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Comparative Study of the Effects of Light on Photophore Ultrastructure from Two Families of Deep-Sea Decapod Crustaceans: Oplophoridae and Sergestidae

Jamie E. Sickles

Thesis of
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Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science
M.S. Marine Biology

Nova Southeastern University
Halmos College of Natural Sciences and Oceanography

April 2020

Approved:
Thesis Committee

Major Professor: Tamara Frank, Ph.D.

Committee Member: Patricia Blackwelder, Ph.D.

Committee Member: Heather Bracken-Grissom, Ph.D.

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By
Jamie Elizabeth Sickles

Submitted to the Faculty of Nova Southeastern University

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Abstract

Counterillumination, the mechanism by which pelagic species produce bioluminescence to replace the light blocked by their bodies to hide their silhouettes, has been known for over 100 years. However, little is known about how these animals are able to so precisely replicate the intensity of downwelling light. The recent discovery of opsins in photophores (Bracken-Grissom *et al.* 2020) suggests that these autogenic organs (i.e. non-bacterial) may be sensitive to light, in addition to their function of emitting visible light. The study presented here is 1) the first ultrastructural assessment of photophores in species *Systellaspis debilis, Janicella spinicauda, Parasergestes armatus,* and *Allosergestes sargassi* and 2) the first study to examine ultrastructural changes in photophore organelles in response to light. The results of this study, demonstrate that photophore organelles exhibit changes in response to light similar to that seen in crustacean photoreceptors, and provides strong support for the hypothesis that the photophores themselves are sensitive to light.

Key words: Mesopelagic, Oplophoridae, Sergestidae, Photophores, Counterillumination, Ultrastructure

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INTRODUCTION

Light in the Deep Sea

When light penetrates a seawater medium, long wavelengths of light such as those at the red end of the spectrum (600-700 nm) are rapidly absorbed, while ultraviolet (UV) wavelengths (300-400 nm) are rapidly scattered (Atkins and Poole 1952, Jerlov 1976). Therefore, only blue light remains below 200 m depth and most deep-sea species have a single, blue sensitive visual pigment to maximize sensitivity to the available light (Clarke and James 1939; Douglas and Partridge 1997; Marshall *et al.* 2003; reviewed in Warrant and Locket 2004).

In the clearest ocean water, there is enough downwelling light for vision to the bottom of the mesopelagic zone (200-1000 m) (reviewed in Warrant and Locket 2004). The lack of any substrate in which to hide behind in the mesopelagic zone makes it difficult for prey to avoid detection from predators that hunt from below using large eyes and sometimes sophisticated visual systems (reviewed in Haddock *et al.* 2010). To avoid detection, some pelagic species utilize a mechanism of camouflage called counterillumination. This involves ventrally, laterally, or internally positioned light organs that produce a bioluminescent emission that mimics the wavelength, intensity, and angular distribution of ambient light blocked by the body (Latz and Case 1982; Herring 2001). Consequently, since blue wavelengths dominate in the mesopelagic zone, almost all marine bioluminescence is blue (reviewed in Haddock *et al.* 2010).

Bioluminescence

Bioluminescence is found throughout terrestrial and aquatic environments, may have independently evolved more than 50 times, and is represented in 700 genera, of which – 80% are marine (reviewed in Haddock *et al.* 2010, Widder 2010). Mesopelagic inhabitants are especially dependent on bioluminescence to search for food, attract prey, reproduce, and avoid predation (Douglas 2001, reviewed in Haddock *et al.* 2010). Visible bioluminescent light is produced by means of chemical reactions always involving the oxidation of a luciferin, the light-emitting molecule, and catalyzed by a luciferase enzyme (reviewed in Haddock *et al.* 2010). Some bioluminescence uses a photoprotein, a protein that requires a specific ion to trigger light production, in addition to luciferin and luciferase. In the mesopelagic zone bioluminescent

emissions are typically between 470-550 nm, but this varies between species and the type of emission, i.e. a spew, a slime, a simple light organ, or a complex light organ(s) (Herring 1983, 1985; Latz *et al.* 1988; reviewed in Widder 2010). Complex light organs, also known as photophores, are common in fish, crustaceans, and cephalopods (reviewed in Haddock *et al.* 2010).

Photophores

Photophores are of two types – bacteriogenic or autogenic (reviewed in Haddock *et al.* 2010). Bacteriogenic light organs are simple cell clusters that require symbiotic bacteria to produce bioluminescence. In contrast, autogenic light organs do not contain bacteria, but contain chemicals that mix to produce light (luciferin and luciferase) and one or all of the following structures; light producing cells (photocytes), optical structures (reflectors and lenses), and associated components (paracrystalline bodies and screening pigments) (Dennell 1940; Arnold and Young 1974; Herring 1981, 1985; Denton *et al.* 1972, 1985; Nowel *et al.* 1998). These structures, along with accompanying organelles, participate in modifying the direction, intensity, angular distribution, refraction, and reflection of emitted bioluminescent light. The three known types of autogenic photophores are 1) dermal, 2) cuticular, and 3) internal organs of Pesta (Burkenroad 1937; Vereshchaka 1994; Poore 2004).

Photophore Structure

Cuticular and Dermal Photophores

The two oplophorid species in this study, *Systellaspis debilis* and *Janicella spinicauda*, possess several pigmented cuticular and dermal photophores over the entire body (Fig. 1A). Cuticular photophores are distinguished from dermal by a concavo-convex lens that is visibly raised when viewed laterally (Kemp 1910; Dennell 1940; Nowel *et al.* 1998; Poore 2004). Dermal photophores may have a lens (Poore 2004) but are continuous with the epidermis (pers. obs.). Most studied in oplophorids and the photophore of interest in the current study is the cuticular pleopod photophore. Ventrally positioned on the coxal segment of the pleopod, these photophores face downwards (Fig. 1A, arrows) and are covered by a trilaminar concavo-convex lens that is colored deep blue in *S. debilis* (Denton *et al.* 1985) and red/orange in *J. spinicauda*

(pers. obs.). Attached to the cuticular lens are large membrane-bound, photogenic cells known as photocytes. The structure of fully-developed photocytes includes a large distal nucleus, central clear area, granular material, apical membrane-bound paracrystalline bodies (that may aid in light production), and a proximal reflecting pigment cap (Fig. 1B, Nowel *et al.* 1998). Laterally surrounding photocytes are sheath cells composed of electron dense, absorbing pigments that prevent lateral leakage of bioluminescent light. New photocytes form outside of the carotenoid pigment sheath and migrate to the center as they mature (Nowel *et al.* 1998). Beyond sheath cells is granulated cytoplasm that houses vesicles, mitochondria, endoplasmic reticulum (ER), Golgi, and lipids.

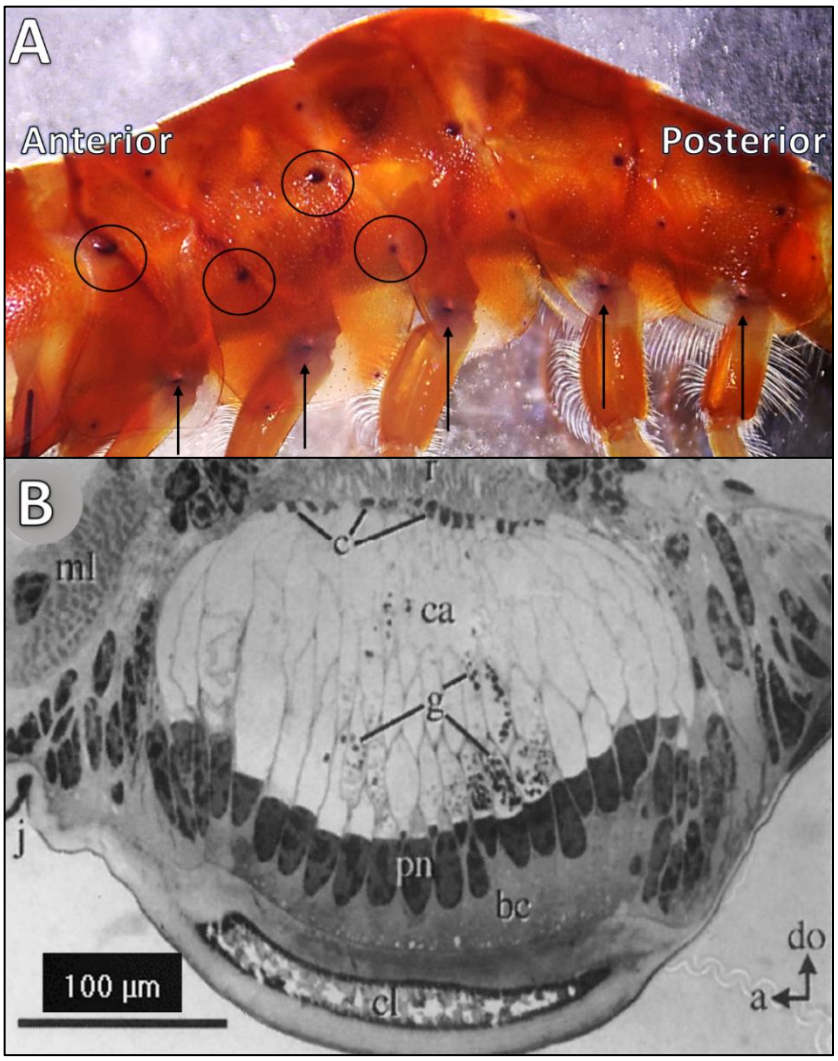

Figure 1. A. Light micrograph of *Systellaspis debilis* autogenic photophores. Cuticular and dermal photophores are present on the midsection (Arrows indicate cuticular pleopod photophores and circles dermal photophores). B. From Nowel *et al.* 1998. Light micrograph of *Oplophorus spinosus* cuticular pleopod photophore. The concavo-convex shaped

cuticular lens distinguishes cuticular photophores from dermal photophores (*CL* cuticular lens, *BC* granulated basal cytoplasm, *PN* mature photocyte nuclei, *G* granulated material, *CA* clear area, *C* paracrystalline bodies, *R* reflecting pigment, *a* anterior, *do* dorsal).

Internal Organs of Pesta

Organs of Pesta were first described by Pesta (1918) in species of *Sergestes* (now revised to *Allosergestes*, *Deosergestes*, *Eusergestes*, *Neosergestes*, and *Parasergestes* – Judkins and Kensley 2008; Vereshchaka 2009) and are found in the cephalothorax (Fig. 2A) of these slightly pigmented, semi-transparent deep-sea shrimp (Burkenroad 1937). Also referred to as internal or hepatic photophores, organs of Pesta evolved from the hepatopancreas, gastrohepatic glands, or specialized liver tubules, and although they are sometimes embedded in liver tubules, they remain distinct from digestive glands (Burkenroad 1937; Dennell 1940). Internal photophores have a species specific arrangement of anterolateral and posterolateral pairs that are sometimes associated with medial organs (Fig. 2B, Foxton 1979). Each organ is composed of several individual translucent tubules that are divided into proximal, medial, and distal regions (Dennell 1940; Herring 1981, reviewed in Latz 1995). Each tubule is capped by the proximal region which is composed of lipids that function as a diffuse reflector, as well as some carotenoid screening pigments that predominantly border tubules on their lateral sides (Fig. 2C). This arrangement gives internal photophores their parabolic shape and directly influences ventrally emitted bioluminescent light. The medial region is thought to be the photogenic area due to the abundance of Golgi, rough and smooth endoplasmic reticulum (RER, SER, respectively), mitochondria, lipids, and paracrystalline platelets, where platelets are presumed to be directly associated with luminescence (Smalley *et al.* 1980; Herring 1981; reviewed in Renwart 2005). Blue distal tips aid to filter bioluminescent light and is composed of amorphous cytoplasm, nuclei, mitochondria, and ER. The tubules are lined by a single layer of columnar epithelial cells comprised of a distal microvilli brush border that surrounds a central lumen (Dennell 1940; Herring 1981, reviewed in Latz 1995). Based on organ location and species, tubules can vary in quantity, shape, size, pigmentation, organelle placement or content (Burkenroad 1937; Dennell 1940; Foxton 1972; Herring 1981, reviewed in Latz 1995).

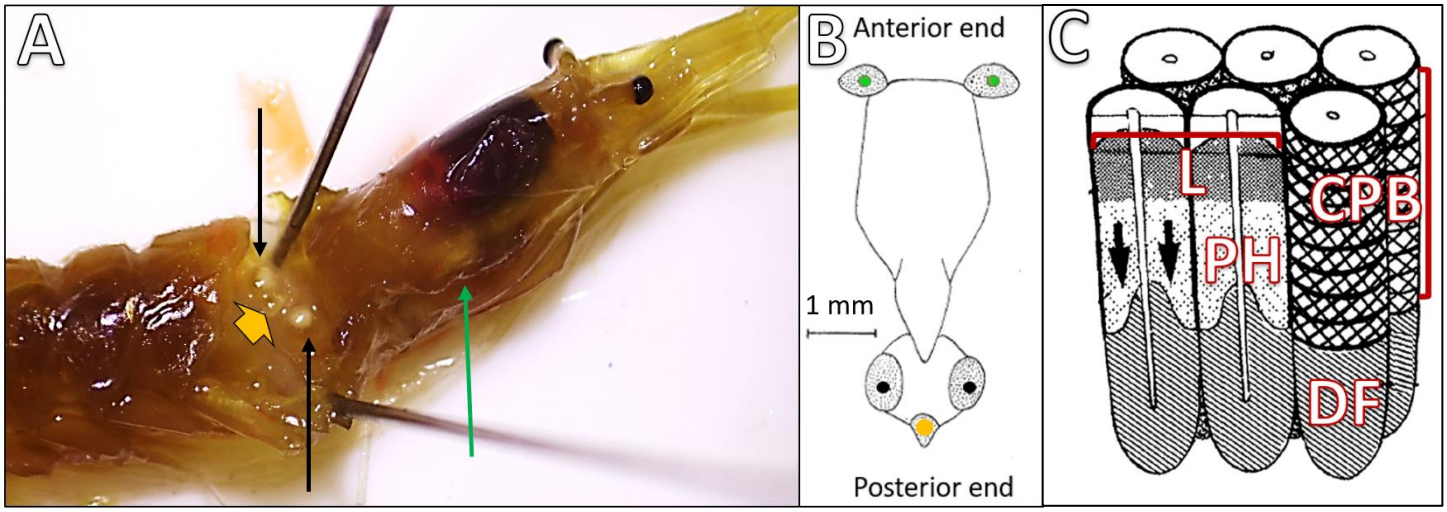

Figure 2. A. Internal photophores in the sergestid specie *Allosergestes sargassi.* Anterolateral organs can be seen from lateral positions while posterolateral and medial organs are best seen from a dorsal view (Green arrows indicate anterolateral organ, black arrows posterolateral organs, and orange arrow posteromedial organ). B. From Foxton 1972. Schematic drawing of internal photophore arrangement in the sergestid specie *Allosergestes sargassi*. Colored dots coordinate with arrows used in image 2A. C. From Denton *et al.* 1985. Schematic drawing of tubule structure in hepatic photophores (C*PB* carotenoid pigment border, *L* diffuse lipid layer, *PH* photogenic region, *DF* distal light filter, *arrows* direction of emitted bioluminescent light).

Counterillumination

Species utilize bioluminescence for various (some hypothesized) biological functions such as mating, conspecific communication, attracting prey, escaping predators, or counterillumination (Clarke 1963; Herring 1976, 1981; reviewed in Herring 2000, Haddock *et al.* 2010). Counterillumination is especially valuable to mesopelagic organisms as their environment lacks substrate to hide behind and as their bodies block incoming downwelling light, resulting in a silhouette that is easily seen by predators that hunt from below using sophisticated vision and dorsally placed eyes. Counterillumination uses ventral, lateral, or internal photophores to emit bioluminescent light downward that exactly matches the physical characteristics, i.e., wavelength, intensity, and angular distribution, of ambient light (reviewed in Warrant and Locket 2004, Haddock *et al.* 2010). This mechanism is triggered by an animal's detection of downward-directed visible light and its efficacy is affected by 1) water clarity and depth (Johnsen *et al.* 2004), 2) unpredictable characteristics of incoming light, 3) surface light intensity based on the time of day, 4) the vision and location of both predators (Munz 1976) and

counterilluminating species in the water column (Johnsen *et al.* 2004), 5) body tilt of counterilluminating species (Latz and Case 1982), and 6) the number of photophores used for counterillumination.

A counterilluminating species must 1) use a photoreceptive organ to accurately interpret ambient light levels (Young *et al.* 1979), 2) possess photophores that can replicate and respond to varying spectral characteristics of downwelling light (Warner *et al.* 1979; Young *et al.* 1980; Latz and Case 1982; Johnsen *et al.* 2004; Jones and Nishiguichi 2004; Claes *et al.* 2010), and 3) maintain luminescence for prolonged periods. The production and emission of bioluminescence is a slow and complicated process because it is linked to hormonal and neural functions (reviewed in Haddock *et al.* 2010), so incoming light must remain constant for counterillumination to occur (reviewed in Latz 1995; Jones and Nishiguchi 2004). Despite its importance, little else is known about the mechanism for counterillumination (Latz and Case 1992). However, the recent discovery of visual opsin proteins in the photophores of decapod crustaceans belonging to the family Oplophoridae has led to the hypothesis that photophores themselves might be light-sensitive (Bracken-Grissom *et al.* 2020). While photosensitivity has been demonstrated in a bacteriogenic light organ in the squid *Euprymna scolopes* (Tong *et al.* 2009), it has never been demonstrated in an autogenic light organ. Determining if there is ultrastructural evidence of photosensitivity in an autogenic light organ, by assessment of the effects of light on photoreceptor organelles (in eyes) as the standard for comparison, is the primary goal of this thesis.

Photosensitive Structures in Photoreceptors

Photoreceptor membrane turnover has been extensively documented in the photoreceptors of many crustacean species. It is a cellular process that removes degraded, aged, or photodamaged photosensitive structures during the onset of light and synthesizes new structures during dark adaptation (Eguchi and Waterman 1967; Itaya 1976; Stowe 1980; Schwemer 1989; Gaten 2013). However, photoreceptors adapted to low-light environments (i.e. night active or deep-sea) have a maximum light-absorption level that, if exceeded, will disrupt photoreceptor membrane turnover and result in cellular damage (Loew 1976; Nilsson and Lindström 1983; Chamberlain *et al.* 1986; Meyer-Rochow 1994). The cellular components known to be involved in the turnover process inclue, but are not limited to, mitochondria,

microvilli, lysosomes, nuclei, ER, and Golgi, and many of these organelles are also found in photophores (Arnold and Young 1974; Herring 1981; Nowel *et al.* 1998). Therefore, if photophores are indeed sensitive to light, then light exposure should elicit cellular responses and organelle morphologies that are similar to those documented in photoreceptors. The research presented here focuses on carotenoid and ommochrome absorbing pigments, basal cytoplasm, mitochondria, microvilli, nuclei, endoplasmic reticulum, Golgi, and lipids, all of which demonstrate light induced changes in position and/or structure in photoreceptors.

METHODS

Sample collection and maintenance

Samples were collected in the Florida Straits during the 2016 (I) and 2017 (II) Bioluminescence and Vision research missions aboard the R/V *Walton Smith*. Specimens were obtained using a $9m^2$ Tucker Trawl equipped with a light tight, thermally insulated, cod-end that closes at depth, to ensure animals are brought to the surface without damage caused by surface light levels or temperatures (Meyer-Rochow and Tiang 1979; Frank and Case 1988). Once on deck, the closed cod-end was detached, taken into a dark room, and specimens were transferred into maintenance containers under dim red light, as previous experiments have demonstrated virtually no sensitivity to dim red light (Frank and Case 1988; Frank and Widder 1999). Inside Koolatrons ©, species were maintained in black, light tight, aerated, 6.5L maintenance containers held at temperatures equivalent to their ambient daytime depths, between 9°C and 10°C, as temperatures warmer than these have demonstrated membrane breakdown and organelle deformities in mesopelagic crustaceans (Meyer-Rochow and Tiang 1979). Pressure changes are generally not harmful for animals living shallower than 2000 m (reviewed in Brown and Thatie 2013) who are not equipped with air-filled spaces (i.e., any animal without a swim bladder or air-filled float), and therefore temperature and light exposure are the major parameters that need to be controlled.

Species Utilized in Study

The focus of this study was cuticular pleopod photophores found in Oplophoridae species *Systellaspis debilis* and *Janicella spinicauda* and internal organs of Pesta found in Sergestidae species *Parasergestes armatus,* and *Allosergestes sargassi.* Species were chosen based on their abundance in the Straits of Florida.

Experimental procedure

On board the R/V *Walton Smith*, oplophorids were divided into a control group that was kept in the dark and two experimental groups that were exposed to two different irradiances: 1) dim white light at 1 $\mu W\ cm^{-2}$ and 2) bright white light at 16 $\mu W\ cm^{-2}$ (fluorescent bulb room lighting), henceforth referred to as either dark/control, dim, or bright. Irradiance was measured using a portable optometer (Gamma Scientific Model S471 with a Nidek 247 Sensor Head). Oplophorid experimental (i.e. non-control) groups underwent one of the two aforementioned light exposures, for either 30 minutes or 60 minutes. Due to a smaller sample size, sergestids were divided into a control group and one experimental group at the bright light exposure, for one timed interval at 60 minutes. Light levels and timed intervals were chosen based on a study from Latz and Case (1992) that demonstrated that dark-acclimated sergestids *Eusergestes similis,* subsequently exposed to a light intensity of $1\ x\ 10^{-3}\ \mu W\ cm^{-2}$, took a minimum of two minutes to counterilluminate and 25 minutes to reach a constant maximum bioluminescent output that matched the experimental illumination level. Light levels considered "dim" mimicked the light levels used by Latz and Case (1992) and bright light levels were chosen with the intention to damage any photosensitive tissues as shown in dim-light adapted crustacean photoreceptors (see discussion).

Before experiments began, dim red light was used to move species from maintenance containers to a sorting tray inside Koolatrons. Species spent 20 minutes acclimating back to the dark (Latz and Case 1992) before the Koolatron lid was opened for dim or bright light exposures. For dim exposures, blocking filters (layers of white mesh) placed over opened Koolatrons were used to achieve the appropriate light intensity, whereas bright light exposures did not use blocking filters and animals were subjected to the intensity of fluorescent bulb room lighting. Control specimens remained in dark, light-tight containers during each timed interval to serve as

the control to validate that it was not collection or maintenance techniques causing any potential organelle disruption. At the end of timed trials, animals were transferred into a 2% glutaraldehyde, 0.05M sodium cacodylate buffered, filtered seawater fixative. Samples spent a minimum of seven days in fixative held at 2° C, to better penetrate and harden tissues to prevent photophores from falling apart during dissection and electron microscopy fixation.

Transmission Electron Microscopy (TEM) Fixation

Animals fixed in 2% glutaraldehyde in sodium cacodylate buffered seawater were removed from the fixative and photophores from the coxal segment in oplophorids and from the cephalothorax in sergestids were dissected out, washed with 0.05M sodium cacodylate buffered filtered seawater for three 15 minute changes, post-fixed in 1% osmium tetroxide in buffer for 60 minutes. Specimens were then rinsed for three changes of the buffer, dehydrated in three changes each of a graded series of ethanol (20%, 50%, 70%), maintained in 70% ethanol for a minimum of 48 hours. Specimens were then further dehydrated in three changes of ethanol (95%, 100%), infiltrated with three changes of Spurr embedding resin, and placed into a VWR drying oven at 60° C for a minimum of 72 hours. After trimming, sections of tissue were cut off Spurr resin blocks using a Sorvall Porter-Blum MT2-B ultramicrotome fitted with a DKD-diamond knife. Thin sections (90 nm gold) were transferred to formvar/carbon coated 200 mesh grids and viewed with a Phillips CM300 TEM scope at NSUOC. Since the NSUOC scope does not have digital image capture capabilities, micrographs were taken at the TEM Core, University of Miami, Miller School of Medicine, using a Phillips CM10 or a Joel 1400 transmission electron microscope, under the direction of Dr. Patricia Blackwelder.

DATA ANALYSIS

Photophore photosensitivity

To identify photophore photosensitivity in oplophorids and sergestids, organelles from the same regions of dark controls and light exposed tissues were measured, observed, and compared. In oplophorids, changes in the area of basal cytoplasm, vacuolated area within the basal cytoplasm, sheath cell widths, and pigment granule diameters were quantified. In addition,

changes in the Golgi apparatus and endoplasmic reticulum (ER) size (μm), level of vesiculation, and fragmentation, were also observed. In sergestids, the formation of cytoplasmic organelles identical to those formed during photoreceptor membrane turnover and lipid to organelle contact sites were identified, and lipid diameters in medial and distal tubule regions were also measured along their major axis and compared between the controls and bright light exposed tissues. If a Shapiro-Wilk test determined the data were normally distributed and if a Bartlett test showed that variances were homogeneous, then a One-Way ANOVA was used to analyze organelle measurements and determine if significant differences were present. If data were not normally distributed and/or if variances were not homogeneous, then a non-parametric Kruskal-Wallis test was used for statistical analysis. Images were uploaded into the free software ImageJ (Schneider *et al.* 2012) and measurements were taken three times and averaged to minimize human error. All statistical analyses were performed in the statistical software R and test statistics were considered significantly different at $p \leq 0.05$.

RESULTS

Oplophorids

Basal Cytoplasm

Data for 30 and 60 minute groups was combined for *Systellaspis debilis* and *Janicella spinicauda* to obtain a larger sample size since only cuticular pleopod photophores were analyzed and results from individual species were consistent. The organelles of interest in pleopod photophores includes the basal cytoplasm, vacuoles in the basal cytoplasm, sheath cells, endoplasmic reticulum (ER), and Golgi. The basal cytoplasm is composed of electron dense granular material, mitochondria, and ER. The area of basal cytoplasm included any cytoplasm located between the cuticle, photocyte nuclei, and sheath cells. Basal cytoplasm area was measured to determine if light caused an expansion or reduction of cytoplasm and the vacuolated area in the basal cytoplasm was also measured to determine if light caused variability in the area of vacuolated basal cytoplasm.

Observed was an increase in the area of basal cytoplasm and vacuolation in 30 minute groups in dim and bright light exposures compared to the controls, but these differences were not

significantly different between tissues (Table 1, 2). The area of basal cytoplasm was significantly greater in 60 minute dim light and bright light exposures compared to the controls, but in 60 minute dim-light exposures, vacuolation significantly decreased compared to the controls (Table 3, 4; Fig. 3A, B, C). However, exposure to bright light for 60 minutes significantly increased vacuolation compared to control tissue. In these specimens, exposure to bright light for 60 minutes resulted in the greatest expansion and vacuolation of the basal cytoplasm area.

Sheath Cells

Sheath cell widths were measured to determine if light exposures influenced sheath cell size or arrangement. Observed was a significant increase in sheath cell widths in 30 and 60 minute groups in dim and bright light exposures compared to the controls (Table 1-4; Fig. 3D, E. F). Sheath cells in light exposed tissues had broad and robust arrangements compared to the thin linear arrangements seen in the controls. In these specimens, exposure to bright light for 60 minutes resulted in the widest sheath cell widths.

Sheath cells from all tissue types predominately surround exterior photocyte nuclei that are tightly bordered by microtubule channels that sometimes contain sheath cells or other organelles such as mitochondria (Fig. 4) or lipids. Occasionally, control tissues displayed electron lucent granules migrating from photocyte nuclei centers to bordering microtubules (Fig. 5). Additionally, lipids were often seen in close proximity to sheath cells and sometimes within a sheath cell (Fig. 6).

Table 1. Organelle characteristics from oplophorids *J. spinicauda* and *S. debilis* exposed to low intensity light for 30 minutes. The control group (dark) was not exposed to light. Sample size (n) refers to the number of photophores that were analyzed.

Photophore Organelle	Light Level		Test/*p* value
	Dark (n=14)	Dim (n=6)	
	µm ± S.E.		
Basal Cytoplasm Area	254.44 ± 45.16	473.99 ± 212.83	One-Way ANOVA p =0.06
Vacuole Area	45.12 ± 10.10	54.98 ± 14.94	One-Way ANOVA p =0.13
Sheath Cell Width	0.81 ± 0.02	1.01 ± 0.01	Kruskal-Wallis, p <0.001

Table 2. Organelle characteristics from oplophorids *J. spinicauda* and *S. debilis* exposed to high intensity light for 30 minutes. The control group (dark) was not exposed to light. Sample size (n) refers to the number of photophores that were analyzed.

Photophore Organelle	Light Level		Test/*p* value
	Dark (n=14)	Bright (n=14)	
	µm ± S.E.		
Basal Cytoplasm Area	254.44 ± 45.16	710.60 ± 98.22	One-Way ANOVA *p* =0.06
Vacuole Area	45.12 ± 10.10	76.47 ± 16.45	One-Way ANOVA *p* =0.13
Sheath Cell Width	0.81 ± 0.02	1.52 ± 0.03	Kruskal-Wallis *p* <0.001

Table 3. Organelle characteristics from oplophorids *J. spinicauda* and *S. debilis* exposed to low intensity light for 60 minutes. The control group (dark) was not exposed to light. Sample size (n) refers to the number of photophores that were analyzed.

Photophore Organelle	Light Level		Test/*p* value
	Dark (n=14)	Dim (n=6)	
	µm ± S.E.		
Basal Cytoplasm Area	297.2 ± 31.2	317.0 ± 85.6	One-way ANOVA, *p* =0.82
Vacuole Area	76.4 ± 11.4	45.4 ± 12.5	One-way ANOVA, *p* <0.001
Sheath Cell Width	0.85 ± 0.03	1.1 ± 0.07	Kruskal-Wallis, *p* <0.001

Table 4. Organelle characteristics from oplophorids *J. spinicauda* and *S. debilis* exposed to high intensity light for 60 minutes. The control group (dark) was not exposed to light. Sample size (n) refers to the number of photophores that were analyzed.

Photophore Organelle	Light Level		Test/*p* value
	Dark (n=14)	Bright (n=14)	
	µm ± S.E.		
Basal Cytoplasm Area	297.2 ± 31.2	1518.9 ± 273.5	One-way ANOVA, *p* =0
Vacuole Area	76.4 ± 11.4	548.5 ± 192.5	One-way ANOVA, *p* <0.001
Sheath Cell Width	0.85 ± 0.03	1.8 ± 0.04	Kruskal-Wallis, *p* <0.001

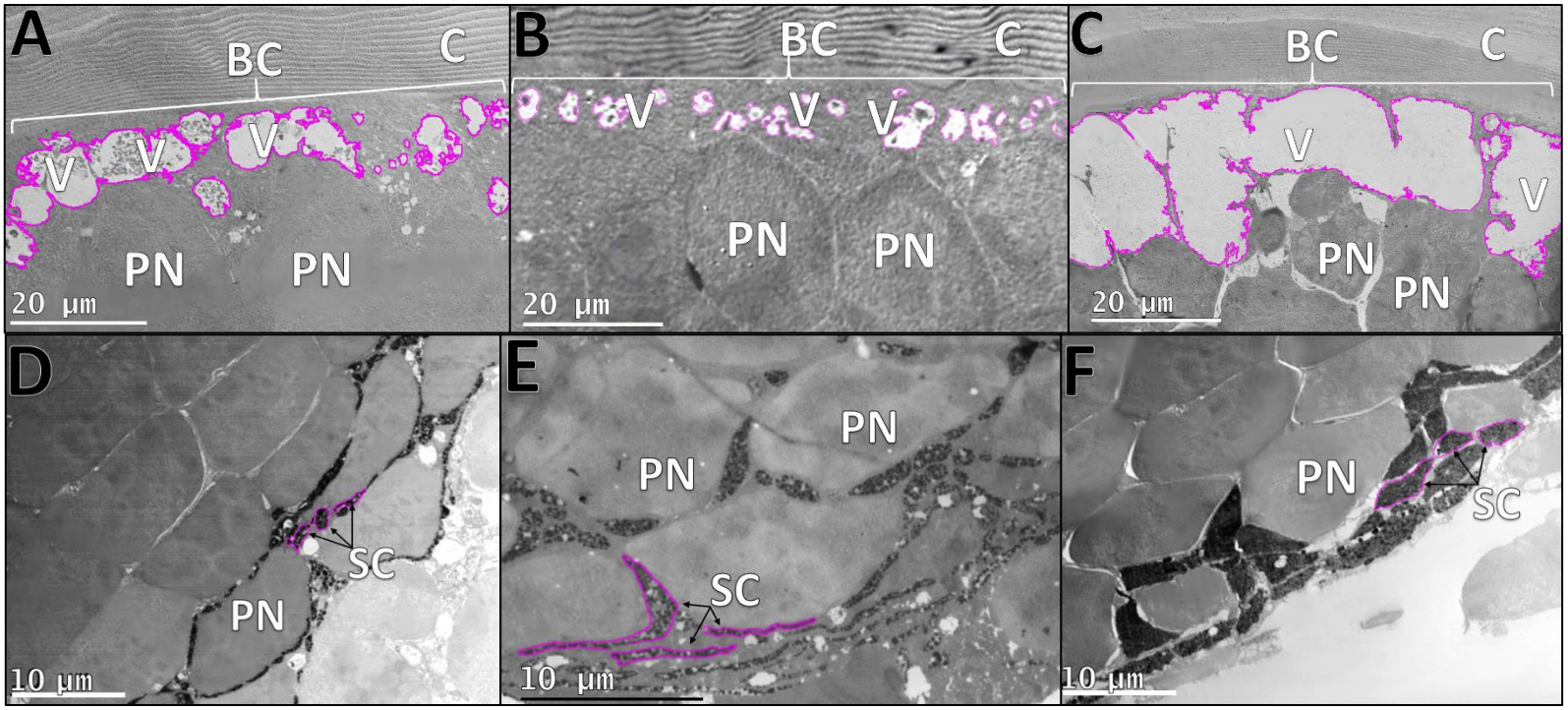

Figure 3. Cellular characteristics analyzed in oplophorid tissues from 60 minute groups. Basal (bracket) and vacuolated (outlined in pink) basal cytoplasm in (A) control (1,950X), (B) dim light (1,950X), and (C) bright light (1,450X) exposed tissues. Sheath cells (outlined in pink) in (D) control (2,500X), (E) dim light (4,000X), and (F) bright light (2,600X) exposed tissues (*C* cuticle, *V* vacuole, *BC* basal cytoplasm, *PN* photocyte nuclei, *SC* pigment sheath cell).

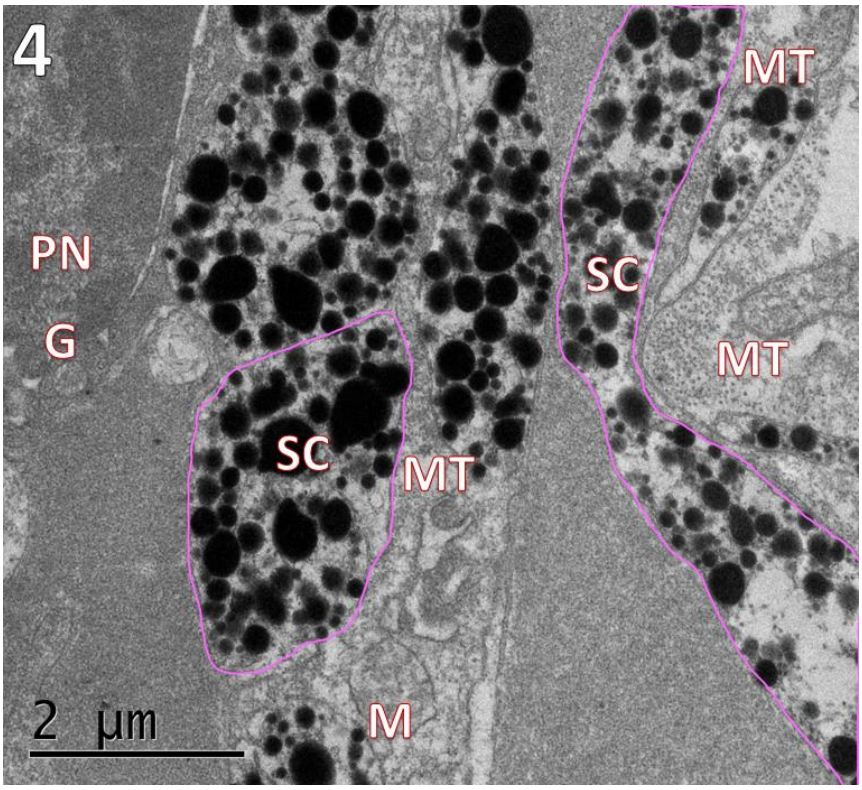

Figure 4. Microtubules in 60 minute oplophorid control tissue. Microtubule channels border photocyte nuclei and can contain sheath cells and mitochondria (15,000X) (*MT* microtubules, *PN* photocyte nuclei, *SC* sheath cells, *M* mitochondria).

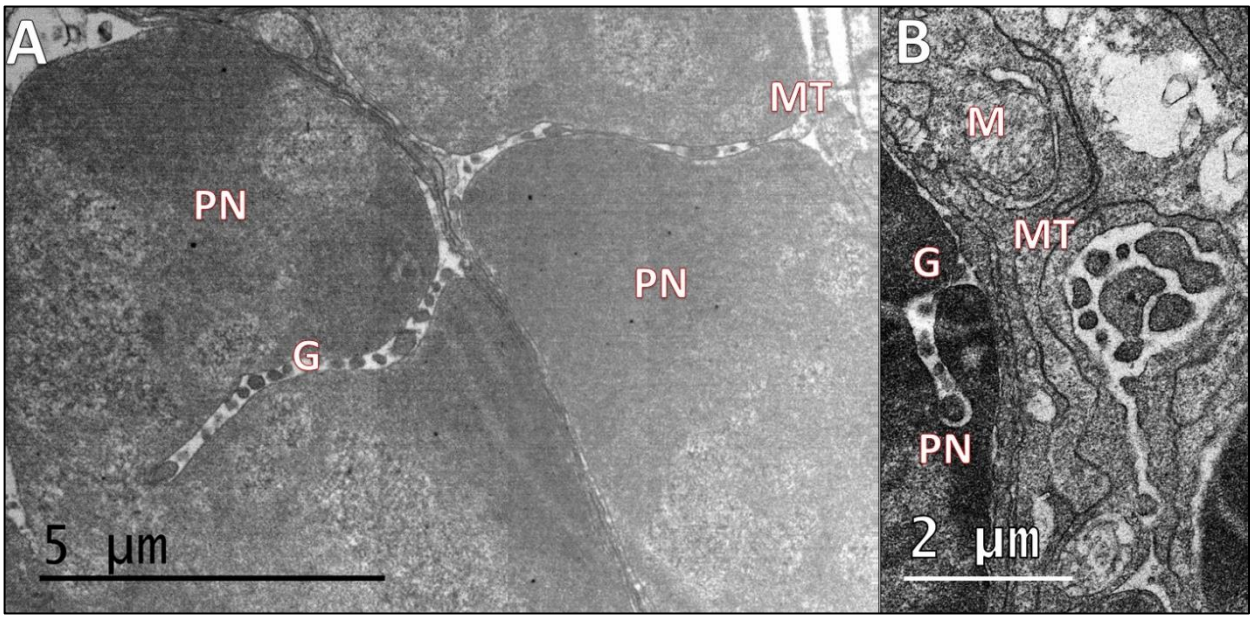

Figure 5. A, B. Oplophorid 60 minute control tissue. Translucent granules found migrating from the center of photocyte nuclei into surrounding microtubule channels (7,900X; 15,000X) (*G* granules, *PN* photocyte nuclei, *MT* microtubules).

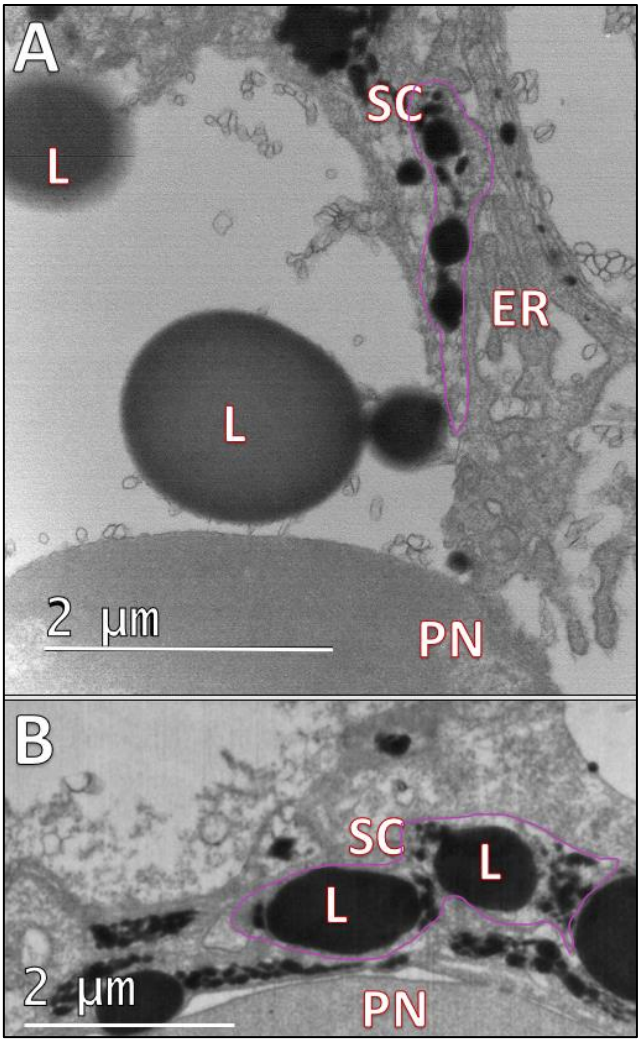

Figure 6. Lipids were found in proximity to sheath cells in all tissue types. A. Lipids in 60 minute control tissue are seen adjacent to sheath cells and in close contact with the endoplasmic reticulum (13,500X). B. Lipids in 60 minute bright light exposed tissue are seen within sheath cells (19,000X) (*PN* photocyte nuclei, *SC* sheath cell, *ER* endoplasmic reticulum, *L* lipid).

Golgi and Endoplasmic Reticulum (ER)

Golgi and ER organelles increased in number and size in tissues exposed to light. Non-vesiculated, small (0.5 µm) Golgi bodies were seen in control tissues, were slightly larger (1 µm) with vesiculated edges in dim light exposed tissues and were the largest (2 µm) and most severely vesiculated in bright light exposed tissues (Fig. 7A, B, C). Golgi were found in the cytoplasm surrounding photocytes, but ER was ubiquitous throughout the photophore, found in various portions of cytoplasm and throughout areas of photocyte nuclei. The controls had dense

patches of ER that made it hard to distinguish smooth ER from rough ER and this was also the case in bright light exposed tissues but due to loosened and fragmented ER morphology (Fig. 7D, F). ER in dim light exposures was the most organized, well-defined, and prominent (Fig. 7E).

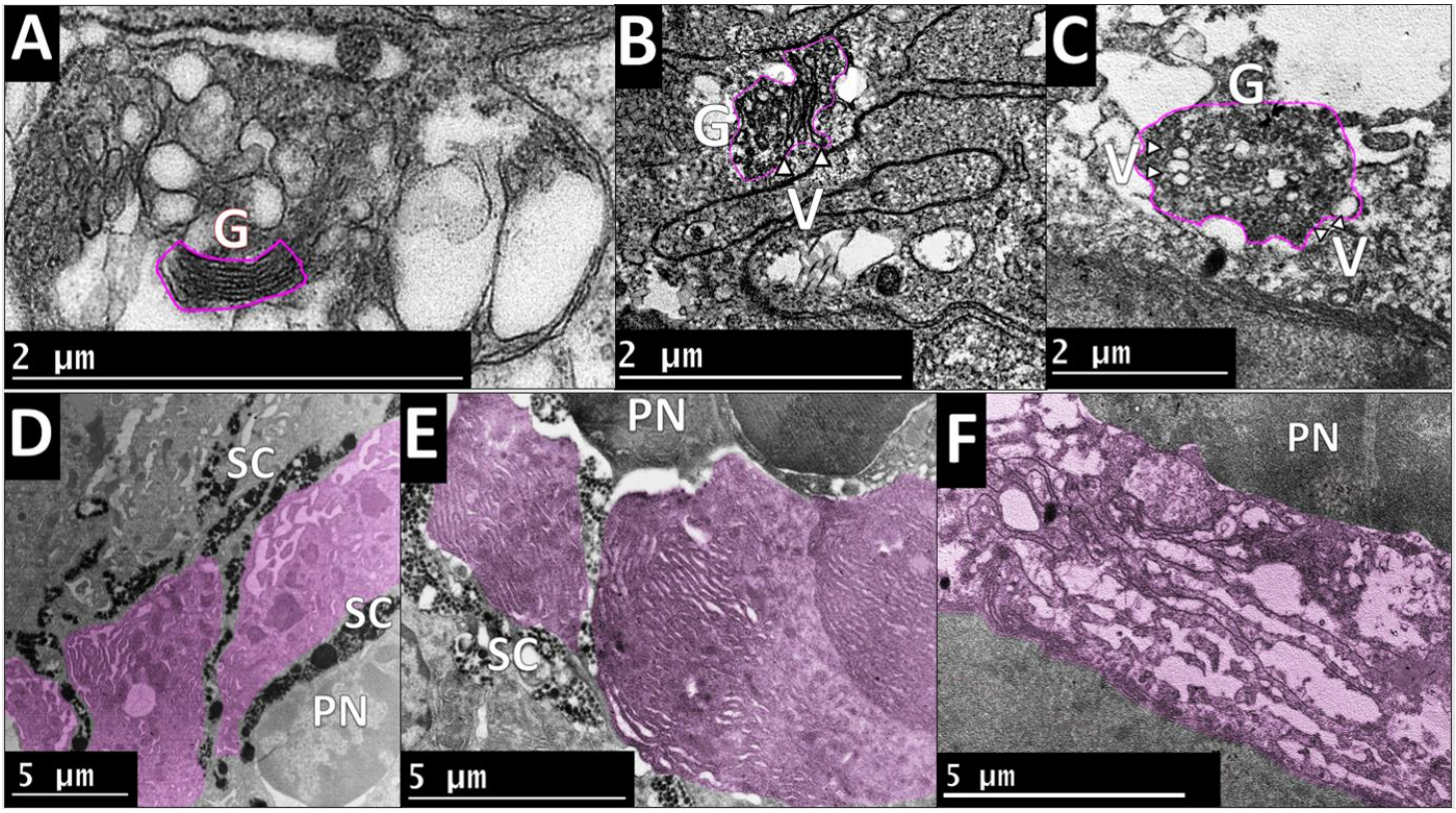

Figure 7. Cellular characteristics analyzed in oplophorid tissues from 60 minute groups. Golgi bodies (outlined in pink) in (A) control (8,000X), (B) dim light (25,000X), and (C) bright light (10,500X) exposed tissues. Golgi bodies were largest and most vesiculated (white arrowheads) in bright light exposed tissues. Endoplasmic reticulum (ER) (pink shading) in (D) control (4,600X), (E) dim light (5800X), and (F) bright light (5,800X) exposed tissues. Prominent and well-organized ER in dim light exposed tissues suggests an increase in cellular activity and efficient cellular function (*PN* photocyte nuclei, *G* Golgi, *V* vesicle, *SC* sheath cell, *white arrowheads* vesicles).

Pigment Granules

Due to the fact that pleopod photophores in oplophorid species used in this experiment are different colors, blue in *S. debilis* and red/orange in *J. spinicauda*, pigment granule diameters were measured and compared between species to determine if pigment granules differed in size.

Pigment granule diameters were significantly larger in *S. debilis* (n=15, mean=0.190 µm) than *J. spinicauda* (n=15, mean=0.096 µm; Mann-Whitney Wilcoxon, W=24200, p <0.001; Fig. 8A, B).

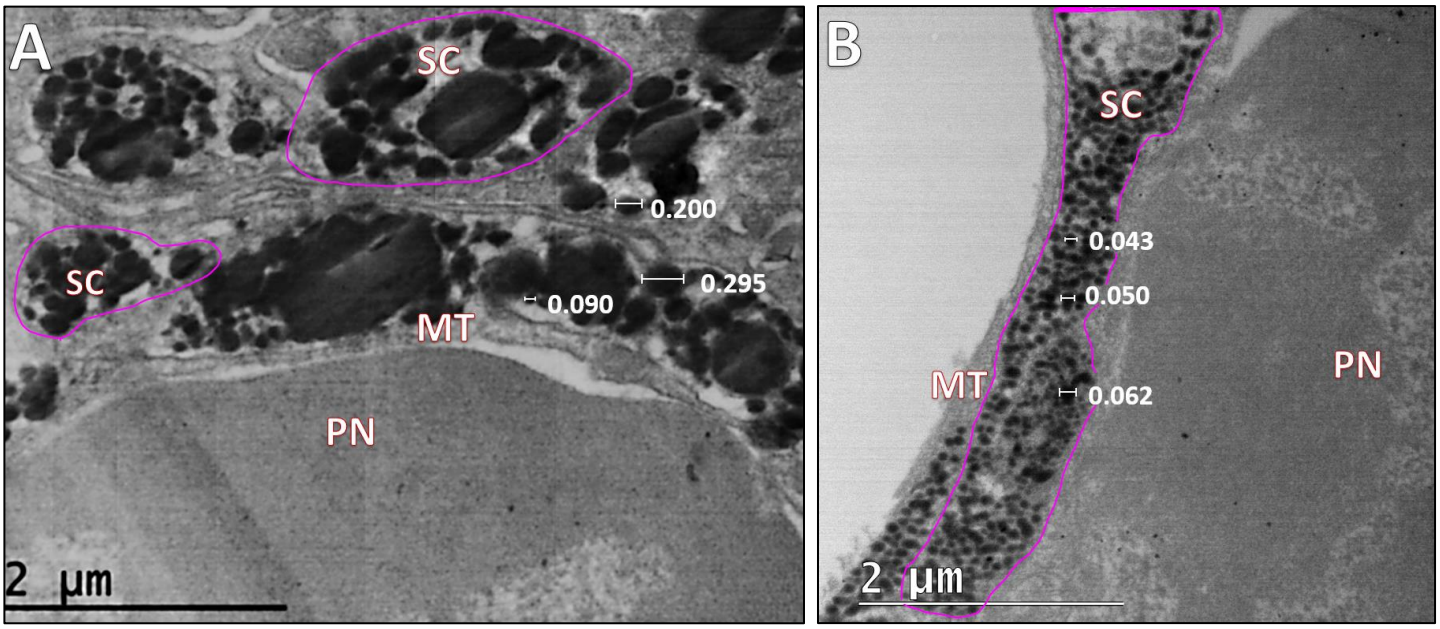

Figure 8. A, B. Pigment granule diameters in sheath cells from oplophorid control tissues. Pigment granules in (A) *Systellaspis debilis* were significantly larger than pigment granules in (B) *Janicella spinicauda* (19,000X) (*PN* Photocyte nuclei, *SC* sheath cell, *MT* microtubule).

Sergestids

Cytoplasmic Organelles

Due to the fact that only internal photophores were analyzed and findings were identical between organs and species, the data from *Parasergestes armatus* and *Allosergestes sargassi* were combined for this analysis. Organs of Pesta are composed of translucent tubules that contain epithelial cells, mitochondria, ER, Golgi, nuclei, lipids, microvilli, and microtubule channels that run parallel along tubule exteriors (Fig. 9). In all tissue types, amorphous material was occasionally present in central tubule lumens (Fig. 10). Only found in bright light exposed tissues were the presence of cytoplasmic organelles identical to those formed during photoreceptor membrane turnover in compound eyes of species from dim light environments. These organelles were also not found in proximal regions, and therefore only medial and distal regions are included in this analysis.

Microvilli brush borders in bright light exposed tissues produced a substantial amount of pinocytotic vesicles (Fig. 11A) that were associated with mitochondria and dense lipids. Pinocytotic vesicles were internalized into single membrane multivesicular bodies (MVBs, Fig. 11B), that ranged in size between 0.2–3 µm. MVBs were concentrated near microvilli sites but could be found throughout tubule cytoplasm. Secondary lysosomes known as multilamellar bodies (MLBs) were less common than MVBs and ranged in sized between 0.5-10 µm. Small MLBs (0.5 µm) were found in all tissue types but were associated with amorphous and dense bodies in bright light exposed tissues (Fig. 12A, B). Additionally, only bright light exposed tissues contained large MLBs (5-10 µm) found in tubule lumens between the lipid diffuse layer and paracrystalline bodies (Fig. 13).

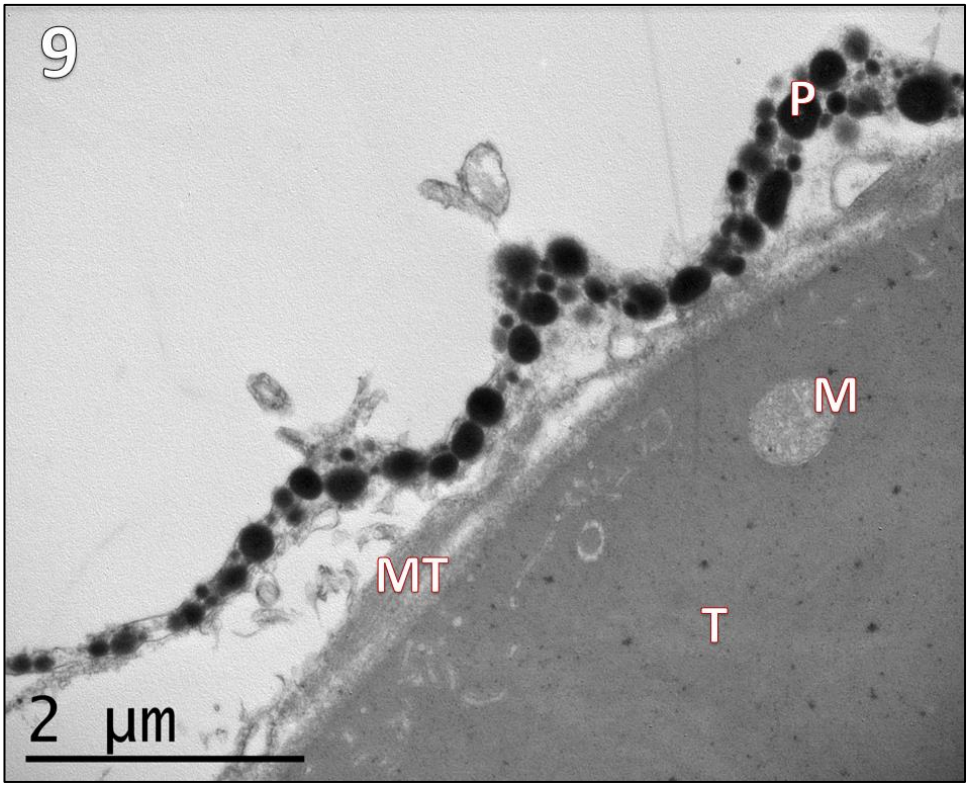

Figure 9. A thin strand of pigments can sometimes be found adjacent to microtubules that border translucent tubule exteriors (13,500X) (*P* pigment, *T* tubule, *M* mitochondria, *MT* microtubule).

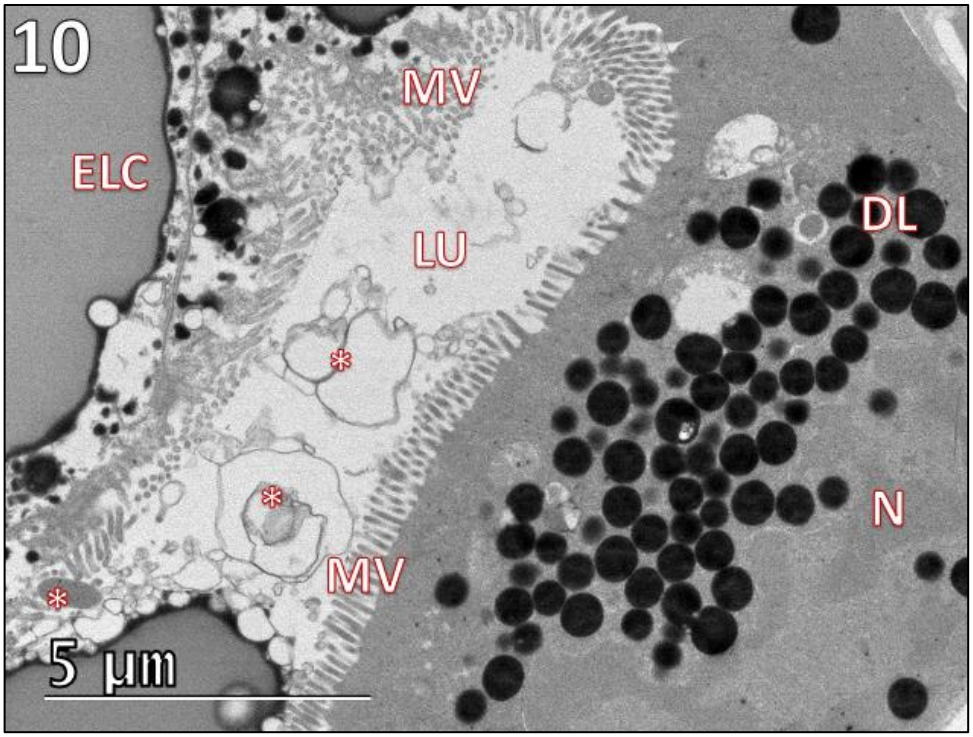

Figure 10. Vacuolar material was often seen in tubule central lumens. Dense lipids are seen within the nuclear envelope and nucleoplasm in an epithelial cell nucleus (7,900X) (*MV* microvilli, *ELC* lipids with an electron dense border and an electron lucent core, *DL* dense lipid, *N* nucleus, *LU* central lumen, *asterisks* vacuolar material).

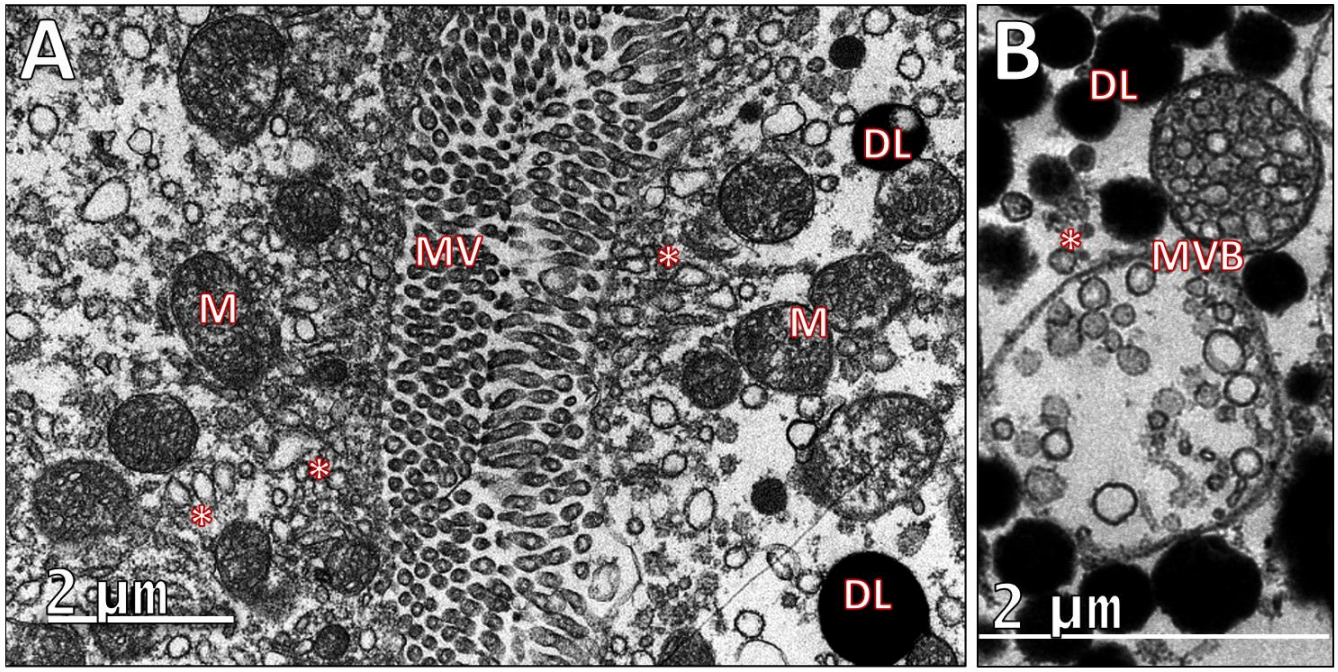

Figure 11. Cytoplasmic organelles formed in 60 minute bright light exposed organs of Pesta. A. Sites of pinocytotic vesicle formation at microvilli brush borders also contained abundant mitochondria and dense lipids (20,000X). B. Two MVBs are surrounded by dense

lipids and some small pinocytotic vesicles. Vesicles in the smaller MVB are more compact than in the larger MVB (20,000X) (*M* mitochondria, *MV* microvilli, *DL* dense lipid, *MVB* multivesicular body, *asterisks* pinocytotic vesicles).

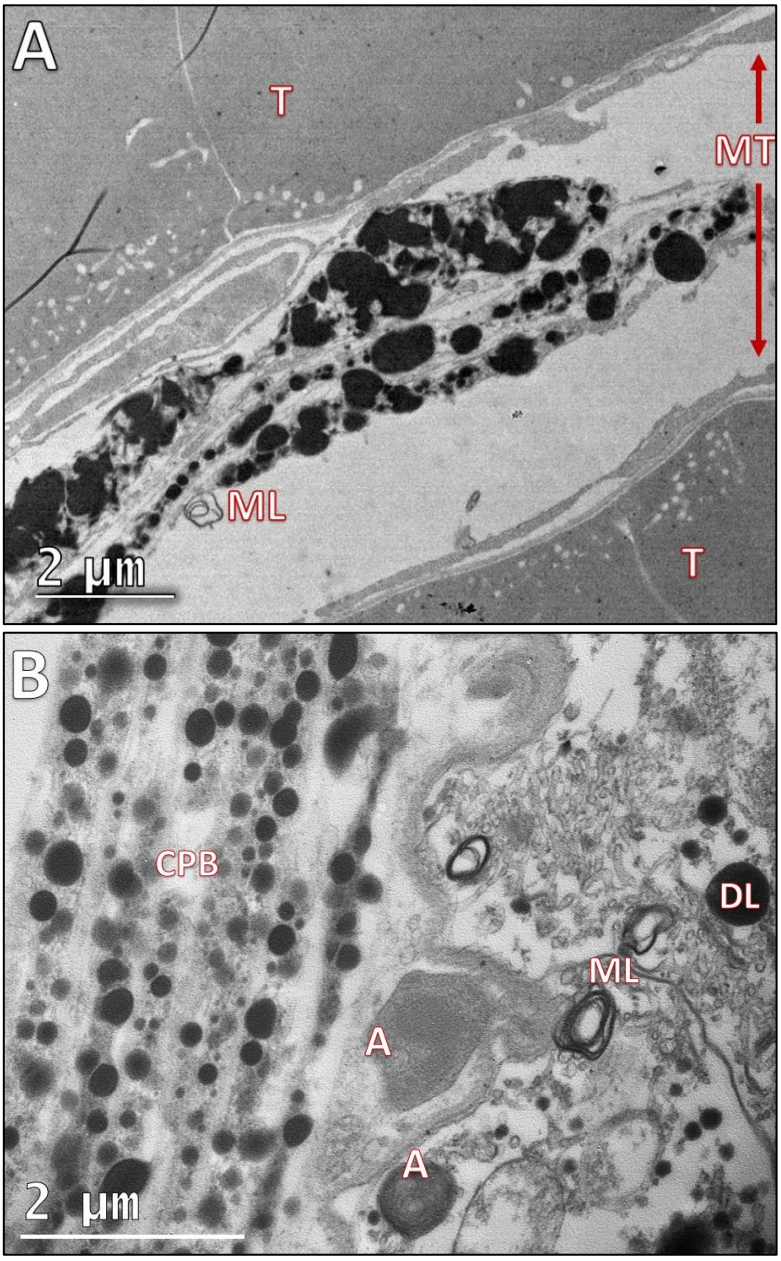

Figure 12. Small multilamellar structures. A. A multilamellar structure attached to membrane-enclosed pigment granules is seen between two tubules in control tissue (15,000X). B. Small multilamellar structures in bright light exposed tissues were associated with amorphous and dense bodies (presumably DLs) adjacent to the carotenoid pigment border (13,500X) (*ML* multilamellar structure, *A* amorphous body, *DL* dense lipid, *T* translucent tubule, *MT* microtubule, *CPB* carotenoid pigment border).

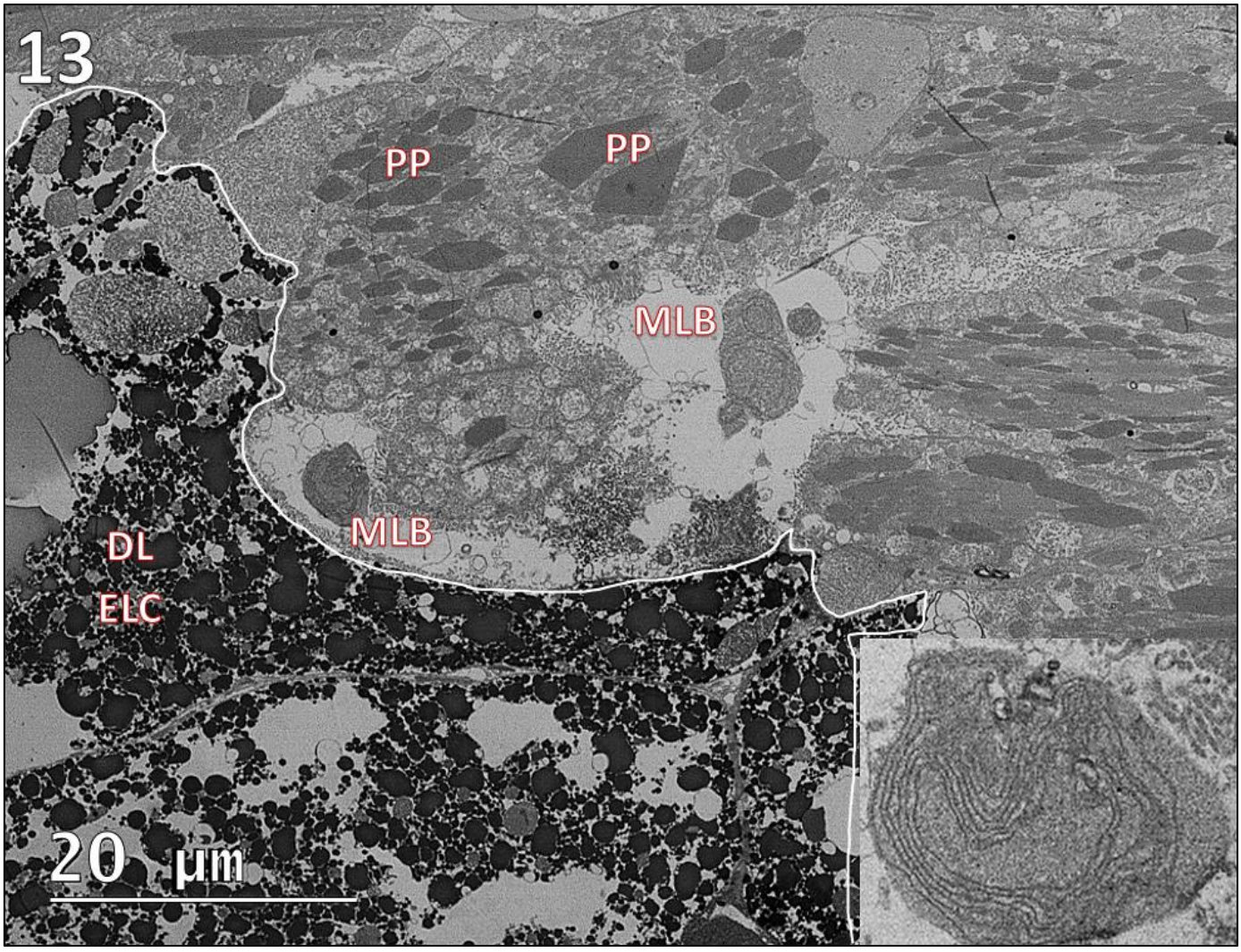

Figure 13. Large multilamellar bodies in the medial region of a bright light exposed sergestid photophore. Multilamellar bodies formed in the central lumen of translucent tubules. To the left of the white outline, multilamellar bodies are surrounded by a mixture of lipids (dense lipids and lipids with an electron dense border and an electron lucent core) and are surrounded by paracrystalline platelets to the right. Insert details multilamellar membranes and amorphous material in MLBs (2,600X) (*MLB* multilamellar body, *PP* paracrystalline platelet, *DL* dense lipid, *ELC* lipid with an electron dense border and an electron lucent core).

Lipids

In all tissue types lipids were the most common organelle found throughout tubules and the only organelle to come in contact with several structures including microvilli, mitochondria, tubule nuclei, ER, and Golgi. Due to morphological variations, lipids were grouped and analyzed according to electron density and will henceforth be referred to as either 1) dense lipids (DLs), 2)

lipids with an electron dense phospholipid layer and an electron lucent core (ELCs), or 3) electron lucent lipids (LLs) (Fig. 14). Lipid diameters were measured in control and bright light exposed tissues and were analyzed between medial and distal tubule regions.

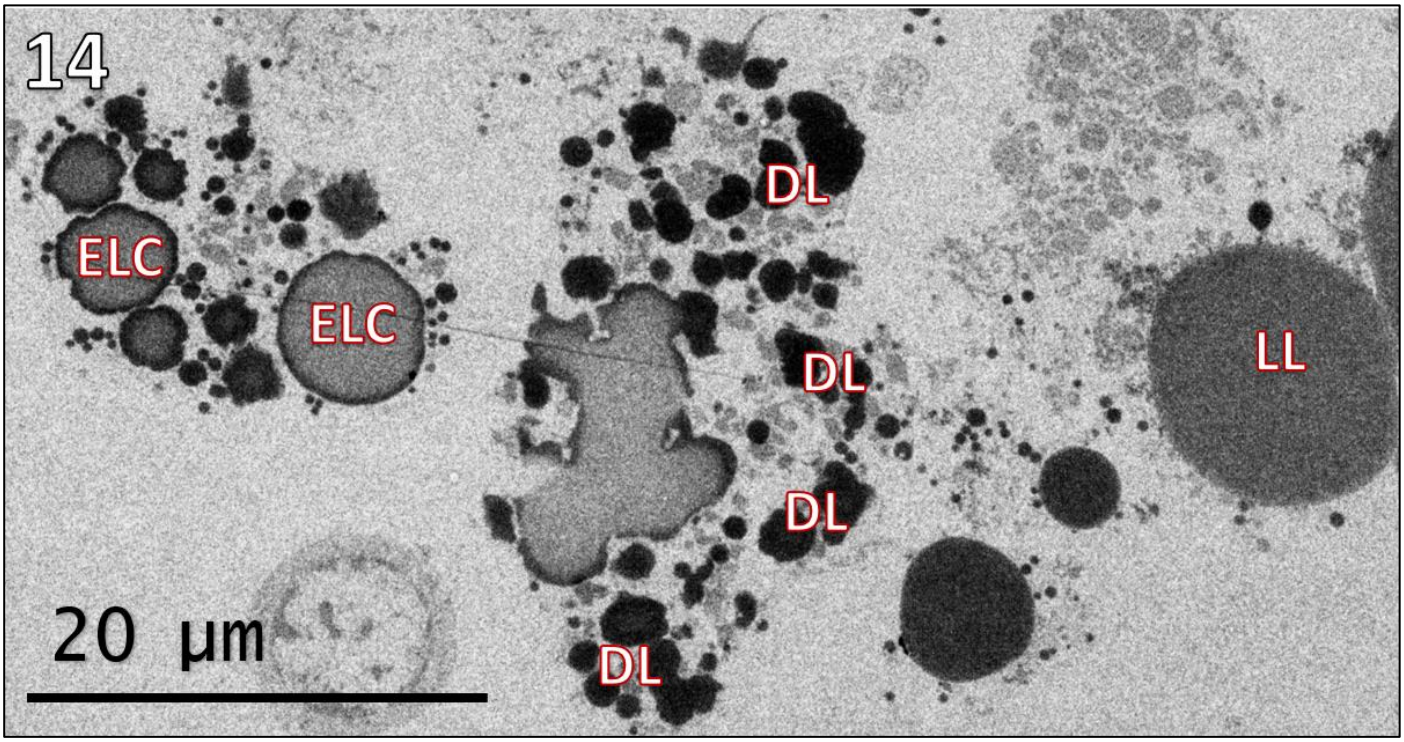

Figure 14. A rare occurrence of all three lipid types was seen in the distal region of bright light exposed tissue. This accurately depicts the abundance of lipid types where DLs were the most abundant, ELCs were moderately abundant, and LLs were the least abundant (2,000X) (*DL* dense lipid, *ELC* lipid with an electron dense border and an electron lucent core, *LL* electron lucent lipid).

Electron dense lipids (DLs)

DLs were the most ubiquitous and diversely shaped, being either circular, oblong, or amoeboid. DL diameters were significantly larger in bright light exposed medial and distal regions, compared to the controls (Table 5, 6). DL diameters were consistent in tubule regions as more than 90% of DLs in medial regions had diameters smaller than 1.0 µm and about half of the DLs in distal regions had diameters greater than 1.0 µm. The largest measured DL diameter (15 µm) was found in the distal region of bright light exposed tissue surrounded by other large DLs that appeared to be sharing electron dense material (Fig 15).

Table 5. Diameters (µm) of dense lipid (DL) droplets in control and bright light exposed medial tissue regions in the sergestids *P. armatus* and *A. sargassi*. Sample size (n) is the number of photophores that were analyzed.

DLs - Medial Region				
Mann-Whitney Wilcoxon, W = 78544, *p* < 0.001 *				
Dark (n=4)			**Bright (n=9)**	
Mean (µm) = 0.549			**Mean (µm) = 0.770**	
Diameter (µm)	*Percent (%)*		*Diameter (µm)*	*Percent (%)*
0.06-0.1	6.3		0.06-0.1	22.5
0.2-0.6	65.3		0.2-0.6	61.9
0.7-1.0	21.1		0.7-1.0	8.2
≥1.1	7.3		≥1.1	7.4

Table 6. Diameters (µm) of dense lipid (DL) droplets in control and bright light exposed distal tissue regions in the sergestids *P. armatus* and *A. sargassi*. Sample size (n) is the number of photophores that were analyzed.

DLs - Distal Region				
Mann-Whitney Wilcoxon, W = 141850, *p* < 0.001 *				
Dark (n=4)			**Bright (n=9)**	
Mean (µm) = 0.630			**Mean (µm) = 1.174**	
Diameter (µm)	*Percent (%)*		*Diameter (µm)*	*Percent (%)*
0.1-0.5	50		0.1-0.5	38.9
0.6-1.0	44.7		0.6-1.0	34.1
1.1-1.5	5.1		1.1-1.5	15.5
≥1.6	0.2		≥1.6	11.5

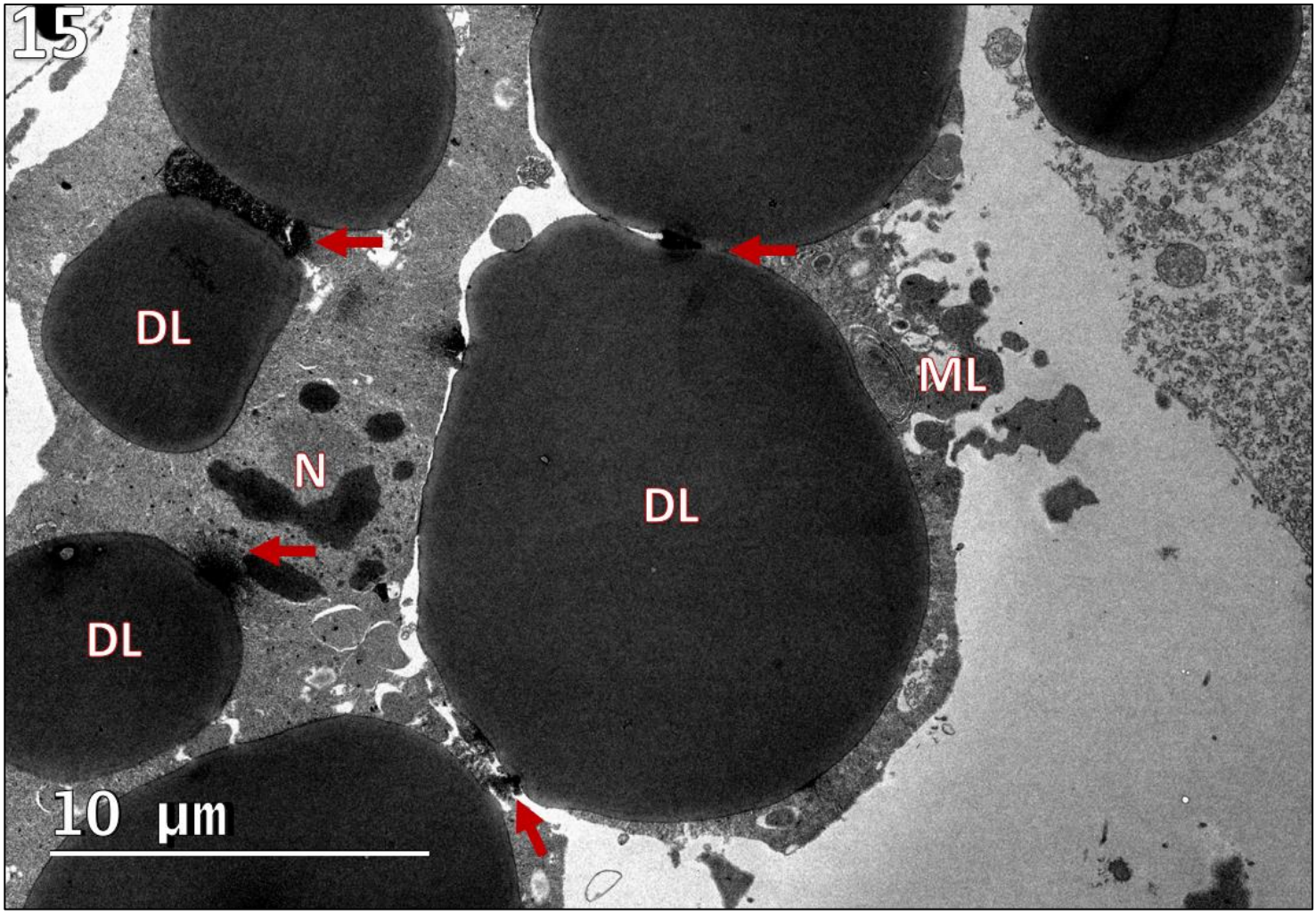

Figure 15. DLs in the distal region from bright light exposed tissue appear to be sharing electron dense material with other dense lipids. A lipid in the bottom left appears to be sharing this electron dense material with a tubule nucleus (2,000X) (*DL* dense lipid, *ML* multilamellar structure, *N* nucleus, *red arrows* electron dense material).

Lipids were abundant throughout hepatic photophores in both the controls and bright light exposures, but only in bright light exposed tissues were lipids, mainly DLs, seen in contact with other organelles. Cellular membranes from mitochondria, microvilli, ER, Golgi, and tubule nuclei were sometimes undulated, concentric, or fragmented when in contiguity with DLs. DLs interacted with microvilli and mitochondria at sites of pinocytotic vesicle formation (Fig. 16). DLs in contact with ER displayed vesicles on the phospholipid layer, but it is unclear if vesicles were forming and pinching off into the surrounding cytoplasm or if they were coming from the cytoplasm and attaching onto DL exteriors (Fig. 17). The greatest numbers of Golgi bodies were

seen when found with DLs, although some appeared to be fragmented (Fig. 18). Finally, DLs were also seen surrounding the nuclear envelope and entering the nucleoplasm of tubule nuclei (Fig. 19). In these instances, nuclear membranes were undulated and broken.

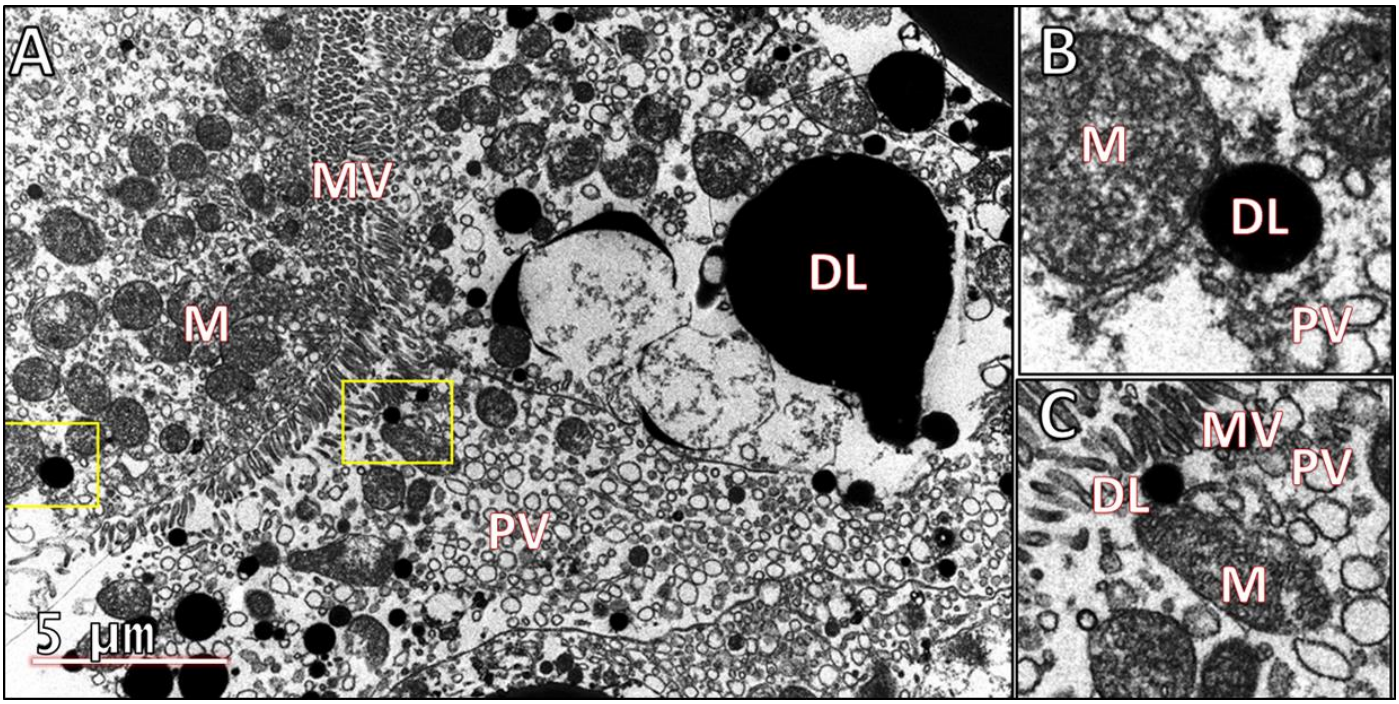

Figure 16. Cellular interaction between DLs and other organelles in the medial region of a bright light exposed sergestid photophore. A. Sites of pinocytotic vesicles are surrounded by substantial numbers of mitochondria and dense lipids. Yellow boxes represent insets (B, C). B. A dense lipid in direct contact with mitochondria. C. A dense lipid is in direct contact with microvilli and mitochondria (8000X) (*PV* pinocytotic vesicles, *MV* microvilli, *DL* dense lipid, *M* mitochondria).

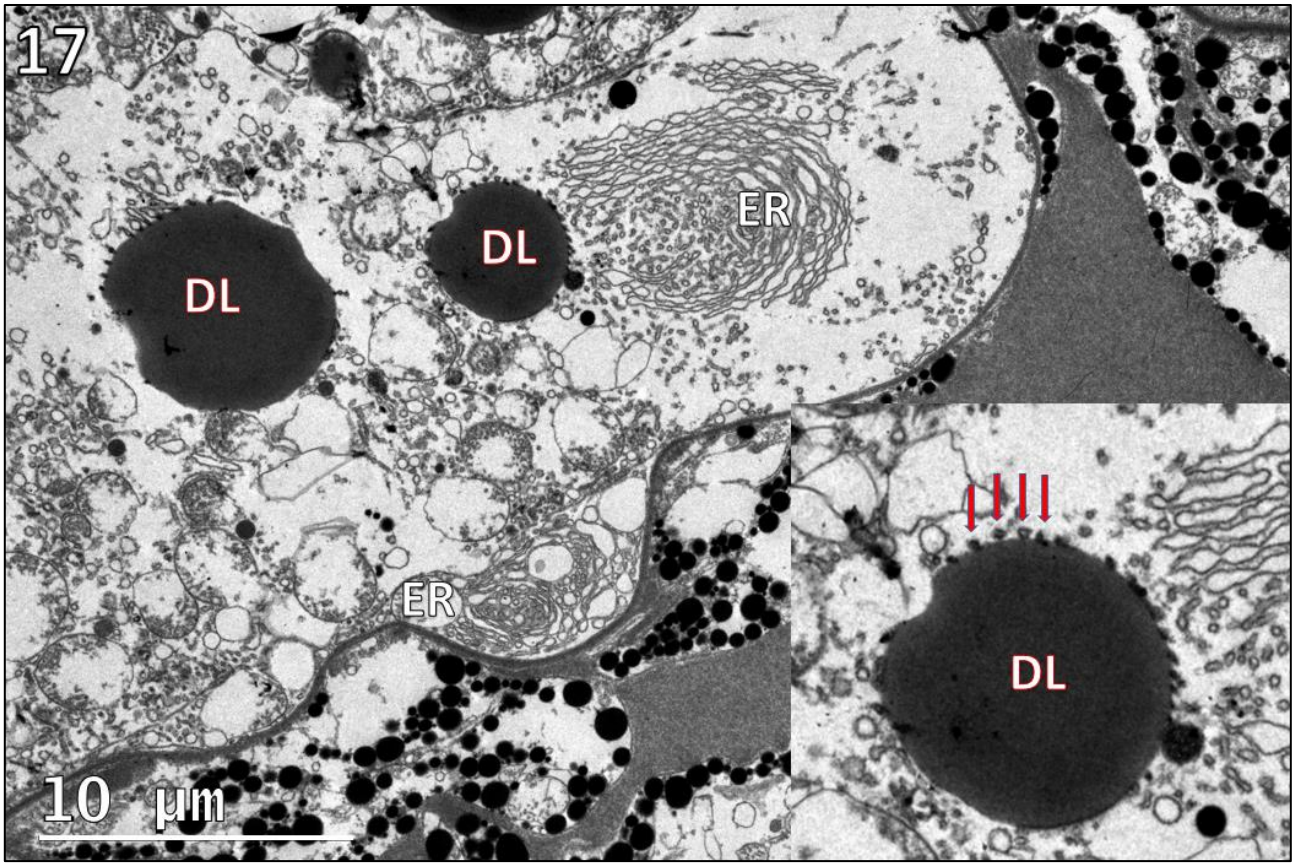

Figure 17. Distal Tubule region in a bright light exposed sergestid photophore. ER networks are arranged in membrane whorls (5,000X). Insert details vesicles seen on lipid exteriors (*ER* endoplasmic reticulum, *DL* electron dense lipids, *arrows* vesicles).

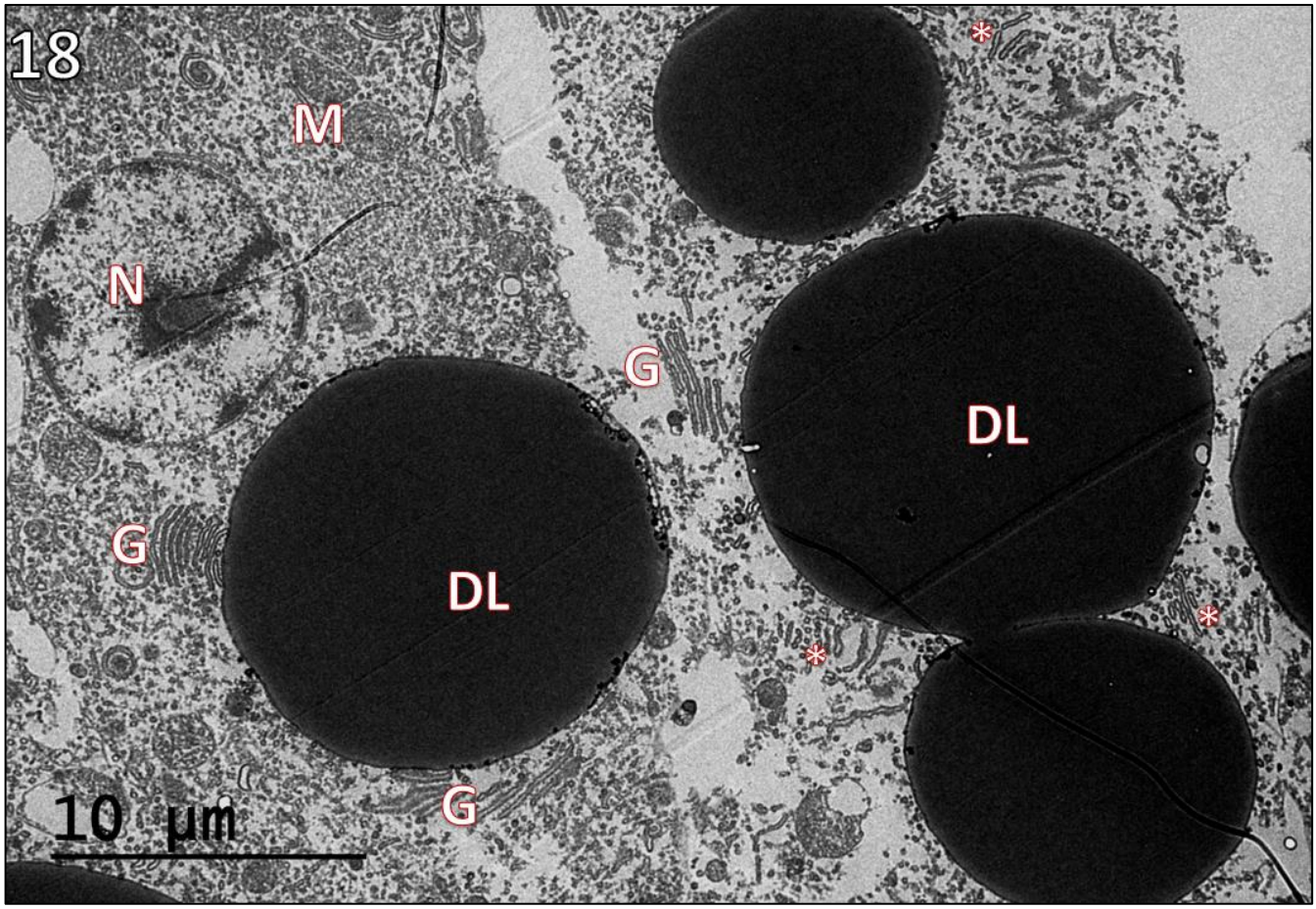

Figure 18. Tubule medial region in bright light exposed sergestid tissue. Golgi are in direct contact with large dense lipids. The surrounding cytoplasm contains some fragmented Golgi, mitochondria, and a nucleus (5,000X) (*G* Golgi, *M* mitochondria, *DL* dense lipid, *N* nucleus, *asterisk* fragmented Golgi).

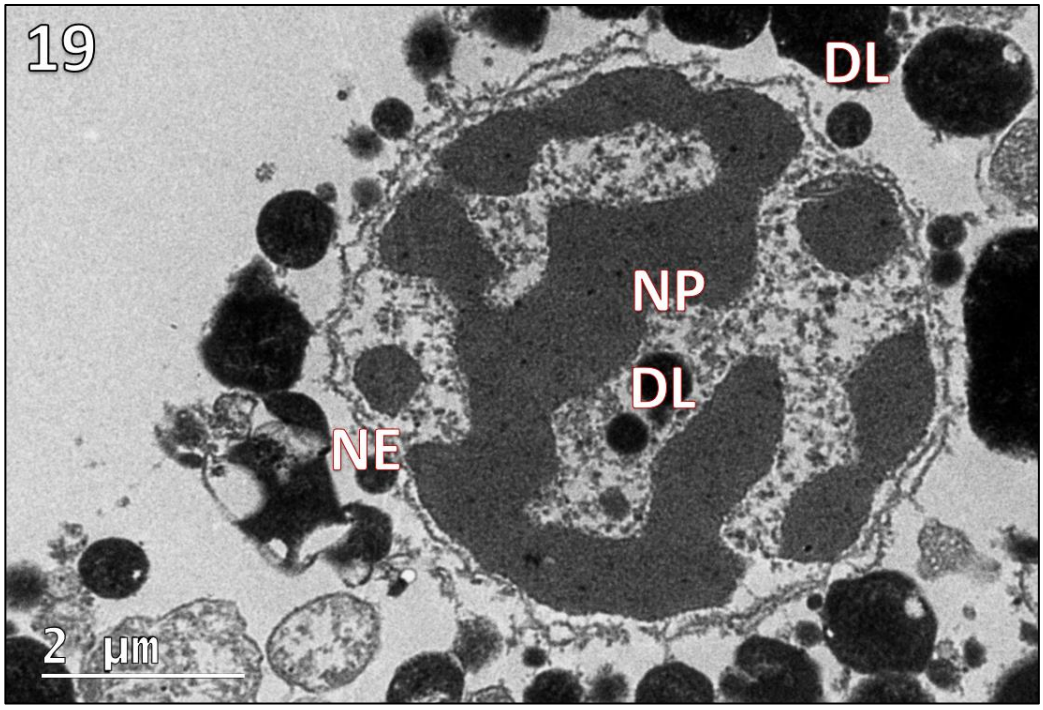

Figure 19. Tubule medial region in sergestid tissue exposed to bright light. A nucleus is surrounded by dense lipids, is in contact with dense lipids at the nuclear envelope and contains dense lipids in the nucleoplasm (20,000X). (*DL* dense lipid, *NE* nuclear envelope, *NP* nucleoplasm).

Lipids with an electron dense phospholipid layer and an electron lucent core (ELCs)

ELCs were found throughout translucent tubules or within the lumen and displayed a mostly ameboid and fluid morphology. ELC diameters in medial regions were significantly greater in bright light exposed tissue compared to the controls but diameters in distal regions were not significantly different between tissues (Table 6-7). More than 50% of ELC diameters in distal regions were larger than 1 µm. Despite distal ELC diameters not being significantly different between control and bright light exposed tissues in distal regions, they were smaller than 5 µm in the controls and could measure up to 15 µm in bright light exposed tissues.

Like DLs, ELCs could be seen surrounding tubule nuclei, but this interaction was never seen causing undulated or fragmented membranes and ELCs were never found within the nucleoplasm. However, in bright light exposed tissues, ELCs were often seen replacing dense tubule tissue (Fig. 20).

Table 7. Diameters (µm) of lipid droplets with an electron dense phospholipid layer and an electron lucent core (ELC) in control and bright light exposed medial tissue regions in the sergestids *P. armatus* and *A. sargassi*. Sample size (n) refers to the number of photophores that were analyzed.

ELCs - Medial Region				
T-test, t = 8.148, *p* < 0.001 *				
Dark (n=4)			**Bright (n=9)**	
Mean (µm) = 2.707			**Mean (µm) = 3.616**	
Diameter (µm)	*Percent (%)*		*Diameter (µm)*	*Percent (%)*
0.7-1.9	35.2		0.7-1.9	13.8
2.0-2.9	34.3		2.0-2.9	32.1
3.0-3.9	17.2		3.0-3.9	23.4
≥4.0	13.3		≥4.0	30.7

Table 8. Diameters (µm) of lipid droplets with an electron dense phospholipid layer and an electron lucent core (ELC) in control and bright light exposed distal tissue regions in the sergestids *P. armatus* and *A. sargassi*. Sample size (n) refers to the number of photophores that were analyzed.

ELCs - Distal Region				
Mann-Whitney Wilcoxon, W = 66063, *p* = 0.3163				
Dark (n=4)			**Bright (n=9)**	
Mean (µm) = 1.372			**Mean (µm) = 1.776**	
Diameter (µm)	*Percent (%)*		*Diameter (µm)*	*Percent (%)*
0.1-1.0	49.2		0.1-1.0	50.2
1.1-2.0	37.3		1.1-2.0	22.9
2.1-3.9	11.7		2.1-3.9	19.8
≥4.0	1.8		≥4.0	7.1

Electron Lucent Lipids (LLs)

LLs were exclusively located in distal tubule regions, had the largest measured lipid diameters in this study, and were significantly larger in tissues exposed to bright light than in control tissues (Table 9). LL diameters in the controls were less than or equal to 1.7 µm and nearly half of LL diameters in bright light exposures measured greater than 5 µm, with the largest diameter measuring at 25 µm.

Table 9. Diameters (µm) of electron lucent lipid (LL) droplets in control and bright light exposed distal regions in the sergestids *P. armatus* and *A. sargassi*. Sample size (n) refers to the number of photophores that were analyzed.

LLs - Distal Region				
Mann-Whitney Wilcoxon, W = 18987, *p* < 0.001 *				
Dark (n=4)			**Bright (n=9)**	
Mean (µm) = 0.830			**Mean (µm) = 5.880**	
Diameter (µm)	*Percent (%)*		*Diameter (µm)*	*Percent (%)*
0.1-0.6	37.8		0.1-0.6	0.8
0.7-1.0	46.4		0.7-1.0	2.3
1.1-1.6	15.3		1.1-1.6	11.4
≥1.6	0.5		≥1.6	85.5

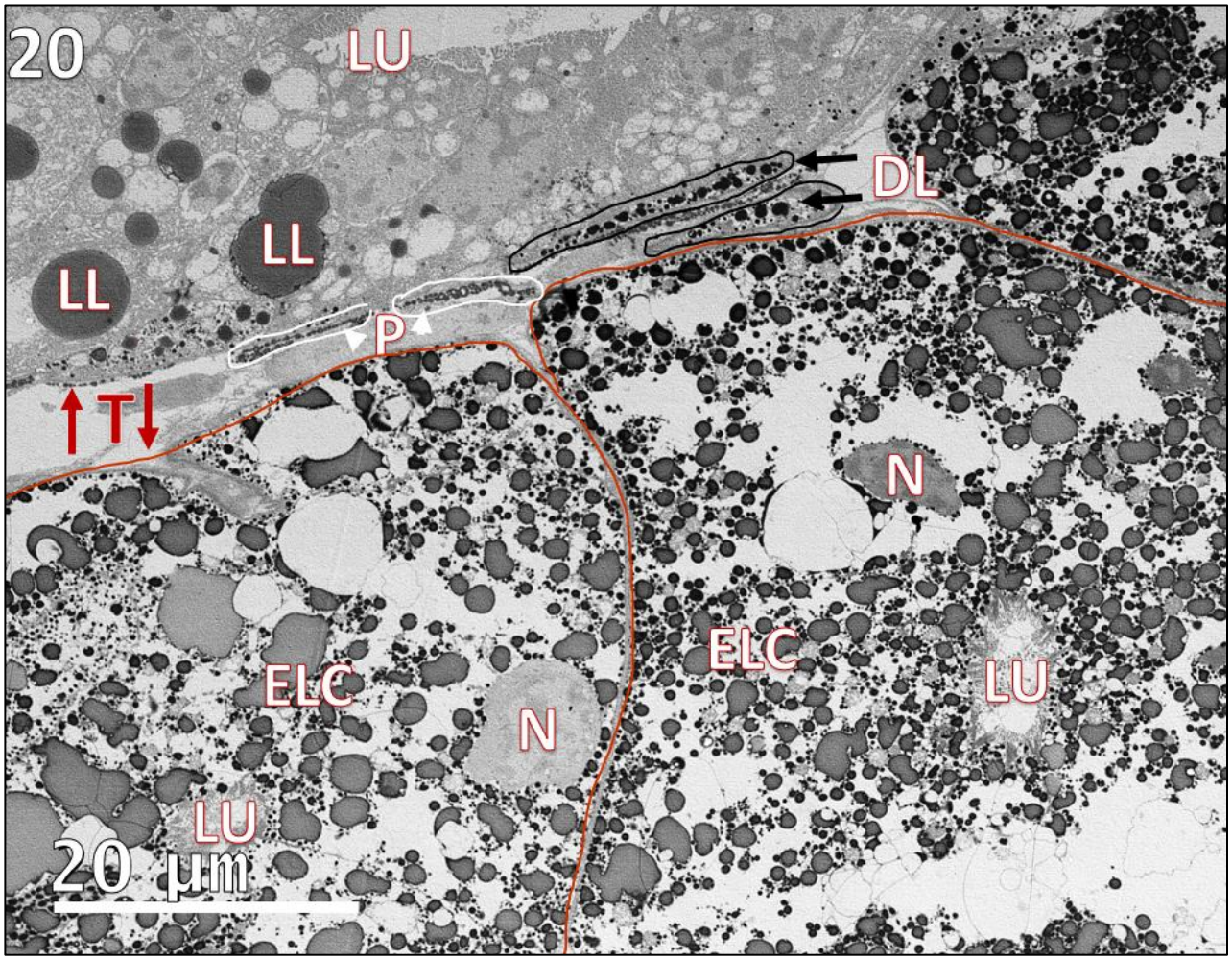

Figure 20. Outlined in red are two partial tubules from bright light exposed tissues that contained abundant lipids with an electron dense border and an electron lucent core in place of tubule tissue. Between these two tubules and an additional tubule at the top, there are pigments and dense lipids enclosed in a membrane structure. The nuclei in this image

may have belonged to epithelial cells if lipids are indeed replacing tubule tissue (1450X) (*ELC* lipids with an electron dense border and an electron lucent core, *T/red arrows* tubule, *P/white arrowheads* pigments, *DL/black arrows* dense lipids, *LL* electron lucent lipids, *N* nuclei, *LU* tubule lumen).

DISCUSSION

Evidence of Photophore Photosensitivity in Cuticular Pleopod Photophores

Basal Cytoplasm Area and Vacuolation

The basal cytoplasm and vacuolated areas were not significantly different between 30 minute control and dim light exposed tissues, but vacuolation significantly decreased in 60 minute dim light exposed tissues compared to the controls. It has been suggested that an increase in vacuolation during dark adaptation is a mechanism cells use to store materials needed during the onset of light (Eguchi and Waterman 1967). This may explain why vacuolated area decreased significantly under dim light adaptation. The basal cytoplasm and vacuolated areas were also not significantly different between the controls and bright light exposed tissues in 30 minute groups, while tissues exposed to bright light for 60 minutes had a significant increase in both areas compared to the controls. This is similar to what has been found in the retinular cytoplasm of *Libinia emarginata* (Eguchi and Waterman 1967), *Cirolana borealis* (Nilsson and Lindström 1983) and *Jasus edwardsii* (Meyer-Rochow and Tiang 1984), where vacuolation was greatest in tissues exposed to high-intensity light. Greater vacuolation in 60 minute bright light exposed tissues could indicate a depletion of resources or cellular stress. Exposure to bright light for 30 minutes did not cause any significant changes in the area of basal cytoplasm or vacuolated area, suggesting that 30 minutes is not enough time to disrupt the basal cytoplasm. This is important to note because in a study conducted on a sergestid shrimp, a minimum of 25 minutes of dim light exposure (of the same intensity used in the current study for "dim" light exposures) was required to reach a maximum bioluminescent output during counterillumination (Latz and Case 1992). An expansion of retinular cytoplasm has been documented in *Libinia emarginata* (Meyer-Rochow and Tiang 1984), but the authors do not discuss the relevance of this. Since the optical properties of the basal cytoplasm in photophores remains inconclusive, there is little discussion that can be

made at this time concerning the significance of the expansion of basal cytoplasm in tissues exposed to high-intensity light for 60 minutes.

Sheath cells

Sheath cell widths were significantly wider in all light exposed pleopod photophores than in the controls, regardless of exposure time. Sheath cells are composed of ommochrome and carotenoid pigments (Nowel *et al.* 1998), the same light absorbing pigments found in crustacean retinas. Migration of absorbing pigments is a well-known indicator of photosensitivity since they readily respond to changes in illumination during photoreceptor membrane turnover. In photoreceptors, pigments promptly respond to the onset of light to regulate visual input and protect photosensitive structures from photodamage, photo-oxidation, and lipid peroxidation (Kleinholz 1966; Eguchi and Waterman 1967; Frixione *et al.*1979; Stavenga 1989; Rao 1985; Meyer-Rochow and Eguchi 1986; Shelton *et al.* 1986; reviewed in Hallberg and Elofsson 1989; Dontsov *et al.* 1999; Meyer-Rochow 2001; reviewed in Gruszecki and Strzalka 2005). Absorbing pigment granules in photophores likely use microtubules as their main method of transportation into sheath cells as they were consistently seen in, or adjacent to, microtubules that border all photocyte nuclei. Microtubules are an accepted mode of transportation for several cellular structures, including photoreceptor pigments that must be mobile to adjust their proximity to photosensitive structures (Frixione *et al.* 1979; Schliwa and Euteneuer 1983; Meyer-Rochow and Eguchi 1986).

Electron lucent granules sometimes seen migrating from photocyte nuclei centers to exterior microtubule channels may indicate a possible pathway for pigment granule or photoprotein synthesis. The endoplasmic reticulum (ER) is crucial for pigment granule production and photoprotein synthesis (White and Sundeen 1967; Behrens and Krebs 1976; Itaya 1976; Meyer-Rochow and Lindström 1988; Schwemer 1989), which possibly explains its prominence across photocyte nuclei. The proteins and enzymes used to synthesize these components may vary between species as this study raises the possibility that oplophorids may possess several different types of screening pigments. This supposition is based on 1) different colored cuticular lenses, i.e. dark blue/violet in *Systellaspis debilis* and orange/red in *Janicella spinicauda* and 2) significantly larger and various sized pigment granules in *S. debilis*. Additionally, carotenoids are a diverse secretory granule with over 750 different types (reviewed

in Gruszecki and Strzalka 2005) that vary between antioxidant properties, photodamage protection, photon wavelength absorption, and cellular responses to light (Dontsov *et al.* 1999).

Lipids remained in proximity to sheath cells in all tissue types. Like carotenoids, lipids also display incredible diversity as over 1,000 different lipid types exist in eukaryotic cells (reviewed in Helle *et al.* 2013). In addition to the ER, lipids may also influence the production of pigments or photoproteins since lipids and carotenoids have morphological similarities which include a hydrophobic core surrounded by a phospholipid layer. The phospholipid layer can contain any number or assortment of proteins and enzymes (reviewed in Murphy and Vance 1999, Yang *et al.* 2012) which profoundly influences the synthesis and regulation of cellular membranes (reviewed in Gruszecki and Strzalka 2005, Schuldiner and Bohnert 2017). Identifying the impact of lipids on cellular processes, gene expression in lipids and pigments, and how proteins and enzymes are signaled to bind to phospholipid layers, may identify cellular pathways and contribute to the mechanism of counterillumination.

Golgi apparatus and Endoplasmic Reticulum (ER)

Golgi bodies were present in all tissue types. However, they became larger and more vesiculated as light intensity and exposure time increased. Also seen in all tissue types was ER that ranged in morphology from dense aggregations in the controls, to well-organized and well-defined in dim light exposed tissues, to swollen, fragmented, and loosely arranged in tissues exposed to bright light. Golgi and ER typically multiply during the onset of light in photoreceptors due to elevated levels of cellular activity and energy requirements (White and Sundeen 1967; Behrens and Krebs 1976; Itaya 1976; Blest and Day 1978; Blest *et al.* 1980; Nilsson and Lindström 1983; Shelton *et al.* 1986; Meyer-Rochow and Lindström 1988) to synthesize membranes, lipids (Thiam 2017), visual pigments, and proteins (Eguchi and Waterman 1967; Itaya 1976; Stowe 1980; Meyer-Rochow and Lindström 1988; Schwemer 1989; Meyer-Rochow 2001). However, vesiculated Golgi, fragmented ER, and hypertrophy of both organelles, as seen in 60 minute bright light exposed tissues, indicates disrupted cellular processes likely due to damaging levels of light exposure (Nilsson and Lindström 1983).

Evidence of Photophore Photosensitivity in Internal Organs of Pesta

Complex Cytoplasmic Organelles

Pigments outside the proximal region border external tubules through the medial region on their lateral sides but provided no evidence of change in pigment distribution or production in any tissue. Pigments were consistently arranged as unilateral strands that were not continuous along tubules, but rather sporadically placed. Contrary to pigment distributions, lipids were ubiquitous and diverse throughout tubules, suggesting that hepatic photophores may heavily depend on the production and distribution of lipids rather than pigments. While organs of Pesta did not show any indication of pigment migration, the presence of complex cytoplasmic organelles found only in bright light exposed tissues are morphologically identical to those formed during photoreceptor membrane turnover in visual systems, as discussed below.

Microvilli brush borders at tubule centers produced a substantial number of pinocytotic vesicles in bright light exposed tissues. These sites had an abundance of associated organelles such as mitochondria, dense lipids, and multivesicular bodies (MVBs). In crustacean retinas, exhausted photosensitive membranes composed of microvilli break down into pinocytotic vesicles, enter the retinular cytoplasm, and are taken up by MVBs via secondary endocytosis (Eguchi and Waterman 1976; Blest *et al.* 1980; Cronin and Goldsmith 1982). Photosensitive membranes continue to break down in MVBs with the help of degrative enzymes. Once MVBs acquire lysosomal enzymes derived from the ER and Golgi, multilamellar bodies (MLBs) form (Eguchi and Waterman 1967, 1976; Blest 1978; Blest *et al.* 1980; Hafner *et al.* 1980; Stowe 1980; Nilsson and Lindström 1983; Schraermeyer and Stieve 1991). The formation of MVBs and MLBs in bright light exposed hepatic photophores suggests that microvilli brush borders may contain photosensitive proteins.

Two types of multilamellar structures were seen in organs of Pesta: 1) small MLBs (0.5 μm), found in tubule cytoplasm in all tissues, that had less than four lamellar inclusions and a virtually empty center and 2) large MLBs (5–10 μm) that were exclusively seen in bright light exposed tissues, located in tubule centers between the diffuse lipid layer and paracrystalline platelets, contained several lamellar inclusions and electron lucent material. In photoreceptor retinas, MLBs further degrade photosensitive membranes using a series of enzymes (Blest *et al.* 1980), but evidence also suggests that they aid in photopigment synthesis, i.e. ommochromes

used in photoregeneration of metarhodopsins back to rhodopsins (Eguchi and Waterman 1967; Meyer-Rochow 1985; Schraermeyer and Stieve 1991), and energy storage (Meyer-Rochow 1985), which may explain the presence of MLBs in control tissues. In the late stages of degradative membrane turnover, additional enzymes in MLBs accumulate, condense, and undergo a series of electron dense reactions to form amorphous and dense bodies (Eguchi and Waterman 1967; Itaya 1976; Blest *et al.* 1980; Hafner *et al.* 1980; Stowe 1983; Doughtie and Rao 1984). This same sequence was found in internal photophores exposed to bright light. The true nature of these 'dense bodies' has been hypothesized to be either lipids or lipofuscin granules (Schraermeyer and Stieve 1991) but this has yet to be confirmed. The complex cytoplasmic organelles formed in bright light exposed internal organs are identical to those formed during photoreceptor membrane turnover and provide ultrastructural evidence of photosensitivity in internal photophores.

Photoreceptor Membrane Turnover

Photoreceptor membrane turnover is a widely accepted and extensively documented process in dim-light adapted arthropod compound eyes. This process is initiated by shifts in illumination and alters ultrastructure in several ways for the purpose of protecting, removing, and renewing photosensitive structures (Eguchi and Waterman 1967, 1976; White and Sundeen 1967; Behrens and Krebs 1976; Itaya 1976; Loew 1976; Blest and Day 1978; Blest *et al.* 1980; Hafner *et al.* 1980; Stowe 1981; Toh and Waterman 1982; Nilsson 1982; Nilsson and Lindström 1983; Stowe 1983; Doughtie and Rao 1984; Meyer-Rochow 1985; Shelton *et al.* 1985; Ball *et al.* 1986; Meyer-Rochow and Lindström 1988; Schwemer 1989; Stowe *et al.* 1990; Schraermeyer and Stieve 1991; Meyer-Rochow 2001). The turnover process is identified by the following sequential phases: 1) screening pigment migration, 2) pinocytotic vesicle formation via microvilli, Golgi, and ER (Novikoff *et al.* 1964; Blest *et al.* 1980), 3) secondary endocytosis of pinocytotic vesicles into multivesicular bodies (MVBs), 4) the accumulation of acid phosphatase in the late stages of MVBs to form secondary lysosomes, 5) such as combination bodies (CBs), and 6) multilamellar bodies (MLBs), that condense to form 7) amorphous bodies, 8) dense bodies, and finally, 9) lipids. This process naturally occurs in sync with an animal's circadian rhythm, but high-intensity light, i.e. light levels that exceed those in an animal's natural habitat, dramatically increases the formation of cytoplasmic organelles and disruption in the morphology

of photosensitive organelles. Cytoplasmic organelles representing those formed during photoreceptor membrane turnover were found in tissues exposed to bright light from anterolateral, posteromedial, and posterolateral organs of Pesta, but were not seen in the controls, and therefore provides further evidence of photosensitivity in internal photophores.

Golgi and Endoplasmic Reticulum (ER)

Golgi and ER were not prominent in the controls but were abundant in bright light exposed tissues. As previously mentioned, the increased quantities of Golgi and ER indicate high energy levels needed for cellular activity. Unlike cuticular photophores, these structures in hepatic photophores did not show signs of hypertrophy, vesiculation in Golgi, or fragmentation in the ER. However, ER in bright light exposed tissues commonly displayed membrane whorls which is an indicator of light-induced stress in photoreceptors (Behrens and Krebs 1976; Itaya 1976; Loew 1976; Blest and Day 1978; Nilsson and Lindström 1983; Meyer-Rochow 1985; Shelton *et al.* 1985) linked to imbalances between membrane destruction and renewal (Behrens and Krebs 1976; Stowe 1981), protein and lipid synthesis, and/or the disturbance of membrane precursors or lysosome functions (Hafner *et al.* 1980). Additionally, these structures were always associated with dense lipids.

Lipids

Lipids were the most common and diverse organelle found throughout tubule regions in all tissue types. Bright light exposed tissues resulted in significantly larger lipid diameters (with the exception of ELCs from distal regions), an increase in the number and size of ER and Golgi bodies, and widespread lipid to organelle contact. Lipid diversity would allow for a greater refractive index of light (Meyer 1979) which may be enhanced by increasing lipid diameters during the onset of light (Johnsen and Widder 1999). DLs had extensive contact with organelles including, but not limited to, mitochondria, microvilli, tubule nuclei, ER, Golgi, and other lipids. Lipid to organelle associations may be physiologically important for cellular activity, as recently suggested in several proteomic reviews in cellular biology (reviewed in Gao and Goodman 2015, Schuldiner and Bohnert 2017).

Golgi and ER organelles were only found when they were in contact with, or in proximity to, dense lipids. Since the ER is the main site of lipid body formation in plants, animals, and microorganisms due to the enzymes and proteins produced in ER systems (reviewed in Murphy and Vance 1999), lipids remain in contact with the ER throughout the lipid lifecycle, for the purpose of lipid and protein trafficking and to respond to ER stress and ER-associated degradation (reviewed in Gao and Goodman 2015). Additionally, the association of Golgi and lipids is a known indicator of increased cellular activity (reviewed in Schuldiner and Bohnert 2017; Thiam and Beller 2017). However, 60 minute exposures to bright light resulted in Golgi bodies that were partially fragmented and ER that displayed membrane whorls, morphologies that are both indicative of cellular stress (Nilsson and Lindström 1983) and may possibly indicate disturbances in either vesicular trafficking or the transport of cellular materials (reviewed in De Matteis and Rega 2015, Thiam and Beller 2017). Therefore, under exposure to high intensity light, ER and Golgi bodies may rely on lipids to carry out cellular processes.

Vesicular trafficking relies on communication between the ER and Golgi. If interrupted then a non-vesicular trafficking method is initiated to continue cellular functions, i.e. organelle communication, material exchange, etc. (reviewed in Schuldiner and Bohnert 2017). Non-vesicular trafficking uses lipids to establish contact sites with organelles to maintain cellular homeostasis, membrane biosynthesis, metabolism, and protein regulation (reviewed in Helle *et al.* 2013, De Matteis and Rega 2015, Gao and Goodman 2015, Thiam and Beller 2017) and may explain the vast contact between lipids and organelles in bright light exposed tissues.

In all tissues DLs and ELCs were seen in contact with nuclear envelopes, but DLs could also be found within the nucleoplasm. This may be a normal occurrence since 1) this was seen in all tissue types, 2) the ER is continuous with the nucleus of a cell and responsible for lipid-biogenesis, and 3) there are shared proteins between lipids and nuclei for nuclear-droplet communication and chromatin remodeling (reviewed in Gao and Goodman 2015, Welte 2015). DLs and ELCs were also in contact with mitochondria, a vital organelle for cellular function. It was recently discovered that despite the ability of mitochondria to synthesize most of its own proteins, some are only obtained through contact with the ER, vacuoles, or lipids (reviewed in Lahiri *et al.* 2016). Mitochondria may also be capable of transferring lipids to other organelles, such as plasma membranes, Golgi, and melanosomes, and be used as an additional source of non-vesicular trafficking.

Non-vesicular trafficking is driven by the enzymes and proteins in the phospholipid monolayer of lipids which directly influence 1) lipid synthesis, morphology, and size, 2) energy storage, 3) organelle degradation (including neutral lipid degradation), 4) the removal of inclusion bodies, 5) organelle transport, 6) organelle associations, 7) cellular and lipid metabolism, and 8) membrane synthesis (reviewed in Walther and Farese Jr. 2012, Yang *et al.* 2012, Schuldiner and Bohnert 2017). Microvilli membranes in crustacean rhabdoms are composed of photopigment and phospholipids (Eguchi and Waterman 1976; Meyer-Rochow and Eguchi 1984; Meyer-Rochow 2001). When these components are stimulated by photons, they enable vision through interactions with proteins, such as G-proteins, and ion channels, especially Ca^{2+}-ions that are stored in visual pigments (Meyer-Rochow 2001; reviewed in Kingston and Cronin 2016). The phospholipid monolayer in lipids contains enzymes and up to 160 different types of proteins (Bartz *et al.* 2007) that influence lipid to organelle contact (reviewed in Walther and Farese Jr. 2012) and may suggest that lipids contribute to a number of cellular processes in hepatic photophores.

The proposed role of lipids in photoreceptor membrane turnover has typically been that of membrane breakdown via lipid peroxidation (Blest *et al.* 1980; Doughtie and Rao 1984), but Kashiwagi *et al.* (1997) determined that the fatty acids most likely to cause lipid peroxidation were reduced within the first two hours of light exposure and were not responsible for long-term damage to photosensitive membranes. New hypotheses consider lipid peroxidation in photoreceptors to be a complex process influenced by a combination of factors such as the number of visual proteins (G-proteins), Ca^{2+} ions, and phospholipid composition in membrane-bound organelles (Meyer-Rochow *et al.* 2000). Additionally, current biological research indicates that lipids are crucial components for cellular activity (reviewed in Schuldiner and Bohnert 2017), and therefore should be further investigated in autogenic photophores to better develop our understanding of the mechanisms of counterillumination. While lipid diameters in dense lipids (DLs), lipids with an electron dense phospholipid layer and an electron lucent core (ELCs), and electron lucent lipids (LLs) were significantly larger in tubule regions in tissues exposed to bright light, no conclusions can be drawn about these results without confirming the identity of proteins in the lipid membrane or of amino acids contained in the hydrophobic core. Although, most of the evidence pertaining to the effects of light on lipids in visual systems

remains inconclusive, the interaction of lipids with several organelles in organs of Pesta suggests that lipids play an important role in the function of autogenic photophores.

CONCLUSION

In both *Janicella spinicauda* and *Systellaspis debilis*, basal and vacuolated basal cytoplasm areas and sheath cell widths significantly increased in size in response to bright light exposure for 60 minutes. In addition, Golgi bodies and the endoplasmic reticulum increased in number and size with increasing light intensity and exposure times, but at the greatest light intensity and exposure time, Golgi became severely vesiculated and the endoplasmic reticulum was swollen and fragmented. These findings in Oplophoridae species provides strong ultrastructural evidence of light sensitivity in cuticular pleopod photophores. The presence of complex cytoplasmic organelles in bright light exposed hepatic photophores (organs of Pesta) in two *Sergestidae* species, *Parasergestes armatus* and *Allosergestes sargassi*, presents strong evidence of photosensitive tissue since organelles were identical to those formed at the onset of light during photoreceptor membrane turnover. The results of this study also indicate that organs of Pesta may rely on lipids for several cellular processes during light exposure, as the lipids moved into direct contact with several key organelles (microvilli, mitochondria, endoplasmic reticulum, Golgi apparatus, and tubule nuclei) during the onset of light. Further information on the mechanisms of counterillumination may reside in the formation, transportation, function, and organelle association of lipids as this structure was extensively found in all photophores investigated in this study.

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