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MORPHOLOGICAL, ELECTROPHYSIOLOGICAL AND BEHAVIORAL
INVESTIGATION OF VISUAL ACUITY OF THE JUVENILE
LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*)

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

By
Soraya Moein Bartol
1999

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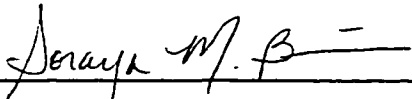
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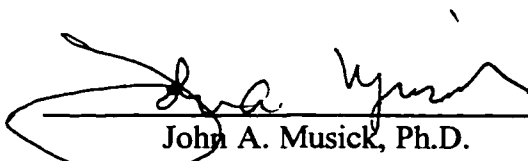
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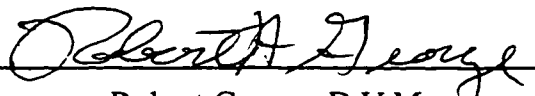


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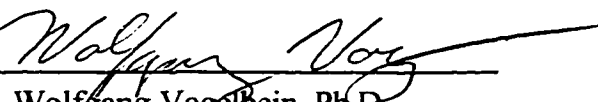
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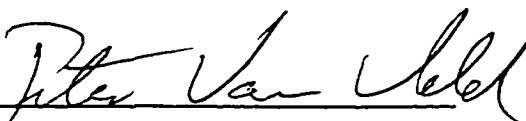
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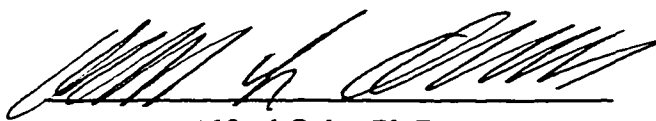
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To my husband, Ian, for his patience, guidance, and love

TABLE OF CONTENTS

	<u>Page</u>
ACKNOWLEDGEMENTS.....	vi
LIST OF TABLES.....	vii
LIST OF FIGURES	ix
ABSTRACT	xiii
INTRODUCTION	2
Morphology.....	6
Visual evoked potentials.....	8
Behavioral psychophysics.....	9
Subject animals	11
Literature Cited	12
CHAPTER 1	15
Abstract.....	16
Introduction.....	17
Materials and Methods.....	20
Statistics	22
Results.....	24
Morphology.....	24
Topography	29
Discussion.....	48
Literature Cited	53
CHAPTER	55
Abstract.....	56
Introduction.....	57
Materials and Methods.....	61
Subject animal.....	61
Experimental design.....	61

	<u>Page</u>
Calculation of visual acuity	63
Statistical analysis	63
Results.....	66
Waveform characteristics.....	66
Visual evoked potentials.....	66
Discussion.....	79
Literature Cited	82
CHAPTER 3	85
Abstract.....	86
Introduction.....	87
Materials and Methods.....	91
Subject animal.....	91
Tank and apparatus	91
Training and experimental procedures.....	92
Calculation of visual acuity and statistical analysis.....	95
Results.....	97
Training and general aspects of behavior	97
Threshold trials	99
Discussion.....	107
Literature Cited	111
CONCLUSIONS.....	114
VITA	118

ACKNOWLEDGEMENTS

I would like to thank my major advisor, Jack Musick, for his guidance and support throughout this project. Jack gave me the freedom to pursue a topic of my own interest and enthusiasm, and for that I will be forever grateful.

I would like to thank my committee members, Bob George, Roger Mellgren, Al Ochs, Wolfgang Vogelbein, and Pete Van Veld for helpful comments and essential suggestions throughout this doctoral process. Both Al Ochs and Wolfgang Vogelbein provided me with necessary equipment and supplies from their lab, at no cost to me (an important point, when your project is unfunded!).

I would also like to acknowledge Pat Blake and Dave Zwerner for their patient help and instruction in the histology lab. I am extremely grateful for the help of Mark Patterson, the creator of the Mathematica program to plot the retina cells on a spherical plot. What initially looked like an easy task, instead took up many hours of his valuable time.

Several groups were instrumental in helping hold turtles over-winter here at VIMS. Gene Burresson was generous with both laboratory space as well as funds so that I could hold turtles in a non-greenhouse facility. Furthermore, Valerie Harmon and her staff graciously housed my turtles in the hatchery facility one winter.

I am especially grateful to the sea turtle crew, both past and present. Deb Barnard and John Keinath taught me everything I needed to know to handle live and dead sea turtles (as well as how to handle VIMS!). I would also like to thank Roy Pemberton, Barbara Bell, Kate Mansfield, Sarah Muffelman, and Sabrina Maynard for their dedication to the sea turtle project. I would like to particularly thank Fred Jett, a poundnetter from the Potomac River, who provided me with every live healthy turtle he caught; this project could never have been completed without his help and generosity.

Finally, no endeavor, such as this, is possible without friends and family. To my friends at VIMS, thank you for listening to me complain, giving me valuable advice and suggestions, and on occasion, helping me wrestle turtles. I would like to thank my family for supporting me at every step along the way. Finally, I would like to thank Ian Bartol, for keeping me laughing, and for providing me with an amazingly wonderful life.

LIST OF TABLES

CHAPTER 1

<u>Table</u>	<u>Page</u>
1. Retinal dimensions of the juvenile loggerhead sea turtle.	26
2. Two-factor ANOVA performed on cone receptor cell counts recorded from the left eye of the juvenile loggerhead sea turtle.....	30
3. Two-factor ANOVA performed on ganglion cell counts recorded from the left eye of the juvenile loggerhead sea turtle.....	31
4. Two-factor ANOVA performed on rod receptor cell counts recorded from the left eye of the juvenile loggerhead sea turtle.....	34
5. Two-factor ANOVA performed on cone receptor cell counts recorded from the right eye of the juvenile loggerhead sea turtle.	39
6. Two-factor ANOVA performed on ganglion cell counts recorded from the right eye of the juvenile loggerhead sea turtle.	40
7. Two-factor ANOVA performed on rod receptor cell counts recorded from the right eye of the juvenile loggerhead sea turtle.	43
8. Average density of cones, rods, and ganglion cells in regions of high, average, and low concentration.....	46
9. Linear correlation performed on left and right eyes for cone and rod receptor cells and ganglion cells.....	47

CHAPTER 2

<u>Table</u>	<u>Page</u>
1. Linear regression results from six sea turtles, and all six turtles combined, when P1-N1 amplitudes are plotted based on stripe size of stimulus.....	76

CHAPTER 3

<u>Table</u>		<u>Page</u>
1	Linear regression analysis results from five sea turtles, and all five turtles combined, when percent correct responses were plotted based on stripe size of stimulus.	105
2	Visual acuities, in minutes of arc, of various aquatic species measured using psychophysical techniques.....	110

LIST OF FIGURES

CHAPTER 1

<u>Figure</u>		<u>Page</u>
1	Light micrograph of a slice sectioned from the eye of a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).....	21
2	Light micrograph of the retina of the juvenile loggerhead sea turtle (<i>Caretta caretta</i>).....	25
3	Light micrograph of the photoreceptor layer of the juvenile loggerhead sea turtle (<i>Caretta caretta</i>).....	28
4	Mean cone photoreceptor cell counts, collected from the retinas of juvenile loggerhead sea turtles (<i>Caretta caretta</i>), for the eight latitudes of the left eye in both the ventral and dorsal hemispheres.....	32
5	Mean ganglion cell counts, collected from the retinas of juvenile loggerhead sea turtles (<i>Caretta caretta</i>), for the eight latitudes of the left eye in both the ventral and dorsal hemispheres.....	33
6	Mean rod photoreceptor cell counts, collected from the retinas of juvenile loggerhead sea turtles (<i>Caretta caretta</i>), for the eight latitudes of the left eye in both the ventral and dorsal hemispheres.....	35
7	Spherical plot of cell counts for the left eye of the juvenile loggerhead sea turtle (<i>Caretta caretta</i>).....	36
8	Mean cone photoreceptor cell counts, collected from the retinas of juvenile loggerhead sea turtles (<i>Caretta caretta</i>), for the eight latitudes of the right eye in both the ventral and dorsal hemispheres.	40

<u>Figure</u>	<u>Page</u>
9 Mean ganglion cell counts, collected from the retinas of juvenile loggerhead sea turtles (<i>Caretta caretta</i>), for the eight latitudes of the right eye in both the ventral and dorsal hemispheres.....	42
10 Mean rod photoreceptor cell counts, collected from the retinas of juvenile loggerhead sea turtles (<i>Caretta caretta</i>), for the eight latitudes of the right eye in both the ventral and dorsal hemispheres.....	44
11 Spherical plot of cell counts for the right eye of the juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	45

CHAPTER 2

<u>Figure</u>	<u>Page</u>
1 Schematic diagram for the collection of visual evoked potentials from a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).....	64
2 Shape of a visual evoked potential waveform for a suprathreshold alternating stripe trial collected from a loggerhead sea turtle (<i>Caretta caretta</i>) using subdermal platinum electrodes.....	67
3 Visual evoked potential recording for a session with one turtle using eight stimulus sizes (ranging from 68.7 to 5.7 minutes of arc).....	68
4 Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 1, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	70
5 Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 2, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	71

<u>Figure</u>	<u>Page</u>
6 Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 3, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	72
7 Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 4, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	73
8 Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 5, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	74
9 Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 6, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	75
10 Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for every trial performed on all loggerhead sea turtles tested.	77
11 Comparison of waveforms collected, using the 45.8 minutes of arc stripe stimulus, for both in air and in water trials.	78

CHAPTER 3

<u>Figure</u>	<u>Page</u>
1 Schematic diagram of tank design used to test the behavioral visual acuity thresholds of the juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	93
2 Four examples of stimuli panels used in the training and testing of visual acuities of juvenile loggerhead sea turtles (<i>Caretta caretta</i>).	94
3 Learning curves of five loggerhead sea turtles (<i>Caretta caretta</i>) trained to discriminate between an illuminated gray panel	

	and 45mm striped panel.	98
4	Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 1, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	100
5	Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 2, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	101
6	Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 3, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	102
7	Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 4, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	103
8	Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 5, a juvenile loggerhead sea turtle (<i>Caretta caretta</i>).	104
9	Percent correct responses to stimulus panels of decreasing stripe size for all juvenile loggerhead sea turtles (<i>Caretta caretta</i>) tested.....	106

ABSTRACT

A majority of the research on sea turtle vision focuses on the turtle's ability to perceive shapes, colors and brightness cues on land. However, aerial vision is a minor component of the visual ability of sea turtles, potentially used only when surfacing to breathe, while basking, and during female reproductive activities. For my doctoral dissertation, I examined the aquatic visual acuity of juvenile loggerhead sea turtles (*Caretta caretta*) by investigating the 1) morphology of the eye, 2) electrophysiology of response to stimuli, and 3) operant conditioning behavior to specific stimuli.

Resolution of the retina was examined by describing the absolute density of the cones as well as the regionalization of cone density. Fresh eyes were obtained from loggerhead sea turtles, euthanized due to injury or illness, and histological techniques were used to identify cones, rods and ganglion cells. Higher concentrations of both cone photoreceptor cells and ganglion cells were found in the dorsal region of the eye. Furthermore, the cone and rod cell densities throughout the eye indicate that this animal has not completely sacrificed sensitivity for acuity, and is capable of vision in a dim environment.

The collection of visual evoked potentials (VEPs), electric responses of any neural tissue identified to correspond to a visual stimulus, was used to non-invasively assess visual resolution in sea turtles. Testing was performed out of water; each animal was fitted with a water-filled goggle constructed of 1/8 inch Plexiglas. Two sets of subdermal platinum electrodes were implanted above the optic nerve and contralateral optic tectum. Stimuli consisted of black and white bars of equal width; flickering angle was set for a fixed exchange of the bars. Intensity of the stimuli remained constant but width of the stripes varied with each trial. Clear and repeatable electrophysiological responses were collected using an averaging computer. A visual acuity threshold of 5.4 minutes of arc was calculated for the turtles tested.

Finally, to illustrate the interlocking relationships among anatomical, electrophysiological and behavioral data, psychophysical experiments were performed. Visual acuity was measured from juvenile loggerheads using a two response forced-choice method. Loggerheads were trained, in a 500-gallon tank, to discriminate between a vertically striped panel and a 50% gray panel. Test panels were illuminated simultaneously and a correct response (contact with PVC pipe below the striped panel) was reinforced with presentation of a food reward. Training continued until the turtle selected the striped panel greater than 80% of the time. Once training was achieved, stripes were methodically reduced in size until they could no longer be resolved. This study recorded a visual acuity threshold for the juvenile loggerhead to be approximately 12.9 minutes of arc.

These visual acuity measurements indicate that vision does play an integral role in the juvenile loggerhead's perception of its surroundings. The three techniques described above suggest that the juvenile loggerhead sea turtle has an effective visual acuity, ranging between 5.4 and 12.9 minutes of arc. Furthermore, the greatest area of resolution is in the dorsal region of the eye. The thresholds recorded are suitable levels for foraging, predator avoidance, territory selection and defense, and other basic behaviors in their aquatic surroundings.

MORPHOLOGICAL, ELECTROPHYSIOLOGICAL AND BEHAVIORAL
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LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*)

INTRODUCTION

Sea turtles reside in the marine and estuarine environment for the majority of their life. The only remaining terrestrial link is reproduction. Females of all species must come ashore to lay their eggs on the beach and thus all hatchlings must also navigate once towards the sea. Otherwise, these turtles are largely adapted to the aquatic environment. In most cases sea turtles have three distinct life history stages (hatchling, juvenile, and adult) and this is true also for the Atlantic loggerhead sea turtle (*Caretta caretta*). These sea turtles use a diversity of habitats corresponding to ontogenetic stages (Musick and Limpus, 1997) and consequently their sensory environment varies throughout these stages.

Loggerhead hatchlings emerge from the nest, find their way to the sea, and immediately begin swimming offshore, becoming pelagic and usually oceanic. Neonate loggerheads have been reported to associate with floating mats of vegetation and are thought to use these areas for both feeding and avoidance of predation (Fletmeyer, 1978). Evidence has confirmed that these hatchlings utilize oceanic currents, such as the Gulf Stream and North Atlantic Gyre, to travel in a circular pattern around the North Atlantic, (Carr et al., 1966; Eckert and Martins, 1989; Witham, 1980; 1991).

After a period of time, thought to be between three and ten years, a critical ontogenetic habitat shift occurs and loggerhead sea turtles actively recruit as juveniles to a demersal, neritic habitat. Frequently this habitat is distinct from the adult areas, either completely or for a period of time. In the western Atlantic, juvenile loggerheads make

seasonal migrations into temperate areas, such as the Mid-Atlantic coast, Delaware Bay and the Chesapeake Bay (Musick, 1988; Shoop and Kenney, 1992). Loggerheads use these areas as seasonal foraging grounds in the summer. It has been estimated that up to 10,000 juvenile loggerheads inhabit the Chesapeake Bay each year (Musick, 1988). These turtles enter the Bay in late May and early June when the water temperature is 18°C or greater. Juvenile loggerheads appear to establish home ranges, usually at the edges of channels (depth less than 13m), the site of most foraging behavior (Keinath et al., 1987; Byles, 1988). Sea turtles leave the Bay area in the autumn with the decline in water temperature, migrate along the coast, and winter in warm coastal waters of Georgia and Florida or offshore in the Gulf Stream waters of North Carolina (Keinath, 1993).

Finally, upon reaching maturity, loggerhead sea turtles occupy a discrete foraging and inter-nesting habitat. Two nesting populations have been identified in the western North Atlantic, one in southern Florida and one from Georgia/South Carolina. Nesting typically occurs between May and September after which the adult turtles disperse to feeding grounds. These feeding grounds range from the Caribbean Sea, Cuba, Bahamas and north along the US East Coast; typically these feeding grounds are distinct from nesting areas.

The visual habitat of these three stages can be very different. The inshore waters of the juveniles and nesting adult stages can have a high sediment load that limits the penetration of light into waters deeper than the first 20 meters. Conversely, the offshore waters of the hatchlings or non-nesting adults have a greater penetration of light into the water column, providing a clearer environment. (Pinet, 1992). Finally hatchlings and adult females must function in a terrestrial environment, albeit briefly, and process aerial visual stimulation.

A majority of the research performed on sea turtle visual systems is behavioral, focusing on the use of their visual capabilities on land. Frequently, the only encounters researchers have with healthy sea turtles are when the turtle is on land. The capacity of nesting females to find a suitable site and hatchling turtles to find water has been the subject of many scientific endeavors. Visual cues were found to be important for these animals. Blindfolded turtles were unable to find the sea (Ehrenfeld and Carr, 1967). Further studies have demonstrated that sea finding behavior is dependent on spectral frequencies, brightness of illumination, and horizon shapes (Anderson, 1958; Daniel and Smith, 1947; Ehrenfeld, 1968; Mrosovsky and Shettleworth, 1968; van Rhijn, 1979a; 1979b; Witherington and Bjorndal, 1991; Salmon and Wyneken, 1994).

Interestingly, even though Ehrenfeld and Carr (1967) found that sea finding orientation was primarily visual, blurring their vision did not impede orientation of the adult females. In fact, it has been a widely held belief that sea turtles are able to distinguish only diffuse images (Walls, 1942). The fact that these animals do not form sharp retinal images on land, however, does not come as a surprise. Preliminary ophthalmological studies of green turtles indicate that they have a refractive error of 40 diopters in air and thus these turtles are highly myopic on land (images are focused between the lens and the retina and thus only close images are resolved) (Ehrenfeld and Koch, 1967). However, when submerged in water, these green turtles had a refractive error of 0 diopters and were emmetropic (images correctly focused onto the retina over a greater range of distances from the turtle). This dichotomy of refractive errors in air and in water was not observed in the freshwater turtle (*Clemmys insculpta*) (Ehrenfeld and Koch, 1967); in fact this turtle was found to be emmetropic in both media.

A comparison of the eye anatomy for both the sea turtle and semi-aquatic turtle helps explain this discrepancy in accommodative ability. The anatomy of the sea turtle eye is discussed only briefly in the literature (Granda, 1979; Walls, 1942). Fortunately, the basic anatomy of the turtle eye appears to be typical of that found in all vertebrates. The eyeball is filled with ocular fluids, aqueous and vitreous humors, and is composed of three tunics: 1) outermost layer comprising the sclera and cornea, 2) middle layer consisting of the choroid, ciliary body and iris, and 3) the inner layer comprising the retina. The sclera is inelastic and is responsible for the eyeball's static shape, while the aqueous humor keeps the fibrous tunic distended. The anterior portion of the sclera, the cornea, is transparent and responsible for much of the refraction of light in air, yet is non-refractive in water. The middle layer choroid is highly pigmented and vascularized; the pigmentation deflects stray light from entering the eye as well as prevents internal reflections. Both the ciliary body and the iris of the middle layer consist of smooth muscle. The inner layer of the eyeball, the retina, contains the visual cell, bipolar, and ganglion cell layers which are continuous with the optic nerve (Ali and Klyne, 1985; Copenhaver, 1964; Granda, 1979; Walls, 1942).

Freshwater turtles have developed an advanced means of accommodation through the contraction of the sphincter iridis muscle, formed by the ciliary body and the annular pad (*ringwulst*). These muscles squeeze upon an extremely pliable lens to adjust the curvature, and thus the refractive angle, of the lens (Duke-Elder, 1958, Ehrenfeld and Koch, 1967, Granda, 1979, Walls, 1942). Sea turtle visual systems, on the other hand, vary from their freshwater relatives. The lens of the green sea turtle (*Chelonia mydas*) is nearly spherical and rigid (Ehrenfeld and Koch, 1967; Granda, 1979; Walls, 1942). The ciliary processes do not reach the lens and the *ringwulst* is weakly developed; thus active accommodation does

not appear to be possible (Ehrenfeld and Koch, 1967). However, the spherical lens is ideal for underwater vision. In the absence of corneal refraction, the refractive index of the cornea is nearly identical to that of seawater, the lens is the only structure responsible for the refraction of incoming light. The spherical lens compensates for this situation with a high refractive index (Sivak, 1985; Fernald, 1992).

From these anatomical studies, we know that visual resolution for sea turtles should be significantly different in air versus water media. This dissertation explores the loggerhead sea turtle's ability to detect details of objects in the marine environment. Specifically, I have investigated the visual acuity, or resolution thresholds, of the juvenile loggerhead sea turtle by examining the morphology of the retina, electrophysiology of response to stimuli, and learned behavior to specific stimuli.

Morphology

Typically, the literature abounds with morphological studies of a given species; retinal morphology and topography provide an initial view of the potential resolving power of an eye. In the case of sea turtles, however, their protected status has prevented researchers from obtaining fresh samples. For this study, samples were obtained through the National Marine Fisheries Service (NMFS) Sea Turtle Stranding and Salvage Network (STSSN).

The first goal of this morphological investigation was to identify components of the retina necessary for image processing. The retina is the site of transformation of photic stimulation into an electrochemical signal. The vertical organization of the retina, in order of conduction, usually begins with the rods and cones as the photo-receptive layer. These visual cells are arranged parallel to each other, perpendicular to the surface of the retina. The base

of the visual cells is adjacent to the dendrites of a bipolar cell, allowing a transfer of stimulus activity. The axons of the bipolar cell are in contact with the dendrites of ganglion cells, where finally their axons converge to form the optic nerve. Each bipolar cell can be in contact with more than one visual cell and each ganglion cell can receive information from more than one bipolar cell so that information from the stimulus spreads horizontally through the layers as well as vertically (Copenhaver, 1964; Davson, 1972; Walls, 1942).

Visual acuity can be affected by many factors along the entire visual pathway, including intensity of the stimulus, optical parameters of the eyeball, and cerebral pathways. However, it is the resolving power of the retina which ultimately determines the extent of acuity (Walls, 1942). Resolution of the retina can be described by examining the size and density of the cone mosaic (Walls, 1942). If the photoreceptors are so large or far apart that object points fall on adjacent photoreceptors or between photoreceptors, then the points are not distinguishable, indeterminate of the accommodation ability (Walls, 1942; Heuter and Gruber, 1982). Though many articles will describe the rod to cone density as a measure of the retina, it is actually the absolute density of the cones as well as the regionalization of cone density which should be used in the prediction of visual acuity (Heuter 1991; Heuter and Gruber, 1982).

Resolution power of the retina also can be a factor of summation. Summation can be useful in retinal structuring; sensitivity in diffuse light is accomplished through the summation of many rods. If the stimulus is weak, then more than one rod converging on a bipolar cell will subsequently multiply intensity of the stimulus. However, for an individual cone to be used as an indication of acuity, the cone must encounter a relatively low level of summation. If several cones are connected to a bipolar cell or several bipolar cells are

connected to a ganglion cell, the information relayed to the optic tectum is not characteristic of one cone, but rather a summation of many. By definition, the more summation occurring among the cones, the more diffuse the image will appear (Walls, 1942).

The objectives of the morphological section were to first identify the vertical structure of the retina of the juvenile loggerhead sea turtle. Secondly, the density of rods, cones, and ganglion cells were measured. The topographical relationships of these densities were used to determine the extent of summation.

Visual evoked potentials

The second phase of this project investigated the visual acuity of sea turtles by recording visual evoked potentials (VEPs). Evoked potentials are electric responses of any neural tissue identified to correspond to a stimulus. In this experiment, the stimuli were presented to the retina as a series of alternating striped patterns. The neural response was then detected by an array of electrodes placed extracranially on the skull. Because the electrodes are over, but not on, the optic tectum, responses are very small when compared to background noise. Excessive biological noise of ongoing neural and muscular electrical activity introduces components unrelated to the stimulus that often “drown out” the response (Rubin and Walls, 1969; Spehlmann, 1985). This obstacle may be overcome by averaging single responses. The background noise is random (positive and negative activity) at any one moment and averaging of this noise will produce a straight line. Alternatively, if a neural discharge occurs at a certain time (latency) as the visual stimulus is presented, then averaging many responses at the same rate as stimulus presentation will produce a summation of the single response (Rubin and Walls, 1969; Moein, 1994; Bartol, 1999). These VEP methods

are noninvasive and are the best means of measuring electrophysiological responses in non-communicative subjects (McCormack and Tomlinson, 1979).

The objectives of the visual evoked potential section were to first determine a methodology for collecting evoked responses from sea turtles. Due to the necessity of recording visual evoked potentials out of water, a procedure for submerging only the stimulated eye was devised. Next, VEPs were collected from alternating stripe stimuli that were reduced in size incrementally. From these data, the threshold of visual acuity was extrapolated.

Behavioral psychophysics

The final phase of this research project assesses the acuity of juvenile loggerheads using behavioral psychophysics. Psychophysical research on a subject animal illustrates the interlocking relationships among anatomical, electrophysiological and behavioral data. To understand the response of the whole animal (in this case, juvenile loggerhead sea turtle) to stimulation, if the pathway from receptor organ to the optic tectum translates into a prescribed behavior, psychophysical experiments are performed.

Behaviors explored in psychophysical experiments usually fall into two categories: innate behaviors and learned behaviors. Innate behaviors are automatic responses to stimuli, such as eye movement, increased heart/breathing rate, aggressive/flight responses, etc. Innate behavior experiments, however, are prone to subjectivity by the researcher as well as habituation of the subject. Learned psychophysical experiments represent behavior imposed by the experimenter. This technique eliminates bias; the experimenter can record both

incorrect responses as well as failures to respond (Blough, 1971; Blough and Blough, 1977; Douglas and Hawryshyn, 1990).

One form of learned experiments, operant conditioning, maintains a learned response with the subject through either positive reinforcement or adverse stimulation. The most commonly used technique when examining visual ability of a subject animal is the two-response forced-choice method. The subject is presented with two stimuli and is reinforced to choose the "correct" one by the presentation of an associated reward. The position of the correct stimulus exchanges with the incorrect stimulus randomly to ensure that the learned behavior is in connection with the stimulus and not the location (Blough and Blough, 1977; Douglas and Hawryshyn, 1990).

Threshold of visual acuity can be obtained with the two-response forced-choice method. Threshold is deemed as occurring when the stimulus elicits a correct response 50% of the time. Starting with a stimulus known to be detectable, the experimenter proceeds in regular intervals in descending order until threshold is achieved. Each stimulus is usually presented in blocks of several trials to account for any variation and provide a reliable indicator of performance (Hodos and Bonbright, 1972; Bough and Blough, 1977).

The objectives of the behavioral psychophysical section were to first train the juvenile turtle to enact a specific response to a suprathreshold stimulus. Once training was achieved, operant conditioning methods were utilized to obtain visual acuity thresholds from the loggerhead sea turtle in an underwater environment.

Subject animals

For all three sections of this project, the subject animal is the juvenile loggerhead sea turtle, *Caretta caretta*. These animals were obtained through the Virginia Institute of Marine Science (VIMS) Sea Turtle Stranding Project. This project is the state letter holder for the National Marine Fisheries Service and is responsible for all stranded and incidentally caught sea turtles in Virginia. Loggerhead sea turtles inhabit the Chesapeake Bay during the summer months, and almost the entire population is composed of juveniles. Through a well-established network of local watermen, the VIMS Sea Turtle Stranding Project is able to obtain healthy juveniles. Turtles frequently are entrapped in the pound enclosure of a poundnet. The mesh of this enclosure is small enough so that the sea turtles do not usually become entangled. Consequently these animals go through minimum stress when captured.

Tissue samples for the morphology section are also obtained through the Sea Turtle Stranding Project at VIMS. All animals used for histological evaluation were either injured or chronically ill when recovered by the stranding network. After examination by the staff veterinarian, these animals were euthanized based on the extent of their existing injuries and tissue samples were immediately collected.

LITERATURE CITED

- Ali, M.A. and M.A. Klyne. 1985. *Vision in Vertebrates*. New York: Plenum Press. Pp.272.
- Anderson, P.K. 1958. The photic responses and water approach behavior of hatchling turtles. *Copeia*. 211-215.
- Bartol, S. M., J. A. Musick, and M. L. Lenhardt. 1999. Auditory evoked potentials of the loggerhead sea turtle (*Caretta caretta*). *Copeia*.
- Blough, P.M. 1971. The visual acuity of the pigeon for distant targets. *J. Exp. Anal. Behav.* 15: 57-67
- Blough, D. and Blough, P. 1977. *Animal Psychophysics*. In: W.K. Honig and J.E.R. Staddon (eds.) *Handbook of Operant Behavior*. Englewood Cliffs, NJ: Prentice- Hill Inc. pp. 514-539.
- Byles, R. A. 1988. Behavior and ecology of sea turtles from Chesapeake Bay, VA. Ph.D. dissertation, College of William and Mary, Williamsburg, VA.
- Copenhaver, W.M. 1964. *Bailey's Textbook of Histology*. Baltimore: The Williams and Wilkins Co. Pp. 679.
- Daniel, R.S. and K.U. Smith. 1947. The sea-approach behavior of the neonate loggerhead turtle (*Caretta caretta*). *J. Comp. Physiol. Psychol.* 40: 413-420.
- Davson, H. 1972. *The Physiology of the Eye*. New York: Academic Press. Pp. 643.
- Douglas, R.H. and C.W. Hawryshyn. 1990. Behavioral studies of fish vision: an analysis of visual capabilities. In R.H. Douglas and M.B.A. Djamgoz (eds.) *The Visual System of Fish*. London: Chapman and Hall Ltd. pp. 373-418.
- Ehrenfeld, D.W. 1968. The role of vision in the sea-finding orientation of the green turtle (*Chelonia mydas*). 2. Orientation mechanism and range of spectral sensitivity. *Anim. Behav.* 16: 281-287.
- Ehrenfeld, D.W. and A. Carr. 1967. The role of vision in the sea-finding orientation of the green turtle (*Chelonia mydas*). *Anim Behav.* 15: 25-36.

- Ehrenfeld, D.W. and A. L. Koch. 1967. Visual accommodation in the green turtle. *Science*. 155: 827-828.
- Eckert, S. A. and H. R. Martins. 1989. Transatlantic travel by a juvenile loggerhead turtle. *Mar. Turtle Newsl.* 45: 15.
- Fernald, R. D. 1990. The optical systems of fishes. In: R. H. Douglas and M. B. A. Djamgoz (eds.) *The Visual System of Fish*. London: Chapman and Hall. pp. 45-61.
- Fletmeyer, J. R. 1978. Underwater tracking evidence that neonate loggerhead sea turtles see shelter in drifting Sargassum. *Copeia*. 1: 148.
- Granda, A.M. 1979. Eyes and their sensitivity to light of differing wavelengths. In M. Harless and H. Morlock (eds.) *Turtles: Perspectives and Research*. New York: John Wiley and Sons. pp. 247-266.
- Heuter, R.E. 1991. Adaptations for Spatial Vision in Sharks. *J. Exp. Zool. Suppl.* 5: 130-141.
- Heuter, R.E. and S.H. Gruber. 1982. Recent advances in studies of the visual system of the juvenile lemon shark (*Negaprion brevirostris*). *Florida Sci.* 45: 11-25.
- Keinath, J. A. 1993. Movements and behavior of wild and head-started sea turtles. Ph.D. dissertation, College of William and Mary, Williamsburg, VA.
- Keinath, J. A., J. A. Musick, and R. A. Byles. 1987. Aspects of the biology of Virginia's sea turtles: 1979-1986. *Va. J. Sci.* 38(4): 329.
- McCormack, G. L. and A. A. Tomlinson. 1979. Human visual acuity assessment through linear extrapolation to threshold of bar grating VERs. *Am. J. Optom. And Physio. Optics.* 56(8): 480-489.
- Moein, S.E. 1994. Auditory Evoked Potentials of the Loggerhead Sea Turtle (*Caretta caretta*). M.A. Thesis. College of William and Mary, Gloucester Point, VA. 66 pp.
- Mrosovsky, N. and S. Shettleworth. 1968. Wavelength preferences and brightness cues in the water-finding behavior of sea turtles. *Behavior.* 32: 211-257.
- Musick, J. A. 1988. *The Sea Turtles of Virginia*. Virginia Sea Grant Program, Virginia Institute of Marine Science, Gloucester Point, VA. Pp.22.
- Musick, J. A. and C. J. Limpus. 1997. Habitat utilization and migration in juvenile sea turtles. In: P. L. Lutz and J. A. Musick (eds.) *The Biology of Sea Turtles*. Boca Raton: CRC Press. Pp. 137-163.

- Pinet, P. R. 1992. Oceanography: an Introduction to the Planet Oceanus. West Publishing Company: St. Paul, MN. Pp. 572.
- Salmon, M. and J. Wyneken. 1994. Orientation by hatchling sea turtles: mechanisms and implications. *Herp. Nat. His.* 2: 13-24.
- Sivak, J. G. 1985. The Glenn A Fry award lecture: optics of the crystalline lens. *Am. J. Optom. and Physiol. Optics.* 62: 299-308.
- Shoop, C. R. and R. D. Kenney. 1992. Seasonal distribution and abundances of loggerhead and leatherback sea turtles in waters of the northeastern United States. *Herpetol. Monogr.* 6: 43.
- Sphelmann, R. 1985. Evoked Potentials Primer: Visual, Auditory, Somatosensory Evoked Potentials in Clinical Diagnosis. Butterworth and Heinemann: Boston.
- van Rhijn, F.A. 1979a. Optic orientation in hatchlings of the sea turtle *Chelonia mydas* I. Brightness: not the only optic cue in sea-finding orientation. *Mar. Behav. Physiol.* 6: 105-121.
- van Rhijn, F.A. 1979b. Optic orientation in hatchlings of the sea turtle *Chelonia mydas* II. Apparatus for prolonged recording of orientation in turtles walking or swimming on the spot. *Mar. Behav. Physiol.* 6: 243-255.
- Walls, G.L. 1942. The Vertebrate Eye and its Adaptive Radiation. Bloomfield Hills: The Cranbrook Institute of Science. 785 pp.
- Witham, R. M. 1980. The "lost years" question in young sea turtles. *Am. Zool.* 20: 525.
- Witham, R. M. 1991. On the ecology of young sea turtles. *Fl Sci.* 54: 179.
- Witherington, B. E. and K. A. Bjorndal. 1991. Influences of wavelength and intensity on hatchling sea turtle phototaxis: implications for sea-finding behavior. *Copeia.* 4: 1060-1069.

CHAPTER 1

Morphology and Topographical Organization of the Retina as an Indication of the Visual Acuity of Juvenile Loggerhead Sea Turtles (*Caretta caretta*)

ABSTRACT

Main features of the structure of the retina of the juvenile loggerhead sea turtle (*Caretta caretta*) are described for both vertical organization and spatial variation. The retina is duplex in nature, containing both cone and rod photoreceptor cells throughout the photoreceptor layer. Moreover, the schematization of the neural layers indicates that this eye is adapted for diurnal functioning. Topographical organization of cells along the retina is also investigated for the presence of an area centralis. A higher concentration of both cone photoreceptor cells and ganglion cells are found in the dorsal region of the eye, and their numbers are positively correlated. These findings suggest that the loggerhead sea turtle possess a higher acuity in its dorsal region, an attribute that could be beneficial for the loggerhead's benthic lifestyle. Furthermore, the cone and rod cell densities throughout the eye indicate that this animal has not completely sacrificed sensitivity for acuity, and is capable of vision in a dim environment.

INTRODUCTION

The mechanisms by which the loggerhead sea turtle gathers visual information from its surrounding environment are still largely unknown, and much of the morphological research on visual systems of turtles has centered on freshwater species (see Peterson, 1992 for review). One structure of particular importance when studying visual acuity, the ability to see details of an object, from a morphological standpoint is the retina. Retinas are the first processors of visual information for most vertebrates and transform photic stimulation into an electrochemical signal that can be interpreted by the brain. By identifying the organization of retinal cells within this tissue, many visual properties and limitations can be described for a species.

The vertical organization of the retina, in order of conduction, begins with the photoreceptive layer, which is composed of cone and rod cells. These visual cells are arranged parallel to each other and perpendicular to the surface of the retina. Though these cell types have similar morphological features, the function of the rod is to maximize sensitivity of the eye to dim stimuli and the function of the cone is to resolve details of a visual object. The base of the visual cells is adjacent to the dendrites of bipolar cells, allowing a transfer of stimulus activity. The axons of the bipolar cell are in contact with the dendrites of ganglion cells, where finally these ganglion axons converge to form the optic nerve, the pathway to the optic tectum of the brain. Each bipolar cell can be in contact with more than one visual cell and each ganglion cell can receive information from more than one

bipolar cell so that information from the stimulus spreads horizontally through the layers of the retina as well as vertically (Walls, 1942; Copenhaver, 1964; Davson, 1972; Stell, 1972).

Historically, turtles were thought to possess a pure cone retina (Schultze, 1873; Walls, 1942). Although the retinal anatomy is virtually unstudied in sea turtles, rods have been found in the retina of *Chelonia mydas* (Liebman, 1972; Granda, 1979), and it is generally thought that all sea turtles contain some form of a duplex retina that have both rods and cones. Sea turtles, consequently, contain a device for both sensitivity (rods) and acuity (cones). Both of these mechanisms are affected by many factors along the entire visual pathway, such as intensity of the stimulus, optical parameters of the eye, cerebral pathways, etc. However, there are two factors within the retina itself that can limit the visual abilities of an animal: summation of the photoreceptor cells upon the optic nerve and portioning of photoreceptor cell densities along the surface of the retina (Walls, 1942).

Summation of the photoreceptor cells can be useful in retinal structuring; sensitivity in diffuse light is accomplished through the summation of many rods. If the stimulus is weak, then more than one rod converging onto one bipolar cell or ganglion cell will subsequently multiply the intensity of the stimulus. However, for an individual cone to be used as an indication of acuity, summation must remain relatively low. If more than one cone is converged onto a single ganglion cell then the information relayed to the optic tectum is not characteristic of one cone, but rather a summation of many resulting in a more diffuse image (Walls, 1942).

Examining the density of photoreceptor cells and neurons along the topography of the retina can also describe the resolving ability of the retina. Though many articles will describe the rod to cone density as a measure of the retina, it is the absolute density of the

cells as well as the regionalization of cell density that is most informative in the prediction of visual acuity (Brown, 1969; Heuter and Gruber, 1982; Gruber and Cohen, 1985; Heuter, 1991; Peterson, 1992). The retinas of many vertebrates have regions of higher cell densities, often called area centralis, which act as a site of increased acuity. Images falling on these regions of high cell density are resolved more clearly than those falling on adjacent areas. The area centralis can vary in shape and location along the retina among species, and this variation is often an indication of behavior and life history attributes of the animal (Walls, 1942; Brown, 1969; Heuter, 1991).

The objectives of this chapter are to describe the main features of the organization of the retina of the juvenile loggerhead sea turtle. Layers of the retina, and their corresponding cells, will be identified. Secondly, topographical organization of cells in the retina will also be explored. Special emphasis will be placed on the identification of an area centralis along the surface of the retina.

MATERIALS AND METHODS

Eyes were obtained from three juvenile loggerhead sea turtles (*Caretta caretta*) incidentally captured in the Chesapeake Bay waters of Virginia and Maryland. All animals were either injured or chronically ill when recovered by the Virginia Institute of Marine Science stranding network. After examination by the staff veterinarian, these animals were euthanized based on the extent of their injuries and according to National Marine Fisheries Service Endangered Species Permit no. 929. The eyes were immediately excised; a slice was made in the cornea to allow influx of fixative, and submerged in Bouin's solution for 48 hours. The eyes were then rinsed over night, infused with lithium carbonate and stored in 70% alcohol.

Each eye was cut into eight equal wedges, from cornea to back of eye, using the cornea and optic nerve as references. This slicing method kept the retina attached to the choroid. These eight wedges were cut identically for each eye. The wedges were then embedded in paraffin, cut side down, and sectioned at 5 μ m using a rotary microtome (Reichert-Jung). Four randomly selected sections (from a random table) were taken from each wedge, placed on a slide, and stained with hematoxylin and eosin.

Each slide was examined using light microscopy techniques. The slice, from the back of the eye to the cornea, was measured under a dissecting microscope with an image processor program (Optimas). This individual slice was divided into eight equal sections (Figure 1) and these eight sections were examined individually under a compound

Figure 1. Light micrograph of a slice sectioned from the eye of a juvenile loggerhead sea turtle (*Caretta caretta*). Slices were cut at 5 μm using a rotary microtome and stained with hematoxylin and eosin. This slice, from the back of the eye to the cornea, was measured under a dissecting microscope using an image processor program (Optimas) and then was divided into eight equal sections (indicated by the black bars). In each section cones, rods, and ganglion cells were counted.



microscope. Within each section, cones, rods and ganglion cells were counted.

Morphological measurements of the layers of the retina and size of the photoreceptive cells were also noted.

Statistics

Measurements of each layer in the retina and width and heights of rods and cones were collected from the retina proper as well as from the peripheral regions of the retina bordering the cornea. These measurements were averaged for all six eyes. Percentages based on total retina size were calculated for each layer.

Statistical analyses were performed on the cell counts from each section. Eyes were group based on side orientation (left and right). Two-factor analysis of variance (ANOVA), examining the effect of latitudinal location along the eye and hemispherical differences (dorsal and ventral), were rendered for cones, rods and ganglion cells. In cases where significance occurred, Student-Newman-Keuls (SNK) multiple comparison test was used to examine individual factor effect. If an interaction between the factors was detected, SNK multiple comparisons were run on the interaction itself (Sokal and Rolf, 1982; Underwood, 1997).

The relationships among these three cells were of interest and a linear correlation analysis was executed using mean data from left and right eyes (Sokal and Rolf, 1982). Also, cell densities were calculated for each of the three cell types in the main regions of the retina as well as in the periphery.

The topography data is displayed using a computer program (Mathematica, Wolfram Research, Inc.) to plot cones, rods, and ganglion cells on a sphere (Patterson and Bartol,

1999). Left and right eyes are portrayed separately, and data were plotted as percentages of the highest concentration of each cell type. Each polygon on the sphere represents the average count for that section, and is colored accordingly.

RESULTS

Morphology

The retina of the juvenile loggerhead is consistent with the generalized vertebrate plan, consisting of seven layers: pigment epithelium, photoreceptor layer, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, and ganglion layer (Figure 2). Measurements were taken from each of the seven layers and the subsequent results are summarized in Table 1. The retina layer's width measurements were the same for sections of the retina proper and the average total width was 238.18 μm . However, measurements were reduced along the outer edges of the retina (near the cornea) with the average total width of the retina of only 177.25 μm .

The pigment epithelium, the outermost layer, was firmly connected to the inner layer of the choroid, the lamina vitrea. The inner section of the pigment epithelium contained heavy pigment laden processes. These processes were intertwined with the outer segment of the photoreceptor cells. The pigment epithelium and lamina vitrea was measured as one, and averaged 32.68 μm thick, comprising 13.7% of the overall retinal thickness. The pigment epithelium and photoreceptor layers were the only two layers that overlapped in the retina. In processing these eyes for histology, the retina frequently disassociated from the rest of the eyeball. In every case, this split occurred in the photoreceptor cell layer with the pigment epithelium remaining firmly attached to the choroid.

Figure 2. Light micrograph of the retina of the juvenile loggerhead sea turtle (*Caretta caretta*). Transverse sections were cut at 5 μm and stained with hematoxylin and eosin. Abbreviations: G = ganglion layer, IN = inner nuclear layer, IP = inner plexiform layer, ON = outer nuclear layer, OP = outer plexiform layer, PE = pigment epithelium, P = photoreceptor layer. (magnification = 250x)

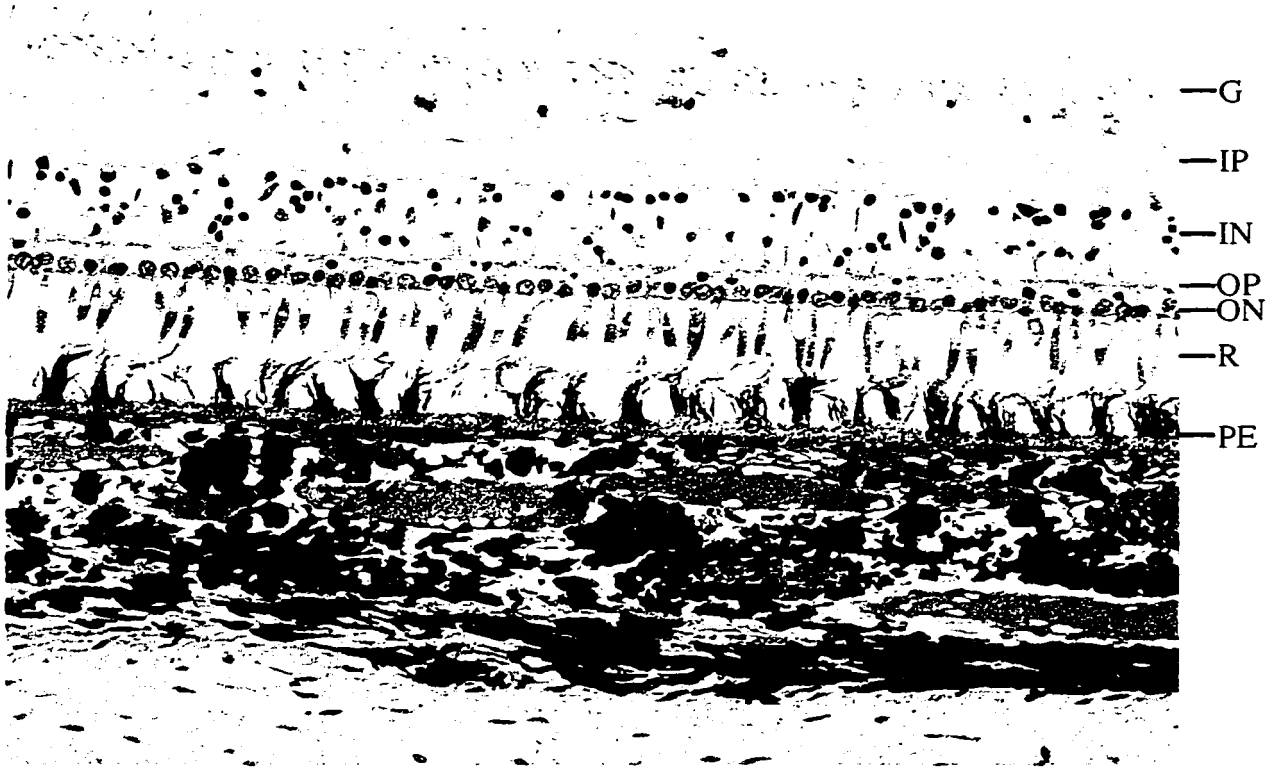


Table 1. Retinal dimensions of the juvenile loggerhead sea turtle. All measurements are in μm .

	Pigment epithelium	Receptor layer	Outer nuclear layer	Outer plexiform layer
Main section	32.68 ± 7.7	41.08 ± 4.4	19.68 ± 3.9	9.67 ± 3.6
% of Total	13.7%	17.2%	8.3%	4.1%
Near Cornea	23.25 ± 3.9	31.5 ± 4.4	13.75 ± 4.1	5.0 ± 2.8
% of Total	13.1%	17.8%	7.8%	2.8%

	Inner nuclear layer	Inner plexiform layer	Ganglion Layer	Total
Main section	32.28 ± 6.0	52.16 ± 8.3	55.06 ± 15.9	238.19 ± 27.6
% of Total	13.6%	21.9%	23.1%	100%
Near Cornea	23.5 ± 4.7	44.25 ± 8.3	41.0 ± 10.6	177.25 ± 11.9
% of Total	13.3%	25.0%	23.1%	100%

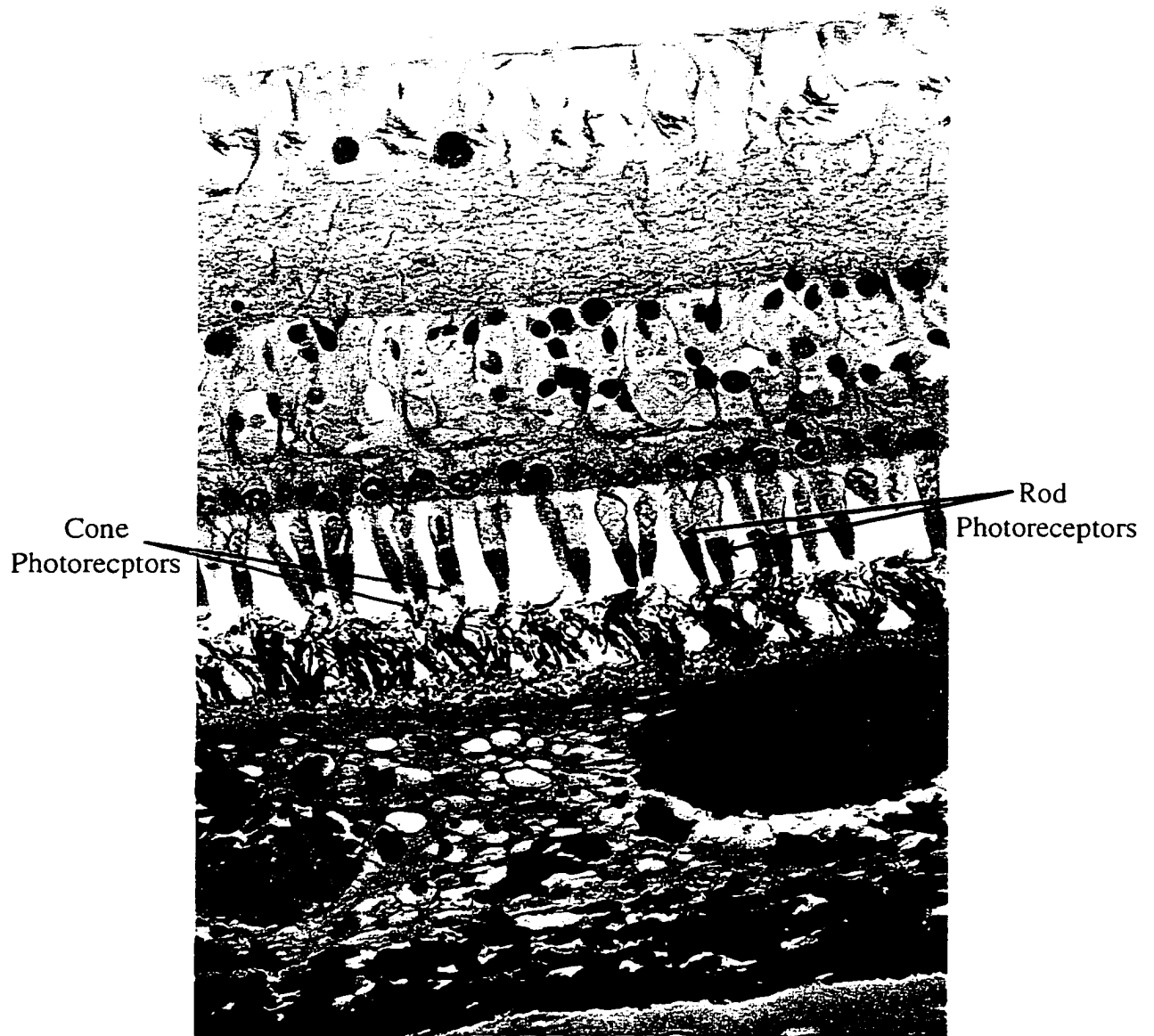
The photoreceptor cell layer was duplex in nature, consisting of both rods and cones. Rods and cones were discriminated based on oil droplet and outer segment morphology. Rods of the loggerhead retina did not have an oil droplet above the ellipsoid element and the outer segment of the rod was longer and more cylindrical than the cone (Figure 3). Both photoreceptor cells were similar in diameter (all measurements taken at the widest point); mean cone diameter measured $5.3 \mu\text{m} \pm 1.04$ S. D. and rod diameter measured $4.1 \mu\text{m} \pm 1.13$ S. D. Double cones were also identified in the retina, but randomly dispersed. The photoreceptor layer was measured from the tip of the outer segment to the outer nuclear layer and measured $41.08 \mu\text{m}$ in thickness (17.2% of the retina). Cone and rod nuclei were found in the outer nuclear layer, lying directly against the extra limiting membrane. This outer nuclear layer averaged $19.68 \mu\text{m}$ thick, or 8.3% of the total retinal thickness.

The outer plexiform layer in the loggerhead retina was invariant and the synaptic connections between the outer and inner nuclear layer could not be distinguished. This layer comprised 4.1% of the overall retinal thickness.

The inner nuclear layer of the vertebrate retina is comprised of the bipolar, horizontal, and amacrine cells, though these cells were not differentiated in this study. This layer averaged $32.28 \mu\text{m}$ (13.6%) in thickness and was highly cellular.

The inner plexiform layer was comprised of the cellular processes of the cells of the inner nuclear layer and the ganglion layer. This layer was broad in the loggerhead retina ($52.16 \mu\text{m}$ or 21.9%) but relatively unremarkable in structure. A nucleus from another layer, however, was occasionally found in the inner plexiform layer.

Figure 3. Light micrograph of the photoreceptor layer of the juvenile loggerhead sea turtle (*Caretta caretta*). Sections were cut at 5 μm and stained with hematoxylin and eosin. Both the cone and rod photoreceptor cells were similar in width and height. Also note the oil droplet above the ellipsoid element in the cone photoreceptor that is absent in the rod photoreceptor.



The ganglion cell layer, the innermost layer of the retina, was also the thickest layer of the retina (55.06 μ m or 23.1%) and measured the largest layer of the retina. Though the layer itself was relatively thick, the ganglion nuclei were usually confined to a single row. These ganglion nuclei varied greatly in size and density. The axons of the ganglion cells comprised the bulk of this layer and converged at the optic nerve. Those sections that cut through the nerve displayed a high concentration of ganglion cells and nerve fibers.

Topography

Eyes were group based on location on the turtle (left vs. right) for all statistical analyses. Initial two-factor ANOVAs were run to examine differences along latitude (from back of eye to cornea) and between hemisphere (dorsal vs. ventral). A significant latitude and hemisphere interaction was detected for both cone photoreceptor cells and ganglion cells for the left eye (Table 2 and 3). A post-hoc Student-Newman-Keuls (SNK) test revealed that the latitudes decrease in cell concentration from the back of the eye to the cornea (Figure 4 and 5). The SNK test also shows that the dorsal hemisphere has greater cell concentration than the ventral hemisphere, but only in the first three latitudes, starting with the back of the eye (Figure 4 and 5). There was also a significant effect of latitude and hemisphere on rod photoreceptor cells in the left eye, though with no interaction between the two factors (Table 4). The post-hoc SNK test reveals that dorsal hemisphere had a higher rod cell concentration than the ventral hemisphere. Also, the latitudes progressed from high cell count to low in three stages (Back of eye through latitude 4 > latitude 5 > 6 > latitude 7 & cornea) (Figure 6, Table 4). These trends for all three cell types are easily discerned when plotted on a sphere (Figure 7a, b, c).

Table 2. Two-factor ANOVA performed on cone receptor cell counts recorded from the left eye of the juvenile loggerhead sea turtle.

	df	Sum of Squares	Mean Square	F-value	P-value
Hemisphere	1	4138.463	4138.463	19.177	<.0001
Latitude	7	326404.094	46629.156	216.076	<.0001
Hemisphere * latitude	7	5296.464	756.638	3.506	.0044
Residual	45	9710.996	215.8		

Student-Newman-Keuls Test

Latitude

Ventral: Back of Eye > Latitude 2–4 > Latitude 5 > Latitude 6 > Latitude 7–Cornea

Dorsal: Back of Eye > Latitude 2 > Latitude 3 > Latitude 4 > Latitude 5 >
Latitude 6 > Latitude 7–Cornea

Hemisphere

Back of Eye–Latitude 3: Dorsal > Ventral

Latitude 4–Cornea: Dorsal = Ventral

Table 3. Two-factor ANOVA performed on ganglion cell counts recorded from the left eye of the juvenile loggerhead sea turtle.

	df	Sum of Squares	Mean Square	F-value	P-value
Hemisphere	1	3868.107	3868.107	63.639	<.0001
Latitude	7	52582.333	7511.762	123.584	<.0001
Hemisphere * latitude	7	4759.394	679.913	11.186	<.0001
Residual	45	2735.209	60.782		

Student-Newman-Keuls Test

Latitude

Ventral: Back of Eye–Latitude 2, Latitude 2–4 > Latitude 5 > Latitude 6 > Latitude 7–Cornea

Dorsal: Back of Eye > Latitude 2 > Latitude 3 > Latitude 4 > Latitude 5 > Latitude 6 > Latitude 7–Cornea

Hemisphere

Back of Eye–Latitude 3: Dorsal > Ventral

Latitude 4–Cornea: Dorsal = Ventral

Figure 4. Mean cone photoreceptor cell counts, collected from the retinas of juvenile loggerhead sea turtles (*Caretta caretta*), for the eight latitudes of the left eye in both the ventral and dorsal hemispheres. All error bars denote + 1 S. D.

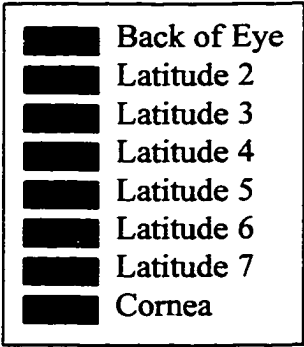
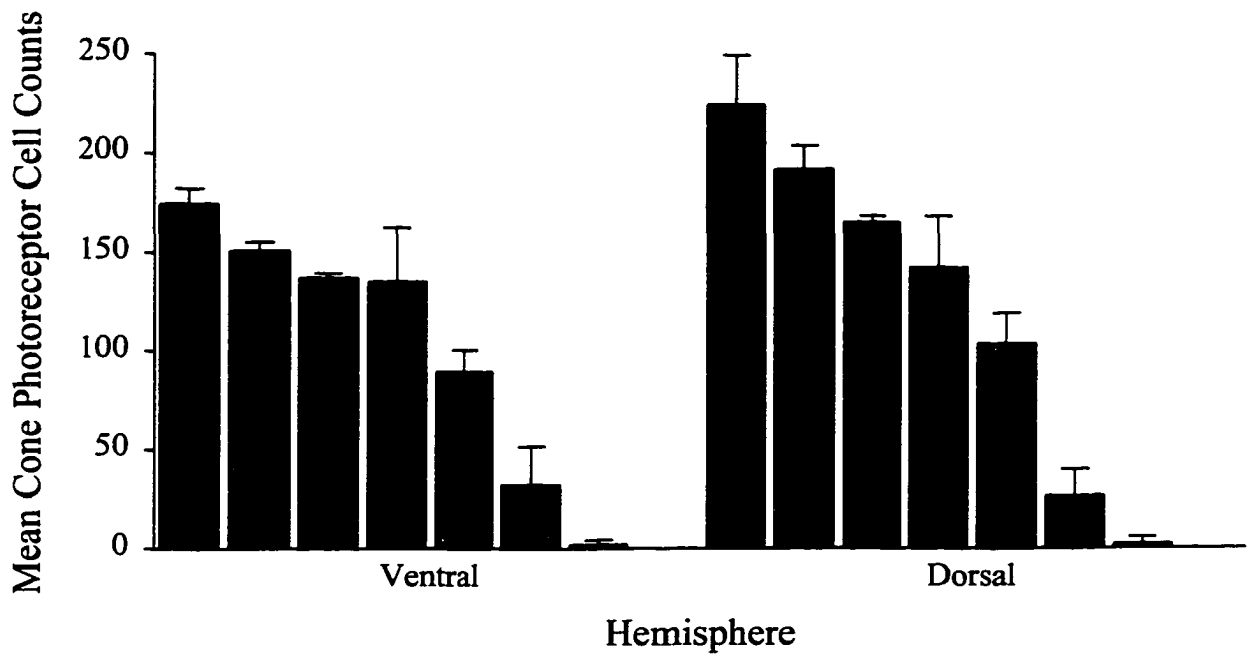


Figure 5. Mean ganglion cell counts, collected from the retinas of juvenile loggerhead sea turtles (*Caretta caretta*), for the eight latitudes of the left eye in both the ventral and dorsal hemispheres. All error bars denote + 1 S. D.

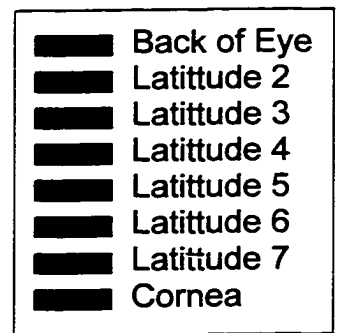
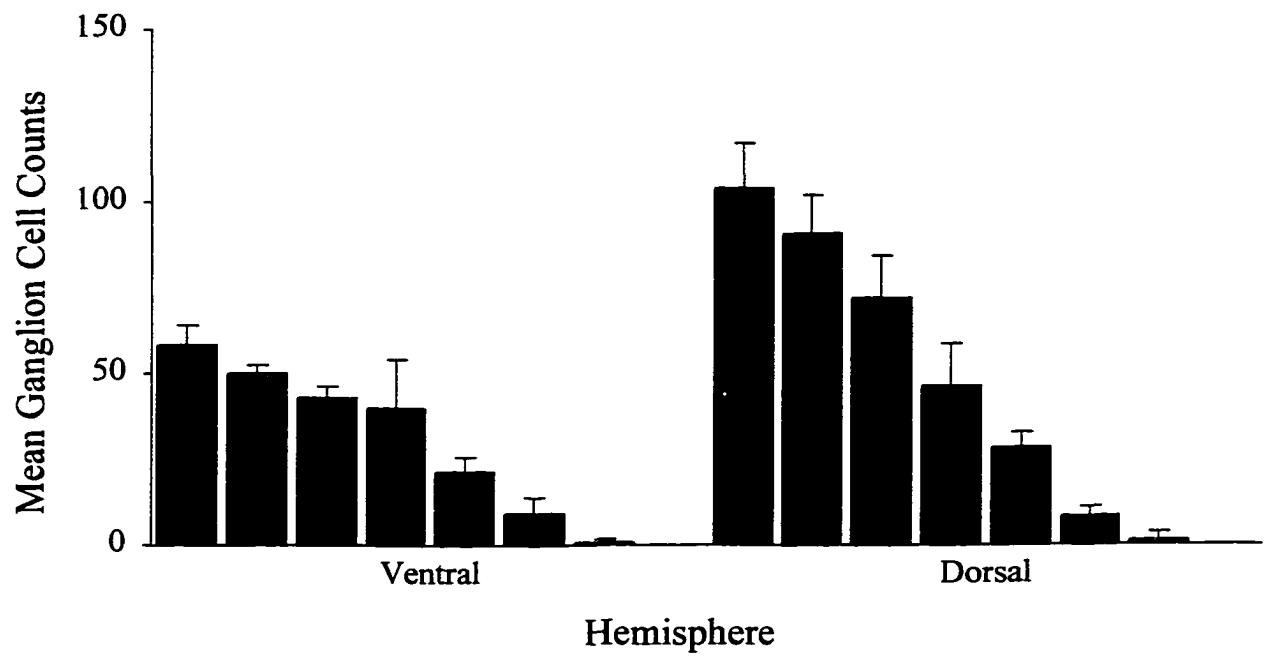


Table 4. Two-factor ANOVA performed on rod receptor cell counts recorded from the left eye of the juvenile loggerhead sea turtle.

	df	Sum of Squares	Mean Square	F-value	P-value
Hemisphere	1	1706.395	1706.395	8.634	.0052
Latitude	7	217700.632	31100.090	157.357	<.0001
Hemisphere * latitude	7	1856.008	265.144	1.342	.2535
Residual	45	8893.792	197.640		

Student-Newman-Keuls Test

Latitude

Back of Eye--Latitude 4 > Latitude 5 > Latitude 6 > Latitude 7--Cornea

Hemisphere

Dorsal > Ventral

Figure 6. Mean rod photoreceptor cell counts, collected from the retinas of juvenile loggerhead sea turtles (*Caretta caretta*), for the eight latitudes of the left eye in both the ventral and dorsal hemispheres. All error bars denote + 1 S. D.

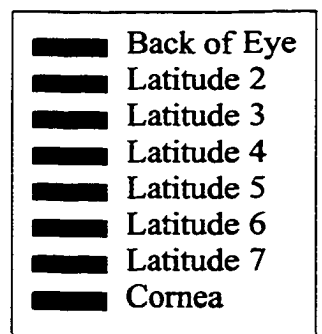
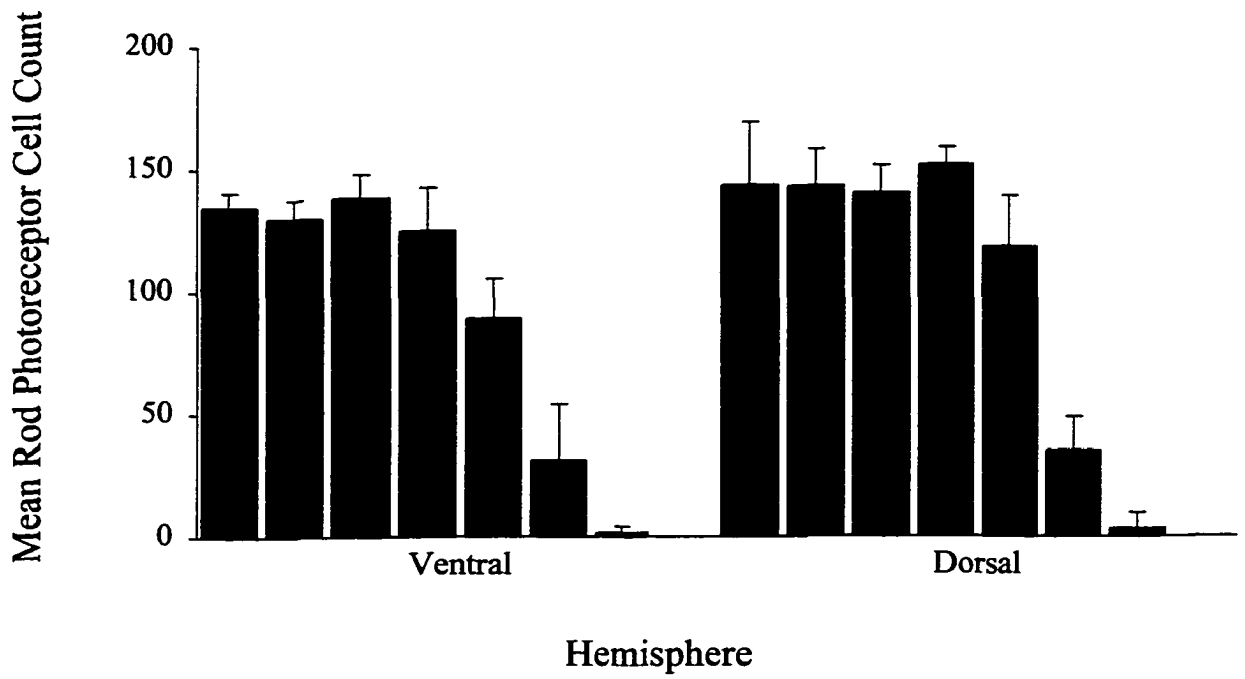
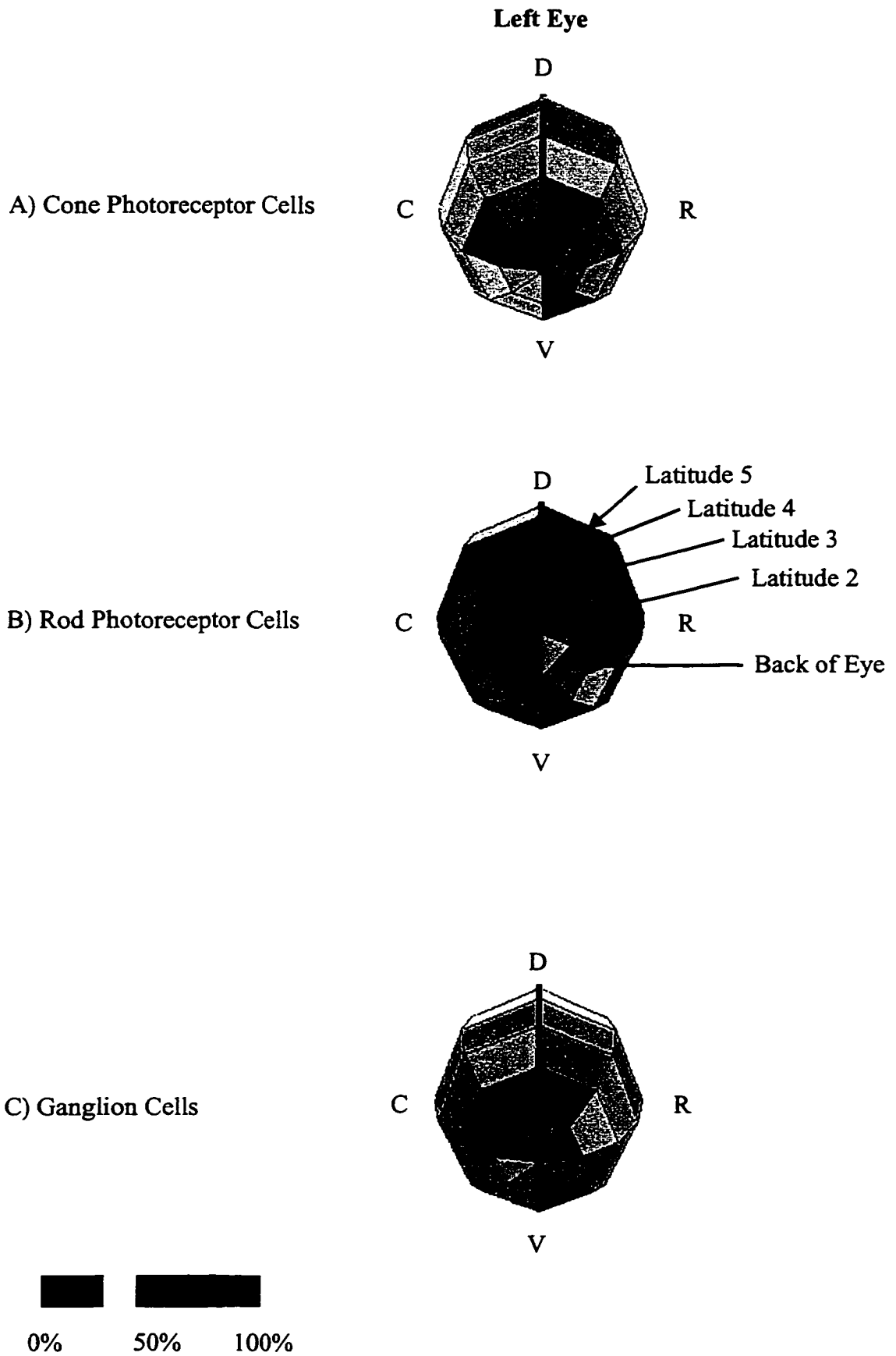


Figure 7. Spherical plot of cell counts for the left eye of the juvenile loggerhead sea turtle (*Caretta caretta*) for A) cone photoreceptors, B) rod photoreceptors, and C) ganglion cells. Each of the eight wedges and eight latitudes are plotted to form sixty-four polygons. Each polygon represents the average cell count for all left eyes, and is plotted (as a color of the spectrum) as a percentage of the highest cell count (Red = 0%, Violet = 100%). Orientation of each of the spheres is from the posterior of the eye; latitudes for all spheres are represented on the rod photoreceptor plot. Abbreviations: C = caudal, D = dorsal, R = rostral, V = ventral.



Similar patterns were found for cones, rods, and ganglion cells of the right eye. There was a significant effect of latitude and hemisphere on cone photoreceptor cell concentration in the right eye (Table 5). SNK test revealed that dorsal hemisphere had a higher cone cell concentration than the ventral hemisphere. The density of cone photoreceptor cells also decreased generally from the back of the eye to the cornea, with some overlap of the latitudes (Figure 8, Table 5). A significant latitude and hemisphere interaction was detected for ganglion cells in the right eye (Table 6). The SNK test revealed a decrease in cell concentration from back of the eye to cornea in both dorsal and ventral hemispheres. However, the dorsal hemisphere had a greater cell concentration than the ventral hemisphere, but only in the first three latitudes from the back of the eye (Figure 9, Table 6). Only latitude was found to have a significant effect on the concentration of rod photoreceptor cells in the right eye. Rod cell concentrations remained similar for the first four latitudes from the back of the eye and then drastically dropped in concentration near the cornea (Table 7, Figure 10). These trends for all three cell types are easily discerned when plotted on a sphere (Figure 11 a, b, c).

Average cell densities of the three considered cell types were calculated for regions of high, average and low density. Densities of cones ranged from 18,200 cells/mm² within the regions of high density, 8,400 cells/mm² within regions of average density, and 1,800 cells/mm² within regions of low density (along the periphery of the retina). Ganglion cells mimicked this same pattern, cell densities ranging from 9,200 cells/mm² to 650 cells/mm². Finally, rod photoreceptor cells remain at a constant density throughout most of the retina at 9,000 cells/mm², falling to 1,700 cells/mm² at the periphery (Table 8). Correlations of

concentration of these three cell types were also calculated. For both the left and right eye, significant correlation ($p < .0001$) was found among these three cell types (Table 9).

Table 5. Two-factor ANOVA performed on cone receptor cell counts recorded from the right eye of the juvenile loggerhead sea turtle.

	df	Sum of Squares	Mean Square	F-value	P-value
Hemisphere	1	2790.811	2790.811	4.756	.0341
Latitude	7	358721.360	51245.909	87.336	<.0001
Hemisphere * latitude	7	8624.824	1232.118	2.100	.0615
Residual	48	28164.893	586.769		

Student-Newman-Keuls Test

Latitude

Back of Eye > Latitude 2 > Latitude 3–Latitude 4, Latitude 4–5 >
Latitude 6–Cornea

Hemisphere

Dorsal > Ventral

Figure 8. Mean cone photoreceptor cell counts, collected from the retinas of juvenile loggerhead sea turtles (*Caretta caretta*), for the eight latitudes of the right eye in both the ventral and dorsal hemispheres. All error bars denote + 1 S. D.

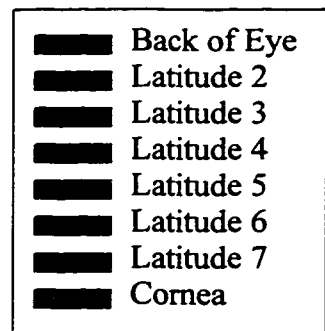
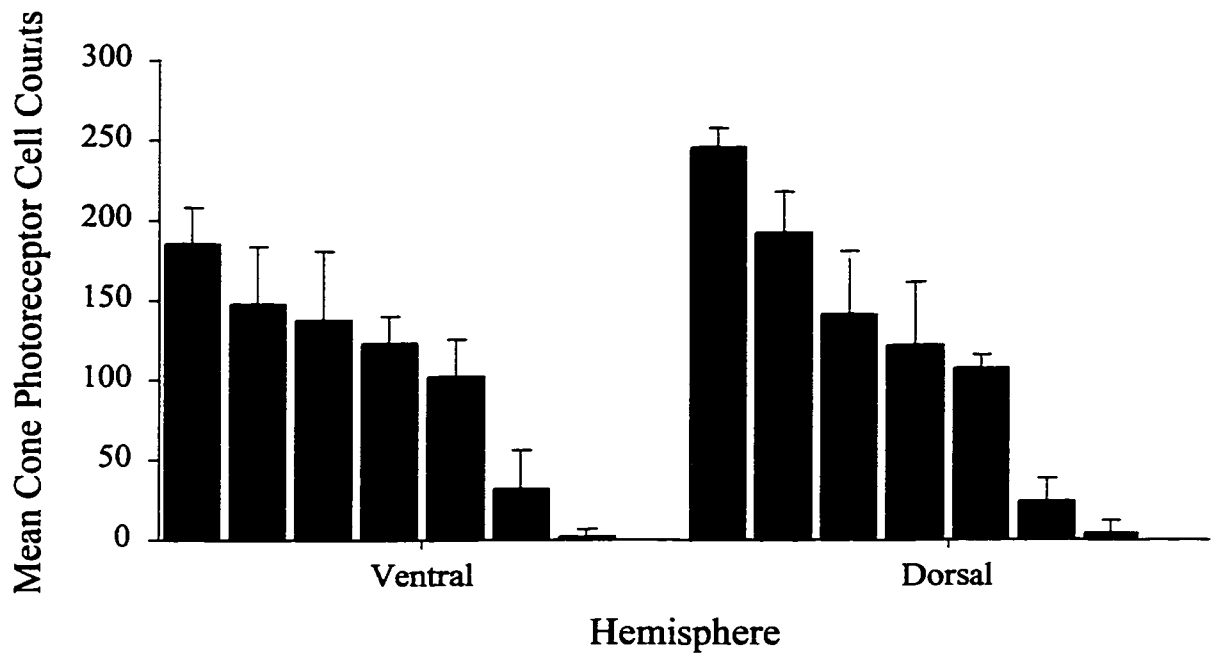


Table 6. Two-factor ANOVA performed on ganglion cell counts recorded from the right eye of the juvenile loggerhead sea turtle.

	df	Sum of Squares	Mean Square	F-value	P-value
Hemisphere	1	4484.813	4484.813	66.799	<.0001
Latitude	7	70018.653	10002.665	148.985	<.0001
Hemisphere * latitude	7	4796.330	685.190	10.206	<.0001
Residual	48	3222.657	67.139		

Student-Newman-Keuls Test

Latitude

Ventral: Back of Eye–Latitude 3 > Latitude 4 > Latitude 5–6, Latitude 6–Cornea

Dorsal: Back of Eye–Latitude 2 > Latitude 3 > Latitude 4 > Latitude 5 >
Latitude 6 > Latitude 7–Cornea

Hemisphere

Back of Eye–Latitude 3: Dorsal > Ventral

Latitude 4–Cornea: Dorsal = Ventral

Figure 9. Mean ganglion cell counts, collected from the retinas of juvenile loggerhead sea turtles (*Caretta caretta*), for the eight latitudes of the right eye in both the ventral and dorsal hemispheres. All error bars denote + 1 S. D.

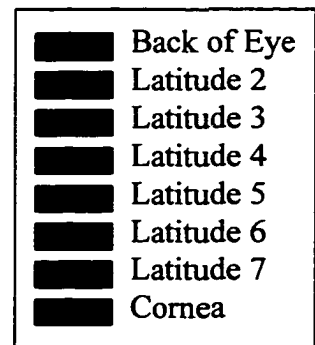
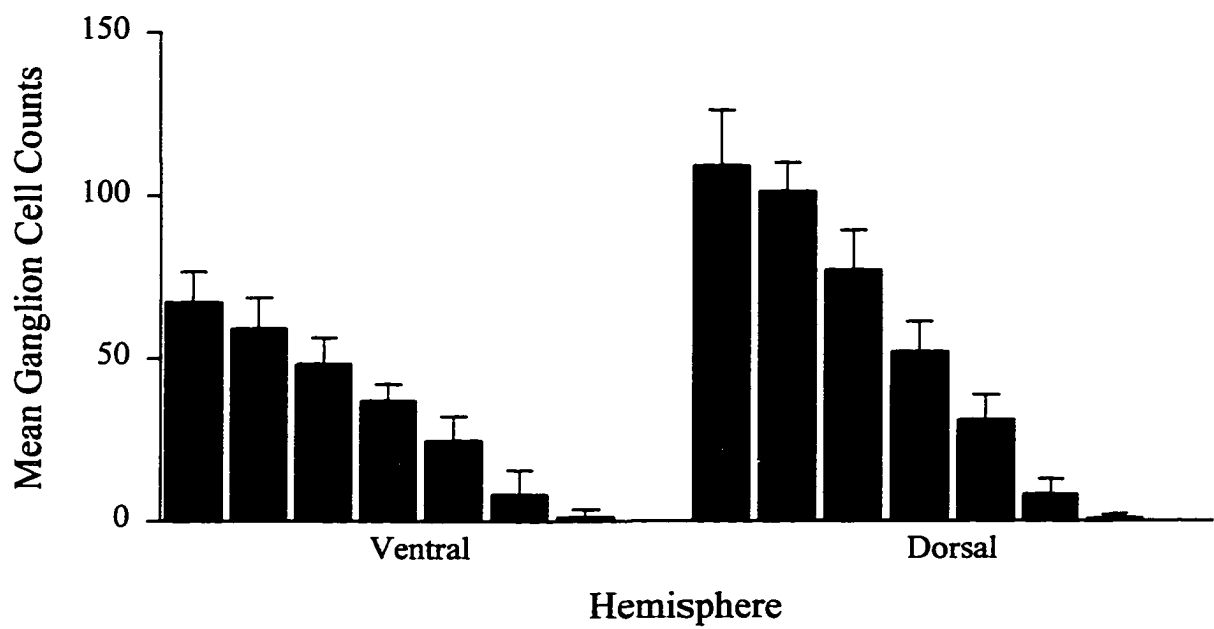


Table 7. Two-factor ANOVA performed on rod receptor cell counts recorded from the right eye of the juvenile loggerhead sea turtle.

	df	Sum of Squares	Mean Square	F-value	P-value
Hemisphere	1	654.065	654.065	2.725	.1053
Latitude	7	262232.673	37461.81	156.06	<.0001
Hemisphere * latitude	7	1611.395	230.190	.959	.4715
Residual	48	1152.285	240.048		

Student-Newman-Keuls Test

Latitude

Back of Eye–Latitude 4 > Latitude 5 > Latitude 6 > Latitude 7–Cornea

Figure 10. Mean rod photoreceptor cell counts, collected from the retinas of juvenile loggerhead sea turtles (*Caretta caretta*), for the eight latitudes of the right eye in both the ventral and dorsal hemispheres. All error bars denote + 1 S. D.

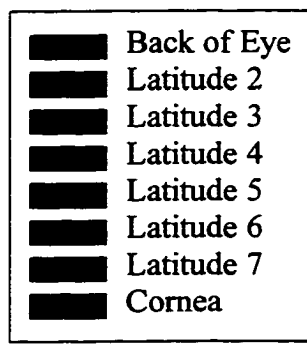
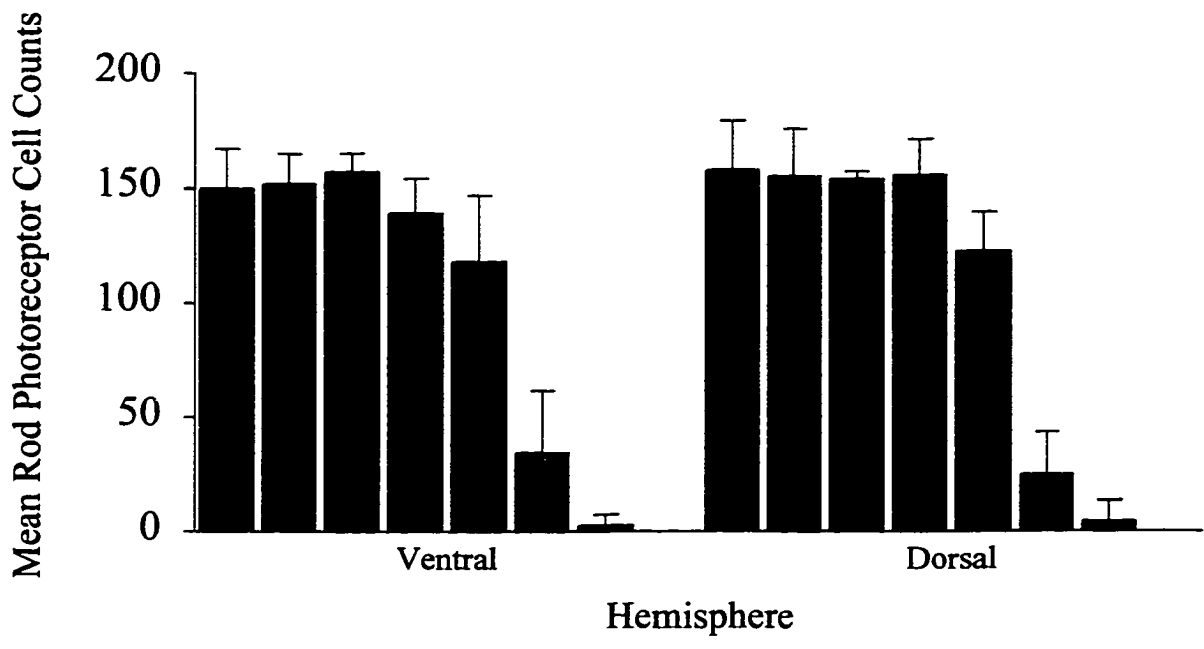


Figure 11. Spherical plot of cell counts for the right eye of the juvenile loggerhead sea turtle (*Caretta caretta*) for A) cone photoreceptors, B) rod photoreceptors, and C) ganglion cells. Each of the eight wedges and eight latitudes are plotted to form sixty-four polygons. Each polygon represents the average cell count for all right eyes, and is plotted (as a color of the spectrum) as a percentage of the highest cell count (Red = 0%, Violet = 100%). Orientation of each of the spheres is from the posterior of the eye; latitudes for all spheres are represented on the rod photoreceptor plot. Abbreviations: C = caudal, D = dorsal, R = rostral, V = ventral.

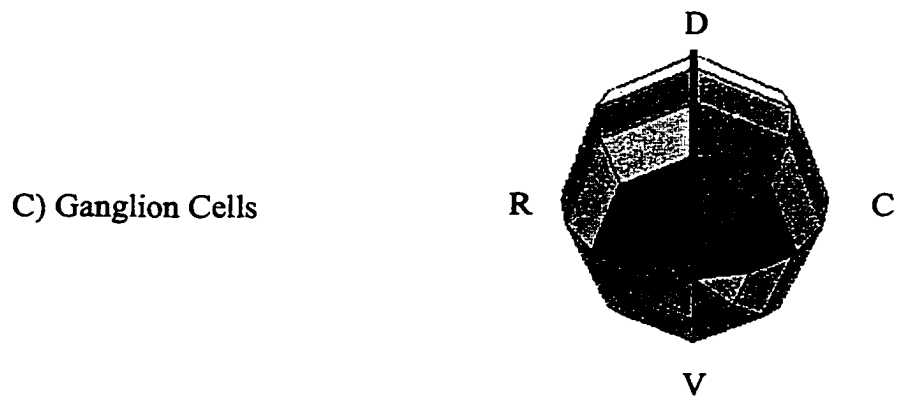
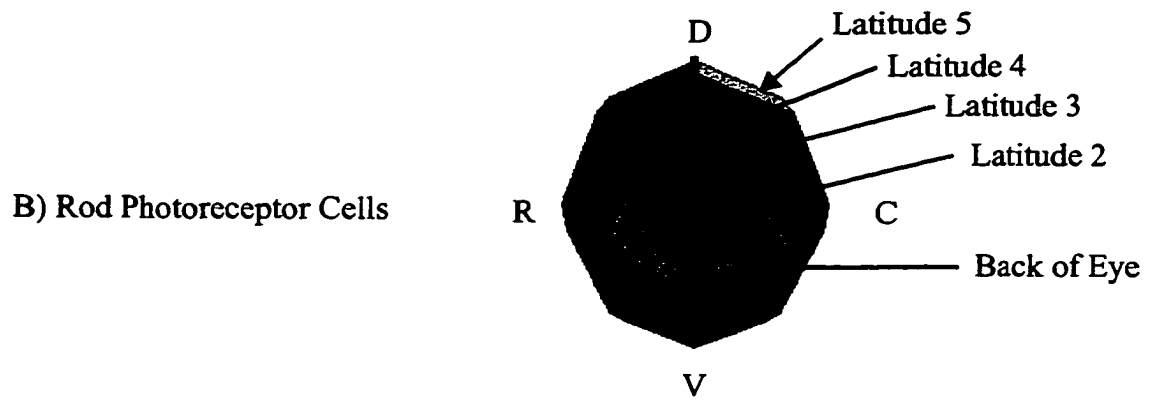
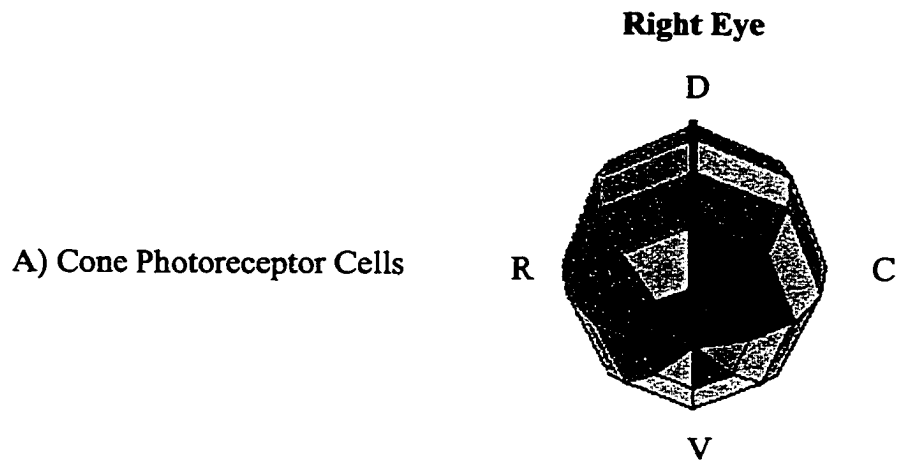


Table 8. Average density of cones, rods, and ganglion cells in regions of high, average, and low concentration.

Cone Photoreceptor Cells		
High Density	Average Density	Low Density
18,200 cones/mm ²	8,400 cones/mm ²	1,800 cones/mm ²

Rod Photoreceptor Cells		
High Density	Average Density	Low Density
9,500 rods/mm ²	9,000 rods/mm ²	1,700 rods/mm ²

Ganglion Cells		
High Density	Average Density	Low Density
9,200 cells/mm ²	3,800 cells/mm ²	650 cells/mm ²

Table 9. Linear correlation performed on left and right eyes for cone and rod receptor cells and ganglion cells.

Left eye	Correlation coefficient	Z-value	P-value
Cones, ganglion	.932	19.546	<.0001
Cones, rods	.912	18.183	<.0001
Ganglion, rods	.789	12.505	<.0001

Right eye	Correlation coefficient	Z-value	P-value
Cones, ganglion	.924	21.474	<.0001
Cones, rods	.904	19.822	<.0001
Ganglion, rods	.785	14.030	<.0001

DISCUSSION

The basic organization of the retina of the juvenile loggerhead sea turtle closely follows the general vertebrate model. The primary functions of the retina are sensitivity in low light conditions and spatial resolution. The necessary requirements for both processes, photoreceptor cells and appropriate neural organization, are found in the loggerhead retina. Retinas are often classified, based on overall functional ability, as either nocturnal or diurnal in nature. Typically, a diurnal eye adapts for vision in daylight through the multiplication of cone photoreceptor cells and the reduction in the degree of summation. Thus, the diurnal eye demonstrates a marked increase in visual acuity. Conversely, the nocturnal eye maximizes sensitivity in low light environments through the aggregation of rod photoreceptor cells coupled with the intensification in the degree of summation (Walls, 1942). The structures and vertical organization of the retinal layers of the loggerhead eye clearly that the overall design is diurnal.

The photoreceptor layer contains both cones and rods throughout the loggerhead retina. Both of these cells are similar in width and height. Though this homogeneity of cell size is unusual, rod photoreceptor cells of vertebrates are typically much longer and more slender than cones (Walls, 1942; Wagner, 1990; Peterson, 1992), these two photoreceptor cells closely resemble the rods and cones of the common snapping turtle (*Chelydra serpentina*) (Walls, 1942). Morphological differences of the loggerhead photoreceptor cells were observed in the shape of the outer segment of the photoreceptor cells as well as in the

presence or absence of oil droplets. The rod's outer segment was more cylindrical than the conical shape of the cone, while the cones contained a large distinctive oil droplet that is missing in the rod. These oil droplets also have been identified in *Chelonia mydas* and are thought to contribute to the collection of light by the cones (Granda and Haden, 1970; Granda, 1979; Peterson, 1992). Though the retina is not comprised uniformly of cones, a predominance of cones was observed in several regions of the retina indicating that these eyes are diurnal in nature.

Further diurnal features are also observed in the properties of the neural layers (outer nuclear layer, inner nuclear layer, and ganglion layer). The outer nuclear layer, formed of nuclei of both rods and cones, is characteristically thin in a diurnal eye. The width of the cones usually prevents the characteristic "stacking" of nuclei found when long, thin rods dominate a retina (Walls, 1942). In loggerheads, the rod photoreceptor has as wide a shape as the cones (Figure 2). Thus the thin outer nuclear layer could not solely be used as an indication of diurnality. The two remaining nuclear layers, the inner nuclear layer composed of bipolar, horizontal, and amacrine cells and the ganglion layer, are conversely thick in diurnal animals (Walls, 1942). In the juvenile loggerhead, both of these layers were found to be broad compared to the overall width of the retina, comprising approximately 37% of the total retina. The width of these layers indicates a high number of neurons per photoreceptor cell and thus a reduction in the degree of summation for the eye.

The adoption of diurnality corresponds to an increase in acuity. The prevalence of cones coinciding with a low summation level in the nuclear layers provides the necessary requirements for the conditions of sharp vision. However, the transverse sections further suggest that these eyes are capable of both spatial resolution and sensitivity in dim

illumination. Both rods and cones are present throughout the eye. Furthermore, correlation analysis shows that the density of photoreceptor cells and ganglion cells are highly positively correlated to each other. Because of this duplex nature, identification of specialized regions devoted to each mechanism was investigated.

From the spherical plots (Figures 4 and 6), two areas of interests were identified to explore further: densities of cells along the latitudes of the entire eye and density differences between the dorsal and ventral regions. All three cell types progressed from high to low density, starting with the back of the eye. This pattern was distinct for cones and ganglion cells; the highest region was always the back of the eye with a staircase decrease as the cornea was approached. Rods, for both the left and right eye, were more likely to maintain a constant level of cell density for the first four latitudes, with a rapid decline as the cornea was approached. Differences between the hemispheres (dorsal and ventral) were also observed for all three cell types. Cone photoreceptor cells were significantly higher in concentration in the dorsal hemisphere. This pattern is mimicked in the plots of ganglion cells. Once again, there is a clear distinction between dorsal and ventral hemispheres, with the dorsal hemisphere containing significantly more ganglion cells. Though the left eye shows a hemispheric difference for the rods, from the plots, rod photoreceptor cells appeared to be ubiquitously distributed throughout the entire eye.

Diversity of retinal topography has been identified in most vertebrates, with variation occurring in size, shape, density, and frequency of many cell types. One way these irregularities can be explained is by examining the planes at which visual information resides. Visual objects along the horizontal and vertical axes contain information of various significance to an animal; for example, objects in the forward field of vision might be of

more interest that those behind the animal. Consequently, regional differences along the retina are often correlated to the behavior of the animal: such as habitat preference, predation, predator avoidance, etc. Regional differences in the retina have been identified in the western painted turtle *Pseudemys scripta elegans* (Brown, 1969). A linear area centralis, region of high cone density, spans the width of the retina rostrocaudally and is found prominently in the ventral region of the eye. Brown (1969) hypothesized that this ventral streak provided greater horizon acuity for the turtle. Furthermore, the location of this streak in the ventral region would aid in avoidance of predators approaching from above. A marine species, the lemon shark (*Negaprion brevirostris*), has also been reported to have a linear visual streak (Heuter, 1990; Heuter and Gruber, 1982; Gruber and Cohen, 1985). Heuter (1990) hypothesized that this streak of cones and ganglion cells provided this animal with proficient spatial resolution along the horizon and aids in the capture of benthic prey.

A diverse topography within the loggerhead retina was distinctly observed. By plotting these cells on a model eye, the density levels partition the loggerhead eye into regions of high and low acuity. Clearly the dorsal region is the area of increased acuity. Though a horizontal streak could not be unequivocally identified using these methods, the edges of the dorsal region (along the equator of the eye) do appear to play some role, especially in the density of the ganglion cells. Nonetheless, an increase in acuity in the dorsal region would be advantageous for the loggerhead sea turtle. When not migrating, the juvenile loggerhead sea turtle occupies shallow water habitats, feeding benthically on a variety of organisms. The regionalization of acuity in the dorsal hemisphere, documented in this paper, would aid the loggerhead in the finding of prey items along the benthos. This animal, however, has not completely sacrificed sensitivity to maximize acuity. Though this

eye can be classified as a diurnal model, rods are present throughout the eye in constant numbers. Considering that the habitat of the loggerhead is often a low light environment (including the Chesapeake Bay), this duplex retina seems perfectly suited to perform the roles of both resolution and sensitivity.

LITERATURE CITED

- Brown, K.T. 1969. A linear area centralis extending across the turtle retina and stabilized to the horizon by non-visual cues. *Vision Res.* 9: 1053-1062.
- Copenhaver, W.M. 1964. *Bailey's Textbook of Histology*. Baltimore: The Williams and Wilkins Co. 679 pp.
- Davson, H. 1972. *The Physiology of the Eye*. New York: Academic Press. 643 pp
- Granda, A.M. 1979. Eyes and their sensitivity to light of differing wavelengths. In M. Harless and H. Morlock (eds.) *Turtles: Perspectives and Research*. New York: John Wiley and Sons. pp. 247-266.
- Ganda, A.M. and K.W. Haden. 1970. Retinal oil droplet counts and distributions in two species of turtles: *Pseudemys scripta elegans* (Weid) and *Chelonia mydas* (Linnaeus). *Vision Res.* 10: 79-84.
- Gruber, S.H. and J.L. Cohen. 1978. Visual system of the elasmobranchs: state of the art 1960-1975. In: E.S. Hodgson and R.F. Mathewson (eds.) *Sensory Biology of Sharks, Skates, and Rays*. Arlington: Office of Naval Research, Department of the Navy. Pp. 11-105.
- Gruber, S.H. and J.L. Cohen. 1985. Visual system of the white shark, *Carcharodon carcharias*, with emphasis on retinal structure. *Mem. S. Cal. Acad. Sci.* 9: 61-72.
- Heuter, R.E. 1991. Adaptations for Spatial Vision in Sharks. *J. Exp. Zool. Suppl.* 5: 130-141.
- Heuter, R.E. and S.H. Gruber. 1982. Recent advances in studies of the visual system of the juvenile lemon shark (*Negaprion brevirostris*). *Florida Sci.* 45: 11-25.
- Liebman, P.A. 1972. Microspectrophotometry of photoreceptors. In H.J.A. Dartnall (ed.), Photochemistry of vision. Vol VII/1. *Handbook of sensory physiology*, pp. 481-528. New York: Springer-Verlag.
- Patterson, M. and S. M. Bartol. 1999. Turtle eyeball data plotter. *Mathematica*: Wolfram Research, Inc.

- Peterson, E.H. 1992. Retinal structure. In: C. Gans and P.S. Ulinski (eds.) *Biology of the Reptilia: Sensorimotor Integration Volume 17, Neurology C*. Chicago: The University of Chicago Press. Pp. 1-135.
- Sokal, R. R. and F. J. Rohlf. 1981. *Biometry*, 2nd edition. New York: W. H. Freeman and Co. 859pp.
- Stell, W.K. 1972. The morphological organization of the vertebrate retina. In: M.G.F. Fuortes (ed.) *Handbook of Sensory Physiology, Physiology of Photoreceptor Organs*, Vol. VII/2. Berlin: Springer-Verlag. Pp. 111-213.
- Underwood, A.J. 1997. *Experiments in Ecology*. Cambridge: Cambridge University Press. 504pp.
- Wagner, H. 1990. Retinal structure of fishes. In: R.H. Douglas and M.B.A. Djamgoz (eds.) *The Visual System of Fish*. New York: Chapman and Hall. Pp. 109-157.
- Walls, G.L. 1942. *The Vertebrate Eye and its Adaptive Radiation*. Bloomfield Hills: The Cranbrook Institute of Science. 785 pp.

Chapter 2

Visual Acuity Thresholds of the Juvenile Loggerhead Sea Turtle (*Caretta caretta*): An Electrophysiological Approach

ABSTRACT

Visual evoked potentials (VEPs) measure dynamic properties of the visual system by recording transient electric responses of any neural tissue identified to correspond to a specific stimulus. This study used VEPs to test the visual acuity thresholds, or resolution capability, of juvenile loggerhead sea turtles (*Caretta caretta*) in the aquatic medium. Stimuli of black and white striped gratings were presented using a slide projector directing an image onto a screen via a rotatable mirror that moved the pattern $\frac{1}{2}$ cycle. Bioelectric activity was collected using subdermal platinum electrodes and a digital averaging computer. The resulting waveforms displayed a positive-negative compound (P1-N1) that was used to track the turtle's response to stimulation. Acuity thresholds for these sea turtles were derived from linear regression analysis of the P1-N1 amplitudes to stripe size, and ranged from .130-.215. This acuity level is comparable to other inshore, shallow water marine species.

INTRODUCTION

Sea turtle visual mechanisms have been examined extensively in their role in orientation and migration. Both hatchlings and adults have been used in behavioral studies to investigate the sea turtle's ability to perceive shapes, colors and brightness cues in air (Daniel and Smith, 1947; Anderson, 1958; Ehrenfeld and Carr, 1967; Ehrenfeld, 1968; Mrosovsky and Shettleworth, 1968; van Rhijn, 1979a; 1979b). Sea turtles have been erroneously classified as possessing all-cone retinas (Walls, 1942; see morphology chapter), and this classification implied that these turtles are strictly diurnal with the capability of sharp vision. Even with this erroneous classification, sea turtles are now known to have a retina composed of both rods and cones, only a few research studies have examined the extent of their resolution abilities. Walls (1942) remarks that the visual acuity of sea turtles on land is "hazy" and Ehrenfeld and Carr (1967) reported that the orientation of adult green turtles (*Chelonia mydas*) on the beach was not hindered by actively blurring the visual images using filtered goggles. The fact that these animals do not form sharp retinal images on land, however, does not come as a surprise. Sea turtles spend very little time on land; in fact once hatchlings enter the ocean, males spend the rest of their lives in water and females only emerge for brief nesting periods.

Acuity of sea turtles on land can not easily be applied to the visual abilities of these animals in water. The optical characteristics of the sea turtle eye differ in water than in air and refraction, refractive errors and accommodation must all be examined to determine how

the eye functions in different media. Refraction is the angle at which light is bent when traveling through the interface between two transparent objects (of different refractive indices) and can be calculated for individual optical features. The refraction of light by each interface combines to bring an image into focus within the eye. Refractive error measurements describe the position of the focal point within the eye, whether it is in front (myopia), behind (hypermetropia), or on (emmetropia) the retinal surface. Finally, accommodation is the ability of the eye to actively change the focal point of images at a variety of distances. For sea turtles, the refractive indices of cornea and ocular fluids are almost identical to seawater. In the absence of corneal refraction, the eye can no longer benefit from the air/cornea interface, which provides considerable dioptric strength. Instead, the lens is the only feature that brings the image into focus. For sea turtles, as is the case for many species of teleosts, a spherical lens is ideal for these conditions (Walls, 1942; Granda, 1979; Fernald, 1990). The high degree of convexity of the lens elevates the overall refractive power (Sivak, 1985; 1990).

Preliminary morphological studies, moreover, describe the sea turtle eye as lacking a mechanism for active accommodation (Walls, 1942; Granda, 1979). Without an apparent means of accommodation, the refractive error of the sea turtle lens should describe the focal point of an image. In fact, an ophthalmological study, which measured the refractive error of green turtles eyes, found them to be highly myopic on land but emmetropic in water (Ehrenfeld and Koch, 1967).

Much of the sea turtle research has been dictated by the degree of difficulty in performing behavioral studies in the aquatic environment. An alternative to psychophysical investigations when examining the visual mechanisms of sea turtles is electrophysiological

experiments. Recording electrical responses from the visual system can provide an objective measure of a variety of stimulus parameters and can reflect the function of underlying processes (Riggs and Wooten, 1972, Bullock et. al, 1992; Jeffreys, 1997). Most of the electrophysiological work on freshwater turtles (and one study on green sea turtles) have used electroretinograms (ERGs) to investigate the spectral sensitivities of these animals (Armington, 1954; Deane et. al, 1958; Granda, 1962; Granda and O'Shea, 1972). An ERG is a recording of rapid action potentials between the cornea and retina when the eye is stimulated and is a robust measurement of early retinal stages in the visual pathway (pre-ganglion cell responses) (Davson, 1972; Riggs and Wooten, 1972; Ali and Klyne, 1985). The ERG may easily be recorded from excised eyes, though it also can be obtained from intact eyes through paralysis of the subject animal and placement of one electrode on the cornea and one reference electrode on the surface of the head (Ali and Klyne, 1985).

One difficulty in performing ERGs on sea turtles is the need to execute non-invasive, benign research on an endangered species while maintaining a reasonable sample size. Placing an electrode on the cornea of an alert sea turtle can be both difficult and invasive in that the process may accidentally damage the eye and anesthetizing the animal is inherently invasive. An alternative in the collection of electrophysiological data is visual evoked potentials. Visual evoked potentials (VEPs) differs from ERGs by recording the compound field potentials of any neural tissue in the visual pathway (post-ganglion cell responses). Visual evoked potentials can be obtained through the use of surface electrodes placed on the head directly over the optic nerve and corresponding optic tectum. A number of measurements can be extracted from the VEPs, including latency of response and dependency of response on stimulus intensity. Responses are identified through the use of

signal averaging techniques that isolate the signal from the noise (Riggs and Wooten, 1972; Spehlmann, 1985; Bartol et. al, 1999).

This project was designed to test the visual acuity of juvenile loggerheads in the aquatic medium. To accomplish this objective, I first characterized the evoked response of the loggerhead, with its eye submerged in water, to a suprathreshold alternating stripe pattern stimulus. After identifying a recording procedure that elicited a consistent response, I systematically decreased the stimulus size to determine the limit of the loggerhead's resolving capability.

MATERIALS AND METHODS

Subject animal

All turtles utilized in this study were juvenile loggerhead sea turtles (*Caretta caretta*), averaging approximately 60 cm straight notch to notch carapace length. These loggerheads were incidentally captured in poundnets in the Potomac River, a tributary of the Chesapeake Bay. The animals were immediately transferred to holding facilities at the Virginia Institute of Marine Science and placed in individual recirculating riverwater tanks. Temperature was maintained between 24 and 27 degrees Celsius. After at least 24 hours of acclimation, the animal was examined to determine its health status and then considered ready for testing. All testing was conducted under the National Marine Fisheries Services sea turtle permit no. 929.

Experimental Design

Experiments occurred out of water in a dry laboratory. The loggerhead first was confined with a canvas restraining device. This device restricted flipper movement while isolating the head. In all cases, the animal stopped resisting the restraint in a few minutes. A goggle was then attached over the animal's right eye. The goggle was constructed of 3.2 mm thick Plexiglass with foam strips lining the attachment side, and the viewing surface of the goggle followed parallel to the surface of the eye. The goggle was attached to the skin around the eye with a low temperature, non-toxic, non-vaporous glue and further sealed to the skin with a dental adhesive cream. This attachment process allowed the goggle to be

filled with filtered seawater. The contralateral eye was completely covered with a dark towel and kept moist during the trials. Trials were also executed without the goggle, with all other parameters identical to the goggle trials, to compare both in air and in water responses.

Bioelectric activity was collected using a Nicolet Compass averaging system. Subdermal platinum needle electrodes (13 mm x .4 mm) were implanted, under the head scutes, directly over the optic nerve and the contralateral optic tectum. A ground electrode was inserted in the inactive skin of the lateral neck. Implantation of the electrodes under the head scutes did not require surgery and the animals were never anesthetized. Recording of electroencephalographic activity (EEG) was amplified and filtered (5-250 Hz). Due to the ongoing brain electrical activity, the distance of the electrodes from the visual system electrical generators, and the intervening muscle and bone, the signal to noise ratio was low; thus, signal averaging techniques were used. The recording of VEPs was time-locked to the delivery of the stimulus, allowing for collection and averaging of single responses at the same rate as stimulus presentation. This technique isolates the single response by reducing biological noise associated with unrelated neural and muscular activity. For each trial, 250 responses were averaged, the point at which the waveform stabilized.

Slides of black and white stripes were used as stimuli. Four stripe widths were used at two distances from the turtle, resulting in eight stripe stimuli (68.7, 45.8, 34.4, 22.9, 17.2, 11.5, 8.6, and 5.7 minutes of arc). These stimuli were presented using a slide projector focused onto the back of a translucent screen via a rotatable single surface mirror. The mirror, controlled by an amplifier, fluctuated at a small angle and moved the striped pattern from side to side across the screen. This angle was controlled for each stripe size so as to

displace the vertical pattern one-half cycle and produce a complete reversal of the pattern.

The pattern reversal presentation rate was 2.1 per second for every trial.

At the initiation of the trials, ambient light was reduced to compel the turtle's attention onto the stimulus. The stimulus screen was placed either .5 or .75 meters from the surface of the turtle's eye, parallel to the face of the goggle (Figure 1). Stimulus presentation randomly varied in order of presentation. A test consisted of two presentations of the same stimulus. Trials lasted until every stimulus size was tested, or as long as the goggle remained attached.

Calculation of Visual Acuity

Visual acuity is the reciprocal of the visual angle and is a measure of the animal's ability to resolve details of an object. Visual angle, measured in minutes of arc, is the angle subtended at the eye by the size of the viewed object and is calculated as follows:

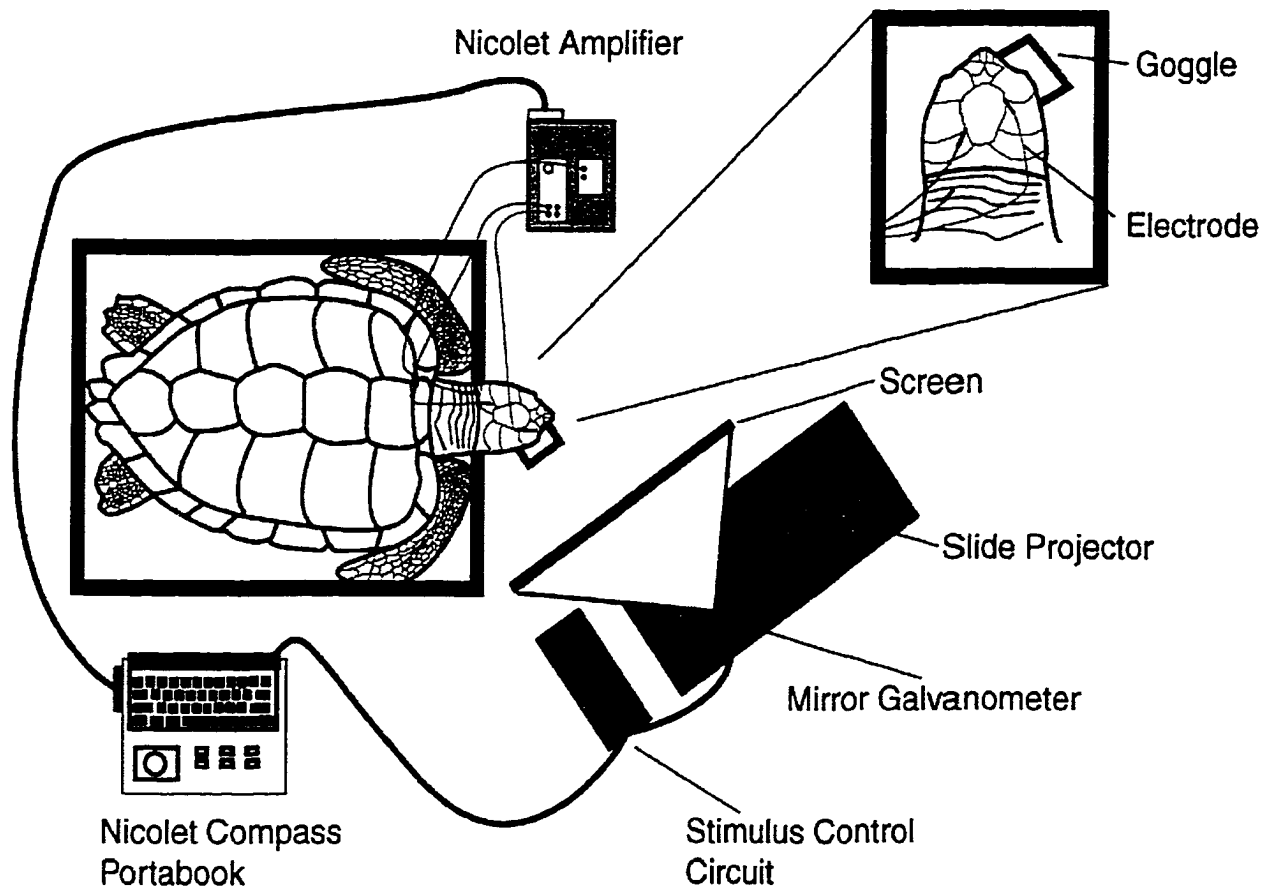
$$\text{Visual angle} = \tan^{-1} \frac{\text{width } \frac{1}{2} \text{ cycle}}{\text{distance between stimulus and turtle's eye}}$$

The width of one half cycle is the distance from the middle of one black stripe to the middle of one white stripe. The distance between the stimulus and the turtle was always either .5 or .75 meters.

Statistical Analysis

In each test, two waveforms, each an average of 250 responses, were collected for the individual stimulus. These two waveforms were then averaged together, using the

Figure 1. Schematic diagram for the collection of visual evoked potentials from a juvenile loggerhead sea turtle (*Caretta caretta*). Subdermal electrodes were inserted under the scutes of the sea turtle's head, above the optic nerve and contralateral optic tectum. The signal from these electrodes was amplified and averaged by the Nicolet Compass. Stimuli were slides of black and white stripes projected onto the back of a translucent screen via a rotatable mirror. This mirror was triggered by the computer to present the stimuli time locked to the collection of the electrophysiological responses.



Nicolet Compass signal averaging computer, and the resulting waveform was used for all further analysis. Amplitudes of the principal positive peak (P1) and the following principal negative peak (N1) were measured and the amplitude difference was calculated using the Nicolet Compass computer software. These amplitudes were plotted based on stripe size for both each individual turtle and all turtles combined and linear regression analysis was performed on these data (Sokal and Rohlf, 1981). The y-intercept of the regression line was used to approximate acuity threshold (McCormack and Tomlinson, 1979).

RESULTS

Waveform Characteristics

The VEP waveform, using a suprathreshold stimulus and collected with subdermal electrodes and a water filled goggle, was similar in shape and form to VEPs recorded in other studies on a variety of species (Riggs and Wooten, 1973; Bullock et al, 1991). The first large deflection of this waveform occurred in latency between 60 and 99 msec. This positive-negative compound was always present when testing at suprathreshold levels, was easily identifiable, and continued at approximately the same latency for each session with the individual turtle (though variation was observed among subject animals). For the purposes of this study, the positive peak was labeled as P1 and the negative peak as N1 (Figure 2). This landmark was used as a reference in all threshold trials. However, due to the extracranial recording methods, this waveform response is defined only as diffuse activity due to visual stimulation.

Visual Evoked Potentials

A dependence of P1-N1 amplitude difference on stripe size was observed in all trials when the eye was in water. As the stripe size decreased, the difference in amplitude of this complex also decreased (Figure 3). Calculating the difference in amplitude became increasingly difficult as the acuity threshold was approached and P1-N1 became obscured by the background noise. To circumvent this problem, amplitude differences were plotted based

Figure 2. Shape of a visual evoked potential waveform for a suprathreshold alternating stripe trial collected from a loggerhead sea turtle (*Caretta caretta*) using subdermal platinum electrodes. P1 and N1 indicate the first major positive and negative deflection that could be easily identified in the evoked potential. The amplitude difference was measured and tracked for each trial. This wave is an average of 250 responses; time zero is the start of stimulation.

Representative Waveform

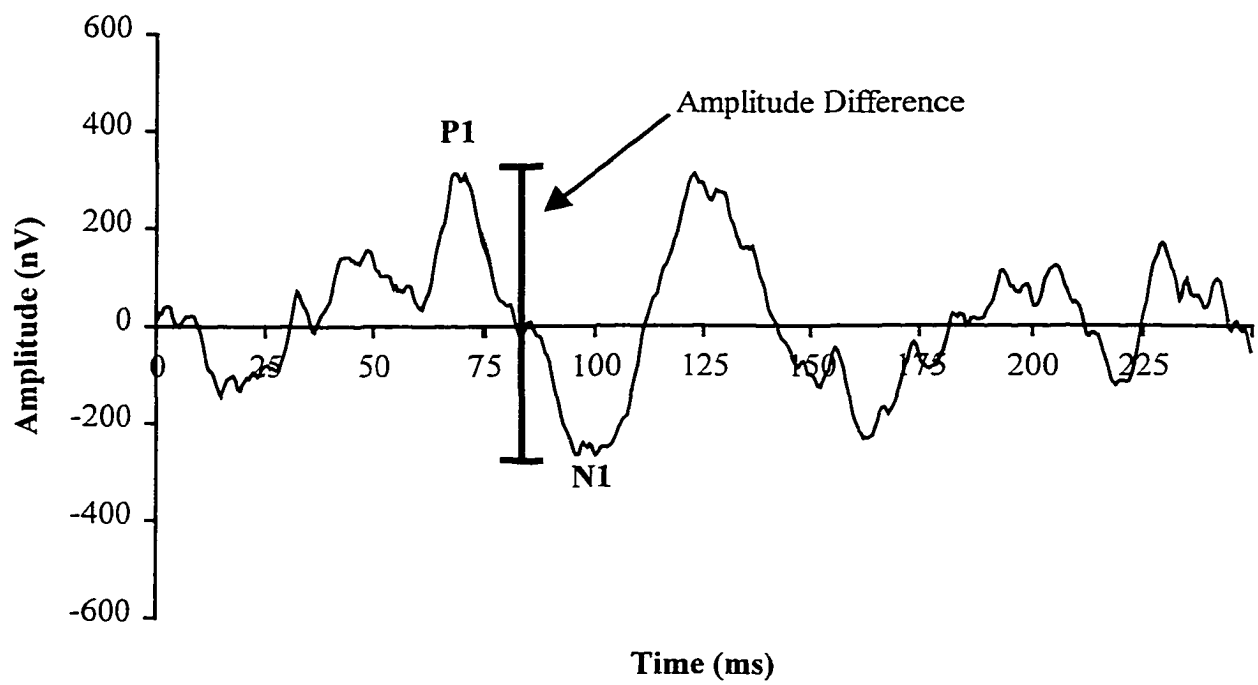
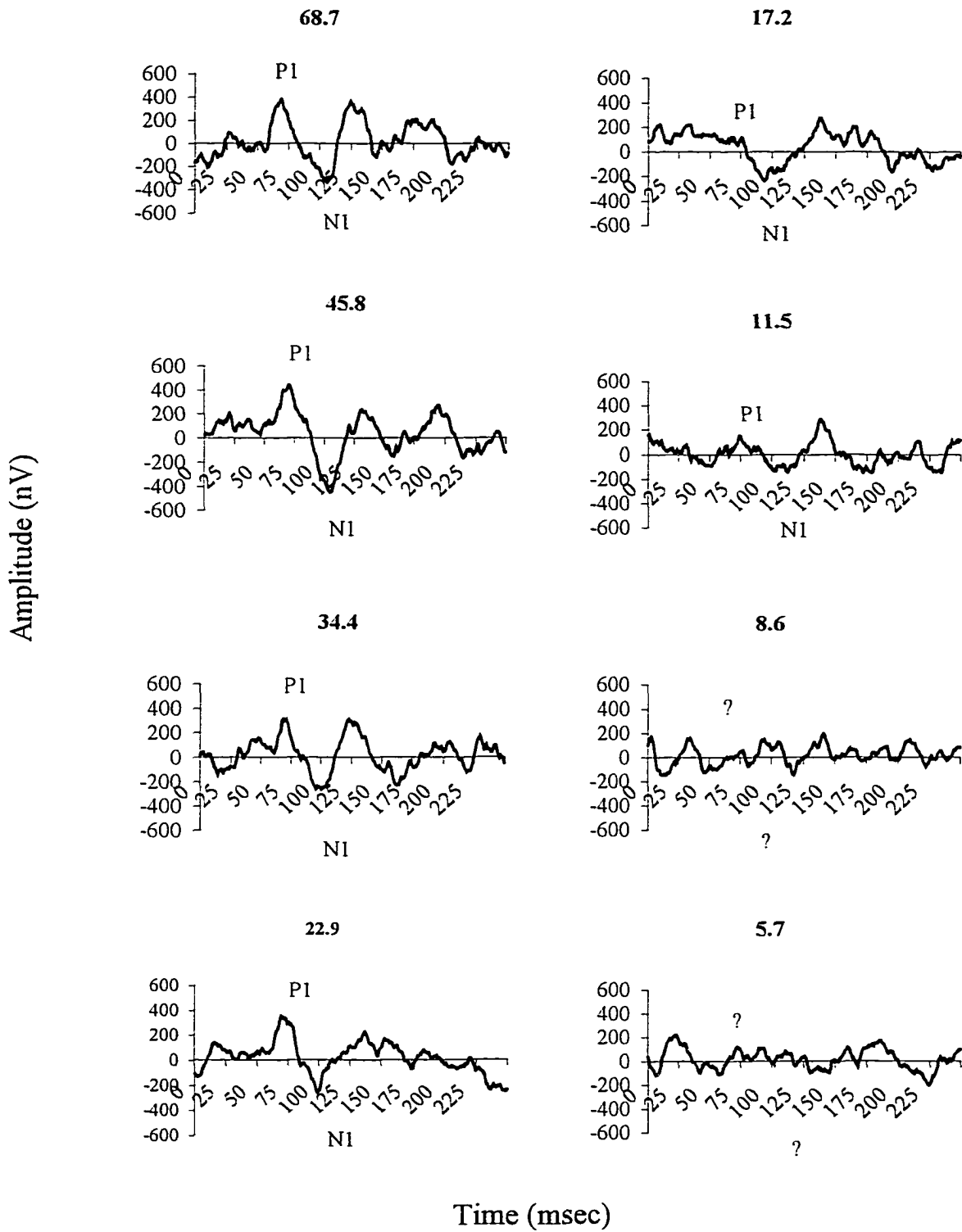


Figure 3. Visual evoked potential recording for a session with one turtle using eight stimulus sizes (ranging from 68.7 to 5.7 minutes of arc, visual angle). Notice that the amplitude difference between P1 and N1 decreases with a decrease in stripe angle, until it can no longer be identified. Each wave is an average of 250 responses; time zero is the start of stimulation.



on stripe size and linear regression analysis was executed to approximate threshold from the x-intercept value (McCormack and Tomlinson, 1979). For all six turtles, the regression line explains a significant portion of the variance of amplitude on stripe size (Figure 4-9, Table 1). The range of approximate acuity thresholds was from 0.130–0.214 (visual angle between 7.72–4.46 minutes of arc). Furthermore, the data from the six turtles were combined, and the x-intercept of the regression line approximated a mean threshold of 0.187 (visual angle of 5.34 minutes of arc) (Figure 10, Table 1).

A well-defined P1-N1 complex was not easily definable when testing occurred in air without the goggle (Figure 6). Behaviorally, the turtles were less likely to stay attentive on the stimulus when they were not wearing the goggle. Moreover, when recordings were collected and compared to goggle trials, the difference in the P1-N1 complex amplitude was distinct (Figure 11). No visually evoked response was elicited from a turtle when the eye was in air, regardless of the stripe width.

Figure 4. Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 1, a juvenile loggerhead sea turtle (*Caretta caretta*). The graph is a combination of multiple trials on separate days for the turtle. Amplitude differences consistently decrease with visual angle. The x-intercept of the linear regression line is an approximation of threshold.

Turtle 1

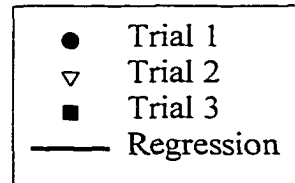
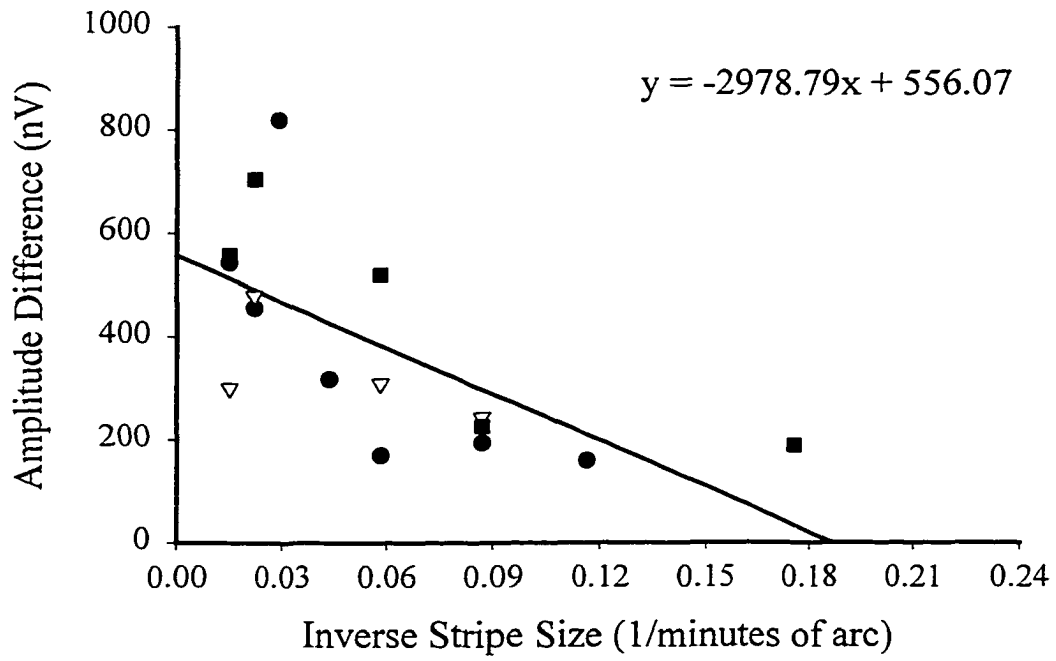


Figure 5. Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 2, a juvenile loggerhead sea turtle (*Caretta caretta*). The graph is a combination of multiple trials on separate days for the turtle. Amplitude differences consistently decrease with visual angle. The x-intercept of the linear regression line is an approximation of threshold.

Turtle 2

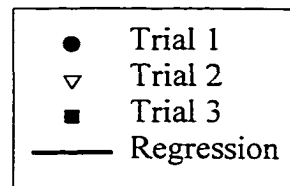
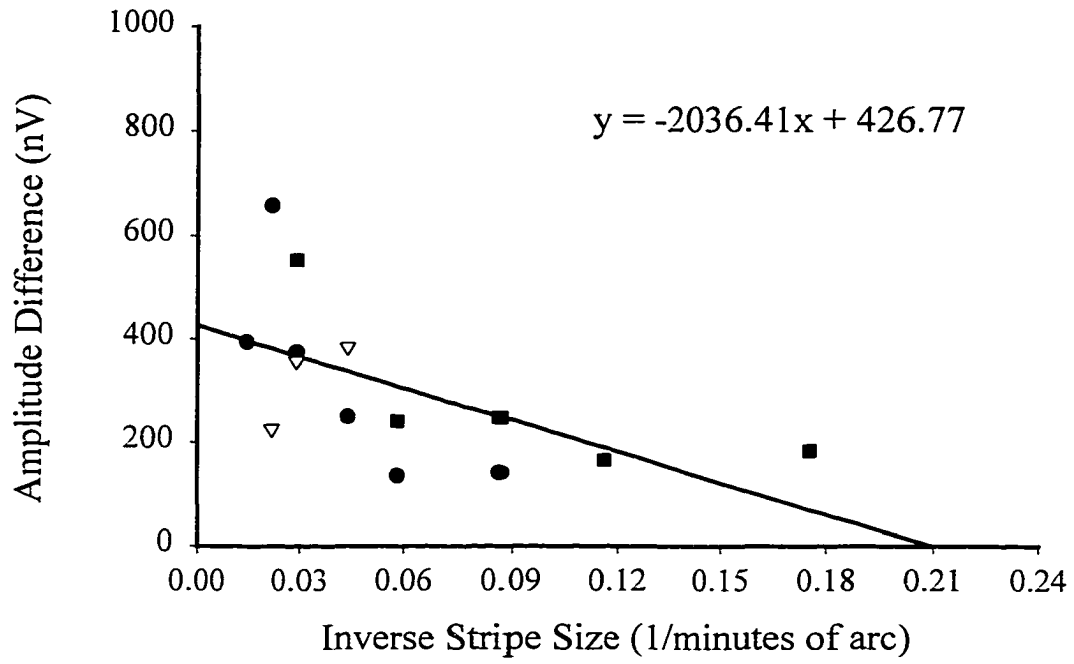


Figure 6. Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 3, a juvenile loggerhead sea turtle (*Caretta caretta*). The graph is a combination of multiple trials on separate days for the turtle. Amplitude differences consistently decrease with visual angle. The x-intercept of the linear regression line is an approximation of threshold.

Turtle 3

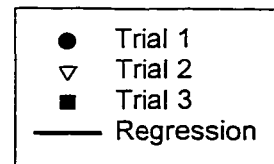
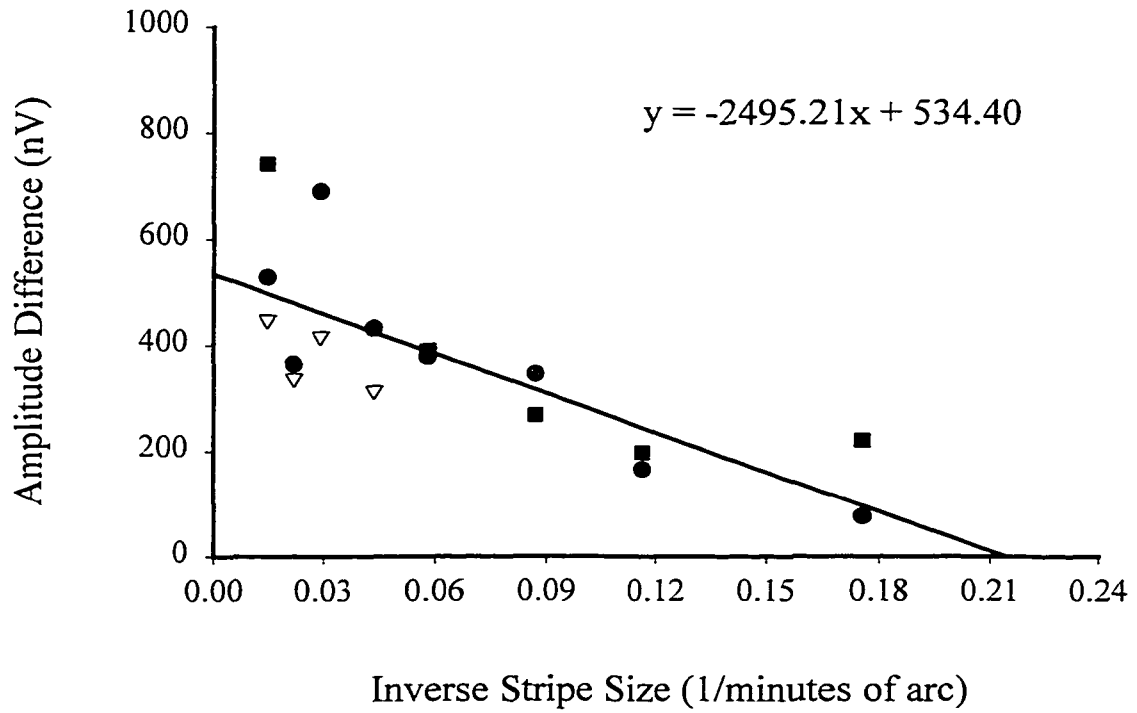


Figure 7. Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 4, a juvenile loggerhead sea turtle (*Caretta caretta*). The graph is a combination of multiple trials on separate days for the turtle. Amplitude differences consistently decrease with visual angle. The x-intercept of the linear regression line is an approximation of threshold.

Turtle 4

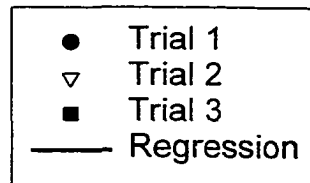
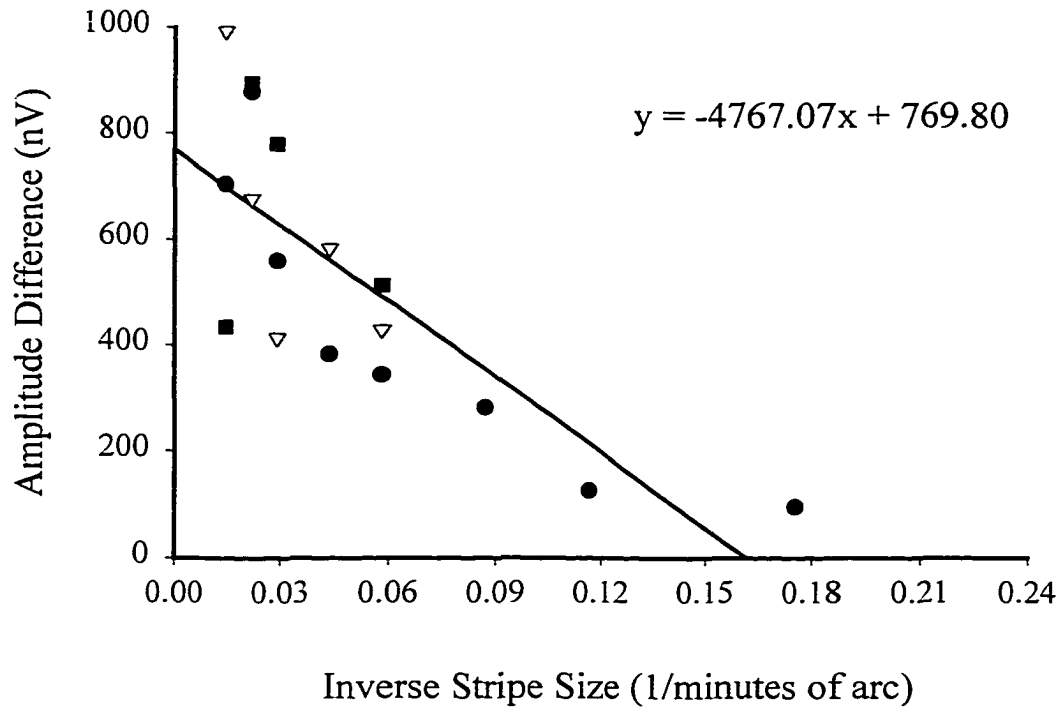


Figure 8. Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 5, a juvenile loggerhead sea turtle (*Caretta caretta*). The graph is a combination of multiple trials on separate days for the turtle. Amplitude differences consistently decrease with visual angle. The x-intercept of the linear regression line is an approximation of threshold.

Turtle 5

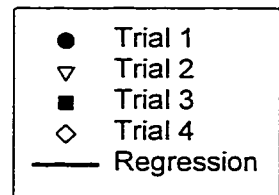
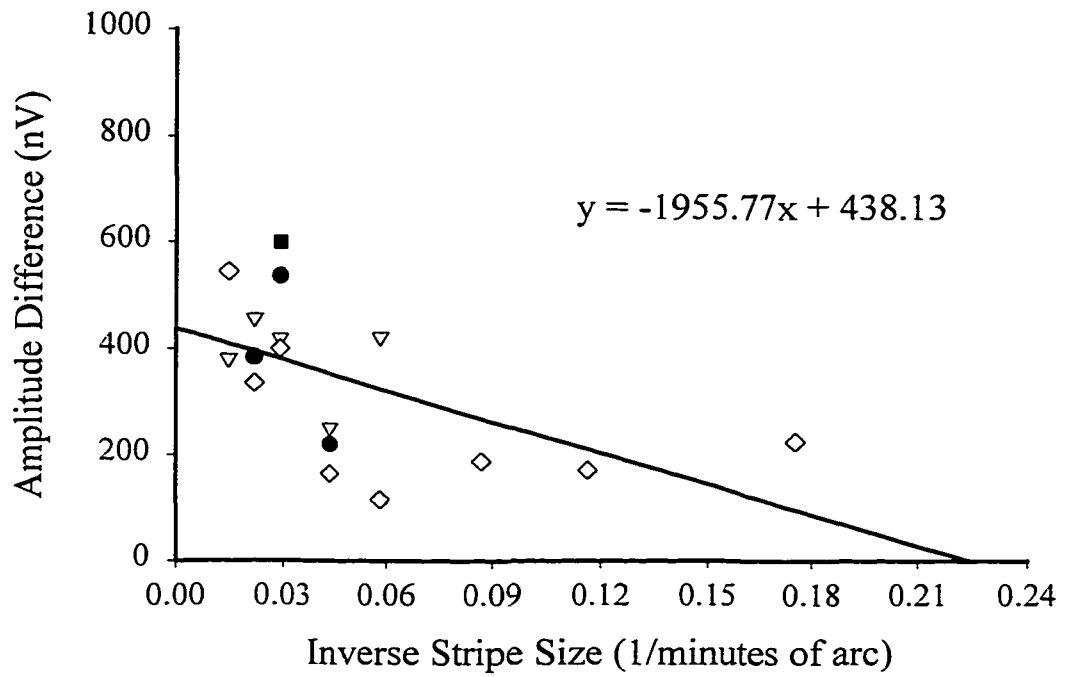


Figure 9. Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for Turtle 6, a juvenile loggerhead sea turtle (*Caretta caretta*). The graph is a combination of multiple trials on separate days for the turtle. Amplitude differences consistently decrease with visual angle. The x-intercept of the linear regression line is an approximation of threshold.

Turtle 6

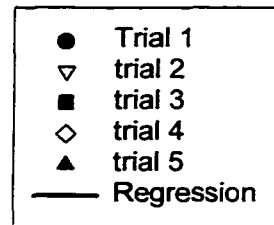
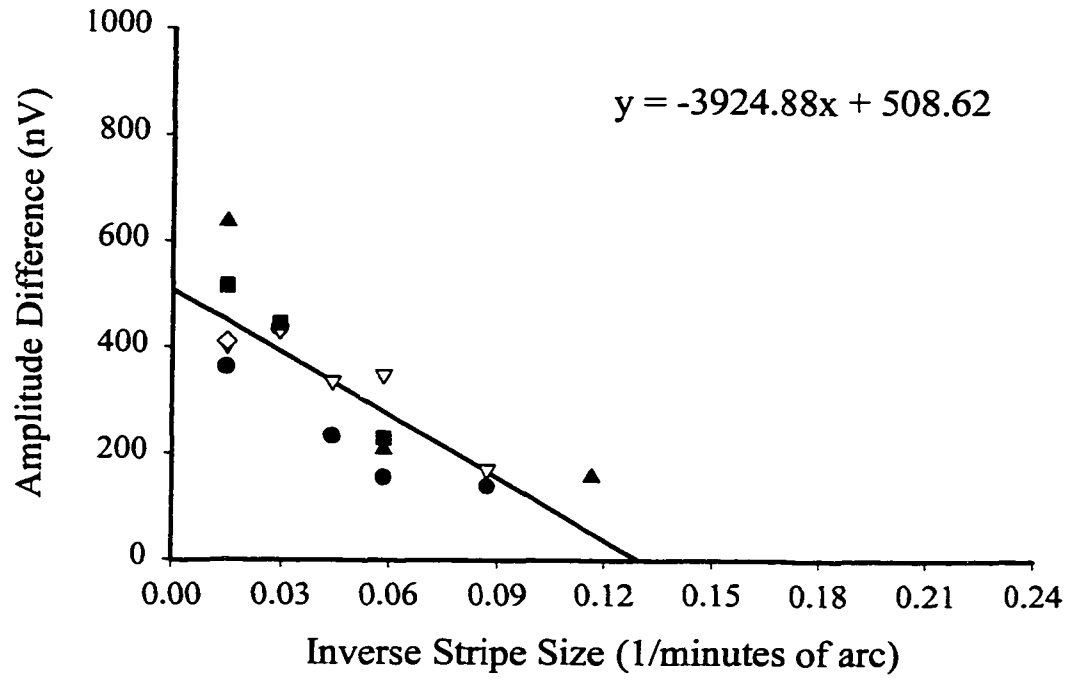


Table 1. Linear regression results from six sea turtles, and all six turtles combined, when P1-N1 amplitudes are plotted based on stripe size of stimulus. The x-intercept approximates threshold.

Turtle	P-value	r^2	x-intercept (minutes of arc)	Visual Acuity
Turtle 1	.005	.438	5.36	.187
Turtle 2	.027	.348	4.77	.210
Turtle 3	<.001	.599	4.67	.214
Turtle 4	<.001	.608	6.19	.161
Turtle 5	.020	.310	4.46	.224
Turtle 6	<.001	.682	7.72	.130
All Turtles Combined	<.001	.402	5.38	.186

Figure 10. Amplitude difference (nV) of P1 and N1 plotted as a function of stimulus stripe size for every trial performed on all loggerhead sea turtles tested. Amplitude differences consistently decrease with visual angle. The x-intercept approximated threshold at a visual angle of 5.38.

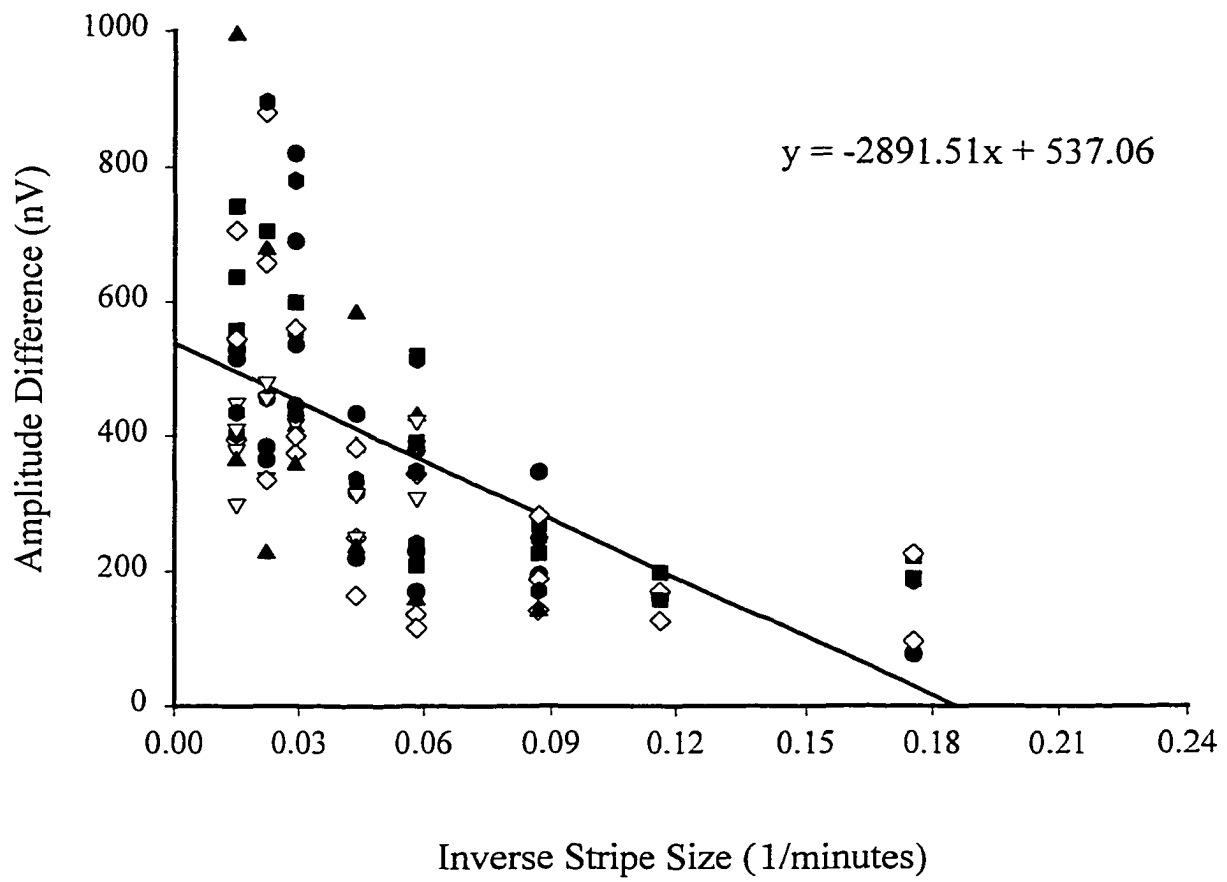
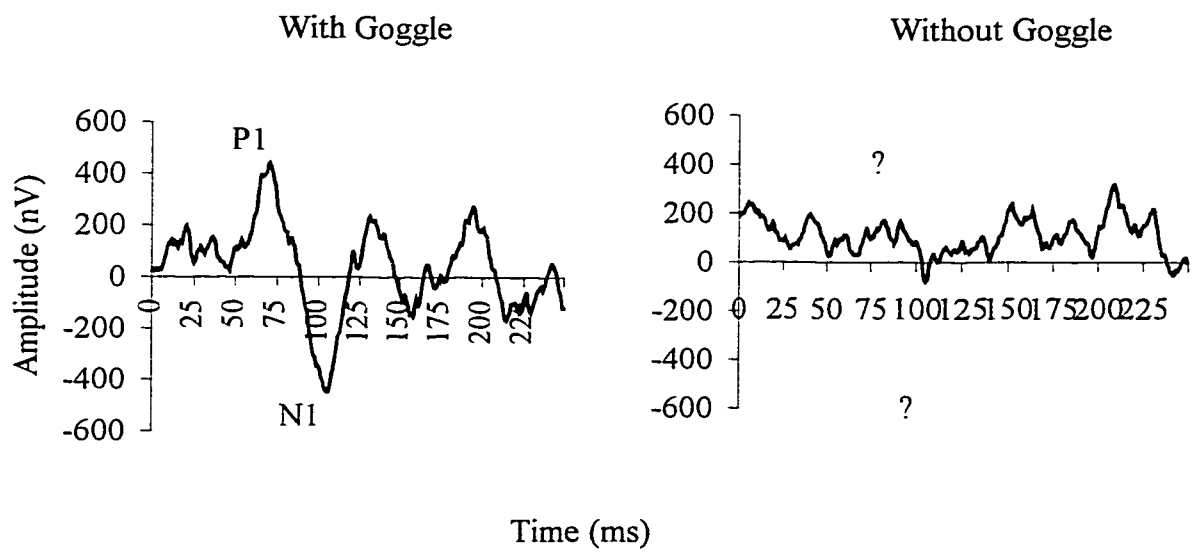


Figure 11. Comparison of waveforms collected, using the 45.8 minutes of arc stripe stimulus, for both in air and in water trials. The trial with the goggle resulted in a distinct P1-N1 complex, and amplitude difference between these peaks was calculated. Without the goggle, and the eye tested in air, neither peak is identifiable and amplitude difference could not be measured.



DISCUSSION

To date, visual electrophysiological research on sea turtles has been restricted to examination of spectral sensitivities of the green sea turtle using electroretinograms (Granda and O'Shea, 1972). The invasive methods associated with ERG procedures, however, have severely limited the progress of electrophysiological research on sea turtles. An extensive review has been made of the visual system of freshwater turtles (primarily *Pseudemys scripta elegans*). However, we know from morphological data, that sea turtles do not have the same accommodation mechanisms of semi-aquatic species, curtailing interpretation of these data across species. Thus the first objective of this paper was to determine if visual evoked potentials could be collected extracranially from unanesthetized sea turtles. Using the methods described in this paper, I found that visual evoked potentials are not only a viable alternative to the collection of electroretinograms, but can also provide insight into state dependent processes of the visual system.

Visual evoked potentials are compound field potentials that result from the stimulation of the retina. These potentials represent the net activity of the visual system and can be examined in regards to shape of waveform, latencies and dependence on stimuli, all of which cannot be predicted from the single unit recordings of ERGs (Riggs and Wooten, 1972; Bullock et al, 1991). Furthermore, because of the diffuse property of the recordings, electrodes were inserted just under the head scutes of the loggerhead without performing surgery, and the signal was recorded and amplified using standard signal averaging

techniques. One peak complex (P1-N1) was found to be pervasive in suprathreshold recordings and was easily tracked to stimulus intensity. It was on this peak that all threshold readings were based.

Visual evoked potentials recorded from juvenile loggerheads, using black and white striped stimuli, elucidated a dependence of stripe size on P1-N1 amplitude. This dependence was tracked to threshold using linear regression analysis. Several studies on mammals have found that threshold levels extrapolated from VEP responses to suprathreshold stimuli were highly correlated to behavioral thresholds. Thus, these methods have been shown to be a valid means of approximating threshold (Berkeley and Watkins, 1971; Parker and Salzen, 1977; McCormack and Tomlinson, 1979). In this study, I found the extrapolated threshold to be between 4.46 and 7.72 minutes of arc.

The threshold recorded from juvenile loggerheads is certainly much lower than alluded to in the sea turtle literature. Much of the literature, however, is centered on sea turtle vision in air. When I compared the response in air and water for the same stimulus, the P1-N1 complex was substantially different. In fact, responses were difficult to record when the eye was not submerged because the turtle often would be inattentive to the stimulus. When attention was held by the stimulus, the P1-N1 complex could not be produced by even the largest of stimulus stripes.

To assess the relative importance of these visual acuity thresholds collected with the eye submerged, these data can be examined in relation to the environment in which the juvenile loggerhead resides. The juvenile western Atlantic loggerhead sea turtle, used in the project, recruit to nearshore demersal habitats at an age of about 7-10 years. These juveniles proceed to make yearly migrations to temperate latitudes (such as the Chesapeake Bay) to

forage, inhabiting the shallow waters along the channel edges (Musick and Limpus, 1997). This level of visual acuity, approximately 5.38 minutes of arc recorded from all turtles combined, could provide the animal with information regarding prey location as well as predator presence. In fact, when this threshold is compared to other benthic, shallow water species, juvenile loggerhead acuity levels are analogous (Tamura, 1957; Heuter and Gruber, 1982; Heuter, 1991). For example, the lemon shark (*Negaprion brevirostris*), which is similar to the loggerhead in that it feeds benthically in shallow inshore waters, has a morphological acuity of 4.1 minutes of arc (Heuter and Gruber, 1982; Heuter, 1991).

Visual evoked potentials provide the researcher with a convenient and noninvasive technique to test the visual capabilities of unanesthetized turtles. However these results may be dependent on factors other than the stimulus itself. Certain psychological variables, such as attention of the subject animal on the stimulus and habituation to the stimulus presentation, are difficult to measure and can confound the results. Consequently, visual evoked potential research can provide both a conservative estimate of electrophysiological acuity as well as a glimpse into the underlying processes, but should be used as one of many tools in the description of an animal's sensory system. Traditional morphological studies elucidating the overall capacity of the system and psychophysical studies documenting visually mediated behaviors should be combined with evoked potentials to thoroughly explore the visual niche occupied by a species.

LITERATURE CITED

- Ali, M. A. and M. A. Klyne. 1985. Vision in Vertebrates. New York: Plenum Press. 272 pp.
- Anderson, P. K. 1958. The photic responses and water approach behavior of hatchling turtles. *Copeia*. 211-215.
- Armington, J.C. 1954. Spectral sensitivity of the turtle, *Pseudemys*. *J. Comp. Physiol. Psychol.* 47: 1-6.
- Bartol, S. M., J. A. Musick, and M. L. Lenhardt. 1999. Auditory evoked potentials of the loggerhead sea turtle (*Caretta caretta*). *Copeia*.
- Berkeley, M. A. and D. W. Watkins. 1971. Visual acuity of the cat estimated from evoked cerebral potentials. *Nat. New Biol.* 234: 91.
- Bullock, T. H., M. H. Hofmann, J. G. New, and F. K. Nahm. 1991. Dynamic properties of visual evoked potentials in the tectum of cartilaginous and bony fishes, with neuroethological implications. *J. Exp. Zool. Supp.* 5: 142-155.
- Daniel, R. S. and K. U. Smith. 1947. The sea-approach behavior of the neonate loggerhead turtle (*Caretta caretta*). *J. Comp. Physiol. Psychol.* 40: 413-420.
- Davson, H. 1972. *The Physiology of the Eye*. New York: Academic Press. 643 pp.
- Deane, H. W., C. Enroth-Cugell, C. Gongaware, M. S. Neyland, and A. Forbes. 1958. Electroretinogram of freshwater turtle: form and spectral sensitivity. *J. Neurophysiol.* 21: 45-61.
- Ehrenfeld, D. W. 1968. The role of vision in the sea-finding orientation of the green turtle (*Chelonia mydas*). 2. Orientation mechanism and range of spectral sensitivity. *Anim. Behav.* 16: 281-287.
- Ehrenfeld, D. W. and A. Carr. 1967. The role of vision in the sea-finding orientation of the green turtle (*Chelonia mydas*). *Anim Behav.* 15: 25-36.
- Ehrenfeld, D. W. and A. L. Koch. 1967. Visual accommodation in the green turtle. *Science.* 155: 827-828.

- Fernald, R. D. 1990. The optical systems of fishes. In: R. H. Douglas and M. B. A. Djamgoz (eds.) *The Visual System of Fish*. London: Chapman and Hall. pp. 45-61.
- Granda, A.M. 1962. Electric responses of the light- and dark-adapted turtle eye. *Vision Res.* 2: 343-356.
- Granda, A.M. 1979. Eyes and their sensitivity to light of differing wavelengths. In M. Harless and H. Morlock (eds.) *Turtles: Perspectives and Research*. New York: John Wiley and Sons. pp. 247-266.
- Granda, A.M. and P. J. O'Shea. 1972. Spectral sensitivity of the green turtle (*Chelonia mydas mydas*) determined by electrical responses to heterochromatic light. *Brain Behav. Evol.* 5: 143-154.
- Heuter, R. E. 1991. Adaptations for Spatial Vision in Sharks. *J. Exp. Zool. Suppl.* 5: 130-141.
- Heuter, R. E. and S. H. Gruber. 1982. Recent advances in studies of the visual system of the juvenile lemon shark (*Negaprion brevirostris*). *Florida Sci.* 45: 11-25.
- McCormack, G. L. and A. A. Tomlinson. 1979. Human visual acuity assessment through linear extrapolation to threshold of bar gratings VERs. *Am. J. Optom. And Physiol. Optics.* 56(8): 480-489.
- Mrosovsky, N. and S. Shettleworth. 1968. Wavelength preferences and brightness cues in the water-finding behavior of sea turtles. *Behavior.* 32: 211-257.
- Musick, J. A. and C. J. Limpus. 1997. Habitat utilization and migration in juvenile sea turtles, p. 137-163. In: *The biology of sea turtles*. P. L. Lutz and J. A. Musick (eds.). CRC Press, Boca Raton.
- Parker, D. M. and E. A. Salzen. 1977. The spatial selectivity of early and late waves within the human visual evoked response. *Perception.* 6: 85-95.
- Riggs, L. A. and B. R. Wooten. 1972. Electrical measures and psychophysical data on human vision. In: D. Jameson and L. M. Hurvich (eds.) *Handbook of Sensory Physiology, Visual Psychophysics Vol. VII No. 4*. Berlin: Springer-Verlag. Pp. 690-731.
- Spehlmann, R. 1985. *Evoked Potentials Primer: Visual, Auditory, Somatosensory Evoked Potentials in Clinical Diagnosis*. Butterworth and Heinemann: Boston.
- Sivak, J. G. 1985. The Glenn A Fry award lecture: optics of the crystalline lens. *Am. J. Optom. and Physiol. Optics.* 62: 299-308.

- Sivak, J. G. 1990. Optical variability of the fish lens. In: R. H. Douglas and M. B. A. Djamgoz (eds.) *The Visual System of Fish*. London: Chapman and Hall. Pp 63- 80.
- Sokal, R. R. and F. J. Rohlf. 1981. *Biometry*, 2nd edition. New York: W. H. Freeman and Co. 859pp.
- Tamura, T. 1957. A study of visual perception in fish, especially on resolving power and accommodation. *Bull. Jap. Soc. Sci. Fish.* 22: 536-557.
- van Rhijn, F. A. 1979a. Optic orientation in hatchlings of the sea turtle *Chelonia mydas* I. Brightness: not the only optic cue in sea-finding orientation. *Mar. Behav. Physiol.* 6: 105-121.
- van Rhijn, F. A. 1979b. Optic orientation in hatchlings of the sea turtle *Chelonia mydas* II. Apparatus for prolonged recording of orientation in turtles walking or swimming on the spot. *Mar. Behav. Physiol.* 6: 243-255.
- Walls, G. L. 1942. *The Vertebrate Eye and its Adaptive Radiation*. Bloomfield Hills: The Cranbrook Institute of Science. 785 pp.

Chapter 3

Visual Acuity of the Juvenile Loggerhead Sea Turtle (*Caretta caretta*):

A Behavioral Approach

ABSTRACT

Behavioral studies that have examined the visual cues sea turtles use to navigate between the nesting site and the sea have found these cues to be diffuse images and have concluded that sea turtles are highly myopic on land. This study explores the visual acuity of juvenile loggerhead sea turtles (*Caretta caretta*) in the marine environment by performing operant conditioning experiments. Turtles were trained, in a tank setting, to distinguish between a striped panel and gray panel by using a food reward. Once training was achieved, the stripes were reduced in size until the turtle choose the correct panel based on chance. Mean threshold level for all turtles tested was found to be 0.078 (visual angle of 12.89 minutes of arc). These results indicate that sea turtles are capable of using distinct visual cues in the aquatic environment.

INTRODUCTION

Sea turtles have been the subjects of many behavioral studies that have explored the perceptions of these animals as they search for a suitable nesting site or orient towards water. As Ehrenfeld and Carr (1967) pointed out in their investigation of sea-finding orientation by turtles, the nesting female and recently emerged hatchling are the two life history stages of the sea turtle where behavior can be easily studied in the natural environment. Many of these terrestrial studies have tested brightness cues, shapes, silhouettes, wavelength, and the horizon as mechanisms for sea turtles to find water. Though many researchers have found vision to be the primary cue in land orientation, these cues are often diffuse images or brightness contrasts (Ehrenfeld and Carr, 1967; Mrosovsky and Shettleworth, 1968; Witherington and Bjorndal, 1991; Salmon and Wyneken, 1990; 1994).

Sea turtles, however, are primarily adapted to aquatic living, making it necessary for researchers discern their basic behavioral conduct in water. The visual capabilities of sea turtles in water are very different from those on land. Though many semiaquatic species have developed adaptations for both media, the sea turtle spends very little time on land and the eye is largely adaptive to the aquatic environment. The lens is nearly spherical and morphological studies have shown it to be static and unpliant. Moreover, from preliminary studies on the morphology of the eyeball, sea turtles do not have the musculature needed for accommodation (Walls, 1942; Ehrenfeld and Koch, 1967; Granda, 1979). Focusing of the lens is often achieved either by changing the shape of the lens (as in freshwater turtles)

(Walls, 1942; Granda, 1979) or by moving the lens along a rostral-caudal axis (as in most teleosts) (Walls, 1942; Munk, 1973; Fernald, 1990). For sea turtles, however, the sphincter muscle, needed to deform the lens shape, is weakly developed and the ciliary processes, needed in the movement of the lens, does not come in contact with the lens itself (Ehrenfeld and Koch, 1966; Granda, 1979).

Lens shape and an apparent lack of accommodative mechanism result in the sea turtle being highly myopic on land. A myopic state is caused by the image coming into focus between the lens and the retina, and thus, only close objects are in focus. In fact, most of the behavioral work on land has shown sea turtles to rely on shapes and silhouettes rather than distinct visual cues in the search for water. However, when the refractive index of the green sea turtle (*Chelonia mydas*) eye was tested in water, these animals were found to be emmetropic (the image is focused onto the photoreceptive elements of the retina) (Ehrenfeld and Koch, 1966). When the eye is submerged in water, the refractive indices of cornea and ocular fluids are almost identical to seawater. In the absence of corneal refraction, the eye can no longer benefit from the air/cornea interface, which provides considerable dioptric strength. Instead, the lens is the only feature that brings the image into focus. For sea turtles, as is the case for many species of teleosts, a spherical lens is ideal for these conditions (Walls, 1942; Granda, 1979; Fernald, 1990). The high degree of convexity of the lens elevates the overall refractive power (Sivak, 1985; 1990), providing, in the case of the green sea turtle, an emmetropic state (Ehrenfeld and Koch, 1966).

One manner of exploring the aquatic vision of sea turtles is through psychophysical experiments, techniques frequently used to test the sensory capacities of non-verbal animals. Behaviors explored in psychophysical experiments usually fall into two categories: innate

behaviors and learned behaviors. Innate behaviors are automatic responses to stimuli, such as eye movements, increased heart/breathing rate, aggressive/flight responses, etc. However, many visual functions, such as visual acuity, often do not elicit an innate response to the stimuli of interest, thus limiting the applicability of this technique. Learned psychophysical experiments represent behavior imposed by the experimenter. Specific responses by the subject animal are maintained to a controlled stimulus. This technique eliminates bias; the experimenter can record both incorrect responses as well as failures to respond from the subject animal (Blough, 1971; Blough and Blough, 1977; Douglas and Hawryshyn, 1990).

One form of learning experiment utilizes operant conditioning techniques where a learned response is established through either positive reinforcement or aversive stimulation. The most commonly used technique when examining the visual ability of a subject animal is the two-response forced-choice method. The subject is presented with two stimuli and is reinforced to choose the “correct” one by the presentation of an associated reward. Environmental factors that could bias the response are identified and eliminated from the experimental design. For example, the position of the correct stimulus is exchanged with the incorrect stimulus randomly to ensure that the learned behavior is in connection with the stimulus and not the location (Blough and Yager, 1972; Blough and Blough, 1977).

Psychophysical methods have been used successfully with hatchling sea turtles in a tank environment. Fehring (1972) trained hatchling loggerhead sea turtles to discriminate between wavelengths of light in a submerged y-maze. This study used operant conditioning methods, maintaining a learned response with the subject animal through positive food reward, to train and tests the hatchlings. Furthermore, behavioral methods have been used to investigate various aspects of hatchling sea turtles’ capacity to learn. In a large tank,

hatchlings were tested for their ability to associate environmental conditions or social interactions with foraging opportunities (Mellgren et al, 1994; Mellgren and Mann, 1996; 1999). These studies clearly indicate that sea turtles are appropriate subjects for behavioral work.

This project proposed to use psychophysical methods to investigate the visual acuity, the ability to distinguish details of an object, of juvenile loggerhead sea turtles (*Caretta caretta*) in the aquatic medium. To accomplish this objective, operant conditioning methods were developed to train juvenile sea turtles to identify a suprathreshold stimulus. Once training was achieved, visual acuity thresholds were tested using similar methods but varying the visual angle of the stimulus until threshold was identified.

MATERIALS AND METHODS

Subject animal

All turtles utilized in this study were juvenile loggerhead sea turtles (*Caretta caretta*), averaging approximately 63 cm straight notch to notch carapace length. These loggerheads were incidentally captured in poundnets in the Potomac River, a tributary of the Chesapeake Bay. The animals were immediately transferred to holding facilities at the Virginia Institute of Marine Science and placed in individual recirculating riverwater tanks. Temperature was maintained between 23 and 27 degrees Celsius. After at least 24 hours of acclimation, the animal was examined to determine its health status and then considered ready for testing. All testing was conducted under the National Marine Fisheries Services sea turtle permit no. 929.

Tank and apparatus

Testing was performed in a rectangle tank 2.5 m long by 1.3 m wide and .6 meters deep, and filled with filtered riverwater. At one end of the tank, running the width of the tank, was a plywood barrier. Two cutouts (9cm by 9cm) were equally spaced on the barrier, .5 m apart from each other, and covered with Plexiglas; these were the sites for the stimuli. Below each stimulus extruded the end of a PVC pipe, and this pipe extended behind the barrier to connect with the food chute. Lights of equal intensity were mounted behind each stimulus panel. These lights were controlled simultaneously by a single on/off switch. Finally, an end of PVC pipe protruded between the stimuli and acted as an observing key

(Figure 1). The entire tank was covered prior to testing and a small strip was cut out of the covering to allow for observation of the turtle's responses.

Stimuli were both black and white stripes of varying size and a 50% gray panel. All stimuli were printed on transparencies and mounted onto Plexiglas. Stimulus panels were attached to the plywood barrier with clips so those stimuli could easily change side location. Contrast ratios between black and white stripes exceeded 90% for all patterns. Eight gratings were used, with stripe widths of 45.0, 22.5, 11.3, 5.6, 2.8, 1.4, 0.7, and .035 mm (Figure 2).

Training and experimental procedures

Training, and all ensuing trials, proceeded using the two-response, forced-choice method of operant conditioning. This method utilized positive reinforcement that was closely associated with the correct stimulus (Blough and Blough, 1977). The subject animal was first trained to bite the observing key, the pipe spaced equidistant from each stimulus panel. This action by the turtle turned on both stimulus lights simultaneously, thus illuminating both panels. The purpose of the observing key was to place the turtle equidistant between each panel at the start of a trial. Once the lights were switched on, the turtle had three possible choices: 1) if the turtle bit the pipe under the striped panel, it immediately received a piece of squid through that very pipe via the food chute, and then the lights were extinguished; 2) if the turtle bit the pipe under the 50% gray panel, both lights were immediately turned off; and 3) if the turtle failed to respond at all within a predetermined period of time, both lights were turned off. Irrespective of the response, once the lights were extinguished, they could not be re-illuminated by the turtle biting the observing key until 30 seconds had lapsed. This period of time was needed to change

Figure 1. Schematic diagram of tank design used to test the behavioral visual acuity thresholds of the juvenile loggerhead sea turtle (*Caretta caretta*).

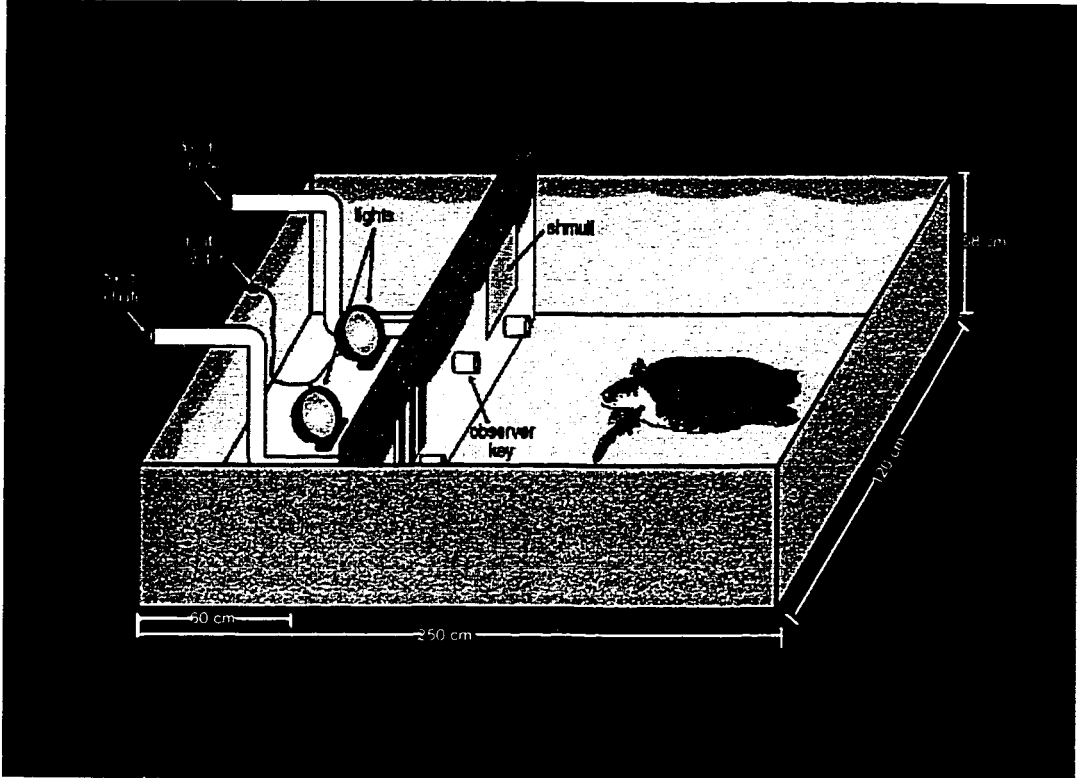
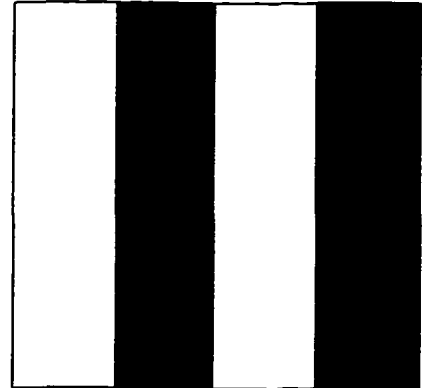


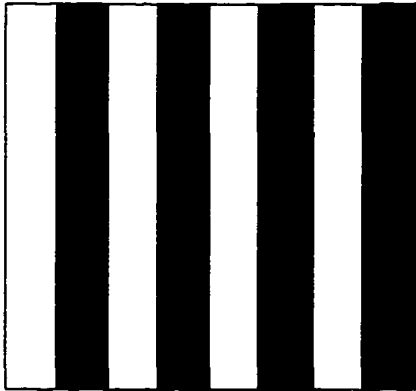
Figure 2. Four examples of stimuli panels used in the training and testing of visual acuities of juvenile loggerhead sea turtles (*Caretta caretta*). During training, the 45mm and gray panels were always used. Once threshold trials began, the gray panel was paired with varying striped panels of descending size. Contrast ratios between black and white stripes exceeded 90% for all patterns. A) 50% gray panel B) 45mm stripe panel C) 22.5mm stripe panel D) 11.3mm stripe panel



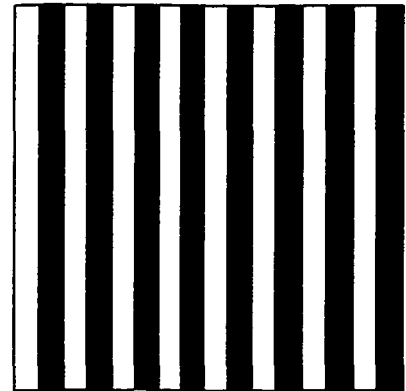
A)



B)



C)



D)

position of the stimuli and to refill the reward chutes (if necessary). Squid was always present in both reward chutes so that olfaction could not bias the response. In the training sessions, the panels used were always the 50% gray panel and the 45mm stripe panel. Side position of each stimulus on the barrier exchanged randomly with each trial.

Training duration was based on our guidelines for holding these animals in captivity. The juvenile loggerhead's diet was restricted to one to three percent of their total body weight. Thus training occurred only every other day for one to two hours per turtle, or until all of the allotted squid was consumed (20 presentations of the stimuli panels). The turtle was deemed trained when it chose the 45mm stripe panel at least 80% of the time.

Once training was achieved, threshold trials began for each animal using a block method of testing. Each day consisted of a warm-up period using the 45mm stripe panel vs. the gray panel and then eight blocks of 10 tests (each stripe size represented by a block). As the block of tests progressed in the trial, the stripe width decreased. Multiple threshold trials were performed on each turtle.

Calculation of visual acuity and statistical analysis

Visual acuity is the reciprocal of the visual angle and is a measure of the ability to resolve details of an object. Visual angle, measured in minutes of arc, is the angle subtended at the eye by the size of the viewed object and is calculated as follows:

$$\text{Visual angle} = \tan^{-1} \frac{\text{width } \frac{1}{2} \text{ cycle}}{\text{distance between stimulus and turtle's eye}}$$

The width of one half cycle is the distance from the middle of one black stripe to the middle of one white stripe. The distance between the stimulus and the turtle was standardized at 150 mm (the distance from each stimulus when the turtle was biting the observing key).

Therefore, the stimuli ranged from 1000 minutes of arc (the 45 mm panel) and 8 minutes of arc (the .035 mm panel).

Percent correct responses for each block of tests were plotted based on the reciprocal of visual angle for each turtle. Linear regression analysis was performed on these data (Sokal and Rohlf, 1981). Threshold was designated to occur when the turtle chose the panels based on chance. The x-intercept of the regression line at the 50% correct level was used to approximate acuity threshold (Blough and Blough, 1977).

RESULTS

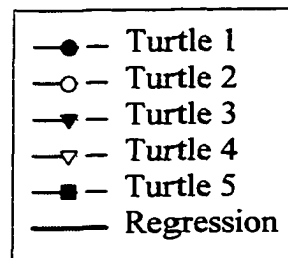
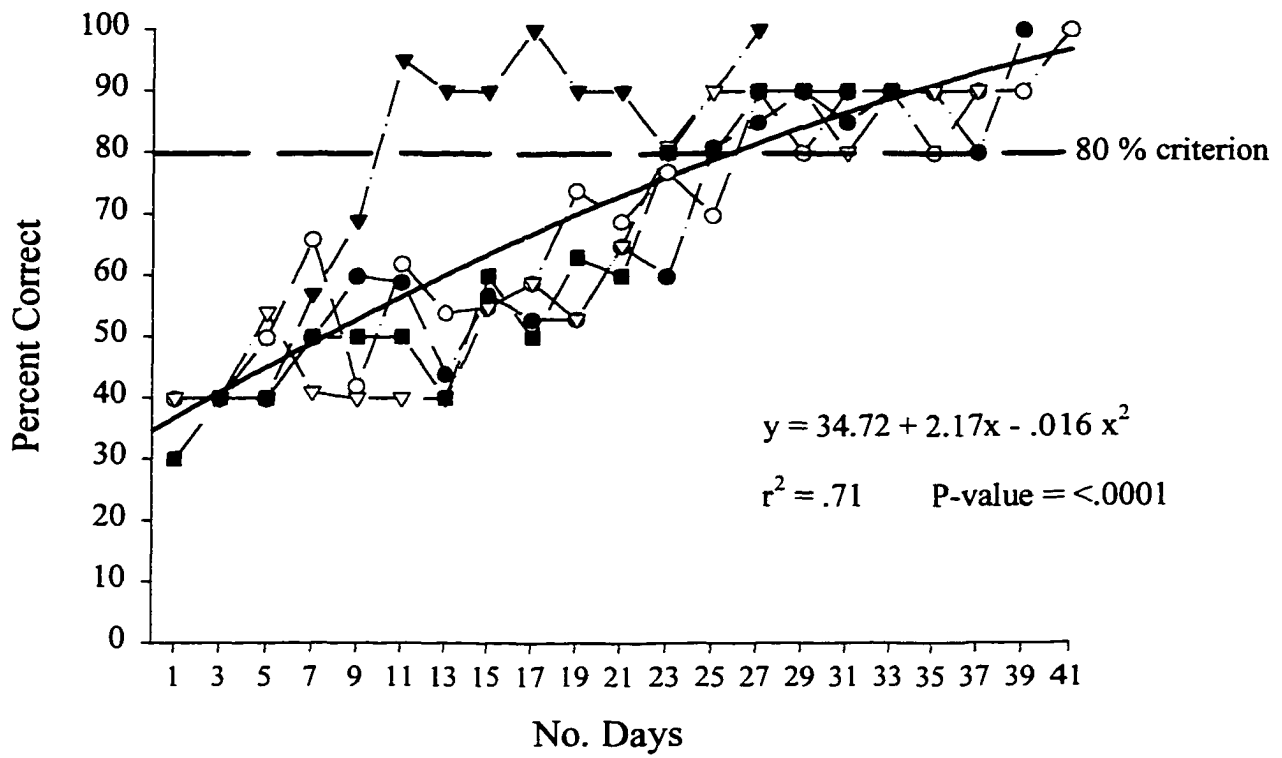
Training and general aspects of behavior

Very little behavioral work has been performed on non-hatchling aged sea turtles, and thus the suitability of these animals for experimental study was first examined. During the training portion of this project, several response behaviors were tested; the action of biting a pipe was by far the most consistent response made by the naive turtle. Because the untrained turtles would bite repeatedly any pipe in the tank, the observing key acted to focus the animals' attention onto the stimulus panels. Furthermore, all turtles were highly motivated by the food reward of squid and fasting prior to testing was not necessary.

Five turtles were successfully trained during the span of these experiments (three months). For those five turtles, training occurred in approximately 26 days (Figure 3). Because training occurred every other day, with 20 presentations for training session, these turtles were trained to the 45 mm panel in 270 presentations of the stimuli. One turtle (Turtle 3), however, was trained in only 11 days, or 120 presentations of the stimuli (Figure 3). After training was achieved, no turtle dropped below the 80% correct criterion for the 45mm stripe panel during any training or threshold trials (Figure 3).

It was further noted that the behavior of the animal drastically changed upon nearing threshold. In suprathreshold trials, the response of the animal was swift, frequently occurring less than 10 seconds after biting the observing key. However, in all five cases, as threshold was approached, the turtle often would not choose either pipe. In fact, the turtle would

Figure 3. Learning curves of five loggerhead sea turtles (*Caretta caretta*) trained to discriminate between an illuminated gray panel and 45mm striped panel. Training occurred every other day for one to two hours per day. The turtle was successfully trained when it chose the striped panel at least 80% of the time.



“pace” back and forth in front of the two panels. If the turtle did not chose at all within 30 seconds, the response was recorded as incorrect.

Threshold Trials

The percentage of correct responses for each test within a trial was calculated and plotted based on the reciprocal of the visual angle of each stimulus. All trials executed were combined for each turtle and linear regression analysis was performed on the data. The intercept of the regression line at the 50% correct criterion was deemed threshold (Figure 4-8). For all five turtles, the regression line explains a significant portion of the variance of responses (Table 1). The range of approximate visual acuity thresholds was from 0.069–0.088 (visual angle between 14.50–11.36 minutes of arc). Furthermore, the data from all five turtles were combined, and the intercept at the 50% correct criterion approximated a mean threshold of 0.078 (visual angle of 12.89 minutes of arc) (Figure 9, Table 1).

Figure 4. Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 1, a juvenile loggerhead sea turtle (*Caretta caretta*). The regression line explains a significant portion of the variance of response to stripe size and the intercept at the 50% correct level approximates acuity threshold for each turtle.

Turtle 1

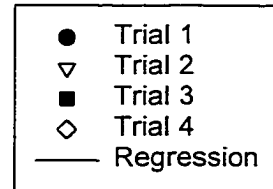
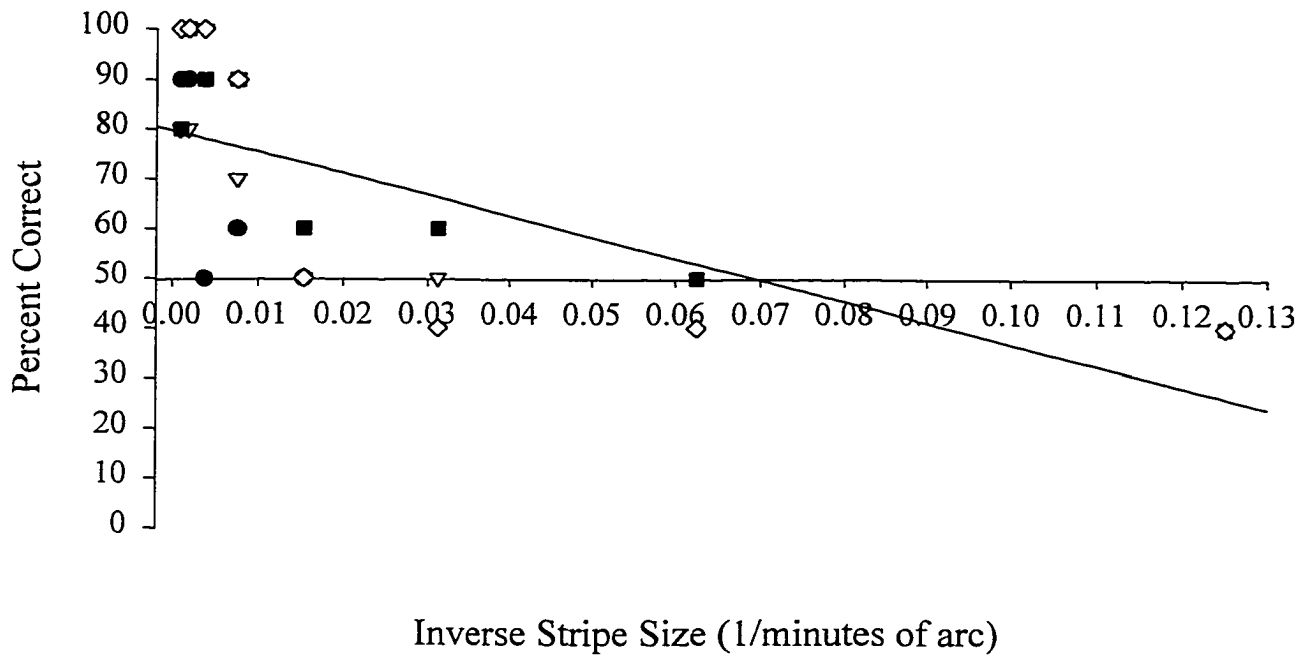


Figure 5. Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 2, a juvenile loggerhead sea turtle (*Caretta caretta*). The regression line explains a significant portion of the variance of response to stripe size and the intercept at the 50% correct level approximates acuity threshold for each turtle.

Turtle 2

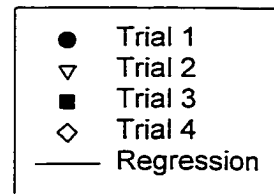
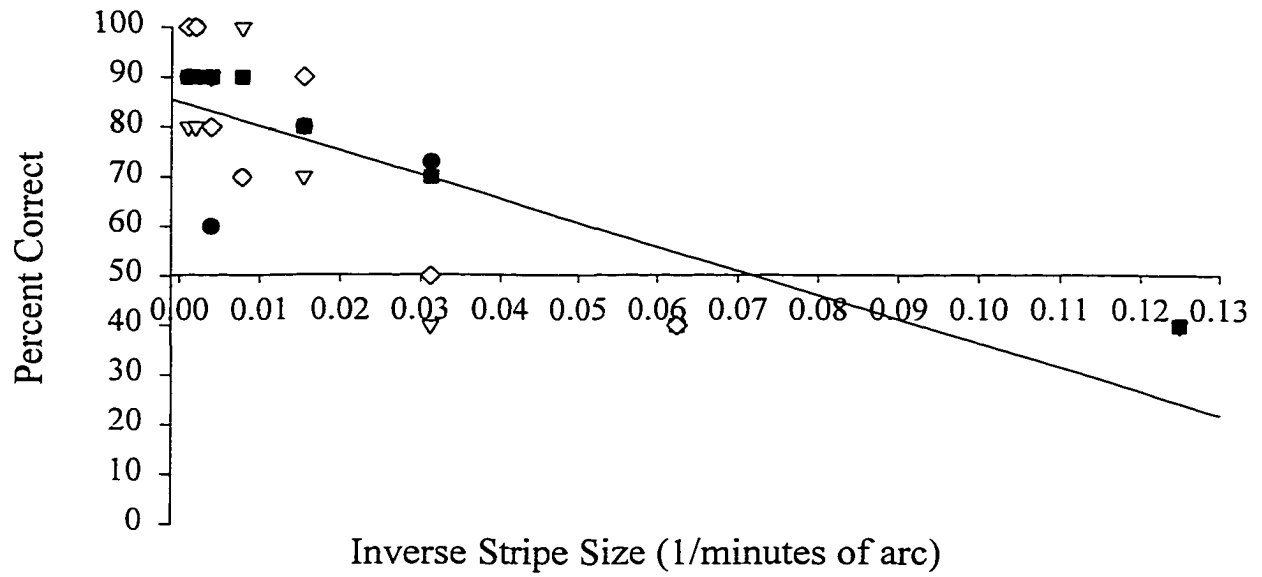


Figure 6. Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 3, a juvenile loggerhead sea turtle (*Caretta caretta*). The regression line explains a significant portion of the variance of response to stripe size and the intercept at the 50% correct level approximates acuity threshold for each turtle.

Turtle 3

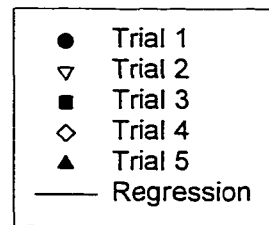
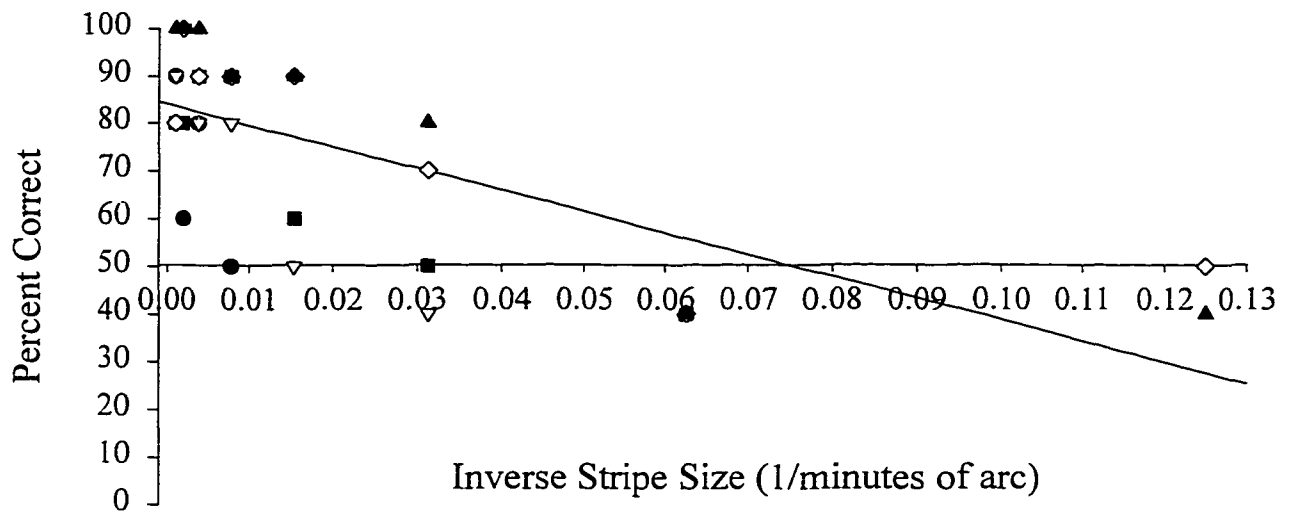


Figure 7. Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 4, a juvenile loggerhead sea turtle (*Caretta caretta*). The regression line explains a significant portion of the variance of response to stripe size and the intercept at the 50% correct level approximates acuity threshold for each turtle.

Turtle 4

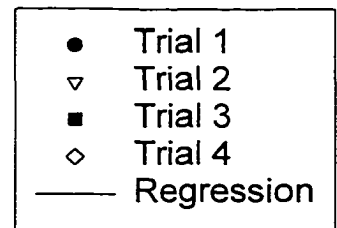
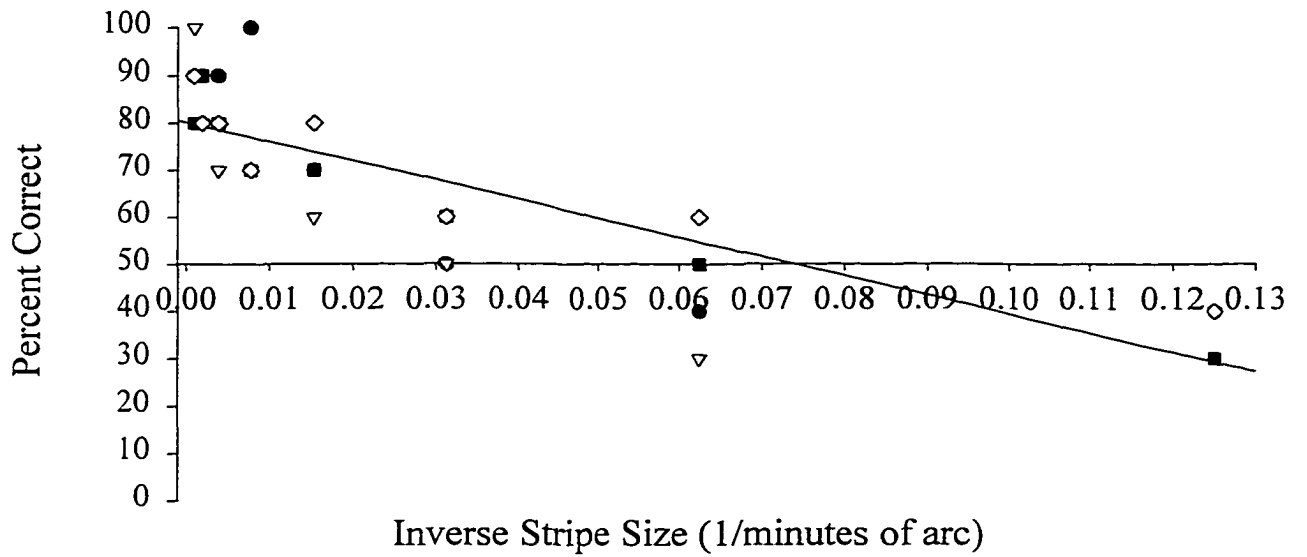


Figure 8. Plot of percent correct responses to stimulus panels of decreasing stripe size for multiple acuity threshold trials performed on Turtle 5, a juvenile loggerhead sea turtle (*Caretta caretta*). The regression line explains a significant portion of the variance of response to stripe size and the intercept at the 50% correct level approximates acuity threshold for each turtle.

Turtle 5

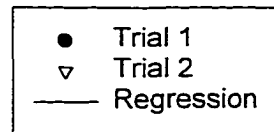
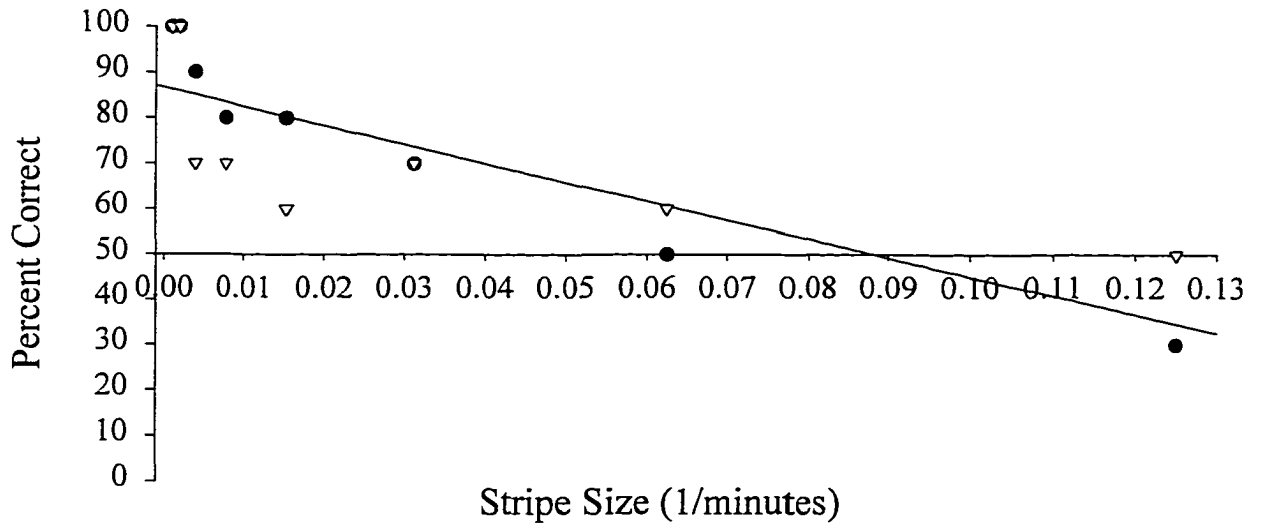
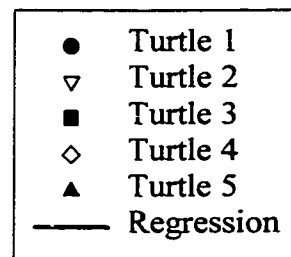
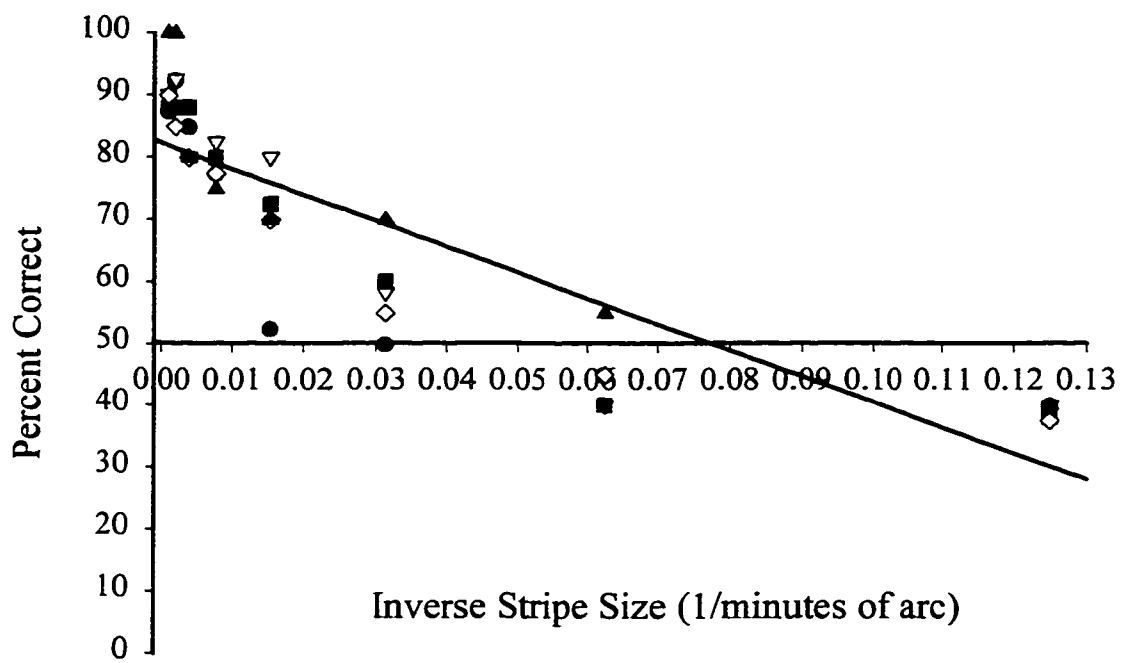


Table 1. Linear regression analysis results from five sea turtles, and all five turtles combined, when percent correct responses were plotted based on stripe size of stimulus. The regression line intercept at the 50% correct level approximates threshold.

Turtle	P-value	r^2	50% intercept (minutes of arc)	Visual Acuity
Turtle 1	<.001	.430	14.50	.069
Turtle 2	<.001	.610	13.89	.072
Turtle 3	<.001	.474	13.33	.075
Turtle 4	<.001	.688	13.51	.074
Turtle 5	<.001	.691	11.36	.088
All Turtles Combined	<.001	.747	12.89	.078

Figure 9. Percent correct responses to stimulus panels of decreasing stripe size for all juvenile loggerhead sea turtles (*Caretta caretta*) tested. Percentage of correct responses by the turtles consistently decreased with visual angle. The intercept of the regression line at the 50% correct level approximated acuity threshold to be .078 (12.89 minutes of arc).



DISCUSSION

Juvenile loggerhead sea turtles proved to be a suitable subject animal for in-tank behavior studies. Adapting an appropriate response to these loggerheads was relatively simple; these turtles readily bit any protuberance in a tank. Consequently, the main aspect of training involved directing the animals to associate the pipe with the above stimuli. Several methods were attempted, but the observing key proved to focus the turtle's attention on to the "game" at hand. Even before the observing key was introduced into these experimental procedures, the turtle associated the presence of light with the possibility of finding squid. By adding the extra step of biting the observing key to turn on the lights, the turtles became more focused on finding the squid. Furthermore, this step positioned the turtle equidistant from both stimuli at the start of each trial.

Squid was a strong motivating force for these animals, and it was never necessary to withhold food prior to training sessions or trials. One limitation in the duration of training was the restriction in diet for sea turtles maintained in captivity. The juvenile loggerhead's diet was limited to 1-3% of their body weight per week; this diet restriction reduces the possibility of obesity often associated with captive sea turtles. Moreover, squid is not a complete diet for sea turtles, and outside of the training sessions their diet was augmented with blue crabs and dietary supplements (two items that would not work as a reward item) (George, 1997). Even though the subject animal was motivated to continue with the training, these sessions were terminated when the allotted squid was consumed.

The reaction time variance mentioned in this study was unexpected. Over the course of the trials, as stripe width decreased, latency of response by the turtle increased. In the suprathreshold trials, the response by the turtle (biting the pipe under the stimulus panel) occurred almost without delay. Yet as the trials approached the threshold level, response time increased. The turtle would bite the observing key and then pace back and forth between the two stimulus panels. A time limit for choosing a response had to be imposed, and if the turtle did not choose at all during this time, the response was marked as incorrect. Though this stimulus intensity-response latency correlation has been recorded in mammals and birds, this relationship has not been documented in any behavioral studies of turtles. This response-latency correlation needs to be explored in future trials with sea turtles. If this association were confirmed, it would be possible to use this response as a technique for evaluating not only thresholds but also the similarity of suprathreshold stimuli. Equal visual stimuli could be derived from equal latencies of response (Blough and Yager, 1972).

Using operant conditioning threshold methods, this study estimates the acuity threshold for the juvenile loggerhead to be approximately .078 (visual angle of 12.89 minutes of arc). Comparison of these results to previous sea turtle work is problematic. Most prior research examined behavior of these animals directly on the beach, and provides an estimate of natural behavior by the turtle on land. Visual stimuli are the primary cues used by hatchling and adult sea turtles between the nest site and the sea. These cues, however, are restricted to diffuse images and/ or brightness contrasts and did not require the sea turtle to use a high degree of resolution (Salmon and Wyneken, 1994). However, the eye is more suited for aquatic than aerial vision, and it is not surprising that the results of this study

demonstrate the juvenile sea turtle to have a significantly higher degree of acuity in water than on land.

When these results are weighted against the visual acuity of other aquatic species that were tested using psychophysical methods, these acuity values are comparable (Table 2). Though Table 2 encompasses a wide range of species, habitats, and experimental procedures, studies performed on other marine species provide a frame of reference when evaluating the acuity of loggerhead sea turtles. All of these animals, except for the nautilus whose eye acts as a simple pin-hole camera, possess an acuity threshold between 5-20 minutes of arc and all are hypothesized to use visual cues extensively in the aquatic environment. It is apparent from these comparisons that juvenile loggerheads are also using distinct visual cues to function in the marine environment. From the results of this study, juvenile loggerheads have been shown to use a high degree of resolution to forage for food. Moreover, with this acuity level, the juvenile loggerhead could be using visual cues for predator avoidance, locomotion, territory selection and defense, and other basic behavior in their aquatic surroundings.

Behavioral experiments provide a unique opportunity to examine the response of the whole animal to sensory stimulation. Morphological and electrophysiological studies on the vision of sea turtles have elucidated the underlying visual mechanisms. Both of these methods have mapped the pathway from stimulus to receptor organ to optic tectum. Psychophysical experiments, however, depict a prescribed behavior, or lack thereof, associated with stimulation. To further our understanding on how the sea turtle perceives its surroundings, more behavioral research of sensory systems needs to be performed on these animals in the aquatic environment.

Table 2. Visual acuities, in minutes of arc, of various aquatic species measured using psychophysical techniques, with one exception (*Negaprion brevirostris*).

	Species	Visual Acuity (min. of arc)	Reference
Mammals	<i>Phoca vitulina</i>	8.3	Schusterman and Balliet, 1970
	<i>Eumetopias jubata</i>	7.1	Schusterman and Balliet, 1970
Teleost Fishes	<i>Katsuwonus pelamis</i>	5.6	Nakamura, 1968
	<i>Euthynnus affinis</i>	7.4	Nakamura, 1968
	<i>Thunnus albacares</i>	3.7	Nakamura, 1968
	<i>Scopthalmus maximus</i>	11	Neave, 1984
	<i>Salmo gairdneri</i>	14	Rahmann et al., 1979
	<i>Lepomis macrochirus</i>	14.2 17.0	Hairston et al. 1982; Breck and Gitter, 1983
Elasmobranchs	<i>Negaprion brevirostris</i>	4.1	Heuter and Gruber, 1982; Heuter, 1991 (morphological acuity)
Cephalopods	<i>Nautilus pomppilius</i>	330-670	Muntz and Raj, 1984
	<i>Octopus pallidus</i>	9.7	Muntz and Gwyer, 1988
	<i>Octopus australis</i>		
Reptiles	<i>Caretta caretta</i>	12.9	Bartol, 1999

LITERATURE CITED

- Blough, P.M. 1971. The visual acuity of the pigeon for distant targets. *J. Exp. Anal. Behav.* 15: 57-67
- Blough, D. and Blough, P. 1977. Animal Psychophysics. In: W.K. Honig and J.E.R. Staddon (eds.) *Handbook of Operant Behavior*. Englewood Cliffs, NJ: Prentice-Hill Inc. pp. 514-539.
- Blough, D. S. and D. Yager. 1972. Visual psychophysics in animals. In: D. Jameson and L. M. Hurvich (eds.) *Handbook of Sensory Physiology, Visual Psychophysics Vol. VII No. 4*. Berlin: Springer-Verlag. Pp. 733-763.
- Breck, J. E. and M. J. Gitter. 1983. Effect of fish size on the reactive distance of bluegill (*Lepomis macrochirus*) sunfish. *Can. J. Fish. Aquat. Sci.* 40: 162-167.
- Douglas, R.H. and C.W. Hawryshyn. 1990. Behavioral studies of fish vision: an analysis of visual capabilities. In: R.H. Douglas and M.B.A. Djamgoz (eds.) *The Visual System of Fish*. London: Chapman and Hall Ltd. pp. 373-418.
- Ehrenfeld, D.W. and A. Carr. 1967. The role of vision in the sea-finding orientation of the green turtle (*Chelonia mydas*). *Anim Behav.* 15: 25-36.
- Ehrenfeld, D.W. and A. L. Koch. 1967. Visual accommodation in the green turtle. *Science.* 155: 827-828.
- Fehring, W. K. 1972. Hue discrimination in hatchling loggerhead turtles (*Caretta caretta caretta*). *Anim. Behav.* 20: 632-636.
- Fernald, R. D. 1990. The optical systems of fishes. In: R. H. Douglas and M. B. A. Djamgoz (eds.) *The Visual System of Fish*. London: Chapman and Hall. pp. 45-61.
- George, R. H. 1997. Health problems and diseases of sea turtles. In: P. L. Lutz and J. A. Musick (eds.) *The Biology of Sea Turtles*. CRC Press: Boca Raton. Pp. 363-385.
- Granda, A.M. 1979. Eyes and their sensitivity to light of differing wavelengths. In M. Harless and H. Morlock (eds.) *Turtles: Perspectives and Research*. New York: John Wiley and Sons. pp. 247-266.

- Hairston, N. G., T. L. Kao, and S. S. Easter. 1982. Fish vision and the detection of planktonic prey. *Nature*, London. 218: 1240-1242.
- Mellgren, R. L. and M. A. Mann. 1996. Comparative behavior of hatchling sea turtles. In: Keinath, J. A., D. E. Barnard, J. A. Musick, and B. A. Bell (Compilers) Proc. 15th Ann. Symp. Sea Turtle Bio. and Conserv. NOAA Tech. Mem. NMFS-SEFSC-387. Pp. 202-204.
- Mellgren, R. L., M. A. Mann, and J. C. Zurita. 1994. Feeding on novel food in green (*Chelonia mydas*) and hawksbill (*Eretmochelys imbricata*) hatchling sea turtles. In: Schroeder, B. A., B. E. Witherington (Compilers) Proc. 13th Ann. Symp. Sea Turtle Bio. and Conserv. NOAA Tech. Mem. NMFS-SEFSC-341. Pp. 105-106.
- Mrosovsky, N. and S. Shettleworth. 1968. Wavelength preferences and brightness cues in the water-finding behavior of sea turtles. *Behavior*. 32: 211-257.
- Munk, O. 1973. Early notions of dynamic accommodatory devices in teleosts. *Vidensk. Meddr. Dansk Naturh. Foren.* 136:7-28.
- Muntz, W. R. A. and J. Gwyther. 1989. The visual acuity of octopuses for gratings of different orientations. *J. Exp. Bio.* 142: 461-464.
- Muntz, W. R. A. and U. Raj. 1984. On the visual system of *Nautilus pompilius*. *Biol. Bull.* 173: 387-397.
- Nakamura, E. L. 1968a. Visual acuity of two tunas, *Katsuwonus pelamis* and *Euthyunus affinis*. *Copeia* 1: 41-49.
- Nakamura, E. L. 1968b. Visual acuity of yellowfin tuna, *Thunnus albacares*. *FAO Fish Rep.* 62: 463-468.
- Neave, D. A. 1984. The development of visual acuity in larval plaice (*Pleuronectes platessa* L.) and turbot (*Scophthalmus maximus* L.). *J. Exp. Mar. Biol. Ecol.* 78: 167-175.
- Rahmann, H., G. Jeserich, and I. Zeutzius. 1979. Ontogeny of visual acuity of rainbow trout under normal conditions and light deprivation. *Behav.* 68: 315-322.
- Salmon, M. and J. Wyneken. 1990. Do swimming loggerhead sea turtles (*Caretta caretta* L.) use light cues for offshore orientation? *Mar. Behav. Physiol.* 17: 233-246.
- Salmon, M. and J. Wyneken. 1994. Orientation by hatchling sea turtles: mechanisms and implications. *Herp. Nat. His.* 2: 13-24.
- Schusterman, R. J. and R. F. Balliet. 1970. Visual acuity of the harbour seal and the stellar sea lion underwater. *Nature*, London. 226: 563-564.

- Sokal, R. R. and F. J. Rohlf. 1981. *Biometry*, 2nd edition. New York: W. H. Freeman and Co. 859pp.
- Walls, G.L. 1942. *The Vertebrate Eye and its Adaptive Radiation*. Bloomfield Hills: The Cranbrook Institute of Science. 785 pp.
- Witherington, B. E. and K. A. Bjorndal. 1991. Influences of wavelength and intensity on hatchling sea turtle phototaxis: implications for sea-finding behavior. *Copeia*. 4: 1060-1069.

CONCLUSION

The western Atlantic loggerhead undergoes three ontogenetic shifts during its life history: hatchling, juvenile, and adult. These three shifts also correspond with a shift in the visual niche occupied by these animals. This project explored the visual system of one of the three life stages, the juvenile loggerhead sea turtle. More specifically, morphology, electrophysiology and behavioral psychophysics were used to describe the visual acuity thresholds for these turtles in the aquatic environment.

Retinal morphology studies reveal the maximum capability of a visual system. Certain cells and structures must be present for the retina of a typical vertebrate eye to process visual stimulation. Consequently predictions can be made from identifying cell type and size, describing pathways from one cell layer to the next, and mapping regions within the retina of high and low density cell counts. Morphological studies are usually the first step in exploring the visual ability of an animal, yet very little work had been accomplished on sea turtle visual systems.

The retina of the juvenile loggerhead was found to be duplex, containing both cone and rod receptor cells. The overall proportion of these two cell types indicate that this eye is capable of both sensitivity (vision in dim light) as well as acuity (resolution of details of an object). Topographical organization of cones, however, points to a type of area centralis, a region of increased resolving power, in the juvenile loggerhead eye. A higher concentration

of both cones and ganglion cells in the dorsal area of the eye indicates a greater acuity in this region.

Visual evoked potentials also were used in this project; VEPs provide the researcher with a noninvasive method of collected electrophysiological data from an unanesthetized animal. Responses to visual stimuli are collected from an electrode array on the scalp of the animal. By identifying shape and latency of these responses, the researcher can infer underlying visual processes.

For this project, visual evoked potentials were collected to test the visual acuity of the juvenile loggerhead sea turtle. Though all trials were performed out of water, the stimulated eye was always submerged within a reverse goggle. Using pattern-reversal stripe stimuli, bioelectric activity was recorded from the subject. Stripes were reduced in size until the evoked potentials displayed no response. Acuity thresholds were extrapolated from the data and the mean visual acuity derived for the juvenile loggerhead was approximately 5.4 minutes of arc.

The final stage of this project was to examine the behavioral responses to visual stimuli for these turtles. Behavioral studies are unique in that they document the response of the whole animal to stimulation, not just a single system. This behavior study used operant conditioning techniques to train the sea turtle to respond, in a prescribed manner, to a stripe stimulus using positive reinforcement. Once training was achieved, stripes were methodically reduced in size until they could no longer be resolved by the loggerhead. This study recorded a visual acuity threshold for the juvenile loggerhead to be approximately 12.9 minutes of arc.

These three techniques combined describe the juvenile loggerhead sea turtle as having an effective visual acuity, ranging between 5.4 and 12.9 minutes of arc, with the greatest region of resolution in the dorsal region of the eye. From the literature, the acuity of the juvenile loggerhead is found to be very similar to other species in the aquatic environment (see Chapter 3, Table 2). Furthermore, when this visual acuity threshold is compared to other benthic, shallow water species, juvenile loggerhead acuity levels are analogous. For example, the lemon shark (*Negaprion brevirostris*), which is similar to the loggerhead in that it feeds benthically in shallow inshore waters, has a morphological acuity of 4.1 minutes of arc. Moreover, this shark has also been reported to have a linear visual streak. Heuter (1990) hypothesized that this streak of cones and ganglion cells provided this animal with proficient spatial resolution along the horizon and aids in the capture of benthic prey.

These visual attributes can be beneficial in the detection of objects by the juvenile loggerhead in the aquatic medium. The visual environment occupied by the juvenile turtle can be varied throughout its annual migrations. However, the spatial characteristic of their environment remains the same at all locations; the loggerhead is a shallow water, benthic feeder. For example, in the Chesapeake Bay, the juvenile loggerhead takes up residence along the edges of channels, foraging passively with the tide. The juvenile's diet includes bivalves, gastropods, shrimp, and crabs, among others. In the Chesapeake Bay the loggerhead feeds mainly on horseshoe crabs (*Limulus polyphemus*). The acuity levels recorded for juvenile loggerheads are sufficient for foraging activities. Furthermore, the regionalization of acuity in the dorsal hemisphere of the eyes, documented in this paper, acts to increase the resolutions of objects when the animal is looking downward and thus also aids in the capture of prey. Finally the apparent lack of active accommodation in this

species may be inconsequential for their feeding ecology. By actively foraging back and forth along the bottom, the sea turtle has reduced the need for active accommodation within its visual niche.

This study provides a detailed account of the acuity of one ontogenetic stage of sea turtles. However, more basic research needs to be performed to understand how sea turtles are gathering visual information from their environment. Is there a change in morphology among the life history stages? Does the design of the retina change among species due to a divergence of habitats? Do visual traits other than spatial vision play a role in their sensory niche? All of these questions, and more, can be examined using the three techniques outlined in this research project.

VITA

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