were overexpressed (coral) during the day (top right cluster; putative annotations in red box), while the remaining 85 genes were overexpressed (teal) at night (bottom left cluster; putative annotations in blue box). All available gene annotations are shown in the boxes to the right. Number prefacing annotation indicates row number for reference. Photograph credit: ©DantéFenolio

(GO_MWU) revealed significant enrichment (q-value < 0.05) associated with various metabolic processes (Figure S6), proteolysis and RNA processing.

Among the genes significantly overexpressed in the photophores of the shallower, night samples were a cuticle protein-coding gene (proresilin), a mitochondrial enzyme associated with energy production (HADHB), genes associated with the regulation of circadian rhythms (histone demethylase KDM5C) and cellular responses to UV light (CREB-binding protein). Corresponding KEGG pathway analyses (Table S3) revealed various genes were further linked to unfolded protein binding and modification. Enrichment analysis (GO_MWU) revealed significant enrichment (q-value < 0.05) associated with signal transduction and organelle, chromosome and cytoskeletal organization (Figure S6).

4 | DISCUSSION

In this study, we conducted the first transcriptomic investigation into the influence of diel vertical migration on the visual system of a deep-sea invertebrate. Phylotranscriptomic methods were used to characterize the diversity of putative visual opsins in the photoreceptors (ocular and extraocular) of *S. debilis* and expose diel fluctuations in opsin co-expression that may be associated with spectral tuning during the migratory process. As opsins are responsible for photoreceptor spectral tuning (Carleton & Kocher, 2001), it is possible that higher opsin diversity is linked to the diel migratory lifestyle of these shrimp as they transverse different photic environments. Differential gene expression analyses were further conducted to reveal the cellular processes and pathways that are impacted by long-distance (hundreds of metres) vertical migratory behaviour. Together, these results illuminate the visual adaptations of a deep-sea vertical migrator from a genomic perspective and the influence of diel vertical migration on sensory systems. These data also add to the sparse amount of high-throughput sequencing data for deep-sea fauna and nonmodel invertebrates.

Among the eyes of *S. debilis*, phylogenetic analyses revealed opsins corresponding to both medium- and long-wavelength-sensitive classes, providing further support that oplophorid shrimp have an expanded opsin repertoire supportive of *at least* a "dual-sensitivity" system. It is possible that we are finding a higher diversity of opsins in the eyes than expected based on physiological measurements because there is some overlap between the range of spectral sensitivities/bandwidths of the putative opsins, which could collectively expand the range of (mid)wavelengths to which the photoreceptors are sensitive (Bowmaker & Hunt, 1999). These opsins were near identical to the MWS and LWS opsins recovered in the oplophorid *J. spinicauda* (Bracken-Grissom et al., 2020). But, unlike past physiological and behavioural studies on *S. debilis* (Frank & Case, 1988; Frank & Widder, 1994b), and the molecular/NGS studies on *J. spinicauda*, that assessed the visual sensitivities of oplophorid shrimp, a putative UV-shifted short-wavelength opsin in the eye was not recovered in this study. The unexpected absence of a UV/SWS opsin was similarly found for *Euphasia superba*, a species of shallow water migrating krill (Biscontin et al., 2016), and was attributed to an insufficient sequencing depth. Based on the lower relative expression of the UV/SWS opsin in *J. spinicauda* (Bracken-Grissom et al., 2020), it is therefore possible that deeper sequencing would reveal additional opsin(s) with maximal sensitivity to short wavelengths.

4.1 | Opsin expression among ocular photoreceptors of migrating shrimp

Co-expressing opsins with different spectral sensitivities can broaden the spectral range of wavelengths in which a photoreceptor is sensitive (e.g. cichlids, Hofmann & Carleton, 2009). For deep-sea fauna, an increased sensitivity to wavelengths in the middle range of the visible spectrum is particularly important as the deep sea is light-limited and primarily consists of blue (mid-wavelength) light either penetrating from the surface (Cronin, 1986; Dartnall, 1975) or in the form of bioluminescence (Herring, 1983; Latz et al., 1988; Widder et al., 1983). Expression of opsin sequences belonging to two distinct MWS clades in the eyes of *S. debilis* suggests they have an expanded spectral sensitivity to mid-wavelengths. Our findings are identical to what has been recovered in the vertically migrating oplophorid *J. spinicauda* (Bracken-Grissom et al., 2020) and the deep-sea lophogastrid, *Neognathophausia ingens* (Frank et al., 2009). Dual overlapping mid-wavelength peaks with slightly different λ_{max} in the eyes of *S. debilis* could broaden the spectral sensitivity of these photoreceptors to mid-wavelengths, as was suggested for other arthropods (crab *Hemigrapsus sanguineus*, Sakamoto et al., 1996; butterfly *Papilio Xuthus*, Kitamoto, Sakamoto, Ozaki, Mishina, & Arikawa, 1998; ostracods, Oakley & Huber, 2004). This difference would not necessarily be detectable due to the limited sensitivity of the electrophysiological methods previously used to characterize the visual sensitivity of *S. debilis* (Frank & Case, 1988). The study by Frank and Case (1988) found only 2 distinct peaks with sensitivity maxima at 400 and 500 nm (i.e. short and long wavelengths), though some variability was observed in the number and relative sizes of the peaks. Further, Sakamoto et al. (1996) found two related MWS opsins expressed in the eyes of *H. sanguineus*, but only one corresponding sensitivity peak (~480 nm) suggesting the opsins are maximally sensitive to similar wavelengths. It is possible that dual MWS visual pigments allows for an enhanced visual sensitivity to mid-wavelengths which could be beneficial as they migrate through different depth zones and light environments. As oplophorids also exhibit dual modes of bioluminescence (secretion and photophore) that differ slightly in their emission spectra and spectral bandwidths (Herring, 1983; Latz et al., 1988), this expanded opsin repertoire may allow the shrimp to discriminate between differences in surrounding spectral characteristics (Cronin & Frank, 1996; Gaten et al., 2004), like the bioluminescent emissions of congeners and/or between bioluminescent light and that of downwelling blue light.

4.2 | Diel fluctuations in ocular opsin expression during vertical migration

Opsin expression is influenced by spectral changes in the environment (Carleton & Kocher, 2001; Fuller & Claricoates, 2011) and can fluctuate daily (Arikawa et al., 1987, 1988). By investigating diel fluctuations in opsin (co)expression, or lack thereof, in vertical migrators we can better understand the impacts of opsin expression patterns on spectral tuning during the migratory process. For diel vertical migrators, particularly those that migrate long distances across different photic zones, changes in relative opsin expression may allow for a diversified visual response. For *S. debilis*, relative opsin expression was highest for the LWS opsin, followed by the MWS2 opsin, and LWS expression remained relatively stable across replicates regardless of their stage of vertical migration (day and night). Consistent LWS expression, despite differences in depth and corresponding environmental factors (e.g. temperature, pressure, light availability), could be linked to similarities in light availability between shallower waters at night (i.e. moonlight) and deeper waters during the day (i.e. downwelling sunlight and bioluminescent sources). According to Herring (1996), in clear waters, moonlight (and starlight) can make similar contributions to ambient light at 400 m as sunlight at 800 m; the intensities of certain bioluminescent sources can also reach the intensity of moonlight. Since *S. debilis* is thought to inhabit average daytime depths between ~650 and 900 m and night-time depths between 100 and 300 m (Ziemann, 1975), it is possible that they experience comparable light environments within these particular depth ranges albeit by different light sources. For example, downwelling moonlight and bioluminescence may expose *S. debilis* to comparable levels of blue/green light in shallow waters at night as downwelling sunlight and bioluminescent point sources do in deeper waters during the day. However, this study represents only a snapshot of the conditions/environmental influences experienced by *S. debilis* during the migratory process and it is possible that LWS opsin expression varies at other times/depths.

Conversely, MWS2 expression was significantly higher in the eyes of *S. debilis* collected at night. Daily cyclic fluctuations in opsin expression have been previously described in the compound eyes of arthropods (crabs, Arikawa et al., 1987; Arikawa et al., 1988; horseshoe crabs, Katti et al., 2010). This diel fluctuation has probable ecological relevance and may function to fine-tune the visual sensitivity of *S. debilis* while feeding in shallow waters at night. The use of two or more visual pigments (i.e. MWS and LWS) that are offset from the peak wavelength of downwelling light is thought to improve contrast detection (Gaten et al., 2004; Lythgoe, 1979). Therefore, it is likely that the contrast detection of *S. debilis* is improved, not only by possessing multiple opsins with different spectral sensitivities, but by altering the expression patterns of those opsins during vertical migration. Selective expression of different opsin subsets was also shown to alter the visual responses of cichlid fish (Carleton & Kocher, 2001). As *S. debilis* is thought to migrate as shallow as 100 m at night (Frank & Case, 1988), differential opsin expression may fine-tune the photoreceptors and improve contrast detection during migration, possibly to detect changes in the spectral characteristics of surrounding light or in the carapace radiance characteristics of migrating congeners and/or planktonic prey (Gaten et al., 2004).

Diel fluctuations in opsin co-expression are also thought to produce diel changes in the spectral sensitivity of the horseshoe crab *Limulus* (Katti et al., 2010). Further, these fluctuations are believed to be linked to diel changes in photoreceptor function. Katti et al. (2010) showed opsin co-expression can be differentially regulated and that relative levels of opsin expression are influenced by circadian rhythms. The authors proposed that shedding mechanisms, of photosensitive membranes, are responsible for the observed reduction in daytime opsin levels, and subsequent photosensitivity, of *Limulus*. One such shedding mechanism, observed in animals living in variable light environments, is light-driven shedding (LDS). LDS is triggered by a prolonged exposure to light and involves clathrin-mediated endocytosis (Sacunas et al., 2002). As genes associated with clathrin-binding and coat assembly were overexpressed in the eyes of *S. debilis* at night, it is possible that similar mechanisms are at play during their migratory process. In this case, the prolonged exposure may be to moonlight or a higher intensity of downwelling sunlight as these animals begin to migrate down to deeper waters.

4.3 | Expression of opsins and light interaction genes among bioluminescent light organs

The functional role of photophore photosensitivity is still unknown, although a recent study by our group suggests a possible role in the fine-tuning of counterillumination (Bracken-Grissom et al., 2020). This current study builds upon previous work by examining opsin expression among light organs, as well as the expression of other light interaction genes, to gain valuable insight into their putative ecological role during diel vertical migration. Similar to the eyes, the photophores of *S. debilis* contained opsin sequences corresponding to both medium- and long-wavelength-sensitive classes. As putative extraocular photoreceptors, this work further suggests oplophorid photophores have a "dual sensitivity" system, similar to the ocular photoreceptors (eyes). The MWS2 and LWS2 opsin sequences are near identical to the opsins found in the eyes, in contrast to previous studies that recovered different opsins in the compound eyes of arthropods and their more simplified photoreceptors (Oakley & Huber, 2004; Pollock & Benzer, 1988; Smith, Price, Greenberg, & Battelle, 1993). LWS expression was also relatively stable in the photophores regardless of their stage of vertical migration (day/night), but interestingly—in contrast to the eyes—MWS2 expression was highest during the day when *S. debilis* was in deeper waters. Bracken-Grissom et al. (2020) proposed that photophore photosensitivity may be functioning to sense and fine-tune bioluminescent emissions during counterillumination, which is crucial during the migratory process of these animals. It is possible that higher expression of the MWS opsin in the photophores during the day is correlated with this emission sensing/matching mechanism as the shrimp descend or ascend in the water column. However, MWS2

expression was only detected in 60% of the replicates which likely led to a lack of significance during differential expression testing. This is potentially associated with unknown variations in the timing of migration (ascending versus. descending at depth) between the *S. debilis* replicates. Further, the photophores did not contain the MWS1 opsin sequence found among *S. debilis* eyes. This may be due to the functional differences between the ocular versus extraocular photoreceptors, though it is also possible that an opsin similar to MWS1 would be recovered with deeper sequencing. Oakley and Huber (2004) proposed that differential sensitivities among different photoreceptor types, called brightness range refraction, may serve a functional purpose in the ecology of the ostracod *S. lerneri* that are most active during periods of transitional light intensities. Differing sensitivities between the eyes and photophores of *S. debilis* may serve a similar functional purpose as they vertically migrate through different depth zones and light environments.

The photophores of *S. debilis* also contain numerous visual, light interaction and developmental genes commonly found among visual sensory organs. This includes genes essential to eye development (described in Fu & Noll, 1997) that were similarly identified in the light-sensitive (embryonic) photophores of the bioluminescent squid *E. scolopes* (Peyer, Pankey, Oakley, & McFall-Ngai, 2014). Interestingly, expression of the gene eyegone (eyg), which functions in the repression of eye development in other (postembryonic) arthropods (ZarinKamar et al., 2011), was unique to the photophores. These results add to prior evidence presented by Bracken-Grissom et al. (2020) that oplophorid photophores are capable of both emitting and detecting light, functioning as extraocular photoreceptors. Bracken-Grissom et al. (2020) suggest photophore photosensitivity may enable shrimp to detect and fine-tune their own bioluminescent emissions to closely match downwelling light. This ability to counterilluminate is vital to avoiding predation during the migratory process (Herring, 1976). However, it is also plausible that these organisms are using extraocular photosensitivity for orientation during vertical migration. As mentioned previously, photophore-bearing shrimps migrate hundreds of metres each night into shallower waters to feed and mate. These vertical migrations (and other fast movements of the tail fan in response to) require the shrimp to be nonhorizontal, either positioned near vertical during the upward migration or pointed downward during the downward migration, as witnessed in krill (Grinnell, Narins, Awbrey, Hamner, & Hamner, 1988). As the photophores of *S. debilis* are oriented across the entire body (dorsally, laterally and ventrally), light sensitivity may aid in maintaining proper orientation during long migrations.

4.4 | Ecological relevance of diel changes in gene expression

In this present study, genes associated with lateral inhibition, a process by which an excited neuron reduces the activity of neighbouring neurons to enhance contrast and improve sensory perception (Formosa-Jordan, Ibañes, Ares, & Frade, 2013), were significantly underexpressed in *S. debilis* eyes at night, relative to high expression at depth during the day. Reduced visual lateral inhibition, in combination with stable LWS opsin expression, may be associated with the circadian clock of this vertically migrating shrimp. Visually guided behaviour has previously been described in insects that have adapted motion-sensitive mechanisms to efficiently encode, process and respond to natural stimuli (reviewed in Rieke, Warland, Van Steveninck, & Bialek, 1999). The horseshoe crab *Limulus* has various retinal mechanisms that increase the sensitivity of the eye at night, and suppress noisy signals, in order to respond to stimuli and detect potential mates. This includes a circadian clock that increases visual sensitivity at night by stabilizing opsin expression, weakening lateral inhibition and increasing photoreceptor gain (Barlow, Hitt, & Dodge, 2001). Various circadian clock genes were also detected in the (ocular and extraocular) photoreceptors of *S. debilis*. It is therefore possible that oplophorid shrimp possess similar sensory mechanisms that enhance their visual sensitivity at night when they migrate to shallower waters to feed and mate. Moreover, overexpression of genes associated with cellular responses to UV/light stimulus in the eyes during the day suggests *S. debilis* may be using visual cues, possibly in conjunction with a circadian clock, as a migratory signal and/or control at some point during their migration. Since shifts in the spectral distribution of underwater light do not appear to be apparent at depths > 150 m (Frank & Widder, 1996), it is likely that this signal occurs during the daytime migratory decent as the shrimp gauge their depth by monitoring the relative decrease in the spectral bandwidth of downwelling light as previously proposed by Frank and Widder (1996). As deep-sea plankton were shown to form dense aggregations at depth during the day, relative to a more sparse distribution a night closer to the surface (van Haren & Compton, 2013), it is also plausible that the shrimp are responding to surrounding bioluminescent stimuli.

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AUTHOR CONTRIBUTIONS

The project was conceptualized by D.D. and H.B.G. All wet laboratory procedures and analyses were done by D.D., and the manuscript was written by D.D. with guidance and input from H.B.G. Both authors helped with the collection of specimens.

DATA AVAILABILITY STATEMENT

Raw RNA sequencing data used for these analyses are publicly available through NCBI's Sequence Read Archive (SRA) database (BioProject ID: PRJNA605562). The tissue-specific

assemblies and associated metadata are available on the Dryad Digital Repository: https://doi.org/10.5061/dryad.rjdfn2z81

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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