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Comparative Study of Spectral Sensitivity, Irradiance Sensitivity, Spatial Resolution and Temporal Resolution in the Visual Systems of Aratus pisonii and Ocypode quadrata

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Nova Southeastern University
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Comparative Study of Spectral Sensitivity, Irradiance Sensitivity, Spatial Resolution and Temporal Resolution in the Visual Systems of *Ocypode quadrata* and *Aratus pisonii*

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To my friends, Marshall and Kathryn, thank you so much for driving me all the way down to the campus when I did not own a car.

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Abstract

Comparative Study of Spectral Sensitivity, Irradiance Sensitivity, Spatial Resolution and Temporal Resolution in the Visual Systems of *Ocypode quadrata* and *Aratus pisonii*

Autrum's studies (1950, 1958) on terrestrial arthropods first revealed that the visual systems of arthropods reflected their lifestyles and habitats, demonstrating that rapidly moving predatory diurnal species tend to have better temporal resolution than slower moving nocturnal species. In order to test Autrum's hypothesis that visual adaptions are driven by predator/prey interactions, the visual physiology of a nocturnal fast-moving predatory crab, the Atlantic ghost crab (*Ocypode quadrata)*, and a diurnal herbivorous crab, the mangrove tree crab (*Aratus pisonii*), was examined and compared. Spectral sensitivity, irradiance sensitivity, and temporal resolution of the crabs were quantified using the electroretinogram (ERG), while the spatial resolution was calculated utilizing morphological methods. Both *O. quadrata* and *A. pisonii* had a single dark-adapted spectral sensitivity peak (494 and 499 nm respectively) and chromatic adaptation had no effect on their spectral sensitivity, indicating that both species have monochromatic visual systems. The temporal resolution of *O. quadrata* was not significantly different from that of *A. pisonii*, but *O. quadrata* did possess a significantly greater spatial resolution and irradiance sensitivity. Both species possess an acuity zone in the anterior region of their eyes. The data presented in this study will aid in the current understanding of the correlation between visual physiology and the life history of the animal.

Key words: Crustacean vision, Terrestrial decapod, Visual adaptation, Visual physiology

1. Introduction

1.1 Background

The visual systems of most animals are highly evolved to efficiently extract visual signals from the background noise. Since the photons in the visual signals are limited in dim-light environments, inhabitants of these environments face the classic trade-off between sensitivity and resolution. The evolution to reach the best balance between sensitivity and resolution can occur in the composition of the visual pigments (Forward et al., 1988), the membrane properties of the photoreceptor cells (de Souza & Ventura, 1989; Laughlin & Wickström, 1993), and the structure of the eye (rev in Meyer-Rochow, 2001). Nocturnal animals and deep-sea animals require higher sensitivity (and the resultant lower resolution) to see in dim light (Frank, 2000; Johnson et al., 2002), while carnivorous (usually diurnal) predatory animals require higher temporal resolution (resulting in lower sensitivity) to track their prey (Howard et al., 1984; de Souza & Ventura, 1989; Laughlin & Weckström, 1993). It has been hypothesized that differences in organisms' visual systems result mostly from differences in their ecology – primarily habitat and lifestyle, which includes prey preferences and activity cycles (Autrum, 1958).

The animals in this study belong to the subphylum Crustacea, which consists of around 52,000 species worldwide (Cronin & Porter, 2008). Crustaceans occupy almost every conceivable niche within marine ecosystems (Cronin & Porter, 2008), and the visual systems of crustaceans are very diverse with respect to their eye morphology and physiology. This study sought to compare the visual physiology of *Ocypode quadrata* (Atlantic ghost crab) and *Aratus pisonii* (mangrove tree crab), two decapod crustaceans occupying different niches in South Florida, in terms of their spectral sensitivity, irradiance sensitivity, spatial resolution, and temporal resolution. These two species were chosen because of their vastly different feeding ecologies, providing an excellent test of Autrum's hypothesis that visual physiology is correlated with the life history of an animal.

1.2 Structure of Crustacean Compound Eyes

The two species in this study are decapod crustaceans, which all possess a compound eye consisting of tens to thousands of repeating units called ommatidia (Hodierna, 1644; Muller, 1826; rev in Land & Nilsson, 2012). Each ommatidium contains a corneal facet, a crystalline cone, and a rhabdom comprised of differing numbers of retinula cells (Figure 1). The corneal facet functions as a lens and composes the surface structure of compound eyes. A crystalline cone, functioning as a light guide, sits right below the corneal facet. Photosensitive pigments are located in retinula cells in the rhabdom.

There are two existing configurations, apposition and superposition, in the compound eye of modern crustaceans (Horridge, 1971; rev in Meyer-Rochow, 2001). In apposition eyes, each ommatidium is isolated from its neighboring ommatidia by screening pigment cells. The end of the crystalline cone is directly connected with the rhabdom, and the rhabdom only receives light from its corresponding facet (Figure 1A). Contrary to this, superposition eyes have a distinct clear-zone between the end of the crystalline cones and the rhabdom, allowing light from multiple facets to be focused on a single rhabdom (Horridge, 1971; rev in Cronin & Porter, 2008; Figure 1B). Compared to the apposition eye, superposition eyes have higher sensitivity, but due to the superposition of light from multiple facets, they sacrifice acuity. Apposition eyes can be mostly found in diurnal (day active) species, while superposition eyes are usually possessed by nocturnal (night active) species and deep-sea species (Land, 1984; rev in Meyer-Rochow, 2001).

Retinula cells are fragile and can be permanently damaged by strong light when there is a massive bleaching of the photopigment that spans the cell membranes (Shelton et al., 1985). The apposition optics that are found in most diurnal species protect the retinula from being exposed to too much light. However, some diurnal and shallow-water species also have superposition eyes, and during the day, the screening pigments migrate up between the crystalline cones, essentially turning these superposition eyes into functionally apposition eyes. At night, the screening pigment migrates back down below the retinula cells, functioning similarly to pupillary dilation of the human eye, to increase light sensitivity in dark

environments. However, the screening pigments are energetically expensive, thus many nocturnal and deep-sea crustaceans do not have screening pigments.

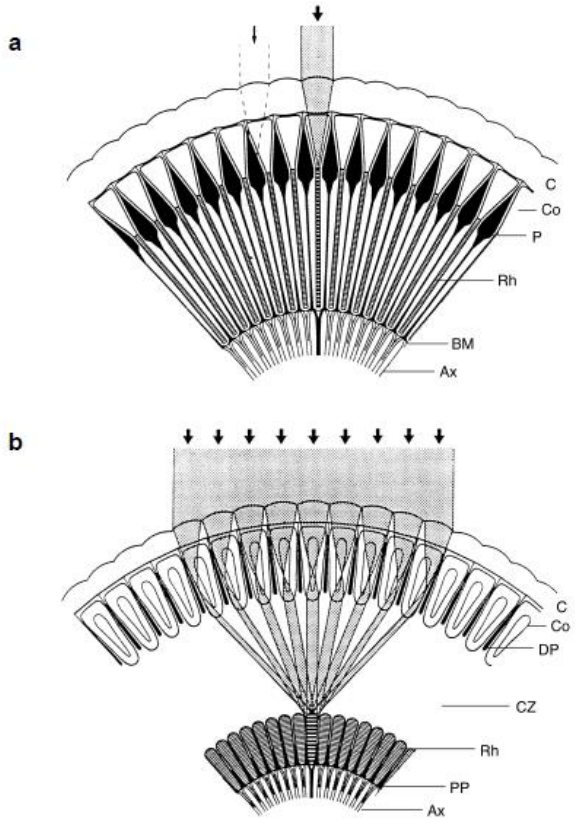

Figure 1. From Meyer-Rochow, 2001. Pathway of the light and structural elements in a model crustacean compound eye. (a) apposition eye. (b) superposition eye. The shaded areas show the light entering the rhabdom from a single facet in apposition eyes and multiple facets in superposition eyes. C=cornea, Co=crystalline cones, DP=distal screening pigment, CZ=clear-zone, Rh=rhabdoms, PP=proximal screening pigment, Ax=axons.

1.3 Spatial Resolution

The rhabdom is the location where the light stimulus is transduced into an electrical signal. It contains high concentrations of light-sensitive pigments called rhodopsins. Rhodopsins absorb light and convert the energy into electrochemical energy, which sensory cells transduce into electrical signals that transmit the visual information to the optic neuropils of the brain (rev in Rockstein, 2013). Johannes Muller's study (1826) on compound eye vision was the first to provide a hypothesis that described the image formation in the compound eye - Muller's mosaic theory. In this hypothesis, which was developed for insects with apposition optics, each ommatidium only detects the light at a small angle to its axis, and the visual fields of adjacent

ommatidia do not significantly overlap. Consequently, the contribution of all individual ommatidia in a compound eye results in a mosaic image. Modern studies on compound eye verified the mosaic hypothesis in terms of optics, elevating it to the level of a theory, but these studies also demonstrated that due to post-ommatidial neural processing, ommatidial visual fields do overlap (Wiitanen & Varela, 1971; rev in Rockstein, 2013), and the final image is not a mosaic.

Spatial resolution is the amount of detail that an eye can capture, and it can be directly quantified by measuring the interommatidial angle (△Φ), which is the angle of separation between adjacent ommatidial axes (Barlow, 1952; rev in Caves et al., 2016). Another parameter that can be used for studying visual acuity is cycles per degree (CPD), the angle subtended at the eye by two stripes in a grating composed of equal light and dark stripes, each pair of stripes being one cycle (rev in Feller et al., 2021). In other words, animals with high spatial acuity can distinguish very small stripes (small minimum separable threshold), while those with low spatial acuity can only distinguish very broad stripes. △Φ is calculated by dividing the facet diameter by the radius of curvature of the eye (Snyder, 1976; Figure 2), and CPD is the reciprocal of 2△Φ. The radius of the eye curvature and the radius of the whole eye are generally different in the arthropods with ellipsoid-shaped eyes, while in arthropods with spherical eyes, those values can be the same (Stavenga, 1979).

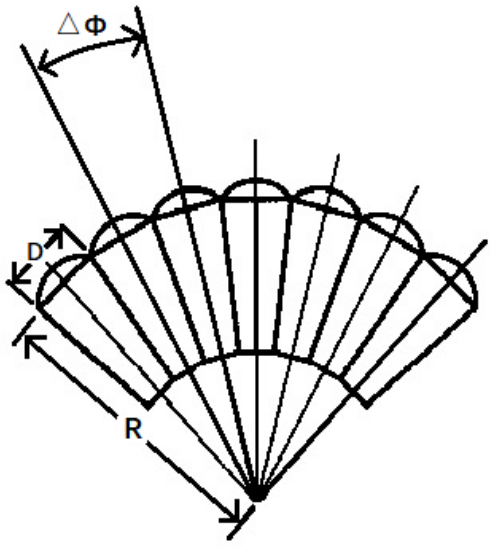

Figure 2. Parameters for measuring spatial resolution. Sagittal section of several ommatidia, where △Φ is the interommatidial angle, D is the facet diameter, and R is the radius of curvature.

Spatial resolution can also be measured through the behavioral method. For example, Caves et al. (2016) tested the spatial resolution of cleaner shrimps by measuring their optomotor responses (OMR). The study recorded the optomotor response by placing cleaner shrimps inside a rotating drum lined with vertical black and white strips. The shrimp will rotate in the same direction as the drum rotation if it can resolve the strip. The finest detail a shrimp can resolve will be the thinnest strip width to which the shrimp responds, and two times that width (one black + one white stripe) is interpreted as the animal's minimal spatial resolution.

Spatial resolution is inversely proportional to the $\triangle\Phi$, as an eye with a small $\triangle\Phi$ can resolve more details. Meanwhile, $\triangle\Phi$ can directly affect photosensitivity, because the light acceptance angle of each ommatidia can affect the number of entering photons. In eyes of the same shape and size, the larger the $\triangle\Phi$, the lower the spatial resolution, and the greater the photosensitivity. *Cirolana borealis*, as an example, is a marine isopod with extraordinary huge facets. This feature gives this species one of the most sensitive crustacean eyes known, but with poor spatial resolution (Nilsson & Nilsson, 1981). However, one way to increase spatial resolution without decreasing photosensitivity is to have a larger eye, or an ellipsoid eye rather than a spherical eye (Caves et al., 2018).

A pseudopupil is a dark spot seen around the center of a compound eye and can be an indicator of spatial resolution as well. This dark spot is caused by the absorption of incident light by the ommatidium directly facing the observer (Stavenga, 1979; Figure 3). The location of pseudopupil is not fixed as it moves across the facets of compound eye when the eye is rotated or the observer is moved (Zeil & Al-Mutairi, 1996). The size of the pseudopupil is directly proportional to the number of ommatidium that are viewed "head-on" and it sometimes varies markedly at different parts of the eye, which means spatial resolution is not evenly distributed on the entire eye (Zeil & Al-Mutairi, 1996). Many arthropods possess an acuity zone with extraordinarily high spatial resolution. The size of pseudopupil is much larger in the acuity zone and this feature can be used to locate the acuity zone of the eye. The spatial resolution could also be different in vertical vision and horizontal vision. For example, the pseudopupil increased in size vertically but not horizontally in the acuity zone of *Uca lactea annulipes* (Zeil & Al-Mutairi, 1996; Figure 3), which means that the spatial resolution in the

acuity zone is higher in vertical vision, but the same as that of the other parts of the eye in horizontal vision.

Figure 3. From Zeil & Al-Mutairi, 1996. The pseudopupils varied in shape and size in different parts of the compound eye of *Uca lactea annulipes* (Zeil & Al-Mutairi, 1996). Most members in the family Ocypodidae, including this species, has an acuity zone in the middle part of the eye.

1.4 Temporal Resolution

The temporal resolution of photoreceptors is a measure of how long the photoreceptors collect light before an electrical signal is sent to the brain (the integration time), and it is directly related to the speed of moving targets that the photoreceptor can track. A high temporal resolution requires responses being delivered to the brain in high frequency, which means less time to collect photons for each response. An animal with a higher temporal resolution will be great at tracking fast moving objects but will have trouble discerning contrast between an

animal and the background light in a dim light (nighttime or deep-sea) environment. Conversely, a low temporal resolution indicates that the eye takes more time to collect photons before sending a signal to the brain. Rapidly moving objects will look blurred for the animals with a lower temporal resolution, but the animals will have better contrast resolution in dim light environments, which means a greater photosensitivity (Laughlin & Weckström, 1993; Frank, 1999).

Photosensitivity is directly proportional to the size of facets, the length of rhabdoms, the $\triangle\Phi$, and the integration time. Therefore, photosensitivity is inversely proportional to both spatial resolution and temporal resolution, and an increase in photosensitivity is usually correlated with a decrease in temporal resolution and/or spatial resolution in eyes of the same size (Ruck, 1958; Frank, 2017; rev in Goldsmith & Bernard, 1974). The evolution of animals' eyes involved reaching the best balance between the resolution and irradiance sensitivity (Srinivasan & Bernard, 1975).

It is energetically expensive to have a good temporal resolution because the faster ion channels required for higher temporal resolution are metabolically more expensive than slower ion channels (Laughlin & Weckström, 1993). Therefore, evolution dictates that animals that are nocturnally active and need better contrast resolution have lower temporal resolution, as do those species that feed on slow moving or non-moving prey. Animals that are active predators and feed on fast-moving prey require higher temporal resolution, and in general, their prey provide sufficient energy to maintain the higher metabolic requirements of the fast ion channels required for higher temporal resolution. If an animal has reached the best balance between resolution and sensitivity, any additional improvement in temporal resolution will result in greater cost than benefit (Laughlin & Wickström, 1993). Therefore, evolution will not drive animals to unnecessarily improve temporal resolution.

Insects and crustaceans that have been studied to date show an improvement in the temporal resolution under light-adaptation (Laughlin & Weckström, 1993; Frank, 2003), with the exception of a few deep-sea crustaceans that show no change or even a slight decrease in their temporal resolution upon being light-adapted (Frank, 2003). In addition, the irradiance of the stimulus light used to measure the temporal resolution also affects the measurements

(Glantz, 1968; Laughlin & Weckström, 1993; Frank, 2017). As the stimulus light irradiance increases, the temporal resolution of most crustaceans increases gradually to a maximum, referred to as the critical flicker fusion frequency maximum (CFF_{max}) (Glantz, 1968; Figure 4). This is the maximum flicker rate of a light stimulus the eye is capable of following at any light intensity when measured electrophysiologically; if the flicker rate is above the CFF_{max}, then it looks like a steady glow rather than a flickering light, and the only response is when the stimulus is initially turned on. This parameter has often been used to determine the temporal resolution in crustaceans (Frank, 1999; Figure 4). The response latency is another indicator of temporal resolution, revealing the speed of light transduction. The response latency is defined as the elapsed time between the onset of the light stimulus and the onset of the photoreceptor response, and therefore it is inversely correlated with the critical flicker fusion frequency (Laughlin & Weckström, 1993; Figure 5A). Since the response latency also varies with light irradiance, it is usually measured with an irradiance that produces a response that is 50% of the maximum amplitude that the eye is capable of generating to the highest light irradiance.

Temporal resolution of an individual animal can also vary due to temperature (higher temperature results in higher temporal resolution in many species (Frank, 2017)) and ontogeny (in certain species). Frank's (2017) study on deep sea crustaceans demonstrated the significant effects of temperature and ontogeny on temporal resolution. The study tested the temporal resolution of juvenile and adult stages of *Gnathophausia ingens* and *Systellaspis debilis* under three different temperatures. Results indicated that the temperature significantly increased the CFF_{max} of adults and juveniles of both species. At the same temperature, the CFF_{max} of juveniles and adults was significantly different in *S. debilis* but not in *G. ingens*. Therefore, for any kind of comparative study of temporal resolution, it is important that the CFF_{max} is measured in individuals that have been thoroughly dark-adapted, that the temperature remains constant, and that experimental subjects are all around the same stage of their live history (i.e., either all juveniles or all adults).

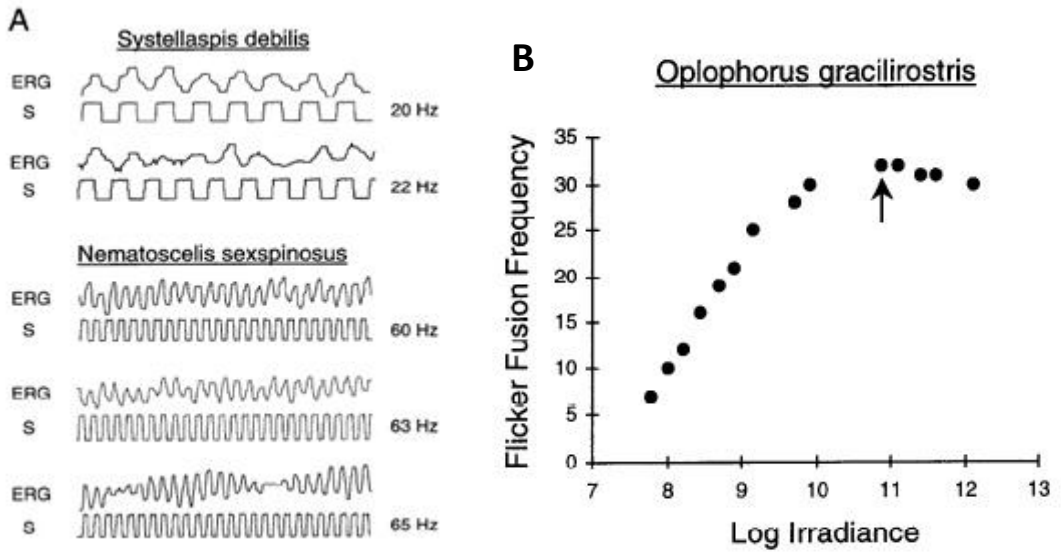

Figure 4. From Frank, 1999. (A) Examples of species with different the maximum flicker fusion frequency. Top trace (ERG) is the electroretinogram response recorded from the eye; lower trace (S) is the flicking light stimulus. (B) Flicker fusion frequency (Hz) as a function of irradiance (photons cm^{-2} s^{-1}) for *Oplophorus gracilirostris.* As irradiance increases, flicker fusion frequency increases up to the maximum critical flicker fusion frequency (arrow), the point at which further increases in irradiance do not lead to any increase in flicker fusion frequency.

1.5 Irradiance Sensitivity

Light irradiance is defined as the radiant flux (i.e., the power of light or photons per second) received by a surface per unit area, and the irradiance sensitivity is the characteristic that determines the photosensitivity of an eye. The irradiance sensitivity is usually different in animals living in different light levels. For example, crustaceans living in the deep sea generally have a higher photosensitivity for better vision in darkness (Frank, 2003). Irradiance sensitivity in the compound eye is determined by the eye structure, aperture size, and rhabdom properties (rev in Meyer-Rochow, 2001). Visual structures such as reflective tapetum or reduced lens can significantly increase the number of photons received by photoreceptors (Meyer-Rochow & Tiang, 1984; Palmer et al., 2018).

Aperture size of each ommatidium is another important factor that can affect irradiance sensitivity, as an aperture with a large surface area can receive more light. The aperture in an apposition eye is equal to the surface area of each corneal facet, while in the superposition, it

is equal to the surface area of multiple facets because the rhabdom in a superposition eye can receive light from neighboring facets (Horridge, 1971; rev in Meyer-Rochow, 2001; Figure 1). Therefore, superposition eyes generally have a better photosensitivity than apposition eyes, with the exception of apposition eyes with unusually large facets, such as *Cirolana* mentioned above. The size of facets is directly correlated with the size of ommatidia and the $\triangle\Phi$. Since the $\triangle\Phi$ is inversely proportional to the spatial resolution, irradiance sensitivity is often inversely correlated with the spatial resolution for eyes of the same size.

Irradiance sensitivity is also directly proportional to the response latency of the photoreceptor cell (Frank, 2003; Figure 5B) and therefore often inversely correlated with the temporal resolution (explained in section 1.4). The irradiance sensitivity in most crustaceans is affected by the dark/light adaptation (Meyer-Rochow & Tiang, 1984), because light adaptation can reduce response latency. Most crustaceans will maintain a greater irradiance sensitivity when dark adapted, but as the light level increases, many predatory species will sacrifice their irradiance sensitivity by decreasing the response latency to increase their temporal resolution.

Visual transduction, the process of light being converted into electrophysiological signal, is caused by the absorption of photons by photopigments and photons (rev in Meyer-Rochow, 2001). The absorption of photons results in a conformational change in the photopigment, opening ion channels, with the resulting ion flow producing an electrical signal. The electroretinogram is an extracellular signal that results from the summed mass response from multiple photoreceptor cells in response to a light stimulus. As the light irradiance increases, the amplitude (voltage) of this signal will gradually increase until it reaches the maximum amplitude (V_{max}). A response/stimulus curve, called V/log I curve, is usually generated to compare the irradiance sensitivities between different species (Figure 6). The logK, the log irradiance required to elicit a response that is 50% of V_{max}, and the dynamic range, log irradiance range between response limits of 5–95% V_{max}, are also additional measures of the photosensitivity (Laughlin & Hardie, 1978).

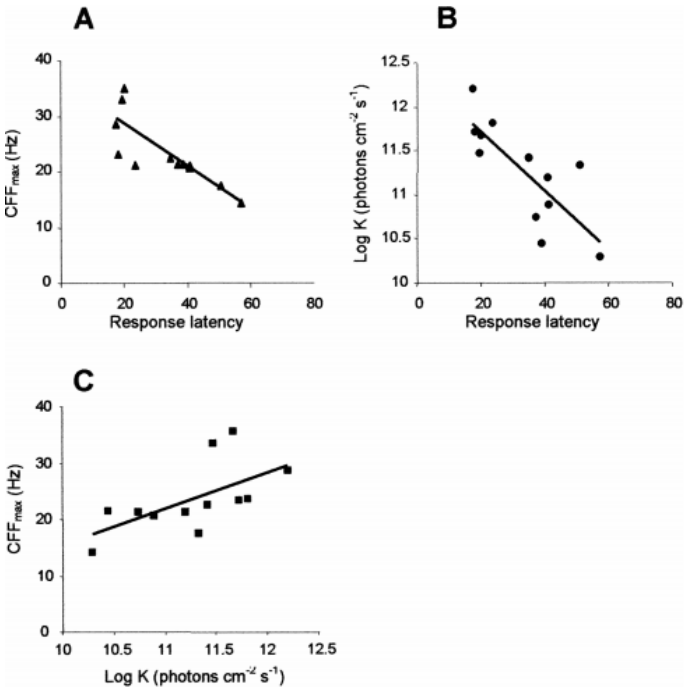

Figure 5. From Frank, 2003. The irradiance sensitivity and temporal resolution of 13 species of deep-sea crustaceans. (A) Correlation between response latency and CFFmax of dark-adapted eyes. Line is linear trend line fit to the data. (B) Correlation between response log K (irradiance required to generate 50% V_{max} amplitude response) and response latency. (C) Correlation between log K and CFF_{max}.

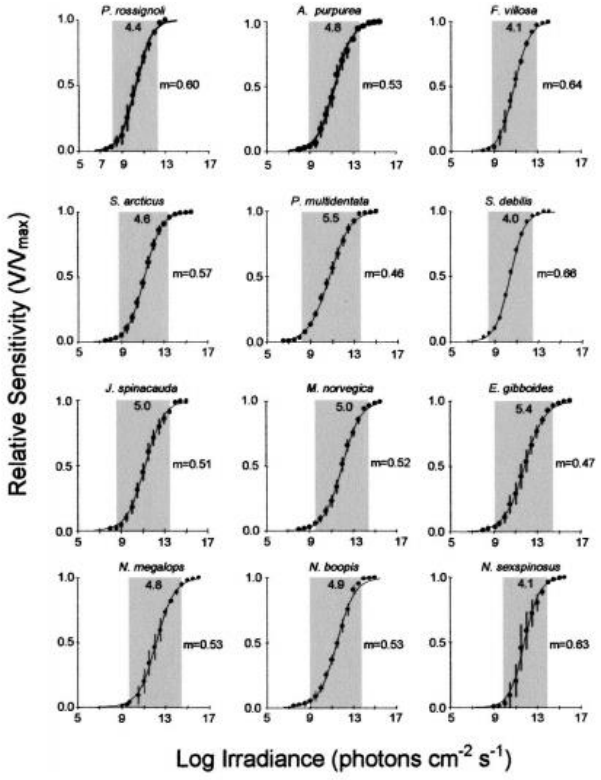

Figure 6. From Frank, 2003. The V/logI curves of 12 species of deep-sea crustaceans. Shaded boxes indicate dynamic range; numbers at the top indicate the size. m = slope of curve.

1.6 Spectral Sensitivity

Most decapod crustaceans have two classes of photoreceptor cells. Seven anatomically similar photoreceptor cells (called R1-7) form the main portion of the rhabdom, and one separate cell (called R8) is located at the distal part of the rhabdom (Eguchi & Waterman, 1967; Cummins & Goldsmith, 1981). R1-7 cells contain blue-green sensitive visual pigments, while the R8 cell contains violet-ultraviolet sensitive visual pigment in those species for which these data are available (rev in Marshall et al., 2003). R8 cells in some decapods are reduced or lost, resulting in these species not being able to sense violet or near-UV light (Cummins & Goldsmith, 1981; rev in Marshall et al., 2003).

Spectral sensitivity is the ability of an eye to detect light as a function of wavelength. Animals are more sensitive to the light (i.e., it takes less light to generate a response) with a wavelength closer to the wavelength maximally absorbed by their visual pigments. There are two existing hypotheses explaining the relationship between spectral sensitivity and the light spectrum in the habitat. The Sensitivity Hypothesis states that the photopigment matches the spectral composition of light in its habitat for maximum sensitivity to the available light (Clarke, 1936; Munz, 1958). The Contrast Hypothesis states that photopigment has evolved to maximize the contrast between the light reflected from objects of interest and the background light (Lythgoe, 1968).

The Sensitivity Hypothesis can be best explained by comparing the sensitivities of crustaceans living in different habitats (Figure 7). Even though a small amount of water is colorless, rivers and oceans appear to have color because of the selective absorption and scattering effects of water. Longer wavelengths of light, such as red and orange, are the wavelengths most strongly absorbed by water. As a result, long wavelength light disappears first as the sunlight travels through the water, and even the green light will disappear if the light travels for a longer distance. The wavelength of light that penetrates best in clear oceanic water is 475 nm, which is blue, and the spectrum shifts toward green in more coastal, turbid water. The spectral range might even shift toward yellow in freshwater environments (Jerlov, 1976). R1-7 spectral sensitivity of coastal crabs that live in greener water have maxima between 483

to 516 nm (Forward et al., 1988), while the crustaceans that live in bluer open water have sensitivity maxima between 460 and 490 nm (Frank & Widder, 1999).

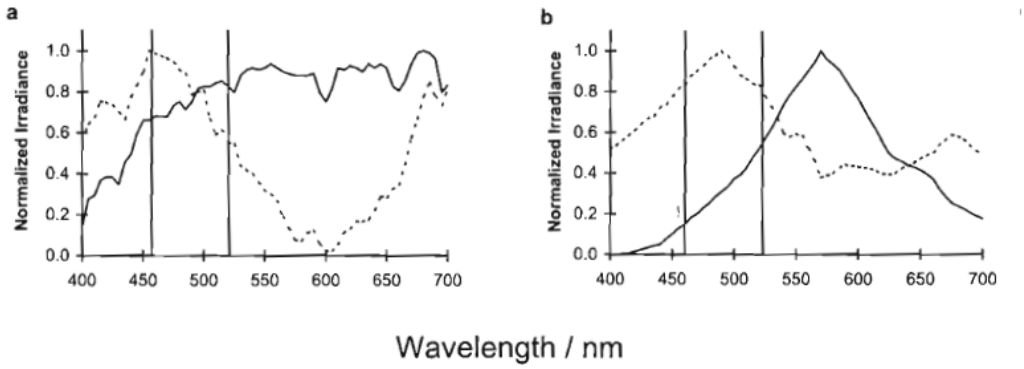

Figure 7. From Munz & McFarland, 1977. The Sensitivity Hypothesis states that the spectral sensitivity is matched to the spectral composition of light in its habitat. (Clarke, 1936; Munz, 1958) (a) Light irradiance at 3m in clear oceanic reef water at noon (solid line) and after sunset (dotted line). (Data from Eniwetok atoll in the Pacific Ocean - Munz & McFarland, 1973.) R1-7 cell sensitivities in most monochromatic and dichromatic marine crustaceans range between the vertical solid lines. (b) Light at noon (solid line) and just after dark (dotted line) in an estuary. Crab R1-7 spectral sensitivities lie between the vertical solid lines. (Light data from Munz & McFarland, 1977)

A spectral sensitivity that is exactly matched to the background light is good for detecting a dark item against the brighter background. On the other hand, the spectral sensitivity maximum should be offset from the background light in order to detect bright targets against a dark background, in order to maximize the contrast between the light reflected from the target and the background light (Lythgoe, 1968). The spectral sensitivity of oplophorids and sergestids, two groups of deep-sea crustaceans, is offset from the downwelling light maximum and bioluminescent sources (Frank & Widder, 1999), which can potentially be explained by the Contrast Hypothesis (Figure 8).

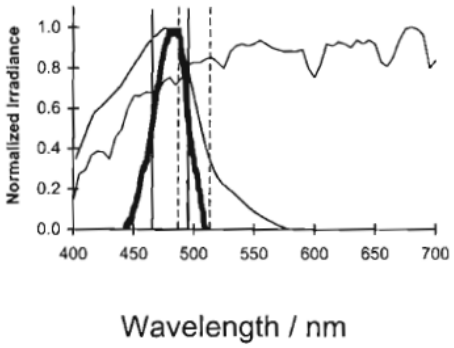

Figure 8. From Marshall et al., 2003. A spectral sensitivity offset from the maximum irradiance is useful to detect light objects against a darker background. Sensitivity of oplophorids and sergestids is offset from the light in their habitats. Normalized light irradiance at 3 m, 60 m, and 335 m (progressively thicker lines) in clear oceanic water. Spectral sensitivity maxima of euphausiids lies between solid vertical lines; spectral sensitivity maxima of oplophorid and sergestid shrimps lies between dotted vertical lines

1.7 Methods of Investigating Visual Systems

There are several methods for testing an organism's visual capabilities. Behavioral studies seek to observe a behavioral response of an animal to a stimulus light. The results can directly illustrate the relationship between the stimuli and responses. However, the result might not be accurate since captive environments can alter the behavior of most wild animals (Okuno, 1963).

Histological studies illustrate the detailed physical structure of an eye. Images from light microscopy can provide multiple important characteristics of an eye. The presence of a clear-zone can be used as evidence for determining if the eye is of the superposition type (Horridge, 1971), while the density of facets will be used for calculating the spatial resolution. Histological studies do not require live animals, but only well-preserved tissues. However, the result is limited to the structure of the eye and cannot illustrate any physiological characteristic, such as temporal resolution.

Spectrophotometry is a technique to measure the spectral absorbance of liquid solutions. It has been widely used to assess the spectral sensitivity of compound eyes. Visual pigments in compound eyes are extracted with a solution and examined by a spectrophotometer. The result will not be affected by other factors, such as the animal's behavior and the environmental conditions. Therefore, this study can be extremely precise. However, the results might be offset

from whole eye spectral sensitivity because extracted photopigments might not behave the same *in situ* as they do in solution (Bruno & Goldsmith, 1974; rev in Johnson et al., 2002), and the effects of any screening pigments are not taken into account.

Microspectrophotometry (MSP) is a technique similar to spectrophotometry but measures the spectral absorbance of visual pigments *in situ* (i.e., on frozen sections of the rhabdom). MSP directly measures intact rhabdoms and provides excellent information on the absorbance of the photopigments within the eyes. However, MSP may not show the accurate spectral sensitivity of the eye, as any filtering effects of screening pigments and/or pre-retinal tissues are eliminated in isolated rhabdoms (Wald, 1967; Cummins & Goldsmith, 1981; Frank & Case, 1988).

An electroretinogram (ERG) records electrical activity of retinal cells in response to the absorption of photons. A photoreceptor cell will provide an electrical response to a stimulus light flash, and the ERG, being an extracellular signal, records the summed mass response of multiple photoreceptor cells simultaneously. ERG is a very efficient way to study an animal's visual capabilities, because it directly records the responses in the receptor layer that takes into account any changes caused by pre-retinal filtering (Bryceson, 1986) and can be used to measure photosensitivity, spectral sensitivity and temporal resolution. However, the drawback of electrophysiological studies is that they need to be conducted in living animals.

Each method has its own strengths and weaknesses. An ERG was used to determine the spectral sensitivity of crustaceans in this study, because the ERG provides more accurate data than either spectrophotometry or MSP. Another reason for using the ERG is that it is also able to measure the temporal resolution and irradiance sensitivity in addition to spectral sensitivity. In addition, histological techniques were used to study the spatial resolution and eye structure of crustaceans. The combination of electrophysiology and histology has been used in many previous studies of the visual physiology of crustacean eyes (Bernhard et al., 1963; Jacklet, 1969; Caves et al., 2016). The advantage of this combination is that the aspects of the visual system that need to be known to understand how well these visual systems are adapted to the habitat and life history of the organisms - spatial resolution, temporal resolution, irradiance sensitivity, and spectral sensitivity, can all be determined with these two techniques.

1.8 Objectives

Vision provides vital information to all animals with photoreceptors, and different species have different visual systems depending on their lifestyles and the environments they live in. Therefore, the study of visual physiology provides important information on the ecology of a species. This study will conduct research on the vision of crustaceans. Crustaceans serve as an indispensable food source of many marine organisms, including many commercial fishes (Szaniawska, 2018). Many crustaceans are opportunists and have a wide range of food preferences, consequently increasing the stability of an ecosystem (Covich et al., 2010; Szaniawska, 2018). Crustaceans can also serve as indicator animals for accessing the impact of human activity and climate change, as they are very sensitive to the influence of biotic and abiotic factors (Hayden & Dolan, 1974; Abduho & Madjos, 2018). This study will contribute to the understanding of the visual ecology and physiology of crustaceans, therefore benefitting marine conservation efforts.

2. Methods

2.1 Species

Figure 9. The Atlantic ghost crab (A) and the mangrove tree crab (B).

The Atlantic ghost crab, *Ocypode quadrata*

The Atlantic ghost crab, *Ocypode quadrata* (Figure 9A), is a carnivorous crab of the family Ocypodidae inhabiting the sandy beaches from Block Island, Rhode Island, USA to Santa Catarina, Brazil (Williams, 1984). The ghost crab is nocturnal and forages actively from sunset till dawn (Wolcott, 1978). Ghost crabs are sand burrowers in the intertidal zone (Figure 10A) and will only get into the water for moistening their gills (Williams, 1965), as they will drown if submerged in water for too long. The muscles of their leg have high tetanus fusion-frequencies, of the order of 90 Hz, which allows for extremely rapid locomotion (Hafemann & Hubbard, 1969). The speed of ghost crabs is correlated with their body size and most of the crabs can reach a speed of over 2m/s (Burrows & Hoyle, 1973). The fast speed of ghost crabs allows them to escape from predation (Burrows & Hoyle, 1973) and actively hunt for prey (Wolcott, 1978).

Ghost crabs are mainly active predators and feed exclusively on other smaller crustaceans and mollusks (Wolcott, 1978). In addition, ghost crabs are also scavengers and will consume dead marine animals washed up on the coasts. As the ghost crabs occupy an important role in the food web of intertidal habitats, ghost crabs can be a great biological indicator for the health status of the coastal areas and their population density is correlated with the level of anthropogenic impacts on beaches (Aheto et al., 2011; Noriega et al., 2012). Many studies have

shown a reduction in the population densities of the Atlantic ghost crab in regions disturbed by humans (Barros, 2001; Neves & Bemvenuti, 2006; Magalhães et al., 2009).

Most crabs in the genus Ocypode, including the *Ocypode quadrata*, have sensitive auditory systems for interspecific communication and prey detection (Horch & Salmon, 1972; Clayton, 2008). During courtship, the male ghost crab hits the ground with the major cheliped to produce long vibrational sounds. If attracted to the male, a female ghost crab will approach the male by moving toward the sound source (Clayton, 2008). The ghost crab also has a well-developed olfactory system and can distinguish a wide range of odor cues, which allows the crab to be an excellent predator and scavenger (Wellins et al., 1988).

Forward et al. (1988) attempted to use MSP to investigate the spectral sensitivity of *Ocypode quadrata* but were not able to collect any data because of the heavy coatings of screening pigment granules. The main function of screening pigment granules is to absorb unwanted stray light (Stavenga, 1989). The screening pigment in the ghost crab is mobile, moving up between the ommatidia during the day to protect the rhabdoms from too much light. At night, the screening pigments retract, allowing rhabdoms to receive more light, which results in increased photosensitivity. These screening pigment granules, located in the pigment cells between retinula cells (Insausti, 2013), interfere with measurements of the absorption spectra of the visual pigments.

Figure 10. Dwelling habitats of *O. quadrata* and *A. pisonii*. *O. quadrata* is usually buried in sandy beaches during the daytime (A), while *A. pisonii* primarily lives in mangrove forests (B).

The mangrove tree crab, *Aratus pisonii*

The mangrove tree crab, *Aratus pisonii* (Figure 9B), is an arboreal crab of the family Grapsidae inhabiting the mangrove forests of the western Atlantic from central Florida to Brazil and the eastern Pacific from Sonora to Peru (Rathbun, 1917). This crab is diurnal and lives above water on the root, trunk and branches of mangrove trees, especially the red mangrove (Conde & Diaz, 1989, Figure 10B). The mangrove tree crab is great climber and can even jump from tree to tree. The dorsal side of the crab's carapace has a similar color to tree bark, which gives them great camouflage in mangrove forests. However, if spotted by a predator, the crab is not able to move rapidly. Instead, its defense mechanisms against predation will be either moving to the back side of the tree or falling into the water.

Studies on the diet of the mangrove tree crab indicate that they are herbivore and mainly feed on mangrove leaves and arboreal algae (Beever et al., 1979). However, the study by Conde & Diaz (1989) suggested that the mangrove tree crab might also be an omnivorous scavenger, since the crab is able to survive on an omnivore diet for a very long time in laboratory. There is no direct observation of a mangrove tree crab scavenging on an animal corpse in the wild, but researchers found that fish meat bait could occasionally attract mangrove tree crabs. Animal corpses are very rare in mangrove forests but as they contain high concentrations of nutritious proteins, they would be extremely valuable for mangrove tree crabs. Upon an incidental encounter of an animal corpse, the crab may switch briefly from being an herbivore to being a scavenger.

The mangrove tree crab is a keystone species in the mangrove forest ecosystem (Beever et al., 1979). It is one of the very few species that can exclusively feed on mangrove leaves and then bring the energy to the higher trophic levels. They constantly transfer the energy and biomass from arboreal mangrove habitat to the surrounding aquatic system in the form of frass (the excrement of plant-eating invertebrates) and offspring (Beever et al., 1979). In Florida, the average output of eggs of mangrove tree crab in mangrove forest, with a density of 2.8 mature females per m^2, is 207 eggs per day per m^2, with 99.959% of the larvae and young crabs being consumed by other aquatic organisms. After an egg successfully reached adulthood, a single adult crab can consume 35.3 cm^2 of leaf area per month and produce about 0.25 cm^3 of frass/

cm^2 of red mangrove. This eventually results in one mangrove tree crab introducing 8.8 cm^3 of frass into the aquatic system each month.

The vision of *Aratus pisonii* has never been studied. The evolution of the visual system of the mangrove tree crab should mainly be driven by the predation pressure, since most of the mortality in the population is caused by predation. Foraging might not be a strong factor acting on the evolution on the visual system of this crab, because there is never any shortage of mangroves leaves in mangrove forests.

2.2 Specimen Collection and Maintenance

Aratus pisonii were collected from the mangrove forests, and *Ocypode quadrata* were collected from the intertidal zones of exposed sandy beaches in Hollywood, Florida. All animals were transported to the laboratory in plastic containers containing seawater from the collection site and were kept in complete darkness until the experiments. The temperature of the laboratory remained constantly at 21.8 Celsius. Fresh mangrove leaves were supplied in abundance in the container of *Aratus pisonii*, while fish meat was provided to *Ocypode quadrata* once every two days. The seawater was changed daily, and all animals were used for experiments within 10 days of collection.

2.3 Electrophysiological Recordings

The electrophysiological recording method was based on methods used by Frank et al. (2012). All animals were dark adapted in a dark room for seven hours prior to the experiment. Animals were prepared for recordings under dim red light and remained alive during the experiments. For both species, the chelipeds were autotomized by applying pressure to the merus with a hemostat in order to prevent the animals from pulling the electrode out from their eyes. A drop of Cyanoacrylate glue was applied to the base of eye stalk to stabilize the eye, and the carapace was glued to a plastic holder. Animals were suspended in a seawater bath inside a Faraday cage that was covered with a lightproof sheet. The animal body was

submerged in the seawater with only the dorsal surface of the eye slightly above the level of the water. A glass insulated tungsten microelectrode (Frederick Haer Corp. Inc.) was placed into the eye with the aid of a dissecting microscope (Olympus Corp.). A silver chloride electrode was placed in the water near the animal to ground out the background electrical noise in the water bath. Once prepared, animals were allowed to dark-adapt for 1 hour prior to experiments, since the animals might be slightly light-adapted under the dim red light.

Signals were amplified with an X Cell-3 Microelectrode Amplifier (FHC, Inc.) with a high impedance probe. Level of amplification was set to 2000X, and filters were set between 1-1000 Hz. Data were displayed on a laptop computer (PowerBook Titanium G4, Apple Inc.) and then digitized using a program written in LabView (National Instructions, Austin, TX, USA) and stored for later analysis.

2.4 Optical Apparatus and Light Stimuli

The pathway of a light stimuli from the light source to the tested eye was in the following order: Light source, monochromator, shutter, neutral-density filter wheel, light guide, and the eye. The full spectrum light was first adjusted to monochromatic light at the tested wavelength by a monochromator (CM110 monochromator, Spectral Products, Putnam, CT, USA). Flash duration was regulated by a computer-controlled shutter (Model VS14, Vincent Associates, Rochester, NY, USA), and light irradiance was controlled with a neutral-density filter wheel driven by a computer-controlled stepper motor (all control programs were written in LabView). Test flashes of light were transmitted to the eye through a one end of a bifurcated light guide composed of randomized silica fibers that had been positioned close to the eye so that the entire eye was bathed in light.

The light irradiance was measured at each wavelength with an optometer (UDT Instruments, San Diego, CA, USA). These light calibration data were in μW cm^{-2} and then were converted to photons cm^{-2} s^{-1}, because the number of photons within 1 microwatt of light varies at different wavelength and the response of photoreceptor cell is based on the number of photons absorbed. The conversion was done using the following equation: irradiance [in

photons cm^{-2} s^{-1}] = 5035000000* wavelength [in nm] * irradiance [in μW cm^{-2}]

2.5 Electrophysiological Experiments Procedure

Spectral Sensitivity

A dim test flash of constant wavelength and irradiance was given at the beginning of the experiment, and the measurements began when the response to the test flash had not changed for 1 hour. The eye was then stimulated with stimulus flashes of monochromatic light, and flash irradiance was adjusted until the eye produced a criterion response of 50 μV. Duration of each flash was 0.1 second, and the wavelengths of the flashes were in random order, ranging from 380-600 nm in increments of 10 nm. A dim test flash was given after testing each wavelength in order to ensure the eye remains fully dark-adapted. Data were plotted as the inverse of the irradiance required to evoke the criterion response at each wavelength and normalized to the wavelength of maximum sensitivity.

Irradiance Sensitivity

Voltage versus log irradiance (V/logI) curves were generated from measurements made in dark-adapted eyes to compare the irradiance sensitivity of two species. The dark-adapted eyes were stimulated with 0.1 second test flashes of increasing irradiances of 490 nm monochromatic light. The first stimulus flash was the dimmest flash and the irradiance started at around 10^8 photons cm^{-1} s^{-1}. The irradiance of each following flash was increased by half log unit until the response was saturated. To ensure that the stimulus was given to a fully dark-adapted eye, a dim test flash was administered after each stimulus, and no further stimulus flashes were given until the response to the test flash had recovered to the dark-adapted level. The V/log I curves were plotted with the Zettler modification of the Naka-Rushton equation (Naka and Rushton, 1966; Zettler, 1969): $V/V_{max}=I^m/(I^m+K^m)$, where I is stimulus irradiance, V_{max} is maximum response amplitude the eye is capable of generating, and K is the irradiance yielding a response that is 50% V_{max}. The dynamic range of 5-95% V_{max} was also labeled in the curves.

Temporal Resolution

Temporal resolution was examined by determining the CFF_{max} and the response latency. CFF_{max} was tested under both dark-adapted and light adapted condition, while the response latency was tested in dark-adapted eyes. In the dark-adapted test, all animals remained fully dark-adapted, because light adaptation could affect the temporal resolution in certain species. A stimulus of 490 nm flickering monochromatic light was presented to the dark-adapted eye, and the flicker frequency was increased until critical flicker fusion was achieved. The flickering light stimulus was generated by a computer-controlled electromagnetic shutter with a constant 50% duty cycle (50:50 light: dark ratio). To ensure that the eye remains dark-adapted, a dim test flash was given between each measurement, and subsequent flickering stimuli would not be given until the amplitude of the test flash had returned to its dark-adapted level. Irradiance was then increased by one log unit, and the flicker rate of the stimulus light was increased until critical flicker fusion is again achieved. CFF_{max} was determined as the point at which the eye could no longer respond to each individual flash of light. The response latency, equal to the elapsed time between the onset of stimulus and the onset of response, was measured under a test flash with an irradiance of K (measured in irradiance sensitivity test), which is the irradiance that produced a response that was 50% of the V_{max}.

2.6 Light and Electron Microscopy

Scanning Electron Microscopy (SEM)

After the electrophysiology experiments, animals were anesthetized by placing them in seawater at 4°C for 3–4 minutes. A scalpel was used to cut off the eyes for SEM and histology studies. Samples were stored in 2% glutaraldehyde in seawater at 4°C.

Post-fixation for scanning electron microscopy was in 1% OsO4 (osmium) in phosphate-buffered saline (PBS) for 1.5 hours, followed by three 15-minute washes in buffer. Samples were dehydrated through a sequence of 20%, 50%, 70%, 95%, and 100% ethanol for 15 minutes each. The samples were then dried in 3 changes of hexamethyldisilazane (HMDS) for 5 minutes each. Following drying, samples were coated with a thin (20 nm) layer of Pd in a

Cressingtin 108 Manual Sputter Coater. Imaging was in a Philips XL-30 Field Emission Scanning Electron Microscope at varying magnifications. Images were digitally recorded.

Spatial Resolution and Histology Experiments

It was not known whether the animals have apposition or superposition eyes prior to the experiment. The clear-zone in superposition eyes would be heavily covered with screening pigments if the eyes were light adapted, which will make it extremely difficult to differentiate apposition from superposition eyes. Therefore, the collection of the eyes for histology was conducted under red light so that all specimens were fully dark-adapted.

To make sure the fixative penetrated to the center of the eye, each eye was cut into two equal sagittal sections before fixation in a mixture of 2.5% glutaraldehyde and 3.7% formaldehyde in seawater at room temperature (Alkaladi & Zeil, 2014). Samples were subsequently washed in three changes of seawater for 5 minutes each. The eyes were dehydrated through a sequence of 20%, 50%, 70%, 90%, 95%, and 100% ethanol for 15 minutes each. Samples were embedded in paraffin wax and sectioned at a thickness of 5 μm.

The tissue sections were then deparaffinized, hydrated with water, and then stained for 5 minutes using Mallory-Heidenhain Stain, which consists of 1.0 gram of Phosphomolybdic or phosphotungstic acid, 2.0 grams of Orange G, 1.0 gram of water-soluble aniline blue, and 3.0 grams of acid fuchsin in 200 mL distilled water.

Sections were viewed and photographed under a light microscope, and the digital images were analyzed using Image J. The following structures were examined histologically: screening pigments, the presence or absence of clear-zone, rhabdoms and facets. The interommatidial angle ($\triangle\Phi$) was used to quantify spatial resolution and was calculated by dividing the facet diameter by the radius of the eye curvature. The local curvature was measured by fitting circles to the images of eyes, with the radius of the eye curvature being equal to the radius of the circle (following Baldwin Fergus et al., 2015).

In order to determine if the animal possessed an acuity zone, the pseudopupils of both species were examined visually. The live animal was placed 20 cm in front of the eyes of the

observer and rotated 360° vertically and then 360° horizontally. Any increase in the size of pseudopupil would indicate the presence of an acuity zone. After an acuity zone was found, the spatial resolution was quantified from the $\triangle\Phi$ in the acuity zone, using the same measurement for calculating the $\triangle\Phi$ in non-acuity zone. Photos of pseudopupils in the anterior, dorsal, and posterior of the eye were taken with a camera (Canon Inc.) connected to a stereo microscope (Meiji Techno).

2.7 Statistics

The CFF_{max}, the response latency, the interommatidial angle and the cycles per degree in the non-acuity zone, the interommatidial angle and the cycles per degree in the acuity zone, the rhabdom length, and the facet diameter were analyzed statistically to determine if there were significant differences between *O. quadrata* and *A. pisonii*. A Shapiro-Wilk test was used to test for normality, and a two-sample t-test was used to analyze normally distributed data, while a two-sample Mann-Whitney Wilcoxon test was used to analyze non-normal data. All statistical analyses were conducted using the statistical software package R and null hypotheses (no difference between the two species for each of the factors mentioned above) were rejected when $p \leq 0.05$.

3. Results

3.1 Spectral sensitivity

Each species showed only one peak in the dark-adapted spectral sensitivity curve, with peak sensitivity of 494 nm (*O. quadrata*) and 499 nm (*A. pisonii*). Chromatic adaptation at a wavelength of 500 nm was used to determine whether the animals have additional photopigments. The chromatic-adapted spectral sensitivity curves of both species are the same as their dark-adapted curves, which indicates that each species has only one blue sensitive photopigment. Any flashes at a wavelength below 400 nm or above 600 nm cannot evoke a criterion response of 50 μV, and therefore the range of the sensitivity curves is between 400 and 600 nm (Figure 11).

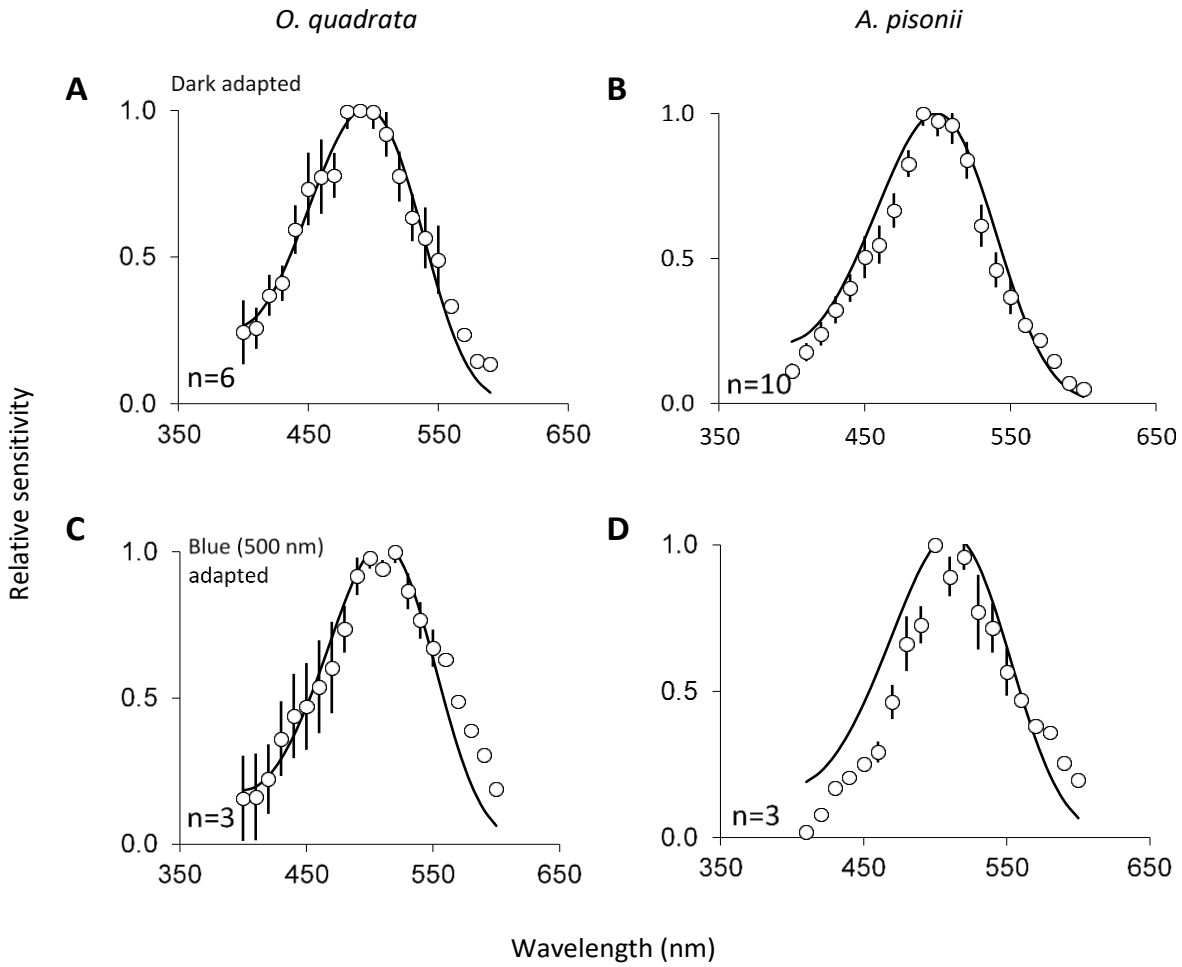

Figure 11. Spectral sensitivity curves for *O. quadrata* and *A. pisonii*. The dark-adapted spectral sensitivity curves for both *O. quadrata* (A) and *A. pisonii* (B) show a single sensitivity peak in the blue wavelengths. Under 500 nm chromatic adaptation, there was no change in the

shape of the spectral sensitivity curve for either *O. quadrata* (C) and *A. pisonii* (D). Data points represent the inverse of the irradiance required to evoke the criterion response at each wavelength and normalized to the wavelength of maximum sensitivity. Error bars represent standard error of the mean (S. E. M). Solid lines are the best-fit absorbance curves.

3.2 Temporal Resolution and Irradiance Sensitivity

The dark-adapted CFF_{max} of both species was the same, and both species had a higher CFF_{max} when light-adapted than when dark-adapted (Table 1). The light-adapted CFF_{max} is statistically different between *O. quadrata* and *A. pisonii*, even though the absolute difference was only 1.5 Hz. There is also no significance difference in response latency between the two species.

Table 1. CFF_{max}, latency of 50% V_{max} response, and logK in *O. quadrata* and *A. pisonii*.

	O. quadrata	*A. pisonii*	
CFF_{max}: dark-adapted (Hz)	33.7±0.3 (n=3)	33.5±0.2 (n=6)	p=0.89
CFF_{max}: light-adapted (Hz)	41.8±0.4 (n=5)	43.3±0.2 (n=6)	p=0.016 *
Latency of 50% V_{max} (s)	0.0237±0.0003 (n=3)	0.0228±0.0007 (n=5)	p=0.51
logK (log photons cm^{-2} s^{-1})	12.48 (n=3)	13.62 (n=4)	p=0.00005 *

Sample size (n) indicates number of individuals per species, and values are means±S.E.M. Asterisk indicates a statistically significant difference.

The logK value was significantly lower in *O. quadrata* than in *A. pisonii*. The dynamic ranges of both *O. quadrata* and *A. pisonii* were both approximately 3 log units (Figure 12).

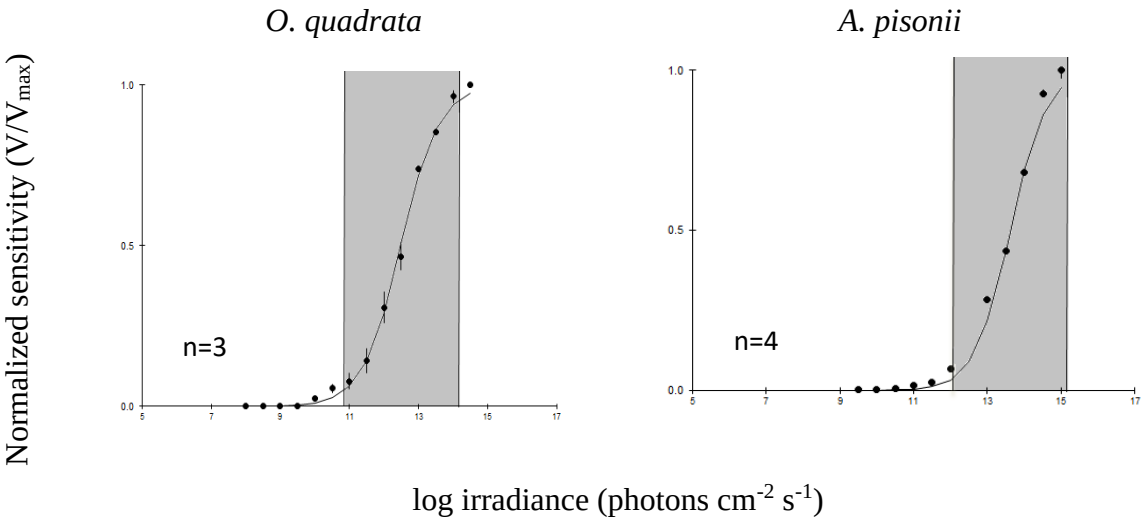

Figure 12. V/logI curves for *O. quadrata* and *A. pisonii*. Data points represent the mean of irradiance sensitivity data normalized to V_{max} for each individual; error bars are S. E. M. Solid curves are best fit (Excel solver) to the Naka–Rushton equation, and the shaded areas represent the dynamic range between 5% V_{max} and 95% V_{max}.

3.3 Eye Structures and Spatial Resolution

O. quadrata has apposition eyes, as the end of the crystalline cone is directly connected to the rhabdom (Figure 13A). The shape of the eyes is between a hemisphere and an ellipsoid (Figure 13B). The diameter of facets does not change within the eye, while the radius of eye curvature and the size of pseudopupil increased in the middle anterior region of the eye, indicating that the *O. quadrata* eye possesses an acuity zone (Figure 13B). The pseudopupil of *O. quadrata* in the acuity zone only elongated vertically but not horizontally (Figure 14), and therefore the spatial resolution varied between their vertical vision and horizontal vision. The $\triangle\Phi$ measured horizontally was 1.36°, which is the same as the $\triangle\Phi$ measured vertically in the bottom and top parts of the eye. In the middle anterior region of the eye, where the acuity zone is found, the $\triangle\Phi$ is 0.42°.

A. pisonii also has apposition eyes (Figure 13C). The body size of the male crabs is slightly

larger than that of the females, and consequently the eye radius of most males was slightly larger than that of the females. Nevertheless, in *A. pisonii*, any increase in eye radius also proportionally increased the diameter of facets, and therefore interommatidial angle and spatial resolution were not affected by the variation in body sizes. The shape of the eyes is a hemisphere (Figure 13D), with a $\triangle\Phi$ of 1.87°. An acuity zone with a $\triangle\Phi$ of 1.21° was found in the anterior region of the eye. Spatial resolution does not vary between the vertical and horizontal vision in *A. pisonii*, and the pseudopupil in the acuity zone increased in size both vertically and horizontally (Figure 14).

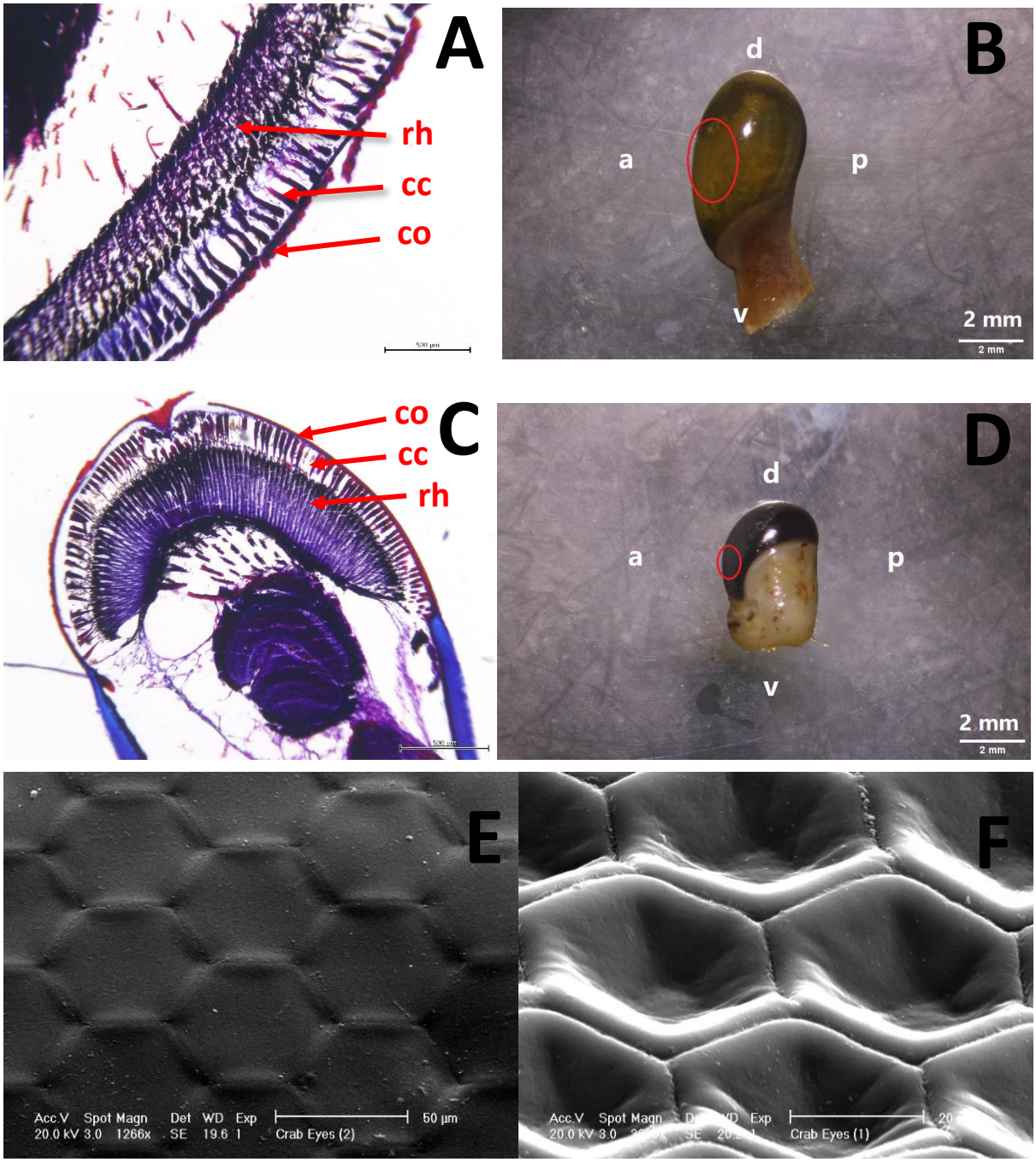

Figure 13. Light (A,B,C,D) and Scanning Electron Microscopy (E,F) of *O. quadrata* (A,B,E) and *A. pisonii* (C,D,F). There is no clear-zone between the crystalline cones and rhabdoms in *O. quadrata* (A) or *A. pisonii* (C). *O. quadrata* has ellipsoid eyes (B), while *A. pisonii* has spherical eyes (D). In both species, the shape of the facets (E,F) is a hexagon. a, anterior; p, posterior; d, dorsal; v, ventral; cc, crystalline cones; co, cornea; rh, rhabdom. Red circle indicates the position of the acuity zone.

Figure 14. Pseudopupils of *O. quadrata* and *A. pisonii.* The pseudopupil is the dark spot near the center of each view, and it increased in size in the anterior region of the eye in both species.

The shape of the facets in both species is hexagonal, as seen in the SEM images (Figure 13 E, F). The average length of rhabdom and the diameter of facets in *O. quadrata* were significantly larger than those in *A. pisonii*. The △Φ in the acuity zone and non-acuity zone of *O. quadrata* were significantly smaller than those in *A. pisonii* (table 2).

Table 2. Measures of spatial resolution in *O. quadrata* and *A. pisonii*.

	O. quadrata	*A. pisonii*	t-test
Facet diameter (μm)	53.83±1.49 (n=4)	31.38±2.72 (n=5)	p=0.00034 *
Rhabdom length (μm)	436±5 (n=3)	368±14 (n=4)	p=0.016 *
△Φ in non-acuity zone (deg)	1.36+0.05 (n=4)	1.87±0.13 (n=5)	p=0.013 *
Cycles/degree in non-acuity zone	0.37±0.01 (n=4)	0.27±0.02 (n=5)	p=0.0040 *
△Φ in acuity zone (deg)	0.42±0.01 (n=4)	1.21±0.01 (n=4)	p=0.00075 *
Cycles/degree in acuity zone	1.18±0.03 (n=4)	0.41±0.02 (n=4)	p=0.0000022 *

Sample size (n) indicates number of individuals per species, and values are means±S. E. M. Asterisk indicates a statistically significant difference.

4. Discussion

4.1 Spectral Sensitivity

The results of the dark-adapted sensitivity experiments showed that there is no difference in the spectral sensitivity between *O. quadrata* and *A. pisonii* – both peaked in the blue region of the spectrum (494 nm and 499 nm respectively). This is consistent with earlier studies that demonstrated that most terrestrial and shallow-water decapods are maximally sensitive to wavelengths between 480 and 540 nm (rev in Marshall et al., 1999; Johnson et al., 2002). The light spectrum in terrestrial and shallow-water areas peak in the blue-green region of the light spectrum (rev in Marshall et al., 2003**;** Ciocca & Wang, 2013), and the maximum sensitivity of photopigments of both Atlantic ghost crabs and mangrove tree crabs is matched for maximum sensitivity to the light spectrum, which is consistent with the Sensitivity Hypothesis.

Being diurnally active vs. nocturnally active did not play an important role in their spectral sensitivity since the spectrum of nightlight is very similar with the daylight with only a slightly shift toward the longer wavelengths (Ciocca & Wang, 2013). Atlantic ghost crabs and mangrove tree crabs are both terrestrial species, and the light that passed through their eyes has been scattered similarly by the atmosphere. Consequently, the background light in their habitats have the same spectrum, which caused a similar evolution in their spectral sensitivity.

The spectral sensitivity curves did not change between dark-adaptation and chromatic-adaptation, which indicated that both the Atlantic ghost crab and the mangrove tree crab have only one photopigment. The absence of photopigments at the UV wavelength indicates that their R8 rhabdom cells are either lost or reduced. The flat sandy beach is a relatively monotone environment, and a monochromatic visual system should provide sufficient contrast detection for an Atlantic ghost crab to spot prey (Figure 10A). The mangrove forest habitat of the mangrove tree crab is a complex three-dimensional environment (Figure 10B). However, since the mangrove tree crab is an herbivore, a monochromatic visual system is adequate for it to recognize mangrove leaves in mangrove forests. Color vision may not result in an advantage that is significant enough to drive evolution in either species.

4.2 Temporal Resolution and Irradiance Sensitivity

The dark-adapted CFF_{max} of the Atlantic ghost crab was virtually identical to that of the mangrove tree crab (33.7 vs. 33.5 Hz), indicating that both species have the same temporal resolution. While the light-adapted CFF_{max} of the Atlantic ghost crab was significantly higher than that of the mangrove tree crab, the difference of only 1.5Hz is too small a difference to have an effect on their ability to track moving objects, and this small difference, together with no difference in their dark-adapted CFF_{max} or response latency, indicates that both species have the same temporal resolution.

Their feeding ecology could play an important role on the evolution of their temporal resolution, and based on feeding ecology alone, the Atlantic ghost crab would be expected to have a higher temporal resolution since it is a predator, eating mobile prey, while the mangrove tree crab eats non-motile leaves. However, the day/night activity pattern can also affect temporal resolution, and the nocturnal Atlantic ghost crab, in this case, would need a lower temporal resolution correlated with a higher sensitivity, than the diurnal mangrove tree crab. The effects of the predator/prey interaction and day/night activity pattern may have counterbalanced each other, resulting in similar temporal resolution in this nocturnal predator and this diurnal herbivore.

Irradiance sensitivity of apposition eyes is a function of integration time and facet size. The response latency of the Atlantic ghost crab is about the same as that of the mangrove tree crab, suggesting no difference in their integration time of light transduction. However, the logK value of the Atlantic ghost crab is significantly smaller than that of the mangrove tree crab, meaning that it takes significantly less light to produce a 50% Vmax, which indicates a significantly higher irradiance sensitivity in the Atlantic ghost crab. As there is no difference in temporal resolution, this difference in the irradiance sensitivity must result from the significant differences in their optics or eye sizes. Both species have apposition eyes, but the Atlantic ghost crab has a significantly larger corneal facet (53.8 vs. 31.4 μm), meaning that each rhabdom has a larger aperture, collecting more light and thus providing a partial explanation for the significantly higher photosensitivity in the Atlantic ghost crab.

This is similar to what has been found in two other species of crabs with similar activity

levels – one nocturnal and one diurnal. The facet diameter of *Leptograpsus variegatus,* a nocturnally active crab living by the shoreline (similar to the Atlantic ghost crab), has a facet diameter of 45 μm (Stowe 1980), which is considerably larger than the 19-36 μm of *Uca lacteal,* a low-tide diurnal crab (Alkaladi & Zeil, 2014).

Enlarged corneal facets have also been found in many other nocturnal and deep-sea arthropods. Only 0.0001% of the surface light remains at a depth of 400 m in clearest ocean water (Jerlov, 1976) and some deep-sea crustaceans have evolved extraordinarily huge facets for increased photosensitivity (rev in Land & Nilsson, 1990). For example, both the isopod *Cirolana* (a shallow water species but in water so murky that the light intensity is equivalent to that at 600 m in clear ocean water) and the deep-sea amphipod *Phronima* have apposition eyes with a facet diameter of 150 μm and 100-135 μm respectively (Nilsson & Nilsson, 1981; Land, 1981). Warrant et al. (2004) found similar adaptations in several species of bees. Like all other bees, the nocturnal sweet bee, *Megalopta genalis,* has apposition eyes, but the photosensitivity of their eyes is almost 30 times greater than the eyes of diurnal honeybees. This is primarily due to the relatively large facets - 36 μm average diameter in the nocturnal species, whereas the diurnal species have an average facet diameter of 20 μm. All these studies including the current study support the hypothesis that in general, nocturnally active species would have larger corneal facets than diurnally active species.

4.3 Eye Structures and Spatial Resolution

The absence of the clear-zone between crystalline cones and rhabdoms indicates that both Atlantic ghost crabs and mangrove tree crabs have apposition eyes. No tapetal reflection was observed in the Atlantic ghost crab or the mangrove tree crab when tested with a beam of light at night, another indication of apposition optics, as the tapetal reflection is a feature that can only be found in superposition eyes (Kunze, 1979).

Apposition eyes in these two species is consistent with what has been described for other species in the families Ocypodidae and Grapsidae, all of which have been found to possess apposition eyes (Arikawa et al., 1987; Alkaladi & Zeil, 2014; rev in Gaten, 1998). This eye

structure in the mangrove tree crab is consistent with their lifestyle - they are active during the day and apposition eyes allow less light to get to the retinula cells, protecting them from possibly damaging light levels. The apposition eye type of Atlantic ghost crabs is not consistent with most nocturnally active species, because apposition eyes generally are not as sensitive as superposition eyes at night. However, the results presented here show the Atlantic ghost crab has a large facet diameter, which makes their eyes as sensitive as a superposition eye at night. There are also nocturnally active insects with apposition optics, including cricket, locust, and cockroach (rev in Honkanen et al., 2016), and thus the superposition vision is beneficial but not necessary for nocturnally active species.

Even though the Atlantic ghost crab has larger facets, which is normally associated with a lower spatial resolution, it has a larger eye than the mangrove tree crab (an averaged diameter of 3.18 vs 1.87 mm), and therefore possess a larger number of ommatidia with longer rhabdoms and a longer focal length. Longer rhabdoms increase photosensitivity, as there is a greater chance that photons will be absorbed by the visual pigment during their trip through a long rhabdom, while the longer focal increases spatial resolution, as the focal length is directly proportional to the radius of eye curvature (Caves et at. 2018). The $\triangle\Phi$, the ratio of facet diameter to the radius of eye curvature, is significantly smaller in the Atlantic ghost crab than that in the mangrove tree crab, giving them the higher spatial resolution that they would need to identify motile prey, while the larger eye diameter counteracts the usual reduction in sensitivity that is associated with a better spatial resolution.

The larger radius of eye curvature in the Atlantic ghost crab compared to the mangrove tree crab mainly originates from the difference in the shape of their eyes. The Atlantic ghost crab has an ellipsoid shaped eye, which is a divergent trait in the family Ocypodidae. Ocypodidae crabs have an acuity zone in the middle anterior region of their eyes (Zeil & Al-Mutairi, 1996; Figure 3) and their vertical spatial resolution is extremely high compared to other arthropods (rev in Feller et al., 2021). The eyes of Ocypodidae crabs have great distance perception in a flat environment. However, the vertical resolution and horizontal resolution are very different in their eyes, resulting in a lack of stereopsis, the perception of three-dimensional structure (rev in Schwind, 1989; Alkaladi & Zeil, 2014). Therefore, most Ocypodidae crabs,

including the Atlantic ghost crab, are ground dwellers living in a flat environment. The mangrove tree crabs, on the other hand, have a spherical eye, which is common in the family Grapsidae. The eyes of the mangrove tree crabs are wide apart (Figure 9B), offering them great stereopsis (rev in Schwind, 1989), and this should enhance their binocular vision, assisting them in navigating in a three-dimensional mangrove forest.

4.3 Sensitivity vs Resolution.

This study has demonstrated that the hypothesized inverse relationship between sensitivity and resolution might not always be valid. The Atlantic ghost crab has not only a greater irradiance sensitivity, but also a higher spatial resolution. The irradiance sensitivity is usually inversely corelated with the spatial resolution because the aperture size is directly proportional to the irradiance sensitivity but inversely proportional to the spatial resolution. Any change in the aperture size will result in an increase in one of these two visual characteristics and a decrease in the other. However, the spatial resolution is not only determined by the aperture size but also by the shape and size of the eye. Therefore, factors such as radius of eye curvature and shape of the eye can weaken the inverse relationship between irradiance sensitivity and spatial resolution. In this study, both radius of eye curvature and eye shape had a huge effect on the spatial resolution, and therefore the inverse relationship between irradiance sensitivity and spatial resolution was not significant.

5. Conclusion

When attempting to correlate visual adaptations with environmental characteristics, it is important to not only look at activity cycles (diurnal vs. nocturnal), but also feeding ecology. The ghost crab is an active predator, needing higher spatial resolution, but is also nocturnal, needing greater sensitivity, and the two requirements seem to conflict with each other. This study demonstrated the importance of using both histological and electrophysiological methods to study visual adaptations, as the electrophysiological results demonstrated that the nocturnal ghost crab was significantly more photosensitive than the diurnal mangrove crab, as expected, but the lack of differences in temporal resolution would have made this a puzzling result. However, the histological studies demonstrated that this difference in photosensitivity originated in differences in the eye morphology of the two species. Larger eyes together with larger facet diameters can produce both an increase in spatial resolution and an increase in photosensitivity, which a nocturnal active predator would need, while the smaller facet diameters and smaller eyes are sufficient for a diurnally active species specializing on non-motile prey. The prey differences also explain why the ghost crab can afford to have a metabolically more expensive larger eye, while the less active mangrove crab can get sufficient nutrients from its herbivorous diet to support its smaller eyes. This study emphasized the relationship between pseudopupil and spatial resolution, and it is an excellent technique for comparing the vertical and horizontal resolutions.

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