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The organization of the visual system in the bonnethead shark (Sphyrna tiburo)

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The Organization of the Visual System in the Bonnethead Shark (Sphyrna tiburo)

by

Amy L. Osmon

A thesis submitted in partial fulfillment
Of the requirements for the degree of
Cognitive and Neural Sciences
Department of Psychology
College of Arts and Sciences
University of South Florida

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The Organization of the Visual System in the Bonnethead Shark (*Sphyrna tiburo*)

Amy L. Osmon

ABSTRACT

The goal of this project was to examine the visual system of the bonnethead shark (*Sphyrna tiburo*). The eyes of this shark are located at the extreme lateral ends of a broad, elongated cephalofoil. Better understanding of their visual system may aid in determining the adaptive benefits of their usual head shape. The proposed project examined one specific aspect of their visual system: the organization of retinal ganglion cells and identification of areas of increased resolution. Two experiments were conducted to realize these aims: (1) staining of retinal ganglion cells, to examine their distributional pattern, and (2) retrograde staining of retinal ganglion cells to determine morphology.
Chapter One

Introduction

Elasmobranchs had long been thought to possess poor vision and utilize other sensory systems for navigation and detection of both prey and predators (Gruber, 1977). However, during the 1960’s and 1970’s the scientific community began to publish both anatomical and physiological research indicating that the visual systems of sharks and rays were capable of higher visual resolution than previously believed (Ali and Anctil, 1974a; Gruber, 1977; Gruber, Gulley, and Brandon, 1975; Gruber, Hamasaki, and Bridges, 1963; Hamasaki and Gruber, 1965; Stell, 1972; Stell and Witkovsky, 1973). Concurrent studies also attempted to test the visual acuity and learning ability of sharks by investigating visually mediated behaviors (Graeber, 1978; Tester and Kato, 1966; Wright and Jackson, 1964). These studies revealed complexity within sharks’ visual system and provided insight into the significance of the visual system in their daily existence.

Research regarding retinal anatomy in sharks has shown variability in rod-to-cone ratios, the distribution of ganglion cells within the retina, as well as the presence of visual streaks (Bozzano and Collin, 2000; Gruber, 1977; Gruber et al., 1975; Gruber et al., 1963; Hamasaki and Gruber, 1965; Hueter, 1988; Peterson and Rowe, 1980; Stell, 1972; Stell and Witkovsky, 1973). The implications of these retinal variations have not yet been thoroughly explored. However, several authors (Bonazzo and Collin, 2000; Gruber et al., 1975; Hueter, 1989; Hueter and Gruber, 1982) have related the variability of sharks’ visual systems to their feeding behaviors and habitats. This study examined the retinal anatomy of one shark species, the bonnethead shark.

The bonnethead shark is one of nine species in the Sphyrnidae family, commonly referred to as “hammerhead” sharks, possessing a broad and elongated head shape. The bonnethead shark inhabits clear to turbid inshore waters of the Gulf of Mexico, the Atlantic Ocean, and along the coasts of Central and South America (Cortes and Parsons, 1996; Hoese and Moore, 1958). Their
diet consists of a variety of swift-moving crabs and cephalopods (Cortes, Manire, and Hueter, 1996; Motta and Wilga, 2000). As this shark species adjusts well to captivity (Cortes et al., 1996; Cortes and Parsons, 1996) and their ecology is well documented (Cortes et al., 1996; Cortes and Parsons, 1996; Hoese and Moore, 1958; Myreberg and Gruber, 1974), it is an excellent subject for an investigation into the relationship between retinal anatomy and ecological niche.

Theories regarding the function of Sphyrididae sharks’ unique cephalofoil focus on their head shape providing increased hydrodynamic lift, an area useful for capture of large prey items, and/or an enlarged area for electrorception and olfaction (Antcil and Ali, 1976; Compagno, 1984; Johnsen and Teeter, 1985; Kajiura and Holland, 2002; Martin, 1993; Nakaya, 1995; Strong, Gruber, and Snelson, 1990). Only two studies have examined the visual system of Sphyrididae sharks (Antcil and Ali, 1974b; Gruber et al., 1963). Antcil and Ali (1974b) investigated the retinal morphology of the scalloped hammerhead shark (*Sphyrna lewini*) and designated some retinal ganglion cells as giant ganglion cells due to their large soma size. Gruber et al. (1963) reported similarities in the morphology of cones between the lemon shark (*Negaprion brevirostris*) and the great hammerhead shark (*Sphyrna mokarran*). However, no research has been conducted regarding the visual system of the bonnethead shark. Information pertaining to the bonnethead shark’s visual system may lead to a better understanding of the true function and significance of this shark’s unusual head shape compared to other shark species.

Organization of retinal ganglion cells in non-shark species

How does retinal cell topography relate to the diverse habitats of different vertebrate species? According to Hughes’ (1977) terrain theory, animals with a predominantly two-dimensional horizon in their visual environment (e.g., the ocean with a sand-water boundary for a benthic aquatic species) gain an advantage from possession of a visual streak. A visual streak is an elongated area of increased ganglion cell density relative to other areas within the retina.
(Bozzano and Collin, 2000). The advantage of possessing a visual streak is higher resolving power in the visual fields corresponding to the area of increased cell density (Bozzano and Collin, 2000). A visual streak may also negate the necessity of utilizing distinctive eye movements while an animal is gazing across an expansive horizon (Collin and Pettigrew, 1988b).

Hughes’ theory (1977) has been tested and validated in many non-aquatic vertebrates including the fat-tailed dunnart (Sminthopsis crassicaudata) (Arrese, Dunlop, Harman, Braekevelt, Ross, Shand, and Beazley, 1999), several ungulates (e.g. the pig, sheep, ox, dog, and horse) (Hebel, 1976), the tammar wallaby (Macropus eugenii) (Wong, Wye-Dvorak, and Henry, 1986), and the African elephant (Loxodonta africana) (Stone and Halasz, 1989). Visual streaks have also been found in teleosts occupying open areas with a distinct visual horizon such as the blue tuskfish (Choerodon albigena), red-throated emperor (Lethrinus chrysostomas), collared sea bream (Gymnocranius bitorquatus), clown triggerfish (Balistoides conspicillum), and painted flutemouth (Aulostoma chinensis) (Collin and Pettigrew, 1988b). In addition, Hughes’ theory has been applied to other marine animals, including the loggerhead (Caretta caretta), leatherback (Dermochelys coriacea), and green (Chelonia mydas) turtles (Oliver, Salmon, Wyneken, Hueter, and Cronin, 2000), and marine mammals including the sea otter (Enhydra lutris) (Mass and Supin, 2000). However, there is scant information regarding elasmobranch species (Bozzano and Collin, 2000; Hueter, 1989; Peterson and Rowe, 1980).
Table 1: Comparison of elasmobranch species

<table>
<thead>
<tr>
<th>Environmental Factors</th>
<th>Behavioral Factor</th>
<th>Visual Streak</th>
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<tr>
<td></td>
<td>Illumination</td>
<td>Habitat and depth</td>
</tr>
<tr>
<td>Tiger shark</td>
<td>Fair</td>
<td>Pelagic 0-140m</td>
</tr>
<tr>
<td>Epaulette shark</td>
<td>Not recorded</td>
<td>Benthic 0-50m</td>
</tr>
<tr>
<td>Small-spotted dogfish shark</td>
<td>Dim</td>
<td>Benthic 50-400m</td>
</tr>
<tr>
<td>Black-mouth dogfish shark</td>
<td>Dim</td>
<td>Benthopelagic 300-2,000m</td>
</tr>
<tr>
<td>Velvet-belly shark</td>
<td>Dim</td>
<td>Meso-pelagic 500-2,000m</td>
</tr>
<tr>
<td>Lemon shark</td>
<td>Fair to Good</td>
<td>Benthic 0-90m</td>
</tr>
<tr>
<td>Bigelow’s ray</td>
<td>Not recorded</td>
<td>Benthic 650-2200m</td>
</tr>
</tbody>
</table>

(table adapted from Bonazzo et al., 2000; Hueter, 1991; Compagno, 1984; Motta, Tricas, Hueter, and Summer, 1997)

There are multiple factors which appear to relate to the width, length, and location of the visual streak (Bozzano and Collin, 2000; Hueter, 1991). These factors include: (1) the amount of light available to an animal, (2) the habitat of the animal (e.g., whether the horizon is completely open or partially obstructed), and (3) how an animal utilizes its visual streak, which includes foraging and prey detection strategies.
Illumination

In regards to the variability of illumination, this factor appears to relate to the density of the ganglion cells within the visual streak and the extent of the visual streak horizontally across the retina. This variability in the ganglion cell density within the visual streak has been evident between nocturnal and diurnal species, including ungulates and primates (Hughes, 1977; Lima, Silveira, and Perry, 1996). These studies revealed that the visual streak of nocturnal animals, if they possess a visual streak, generally contains lower cell densities than that of diurnal animals (Hughes, 1977; Lima et al., 1996).

Within the realm of aquatic animals, Oliver et al. (2000) revealed that green sea turtles, inhabiting areas with clear water and a high level of illumination, have strong visual streaks (e.g. visual streaks with the highest number of retinal ganglion cells and longest horizontal extent). In contrast, the loggerhead and leatherback sea turtles, both inhabiting areas with highly varied illumination, possess weaker visual streaks with lower cell densities and shorter horizontal extents.

Habitat

Referring to habitat, the visual streak generally extends the farthest horizontally in species living in open habitats with an unobstructed view of the horizon (Collin and Pettigrew, 1988b; Oliver et al., 2000). For instance, Collin and Pettigrew (1988b) investigated several teleost species inhabiting coral reefs and found that species with completely unhindered views of the horizon, such as the clown triggerfish and blue tuskfish, possess horizontal visual streaks extending across most of their retinal meridians. Whereas the Australian frogfish, inhabiting a more “closed environment” (e.g. with an obstructed view of the visual horizon) possess a weaker visual streak extending a much shorter distance across the retinal meridian (Collin and Pettigrew, 1988a).
Behavior

The third factor which may relate to an animal’s visual streak concerns how an animal behaviorally utilizes its visual system. Collin and Pettigrew’s (1988b) study of coral reef fishes revealed that the differences in location of peak cell density within the visual streak and actual location of the visual streak may vary due to the way these fish species utilize them. More specifically, the topography of the visual streak may vary in association with the behavioral needs of the teleost (e.g. the visual streak may be more important for predatory behavior and/or for predator surveillance).

For example, the blue tuskfish and painted flutemouth both possess a horizontal visual streak along their retinal meridians (Collin and Pettigrew, 1988b). However, the visual streak of these fish species differs in regards to whether a temporal area of increased ganglion cell density is separate from (blue tuskfish) or extends into (painted flutemouth) the visual streak. The variations in retinal topography possessed by these fish species may indicate whether their visual streak is useful primarily for predator surveillance or for predatory behavior (Collin and Pettigrew, 1988b). The blue tuskfish forages by searching through and moving coral debris on the substrate in search of food. The authors believe that the visual streak of this fish species may be useful for predator detection, as possessing a visual streak congruent with the environmental horizon may help it to watch for predators while it forages (Collin and Pettigrew, 1988b). The temporal area of increased cell density in the blue tuskfish appears better suited to foraging for prey (Collin and Pettigrew, 1988b). The temporal area subtends the visual region directly in front of the blue tuskfish, and should increase its resolving power in the area where the fish would be searching for invertebrates within the substrate (Collin and Pettigrew, 1988b).

The painted flutemouth, however, shadows other fish species to approach its prey (swift-moving fishes) by surprise (Collin and Pettigrew, 1988b). Possession of a visual streak correlated with the environmental horizon may be more useful to detect and approach unsuspecting prey,
rather than guard for predators in this fish species. The authors also state that this fish’s retinal
topography (e.g. having the temporal retinal specialization “extend” into a visual streak across the
retinal meridian) is similar to the retinal topography of other species where vision is more
valuable for prey detection than predator surveillance (Collin and Pettigrew, 1988b).

The study conducted by Oliver et al. (2000) also supports the idea that differences in
feeding behaviors may be correlated with visual streak length. Of the three species examined in
the study, green turtles possessed the strongest and longest visual streak, likely due to their well-
illuminated habitat containing an unobstructed view of the visual horizon (Oliver et al., 2000).
Both the loggerhead and leatherback turtles possess weaker visual streaks than the green turtle,
with the leatherback turtle possessing the weakest visual streak of the three (Oliver et al., 2000).
Although the visual streak of the loggerhead turtle was wider than that of the green turtle, its
visual streak was less horizontally extensive and contained a lower ganglion cell density (Oliver
et al., 2000). Loggerhead turtles forage for prey such as snails, sea anemones, and crustaceans
contained within and around the mats of sea grasses or algae this turtle hides amongst (Oliver et
al., 2000). The sea grasses and algae mats loggerhead turtles feed within would hinder much of
their vision, with the exception of objects located directly in front of them. Therefore, it is likely
they would not need to possess a visual streak extending across their entire retinal meridian to
provide them with increased sampling of visual targets in their lateral/ peripheral visual fields
(Oliver et al., 2000).

Of all three turtle species in the study, the leatherback turtle possessed the weakest (least
elongated across the retinal meridian and containing the lowest density of retinal cells) visual
streak (Oliver et al., 2000). The leatherback turtle was also the only species in the study to
possess an area centralis (e.g. a small area of increased retinal cell density) separate from their
visual streak (Oliver et al., 2000). Leatherback turtles feed primarily on jellyfish and other
jellylike prey they capture via diving. The weakness of the leatherback turtles’ visual streak
indicates that this type of visual adaptation may not be as beneficial to detect prey as their well-developed area centralis (Oliver et al., 2000). The lack of a lengthy and strong visual streak in the leatherback turtle could also be a result of their predilection for capturing prey in open water where the environmental horizon may be vague or even absent (Oliver et al., 2000).

Other examples regarding how the location of the visual streak may reveal its importance in a species daily survival, and how the visual streak may relate to an animal’s behavior (e.g. foraging or predator surveillance behaviors) are the striped panchax (*Apolcheilus lineatus*) and Graham’s Hechtling (*Epiplatys grahami*), two freshwater fish species. Both the striped panchax and Graham’s Hechtling possess two “band-shaped” thickenings extending across their retinal meridian. One band traverses the retinal meridian, and the other “band” extends across the retina, just ventral to the retinal meridian (Collin and Pettigrew, 1988b). These band-shaped thickenings are the equivalent of visual streaks (Collin and Pettigrew, 1988b). These fish species feed on insects and other small prey items living upon or just below the water surface where higher resolution power in the upper visual fields would aid these fish species in locating prey (Collin and Pettigrew, 1988b). Therefore, the authors believe that the ventral thickening is likely useful for detection of prey located just above the fish and the thickening of the central retinal meridian is congruent with the lateral visual field of these fishes and useful for predator detection (Collin and Pettigrew, 1988b).

**Organization of retinal ganglion cells in sharks**

The visual surroundings of many shark species relate well to the terrain theory, as their environments are composed of a two-dimensional setting containing a sand-water boundary or a boundary containing a “horizontal gradation of light within the water column in the clear waters of the open ocean” (Bozzano and Collin, 2000). Therefore, most shark species, especially those living in relatively well-illuminated benthic, pelagic, or mesopelagic habitats, could possess a
visual streak. This appears to be true (see Table 1) as shark species investigated thus far (e.g., the tiger, epalouette, black-mouthed, velvet-belly, lemon, small-spotted dogfish, and California horn shark) all possess a visual streak (Bozzano and Collin, 2000; Hueter, 1989; Hueter, 1991).

Shark species that have been investigated also show species-specific variations in the width, length, and location of their visual streaks, similar to that found in teleosts and terrestrial vertebrates (Bozzano and Collin, 2000; Hueter, 1991; Peterson and Rowe, 1980). These variations in visual streak organization appear to be associated with environmental factors as well as predatory or surveillance behavior, and not with phylogenetic relationships between shark species.

Illumination

In contrast to sea turtle hatchlings, there appears to be no apparent difference regarding overall ganglion cell density within the visual streak between deep-sea (low illumination) and shallow-water (higher illumination) shark species (Bozzano and Collin, 2000). However, there does appear to be a difference in the percentage of giant ganglion cells contained within the retina of shallow water versus deep-sea sharks (Bozzano and Collin, 2000). Giant ganglion cells are characterized by a larger soma (e.g. two-to-three times the soma size of other ganglion cells) and are thought to possess larger receptive fields than the normal ganglion cells (Bozzano and Collin, 2000). Shallow water species, (e.g. the tiger and epaulette sharks), regardless of whether they are benthic or pelagic, have the lowest percentage of giant ganglion cells, whereas deep-sea species (e.g. the blackmouth dogfish and velvet belly sharks) have the highest percentage (Bozzano and Collin, 2000). This difference in the overall percentage of giant ganglion cells may actually result from differences in the distinctiveness of the visual horizon between deep-sea and shallow water species (Bozzano and Collin, 2000).
Habitat

Bozzano and Collin (2000) suggest that more pelagic than benthic species should possess a broad visual streak. This may be generally true, as the small-spotted dogfish and Bigelow’s ray are benthic and possess a narrow visual streak, whereas the black-mouth dogfish shark is benthopelagic and possesses a broader visual streak (Bozzano and Collin, 2000). However, the lemon shark, a shallow-water benthic species with a broad visual streak, may be an exception.

The visual streak of the lemon shark forms a fairly wide horizontal band of increased cell density running along the retinal meridian (Hueter, 1989). This form of visual streak may aid the lemon shark in both prey and predator detection. Both diurnal and nocturnal in activity, the lemon shark pursues swift-moving crustaceans and fish (Hueter, 1991). It hunts via patrolling over a sandy substrate or sea grass flats sweeping its body from side-to-side (Hueter, 1991; Oliver et al., 2000). Possession of a broad and lengthy visual streak may allow this shark to detect movement of visual objects both directly in front of it and within the lateral periphery of this visual field (Hueter, 1991). Therefore, the visual streak of the lemon shark may play a role in detection of prey. Although hunters themselves, lemon sharks are also occasionally predated upon by larger sharks. The presence of a sizeable visual stimulus has been found to elicit a “rapid withdrawal response” in lemon sharks (Hueter, 1991). Therefore, it is also likely that the visual streak of the lemon shark may be useful for detection of predators as well.

Behavior

Whether a visual streak is broad or narrow may also be associated with whether detection of predators or prey is visually important to a shark species. The location of the visual streak either across the retinal meridian, or just dorsal or ventral to the retinal meridian, may vary in relation to the different types of predatory behaviors employed by shark species.
All shark species examined possessed rather centrally located visual streaks with the exception of the tiger shark (Bozzano and Collin, 2000). The visual streak of this shark is located ventrally to the retinal meridian (Bozzano and Collin, 2000), and would subtend vision in the upper visual field. The tiger shark is a large predator generally found in shallow water, near the surface (Bozzano and Collin, 2000). This shark likely has the most varied diet of all shark species, as it feeds on bony fish, sea turtles, sea snakes, mollusks, and mammals, among other items (Compagno, 1984). As this shark generally attacks via a bump-and-bite or ambush-style predatory technique (Compagno, 1984), a visual streak allowing it to swim unnoticed underneath potential prey, such as one subtending the upper portion of the shark’s visual field, would be advantageous.

In regards to predatory behavior and increases in ganglion cell density, an increased area of retinal ganglion cells was found in the center of the epaulette shark’s visual streak (Bozzano and Collin, 2000). This benthic shark inhabits relatively shallow waters and preys upon benthic invertebrates such as crustaceans and mollusks (Compango, 1984). An increase in the resolving power within the central area of their frontal visual field may aid them in locating their prey (Bozzano and Collin, 2000).

The benthopelagic blackmouth dogfish shark possesses two areas of increased cell density within the nasal and temporal areae of their visual streak (Bozzano and Collin, 2000). This shark consumes swift-moving prey such as bony fishes and cephalopods via sweeping its head and body from side-to-side (Bozzano and Collin, 2000). The two areas of increased cell density should increase the sampling of visual targets within the shark’s frontal and caudal visual fields and are likely useful for detection of prey (Bozzano and Collin, 2000). This sharks’ retinal topography is also congruent with the idea proposed by Collin and Pettigrew (1988b) that species possessing temporal areas of increased cell density which extend into a visual streak are more likely to utilize these areas for prey detection.
Possible retinal ganglion cell topography of the bonnethead shark

The bonnethead shark is likely to possess a visual streak extending across the entire length of their retinal meridian, congruent with the visual horizon, due to its potentially well-illuminated shallow-water habitat which should possess a rather distinct visual horizon.

Similar to the lemon shark, the visual streak of the bonnethead shark may be useful for both detection of predators and prey. However, the primary use of this shark’s visual streak should not be prey detection, as it would appear from the location of this shark’s eyes on its broad, elongated head, that this shark may lack a frontal visual field. This potential blind spot would negate the bonnethead shark’s ability to visually locate prey directly in front of it, though it may possess the ability to locate prey within its lateral visual fields. The sweeping side-to-side motion of this shark’s head while it patrols for prey may also aid it to detect prey within its peripheral visual fields.

Even though pelagic sharks may, in general, possess wider visual streaks than benthic species, the bonnethead shark, like the lemon shark, may be an exception. Due to the location of this shark’s eyes within the extreme edges of its broad, shovel-shaped head, the bonnethead shark may also possess an elongated lateral visual field. This potential increase in the lateral visual field should allow the bonnethead shark to develop a broad horizontal visual streak extending across their entire retinal meridian. This type of visual streak should aid the bonnethead shark in avoiding predation by larger shark species, as it would provide the bonnethead shark with full visual access to the areas along its sides. Prey species of this shark are primarily crustaceans with the ability to change direction rapidly, thus possessing a broad visual streak may also aid them in locating potential prey within their lateral visual fields.
Eight retinas, from eight individual sharks, were utilized for this study. The sharks were obtained with help from Mote Marine Laboratory in Sarasota, Florida. Each shark was caught within the Tampa Bay region (Charlotte Harbor) using gill nets. Measurements of length and weight were taken before the sharks were placed into a cooler containing ice. A preservative (4% paraformaldehyde solution in 0.5 M phosphate buffer, 1.00 cc per eye) was injected intraocularly to prevent disturbing the retina within ten minutes of the sharks expiring to preserve the eyes. The eyes were then removed and immersed in a 4% paraformaldehyde solution in 0.1 M PB solution, Ph 7.4; Huxlin and Goodchild, 1997) in small containers and placed into a cooler for transport back to the lab. Excess tissue (e.g. connective tissue) was removed from the eye before the eye was placed into the preservative solution. The retinas were removed from the eyes and wholemounted within 24 hours of collection. Eight of the retinas were used for Nissl staining and two eyes (without the retina removed for the procedure) were used for the retrograde tracing with DiI. Though eyes were to be counterbalanced between left and right for this project, seven were right eyes and one was from the left eye. This discrepancy was due to selection of the best wholemounts from the retinas available for topographic analysis of ganglion cells.

Nissl Staining

Before removal of the retina from the eyecup, each eye was marked with a small indentation, using a # 11 scalpel (Hueter, 1988), to maintain the dorsal/ventral and anterior/posterior orientations of the eye. The eyes were then removed and placed in a deep petri dish containing the preservative solution (4% paraformaldehyde solution in 0.1 M phosphate buffer). The preservative solution covered each eye to prevent them from becoming dehydrated. After fixation, an adaptation of Hueter’s (1988) retinal wholemount technique was utilized. To
remove the retina, the eye was placed in a petri dish filled with the 4 % paraformaldehyde solution. Using a scalpel, the eyes were cut open at the choroidal-scleral boundary to gain access to the retina. Cutting ceased when there was a small amount of tissue, forming a ‘lid’, left on the dorsal portion of the eyecup. The lens and vitreous humor were then lifted from the eyecup and removed. Eye orientation (dorsal vs. ventral) was maintained while the retina was removed from the eyecup using small indentations made with a scalpel blade on the dorsal and ventral margins of the retina. Using a soft brush (camel hair, #0), the retina was then transferred unto a glass slide.

The retina was then flattened against the glass slide. If the retina did not lie flat against the glass slide, small incisions were made around the retina’s circumference to help it to lie flat.

Any additional preservative solution and vitreous material was then carefully removed by touching filter paper to the coverslide to absorb them. Each retina was then placed into a dust-free container for at least 12 hours to fully adhere to the slide before being taken out and gently washed with DH₂O to prepare it for Nissl staining.

Each retina was stained for 10-15 minutes in 0.05% Cresyl Violet. Each slide was then dehydrated, cleared, and coverslipped. Once the retinas were stained, the retinal ganglion cell layer was examined microscopically.

Topographic Mapping

Before taking pictures, each retina was traced into the Canvas7 computer software program using a computerized Wacom drawing tablet, then divided into 1mm sections. A starting point for pictures was pinpointed, then the coordinates for each 1mm section were labeled on the retinal drawings from readings taken from a Nikon microphot-FXA microscope using an X,Y grid system on its stage micrometer. A micro-photograph was taken of the lower right corner intersection between each 1 mm square with a Lucida camera attached to the Nikon
microscope. Retinal ganglion cell counts were taken from a 200 micron square area in the center of each microphotograph. Cell counts were not taken from the very edges of the retina, as retinal shrinkage (usually between 2 and 20%; Collin, 1988; Oliver et al., 2000) can occur in these areas (Bozzano and Collin, 2000). Retinal ganglion cell counts were noted for each 1mm section of each retina. These raw counts were then converted to the number of cells per 1mm² and topographic maps were composed to represent the visual topography.

In the ganglion cell layer, ganglion cells were differentiated using morphological standards of Collin (1988). The author found retinal ganglion cells to be large, irregularly shaped with darkly stained somas (Collin, 1988). Ganglion cell morphology was to be assured by labeling ganglion cells within the retina using DiI for retrograde tracing.

Retrograde ganglion cell labeling

In order to assure correct identification of ganglion cell morphology, four retinas were to be examined using retrograde labeling of ganglion cells. Two eyes from one bonnethead shark were used to test whether the crystal form of 1,1’ –dioctadecylic-3,3,3’,3’ – tetramethylindocarbonocyanine perchlorate (DiI) would be suitable for retrograde labeling of ganglion cells. DiI is a lipophilic carbanocyanine which attaches to and stains the plasma membrane. It is then is diffused laterally through the cell, eventually staining the entire cell.

The optic nerve ending of the eyes utilized for retrograde labeling were cut to make the ends level and a small incision was made at into the end of the optic nerve. A small crystal of DiI was then placed into the incision. As DiI is sensitive to light, this procedure was performed under minimal light conditions in the lab. Once the DiI crystal was securely placed into the incision at the end of the optic nerve, the eye was returned to the container of preservative solution, with the optic nerve tip containing the DiI crystal supported above the preservative to keep it dry. In both cases, the eyes were placed into small containers filled with the 4% paraformaldehyde in 0.1M
PB buffer solution, Ph 7.4. The containers were then wrapped in aluminum foil to protect them from light contamination and placed in a dry, dark area. Dil was allowed to absorb into the cells of the eyes in one case for two weeks and for four weeks for the second case. In both cases, after the allotted time for Dil absorption, the retinas were removed and wholemounted using the aforementioned procedure with the exception of the retinas being removed under low light conditions. Once the retinas were wholemounted, several drops of glycerin were placed on the retinas and a coverslip applied. The retinas were then examined under a Nikon microscope under fluorescent lighting for evidence of Dil staining. Unfortunately, the Dil did not completely stain the entire ganglion cell bodies, making it impossible to use Dil to confirm ganglion cell morphology. In lieu of the Dil retrograde labeling, morphology of ganglion cells was confirmed using the descriptions from Collin (1988) and Hueter (1991).

Data Analysis

Ganglion cells

Wholemounted retinas were used to identify ganglion cells. The morphological criteria for identification of ganglion cells by Collin (1988) and Hueter (1991) was used. An average of 333 regions were sampled from each retina (see Table 1 in the results section for individual sampling data). A visual streak was to be defined in this study as any area of the retina where a significant increase in ganglion cell density was found.

Nissl stains

Eight retinas, from both male and female bonnethead sharks were whole mounted, stained with Nissl substance, and photographed. After completion of retinal counts, each retina was mapped. The resulting maps were divided into four quadrants (dorso-nasal and temporal and ventro-nasal and temporal) to aid the descriptive process. Retinal counts were divided into four
categories, low (under 25%), medium (26-50%), high (51-75%), and highest (over 75%) number of retinal ganglion cells. These categories were based on dividing the maximum and minimum counts averaged across the eight retinas into four equal quartiles and used to measure differences in retinal ganglion cell numbers across each retina.

Expected results of these experiments

The bonnethead shark was expected to possess a wide horizontal visual streak (covering at least one-third of the longitudinal retinal diameter) across the entire length of the retinal meridian. This streak should mediate vision in their panoramic lateral visual field. If this visual streak was not found, then vision may not be important to the daily survival of this shark species.

If retinal specializations were found (increased areas of peak retinal ganglion cell density) in the nasal region of the retina, then the bonnethead shark may use this area of increased resolution to detect predators coming from behind, and therefore, predator detection would likely be the primary function of their visual system, and its subsequent organization. If a retinal specialization was found within the temporal region of the bonnethead shark retina, then this shark species may utilize their visual sense to detect prey in front of or to the sides of the shark’s head. A visual streak in this retinal area would likely be aided by the head movements of the bonnethead shark, as they sweep their heads from side to side while patrolling for prey.
Chapter Three

Results

For the eight retinas utilized in this study pictures (0.5mm by 0.5mm² each) were taken at 1mm intervals across the entire area of each retina. This cumulated in a total of 2,660 photographs. The number of pictures for each retina varied between 266 (case # IMF2) and 413 (case # 2625-2). The average number of photographs taken per retina was 332.5. For each picture, retinal ganglion cells were identified and counted and this information was used to create topographic maps of ganglion-cell density across each individual retina (Figures 2 through 9). See Table 2 for individual data regarding the number of counted areas for each retina. A typical photographic image utilized for analysis is shown in Figure 1.

Figure 1. Typical photographic image showing retinal ganglion cells
Darkly stained irregularly shaped cells (solid arrows) were counted as ganglion cells. The smaller circular cells (open arrow) were considered possible amacrine cells and not counted.

In general, topographical maps revealed heterogeneous characteristics of retinal ganglion cell distribution. Therefore, some areas within each retina showed higher ganglion cell density than the rest of the retina. In most cases, these areas of higher density formed a band-like shape
across the retinal meridian. The term “band” was used to describe them instead of “visual streak” for the results section. For the reasoning behind this terminology, see the discussion section.

Of the eight retinas used for this study, six were from adult animals and two were from immature individuals that are referred to as IMF1 and IMF2. However, since no apparent differences regarding the cell distribution pattern were revealed between the adult and juvenile sharks, all results, including those from both immature and adult specimens, were combined and analyzed together.

DiI crystals were placed into the optic nerve to stain and identify ganglion cells. However, the DiI substance did not fully stain the ganglion cells, making it impossible to discern ganglion cell morphology. Descriptions of ganglion cell morphology from Hueter (1991) and Collin (1988) were used to identify and count ganglion cells in lieu of DiI staining.

General Results

Cell Numbers/Density

In all cases combined (from the 2,660 individual photographs), a total of 402,372 cells were identified as retinal ganglion cells. The minimum number of ganglion cells found was 127 cells per mm² (case # 2625-2), whereas the maximum number equaled 1571 cells per mm² (case # 2626-8). The mean ganglion cell density was 693 cells per mm² with a standard deviation of 43. Figures 2 through 9 show the frequency distribution for each individual case.

For the results section, cell numbers were categorized into quartiles in order to demarcate the general trend of retinal ganglion cell distribution as well as utilize all data collected from the eight cases. The quartiles were obtained by averaging the minimum and maximum cell counts from each individual retina. These averaged minimum and maximum counts were then used to establish the quartiles utilized for this study. These quartiles are defined as follows: Low (0-480 cells per mm²), Medium (481-747 cells per mm²), High (748-1010 cells per mm²) and Highest
High Density Band

Out of the eight cases, two retinas revealed no clear heterogeneous pattern of ganglion cell distribution (cases 2625-2 and 2626-6). However, the remaining six cases (2627-1, 2626-4, 2626-5, 2626-8, IMF1 and IMF2) possessed the area of increased ganglion cell density categorized as “higher density” along the retinal meridian (see Figures 2, 3, 4, 5, 6, and 7). These bands, though, varied in length between the individual cases. Four of the retinas (cases 2626-5, 2626-8, IMF1, and IMF2) contained areas of “higher” cell density running at least two-thirds of the length of the entire retinal meridian. In two cases (2626-4 and IMF2) the band extended across the entire length of the retinal meridian, and in one case (2627-1) the area of “higher” cell density covered at least half the length of the retinal meridian.

Variations in the width of this “higher” density band were also observed. In four cases (2626-4, 2626-5, IMF2 and 2626-8; see Figures 3, 4, 5, and 8), the “higher” density band covered approximately one-third of the dorsal-to-ventral expanse of the retina. The width of the “higher” density band was widest in one case, 2627-1 (see Figure 2), where it covered at least half of the dorsal-to-ventral expanse of the retina.

High density dorso-temporal area

In addition to the band of “higher” density, there was a distinct area of increased ganglion cell density in the dorso-temporal retina in six cases (2627-1, 2626-4, 2626-5, 2626-8, IMF1 and IMF2). Shape and density of this dorso-temporal increase varied between all six cases (see Figures 2, 3, 4, 5, 6, and 7). Three cases, (2627-1, 2626-5, and 2626-8) had fairly small dorso-temporal areas compared to the rest of the cases. In all five cases, density within the dorso-
temporal area was categorized as “High”. In three cases (2627-1, IMF1 and IMF2), this dorso-temporal higher-density area appeared to be an extension of the “higher” density band, whereas in the other four cases (2627-1, 2626-4, 2626-5, and 2626-8) this area was not connected to the “higher” density band.

Table 2  Retinal ganglion cell counts and statistics (all cell counts in cells/mm²)

<table>
<thead>
<tr>
<th>Case Number</th>
<th>2627-1</th>
<th>2626-4</th>
<th>2626-5</th>
<th>2626-8</th>
<th>IMF1</th>
<th>IMF2</th>
<th>2625-2</th>
<th>2626-6</th>
<th>Overall Mean</th>
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<td>634</td>
<td>751</td>
<td>720</td>
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<td>706</td>
<td>797</td>
<td>566</td>
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<td>339</td>
<td>368</td>
<td>266</td>
<td>413</td>
<td>344</td>
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</tr>
</tbody>
</table>
Individual Results

Retina 2627-1

This retina came from the right eye of an adult female shark. The band of “higher” density (outlined in figure), starting in the mid-nasal portion of the retina, was found running along most of the retinal meridian in this retina (Table 2, Figure 2).

The dorso-nasal portion of the retina contained both low and medium cell counts. The dorso-temporal portion of the retina also contained mostly medium cell counts, with the exception of an area of high counts located just above the retinal meridian in the extreme temporal edge of the retina. The band of “higher” cell density started just nasally of the optic disc (along the equator of the retina) and ran from this area across to the temporal portion of the retinal meridian.
as well as down toward the ventro-central portion of the retina. The ventro-temporal portion of 
the retina contained mostly low and medium cell counts.

Figure 3. Topographic map of case 2626-4
(areas marked with e= edge, nc= not countable)

Retina 2626-4

This retina came from the right eye of an adult female shark. A band of “higher” cell
density was observed along part of the retinal meridian, starting at the nasal edge of the retina and 
running across the entire retinal meridian (Table 2, Figure 3).

The dorso-nasal portion of the retina contained mostly medium cell counts with a few 
randomly interspersed high counts. The dorso-temporal portion of the retina also contained some
medium and mostly high cell counts, as well as an area of High cell counts located just above the 
retinal meridian running from the mid-retina to the extreme temporal edge of the retina. The 
ventro-nasal portion of the retina contained mostly medium cell counts with a few low and high
counts at the extreme ventro-nasal edge. The ventro-temporal portion of the retina contained mostly high cell counts.

Figure 4. Topographic map of case 2626-5 (areas marked with e= edge, nc= not countable)

Retina 2626-5

This retina came from the left eye of an adult female shark. A band of “higher” ganglion cell density (outlined in figure) began in the mid-nasal portion of the retina and ran across the rest of the retinal meridian (Table 2, Figure 4).

The dorso-nasal portion of the retina contained mostly medium and low cell counts. The dorso-temporal portion of the retina contained mostly medium cell counts. The ventro-nasal portion of the retina contained mostly medium cell counts whereas the ventro-temporal portion of the retina contained mostly medium and some high cell counts in the most dorsal part of this area.
Retina 2626-8

This retina came from the right eye of an adult female shark. A band of “higher” cell density (outlined) was located along the retinal meridian. This band started in the nasal portion of the retina and ran across the majority of the retinal meridian as well slightly into the ventro-temporal portion of the retina (Table 2, Figure 5).

The dorso-nasal portion of the retina contained mostly medium cell counts. The dorso-temporal portion of the retina contained mostly medium cell counts with a small area of high counts within the dorsal portion of this area. The ventro-nasal portion of the retina contained mostly medium cell counts interspersed with a few high counts. The ventro-temporal portion of
the retina contained mostly high cell counts with the very extreme ventral edges containing medium cell counts.

Figure 6. Topographic map of case IMF1

Retina IMF1

This retina came from the right eye of an immature female. A band of “higher” cell density (outlined) was located along the retinal meridian and started at the nasal edge of the retina and ran into both the dorso-temporal and ventro-nasal portions of the retina (Table 2, Figure 6).

The dorso-nasal portion of this retina contained mostly medium cell counts with some a few high cell counts located at within the central portion of this area. The dorso-temporal portion of the retina contained high density cell counts within its mid-to-dorsal portion and medium and low counts at the extreme temporal edge of the retina. The ventro-nasal portion of the retina
contained mostly high cell counts, whereas the ventro-temporal portion contained mostly medium cell counts with a small area of high counts in the most ventro-temporal portion of this area.

Figure 7. Topographic map of case IMF2
(areas marked with e= edge, nc= not countable)

Retina IMF2

This retina came from the right eye of an immature female. A narrow band of “higher” cell density, starting at the nasal edge of the retina and running across the entire retina, was located along the retinal meridian, just above the optic disc (Table 2, Figure 7).

The dorso-nasal portion of the retina some medium cell counts with an area of high cell counts. The dorso-temporal portion of the retina contained mostly high cell counts. The ventro-nasal portion of the retina contained mostly medium and a scattering of high cell counts whereas
the ventro-temporal portion of the retina contained a mixture of high and medium cell counts with no distinguishing pattern.

Figure 8. Topographic map of case 2625-2
(areas marked with e= edge, nc= not countable)

Retina 2625-2

This retina came from the right eye of an adult male. This retina did not contain any distinct pattern of cell distribution. However, there were scattered small areas containing high cell counts located in areas along the retinal meridian (Table 2, Figure 8).

Both the dorso-nasal and dorso-temporal portions of the retina contained mostly medium and low cell counts without any definite pattern to their distribution. Both the ventro-nasal and
ventro-temporal portions of the retina contained mostly medium cell counts. There were scattered areas of high counts along the retinal meridian, however these counts were not distributed in a way that would allow for the establishment of an observable cell distribution pattern.

Retina 2626-6

This retina came from the right eye of an adult female shark. This retina showed absolutely no distinguishable pattern of cell distribution along the retinal meridian (Table 2, Figure 9).

The dorso-nasal and dorso-temporal portions of the retina contain mostly medium cell counts. Both the ventro-nasal and ventro-temporal portions of the retina contained mostly
medium cell counts with some high and low counts interspersed across the retina in no distinguishable pattern.
Chapter Four

Discussion

The current results revealed that bonnethead sharks had some heterogeneity in retinal ganglion cell distribution. The results also showed that the bonnethead possessed a higher-density “band” of ganglion cells traversing the central potion of the retinal meridian. Finally, small areas of higher cell density in the dorso-temporal area were found in several cases.

Although a higher density “band” of retinal ganglion cells was found within the retinal meridian of this species, the ratio between cell counts within the retinal meridian and other areas outside of the retinal meridian were not appreciably different enough to warrant calling this area a visual streak. The term “band” was used in the present study, even though there was a high degree of individual variation in its overall shape between cases, because of its fairly elongated shape in all cases.

The results of this study may be explained by two factors (habitat openness and predatory behavior) that appear to be related to ganglion cell topography in sharks as well as other species. Although bonnethead sharks used in this study lived in fairly well-lit, shallow water habitat, their surroundings were likely obstructed, to some extent, due to particulate matter in the water. This factor may be related to the relatively short length and low ratio between cell density within and outside of the “band” of higher cell density within their retinal meridian. The low ratio between cell counts within and outside of the “band” as well as the low overall density of ganglion cells within the bonnethead retina may also be explained by behavior. Though the diet of these animals is well-known, when they are most active during a 24-hour cycle is not. Therefore, the results from this study may also be explained by these sharks being predominantly nocturnal in nature. If the bonnethead shark is a predominantly nocturnal predator, then a visual streak may not be necessary to aid them in prey detection. Sensitivity to light, rather than visual acuity, would likely be more important to a nocturnal predator. Thus, if this species is predominately
nocturnal, this may explain their lack of a visual streak. The dorso-temporal area of increased
ganglion cell density found in several cases could also be potentially associated with the
predatory behavior of this species.

Habitat openness

Habitat openness may influence both the length of areas of higher ganglion cell density as
well as the width of these areas. Hughes terrain theory (1977) predicts that animals with a
distinct visual horizon should possess a visual streak or areas of higher ganglion cell density
along their retinal meridian. More specifically, species with unobstructed views of their
surroundings generally possess lengthy visual streaks which extend across the entire length of
their retinal meridian (Collin and Pettigrew, 1988b). Species inhabiting areas with partially
obstructed views of their habitat generally possess visual streaks that do not traverse the entire
length of their retinal meridian (Oliver et al., 2001).

Results of this study revealed that the “band” of increased cell density in the bonnethead
shark was rather narrow in width and did not traverse the entire length of the retinal meridian.
The increase in overall ganglion cell density across the retinal meridian was expected because of
the fairly-well illuminated, shallow water habitat of the bonnethead shark. Additionally, several
other shark species (lemon, tiger, epaulette, small-mouth dogfish, etc.) all possessed a visual
streak. Although it does not meet the requirements of a visual streak, the existence of a short and
weak “band” found in the present study is consistent with predictions from Hughes terrain theory.

Why did the bonnethead sharks in the present study not possess a visual streak? One
possibility may be their activity cycle. If this species is predominately nocturnal in nature, that
could explain the lack of a strong visual streak as well as why their ganglion cell density is rather
low considering their habitat. Nocturnal predators require a visual system that is more sensitive
to light (and would possess lower ganglion cell densities across their retinal meridians) than able
to resolve visual images with high acuity. Another possibility may be related to the water quality of their habitat. Sharks used in this study were collected from the waters of Charlotte Harbor, which contains rather cloudy water (Humphreys and Grantham, 1995; Tomasko, 2001). Because of murkiness of the water, the view of the visual horizon was likely obstructed, to some extent, for this shark species, potentially making possession of a visual streak not useful to them. Therefore, sharks in this habitat may not have developed a visual streak, dependent on the amount of time they spend within this habitat during the year.

A potential answer as to whether the water quality of the bonnethead sharks collected for this study or their behavioral patterns over a 24 hour-cycle affected the ganglion cell density within their retinas could be found from a comparison of bonnethead sharks living in the waters of Charlotte Harbor to those inhabiting Florida Bay. Florida Bay is located near the Florida Keys and contains less particulate matter which can interfere with overall water clarity (Cortes et al., 1996; Cortes and Parsons, 1996). Preliminary results from mitochondrial DNA testing have also revealed that there is no significant difference between the mitochondrial DNA of bonnethead sharks inhabiting either region (Lombardi-Carlson, Cortes, Parsons, and Manire, 2003). This comparison would be necessary to ascertain whether a relatively narrow and short area of increased ganglion cell density is found species-wide or is due to nocturnal behavior or even potentially regional differences in habitat.

Behavior

Behavior may also be an influential factor affecting retinal ganglion cell topography. This factor may influence both the width as well as the location of the visual streak and any associated specialized areas within the retina (Bozzano and Collin, 2001; Collin and Pettigrew, 1988a and 1988b).
In the bonnethead shark, their higher density “band” was found in a fairly central location within the retina. This higher density “band” did not transverse the entire retinal meridian for the majority of cases. The higher density “band” likely subtends vision for this species visual horizon. Nonetheless, ganglion cell density may not be high enough, within the higher density “band”, to provide this species with anything more than increased motion detection in this area of their visual environment.

However, the areas of higher ganglion cell density located within the temporal retina were not an expected finding, and could be related to the predatory behavior of this shark species. Species who have specialized methods of capturing prey may have specializations that provide them with a visual advantage in locating prey (Bozzano and Collin, 2001; Collin and Pettigrew, 1988a and 1988b; Oliver et al., 2001).

Six of the bonnethead sharks from this study possessed a dorso-temporal area of increased ganglion cell density. According to Collin and Pettigrew (1988b), species possessing areas of increased ganglion cell density in their temporal retina likely use these areas for prey detection. Bonnethead sharks primarily predate upon swift moving blue crabs (Cortez et al., 1996). These crabs may be located moving along the substrate in front and slightly below the visual horizon of this shark. Therefore, an area of higher ganglion cell density located within the dorso-temporal retina may be advantageous to this shark species. An increased sensitivity to motion detection along the substrate would likely aid the bonnethead shark in locating potential prey items. Capture of these prey items would possibly then fall to other sensory systems, such as movement detected by the lateral line and electroreception of prey location detected through use of the ampullae of Lorenzini.

Although results from the lone male retina utilized in the study did not reveal any topographic pattern, possibly due to methodological issues, the lack of a definitive pattern could also be related to sexually dimorphic differences in the head shape of these sharks between males.
and females. Further investigation regarding the retinal topography of male bonnethead sharks is necessary to establish whether this is a possibility.

Technical problems in the present study and possible solutions

No definitive pattern of retinal ganglion cell distribution was found in two of the retinas (2625-2 and 2626-6) and considerable individual differences were observed in the rest of the retinas. This absence and variation of the retinal ganglion cell pattern may be due to methodological problems. In particular, in the field, it is possible that the eyes did not receive enough preservative to fix them properly or too much time passed before preservative was added to the eyes to conserve them. If the eyes did not receive preservative in a timely matter, this could have resulted in ischemic damage which could explain the absence of a topographic pattern in two of the cases as well as the considerable individual differences between the “band” of higher cell density as well as location and size of the dorso-temporal area between all cases.

Retinal counts in this study may have also been underestimated. The tracer DiI was to be utilized as a medium with which ganglion cell morphology in the bonnethead shark could be documented. However, DiI does not appear to be compatible with elasmobranch body chemistry and was unable to be used to document retinal ganglion cell morphology. Ganglion cell counts were then based on descriptions from both Hueter (1991) and Collin (1988). All ganglion cell counts in this study were based on darkness of the Nissl stain, cell-body shape, and presence of axon bodies. It is possible that the actual number of ganglion cells were undercounted.

To correct for both problems, it may be better to watch the sharks after collection and inject preservative into them immediately after the shark expires, instead of attempting to inject preservative into the eyes within 15 minutes of the shark expiring. As of now, sacrificing the shark and immediately harvesting the eyes still appears to be the most effective way to conduct a
study of retinal topography. It would also be helpful to find a suitable tracer that works well with the body chemistry of elasmobranches.

Future directions

To further elaborate upon and better understand the significance of retinal ganglion cell topography in the bonnethead shark, an investigation of the visual threshold and visual capabilities of this shark species would be beneficial. These types of studies should also take the differences in head shape between males and females into account. If this species is unable to detect targets or objects located in front and slightly below them, then vision is likely not as important to their daily survival as other sensory systems.

Conducting an analysis of retinal ganglion cell topography on bonnethead sharks living in Florida Bay may also help to confirm the findings from this project. Findings from the same species of shark inhabiting a more illuminated and open habitat may shed light on whether or not the overall ganglion cell concentration as well as width and length of the higher density “band” is common to all bonnetheads living in Florida or varies according to location (i.e. is influenced by environmental factors). If retinal topography between the two populations of bonnethead sharks is the same, then a study investigating whether or not these sharks are nocturnal in nature would also aid in better understanding of their retinal topography. Physically measuring and behaviorally testing the extent and limits of the visual field of the bonnethead shark (from either region) would also help to ascertain whether the retinal topography of this shark could be related to its predatory behavior.

Conclusions

Even though a higher density “band” was revealed within the retinal meridian of this species, the ambiguity of the shape of this “band” as well as the low ratio between the density of
this “band” may signify that vision is not as important to this species as other sensory systems or that this shark could be more nocturnal than diurnal in its activity patterns. This region could also be left over from before the evolution of this species unique head shape or could be used to lower the threshold for detection of disturbances within the shark’s visual horizon. Further research regarding the visual capabilities of this species could reveal how, if at all, this area of increased cell density is utilized. Comparisons between this and other hammerhead species could also reveal whether or not retinal topography differs between the bonnethead shark and other hammerhead species. If there is a difference in retinal ganglion cell topography within the other species of hammerhead sharks, it could signify that behavioral utility is the most influential factor behind retinal ganglion cell topography in these sharks.
References


Appendix A: Raw data retinal counts (in cells per mm²)

Figure 10. Raw data retina 2627-1
Figure 11. Raw data retina 2626-4

Figure 12. Raw data retina 2626-5
Figure 13. Raw data retina 2626-8

Figure 14. Raw data retina IMF1
Figure 15. Raw data retina IMF2
Figure 16. Raw data retina 2625-2

Figure 17. Raw data retina 2626-6