Vision in Deep-sea Oegopsid Squids

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Abstract

Squids of the order Oegopsida are a lineage of coleoid cephalopods with over 300 species that often inhabit the light-limited deep sea (>200m deep). Despite their dim surrounding environments, oegopsids use vision as their primary sense to navigate and possess camera-type eyes that evolved convergently with vertebrates. However, oegopsid vision remains understudied when compared to near-shore and shallow water cephalopod species. Specifically, there is little information available on the basic anatomy and functions of their retinas. Therefore, this thesis aimed to increase understanding of the oegopsid retina and determine if any correlations exist between any findings and the life-histories and behaviours of the studied species. Overall, there were four main aims of this study: 1) Investigate the retinal anatomy of oegopsids and identify any specialisations that may be beneficial for vision in the deep sea; 2) to determine whether screening pigment migrates in oegopsids despite many species living in relatively dark conditions; 3) measure the spectral sensitivity curves of the retinal visual pigments and determine whether any correlations exist between those characteristics and known behaviours of the studied species; and 4) sequence the rhodopsin gene of the studied species and create a rhodopsin phylogenetic tree to identify any traits that may drive its spectral sensitivity specialisation. The results revealed several trends that suggest that certain regions of their retinas typically contain longer photoreceptors that may aid individuals to recognise prey, predators and conspecifics. The photoreceptor lengths observed in adult oegopsids, relative to age-related increased length previously reported in juveniles, suggest a possible slowing or cessation of photoreceptor growth during adulthood. Furthermore, it was experimentally shown that screening pigment within the retina does migrate to the distal ends of the photoreceptors in oegopsids, a feature that reduces the amount of light that contacts the retina, which prevents photoreceptors from becoming oversaturated in bright ambient light conditions. Finally, by characterising the spectral sensitivity curves of the retinal visual pigments and sequencing the opsin gene, the absorbance profiles obtained from retinal pigment extracts suggest differences among species that may be associated with bioluminescent capability. Specifically, the spectral sensitivity curves of *Histioteuthis miranda, Taningia* spp., and *Mesonychoteuthis hamiltoni* – all species that are bioluminescent – exhibited broader sensitivity curves, potentially indicating increased sensitivity to additional wavelengths of light when compared to non-bioluminescent species. However, because cephalopod rhodopsin and metarhodopsin possess overlapping absorbance spectra, contributions from both photopigment states cannot be completely excluded. Consequently, the reported absorbance characteristics should be interpreted as estimates of visual pigment spectral sensitivity rather than definitive measurements of rhodopsin alone. Overall, this thesis showcases the complexity of oegopsid vision, and that additional research is required to fully comprehend the mechanisms that drive their success as voracious predators in light limited deep-sea environments.

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Attestation of Authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor used artificial intelligence tools or generative artificial intelligence tools (unless it is clearly stated, and referenced, along with the purpose of use), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

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Co-Authored Works

STUDENT AND SUPERVISOR APPROVALS

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Chapter 2 in this thesis has been prepared for publication in a peer-reviewed journal.

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Intellectual Property Rights

There are no intellectual property rights associated with this thesis.

Ethics Approval

Collection of all material used in this thesis was carried out under existing permits and ethical approvals held by the National Institute for Water and Atmospheric Research, Ltd (NIWA) (Chapters 2 & 4) and the Monterey Bay Aquarium Research Institute (MBAR) (Chapter 3).

Confidential Material

There is no confidential material associated with this thesis.

Chapter 1 – General Introduction

1.1 Cephalopod Evolution and Biodiversity

For a half-billion years cephalopods have evolved in competition with fishes to inhabit a variety of marine environments from nearshore coral reefs to deep-sea hydrothermal vent communities (Doubleday et al., 2016; González et al., 1998; Packard, 1972). The first cephalopods appear in the fossil record about 530 million years ago, having likely evolved from a monoplacophoran-like mollusc (Kröger et al., 2011). They can be distinguished from their ancestors by their external shells that have been modified into a chambered buoyancy apparatus to aid in locomotion. Otherwise, the cephalopod form is generally defined by a bilateral symmetry, an unsegmented mantle, a funnel for propulsion, and the direct attachment of a head to many tentacles and/or arms, which have been modified from the ancestral molluscan foot, surrounding a beaked mouth. Indeed, the etymological origin of cephalopod is derived from the Greek words kephalé́ (cephal) and poús (pod), which mean head and foot, respectively. Alongside other marine molluscan groups, cephalopods emerged during the Cambrian Explosion and radiated into five lineages (Ammonoidea, Orthoceratoidea, Belemnoidea, Nautiloidea, and Coleoidia) of which only two remain extant, nautiloids and coleoids.

Nautiloids are reminiscent of ancient cephalopod groups that originated in the Late Cambrian and are characterised by their buoyant external shells used for vertical migrations (Dunstan et al., 2011). At the height of nautiloid prevalence, the fossil record depicts a diverse group. Today, fewer than 10 species exist spread over two genera (*Nautilus* and *Allonautilus*). More primitive in anatomy compared to coleoids, nautiloids are visually guided by simple pit eyes that generate images similar to pinhole cameras, but more heavily rely on their numerous tentacles for chemoreception and navigation (Basil et al., 2000). Due to the limitations of their shells' buoyancy, nautiloids typically reside within the water column between 100-700 m deep.

The first coleoids arose about 330 million years ago and obviously differed from nautiloids by their shells that were either internalised or lost entirely. The latter is true for most members of the superorder Octopodiformes, which consists of the shell-less octopods (Order: Octopoda) and a single extant vampyroteuthid species that retains a modified internal shell, or gladius (Order Vampyromorphida, also known by the misnomer, vampire squid). While octopods and vampyromorphs share a common feature of eight arms, vampyromorphs have an additional pair of unique feeding filaments. The filaments are useful for their languid lifestyle in the deep pelagic aphotic zone where they feed on marine snow and zooplankton with the additional aid of bioluminescent photophores to attract prey (Hoving & Robison, 2012). They are also known for inverting their webbed arms over their head for protection when threatened. In contrast, octopuses are typically demersal whether residing in nearshore or deep-sea benthic ecosystems, although certain, mostly gelatinous, groups do inhabit the pelagic zone (Alejo-Plata et al., 2019; Quetglas et al., 2013; Rosa et al., 2019; Voight, 2000). Many octopod species are notable for their unique cognitive abilities, which are likely at least partially derived from the need to navigate complex coral reef ecosystems primarily using chemotactile exploration (Kang et al., 2023; Maselli et al., 2020).

However, octopuses, like most coleoids, have acute vision that may aid in controlling one of their most striking features, their rapidly adaptive colour changing chromatophores.

Finally, the superorder Decapodiformes comprises all remaining orders including the ram's horn squid (Order: Spirulida), cuttlefishes (Order: Sepiida), bobtail squids (Order: Sepiolida), pygmy squids (Order: Idiosepida), bathyteuthoids (Order: Bathyteuthida), the commercially important shallow-water myopsids (Order: Myopsida) and deep-sea oegopsids (Order: Oegopsida). Decapodiformes is believed to have split from other dibranchiates about 250 million years ago when the fourth arm-pairs of their cephalopod ancestors evolved into feeding tentacles (Kröger et al., 2011), which is a common feature among the seven orders.

Focusing on the most abundant and species rich orders within Decapodiformes, cuttlefishes have an internalised modified shell called the sepion (or 'cuttlebone') within the mantle, which supports buoyancy and locomotion. They are typically coastal species that occasionally inhabit depths of up to 200 m and are located along most coasts except along the Americas and New Zealand (Chembian & Mathew, 2011). Bobtail squids are relatively small (generally, <10 cm total length) and inhabit benthic communities from coastal ecosystems down to 1600 m deep (P. Boyle & Rodhouse, 2005; Jereb & Roper, 2005). Many species are nocturnal, burying in the substrate during the day then hunting at night, often aided with bioluminescent countershading provided by symbiotic bacteria (Jones & Nishiguchi, 2004). Therefore, bobtails tend to prefer muddy or sandy substrate (Anderson & Mather, 1996).

Often referred to as the "true squids" (perhaps incorrectly) myopsids and oegopsids can be found in all major marine ecosystems and tend to be fast-moving predators. About 350 species are known across the two groups, with potentially dozens left to be formally described. One key feature that differentiates these two orders is the presence/absence of a cornea (pseudocornea), an external layer that protects the eye (Young et al., 1998). The myopsids, which possess a cornea, include over 50 species found in shelf-water habitats. Oegopsida is the more diverse order with over 300 species that inhabit oceanic and neritic waters; their eyes have no cornea and are therefore in direct contact with the surrounding water.

For over 500 million years cephalopods have been fierce competitors for marine resources and have successfully occupied a wide range of ecological oceanic niches. Among cephalopods, squids are the most important for human fisheries due to their abundance and appear to be ecologically significant by maintaining trophic assemblages and contributing to carbon sequestration in marine ecosystems (Arkhipkin et al., 2015; Hoving et al., 2017; Navarro et al., 2013). Due to their ecological and economic importance, their unique morphology, and their connection to the "mysterious" deep sea, squids have broached the zeitgeist of pop culture.

1.2 Squid Ecology

Squids have long captured human imagination. Norse myths told of the gargantuan Kraken that terrorised sailors off the coast of Norway. *20,000 Leagues Under*

the Sea by Jules Verne concludes with its protagonists coming under siege by a giant squid. Even in modern media, a giant squid-like creature attacks New York in the graphic novel *Watchmen.* Though merely folklore and science fiction, these exaggerations are ingrained with truth. The giant squid (*Architeuthis dux*) and colossal squid (*Mesonychoteuthis hamiltoni*) both exhibit deep-sea gigantism. However, the term "squid" is not well defined and thus squids are a diverse polyphyletic clade that include groups like the pygmy squids, with one species maturing at just 15-mm mantle length (Nishiguchi et al., 2014). The diversity highlighted here has contributed to squids occupying a wide variety of marine ecosystems. Though their populations are difficult to measure, squid make up a significant amount of the biomass in marine ecosystems (Amaratunga & Caddy, 1983). This is exemplified by squid fisheries that can serve as a proxy for measuring squid abundance over the decades, and which generally target the more commercially viable myopsid and oegopsid squids.

For the past 60 years, commercial cephalopod catches have surged 10-fold reaching nearly 5 million tonnes at their apex in 2014 (FAO, 2020). Squid catches in particular have increased substantially (Moustahfid et al., 2021). Though catches have undoubtedly risen due to improved fishing technology and heightened efforts, Doubleday et al. (2016) also calculated using fisheries independent time-series survey data, that squid populations have grown across all their habitats. This is most likely due to anthropogenic effects like global warming and the drastic reduction of fish as predators and resource competitors due to fishing pressure (Caddy & Rodhouse, 1998; Field et al., 2007; Piatkowski et al., 2001; Rodhouse et al., 2014). Oceanic warming may accelerate the growth of certain species, as seen in *Loligo forbesii*, which are found to be three times larger at 90 days post-hatching when raised in the laboratory and warmer water (Forsythe & Hanlon, 1989). Additionally, rising sea surface temperatures (SST) can shift their migration patterns to occur earlier (Sims et al., 2001). Changes to SST also favour increased hatching ratios in *Illex argentinus* (Waluda et al., 1999). These benefits from environmental changes paired with their short lifespans and high fecundity, enables squids to utilise niches vacated by overfished teleost species (Balguerías et al., 2000; Caddy & Rodhouse, 1998; Jackson & O'dor, 2001). These trends suggest the ecological importance of squids in marine ecosystems will continue to grow, thus making them a priority for studying and understanding their ecological functions.

Squids show rapid growth during their short lifespans (Arkhipkin & Roa-Ureta, 2005; Jackson, 1994) maintained by their voracious consumption of fish, crustaceans, zooplankton, and other squids (Collins et al., 1994; Kear, 1992; Wangvoralak et al., 2011). They also serve as a substantial food source for fishes, seabirds, and marine mammals (Clarke, 1996; Croxall & Prince, 1996; Klages, 1996; Smale, 1996). Consequently, squids tend to occupy mid to upper positions within trophic assemblages (Coll et al., 2013), and since they inhabit many oceanic ecosystems (Arkhipkin et al., 2015; Rosa et al., 2019), they play a significant role in transferring energy between trophic levels and marine communities. For example, aggregations of post-spawning senescent adults create large squid falls that may contribute substantial nutrients to benthic communities (Hoving et al., 2017). Despite their relevance in maintaining ecosystem stability through nutrient transport, and their upward population trends, squids remain understudied, especially deep-sea oegopsids.

The occurrence of squid species in pelagic or deep-sea communities makes them difficult to sample and study; research expeditions to these remote locations are expensive and relatively rare compared with research in other marine habitats. As a result, basic ecological information required to understand how squid influence marine ecosystems remains unknown for many species. For instance, accurately placing species within taxonomic groups is a priority; many undiscovered species remain elusive while some 'known' taxonomic groups (and teuthofaunal assemblages of certain geographic regions) require revision (Braid et al., 2014, 2017; Braid & Bolstad, 2019; Fernandez-Álvarez et al., 2020). Additionally, many reproduction strategies including mating and spawning tactics remain a mystery (Murai et al., 2021). Many squid have multiple small paralarval stages that are difficult to match to their juvenile, sub adult, or adult forms, and can be dispersed across hundreds of kilometres (Evans & Bolstad, 2015; Robin et al., 2014). Determining the integration of each ontological stage and trophic assemblage is necessary to understand a species' broader impact on its ecosystem. For this process, gut analysis is helpful, but not always obtainable (especially for paralarvae).

With so much left to be discovered regarding the role of squid in marine ecosystems it is prudent to study any factors that may give insight into the ecological niche and behaviours of squids, especially for rare deep-sea species. One such understudied area of research that can give insight into some of the missing ecological information on deep-sea squids is their vision. However, before reviewing squid vision and visual ecology, it is important to understand the physical properties of light as it interacts with water, its influence on aquatic ecosystems, and how marine fauna react to varying light conditions.

1.3 Light in the Ocean

Ambient light conditions drive the largest migration of biomass on Earth each day in the world's oceans (Brierley, 2014). Daily, at dusk, zooplankton migrate to near-surface waters to feed on phytoplankton, hidden from potential predators by cover of darkness, though some migration does happen in reverse (Cohen & Forward, 2016). This demonstrates the significance of light in marine ecosystem dynamics and the influence it has on animal behaviour. Light is so important that one way to define ocean strata is by light availability.

Light conditions within the ocean follow consistent and predictable patterns with depth. During daylight hours, the bright euphotic zone (or 'sunlight zone') encompasses the top 200 m of the ocean (on average worldwide). Here, all wavelengths of light are present but attenuate at varying depths according to the frequency and wavelength of the light (Denton, 1990). Attenuation is the process by which light is absorbed and scattered by water. Wavelengths with high energy are more easily absorbed by water molecules and thus violet and ultraviolet light quickly attenuate in the first 20-30 m of oceanic depth. Conversely, light with long wavelengths, such as yellow, orange, red and infrared light, are more likely to collide with water molecules, scattering the light into unpredictable directions, making the light's journey to deeper water improbable as the light is eventually absorbed while still within shallow water. This is why many deep-sea creatures are reddish in color; since there's no red light, being red is functionally equal

to being black and essentially invisible, in line with the principle – “it is better to be red than dead.”

Past 200 m the euphotic zone gives way to the dysphotic zone (or ‘twilight zone’). Here, only blue-green light penetrates, giving the ocean its unique color. That light typically has a wavelength of about 480 nm, which can penetrate down to 1000 m deep (Johnsen, 2012). The conditions generated by light in the twilight zone drive many of the behaviours and unique adaptations seen there. For example, many species have ventral photophores that counterilluminate and disrupt any silhouettes created by the downwelling light as a mechanism to prevent potential predators from identifying them against the background (Young et al., 1979, 1980). The counterillumination light created by the photophores is a product of bioluminescence, a chemical reaction between a light emitting molecule (luciferin) that is oxidised by either an enzyme (luciferase) or a photoprotein. In response, some predators have yellow lenses that help to discriminate the blue bioluminescent photophores against the downwelling blue-green light (Thomas et al., 2017). It is an evolutionary arms race even in the deep sea.

Deeper still, beyond 1000 m is the aphotic zone (or ‘midnight’ zone), where no vestiges of sunlight can reach. The ocean here would be devoid of light if not for bioluminescence. Some estimates suggest that 79% of deep-sea macroscopic pelagic marine life are bioluminescent (Martini & Haddock, 2017), with most organisms generating their own luciferase to oxidise a luciferin that can be self-created or acquired through predation (Haddock et al., 2010). Alternatively, bioluminescent bacteria may form symbiotic relationships with larger organisms and reside within a light organ (photophore) as seen in bobtail squids (Jones & Nishiguchi, 2004; Osborn et al., 2009). Bioluminescence can be displayed in an array of colors including green (Kubodera et al., 2007; Osborn et al., 2009), blue (Haddock & Case, 1999), yellow (Francis et al., 2014), and red (Herring & Cope, 2005; Kenaley et al., 2014), each with their own evolutionary history, and may be used for hunting, predator avoidance, and intraspecific communication (Haddock et al., 2010).

1.4 Sensing in the Ocean and the Evolution of Eyes

The Cambrian Explosion sparked the greatest diversification of metazoans ever, resulting in the evolution of nearly all major animal phyla over 500 million years ago (Marshall, 2006). An evolutionary arms race ensued driven by competition for limited resources and predator avoidance (Sperling et al., 2013). This era of rapid marine evolution facilitated the creation of dominant sensory modalities still used by organisms today.

One possible scenario for the evolution of the earliest sensory modalities is the specialisation of a single cell type that diversified many times over to produce sister cells capable of mechanoreception, chemoreception, and photoreception. This scenario is supported by the similar components incorporated into across the three sensory transduction cascades (Schlosser, 2018). Nevertheless, the evolution of each modality significantly shaped the trajectory of animal behaviour and evolution. Mechanoreceptors can detect environmental stimuli such as gravity; some organisms use specialised organs (statocysts) to facilitate orientation in the pelagic zone’s three-

dimensional structure (Wiederhold et al., 1989). Mechanoreceptors are also used for detecting sound; cephalopods can use their statocysts to hear low-frequency acoustic particle motion (Kaifu et al., 2008; Mooney et al., 2010). Pressure can also be detectable; the lateral lines of fish and analogous structures in cephalopods perceive nearby hydrodynamic variations generated by predator and prey movements (Bleckmann, 2006; Budelmann & Bleckmann, 1988). Finally, haptic feedback can help manipulate food or inanimate objects (Brown, 2012; Scheel et al., 2018). Chemoreceptors allow users to navigate over great distances underwater. Chemicals readily dissolve in saltwater, giving organisms access to thousands of compounds distributed throughout their environments (Derby & Sorensen, 2008). Most are unspecialised metabolic products or pheromones that can be detected through olfactory or gustatory sense organs (Atema, 1988; Carr, 1988; Sorensen & Stacey, 1999). Sensing these chemicals allows organisms to navigate to breeding grounds (Johnstone et al., 2011; Ueda, 2014), recognise conspecifics (Wood et al., 2008; Schneider et al., 1999), track prey (Premke et al., 2003), and detect predators (Boal & Golden, 1999). Photoreception, however, has potentially had a great influence on evolution during the Cambrian Period.

Photoreception provides a critical source of sensory information for many metazoan lineages and became a prominent sense during the Cambrian Period. Increased oxygen levels supplied organisms with the necessary prerequisites for metazoan life to diversify (Jiang et al., 2022) but also contributed to atmospheric changes that potentially increased light availability. The “Light Switch” theory postulates the rapid growth in adaptations witnessed in fossil records during this time was also driven by the evolution of vision, which predators could use to identify prey and potential threats quickly; in response competitors evolved hard bodies for protection or to help burrow out of sight (Parker, 2011). The first true eyes evolved during these rapid environmental changes about 521 million years ago in trilobites, but the precursors to eyes originated millions of years prior.

The first photosensitive organs were eye spots, which consist of screening pigment within an open cup and a few photoreceptors to aid in detecting the shadows of nearby predators (Schnitzler et al., 2012). Later, the cup became increasingly invaginated, which allowed for better determination of directionality of any passing shadow, until a simple optic cup was formed with epithelial tissue to create a water filled cavity. Though simple, this eye design included the first retina and functions similarly to a pinhole camera, which can be found in extant *Nautilus* species (Muntz, 1991). Next came the development of the lens. First, a primitive refractive lens evolved which improved visual acuity, then a finely tuned lens with ciliary muscles to focus. In addition, an iris evolved from epithelial tissue that helps modulate the amount of light entering the eye through a pupil, and a cornea, which gave additional protection to the eye. Coleoids have this type of complex eye. Today, by their morphology eyes can be broken down into two major groups, chambered and compound eyes.

Both chambered and compound eyes are found in marine ecosystems, with seven of the eight major optic types represented. Compound eyes are exclusive to invertebrates and are comprised of many ommatidia, each unit with its own photoreceptor, lens, and cornea (Warrant & Locket, 2004). The simplest compound eye

is found in bivalve molluscs, which rely on shadows to produce images (Morton, 2008). Refracting compound eyes are found in crabs with their ommatidia appositionally assembled (Alkaladi & Zeil, 2014), and in krill with their ommatidia superpositionally assembled (Hallberg & Nilsson, 1983). Finally, shrimp and lobsters have superposition compound eyes that rely on reflection (Johnson et al., 2015).

Chambered eyes are present in vertebrates and invertebrates alike. They consist of a retina with ciliary or rhabdomeric photoreceptors and may include a single lens and cornea. Simple pit eyes are found in chambered nautiluses that rely on shadows to create images (Muntz, 1991). Chambered eyes with circular lenses that focus light through refraction to direct light can be found in most cephalopods and all fishes (Sweeney et al., 2007). Absent from marine environments are the semi-circular lensed chambered eyes of terrestrial vertebrates. Scallops possess the final major optic type, defined by chambered eyes with refracting concave mirrors for directing light towards the retina (Speiser & Johnsen, 2008). Of all these visual strategies, lensed chambered eyes are particularly intriguing because they have evolved independently multiple times (Serb & Eernisse, 2008).

In *On the Origin of Species,* Charles Darwin acknowledged "...that the eye with all its inimitable contrivances... could have been formed by natural selection, seems, I freely confess, absurd in the highest degree." Yet the narrative behind eye evolution becomes even more astonishing when it is considered that camera-type eyes emerged not just once in vertebrates, but a second time in cephalopods. Both lineages share common ancestry from over 600 million years ago and despite evolving independently, their eyes are strikingly similar, providing a superb example of convergent evolution (Kröger et al., 2011; Serb & Eernisse, 2008). In cephalopods the evolutionary pressure to develop sophisticated eyes that exceed the visual acuity of all other invertebrates and some vertebrates (Messenger, 1981; Serb & Eernisse, 2008) is, in part, derived from their direct competition for resources with fishes (Packard, 1972). These revelations have led to numerous studies on the ocular anatomy and visual capabilities of cephalopods to better understand how eye development and adaptations influence their behaviours and ecology.

1.5 Cephalopod Vision

While there are many similarities between the coleoid and vertebrate eye, considerable differences exist. Vertebrate eyes derive from extrusions of the central nervous system and neurectoderm, but cephalopod eyes develop from invaginations of the epidermis from the placode and placode lips during ontogenesis (Arnold, 1965). This process of eye formation begins early in development, as observed in *Doryteuthis pealeii* (Koenig et al., 2016). Most coleoid cephalopod eyes consist of a cornea, an iris delimiting a pupil, a nearly circular lens, a vitreous cavity, and photoreceptors that compose a retina.

If present, the cornea is the first structure light travels through. In cephalopods, it is separate from the rest of the eye and is able to regenerate quickly if damaged (~10 days) (Hanke & Kelber, 2020). This is a useful function to ensure the inner eye is constantly protected and maintains a smooth hydrodynamic surface to ease drag

(Dingerkus & Santoro, 1981; Talbot et al., 2012). The cornea has a dorsal opening allowing water to flow over the lens (Hanke & Kelber, 2020). Temperature and salinity maintain the refractive index of water between 1.392 and 1.343 (Quan & Fry, 1995; Temple, 2007), and therefore the cornea is not required to focus light as in vertebrate eyes (Talbot et al., 2012). Nipple-array-structured microprojections along the cornea increase cell surface area and help stabilise the mucous film that protects the eyes during foraging, hunting, or burrowing, and may also aid in cellular metabolic processes through gas exchange and nutrient acquisition (Talbot et al., 2012).

Light then passes through the pupil formed by the iris, which regulates the amount of incoming light reaching the retina by constricting or dilating around the lens. Froesch (1973) showed that the iris of *Octopus vulgaris* consists of 5 layers: the external epithelium, a chromatophore layer, the iridocyte layer, a layer of muscle strands and fibres, then the pigmented epithelium. Though these layers are not consistent throughout all species the basic arrangement remains the same. However, the irregular shapes of many coleoid pupils are striking. The W-shaped pupils of cuttlefishes help to even out the vertical light present in shallow waters, which improves the distribution of intensity across the retina (Mäthger et al., 2013). The shape also reduces scattering from direct sunlight to enhance visual acuity (Mäthger et al., 2013). In contrast, circular pupils allow the maximum amount of light and are frequently found in low-light environments. Squids have circular or crescent-shaped pupils with a reflective iris that facilitates passive camouflage (Holt et al., 2011). Octopus pupils typically remain horizontal regardless of the animal's body position, a behaviour facilitated by the statocysts, which help maintain proper eye alignment for effective visual processing (Boycott, 1960). This same mechanism is assumed to maintain body and eye orientation for many species, whether nearshore, deep-sea, or pelagic.

Coleoids refract the inbound light through their ellipsoidal lenses. The lenses are divided into two hemispherical segments, a posterior and anterior section. They meet at a septum along the equator (Budelmann, 1996). Both lens segments derive from specialised cells that excrete cytoplasmic processes that fuse (Arnold, 1967; West et al., 1995). The anterior portion is excreted from an outer ectodermal layer of lentogenic cells, while the posterior portion is excreted from an inner ectodermal layer (Jacob & Duncan, 1981; Sivak, 1991). This process produces a lens with a refractive index gradient, which may reduce spherical aberration (West et al., 1995). Another unique adaptation is the ability of the lens to accommodate ametropia by adjusting its distance relative to the retina thereby correcting focus (Land & Nilsson, 2012).

After refracting through the lens, light travels through the vitreous humour. The amount of research on the vitreous is limited, but Balazs et al. (1959) completed a biochemical analysis on *Doryteuthis pealeii*. He found that the vitreous cavity was filled with a low-viscosity liquid, rather than the common gel-like substance found in the eyes of other animals (i.e., amphibians, reptiles, mammals, and most birds) indicating collagen was not abundantly present. The study also showed the blood vessel system was distinct and envelops the vitreous, as shown by the reduced amount of dissolvable protein within the liquid medium.

Finally, the inbound light reaches the retina. Cephalopod retinas are everted with the distal ends of the photoreceptors directed anteriorly, thus being the first structure the light influx contacts. The exact opposite is true in vertebrates, where the inverted retina requires light to pass through multiple cell layers before stimulating phototransduction (Fröhlich, 1914). Another distinction between invertebrate and vertebrate retinas is the use of rhabdomeric opposed to ciliary photoreceptors, respectively. The rhabdomeres in cephalopods are the microvillar structure located at the distal end of the photoreceptor; four rhabdomeres form a square bundle with a central supporting cell which together comprise a rhabdom (Hanke & Kelber, 2020). A basal membrane separates the proximal and distal ends of the photoreceptors. Photoreceptor nuclei lie in the proximal end, while supporting cell nuclei lie distally, adjacent to the basal membrane (Hanke & Kelber, 2020; Young, 1960). In vertebrates, the inverted orientation of photoreceptors causes the brain-contacting nerves to pass over the retina before they converge at the optic disc, creating a blind spot. In contrast, the photoreceptor proximal segments in cephalopods directly innervate the optic lobe within the retinal nerve plexus (Hanke & Kelber, 2020).

Cephalopod vision follows the same principles of phototransduction that all visual organisms adhere to, converting light photons into chemical and electrical signals that enact a physiological response. This process is achieved in the rhabdomeric photoreceptors, which use rhodopsin, a photosensitive pigment (Hanke & Kelber, 2020; Hubbard & St George, 1957; Shichida & Imai, 1998; Yarfitz & Hurley, 1994). Rhodopsin has two components, the chromophore, 11-cis-retinal or often a hydroxylated or dehydroretinal derivative in squids (dependent upon the species), and the G-protein-coupled receptor (GPCR) protein, opsin (Mitchell & Swardfager, 2010). Upon absorption of a photon, the chromophore undergoes isomerisation from the cis to all-trans configuration, initiating a series of conformational changes that produce several photochemical intermediates before forming metarhodopsin (Hubbard & St George, 1957; Shichida & Imai, 1998). Unlike vertebrate visual systems, where metarhodopsin is rapidly degraded and regenerated through enzymatic pathways (Baumann, 1972), cephalopod metarhodopsin remains photosensitive and can be photoconverted back to rhodopsin through the absorption of light of appropriate wavelengths (Hara & Hara, 1967; Hubbard & St George, 1957). Consequently, cephalopod visual pigments exist within a photoreversible rhodopsin–metarhodopsin system. The relative proportions of rhodopsin, metarhodopsin, and the intermediary photoproducts reflect the recent light exposure and the dynamic photoconversion equilibrium, which has important implications for the interpretation of visual pigment absorbance measurements. Ultimately, once rhodopsin is photoactivated, it activates a heterotrimeric G-protein by catalysing nucleotide exchange, initiating a phospholipase C–mediated phototransduction cascade that eventually signals photic stimulation to optic lobe (Terakita et al., 1998). This pathway is used by many GPCRs thus implying that invertebrate rhodopsin is prototypical (Murakami & Kouyama, 2008). The rhodopsin molecule forms the photoreceptors' rhabdomeric structure via dimerisation between amino-terminal polypeptides of abutting microvilli membranes, contributing to their hexagonal organisation, as seen in *Todarodes pacificus* (Murakami & Kouyama, 2008).

The photoreceptor's microvilli within the rhabdomeres are orthogonally arranged, enabling polarised vision (Shashar et al., 2002).

Peculiarly, nearly all species of cephalopods studied to date have only one sensory opsin. Therefore, cephalopods appear to be colour-blind, even though they are known for their dynamic colour displays and can live in vibrant environments. Nevertheless, determining the spectral absorbance of their visual pigments can give insight into their ecology. Typically, the retinal pigments of deep-sea animals are attuned to absorbance in the blue spectra to match the downwelling light and/or bioluminescence of organisms ecologically significant to the species (Crescitelli, 1990). This holds true for cephalopods. The λ_{max} absorbance for opsins described in cephalopods typically lies between 480 and 500 nm (Bellingham et al., 1998; Hall et al., 1991; Hara-Nishimura et al., 1993; Morris et al., 1993; Ovchinnikov et al., 1988; Tong et al., 2009). A correlation between depth and peak spectral absorbance of their photopigments is apparent, with lower peak values associated with deeper depths (Messenger, 1981; Morris et al., 1993). Having the photopigment absorbance match the spectra of the downwelling light achieves greater visual sensitivity for species (Crescitelli, 1990). However, there are exceptions. Of note, three photopigments are found in *Watasenia scintillans*, which peak at 470, 484, and 500 nm (Matsui et al., 1988; Seidou et al., 1990). It is believed these photopigments are attuned to the colours of their complex bioluminescent displays.

Most cephalopod vision research has been produced studying octopuses or nearshore decapod species. The goal of this study is to characterise the retina of deep-sea oegopsid squids, for which little research has been conducted. However, Evans et al. (2015) reported that photoreceptors in the outer segments of the eyes in cranchiid squid (*Teuthowenia pellucida)* grew disproportionately rapidly, relative to overall body growth. This occurred despite eye diameter and mantle length maintaining a linear ratio. This accelerated photoreceptor growth coincided with the species' shift into deeper and darker waters. That is, as *T. pellucida* matured they descended into deeper mesopelagic zones, which may have been facilitated (or driven) by their accelerated photoreceptor growth. Photoreceptor length determines the amount of light able to activate phototransduction. Since the ventral photoreceptors (which absorb downwelling light) experienced accelerated growth, it is hypothesised this growth may trigger the squid to migrate deeper into the ocean as low-light environments become preferable.

Though many similarities exist amongst the retinal structures of many cephalopods, there is no entirely consistent morphology; critical differences occur in retinal composition that can correspond to visual adaptations that usually emerge from environmental pressures. An early discovery using histological techniques was the presence of pigment granules within the supporting cells of the cephalopod retina (Young, 1962). Identified as ommin (Butenandt, 1959), the pigment was determined to migrate distally once isomerised (Daw & Pearlman, 1974). Therefore, in bright ambient light conditions, this migration blocks intense light from oversaturating the photoreceptors (Young, 1963). Migration of screening pigment has been found in many nearshore and shallow pelagic species but is typically reduced in deep-sea oegopsid species maximising light reaching the retina (Chung & Marshall, 2017). However, pigment migration was identified in juveniles of the deep-sea squid *T. pellucida* (Evans

& Bolstad, 2015).The study reports their findings on juveniles when individuals are likely near surface waters and exposed to day/night light cycles. Therefore, further investigation is required on the relationship between screening pigment migration and deep-sea oegopsids to determine whether the mechanisms are conserved into adulthood.

Similar histological examinations of *Sepioteuthis lessoniana* and *Idiosepius notoides*, two coastal species, revealed retinal deformations that blur a large portion of their visual field (Chung & Marshall, 2014; Chung & Marshall, 2017). This blurred image, combined with a head bobbing technique, moves potential prey in and out of focus; that is, until the prey is within striking distance of the tentacles and the focus differential of the retinal bump no longer blurs the prey, signalling the individual to attack. Chung and Marshall (2017) also discovered a foveal pit within the retina of the deep-sea bathyteuthoid *Bathyteuthis abyssicola*. The fovea consists of an indentation within the retina that can accommodate long photoreceptors, thereby drastically increasing visual acuity over a restricted area. This enhanced visual field may be critical for finding mates or hunting. Another surprising anatomical anomaly was identified in *Abraliopsis falco*, which has a dual-layered inner segment within the retina. It is theorised that the dual-layer increases light sensitivity but decreases spatial resolution. This may be an acceptable trade-off for the squid and is a similar adaptation found in nocturnal or crepuscular insect species (Agi et al., 2014; Chung & Marshall, 2017; Land, 1981; Warrant & Locket, 2004). By histologically examining the eye of deep-sea species similar adaptations may be found. It can be difficult to differentiate objects in the open ocean due to a lack of visual references, especially while organisms are attempting to blend into the environment, so evolving adaptations like the foveal pit of *B. abyssicola,* the retinal deformations in *S. lessoniana* and *I. notoides*, or the dual-retinal layer of *A. falco,* would not be surprising.

Retinal topography reveals the arrangement and density of photoreceptors, providing insight into a species' visual orientation and how it may relate to their visual ecology. Research conducted on the retinal topography of three near-shore coleoid species, a cuttlefish (*Sepia plangon*), an octopus (*Octopus cynaea*), and a myopsid squid (*Sepioteuthis lessoniana*) revealed an area of highly dense photoreceptor cells arranged as a horizontal streak along the retina (Talbot & Marshall, 2010). This horizontal streak aligns with the region of highest visual acuity with the horizon, where approaching predators or prey are most likely to appear, an adaptation also observed in some species of fish (Collin & Pettigrew, 1988; Muntz, 1999). *Sepioteuthis lessoniana* had the least dense horizontal streak, which is most likely indicative of its life above the benthic environment and therefore a need to detect objects from all directions. Topographical studies on five decapod coleoids revealed the visual orientation of each species and allowed comparisons with their respective environments (Makino & Miyazaki, 2010). It was shown that in the two coastal species (*Euprymna morsei* and *S. lessoniana)* had high photoreceptor densities slightly dorsal to the retinal midline, indicating a downward visual orientation, an adaptation useful for hunting crustaceans on the seafloor. All three oceanic species studied (*Todarodes pacificus*, *Eucleoteuthis luminosa*, and *Thysanoteuthis rhombus*) had increased photoreceptor density in the ventral part of the retina. For *E. luminosa* the density was significantly greater ventrally, allowing

individuals of this species to direct their highest visual acuity upward, which likely enables detection of prey against downwelling light. The less exaggerated high-photoreceptor density of *T. pacificus* and *T. rhombus* in the ventral region of their retinas directs their gaze mostly forwards and is useful for detecting prey and predators in the mesopelagic environment. Topographically mapping the retina of other deep-sea squids can likewise show their highest visual acuity area and give insight into their hunting and predator avoidance strategies.

Since little research exists on the retinal anatomy of deep-sea squids and its effects on development, examining the adaptations of the retina in deep-sea lanternfish (Order: Myctophiformes) may give insight on the validity of this hypothesis. Vertebrates have camera-type chambered eyes like those of cephalopods; thus, myctophids make excellent comparison species. They inhabit the same environments, are subject to similar ecological pressures, and migrate deeper with ontological development. A study on the eye development of three species of lanternfishes found that during larval development cones located in the retina were progressively lost, and nearly completely absent in adult forms (Bozzano et al., 2007). Cones are responsible for colour vision in vertebrates, a visual property less necessary in the aphotic zone. Conversely, in vertebrates, rod photoreceptors allow for detection of dim light levels and can undergo gradual extension of the outer segments length to adapt to this reduced light level. The researchers interpreted the retinal compositional changes as an adaptive response to occupying deeper mesopelagic waters. Similar to observations in *T. pellucida,* lanternfish experienced rapid growth of the outer segments of the rod photoreceptors. This indicates an increased photosensitivity that captures more light in darker waters and possibly helps lanternfish to determine the appropriate depth to inhabit. This suggests photoreceptor outer segment or microvilli growth as a mechanism for downward migration into aphotic zones and may be an adaptation that has evolved independently many times in the ocean.

1.6 Rationale for this Study

Aotearoa New Zealand is an area with high oegopsid squid diversity with more than 120 species known to date. Due to fishing surveys conducted by organisations like the National Institute for Water and Atmospheric Research (NIWA) a wealth of fresh deep-sea squid samples are accessible for examination. Many of these species hold critical ecological and economical importance to Aotearoa New Zealand, such as *Nototodarus* spp. that support local fisheries. Since these surveys have been conducted for decades, numerous projects have investigated their diet and trophic interactions (Braid & Bolstad, 2014; Dunn, 2009; Phillips et al., 2001; Phillips et al., 2002; Phillips et al., 2003). This provides ecological context for the visual abilities of species this project plans to examine and characterise. Taking advantage of these resources will also give a unique opportunity to compare the ocular morphology and visual adaptations of closely and more distantly related taxa. Furthermore, as interest in deep-sea exploration increases it is valuable to understand how these animals visually interpret their environment to refine our abilities to observe them without causing long-term retinal damage and invasive monitoring.

Chapter 2 – Retinal Anatomy of Deep-sea Oegopsid Squids of Aotearoa New Zealand

2.1 Abstract

Oegopsid squids navigate the light-limited deep sea primarily through vision. To facilitate their visual navigation, squids have evolved camera-type eyes, which are strikingly similar to eyes found in vertebrates and are generally associated with high visual acuity. With over 300 oegopsid species worldwide, the retinal anatomy of many have not been investigated, even though recent research has discovered retinal specialisations in several species. Therefore, this study aimed to describe the retinal anatomy of eight oegopsid squids found within the exclusive economic zone of Aotearoa New Zealand. In particular, the lengths of the various photoreceptor segments were measured and compared among species and compared to known life history traits and behaviours, such as the depth a species inhabits. Intriguingly, our results did not show any obvious unique retinal specialisations of the species studied that may aid with vision in the deep sea. However, certain trends were observed that suggest photoreceptor growth may slow significantly during adult life stages and that longer photoreceptors are found in species that inhabit deeper oceanic strata, which likely increases light sensitivity in darker habitats further away from the day/night light cycle.

2.2 Introduction

Oegopsid squids inhabit the ocean from the surface to over 6000 m deep (Jamieson & Vecchione, 2022), with adults mostly living beyond the euphotic zone (200 m) and characterised as 'deep-sea' squids. This broad range of depth distribution exposes oegopsids to a wide variety of light conditions driven foremost by the day/night cycle that fluctuates light availability over a 24 h period. The effects of daylight can be sensed down to 1000 m, where the aphotic zone begins, and sunlight cannot penetrate. For some oegopsids, daily vertical migrations towards the surface occur in rhythm with the day/night cycle, prompting species to ascend hundreds of meters in a single night to feed (Cones et al., 2022; Gilly et al., 2006; Watanabe et al., 2006). Ocean depth also plays a significant role on ambient light conditions. Light is readily absorbed by water and scattered by particulate matter, making it difficult for short and long wavelength light to penetrate deep into the ocean. In daylight conditions, light entering the water at 480 nm can reduce in intensity by 2.5 log units within the first 100 m of depth, and by 1.5 orders of magnitude every 100 m thereafter (Warrant & Johnsen, 2013). Depth-dependent light variation in the sea appears to influence the behaviours and development of some squid species (Turnbull et al., 2015; Young, 1991). Species can have depth distributions that cover thousands of metres over their lifespan; for example, the hatchlings of some species inhabit surface layers, and migrate deeper as they mature into adults (Hunt & Seibel, 2000). However, despite inhabiting a low-light environment and being exposed to a wide variety of light conditions, oegopsids remain successful visual predators.

Squids possess camera-type eyes, a configuration that evolved convergently with fishes, demonstrating their practicality for deep-sea environments, and resulting in both lineages sharing many ocular features (Packard, 1972). In camera-type eyes, light passes through the pupil, which acts as an aperture and moderates light intake. The light is then refracted through the lens and focused onto the retina. However, in coleoid cephalopods, the retina is comprised of rhabdomeric photoreceptors that consist of an inner and outer photoreceptor segment and a basal membrane between the two (Hanke & Kelber, 2020). In contrast, fishes have rod photoreceptors that allow for perceiving light in dim conditions and cone photoreceptors allowing for colour vision, though some species shed their cones as adults when they occupy deeper and darker waters where colour vision becomes less necessary (Lupše et al., 2021). Nevertheless, the retinas of both fishes and squids have evolved in intriguing ways to aid their visually guided behaviours, typically by increasing light sensitivity.

Due to the low-light environment and presence of small bioluminescent light sources in the deep sea, animals inhabiting these habitats generally rely on mechanisms that increase light sensitivity, sometimes at the detriment of visual acuity. In many fish, a thin reflective layer called the tapetum lucidum lines the back of the interior eye, which reflects light back through the retina, giving a second opportunity for the photoreceptors to be triggered by the reflected light (Douglas & Marshall, 1999). Additionally, some fish possess aphakic gaps that allow light to reach the retina passing on the side rather than through the lens (de Busserolles et al., 2020). The unfocused light creates an image with less visual acuity but increases light sensitivity (Warrant et al., 2003), which seems to be advantageous in dark environments. However, squids do not possess tapeta lucida or aphakic gaps. Instead, squids (along with many vision-dependent animals) rely on a multitude of other physiological and anatomical factors to increase their light sensitivity (Nilsson et al., 2014), including but not limited to the underlying neural circuitry facilitating visual processes, eyeball and pupil size (Nilsson et al., 2012), photoreceptor density (Makino & Miyazaki, 2010; Talbot & Marshall, 2011), and individual receptor length and width (Chung, 2014). Previous research investigating oegopsid vision has recorded photoreceptors longer than 400 µm (Chung, 2014), with some related species, such as the bathyteuthid *Bathyteuthis abyssicola* (in a sister clade to the oegopsids), additionally possess fovea that accommodate elongated photoreceptors at greater densities that improve visual acuity and decreases the threshold to detect small flashes of bioluminescence (Chung & Marshall, 2017). In comparison, myctophids or 'lanternfishes', a branch of common deep-sea fish that often inhabit the same environments as oegopsids but typically possess tapeta lucida and aphakic gaps, have rod photoreceptors that range from about 30µm to 100µm in length (de Busserolles et al., 2014). This may indicate that squids have a greater reliance on relatively longer photoreceptors to improve light sensitivity in the deep sea, and therefore photoreceptor length was a focus of this study.

In addition to long photoreceptors, histological investigations of squid retinas have identified other mechanisms to aid their visually dependent behaviours. The retina of *Sepioteuthis lessoniana* incorporates a "retinal bump" that likely blurs potential prey until they move within striking distance (Chung & Marshall, 2014). Similarly, *Spirula spirula* has an anterior-ventral retinal ridge that may be used to spot prey overhead

(Chung & Marshall, 2017). To improve vision in brighter near-surface waters, screening pigment in the form of an ommin layer (OL) has been found in nearshore myopsid squids, octopuses, and more recently in oegopsid species (Evans et al., 2015; Gleadall et al., 1993; Howard et al., 2024; Young, 1963). Identified as ommin pigment granules by Butenandt (1959), the OL is embedded at the base of the outer segment of the photoreceptors (OSP) in dark-adapted eyes that have had little exposure to light. In brighter conditions, the granules from the OL migrate towards the end of the OSP to block incoming light and stop oversaturation of the photoreceptors, potentially preventing damage (Daw & Pearlman, 1974). This study aimed to identify any analogous retinal modifications in the examined squid species that may indicate similar visually dependent behaviours.

Despite recent advancements in observation and collection technology in the deep sea, oegopsids' eyes remain relatively understudied compared to their near-shore and shallow-water counterparts, even though they comprise the most diverse clade in Cephalopoda. In this study, the retinas of eight oegopsid species native to Aotearoa, New Zealand were investigated using histological examinations. Specifically, photoreceptor length was measured as a proxy for determining light sensitivity across the retina.

2.3 Methods

2.3.1 Specimen Acquisition

Adult squids were collected aboard the *RV Tangaroa* during three research surveys conducted by the National Institute for Water and Atmospheric Research, Ltd. (NIWA) within the exclusive economic zone of Aotearoa New Zealand. Specimens were collected in January 2020 during the TAN2001 Chatham Rise Trawl Survey along the Chatham Rise (Figure 1, Site A) and in July 2021 during the TAN2107 West Coast South Island Trawl Survey along the northwest coast of Te Waipounamu (Fig. 1, Site B). Squids were trawled at a depth range of 200-1200 m. Once aboard, the squids were euthanised, and their eyes were enucleated and preserved in 5% buffered formalin in filtered seawater until further analysis.

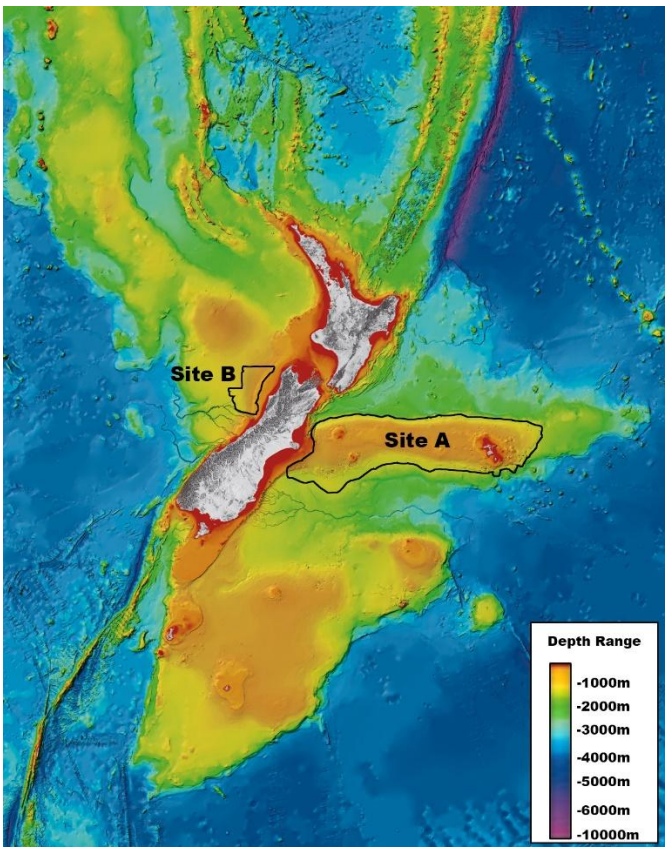


Figure 1. *A bathymetric map of the specimen collection sites around Aotearoa New Zealand. Site A) Chatham Rise. Site B) Te Waipounamu west coast. This image has been modified with permission from NIWA (Mitchell et al., 2012).*

The *Taningia danae* specimen was collected aboard fisheries vessels near the west coast of Te Ika-a-Maui (north island) as part of the New Zealand Ministry for Primary Industries (MPI) fisheries observer services. The squid was frozen whole aboard the ship at -20°C before being transferred to the laboratory for processing. The right eye was then enucleated and preserved in 70% ethanol until further analysis. In total, 11 species were investigated in this study (Table 1).

Table 1. Squid species collected for this study by NIWA and the MPI Scientific Observer Programme, during NIWA fish surveys and MPI observer services.

Common and Family Name	Species	Mantle Length Range (mm)	Sample Size	Collection Site
'Arrow Squids' Ommastrephidae	*Nototodarus sloanii*	130-410	12	Site A
	Nototodarus gouldi	156-256	6	Site B
'Flying Squids' Ommastrephidae	*Todarodes filippovae*	223-313	3	Site A
'Hooked Squids' Onychoteuthidae	*Moroteuthopsis ingens*	140-554	10	Site A
	Onykia robsoni	397-575	4	Site A
'Cock-eyed Squids' Histioteuthidae	*Histioteuthis miranda* (Right Eye)	232	1	Site A
	Histioteuthis miranda (Left Eye)	232	1	Site A
'Octopus Squids' Octopoteuthidae	*Taningia danae*	989	1	-
'Giant Squid' Architeuthidae	*Architeuthis dux*	1770	1	Site A

2.3.2 Histological Processing of Retinal Tissues

To obtain histological sections of the retinal tissues, the right eye from each specimen was preserved in a 5% formalin solution in filtered seawater, as well as one left eye from *H. miranda*. Each eye was processed separately and initially rinsed rapidly with 0.1 M phosphate buffer solution (PBS) twice, then submerged for 30 min in PBS. The eye was then placed in a fresh solution of 0.1 M PBS and stored overnight at 4°C. For cryoprotection, over three consecutive days, the eye was gradually infiltrated with increasing concentrations of 10%, 20%, and 30% sucrose in 0.1 M PBS; each step was performed overnight at 4°C.

The eyes were first placed in the lowest sucrose concentration to gradually replace the water content with a solution suitable for cryosectioning. Due to the difference in fluid density, the eyes initially floated in the sucrose solution. Once equilibrium was reached, the eyes sank, indicating readiness for transfer to the next more concentrated sucrose solution. Most eyes sank overnight, but larger eyes such as those from *T. danae* and *A. dux,* required multiple days in each sucrose solution to ensure complete water replacement.

Following cryoprotection, the eye was dissected. The lens was removed, and the eye was inverted to remove any residual vitreous fluid. A custom-made container was made to match the volumetric size of the eye and was filled with a clear Optical Cutting Temperature (OCT) embedding medium (Scigen Tissue Plus O.C.T. Compound, Paramount, U.S.A.). The eye was placed within the container; the eyecup was filled with OCT and then rapidly frozen at -80°C (Fig. 2A).

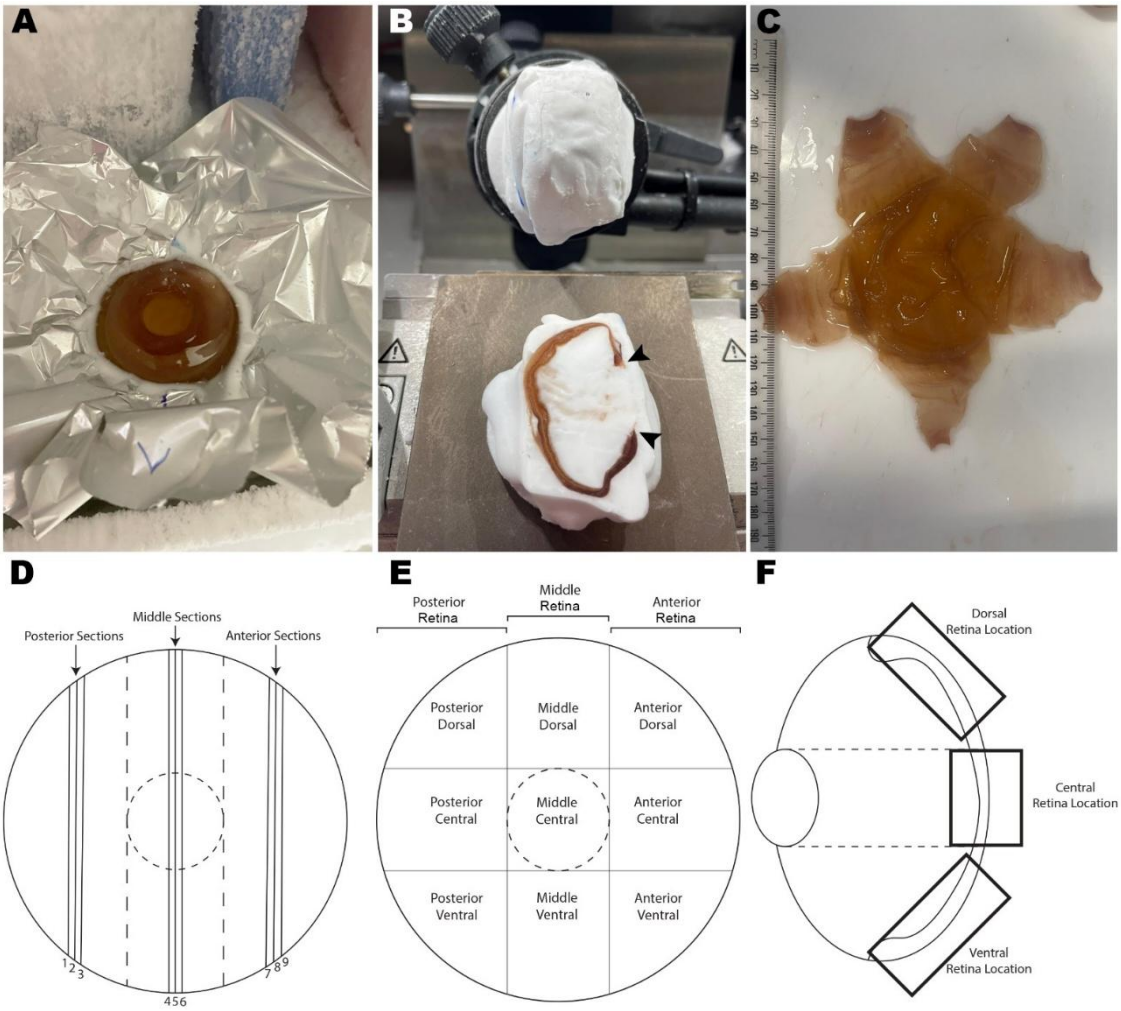


Figure 2. *A) Aluminium foil was used to create containers for submerging the eyes in OCT medium before freezing at -80°C. B) The top half of the image shows the OCT block before sectioning, with the anterior half of the eye attached to the holding puck, locked into the sectioning arm, and prepared for sectioning. The bottom half of the image shows the posterior segment of the eye already sectioned down to the middle retina. The black arrowheads indicate the upper and lower limit of the aperture. C) For large eyes that could not fit on the holding puck, namely T. danae, A. dux, and H. miranda, the retina was cut and laid flat before the middle central area was separated, embedded in OCT, and sectioned. D) A diagram illustrating the retinal areas where sections were taken. The solid outer circle represents the edge of the eye, while the dashed inner circle marks the edge of the pupil (aperture). The solid vertical lines indicate the nine retina sections taken, and the vertical dashed lines mark the borders aligned with the pupil, considered central retinal area in this study. The three posterior sections were taken from halfway between the far-left edge of the eye and the left vertical border created by the aperture. The three middle sections (central retina) were taken from the centre of the eye. The three anterior sections were taken from halfway between the far-right edge of the eye and the right vertical border created by the aperture. E) A diagram of the three defined segments of the eye (posterior, middle, anterior) and the name designations and abbreviations of each of the nine measured retinal regions. The posterior segment was defined as the retina from the left edge of the eye to the leftmost vertical border created by the aperture. The middle segment spanned the width of the aperture, between the borders created by its edge. The anterior segment extended from the right edge of the eye to the rightmost vertical border created by the aperture. The nine measured areas were named based on their position along the retina. F) A diagram depicting the three defined locations along the retinal sections that were imaged for analysis. The peripheral dorsal*

retina was defined as any retinal area above the uppermost horizontal border created by the edge of the aperture (for midsegment sections) or as above the horizontal border created by the edge of the scleral tissue. The central retina was defined as the location between the two horizontal borders created by the edge of the aperture (for sections from the middle retina) or the edge of the scleral tissue. The peripheral ventral retina location was defined as any retina below the lowermost horizontal border created by the edge of the aperture (for midsegment sections) or the edge of the scleral tissue.

The OCT-embedded tissue was mounted on to a holding puck and sectioned at 20 µm or 50 µm using a cryostat (Leica CM3050 S, Wetzlar, Germany; Fig. 2B). Section thicknesses were determined through trial and error, with 20-50 µm sections found to be optimal for producing intact histological sections suitable for morphological analyses of the relatively large eyes of adult oegopsid squids.

The OCT embedding protocol was modified for larger eyes, such as those from *A. dux*, to accommodate the limited space available on the holding puck used during sectioning. For these samples, the lens and fluid were removed following the same procedure as described for smaller eyes. However, additional incisions were made to flatten the eye (Fig. 2C). The middle central area of the retina was removed from the rest of the retina, covered in OCT, frozen at -80°C, and then vertically mounted onto the holding puck for sectioning.

For most eyes, three consecutive sections were taken from the posterior retina, three from the middle retina, and three from the anterior retina, yielding nine distinct regions from each eye sample (Fig. 2D). The mid-retina was defined as any retinal tissue within the vertical boundaries created by the width of the aperture. The peripheral retinal areas were defined as any retinal tissue located posterior or anterior to the mid-retina, respectively (Fig. 2E). Sections from the mid-retina were taken from the central part of the eye, while sections from the posterior and anterior retinal segments were taken at the midpoint between the vertical aperture boundaries and the outer edge of the eye (Fig. 2D). For large eyes, three consecutive sections were taken from the excised and separated middle central retinal region.

All sections were mounted on hydrophilic surface adhesion microscope slides (Haimen Changlong Instrument Co., Ltd., Haimen City, China) and stored at -20°C until stained and imaged.

2.3.3 Staining and Imaging

Sections were stained with toluidine blue (TB) prior to imaging. The slides were removed from the freezer and immediately soaked in two consecutive 15 min baths of 0.1 M PBS to remove the frozen OCT. Next, 0.025% TB prepared in 0.1 M PBS was placed to the retinal sections for 30-45 sec, and then briefly resubmerged in 0.1 M PBS to remove excess TB solution. A coverslip was placed on the slides with mounting solution (Electron Microscopy Science Citi Fluor AF1 Glycerol/PBS Solution, Hatfield, U.S.A.), and the coverslip was sealed with nail polish to prevent evaporation.

All images were captured with a Nikon Digital Sight DS-5Mc camera (Tokyo, Japan) attached to a Leica DMR Microscope (Wetzlar, Germany) using a 10x ocular lens

and 10x low power dry objective lens (0.30 A) and NIS-Elements AR software (Version 3.2). Images were taken from the dorsal, central, and ventral locations of the nine retinal sections from each eye, producing 27 images per eye. The central location of the retina was defined as any retinal tissue located between the horizontal borders created by either the iris (posterior and anterior sections) or the aperture (middle sections), while the dorsal and ventral locations were defined as either above or below the iris/aperture borders, respectively (Fig. 2F). For larger eyes (e.g. *A. dux*), one image from each of the three middle central retina sections was taken, producing three images.

2.3.4 Statistical Analyses

To analyse the histological data, images of the dorsal, central, and ventral retina from the three different retinal portions (posterior, middle, and anterior) were divided into nine measurable retinal regions (Fig. 2E). The images were analysed to measure the whole length of the photoreceptors, the outer segment (OSP) and inner segment of the photoreceptors (ISP), and the depth of the screening pigment in the ommin layer (OL) concentrated at the base of the outer segment, when applicable.

Images were uploaded to FIJI (Image J Version 1.54m) and the image-scale was calibrated to 2.8833 µm/pixel. Retinal layers (OSP, ISP, OL) were visually identified and measured using the straight-line tool. The OSP length was measured from the basal membrane-OSP boundary (including the OL resting at the base of the OSP in dark-adapted squid) to the apical tip of the OSP at the limiting membrane. The ISP length was measured from the basal membrane-ISP boundary to the boundary of the retinal plexus layer and the distal end of the ISP. The OL layer was measured as the depth of the visible screening pigment granules concentrated at the base of the OSP in specimens where it was visible.

Each retinal region was measured three times and regions were defined by their dorsal, central, ventral location and the anterior, middle or posterior part of the retina, resulting in 81 measurements per specimen for a single retina. However, occasional damage to retinal sections meant that ISP measurements were not always taken. Additionally, the eye preservation method and light exposure prior to fixation often caused the dissolution or movement of the ommin pigment, resulting in only a few OL measurements available for analysis. Interspecific comparisons of OSP length, ISP length, and OL depth were conducted using linear mixed-effects models due to the nested nature of the data. The models were fitted using R (Version 4.4.1) with RStudio (Cranberry Hibiscus, Version 2024.09.0 Build 375) and the package Linear Mixed-Effects Models using ‘Eigen’ and S4 (Version 1.1-35.5). Linear mixed-effects models were chosen because they account for the hierarchical structure of the data, allowing for variability at different levels while controlling for correlation among nested observations, thus avoiding inflation of Type I errors.

To determine whether variables were continuous and approximately normally distributed, and had a linear relationship, Pearson’s correlation tests were conducted using R (Version 4.4.1) with RStudio (Cranberry Hibiscus, Version 2024.09.0 Build 375). Retinal measurements of the OSP and ISP were analysed against the mantle length. To

avoid pseudo-replication confounding the results for Person's correlation tests, measurements from all nine retinal regions were averaged for each specimen.

Heatmaps were created to visualise the differences of OSP length amongst the nine retinal regions for each species by averaging OSP measurements from individuals. The heatmaps for both eyes of *H. miranda* were generated from a single specimen. OSP lengths were compared amongst the nine retinal regions within each species individually for species with a sample size greater than one using linear mixed-effects models and Tukey's HSD post-hoc tests for pairwise comparisons using R (Version 4.4.1) with RStudio (Cranberry Hibiscus, Version 2024.09.0 Build 375) and the package Linear Mixed-Effects Models using 'Eigen' and S4 (Version 1.1-35.5).

Species were grouped into depth range categories based on their observed inhabited depths (see Table 3) and the OSP and ISP lengths were compared using one-way ANOVA analyses in R (Version 4.4.1) with RStudio (Cranberry Hibiscus, Version 2024.09.0 Build 375). Species were classified into one of six potential depth range categories based on the oceanic strata they are reported to inhabit, to better understand whether ambient light conditions affect OSP length. Species were grouped according to whether adults inhabit any or all the following: epipelagic layers, 0-200 m (sunlight zone), or from 200-1000 m deep (twilight zone), or below 1000 m deep (midnight zone). Some species were assigned a category that encompasses more than one zone (Table 2). No species in this study satisfied the conditions to be grouped in the sunlight zone (0-200 m) or in the deepest category encompassing only the midnight zone (+1000 m; Table 3).

Table 2. *The six depth range categories (DRC) and their corresponding depth ranges and oceanic strata.*

Depth Range (m)	Oceanic Strata
0-200	Sunlight Zone Only
0-1000	Sunlight and Twilight Zones
0-1000+	Sunlight, Twilight, and Midnight Zones
200-1000	Twilight Zone Only
200-1000+	Twilight and Midnight Zones
1000+	Midnight Zone Only

Table 3. *The depth ranges for each species.*

Species	Depth Range (m)	References
N. sloanii	0-600	(Roper, Nigmatullin, et al., 2010)
N. gouldi	0-825	(Roper, Nigmatullin, et al., 2010)
T. filippovae	0-1200	(Roper, Nigmatullin, et al., 2010)
M. ingens	300-1400	(Roper, Nigmatullin, et al., 2010)
O. robsoni	250-800	(Roper, Nigmatullin, et al., 2010)
*A. dux**	200-900	(Roper, Nigmatullin, et al., 2010)
*T. danae**	240-2157	(Kubodera et al., 2007)
*H. miranda**	300-1200	(Roper, Nigmatullin, et al., 2010)

*Due to the small samples size (n=1), *A. dux*, *T. danae*, and both eyes of *H. miranda* were not included in analyses that required species grouping.

2.4 Results

2.4.1 Retinal Morphology

Histological sections taken from the retinal mid-region of each species were examined to identify presence of structures that may influence visual acuity, such as fovea, retinal bumps, or double-nucleated photoreceptors, as described in other species (Chung & Marshall, 2017). Sections of the dorsal, central and ventral mid-retina were compared among species to identify structural differences. No specialisations were observed.

There was a similar retinal layer organisation across species, resembling the coleoid retina structure previously described (Hanke & Kelber, 2020;). The rhabdomeric photoreceptors were visible as distinct outer and inner photoreceptor segments connected by passing through the basal membrane. At the distal end of the OSP a limiting membrane capped the photoreceptors. In some specimens, screening ommin pigment granules were visible at the base of the OSP (Fig. 3I). The ISP was continuous with the retinal plexus layer that connected to afferent nerve fibres, which travel to the optic lobe through gaps in the rudimentary cartilage (Fig. 3C).

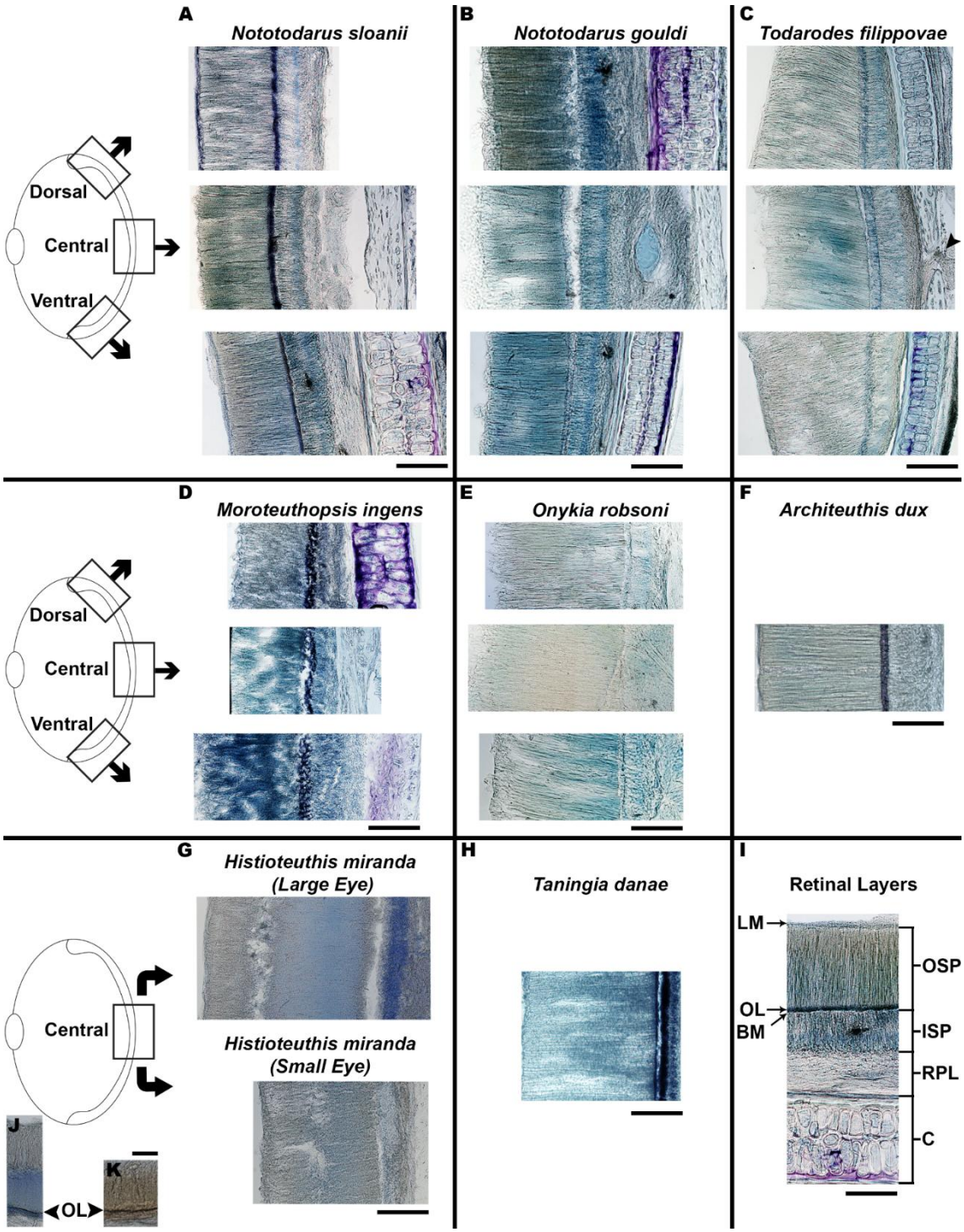


Figure 3. *Histological sections from the middle of the retina stained with toluidine blue (TB). A-E) Dorsal, central and ventral retinal sections. In the central section of* Todarodes filippovae*, note the gap in the rudimentary cartilage with afferent nerve fibres passing through (black arrowhead). F-H) Central retinal sections. I) Diagram of the squid retina. J) Peripheral retinal from large eye of* H. miranda *with intact screening ommin pigment. K) Peripheral retinal of the small eye of* H. miranda *with intact screening ommin pigment. Abbreviations: limiting membrane (LM); outer segment of the photoreceptor (OSP); screening ommin pigment layer (OL); basal membrane (BM); inner segment of the photoreceptor (ISP); retinal plexus layer (RPL); cartilage (C). Scale bars = 100µm.*

2.4.2 Outer Segments of the Photoreceptors

Measurements of the OSP were taken from each of the nine retinal regions to determine whether significant differences exist in OSP length within a species. The OSP lengths for each region were calculated by averaging the OSP lengths from individual specimens for each species, separately. OSP length serves as a proxy for light sensitivity, which may inform the regions of the retina important for scanning the environment. Heatmaps were constructed depicting the OSP lengths of the nine regions along the retina and compared using mixed linear modelling and Tukey's HSD post-hoc test for each species (Fig. 4-6). Heatmaps of *A. dux* and *T. danae* retinal OSP lengths were not constructed since measurements were only take from the middle-central region for these two species, and both eyes of *H. miranda* were not included in the statistical analysis due to their small sample size (n=1). For the species included in the analysis, the longest OSPs were concentrated in five retinal regions: posterior-dorsal (PD), anterior-dorsal (AD), posterior-central (PC), middle-central (MC), and anterior-central (AC). The shortest OSPs were typically observed in ventral regions and the middle-dorsal (MD) region. Post-hoc test statistics and probability metrics comparing the retinal regions are listed in the appendix (Appendix A, Tables I-V).

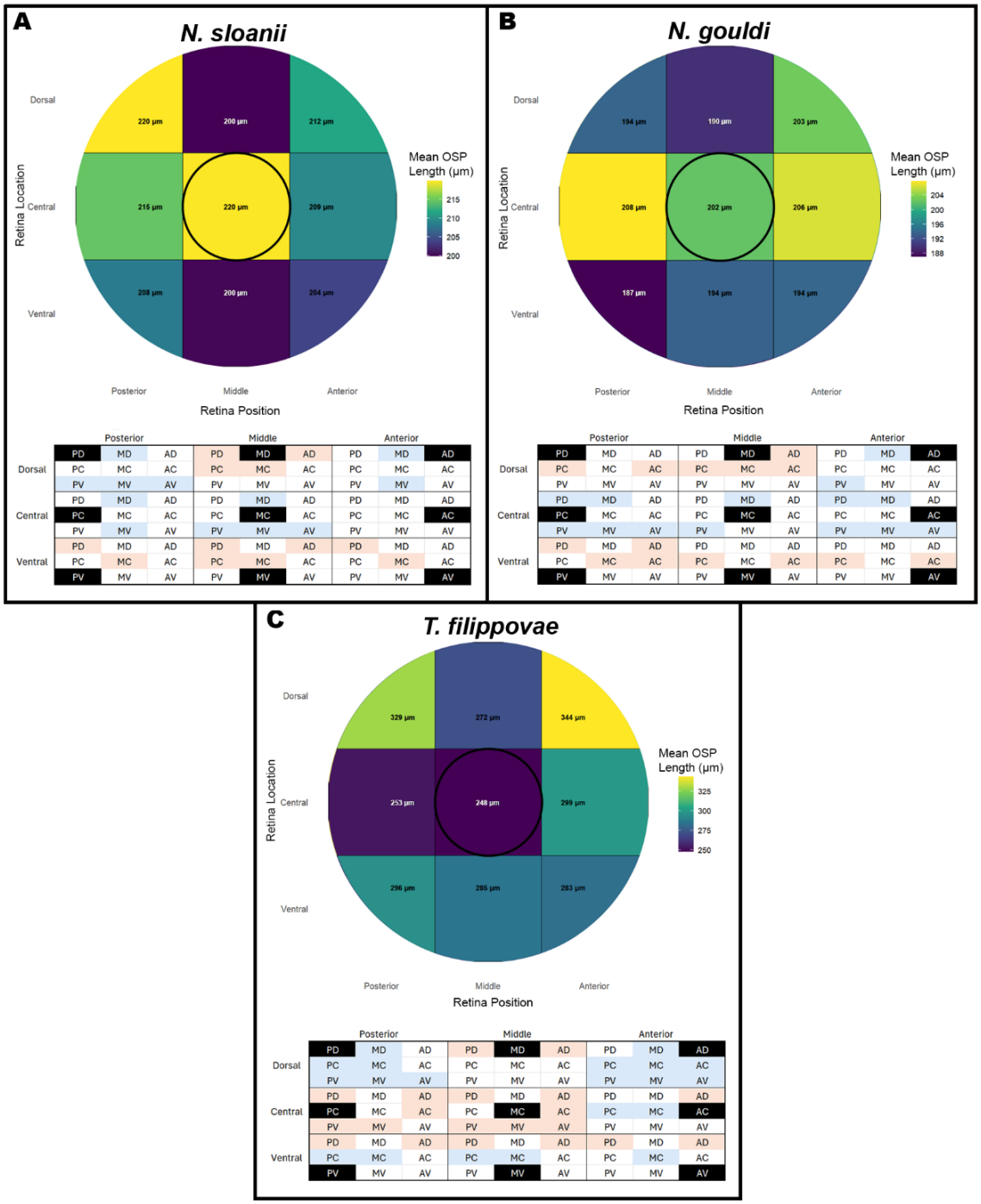


Figure 4. *(A-C) Heatmaps of retinal OSP length for species in the family Ommastrephidae. The colour-scale on the side of each heatmap is species-specific. Matrices indicate significance differences of OSP length among retinal regions from mixed linear modelling analysis and Tukey's HSD post-hoc test for pairwise comparisons. Each matrix corresponds to the heatmap, and species located directly above. Small, coloured boxes in the matrices indicate significant differences (p <0.05) in OSP length from other retinal regions (longer in blue, shorter in red). For example, using the matrix representing* T. filippovae *(C), the anterior-ventral retinal area had significantly longer OSP lengths than the middle-central area, and significantly shorter OSP lengths than the posterior-dorsal and anterior-dorsal areas. Post-hoc test statistics and probability metrics are listed in the appendix (Appendix A, Tables I-III). Sample sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3). Abbreviations: posterior-dorsal (PD), posterior-central (PC), posterior-ventral (PV), middle-dorsal (MD), middle-central (MC), middle-ventral (MV), anterior-dorsal (AD), anterior-central (AC), anterior-ventral (AV).*

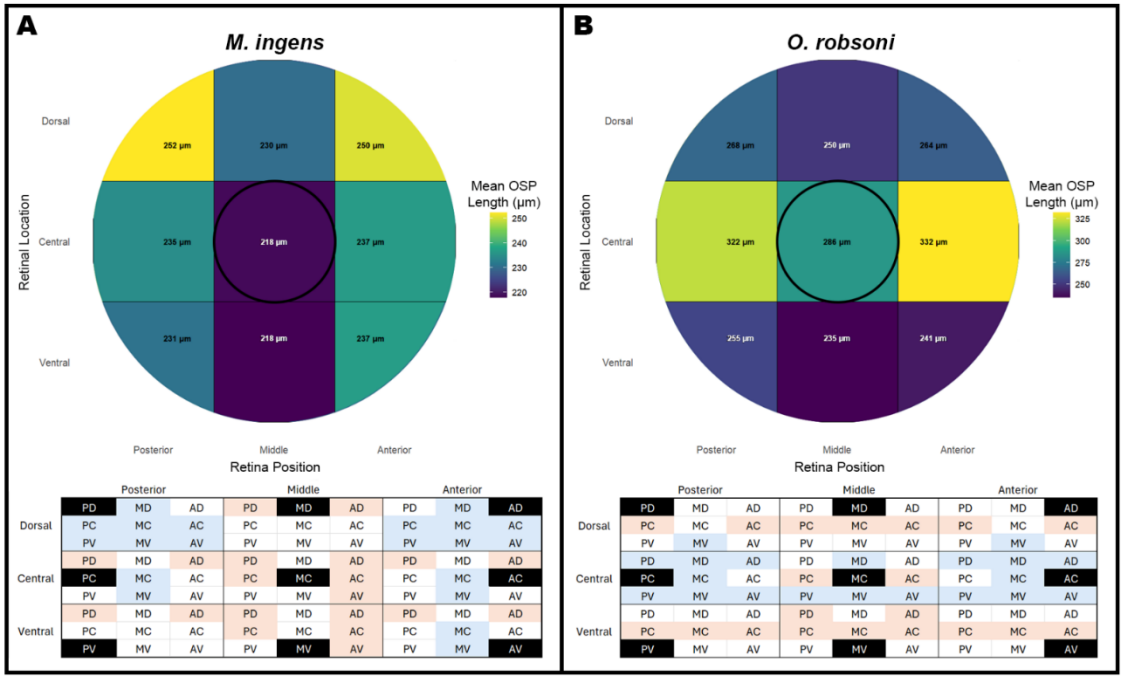


Figure 5. *(A & B) Heatmaps of retinal OSP length for species in the family Onychoteuthidae. Colour scales are species specific. Matrices indicate significance differences of OSP length among retinal regions from mixed linear modelling analysis and Tukey's HSD post-hoc test for pairwise comparisons. Each matrix corresponds to the heatmap, and species located directly above. Small, coloured boxes in the matrices indicate significant differences ($p < 0.05$) in OSP length from other retinal regions (longer in blue, shorter in red). For example, using matrix representing* O. robsoni *(B), the posterior-dorsal retinal area had significantly longer OSP lengths than the middle-ventral area, and significantly shorter OSP lengths than the posterior-central and anterior-central areas. Post-hoc test statistics and probability metrics are listed in the appendix (Appendix A, Tables IV-V). Sample sizes:* M. ingens *(n = 10),* O. robsoni *(n = 4). Abbreviations: posterior-dorsal (PD), posterior-central (PC), posterior-ventral (PV), middle-dorsal (MD), middle-central (MC), middle-ventral (MV), anterior-dorsal (AD), anterior-central (AC), anterior-ventral (AV).*

The large eye of *H. miranda* had the longest OSP concentrated in the middle-central and anterior-central retinal regions (Fig. 6A). In contrast, the small eye had the longest OSP in the posterior-central region. Additionally, the anterior-dorsal and anterior-ventral areas had OSPs of similar length, which were shorter than the posterior central region, but longer than all other regions (Fig. 6C). Since squid species in the family Histioteuthidae are frequently observed to position themselves vertically in the water column to optimise the function of their dimorphic eyes, the data was

recalculated taking into consideration this orientation. This behaviour must be considered when interpreting their retinal composition (Fig. 6B & 6D).

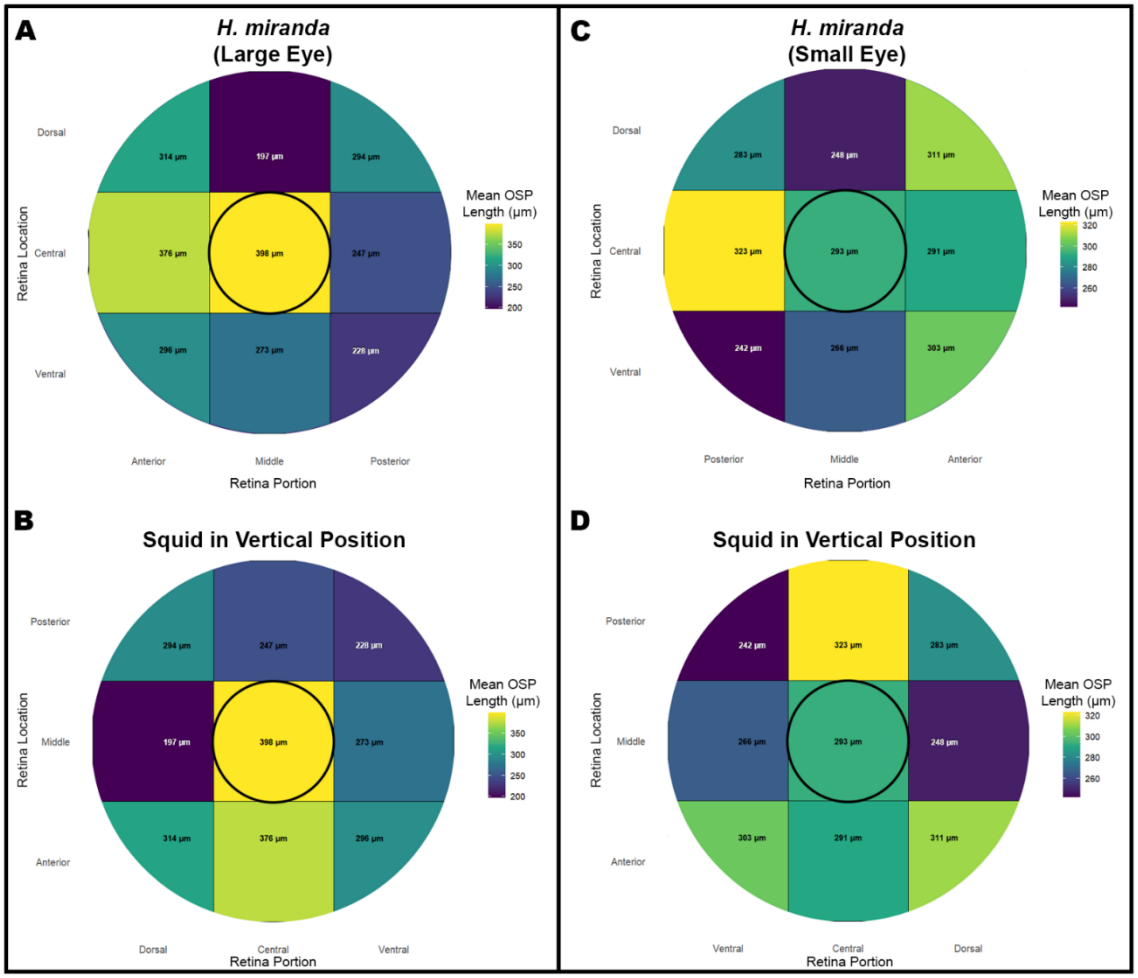


Figure 6. *(A-D) Retinal heatmaps depicting OSP lengths for* H. miranda*. Colour scales are eye specific. No statistical analyses were conducted due to the small sample size (n = 1). (A & C) Retinal heatmaps oriented for the species in the horizontal position. (B & D) Retinal heatmaps oriented for the species in the vertical position. Sample sizes: Large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1).*

To determine whether there were significant differences in OSP length among the dorsal, central, and ventral retinal locations within a species, measurements from each location were grouped and compared using mixed linear modelling for individual species (Fig. 7). *A. dux* and *T. danae* were not included in this analysis since measurements were only taken from the middle-central retinal region. Additionally, statistical analyses were not conducted on the small and large eye of *H. miranda* because of their small sample sizes (n = 1).

This analysis showed that, for most species, the ventral retinal location had the shortest OSP. For *N. sloanii* and *O. robsoni*, the ventral location OSP was significantly shorter than the dorsal and central locations. In *N. gouldi*, the OSP lengths from the ventral and dorsal locations were statistically similar but shorter than the central location. Conversely, in *M. ingens*, the OSP lengths from the ventral and central locations were statistically similar but shorter than the dorsal location. The pattern was different for *T. filippovae*, which showed the ventral location had a shorter OSP than the dorsal

location, but longer than the central location. For both the small eye and large eye of *H. miranda* the central region had the longest OSP. Post-hoc test statistics and probability metrics comparing OSP lengths from different retinal locations are listed in the appendix (Appendix A, Table VI).

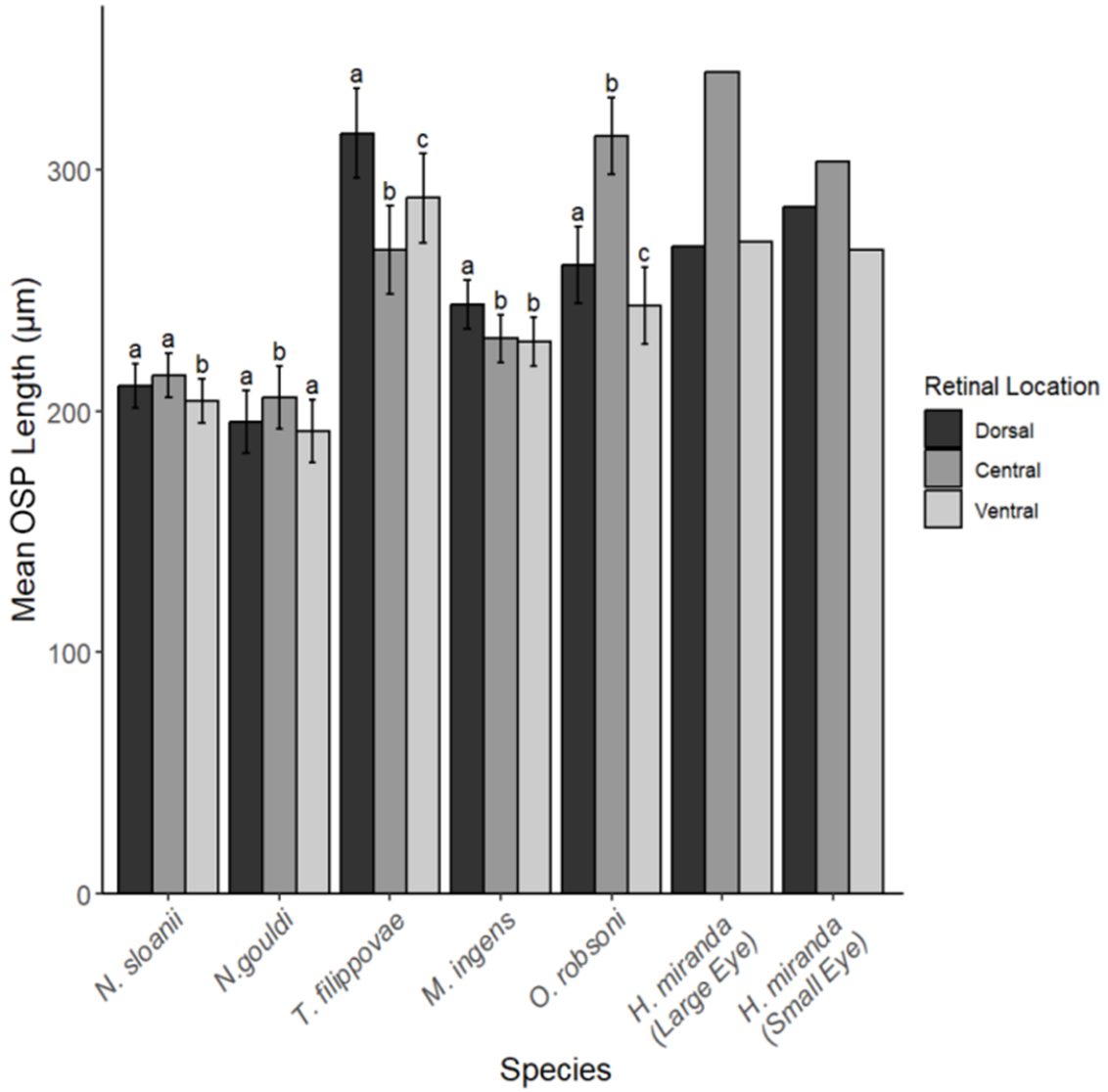


Figure 7. *The mean OSP length of the dorsal, central and ventral retinal locations for each species. Retinal locations sharing the same letter within a species do not have significantly different OSP lengths (p > 0.05). Analyses were conducted by mixed-effect linear model and Tukey's HSD post-hoc tests for pairwise comparisons. Post-hoc test statistics and probability metrics comparing OSP lengths of retinal locations are listed in the appendix (Appendix A, Table VI). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 10),* O. robsoni *(n = 4), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1). Error bars represent the standard error of the mean.*

To determine if there were significant differences in OSP length among the posterior, middle, and anterior retinal segments within a species, measurements from each segment were grouped and compared using mixed linear modelling (Fig. 8). *A. dux* and *T. danae* were not included in this analysis since measurements were only taken from the middle-central retinal region. Additionally, statistical analyses were not

conducted on the small and large eye of *H. miranda* because of their small sample sizes (n = 1).

This analysis showed that for most species the middle segment often had the shortest OSP. This was true for *T. filippovae*, *M. ingens* and *O. robsoni*, where the middle retinal segment had significantly shorter OSPs than their posterior and anterior regions. In *N. sloanii*, the middle and anterior retina were statistically similar but significantly shorter than the posterior retina. In *N. gouldi* the middle retina also had the shortest OSP, which was significantly shorter than the anterior retina but statistically similar to the posterior retina. The posterior segment for *N. gouldi* was also statistically similar to the anterior retina. Post-hoc test statistics and probability metrics comparing OSP lengths of eye regions are listed in the appendix (Appendix A, Table VII).

For the large eye of *H. miranda*, the middle retina had a longer OSP than the posterior retina but was shorter than the anterior retina. More in line with the overall trend observed across species, the small eye of *H. miranda* had a middle retina that was shorter than both the anterior and posterior retina.

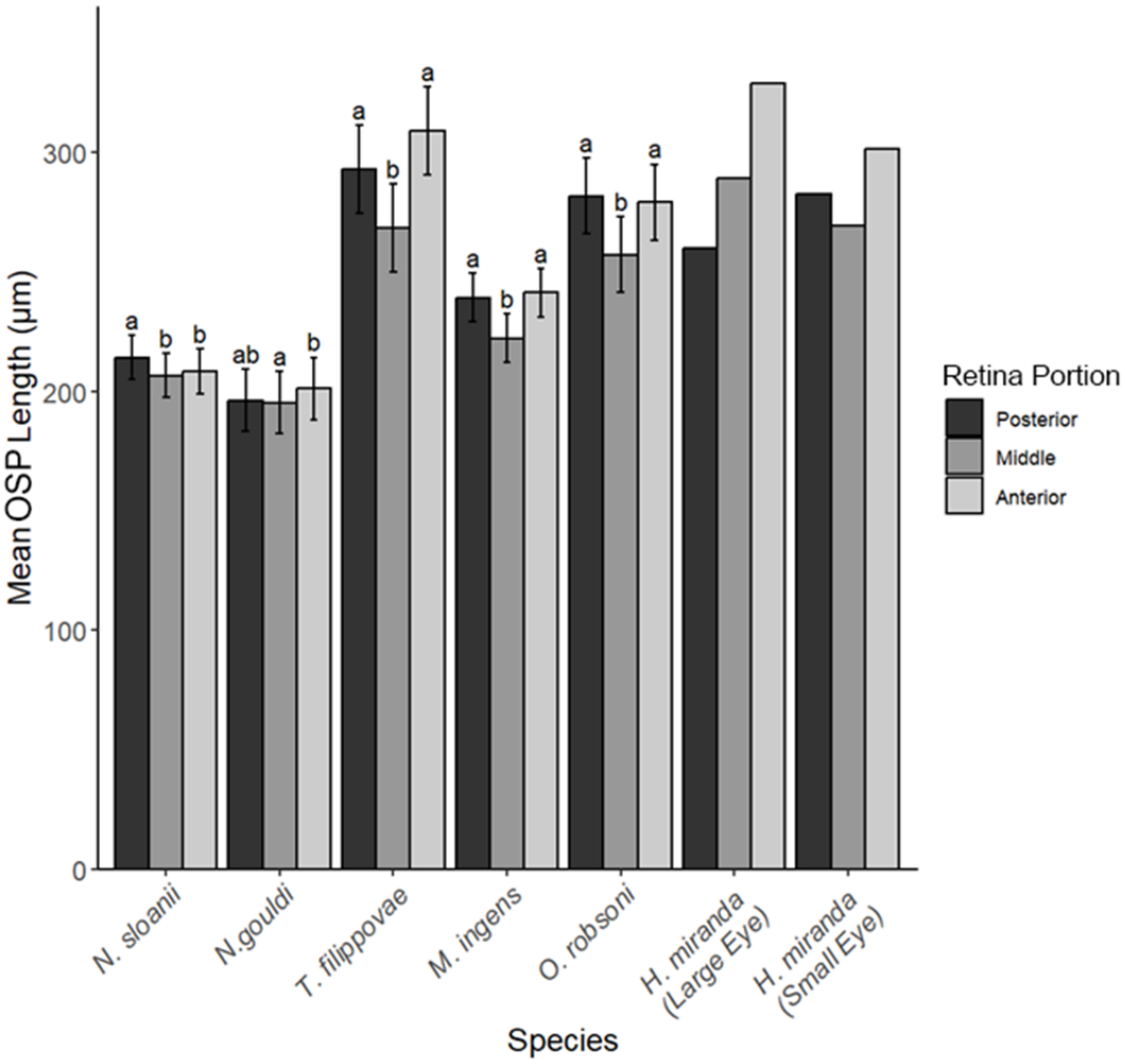


Figure 8. *The mean OSP length of the posterior, middle and anterior retina for each species. Retinal regions sharing the same letter within a species do not have significantly different OSP lengths (p > 0.05). Analyses were conducted by mixed-effect linear model and Tukey's HSD post-hoc tests for pairwise comparisons. Post-hoc test statistics and probability metrics comparing OSP lengths of eye segments are listed in the appendix (Appendix A, Table VII). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 10),* O. robsoni *(n = 4), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1). Error bars represent the standard error of the mean.*

The OSP lengths were averaged across the entire retina and compared among species. Mixed linear modelling followed by Tukey's HSD post-hoc tests identified three distinct groups with significant differences in mean OSP length (Fig. 9). *Nototodarus sloanii* (209.74 ± 9.60µm) and *N. gouldi* (197.51 ± 13.58µm) had significantly shorter OSPs compared to *T. filippovae* (289.90 ± 19.20µm) and *O. robsoni* (272.59 ± 16.63µm), while the mean OSP length of *M. ingens* (234.16 ± 10.52µm) was statistically similar to all four analysed species. The large eye (292.64 ± 33.31µm) and small eye (286.08 ± 33.44µm) of *H. miranda* had mean OSP lengths comparable to *T. filippovae* and *O. robsoni*. Similarly, *A. dux* (251.67 ± 35.52µm) and *T. danae* (251.33 ± 35.52µm) had comparable OSP lengths. Post-hoc test statistics and probability metrics comparing OSP length among species are listed in the appendix (Appendix A, Table VIII).

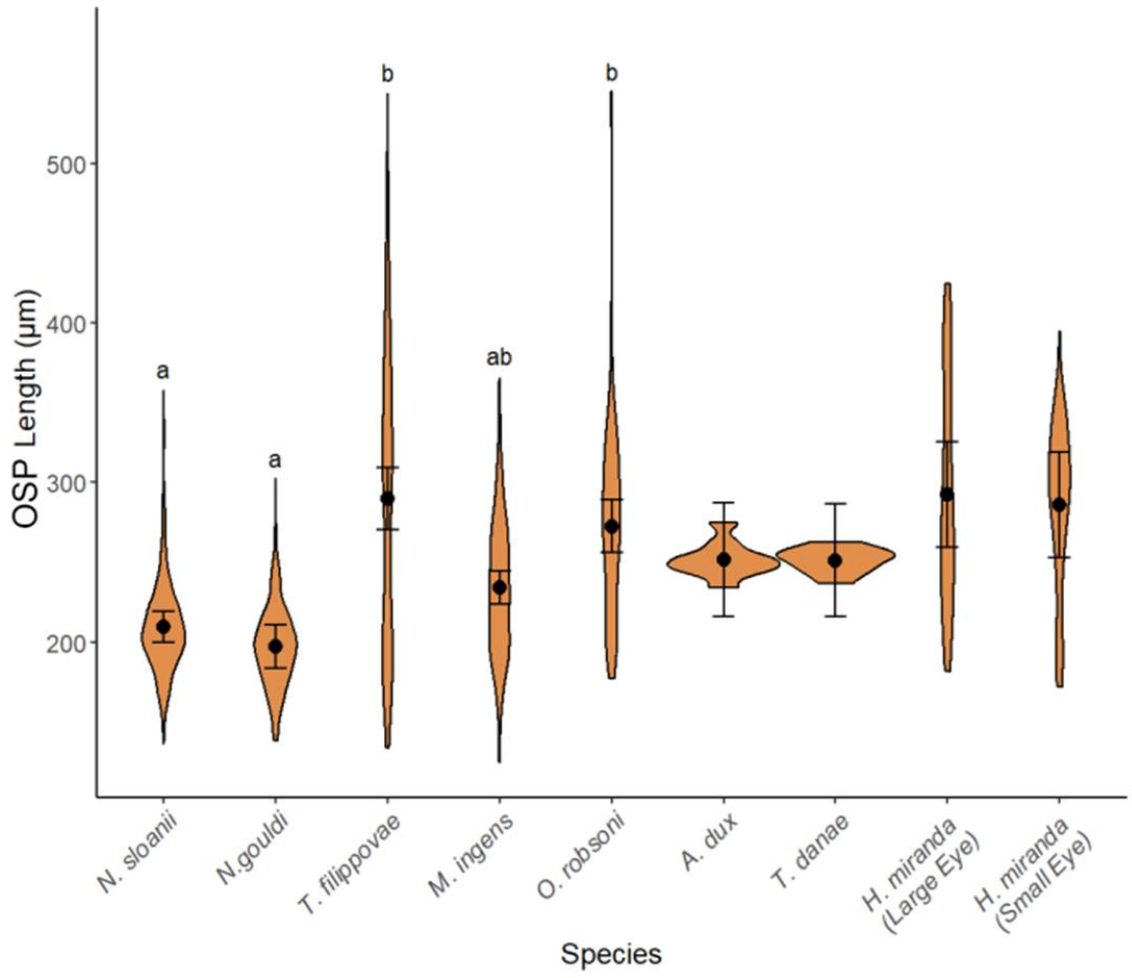


Figure 9. *The mean OSP length across the entire retina for each species. Black dots represent the mean OSP lengths. Error bars represent the standard error of the mean (SEM). Species sharing the same letter do not have significantly different OSP lengths (p > 0.05). For example, groups labelled with 'a' are not significantly different from each other but differ from groups labelled with 'b'. Groups with multiple letters (e.g., 'ab') are not significantly different from groups labelled with either 'a' or 'b'. Statistical differences are based on Tukey's HSD post-hoc test following a mixed-effect linear model. Post-hoc test statistics and probability metrics are listed in the appendix (Appendix A, Table VIII). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 10),* O. robsoni *(n = 4), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1).*

The OSP lengths were averaged across the entire retina for individual specimens within a species (Fig. 10). Pearson's correlation tests were conducted to determine the relationship between ML and OSP length for each species. No significant correlation was observed between ML and OSP for the five species with sample sizes of n ≥ 3.

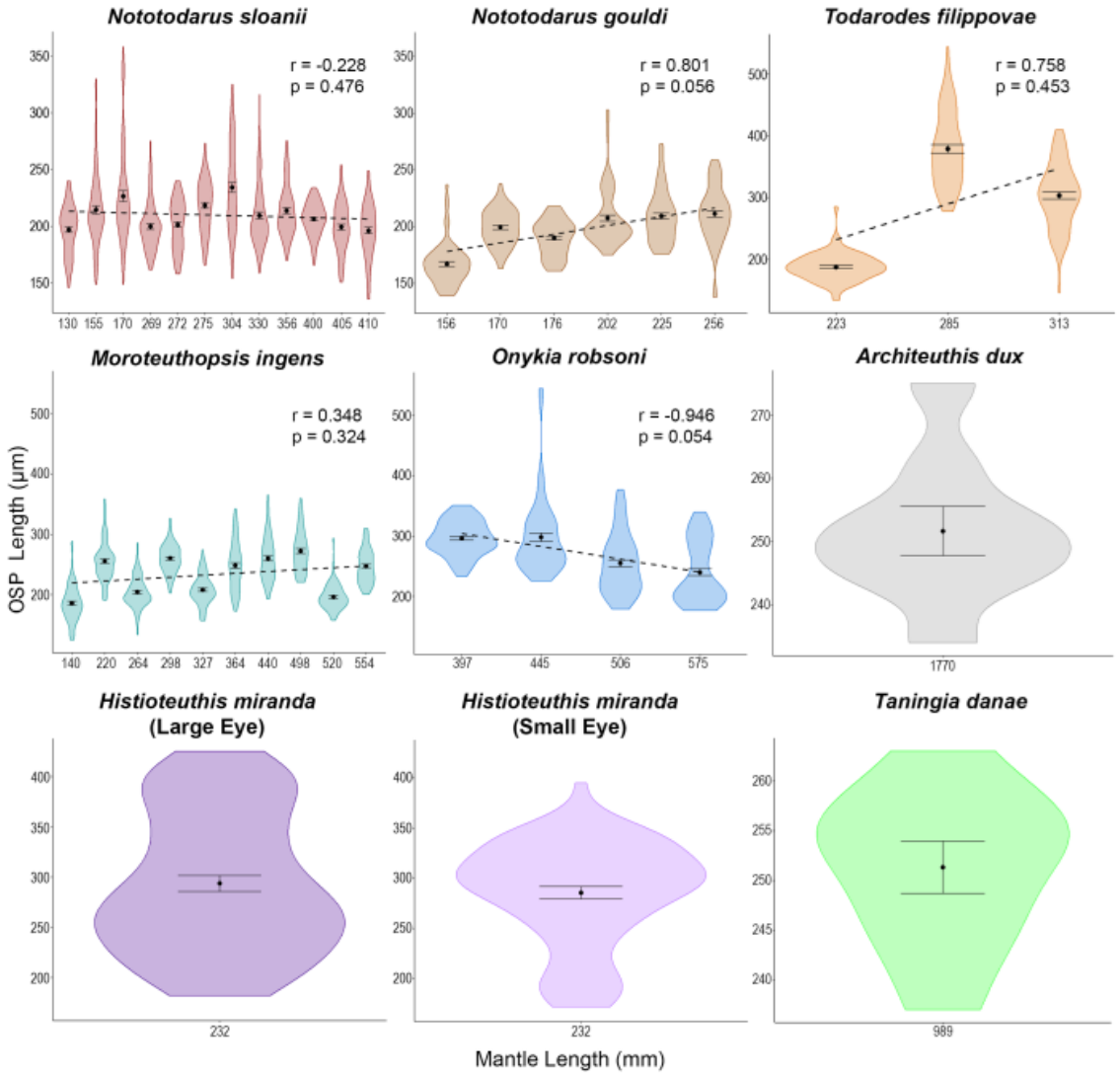


Figure 10. *Violin plots of the outer segment of the photoreceptor (OSP) length from individual specimens. The r-values indicate the correlation coefficient from Pearson's correlation tests and the p-values indicate the statistical significance. No significant correlation between mantle length and OSP length were observed. Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 10),* O. robsoni *(n = 4),* A. dux *(n = 1), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1),* T. danae *(n = 1).*

The correlation between the ML and OSP length across species was investigated but no significant correlation was found (Pearson's correlation test, r = 0.207, p = 0.206; Fig. 11). However, to determine whether gigantism in squids (*A. dux* and *T. danae*) could bias the data towards showing no correlation a second correlation analysis was conducted after removing these two species, but still no significant correlation between ML and OSP length was observed (Pearson's correlation test, r = 0.286, p = 0.086; Fig. 12).

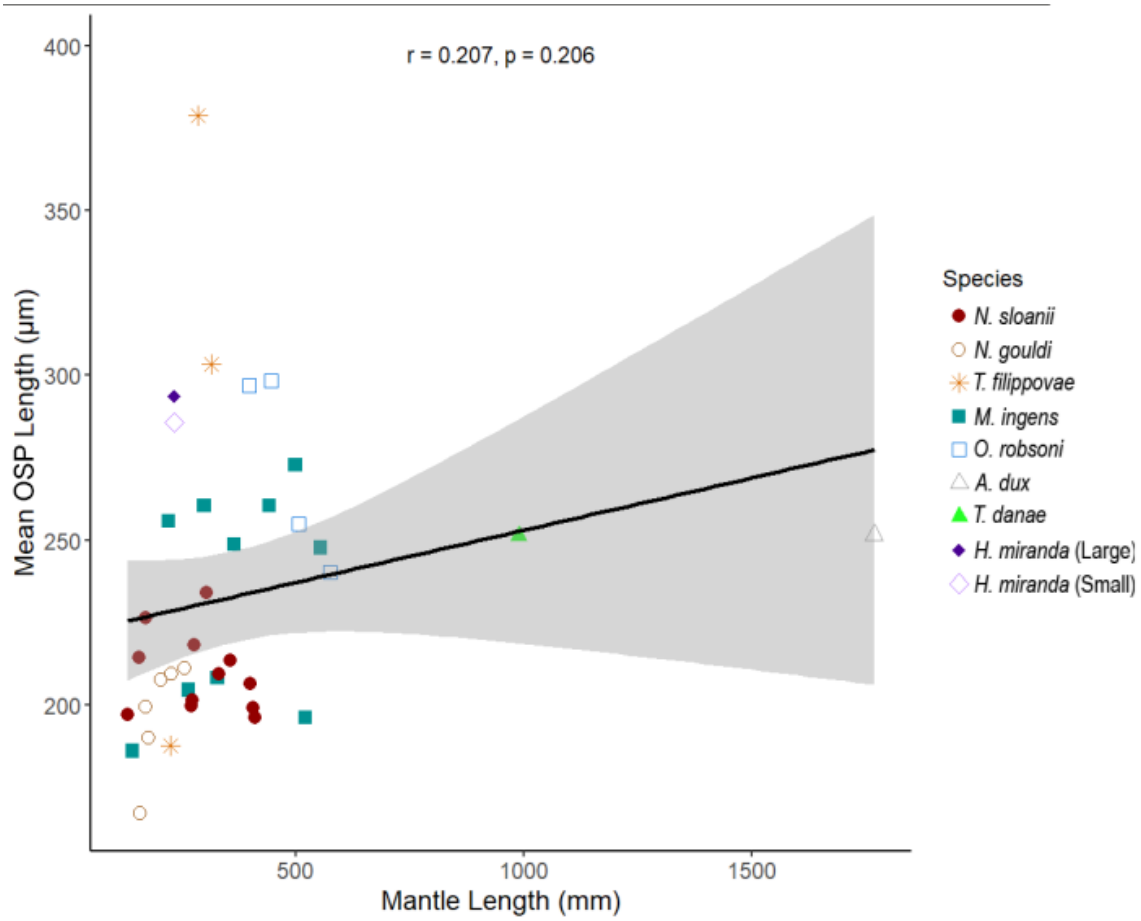


Figure 11. Mean OSP length of each specimen plotted against its ML. The black line represents the linear model correlation between the two variables (r = 0.207), and the grey area depicts the 95% confidence interval of the linear model (-0.116 < r < 0.491). Pearson's correlation test showed no significant correlation between the OSP length and ML (p = 0.206). Sample Sizes: N. sloanii (n = 12), N. gouldi (n = 6), T. filippovae (n = 3), M. ingens (n = 10), O. robsoni (n = 4), A. dux (n = 1), large eye of H. miranda (n = 1), small eye of H. miranda (n = 1), T. danae (n = 1).

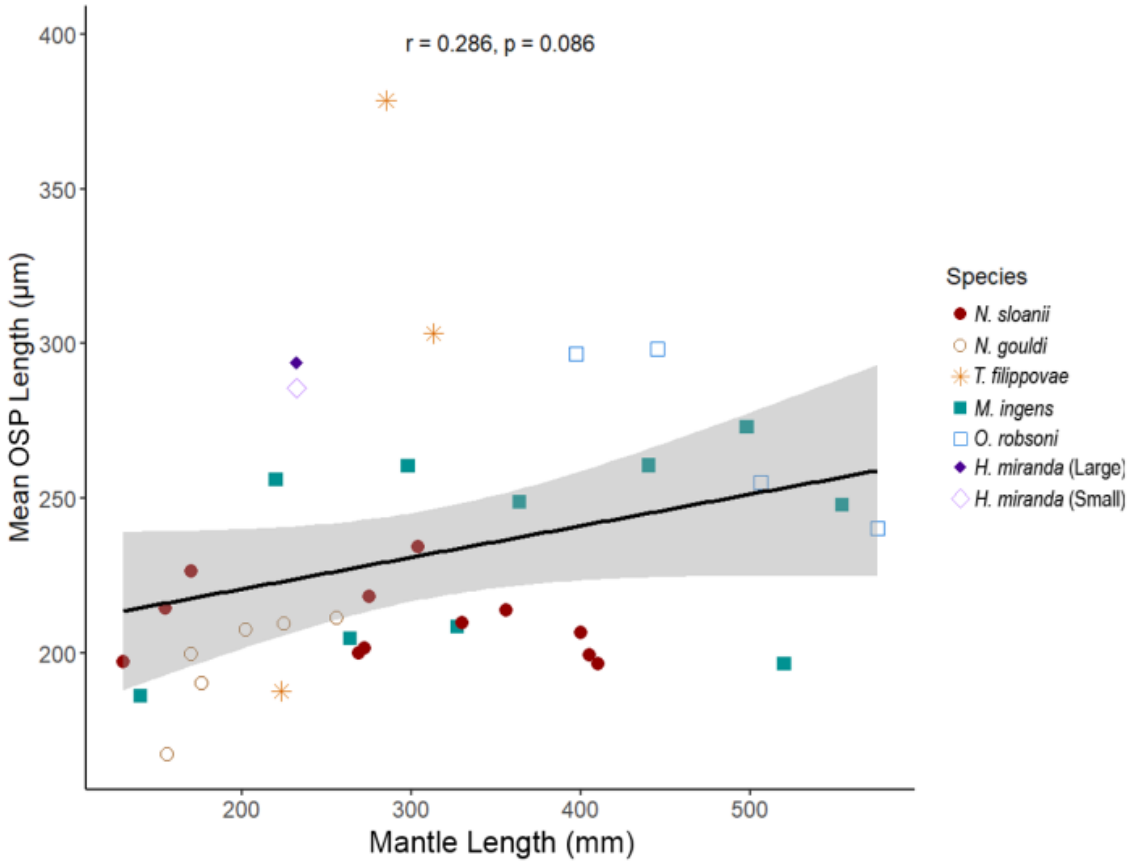


Figure 12. *Mean OSP length of each specimen plotted against its ML after species that exhibit gigantism were removed from the analysis (A. dux and T. danae). The black line represents the linear model correlation between the two variables (r = 0.286), and the grey area depicts the 95% confidence interval of the linear model (-0.042 < r < 0.558). Pearson's correlation test showed non-significant correlation between the OSP length and ML (p = 0.086). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 10),* O. robsoni *(n = 4), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1).*

The mean OSP length of individuals grouped by depth range categories showed significant variation (one-way ANOVA, $p < 0.001$, Fig. 13). Species that inhabit the sunlight and twilight zones (0-1000m) had significantly shorter OSPs than species that inhabit depth ranges that include the midnight zone (Tukey's HSD post-hoc test, 0-1000+ and 200-1000+ metres, $p = 0.002$ for both comparisons). While no significant difference was seen between the 0-1000m and 200-1000m depth ranges, only one *A. dux* represented the 200-1000m depth range had an OSP length similar to the 0-1000+ and 200-1000+ metres depth ranges. Post-hoc test statistics and probability metrics comparing OSP length of among species grouped by depth range categories are listed in the appendix (Appendix A, Table IX).

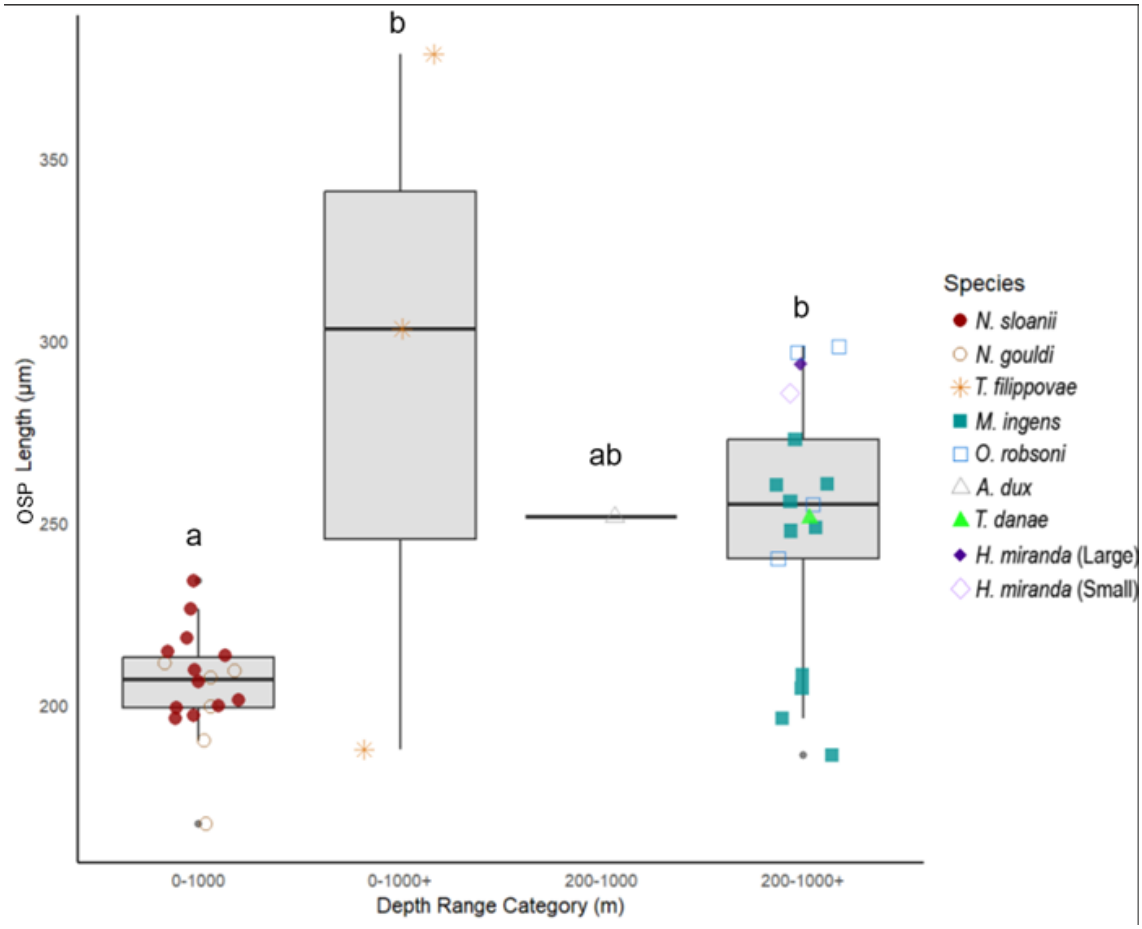


Figure 13. *OSP lengths of species grouped by their habitat depth. Depth range categories sharing the same letter do not have significantly different OSP lengths ($p > 0.05$ Statistical differences are based on Tukey's HSD post-hoc test following a one-way ANOVA ($p < 0.001$). Post-hoc test statistics and probability metrics are listed in the appendix (Appendix A, Table IX). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 10),* O. robsoni *(n = 4), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1).*

2.4.3 Inner Segment of the Photoreceptors

The ISP length measurements were grouped by retinal location and compared among species using a mixed linear model followed by Tukey's HSD post-hoc tests for pairwise comparisons (Fig. 14). No clear trend was observed across species when comparing the dorsal, central and ventral retina. The dorsal location had a significantly shorter mean ISP compared to the central and ventral locations in *N. sloanii* specimens. All three locations were statistically similar for *N. gouldi* and *T. filippovae*. For *M. ingens*, the ventral location had a significantly longer ISP than both the dorsal and central locations. The central location had a significantly longer ISP than the ventral location, while the dorsal location was not significantly different to both the central and ventral locations. Though not statistically analysed, the large eye of *H. miranda* had the longest ISP in the dorsal retinal location, but the small eye had the longest ISP in the central location. Post-hoc test statistics and probability metrics comparing ISP lengths from different retinal locations are listed in the appendices (Appendix A, Table X).

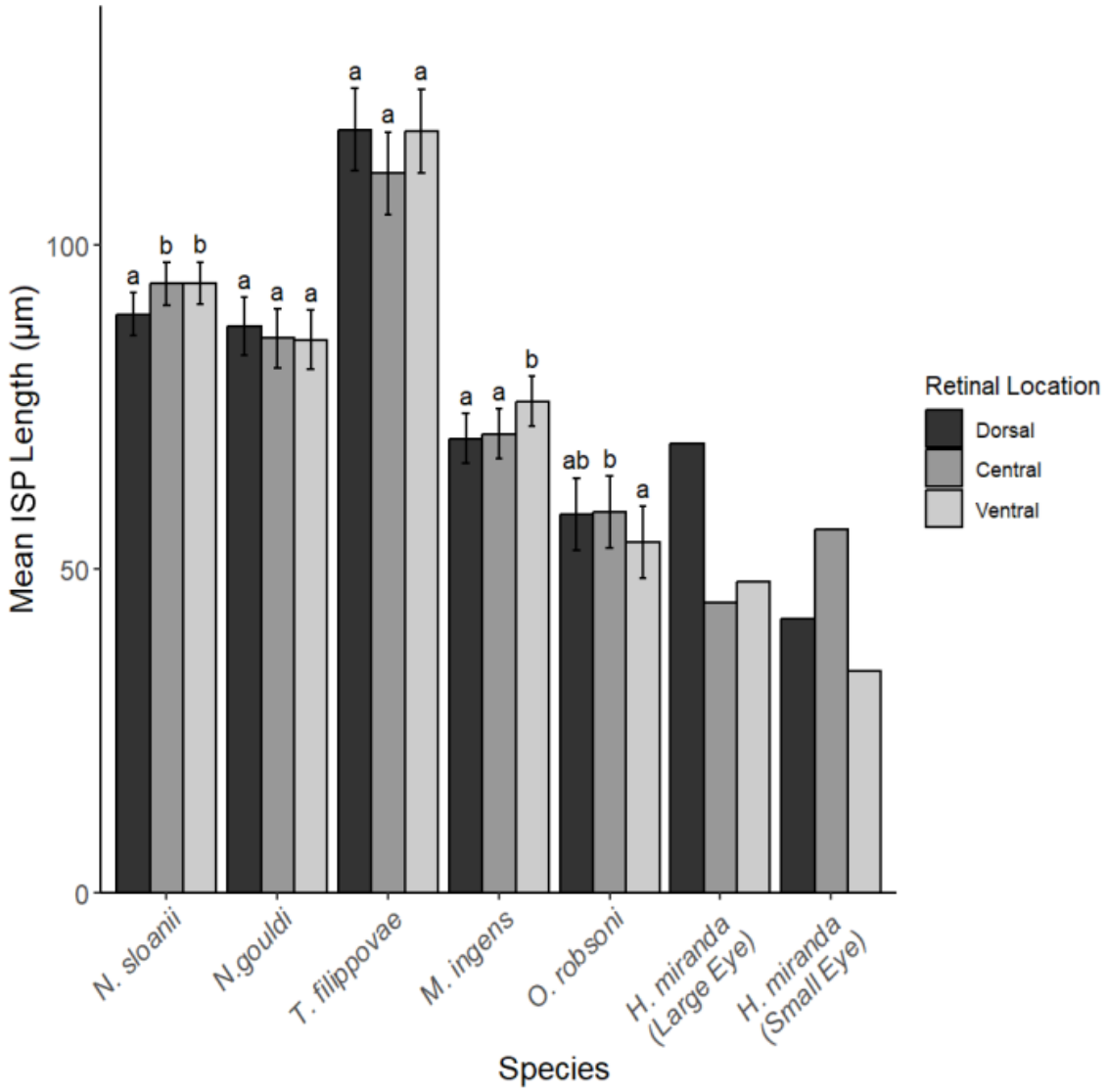


Figure 14. *Mean ISP length of the dorsal, central and ventral retinal locations for each species. Retinal locations sharing the same letter within a species do not have significantly different OSP lengths (p > 0.05). Analyses were conducted by mixed-effect linear model and Tukey's HSD post-hoc tests for pairwise comparisons. Post-hoc test statistics and probability metrics comparing ISP lengths from different retinal locations are listed in the Appendices (Appendix A, Table X). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 9),* O. robsoni *(n = 4), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1). Error bars represent the standard error of the mean.*

The mean ISP length in each retinal region was also compared using mixed-effect linear modelling and Tukey's HSD post-hoc tests (Fig. 15). In most species the ISP in the middle segment was significantly shorter than in the posterior and anterior segments (*N. sloanii*, *T. filippovae* and *O. robsoni*). However, the ISP was significantly longer in the anterior segment in *N. gouldi* than the other segments. For *M. ingens*, the middle segment ISP was significantly shorter than the posterior segment, but there was no significance between the anterior segment and both the posterior and middle segments. The posterior segment had the shortest ISP for the both the large and small eye of *H. miranda*. Post-hoc test statistics and probability metrics comparing ISP lengths from different eye segments are listed in the Appendices (Appendix A, Table XI).

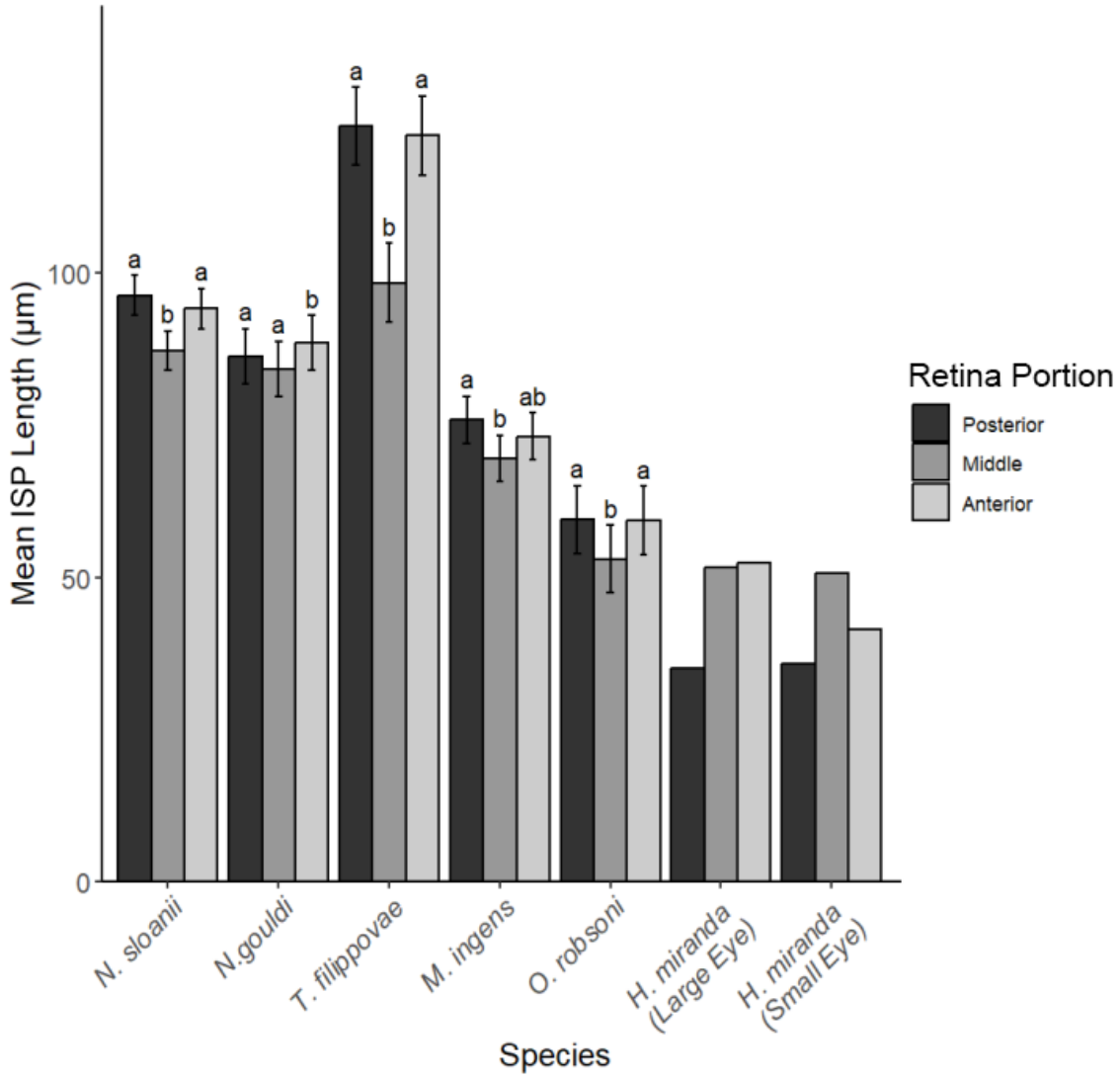


Figure 15. *The mean ISP length of the posterior, middle and anterior retinal segments for each species. Eye segments within a species sharing the same letter do not have significantly different OSP lengths (p > 0.05). Analyses were conducted by mixed-effect linear model and Tukey's HSD post-hoc tests for pairwise comparisons. Post-hoc test statistics and probability metrics comparing ISP lengths from different eye segments are listed in the Appendices (Appendix A, Table XI). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 9),* O. robsoni *(n = 4), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1). Error bars represent the standard error of the mean.*

The ISP lengths were averaged across the entire retina and compared among species. Mixed-effect linear modelling and Tukey's HSD post-hoc tests identified significant differences in ISP length (Fig. 16). *T. filippovae* had the longest ISP (113.81 ± 6.12µm) but was not significantly different to *N. sloanii*. *N. sloanii* (92.05 ± 3.10) and *N. gouldi* (86.11 ± 4.31µm) had significantly longer ISP than *O. robsoni* (57.51 ± 5.31µm), while the mean ISP length of *M. ingens* (72.44 ± 3.69 µm) was statistically similar to *N. gouldi* and *O. robsoni*. The large eye (46.18 ± 10.71µm) and small eye (42.73 ± 10.79µm) of *H. miranda* had ISP lengths similar to *A. dux* (49.11 ± 12.16 µm). *T. danae* had the shortest ISP (20.67 ± 12.16µm). Post-hoc test statistics and probability metrics comparing ISP lengths among species are listed in the Appendices (Appendix A, Table XII).

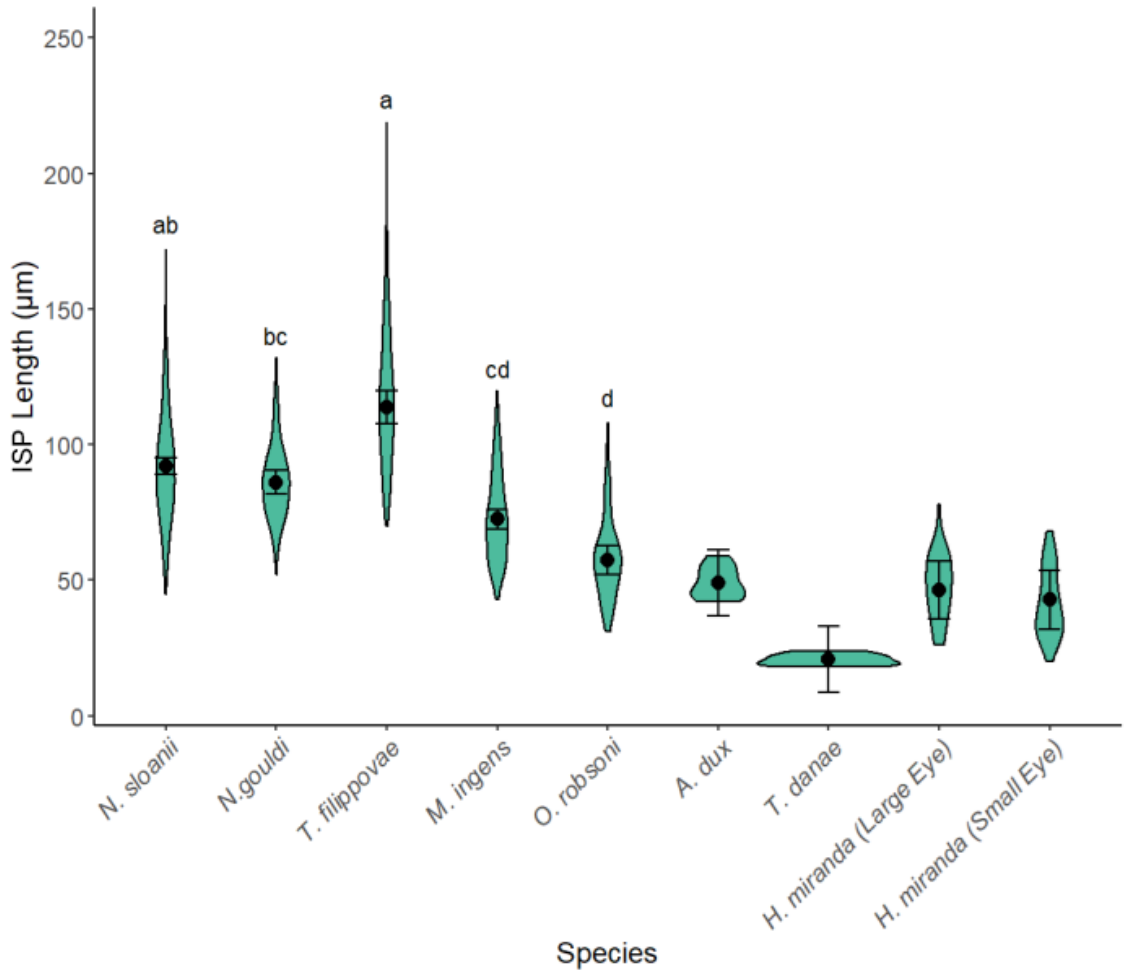


Figure 16. *Mean ISP length across the entire retina for each species. Black dots represent the ISP mean and error bars represent the standard error of the mean (SEM). Species sharing the same letter do not have significantly different OSP lengths (p > 0.05). Statistical differences are based on Tukey's HSD post-hoc test following a mixed-effect linear model. Post-hoc test statistics and probability metrics are listed in the Appendices (Appendix A, Table XII). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 9),* O. robsoni *(n = 4), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1).*

The mean ISP length was calculated by averaging the measurements across the entire retina for individual specimens within a species and plotted against ML to determine if a correlation existed (Fig. 17). Among all species analysed, *N. sloanii* had a significant negative correlation between ISP length and ML (Pearson's correlation test, p = 0.045). All other species showed no correlation.

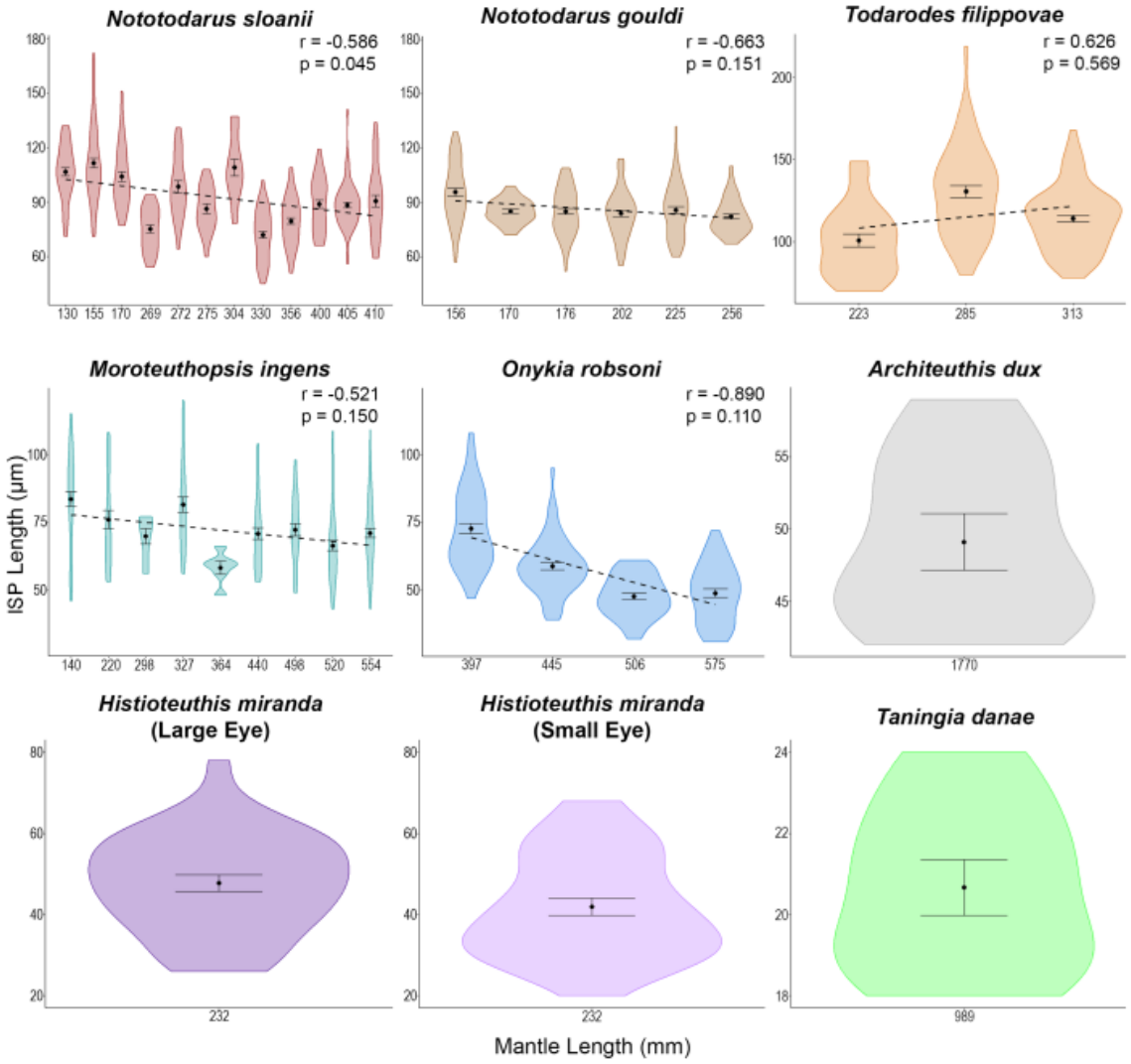


Figure 17. *Violin plots of ISP measurements from individual specimens for all species. The r-values indicate the correlation coefficient from Pearson's correlation tests and the p-values indicate their significance. Only* N. sloanii *showed a significant negative correlation between ISP length and ML. Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 9),* O. robsoni *(n = 4),* A. dux *(n = 1), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1),* T. danae *(n = 1).*

The ISP length for all specimens was compared against ML to determine whether a correlation existed (Fig. 18). A strong negative correlation was observed (Pearson's correlation test, r = -0.523, p = 0.001). However, squids that exhibit gigantism (*A. dux* and *T. danae*) may bias the data towards a negative correlation. Therefore, these specimens were removed, and a new correlation analysis was conducted yet still showed a moderate negative correlation between ML and ISP length (Pearson's correlation test, r = -0.456, p = 0.004; Fig. 19).

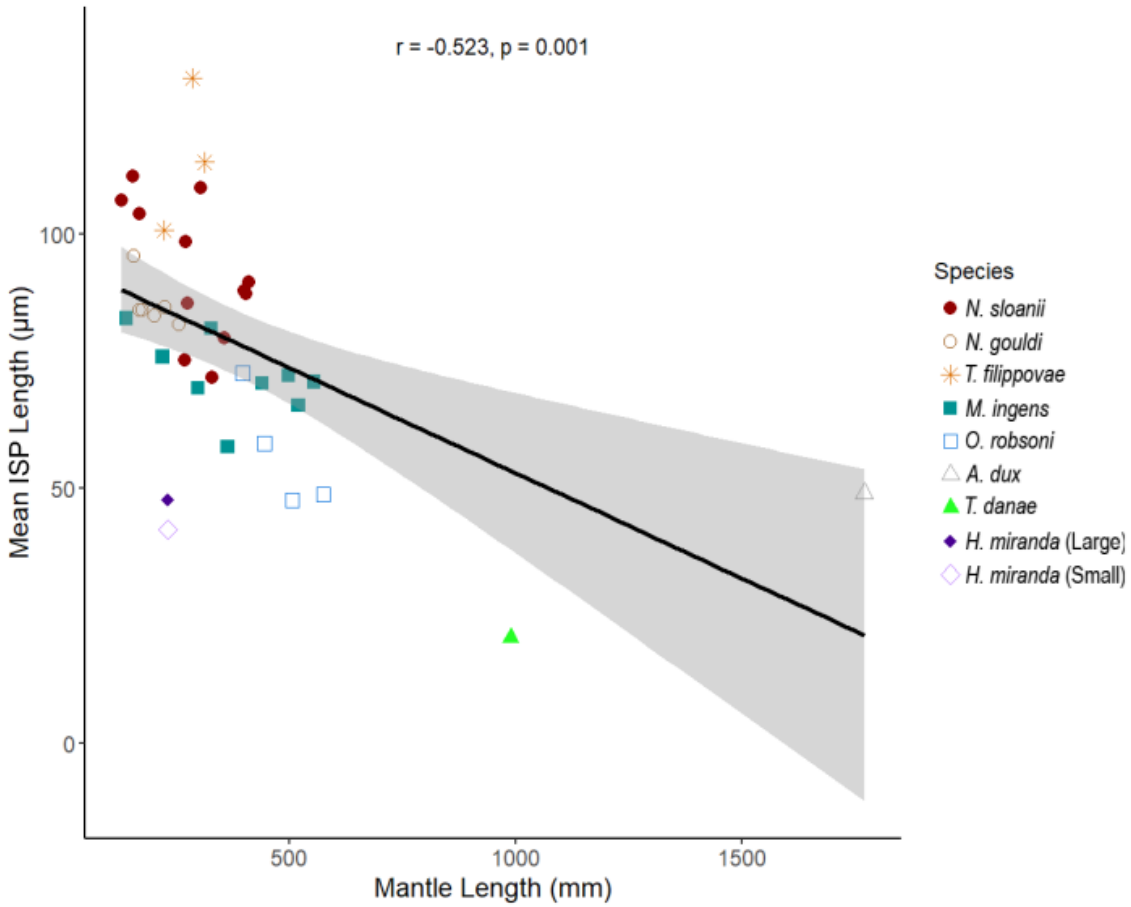


Figure 18. *ISP length of each specimen plotted against their ML. The black line represents the linear model correlation between the two variables (r = -0.523), and the grey area depicts the 95% confidence interval of the linear model (-0.722 < r < -0.244). Pearson's correlation test indicated a significant strong negative correlation between the ISP length and ML (p = 0.001). Sample Sizes: N. sloanii (n = 12), N. gouldi (n = 6), T. filippovae (n = 3), M. ingens (n = 10), O. robsoni (n = 4), A. dux (n = 1), large eye of H. miranda (n = 1), small eye of H. miranda (n = 1), T. danae (n = 1).*

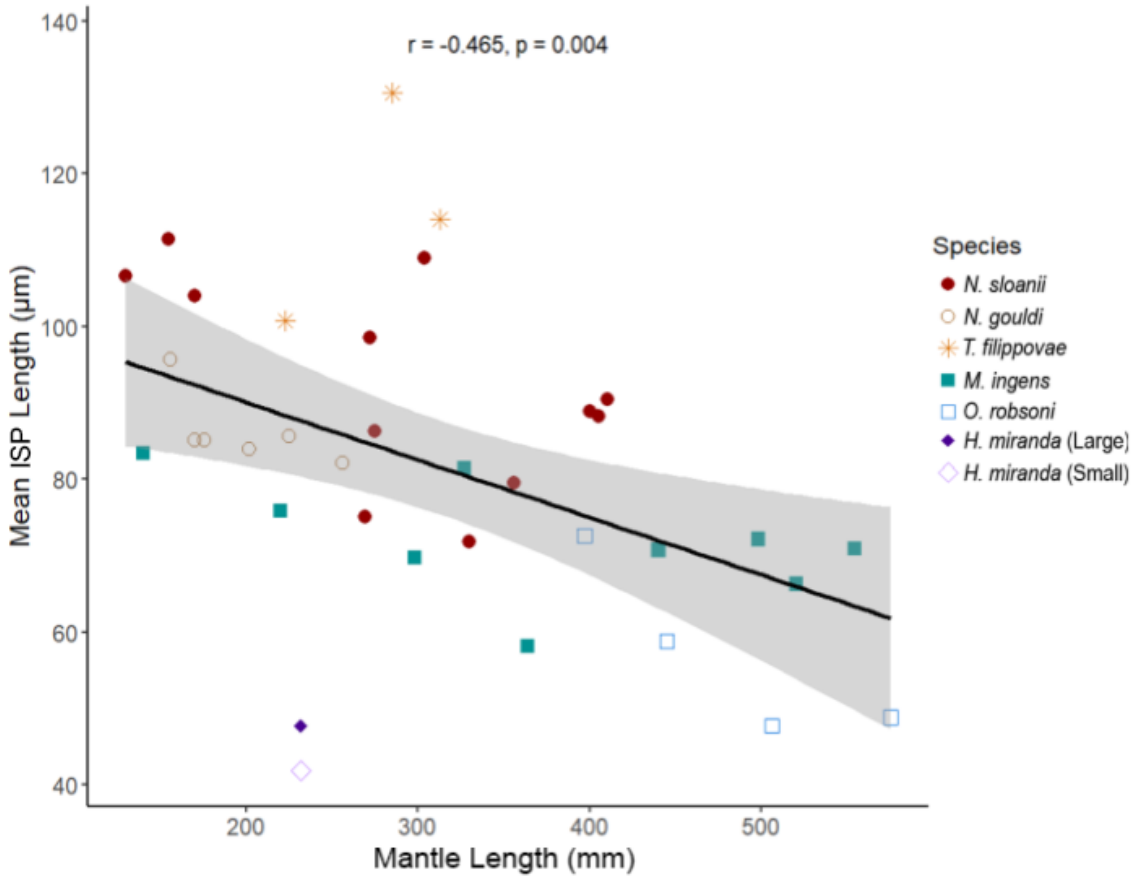


Figure 19. *Mean ISP length of each specimen plotted against their ML after removing specimens that exhibit gigantism (A. dux and T. danae). The black line represents the linear model correlation between the two variables (r = -0.465), and the grey area depicts the 95% confidence interval of the linear model (-0.688 < r < -0.161). Pearson's correlation test indicated a significant moderate negative correlation between the ISP length and ML (p = 0.004). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 10),* O. robsoni *(n = 4), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1).*

The mean ISP length of individuals grouped by depth range categories showed significant variance (one-way ANOVA, $p < 0.001$, Fig. 20). Species that inhabit all light zones (0-1000+ metres) had significantly longer ISP lengths than those in other depth range categories (Tukey's HSD test, 0-1000m, $p = 0.04$; 200-1000m, $p = 0.002$; 200-1000+ metres, $p < 0.001$). Additionally, species that inhabit 0-1000m had significantly longer ISP lengths than the 200-1000m category (Tukey's HSD test, $p = 0.04$) and the 200-1000+ metre category (Tukey's HSD test, $p < 0.001$). There was no significant difference in ISP length between the 200-1000m and 200-1000+ metre categories. However, it is important to note that only *A. dux* represented the 200-1000m depth range category, potentially skewing the post-hoc tests it was included in. Post-hoc test statistics and probability metrics comparing ISP length of among species grouped by depth range categories are listed in the Appendices (Appendix A, Table XII).

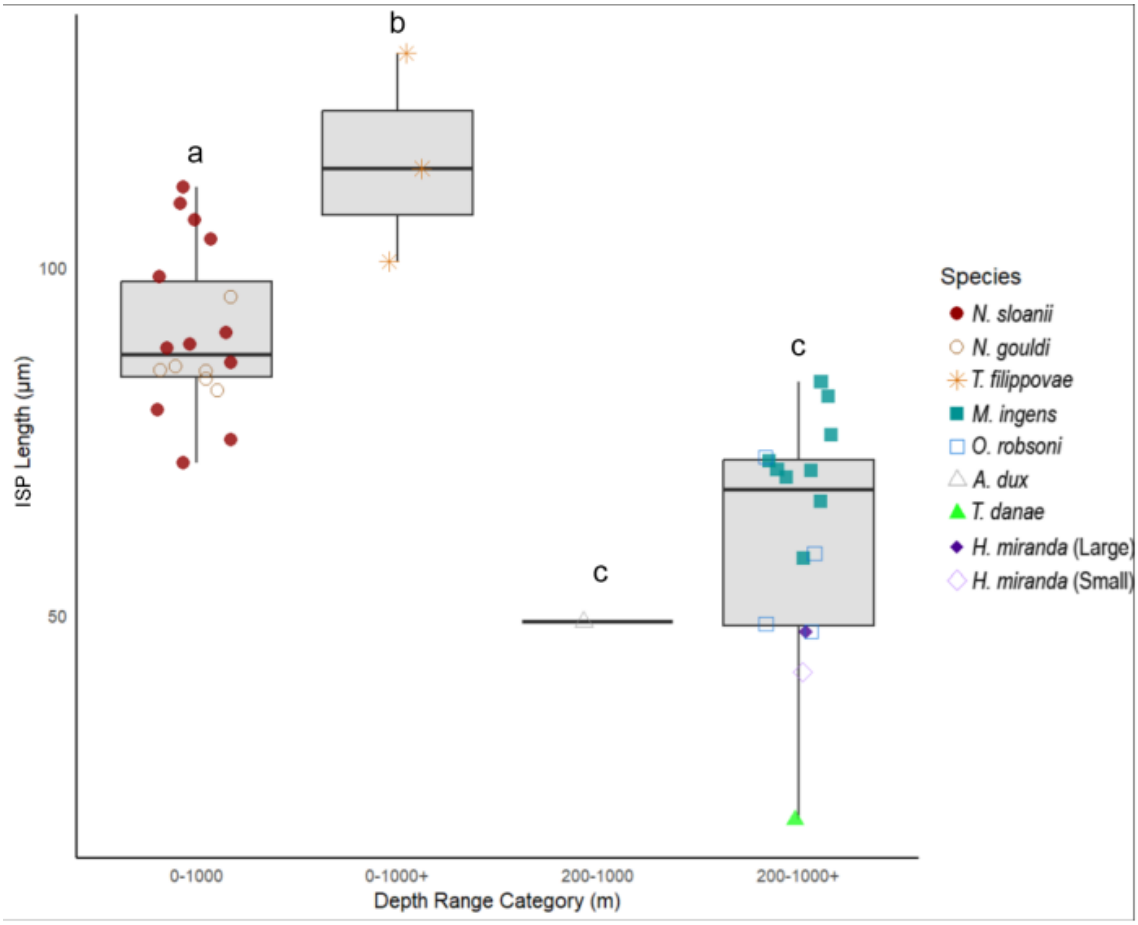


Figure 20. *ISP lengths of species grouped by their inhabited depth. Depth range categories sharing the same letter do not have significantly different OSP lengths ($p > 0.05$). Statistical differences are based on Tukey's HSD post-hoc test following a one-way ANOVA ($p < 0.001$). Post-hoc test statistics and probability metrics are listed in the Appendices (Appendix A, Table XII). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 9),* O. robsoni *(n = 4), A. dux (n = 1), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1),* T. danae *(n = 1).*

2.4.4 Screening Pigment of the Ommin Layer

Screening pigment in the ommin layer (OL) was observed at the basal membrane layer in *N. sloanii*, *T. filippovae*, *M. ingens, O. robsoni*, *A. dux, T. danae* and both eyes of *H. miranda*. Preservation methods and light exposure before fixation often caused the OL to shift or dissolve, limiting the specimens included in the pigment analyses. Mixed-effect linear modelling and Tukey's HSD post-hoc tests showed no significant difference in OL thickness among species (Fig. 21). The large variability in *O. robsoni* is due to the small number of specimens that had OL present after fixation. Post-hoc test statistics and probability metrics comparing OL depth among species are listed in the Appendices (Appendix A, Table XIII).

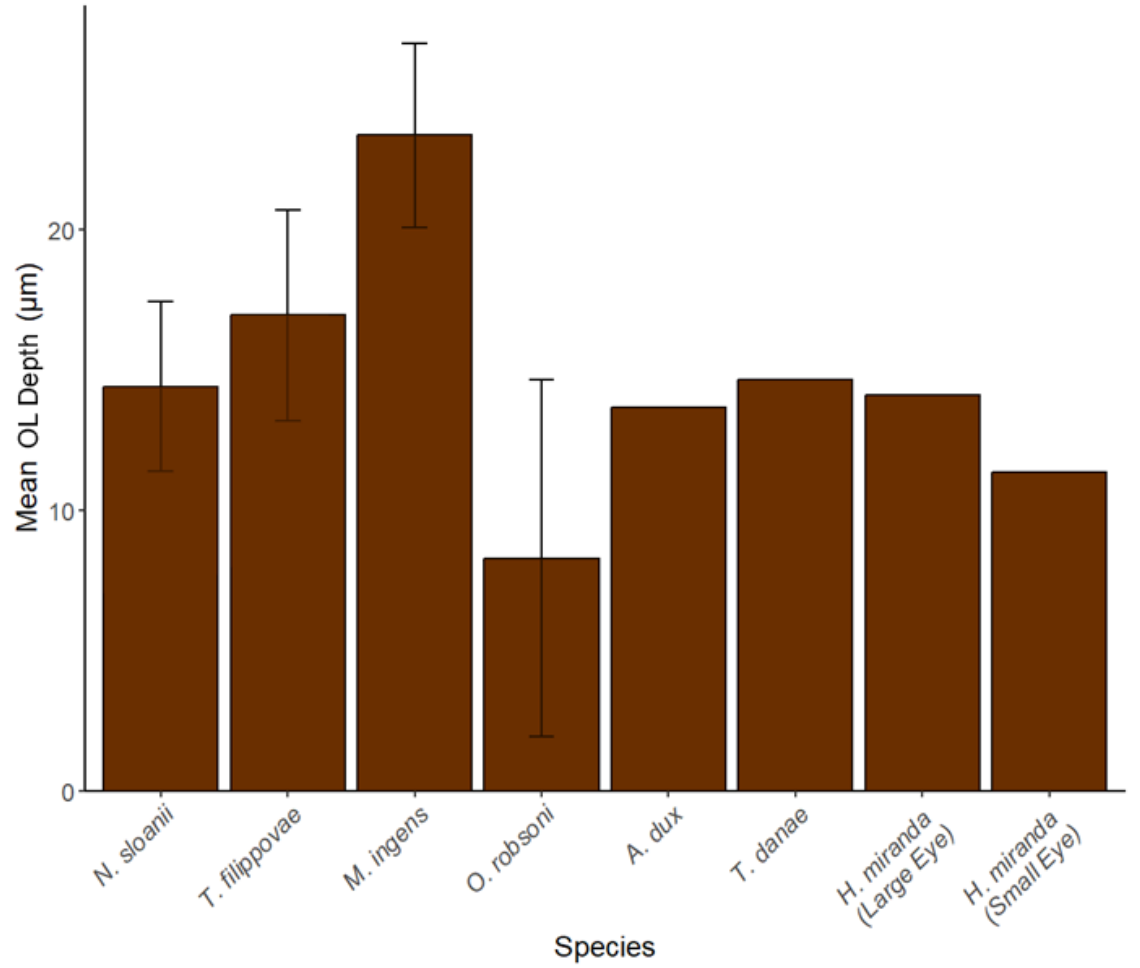


Figure 21. *Comparative analysis of mean OL depth across species. No significant differences were observed among species (linear mixed-effects model, Tukey's HSD post-hoc test). Error bars represent the standard error of the mean. Post-hoc test statistics and probability metrics comparing OL depth among species are listed in the Appendices (Appendix A, Table XIII).*

2.4.5 Total Photoreceptor Length

The OSP and ISP lengths were measured to calculate the mean total photoreceptor length for each species (Table 4). The depth of the OL was also measured but was included as part of the base of the OSP. *Todarodes filippovae* had the longest photoreceptors and largest variability in photoreceptor length (407.02 ± 95.31µm), while *T. danae* had the shortest photoreceptors and the smallest variability in photoreceptor length (272.00 ± 9.52µm). All other species had a mean total photoreceptor length between 300 and 400µm.

Table 4. *The mean value of the photoreceptor layers and total photoreceptor length for all species (± SD). Note that no OL was observed in any* N. gouldi *specimens.*

Species	Photoreceptor Length (µm)	OSP Length (µm)	ISP Length (µm)	OL Depth (µm)
N. sloanii	301.91 ± 36.19	209.74 ± 29.56	92.17 ± 20.88	17.76 ± 7.40
N. gouldi	284.00 ± 29.38	197.51 ± 25.92	86.48 ± 13.84	-
T. filippovae	407.02 ± 95.31	289.90 ± 91.98	117.12 ± 24.99	16.38 ± 3.13
M. ingens	307.48 ± 45.75	234.16 ± 42.73	73.32 ± 16.35	23.41 ± 8.52
O. robsoni	330.81 ± 57.56	272.59 ± 55.57	58.22 ± 15.00	± 1.31
A. dux	300.78 ± 15.06	251.67 ± 11.67	49.11 ± 5.88	13.67 ± 1.80
T. danae	272.00 ± 9.52	251.33 ± 7.91	20.67 ± 2.06	14.67 ± 1.87
H. miranda (Large Eye)	341.48 ± 71.72	293.73 ± 70.22	47.75 ± 12.17	13.78 ± 3.69
H. miranda (Small Eye)	327.51 ± 53.39	285.59 ± 51.85	41.92 ± 12.76	11.36 ± 3.15

The mean total photoreceptor lengths were compared among species using mixed-effect linear modelling and Tukey's HSD post-hoc test for pairwise comparisons (Fig. 22). *Todarodes filippovae* had significantly longer photoreceptors than all other species but showed no significant statistical difference to O. *robsoni. Taningia danae* (n=1) was identified as the specimen with the shortest photoreceptor, but this was due to the thinner ISP measured in this specimen. Post-hoc test statistics and probability metrics comparing total photoreceptor length among species are listed in the Appendices (Appendix A, Table XIV).

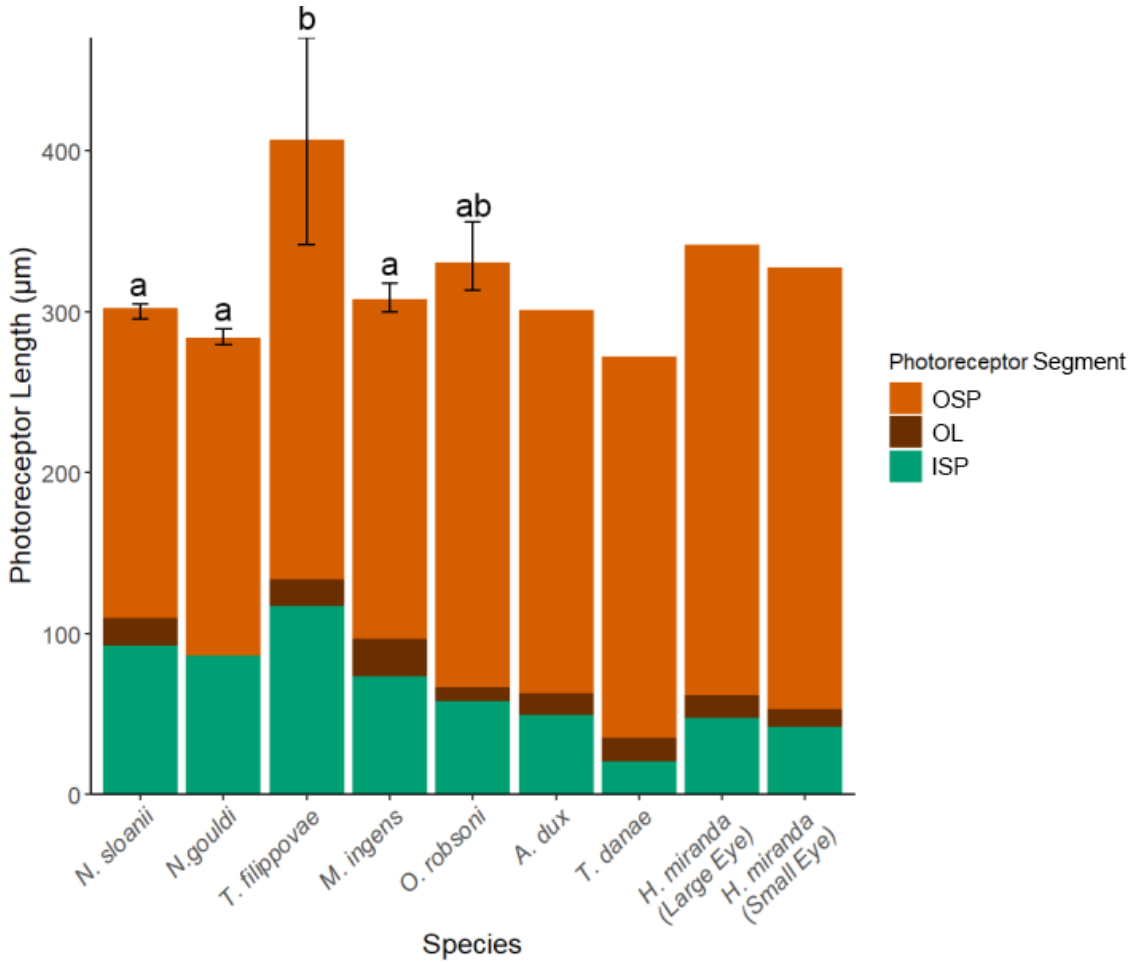


Figure 22. *Mean total photoreceptor lengths in each species. The OL is embedded at the base of OSP; therefore, the OL depth measurements were not included when calculating the total photoreceptor length. Species sharing the same letter do not have significantly different total photoreceptor lengths (p > 0.05). Analyses were conducted by mixed-effect linear model and Tukey's HSD post-hoc tests for pairwise comparisons.* T. filippovae *had the longest total photoreceptor length but showed no significant difference to the total photoreceptor length of* O. robsoni*. Error bars represent the standard error of the mean of the total photoreceptor length. Post-hoc test statistics and probability metrics comparing total photoreceptor lengths among species are listed in the Appendices (Appendix A, Table XIV). Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 10),* O. robsoni *(n = 4),* A. dux *(n = 1), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1),* T. danae *(n = 1). Abbreviations: outer segment of the photoreceptors (OSP); ommin layer (OL); inner segment of the photoreceptors (ISP).*

To determine the relative lengths of each photoreceptor layer within a species, each layer length (OSP, ISP, OL) was converted to a percentage of the mean total photoreceptor length (Table 5 and Fig. 23). *Taningia danae* had the longest relative OSP compared to its total photoreceptor length (92.40%) and shortest relative ISP (7.60%). *Nototodarus gouldi* had the shortest relative OSP compared to its total photoreceptor length (69.55%) and the longest relative ISP (30.45%). The percent of total photoreceptor length occupied by the OL layer (in those specimens it was visible) remained fairly consistent among species and ranged from 2.51% (*O. robsoni)* to 7.61% (*M. ingens*).

Table 5. *Relative percentage values of each photoreceptor layer across species. The OSP% and ISP% make up 100% of the total photoreceptor length. The OL depth percentage is the proportion of the total photoreceptor length covered by the OL. Note that no OL was visible in any* N. gouldi *specimens.*

Species	OSP Length %	ISP Length %	OL Depth %
N. sloanii	69.44 ± 4.96	30.56 ± 4.96	6.66 ± 2.70
N. gouldi	69.46 ± 4.52	30.54 ± 4.52	-
T. filippovae	71.78 ± 5.09	28.22 ± 5.09	3.91 ± 0.91
M. ingens	75.51 ± 5.56	24.49 ± 5.56	8.74 ± 3.73
O. robsoni	82.71 ± 3.31	17.29 ± 3.31	2.62 ± 0.47
A. dux	83.70 ± 1.49	16.30 ± 1.49	4.57 ± 0.74
T. danae	92.41 ± 0.55	7.59 ± 0.55	5.38 ± 0.57
H. miranda (Large Eye)	86.41 ± 2.94	13.59 ± 2.94	4.66 ± 1.31
H. miranda (Small Eye)	87.24 ± 2.41	12.76 ± 2.41	3.59 ± 1.21

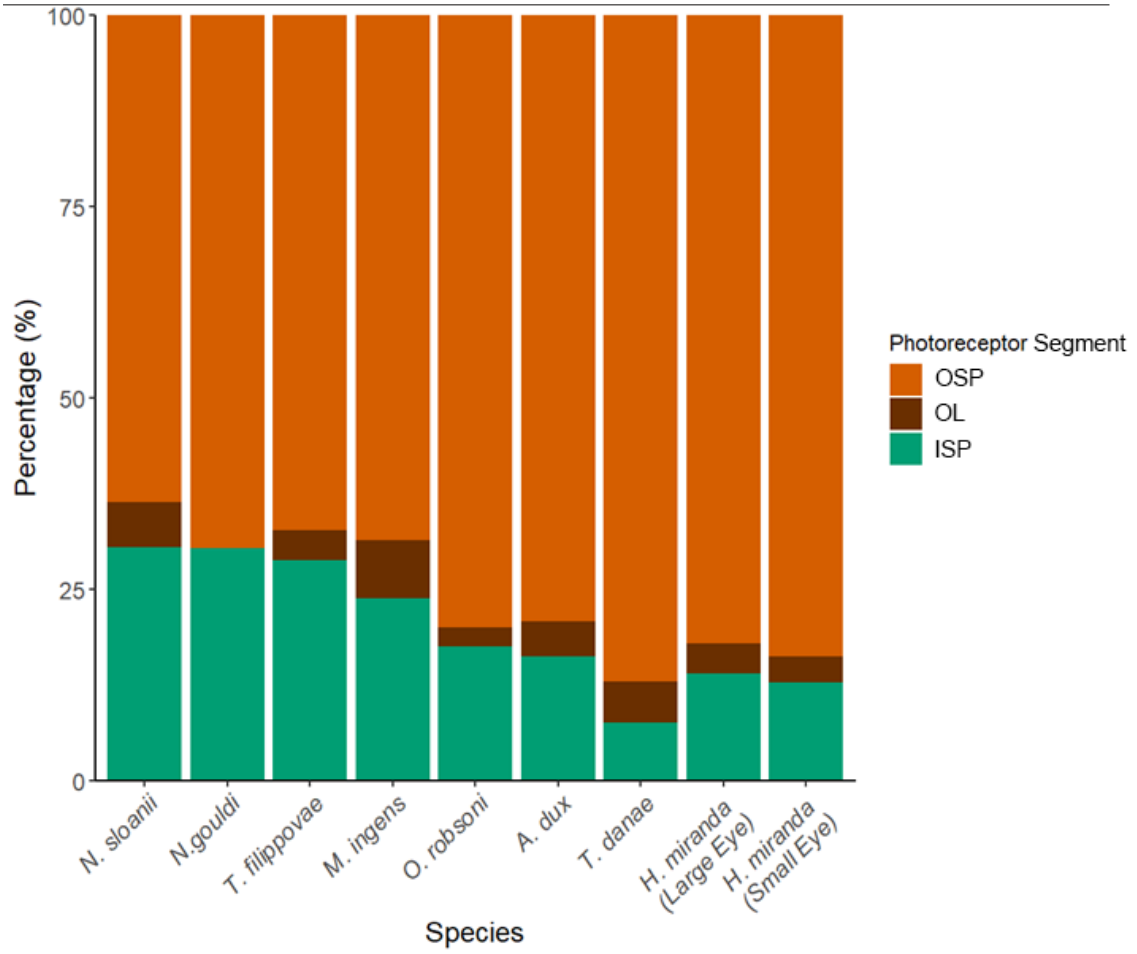


Figure 23. *The relative coverage of photoreceptor layers as a percentage of the total photoreceptor length. The OSP% and ISP% make up 100% of the total photoreceptor length. OL depth percentage is the proportion of the total photoreceptor length. Sample Sizes:* N. sloanii *(n = 12),* N. gouldi *(n = 6),* T. filippovae *(n = 3),* M. ingens *(n = 10),* O. robsoni *(n = 4),* A. dux *(n = 1), large eye of* H. miranda *(n = 1), small eye of* H. miranda *(n = 1),* T. danae *(n = 1). Abbreviations: outer segment of the photoreceptors (OSP); ommin layer (OL); inner segment of the photoreceptors (ISP).*

2.5 Discussion

The primary objective of this study was to characterise the retinal morphology of eight deep-sea oegopsid squids. This goal was divided into four aims: (1) to assess photoreceptor length variability among adult squids and determine whether photoreceptor growth continues during adulthood; (2) to map the variation in photoreceptor length across the retina and infer correlation with light sensitivity; (3) to investigate the relationship between inhabited depth and photoreceptor length; and (4) to identify any morphological modifications to the retina that may enhance vision in the deep sea, including the presence or absence of screening pigment.

2.5.1 Photoreceptor Growth in Adult Oegopsids

Previous research by Evans et al. (2015)-demonstrated that the ‘glass’ (cranchiid) squid *Teuthowenia pellucida* exhibits accelerated growth of the OSP when transitioning from a near-surface juvenile stage to a deeper-dwelling adult stage. Since the OSP contains visual pigments, its elongation likely enhances light sensitivity in darker

environments. Similar habitat-driven modifications to photoreceptors have been widely documented in other deep-sea organisms, including whales, fishes, and crustaceans (de Busserolles et al., 2014; Frank, 2017; Shand et al., 1999). However, whether photoreceptor growth persists into adulthood in squids remains unknown.

This study, limited to adult specimens, suggests that OPL length does not scale linearly with ML within this life stage, suggesting that significant photoreceptor growth occurs primarily during early developmental stages and slows in adulthood. Disproportionate elongation likely coincides with critical life stages, such as habitat shifts, rather than ongoing growth throughout life (Evans et al., 2015). When comparing OSP length to ML across species, no correlation was found but the absolute length of the photoreceptors in some of the smaller species were longer than those in the 'giants'. This result is somewhat surprising given the exceptionally large eyes of species like *A. dux* and *T. danae*, which could theoretically accommodate longer photoreceptors. Instead, these species appear to maximize light capture by enlarging the eye itself (Nilsson et al., 2012), containing more photoreceptors and allowing more light to reach them rather than relying on increased photoreceptor length. Excessive photoreceptor elongation could result in diminishing returns, as light may fail to saturate the entire OSP, creating an unnecessary energetic burden. Since cephalopods already devote substantial neural resources to vision, as evidenced by their large optic lobes (Nixon & Young, 2003), there may be an upper limit to photoreceptor elongation that balances sensitivity with efficiency.

Notably, *N. sloanii* was the only species to exhibit a moderate correlation between ISP length and ML, with larger individuals having shorter ISPs. A similar trend was observed across all studied species. Since the ISP houses mitochondria, a shorter ISP could indicate reduced energetic demands, as suggested by de Busserolles et al. (2014). However, it remains unclear why larger squids would require less energy for vision. Theoretically, larger individuals should require more energy for signal processing due to increased light capture. Further investigation is needed to clarify this relationship.

The results suggest that photoreceptor growth slows as OSP length does not appear to continue growing in adulthood, while ISP length may be shorter in larger squids. However, due to small sample sizes for species such as *T. filippovae* and *O.* robsoni, further analyses are required before drawing definitive conclusions.

2.5.2 Mapping Photoreceptor Length

The observed distribution of longer OSPs in the dorsal and central retinal regions of most studied species suggests an adaptive advantage for detecting light sources from below and along the horizontal plane. In the open ocean, light is primarily present as either downwelling sunlight from the surface, or bioluminescence, which could be present in any direction. The ventral portion of the retina, which interacts with downwelling light, may not require elongated photoreceptors to detect this relatively stable light source, except during nighttime or rapid depth changes. In contrast, detecting bioluminescence requires rapid response times to perceive moving organisms or spontaneous flashes. Since bioluminescent signals are often easier to detect against a dark background rather than against downwelling light (Warrant & Locket, 2004b),

having longer photoreceptors in the dorsal and central retina may enhance sensitivity to these fleeting signals.

Comparisons of OSP length among posterior, middle, and anterior retinal areas revealed that the middle retina generally exhibited significantly shorter OSPs. Functionally, this may enhance vision in forward and backward directions, aligning with squid locomotion, which relies on jet propulsion for bidirectional movement. Longer OSPs in these regions could improve environmental awareness, aiding both predatory and escape behaviours.

Squids from the family Histioteuthidae provide an intriguing model for retinal studies due to their dimorphic eyes, which have sensitivity to different light sources (Thomas et al., 2017). These squids often adopt a vertical posture, and the position of their large left eye can detect silhouettes against downwelling light, while their smaller right eye is oriented downward and may better perceive bioluminescence (Thomas et al., 2017). Vertical postures have also been suggested to reduce detectability by diving whales that hunt for squids (Hoving & Visser, 2024). Observations of *H. miranda's* OSP length show the longest OSPs in the large eye were located in the middle-central and anterior-central regions, aligning with the expected angle for detecting light from above when the squid is vertically positioned. Conversely, the small eye exhibited its longest OSPs in the posterior-central retina, positioning its most sensitive region to detect bioluminescence from below. This suggests that the left and right retina is specialised for their specific light sources. Although some cephalopods can rotate their eyes independently to maintain a level gaze (Budelmann & Young, 1984; Williamson, 1995), studies suggest that the large eye of *Histioteuthis* remains fixed when the squid is vertically positioned, while no rotation has been observed in the small eye (Thomas et al., 2017). This suggests each eye has evolved a distinct retinal organisation optimized for its specialised function.

2.5.3 Depth-related Variation in Photoreceptor Length

Comparisons of OSP length across species revealed that squids inhabiting deeper waters tend to have longer photoreceptors. This trend aligns with expectations and corroborates previous findings (Chung, 2014; de Busserolles et al., 2014), as extended photoreceptors enhance sensitivity to faint light signals in dark environments. However, *N. sloanii* and *N. gouldi* primarily inhabit the upper 300 m of the water column, a relatively shallow range compared to other studied species (Roper, De Angelis, et al., 2010). All other species examined reach or exceed 1000 m depths, where light is minimal. Interestingly, no significant differences in OSP length were found among these deeper-dwelling species, suggesting an upper limit to photoreceptor elongation, beyond which further increases may not confer additional advantages.

In contrast, ISP length was found to be shorter in species occupying deeper habitats, mirroring trends observed in lanternfish, where inner rod segments are shorter in deeper-dwelling species (de Busserolles et al., 2014). As previously mentioned, a shorter ISP may indicate lower energetic demands for vision. This hypothesis is supported by the finding that *T. filippovae*, *N. sloanii*, and *N. gouldi*—the three species most frequently found near the surface—had longer ISPs, potentially reflecting higher metabolic

demands for processing photic signals in well-lit environments. Additionally, supporting cells in this layer can contribute to the increase in thickness, and the speculation is that, as in vertebrates, the complexity of this layer is associated with a variety of specialised cells for visual signal tuning. In contrast, species residing in the aphotic zone exhibited significantly thinner ISPs, suggesting that energetic efficiency plays a role in deep-sea visual adaptations.

2.5.4 Screening Pigment in the Ommin Layer

Screening pigment in the ommin layer in cephalopods has been documented since the late 19th century (Rawitz, 1891), with early studies primarily focusing on shallow-water species. As research expands into oegopsid squids, it is becoming evident that screening pigment migration is more widespread than previously assumed (Chung, 2014; Evans et al., 2015; Howard et al., 2024). These recent findings suggest that most, if not all, oegopsid squids possess this feature, though its functional role in deep-sea environments remains unclear. In the current study, specimens were fixed under varying light/dark conditions, resulting in some specimens having a more visible OL than others. Additionally, some screening pigment may have been lost during the preservation of the retina. This is likely why no OL was observed in *N. gouldi*, and only a few specimens for other species had visible OLs.

Experimental evidence has confirmed screening pigment migration in juvenile oegopsids that inhabit shallow waters, where pigment movement would help regulate light exposure in fluctuating conditions (Howard et al., 2024). However, whether screening pigment migration persists in adults that inhabit perpetual darkness is unknown. An alternative hypothesis is that the OL plays a role in rhodopsin regeneration, aiding photoreceptor recovery and maintaining sensitivity in low-light environments (Stavenga & Hardie, 2011). If true, this would suggest a broader physiological function for screening pigment beyond light modulation. Future research, particularly in vivo studies of deep-sea oegopsids, is necessary to determine the persistence and adaptive significance of SP migration in adulthood.

2.6 Conclusion

This study provides new insights into the retinal morphology of deep-sea oegopsid squids, highlighting key adaptations related to photoreceptor growth, spatial distribution, depth variation, and screening pigment presence. While photoreceptor elongation appears to be developmentally regulated rather than continuous in adulthood, variations in OSP and ISP length suggest species-specific adaptations to different light environments. It is also worth mentioning that two of the species included in this study were bioluminescent (*H. miranda* and *T.* danae) and only had a sample size of one. Therefore, Future studies should include more bioluminescent species, such as enoploteuthids and cranchiids. Additionally, it would be interesting to investigate species known to inhabit very deep depths – possibly only very deep depths – like *Magnapinna* and *Bathyteuthis*. These findings reinforce the importance of visual specialization in deep-sea squids and underscore the need for further research, particularly on the functional role of screening pigment and the physiological mechanisms underlying retinal modifications.

Chapter 3 – Prelude

3.1 Introduction to Biological Sunglasses in a Deep-Sea Squid: Pigment Migration in the Retina of *Gonatus onyx*

One of the most interesting findings from Chapter 2 – The Retinal Anatomy of Deep-sea Oegopsid Squids of Aotearoa New Zealand, was the presence of ommin pigment granules within the retinas of most of the studied species. Screening pigment has long been known to exist in nearshore and shallow water octopuses and myopsids and has been demonstrated to likely be a mechanism that blocks light in bright ambient conditions. This is understandable for species that are near the surface and are often exposed to bright conditions during daylight hours. However, recent research has indicated that ommin granules are present in the retinas of oegopsid species. Chung (2014) and Evans et al. (2015) both identified screening pigment in deep-sea oegopsids, including species that may not typically be exposed to near-surface light conditions during the day at any point in their lives. Both studies found ommin granules arrested at various positions throughout the outer photoreceptor segments among the individuals examined. This evidence suggests that ommin granules continue to serve a purpose for even deep-sea oegopsids and likely maintain the ability to migrate through the outer photoreceptor layer. However, the migration of ommin pigment in oegopsids has never been experimentally investigated. Therefore, with the help of the Monterey Bay Aquarium Research Institute, juvenile *Gonatus onyx* specimens were exposed to various light conditions to determine whether the screening pigment does indeed migrate in oegopsids, and to assess the neurotransmitters that may help facilitate this process. This chapter is modified (in format only, to align with the rest of this thesis) from the published version (Howard et al., 2024), which appears in its original format in Appendix B.

Chapter 3 – Biological sunglasses in a deep-sea squid: Pigment migration in the retina of *Gonatus onyx*

3.2 Abstract

Migration of ommin pigment granules in response to light exposure has been reported within the retinas of several (mostly coastal) squid species. Here, we investigated whether light/dark exposure induces ommin pigment migration in juvenile individuals of the deep-sea oegopsid squid *Gonatus onyx*. Following light/dark exposure treatments, we collected and processed retinal tissue for histological assessment, comparison of ommin pigment migration between treatments, and identification of neurotransmitter expression. We observed distal migration of the ommin pigment in response to light, varying according to duration of light and dark exposure patterns, as well as associated changes in glutamate, glycine, and gamma aminobutyric acid (GABA) distribution. These findings support the presence and migration of retinal ommin pigment granules as a mechanism of incoming light regulation in the eyes of deep-sea squids.

3.3 Introduction

Eye evolution in animals that are primarily active under dark conditions has followed several strategies. The ability to moderate the intensity of light entering the eye may be particularly important for species exposed to a wide range of light conditions, such as shallow-dwelling organisms exposed to regular day/night cycles, or species that inhabit diverse oceanic layers across their lifespan. One physiological response to changing light conditions, reported in several cephalopods to date, is the migration of ommin pigment granules within the retina, providing a visual screening effect akin to 'biological sunglasses.'

In cephalopods, evidence of ocular screening pigment has existed since the late 19th century (Rawitz, 1891). In 1959, Butenandt (1959) identified the ommochrome pigmentation as ommin in the retinas of two squids (*Todarodes sagitattus* and *Alloteuthis subulata*), the European common cuttlefish (*Sepia officinalis*) and two octopuses (*Octopus vulgaris* and *Eledone moschata*). Daw and Pearlman (1974) showed that in the coastal market squid *Doryteuthis pealeii*, ommin granules could migrate to the distal end of the of the outer retinal layer, providing a filter equivalent to 0.6 log units. This outward migration had a spectral sensitivity equivalent to rhodopsin and occurred in 5–15 minutes, once 3–10% of the rhodopsin had isomerized. After the light stimulus was removed, the pigment granules returned to their original basal position across a further 5–15 minutes. However, if more than 10% of the rhodopsin had isomerized, the screening pigment instead took several hours to fully migrate inward under restored dark conditions. Notably, pigment migration occurred only when light fell directly on the rhabdomeres, and not during illumination of the granules themselves (Hagins & Liebman, 1962). These observations supported the prevailing hypothesis that outward ommin migration occurs during bright ambient light conditions to block intense

light and prevent photoreceptor oversaturation, with a quick return to the ommin layer upon return to darkness.

In cephalopods, screening pigments have mostly been found in better-studied nearshore and shallow-water species, which experience changing light conditions due to daylight cycles (Daw & Pearlman, 1974; Gleadall et al., 1993; Wolken, 1958; Young, 1963). However, screening pigment granules were also recently recognized in the oegopsid (deep-sea) 'glass' squid *Teuthowenia pellucida*, and their position within the retina suggested different stages of light/dark adaptation at the time of specimen preservation (Evans et al., 2015b). In deep-sea squids, migratory pigment granules could provide an advantage during diel vertical migrations, or during descent into deeper layers as they mature. The mechanisms governing pigment migration during dark adaptation are different from the mechanisms controlling migration during light adaptation (Kier & Chamberlain, 1990) and may even differ among species of the same group.

In the present paper, we investigated the timing of ommin pigment migration under dark conditions, and whether glutamate neurotransmitter levels change during dark adaptation believed to be under dopaminergic efferent innervation controlling the retraction of ommin. Enzymes that synthesize glutamate, an excitatory neurotransmitter (the same neurotransmitter used by vertebrate photoreceptors) have been found within the photoreceptors of the hummingbird 'bobtail squid', *Euprymna berryi* (Gavriouchkina et al., 2022). Additionally, glutamate levels in the retina of *Sepia officinalis* can be influenced by the light/dark environment (D'Aniello et al., 2005).

This study investigates retinal screening pigment migration in a pelagic oegopsid squid species primarily found throughout the North Pacific. *Gonatus onyx*, known as the 'black-eyed' squid, is the most encountered gonatid off California, and is frequently observed at depths of 375–925 m (maximum 1975 m) by remotely operated vehicles operating in the Monterey Canyon (*MBARI's Deep Sea Guide - Data Artifacts for Gonatus Onyx*, 2018). Like many deep-sea oegopsids, *G. onyx* inhabits deeper oceanic strata as it matures (Hunt & Seibel, 2000); females have been observed brooding egg masses at depths of 1250–2500 m (Hunt & Seibel, 2000; Seibel et al., 2005). This species therefore experiences a range of light conditions across its lifespan (and potentially across shorter timeframes), prompting us to investigate the possibility of screening pigmentation migration in a cohort of juveniles under a controlled series of light/dark exposures.

3.4 Materials and Methods

3.4.1 Specimen Acquisition

Live juvenile gonatid squid (*Gonatus onyx*, ML 10–20 mm; Fig. 24) were trawled after sundown at a depth of 350 m by the Monterey Bay Aquarium Research Institute (MBARI) aboard the RV *Western Flyer* over the Monterey Canyon (36°41.4–42.8'N, 122°10.3–13.3'W for individuals G01, G12-G13; 36°32.3–35.4'N, 122°31.8–35.3'W for G02–G11) in March 2013. Thirteen individuals were used in this study.

Figure 24. *Juvenile 'black-eyed' squid, Gonatus onyx, feeding on a myctophid. © Monterey Bay Aquarium Research Institute. Scale bar = 1 cm.*

3.4.2 Experimental Design

After net retrieval, the 13 juveniles were sorted while exposed to ambient light under bright fluorescent bulbs (F24T12 35W linear fluorescent tube, 4200 k, 380-760 nm, OSRAM Sylvania, Wilmington, NC, USA) with an illuminance of 350 lux measured with a LX-103 Digital Light Meter (Lutron Electronic Enterprise, Taipei City, Tawain). During experiments, each specimen was sorted from the trawl catch and placed into a small container of seawater, in which it was exposed to light for a total (including sorting time) of 20, 30 or 90 min, and subsequently moved into a lightless room for variable periods of time. Of the 13 juvenile G. onyx processed under light, 11 specimens were selected to assess the migration and return of the pigment granules within the retina after subsequent dark adaptation. The remaining two specimens were used to identify the presence of the neurotransmitter glutamate in the retina (Table 6).

Table 6. *List of* G. onyx *identification numbers (ID) with corresponding mantle lengths (ML), light-exposure and dark-adaptation times for each specimen and their use in the analysis of pigment migration (PM) or neurotransmitter expression (NE). G02-G06 were grouped together for PM analysis (Group 1) and G07-G11 were in Group 2. G01 served as the negative control (NC) for PM analysis.*

ID	ML (mm)	Analysis Conducted	Light Exposure (min)	Dark Adaptation (min)
G01	30	PM, NC	90	1440 (24 hrs)
G02	15	PM, Group 1	20	15
G03*	15	PM, Group 1	20	30
G04	15	PM, Group 1	20	45
G05	15	PM, Group 1	20	60
G06	15	PM, Group 1	20	75
G07	10	PM, Group 2	30	20
G08	10	PM, Group 2	30	30
G09*	20	PM, Group 2	30	40
G10	10	PM, Group 2	30	50
G11	10	PM, Group 2	30	60
G12	15	NE	30	90

G13	15	NE	30	0

*G03 and G09 were excluded from analysis

3.4.3 Pigment Migration

Five individuals were exposed to 350 lux of fluorescent lighting for 20 min (G02-G06, Group 1), while five individuals were exposed to the same light for 30 min (G07-G11, Group 2). The light-exposure paradigm was determined by the time required to sort the catch. The 10 min difference in processing time provided an opportunity to compare pigment migration differences; an extra 10 min light exposure could have a significant impact according to a previously reported dark/light adaptation study (Daw & Pearlman, 1974). After light exposure, specimens were then placed into individual containers within a darkened cold room (<60 lux), with one individual euthanized at 15 min and every 15 min interval thereafter until 75 min (Group 1) or after 20 min and every 10 min thereafter until 60 min (Group 2). Two specimens appeared moribund at the time of euthanasia and were subsequently removed from the results (Group 1, 30 min dark adaptation, G03; Group 2, 40 min dark adaptation, G09).

Specimens were immediately fixed whole after euthanasia in 5% buffered formalin to preserve the position of ommin pigment granules. Later, a single eye from each fixed specimen was removed and fixed again in 4% formaldehyde in phosphate buffer solution (PBS) for 30 min, rinsed in PBS and stored until sectioning. The contralateral eye was fixed in glutaraldehyde. Unfortunately, glutaraldehyde fixation did not preserve the ommin pigment well, and these samples were not processed further.

One specimen (G01, ML 30 mm) served as the negative control for pigment migration analysis; this individual was exposed to 90 min of light at 350 lux, then dark adapted for 24 h, then euthanized. However, the PFA fixed eye was damaged beyond use and only the contralateral eye preserved in 2.5% glutaraldehyde was used, which resulted in the poor preservation of the ommin pigment granules, although some pigment granules survived in the negative control.

Prior to sectioning, all formaldehyde-fixed tissues for pigment migration analysis were immersed in 30% sucrose solution for at least 24 h before embedding them in optimum-cutting-temperature medium and sectioning at 12 µm thickness using a cryostat (Leica CM3050 S, Wetzlar Germany). Dorsal, central, and ventral sections were obtained from the specimen that dark adapted for the longest duration (G06, 20 min light/ 75 min dark) and from the specimen with the most exposure to light and shortest dark adaptation (G07, 30 min light/20 min dark). For comparison among individuals in a group, vertical sections were obtained from the retina's central region. All samples were stained with toluidine blue.

3.4.4 Neurotransmitter Expression

To determine whether changes in neurotransmitter presence are discernible between different dark-adaptation paradigms (i.e., less than a day of dark adaptation), two juvenile squids (*G. onyx*, G12 and G13, ML 15 mm) were exposed to 30 min of light at 350 lux. G13 was immediately euthanized after light exposure with no subsequent

dark adaptation. After light exposure, G12 was placed in a darkened container for 90 min to allow for partial or full dark adaptation of the retina. The eyes were then preserved in conditions identical to those for specimens analysed for pigment migration. Samples were placed in 2.5% glutaraldehyde with 1% formaldehyde in PBS for 1 h and dehydrated in an ethanol series of increasing concentration. Fixation in glutaraldehyde rendered most of the ommin pigment granules unperceivable. These glutaraldehyde-fixed samples were then processed for epoxy resin (EPON) embedding following a standard protocol (Kalloniatis et al., 2013; Marc et al., 1990) and placed in a 3:1 resin:acetone mixture overnight. Next, the tissues were submerged in 100% EPON and placed in an oven at 60°C for at least 24 h to polymerize the resin. The resin-embedded samples were cut into 250–500 nm thick vertical sections using a glass knife (made with a Leica EM KMR2, Wetzlar, Germany) and an ultramicrotome (Leica Ultracut UCT, Wetzlar, Germany). Sections were placed on Teflon-coated slides and processed for immunogold labelling of glutamate.

The retinas were prepared for the identification of neurotransmitters following the silver-intensified immunogold-labelling procedure described by Marc et al. (Acosta & Kalloniatis, 2005; Marc et al., 1990). Single consecutive 250 nm sections were collected in separate wells and labelled with antibodies. The primary antibody, rabbit anti-glutamate (1:1000, ab9440, Abcam, Cambridge, UK) was used. Controls for each treatment were produced by taking retinal sections and processing them under the same conditions as the experimental samples, excluding the primary antibodies. This allowed assessment of any non-specific staining or background signals present without specific antibody binding. A 1.4 nm Nanogold® conjugate goat anti-rabbit secondary antibody (1:100, #2003; Nanoprobes, New York, NY, USA) was used to detect the primary antibody in a silver-intensified reaction procedure (Acosta & Kalloniatis, 2005; Nivison-Smith et al., 2013).

3.4.5 Imaging

All images were captured with a Leica CTR MIC microscope (Wetzlar, Germany) and a Leica DC 500 camera (Wetzlar, Germany) using either 10×, 20× or 40× objectives. Images were acquired using the same parameters of exposure, contrast and brightness.

3.4.6 Barcoding

Small tissue snips were taken from the mantle of each specimen and stored separately in 95% ethanol. Due to the presence of multiple *Gonatus* species in this region, DNA barcoding (cytochrome c oxidase subunit 1 [CO1]) was used to confirm this material as *G. onyx (*>99% match to other *G. onyx* material from the same region, using the Barcode of Life Database [BOLD] (Ratnasingham & Hebert, 2007). DNA was extracted and prepared at the AUT Lab for Cephalopod Ecology & Systematics (ALCES) following the protocols outlined by Braid & Bolstad (2019), then sequenced at Macrogen, Korea.

3.5 Results

3.5.1 Pigment Migration

The transverse cross-sections of the juvenile *G. onyx* eyes revealed a typical cephalopod eye structure (Fig. 25), consisting of an everted retina with the distal ends of the outer segment of the photoreceptors directed anteriorly (Fig. 25a). A basal membrane and supporting cells separated the outer and inner segments of the photoreceptor cells, while a limiting membrane separated the outer segment of the photoreceptors from the vitreous body (Fig. 25b). In the dark-adapted eye, the ommin layer would be expected to be concentrated at the base of the outer segment of the photoreceptors. In the ventral and dorsal anterior sections of the eye, the retina was supported by a cartilaginous sclera (Fig. 25d), which continued anteriorly to the body of the iris (Fig. 25c).

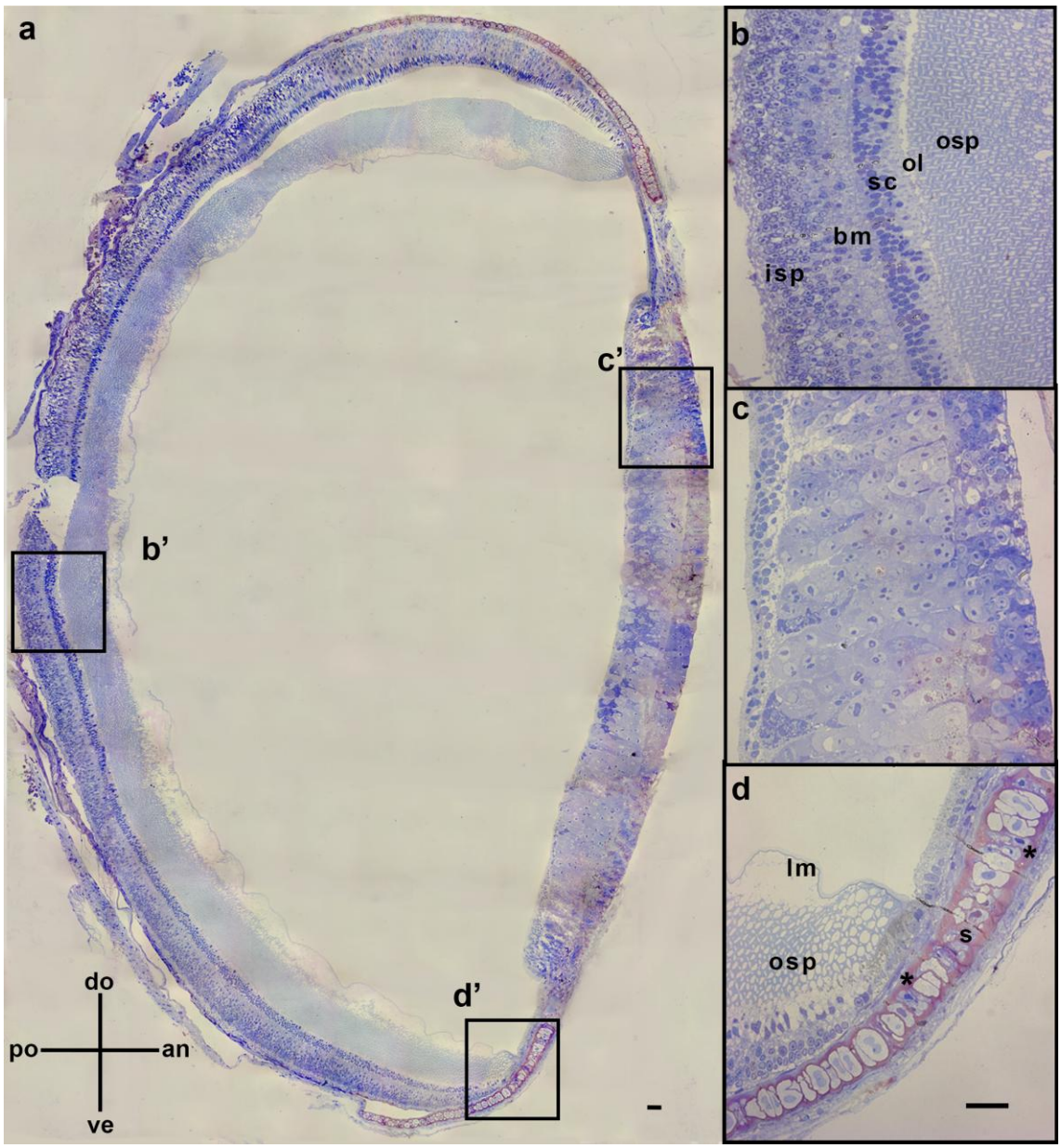


Figure 25. *(a) Transverse cross-section of juvenile* G. onyx *(15 mm ML) eye. (b) Enlarged section of b' depicting retinal layers. (c) Enlarged section of c' depicting the iris. (d) Enlarged section of d' depicting the transition from the retina to the ciliary body. The asterisks indicate the cartilaginous parts of the sclera (s). Ommin granules are not visible due to glutaraldehyde preservation (see Section 3.5). Abbreviations: dorsal (do), ventral (ve), posterior (po), anterior (an), inner segment of photoreceptors (isp), basal membrane (bm), supporting cells (sc), expected location of ommin layer (ol), outer segment of photoreceptors (osp), limiting membrane (lm), sclera (s). Scale bars = 20 µm.*

The photoreceptors were arranged in groups of four, forming rhabdomeres, appearing as a lattice arrangement (Fig. 26). In the negative control specimen (G01), exposed to 90 min light followed by 24 h in darkness, the ommin pigment granules were dispersed throughout the outer photoreceptor layer, with most of the pigment granules found near the base of the ommin layer and in the layer of supporting cells (Fig. 27).

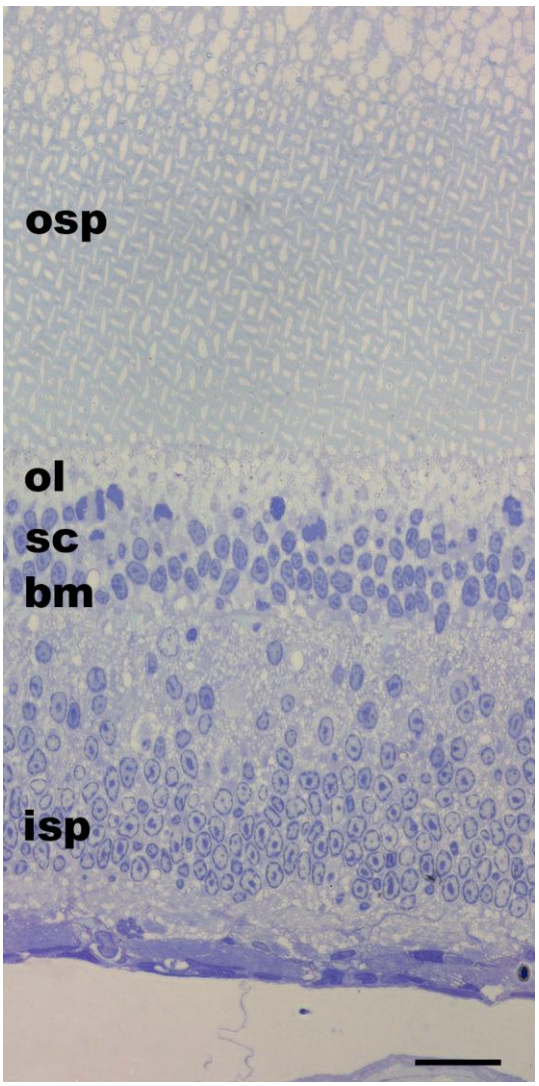


Figure 26. *Toluidine blue staining of a horizontally sectioned juvenile Gonatus onyx retina at the photoreceptor level. The lattice-like arrangement of rhabdomeres in the outer segment is typical of cephalopods. Ommin granules are not visible due to glutaraldehyde preservation (see Section 3.5), but the ommin layer (ol) should be near the base of the outer segment of the photoreceptors (osp). Abbreviations: inner segment of the photoreceptors (isp), basal membrane (bm), supporting cells (sc), expected location of ommin layer (ol), outer segment of the photoreceptors (osp). Scale bar = 20 µm.*

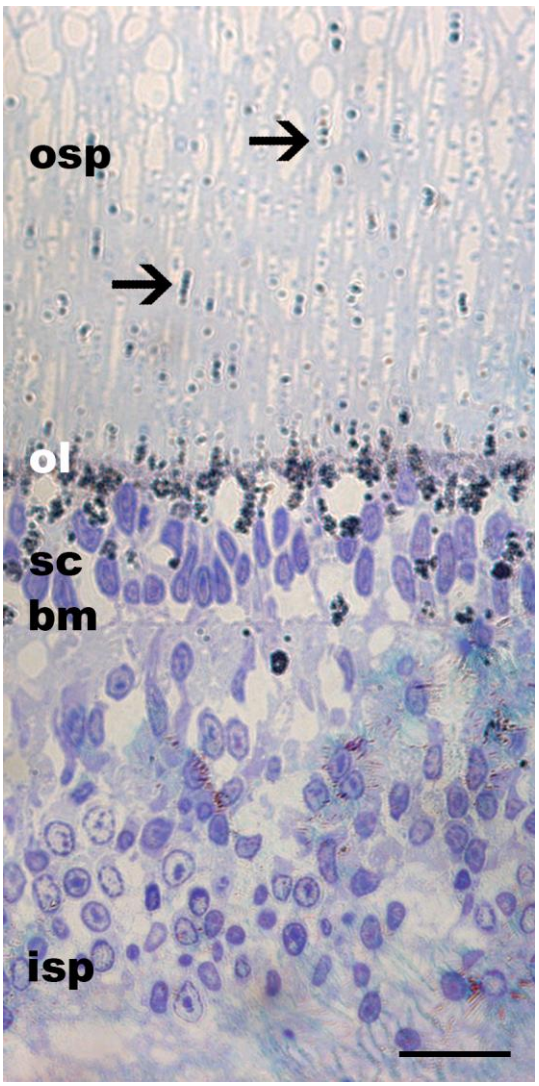


Figure 27. *Ommin pigment location in the retina of a juvenile* G. onyx *exposed to 90 min of light at 350 lux then subjected to darkness for 24 h. The screening pigment of this specimen was preserved, revealing the single-cell resolution and location of ommin pigment granules. No pigment was observed at the distal tip of the photoreceptors. Arrows indicate location of migrating ommin pigment. Abbreviations: inner segment of the photoreceptors (isp), basal membrane (bm), supporting cells (sc), ommin layer (ol), outer segment of the photoreceptors (osp). Scale bar = 20 µm.*

From specimens G06 (20 min light/75 min dark) and G07 (30 min light/20 min dark), the dorsal, central and ventral horizontal sections were obtained to determine whether the return of pigment to the ommin baseline was uniform across the retinal regions.

The specimen exposed to the longest light and shortest dark treatments (G07), showed scattered ommin granules distributed along the photoreceptors after 20 min of dark adaptation (Fig. 28). However, all regions of the retina showed similar stages of pigment return, as nearly all the ommin granules had returned to the baseline.

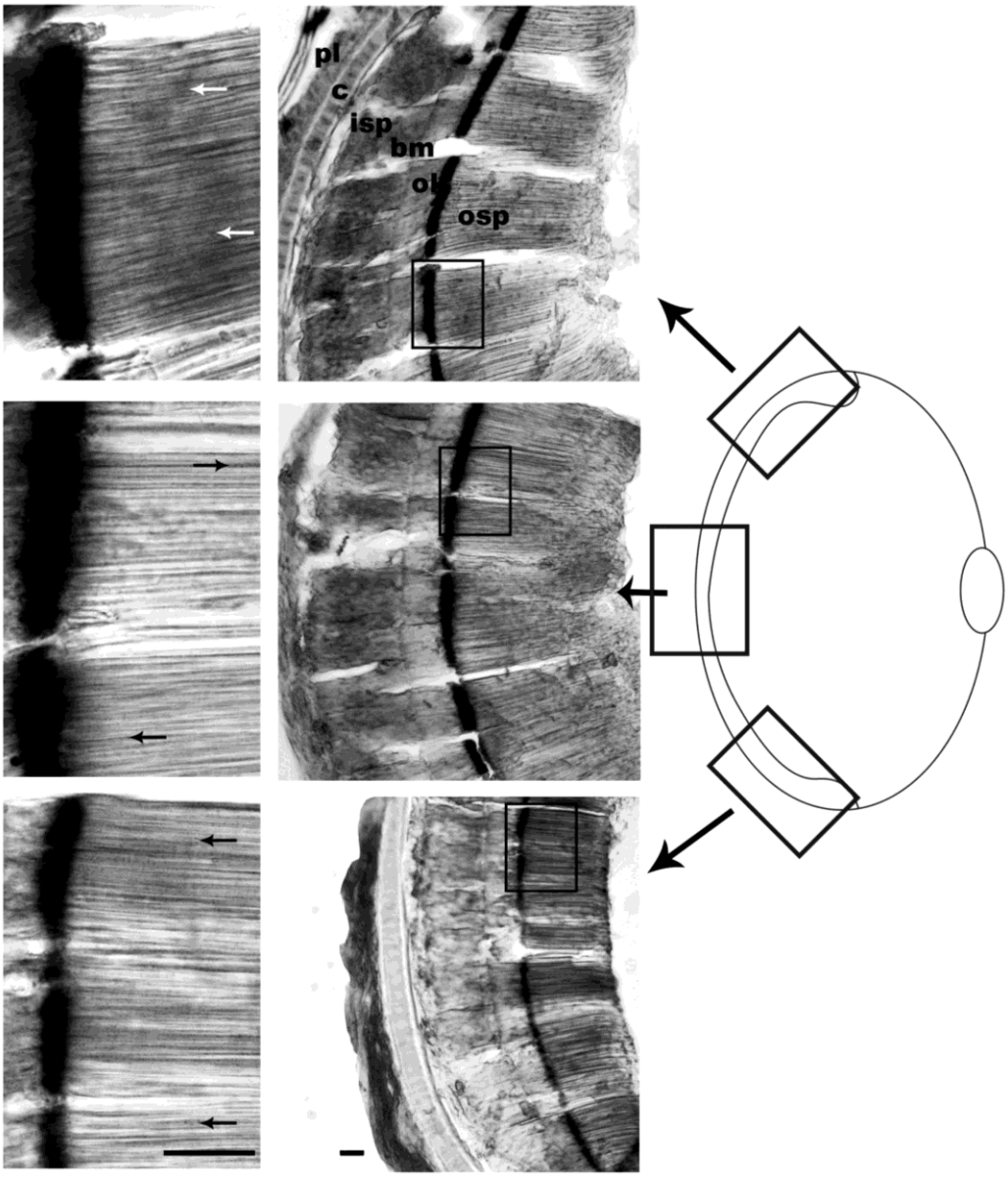


Figure 28. *Ommin pigment location in the specimen exposed to 30 min of light at 350 lux then subjected to darkness for 20 min (G07). The screening pigment of this specimen was preserved, revealing the location of ommin pigment granules close to the basal ommin layer and absent from the tip of the photoreceptors for all retinal sections. The top row of images depicts the dorsal section of the retina, the middle row depicts the central section, and the bottom row depicts the ventral section. Arrows indicate location of migrating ommin pigment. Abbreviations: outer segment of the photoreceptors (osp), ommin layer (ol), basal membrane (bm), inner segment of the photoreceptors (isp), rudimentary cartilage (c), plexiform layer (pl). Scale bars = 20 µm.*

In the two specimens with the longest dark adaptation periods (G06, 75 min, Fig. 29; and the negative control, G01, 24 h, Fig. 27), most of the ommin granules appeared to return to the basal layer, and only a few remained distributed along the photoreceptor length based on the percent length of migration (more in G06 than in G01). The visual observations for each segment of G06 showed that this trend was consistent throughout all the retinal regions.

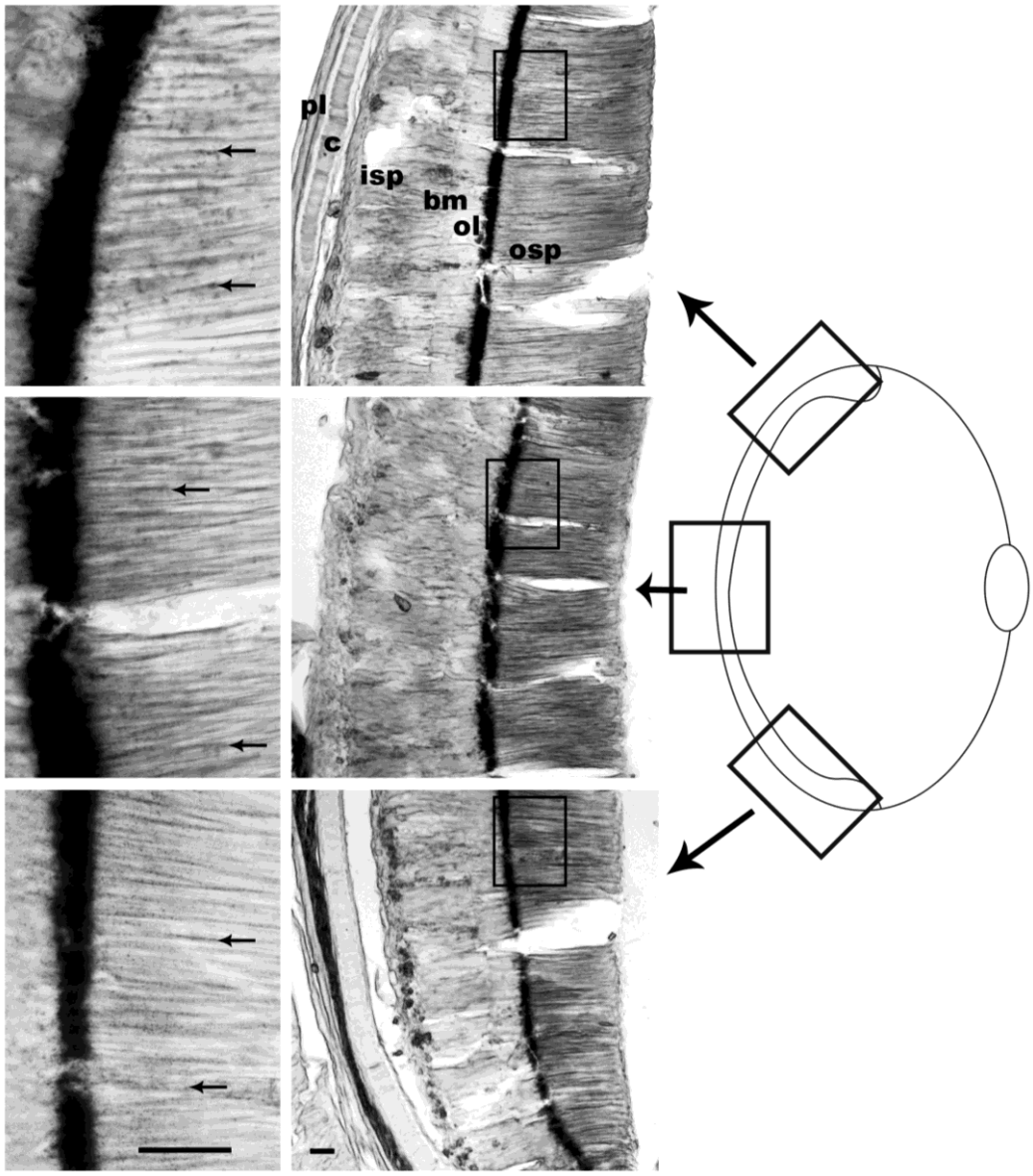


Figure 29. *Ommin pigment location in the* G. onyx *specimen exposed to 20 min of light at 350 lux then subjected to darkness for 75 min. The screening pigment of this specimen was preserved, revealing the location of ommin pigment granules close to the ommin layer and absent from the tip of the photoreceptors. The top row of images depicts the dorsal section of the retina, the middle row depicts the central section, and the bottom row depicts the ventral section. Arrows indicate location of migrating ommin pigment. Abbreviations: outer segment of the photoreceptors (osp), ommin layer (ol), basal membrane (bm), inner segment of the photoreceptors (isp), rudimentary cartilage (c), plexiform layer (pl). Scale bars = 20 µm.*

In the two individuals where the dorsal, central and ventral retinal sections were analysed (G06, G07), the proportions of ommin granules in the basal layer and along the photoreceptor length appeared to be consistent within each eye, regardless of their location.

In the central retinal section of all the experimental samples (G02–G11), a substantial proportion of the ommin pigment granules was concentrated within the basal layer of the photoreceptors (Group 1, Fig. 30; Group 2, Fig. 31). In all the samples, some granules were also distributed along the photoreceptors' length, with some variation observed among individuals.

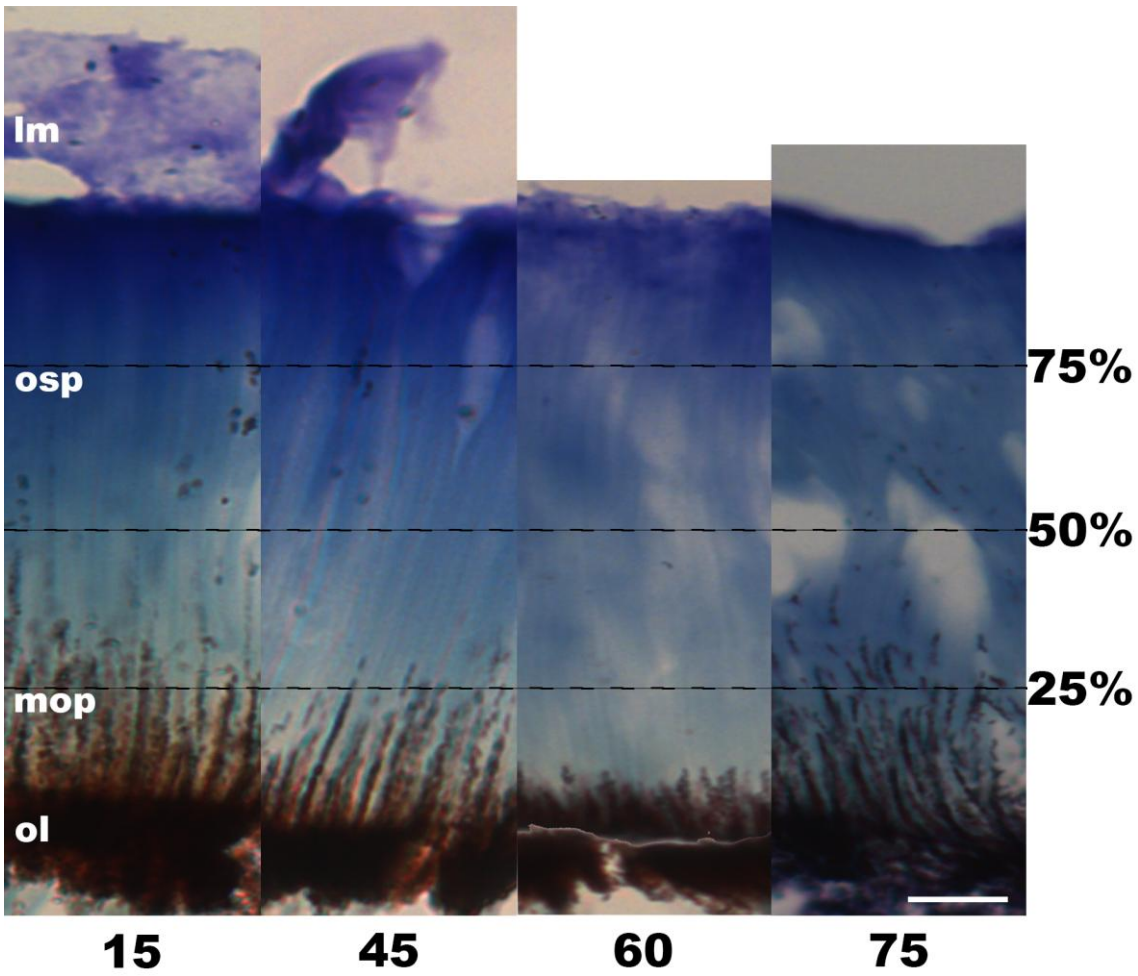


Figure 30. *Ommin pigment granule migration in the outer segment of the photoreceptors of Group 1 juvenile (ML 10–20 mm) G. onyx retinas following exposure to light (350 lux) for 20 min, followed by dark (minutes indicated below each cross-section). Percentage values to the right of the image and dotted lines refer to the outer segment of the photoreceptors. Abbreviations: ommin layer (ol), migrating ommin pigment (mop), ommin-free outer segment of the photoreceptors (osp), limiting membrane (lm). All images depicted at 40× magnification. Scale bar = 20 µm.*

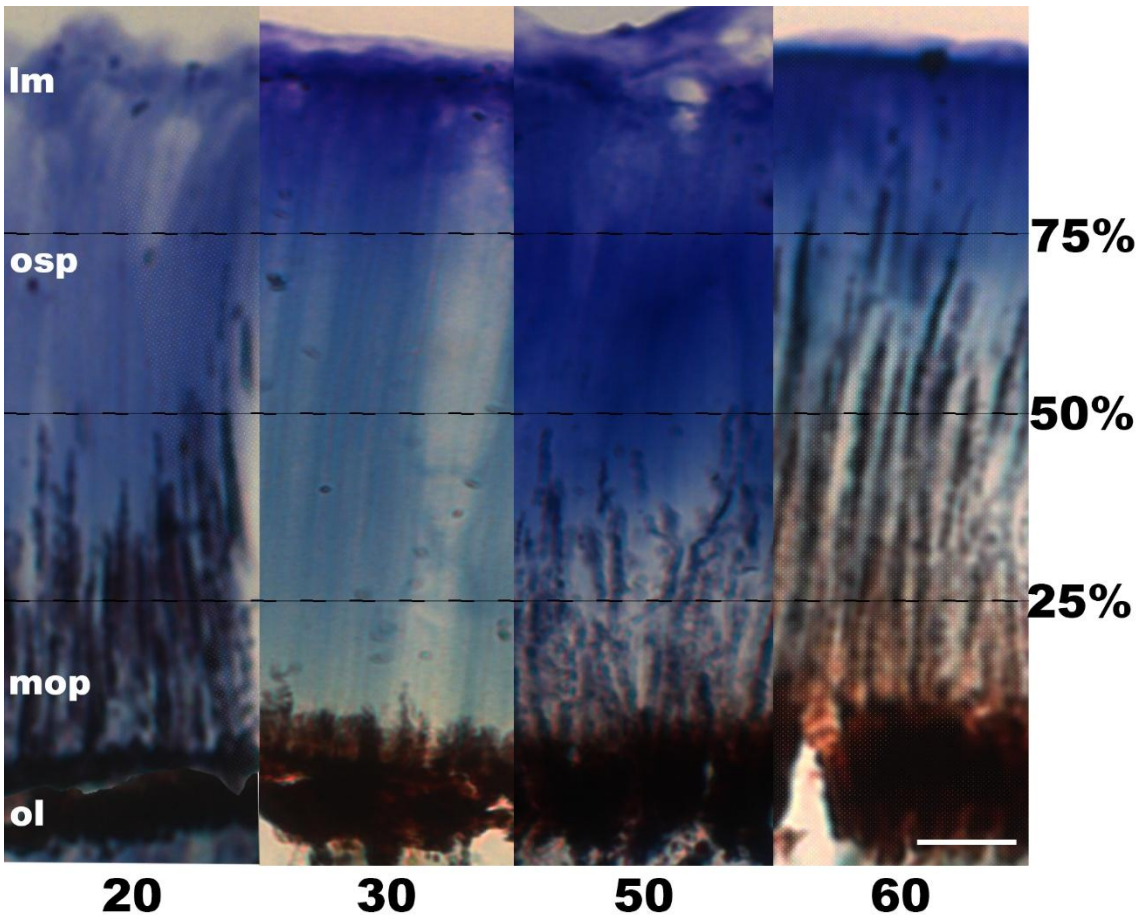


Figure 31. *Ommin pigment granule migration in the outer segment of the photoreceptors of Group 2 juvenile (ML 10–20 mm)* G. onyx *retinas following exposure to light (350 lux) for 30 min, followed by dark (minutes indicated below each cross-section). Percentage values on the side of the image and dotted line refer to photoreceptor length. Abbreviations: ommin layer (ol), migrating ommin pigment (mop), ommin-free outer segment of the photoreceptors (osp), limiting membrane (lm). All images depicted at 40× magnification. Scale bar = 20 µm.*

In Group 1 (individuals exposed to 20 min of light and then periods of darkness increasing by 15 min; Fig. 30), similar results were observed among the individuals returned to darkness for 15, 45 and 75 min, with most of the pigment evident in the basal 25% of the photoreceptor length. In the individual that was subjected to darkness for 60 min after light exposure (G05), the returning granules were more concentrated and positioned closer to the ommin layer, within the basal 10% of the photoreceptor length.

In Group 2 (individuals exposed to 30 min of light and then periods of darkness increasing by 10 min; Fig. 31), most of the specimens showed widely distributed ommin pigment granules in the photoreceptors. The individuals that were returned to darkness for 20 and 50 min post light exposure (G07 and G10, respectively) exhibited granules across the basal 40–50% of the photoreceptor length; in the 60 min dark-exposed individual (G11), the migrated granules spanned the majority (basal ~80%) of the photoreceptor length. In the individual exposed to 30 min of darkness (G08), the granules were more concentrated near the ommin layer, within the basal 10% of the photoreceptor length.

3.5.2 Neurotransmitter Expression

The G12 and G13 retinas were labelled with an anti-glutamate antibody, and both specimens exhibited glutamate labelling within both the inner and outer segments of the photoreceptors (Fig. 32). This technique involves the deposition of silver in cells in proportion to the quantity of antibody present, leading to the formation of various shades of grey in different areas. To ensure the specificity of the labelling, each tissue sample was processed with and without the anti-glutamate antibody. The 'silver only' negative controls in specimens G12 and G13 showed uniformly low and weak silver staining (top and bottom row, Fig.32a). However, the samples that were treated with the anti-glutamate antibody showed strong staining through the tissue and clearly identified cells were positive for glutamate labelling, with the highest concentration of glutamate appearing within the inner photoreceptor layer (top and bottom row, Fig. 32b). The qualitative assessment of the two conditions appears to show that in the specimen allowed to adapt to darkness for 90 min (G12, top row, Fig. 32b), glutamate labelling occurred in discrete cells; in comparison, the specimen with no subsequent dark adaptation period after light exposure (G13, bottom row, Fig. 32b) exhibited similar labelling in all its inner photoreceptor cells.

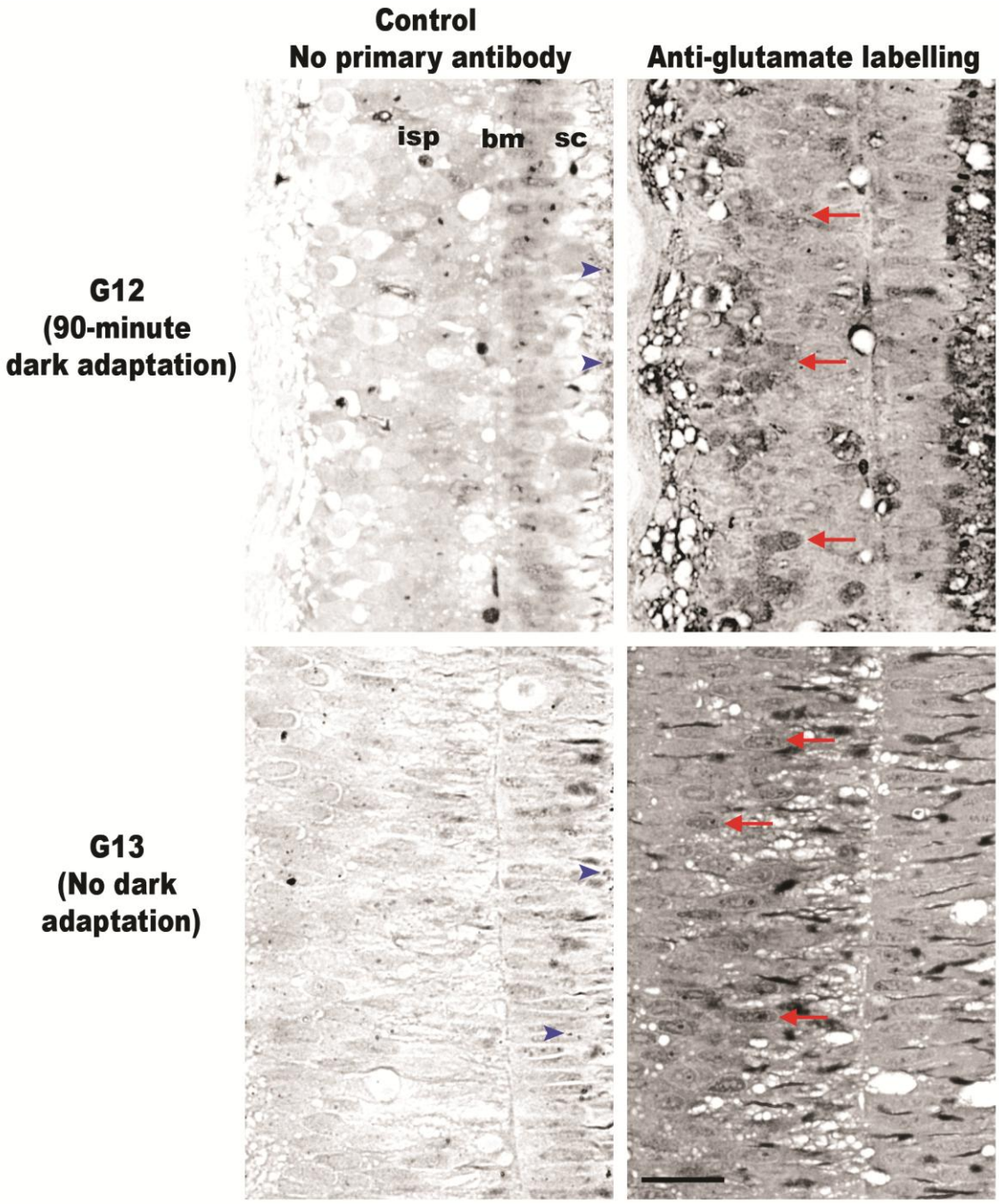


Figure 32. *Transverse retinal cross-sections of the inner photoreceptor layer of juvenile G. onyx (G12–G13, both 15 mm ML). (a) Retinal sections without primary anti-glutamate serve as negative controls. (b) Retinal sections labelled with antibodies indicate the expression of glutamate neurotransmitters (dark grey areas). Glutamate expression in retinas subjected to darkness for 90 min (G12, top row) or without dark adaptation (G13, bottom row) following light exposure (350 lux for 30 min). Arrows indicate examples of cells reactive to the antibody. Arrowheads indicate ommin pigment granules within the supporting cells not destroyed by the preservation process. Abbreviations: supporting cells (sc), basal membrane (bm), inner layer of photoreceptors (isp). Scale bar = 20 µm.*

3.6 Discussion

Recent studies have revealed an intriguing phenomenon in the migration dynamics of ommin pigment within cephalopod photoreceptors. While the migration of pigment to the distal tips is well documented, the return of pigment to the baseline is

an area of growing interest. This return to the baseline may indicate a regulatory mechanism that maintains pigment distribution within the photoreceptor outer segments, ensuring optimal visual performance under varying light conditions. This study aimed to investigate the mechanisms underlying this pigment redistribution to improve our understanding of the adaptive strategies of cephalopod visual systems.

The results support the presence of migratory ommin pigment within the retinas of juvenile *Gonatus onyx*, suggesting that these animals can screen light in illuminated conditions. Unlike in coastal specimens, where dark adaptation results in the return of all pigment granules to the ommin layer, some pigment granules were clearly visible within the photoreceptors even after prolonged periods of darkness, providing the first experimental result of this mechanism and its potential time delay within a deep-sea oegopsid squid.

The use of ommochromes as a screening pigment has been reported in the retinas of major invertebrate groups including insects (*Drosophila melanogaster* [Lo & Pak, 1981]; *Deilephila elpenor* [Hamdorf et al., 1986]; *Manduca sexta* [White et al., 1983]), crustaceans (*Procambarus clarkia* [Arechiga et al., 1990]; *Orconectes limosus* [Schraermeyer, 1990]), and chelicerates (*Limulus polyphemus* [Kier & Chamberlain, 1990]), all of which are shallow-water or terrestrial species with pigments that migrate in coordination with the day/night cycle. Extensive studies, mostly on *Drosophila melanogaster*, have shown that ommochromes are photosensitive coloured compounds derived from tryptophan metabolism (Summers et al., 1982). They are synthesized and stored in lysosome-related organelles called ommochromasomes, and are categorized into three groups, ommatin, ommin, and ommidin; for the latter two, little is understood of their chemistry and structure (Figon & Casas, 2019).

In juvenile *Gonatus s*pecimens exposed to light for 20 or 30 min, most pigment (but not all) returned to the basal layer under dark conditions, suggesting that an extended light response occurs, and may take more than 24 h to reverse. If the mechanisms controlling the return of the pigment granules to the photoreceptor bases are the same in *G. onyx* as in *Doryteuthis pealeii,* as described by Daw and Pearlman (1974), then it is likely that the light adaptation conditions of 350 lux were strong enough to isomerize more than 10% of rhodopsin, which would elongate the time required for the pigment to fully return to its basal layer. Since *G. onyx* juveniles are typically distributed around 300-400 m deep (Hunt & Seibel, 2000), illumination at 350 lux is likely far brighter than any natural condition they would encounter. Furthermore, the spectral distribution of light emitted from fluorescent bulbs, such as those used in this study, typically has a spectral peak near 480-500 nm, aligning well with the spectral absorbance of most cephalopod photopigments, which may be linked to inducing pigment migration (Hansen et al., 2013). Therefore, the emitted light with an illuminance of 350 lux for 20-30 min from the fluorescent lighting does appear sufficient to trigger some light adaptation in *G. onyx*, but the restoration of full dark adaptation may take far longer than the duration of our trials. Additionally, dark adaptation may take significantly longer in deep-sea cephalopods exposed to abrupt bright light. It is also possible that the duration and intensity of light conditions in this study irreversibly damaged the retina, preventing a return to full dark adaptation. Alternatively, some ommin granules

may consistently be found within the outer photoreceptor layer under any conditions. In some species of blowflies (*Lucilia* sp.), ommochromes help maintain the photoreceptor by transporting degraded rhabdomere membranes (Figon & Casas, 2019).

This study also provided novel evidence that glutamate is present within the retina of *G. onyx*. Glutamate does appear to decrease after a longer duration in the dark, which agrees with the findings of D'Aniello et al. (2005). They identified significant levels of neurotransmitters in the retina of cephalopods; glutamate was shown to decrease under dark conditions. However, D'Aniello et al. (2005) examined adults of shallow-water species, with a relatively different eye structure, that were dark adapted for five days, making their results and the results of this study difficult to compare directly. Whether neurotransmitters used in cephalopod vision differ among lineages, among species occupying different environments with distinct light regimes, or between adults and juveniles, remains to be seen.

The life history of *G. onyx* makes it an interesting study subject for light/dark adaptation. Females brood their eggs for six to nine months at depths of up to 2500 m where they sometimes release their hatchlings but have also been observed doing so near the surface (Okutani et al., 1995; Seibel et al., 2000, 2005). Brooding at such depths under aphotic conditions may reduce predation risks for the female and her eggs/hatchlings, while the extended post-spawning egg care may allow relatively large and precocious young to hatch, capable of migrating vertically to nutrient-rich shallow water (Mangold, 1983). Retinal screening pigments could be advantageous as the young squid traverse a range of light conditions between the aphotic zone and the nutrient-rich euphotic zone, and across their subsequent exposure to regular day/night cycles.

As hatchlings mature into juveniles, they appear to settle into a regular diel cycle, distributed across a broad depth range during the day (100–800 m, peaking at 300–400 m) and a narrower range during the night (0–400 m, peaking at 200–300 m) (Hunt & Seibel, 2000). Juvenile *G. onyx* have higher metabolic rates than their adult counterparts, which may influence their schooling behaviors and daily vertical migrations (Hunt & Seibel, 2000). Their screening pigment may support visual acuity across the broad range of light conditions to which they may be exposed throughout the day and night and may protect the retina during ascents into shallower water during crepuscular hours.

At about 30–35 mm ML, juveniles begin to descend into greater depths. Their metabolisms slow to accommodate a less mobile, more solitary lifestyle, and they are most frequently observed at 300–1000 m (peaking at 700–800 m) during the day, and 100–800 m at night (peaking at 400–500 m [Hunt & Seibel, 2000]). Thus, adult *G. onyx* may not experience the same diel range of light conditions, and screening pigment may play a less significant role. One exception may be females releasing hatchlings, which have been observed in near-surface waters (Okutani et al., 1995). It has been theorized that observations of deep-sea releases are the result of external stressors, such as net capture or predation, forcing the brooder to release her clutch prematurely, which has also been observed in octopods (Seibel et al., 2000; Young, 1972a). Females that release in shallow water may benefit from screening pigment as they pass potential predators while migrating through brighter light conditions. To determine whether ommin

pigment migration occurs past the juvenile stage, more morphological investigations of adult *G. onyx* must be conducted.

The migration of these pigment granules in octopuses is controlled by melatonin circadian rhythms and varies during dark and light exposure (Muñoz et al., 2011). Dopaminergic signals also act to modulate basal migration of the granules, simulating dark-adaptation conditions; efferent nerves cells release dopamine that acts on the retina and triggers inward migration of the screening pigment, allowing maximum photoreceptor activity (Gleadall et al., 1993). This study provides the first report of confirmed ommin pigment migration in an oegopsid squid. Evans et al. (2015) reported an observation of apparent arrested screening pigment migration in the retinas of preserved juvenile *Teuthowenia pellucida*. This glass squid similarly inhabits well-lit shallow waters during its early-life stages, then descends to greater depths as adults. Evans et al. (2015) did not observe any ontogenetic variation in thickness of the ommin layer, despite a disproportionate increase in photoreceptor length compared to overall growth, and it remains unclear whether screening pigment migration remains possible and/or advantageous for deep-sea dwelling, adult *T. pellucida*. However, findings from the current study and Evans et al. (2015) suggest that ommin migration does occur in at least some deep-sea squids across life stages that dwell primarily below the photic zone.

More research is required to fully understand the complex mechanisms governing screening pigment migration in the retina of deep-sea squids and the role neurotransmitters play. Future research on *G. onyx* screening pigment and its potential correspondence to dopamine levels should aim to determine a more precise time scale for light and dark adaptations in the retina and investigate a wider range of ontogenetic stages. Additionally, if interindividual variation exists, and if the mechanisms controlling ommin migration undergo ontogenetic modifications that are linked to visual transmission, those phenomena can start to be deciphered. More broadly, future research may aim to identify the variation of screening pigments among closely related species, and whether this mechanism is present in other oegopsids.

Chapter 4 – Prelude

4.1 Introduction to Chapter 4 – The Influence of Bioluminescence on the Spectral Sensitivity of Visual Pigments in Deep-sea Oegopsid Squids

In Chapter 3 – Biological sunglasses in a Deep-Sea Squid: Pigment Migration in the Retina of *Gonatus onyx*, the molecular mechanisms that facilitate pigment migration through the outer segment of the photoreceptors were investigated. The results revealed that glutamate is present in the retina suggesting that, as in the majority of animal species, it is a key neurotransmitter for this process. As Daw & Pearlman (1974) showed, when 3% or more of the visual pigment rhodopsin is isomerised, the screening pigment migrates to the distal tips of the outer photoreceptor segments to then return to its basal position under dark conditions. The screening pigment's return to the basal position can take many hours, depending on the amount of rhodopsin that is isomerised. Their research and the results from the previous chapter suggest there may be a link between the isomerisation of rhodopsin in the microvilli and glutamate release at the synaptic end for facilitating pigment migration. Therefore, it would be interesting to determine whether different light regimes, that is – light of differing wavelengths – may trigger pigment migration differentially-depending on the wavelength of peak spectral absorbance of rhodopsin in a particular species. Previous research has demonstrated that that the wavelength of peak spectral absorbance lies within a range of 470-510 nm in cephalopods, which seems to be correlated with the light spectrum at the depth and environment a species inhabits. However, to investigate the impact of different light regimes on pigment migration in oegopsid species, the peak spectral absorbance wavelength of rhodopsin needs to be identified for each species. Currently, peak absorbance measurements of oegopsids' rhodopsin are largely unavailable. Therefore, this chapter focuses on identifying the wavelength of peak spectral absorbance of rhodopsin in ten oegopsid species found in Aotearoa New Zealand. Furthermore, the chapter aims to better understand the environmental and behavioural factors that characterise the spectral sensitivity of rhodopsin in different species. The following research was conducted by first extracting and isolating rhodopsin from the retina and characterising its spectral sensitivity through spectrophotometric analyses. Then, the rhodopsin gene was sequenced and compared across oegopsid squid species to identify factors that may influence genetic variation.

Chapter 4 – The Influence of Bioluminescence on the Spectral Sensitivity of Visual pigments in Deep-sea Oegopsid Squids

4.2 Abstract

Cephalopods possess a visual system that is typically considered monochromatic and is mediated primarily by rhodopsin-based visual pigments. In deep-sea oegopsid squids, visual pigment spectral sensitivity is generally reported to exhibit peak absorbance values near 480 nm, corresponding to the wavelength of downwelling light that penetrates deepest into oceanic waters. However, some studies have suggested that spectral tuning may vary among species in response to ecological and environmental factors, including habitat depth and bioluminescence. Furthermore, cephalopod visual pigments exist within a photoreversible rhodopsin–metarhodopsin system, where absorbance measurements may be influenced by varying proportions of rhodopsin, metarhodopsin, and phototransduction intermediates. Therefore, this study aimed to characterise the absorbance profiles of retinal visual pigment extracts from oegopsid squids collected within the exclusive economic zone of Aotearoa New Zealand using spectrophotometric analyses. In addition, the rhabdomeric opsin (r-opsin) gene encoding rhodopsin was sequenced and phylogenetically compared among species to investigate factors that may contribute to variation in visual pigment absorbance characteristics. The results suggest that most species possess visual pigment absorbance profiles with peak absorbance values near 480 nm, although some species capable of bioluminescence exhibited absorbance maxima closer to 490 nm and broader absorbance bandwidths. Phylogenetic analyses further revealed that rhodopsin sequences from bioluminescent species were generally more closely related to one another than to those of non-bioluminescent species. While contributions from metarhodopsin and other photoproducts cannot be completely excluded from the absorbance measurements, the observed patterns are consistent with the hypothesis that ecological factors, including bioluminescence, may influence visual pigment spectral characteristics in deep-sea oegopsid squids.

4.3 Introduction

Coleoid cephalopods have rapid adaptive colour-changing abilities. In milliseconds, they can dynamically adjust the colour and texture of their skin to camouflage, communicate, and hunt despite overwhelming evidence suggesting most species are colour-blind (Hanlon et al., 2011; Marshall & Messenger, 1996; Messenger, 1977). Though the retinal structure of coleoids allows detection of polarised light – a possible substitute for colour vision – they consistently fail colour discrimination tests (Mäthger et al., 2006). The rhabdomeric design that enables polarised vision in cephalopods is shared across many invertebrate species in both terrestrial and aquatic environments with examples demonstrating rhabdomeric photoreceptors are capable of colour vision (Osorio & Vorobyev, 2008; Seidou et al., 1990; Thoen et al., 2014). Yet, most cephalopods have only one photoreceptor type mediated primarily by one visual pigment.

Coleoids possess camera-type eyes that allow for visual function similar to the eyes of vertebrate (Serb & Eernisse, 2008). However, instead of the retina possessing ciliary rod- and cone-type photoreceptors as in vertebrates, including deep-sea fishes, coleoids possess a single comb-like photoreceptor type (Young, 1962). The photoreceptors have densely packed microvilli—tiny finger-like projections that increase the surface area for light absorption. The microvillar arrangement into a rhabdomere makes these photoreceptors sensitive to the orientation of light waves, allowing these species to detect polarised light (Cartron et al., 2013; Pignatelli et al., 2011; Talbot & Marshall, 2010, 2011). The microvilli of the photoreceptors house the photosensitive pigment molecules (visual pigment) necessary for phototransduction. The primary visual pigment used by coleoids, called rhodopsin, allows detection of light intensity.

Rhodopsin consists of an opsin protein linked to a species-dependent retinal-derivative chromophore that is photoactivated when the photoreceptor is exposed to light. This interaction initiates phototransduction, involving Gq protein-PLC based reactions that generates an electrical response in photoreceptors that is processed by downstream neural circuits (Terakita et al., 1998). The spectral sensitivity of rhodopsin from a particular-species – or simply, the wavelengths of light capable of photoactivating the chromophore – is likely influenced by the specific opsin sequence and binding site of the chromophore (Hubbard & St George, 1957; Morris et al., 1993; Shichida & Imai, 1998). Variations in rhodopsin light absorbance properties will largely be due to difference in the opsin protein sequence. To investigate these properties, rhodopsin can be extracted and isolated; its spectral sensitivity can be measured via spectrophotometry and characterised by determining the peak spectral absorbance (λ_{max}) and bandwidth of the spectral absorbance curve. However, visual phototransduction in cephalopods differs substantially from that of vertebrates and is characterised by a photoreversible rhodopsin–metarhodopsin system (Hara & Hara, 1972). In the dark-adapted state, rhodopsin consists of an opsin protein bound to a cis-form of a retinal-based chromophore (Mitchell & Swardfager, 2010). Absorption of a photon initiates a conformational change in the chromophore, causing isomerisation to the all-trans form and triggering a phototransduction cascade that ultimately generates a neural response (Hubbard & St George, 1957; Shichida & Imai, 1998). During this process, rhodopsin passes through several intermediate states before forming metarhodopsin, a relatively stable photoactive state (Hara & Hara, 1972). Unlike vertebrate metarhodopsin, which is rapidly degraded and regenerated through enzymatic pathways (Baumann, 1972), cephalopod metarhodopsin remains photosensitive and can be converted back to rhodopsin through subsequent absorption of light of appropriate wavelengths (Hara & Hara, 1967; Hubbard & St George, 1957). This photoreversible system enables cephalopods to photochemically regenerate their visual pigments without relying on a classical enzymatic visual cycle. Consequently, the relative proportions of rhodopsin and metarhodopsin vary dynamically depending on previous light exposure, which creates challenges when interpreting absorbance measurements because these photostationary molecules contribute to the overall spectral profile. Additionally, metarhodopsin does not undergo significant dark-driven reconversion to rhodopsin, instead pigment-state transitions are primarily governed by wavelength-dependent photoconversion rather than time-dependent dark

regeneration. To control for this in the current study, specimens were dark-adapted (as much as possible), and spectral analyses of the visual pigments were measured in dark conditions. However, the potential for the analysed visual pigments to contain residual metarhodopsin remained, depending on the prior light history and conversion state at capture for each animal.

Given that the characteristics of the spectral absorbance curve of photopigments found in deep-sea animals is largely influenced by the properties and physics of light in the ocean (Warrant & Adam Locket, 2004; Warrant & Johnsen, 2013), we hypothesise that the spectral tuning of the visual pigments in these species is an adaptive response to their specific light environment. When sunlight contacts water, short- and long-wavelength light is quickly absorbed by the upper layers, allowing only downwelling blue-green light (~480 nm) to penetrate up to 1000 m deep (McFarland, 1990; Yentsch, 1962). Beyond 1000 m, natural light is only present as bioluminescence. Typically, the retinal pigments of deep-sea animals absorb light in the blue-green spectra that matches the colour of downwelling light, which achieves greater visual sensitivity for the species; otherwise, the photopigment may react to the bioluminescence of organisms ecologically significant to the species (Crescitelli, 1990). Cephalopods are no exception and exhibit rhodopsin spectral absorbances peaking between 480 and 500 nm (Bellingham et al., 1998; Hall et al., 1991; Hara-Nishimura et al., 1993; Morris et al., 1993; Ovchinnikov et al., 1988; Tong et al., 2009). Research suggests that species inhabiting deeper strata may experience a hypochromic (blue) shift in their spectral absorbances (Messenger, 1981; Morris et al., 1993). For instance, Morris et al. (1993) compared the λ_{max} and rhodopsin opsin gene sequences of two shallow-water myopsid squids. *Loligo forbesi*, is found at a maximum known depth of 360 m, showed a 5 nm λ_{max} hypsochromic shift compared to *Alloteuthis subulata*, which inhabits a maximum depth of 200 m. The study identified amino acid substitutions of the opsin protein at sites where the chromophore binds. This suggests that substitutions within the chromophore binding pocket may be one factor responsible for shifting the peak spectral absorbance of rhodopsin in cephalopods. Most oegopsid species spend the majority of their lifespan in the deep sea (beyond 200 m) where sunlight is severely limited, which makes them a key group for studying vision adaptations. In dark-adapted conditions, the predominant pigment is rhodopsin; and therefore, the absorbance reflects the intrinsic spectral tuning of opsin. Dark-adapted specimens are commonly used to estimate λ_{max} of rhodopsin because this minimises the contribution of photoproducts to the measured spectral sensitivity. Alternatively, measuring the spectral sensitivity of specimens adjusted to bright conditions would produce a photostationary mixture whose spectral profile depends on illumination conditions rather than intrinsic pigment properties. Additionally, sequencing the opsin gene that codes for the rhodopsin visual pigment is a useful approach for exploring genetic modifications that may determine their visual pigment spectral sensitivity. We hypothesise that, paired with the visual pigments' dark-adapted spectral absorbance and the optical habitat of the species, we may be able to infer what drives spectral absorbance shifts; correlations that are less readily available in the literature.

The goal of this study was to measure the spectral sensitivity of the visual pigments in a standardised (dark-adapted) biochemical state of ten oegopsids from

Aotearoa New Zealand and to compare their rhodopsin gene sequences to identify potential ecological factors influencing spectral tuning.

4.4 Methods

4.4.1 Specimen Acquisition

Specimens were collected within the exclusive economic zone of Aotearoa New Zealand with support from the National Institute for Water and Atmospheric Research (NIWA). Aboard the *RV Tangaroa*, squids were trawled at a depth range of 200-1200 m during three fisheries surveys. Specimens were collected in January 2020 during the Chatham Rise Trawl Survey (TAN2001) along the Chatham Rise (Fig. 33, Site A), in December 2020 during the Sub Antarctic Trawl Survey (TAN2014) around the Campbell Plateau (Fig. 33, Site D) and near Puysegur Bank (Fig. 33, Site C), and in July 2021 during the West Coast South Island Trawl Survey (TAN2107) along the west coast of Te Waipounamu (Fig. 33, Site B). The specimens were dark-adapted in sealed light containers then immediately decapitated and stored at -20°C until later use. Tissue was also collected and stored in 99% ethanol to facilitate partial sequencing of the rhodopsin gene.

Taningia danae, *Taningia fimbria*, and *Mesonychoteuthis hamiltoni* specimens were collected aboard fisheries vessels as part of the New Zealand Ministry for Primary Industries Scientific Observer Programme within the exclusive economic zone of Aotearoa New Zealand. Squids collected by fisheries observers were frozen whole aboard the ship at -20°C until transferred to the lab for processing for spectral analysis. In the lab, tissue samples were collected and stored in 99% ethanol for later rhodopsin sequencing.

In total, the spectral sensitivity of the visual pigments were analysed for ten species, and rhodopsin was partially sequenced for 11 (Table 7).

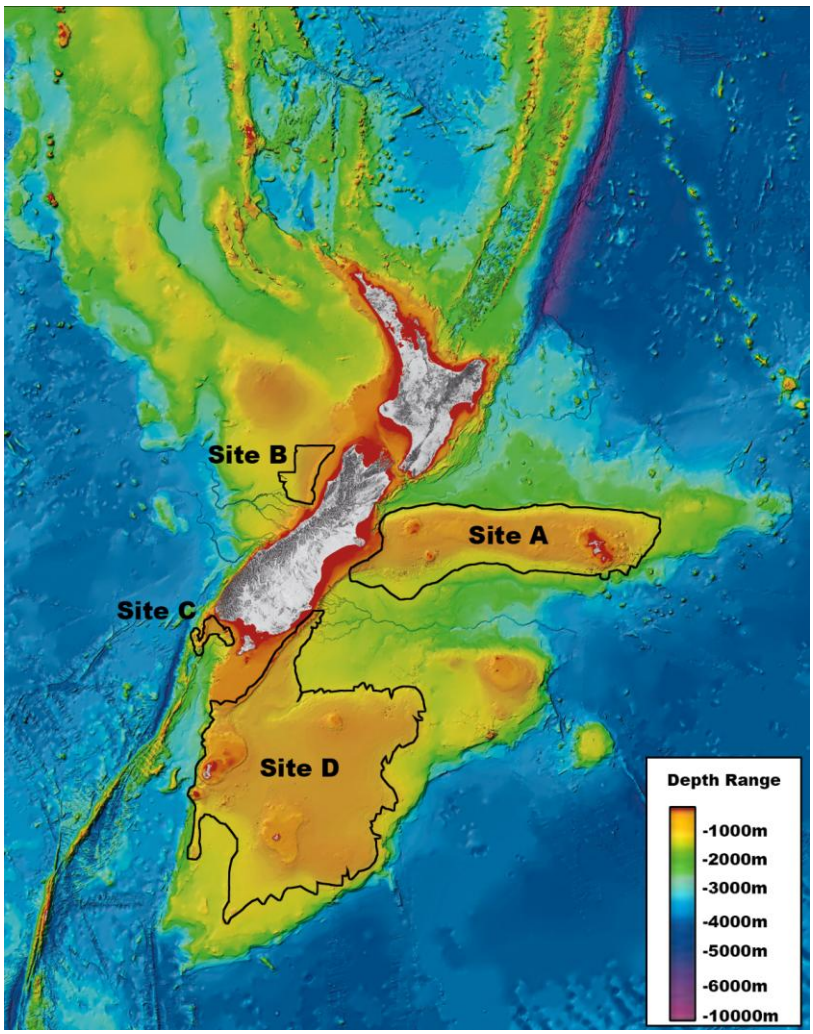


Figure 33. *A bathymetric map of the specimen collection sites around Aotearoa New Zealand. Site A) Chatham Rise. Site B) Te Waipounamu west coast. Site C) Puysegur Bank. Site D) Campbell Plateau. This image has been modified with permission from NIWA (Mitchell et al., 2012).*

Table 7. *A list of the species, their catch data and analyses conducted. Rhodopsin sequencing was conducted on one individual for all species. Fishery observer (FO) samples were collected from unknown locations around New Zealand.*

Species	Mantle Length Range (mm)	Spectral Analysis Sample Size	Rhodopsin Spectral Analysis	Rhodopsin Sequencing	Sample Collection Location
Nototodarus sloanii	245-270	6	Yes	Yes	Site A
Nototodarus gouldi	NA	0	No	Yes	Site B
Todarodes angolensis	375-380	3	Yes	Yes	Site B
Todarodes filippovae	279-390	3	Yes	Yes	Site A
Moroteuthopsis ingens	172-475	10	Yes	Yes	Site A
Onykia robsoni	329-552	6	Yes	Yes	Site A
Onykia aff. robsoni	670-810	4	Yes	Yes	Site B
Histioteuthis miranda	242-251	3	Yes	Yes	Site A, C & D
Taningia danae	989-1030	2	Yes	No	FO
Taningia fimbria	*	1	Yes	Yes	FO
Architeuthis dux	1770	1	No	Yes	Site A
Mesonychoteuthis hamiltoni	2500	1	Yes	Yes	FO

* Indicates species that include specimens of unknown mantle length.

4.4.2 Tissue Dissection

Rhodopsin, metarhodopsin and their intermediaries are light-sensitive molecules embedded in the densely packed microvilli of the outer segment of the photoreceptor in the retina. Rhodopsin is relatively stable when stored at low temperatures, particularly in the dark and if properly frozen, such as in liquid nitrogen or at -20°C (Hara & Hara, 1976). Maintaining rhodopsin in dark conditions prevents its conversion to intermediary forms that have transient spectral absorbance in the photochemical cycle (Tsuda, 1979). However, isolating rhodopsin with minimal contribution from other photopigment intermediaries depends on the dark-adapted state of the specimens. Since specimens were collected under variable environmental light conditions (depth, time of day, etc.). It was not possible to confirm that specimens were fully dark-adapted at the time of capture. To minimise this limitation, specimens were transferred immediately into light-sealed containers and maintained in darkness for at least an hour prior to decapitation. To further limit photoconversion and stabilise

the intermediary forms of the visual pigments, the decapitated heads were immediately frozen at -20°C. These procedures were implemented to bias the extracted pigment towards the rhodopsin state and reduce the contribution of metarhodopsin and other photointermediates. Residual photoproducts cannot be excluded from this protocol, but dark adaptation of specimens and rapid preservation of the heads via freezing are expected to minimise their relative abundance, thereby allowing the measured absorbance spectra to predominantly reflect the intrinsic spectral properties (λ_{max}) of the species.

To isolate the microvilli and extract the visual pigments, the dark-adapted eyes were first enucleated from the heads of frozen squids. All dissections and extraction procedures were performed under minimal light exposure. Frozen specimen heads were thawed only sufficiently to permit tissue handling and were otherwise maintained in light-sealed containers throughout processing. Between each extraction step, samples were kept on ice within opaque containers to minimise photoconversion of rhodopsin to metarhodopsin and other photointermediates. The frozen squid heads collected during the NIWA surveys were placed in a light sealed container and partially thawed for up to 15 min, which allowed the eyes to be enucleated and dissected. Enucleation was done by first cutting away the eyelids with dissecting scissors to reveal the eye, which was carefully displaced from the orbital cavity with a small spoon until the optical nerve was visible to cut and detach the eye. Whole squids collected by fisheries observers were partially thawed until the eyes could be enucleated using similar methods. However, the eyes were immediately refrozen at -20°C until the visual photopigment extraction protocol could be performed later. Finally, immediately prior to performing the rest of the extraction protocol, the eyes were partially thawed for several minutes in a light sealed container to enable their dissection.

4.4.3 Rhodopsin Extraction Protocol

To isolate the outer photoreceptor segments' microvilli, the eyes were first hemisected to separate the eyecups from the anterior segment and lens. A beaker was filled with at least 1.5 ml of 0.1 M phosphate buffer solution (PBS) or with larger volumes of PBS for bigger eyes. The eyecups were placed into the solution so that the interior of the eyecups faced down, and the retinas contacted the PBS. The beaker was shaken vigorously to mechanically separate the outer segment of the photoreceptors from the inner segment along the basal membrane, using a modified protocol described by Hara and Hara (1972). However, the inner and outer segments of the photoreceptors rarely separated perfectly, requiring the retina to be scraped from the eye cup manually with a dissecting razor before being added to the beaker of PBS. The procedure resulted in the PBS containing a mix of various retinal components including the inner and outer photoreceptor segments, supporting cells, screening pigment granules and vitreous fluid (Fig. 34A).

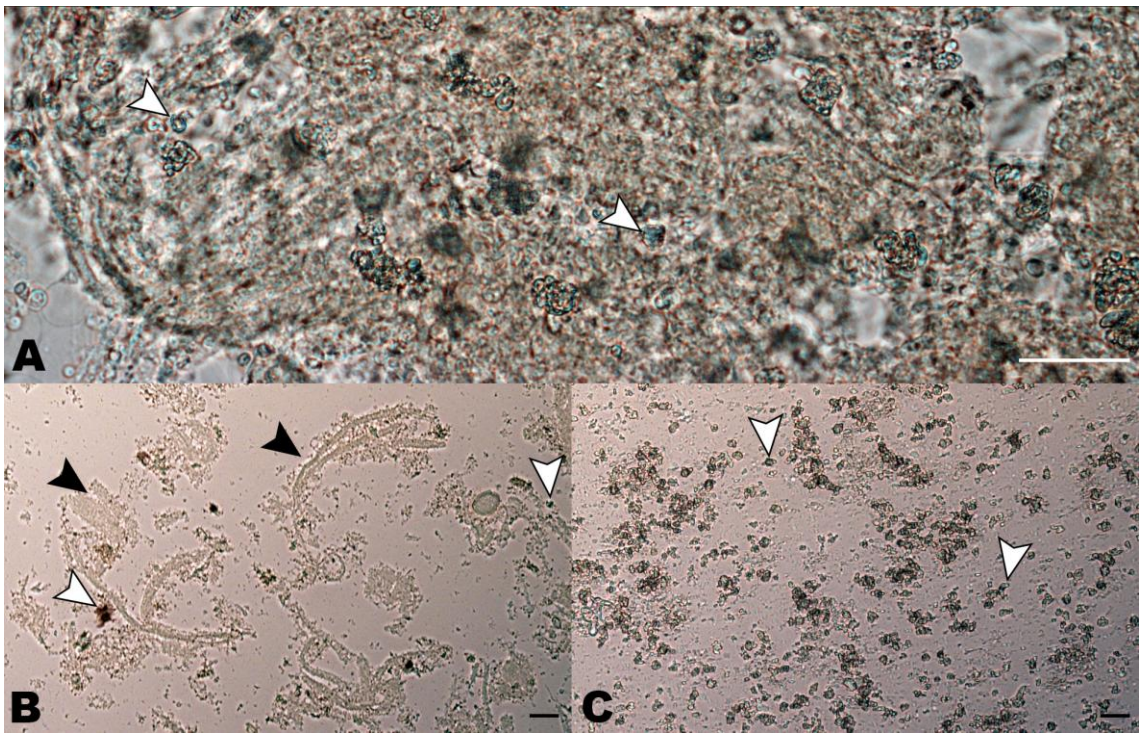


Figure 34. *Microscopy images depicting the various retinal components mixed within the PBS at consecutive steps of the rhodopsin extraction protocol. A) Prior to homogenisation, large retinal components including the inner and outer photoreceptor segments and smaller components such as the screening pigment (white arrows) and vitreous fluid were collected in PBS. Image taken at 40x magnification. B) The outer photoreceptor segments (black arrowheads) were separated during homogenization, where rhodopsin is located. Image taken at 10x magnification. C) Differential centrifugation isolated the microvilli from the larger retinal components. Note the absence of the outer photoreceptor segments (Fig. 34B). Microvilli are not visible with standard microscopy but are contained within the supernatant sucrose solution along with the black pigment (white arrows). Image taken at 10x magnification. All scale bars = 20 µm.*

The retinal mixtures from both eyes of a specimen were transferred to a single vial and manually homogenized with a glass homogeniser (PYREX®, 1 mL Potter-Elvehjem Tissue Grinder with PTFE Pestle). For relatively small eyes, retinal mixtures acquired from multiple specimens of the same species were homogenised together to ensure an adequate amount of the visual pigments were extracted for analysis. In the case of the dimorphic eyes of *Histioteuthis miranda*, the mixtures retrieved from each eye were kept separate. The homogenised solution was transferred into a 2 mL Eppendorf tube and topped up with additional 0.1 M PBS, if needed, to ensure a balanced centrifuge. Between each of the following steps, the solutions were kept on ice in a light sealed container.

Differential centrifugation was used to further isolate the microvilli. Each Eppendorf tube was inverted to mix the homogenized solution then centrifuged at 16,000 g for 30 min at 4℃ (5415R Refrigerated Centrifuge, Eppendorf, Hamburg, Germany). The supernatant was decanted, and the remaining pellet was resuspended in 36% sucrose in 0.1 M PBS by pipetting, then centrifuged again at 1,800 g for 5 min at

4℃. This step produced tissue homogenates (Fig. 34B). The sucrose created a density gradient which suspended the microvilli within the supernatant while the large retinal components precipitated to the bottom of the tube as described in Hara et al. ([1995]; Fig 34C). The supernatant was transferred to a new 2 mL Eppendorf tube, filled to the top with 0.1 M PBS, then centrifuged at 16,000 g for 20 min at 4℃. At this step, precipitated pellet contained the microvilli and the black screening pigment (Fig. 35A).

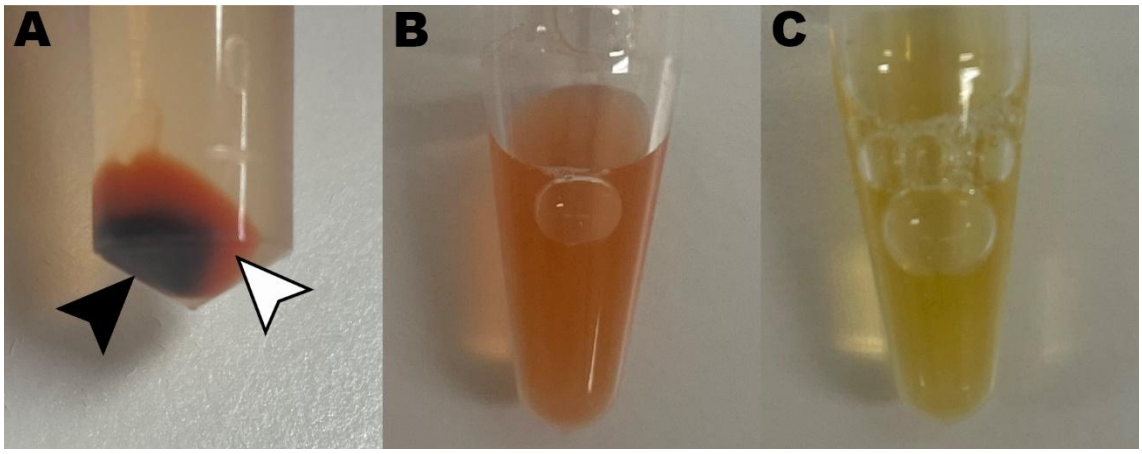


Figure 35. *A) An image of the precipitated pellet after the sucrose gradient centrifugation, which is comprised of the microvilli (white arrowhead) and screening pigment (black arrowhead). The reddish-orange colour of the microvilli derives from the rhodopsin photopigment molecules. The screening pigment was washed away with Na_2PO_4 in subsequent rhodopsin purification steps. B) Isolated squid rhodopsin is orange in colour. C) Adding 0.4 M HCl bleaches rhodopsin to yellow, confirming its presence.*

The supernatant was decanted, and 1.5 mL of Na_2PO_4 was added to wash away the unwanted screening pigment. The pellet was resuspended by pipetting and was centrifuged at 16,000 g for 5 min at 4℃. This step was repeated at least twice and until all the screening pigment was removed. After the final Na_2PO_4 wash and centrifugation the supernatant was decanted. To remove any excess Na_2PO_4 from the pellet, 1.5 mL of 0.1 M PBS was added, and the solution was centrifuged at 16,100 g for 5 min at 4℃.

To extract the rhodopsin from the remaining tissue, a detergent was employed to solubilise the microvilli membranes. For this experiment, 0.6% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS; Sigma-Aldrich, Burlington, MA, U.S.A.) in PBS was used. (Miyazaki & Osumi, (2022) showed that 0.6% CHAPS enabled the production of the expected rhodopsin absorption curves, demonstrating the effectiveness of CHAPS solubilising and preserving rhodopsin's optical properties. 500 µL of 0.6% CHAPS was added to the final precipitate, and the tubes were rotated for 1.5 -2 h at 4℃, then centrifuged at 800 g for 5 min at 4℃. The final product of isolated photopigments appear as a reddish-orange colour that bleaches yellow after adding a few drops of 0.4 M HCl, which confirms the presence of rhodopsin ([Kito et al., 1968; Miyazaki & Osumi, 2022] Fig. 35B & 35C). The samples were kept on ice and immediately brought to a spectrophotometer for absorbance measurements.

4.4.4 Rhodopsin Spectral Sensitivity Measurements

The isolated photopigment samples and a negative control (pure 0.6% CHAPS) were placed into 1 mm wide wells of a transparent 96-well microplate (Nunclon Delta

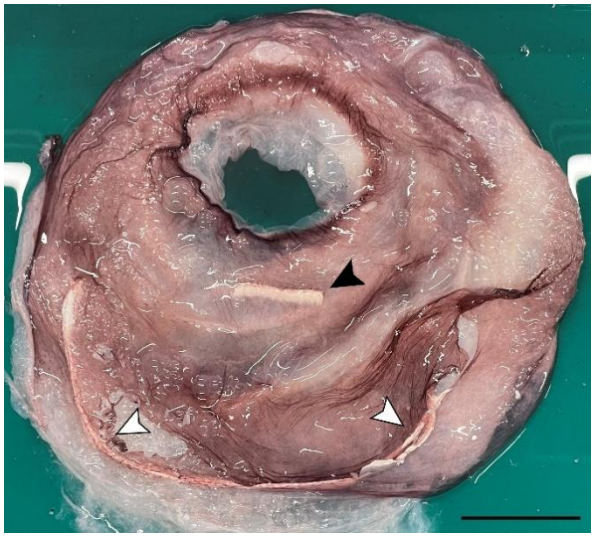
Surface 96-well Microplate, Thermo Fisher Scientific, Roskilde, Denmark) under dark conditions and measured for their spectral sensitivity using a spectrophotometer (CLARIOstar Plus, BMG LABTECH, Ortenberg, Germany). Absorbance spectra were recorded from 400 nm to 600 nm at 2 nm intervals using SMART Control software (BMG LABTECH, Ortenberg, Germany) and corrected against the negative control.

4.4.5 Rhodopsin Spectral Sensitivity Analyses

The absorbance curves were normalised against each other on a zero to one scale using OriginPro software (OriginPro 2021, OriginLab Corporation, Northampton, MA., U.S.A.). The peak absorbance wavelength (λ_{max}) and absorbance bandwidth was measured from each specimen's normalised absorbance curves. The absorbance bandwidth was measured as the full width at half-maximum height (FWHM) of the absorbance curve. Variation in both λ_{max} and FWHM measurements are not unexpected in cephalopod visual pigment studies, as the photoreversible relationship between rhodopsin and metarhodopsin can influence the absorbance characteristics of visual pigment extracts. Unlike vertebrates, cephalopod retinal tissues may contain varying proportions of rhodopsin, metarhodopsin, and other phototransduction intermediates, depending on the recent light history and physiological state of the animal. Although specimens and visual pigment extracts were maintained under light-protected conditions whenever possible, the relative abundance of these pigment states could not be quantified. Consequently, some of the observed variation among specimens may reflect differences in the proportions of visual pigment states present within the extracts at the time of measurement. To account for this variability while facilitating interspecific comparisons, λ_{max} and FWHM values were averaged for each species, and these mean values were used as estimates of the visual pigment absorbance characteristics in all subsequent analyses. The mean λ_{max} and FWHM for each species were compared across species by linear regression modelling and Tukey's HSD Posthoc Test was used for pairwise comparisons using R package EMMEANS (v1.10.6).

Species were also categorised by ability or inability to produce bioluminescence (Table 8). Both groups were compared to determine significant variance for λ_{max} and FWHM using one-way ANOVA analyses. A MANOVA analysis was used to determine the relationship between λ_{max} and FWHM for bioluminescent and non-bioluminescent species. Investigations of the large eye of *H. miranda* suggested it had similar λ_{max} and FWHM measurements to non-bioluminescent species. Therefore, the MANOVA analysis was calculated again with the large *H. miranda* eye moved to the "non-bioluminescent" category. The photopigment spectral sensitivity curve statistical analyses were performed using R (Version 4.4.1) with RStudio (Cranberry Hibiscus, Version 2024.09.0 Build 375).

Table 8. *Collected species with bioluminescent capabilities, a brief description and accompanying image of select photophores.*

Species	Description	Picture
T. danae & T. fimbria	The genus *Taningia* has the largest known photophores in the animal kingdom (Kubodera et al., 2007). A large photophore is embedded within the L2 and R2 arm tips. Skin flaps that cover each photophore open and close (similar to the action of an eyelid) to reveal or conceal the emitted light. The bioluminescence appears to be yellowish green (Kubodera et al., 2007). Scale bar = 10 mm	
M. hamiltoni	Colossal squids, like other species in the family Cranchiidae, have two photophores located on the ventral hemisphere of the external surface of the eye (Rosa et al., 2017). A smaller photophore (black arrowhead) lies dorsally to the larger crescent-shaped photophore (white arrowheads). The colour of the emitted light is unknown. Scale bar = 50 mm	

H. miranda	The family Histioteuthidae are also known as strawberry squid because of the numerous photophores that cover the body primarily the ventral surface of the mantle, head and arms) and fluoresce orange under UV light. Photophore density is higher on the right side of the body, where the smaller eye is located and surrounded by photophores (add arrowheads. Scale bar = 10 mm	

4.4.6 Rhodopsin DNA Barcoding: Lysis and DNA Extraction

Tissue samples stored in 99% ethanol were lysed to extract DNA for sequencing. Using sterile dissection scissors (wiped clean, dipped in three washes of deionised water, swirled in DNA-ExitusPlus (Qiagen) and wiped again with a clean paper towel), a small section of tissue was cut and placed into the Eppendorf tube. 180 µL of animal tissue lysis buffer (Buffer ATL, Qiagen, Germany) and 20 µL of proteinase K (PCR grade recombinant proteinase K, Thermo Fisher Scientific) were pipetted (Research plus Pipette, Eppendorf, Hamburg, Germany) into a 1.5 mL Eppendorf tube for each sample and incubated overnight at 56°C (MinIT-100 Aosheng Incubator, Hangzhou Allsheng Instruments Co., LTD., Hangzhou, Zhejiang, China).

The next day, the samples were centrifuged for 30 sec at 3,000 g to ensure no solution was left on the underside of the lid (Centrifuge 5424R, Eppendorf, Hamburg, Germany). In a falcon tube, a 1:1 ratio of animal lysis buffer (Buffer AL, Qiagen) and 100% ethanol were combined and mixed. 400 µL of this mixture was added to the Eppendorf tube containing the lysed tissue sample and pipetted up and down to mix the contents together.

All 600 µL of the sample was transferred to a spin column (EconoSpin® All-In-One DNA/RNA Mini Spin Columns, Epoch Life Science Inc., Missouri City, TX, USA) and centrifuged at 6,000 g for 1 min. The flow-through liquid contained within the collection tube of the spin column was discarded. With the spin column placed back into the collection tube, 500 µL of wash buffer 1 (Buffer AW1, Qiagen) was added before the tube was centrifuged again at 6,000 g for 1 min. The flow-through liquid was discarded again, then the spin column was placed back into the collection tube. 500 µL of wash buffer 2 (Buffer AW2, Qiagen) was added to the spin column, and it was centrifuged at 20,000 g for 3 min. The flow-through liquid was discarded for a final time before the spin

column was centrifuged again at 20,000 g for 1 min to fully dry the column and ensure no ethanol remains with the isolated DNA.

The spin column was transferred into a new 1.5 mL Eppendorf tube and 50 µL of elution buffer (Buffer AE, Qiagen) was added to the column for 1 min at room temperature then centrifuged at 6,000 g for 1 min. Afterwards, another 50 µL of elution buffer was added to the spin column, which rested for 1 min at room temperature and then centrifuged at 6,000 g for 1 min again. The isolated DNA collected in the Eppendorf tube was stored at -20°C until it could be amplified by polymerase chain reaction (PCR) and sequenced.

4.4.7 Primer Design

Sequencing primers were designed using Geneious Prime software (Version 2024.0.7, GraphPad Software LLC) and synthesised by Integrated DNA Technologies (Danaher Corp., Coralville, Iowa, USA). The primers were designed based on oegopsid rhodopsin sequences uploaded to GenBank of species representing the six families included in this study (Table 9). Ten primer sets were designed to partially sequence the rhodopsin gene, targeting the 5^{th}-7^{th} transmembrane alpha helices where the chromophore is reported to bind (Chung & Marshall, 2016; Morris et al., 1993).

Table 9. *Reverse and forward primers used for rhodopsin (opsin) DNA sequencing.*

Family & Species	Forward Primer	Melting Temperature (°C)	Reverse Primer	Melting Temperature (°C)	Primer Set
Ommastrephidae					
N. sloanii	AGGGAGTCCTCTGCAAYTGC	66.1	CCAKGAGAGCAAGAACTGGG	65.8	1
N. gouldi	CCCAGTTCTTGCTSTCMTGG	66.2	TGGATRGCAGADGCCTTGGC	70.4	2
T. angolensis	TCATGTTCGCCAAGGCHTCT	68.4	GTGGGTAWCCTTGTGGTGGG	66.6	3
T. filippovae					
Onychoteuthidae					
M. ingens	ARGGMGTCCTCTGCAATTGC	67.5	CCAKGAGAGCAAGAACTGGG	65.8	4
O. robsoni	CCCAGTTCTTGCTSTCMTGG	66.2	TGGATRGCAGARGCCTTGGC	70.5	5
O. aff. *robsoni*	TCATGTTCGCCAAGGCHTCT	68.4	GTGGGTATCCYTGTGGTGGG	67.8	6
Histioteuthidae					
	AGGGAGTYCTCTGCAAYTGY	63.5	CCAKGAGAGCAAGAACTGGG	65.8	7*
H. miranda	CCCAGTTCTTGCTSTCMTGG	66.2	TGGATAGCWGAAGCCTTGGC	66.7	8
	TCATGTTCGCCAAGGCTTCW	68.3	GTGGGTAWCCTTGTGGTGGG	66.6	9*
Octopoteuthidae					
	AGGGAGTCCTCTGCAAYTGC	66.1	CCAKGAGAGCAAGAACTGGG	65.8	1
T. fimbria	CCCAGTTCTTGCTSTCMTGG	66.2	TGGATRGCAGARGCCTTGGC	70.5	5
	TCATGTTCGCCAAGGCHTCT	68.4	GTGGGTAWCCTTGTGGTGGG	66.6	3*
Architeuthidae					
	AGGGAGTCCTCTGCAAYTGC	66.1	CCAKGAGAGCAAGAACTGGG	65.8	1
A. dux	CCCAGTTCTTGCTSTCMTGG	66.2	TGGATRGCAGARGCCTTGGC	70.5	5
	TCATGTTCGCCAAGGCHTCT	68.4	GTGGGTAWCCTTGTGGTGGG	66.6	3
Cranchiidae					
	AGGGAGTCCTCTGCAAYTGC	66.1	CCAKGAGAGCAAGAACTGGG	65.8	1
M. hamiltoni	CCCAGTTCTTGCTSTCMTGG	66.2	TGGATRGCAGARGCCTTGGC	70.5	5
	TCATGTTCGCCAAGGCHTCT	68.4	GTGGGTATCCYTGKGGTGGG	69.2	10

* Indicates a primer set that did not produce a sequence and is not represented in the electrophoresis figure (see Appendix B, Fig. I)

4.4.8 Polymerase Chain Reaction

The isolated DNA samples were amplified by PCR. To begin, 10.5 µL of a PCR 'master mix' solution was added to 200 µL well strips, one filled well for each sample (8-Strip 0.2 mL PCR Tubes with Attached Dome Caps, Neptune, USA). The PCR 'master mix' consisted of 6.25 µL of 10% Trehalose (Invitrogen), 2 µL of deionised water, 1.25 µL of 10x PCR Rxn buffer (Invitrogen), 0.625 µL of $MgCl_2$ (50 mM $MgCl_2$, Invitrogen), 0.625 µL of deoxynucleotide triphosphates (dNTP Solution Mix, New England BioLabs), and 0.06 µL of Platinum Taq DNA polymerase (Invitrogen). Then, 0.2 µL of the appropriate forward and reverse primers and isolated DNA were added to each well. Negative controls were primer sets and 'master mix' without DNA into additional 50 µL wells. All samples and negative controls were centrifuged for 30 sec at 3,000 g to ensure the solutions were collected at the bottom of the tubes.

The well strips containing the DNA samples and negative controls were placed into a thermocycler for PCR (Vapo.Protect Mastercycler Pro, Eppendorf, Hamburg, Germany). The thermocycler was programmed with the following settings: First, the DNA was denatured at 95°C for 5 min. Then, the samples were denatured for an additional 30 sec at 94°C, annealed for 40 sec at 53°C and extended for 1 min at 72°C; this second step ran for 35 cycles. After the last PCR cycle, the samples were extended a final time for 10 min at 72°C. The thermocycler was set to preserve the samples at 4°C until they were collected and refrigerated at 4°C.

4.4.9 Electrophoresis

The day after the DNA samples were amplified by PCR, electrophoresis tests were performed to determine whether the PCR process was successful. An agarose gel for electrophoresis was created by combining 1 g of agarose powder to 100 mL of 1x Tris-Borate-Ethylenediaminetetraacetic Acid (TBE; UltraPure 10x TBE Buffer, Invitrogen). The solution was microwaved for 30 sec, and then in 10 sec intervals, while making sure to swirl the mixture in between reheating, until the solution was clear (The Genius Sensor1100W Microwave, Panasonic, Osaka, Japan). After cooling for 10 min, 1.5 µL of GelRed (Invitrogen) was added to the agarose solution, mixed, then poured evenly into a gel casting tray with combs inserted to create wells. The agarose gel was allowed to cool for a further 30 min while covered to prevent contamination.

After 30 min, the combs were removed from the gel and the casting tray was placed into an electrophoresis chamber (Owl EasyCast B2 Mini Gel Electrophoresis Systems, Thermo Fisher Scientific, Waltham, Massachusetts U.S.A.). The chamber was filled with 1x TBE, covering the gel completely. Next, 1 µL of DNA solution was mixed with 1 µL of loading dye (Thermo Fisher Scientific) using a pipette. Each 2 µL DNA and loading dye solution was loaded into individual wells within the gel. Negative controls were also mixed with loading dye and loaded into the wells, and 2 µL of a 1 Kb DNA ladder (Thermo Fisher Scientific) was loaded into the first well in the gel to determine the lengths of DNA sequence amplified by PCR.

Once the gel was loaded, the electrophoresis chamber was connected to a power supply (PowerPac Basic, Bio-Rad Laboratories, Auckland, New Zealand) set to 120 V for 10 min. After running, the gel was removed from the casting tray and placed into a UV

transilluminator (Smartview Pro 1100, Major Science Co., Ltd, Taoyuan, Taiwan) for imaging to confirm the presence of bands. The gel was imaged using the Smartview Pro 1100 Imager System (v2015) under UV light and set to a 6 sec exposure (Appendix C, Fig. I).

4.4.10 Sequencing and Analysis

The DNA samples were transferred into a skirted Eppendorf plate and sent to Macrogen, Inc. (Seoul, South Korea) for sequencing. Sequences were uploaded to the Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007) with the public project title, 'NZ Oegopsid Rhodopsins' (project code: ORHO). The sequences will subsequently be uploaded to GenBank via BOLD during publication. A phylogenetic tree was constructed with these sequences in addition to full and partial oegopsid rhodopsin sequences available on GenBank retrieved for analysis (Table 10).

Sequences were aligned using the Multiple Alignment Fast Fourier Transform tool (MAFFT; v2024, European Molecular Biology Laboratory – European Bioinformatics Institute). The aligned sequences were uploaded to the software jModelTest2 (v2016) to determine the best-fit model for building a phylogenetic tree, which suggested using the Hasegawa-Kishino-Yano model. Next, the aligned sequences were uploaded to the software Molecular Evolutionary Genetics Analysis (MEGA v12.0.8, Pennsylvania State University) to generate a phylogenetic tree using the Hasegawa-Kishino-Yano model with 1000 bootstrap replicates. The software TreeGraph2 (v2.15.0-887, GNU General Public License) was used to collapse any branch with a confidence level lower than 70%. Finally, the application FigTree (v1.4, Institute of Evolutionary Biology, University of Edinburgh) was used to ready the phylogenetic tree for publication.

Table 10. *Species used in the phylogenetic analyses, their accession numbers on GenBank and BOLD (for sequences produced by the current study), and references from where the λ_{max} measurements derive.*

Species	λ_{max} (nm)	FWHM (nm)	Bioluminescent	Accession	λ_{max} Reference
Nautilus pompilius	467	-	No	LC021433	(Muntz, 2010)
Taningia fimbria	478	86	Yes	RHO005-25*	Current Study
Joubiniteuthis sp.	-	-	No	AY616914.1	-
Cranchia scabra		-	Yes	AY617061.1	-
Octopoteuthis nielseni	-	-	No	AY617056.1	-
Architeuthis dux	-	-	No	AY617052.1	-
Architeuthis dux			No	ORHO010-25*	Current Study
Histioteuthis oceanica	-	-	Yes	AY617053.1	-
Histioteuthis miranda	487^{L} 467^{S}	79^{L} 91^{S}	Yes	ORHO009-25*	Current Study

Pterygioteuthis microlampas	-	-	Yes	AY616913	-
Abraliopsis pacificus	-	-	Yes	AY617054.1	-
Enoploteuthis higginsi	-	-	Yes	AY617054.1	-
Onychoteuthis sp.	-	-	No	AY617051.1	-
Moroteuthopsis ingens	482	79	No	ORHO006-25*	Current Study
Onykia robsoni	489	80	No	ORHO007-25*	Current Study
Onykia aff. *robsoni*	489	84	No	ORHO008-25*	Current Study
Megalocranchia fisheri	-	-	Yes	AY617059.1	-
Teuthowenia megalops	-	-	Yes	AY617060.1	-
Galiteuthis sp.	-	-	Yes	AY617058.1	-
Mesonychoteuthis hamiltoni	480	91	Yes	ORHO011-25*	Current Study
Illex coindetii	-	-	No	AY617062.1	-
Todaropsis eblanae	-	-	No	AY617063.1	-
Ommastrephes bartramii	482	-	Yes	AY616915.1	Seidou et al. 1990
Sthenoteuthis oualaniensis	-	-	Yes	AY545185.1	-
Todarodes filippovae	480	83	No	ORHO004-25*	Current Study
Todarodes angolensis	475	80	No	ORHO003-25*	Current Study
Nototodarus gouldi	-	-	No	ORHO002-25*	-
Todarodes pacificus	482	-	No	X70498	Seidou et al. 1990
Nototodarus sloanii	478	80	No	ORHO001-25*	Current Study

[L] Indicates measurements that were taken from the large eye of *H. miranda*
[S] Indicates measurements that were taken from the small eye of *H. miranda*
*Indicates an accession number for BOLD from a specimen in the current study

The rhodopsin DNA sequence retrieved from species published to GenBank and used in the phylogenetic analyses, whether they possess bioluminescent capabilities was confirmed through the Tree of Life Web Project (ToLWeb; (Maddison & Schulz, 2007)). Pagel's lambda test was used to estimate the phylogenetic signal to quantify the degree that the λ_{max}, FWHM and bioluminescence trait variation among species corresponds to their phylogenetic relationships; this was done using the R packages APE (v5.8-1) and PHYLOTOOLS (v2.4-4). Due to the two λ_{max} and FWHM measurements for

H. miranda, one set of measurements for each dimorphic eye, Pagel's lambda test was run twice for those traits, once for each eye. All phylogenetic analyses were performed using R (Version 4.4.1) with RStudio (Cranberry Hibiscus, Version 2024.09.0 Build 375).

4.5 Results

4.5.1 Rhodopsin Spectral Sensitivity

Normalised visual pigment spectral sensitivity absorption curves were produced for each target species (Fig. 36), providing insights into variations in light sensitivity across taxa. Two key measurements were obtained from each spectral absorption curve: the wavelength of peak absorbance (λ_{max}) and the full width at half maximum (FWHM) (Fig. 36L). *H. miranda* dimorphic eyes results are plotted in separate figures (Fig. 36 J & K). The figure highlights observed trends potentially indicating spectral tuning, with λ_{max} and FWHM measurements illustrating interspecies differences.

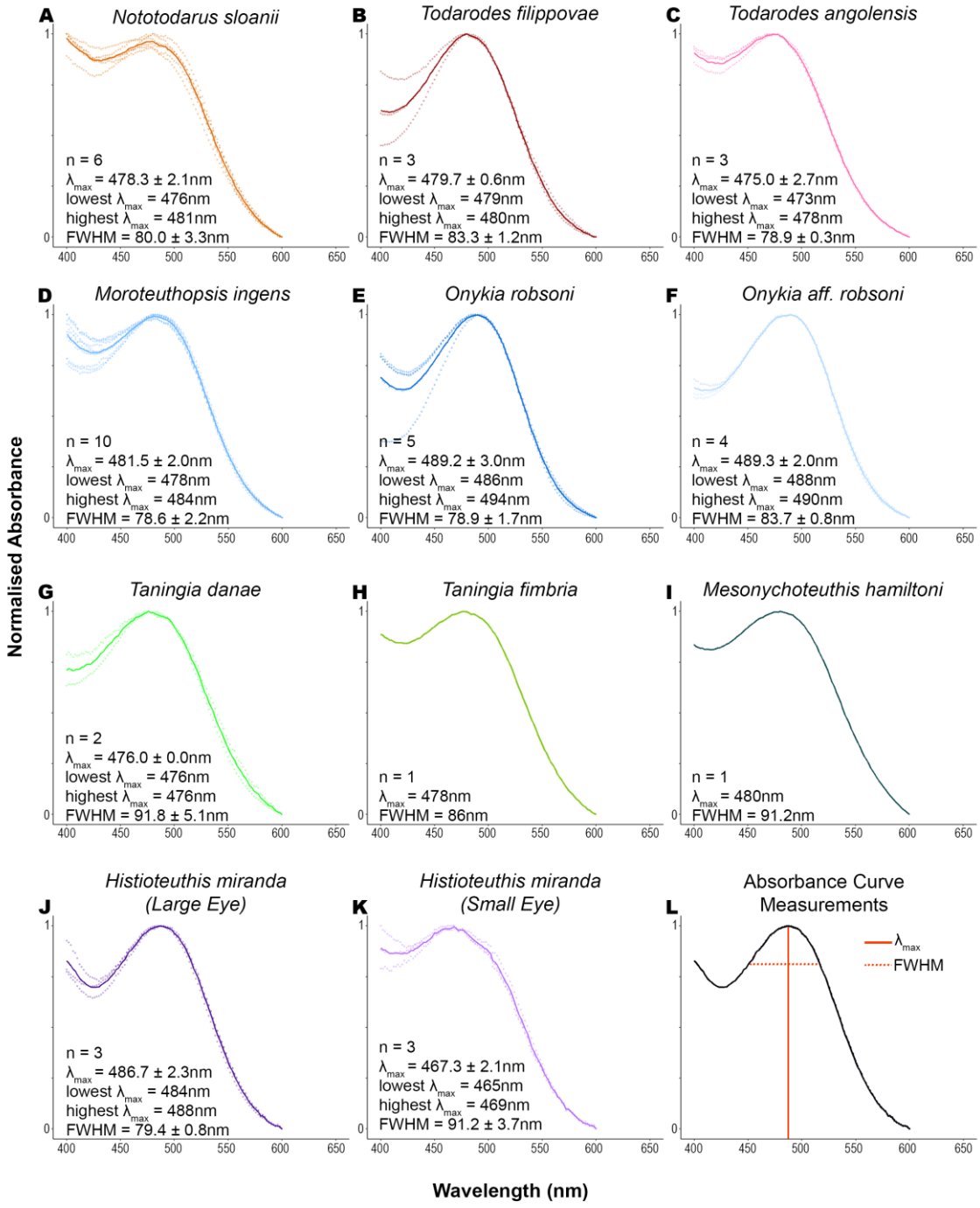


Figure 36. *A-K) Normalised visual pigments spectral sensitivity absorption curves for all species. The dotted lines indicate measured spectral curves for each specimen within a species. Small diamonds represent measurements at 2 nm intervals. Species without diamonds had a sample size of n = 1. Solid lines represent the mean normalised visual pigments spectral sensitivity absorption values. L) Diagram showing the two measurements analysed in each absorption curve. Abbreviations: sample size (n); peak absorbance (λ_{max}); full width at half maximum of the curve (FWHM).*

The mean λ_{max} was calculated to determine the optimal wavelength of light detected by the visual pigments in each species (Fig. 37). Linear modelling revealed a significant effect of species on λ_{max} ($p < 0.001$). Post-hoc comparisons (Tukey's HSD post-hoc tests, p-values in Appendix D, Table XV) were conducted to assess statistical

differences in mean λ_{max} among species. The highest mean λ_{max} values, approaching 490nm, were observed in *O. robsoni* (mean λ_{max} = 489.2 ± 3.0 nm; lowest observed λ_{max} = 488; highest observed λ_{max} = 494; n = 5), *O.* aff. *robsoni* (mean λ_{max} = 489.3 ± 2.0 nm; lowest observed λ_{max} = 489; highest observed λ_{max} = 490; n = 4), and the large eye of *H. miranda* (mean λ_{max} = 486.7 ± 2.3 nm; lowest observed λ_{max} = 484; highest observed λ_{max} = 488; n = 3). Several species, including *N. sloanii* (mean λ_{max} = 478.3 ± 2.1 nm; lowest observed λ_{max} = 476; highest observed λ_{max} = 481; n = 6), *T. filippovae* (mean λ_{max} = 479.7 ± 0.6 nm; lowest observed λ_{max} = 479; highest observed λ_{max} = 480; n = 3), and *T. danae* (mean λ_{max} = 476.0 ± 0.0 nm; lowest observed λ_{max} = 476; highest observed λ_{max} = 476; n = 2) had mean λ_{max} values not significantly different to *T. angolensis* (mean λ_{max} = 475.0 ± 2.7 nm; lowest observed λ_{max} = 473; highest observed λ_{max} = 478; n = 3) and *M. ingens* (mean λ_{max} = 481.5 ± 2.0 nm; lowest observed λ_{max} = 478; highest observed λ_{max} = 484; n = 10), all clustering around 480 nm. However, *T. angolensis* had a significantly lower mean λ_{max} than *M. ingens*. Due to *T. fimbria* (λ_{max} = 478 nm) and *M. hamiltoni* (λ_{max} = 480 nm) having a sample size of 1, these species were not statistically analysed, but their λ_{max} values were also near 480 nm. The small eye of *H. miranda* (mean λ_{max} = 467.3 ± 2.1 nm; lowest observed λ_{max} = 465; highest observed λ_{max} = 468; n = 3) had the significantly lower mean λ_{max}, near 470 nm. The difference between the mean λ_{max} of the small and large eyes of *H. miranda* was 19.4 nm. The calculated mean λ_{max} had a standard deviation less than 3.0 nm.

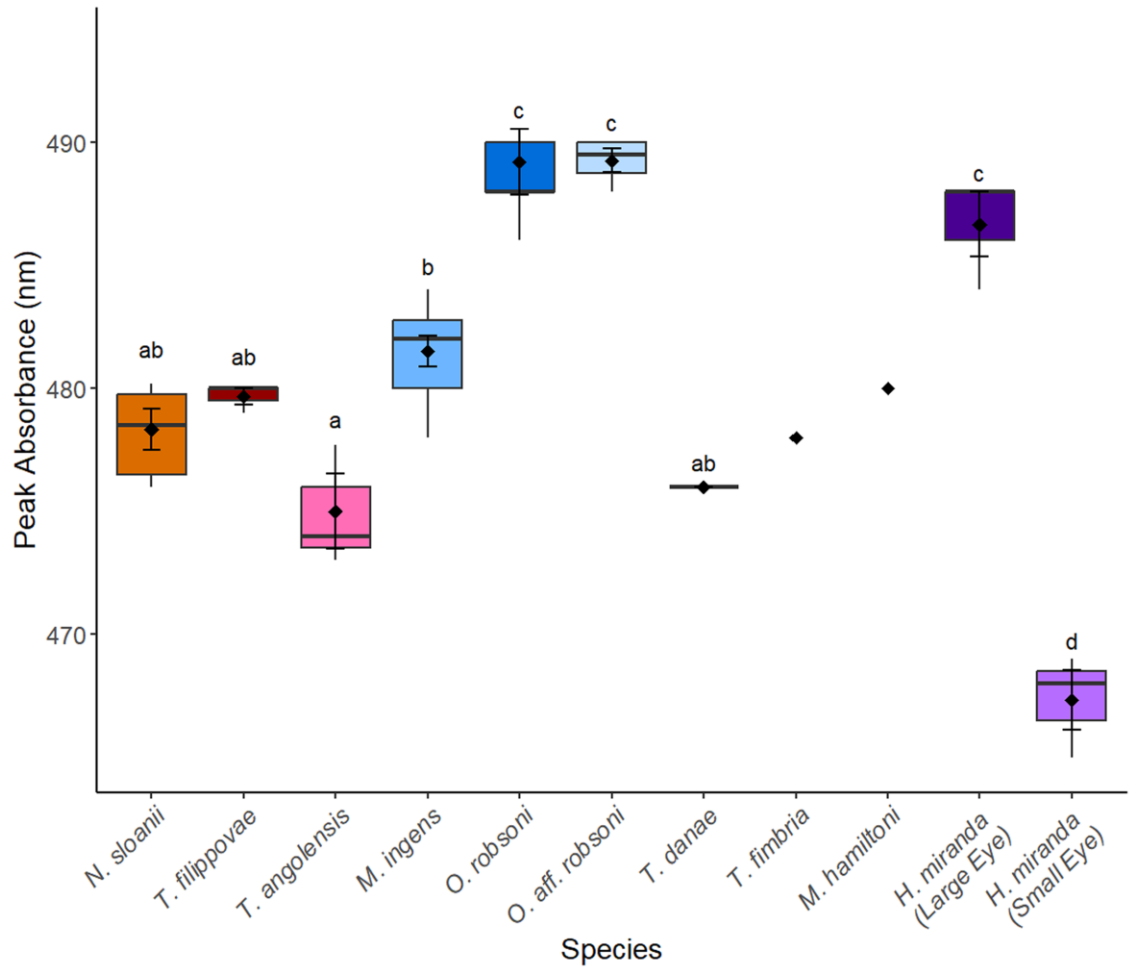


Figure 37. *Mean peak absorbance (λ_{max}) of visual pigments based on the spectral sensitivity curves of each species. Diamonds indicate the mean λ_{max} and error bars represent the standard error of the mean (SEM). The letters over the data indicate statistical significance. Data points labelled with the same letter are not significantly different λ_{max} values ($p > 0.05$). Analyses were conducted by linear modelling and Tukey's HSD post-hoc tests for pairwise comparisons. Post-hoc test statistics and probability metrics comparing λ_{max} values are listed in the Appendices (Appendix D, Table XV). Species with a sample size of 1 were not included in the statistical analysis (*T. fimbria *and* M. hamiltoni*).*

The mean FWHM values of each species were calculated to determine the bandwidth of spectral sensitivity absorbance and the wavelength range most likely to induce activation of the visual pigments' molecules (Fig. 38). Linear modelling revealed a significant effect of species amongst their mean FWHM values ($p < 0.001$). Post-hoc comparisons (Tukey's HSD post-hoc tests, p-values in Appendix D, Table XVI) identified statistical differences in mean FWHM among species. *T. danae* (mean FWHM = 91.8 ± 5.1 nm) and the small eye of *H. miranda* (mean FWHM = 91.2 ± 3.7 nm) had the broadest mean FWHM values, both near 90 nm. *T. fimbria* (FWHM = 86 nm) and *M. hamiltoni* (FWHM = 91.2 nm) also had FWHM values near 90 nm but could not be statistically analysed due to their small sample size (n = 1). Species with mean FWHM values near 80 nm included *N. sloanii* (mean FWHM = 80.0 ± 3.3 nm), *T. filippovae* (mean FWHM = 83.3 ± 1.2 nm), *T. angolensis* (mean FWHM = 78.9 ± 0.3 nm), *O. robsoni* (mean FWHM =78.9 ± 1.7 nm), and the large eye of *H. miranda* (mean FWHM = 79.4 ± 0.8 nm) all of which were statistically similar to *M. ingens* (mean FWHM = 78.6 ± 2.2 nm) and *O.* aff.

robsoni (mean FWHM = 83.7 ± 0.8 nm). However, different mean FWHM values were observed for *M. ingens* and *O.* aff. *robsoni*. Notably, the mean FWHM values of the large and small eye of *H. miranda* differed by 11.8 nm.

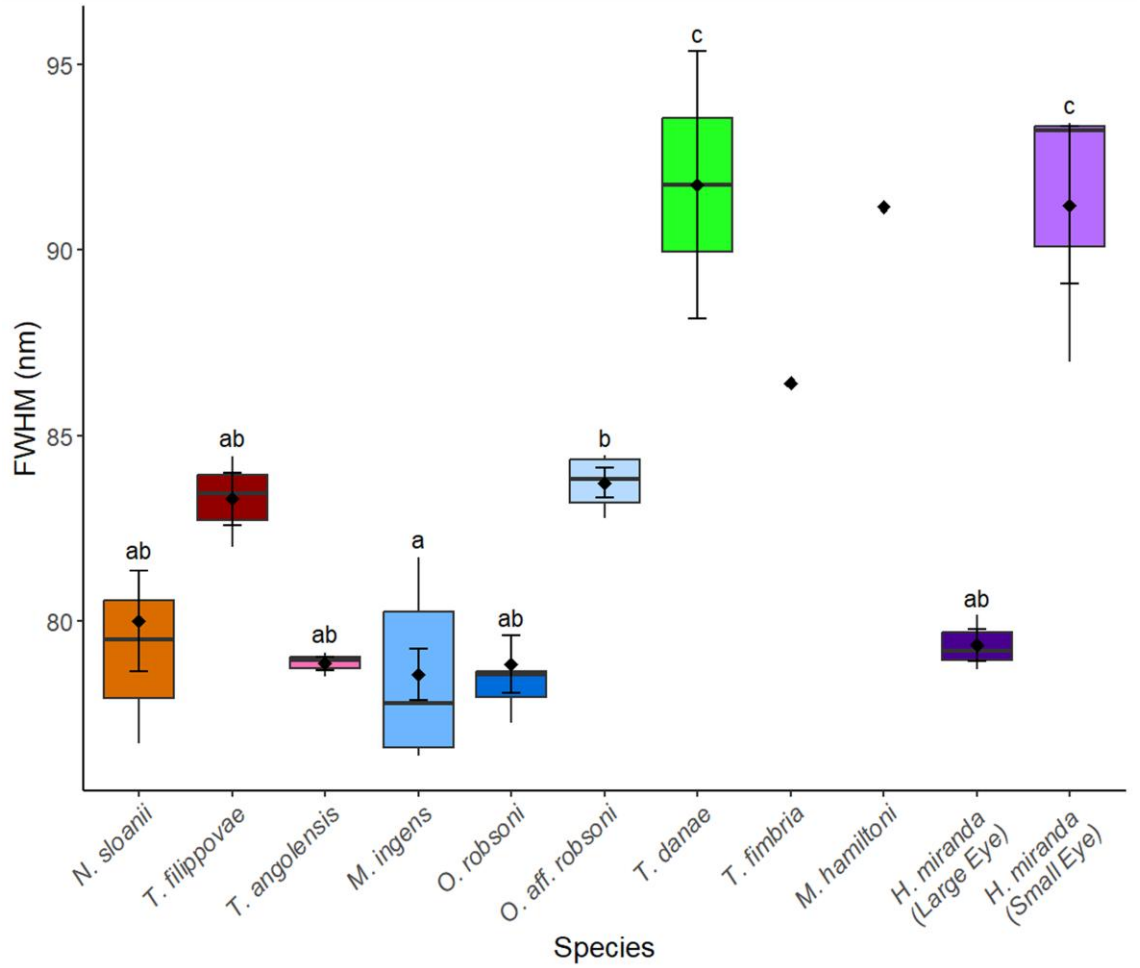


Figure 38. *Mean FWHM of visual pigments derived from the spectral sensitivity curves of each species. Diamonds indicate the mean FWHM values and error bars represent the standard error of the mean (SEM). Species sharing the same letter do not have statistically significantly different FWHM values (p > 0.05). Analyses were conducted by linear modelling and Tukey's HSD post-hoc tests for pairwise comparisons. Post-hoc test statistics and probability metrics comparing mean FWHM values are listed in the Appendices (Appendix D, Table XVI). Species with a sample size of 1 were not included in the statistical analysis (*T. fimbria *and* M. hamiltoni*).*

Species were grouped based on the presence or absence of bioluminescent capabilities. A one-way ANOVA showed a significant difference in λ_{max} between the two groups (p = 0.02; Fig. 39A). Non-bioluminescent species had a significantly higher peak absorbance than bioluminescent species; however, considerable overlap in λ_{max} existed between the two groups, around 480 nm (Fig. 39B).

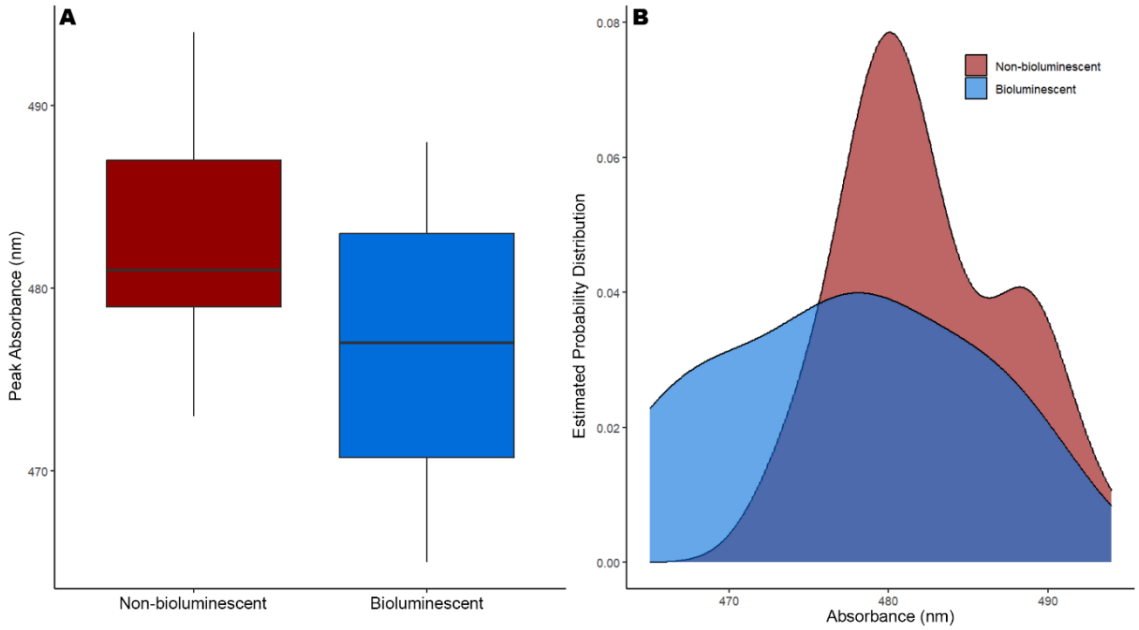


Figure 39. *A) Species with bioluminescent capabilities had significantly lower λ_{max} measurements (one-way ANOVA, p = 0.02) B) λ_{max} values in the two groups overlapped around 480 nm.*

A one-way ANOVA showed a significant difference in FWHM between non-bioluminescent and bioluminescent species ($p < 0.01$, Fig. 40A). Bioluminescent species had significantly broader FWHM values than non-bioluminescent species, with some overlap observed between the groups (Fig. 40B). Notably, the large eye of *H. miranda* had a FWHM of 79.4 nm, aligning more closely with non-bioluminescent species.

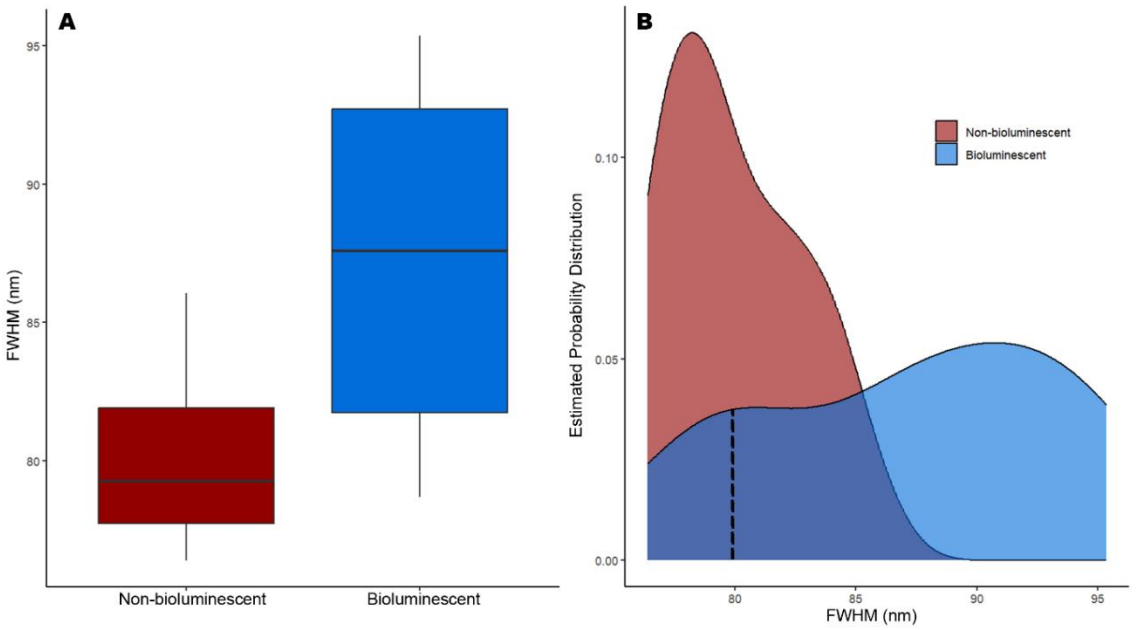


Figure 40. *A) Species with bioluminescent capabilities had significantly wider FWHM values (one-way ANOVA, p < 0.01) than non-bioluminescent ones. B) The FWHM value from the large eye of H. miranda caused additional overlap between the two groups (black dashed line).*

A MANOVA analysis was conducted to compare FWHM and λ_{max} between bioluminescent and non-bioluminescent species, revealing a significant difference between the two groups ($p < 0.001$, Fig. 41). Pillai's trace value (0.414) indicated a moderate-to-strong effect of bioluminescence on the combined spectral characteristics, accounting for 41% of the variance in the dependent variables.

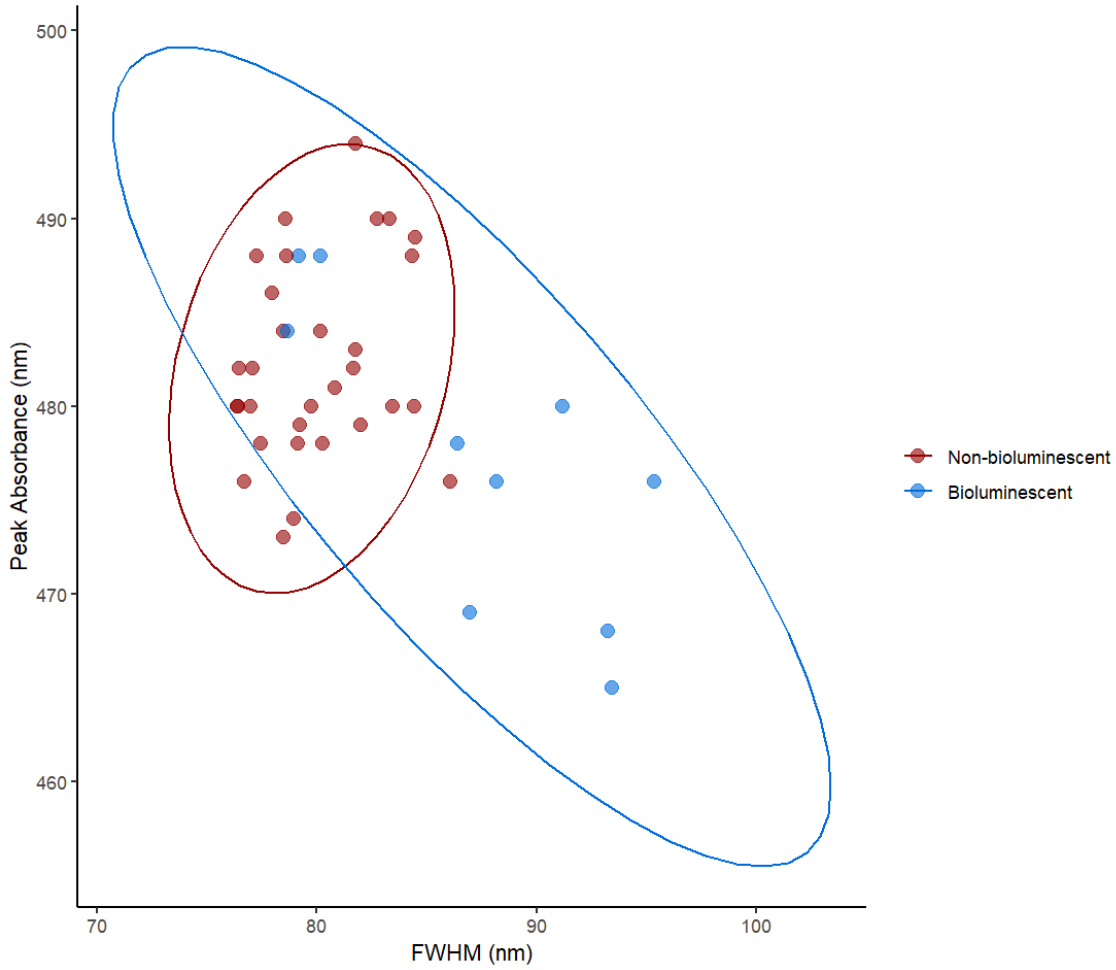


Figure 41. *λ_{max} values plotted against FWHM values for individual specimens, categorised by their bioluminescent capability. The circular areas represent the 95% confidence intervals for each group. Bioluminescent and non-bioluminescent species showed a significant difference in the variance of the combined measurements (MANOVA, $p < 0.001$).*

Notably, the combined FWHM and λ_{max} characteristics of the large eye of *H. miranda* align better with those of non-bioluminescent species. Taking this into consideration, when the large eye of *H. miranda* is reassigned to the non-bioluminescent category, and the MANOVA test is repeated, the differences between the two groups remains statistically significant ($p < 0.001$, Fig. 42). However, Pillai's trace value increases to 0.74, indicating a strong effect of bioluminesence on the combined spectral characteristics of FWHM and λ_{max}, accounting for about 74% of the variance in the dependent variables.

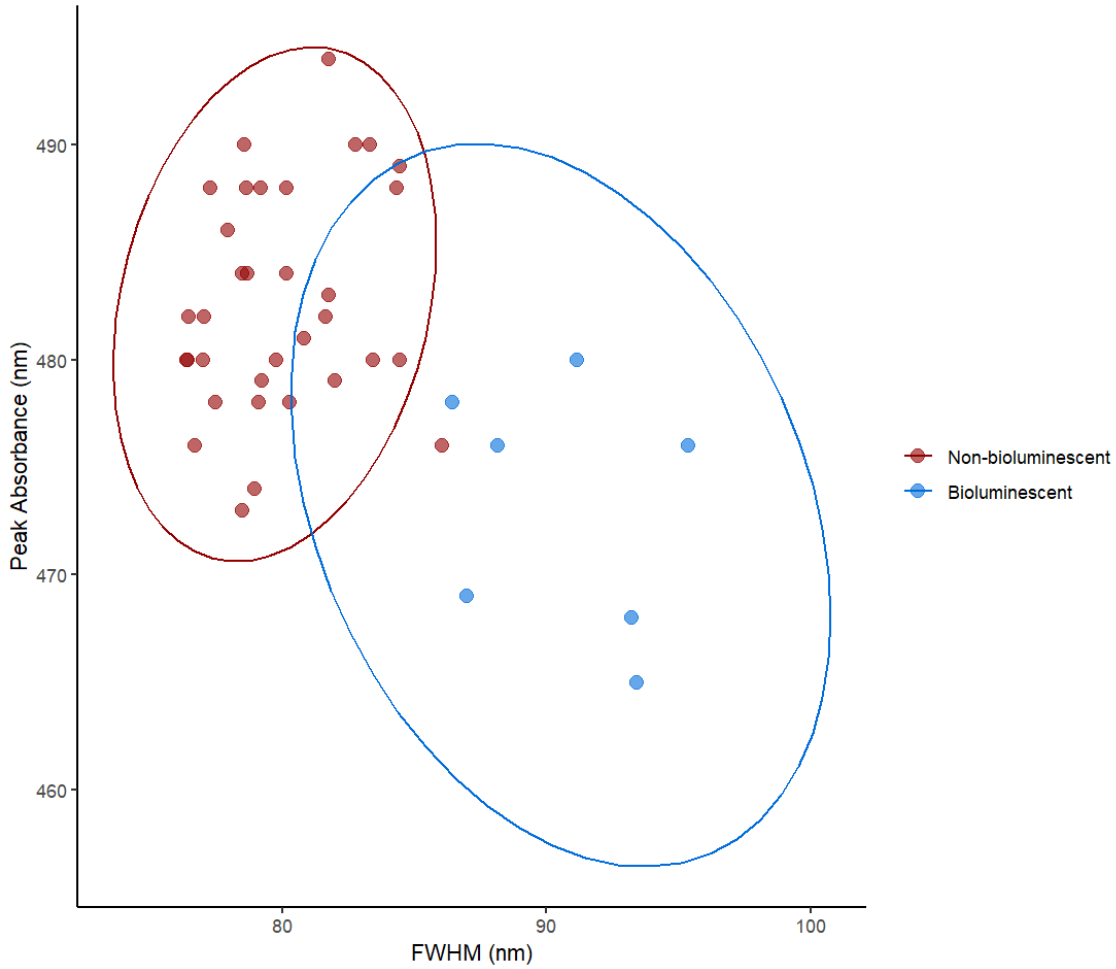


Figure 42. *λ_{max} values plotted against FWHM values for individual species, categorised by bioluminescent capability, but with the large eye of H. miranda re-assigned to the non-bioluminescent group. The circular areas represent the 95% confidence intervals for each category. Bioluminescent and non-bioluminescent species showed a significant difference in the variance of the combined measurements (MANOVA, $p < 0.001$).*

4.5.2 Phylogenetic Analysis

Partial rhodopsin sequences were generated for 11 oegopsid species and combined with 16 additional complete and partial rhodopsin sequences obtained from GenBank to determine the phylogenetic relationships of rhodopsin amongst species. *Nautilus pompilius* was used as the outgroup species (Fig. 43). Amino acid sequence prediction and alignment was used as the base for the phylogenetic reconstruction (Appendix E, Table XVII).

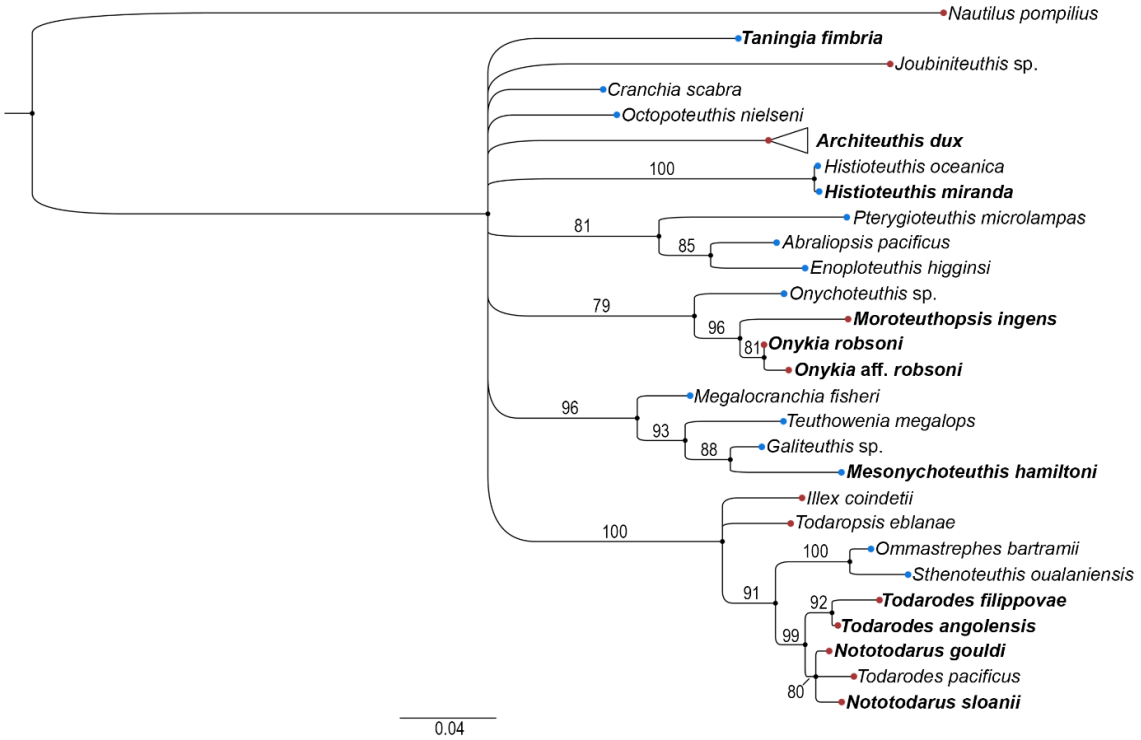


Figure 43. *Maximum-likelihood phylogenetic tree of oegopsid rhodopsin. Species sequenced in this study are shown in bold. Red terminal nodes indicate non-bioluminescent species. Blue terminal nodes indicate bioluminescent species. Bootstrap values are shown at the nodes of their respective branches.*

Pagel's lambda test was used to estimate the phylogenetic signal of λ_{max}, including the λ_{max} measurement for the large eye of *H. miranda.* The test showed a moderate to strong phylogenetic relationship; however, the confidence level did not exceed the 95% confidence interval ($\lambda = 0.78$, $p = 0.10$). Similarly, the phylogenetic signal for FWHM, using the FWHM measurement for the large eye of *H. miranda*, suggested a moderate to strong phylogenetic relationship, but this was also not statistically significant ($\lambda = 0.75$, $p = 0.36$). When the λ_{max} and FWHM measurements from the small eye of *H. miranda* were included, the phylogenetic signals for both traits changed. The phylogenetic signal of Pagel's lambda test for λ_{max} increased, showing a significant phylogenetic relationship ($\lambda = 0.91$, $p = 0.03$). In contrast, the phylogenetic signal for FWHM increased, but remained statistically non- significant ($\lambda = 0.81$, $p = 0.10$). The phylogenetic signal estimation of bioluminescence showed a very strong relationship between the presence or absence of bioluminescence and rhodopsin phylogeny ($\lambda > 0.99$, $p < 0.001$).

4.6 Discussion

A key limitation of the present study is that cephalopod rhodopsin and its photoconverted metarhodopsin possess partially overlapping absorbance spectra, complicating precise determination of the rhodopsin peak spectral absorbance (λ_{max}). Following the absorption of light, rhodopsin is rapidly converted through a series of photochemical intermediates to metarhodopsin, with this process occurring on the order of milliseconds to seconds. Conversely, metarhodopsin can be rapidly photoconverted back to rhodopsin following exposure to light of appropriate

wavelengths. However, in the absence of light, metarhodopsin is thermally stable and does not readily revert to rhodopsin through spontaneous dark regeneration, unlike the visual pigment cycle observed in vertebrates (Baumann, 1972). Consequently, absorbance measurements obtained from retinal photopigment extracts may represent a combination of rhodopsin, metarhodopsin, and intermediary photoproducts generated during the photochemical cycle. Although extensive precautions were taken to minimise photoconversion, including maintaining specimens and extracts in light-sealed containers, conducting dissections under minimal light exposure, and keeping samples chilled throughout processing, contributions from metarhodopsin cannot be completely excluded. Therefore, the reported mean λ_{max} and mean FWHM measurements should be interpreted as characteristics of the extracted visual pigment preparations rather than definitive measurements of dark-adapted rhodopsin alone. Nevertheless, as expected many species from this study exhibited photopigment spectral peak absorbances near 480nm, the wavelength of light that penetrates deepest in clear oceanic waters. This has also been observed in other deep-sea animals, such as fishes, as a visual tuning characteristic that may improve their ability to detect predators and prey in the euphotic and dysphotic zones (Warrant & Locket, 2004). However, *O. robsoni* and *O.* aff*. robsoni* exhibited mean λ_{max} values near 490 nm. *Onykia* aff*. robsoni* has not been officially described and little is known about its life-history and behaviours, making it difficult to surmise the reasons this species' λ_{max} near 490 nm. From personal observations, *O.* aff*. robsoni* appears to inhabit the same environmental niche as *O. robsoni* but with a more southerly distribution but suggest a close genetic relationship between their respective rhodopsin molecules. Adult *O. robsoni* are usually collected in trawls from 700-1100 m (Bolstad, 2007). In comparison, the closely related *M. ingens* has a typical distribution of 400-900 m (Bolstad, 2007), but exhibited a mean λ_{max} near 480 nm. This suggests a nearly 10 nm spectral shift in λ_{max} between these two genera. Perhaps most surprising is that the deeper dwelling *O. robsoni* (and presumably *O.* aff. *robsoni*) experiences a bathochromic (red) shift in comparison to its shallower relative. Typically, species that have rhodopsin attuned to longer wavelengths of light are found in shallow-water, coastal, and so are reef-inhabiting cephalopod species, which exhibit peak spectral absorbance values from 490-510 nm (Crescitelli et al., 1985; Morris et al., 1993). Therefore, additional ecological factors other than ocean depth appear to be vital in determining the possible need for spectral tuning away from the typical 480 nm peak spectral light sensitivity observed in most deep-sea cephalopod species. One possible factor is the difference in hunting behaviours between *M. ingens* and *O. robsoni*. Gut analyses of *M. ingens* stomachs suggest a diet that relies mostly on myctophid species that are associated with midwater environments (Cherel & Duhamel, 2003). In contrast, adult *O. robsoni* are believed to be a typically demersal species, which may require them to prioritise looking towards the surface for potential prey. This suggests that looking towards the surface and into the downwelling light may favour red-shifted visual pigment sensitivity in deep-sea species. This hypothesis is further supported by the results comparing the mean λ_{max} of the dimorphic eyes of *H. miranda*.

Like *O. robsoni, H. miranda* is commonly found around 1,000 m deep during the day, on the boundary of the aphotic and dysphotic zones (Roper et al., 2010). Unlike *O. robsoni, H. miranda* is a pelagic species that hunts high above the ocean floor. The

dimorphic nature of the eyes of *H. miranda* may give insight into the influence that eye orientation in relation to downwelling light has on visual pigment spectral sensitivity. Histioteuthids dedicate their large eye to spotting overhead predators and prey silhouetted by downwelling sunlight, while the small eye detects bioluminescent sources of light from below (Thomas et al., 2017). Surprisingly, the study by Muntz & Johnson (1978) reported a single λ_{max} value of 484 nm in *Histioteuthis meleagroteuthis*, despite the dimorphism of the eyes. The current study suggests a nearly 20 nm difference between the λ_{max} of each eye in *H. miranda*, implying the previously reported λ_{max} in *H. meleagroteuthis* may have been an average of the peak spectral absorbance measured from both eyes. At 486.7 nm, the large eye of *H. miranda* has a similar mean λ_{max} to *O. robsoni* and *O.* aff. *robsoni*. While the typical eye orientation of the onychoteuthid species is unknown, the large eye of *H. miranda* has been observed to orient towards the surface and into the downwelling light, which may have driven the large eye to incorporate a rhodopsin better attuned to longer wavelengths of light. In contrast, the small eye of *H. miranda* had the lowest mean λ_{max} value at 467.3 nm, which is an inverse of the observation we see in the large eye. Since the downward oriented eye faces away from the downwelling light, it is possible that a hypsochromic shift in peak spectral absorbance is favoured. These findings underscore the functional specialisation of their asymmetrical eyes and suggests that previous measurements may have masked these differences. Further comparisons with deep-sea species exhibiting similar upward or downward visual fields, such as the 'barreleye fish' *Macropinna microstoma,* could provide additional insights into these observations and determine whether this trend in spectral tuning in relation to eye orientation and downwelling light is common in the deep-sea.

Measurements of λ_{max} showed a standard deviation of no more than 3 nm across all species. While this may be an acceptable deviation from the mean calculations of λ_{max}, rhodopsin undergoes an isomerisation reaction that is triggered when the chromophore reacts with light. The chromophore transforms from an 11-cis-retinal to all-*trans*-retinal within picoseconds (Schoenlein et al., 1991). The protein itself reacts more slowly, over a few nanoseconds rhodopsin transitions to multiple intermediary molecules until reaching its final state as acid metarhodopsin. To return acid metarhodopsin to its initial state (rhodopsin), a retinal-binding protein serves as a shuttle to recycle all-*trans*-retinal taken from retinochrome proteins typically located at the base of the outer photoreceptor segment (Kingston et al., 2015). These intermediary molecules, and acid metarhodopsin, have been shown to exhibit λ_{max} values shifted to shorter wavelengths of light when compared to rhodopsin λ_{max}. Therefore, it is possible that the λ_{max} values of rhodopsin measured in this study have been slightly altered by the presence of rhodopsin intermediaries generated by phototransduction. However, there are several steps that were taken to avoid this measuring artifact. First, rhodopsin (or the eye containing the visual pigment) was kept on ice and in dark conditions to prevent phototransduction reactions. These precautions were taken in the current study. Second, 1% hydroxylamine, can be added to rhodopsin to prevent the conversion to any intermediaries. However, this step was not included in the current study. Finally, a direct microspectrophotometry analysis of the retina could be conducted in lieu of extracting the rhodopsin, as done by (Chung & Marshall, (2016). Their research showed

rhodopsin λ_{max} values shifted to longer wavelengths of light compared to rhodopsin extraction protocols. Still, Miyazaki & Osumi (2022) pointed out that microspectrophotometry analyses may accidentally be measuring retinochrome photopigments contained within the retina, which may have also altered their results. Nevertheless, while precautions were taken to isolate and measure only rhodopsin, some λ_{max} values may have been altered by the presence of rhodopsin – acid metarhodopsin intermediaries.

Also worthy of note is that visual pigment absorbance spectra are frequently analysed using the Govardovskii–Fyhrquist–Reuter–Kuzmin–Donner (GFRKD) visual pigment template equation (Govardovskii et al., 2000), which mathematically describes the expected absorbance profile of a visual pigment based on its peak absorbance wavelength (λ_{max}). The template was developed from empirical measurements of vertebrate and invertebrate visual pigments and provides a standardised method for estimating λ_{max} and reconstructing absorbance curves when the measured spectrum is assumed to represent a single visual pigment state. Consequently, GFRKD template fitting is commonly applied to microspectrophotometry and visual pigment extraction studies to facilitate comparisons among species and to minimise measurement noise. However, the accuracy of the template relies on the assumption that the absorbance spectrum originates primarily from a single visual pigment with minimal contributions from photoproducts or other visual pigments. When multiple photopigment states are present, such as mixtures of rhodopsin, metarhodopsin, and phototransduction intermediates, deviations from the template may occur, potentially reducing the reliability of λ_{max} estimates derived from template fitting.

Visual pigment template fitting using the GFRKD template equation was considered but was not applied to the absorbance spectra obtained in this study. The specimens analysed were collected during multiple surveys, at different times of day, and under differing ambient light conditions prior to capture. Although specimens and extracted visual pigments were subsequently maintained frozen and under light-protected conditions whenever possible, the proportion of rhodopsin, metarhodopsin, and other photointermediates present within each extract at the time of measurement could not be determined, although the dark-adaptation protocol applied favoured rhodopsin state. Since visual pigment template fitting assumes that absorbance spectra are derived predominantly from a single visual pigment state, applying a GFRKD template to these extracts may have produced λ_{max} estimates that were artificially precise and potentially biologically misleading. Consequently, mean λ_{max} and mean FWHM values were calculated directly from the normalised absorbance profiles and interpreted as comparative characteristics of the extracted visual pigment preparations rather than definitive estimates of dark-adapted rhodopsin spectral sensitivity.

The range of wavelengths over which a photopigment absorbs light (measured here as the FWHM), defines the spectral sensitivity of photoreceptors and influences contrast detection, and overall visual function. Broader bandwidths allow for greater spectral overlap. For example, the absorbance curve bandwidth of vertebrate rod photoreceptors used in night vision is broader than that of cone photoreceptors that are optimal for daytime observations. This helps animals navigate dark environments; the

visual pigments in retinas with rods and cones are triggered by a wide array of light wavelengths, while the specialisation of the cones to specific light wavelengths improves colour discrimination. In the current study, visual pigment extracts obtained from bioluminescent species generally exhibited broader absorbance profiles than those from non-bioluminescent species. One possible explanation is that broader spectral sensitivity may enhance the detection of bioluminescent signals used for communication or other ecological interactions. However, because the absorbance spectra may include contributions from both rhodopsin and metarhodopsin, caution is required when interpreting differences in FWHM as evidence of adaptive spectral tuning. Additional studies using visual pigment template fitting, microspectrophotometry, or methods that distinguish rhodopsin from its photoproducts are required to determine whether the observed differences represent true biological specialisations. It is possible that broader spectral sensitivity curves may also assist in detecting bioluminescent prey, but then it would be expected that species without bioluminescent abilities would have similar FWHM characteristics to aid hunting. Furthermore, the two eyes of *H. miranda* had FWHM values that differed by 11.8 nm. The large eye absorbance curve bandwidth was similar to the values observed in non-bioluminescent species. These findings are logical when the function of the large eye – detecting silhouettes, and not bioluminescent point sources – is taken into consideration. In contrast, the small eye is better suited to detecting bioluminescent point sources and had an absorbance curve bandwidth similar to bioluminescent species. In the case of histioteuthid vision, it seems plausible that the visual pigments incorporated into large eye have specialised to serve a similar function to the eyes of non-bioluminescent squid species, while photopigments found in the small eye exhibits similar visual characteristic observed in other bioluminescent squid species. This idea is supported by the MANOVA analyses, which clearly indicated the spectral characteristics (FWHM and λ_{max}) of the large eyes of *H. miranda* group well with non-bioluminescent species and the small eyes with bioluminescent species.

Overall, the phylogeny of rhodopsin from oegopsids aligns closely with previous phylogenetic taxonomic analyses of oegopsids using 13 manually aligned mitochondrial protein genes (Fernández-Álvarez et al., 2022). The close relationship between these two phylogenetic analyses suggests that rhodopsin generally follows the taxonomic relationships observed among oegopsid species. However, notably, (Fernández-Álvarez et al., (2022) showed onychoteuthid and histioteuthid species are closely related, which was not shown in the rhodopsin phylogeny of the current study. Therefore, it is likely other factors may be driving rhodopsin specialisation, such as physiological and behavioural difference between species. One potential driving factor identified from this study is their bioluminescent capabilities.

The relationship between bioluminescence and spectral sensitivity was further explored in all species that revealed the FWHM, and λ_{max} characteristics of spectral absorbance curves are separated between bioluminescent and non-bioluminescent species. However, this research suggests that FWHM may be the primary characteristic influenced by bioluminescence as the λ_{max} values showed more overlap between the two groups. These results suggest that broadening the rhodopsin spectral sensitivity

curve for bioluminescent species may be advantageous, but that a peak spectral sensitivity near 480 nm is still optimal for most deep-sea species.

The oegopsid rhodopsin phylogenetic tree showed clear groupings of species based on their bioluminescent capabilities. Notably, members of the Ommastrephidae family clustered neatly; *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis* grouped separately from the other species and are the only two species in the family capable of bioluminescence that are included in this study. Similarly, *Onychoteuthis* spp. comprise the only genus in the family Onychoteuthidae that possess photophores (Young, 1972b). Rhodopsin sequences from species in the genera *Onykia* and *Moroteuthopsis*, which are not bioluminescent, are more closely related to each other than *Onychoteuthis* sp. These two examples suggest that bioluminescence may be a driver of rhodopsin modification in deep-sea squids. However, *Cranchia scabra*, a member of the family Cranchiidae, does not appear to possess rhodopsin that is closely related to the other cranchiids in this study, even though all cranchiid species are capable of bioluminescence. This is not surprising, as previous reports have suggested ecological factors other than bioluminescence also likely influence rhodopsin phylogeny, such as the habitat and depth (Chung & Marshall, 2014).

The phylogenetic analysis of mean λ_{max} values in respect to rhodopsin phylogeny suggests that modifications in the rhodopsin sequence may be responsible for adjusting peak absorbance properties. These results align with earlier studies that indicate amino acid substitution sites of the rhodopsin molecule that facilitate spectral tuning in response to environmental and ecological factors (Bellingham et al., 1998; Chung & Marshall, 2016; Muntz, 2010; Muntz & Johnson, 1978). Unfortunately, the partial sequencing of rhodopsin in this study left gaps in the sequences and undermined investigations into substitution sites involved with spectral tuning in oegopsid squids. However, future studies aimed at understanding spectral tuning should consider comparing the closely rhodopsin sequences of *M. ingens* and *O. robsoni,* which exhibit surprisingly different mean λ_{max} values. The analysis of FWHM did not indicate any relationship to the phylogeny of rhodopsin. However, many oegopsid species included in the phylogenetic analysis and retrieved from GenBank did not have comprehensive measurements of λ_{max} and FWHM, limiting the ability to fully assess the impact of these traits on rhodopsin phylogeny.

It seems relevant to discuss the phylogeny of rhodopsin in histioteuthid species. Two phylogenetic analyses of λ_{max} and FWHM were conducted separately with the measured values from each eye. Only when the small eye of *H. miranda* was analysed did a significant relationship emerge between λ_{max} and rhodopsin phylogeny. This leaves one to postulate the mechanisms that are used to incorporate rhodopsin molecules with drastically different spectral sensitivity characteristics separately into each eye. This study found evidence for a single rhodopsin (opsin) gene in *H. miranda*. *Histioteuthis oceanica*, the only other histioteuthid rhodopsin sequence on GenBank, similarly has a single gene reported. Future studies may find that histioteuthids have multiple rhodopsin genes per species, but it is equally possible that post-translation modifications occur. Fishes and amphibians have been shown to adjust their visual pigments during development by substituting the chromophore (Bowmaker, 2008). In

deep-sea fish, many such changes to rhodopsin occur when individuals are transitioning from a shallow water larval stage to an adult life at greater depths (Sugawara et al., 2010; Van Nynatten et al., 2024). Evidence of squids using more than one chromophore-type already exist in the species *Watasenia scintillans* (Seidou et al., 1990). *Watasenia scintillans* is currently the only known cephalopod capable of colour discrimination, which is facilitated by three different chromophores distributed unevenly throughout the retina (Matsui et al., 1988). The most abundant chromophore found in the retina of *W. scintillans* is retinal (A1 chromophore), which produces a λ_{max} of 484 nm. This is the chromophore used by most cephalopods (Seidou et al., 1990). In addition, *W. scintillans* has opsins bound to 3-dehydroretinal (A2 chromophore) and 4-hydroxyretinal (A4 chromophore), which produce a λ_{max} of 500nm and 470nm, respectively. If post-translation modification occurs in histioteuthids, rhodopsin from the small eyes may use an A4 chromophore to shift its spectral sensitivity to be optimised for sensing shorter wavelengths of light, as seen in *W. scintillans*. Further, it is unclear whether histioteuthids are able discriminate colours if each rhodopsin is segregated into separate eyes. Certainly, more research into chromophore use in histioteuthids is needed to fully understand the mechanisms at play.

While this study provides valuable insights into the relationship between bioluminescence and rhodopsin spectral sensitivity, there are several areas that warrant further investigation. Expanding the dataset to include more oegopsid species with comprehensive and confirmed measurements of isolated rhodopsin would allow for more robust phylogenetic analyses and a better understanding of how bioluminescence and spectral sensitivity co-evolve in these squids. Future studies could also aim to identify the presence of rhodopsin post-translation modifications in squids, particularly in histioteuthids. Finally, little is currently known about the spectral sensitivity of other bioluminescent oegopsid families, such as enoploteuthids, other bioluminescent ommastrephids, and lycoteuthids. It would be particularly interesting to gain insight whether ontogenetic changes occur in the spectral sensitivity of rhodopsin in the genera *Thysanoteuthis*, which lose their photophore as they mature. These additional areas of research would allow researchers to determine of whether bioluminescence is truly a driver of spectral sensitivity in oegopsid squids.

Chapter 5 Conclusions and Final Discussion

Cephalopod vision has intrigued scientists for millennia, at least since Aristotle commented on the complexity of cephalopod eyes in *Historia Animalium*. However, most cephalopod vision research has been conducted on near-shore squids and octopuses. Relatively overlooked, are deep-sea oegopsid squids. This is, in part, due to the immense requirements of keeping specimens alive for laboratory research and the difficulty of collecting rarer species from the deep. Much of the information gathered on oegopsids comes from *in situ* observations by cameras mounted to remotely operated vehicles (ROVs) and underwater landers, which have given a deeper look into their behaviours (Chung, 2014; Hoving et al., 2017; Robinson et al., 2021; Thomas et al., 2017). Predominately absent from oegopsid vision research are physiological studies of the eye. The first aim of this thesis was to investigate the retinal anatomy of oegopsids and identify any specialisations that may be beneficial for vision in the deep sea, which was explored in Chapter 2. The second aim was to determine whether screening pigment migrates in oegopsids despite many species living in relatively dark conditions, which is discussed in Chapter 3. In Chapter 4, the third aim sought to characterise the spectral sensitivity curves of the retinal visual pigments and determine whether any correlations exist between those characteristics and known behaviours of the studied species. Finally, the fourth aim investigated in Chapter 4 was to genetically sequence rhodopsin genes of the studied species and create a rhodopsin phylogenetic tree to identify any traits that may drive its specialisation.

5.1 Observed Trends of Oegopsid Retinal Anatomy

The anatomy of a cephalopod retina was first described in 1960 via histological examinations of an octopus. Young (1960) determined the octopus retina is comprised of rhabdomeric photoreceptors that were everted when compared to vertebrate retinas. That same year, Moody & Parriss (1960) discovered the orthogonal arrangement of the photoreceptor microvilli allowed octopuses to discriminate between different angles of polarised light. This early research on octopuses started an investigation into the intriguing complexity of cephalopod vision that continues today. More recently, Chung & Marshall (2014) conducted detailed research on the retinal anatomy of cephalopods that included the less frequently studied oegopsid squids. Their demonstrated that some oegopsid species have evolved modifications to their retinas that likely aid in hunting and improve visual acuity (Chung & Marshall, 2014) In the interest of further investigating the diversity of oegopsid retinas and identifying any unknown retinal specialisations, retinal histological examinations were further conducted on oegopsids from Aotearoa New Zealand in this study.

Surprisingly, the species from this study all exhibited retinas that were similarly organised. No discernible specialisations were observed, such as foveae, eye deformations or modified retinal layers. This was an unexpected result. However, it is possible that the methods used to investigate the retina were not sufficiently detailed to identify these modifications. Oegopsid vision research tends to use juvenile specimens. This is likely due to the relative ease of collecting smaller specimens.

Furthermore, producing histology samples from the retinas of juvenile squids is simpler due to their small eye size. In general, adult cephalopods have large eyes that help capture more light and increase light sensitivity (Chung, 2014). These larger eyes are more difficult to section via traditional cryosectioning techniques because of the limited size of the holding puck the eye is mounted to. To overcome this barrier, this study took three consecutive retinal sections from the posterior, central, and anterior retina, rather than sectioning the entire eye. Therefore, it is possible that any specialisations of the retinas potentially present were not represented in the sections produced. However, measuring the lengths of the outer and inner photoreceptor segments did reveal trends that may align with the needs and behaviours of squids.

In the current study, the outer segments of the photoreceptors were generally longer in the dorsal and central retina than the ventral retina when comparing lengths along the vertical axis of the eye. These findings support previous research that came to similar conclusions and may be indicative of enhanced light sensitivity in those retinal regions to increase the probability of detecting light from sources below or horizontal to individuals (Chung, 2014). Light from above an individual often comes from downwelling sunlight during the day and in the first 1000 m of the ocean. However, light from a source horizontal to or below an individual can either be downwelling light reflected off an object, or from photophores. Downwelling light, which would most often contact the ventral portion of the retina, changes brightness at a slower pace (determined by the day/night cycle) and may be brighter than bioluminescent sources, depending on the time of day and depth. It is possible that the ventral retina, in most species, does not require long outer photoreceptor segments to perceive this relatively stable light source. In contrast, bioluminescence can be displayed for short durations or harder to identify when downwelling light is present or at greater distances. Therefore, oegopsid retinas may have evolved to incorporate longer outer photoreceptor segments in the dorsal and central retinal regions to increase light sensitivity and improve their ability to spot bioluminescent flashes.

It is important to note that the differences in length of the outer photoreceptor segments between the dorsal and central regions compared to the ventral region of the retina is larger for species that inhabit deeper strata. For example, species like the ommastrephid ‘arrow’ squids *N. sloanii* and *N. gouldi*, which are found mostly in the upper 300 m of the water column (Roper, Nigmatullin, et al., 2010), exhibited a 20-21 µm range of OSP length, respectively. Alternatively, species like the Southern flying squid *T. filippovae,* which have been observed in the aphotic zone (>1000 m deep), exhibited a OSP length range of 94 µm. Additionally, the cock-eyed squid *H. miranda,* a species that stays in the aphotic zone during daylight hours (Roper et al., 2010), exhibited a OSP length range of 201 µm for the large eye and 81 µm for the small eye. These differences among species suggests selective pressures to have longer OSP in the dorsal and/or central retinal regions may be greater for species that inhabit deeper waters where ambient light is limited, and bioluminescence is predominant.

The outer segments of the photoreceptors were also generally longer in the anterior and poster retinal regions than the middle portion of the retina when comparing lengths along the horizontal axis. Since squids propel themselves anteriorly

or posteriorly, either via jet propulsion by their funnel or using their fins, longer OSP in those regions of the retina may help to identify predators or prey in the direction they are most likely to move in.

While longer outer photoreceptor segments are believed to correlate with increased light sensitivity by allowing more space for visual pigments to occupy (Chung, 2014; de Busserolles et al., 2014), less is known about the impact the inner photoreceptor segment has on visual acuity or light sensitivity. The inner photoreceptor segment contains the cell's nucleus. It has been demonstrated that certain oegopsid species have double-nucleated inner photoreceptor layers, which has been suggested to increase the visual acuity of the species (Chung & Marshall, 2017). Furthermore, it has been proposed that longer inner photoreceptor segments in myctophids (lanternfishes) may help to meet the energetic demands of phototransduction in species that inhabit shallower waters where ambient light is more available (de Busserolles et al., 2014). However, comparisons of the inner photoreceptor segment lengths among the dorsal, central, and ventral regions of the retina were more variable. Some species, like *N. sloanii,* exhibited longer ISP lengths in the ventral and central regions, while other species, like *T. filippovae,* exhibited significantly shorter ISP lengths in the central retinal region. These discrepancies make it difficult to draw conclusions on the impact the inner photoreceptor segment may have on visual acuity and light sensitivity in oegopsids. In contrast, the inner photoreceptor segment was generally shorter in the middle portion of the retina when compared to the anterior and posterior retina. Similar to OSP length, if ISP length does influence visual acuity and/or light sensitivity, these findings may indicate that the anterior and posterior retina benefits from longer ISPs that could aid to identify predators or prey in the direction individuals tend to move.

5.2 The Correlation Between Photoreceptor Length and Light Conditions

In the ocean, as depth increases the amount of available ambient light decreases (Denton, 1990). At 1000 m, on average, ambient downwelling light is no longer visible due to attenuation caused by particulate matter and water molecules. To overcome these lowlight conditions, many species have a specialised tissue called the tapetum lucidum along the inner eye to reflect light back to the retina and increase light sensitivity (de Busserolles et al., 2014; Douglas & Marshall, 1999; Frederiksen, 1976; McFarland, 1990). No such mechanism has been observed in cephalopods. Instead, it appears cephalopods have evolved relatively long photoreceptors compared to their fish counterparts. For instance, myctophids have been shown to have maximum outer photoreceptor lengths of about 100 µm (de Busserolles et al., 2014), but squids can possess outer segments up to about 500 µm (Chung, 2014). This study sought to determine whether a correlation existed between the lengths of the inner and outer photoreceptor segments and the depth a species inhabited. Overall, OSP length was shown to be longer for species that inhabit deeper strata. This may increase light sensitivity for species that frequent habitats with lower ambient light conditions and/or increased bioluminescent signals. In contrast, the results from this study showed that species that inhabit deeper strata tend to have shorter ISP lengths. This follows similar trends that have been shown in myctophids (de Busserolles et al., 2014). This may indicate that longer ISP lengths may be more beneficial for species that generally inhabit

shallower waters where ambient light conditions are brighter. If ISP length is linked to the energetic input retinas require for facilitating phototransduction, it is possible that long ISPs are unnecessary for species that inhabit deeper habitats where light is less readily present. Alternatively, more specialised supporting cells may be required in the ISP of the retina in squid that inhabit shallower waters to compensate for the broader range of light conditions present, compared to the narrower wavelength of light found in the deep sea.

Though the results presented by this study are intriguing, more research is required to validate these findings before making definitive conclusions. Deep-sea animals are relatively hard to study, even with current ROV and camera technology. Therefore, the true depth range a species inhabits is difficult to confidently determine. As more research improves our understanding of the depth ranges squids inhabit, the interpretation of these results may evolve.

5.3 Photoreceptor Growth in Adult Oegopsids

Some squid species undergo ontogenetic migrations that occur as they mature from juveniles into adults (Evans & Bolstad, 2015; Hunt & Seibel, 2000; Seibel et al., 2000). Juveniles often live near the surface, then migrate into deeper water, potentially to hunt different prey and avoid predation in the brighter ambient light conditions near the surface. It has been suggested that the mechanism triggering the behavioural response to move to deeper habitats is facilitated by a rapid increase in photoreceptor length. However, it has remained unclear whether photoreceptors continue to grow well into adulthood, which would potentially help to further increase light sensitivity throughout their lifespan. In this study, no correlation was observed between mantle length and OSP length in any of the study species, although most of the individuals studied within any species represented similar life stages. Since all specimens were adults or subadults, these findings suggest that OSP growth slows for oegopsids once they reach adulthood. For species that exhibit ontogenetic migrations to different strata, this could indicate that once the migration is complete, these species reduce the energetic input that would otherwise be necessary to continually grow their outer photoreceptor segments. Additionally, large species, such as *T. danae* and *A. dux*, did not show OSP lengths that were significantly longer than other smaller species, despite having much larger eyes. This suggests that it may be inefficient and unnecessary to continually grow their outer photoreceptor segments to increase light sensitivity. Instead, it appears to be more effective to evolve larger eyes that allow more light into the eye to reach the retina.

In contrast, correlation between mantle length and ISP length was found in *N. sloanii*. This negative correlation suggests ISP length may decrease as individuals continue to grow throughout adulthood. However, since this was only exhibited in one species, it is difficult to determine whether this observation commonly occurs among oegopsids. Interestingly, analysing correlation between ISP length and mantle length across species does show that ISP length is shorter in larger species, further supporting the notion that ISP length shortened throughout adulthood. The reasons oegopsid ISP length may shorten requires more investigation.

Once again, definitive conclusions must be reserved until more investigations can be made. Research on deep-sea animals is often limited by the number of specimens that can be collected. While some species in this study, like *N. sloanii*, had an ample sample size (n = 10), others, like *T. filippovae* (n = 3), would benefit from larger sample sizes. To confidently determine whether a photoreceptor growth does significantly slow for adult oegopsids, additional specimens need to be analysed ideally representing different life stages to increase confidence of the analyses conducted.

5.4 The Migration of Screening Pigment in Juvenile Oegopsids

The hypothesised primary function of screening pigment in the retina was notably researched by Daw and Pearlman in the late 1970s (Daw & Pearlman, 1974). They showed that bright ambient light conditions cause the ommin granules to migrate to the tips of the outer photoreceptor segments, which block incoming light, likely preventing the photoreceptors from being oversaturated. Their experiments were conducted on *Doryteuthis pealeii*, similar to more research on retinal screening pigment in cephalopods which has been conducted on nearshore octopuses and squids that experience brighter ambient light conditions in their shallower habitats. Recently, several studies have looked at screening pigment in pelagic squids and showed that it likely migrates despite these species generally inhabiting darker environments. However, it remains unclear how ubiquitous screening pigment is throughout the oegopsids lineage.

The research from this thesis suggests that ommin pigment is a common feature of the oegopsid retina. Screening pigment was observed in the retinas of all species, except for *N. gouldi*. The absence of screening pigment in *N. gouldi* is likely due to the methods used to preserve the eyes, which seemed to cause the ommin granules to be lost. However, while previous studies have shown pigment granules distributed throughout the retinas' outer photoreceptor segments in oegopsids (Chung, 2014; Evans & Bolstad, 2015), it has never been experimentally proven.

In collaboration with the Monterey Bay Area Research Institute, juvenile *G. onyx* specimens were collected and exposed to various light/dark regimes to determine whether screening pigment migrates in oegopsids. The experiments indicated that the screening pigment does migrate distally in the retinas of juvenile *G. onyx*. This is likely a mechanism to reduce the amount of light that reaches the retina and prevent oversaturation of the photoreceptors. This may be beneficial for species that exhibit ontogenetic migrations, like *G. onyx*, which inhabit relatively bright ambient light conditions near the surface of the ocean during their early developmental stages (Hunt & Seibel, 2000). However, it is still unclear whether this mechanism remains functional during adulthood after individuals have migrated to dark habitats where screening pigment may be less useful.

5.5 Visual Orientation and its Impact on the Peak Spectral Sensitivity of Visual pigments

In the three-dimensional environment characteristic of pelagic habitats, hunting, mating, and predator avoidance strategies can influence how an animal orients its head

and eyes (Makino & Miyazaki, 2010b). Most commonly, topographical maps of retina photoreceptor density and length can indicate the direction of incoming light that is most important to a species. For example, the benthic 'bobtail' squid *Euprymna morsei* exhibits higher photoreceptor densities in the posterior-dorsal region of the retina. Since this species typically forages for crustaceans along the seafloor (Carpenter & Niem, 1998), this specialisation of their retina may be beneficial for their hunting behaviours (Makino & Miyazaki, 2010). However, eye and head orientation and its relationship to the spectral sensitivity of visual pigments has never been investigated. It has been suggested that there may be a correlation between depth and peak spectral sensitivity (Fasick & Robinson, 2000; Morris et al., 1993), demonstrating that spectral sensitivity may specialise to pressures created by the behaviours of a species and the habitats it occupies.

The peak spectral sensitivities of visual pigments from ten species were characterised in this study. Most species exhibited a peak sensitivity to light with a wavelength near 480 nm, which is the wavelength of light that penetrates oceanic water the deepest and the most common wavelength for bioluminescence. This sensitivity likely improves the probability of these species to recognise predators or prey illuminated by downwelling light. These findings align well with the Sensitivity Hypothesis which states that retinal photopigments evolve to be optimised to the wavelengths of light predominant in the environment (Crescitelli et al., 1985).

A few species (*O. robsoni, O.* aff. *robsoni*, and the large eye of *H. miranda*) exhibited sensitivity optimised to wavelengths of light near 490 nm. Since it has been demonstrated that the large eye of *H. miranda* is often directed towards the surface to identify predators and/or prey silhouetted by downwelling light (Thomas et al., 2017), this bathochromic shift in their spectral sensitivity may indicate an advantage for identifying predators and prey above the individual. Therefore, this may suggest that *O. robsoni* and *O.* aff. *robsoni* tend to look towards the surface when hunting or avoiding predation. Currently, minimal research has been conducted on the hunting behaviours of these two species, but recent observations indicate they may be demersal species. If proven true, *O. robsoni* and *O.* aff. *robsoni* may have evolved visual pigments that have been specialised to better identify species from below and visual stimuli above them. In contrast, the small eye of *H. miranda* exhibited a hypsochromic shift of its peak spectral sensitivity to wavelengths of light near 470 nm. This suggests that shifting spectral sensitivity to optimally see shorter wavelengths of light may be advantageous for the eyes of species that are often directed towards deeper and darker waters.

5.6 Bioluminescence as a Driver of Rhodopsin Specialisation

Bioluminescence is a major source of light in the deep sea, and many animals have visual pigments that are optimally sensitive to the bioluminescent signals of conspecifics (Crescitelli, 1990; Seidou et al., 1990). However, minimal research has investigated whether bioluminescence may drive visual pigment spectral sensitivity in cephalopods. Characterisation of the bandwidth of the spectral sensitivity curves showed most species had a FWHM narrower than 85 nm. However, a few species exhibited FWHM measurements closer to 90 nm. Further analysis showed a correlation between species

with broader bandwidths and their bioluminescent abilities. That is, species that have photophores appear to have broader spectral sensitivity of their visual pigment. It is possible that this adjustment to their spectral sensitivity curves is advantageous for species that use bioluminescence for intraspecific communication by improving their ability to identify conspecifics showing bioluminescent displays. Interestingly, the large eye of *H. miranda* exhibited a spectral sensitivity curve bandwidth near 80 nm, despite being a bioluminescent species. However, the small eye exhibited a bandwidth near 90 nm, which more aligns with the other bioluminescent species in this study. This difference in their spectral sensitivity curves may be caused by the different purposes of each eye. Since the small eye is likely used to spot bioluminescent sources from below the individual, a broader spectral sensitivity bandwidth of the rhodopsin located in this eye may be advantageous. In contrast, the large eye may not need to exhibit similar bandwidth characteristics because it is more likely used to identify predators and prey above the individual. The link between bioluminescence and the bandwidth of the visual pigments' spectral sensitivity curves was further supported by the phylogenetic analysis of rhodopsin in oegopsids from this study. The partial sequencing of rhodopsin from 11 oegopsid species were determined and combined with rhodopsin sequences of oegopsid species available on GenBank to create a phylogenetic tree of rhodopsin. Most notably, a strong correlation was found between the bioluminescent capabilities of a species and the phylogenetic assemblage of rhodopsin. This indicates that whether a species has bioluminescent photophores may put evolutionary pressure that drives specialisation of their visual photopigment, most likely by increasing the bandwidth of their spectral sensitivity curves.

A limitation of the present study is that the absorbance spectra were obtained from a single measurement of each visual pigment extract without experimentally quantifying the relative contributions of rhodopsin and metarhodopsin. A more robust approach would involve recording an initial dark-adapted absorbance spectrum, followed by exposing the same extract to light of a defined wavelength to photoconvert the visual pigment to a known state before acquiring a second absorbance spectrum. Subtraction of the light-converted spectrum from the initial spectrum would allow the spectral contributions of rhodopsin and metarhodopsin to be more accurately resolved and provide improved estimates of the spectral characteristics of each pigment state. Such an approach has been widely used in visual pigment studies to distinguish photoreversible pigment states and would reduce uncertainty associated with the presence of mixed rhodopsin, metarhodopsin, and intermediate photoproducts within retinal extracts. While this methodology was beyond the scope of the present study, it represents an important consideration for future investigations seeking to characterise the visual pigments of deep-sea cephalopods with greater precision.

5.7 Future Directions

This thesis supports and augments previous work in the field, which together as a body of knowledge demonstrate that the complexity of oegopsid vision is vast, with many potential directions for future research. Through this study, it has been shown that photoreceptor length may be linked to depth and the behaviours of species but that adults may exhibit slowed photoreceptor growth. Furthermore, screening pigment has

been experimentally shown to migrate in oegopsids, which was previously unknown. Finally, the spectral sensitivity curve of the visual pigments is likely influenced by the ocular orientation of species and whether they possess bioluminescent capabilities. Future directions should aim to investigate the retinal anatomy and spectral sensitivity of visual pigments in oegopsids and other closely related deep-sea cephalopod lineages that have not been studied or are undiscovered, such as *Vampyroteuthis infernalis* and bathyteuthids. In particular, more research is required on adult squids, which receive less attention than juveniles that are often exposed to different environments and use different hunting and predator avoidance techniques. It would be especially interesting to compare the visual capabilities of adults and juveniles within a species to determine if ontogenetic changes occur at the cellular and molecular level of the retina. In doing so, research can continue to gain greater insight into the visual capabilities of oegopsids that have evolved for over half a billion years.

References

Acosta, M. L., & Kalloniatis, M. (2005). Short- and long-term enzymatic regulation secondary to metabolic insult in the rat retina. *Journal of Neurochemistry*, *92*(6), 1350–1362. https://doi.org/10.1111/j.1471-4159.2004.02976.x

Agi, E., Langen, M., Altschuler, S. J., Wu, L. F., Zimmermann, T., & Hiesinger, P. R. (2014). The evolution and development of neural superposition. *Journal of Neurogenetics*, *28*(3–4), 216–232. https://doi.org/10.3109/01677063.2014.922557

Alejo-Plata, M. del C., León-Guzmán, S. S., Díaz-Polo, R., & Torres-Huerta, A. M. (2019). Diversity, abundance and frequency of argonauts (Cephalopoda: Argonautidae) in the diet of pelagic fishes of commercial value in Oaxaca, Mexico. *Revista de Biologia Marina y Oceanografia*, *54*(1), 107–117. https://doi.org/10.22370/rbmo.2019.54.1.1651

Alkaladi, A., & Zeil, J. (2014). Functional anatomy of the fiddler crab compound eye (*Uca vomeris*: Ocypodidae, Brachyura, Decapoda). *Journal of Comparative Neurology*, *522*(6), 1264–1283. https://doi.org/10.1002/cne.23472

Amaratunga, T., & Caddy, J. F. (1983). The role of cephalopods in the marine ecosystem. *Advances in Assessment of World Cephalopod Resources*, *231*, 379–415.

Anderson, R. C., & Mather, J. A. (1996). Escape responses of *Euprymna scolopes* Berry, 1911 (Cephalopoda: Sepiolidae). *Journal of Molluscan Studies*, *62*, 543–545. https://doi.org/10.1093/mollus/62.4.543

Arechiga, H., Banuelos, E., Frixione, E., Picones, A., & Rodriguez-Sosa, L. (1990). Modulation of crayfish retinal sensitivity by 5-Hydroxytryptamine. *Journal of Experimental Biology*, *150*, 123–143. https://doi.org/10.1242/JEB.150.1.123

Arkhipkin, A. I., & Roa-Ureta, R. (2005). Identification of ontogenetic growth models for squid. *Marine And Freshwater Research*, *56*(4), 371–386. https://doi.org/10.1071/MF04274

Arkhipkin, A. I., Rodhouse, P. G. K., Pierce, G. J., Sauer, W., Sakai, M., Allcock, L., Arguelles, J., Bower, J. R., Castillo, G., Ceriola, L., Chen, C. S., Chen, X., Diaz-Santana, M., Downey, N., González, A. F., Granados Amores, J., Green, C. P., Guerra, A., Hendrickson, L. C., … Zeidberg, L. D. (2015). World squid fisheries. *Reviews in Fisheries Science and Aquaculture*, *23*(2), 92–252. https://doi.org/10.1080/23308249.2015.1026226

Arnold, J. M. (1965). Normal embryonic stages of the squid, *Loligo pealii* (Lesueur). *The Biological Bulletin*, *128*(1), 24–32. https://doi.org/10.2307/1539386

Arnold, J. M. (1967). Fine structure of the development of the cephalopod lens. *Journal of Ultrasructure Research*, *17*(5–6), 527–543. https://doi.org/10.1016/S0022-5320(67)80139-4

Atema, J. (1988). Distribution of chemical stimuli. In J. Atema, R. R. Fay, A. N. Popper, W. N. Tavolga (Eds.), *Sensory Biology of Aquatic Animals* (pp. 29–56). Springer New York. https://doi.org/10.1007/978-1-4612-3714-3_2

Balazs, E. A., Laurent, T. C., Laurent, U. B. G., DeRoche, M. H., & Bunney, D. M. (1959). Studies on the structure of the vitreous body. VIII. Comparative biochemistry. *Archives of Biochemistry and Biophysics*, *81*(2), 464–479. https://doi.org/10.1016/0003-9861(59)90227-9

Balguerías, E., Quintero, M. E., & Hernández-González, C. L. (2000). The origin of the Saharan Bank cephalopod fishery. *ICES Journal of Marine Science*, *57*(1), 15–23. https://doi.org/10.1006/jmsc.1999.0572

Basil, J. A., Hanlon, R. T., Sheikh, S. I., & Atema, J. (2000). Three-dimensional odor tracking by *Nautilus pompilius*. *Journal of Experimental Biology*, *203*(9), 1409–1414. https://doi.org/10.1242/jeb.203.9.1409

Baumann, C. (1972). The regeneration and renewal of visual pigments in vertebrates. In H. Dartnall (Ed.), *Photochemistry of Vision. Handbook of Sensory Physiology.* (Vol. 7, pp.395–416). Springer, Berlin, Heidelberg. https://10.1007/978-3-642-65066-6_10

Bellingham, J., Morris, A. G., & Hunt, D. M. (1998). The rhodopsin gene of the cuttlefish *Sepia officinalis*: Sequence and spectral tuning. *Journal of Experimental Biology*, *201*(15), 2299–2306. https://doi.org/10.1242/jeb.201.15.2299

Bleckmann, H. (2006). The lateral line system of fish. In T. J. Hara & B. S. Zielinski (Eds.), *Fish Physiology: Sensory Systems Neuroscience* (Vol. 25, pp. 337–376). Academic Press. https://doi.org/10.1016/S1546-5098(06)25010-6

Boal, J. G., & Golden, D. K. (1999). Distance chemoreception in the common cuttlefish, *Sepia officinalis* (Mollusca, Cephalopoda). *Journal of Experimental Marine Biology and Ecology*, *235*(2), 307–317. https://doi.org/10.1016/S0022-0981(98)00187-7

Bolstad, K. S. (2007). Systematics and distribution of the New Zealand onychoteuthid fauna (Cephalopoda: Oegopsida), including a new species, *Notonykia nesisi* sp. nov. *Reviews in Fish Biology and Fisheries*, *17*(2–3), 305–335. https://doi.org/10.1007/s11160-007-9041-2

Bowmaker, J. K. (2008). Evolution of vertebrate visual pigments. *Vision Research*, *48*(20), 2022–2041. https://doi.org/10.1016/j.visres.2008.03.025

Boycott, B. B. (1960). The functioning of the statocysts of *Octopus vulgaris*. *Proceedings of the Royal Society of London. Series B, Biological Sciences, 152*, 78–87. https://doi.org/10.1098/rspb.1960.0025

Boyle, P., & Rodhouse, P. (Eds.). (2005). *Cephalopods: Ecology and fisheries*. Blackwell Publishing. https://doi.org/10.1002/9780470995310

Bozzano, A., Pankhurst, P. M., & Sabatés, A. (2007). Early development of eye and retina in lanternfish larvae. *Visual Neuroscience*, *24*(3), 423–436. https://doi.org/10.1017/S0952523807070484

Braid, H. E., & Bolstad, K. S. R. (2014). Feeding ecology of the largest mastigoteuthid squid species, *Idioteuthis cordiformis* (Cephalopoda, Mastigoteuthidae). *Marine Ecology Progress Series*, *515*, 275–279. https://doi.org/10.3354/meps11008

Braid, H. E., & Bolstad, K. S. R. (2019). Cephalopod biodiversity of the Kermadec Islands: Implications for conservation and some future taxonomic priorities. *Invertebrate Systematics*, *33*(2), 402–425. https://doi.org/10.1071/IS18041

Braid, H. E., Kubodera, T., & Bolstad, K. S. R. (2017). One step closer to understanding the chiroteuthid families in the Pacific Ocean. *Marine Biodiversity*, *47*(3), 659–683. https://doi.org/10.1007/s12526-016-0628-5

Braid, H. E., McBride, P. D., & Bolstad, K. S. R. (2014). Molecular phylogenetic analysis of the squid family Mastigoteuthidae (Mollusca, Cephalopoda) based on three mitochondrial genes. *Hydrobiologia*, *725*(1), 145–164. https://doi.org/10.1007/s10750-013-1775-3

Brierley, A. S. (2014). Diel vertical migration. *Current Biology*, *24*(22), R1074–R1076. https://doi.org/10.1016/j.cub.2014.08.054

Brown, C. (2012). Tool use in fishes. *Fish and Fisheries*, *13*(1), 105–115. https://doi.org/10.1111/j.1467-2979.2011.00451.x

Budelmann, B. U. (1996). Active marine predators: The sensory world of cephalopods. *Marine and Freshwater Behaviour and Physiology*, *27*(2–3), 59–75. https://doi.org/10.1080/10236249609378955

Budelmann, B. U., & Bleckmann, H. (1988). A lateral line analogue in cephalopods: Water waves generate microphonic potentials in the epidermal head lines of *Sepia* and *Lolliguncula*. *Journal of Comparative Physiology A*, *164*(1), 1–5. https://doi.org/10.1007/BF00612711

Budelmann, B. U., & Young, J. Z. (1984). The statocyst-oculomotor system *of Octopus vulgaris*: Extraocular eye muscles, eye muscle nerves, statocyst nerves and the oculomotor centre in the central nervous system. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *306*(1127), 159–189. https://www.jstor.org/stable/2398664

Butenandt, A. (1959). Wirkstoffe des Insektenreiches. *Die Naturwissenschaften*, *46*(15), 461–471. https://doi.org/10.1007/BF00634039

Caddy, J. F., & Rodhouse, P. G. (1998). Cephalopod and groundfish landings: Evidence for ecological change in global fisheries? *Reviews in Fish Biology and Fisheries*, *8*(4), 431–444. https://doi.org/10.1023/A:1008807129366

Carpenter, K. E., & Niem, V. H. (Eds.). (1998). *The living marine resources of the Western Central Pacific. Volume 2: Cephalopods, crustaceans, holothurians, and sharks*. FAO.

Carr, W. E. S. (1988). The molecular nature of chemical stimuli in the aquatic environment. In J. Atema, R. R. Fay, A. N. Popper, W. N. Tavolga (Eds.), *Sensory Biology of Aquatic Animals* (pp. 3–27). Springer New York. https://doi.org/10.1007/978-1-4612-3714-3_1

Cartron, L., Josef, N., Lerner, A., McCusker, S. D., Darmaillacq, A.-S., Dickel, L., & Shashar, N. (2013). Polarization vision can improve object detection in turbid waters by cuttlefish. *Journal of Experimental Marine Biology and Ecology*, *447*, 80–85. https://doi.org/10.1016/j.jembe.2013.02.013

Chembian, A. J., & Mathew, S. (2011). Migration and spawning behaviour of the pharaoh cuttlefish *Sepia pharaonis* Ehrenberg, 1831 along the south-west coast of India. *Indian Journal of Fisheries*, *58*(3), 1–8.

Cherel, Y., & Duhamel, G. (2003). Diet of the squid *Moroteuthis ingens* (Teuthoidea: Onychoteuthidae) in the upper slope waters of the Kerguelen Islands. *Marine Ecology Progress Series*, *250*, 197–203. https://doi.org/10.3354/MEPS250197

Chung, W.-S. (2014). *Comparisons of visual capabilities in modern cephalopods from shallow water to deep sea*. The University of Queensland.

Chung, W.-S., & Marshall, N. J. (2017). Complex visual adaptations in squid for specific tasks in different environments. *Frontiers in Physiology*, *8*, 1–16. https://doi.org/10.3389/fphys.2017.00105

Chung, W.-S., & Marshall, J. (2014). Range-finding in squid using retinal deformation and image blur. *Current Biology*, *24*(2), R64-65. https://doi.org/10.1016/j.cub.2013.11.058

Chung, W.-S., & Marshall, N. J. (2016). Comparative visual ecology of cephalopods from different habitats. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *283*(1838), 20161346. https://doi.org/10.1098/rspb.2016.1346

Clarke, M. R. (1996). Cephalopods as prey. III. Cetaceans. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 351*(1343), 1053–1065. https://doi.org/10.1098/rstb.1996.0093

Cohen, J. H., & Forward, R. B. J. (2016). Zooplankton diel vertical migration — A review of proximate control. In R. N. Gibson, R. J. A. Atkinson, & J. D. M. Gordon (Eds.), *Oceanography and Marine Biology: An Annual Review* (Vol. 47, pp. 77-109). CRC Press. https://doi.org/10.1201/9781420094220

Coll, M., Navarro, J., Olson, R. J., & Christensen, V. (2013). Assessing the trophic position and ecological role of squids in marine ecosystems by means of food-web models. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *95*, 21–36. https://doi.org/10.1016/j.dsr2.2012.08.020

Collin, S. P., & Pettigrew, J. D. (1988). Retinal topography in reef teleosts: II. Some species with prominent horizontal streaks and high-density areae. *Brain, Behavior and Evolution*, *31*(5), 283–295. https://doi.org/10.1159/000116595

Collins, M. A., De Grave, S., Lordan, C., Burnell, G. M., & Rodhouse, P. G. (1994). Diet of the squid *Loligo forbesi* Steenstrup (Cephalopoda: Loliginidae) in Irish waters. *ICES Journal of Marine Science*, *51*(3), 337–344. https://doi.org/10.1006/jmsc.1994.1034

Cones, S. F., Zhang, D., Shorter, K. A., Katija, K., Mann, D. A., Jensen, F. H., Fontes, J., Afonso, P., & Mooney, T. A. (2022). Swimming behaviors during diel vertical migration in veined squid *Loligo forbesii*. *Marine Ecology Progress Series*, *691*, 83–96. https://doi.org/10.3354/meps14056

Crescitelli, F. (1990). Adaptations of visual pigments to the photic environment of the deep sea. *Journal of Experimental Zoology*, *256*(S5), 66–75. https://doi.org/10.1002/JEZ.1402560510

Crescitelli, F., McFall-Ngai, M., & Horwitz, J. (1985). The visual pigment sensitivity hypothesis: Further evidence from fishes of varying habitats. *Journal of Comparative Physiology A, 157*, 323–333. https://doi.org/10.1007/BF00618122

Croxall, J. P., & Prince, P. A. (1996). Cephalopods as prey. I. Seabirds. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *351*(1343), 1023–1043. https://doi.org/10.1098/rstb.1996.0091

D'Aniello, S., Spinelli, P., Ferrandino, G., Peterson, K., Tsesarskia, M., Fisher, G., & D'Aniello, A. (2005). Cephalopod vision involves dicarboxylic amino acids: D-aspartate, L-aspartate and L-glutamate. *Biochemical Journal*, *386*(2), 331–340. https://doi.org/10.1042/BJ20041070

Daw, N. W., & Pearlman, A. L. (1974). Pigment migration and adaptation in the eye of the squid, *Loligo pealei*. *Journal of General Physiology*, *63*(1), 22–36. https://doi.org/10.1085/jgp.63.1.22

de Busserolles, F., Fitzpatrick, J. L., Marshall, N. J., & Collin, S. P. (2014). The influence of photoreceptor size and distribution on optical sensitivity in the eyes of lanternfishes (Myctophidae). *PLoS ONE*, *9*(6), Article e99957. https://doi.org/10.1371/journal.pone.0099957

de Busserolles, F., Fogg, L., Cortesi, F., & Marshall, J. (2020). The exceptional diversity of visual adaptations in deep-sea teleost fishes. *Seminars in Cell and Developmental Biology*, *106*, 20–30. https://doi.org/10.1016/j.semcdb.2020.05.027

de Busserolles, F., Marshall, N. J., & Collin, S. P. (2014). The eyes of lanternfishes (Myctophidae, Teleostei): Novel ocular specializations for vision in dim light. *Journal of Comparative Neurology*, *522*(7), 1618–1640. https://doi.org/10.1002/cne.23495

Denton, E. J. (1990). Light and vision at depths greater than 200 meters. In P. J. Herring, A. K. Campbell, M. Whitfield, & L. Maddock (Eds.), *Light and life in the sea* (pp. 127–148). Cambridge University Press.

Derby, C. D., & Sorensen, P. W. (2008). Neural processing, perception, and behavioral responses to natural chemical stimuli by fish and crustaceans. *Journal of Chemical Ecology*, *34*(7), 898–914. https://doi.org/10.1007/s10886-008-9489-0

Dingerkus, G., & Santoro, E. D. (1981). Cornea regeneration in the Pacific giant octopus, *Octopus dofleini*, and the common octopus, *O. vulgaris*. *Experientia*, *37*(4), 368–369. https://doi.org/10.1007/BF01959867

Doubleday, Z. A., Prowse, T. A. A., Arkhipkin, A., Pierce, G. J., Semmens, J., Steer, M., Leporati, S. C., Lourenço, S., Quetglas, A., Sauer, W., & Gillanders, B. M. (2016). Global proliferation of cephalopods. *Current Biology*, *26*, R406–R407. https://doi.org/10.1016/j.cub.2016.04.002

Douglas, R. H., & Marshall, N. J. (1999). A review of vertebrate and invertebrate ocular filters. In S. N. Archer, M. B. A. Djamgoz, E. R. Loew, J. C. Partridge, S. Vallerga (Eds.), *Adaptive Mechanisms in the Ecology of Vision* (pp. 95–162). Springer Netherlands. https://doi.org/10.1007/978-94-017-0619-3_5

Dunn, M. R. (2009). Feeding habits of the ommastrephid squid *Nototodarus sloanii* on the Chatham Rise, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *43*(5), 1103–1113. https://doi.org/10.1080/00288330.2009.9626533

Dunstan, A. J., Ward, P. D., & Marshall, N. J. (2011). Vertical distribution and migration patterns of *Nautilus pompilius*. *PLoS ONE*, *6*(2), Article e16311. https://doi.org/10.1371/journal.pone.0016311

Evans, A. B., Acosta, M. L., & Bolstad, K. S. (2015). Retinal development and ommin pigment in the cranchiid squid *Teuthowenia pellucida* (Cephalopoda: Oegopsida). *PLoS ONE*, *10*(5), 11. https://doi.org/10.1371/journal.pone.0123453

Evans, A. B., & Bolstad, K. S. R. (2015). Ontogeny of the deep-sea cranchiid squid *Teuthowenia pellucida* (Cephalopoda: Cranchiidae) from New Zealand waters. *Journal of Natural History*, *49*(21–24), 1327–1349. https://doi.org/10.1080/00222933.2013.840747

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. FAO. https://doi.org/10.4060/ca9229en

Fasick, J. I., & Robinson, P. R. (2000). Spectral-tuning mechanisms of marine mammal rhodopsins and correlations with foraging depth. *Visual Neuroscience*, *17*(5), 781–788. https://doi.org/10.1017/S095252380017511X

Fernandez-Alvarez, F. A., Braid, H. E., Nigmatullin, C. M., Bolstad, K. S. R., Haimovici, M., Sanchez, P., Sajikumar, K. K., Ragesh, N., & Villanueva, R. (2020). Global biodiversity of the genus *Ommastrephes* (Ommastrephidae: Cephalopoda): An allopatric cryptic species complex. *Zoological Journal of the Linnean Society*, *190*(2), 460–482. https://doi.org/10.1093/zoolinnean/zlaa014

Fernández-Álvarez, F. Á., Taite, M., Vecchione, M., Villanueva, R., & Allcock, A. L. (2022). A phylogenomic look into the systematics of oceanic squids (order Oegopsida). *Zoological Journal of the Linnean Society*, *194*(4), 1212–1235. https://doi.org/10.1093/zoolinnean/zlab069

Field, J. C., Baltz, K., Phillips, A. J., & Walker, W. A. (2007). Range expansion and trophic interactions of the jumbo squid, *Dosidicus gigas*, in the California current. *California Cooperative Oceanic Fisheries Investigations Reports*, *48*, 131–146.

Figon, F., & Casas, J. (2019). Ommochromes in invertebrates: biochemistry and cell biology. *Biological Reviews*, *94*(1), 156–183. https://doi.org/10.1111/BRV.12441

Forsythe, J. W., & Hanlon, R. T. (1989). Growth of the Eastern Atlantic squid, *Loligo forbesi* Steenstrup (Mollusca: Cephalopoda). *Aquaculture Research*, *20*(1), 1–14. https://doi.org/10.1111/j.1365-2109.1989.tb00437.x

Francis, W. R., Powers, M. L., & Haddock, S. H. D. (2014). Characterization of an anthraquinone fluor from the bioluminescent, pelagic polychaete *Tomopteris*. *Luminescence*, *29*(8), 1135–1140. https://doi.org/10.1002/BIO.2671

Frank, T. M. (2017). Ontogenetic adaptations in the visual systems of deep-sea crustaceans. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *372*(1717). https://doi.org/10.1098/rstb.2016.0071

Frederiksen, R. (1976). Retinal tapetum containing discrete reflectors and photoreceptors in the bathypelagic teleost *Omosudis lowei*. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening*, *139*, 109–146.

Froesch, D. (1973). On the fine structure of the Octopus iris. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, *145*(1), 119–129. https://doi.org/10.1007/BF00307193

Fröhlich, F. W. (1914). Beiträge zur allgemeinen Physiologie der Sinnesorgane. *Zeitschrift für Sinnesphysiologie*, *48*, 28–164.

Gavriouchkina, D., Tan, Y., Ziadi-Künzli, F., Hasegawa, Y., Piovani, L., Zhang, L., Sugimoto, C., Luscombe, N., Marlétaz, F., & Rokhsar, D. S. (2022). *A single-cell atlas of bobtail squid visual and nervous system highlights molecular principles of convergent evolution*. https://doi.org/10.1101/2022.05.26.490366

Gilly, W., Markaida, U., Baxter, C., Block, B., Boustany, A., Zeidberg, L., Reisenbichler, K., Robison, B., Bazzino, G., & Salinas, C. (2006). Vertical and horizontal migrations by the jumbo squid *Dosidicus gigas* revealed by electronic tagging. *Marine Ecology Progress Series*, *324*, 1–17. https://doi.org/10.3354/meps324001

Gleadall, I. G., Ohtsu, K., Gleadall, E., & Tsukahara, Y. (1993). Screening-pigment migration in the octopus retina includes control by dopaminergic efferents. *Journal of Experimental Biology*, *185*, 1–16. https://doi.org/10.1242/jeb.185.1.1

González, A. F., Guerra, A., Pascual, S., & Briand, P. (1998). *Vulcanoctopus hydrothermalis* gen. et sp. nov. (Mollusca, Cephalopoda): An octopod from a deep-sea hydrothermal vent site. *Cahiers de Biologie Marine*, *39*(2), 169–184.

Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G., & Donner, K. (2000). In search of the visual pigment template. *Cambridge University Press*, *17*(4), 509–528. https://doi:10.1017/S0952523800174036

Haddock, S. H. D., & Case, J. F. (1999). Bioluminescence spectra of shallow and deep-sea gelatinous zooplankton: Ctenophores, medusae and siphonophores. *Marine Biology*, *133*, 571–582. https://doi.org/10.1007/s002270050497

Haddock, S. H. D., Moline, M. A., & Case, J. F. (2010). Bioluminescence in the sea. *Annual Review of Marine Science*, *2*(1), 443–493. https://doi.org/10.1146/ANNUREV-MARINE-120308-081028

Hagins, W. A., & Liebman, P. A. (1962). Light induced pigment migration in the squid retina. *The Biological Bulletin (Woods Hole)*, *123*(498).

Hall, M. D., Hoon, M. A., Ryba, N. J. P., Pottinger, J. D. D., Keen, J. N., Saibil, H. R., & Findlay, J. B. C. (1991). Molecular cloning and primary structure of squid (*Loligo forbesi*) rhodopsin, a phospholipase C-directed G-protein-linked receptor. *Biochemical Journal*, *274*(1), 35–40. https://doi.org/10.1042/bj2740035

Hallberg, E., & Nilsson, D. E. (1983). The euphausiid compound eye - A morphological re-investigation (Crustacea: Euphausiacea). *Zoomorphology*, *103*(1), 59–66. https://doi.org/10.1007/BF00312058

Hamdorf, K., Höglund, G., & Juse, A. (1986). Ultra-violet and blue induced migration of screening pigment in the retina of the moth *Deilephila elpenor*. *Journal of Comparative Physiology A*, *159*(3), 353–362. https://doi.org/10.1007/BF00603981

Hanke, F. D., & Kelber, A. (2020). The eye of the common octopus (*Octopus vulgaris*). *Frontiers in Physiology*, *10*. https://doi.org/10.3389/fphys.2019.01637

Hanlon, R. T., Chiao, C.-C., Mäthger, L. M., Buresch, K. C., Barbosa, A., Allen, J. J., Siemann, L., & Chubb, C. (2011). Rapid adaptive camouflauge in cephalopods. In M. Stevens, S. Merilaita (Eds.), *Animal Camouflage: Mechanisms and Function* (pp. 145–163). Cambridge University Press. https://doi.org/10.1017/CBO9780511852053.009

Hansen, A. G., Beauchamp, D. A., & Schoen, E. R. (2013). Visual prey detection responses of piscivorous trout and salmon: Effects of light, turbidity, and prey size. *Transactions of the American Fisheries Society*, *142*(3), 854–867. https://doi.org/10.1080/00028487.2013.785978

Hara, T., & Hara, R. (1972). Cephalopod Retinochrome. In H. J. A. Dartnall (Ed.), *Handbook of Sensory Physiology: Photochemistry of Vision: Vol. VII* (1st ed., pp. 720–746). Springer-Verlag.

Hara, T., & Hara, R. (1976). Distribution of rhodopsin and retinochrome in the squid retina. *Journal of General Physiology*, *67*(6), 791–805. https://doi.org/10.1085/jgp.67.6.791

Hara, T., Hara, R., Kishigami, A., Koshida, Y., Horiuchi, S., & Raj, U. (1995). Rhodopsin and retinochrome in the retina of a tetrabranchiate cephalopod *Nautilus pompilius*. *Zoological Science*, *12*(2), 195–201. https://doi.org/10.2108/zsj.12.195

Hara-Nishimura, I., Kondo, M., Nishimura, M., Hara, R., & Hara, T. (1993). Cloning and nucleotide sequence of cDNA for rhodopsin of the squid *Todarodes pacificus*. *FEBS Letters*, *317*(1–2), 5–11. https://doi.org/10.1016/0014-5793(93)81480-N

Herring, P. J., & Cope, C. (2005). Red bioluminescence in fishes: On the suborbital photophores of *Malacosteus*, *Pachystomias* and *Aristostomias*. *Marine Biology*, *148*(2), 383–394. https://doi.org/10.1007/S00227-005-0085-3

Holt, A. L., Sweeney, A. M., Johnsen, S., & Morse, D. E. (2011). A highly distributed Bragg stack with unique geometry provides effective camouflage for Loliginid squid eyes. *Journal of the Royal Society Interface*, *8*(63), 1386–1399. https://doi.org/10.1098/rsif.2010.0702

Hoving, H. J. T., Bush, S. L., Haddock, S. H. D., & Robison, B. H. (2017). Bathyal feasting: Post-spawning squid as a source of carbon for deep-sea benthic communities. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *284*(1869), 10–12. https://doi.org/10.1098/rspb.2017.2096

Hoving, H. J. T., & Robison, B. H. (2012). Vampire squid: Detritivores in the oxygen minimum zone. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *279*(1747), 4559–4567. https://doi.org/10.1098/rspb.2012.1357

Hoving, H., & Visser, F. (2024). Whales and cephalopods in a deep-sea arms race. *Limnology and Oceanography Letters*, *9*(3), 165–171. https://doi.org/10.1002/lol2.10391

Howard, R. B., Kniller, J., Bolstad, K. S. R., & Acosta, M. L. (2024). Biological sunglasses in a deep-sea squid: Pigment migration in the retina of *Gonatus onyx*. *Vision*, *8*(2), 26. https://doi.org/10.3390/vision8020026

Hubbard, R., & St George, R. C. C. (1957). The rhodopsin system of the squid. *The Journal of General Physiology*, *41*(3), 504–528. https://doi.org/10.1085/jgp.41.3.501

Hunt, J. C., & Seibel, B. A. (2000). Life history of *Gonatus onyx* (Cephalopoda: Teuthoidea): Ontogenetic changes in habitat, behavior and physiology. *Marine Biology*, *136*(3), 543–552. https://doi.org/10.1007/s002270050714

Jackson, G. D. (1994). Application and future potential of statolith increment analysis in squids and sepioids. *Canadian Journal of Fisheries and Aquatic Sciences*, *51*(11), 2612–2625. https://doi.org/10.1139/f94-261

Jackson, G. D., & O'dor, R. K. (2001). Time, space and the ecophysiology of squid growth, life in the fast lane. *Vie et Milieu*, *51*(4), 205–215.

Jacob, T. J. C., & Duncan, G. (1981). Electrical coupling between fibre cells in amphibian and cephalopod lenses. *Nature*, *290*(5808), 704–706. https://doi.org/10.1038/290704A0

Jamieson, A. J., & Vecchione, M. (2022). Hadal cephalopods: First squid observation (Oegopsida, Magnapinnidae, *Magnapinna* sp.) and new records of finned octopods (Cirrata) at depths > 6000 m in the Philippine Trench. *Marine Biology, 169*(11). https://doi.org/10.1007/s00227-021-03993-x

Jereb, P., & Roper, C. (2005). *Cephalopods of the world. An Annotated and illustrated catalogue of cephalopod species known to date. Volume 1: Chambered nautiluses and sepioids* (Nautilidae, Sepiidae, Sepiadariidae, Idiosepiidae and Spirulidae). FAO.

Jiang, L., Zhao, M., Shen, A., Huang, L., Chen, D., & Cai, C. (2022). Pulses of atmosphere oxygenation during the Cambrian radiation of animals. *Earth and Planetary Science Letters*, *590*. https://doi.org/10.1016/j.epsl.2022.117565

Johnsen, S. (2012). *The optics of life: A biologist's guide to light in nature*. Princeton University Press.

Johnson, M. L., Dobson, N., & De Grave, S. (2015). External morphology of eyes and Nebenaugen of caridean decapods-ecological and systematic considerations. *PeerJ*, *3*, Article e1176. https://doi.org/10.7717/peerj.1176

Johnstone, K. A., Lubieniecki, K. P., Koop, B. F., & Davidson, W. S. (2011). Expression of olfactory receptors in different life stages and life histories of wild Atlantic salmon (*Salmo salar*). *Molecular Ecology*, *20*(19), 4059–4069. https://doi.org/10.1111/j.1365-294X.2011.05251.x

Jones, B. W., & Nishiguchi, M. K. (2004). Counterillumination in the Hawaiian bobtail squid, *Euprymna scolopes* Berry (Mollusca: Cephalopoda). *Marine Biology*, *144*(6), 1151–1155. https://doi.org/10.1007/s00227-003-1285-3

Kaifu, K., Akamatsu, T., & Segawa, S. (2008). Underwater sound detection by cephalopod statocyst. *Fisheries Science*, *74*(4), 781–786. https://doi.org/10.1111/j.1444-2906.2008.01589.x

Kalloniatis, M., Loh, C. S., Acosta, M. L., Tomisich, G., Zhu, Y., Nivison-Smith, L., Fletcher, E. L., Chua, J., Sun, D., & Arunthavasothy, N. (2013). Retinal amino acid neurochemistry in health and disease. *Clinical and Experimental Optometry*, *96*(3), 310–332. https://doi.org/10.1111/CXO.12015

Kang, G., Allard, C. A. H., Valencia-Montoya, W. A., van Giesen, L., Kim, J. J., Kilian, P. B., Bai, X., Bellono, N. W., & Hibbs, R. E. (2023). Sensory specializations drive octopus and squid behaviour. *Nature*, *616*(7956), 378–383. https://doi.org/10.1038/s41586-023-05808-z

Kear, A. J. (1992). The diet of Antarctic squid: Comparison of conventional and serological gut contents analyses. *Journal of Experimental Marine Biology and Ecology*, *156*(2), 161–178. https://doi.org/10.1016/0022-0981(92)90243-4

Kenaley, C. P., Devaney, S. C., & Fjeran, T. T. (2014). The complex evolutionary history of seeing red: Molecular phylogeny and the evolution of an adaptive visual system in deep-sea dragonfishes (Stomiiformes: Stomiidae). *Evolution*, *68*(4), 996–1013. https://doi.org/10.1111/evo.12322

Kier, C. K., & Chamberlain, S. C. (1990). Dual controls for screening pigment movement in photoreceptors of the *Limulus* lateral eye: Circadian efferent input and light. *Visual Neuroscience*, *4*(3), 237–255. https://doi.org/10.1017/S0952523800003382

Kingston, A. C. N., Kuzirian, A. M., Hanlon, R. T., & Cronin, T. W. (2015). Visual phototransduction components in cephalopod chromatophores suggest dermal photoreception. *Journal of Experimental Biology*, *218*(10), 1596–1602. https://doi.org/10.1242/jeb.117945

Kito, Y., Suzuki, T., Azuma, M., & Sekoguti, Y. (1968). Absorption spectrum of rhodopsin denatured with acid. *Nature*, *218*(5145), 955–957. https://doi.org/10.1038/218955A0

Klages, N. T. W. (1996). Cephalopods as prey. II. Seals. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *351*(1343), 1045–1052. https://doi.org/10.1098/RSTB.1996.0092

Koenig, K. M., Sun, P., Meyer, E., & Gross, J. M. (2016). Eye development and photoreceptor differentiation in the cephalopod *Doryteuthis pealeii*. *Development (Cambridge)*, *143*(17), 3168–3181. https://doi.org/10.1242/dev.134254

Kröger, B., Vinther, J., & Fuchs, D. (2011). Cephalopod origin and evolution: A congruent picture emerging from fossils, development and molecules. Extant cephalopods are younger than previously realised and were under major selection to become agile,

shell-less predators. *BioEssays*, *33*(8), 602–613. https://doi.org/10.1002/bies.201100001

Kubodera, T., Koyama, Y., & Mori, K. (2007). Observations of wild hunting behaviour and bioluminescence of a large deep-sea, eight-armed squid, *Taningia danae*. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *274*(1613), 1029–1034. https://doi.org/10.1098/rspb.2006.0236

Land, M. F. (1981). Optics and vision in invertebrates. In H. Autrum (Ed *Handbook of Sensory Physiology, Vol. VII/6C, Comparative Physiology and Evolution of Vision in Invertebrates* (pp. 471–592). Springer Verlag. https://doi.org/10.1007/978-3-642-66907-1_4

Land, M. F., & Nilsson, D.-E. (2012). *Animal Eyes* (2nd ed.). Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199581139.001.0001

Lo, M. V. C., & Pak, W. L. (1981). Light-induced pigment granule migration in the retinular cells of *Drosophila melanogaster*: Comparison of wild type with ERG-defective mutants. *Journal of General Physiology*, *77*(2), 155–175. https://doi.org/10.1085/JGP.77.2.155

Lupše, N., Cortesi, F., Freese, M., Marohn, L., Pohlmann, J. D., Wysujack, K., Hanel, R., & Musilova, Z. (2021). Visual gene expression reveals a cone-to-rod developmental progression in deep-Sea fishes. *Molecular Biology and Evolution*, *38*(12), 5664–5677. https://doi.org/10.1093/molbev/msab281

Maddison, D. R., & Schulz, K.-S. (2007). *The Tree of Life Web Project*. https://www.tolweb.org/tree/

Makino, A., & Miyazaki, T. (2010). Topographical distribution of visual cell nuclei in the retina in relation to the habitat of five species of Decapodiformes (Cephalopoda). *Journal of Molluscan Studies*, *76*(2), 180–185. https://doi.org/10.1093/mollus/eyp055

Mangold, K. (1983). Reproduction. In P. R. Boyle (Ed.), *Cephalopod Life Cycles* (Vol. 2, pp. 157–200). Academic Press.

Marc, R. E., Liu, W. L. S., Kalloniatis, M., Raiguel, S. F., & Van Haesendonck, E. (1990). Patterns of glutamate immunoreactivity in the goldfish retina. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *10*(12), 4006–4034. https://doi.org/10.1523/JNEUROSCI.10-12-04006.1990

Marshall, C. R. (2006). Explaining the Cambrian "explosion" of animals. *Annual Review of Earth and Planetary Sciences*, *34*, 355–384. https://doi.org/10.1146/annurev.earth.33.031504.103001

Marshall, N. J., & Messenger, J. B. (1996). Colour-blind camouflage. *Nature*, *382*(6590), 408–409. https://doi.org/10.1038/382408b0

Martini, S., & Haddock, S. H. D. (2017). Quantification of bioluminescence from the surface to the deep sea demonstrates its predominance as an ecological trait. *Scientific Reports*, *7*(1), Article 45750. https://doi.org/10.1038/srep45750

Maselli, V., Al-Soudy, A. S., Buglione, M., Aria, M., Polese, G., & Di Cosmo, A. (2020). Sensorial hierarchy in *Octopus vulgaris's* food choice: Chemical vs. visual. *Animals*, *10*(3). https://doi.org/10.3390/ani10030457

Mäthger, L. M., Barbosa, A., Miner, S., & Hanlon, R. T. (2006). Color blindness and contrast perception in cuttlefish (*Sepia officinalis*) determined by a visual sensorimotor assay. *Vision Research*, *46*(11), 1746–1753. https://doi.org/10.1016/j.visres.2005.09.035

Mäthger, L. M., Hanlon, R. T., Håkansson, J., & Nilsson, D. E. (2013). The W-shaped pupil in cuttlefish (*Sepia officinalis*): Functions for improving horizontal vision. *Vision Research*, *83*, 19–24. https://doi.org/10.1016/j.visres.2013.02.016

Matsui, S., Seidou, M., Horiuchi, S., Uchiyama, I., & Kito, Y. (1988). Adaptation of a deep-sea cephalopod to the photic environment. *Journal of General Physiology*, *92*(1), 55–66. https://doi.org/10.1085/jgp.92.1.55

MBARI's Deep Sea Guide - Data Artifacts for Gonatus onyx. (2018). Monterey Bay Aquarium Research Institute (MBARI). http://dsg.mbari.org/dsg/plots/concept/Gonatus%20onyx/Global

McFarland, W. (1990). Light in the sea: The optical world of elasmobranchs. *Journal of Experimental Zoology*, *256*(5S), 3–12. https://doi.org/10.1002/JEZ.1402560503

Messenger, J. B. (1977). Evidence that *Octopus* is colour blind. *Journal of Experimental Biology*, *70*(1), 49–55. https://doi.org/10.1242/jeb.70.1.49

Messenger, J. B. (1981). Comparative physiology of vision in molluscs. In H. Autrum (Ed.), *Handbook of Sensory Physiology, Vol. VII/6C, Comparative Physiology and Evolution of Vision in Invertebrates* (pp. 93–200). Springer-Verlag.

Mitchell, J. S., Mackay, K. A., Neil, H. L., Mackay, E. J., Pallentin, A., & Notman, P. (2012). Undersea New Zealand, 1:5,000,000. In *NIWA Chart, Miscellaneous Series No. 92*. National Institute of Water & Atmospheric Research Ltd.

Mitchell, J., & Swardfager, W. (2010). Photoresponse in squid. In D. A. Dartt (Ed.), *Encyclopedia of the Eye* (pp. 347–353). Elsevier. https://doi.org/10.1016/B978-0-12-374203-2.00154-8

Miyazaki, T., & Osumi, H. (2022). Application of CHAPS-PC detergent to determine rhodopsin absorbance spectra in squids. *Fisheries Science*, *88*(4), 485–492. https://doi.org/10.1007/S12562-022-01603-3/FIGURES/3

Moody, M. F., & Parriss, J. R. (1960). Discrimination of polarized light by octopus. *Nature*, *186*(4728), 839–840. https://doi.org/10.1038/186839a0

Mooney, T. A., Hanlon, R. T., Christensen-Dalsgaard, J., Madsen, P. T., Ketten, D. R., & Nachtigall, P. E. (2010). Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: Sensitivity to low-frequency particle motion and not pressure. *Journal of Experimental Biology*, *213*(21), 3748–3759. https://doi.org/10.1242/jeb.048348

Morris, A., Bowmaker, J. K., & Hunt, D. M. (1993). The molecular basis of a spectral shift in the rhodopsins of two species of squid from different photic environments. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *254*(1341), 233–240. https://doi.org/10.1098/RSPB.1993.0151

Morton, B. (2008). The evolution of eyes in the Bivalvia: New insights. *American Malacological Bulletin*, *26*(1–2), 35–45. https://doi.org/10.4003/006.026.0205

Moustahfid, H., Hendrickson, L. C., Arkhipkin, A., Pierce, G. J., Gangopadhyay, A., Kidokoro, H., Markaida, U., Nigmatullin, C., Sauer, W. H., Jereb, P., Pecl, G., de la Chesnais, T., Ceriola, L., Lazar, N., Firmin, C. J., & Laptikhovsky, V. (2021). Ecological-fishery forecasting of squid stock dynamics under climate variability and change: Review, challenges, and recommendations. *Reviews in Fisheries Science and Aquaculture*, 1–36. https://doi.org/10.1080/23308249.2020.1864720

Muñoz, J. L. P., Patiño, M. A. L., Hermosilla, C., Conde-Sieira, M., Soengas, J. L., Rocha, F., & Míguez, J. M. (2011). Melatonin in octopus (*Octopus vulgaris*): Tissue distribution, daily changes and relation with serotonin and its acid metabolite. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *197*(8), 789–797. https://doi.org/10.1007/S00359-011-0641-X

Muntz, W. R. A. (1991). Anatomical and behavioral studies on vision in *Nautilus* and *Octopus*. *American Malacological Bulletin*, *9*, 69–74.

Muntz, W. R. A. (1999). Visual systems, behaviour, and environment in cephalopods. *Adaptive Mechanisms in the Ecology of Vision*, 467–483. https://doi.org/10.1007/978-94-017-0619-3_15

Muntz, W. R. A. (2010). Visual behavior and visual sensitivity of *Nautilus pompilius*. In W. B. Saunders, N. H. Landman (Eds.), *Nautilus: The biology and paleobiology of a living fossil* (pp. 231–244). Springer. https://doi.org/10.1007/978-90-481-3299-7_15

Muntz, W. R. A., & Johnson, M. S. (1978). Rhodopsins of oceanic decapods. *Vision Research*, *18*(5), 601–602. https://doi.org/10.1016/0042-6989(78)90210-9

Murai, R., Shiomi, M., Yoshida, M., Tomano, S., Iwata, Y., Sugai, K., & Hirohashi, N. (2021). All the spermatangia on a female were implanted by single-pair copulation in giant squid *Architeuthis dux*. *Deep-Sea Research Part I: Oceanographic Research Papers*, *175*. https://doi.org/10.1016/j.dsr.2021.103585

Murakami, M., & Kouyama, T. (2008). Crystal structure of squid rhodopsin. *Nature*, *453*(7193), 363–367. https://doi.org/10.1038/nature06925

Navarro, J., Coll, M., Somes, C. J., & Olson, R. J. (2013). Trophic niche of squids: Insights from isotopic data in marine systems worldwide. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *95*, 93–102. https://doi.org/10.1016/j.dsr2.2013.01.031

Nilsson, D. E., Warrant, E. J., Johnsen, S., Hanlon, R., & Shashar, N. (2012). A unique advantage for giant eyes in giant squid. *Current Biology*, *22*(8), 683–688. https://doi.org/10.1016/j.cub.2012.02.031

Nilsson, D. E., Warrant, E., & Sönke, J. (2014). Computational visual ecology in the pelagic realm. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1636), 20130038. https://doi.org/10.1098/rstb.2013.0038

Nishiguchi, M. K., Nabhitabhata, J., Moltschaniwskyj, N. A., & Boletzky, S. V. (2014). A review of the pygmy squid *Idiosepius*: Perspectives emerging from an "inconspicuous" cephalopod. *Vie et Milieu*, *64*, (23–34).

Nivison-Smith, L., Collin, S. P., Zhu, Y., Ready, S., Acosta, M. L., Hunt, D. M., Potter, I. C., & Kalloniatis, M. (2013). Retinal amino acid neurochemistry of the Southern Hemisphere lamprey, *Geotria australis*. *PLoS ONE*, *8*(3). https://doi.org/10.1371/JOURNAL.PONE.0058406

Nixon, M., & Young, J. Z. (2003). *The brains and lives of cephalopods*. Oxford University Press. https://global.oup.com/academic/product/the-brains-and-lives-of-cephalopods-9780198527619?cc=us&lang=en&

Okutani, T., Nakamura, I., & Seki, K. (1995). An unusual egg-brooding behavior of an oceanic squid in the Okhotsk Sea. *Japanese Journal of Malacology*, *54*(3), 237–239. https://doi.org/10.18941/venusjjm.54.3_237

Osborn, K. J., Haddock, S. H. D., Pleijel, F., Madin, L. P., & Rouse, G. W. (2009). Deep-sea, swimming worms with luminescent "bombs." *Science*, *325*(5943), 964. https://doi.org/10.1126/science.1172488

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research*, *48*(20), 2042–2051. https://doi.org/10.1016/j.visres.2008.06.018

Ovchinnikov, Y. A., Abdulaev, N. G., Zolotarev, A. S., Artamonov, I. D., Bespalov, I. A., Dergachev, A. E., & Tsuda, M. (1988). Octopus rhodopsin Amino acid sequence deduced from cDNA. *FEBS Letters*, *232*(1), 69–72. https://doi.org/10.1016/0014-5793(88)80388-0

Packard, A. (1972). Cephalopods and fish: The limits of convergence. *Biological Reviews*, *47*(2), 241–307. https://doi.org/10.1111/j.1469-185X.1972.tb00975.x

Parker, A. R. (2011). On the origin of optics. *Optics and Laser Technology*, *43*(2), 323–329. https://doi.org/10.1016/j.optlastec.2008.12.020

Phillips, K. L., Jackson, G. D., & Nichols, P. D. (2001). Predation on myctophids by the squid *Moroteuthis ingens* around Macquarie and Heard Islands: Stomach contents and fatty acid analyses. *Marine Ecology Progress Series*, *215*, 179–189. https://doi.org/10.3354/meps215179

Phillips, K. L., Jackson, G. D., & Nichols, P. D. (2003). Temporal variations in the diet of the squid *Moroteuthis ingens* at Macquarie Island: Stomach contents and fatty acid analyses. *Marine Ecology Progress Series*, *256*, 135–149. https://doi.org/10.3354/meps256135

Phillips, K. L., Nichols, P. D., & Jackson, G. D. (2002). Lipid and fatty acid composition of the mantle and digestive gland of four Southern Ocean squid species: Implications for

food-web studies. *Antarctic Science*, *14*(3), 212–220. https://doi.org/10.1017/S0954102002000044

Phillips, K. L., Nichols, P. D., & Jackson, G. D. (2003). Dietary variation of the squid *Moroteuthis ingens* at four sites in the Southern Ocean: Stomach contents, lipid and fatty acid profiles. *Journal of the Marine Biological Association of the United Kingdom*, *83*(3), 523–534. https://doi.org/10.1017/S0025315403007446h

Piatkowski, U., Pierce, G. J., & Morais da Cunha, M. (2001). Impact of cephalopods in the food chain and their interaction with the environment and fisheries: An overview. *Fisheries Research*, *52*(1–2), 5–10. https://doi.org/10.1016/S0165-7836(01)00226-0

Pignatelli, V., Temple, S. E., Chiou, T.-H., Roberts, N. W., Collin, S. P., & Marshall, N. J. (2011). Behavioural relevance of polarization sensitivity as a target detection mechanism in cephalopods and fishes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *366*(1565), 734–741. https://doi.org/10.1098/rstb.2010.0204

Premke, K., Muyakshin, S., Klages, M., & Wegner, J. (2003). Evidence for long-range chemoreceptive tracking of food odour in deep-sea scavengers by scanning sonar data. *Journal of Experimental Marine Biology and Ecology*, *285–286*, 283–294. https://doi.org/10.1016/S0022-0981(02)00533-6

Prugnor, J. R., & Niell, C. M. (2023). The neural basis of visual processing and behavior in cephalopods. *Current Biology*, *33*(20), R1106–R1118. https://doi.org/10.106/j.club.2023.08.093

Quan, X., & Fry, E. S. (1995). Empirical equation for the index of refraction of seawater. *Applied Optics*, *34*(18), 3477. https://doi.org/10.1364/ao.34.003477

Quetglas, A., Ordines, F., González, M., Zaragoza, N., Mallol, S., Valls, M., & De mesa, A. (2013). Uncommon pelagic and deep-sea cephalopods in the Mediterranean: New data and literature review. *Mediterranean Marine Science*, *14*(1), 69–85. https://doi.org/10.12681/mms.320

Ratnasingham, S., & Hebert, P. D. N. (2007). Barcoding: Bold: The barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes*, *7*(3), 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rawitz, B. (1891). Über Pigmentverschiebungen im Cephalopodenauge. *Zoologischer Anzeiger*, *14*, 157–158.

Robin, J. P., Roberts, M., Zeidberg, L., Bloor, I., Rodriguez, A., Briceño, F., Downey, N., Mascaró, M., Navarro, M., Guerra, A., Hofmeister, J., Barcellos, D. D., Lourenço, S. A. P., Roper, C. F. E., Moltschaniwskyj, N. A., Green, C. P., & Mather, J. (2014). Transitions during cephalopod life history: The role of habitat, environment, functional morphology and behaviour. In E. A. G. Vidal (Ed.), *Advances in marine biology. Advances in cephalopod science: Biology, ecology, cultivation and fisheries* (Vol. 67, pp. 361–437). Academic Press. https://doi.org/10.1016/B978-0-12-800287-2.00004-4

Robinson, N. J., Johnsen, S., Brooks, A., Frey, L., Judkins, H., Vecchione, M., & Widder, E. (2021). Studying the swift, smart, and shy: Unobtrusive camera-platforms for observing large deep-sea squid. *Deep-Sea Research Part I: Oceanographic Research Papers*, *172*. https://doi.org/10.1016/j.dsr.2021.103538

Rodhouse, P. G. K., Pierce, G. J., Nichols, O. C., Sauer, W. H. H., Arkhipkin, A. I., Laptikhovsky, V. V., Lipiński, M. R., Ramos, J. E., Gras, M., Kidokoro, H., Sadayasu, K., Pereira, J., Lefkaditou, E., Pita, C., Gasalla, M., Haimovici, M., Sakai, M., & Downey, N. (2014). Environmental effects on cephalopod population dynamics: Implications for management of fisheries. In E. A. G. Vidal (Ed.), *Advances in marine biology. Advances in cephalopod science: Biology, ecology, cultivation and fisheries* (Vol. 67, 99–233) Academic Press. https://doi.org/10.1016/B978-0-12-800287-2.00002-0

Roper, C. F. E., De Angelis, N., Roper, I., Sweeney, M. J., D'Antoni, E., Kautenberger-Longo, M., Carocci, F., & D'Antoni, E. (2010). *Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume 3. Octopods and vampire squids.* FAO.

Roper, C. F. E., Nigmatullin, C., & Jereb, P. (2010). *Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume 2. Myopsid and oegopsid squids. FAO.*

Rosa, R., Lopes, V. M., Guerreiro, M., Bolstad, K., & Xavier, J. C. (2017). Biology and ecology of the world's largest invertebrate, the colossal squid *(Mesonychoteuthis hamiltoni*): A short review. *Polar Biology*, *40*(9), 1871–1883. https://doi.org/10.1007/s00300-017-2104-5

Rosa, R., Pissarra, V., Borges, F. O., Xavier, J., Gleadall, I., Golikov, A., Bello, G., Morais, L., Lishchenko, F., Roura, Á., Judkins, H., Ibáñez, C. M., Piatkowski, U., Vecchione, M., & Villanueva, R. (2019). Global patterns of species richness in coastal cephalopods. *Frontiers in Marine Science*, *6*, 469. https://doi.org/10.3389/fmars.2019.00469

Scheel, D., Godfrey-Smith, P., Linquist, S., Chancellor, S., Hing, M., & Lawrence, M. (2018). Octopus engineering, intentional and inadvertent. *Communicative & Integrative Biology*, *11*(1), Article e1395994. https://doi.org/10.1080/19420889.2017.1395994

Schlosser, G. (2018). A short history of nearly every sense — The evolutionary history of vertebrate sensory cell types. *Integrative and Comparative Biology*, *58*(2), 301–316. https://doi.org/10.1093/icb/icy024

Schnitzler, C. E., Pang, K., Powers, M. L., Reitzel, A. M., Ryan, J. F., Simmons, D., Tada, T., Park, M., Gupta, J., Brooks, S. Y., Blakesley, R. W., Yokoyama, S., Haddock, S. H. D., Martindale, M. Q., & Baxevanis, A. D. (2012). Genomic organization, evolution, and expression of photoprotein and opsin genes in *Mnemiopsis leidyi*: A new view of ctenophore photocytes. *BMC Biology*, *10*(1), 107. https://doi.org/10.1186/1741-7007-10-107

Schoenlein, R. W., Peteanu, L. A., Mathies, R. A., & Shank, C. V. (1991). The first step in vision: Femtosecond isomerization of rhodopsin. *Science*, *254*(5030), 412–415. https://doi.org/10.1126/science.1925597

Schraermeyer, U. (1990). Further evidence for synthesis of screening pigment granules involved in the photosensory membrane turnover of the crayfish photoreceptor. *Pigment Cell Research*, *3*(6), 279–289. https://doi.org/10.1111/j.1600-0749.1990.tb00299.x

Seibel, B. A., Hochberg, F. G., & Carlini, D. B. (2000). Life history of *Gonatus onyx* (Cephalopoda: Teuthoidea): Deep-sea spawning and post-spawning egg care. *Marine Biology*, *137*(3), 519–526. https://doi.org/10.1007/S002270000359

Seibel, B. A., Robison, B. H., & Haddock, S. H. D. (2005). Post-spawning egg care by a squid. *Nature*, *438*(7070), 929–929. https://doi.org/10.1038/438929a

Seidou, M., Sugahara, M., Uchiyama, H., Hiraki, K., Hamanaka, T., Michinomae, M., Yoshihara, K., & Kito, Y. (1990a). On the three visual pigments in the retina of the firefly squid, *Watasenia scintillans*. *Journal of Comparative Physiology A*, *166*(6), 769–773. https://doi.org/10.1007/BF00187321

Serb, J. M., & Eernisse, D. J. (2008). Charting evolution's trajectory: Using molluscan eye diversity to understand parallel and convergent evolution. *Evolution: Education and Outreach*, *1*, 439–447. https://doi.org/10.1007/s12052-008-0084-1

Shand, J., Archer, M. A., & Collin, S. P. (1999). Ontogenetic changes in the retinal photoreceptor mosaic in a fish, the black bream, *Acanthopagrus butcheri*. *The Journal of Comparative Neurology*, *412*(2), 203–217. https://doi.org/10.1002/(SICI)1096-9861(19990920)412:2<203::AID-CNE2>3.0.CO;2-3

Shashar, N., Milbury, C., & Hanlon, R. (2002). Polarization vision in cephalopods: Neuroanatomical and behavioral features that illustrate aspects of form and function. *Marine and Freshwater Behaviour and Physiology*, *35*(1–2), 57–68. https://doi.org/10.1080/10236240290025617

Shichida, Y., & Imai, H. (1998). Visual pigment: G-protein-coupled receptor for light signals. *Cellular and Molecular Life Sciences*, *54*(12), 1299–1315. https://doi.org/10.1007/s000180050256

Sims, D. W., Genner, M. J., Southward, A. J., & Hawkins, S. J. (2001). Timing of squid migration reflects North Atlantic climate variability. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, *268*(1485), 2607–2611. https://doi.org/10.1098/rspb.2001.1847

Sivak, J. G. (1991). Shape and focal properties of the cephalopod ocular lens. *Canadian Journal of Zoology*, *69*(10), 2501–2506. https://doi.org/10.1139/z91-354

Smale, M. J. (1996). Cephalopods as prey. IV. Fishes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *351*(1343), 1067–1081. https://doi.org/10.1098/rstb.1996.0094

Sorensen, P. W., & Stacey, N. E. (1999). Evolution and specialization of fish hormonal pheromones. In R. E. Johnston, D. Müller-Schwarze, P. W. Sorensen (Eds.), *Advances in Chemical Signals in Vertebrates* (pp. 15–47). Springer. https://doi.org/10.1007/978-1-4615-4733-4_2

Speiser, D. I., & Johnsen, S. (2008). Comparative morphology of the concave mirror eyes of scallops (Pectinoidea). *American Malacological Bulletin*, *26*(1–2), 27–33. https://doi.org/10.4003/006.026.0204

Sperling, E. A., Frieder, C. A., Raman, A. V., Girguis, P. R., Levin, L. A., & Knoll, A. H. (2013). Oxygen, ecology, and the Cambrian radiation of animals. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(33), 13446–13451. https://doi.org/10.1073/pnas.1312778110

Stavenga, D. G., & Hardie, R. C. (2011). Metarhodopsin control by arrestin, light-filtering screening pigments, and visual pigment turnover in invertebrate microvillar photoreceptors. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *197*(3), 227–241. https://doi.org/10.1007/s00359-010-0604-7

Sugawara, T., Imai, H., Nikaido, M., Imamoto, Y., & Okada, N. (2010). Vertebrate rhodopsin adaptation to dim light via rapid meta-II intermediate formation. *Molecular Biology and Evolution*, *27*(3), 506–519. https://doi.org/10.1093/molbev/msp252

Summers, K. M., Howells, A. J., & Pyliotis, N. A. (1982). Biology of eye pigmentation in insects. *Advances in Insect Physiology*, *16*, 119–166. https://doi.org/10.1016/S0065-2806(08)60153-8

Sweeney, A. M., Haddock, S. H. D., & Johnsen, S. (2007). Comparative visual acuity of coleoid cephalopods. *Integrative and Comparative Biology*, *47*(6), 808–814. https://doi.org/10.1093/icb/icm092

Talbot, C., Jordan, T. M., Roberts, N. W., Collin, S. P., Marshall, N. J., & Temple, S. E. (2012). Corneal microprojections in coleoid cephalopods. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *198*(12), 849–856. https://doi.org/10.1007/s00359-012-0755-9

Talbot, C. M., & Marshall, J. (2010). Polarization sensitivity and retinal topography of the striped pyjama squid (*Sepioloidea lineolata* -Quoy/Gaimard 1832). *Journal of Experimental Biology*, *213*(19), 3371–3377. https://doi.org/10.1242/jeb.048165

Talbot, C. M., & Marshall, J. N. (2011). The retinal topography of three species of coleoid cephalopod: Significance for perception of polarized light. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *366*(1565), 724–733. https://doi.org/10.1098/rstb.2010.0254

Temple, S. E. (2007). Effect of salinity on the refractive index of water: Considerations for archer fish aerial vision. *Journal of Fish Biology*, *70*(5), 1626–1629. https://doi.org/10.1111/j.1095-8649.2007.01432.x

Terakita, A., Yamashita, T., Tachibanaki, S., & Shichida, Y. (1998). Selective activation of G-protein subtypes by vertebrate and invertebrate rhodopsins. *FEBS Letters*, *439*(1–2), 110–114. https://doi.org/10.1016/S0014-5793(98)01340-4

Thoen, H. H., How, M. J., Chiou, T. H., & Marshall, J. (2014). A different form of color vision in mantis shrimp. *Science*, *343*(6169). https://doi.org/10.1126/science.1245824

Thomas, K. N., Robison, B. H., & Johnsen, S. (2017). Two eyes for two purposes: In situ evidence for asymmetric vision in the cockeyed squids *Histioteuthis heteropsis* and *Stigmatoteuthis dofleini*. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *372*(1717). https://doi.org/10.1098/rstb.2016.0069

Tong, D., Rozas, N. S., Oakley, T. H., Mitchell, J., Colley, N. J., & McFall-Ngai, M. J. (2009). Evidence for light perception in a bioluminescent organ. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(24), 9836–9841. https://doi.org/10.1073/pnas.0904571106

Tsuda, M. (1979). Transient spectra of intermediates in the photolytic sequence of octopus rhodopsin. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *545*(3), 537–546. https://doi.org/10.1016/0005-2728(79)90162-2

Turnbull, P. R. K., Backhouse, S., & Phillips, J. R. (2015). Visually guided eye growth in the squid. *Current Biology*, *25*(18), R791–R792. https://doi.org/10.1016/j.cub.2015.07.073

Ueda, H. (2014). Homing ability and migration success in Pacific salmon: Mechanistic insights from biotelemetry, endocrinology, and neurophysiology. *Marine Ecology Progress Series*, *496*, 219–232. https://doi.org/10.3354/meps10636

Van Nynatten, A., Duncan, A. T., Lauzon, R., Sheldon, T. A., Chen, S. K., Lovejoy, N. R., Mandrak, N. E., & Chang, B. S. W. (2024). Adaptive evolution of Nearctic deepwater fish vision: Implications for assessing functional variation for conservation. *Molecular Biology and Evolution*, *41*(2). https://doi.org/10.1093/molbev/msae024

Voight, J. R. (2000). The distribution of Octopuses of *Graneledone* (Cephalopoda: Octopodidae) in reference to deep-sea features. *Malacologia*, *42*(1–2), 63–74.

Waluda, C. M., Trathan, P. N., & Rodhouse, P. G. (1999). Influence of oceanographic variability on recruitment in the *Illex argentinus* (Cephalopoda: Ommastrephidae) fishery in the South Atlantic. *Marine Ecology Progress Series*, *183*, 159–167. https://doi.org/10.3354/meps183159

Wangvoralak, S., Hastie, L. C., & Pierce, G. J. (2011). Temporal and ontogenetic variation in the diet of squid (*Loligo forbesii* Streenstrup) in Scottish waters. *Hydrobiologia*, *670*(1), 223–240. https://doi.org/10.1007/s10750-011-0723-3

Warrant, E. J., Collin, S. P., & Locket, N. A. (2003). Eye design and vision in deep-sea fishes. In S. P. Collin & N. J. Marshall (Eds.), *Sensory processing in aquatic environments* (1st ed., pp. 303–322). Springer New York. https://doi.org/10.1007/978-0-387-22628-6_16

Warrant, E. J., & Johnsen, S. (2013). Vision and the light environment. *Current Biology*, *23*(22). https://doi.org/10.1016/j.cub.2013.10.019

Warrant, E. J., & Locket, N. A. (2004). Vision in the deep sea. *Biological Reviews of the Cambridge Philosophical Society*, *79*(3), 671–712. https://doi.org/10.1017/S1464793103006420

Watanabe, H., Kubodera, T., Moku, M., & Kawaguchi, K. (2006). Diel vertical migration of squid in the warm core ring and cold water masses in the transition region of the

western North Pacific. *Marine Ecology Progress Series*, *315*, 187–197. https://doi.org/10.3354/meps315187

West, J. A., Sivak, J. G., & Doughty, M. J. (1995). Microscopical evaluation of the crystalline lens of the squid (*Loligo opalescens*) during embryonic development. *Experimental Eye Research*, *60*(1), 19–35. https://doi.org/10.1016/S0014-4835(05)80080-6

White, R. H., Banister, M. J., & Bennett, R. R. (1983). Spectral sensitivity of screening pigment migration in the compound eye of *Manduca sexta*. *Journal of Comparative Physiology A*, *153*(1), 59–66. https://doi.org/10.1007/BF00610343

Wiederhold, M. L., Sheridan, C. E., & Smith, N. K. R. (1989). Function of molluscan statocysts. In R. E. Crick (Ed.), *Origin, Evolution, and Modern Aspects of Biomineralization in Plants and Animals* (pp. 393–408). Springer Boston. https://doi.org/10.1007/978-1-4757-6114-6_31

Williamson, R. (1995). A sensory basis for orientation in cephalopods. *Journal of the Marine Biological Association of the United Kingdom*, *75*(1), 83–92. https://doi.org/10.1017/S0025315400015216

Wolken, J. J. (1958). Retinal structure. Mollusc cephalopods: *Octopus*, *Sepia*. *The Journal of Biophysical and Biochemical Cytology*, *4*(6), 835–837. https://doi.org/10.1083/jcb.4.6.835

Wood, J. B., Pennoyer, K. E., & Derby, C. D. (2008). Ink is a conspecific alarm cue in the Caribbean reef squid, *Sepioteuthis sepioidea*. *Journal of Experimental Marine Biology and Ecology*, *367*(1), 11–16. https://doi.org/10.1016/j.jembe.2008.08.004

Yarfitz, S., & Hurley, J. B. (1994). Transduction mechanisms of vertebrate and invertebrate photoreceptors. *Journal of Biological Chemistry*, *269*(20), 14329–14332. https://doi.org/10.1016/s0021-9258(17)36620-6

Yentsch, C. S. (1962). Measurement of visible light absorption by particulate matter in the ocean. *Limnology and Oceanography*, *7*(2), 207–217. https://doi.org/10.4319/lo.1962.7.2.0207

Young, J. Z. (1960). The visual system of *Octopus*: (1) Regularities in the retina and optic lobes of octopus in relation to form discrimination. *Nature*, *186*(4728), 836–839. https://doi.org/10.1038/186836a0

Young, J. Z. (1962). The retina of cephalopods and its degeneration after optic nerve section. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *245*(718), 1–18. https://doi.org/10.1098/RSTB.1962.0004

Young, J. Z. (1963). Light and dark adaptation in the eyes of some cephalopods. *Proceedings of the Zoological Society of London*, *140*(2), 255–272. https://doi.org/10.1111/J.1469-7998.1963.TB01863.X

Young, J. Z. (1991). Light has many meanings for cephalopods. *Visual Neuroscience*, *7*(1–2), 1–12. https://doi.org/10.1017/S0952523800010907

Young, R. E. (1972a). Brooding in a bathypelagic octopus. *Pacific Science*, *26*(4), 400–404. http://hdl.handle.net/10125/444

Young, R. E. (1972b). The systematics and areal distribution of pelagic cephalopods from the seas off Southern California. *Smithsonian Contributions to Zoology*, *97*, 1–159. https://doi.org/10.5479/si.00810282.97

Young, R. E., Kampa, E. M., Maynard, S. D., Mencher, F. M., & Roper, C. F. E. (1980). Counterillumination and the upper depth limits of midwater animals. *Deep-Sea Research Part A. Oceanographic Research Papers, 27*(9), 671–691. https://doi.org/10.1016/0198-0149(80)90022-9

Young, R. E., Roper, C. F. E., & Walters, J. F. (1979). Eyes and extraocular photoreceptors in midwater cephalopods and fishes: Their roles in detecting downwelling light for counterillumination. *Marine Biology*, *51*(4), 371–380. https://doi.org/10.1007/BF00389215

Young, R. E., Vecchione, M., & Donovan, D. T. (1998). The evolution of coleoid cephalopods and their present biodiversity and ecology. *South African Journal of Marine Science*, *20*(1), 393–420. https://doi.org/10.2989/025776198784126287

Zulandt Schneider, R. A., Schneider, R. W. S., & Moore, P. A. (1999). Recognition of dominance status by chemoreception in the red swamp crayfish, *Procambarus clarkii. Journal of Chemical Ecology*, *25*(4), 781–794. https://doi.org/10.1023/A:1020888532513

Appendices

Appendix A. *Supplemental material of statistical analyses and probability metrics from Chapter 2 – Retinal Anatomy of Deep-sea Oegopsid Squids of Aotearoa New Zealand.*

Table I. A *list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for OSP heatmaps and matrices of* N. sloanii*.*

Retinal Regions Compared	Estimate	SE	df	t-ratio	p-value
AC-AD	-2.5185	3.61	952	-0.699	0.9988
AC-AV	5.1667	3.61	952	1.433	0.8849
AC-MC	-10.5833	3.61	952	-2.936	0.0817
AC-MD	9.3056	3.61	952	2.581	0.1959
AC-MV	9.037	3.61	952	2.507	0.2299
AC-PC	-5.4074	3.61	952	-1.5	0.8557
AC-PD	-10.6667	3.61	952	-2.959	0.0767
AC-PV	1.0926	3.61	952	0.303	1
AD-AV	7.6852	3.61	952	2.132	0.4521
AD - MC	-8.0648	3.61	952	-2.237	0.3824
AD - MD	11.8241	3.61	952	3.28	0.0296
AD - MV	11.5556	3.61	952	3.205	0.0374
AD - PC	-2.8889	3.61	952	-0.801	0.9968
AD - PD	-8.1481	3.61	952	-2.26	0.3677
AD - PV	3.6111	3.61	952	1.002	0.9858
AV - MC	-15.75	3.61	952	-4.369	0.0005
AV - MD	4.1389	3.61	952	1.148	0.9666
AV - MV	3.8704	3.61	952	1.074	0.9779
AV - PC	-10.5741	3.61	952	-2.933	0.0823
AV - PD	-15.8333	3.61	952	-4.392	0.0004
AV - PV	-4.0741	3.61	952	-1.13	0.9696
MC - MD	19.8889	3.61	952	5.517	<0.0001
MC - MV	19.6204	3.61	952	5.442	<0.0001
MC - PC	5.1759	3.61	952	1.436	0.8839
MC - PD	-0.0833	3.61	952	-0.023	1
MC - PV	11.6759	3.61	952	3.239	0.0337
MD - MV	-0.2685	3.61	952	-0.074	1
MD - PC	-14.713	3.61	952	-4.081	0.0016
MD - PD	-19.9722	3.61	952	-5.54	<0.0001
MD - PV	-8.213	3.61	952	-2.278	0.3565
MV - PC	-14.4444	3.61	952	-4.007	0.0022
MV - PD	-19.7037	3.61	952	-5.465	<0.0001
MV - PV	-7.9444	3.61	952	-2.204	0.404
PC - PD	-5.2593	3.61	952	-1.459	0.8742
PC - PV	6.5	3.61	952	1.803	0.6803
PD - PV	11.7593	3.61	952	3.262	0.0314

Table II. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for OSP heatmaps and matrices of* N. gouldi*.*

Retinal Regions Compared	Estimate	SE	df	t-ratio	p-value
AC - AD	3.593	3.84	472	0.937	0.9908
AC - AV	12.519	3.84	472	3.264	0.032
AC - MC	4.296	3.84	472	1.12	0.9711
AC - MD	16.722	3.84	472	4.36	5.00E-04
AC - MV	12.926	3.84	472	3.37	0.0229
AC - PC	-1.278	3.84	472	-0.333	1
AC - PD	12.685	3.84	472	3.307	0.028
AC - PV	19.407	3.84	472	5.06	1.00E-04
AD - AV	8.926	3.84	472	2.327	0.3279
AD - MC	0.704	3.84	472	0.183	1
AD - MD	13.13	3.84	472	3.423	0.0192
AD - MV	9.333	3.84	472	2.433	0.2683
AD - PC	-4.87	3.84	472	-1.27	0.9396
AD - PD	9.093	3.84	472	2.371	0.3027
AD - PV	15.815	3.84	472	4.123	0.0014
AV - MC	-8.222	3.84	472	-2.144	0.4448
AV - MD	4.204	3.84	472	1.096	0.9747
AV - MV	0.407	3.84	472	0.106	1
AV - PC	-13.796	3.84	472	-3.597	0.0106
AV - PD	0.167	3.84	472	0.043	1
AV - PV	6.889	3.84	472	1.796	0.6849
MC - MD	12.426	3.84	472	3.24	0.0345
MC - MV	8.63	3.84	472	2.25	0.3752
MC - PC	-5.574	3.84	472	-1.453	0.8762
MC - PD	8.389	3.84	472	2.187	0.4158
MC - PV	15.111	3.84	472	3.94	0.003
MD - MV	-3.796	3.84	472	-0.99	0.9867
MD - PC	-18	3.84	472	-4.693	1.00E-04
MD - PD	-4.037	3.84	472	-1.053	0.9803
MD - PV	2.685	3.84	472	0.7	0.9988
MV - PC	-14.204	3.84	472	-3.703	0.0073

MV - PD	-0.241	3.84	472	-0.063	1
MV - PV	6.481	3.84	472	1.69	0.7527
PC - PD	13.963	3.84	472	3.64	0.0091
PC - PV	20.685	3.84	472	5.393	1.00E-04
PD - PV	6.722	3.84	472	1.753	0.7133

Table III. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for OSP heatmaps and matrices of* T. filippovae*.*

Retinal Regions Compared	Estimate	SE	df	t-ratio	p-value
AC - AD	-44.33	10.3	232	-4.324	0.0008
AC - AV	16.11	10.3	232	1.571	0.8193
AC - MC	51.37	10.3	232	5.011	<.0001
AC - MD	27.44	10.3	232	2.677	0.162
AC - MV	14.15	10.3	232	1.38	0.9044
AC - PC	46.52	10.3	232	4.537	0.0003
AC - PD	-30.07	10.3	232	-2.933	0.0862
AC - PV	3.37	10.3	232	0.329	1
AD - AV	60.44	10.3	232	5.896	<.0001
AD - MC	95.7	10.3	232	9.335	<.0001
AD - MD	71.78	10.3	232	7.001	<.0001
AD - MV	58.48	10.3	232	5.704	<.0001
AD - PC	90.85	10.3	232	8.862	<.0001
AD - PD	14.26	10.3	232	1.391	0.9004
AD - PV	47.7	10.3	232	4.653	0.0002
AV - MC	35.26	10.3	232	3.439	0.0195
AV - MD	11.33	10.3	232	1.105	0.973
AV - MV	-1.96	10.3	232	-0.191	1
AV - PC	30.41	10.3	232	2.966	0.0791
AV - PD	-46.19	10.3	232	-4.505	0.0004
AV - PV	-12.74	10.3	232	-1.243	0.946
MC - MD	-23.93	10.3	232	-2.334	0.3264
MC - MV	-37.22	10.3	232	-3.631	0.0103
MC - PC	-4.85	10.3	232	-0.473	0.9999
MC - PD	-81.44	10.3	232	-7.944	<.0001

MC - PV	-48	10.3	232	-4.682	0.0002
MD - MV	-13.3	10.3	232	-1.297	0.9315
MD - PC	19.07	10.3	232	1.86	0.6416
MD - PD	-57.52	10.3	232	-5.61	<.0001
MD - PV	-24.07	10.3	232	-2.348	0.318
MV - PC	32.37	10.3	232	3.157	0.0464
MV - PD	-44.22	10.3	232	-4.313	0.0008
MV - PV	-10.78	10.3	232	-1.051	0.9802
PC - PD	-76.59	10.3	232	-7.471	<.0001
PC - PV	-43.15	10.3	232	-4.209	0.0012
PD - PV	33.44	10.3	232	3.262	0.034

Table IV. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for OSP heatmaps and matrices of* M. ingens*.*

Retinal Regions Compared	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
AC - AD	-13.678	4.24	792	-3.223	0.0356
AC - AV	-0.311	4.24	792	-0.073	1
AC - MC	18.111	4.24	792	4.267	0.0007
AC - MD	6.333	4.24	792	1.492	0.8592
AC - MV	18.744	4.24	792	4.417	0.0004
AC - PC	1.878	4.24	792	0.442	1
AC - PD	-15.644	4.24	792	-3.686	0.0075
AC - PV	5.711	4.24	792	1.346	0.9171
AD - AV	13.367	4.24	792	3.15	0.0445
AD - MC	31.789	4.24	792	7.49	<.0001
AD - MD	20.011	4.24	792	4.715	0.0001
AD - MV	32.422	4.24	792	7.64	<.0001
AD - PC	15.556	4.24	792	3.665	0.008
AD - PD	-1.967	4.24	792	-0.463	0.9999
AD - PV	19.389	4.24	792	4.569	0.0002
AV - MC	18.422	4.24	792	4.341	0.0005
AV - MD	6.644	4.24	792	1.566	0.8232
AV - MV	19.056	4.24	792	4.49	0.0003
AV - PC	2.189	4.24	792	0.516	0.9999

AV - PD	-15.333	4.24	792	-3.613	0.0097
AV - PV	6.022	4.24	792	1.419	0.8905
MC - MD	-11.778	4.24	792	-2.775	0.1244
MC - MV	0.633	4.24	792	0.149	1
MC - PC	-16.233	4.24	792	-3.825	0.0044
MC - PD	-33.756	4.24	792	-7.954	<.0001
MC - PV	-12.4	4.24	792	-2.922	0.0851
MD - MV	12.411	4.24	792	2.924	0.0845
MD - PC	-4.456	4.24	792	-1.05	0.9807
MD - PD	-21.978	4.24	792	-5.179	<.0001
MD - PV	-0.622	4.24	792	-0.147	1
MV - PC	-16.867	4.24	792	-3.974	0.0025
MV - PD	-34.389	4.24	792	-8.103	<.0001
MV - PV	-13.033	4.24	792	-3.071	0.0561
PC - PD	-17.522	4.24	792	-4.129	0.0013
PC - PV	3.833	4.24	792	0.903	0.9928
PD - PV	21.356	4.24	792	5.032	<.0001

Table V. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for OSP heatmaps and matrices of* O. robsoni*.*

Retinal Regions Compared	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
AC—AD	67.81	8.88	312	7.632	<.0001
AC—AV	91.28	8.88	312	10.274	<.0001
AC—MC	45.47	8.88	312	5.118	<.0001
AC—MD	82.06	8.88	312	9.236	<.0001
AC—MV	97	8.88	312	10.918	<.0001
AC—PC	9.67	8.88	312	1.088	0.9756
AC—PD	64.33	8.88	312	7.241	<.0001
AC—PV	76.83	8.88	312	8.648	<.0001
AD—AV	23.47	8.88	312	2.642	0.1738
AD – MC	-22.33	8.88	312	-2.514	0.2294
AD – MD	14.25	8.88	312	1.604	0.8022
AD – MV	29.19	8.88	312	3.286	0.0307
AD – PC	-58.14	8.88	312	-6.544	<.0001
AD – PD	-3.47	8.88	312	-0.391	1
AD – PV	9.03	8.88	312	1.016	0.9842
AV – MC	-45.81	8.88	312	-5.156	<.0001
AV – MD	-9.22	8.88	312	-1.038	0.9819
AV – MV	5.72	8.88	312	0.644	0.9993
AV – PC	-81.61	8.88	312	-9.186	<.0001

AV – PD	-26.94	8.88	312	-3.033	0.0647
AV – PV	-14.44	8.88	312	-1.626	0.79
MC - MD	36.58	8.88	312	4.118	0.0016
MC - MV	51.53	8.88	312	5.8	<.0001
MC – PC	-35.81	8.88	312	-4.03	0.0023
MC – PD	18.86	8.88	312	2.123	0.4595
MC – PV	31.36	8.88	312	3.53	0.014
MD - MV	14.94	8.88	312	1.682	0.7571
MD – PC	-72.39	8.88	312	-8.148	<.0001
MD – PD	-17.72	8.88	312	-1.995	0.5482
MD – PV	-5.22	8.88	312	-0.588	0.9997
MV – PC	-87.33	8.88	312	-9.83	<.0001
MV – PD	-32.67	8.88	312	-3.677	0.0084
MV – PV	-20.17	8.88	312	-2.27	0.3637
PC – PD	54.67	8.88	312	6.153	<.0001
PC – PV	67.17	8.88	312	7.56	<.0001
PD - PV	12.5	8.88	312	1.407	0.8946

Table VI. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for OSP retinal locations (dorsal, central and ventral OSP lengths).*

Species	**Retinal Locations Compared**	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
N. sloanii	Central-Dorsal	4.04	2.12	958	1.902	0.1387
	Central-Ventral	10.43	2.12	958	4.913	<.0001
	Dorsal-Ventral	6.39	2.12	958	3.011	0.0075
N. gouldi	Central-Dorsal	9.99	2.24	478	4.454	<.0001
	Central-Ventral	13.94	2.24	478	6.215	<.0001
	Dorsal-Ventral	3.95	2.24	478	1.761	0.1840
T. filippovae	Central-Dorsal	-48.3	6.86	238	-7.042	<.0001
	Central-Ventral	-21.4	6.86	238	-3.124	0.0057
	Dorsal-Ventral	26.9	6.86	238	3.918	0.0003
M. ingens	Central-Dorsal	-14.33	2.56	798	-5.606	<.0001
	Central-Ventral	1.39	2.56	798	0.542	0.8506

	Dorsal-Ventral	15.71	2.56	798	6.148	<.0001
O. robsoni	Central-Dorsal	53	5.39	318	9.838	<.0001
	Central-Ventral	70	5.39	318	12.987	<.0001
	Dorsal-Ventral	17	5.39	318	3.149	0.0051

Table VII. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for OSP eye segments (posterior, middle, and anterior OSP lengths).*

Species	Retinal Locations Compared	Estimate	SE	df	t-ratio	p-value
N. sloanii	Anterior-Middle	1.70	2.13	958	0.798	0.7402
	Anterior-Posterior	-5.88	2.13	958	-2.753	0.0166
	Middle-Posterior	-7.58	2.13	958	-3.551	0.0012
N. gouldi	Anterior-Middle	5.94	2.32	478	2.562	0.0288
	Anterior-Posterior	4.90	2.32	478	2.113	0.0883
	Middle-Posterior	-1.04	2.32	478	-0.450	0.8946
T. filippovae	Anterior-Middle	40.4	7.06	238	5.719	<.0001
	Anterior-Posterior	16.0	7.06	238	2.267	0.0625
	Middle-Posterior	-24.4	7.06	238	-3.452	0.0019
M. ingens	Anterior-Middle	19.06	2.52	798	7.558	<.0001
	Anterior-Posterior	1.98	2.52	798	0.784	0.7128
	Middle-Posterior	-17.08	2.52	798	-6.774	<.0001
O. robsoni	Anterior-Middle	21.81	6.6	318	3.306	0.0030
	Anterior-Posterior	-2.75	6.6	318	-0.417	0.9087

	Middle-Posterior	-24.56	6.6	318	-3.723	0.0007

Table VIII. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for OSP lengths amongst species.*

Species Compared	Estimate	SE	df	t-ratio	p-value
M. ingens – O. robsoni	-38.43	19.7	30	-1.953	0.4635
M. ingens – N. gouldi	36.65	17.2	30	2.134	0.3599
M. ingens – N. sloanii	24.42	14.2	30	1.715	0.6119
M. ingens – T. filippovae	-55.74	21.9	30	-2.546	0.1793
O. robsoni – N. gouldi	75.08	21.5	30	3.497	0.0224
O. robsoni – N. sloanii	62.85	19.2	30	3.273	0.0382
O. robsoni – T. filippovae	-17.31	25.4	30	-0.681	0.9927
N. gouldi – N. sloanii	-12.23	16.6	30	-0.735	0.9891
N. gouldi – T. filippovae	-92.39	23.5	30	-3.928	0.0076
N. sloanii – T. filippovae	-80.16	21.5	30	-3.734	0.0124

Table IX. *A list of test statistics and probability metrics from one-way ANOVA analysis and Tukey's HSD post-hoc pairwise comparisons for OSP lengths from species grouped by depth range category.*

Statistical Test	**df**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**
One-way ANOVA	3	28864	9621	8.113	0.0003
Statistical Test	**Compared Depth Range Categories**	**Difference**	**Lower Bound**	**Upper Bound**	**Adjusted p-value**
Post-hoc comparison	(0-1000) – (0-1000+)	-84.237	-142.152	-26.320	0.0021
Post-hoc comparison	(0-1000) – (200-1000)	46.002	-49.415	141.419	0.5689
Post-hoc comparison	(0-1000) – (200-1000+)	45.079	13.669	76.488	0.0024
Post-hoc comparison	(0-1000+) – (200-1000)	-38.235	-145.474	69.005	0.7719
Post-hoc comparison	(0-1000+) – (200-1000+)	-39.158	-97.317	19.000	0.2832
Post-hoc comparison	200-1000) – (200-1000+)	-0.924	-96.488	94.641	0.9999

Table X. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for ISP retinal locations (dorsal, central and ventral ISP lengths).*

Species	Retinal Locations Compared	Estimate	SE	df	t-ratio	p-value
N. sloanii	Central-Dorsal	4.743	1.86	507	2.552	0.0296
	Central-Ventral	-0.042	1.89	508	-0.022	0.9997
	Dorsal-Ventral	-4.785	1.68	509	-2.843	0.0129
N. gouldi	Central-Dorsal	-1.78	1.81	329	-0.986	0.5861
	Central-Ventral	0.24	1.88	330	0.128	0.9910
	Dorsal-Ventral	2.02	1.69	329	1.195	0.4568
T. filippovae	Central-Dorsal	-6.689	4.19	157	-1.597	0.2489
	Central-Ventral	-6.540	4.56	157	-1.434	0.3260
	Dorsal-Ventral	0.148	4.50	157	0.033	0.9994
M. ingens	Central-Dorsal	0.527	2.10	329	0.251	0.9660
	Central-Ventral	-5.169	2.16	330	-2.397	0.0449
	Dorsal-Ventral	-5.695	2.07	330	-2.757	0.0169
O. robsoni	Central-Dorsal	0.367	1.95	1.95	0.188	0.9807
	Central-Ventral	4.693	1.83	1.83	2.564	0.0297
	Dorsal-Ventral	4.327	1.98	1.98	2.186	0.0761

Table XI. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for ISP eye segments (posterior, middle and anterior ISP lengths).*

Species	Retinal Locations Compared	Estimate	SE	df	t-ratio	p-value
N. sloanii	Anterior-Middle	6.93	1.68	505	4.126	0.0001
	Anterior-Posterior	-2.25	1.73	506	-1.297	0.3997
	Middle-Posterior	-9.18	1.73	506	-5.308	<.0001
N. gouldi	Anterior-Middle	4.21	1.67	328	2.516	0.0329
	Anterior-Posterior	2.15	1.84	328	1.171	0.4715
	Middle-Posterior	-2.06	1.80	328	-1.146	0.4867
T. filippovae	Anterior-Middle	24.23	3.93	157	6.164	<.0001
	Anterior-Posterior	-1.44	3.81	157	-0.377	0.9249
	Middle-Posterior	-25.66	3.61	157	-7.105	<.0001
M. ingens	Anterior-Middle	3.93	2.06	329	1.904	0.1390
	Anterior-Posterior	-2.57	2.17	330	-1.184	0.4635
	Middle-Posterior	-6.50	2.13	330	-3.058	0.0068
O. robsoni	Anterior-Middle	6.232	1.91	198	3.263	0.0037
	Anterior-Posterior	-0.215	1.93	198	-0.111	0.9932
	Middle-Posterior	-6.447	1.79	198	-3.600	0.0012

Table XII. *A list of test statistics and probability metrics from one-way ANOVA analysis and Tukey's HSD post-hoc pairwise comparisons for ISP lengths from species grouped by depth range category.*

Statistical Test	df	Sum of Squares	Mean Square	F-value	p-value
One-way ANOVA	3	11949	3983	19.68	<.0001
Statistical Test	Compared Depth Range Categories	Difference	Lower Bound	Upper Bound	Adjusted p-value
Post-hoc comparison	(0-1000) – (0-1000+)	-24.727	-48.685	-0.770	0.0410
Post-hoc comparison	(0-1000) – (200-1000)	-41.311	-80.781	-1.841	0.0374
Post-hoc comparison	(0-1000) – (200-1000+)	-28.721	-41.921	-15.521	<.0001
Post-hoc comparison	(0-1000+) – (200-1000)	-66.039	-110.399	-21.678	0.0017
Post-hoc comparison	(0-1000+) – (200-1000+)	-53.449	-77.619	-29.278	<.0001
Post-hoc comparison	200-1000) – (200-1000+)	12.590	-27.010	52.190	0.8259

Table XIII. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for OL depth amongst species.*

Species Compared	Estimate	SE	df	t-ratio	p-value
M. ingens – O. robsoni	15.069	7.16	7.3	2.104	0.4853
M. ingens – N. sloanii	8.959	4.46	3.27	2.008	0.5732
M. ingens – T. filippovae	6.411	4.98	3.81	1.288	0.8664
O. robsoni – N. sloanii	-6.11	7.05	8.24	-0.867	0.9819
O. robsoni – T. filippovae	-8.658	7.39	8.19	-1.172	0.9203
N. sloanii – T. filippovae	-2.548	4.82	4.4	-0.529	0.9985

Table XIV. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons for total photoreceptor lengths amongst species.*

Species Compared	Estimate	SE	df	t-ratio	p-value
M. ingens – O. robsoni	-26.2	22.5	39.6	-1.164	0.7716
M. ingens – N. gouldi	-23.8	19.8	39.7	-1.201	0.7508
M. ingens – N. sloanii	-8	16.6	40.4	-0.481	0.9887
M. ingens – T. filippovae	98.3	25	39.6	3.931	0.0029
O. robsoni – N. gouldi	-50	24.1	39	-2.076	0.251
O. robsoni – N. sloanii	-34.2	21.6	39.2	-1.587	0.5146
O. robsoni – T. filippovae	72	28.5	39.1	2.526	0.1055
N. gouldi – N. sloanii	15.8	18.7	39.1	0.843	0.9152
N. gouldi – T. filippovae	-122	26.4	39	-4.623	<.0001
N. sloanii – T. filippovae	-106.3	24.1	39.2	-4.404	<.0001

Appendix B. *PDF of the original publication of Chapter 3 – Biological Sunglasses in a Deep-Sea Squid: Pigment Migration in the Retina of* Gonatus onyx*.*

vision

MDPI

Communication

Biological Sunglasses in a Deep-Sea Squid: Pigment Migration in the Retina of *Gonatus onyx*

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Abstract: The outward migration of ommin pigment granules from the bases to the tips of the photoreceptors in response to light has been reported in the retina of several (mostly coastal) squid species. Following exposure to light and then dark conditions, we collected and processed retinal tissue from juvenile specimens of a deep-sea oegopsid squid, *Gonatus onyx*. We aimed to determine whether the ommin pigment returns to baseline, and to investigate the presence of glutamate neurotransmitter signaling under both dark and light conditions. We confirmed the presence of ommin granules but observed variability in the return of pigment to the basal layer in dark conditions, as well as changes in glutamate distribution. These findings provide support for the migration of retinal ommin pigment granules as a mechanism for regulating incoming light.

Keywords: cephalopod; squid; pigment migration; ommin; neurotransmitters; retina; vision

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1. Introduction

Eye evolution in animals that are primarily active under dark conditions has followed several strategies. The ability to moderate the intensity of light entering the eye may be particularly important for species exposed to a wide range of light conditions, such as shallow-dwelling organisms exposed to regular day/night cycles, or species that inhabit diverse oceanic layers across their lifespan. One physiological response to changing light conditions, reported in several cephalopods to date, is the migration of ommin pigment granules within the retina, providing a visual screening effect akin to 'biological sunglasses'.

In cephalopods, evidence of ocular screening pigments has existed since the late 19th century [1]. In 1959, Butenandt [2] identified the ommochrome pigmentation as ommin in the retinas of two squids (*Todarodes sagittatus* and *Alloteuthis subulata*), the European common cuttlefish (*Sepia officinalis*) and two octopuses (*Octopus vulgaris* and *Eledone moschata*). Daw and Pearlman [3] showed that in the coastal market squid *Doryteuthis pealeii*, ommin granules could migrate to the distal end of the of the outer retinal layer, providing a filter equivalent to 0.6 log units. This outward migration had a spectral sensitivity equivalent to rhodopsin and occurred in 5–15 min, once 3–10% of the rhodopsin had isomerized. After the light stimulus was removed, the pigment granules returned to their original basal position over a further 5–15 min. However, if more than 10% of the rhodopsin was isomerized, the screening pigment instead took several hours to fully migrate inward under restored dark conditions. Notably, pigment migration occurred only when light fell directly on the rhabdomeres, and not during the illumination of the granules themselves [4]. These observations supported the prevailing hypothesis that outward ommin migration occurs during bright ambient light conditions to block intense light and prevent photoreceptor oversaturation, with a quick return to the ommin layer upon the return to darkness.

In cephalopods, screening pigments have mostly been found in better-studied nearshore and shallow-water species, which experience changing light conditions due to daylight cycles [3,5–7]. However, screening pigment granules were also recently recognized in the oegopsid (deep-sea) 'glass' squid *Teuthowenia pellucida*, and their position within the retina suggested different stages of light/dark adaptation at the time of specimen preservation [8]. In deep-sea squids, migratory pigment granules could provide an advantage during diel vertical migrations, or during descent into deeper layers as they mature. The mechanisms governing pigment migration during dark adaptation are different from the mechanisms controlling migration during light adaptation [9] and may even differ among species of the same group.

In the present paper, we investigated the timing of ommin pigment migration under dark conditions, and whether glutamate neurotransmitter levels change during dark adaptation, which is believed to be under the control of dopaminergic efferent innervation regulating the retraction of ommin. Enzymes that synthesize glutamate, an excitatory neurotransmitter (the same neurotransmitter used by vertebrate photoreceptors), have been found within the photoreceptors of the hummingbird 'bobtail squid', *Euprymna berryi* [10]. Additionally, glutamate levels in the retina of *Sepia officinalis* can be influenced by light/dark environments [11].

This study investigates retinal screening pigment migration in a pelagic oegopsid squid species primarily found throughout the North Pacific. *Gonatus onyx*, known as the 'black-eyed' squid, is the most encountered gonatid near California, and is frequently observed at depths of 375–925 m (maximum 1975 m) by remotely operated vehicles operating in the Monterey Canyon [12]. Like many deep-sea oegopsids, *G. onyx* inhabits deeper oceanic strata as it matures [13]; females have been observed brooding egg masses at depths of 1250–2500 m [13,14]. This species therefore experiences a range of light conditions across its lifespan (and potentially across shorter timeframes), prompting us to investigate the possibility of screening pigmentation migration in a cohort of juveniles under a controlled series of light/dark exposures.

2. Materials and Methods

2.1. Specimen Acquisition

Live juvenile gonatid squid (*Gonatus onyx*, ML 10–20 mm; Figure 1) were trawled after sundown at a depth of 350 m by the Monterey Bay Aquarium Research Institute (MBARI) aboard the RV *Western Flyer* over the Monterey Canyon (36°41.4–42.8′ N, 122°10.3–13.3′ W for individuals G01, G12–G13; 36°32.3–35.4′ N, 122°31.8–35.3′ W for G02–G11) in March 2013. Thirteen individuals were used in this study.

Figure 1. Juvenile 'black-eyed' squid, *Gonatus onyx*, feeding on a myctophid. © Monterey Bay Aquarium Research Institute. Scale bar = 1 cm.

2.2. Experimental Design

After net retrieval, the 13 juveniles were sorted while exposed to ambient light under bright fluorescent bulbs (F24T12 35W linear fluorescent tube, 4200 k, 380–760 nm, OSRAM Sylvania, Wilmington, NC, USA) with an illuminance of 350 lux measured with an LX-103

Digital Light Meter (Lutron Electronic Enterprise, Taipei City, Tawain). During experiments, each specimen was sorted from the trawl catch and placed into a small container of seawater, in which it was exposed to light for a total (including sorting time) of 20, 30 or 90 min, and subsequently moved into a lightless room for variable periods of time. Of the 13 juvenile *G. onyx* processed under light, 11 specimens were selected to assess the migration and return of the pigment granules within the retina after subsequent dark adaptation. The remaining two specimens were used to identify the presence of the neurotransmitter glutamate in the retina (Table 1).

Table 1. List of *G. onyx* identification numbers (ID) with corresponding mantle lengths (ML), light exposure and dark adaptation times for each specimen and their use in the analysis of pigment migration (PM) or neurotransmitter expression (NE). G02–G06 were grouped together for PM analysis (Group 1) and G07–G11 were in Group 2. G01 served as the negative control (NC) for PM analysis.

ID	ML (mm)	Analysis Conducted	Light Exposure (min)	Dark Adaptation (min)
G01	30	PM, NC	90	1440 (24 h)
G02	15	PM, Group 1	20	15
G03 *	15	PM, Group 1	20	30
G04	15	PM, Group 1	20	45
G05	15	PM, Group 1	20	60
G06	15	PM, Group 1	20	75
G07	10	PM, Group 2	30	20
G08	10	PM, Group 2	30	30
G09 *	20	PM, Group 2	30	40
G10	10	PM, Group 2	30	50
G11	10	PM, Group 2	30	60
G12	15	NE	30	90
G13	15	NE	30	0

* G03 and G09 were excluded from analysis.

2.3. Pigment Migration

Five individuals were exposed to 350 lux of fluorescent lighting for 20 min (G02–G06, Group 1), while five individuals were exposed to the same light for 30 min (G07–G11, Group 2). The light exposure paradigm was determined by the time required to sort the catch. The 10 min difference in processing time provided an opportunity to compare pigment migration differences; an extra 10 min light exposure could have a significant impact according to a previously reported dark/light adaptation study [3]. After light exposure, specimens were then placed into individual containers within a darkened cold room (<60 lux), with one individual euthanized at 15 min and every 15 min interval thereafter until 75 min (Group 1) or after 20 min and every 10 min thereafter until 60 min (Group 2). Two specimens appeared moribund at the time of euthanasia and were subsequently removed from the results (Group 1, 30 min dark adaptation, G03; Group 2, 40 min dark adaptation, G09).

Specimens were immediately fixed whole after euthanasia in 5% buffered formalin to preserve the position of ommin pigment granules. Later, a single eye from each fixed specimen was removed and fixed again in 4% formaldehyde in phosphate-buffered solution (PBS) for 30 min, rinsed in PBS and stored until sectioning. The contralateral eye was fixed in glutaraldehyde. Unfortunately, glutaraldehyde fixation did not preserve the ommin pigment well, and these samples were not processed further.

One specimen (G01, ML 30 mm) served as the negative control for pigment migration analysis; this individual was exposed to 90 min of light at 350 lux, then subjected to darkness for 24 h, then euthanized. However, the PFA-fixed eye was damaged beyond use and only the contralateral eye preserved in 2.5% glutaraldehyde was used, which resulted in the poor preservation of the ommin pigment granules, although some pigment granules survived in the negative control.

Prior to sectioning, all formaldehyde-fixed tissues for pigment migration analysis were immersed in 30% sucrose solution for at least 24 h before embedding them in optimum cutting temperature medium and sectioning at 12 µm thickness using a cryostat (Leica CM3050 S, Wetzlar, Germany). Dorsal, central and ventral sections were obtained from the specimen adapted to the dark for the longest duration (G06, 20 min light/75 min dark) and from the specimen with the most exposure to light and shortest duration of dark adaptation (G07, 30 min light/20 min dark). For comparison among individuals in a group, vertical sections were obtained from the retina's central region. All samples were stained with toluidine blue.

2.4. Neurotransmitter Expression

To determine whether changes in neurotransmitter presence are discernable between different dark adaptation paradigms (i.e., less than a day of dark adaptation), two juvenile squids (*G. onyx*, G12 and G13, ML 15 mm) were exposed to 30 min of light at 350 lux. G13 was immediately euthanized after light exposure with no subsequent dark adaptation. After light exposure, G12 was placed in a darkened container for 90 min to allow for partial or full dark adaptation of the retina. The eyes were then preserved in conditions identical to those for specimens analyzed for pigment migration. Samples were placed in 2.5% glutaraldehyde with 1% formaldehyde in PBS for 1 h and dehydrated in an ethanol series of increasing concentration. Fixation in glutaraldehyde rendered most of the ommin pigment granules unperceivable. These glutaraldehyde-fixed samples were then processed by epoxy resin (EPON) embedding following a standard protocol [15,16] and placed in a 3:1 resin–acetone mixture overnight. Next, the tissues were submerged in 100% EPON and placed in an oven at 60 °C for at least 24 h to polymerize the resin. The resin-embedded samples were cut into 250–500 nm thick vertical sections using a glass knife (made with a Leica EM KMR2, Wetzlar, Germany) and an ultramicrotome (Leica Ultracut UCT, Wetzlar, Germany). Sections were placed on Teflon-coated slides and processed for immunogold labeling of glutamate.

The retinas were prepared for the identification of neurotransmitters following the silver-intensified immunogold labeling procedure described by Marc et al. [15,17]. Single consecutive 250 nm sections were collected in separate wells and labeled with antibodies. The primary antibody, rabbit anti-glutamate (1:1000, ab9440, Abcam, Cambridge, UK), was used. Controls for each treatment were produced by taking retinal sections and processing them under the same conditions as the experimental samples, excluding the primary antibodies. This allowed for the assessment of any non-specific staining or background signals present without specific antibody binding. A 1.4 nm Nanogold® conjugate goat anti-rabbit secondary antibody (1:100, #2003; Nanoprobes, New York, NY, USA) was used to detect the primary antibody in a silver-intensified reaction procedure [17,18].

2.5. Imaging

All images were captured with a Leica CTR MIC microscope (Wetzlar, Germany) and a Leica DC 500 camera (Wetzlar, Germany) using either 10×, 20× or 40× objectives. Images were acquired using the same parameters of exposure, contrast and brightness.

2.6. Barcoding

Small tissue snips were taken from the mantle of each specimen and stored separately in 95% ethanol. Due to the presence of multiple *Gonatus* species in this region, DNA barcoding (cytochrome c oxidase subunit 1 [CO1]) was used to confirm this material as *G. onyx* (>99% match to other *G. onyx* material from the same region, using the Barcode of Life Database [BOLD]; [19]). DNA was extracted and prepared at the AUT Lab for Cephalopod Ecology & Systematics (ALCES) following the protocols outlined by Braid and Bolstad [20], then sequenced at Macrogen, Korea.

3. Results

3.1. Pigment Migration

The transverse cross-sections of the juvenile *G. onyx* eyes revealed a typical cephalopod eye structure (Figure 2), consisting of an everted retina with the distal ends of the outer segment of the photoreceptors directed anteriorly (Figure 2a). A basal membrane and supporting cells separated the outer and inner segments of the photoreceptor cells, while a limiting membrane separated the outer segment of the photoreceptors from the vitreous body (Figure 2b). In the dark-adapted eye, the ommin layer would be expected to be concentrated at the base of the outer segment of the photoreceptors. In the ventral and dorsal anterior sections of the eye, the retina was supported by a cartilaginous sclera (Figure 2d), which continued anteriorly to the body of the iris (Figure 2c).

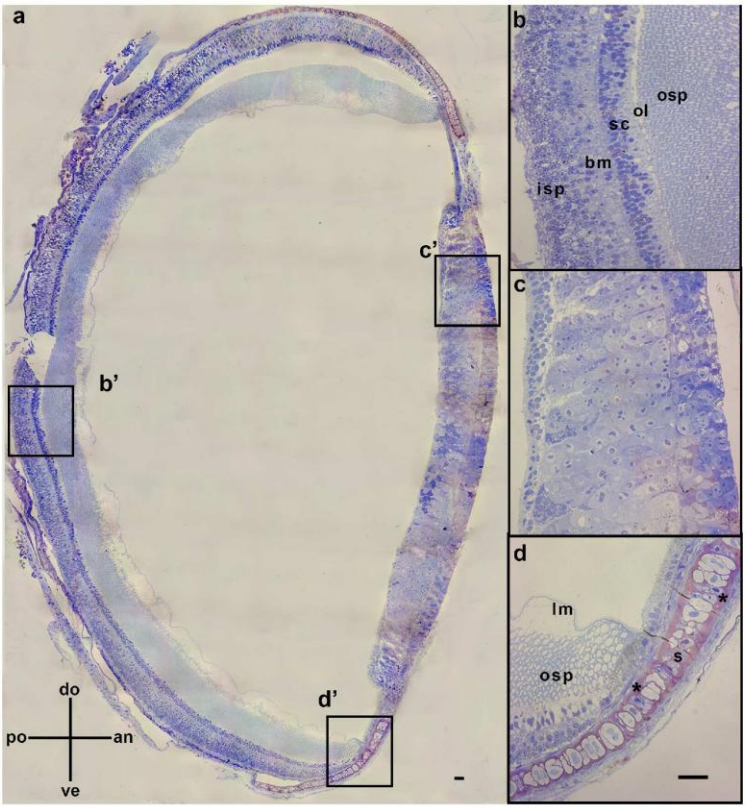

Figure 2. (**a**) Transverse cross-section of juvenile *G. onyx* (15 mm ML) eye. (**b**) Enlarged section of b′ depicting retinal layers. (**c**) Enlarged section of c′ depicting the iris. (**d**) Enlarged section of d′ depicting the transition from the retina to the ciliary body. The asterisks indicate the cartilaginous parts of the sclera (s). Ommin granules are not visible due to glutaraldehyde preservation (see Section 2. Abbreviations: dorsal (do), ventral (ve), posterior (po), anterior (an), inner segment of photoreceptors (isp), basal membrane (bm), supporting cells (sc), expected location of ommin layer (ol), outer segment of photoreceptors (osp), limiting membrane (lm), sclera (s). Scale bars = 20 µm.

The photoreceptors were arranged in groups of four, forming rhabdomeres, appearing as a lattice arrangement (Figure 3). In the negative control specimen (G01), exposed to 90 min light followed by 24 h in darkness, the ommin pigment granules were dispersed throughout the outer photoreceptor layer, with most of the pigment granules found near the base of the ommin layer and in the layer of supporting cells (Figure 4).

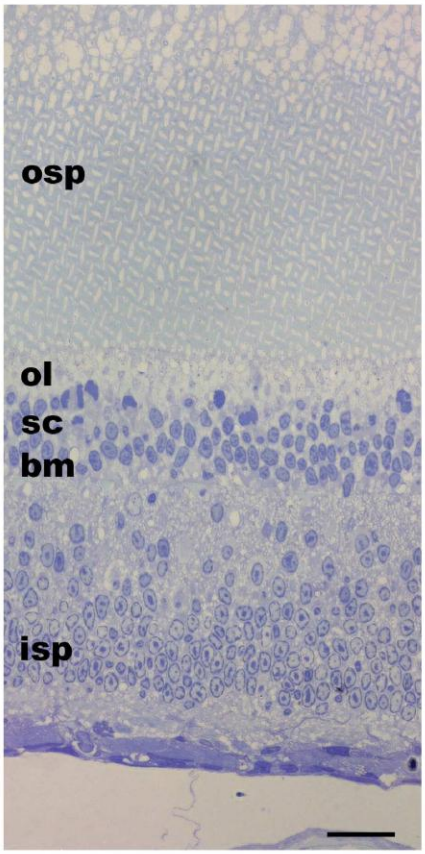

Figure 3. Toluidine blue staining of a horizontally sectioned juvenile *Gonatus onyx* retina at the photoreceptor level. The lattice-like arrangement of rhabdomeres in the outer segment is typical of cephalopods. Ommin granules are not visible due to glutaraldehyde preservation (see Section 2), but the ommin layer (ol) should be near the base of the outer segment of the photoreceptors (osp). Abbreviations: inner segment of the photoreceptors (isp), basal membrane (bm), supporting cells (sc), expected location of ommin layer (ol), outer segment of the photoreceptors (osp). Scale bar = 20 µm.

From specimens G06 (20 min light/75 min dark) and G07 (30 min light/20 min dark), the dorsal, central and ventral horizontal sections were obtained to determine whether the return of pigment to the ommin baseline was uniform across the retinal regions.

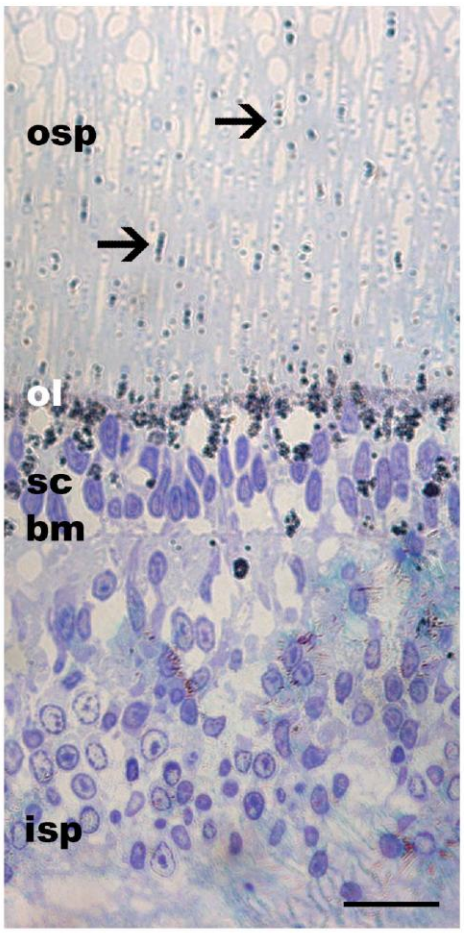

Figure 4. Ommin pigment location in the retina of a juvenile *G. onyx* exposed to 90 min of light at 350 lux then subjected to darkness for 24 h. The screening pigment of this specimen was preserved, revealing the single-cell resolution and location of ommin pigment granules. No pigment was observed at the distal tip of the photoreceptors. Arrows indicate location of migrating ommin pigment. Abbreviations: inner segment of the photoreceptors (isp), basal membrane (bm), supporting cells (sc), ommin layer (ol), outer segment of the photoreceptors (osp). Scale bar = 20 µm.

The specimen exposed to the longest light and shortest dark treatments (G07), showed scattered ommin granules distributed along the photoreceptors after 20 min of dark adaptation (Figure 5). However, all regions of the retina showed similar stages of pigment return, as nearly all the ommin granules had returned to the baseline.

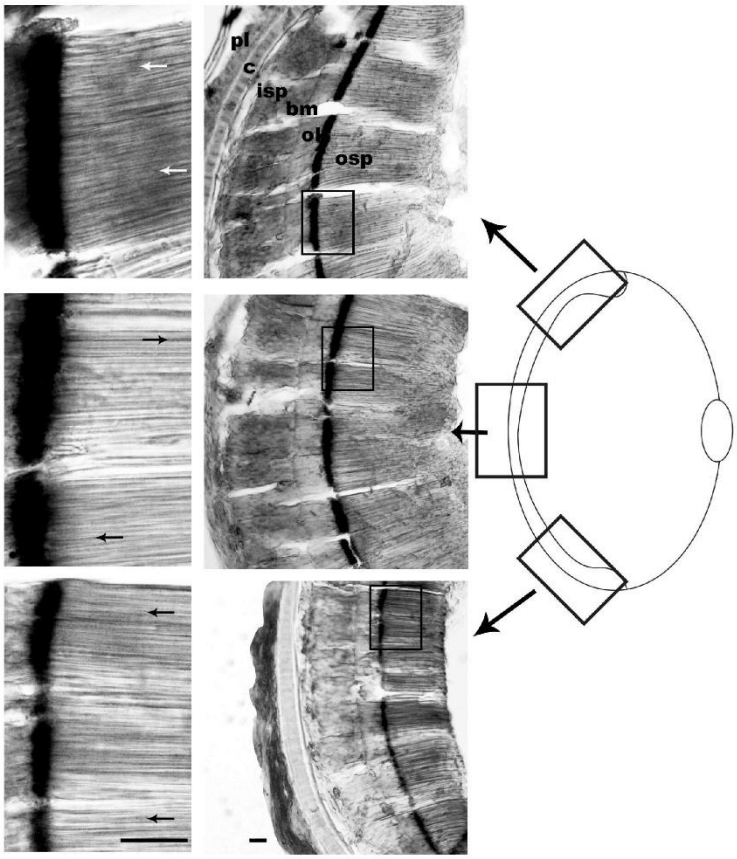

Figure 5. Ommin pigment location in the specimen exposed to 30 min of light at 350 lux then subjected to darkness for 20 min (G07). The screening pigment of this specimen was preserved, revealing the location of ommin pigment granules close to the basal ommin layer and absent from the tip of the photoreceptors for all retinal sections. The top row of images depicts the dorsal section of the retina, the middle row depicts the central section and the bottom row depicts the ventral section. Arrows indicate location of migrating ommin pigment. Abbreviations: outer segment of the photoreceptors (osp), ommin layer (ol), basal membrane (bm), inner segment of the photoreceptors (isp), rudimentary cartilage (c), plexiform layer (pl). Scale bars = 20 μm.

In the two specimens with the longest dark adaptation periods (G06, 75 min, Figure 6; and the negative control, G01, 24 h, Figure 4), most of the ommin granules appeared to return to the basal layer, and only a few remained distributed along the photoreceptor length based on the percent length of migration (more in G06 than in G01). The visual observations for each segment of G06 showed that this trend was consistent throughout all the retinal regions.

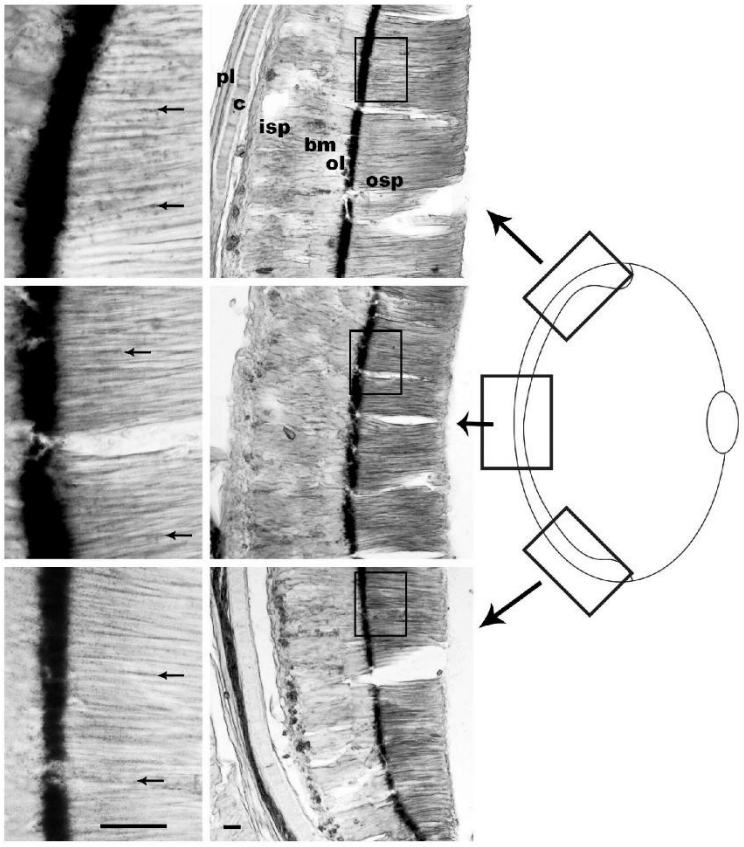

Figure 6. Ommin pigment location in the *G. onyx* specimen exposed to 20 min of light at 350 lux then subjected to darkness for 75 min. The screening pigment of this specimen was preserved, revealing the location of ommin pigment granules close to the ommin layer and absent from the tip of the photoreceptors. The top row of images depicts the dorsal section of the retina, the middle row depicts the central section and the bottom row depicts the ventral section. Arrows indicate location of migrating ommin pigment. Abbreviations: outer segment of the photoreceptors (osp), ommin layer (ol), basal membrane (bm), inner segment of the photoreceptors (isp), rudimentary cartilage (c), plexiform layer (pl). Scale bars = 20 μm.

In the two individuals where the dorsal, central and ventral retinal sections were analyzed (G06, G07), the proportions of ommin granules in the basal layer and along the photoreceptor length appeared to be consistent within each eye, regardless of their location.

In the central retinal section of all the experimental samples (G02–G11), a substantial proportion of the ommin pigment granules was concentrated within the basal layer of the photoreceptors (Group 1, Figure 7; Group 2, Figure 8). In all the samples, some granules were also distributed along the photoreceptors' length, with some variation observed among individuals.

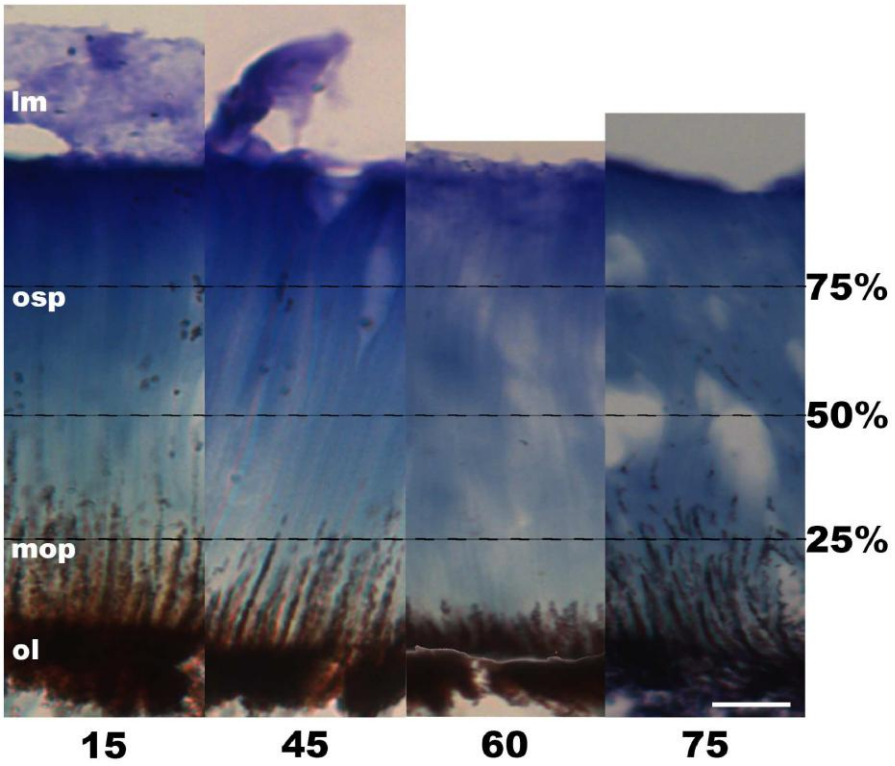

Figure 7. Ommin pigment granule migration in the outer segment of the photoreceptors of Group 1 juvenile (ML 10–20 mm) *G. onyx* retinas following exposure to light (350 lux) for 20 min, followed by dark (minutes indicated below each cross-section). Percentage values to the right of the image and dotted lines refer to the outer segment of the photoreceptors. Abbreviations: ommin layer (ol), migrating ommin pigment (mop), ommin-free outer segment of the photoreceptors (osp), limiting membrane (lm). All images depicted at 40× magnification. Scale bar = 20 μm.

In Group 1 (individuals exposed to 20 min of light and then periods of darkness increasing by 15 min; Figure 7), similar results were observed among the individuals returned to darkness for 15, 45 and 75 min, with most of the pigment evident in the basal 25% of the photoreceptor length. In the individual that was subjected to darkness for 60 min after light exposure (G05), the returning granules were more concentrated and positioned closer to the ommin layer, within the basal 10% of the photoreceptor length.

In Group 2 (individuals exposed to 30 min of light and then periods of darkness increasing by 10 min; Figure 8), most of the specimens showed widely distributed ommin pigment granules in the photoreceptors. The individuals that were returned to darkness for 20 and 50 min post light exposure (G07 and G10, respectively) exhibited granules across the basal 40–50% of the photoreceptor length; in the 60 min dark-exposed individual (G11), the migrated granules spanned the majority (basal ~80%) of the photoreceptor length. In the individual exposed to 30 min of darkness (G08), the granules were more concentrated near the ommin layer, within the basal 10% of the photoreceptor length.

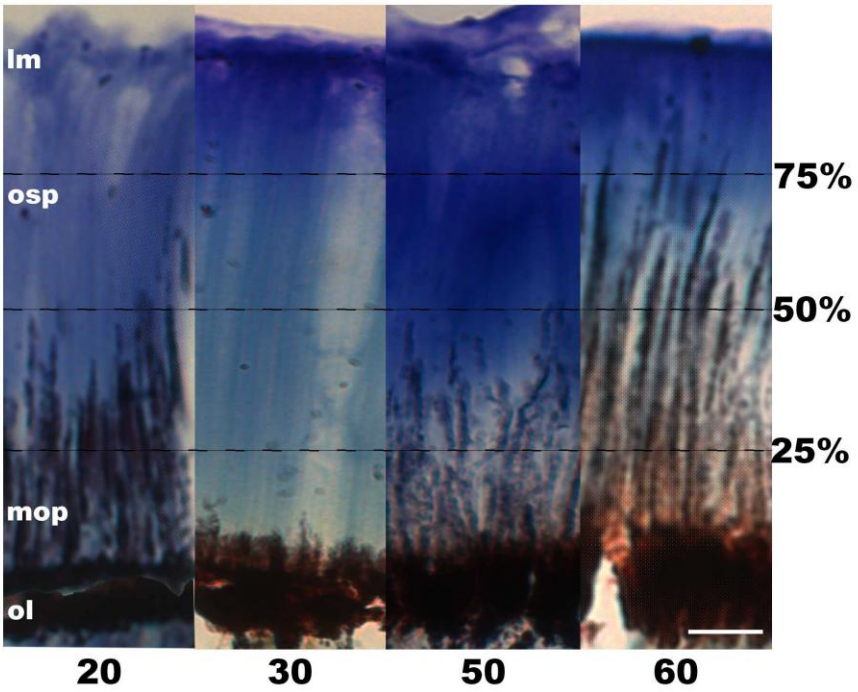

Figure 8. Ommin pigment granule migration in the outer segment of the photoreceptors of Group 2 juvenile (ML 10–20 mm) *G. onyx* retinas following exposure to light (350 lux) for 30 min, followed by dark (minutes indicated below each cross-section). Percentage values on the side of the image and dotted line refer to photoreceptor length. Abbreviations: ommin layer (ol), migrating ommin pigment (mop), ommin-free outer segment of the photoreceptors (osp), limiting membrane (lm). All images depicted at 40× magnification. Scale bar = 20 µm.

3.2. Neurotransmitter Expression

The G12 and G13 retinas were labeled with an anti-glutamate antibody, and both specimens exhibited glutamate labeling within both the inner and outer segments of the photoreceptors (Figure 9). This technique involves the deposition of silver in cells in proportion to the quantity of antibody present, leading to the formation of various shades of grey in different areas. To ensure the specificity of the labeling, each tissue sample was processed with and without the anti-glutamate antibody. The 'silver only' negative controls in specimens G12 and G13 showed uniformly low and weak silver staining (top and bottom row, Figure 9a). However, the samples that were treated with the anti-glutamate antibody showed strong staining through the tissue and clearly identified cells were positive for glutamate labeling, with the highest concentration of glutamate appearing within the inner photoreceptor layer (top and bottom row, Figure 9b). The qualitative assessment of the two conditions appears to show that in the specimen allowed to adapt to darkness for 90 min (G12, top row, Figure 9b), glutamate labeling occurred in discrete cells; in comparison, the specimen with no subsequent dark adaptation period after light exposure (G13, bottom row, Figure 9b) exhibited similar labeling in all its inner photoreceptor cells.

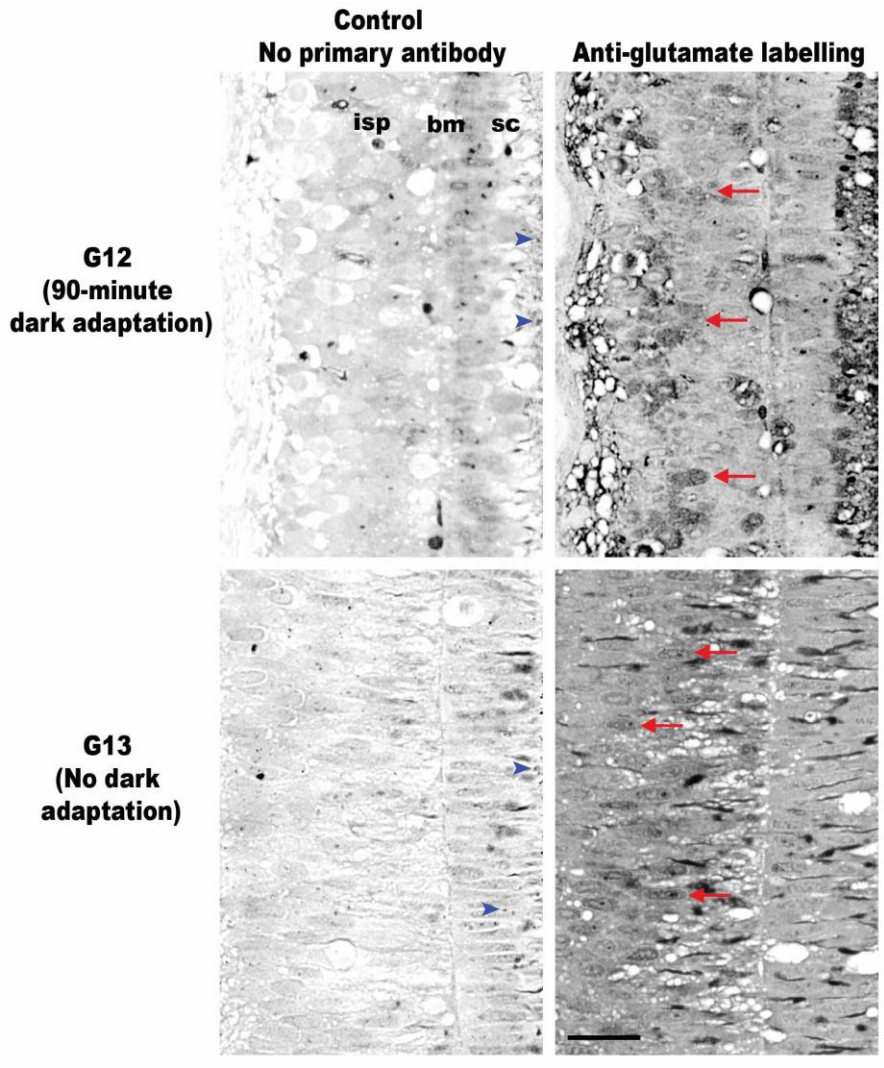

Figure 9. Transverse retinal cross-sections of the inner photoreceptor layer of juvenile *G. onyx* (G12–G13, both 15 mm ML). (**a**) Retinal sections without primary anti-glutamate serve as negative controls. (**b**) Retinal sections labeled with antibodies indicate the expression of glutamate neurotransmitters (dark grey areas). Glutamate expression in retinas subjected to darkness for 90 min (G12, top row) or without dark adaptation (G13, bottom row) following light exposure (350 lux for 30 min). Arrows indicate examples of cells reactive to the antibody. Arrowheads indicate ommin pigment granules within the supporting cells not destroyed by the preservation process. Abbreviations: supporting cells (sc), basal membrane (bm), inner layer of photoreceptors (isp). Scale bar = 20 µm.

4. Discussion

Recent studies have revealed an intriguing phenomenon in the migration dynamics of ommin pigment within cephalopod photoreceptors. While the migration of pigment to the distal tips is well documented, the return of pigment to the baseline is an area of growing interest. This return to the baseline may indicate a regulatory mechanism that maintains pigment distribution within the photoreceptor outer segments, ensuring optimal visual performance under varying light conditions. This study aimed to investigate the mechanisms underlying this pigment redistribution to improve our understanding of the adaptive strategies of cephalopod visual systems.

The results support the presence of migratory ommin pigment within the retinas of juvenile *Gonatus onyx*, suggesting that these animals can screen light in illuminated conditions. Unlike in coastal specimens, where dark adaptation results in the return of all pigment granules to the ommin layer, some pigment granules were clearly visible within the photoreceptors even after prolonged periods of darkness, providing the first experimental result of this mechanism and its potential time delay within a deep-sea oegopsid squid.

The use of ommochromes as a screening pigment has been reported in the retinas of major invertebrate groups including insects (*Drosophila melanogaster* [21]; *Deilephila elpenor* [22]; *Manduca sexta* [23]), crustaceans (*Procambarus clarkia* [24]; *Orconectes limosus* [25]), and chelicerates (*Limulus polyphemus* [9]), all of which are shallow-water or terrestrial species with pigments that migrate in coordination with the day/night cycle. Extensive studies, mostly on *D. melanogaster*, have shown that ommochromes are photosensitive colored compounds derived from tryptophan metabolism [26]. They are synthesized and stored in lysosome-related organelles called ommochromasomes, and are categorized into three groups, ommatin, ommin, and ommidin; for the latter two, little is understood of their chemistry and structure [27].

In juvenile *Gonatus* specimens exposed to light for 20 or 30 min, most pigment (but not all) returned to the basal layer under dark conditions, suggesting that an extended light response occurs, and may take more than 24 h to reverse. If the mechanisms controlling the return of the pigment granules to the photoreceptor bases are the same in *G. onyx* as in *Doryteuthis pealeii*, as described by Daw and Pearlman [3], then it is likely that the light adaptation conditions of 350 lux were strong enough to isomerize more than 10% of rhodopsin, which would elongate the time required for the pigment to fully return to its basal layer. Since *G. onyx* juveniles are typically distributed around 300–400 m deep [13], illumination at 350 lux is likely far brighter than any natural condition they would encounter. Furthermore, the spectral distribution of light emitted from fluorescent bulbs, such as those used in this study, typically has a spectral peak near 480–500 nm, aligning well with the spectral absorbance of most cephalopod photopigments, which may be linked to inducing pigment migration [28]. Therefore, exposure to fluorescent lighting with an illuminance of 350 lux and a spectral emission inclusive of the 480–500 nm range for around 20–30 min does appear sufficient to trigger light adaptation in *G. onyx*, but the restoration of full dark adaptation may take far longer than the duration of our trials. Additionally, dark adaptation may take significantly longer in deep-sea cephalopods exposed to abrupt bright light. It is also possible that the duration and intensity of light conditions in this study irreversibly damaged the retina, preventing a return to full dark adaptation. Alternatively, some ommin granules may consistently be found within the outer photoreceptor layer under all conditions. In some species of blowflies (*Lucilia* spp.), ommochromes help maintain the photoreceptor by transporting degraded rhabdomere membranes [27].

This study also provided novel evidence that glutamate is present within the retina of *G. onyx*. Glutamate does appear to decrease after a longer duration in the dark, which agrees with the findings of D'Aniello et al. [11]. They identified significant levels of neurotransmitters in the retina of cephalopods; glutamate was shown to decrease under dark conditions. However, D'Aniello et al. [11] examined adults of shallow-water species, with a relatively different eye structure, that were subjected to darkness for five days, making

their results and the results of this study difficult to compare directly. Whether neurotransmitters used in cephalopod vision differ among lineages, among species occupying different environments with distinct light regimes, or between adults and juveniles remains to be seen.

The life history of *G. onyx* makes it an interesting study subject for light/dark adaptation. Females brood their eggs for six to nine months at depths of up to 2500 m, where they sometimes release their hatchlings, but have also been observed doing so near the surface [14,29,30]. Brooding at such depths under aphotic conditions may reduce predation risks for the female and her eggs/hatchlings, while extended post-spawning egg care may allow for relatively large and precocious young to hatch, capable of migrating vertically to nutrient-rich shallow water [31]. Retinal screening pigments could be advantageous as the young squid traverse a range of light conditions between the aphotic zone and the nutrient-rich euphotic zone, and across their subsequent exposure to regular day/night cycles.

As hatchlings mature into juveniles, they appear to settle into a regular diel cycle, distributed across a broad depth range during the day (100–800 m, peaking at 300–400 m) and a narrower range during the night (0–400 m, peaking at 200–300 m) [13]. Juvenile *G. onyx* have higher metabolic rates than their adult counterparts, which may influence their schooling behaviors and daily vertical migrations [13]. Their screening pigment may support visual acuity across the broad range of light conditions to which they may be exposed throughout the day and night and may protect the retina during ascents into shallower water during crepuscular hours.

At about 30–35 mm ML, juveniles begin to descend into greater depths. Their metabolisms slow to accommodate a less mobile, more solitary lifestyle, and they are most frequently observed at 300–1000 m (peaking at 700–800 m) during the day, and 100–800 m at night (peaking at 400–500 m) [13]. Thus, adult *G. onyx* may not experience the same diel range of light conditions, and screening pigment may play a less significant role. One exception may be females releasing hatchlings, which have been observed in near-surface waters [30]. It has been theorized that observations of deep-sea releases are the result of external stressors, such as net capture or predation, forcing the brooder to release her clutch prematurely, which has also been observed in octopods [29,32]. Females that release eggs in shallow water may benefit from screening pigment as they pass potential predators while migrating through brighter conditions. To determine whether ommin pigment migration occurs past the juvenile stage, more morphological investigations of adult *G. onyx* must be conducted.

The migration of these pigment granules in octopuses is controlled by melatonin circadian rhythms and varies during dark and light exposure [33]. Dopaminergic signals also act to modulate the basal migration of the granules, simulating dark adaptation conditions; efferent nerve cells release dopamine that acts on the retina and triggers the inward migration of the screening pigment, allowing maximum photoreceptor activity [6]. This study provides the first report of confirmed ommin pigment migration in an oegopsid squid. Evans et al. [8] reported an observation of apparent arrested screening pigment migration in the retinas of preserved juvenile *Teuthowenia pellucida*. This glass squid similarly inhabits well-lit shallow waters during its early life stages, then descends to greater depths as adults. Evans et al. [8] did not observe any ontogenetic variation in the thickness of the ommin layer, despite a disproportionate increase in photoreceptor length compared to the overall growth, and it remains unclear whether screening pigment migration remains possible and/or advantageous for deep-sea dwelling adult *T. pellucida*. However, the findings from the current study and Evans et al. [8] suggest that ommin migration does occur in at least some deep-sea squids across life stages that dwell primarily below the photic zone.

More research is required to fully understand the complex mechanisms governing screening pigment migration in the retina of deep-sea squids and the role neurotransmitters play. Future research on *G. onyx* screening pigment and its potential correspondence to

dopamine levels should aim to determine a more precise time scale for light and dark adaptations in the retina and investigate a wider range of ontogenetic stages. Additionally, if interindividual variation exists, and if the mechanisms controlling ommin migration undergo ontogenetic modifications that are linked to visual transmission, those phenomena can start to be deciphered. More broadly, future research may aim to identify the variation in screening pigments among closely related species, and whether this mechanism is present in other oegopsids.

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Institutional Review Board Statement: Although ethics approval is not needed for cephalopods in the United States of America, special effort was made to minimize suffering and distress in caught specimens. All specimens in this study were collected under valid US State and Federal Scientific Collecting Permits.

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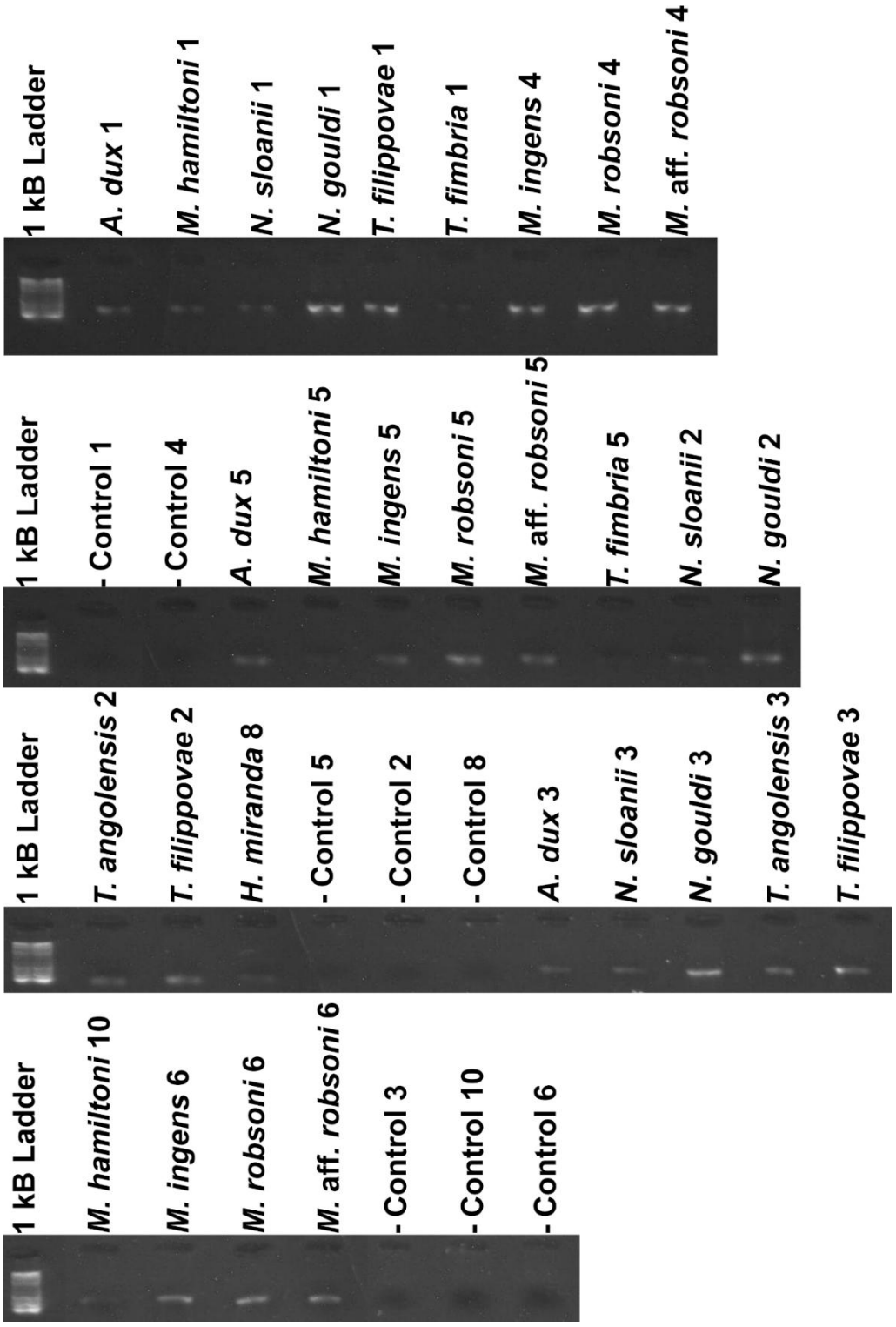
References

1. Rawitz, B. Über Pigmentverschiebungen Im Cephalopodenauge. *Zool. Anz.* **1891**, *14*, 157–158.
2. Butenandt, A. Wirkstoffe Des Insektenreiches. *Naturwissenschaften* **1959**, *46*, 461–471. [CrossRef]
3. Daw, N.W.; Pearlman, A.L. Pigment Migration and Adaptation in the Eye of the Squid, *Loligo pealei*. *J. Gen. Physiol.* **1974**, *63*, 22–36. [CrossRef]
4. Hagins, W.A.; Liebman, P.A. Light Induced Pigment Migration in the Squid Retina. *Biol. Bull.* **1962**, *123*, 498.
5. Young, J.Z. Light- and Dark-Adaptation in the Eyes of Some Cephalopods. *Proc. Zool. Soc. Lond.* **1963**, *140*, 255–272. [CrossRef]
6. Gleadall, I.G.; Ohtsu, K.; Gleadall, E.; Tsukahara, Y. Screening-Pigment Migration in the Octopus Retina Includes Control by Dopaminergic Efferents. *J. Exp. Biol.* **1993**, *185*, 1–16. [CrossRef]
7. Wolken, J.J. Retinal Structure: Mollusc Cephalopods: Octopus, Sepia. *J. Biophys. Biochem. Cytol.* **1958**, *4*, 835–837. [CrossRef]
8. Evans, A.B.; Acosta, M.L.; Bolstad, K.S. Retinal Development and Ommin Pigment in the Cranchiid Squid *Teuthowenia pellucida* (Cephalopoda: Oegopsida). *PLoS ONE* **2015**, *10*, 11. [CrossRef]
9. Kier, C.K.; Chamberlain, S.C. Dual Controls For Screening Pigment Movement In Photoreceptors of The *Limulus* Lateral Eye: Circadian Efferent Input And Light. *Vis. Neurosci.* **1990**, *4*, 237–255. [CrossRef]
10. Gavriouchkina, D.; Tan, Y.; Ziadi-Künzli, F.; Hasegawa, Y.; Piovani, L.; Zhang, L.; Sugimoto, C.; Luscombe, N.; Marlétaz, F.; Rokhsar, D.S. A Single-Cell Atlas of Bobtail Squid Visual and Nervous System Highlights Molecular Principles of Convergent Evolution. *bioRxiv* **2022**, 2022.05.06.490366. [CrossRef]
11. D'Aniello, S.; Spinelli, P.; Ferrandino, G.; Peterson, K.; Tsesarskia, M.; Fisher, G.; D'Aniello, A. Cephalopod Vision Involves Dicarboxylic Amino Acids: D-Aspartate, L-Aspartate and L-Glutamate. *Biochem. J.* **2005**, *386*, 331–340. [CrossRef] [PubMed]
12. MBARI's Deep Sea Guide-Data Artifacts for Gonatus onyx. Available online: http://dsg.mbari.org/dsg/plots/concept/Gonatus%20onyx/Global (accessed on 20 April 2023).
13. Hunt, J.C.; Seibel, B.A. Life History of *Gonatus onyx* (Cephalopoda: Teuthoidea): Ontogenetic Changes in Habitat, Behavior and Physiology. *Mar. Biol.* **2000**, *136*, 543–552. [CrossRef]
14. Seibel, B.A.; Robison, B.H.; Haddock, S.H.D. Post-Spawning Egg Care by a Squid. *Nature* **2005**, *438*, 929. [CrossRef] [PubMed]

15. Marc, R.E.; Liu, W.L.S.; Kalloniatis, M.; Raiguel, S.F.; Van Haesendonck, E. Patterns of Glutamate Immunoreactivity in the Goldfish Retina. *J. Neurosci.* **1990**, *10*, 4006–4034. [CrossRef] [PubMed]
16. Kalloniatis, M.; Loh, C.S.; Acosta, M.L.; Tomisich, G.; Zhu, Y.; Nivison-Smith, L.; Fletcher, E.L.; Chua, J.; Sun, D.; Arunthavasothy, N. Retinal Amino Acid Neurochemistry in Health and Disease. *Clin. Exp. Optom.* **2013**, *96*, 310–332. [CrossRef] [PubMed]
17. Acosta, M.L.; Kalloniatis, M. Short- and Long-Term Enzymatic Regulation Secondary to Metabolic Insult in the Rat Retina. *J. Neurochem.* **2005**, *92*, 1350–1362. [CrossRef] [PubMed]
18. Nivison-Smith, L.; Collin, S.P.; Zhu, Y.; Ready, S.; Acosta, M.L.; Hunt, D.M.; Potter, I.C.; Kalloniatis, M. Retinal Amino Acid Neurochemistry of the Southern Hemisphere Lamprey, *Geotria australis*. *PLoS ONE* **2013**, *8*, e58406. [CrossRef] [PubMed]
19. Ratnasingham, S.; Hebert, P.D.N. Bold: The Barcode of Life Data System (http://www.barcodinglife.org). *Mol. Ecol. Notes* **2007**, *7*, 355–364. [CrossRef] [PubMed]
20. Braid, H.E.; Bolstad, K.S.R. Cephalopod Biodiversity of the Kermadec Islands: Implications for Conservation and Some Future Taxonomic Priorities. *Invertebr. Syst.* **2019**, *33*, 402–425. [CrossRef]
21. Lo, M.V.C.; Pak, W.L. Light-Induced Pigment Granule Migration in the Retinular Cells of *Drosophila melanogaster:* Comparison of Wild Type with ERG-Defective Mutants. *J. Gen. Physiol.* **1981**, *77*, 155–175. [CrossRef]
22. Hamdorf, K.; Höglund, G.; Juse, A. Ultra-Violet and Blue Induced Migration of Screening Pigment in the Retina of the Moth *Deilephila elpenor*. *J. Comp. Physiol. A* **1986**, *159*, 353–362. [CrossRef]
23. White, R.H.; Banister, M.J.; Bennett, R.R. Spectral Sensitivity of Screening Pigment Migration in the Compound Eye of *Manduca sexta*. *J. Comp. Physiol. A* **1983**, *153*, 59–66. [CrossRef]
24. Arechiga, H.; Banuelos, E.; Frixione, E.; Picones, A.; Rodriguez-Sosa, L. Modulation of Crayfish Retinal Sensitivity by 5-Hydroxytryptamine. *J. Exp. Biol.* **1990**, *150*, 123–143. [CrossRef] [PubMed]
25. Schraermeyer, U. Further Evidence for Synthesis of Screening Pigment Granules Involved in the Photosensory Membrane Turnover of the Crayfish Photoreceptor. *Pigment. Cell Res.* **1990**, *3*, 279–289. [CrossRef] [PubMed]
26. Summers, K.M.; Howells, A.J.; Pyliotis, N.A. Biology of Eye Pigmentation in Insects. *Adv. Insect Phys.* **1982**, *16*, 119–166. [CrossRef]
27. Figon, F.; Casas, J. Ommochromes in Invertebrates: Biochemistry and Cell Biology. *Biol. Rev.* **2019**, *94*, 156–183. [CrossRef] [PubMed]
28. Hansen, A.G.; Beauchamp, D.A.; Schoen, E.R. Visual Prey Detection Responses of Piscivorous Trout and Salmon: Effects of Light, Turbidity, and Prey Size. *Trans. Am. Fish. Soc.* **2013**, *142*, 854–867. [CrossRef]
29. Seibel, B.A.; Hochberg, F.G.; Carlini, D.B. Life History of *Gonatus onyx* (Cephalopoda: Teuthoidea): Deep-Sea Spawning and Post-Spawning Egg Care. *Mar. Biol.* **2000**, *137*, 519–526. [CrossRef]
30. Okutani, T.; Nakamura, I.; Seki, K. An Unusual Egg-Brooding Behavior of an Oceanic Squid in the Okhotsk Sea. *Jpn. J. Malacol.* **1995**, *54*, 237–239.
31. Mangold, K. Reproduction. In *Cephalopod Life Cycles*; Boyle, P.R., Ed.; Academic Press: London, UK, 1983; Volume 2, pp. 157–200.
32. Young, R.E. Brooding in a Bathypelagic Octopus. *Pac. Sci.* **1972**, *26*, 400–404.
33. Muñoz, J.L.P.; Patiño, M.A.L.; Hermosilla, C.; Conde-Sieira, M.; Soengas, J.L.; Rocha, F.; Míguez, J.M. Melatonin in Octopus (*Octopus vulgaris*): Tissue Distribution, Daily Changes and Relation with Serotonin and Its Acid Metabolite. *J. Comp. Physiol. A* **2011**, *197*, 789–797. [CrossRef] [PubMed]

Appendix C. *Supplemental material of DNA electrophoresis analysis from Chapter 4 – The Influence of Bioluminescence on the Spectral Sensitivity of Visual pigments in Deep-sea Oegopsid Squids.*

Figure I. *Gel electrophoresis analysis of rhodopsin DNA fragments of oegopsid squids from this study. The numbers denote the primer set used to isolate the DNA fragment analysed (see Chapter 4, Table 9).*



Appendix D. *Supplemental material of statistical analyses and probability metrics from Chapter 4 – The Influence of Bioluminescence on the Spectral Sensitivity of Visual pigments in Deep-sea Oegopsid Squids.*

Table XV. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons of peak absorbance (λ_{max}) among species.*

Species Compared	Estimate	SE	df	t-ratio	p-value
N. sloanii – T. filippovae	-3.2827	1.68	30	-1.95	0.682
N. sloanii – T. angolensis	1.1533	1.68	30	0.685	0.9998
N. sloanii – M. ingens	1.4402	1.23	30	1.171	0.9811
N. sloanii – O. robsoni	1.1655	1.44	30	0.808	0.999
N. sloanii – O. aff. *robsoni*	-3.7131	1.54	30	-2.416	0.3901
N. sloanii – T. danae	-11.7498	1.94	30	-6.044	0.0001
N. sloanii – H. miranda (Large Eye)	0.6557	1.68	30	0.389	1
N. sloanii – H. miranda (Small Eye)	-11.202	1.68	30	-6.653	<.0001
T. filippovae - T. angolensis	4.436	1.94	30	2.282	0.4703
T. filippovae - M. ingens	4.7229	1.57	30	3.013	0.1365
T. filippovae - O. robsoni	4.4482	1.74	30	2.558	0.3132
T. filippovae - O. aff robsoni	-0.4304	1.82	30	-0.237	1
T. filippovae - T. danae	-8.4671	2.17	30	-3.895	0.0183
T. filippovae - H. miranda (Large Eye)	3.9384	1.94	30	2.026	0.6339
T. filippovae - H. miranda (Small Eye)	-7.9193	1.94	30	-4.073	0.0117
T. angolensis - M. ingens	0.2869	1.57	30	0.183	1
T. angolensis - O. robsoni	0.0122	1.74	30	0.007	1
T. angolensis - O. aff. *robsoni*	-4.8664	1.82	30	-2.676	0.2571
T. angolensis - T. danae	-12.9031	2.17	30	-5.936	0.0001

T. angolensis - H. miranda (Large Eye)	-0.4977	1.94	30	-0.256	1
T. angolensis - H. miranda (Small Eye)	-12.3553	1.94	30	-6.355	<.0001
M. ingens – O. robsoni	-0.2747	1.3	30	-0.211	1
M. ingens – O. aff. *robsoni*	-5.1533	1.41	30	-3.658	0.0327
M. ingens – T. danae	-13.19	1.84	30	-7.151	<.0001
M. ingens – H. miranda (Large Eye)	-0.7846	1.57	30	-0.501	1
M. ingens – H. miranda (Small Eye)	-12.6422	1.57	30	-8.066	<.0001
O. robsoni – O. aff. *robsoni*	-4.8786	1.6	30	-3.054	0.1256
O. robsoni – T. danae	-12.9153	1.99	30	-6.483	<.0001
O. robsoni – H. miranda (Large Eye)	-0.5098	1.74	30	-0.293	1
O. robsoni – H. miranda (Small Eye)	-12.3675	1.74	30	-7.112	<.0001
O. aff. *robsoni – T. danae*	-8.0367	2.06	30	-3.897	0.0182
O. aff. *robsoni – H. miranda* (Large Eye)	4.3688	1.82	30	2.402	0.3979
O. aff. *robsoni – H. miranda* (Small Eye)	-7.4889	1.82	30	-4.118	0.0105
T. danae – H. miranda (Large Eye)	12.4055	2.17	30	5.707	0.0001
T. danae – H. miranda (Small Eye)	0.5478	2.17	30	0.252	1
H. miranda (Large Eye) – *H. miranda* (Small Eye)	-11.8576	1.94	30	-6.099	<.0001

Table XVI. *A list of test statistics and probability metrics from Tukey's HSD post-hoc pairwise comparisons of bandwidth (FWHM) among species.*

Species Compared	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
N. sloanii –	-1.333	1.47	30	-0.908	0.9973

T. filippovae					
N. sloanii – T. angolensis	3.333	1.47	30	2.27	0.4777
N. sloanii – M. ingens	-3.167	1.07	30	-2.953	0.1539
N. sloanii – O. robsoni	-10.867	1.26	30	-8.641	<.0001
N. sloanii – O. aff. *robsoni*	-10.917	1.34	30	-8.144	<.0001
N. sloanii – T. danae	2.333	1.7	30	1.376	0.9451
N. sloanii – H. miranda (Large Eye)	-8.333	1.47	30	-5.675	0.0002
N. sloanii – H. miranda (Small Eye)	11	1.47	30	7.491	<.0001
T. filippovae - T. angolensis	4.667	1.7	30	2.752	0.2247
T. filippovae - M. ingens	-1.833	1.37	30	-1.341	0.9532
T. filippovae - O. robsoni	-9.533	1.52	30	-6.286	<.0001
T. filippovae - O. aff robsoni	-9.583	1.59	30	-6.042	0.0001
T. filippovae - T. danae	3.667	1.9	30	1.934	0.6916
T. filippovae - H. miranda (Large Eye)	-7	1.7	30	-4.128	0.0102
T. filippovae - H. miranda (Small Eye)	12.333	1.7	30	7.274	<.0001
T. angolensis - M. ingens	-6.5	1.37	30	-4.755	0.002
T. angolensis - O. robsoni	-14.2	1.52	30	-9.363	<.0001
T. angolensis - O. aff. *robsoni*	-14.25	1.59	30	-8.984	<.0001
T. angolensis - T. danae	-1	1.9	30	-0.527	1
T. angolensis - H. miranda (Large Eye)	-11.667	1.7	30	-6.88	<.0001
T. angolensis - H. miranda (Small Eye)	7.667	1.7	30	4.521	0.0037
M. ingens – O. robsoni	-7.7	1.14	30	-6.769	<.0001
M. ingens –	-7.75	1.23	30	-6.308	<.0001

O. aff. *robsoni*					
M. ingens – *T. danae*	5.5	1.61	30	3.419	0.0571
M. ingens – *H. miranda* (Large Eye)	-5.167	1.37	30	-3.779	0.0244
M. ingens – *H. miranda* (Small Eye)	14.167	1.37	30	10.363	<.0001
O. robsoni – *O.* aff. *robsoni*	-0.05	1.39	30	-0.036	1
O. robsoni – *T. danae*	13.2	1.74	30	7.597	<.0001
O. robsoni – *H. miranda* (Large Eye)	2.533	1.52	30	1.67	0.8384
O. robsoni – *H. miranda* (Small Eye)	21.867	1.52	30	14.418	<.0001
O. aff. *robsoni - T. danae*	13.25	1.8	30	7.367	<.0001
O. aff. *robsoni - H. miranda* (Large Eye)	2.583	1.59	30	1.629	0.8577
O. aff. *robsoni - H. miranda* (Small Eye)	21.917	1.59	30	13.818	<.0001
T. danae – *H. miranda* (Large Eye)	-10.667	1.9	30	-5.627	0.0002
T. danae – *H. miranda* (Small Eye)	8.667	1.9	30	4.572	0.0032
H. miranda (Large Eye) - *H. miranda* (Small Eye)	19.333	1.7	30	11.402	<.0001

Appendix E. *Partial and complete genomic sequences of rhodopsin from oegopsids analysed in Chapter 4– The Influence of Bioluminescence on the Spectral Sensitivity of Visual pigments in Deep-sea Oegopsid Squids.*

Table XVII. *Rhodopsin genetic sequences. Species in bold indicate rhodopsin sequenced in this study. All other species genetic sequences were acquired from GenBank.*

Nucleotides						Species
1	11	21	31	41	50	
|	|	|	|	|	|	
----------	----------	----------	------cagg	tccactggtt		14 *T. pacificus*
----------	----------	----------	----------	----------		0 ***N. gouldi***
----------	----------	----------	----------	----------		0 ***T. filippovae***
----------	----------	----------	----------	----------		0 ***N. sloanii***
----------	----------	----------	----------	----------		0 *T. eblanae*
----------	----------	----------	----------	----------		0 *I. coindetii*
----------	----------	----------	----------	----------		0 ***T. angolensis***
----------	----------	----------	----------	----------		0 *S. oualaniensis*
----------	----------	----------	----------	----------		0 *O. bartramii*
----------	----------	----------	----------	----------		0 ***T. angolensis***
----------	----------	----------	----------	----------		0 ***O. robsoni***
----------	----------	----------	----------	----------		0 ***A. dux***
----------	----------	----------	----------	----------		0 ***T. fimbria***
----------	----------	----------	----------	----------		0 ***O. aff. robsoni***
----------	----------	----------	----------	----------		0 *Onychoteuthis* sp.
----------	----------	----------	----------	----------		0 *C. scabra*
----------	----------	----------	----------	----------		0 *M. fisheri*
----------	----------	----------	----------	----------		0 ***M. hamiltoni***
----------	----------	----------	----------	----------		0 *Galiteuthis* sp.
----------	----------	----------	----------	----------		0 *T. megalops*
----------	----------	----------	----------	----------		0 *Joubiniteuthis* sp.
----------	----------	----------	----------	----------		0 *P. microlampas*
----------	----------	----------	----------	----------		0 *E. higginsi*
----------	----------	----------	----------	----------		0 *A. pacificus*
----------	----------	----------	----------	----------		0 ***H. miranda***
----------	----------	----------	----------	----------		0 *H. oceanica*
----------	----------	----------	----------	----------		0 *O. nielseni*
----------	----------	----------	----------	----------		0 *A. dux*
aggtccgggg	gaatgaacgc	ctagtctggt	cgcgaacaga	ccgagtgcca		50 *N. pompilius*
tactgtagtg	gttcccataa	ggcaggcacc	agtacacaga	gaccaaattt		64 *T. pacificus*
----------	----------	----------	----------	----------		0 ***N. gouldi***
----------	----------	----------	----------	----------		0 ***T. filippovae***
----------	----------	----------	----------	----------		0 ***N. sloanii***
----------	----------	----------	----------	----------		0 *T. eblanae*
----------	----------	----------	----------	----------		0 *I. coindetii*
----------	----------	----------	----------	----------		0 ***T. angolensis***
----------	----------	----------	----------	----------		0 *S. oualaniensis*
----------	----------	----------	----------	----------		0 *O. bartramii*
----------	----------	----------	----------	----------		0 ***T. angolensis***
----------	----------	----------	----------	----------		0 ***O. robsoni***
----------	----------	----------	----------	----------		0 ***A. dux***
----------	----------	----------	----------	----------		0 ***T. fimbria***
----------	----------	----------	----------	----------		0 ***O. aff. robsoni***
----------	----------	----------	----------	----------		0 *Onychoteuthis* sp.
----------	----------	----------	----------	----------		0 *C. scabra*
----------	----------	----------	----------	----------		0 *M. fisheri*
----------	----------	----------	----------	----------		0 ***M. hamiltoni***
----------	----------	----------	----------	----------		0 *Galiteuthis* sp.
----------	----------	----------	----------	----------		0 *T. megalops*
----------	----------	----------	----------	----------		0 *Joubiniteuthis* sp.
----------	----------	----------	----------	----------		0 *P. microlampas*
----------	----------	----------	----------	----------		0 *E. higginsi*
----------	----------	----------	----------	----------		0 *A. pacificus*
----------	----------	----------	----------	----------		0 ***H. miranda***
----------	----------	----------	----------	----------		0 *H. oceanica*
----------	----------	----------	----------	----------		0 *O. nielseni*
----------	----------	----------	----------	----------		0 *A. dux*
gccagtgcag	tttctcgcgc	aacaaagaca	ccgaagccat	ggcgactgcg		100 *N. pompilius*
tgaaaaagaa	ttgtaattcg	ccatgggtag	agatctcaga	gacaatgaga		114 *T. pacificus*
----------	----------	----------	----------	----------		0 ***N. gouldi***

```
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
---------- ---------- ---------- ---------- ----------     0 T. eblanae
---------- ---------- ---------- ---------- ----------     0 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 S. oualaniensis
---------- ---------- ---------- ---------- ----------     0 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
---------- ---------- ---------- ---------- ----------     0 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------     0 C. scabra
---------- ---------- ---------- ---------- ----------     0 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
---------- ---------- ---------- ---------- ----------     0 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------     0 T. megalops
---------- ---------- ---------- ---------- ----------     0 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------     0 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
---------- ---------- ---------- ---------- ----------     0 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- ---------- ---------- ---------- ----------     0 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
caggcgatca ccacaatt-- ---------- -gatccggaa aacatcacgt   137 N. pompilius


cttggtggta taacccttcc atcgtagtcc atcctcattg gagagaattc   164 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
---------- ---------- ---------- ---------- ----------     0 T. eblanae
---------- ---------- ---------- ---------- ----------     0 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 S. oualaniensis
---------- ---------- ---------- ---------- ----------     0 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
---------- ---------- ---------- ---------- ----------     0 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------     0 C. scabra
---------- ---------- ---------- ---------- ----------     0 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
---------- ---------- ---------- ---------- ----------     0 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------     0 T. megalops
---------- ---------- ---------- ---------- ----------     0 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------     0 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
---------- ---------- ---------- ---------- ----------     0 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- ---------- ---------- ---------- ----------     0 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
cggtctggga taacgaagac gtgttcatcc acccgcactg gaaacatttc   187 N. pompilius


gatcaagtgc ctgatgcagt ttactactct ctcggtattt tcatcggtat   214 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
---------- ---------- ---------- ---------- ----------     0 T. eblanae
---------- ---------- ---------- ---------- ----------     0 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 S. oualaniensis
---------- ---------- ---------- ---------- ----------     0 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
---------- ---------- ---------- ---------- ----------     0 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------     0 C. scabra
---------- ---------- ---------- ---------- ----------     0 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
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---------- ---------- ---------- ---------- ----------     0 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------     0 T. megalops
---------- ---------- ---------- ---------- ----------     0 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------     0 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
---------- ---------- ---------- ---------- ----------     0 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- ---------- ---------- ---------- ----------     0 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
cctggggtgc ctgacgcggc ccactacata gtcggcatct ttatcaccgt   237 N. pompilius


ctgtggtatt attggttgcg gtggcaatgg cattgtcata tacctcttca   264 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
---------- ---------- ---------- ---------- ----------     0 T. eblanae
---------- ---------- ---------- ---------- ----------     0 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 S. oualaniensis
---------- ---------- ---------- ---------- ----------     0 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
---------- ---------- ---------- ---------- ----------     0 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------     0 C. scabra
---------- ---------- ---------- ---------- ----------     0 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
---------- ---------- ---------- ---------- ----------     0 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------     0 T. megalops
---------- ---------- ---------- ---------- ----------     0 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------     0 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
---------- ---------- ---------- ---------- ----------     0 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- ---------- ---------- ---------- ----------     0 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
cgtggggata tgcggcgtta tgggaaacgg agtggttatc tacatcttca   287 N. pompilius


ccaagacgaa atccctgcag acaccagcca acatgttcat cattaacttg   314 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
---------- ---------- ---------- ---------- ----------     0 T. eblanae
---------- ---------- ---------- ---------- ----------     0 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 S. oualaniensis
---------- ---------- ---------- ---------- ----------     0 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
---------- ---------- ---------- ---------- ----------     0 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------     0 C. scabra
---------- ---------- ---------- ---------- ----------     0 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
---------- ---------- ---------- ---------- ----------     0 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------     0 T. megalops
---------- ---------- ---------- ---------- ----------     0 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------     0 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
---------- ---------- ---------- ---------- ----------     0 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- ---------- ---------- ---------- ----------     0 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
gcaggacaaa gtctttaaga acaccggcta acatgttcat catcaatctg   337 N. pompilius


gctttctccg atttcacttt ctcattggtg aacggtttcc cgctcatgac   364 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
```

```
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
---------- ---------- ---------- ---------- ----------     0 T. eblanae
---------- ---------- ---------- ---------- ----------     0 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 S. oualaniensis
---------- ---------- ---------- ---------- ----------     0 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
---------- ---------- ---------- ---------- ----------     0 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------     0 C. scabra
---------- ---------- ---------- ---------- ----------     0 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
---------- ---------- ---------- ---------- ----------     0 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------     0 T. megalops
---------- ---------- ---------- ---------- ----------     0 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------     0 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
---------- ---------- ---------- ---------- ----------     0 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- ---------- ---------- ---------- ----------     0 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
gctctaagcg acttgacatt ctccgctgtt aatggattcc ccttgctcag   387 N. pompilius


aatttcttgt ttcttaaaga aatggatctt tggatttgct gcctgcaaag   414 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
---------- ---------- ---------- ---------- ----------     0 T. eblanae
---------- ---------- ---------- ---------- ----------     0 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 S. oualaniensis
---------- ---------- ---------- ---------- ----------     0 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
---------- ---------- ---------- ---------- ----------     0 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------     0 C. scabra
---------- ---------- ---------- ---------- ----------     0 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
---------- ---------- ---------- ---------- ----------     0 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------     0 T. megalops
---------- ---------- ---------- ---------- ----------     0 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------     0 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
---------- ---------- ---------- ---------- ----------     0 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- ---------- ---------- ---------- ----------     0 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
tatttcctcc ttccagaaaa aatggatatt cgggcaaact gcgtgtgaat   437 N. pompilius


tctatggctt catcggtggt attttcggtt ttatgtccat catgactatg   464 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
---------- ---------- ---------- ---------- ----------     0 T. eblanae
---------- ---------- ---------- ---------- ----------     0 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 S. oualaniensis
---------- ---------- ---------- ---------- ----------     0 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
---------- ---------- ---------- ---------- ----------     0 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------     0 C. scabra
---------- ---------- ---------- ---------- ----------     0 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
```

```
---------- ---------- ---------- ---------- ----------     0 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------     0 T. megalops
---------- ---------- ---------- ---------- ----------     0 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------     0 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
---------- ---------- ---------- ---------- ----------     0 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- ---------- ---------- ---------- ----------     0 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
tgtacggtct ggccggaggc atctttggtc tcatgtccat caacaccatg   487 N. pompilius


gctatgatct ccattgatcg atacaatgtc attggaagac caatggcggc   514 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
---------- ---------- ---------- ---------- -gatggcggc     9 T. eblanae
---------- ---------- ---------- ---------- ----------     0 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 S. oualaniensis
---------- ---------- ---------- ---------- ----------     0 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
---------- ---------- ---------- ---------- -tatggctgc     9 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------     0 C. scabra
---------- ---------- ---------- ---------- ----------     0 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
---------- ---------- ---------- ---------- ----------     0 Galiteuthis sp.
---------- ---------- ---------- ---------c cgatggcg--     9 T. megalops
---------- ---------- ---------- ---------- ----------     0 Joubiniteuthis sp.
---------- ---------- ---------- ---------- -catggctgc     9 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
---------- ---------- ---------- ---------- -catggctgc     9 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- ---------- ---------- ---------- ----------     0 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
gctatgatct ccattgaccg ctacaacgtc atcgcccggc cgatggcggt   537 N. pompilius


gtcaaagaaa atgagccaca ggagggcctt cattatgatc atcttcgtat   564 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
atcaaagaaa atgagccaca ggagggcctt cattatgatc atcttcgttt    59 T. eblanae
---------- ---------- ---------- ---------- --------ct     2 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- -------ttn cntcntgatc atcttcgtat    23 S. oualaniensis
---------- --gagccaca ggagggcttt cattatgatc atcttcgtat    38 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
atcaaagaaa atgagccaca ggagggcttt cattatgatc atcttcgtct    59 Onychoteuthis sp.
---------- ---------- ---------- cattatgatc atcttcacct    20 C. scabra
---aaagaaa atgagccaca ggagagcttt cattatgatc atcttcacct    47 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
---------- --gagccaca ggagagcttt cattatgatc atcttcacct    38 Galiteuthis sp.
ctcaaagaaa atgagccaca ggagagcttt cattatgatc atcttcacct    59 T. megalops
---------- ---------- ---------- ---------- ---------t     1 Joubiniteuthis sp.
ttctaagaaa atgagccaca ggagggcttt catcatgatc atcttcacct    59 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
ttctaagaaa atgagccaca ggagggcttt cattatgatc atcttcacct    59 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
---------- --gagccaca ggagggcttt ccttatgatc atcttcgtct    38 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
atcgaagagg atgtcgcaca agaaagcctt tattatgatc atatccgtgt   587 N. pompilius


ggctctggtc agtcttgtgg gctatcggac ccattttcgg atggggcgca   614 T. pacificus
---------- ---------- ---------- ---------- ----------     0 N. gouldi
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---------- ---------- ---------- ---------- ----------     0 T. filippovae
---------- ---------- ---------- ---------- ----------     0 N. sloanii
ggctttggtc agtcttgtgg ggtatcggtc ccatttttgg atggggaatg   109 T. eblanae
ggctttggtc agtcctttgg ggcatcggac ccattttcgg atggggagca    52 I. coindetii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
ggttgtggtc cgtcttgtgg gctattggtc ccatttttgg atggggagca    73 S. oualaniensis
ggttgtggtc agtcttgtgg gctatcggtc ccatttttgg atggggagca    88 O. bartramii
---------- ---------- ---------- ---------- ----------     0 T. angolensis
---------- ---------- ---------- ---------- ----------     0 O. robsoni
---------- ---------- ---------- ---------- ----------     0 A. dux
---------- ---------- ---------- ---------- ----------     0 T. fimbria
---------- ---------- ---------- ---------- ----------     0 O. aff. robsoni
ggatttggtc tgtcctttgg tctatcggcc ccattttcgg ttggggagca   109 Onychoteuthis sp.
gggtctgggc tgtattgtgg tctatcggtc caatttttgg atggggcgca    70 C. scabra
gggtctgggc tgtctgttgg tctatcggtc caatctttgg ctggggcaaa    97 M. fisheri
---------- ---------- ---------- ---------- ----------     0 M. hamiltoni
gggtctgggc atcatgttgg gctatcggtc caatctttgg ttggggcaaa    88 Galiteuthis sp.
gggtatgggc agtctgttgg tctatcggac caatctttgg ttggggagca   109 T. megalops
ggatttgggc tgtcctctgg tctattggtc ccattttcga gtggggaaaa    51 Joubiniteuthis sp.
gggtatggtc cgtcctctgg gccatcggtc ccatctttgg ctggggcgca   109 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
ggttgtggtc tgtcctctgg gccattggtc ccattttcgg ctggggcgca   109 A. pacificus
---------- ---------- ---------- ---------- ----------     0 H. miranda
ggttgtggtc tgtactctgg gctattggtc ccattttcgg atggggagca    88 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
ggatctgggc cgcggtctgg acgctgcccc ctcttttcgg ctggggcgcg   637 N. pompilius


tacaccttag agggagtcct ctgcaattgc tcttttgatt acatcagccg   664 T. pacificus
---------- ---------- ---------- tcttttgatt acatcagccg    20 N. gouldi
---------- ---------- ---------- tcttttgatt acatcagccg    20 T. filippovae
---------- ---------- ---------- tcttttgatt acatcacccg    20 N. sloanii
tntacattgg agggagtcct ctgcaattgc tcatttgatt acatcagccg   159 T. eblanae
tatacattgg agggagttct ctgcaattgc tcttttgatt acatcagccg   102 I. coindetii
---------- ---------- ---------- tcttttgatt acatcagccg    20 T. angolensis
tacaccctag agggagtcct ctgcaactgc tcttttgatt acatcagccg   123 S. oualaniensis
tacaccctag agggagtcct ctgcaattgc tcttttgatt acatcagccg   138 O. bartramii
---------- ---------- ---------- tcttttgatt atatcagccg    20 T. angolensis
---------- ---------- ---------- tcttttgatt atatcagccg    20 O. robsoni
---------- ---------- ---------- tcttttgatt atatcagccg    20 A. dux
---------- ---------- ---------- tcttttgatt anatcagccg    20 T. fimbria
---------- ---------- ---------- tcttttgatt acatcagccg    20 O. aff. robsoni
tacaccttgg aaggcgtcct ctgcaattgc tcttttgatt acatcagccg   159 Onychoteuthis sp.
tacaccctcg agggagtcct ctgcaattgc tcttttgatt acatcagccg   120 C. scabra
tacaccttag agggagtcct ctgcaattgc tcttttgatt acatcagccg   147 M. fisheri
---------- ---------- ---------- tcttttgatt acatcagccg    20 M. hamiltoni
tacaccttgg agggagtcct ctgcaattgc tcttttgatt acatcagccg   138 Galiteuthis sp.
tacaccttgg agggagtcct ctgcaattgc tcttttgatt acatcagccg   159 T. megalops
tatactttgg aaggagtcct ctgcaactgc tcttttgatt acatcagccg   101 Joubiniteuthis sp.
tacaccttgg aaggagtcct ctgcaattgc tcttttgatt acatcagccg   159 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
tacaccctcg agggagtcct ctgcaattgc tcttttgatt acatcagccg   159 A. pacificus
---------- ---------- ---------- tctttcgann atatcagccg    20 H. miranda
tacaccttgg agggagttct ctgcaactgt tctttcgatt atatcagccg   138 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
tacatccccg aaggtttcca gacgtcctgc accttcgact acctgacccg   687 N. pompilius


cgattcaaca accaggtcca acatcttgtg catgtttatt ttgggtttct   714 T. pacificus
tgattcaaca accaggtcca acatcttgtg catgtttatt ttgggtttct    70 N. gouldi
cgattcatct accaggtcca acatcttgtg catgtttatt ttgggtttct    70 T. filippovae
cgattcaaca accaggtcca acatcttgtg catgtttatt ttgggtttct    70 N. sloanii
cgattccaca accaggtcca acatcttgtg catgtttatt tttggctttt   209 T. eblanae
cgattccgta accaggtcca acatcttgtg catgtttatt tttggccttt   152 I. coindetii
cgattcaaca accaggtcca acatcttgtg catgtttatt ttgggtttct    70 T. angolensis
cgattctact accaggtcca acgtcttgtg catgtttatt ttgggtttct   173 S. oualaniensis
cgattctacc accaggncca acatcttgtg catgkttatt ttgggtttct   188 O. bartramii
tgattacaca accaggtcca acatcgtctg catgtacctt ttcgccttta    70 T. angolensis
tgattataca acaaggtcca acatcgtttg catgtacctt ttcgccttta    70 O. robsoni
tgattctacc accaggtcca acgttctctg catgtatatt ttcgccttta    70 A. dux
tgattcnnca acnaggtcca acgttctctg catgtatatt ttcgccttta    70 T. fimbria
tgattataca acaaggtcca acatcgtttg catgtacctt ttcgccttta    70 O. aff. robsoni
tgattcttca accaggtcca acatcatctg catgtacgtt ttcgccttta   209 Onychoteuthis sp.
agatccctca accaggtcca acgtcctctg catgtatatt ttcgccttta   170 C. scabra
tgatccaaca accaggtcca acgttctctg catgtacatt ttcgcctttt   197 M. fisheri
tgattcaatt accaggtcca acgttatgtg catgtacatt ttcgccttca    70 M. hamiltoni
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tgattcagtt accaggtcca acgttctctg catgtacctt ttcgccttca   188 Galiteuthis sp.
tgactcaagt accaggtcta acgttctctg catgtatatt ttcgcctttt   209 T. megalops
tgattcttca accaggtcca acgttctttg catgtttatt ttcggttttt   151 Joubiniteuthis sp.
agatccctca accaggtcca acattctctg catgtacatt ttcgccttta   209 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
agatccttca accaggtcca acatcctctg catgtacgtt ttcgcctttt   209 A. pacificus
cgatcctact accagatcca atgttctctg catgtacctt ttgggctttt    70 H. miranda
cgatcctact accagatcca atgttctctg catgtacctt ttgggctttt   188 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
gaataactac ttcaggtcct acgtcctctg cttgtacctg ttcgggttca   737 N. pompilius


tcggtcctat tctcatcatt ttcttctgct acttcaatat tgtaatgtcc   764 T. pacificus
tcggtccaat tctcatcatt ttcttctgct acttcaacat tgtaatgtcc   120 N. gouldi
tcgggcccat tctcatcatt ttcttctgct acttcaacat tgtaatgtcc   120 T. filippovae
tcggtcctat tctcatcatt ttcttctgct acttcaacat tgtaatgtcc   120 N. sloanii
ttatgccaat tctcatcatt ttcttctgct acttcaacat tgtaatgtcc   259 T. eblanae
tcggtccagt tctcgtcatt ttcttctgct acttcaacat tgtaatgtcc   202 I. coindetii
tcgggccaat tnnnntcatt ntnttctgct acttcaacat tgt-------   113 T. angolensis
tcggtccaat tctcatcatt ttcttctgct acttcaacat tgtaatgtcg   223 S. oualaniensis
tcggtccaat tctcatcatt ttcttctgct acttcaacat tgtaatgtcg   238 O. bartramii
tgggtcccat ccttattatt ttcttctgct acttcaacat cgtcatgtcc   120 T. angolensis
tgggtcccat tcttatcatt ttcttctgtt acttcaacat cgtcatgtcc   120 O. robsoni
tgggccccat tctgatcatt ttcttctgtt acttcaacat tgtcatgtcc   120 A. dux
tgggccccat tcttatcatt ttcttctgct acttcaacat cgtcatgtcc   120 T. fimbria
tgggtcccat tcttatcatt ttcttctgtt acttcaacat cgtcatgtcc   120 O. aff. robsoni
tgggtcccat tcttatcatt ttcttctgtt acttcaacat tgtcatgtct   259 Onychoteuthis sp.
tgtgtcccat tcttatcatc ttcttctgct acttcaacat tgtcatggcc   220 C. scabra
gttgtcccat tcttatcatt ttcttctgct acttcaatat tgtcatggcc   247 M. fisheri
tgactcccat tctaatcatc ttcttctgct acttcaatat cgtcatggcc   120 M. hamiltoni
gttgtcccat tcttatcatc ttcttctgct acttcaatat cgtcatggcc   238 Galiteuthis sp.
gttgtcctat tctcatcatc ttcttctgct acttcaatat cgtcatggcc   259 T. megalops
gtggccctat tcttattatt ttcttctgtt acttcaacat cgttttgtcc   201 Joubiniteuthis sp.
tgatccccat tattatcatt ttcttctgct acttcaacat tgtaatgtct   259 P. microlampas
---------- ---------- ---------- ---------- ----------     0 E. higginsi
gcttccctat tcttatcatt ttcttctgtt acttcaacat tgtcatgtct   259 A. pacificus
gtggacccat tttgatnatc ttcttctgtt acttcaacat tgtcatgtct   120 H. miranda
gtggacccat tttgatcatc ttcttctgtt acttcaacat tgtcatgtct   238 H. oceanica
---------- ---------- ---------- ---------- ----------     0 O. nielseni
---------- ---------- ---------- ---------- ----------     0 A. dux
tcacgccagt gaccatcatc gcgatctgct acttcttcat cttcaaagct   787 N. pompilius


gtgtctaacc acgaaaaaga gatggcagct atggcgaaga ggctgaatgc   814 T. pacificus
gtgtctaacc acgaaaaaga gatggcagct atggcaaaga ggttgaatgc   170 N. gouldi
gtgtctaacc acgaaaaaga gatggcagct atggcgaaga ggcttaatgc   170 T. filippovae
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gtgtccaacc acgaaaaaga gatggcagct atggctaaaa ggttgaatgc   252 I. coindetii
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gtatccaacc acgagaaaga gatggcagct atggccaaaa ggttgaatgc   170 O. aff. robsoni
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---------- ---------- ---------- ---------- ----------     0 A. dux
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gctcaattcg ggcctctcga gtgggttaca ccttatgccg ctc-agttgc   268 T. angolensis
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gcccagttcg gacccatcga atgggtaaca ccttatgcag ctc-agttgc   320 M. hamiltoni
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gcccaattcg gaccactcga gtgggtaaca cgcttcgccg cac-agttgc   421 Joubiniteuthis sp.
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cgcagaagct gaaattgccg cagcagaatc atctgaaaac gcagccagct   415 H. miranda
cgcagaagct gaaattgccc cagcagaatc atctgaaaac gcagccagct   656 H. oceanica
tgcggaagct gacattccag ctggtgaagc ttccggtgat gcaggcg---   176 O. nielseni
tgcagaggct gaaattgctc ctggagaagc atctggcgat gtttcaa---   176 A. dux
aagcaagacg gacgacatgc gggacgagag cagcaccatg agcaaca---  1202 N. pompilius


gtgcggatgc tgcccaaatg aaagaa---- ---atgatgg ctatgatgca  1222 T. pacificus
gtgctgatgc tgctcaaatg aaagaa---- ---atgatgg ctatgatgca   523 N. gouldi
gcgctgatgc tgcccaaatg aaagaa---- ---atgatgg ctatgatgca   512 T. filippovae
gtgctgatgc tgctcaaatg aaagaa---- ---atgatgg ctatgatgca   428 N. sloanii
gtgctgatgc tgcccaaatg aaagaa---- ---atgatgg ctatgatgca   717 T. eblanae
gtgccgatgc tgcccaaatg aaagag---- ---atgatgg ctatgatgca   660 I. coindetii
gtgctgatgc tgcccaaatg aaagaa---- ---atgatgg ctatgatgca   479 T. angolensis
gtgcagatgc tgcccaaatg aaagaa---- ---atgatgg ctatgatgca   681 S. oualaniensis
gtgctgatgc tgcccaaatg aaagaa---- ---atgatgg ctatgatgca   696 O. bartramii
gcgccgatgc tgcccagatg aaggaa---- ---atgatgg ctatgatgca   564 T. angolensis
gcgctgatgc tgcccagatg aaggaa---- ---atgatgg ctatgatgca   560 O. robsoni
gcgctgatgc tgcccagatg aaggaa---- ---atgatgg ccatgatgca   530 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
gcgctgatgc tgcccagatg aaggaa---- ---atgatgg ctatgatgca   531 O. aff. robsoni
ccgctgatgc tgcccagatg aaggaa---- ---atgatgg ctatgatgca   717 Onychoteuthis sp.
gcgttgatgc tgcccagatg aaagaa---- ---atgatgg ctatgatgca   681 C. scabra
gcgctgatgc ygcccagatg aaggaa---- ---atgatgg ctatgatgca   705 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
ccgctgatgc tgcccagatg aaagaa---- ---atgatgg ctatgatgca   696 Galiteuthis sp.
ccgctgatgc cgcccaaatg aaggag---- ---atgatgg ccatgatggc   720 T. megalops
gagctgatgc tgctcagatg aaggaa---- ---atgatgg ctatgatgca   662 Joubiniteuthis sp.
gtgctgatgc tgcccaaatg aaggaa---- ---atgatgg ccatgatgca   720 P. microlampas
ccgctgatgc tgcccagatg aaggaa---- ---atgatgg ctatgatgca   219 E. higginsi
ccgctgatgc tgctcagatg aaggaa---- ---atgatgg ctatgatgca   717 A. pacificus
ccgctgatgc tgcccanatg aaagaa---- ---atgatgg ctatgatggc   458 H. miranda
ccgctgatgc tgcccagatg aaagaa---- ---atgatgg ctatgatggc   699 H. oceanica
gcgctgatgc tgcccagatg aaggaa---- ---atgatgg ctatgatgca   219 O. nielseni
gcgctgatgc tgcccagatg aaggaa---- ---atgatgg ccatgatgca   219 A. dux
tcagcgacgg tggccaggtg gagatgtcca ctcgcggcag acgcggcggc  1252 N. pompilius


gaagatg-ca acaacaacag g--------- ---------- --------cc  1244 T. pacificus
gaagatg-ca acaacaacag g--------- ---------- --------cc   545 N. gouldi
gaagatg-ca acaacaacag g--------- ---------- --------cc   534 T. filippovae
gaagatg-ca acaacaacag g--------- ---------- --------cc   450 N. sloanii
gaagatg-ca acaacaacag g--------- ---------- --------cc   739 T. eblanae
gaagatg-ca agctcaacag g--------- ---------- --------cc   682 I. coindetii
gaagatg-ca acaacaacag g--------- ---------- --------cc   501 T. angolensis
gaagatg-ca agcacaacag g--------- ---------- --------cc   703 S. oualaniensis
gaagatg-ca acaacaacag g--------- ---------- --------cc   718 O. bartramii
gaagatg-ca agcacaacag gcaca----- ---------- ---acagccc   595 T. angolensis
gaagatg-ca agcacaacaa gcaca----- ---------- ---acagccc   591 O. robsoni
gaagatg-ca agcacaacaa gcaca----- ---------- ---acagccc   561 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
gaagatg-ca agcacaacaa gcaca----- ---------- ---acagccc   562 O. aff. robsoni
gaagatg-ca agcacaacaa gcaca----- ---------- ---acaggcc   748 Onychoteuthis sp.
gaaaatg-ca agcacaacag gcacaggc-- ---------- ---accaccc   715 C. scabra
gaagatg-ca acaacaacaa cagca----- ---------- ---acaaccc   736 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
gaagatg-ca agcacaacaa caaca----- ---------- ------accc   724 Galiteuthis sp.
gaagatg-ca agcacaacaa caaca----- ---------- ------acct   748 T. megalops
gaagatg-ca agcacaacaa gcaca----- ---------- ---acagccc   693 Joubiniteuthis sp.
gaagatg-ca ggcacaacaa gctgca---- ---------- ----------   745 P. microlampas
gaagatg-ca agcacaacaa gccca----- ---------- ---acaaccc   250 E. higginsi
gaagatg-ca agcacaacaa gccca----- ---------- ---acaaccc   748 A. pacificus
taagatg-ca agcacaacaa gcaca----- ---------- ---acaggcc   489 H. miranda
taagatg-ca agcacaacaa gcaca----- ---------- ---acaggcc   730 H. oceanica
gaagatg-ca agcacaacaa gcacag---- ---------- ----------   244 O. nielseni
kaagatg-ca agcacaacaa gcaca----- ---------- ---acagccc   250 A. dux
gcggacacca ggtacaacga ccgcggcgac atgggcgtgt ctaacggcga  1302 N. pompilius


gcctac---- --------cc accacaggga tatg------ -----cacca  1271 T. pacificus
gcctac---- --------cc accacaggga tatg------ -----cacna   572 N. gouldi
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gcctac---- --------cc accacaggga tatg------ -----cacna   561 T. filippovae
gcctac---- --------cc accacaggga tatg------ -----cacna   477 N. sloanii
gcctac---- --------cc accacaggga tacg-----c acaaccacca   772 T. eblanae
gcctacccac aaccaccacc accacaggga tacc------ -----cacca   721 I. coindetii
gccta----- ---------- ---------- ---------- ----------   506 T. angolensis
gcctaccc-- -------acc accaccacca cagg------ --gataccca   736 S. oualaniensis
gcctaccc-- -------acc accaccacca cagg------ -----gatac   748 O. bartramii
gcttac---- --------cc accaccacag ggat------ -----accca   622 T. angolensis
gcttac---- --------cc accaccacag ggat------ -----accca   618 O. robsoni
gcttat---- --------cc accacccgct ggat------ -----accca   588 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
gcttac---- --------cc accaccacag ggat------ -----accaa   589 O. aff. robsoni
gcttac---- --------cc accaccacca cagg------ -----gatac   775 Onychoteuthis sp.
gccgga---- --------ta cccaccacag ggat------ -----ac---   739 C. scabra
gccggata-- -------ccc accacccgcc ggat------ -----ac---   763 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
gcctat---- --------cc accacccgcc ggat------ -----accca   751 Galiteuthis sp.
gcctac---- --------cc accaccacag ggat------ -----ac---   772 T. megalops
gcttac---- ---------- -ccacagcag ggat------ -----accca   717 Joubiniteuthis sp.
---------- --------ca accaccagca ggat------ -----ac---   763 P. microlampas
gcatac---- --------cc accaccacag ggat------ -----ac---   274 E. higginsi
gcctac---- --------cc accaccacag ggat------ -----ac---   772 A. pacificus
gcttac---- --------cc accaccacaa nagg------ -----gana-   515 H. miranda
gcttac---- --------cc accaccacaa cagg------ -----gatac   757 H. oceanica
---------- --------gc accaccacag ggat------ -----ac---   262 O. nielseni
gcttat---- --------cc accacccgct ggat------ -----accca   277 A. dux
gattat---- --------cc gcgacctgct aaacgccttc gtcaacgtcg  1340 N. pompilius


ccaccacagg gatac----- ---------- ccaccacagg g------ata  1300 T. pacificus
ccaccacagg gatac----- ---------- ccaccacagg g------ata   601 N. gouldi
ccaccacagg gata------ ---------- ---------- ----------   575 T. filippovae
ccaccacagg gata------ ---------- ---------- ----------   491 N. sloanii
ccaccacagg gatac----- ---------- ccaccacagg g------ata   801 T. eblanae
ccaccacagg gatac----- ---------- ccaccacagg g------ata   750 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
ccaccacagg gatac----- ---------- ccaccacaag g------ata   765 S. oualaniensis
ccaccacagg gatac----- ---------- ccaccacagg g------tta   777 O. bartramii
ccaccacagg gata------ ---------- ---------- ----------   636 T. angolensis
ccaccacagg gatac----- ---------- ccaccacagg g------ata   647 O. robsoni
ccaccacagg gatac----- ---------- caaccacagg g------ata   617 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
ccaccacagg gatac----- ---------- ---------- ----------   604 O. aff. robsoni
ccaccacagg gatac----- ---------- ccaccacagg g------tta   804 Onychoteuthis sp.
ccaccacagg gatac----- ---------- ccaccacagg g------ata   768 C. scabra
ccaccacagg gatac----- ---------- ccaccacagg g------ata   792 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
ccaccacagg gatacccacc acagggatac ccaccacagg g---ggcata   798 Galiteuthis sp.
ccaccacagg gatac----- ---------- ccaccacagg g------ata   801 T. megalops
ccagcacagg gatac----- ---------- ccaccacagg g------ata   746 Joubiniteuthis sp.
ccaccacagg gatac----- ---------- ccaccacagg g------ata   792 P. microlampas
ccaccacagg gataccca-- -------cca ccaccacagg g------ata   309 E. higginsi
ccaccacagg gatac----- -------cca ccaccacaag g------ata   804 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
ccaccacagg gatac----- ---------- ccaccacagg g------ata   786 H. oceanica
ccaccacaag gatac----- ---------- ccaccacagg g------ata   291 O. nielseni
ccaccacagg gatac----- ---------- ccaccacagg g------ata   306 A. dux
tcggcgctca gaaaccccag c---agccat ccaccgtcag cgtggccatg  1387 N. pompilius


c------cca ccacag---g gatacccacc acaagg---a tacccaccac  1338 T. pacificus
c--------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
c------cca ccacag---g gatacccaca acaagg---a tacccaccac   839 T. eblanae
c------cca ccacaa---g gatacccacc acaagg---a tacccaccac   788 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
c------cca ccacag---g gatacccacc acaagg---t tacccaccac   803 S. oualaniensis
c------cca ccacag---g gatacccacc acaagg---t tacccaccac   815 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
t------cca ccacag---g gata------ ---------- ----------   662 O. robsoni
c------cca caacaacagg gata------ ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
c------cca ccacag---g gatacccacc acaagg---a tacccaccac   842 Onychoteuthis sp.
c------cca ccacaa---g gatacccacc acaagg---a tacccaccac   806 C. scabra
c------ccc ccacag---g gatacccacc acaggg---a tacccaccac   830 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
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c------cca ccacaggggg catatccacc acaggggtca tacccacccc   842 Galiteuthis sp.
c------cca ccacagggag catacccacc ccaggggtca tacccacccc   845 T. megalops
c---ccacca gcacag---g gatacccacc acaagg---a tacccaccac   787 Joubiniteuthis sp.
c------cca ccacaa---g gatatgcacc a--------- ---ccaccac   821 P. microlampas
c------cca ccacag---g gatacccacc acaagg---a tatccaccac   347 E. higginsi
c------cca ccacaa---g gatacccacc acaggg---a tacccaccac   842 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
cccaccacca ccacag---g gatacccacc acaggg---a tacccaccac   830 H. oceanica
c------cca ccacag---g gatatcctcc acaggg---a tacccaccac   329 O. nielseni
c---ccacca ccacag---g gatacccacc acaagg---a tacccaccac   347 A. dux
c------cca ccatccc-ga cctacttgcc acccat---g tatccttctc  1427 N. pompilius


aaggttaccc accaccaccc caaggagcac caccccaggg ----------  1378 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
aaggttac-- -ccaccaccc caaggagcac caccccaagg ----------   876 T. eblanae
aaggatac-- -ccaccaccc caaggagcac caccccaagg ----------   825 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
aaggttaccc accaccaccc caaggacccc cacccgcagg gt--------   845 S. oualaniensis
aaggttaccc accaccaccc caaggaccac caccccaagg ----------   855 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
agggatac-- ----ccacca caaggagcac caccccaagg ----------   876 Onychoteuthis sp.
aaggatac-- ----ccacca caaggagcac caccc----- ----------   835 C. scabra
aaggatac-- ----ccaccc caaggagcac caccccaagg ----------   864 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
agggatac-- ----ccaccc caaggagcac caccccaagg ----------   876 Galiteuthis sp.
agggatac-- ----ccaccc cagggagcac caccccaagg ----------   879 T. megalops
agggatac-- ----ccacca caaggagcac cacctcangg ----------   821 Joubiniteuthis sp.
aaggatac-- ----ccacca c--------- ---------- ----------   836 P. microlampas
agggatac-- ----ccacca caaggagcac cacccgcagg ----------   381 E. higginsi
agggatac-- ----ccacca caaggagcac caccccaagg ----------   876 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
aaggatac-- ----ccacca caaggagcac caccccaagg ----------   864 H. oceanica
aaggatac-- ----ccacca caaggatacc caccacaagg ----------   363 O. nielseni
aaggatac-- ----ccaccc caaggagcac caccccaagg ----------   381 A. dux
acgggtacta tccgccgccg c-cggcccac taccccgagg acgccaacag  1476 N. pompilius


------agca cccc---cag ca-------- -------gcc ccaccccagg  1404 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
------agca cctccagcag ca-------- -------gcc ccacccca--   903 T. eblanae
------agca cctccagcag ca-------- -------gcc ccaccccagg   854 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
------ggca ccctcccagg cagg------ ----caggcc ccaccccagg   879 S. oualaniensis
------agca cctccagcag ca-------- -------gcc ccaccccagg   884 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
------accn cccccagcag ca-------- -------gcc ccaccccagg   905 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
------agca cctccagcag ca-------- -------gcc ccacctcagg   893 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
------agca cctccagcag ca-------- -------gcc c---------   896 Galiteuthis sp.
------agca cctccagcag ca-------- -------gcc ccacctcagg   908 T. megalops
------agca cttccagcag ca-------- -------gcc ccnctaatga   850 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
------agca cctccagcag ca-------- -------gcc ccac------   404 E. higginsi
------agca cctccagcag ca-------- -------gcc ccac------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
------agca cccccaaccg ca-------- -------gcc ccacccaccg   893 H. oceanica
------atac ccaccacaag ca-------- -------gcc ccacctcagg   392 O. nielseni
------agca cctccagca- ---------- -------gcc ccacctcagg   407 A. dux
gccctcaccg tatcctgcgg taagacccga cgtctacgcc atttctcacg  1526 N. pompilius


gagttga--- caaccaggct taccaggc-- -------ttg agcaaccaac  1442 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
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---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
gagtt----- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
ggaagtt--- ---------- ---------- ---------- ----------   886 S. oualaniensis
gagtt----- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
gtgtt----- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
ggtt------ ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
gtgtt----- ---------- ---------- ---------- ----------   913 T. megalops
g--------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
gtgtt----- ---------- ---------- ---------- ----------   898 H. oceanica
a--------- ---------- ---------- ---------- ----------   393 O. nielseni
gggtt----- ---------- ---------- ---------- ----------   412 A. dux
gtcccctggc cacctacgac cagcaggtgg ctggcggccc ggcagcggct  1576 N. pompilius


ctaagaactc tgtagac--- ------ttat tttgtagatg ctgcaag---  1480 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
ccgaaacccg ccgaaacggc taaggaccag gctgccgaag tggaaagaat  1626 N. pompilius


-aaagaatta ttctgagaaa aaatatagtt cgatggaacc tgtccgtatc  1529 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
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---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
caaaaaattg gccctggaa- ---------- cgggagcaag tgtacctacc  1665 N. pompilius


ttctaaagag aggcgtccga gtgaagaaga ca-aaaagaa gattgaactt  1578 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
tgcaaacaaa a------cag ccgagggagc cgccaacgaa gacctgcctc  1709 N. pompilius


atcattaaaa cgaagttgca --cgcatgaa aatcgtgcca aaagaaagaa  1626 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
cgcctccgtc ccaaaacaca gccgcagata aaccccaaga agaaaaccag  1759 N. pompilius


gatgatgaag cggtgactaa ca------ta tctgaattat atacagatga  1670 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
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---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
gatgaaaaga agggaattga taataaggcc tttgagaagt aaagaagcaa  1809 N. pompilius


---------- --gctttcac agagaagccg tgtattagga acttttccac  1708 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
aacgtttgac atgatttcgc agaaaaacca agagaaaaac attccttagc  1859 N. pompilius


ctttcctatt tcaatcaaat ttaattaact aatattcagg acattttcgg  1758 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
```

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---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
c--------- ---------- --gaacgata actaattgga aagttagtag  1888 N. pompilius


acatagacaa tt---ttctt gaaaagtaga taaatacatt ttagaaaaaa  1805 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
aaatgtataa ttgcatcctt aaaatttaaa agaatatata tattaaaata  1938 N. pompilius


ttgtgtatat atatatatat atatatatat atatataatc ttgcacgtat  1855 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
atagatagat aattaga--- ---------- ---------- ----------  1955 N. pompilius


tttgaatagt ataagtctgg cgcaaaatcg actcaattac gactttgagc  1905 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
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```
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
ttttggtgac gcatgttcag agtaaagcaa aattaatggt ttttctgg--  2003 N. pompilius


gctgccttaa gtaaatgcgt tgtctacagg taacatcaac aaaaaataaa  1955 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
-------caa agaagcccgt tgctggccgg aatt------ ----------  2030 N. pompilius


caaaagacta tatgcacgag caacatacac gcacataaat gtatacttat  2005 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
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---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
caaagaatct tggg------ ----gtccga tcgcggaagc tatcagttgt  2070 N. pompilius


ttacatacgt atctatttag caatcattat atacatata- -------tat  2047 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
tttcataatt tttaagttgg aaaccaccgc tttcttccaa cagattctct  2120 N. pompilius


cttcgactct tatatgtacg tgcatatatg catatgtatg gatgttggtg  2097 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
tttcgactga ca-------- -gtagataca gattttaatt cacttagaaa  2161 N. pompilius


ggtatgagcg tgagtgtttg cgtgtgtcag tgcatatgat gtatcagtgt  2147 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
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---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
actctccgtg tag------- --------tg tccgtattac cgataagtga  2196 N. pompilius


gcgtatgtgc ttttttttact gtacgatcag aggagttggt ctggctagcg  2197 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
gcaattaatc ctcgacaacc cgattagaat cgatgccttc cggtcaagca  2246 N. pompilius


tctcttcgga attgtgacat cgccacctgg tgtgggtttc tcgcctgaaa  2247 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
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---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
acgtttcagc aaatgcacgt agaagatggt ttcggagttc cctcgtaaaa  2296 N. pompilius


ggacgtggag tgatttttt ctaatggaag atagattttt agaacgtttg  2297 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
cggagaga-- --------ct ctgacagaca gtatagagga tggtatttgg  2336 N. pompilius


tttcattctt cagctgtttc gagtgacctc tctctcattt tttatcagtc  2347 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
ttgtggtttc cagtgacact caacagtact tcttgttatt gttgttgttt  2386 N. pompilius


attccgaatc tatcatcact ggttgatcaa ttgttttgtt tcttcttttt  2397 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
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---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
tattaaagat gacagtttct aaagaaatga ---------- -----ccttt  2421 N. pompilius


ttgatatgtg ttagaaagga aaagagatac aacaataata acaatg-aca  2446 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
tatacgaaag tgagataaaa tacgcattgt gggggtagca aaaaagaaaa  2471 N. pompilius


accagcaata gatcaatact cgattgatga agattttcat cgcgcgaatc  2496 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
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---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
aaaaatggtg ggattgggct caattcatta agat------ ----------  2505 N. pompilius


tataatagag actggtacaa acaaacgaga attgattaca acccctcgga  2546 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
---------- ---------- ---------- ---------a acgcctcag-  2515 N. pompilius


aattgattct aaaatcggtt ttgtatcgta tgcacaatgc tcgataaaag  2596 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
---------- ---------- ---------- ---------- ----------  2515 N. pompilius


caaagaccgt gattgtatct atcggcaggt ttccttccgt ttctaaattt  2646 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
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---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
---------- ---------- ---------- -------cgt ttcta-----  2523 N. pompilius


tgatcatcta ctctaggatt gaaaaaataa aattaagaaa atattgacca  2696 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
-------cta gcctagcagc gcagaag--- ---------- ----------  2543 N. pompilius


aaaaataata ttataacccc ctcaaaaata tatttaaaaa attaaataaa  2746 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
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```
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
---------- ---------- ---------- ---------- ----------  2543 N. pompilius


aataacattt ttggtagcta aattattttt ttctttttaa attgcttaat  2796 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
---------- ---------- ----attcat cggtttttaa tctccgt---  2566 N. pompilius


aatgtggtta tctttcttgc aatttttttt ctct----tt ttaatttttt  2842 T. pacificus
---------- ---------- ---------- ---------- ----------   602 N. gouldi
---------- ---------- ---------- ---------- ----------   575 T. filippovae
---------- ---------- ---------- ---------- ----------   491 N. sloanii
---------- ---------- ---------- ---------- ----------   903 T. eblanae
---------- ---------- ---------- ---------- ----------   859 I. coindetii
---------- ---------- ---------- ---------- ----------   506 T. angolensis
---------- ---------- ---------- ---------- ----------   886 S. oualaniensis
---------- ---------- ---------- ---------- ----------   889 O. bartramii
---------- ---------- ---------- ---------- ----------   636 T. angolensis
---------- ---------- ---------- ---------- ----------   662 O. robsoni
---------- ---------- ---------- ---------- ----------   635 A. dux
---------- ---------- ---------- ---------- ----------   244 T. fimbria
---------- ---------- ---------- ---------- ----------   604 O. aff. robsoni
---------- ---------- ---------- ---------- ----------   910 Onychoteuthis sp.
---------- ---------- ---------- ---------- ----------   835 C. scabra
---------- ---------- ---------- ---------- ----------   897 M. fisheri
---------- ---------- ---------- ---------- ----------   331 M. hamiltoni
---------- ---------- ---------- ---------- ----------   896 Galiteuthis sp.
---------- ---------- ---------- ---------- ----------   913 T. megalops
---------- ---------- ---------- ---------- ----------   851 Joubiniteuthis sp.
---------- ---------- ---------- ---------- ----------   836 P. microlampas
---------- ---------- ---------- ---------- ----------   404 E. higginsi
---------- ---------- ---------- ---------- ----------   899 A. pacificus
---------- ---------- ---------- ---------- ----------   515 H. miranda
---------- ---------- ---------- ---------- ----------   898 H. oceanica
---------- ---------- ---------- ---------- ----------   393 O. nielseni
---------- ---------- ---------- ---------- ----------   412 A. dux
------gttg tccctttatc aaattatctt tccttagctt tggatcttaa  2610 N. pompilius


tagtcagtca gtttttattt cgttttta                         2870 T. pacificus
---------- ---------- --------                          602 N. gouldi
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```
---------- ---------- --------                575 T. filippovae
---------- ---------- --------                491 N. sloanii
---------- ---------- --------                903 T. eblanae
---------- ---------- --------                859 I. coindetii
---------- ---------- --------                506 T. angolensis
---------- ---------- --------                886 S. oualaniensis
---------- ---------- --------                889 O. bartramii
---------- ---------- --------                636 T. angolensis
---------- ---------- --------                662 O. robsoni
---------- ---------- --------                635 A. dux
---------- ---------- --------                244 T. fimbria
---------- ---------- --------                604 O. aff. robsoni
---------- ---------- --------                910 Onychoteuthis sp.
---------- ---------- --------                835 C. scabra
---------- ---------- --------                897 M. fisheri
---------- ---------- --------                331 M. hamiltoni
---------- ---------- --------                896 Galiteuthis sp.
---------- ---------- --------                913 T. megalops
---------- ---------- --------                851 Joubiniteuthis sp.
---------- ---------- --------                836 P. microlampas
---------- ---------- --------                404 E. higginsi
---------- ---------- --------                899 A. pacificus
---------- ---------- --------                515 H. miranda
---------- ---------- --------                898 H. oceanica
---------- ---------- --------                393 O. nielseni
---------- ---------- --------                412 A. dux
tactaaaaca g--------- ---gcatt               2626 N. pompilius
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