COMPARATIVE EYE MORPHOLOGY OF CICONIIFORM BIRDS

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PREFACE

During the course of this investigation I received technical assistance and support from many individuals. I am particularly indebted to the late Dr. Milton R. Curd, Associate Professor of Zoology, student of vertebrate visual systems, ichthyologist, teacher and friend. Dr. Curd undertook the difficult task of converting me from a field biologist to a microscopist in a relatively short period of time. At the onset of this study there must have been many occasions when Dr. Curd was required to exhibit almost superhuman patience as he watched me clean his delicate glassware or adjust his well used and meticulously maintained microscopes.

On other occasions patience was less appropriate and my mentor frequently provided timely, constructive, and sometimes very direct criticism to help aid in what must have seemed to be my interminable apprenticeship.

Coupled with patience and criticism there was always the praise for a job well done. This, Dr. Curd gave sparingly but always with great sincerity.

Dr. Curd died of Leukemia during the last year of this study. He will be sorely missed by myself and many others, and I will always be deeply indebted to him.

I would also like to extend my sincere thanks to the other members of my Graduate Committee. Dr. Bryan P. Glass served as committee chairman and contributed a great amount of encouragement and technical

help. Because Dr. Glass has a very circumspect view of zoology, derived from over 40 years as a student of this discipline, his biological intuition was invaluable. I also thank him for the knowledge of histology I gained as his student and while serving as a graduate teaching assistant in histology.

Dr. Alan Kocan of the Oklahoma State University School of Veterinary Medicine served as a committee member, provided valuable help in formulating the experimental design, and assisted greatly by providing avian eyes which I used to develop the techniques for this study. He also provided financial support for electron micrographs for which I am very grateful. Dr. Rudolph J. Miller, Professor of Zoology, provided expert advice on vertebrate behavior and ecology. The enthusiastic encouragement he always gave me is especially appreciated. Dr. Fritz Knopf served on the committee during the initial phases of this study and provided very helpful advice on ornithology and avian behavior. He also provided a great deal of personal encouragement for which I am very grateful. Dr. Anne Rusoff, Assistant Professor in the Department of Physiological Sciences specializes in vision research, and kindly agreed to serve as a replacement committee member upon Dr. Curd's death. She eagerly accepted this last minute responsibility and provided invaluable technical assistance on vertebrate vision and electron microscopy techniques.

Many other persons provided support for me during this study. Dr. Douglas Mock, Professor of Zoology at the University of Oklahoma, is a nationally known ornithologist and heron expert. He provided valuable biological insight on heron ecology and behavior. My very good friend, Gary W. Sallee, has studied heron ecology in Oklahoma for many years and

provided invaluable assistance in collecting herons. He also provided technical assistance and a great deal of personal encouragement. I am very grateful for his assistance and friendship. I would also like to express my gratitude to Mrs. Helen Murray for her expert assistance in the typing and preparation of the manuscript.

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CHAPTER I

INTRODUCTION

Scope, Justification and Experimental Design

In this study I address questions concerning the morphology of avian eyes and variations in that morphology in closely related birds with different behaviors; particularly, how the arrangement of cells on the retina varies between closely related species. These questions could be asked about species in any vertebrate class, but I chose birds because I have a general interest in ornithology, and also because there is little information available concerning this aspect of avian morphology. Birds are particularly well suited for this study because of the large size of their eyes, and because vision is the most important sensory mode for most avian species.

Many authors consider birds the most visually oriented group of vertebrates (Rochon-Duvigneaud 1943; Prince 1956; and others). This statement is based on an array of anatomical and behavioral evidence. Anatomical support for this statement falls into three general categories: 1) birds have exceptionally large eyes in relation to their body size; 2) they have high densities of photoreceptors in the retina; and 3) the retina is extremely well organized (Walls 1942). Behavorial evidence has been acquired both under laboratory and field conditions (Lehmkuhle and Westendorf 1975). Birds are thought to have excellent visual acuity; however, the term "visual acuity" has an inherent bias,

because we tend to define it in terms appropriate for the human visual system. It is, however, safe to state that birds possess remarkable visual systems. In spite of this fact there is a dearth of information concerning this aspect of avian anatomy. Most available information concerning the anatomy of avian eyes comes from studies conducted by Rochon-Duvigneaud (1933, 1939, 1943, 1950), Wood (1917), and Slonaker (1918) and other anatomists mostly of the period from 1900-1940. General discussions of avian ocular morphology are in Walls 1942; Prince 1956; Polyak 1957; Pumphrey 1961; Rodieck 1973; Sillman 1973; Meyer 1977.

General Information on Avian Eye Morphology

Shape and Position of the Eyes

Early investigators recognized that most predatory birds, including Herons, have eyes in which the axial length to width ratio (Figure 1) closely approaches unity. An axial length to width ratio of 1 means that the eye is globular in shape as opposed to the oval shape found in most birds (Rochon-Duvigneaud 1950). As the axial length of the eye increases, if all else remains the same, the same object at the same distance cast a larger image on the retina (Sillman 1973). This larger image affects more photoreceptors and therefore increases visual acuity. According to Walls (1942) there are three basic shapes of avian eyes which are also correlated with the degree of binocularity (Figure 2). The axial length to width ratio of Heron eyes has not been reported.

The position of the eyes in a Heron's head may reflect the degree of binocularity and its probability of being preyed upon (Lythgoe 1979).

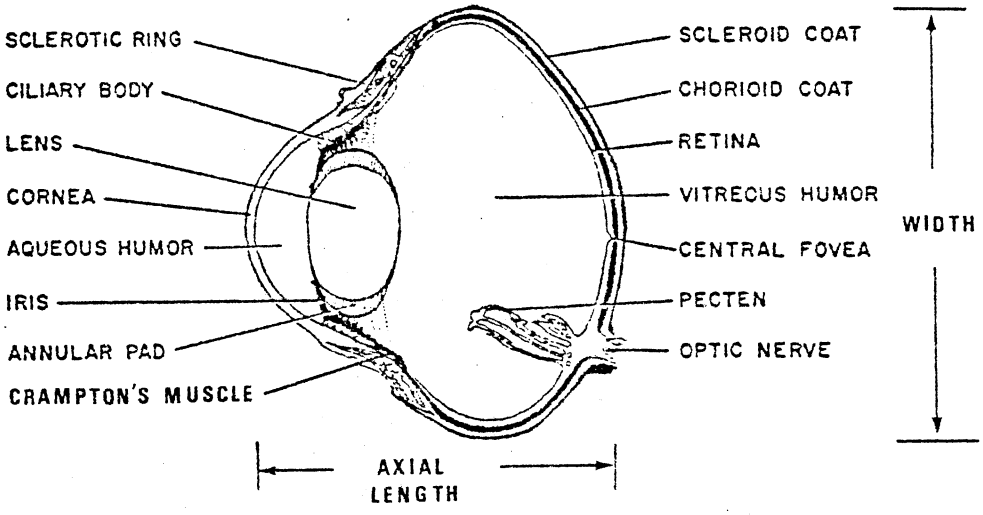

Figure 1. Major morphological features of the avian eye. Modified from Welty 1975.

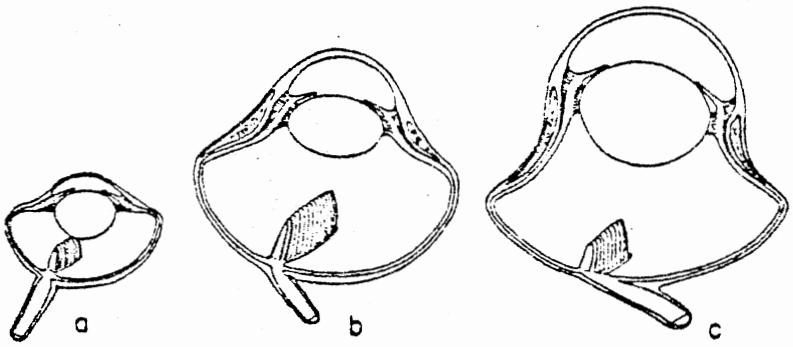

Figure 2. Three characteristic shapes found in avian eyes. a, flattened (most common shape). b, globose (most predatory birds). c, tubular (owls). From Walls 1942.

In some avian species the eyes are directed more toward the front resulting in a higher degree of binocularity and depth perception. This is particularly true of more predaceous species (Welty 1975). The smaller species may have more laterally-positioned eyes to help avoid predation, but this topic has not been adequately investigated.

Accommodation

In birds the cornea plays an important role in accommodation. The contraction of Crampton's muscle (Figure 1) which extends from the cornea to the sclera causes the cornea to increase in curvature, and therefore increases its refractive power. In Herons this system is highly developed and the curvature of the cornea varies greatly.

The Retina

From the outside (against the choroid coat) inward toward the vitreous chamber, the retinae of birds possess the usual sequence of layers (Figure 3) typical of most vertebrates: (1) retinal pigmented epithelium, (2) outer segment layer, (3) inner segment layer, (4) outer limiting membrane, (5) outer nuclear layer, (6) outer plexiform layer, (7) inner nuclear layer, (8) inner plexiform layer, (9) ganglion cell layer, and (10) optic fiber layer.

In birds, it has been noted that the thickness of the retina does not vary much between species even though eye size may be very different (Pearson 1972). For example, the retina of a chickadee (*Parus sp.*) is almost as thick as that of a golden eagle (*Aquila chrysaetos*).

In all birds whose retinae have been examined, the retina is duplex (i.e., contains both rod and cone type visual cells). Rods are usually

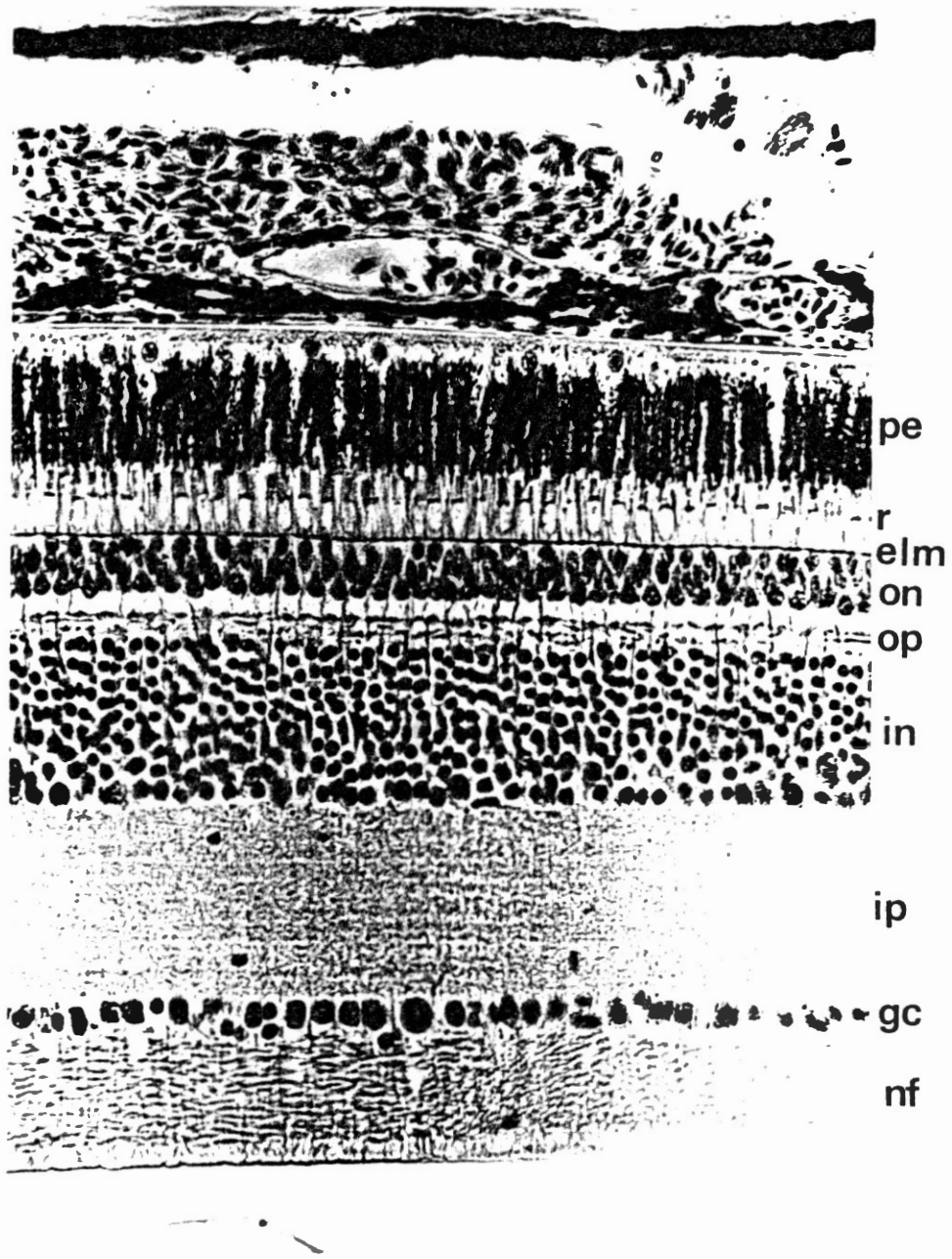

Figure 3. The retina of the Cattle Egret showing layers typical of vertebrate retinae. pe, pigmented epithelium. r, receptor layer (outer and inner segments not clearly visible). elm, external limiting membrane. on, outer nuclear layer. op, outer plexiform layer. in, inner nuclear layer. ip, inner plexiform layer. gc, ganglion cell layer. nf, nerve fiber layer.

not found in the foveal region of the eye. In nocturnal birds cones are far less abundant than rods (Walls 1942; Prince 1956). The distribution of photoreceptors on the retina of birds is understood only in a general way. It has been reported that higher concentrations of photoreceptors occur on the dorsal half of the retina than on the ventral half, at least for some birds such as falconiformes (Rochon-Duvigneaud 1933). Since the image of the world is inverted on the retina, it is easy to understand how birds which scan the ground or water for prey could benefit by having more visual cells in this region. Also, concentrations of photoreceptors progressively decrease from the foveal region of the eye to the ora (Sloanaker 1918; Prince 1956). Recently, the distribution of retinal cells in the pigeon has been extensively studied and it has been shown that the distribution of these cells relates well to the feeding habits of this species (Maturana 1962, 1963; Cohen 1963; Galifret 1968; Binggeli and Paule 1969).

Areas and Foveae

Lythgoe (1979) stated that in many vertebrates certain parts of the retina have high densities of photoreceptors and a correspondingly high number of retinal ganglion cells. These regions are known as area retinae or more simply as areas. A line drawn through the center of the lens and the retina at each area indicates an important direction for vision.

The areae retinae may be of different shapes. Birds which forage over large expanses of water, and mammals which live in open country habitats, have a band-shaped horizontal area sometimes referred to as a visual streak (Wood 1917; Luck 1965; Hughes 1977; Meyer 1977).

Associated with these areas are depressions or foveae formed by the lateral displacement of all cell layers except the photoreceptors. The cone cells attain very high densities in foveal regions. Most of the birds thus far examined (95%) have foveae. Fifty four percent have a single, centrally positioned fovea (Meyer 1977). Many have two foveae, one central and one temporal. Bifoveate avian retinae are characteristic of birds that pursue fast moving prey or feed on the wing (i.e., Hawks, Hummingbirds, Swallows, etc.).

The central fovea of predatory birds is steeper-sided than those of most other vertebrates (Sillman 1973). Figure 4 shows the shape of the central fovea of a golden eagle compared to the central fovea of a human. Contrasting theories exist concerning the function of the steep-sided central foveae found in some birds. Walls (1937, 1942) suggested that the refractive indexes of the retinal tissue and the vitreous humor are such that the index of refraction of the retina is always substantially higher than that of the vitreous humor. Thus, if a light ray should strike the vitreo-retinal boundary at anything but a right angle, it will be refracted at the point of impact resulting in a larger image (Figure 5). He also stated that a convex bulge into the vitreous humor as a result of the development of an area without a fovea would tend to converge the image and make it smaller; an undesirable condition in terms of visual acuity. Walls concluded that the steep-sided shape of some avian foveae could result in a 13% linear increase in the image size in birds.

Pumphrey (1948) took issue with Walls' analysis. He stated that the deep avian fovea would so distort an image as a consequence of aberration that if the fovea did anything at all in terms of visual

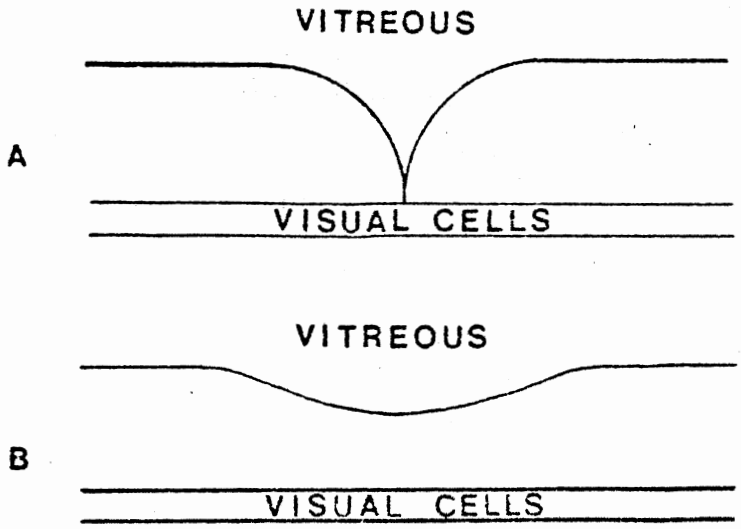

Figure 4. The central fovea of a Golden Eagle compared to the central fovea of a human. Golden Eagle (A), and human (B).

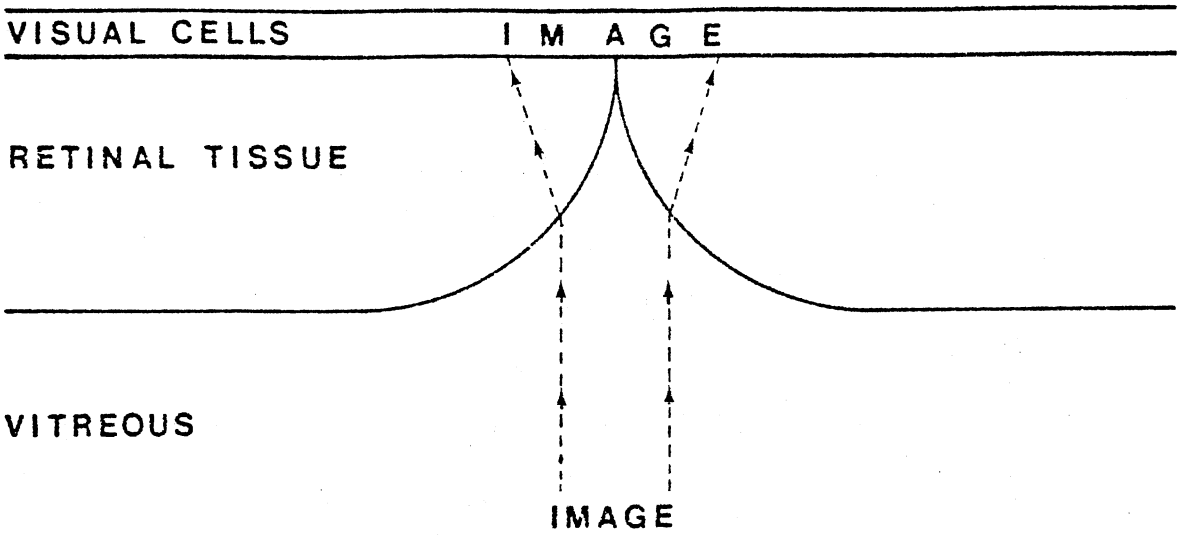

Figure 5. Theoretical diverging effect of a well developed avian fovea.

acuity, it would tend to decrease acuity rather than enhance it. Pumphrey (1948) contended that this type of fovea would be of great help in fixation and detection of movement. Thus, as the image moved across the retina of the bird, it would be maintained in good focus, but as the image passed across the fovea it would become slightly out of focus as a result of the aberration caused by the steep sides of the pits. By always maintaining the image slightly out of focus the bird would be able to "lock" on the object and keep it in sight rather easily.

Owen (1971) contended that a specific arrangement of receptors and post-receptor elements could compensate for distortions in the image so that the animal would see the distorted image clearly and sharply, and that the deep foveae could still give good acuity in spite of the distortion.

The temporal fovea is much shallower than the central fovea and its function is not as controversial. It undoubtedly functions in binocular vision and depth perception. Evidence for this idea is derived from the position of this fovea and the fact that the temporal fovea is only found in birds that pursue fast-moving prey and need good binocular vision (Wood 1917).

The precise functions of the various other retinal areas of acute vision are also poorly understood. Pumphrey (1948) and Rodieck (1973) stated that a visual streak or horizontal band-shaped area might serve to increase sensitivity to objects on the horizon or to aid in orientation. Lythgoe (1979) stated that functions of these areas in birds is not understood. He further stated that until the visual problems of bird flight are better understood the functions of these retinal specializations are likely to remain obscure.

In ciconiiform birds these areas and foveae have been only briefly described. Wood (1917) described the areas and foveae for nine species. The American Bittern (Botaurus lentiginosus) has both a temporal and central fovea while the Black-Crowned Night Heron (Nycticorax nycticorax), the Boat-Billed Heron (Cochlearius cochlearius), and the Great White Heron (Ardea occidentalis) do not have any fovea. Glossy Ibis (Plegadis falcinellus), American Jabaru (Jabaru mycteria), and the Roseate Spoonbill (Ajaja ajaja) were reported to have a single centrally located fovea. An interesting area in the shape of a horizontal band with a slit-like fovea at its center was described for the European Flamingo (Phencopterus rubra).

Oil Droplets

Colored oil droplets are lodged in the cone ellipsoids in many vertebrates; light must pass through these droplets before being absorbed by the visual pigment (Lythgoe 1979). These droplets have been identified in the retinae of chondrosteans, dipnoans, amphibians, reptiles, birds, monotremes, and marsupials (Walls 1942). The presence of colored oil droplets in the avian eye was first discovered by Valentin (1840) and Hannover (1840). In birds oil droplets are usually red or orange, and are usually more common in diurnal birds and absent or relatively rare in nocturnal ones.

The oil droplet mosaic of birds has been interpreted as a mechanism for color hue discrimination (Krause 1894). This idea has been restated by later investigators (Wald and Zussman 1938; Hailman 1964; King-Smith 1969). Additional theories include the possibility that the oil droplets heighten the contrast of colored objects in the field of view

and help pierce haze by holding back glaring short wavelengths (Welty 1975). Martin and Muntz (1978) stated that the presence of a colored oil droplet in a cone narrows the spectral sensitivity curve of the cone, particularly at short wavelengths and will presumably have the effect of sharpening hue discrimination at the expense of sensitivity.

Color Vision

All diurnal birds are thought to have color or chromatic vision (Sturkie 1965). Evidence for color vision is based on information from morphological, physiological and behavioral studies. The high densities of cone cells found in the retinae of many birds are linked with chromatic vision. Because cone densities are known to be high in the retinae of some ciconiiforms, it is probable that their color vision and hue discrimination are well developed. Behavioral and physiological investigations on the subject of color vision in birds has been conducted on domestic fowl, pigeons, and passerine birds (Order Passeriformes) (Porter 1904, 1906; Watson 1915; Lashley 1916; Wood 1925; Hamilton and Coleman 1933; Hess 1956; Alder and Dalland 1959; Ikeda 1965).

Pecten

The pecten, in one form or another, is present in the retinae of all birds and is always located with its base positioned directly over the optic disc. It is highly vascular with many folds (Figure 6).

The first report of the presence of this structure is attributed to Perrault (1676). Since that time there have been at least 30 proposed theories on the function of the pecten (Walls 1942).

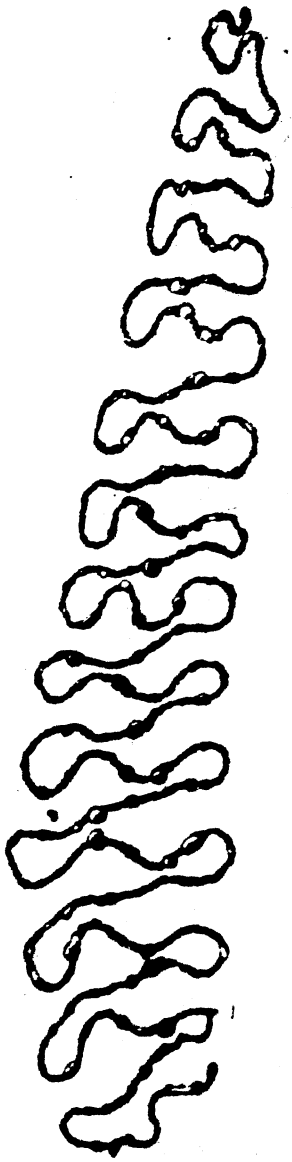

Figure 6. The pecten of the Cattle Egret. Figure shows a horizontal cross section through the pecten.

Recent research conducted by Dunn (1968) and Wingstrand and Munk (1965) has provided overwhelming evidence that the avian pecten functions in the metabolic support of the retina. However, the tremendous variation in its size and complexity indicates that the organ must have a function in addition to that of nutrition.

Non-nutritive functions suggested for the avian pecten include: control of interocular pressure (Beauregard 1876; Rable 1898), control of high ocular temperature (Kajikawa 1923), reduction of glare on the retina (Thomson 1928), navigation during migration (Griffin 1953), and enhancing the detection of moving objects (Menner 1938).

The pectens of ciconiiform birds are variable in size and shape. Nocturnal species have much smaller pectens than diurnal ones.

Visual Acuity

Behavioral studies of certain birds have shown that they have high visual acuity compared to humans (Lehmkuhle and Westendorf 1975).

Current ornithological textbooks (Wallace 1962; VanTyne and Berger 1971; Welty 1975) report that the visual acuity of hawks and eagles is at least 8 to 10 times greater than that of man. No reports on the visual acuity of ciconiiforms have been noted.

Shlaer (1971) determined optical quality of the retinal image in the eye of an African Serpent Eagle (Dryotriochis spectabilis) using a modern ophthalmoscopic method. The performance of the eye was substantially better than that reported for humans. He determined foveal resolving power to be 2.4 times as great as that of the human. This is perhaps the most objective estimate of visual acuity in a bird.

Studies on the physical properties of eyes of ciconiiform birds

have been very superficial. The available information on the visual abilities of herons and their allies has been obtained from direct studies of the eyes rather than by behavioral studies. The main justification for stating that the larger herons have very acute vision is based upon the large size of the eyes and the relatively large image cast upon the retina, the high density of cones, the presence of a temporally located fovea in each eye in addition to the central fovea (at least in some species), and the high ratio of optic nerve fibers to visual cells. Although the acuity of active feeders is generally considered to be quite good, there is no quantified information available on the visual acuity of ciconiiform birds.

To make inferences on the visual acuity of birds without first having a good understanding of the morphology of their eyes seems to be a backward approach to the problem. Authorities such as VanTyne and Berger (1971) and Welty (1975) have stated that cone type photoreceptors and their distribution on the retina are of paramount importance in visual acuity. It is evident from a review of literature on the subject that more comprehensive studies on the comparative ocular morphology of birds are needed.

Some important questions remain to be answered: (1) are there subtle differences in concentration of photoreceptors from point to point on the retina? (2) to what degree does the organization of the retina vary with the ecological lifestyle of the bird? To answer these questions I (1) determined which morphological traits might be correlated with behavior based on available literature (Table 1); (2) devised a method to study those traits; and (3) applied those methods to a study of a group of birds which are closely related but have

Table 1. Morphological features and behavioral correlates of vertebrate eyes.

Morphological feature	Ecological or behavorial correlates	References
Shape of eye (axial length x width)		
Tubular	High binocularity, predatory	Walls 1942; Prince 1956; Martin 1977
Oval	Wide visual fields - infrequently subject to predation	
Position of eyes in head		
Frontally located	High binocularity, predatory	Walls 1942; Prince 1956; Lythgoe 1979
Laterally located	Wide visual fields - infrequently subject to predation	
Pecten		
Large	Diurnal activity	Wood 1917; Walls 1942; Rochon-Duvigneaud 1943; Prince 1956
Small	Nocturnal activity	
Foveae		
Multiple, well developed	Fast active feeding, good at detecting fast small objects, diurnality	Wood 1917; Walls 1942; Rochon-Duvigneaud 1943; Prince 1956; Meyer 1977
Single, shallow or absent	Passive feeding less well adapted to feed on small fast objects, nocturnality or diurnality, general feeding behavior	

Table 1. Continued.

Morphological feature	Ecological or behavorial correlates	References
Receptor concentrations on retinae		
High on dorsal half	Vision oriented toward ground	Walls 1942; Prince 1956; Polyak 1957
High on ventral half	Vision oriented above horizon	
High on temporal half	Vision oriented forward	
High on nasal half	Vision oriented posteriorly	
High per unit area	High acuity	
Low per unit area	Relatively lower acuity	
Relative ocular size		
Large	More nocturnal	Walls 1942; Prince 1956
Small	More diurnal	

diverse lifestyles.

Herons were chosen as subjects for this research because of certain ecological, anatomical and practical considerations: (1) these birds have large eyes which are relatively easy to dissect and handle; (2) they exhibit a wide range of ecological lifestyles; (3) they can be easily collected; and (4) there is a great deal of existing information on ecology, behavior and feeding activities of herons.

The amount of time necessary to collect materials and prepare slides for light microscopy necessitated limitation of the number of species examined. The four species chosen for analysis were the Cattle Egret (Bubulcus ibis), Snowy Egret (Leucophoyx thula), Great Egret (Casmerodius albus), and Great Blue Heron (Ardea herodias). These were selected because they represent certain important behaviors and ecological lifestyles such as nocturnality, diurnality, passive feeding, and active feeding (Table 2).

The selection of the Great Blue Heron as a nocturnal species requires some explanation. Both Black and Yellow-Crowned Night Herons (Nycticorax nycticorax and Nyctonassa violacea, respectively) are truly nocturnal species and occur in Oklahoma (Sutton 1967). However, they are rare and difficult to collect. Great Blue Herons can exhibit high degrees of nocturnality at particular times of the year (Douglas Mock, pers. comm.). Therefore, Great Blue Herons were used to represent a relatively nocturnal heron. Although night herons would probably have been better for this comparison, their rarity in Oklahoma limited their potential usefulness for this study.

In addition to the anatomical features listed in Table 1, other anatomical features that are less clearly related to behavior and

Table 2. Ecological and behavioral characteristics of four species of herons used in this study.

Species	Relative body size rank*	Circadian activity pattern	Type of feeding behavior	Aquatic vs. terrestrial habits	Food	References
Cattle Egret	1	Diurnal	Active	Semiaquatic to terrestrial	Insects particularly grasshoppers; also small vertebrates and arthropods	Kushlan 1976; Palmer 1976
Snowy Egret	2	Diurnal	Active	Aquatic	Aquatic invertebrates, small fish small aquatic vertebrates	Kushlan 1976; Palmer 1976
Great Egret	3	Diurnal	Passive	Aquatic	Aquatic invertebrates, small to medium sized fish and aquatic vertebrates	Cushlan 1976; Palmer 1976

Table 2. Continued.

Species	Relative body size rank*	Circadian activity pattern	Type of feeding behavior	Aquatic vs. terrestrial habits	Food	References
Great Blue Heron	4	Biurnal	Passive	Mostly aquatic	Aquatic and terrestrial invertebrates; small to large sized fish; large aquatic reptiles and amphibians such as frogs, snakes, turtles, etc.	Kushlan 1976; Palmer 1976

*The numeral 1 indicates the smallest species, 4 indicates the largest species. Cattle Egrets and Snowy Egrets overlap in size; therefore, this ranking is arbitrary for these two species.

ecology were also examined. These included relative retinal areas, relative thickness of retinal layers, and variation in photoreceptor size and shape.

Objectives and Hypothesis

The specific objectives of this research were as follows:

A. To determine the distribution of cones, rods, and other retinal cells in key locations in the retinae of four species of Ciconiiformes.

B. To determine the degree to which the distribution of visual cells in the retinae and general ocular morphology of Ciconiiformes is related to the varying life style exhibited by these birds.

C. To describe the general morphological features of the eyes of ciconiiform birds such as relative size, shape, position in head, degree of binocularity and other important anatomical aspects.

The information needed to accomplish objectives A and B requires detailed systematic sampling of retinal cells. This information is much more difficult to obtain than that required to accomplish objective C, because the retinal features are distinguishable only with a microscope. For these reasons the main effort of this study is devoted to describing the spatial distribution of retinal cells (photoreceptors, bipolar cells, and ganglion cells) across the retinae. At the outset the following hypothesis was formulated:

The eyes of closely related birds that have diverse ecological lifestyles and behaviors will be morphologically different,

particularly with respect to the spatial distribution of cells within the retina.

CHAPTER II

METHODS AND MATERIALS

Field Methods

Collecting

Heron specimens were collected in Oklahoma under Federal Scientific Collecting Permit Number PRT 2-2377AQ and Oklahoma Scientific Collecting Permit Number 1759. Herons were collected at feeding areas along rivers and ponds using a .22 caliber rifle. Eyes were removed in the field immediately after collection. Eyes were removed by skinning the head, cutting the eyelids loose, then freeing the borders of the eyes in the orbits with a scalpel. Next, curved scissors were used to cut the ocular muscles and the optic nerve. Eyes were then washed in normal saline to remove blood before being placed in small jars containing a volume of Bouin's Fixative at least ten times greater than the volume of the eyes. If the herons were collected to determine the angle of eye position, the heads were removed and skinned immediately after they were collected and placed in bottles containing Perenyi fixative.

Carcasses of herons were placed in plastic trash bags and were frozen after returning to the laboratory. They were eventually donated to the Oklahoma State University Museum.

Laboratory Methods

Fixation

Fixation in light microscopy is defined as a process which helps prevent postmortum changes in the tissue, coagulates cell contents into insoluble substances, protects tissue against shrinkage and distortion during subsequent treatments, and allows cell parts to be made selectively clear and visible. The particular sampling scheme designed for this study required whole avian eyes fixed in a relatively natural position. Except for very superficial information, there are virtually no published techniques dealing with fixing whole avian eyes. For this reason the results of techniques developed during this study will be dealt with in detail.

The need for good fixation of the retina is very important when dealing with the distribution of photoreceptors. Two important sources of bias which result from using incorrect fixing agents are swelling and wrinkling of the retina. Swelling and wrinkling can cause lateral displacement of retinal cells, and thus cause severe sampling errors. The ideal situation would be to fix the retinal cells in their natural position in relation to the rest of the eye, but unfortunately some degree of shrinking and swelling occurs with all the available fixatives. Severe wrinkling and other artifacts associated with fixation are illustrated in Figure 7. Wrinkling associated with fixation of large whole eyes is a serious problem which has been reported by several investigators (Rochon-Duvigneaud 1943). Unfortunately no methods which can consistently overcome this problem have been presented in any detail in the literature. However, some

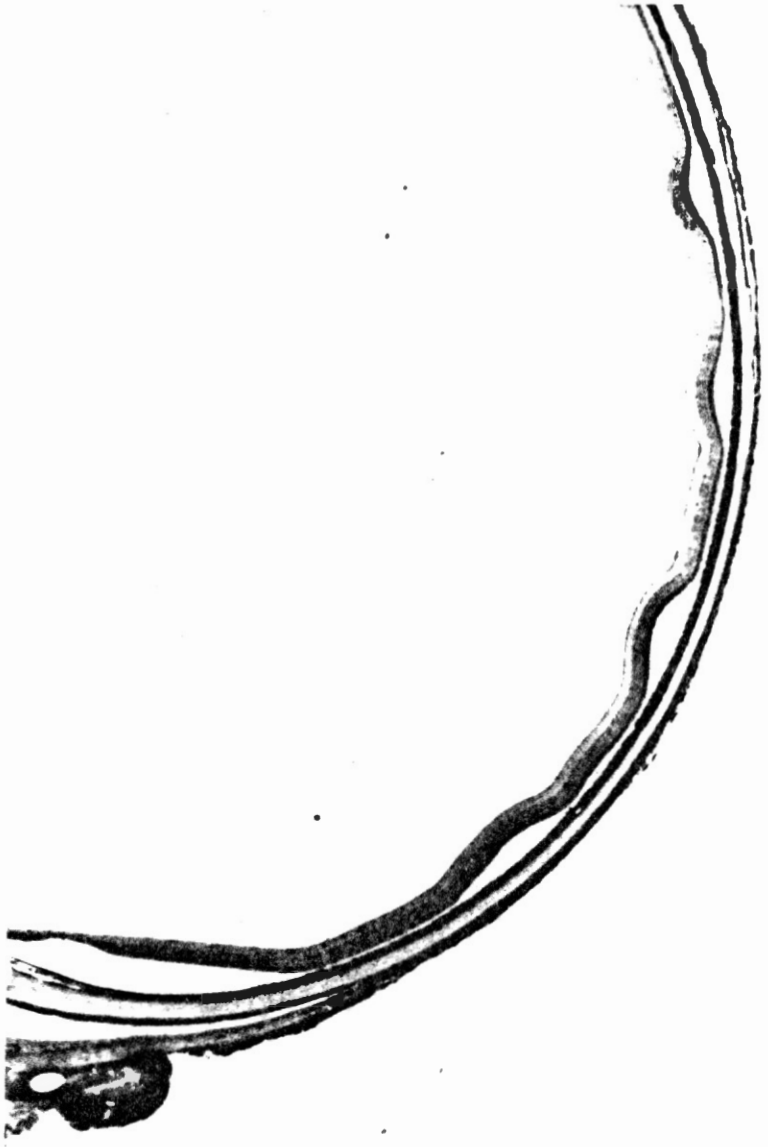

Figure 7. Wrinkling of the retina of a Cattle Egret due to swelling.

authors have reported various methods which tend to minimize this problem. Polyak (1957) reported that the slow injection of fixative into the eye can minimize wrinkling. Humason (1979) reported that holes cut into the ciliary body can allow fixatives to reach the retina faster and preserve it in a more natural state. The causes of wrinkling and swelling are obscure. However, one possible cause may be a differential diffusion of various constituents in the fixative. Most chemical reagents either cause shrinking or swelling of living tissue due to osmotic differentials between the reagent and the tissue. Because of this, most fixatives have a balance between one reagent which swells the tissue and one which shrinks it in order to minimize distortion. If the cartilagenous sclera imposes an osmotic barrier which results in a differential diffusion of the reagents in the fixative then wrinkling or swelling will occur. Since the type of fixative used and the method of getting the fixative into the eye both seem to affect the extent of wrinkling, a series of preliminary experiments were conducted to determine what method and fixative would be most useful. Several fixatives which were reported as suitable for eyes were initially chosen. These included Kohlmer's, Bouin's, Zenker's, 10% formalin, Perenyi and alcoholic Bouin's (Humason 1979). The methods of infiltrating the eyes with fixative included: (1) removing the eyes and immersing them in the fixative; (2) removing the eyes, cutting a window approximately 2 x 5 mm in the sclerotic ring, and immersing the eye in the fixative; and (3) slowly injecting the eye with fixative while allowing the fixative to exit through a small hole cut in the sclerotic ring. These three methods and several fixatives were used to fix approximately 87 avian eyes of various species. Several interesting

observations resulted from these experiments:

1. Eyes fixed in situ were consistently severely wrinkled with all fixatives except Perenyi fixative.
2. Opening the eye to allow penetration of the fixative did not decrease swelling and wrinkling.
3. Unopened eyes removed from the head yielded the most consistent results with all fixatives.
4. Perenyi fixative controlled swelling and wrinkling better than all other fixatives and sometimes fixed the retina with no obvious distortion; however, it also completely destroyed the cytological detail of the retina.
5. Bouin's fixative yielded the best cytological detail and a minimum of wrinkling and swelling when eyes were removed from the head.

Therefore, only eyes fixed in Bouin's fixative were used in sampling receptor densities. Since no fixative yielded perfect results, a minimum amount of wrinkling was considered acceptable. The development of a suitable technique for fixation of large whole eyes would be extremely valuable for studies of this type. The perfect preservation of the retina without wrinkling remains a matter of luck. The best approach in overcoming this problem is to fix a large number of eyes and use only those eyes which are fixed in the most perfect condition.

Embedding, Staining and Sectioning

Two common embedding media used in microtechnique are paraffin and celloidin, or nitrocellulose. Celloidin was chosen because it offers

several advantages over paraffin: (1) it causes less compression, (2) thin sections are easier to cut, and (3) the retina is not exposed to heat. The general schedule used for infiltration and embedding with celloidin is shown in Table 3. The retinal tissue was mounted on a wooden block approximately 1.5 cm square. Identification labels were attached to the blocks with thumb tacks.

Sections were cut on an American Optical model 820 microtome. Sections used in sampling receptor density were 6 micrometers thick. For most species the photoreceptor layer in these sections was 1-2 cell layers thick. Sections 3 micrometers thick were used to study the anatomy of the photoreceptors. When cutting celloidin sections this thin, usually only fragments of the retina could be obtained. These sections were very useful for making drawings and clear photomicrographs. In order to minimize lateral compression the length of the section was always oriented parallel to the microtome knife. Also, every effort was made to cut through the retina at the most perpendicular angle possible, thus minimizing error in sampling. If sections were examined with the light microscope and the angle of sectioning was found to be too oblique, blocks were resectioned to get a more perpendicular angle. The microscopic appearance of the external limiting membrane was used to determine how perpendicular the sections were (Figure 8).

Celloidin sections were stained in eosin y and Heidenhein's hematoxylin, according to the schedule shown in Table 4. Individual sections were moved through this procedure on small tapered glass rods. Some sections were bleached in a solution of 5% hydrogen peroxide and a small quantity of ammonium hydroxide. The addition of the ammonium

Table 3. Infiltration schedule for celloidin.

Step number	Treatment	Time
1	70% Ethanol	2 hours
2	95% Ethanol	2 hours
3	Absolute ethanol	1 hour
4	Absolute ethanol	1 hour
5	Diethyl-ether/absolute ethanol 1:1	4-24 hours
6	4% celloidin	4 days
7	12% celloidin	7 days

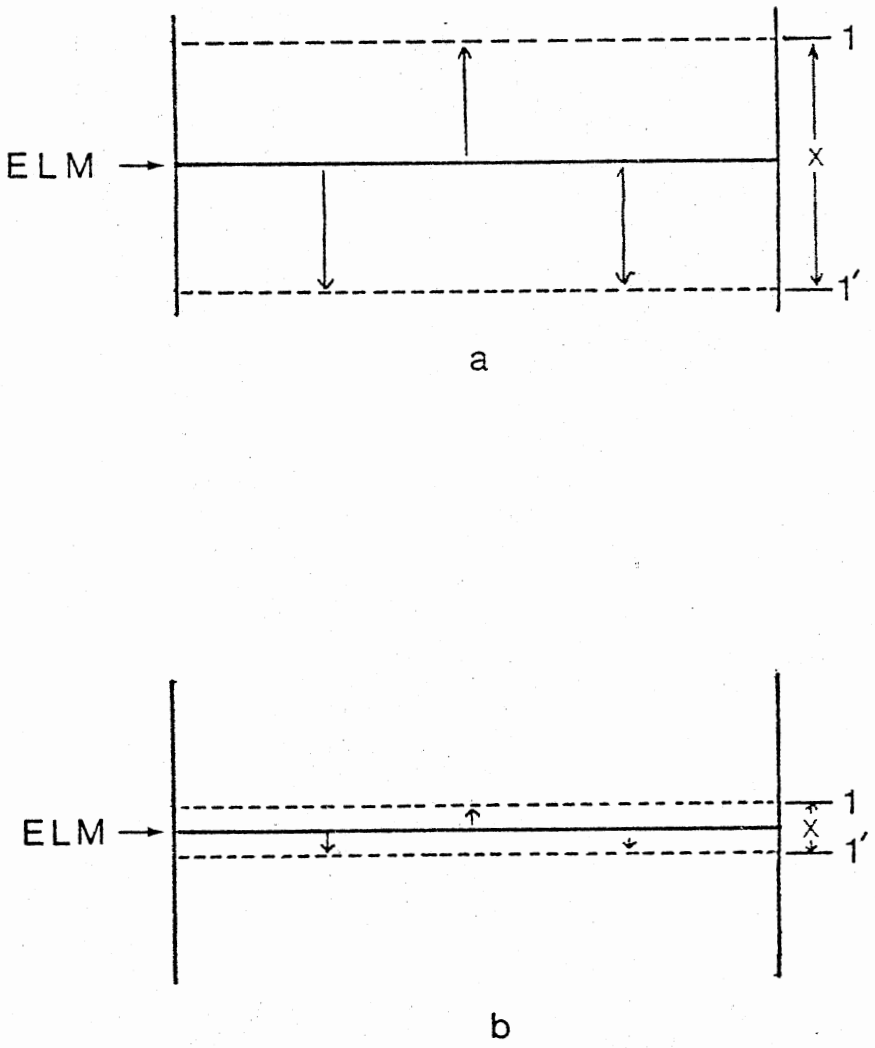

Figure 8. A figure illustrating how to determine if the retina has been sectioned at a perpendicular angle. The distance the external limiting membrane (ELM) appears to move during cross-focusing can be used to determine how perpendicular the section has been cut. In a and b the ELM moves up to 1 and down to 1' when cross-focusing. The distance x in a is greater than in b therefore b has been cut more perpendicular.

Table 4. Staining schedule used for heron retinae.

Step number	Treatment	Time
1	95% Ethanol	3 min.
2	70% Ethanol	3 min.
3	50% Ethanol	3 min.
4	30% Ethanol	3 min.
5	De-ionized water	3 min.
6	3% Iron Alum	45 min.
7	De-ionized water	1 min.
8	Heidenhein's hematoxylin	45 min.
9	De-ionized water	3 min.
10	Sat. aqueous picric acid	2-3 min.
11	Tap water	5-10 min.
12	70% Ethanol	3 min.
13	Eosin y	5 min.
14	95% Ethanol	5 min.
15	100% Isopropyl alcohol	5 min.
16	Terpeniol	5 min.

hydroxide enhanced the bleaching properties of the hydrogen peroxide. Bleaching removed the pigments from the pigmented epithelium layer so that the outer segments of the rods and cones could be observed and studied. It also enhanced the staining properties of the outer segments causing increased uptake of eosin y.

Sampling Receptor Density

The availability of herons is very restricted by state and federal regulations. Therefore, I had to devise a method which would yield the maximum amount of material from a limited number of birds, and also provide a large enough sample size to permit reliable statistical analysis. The following scheme was chosen: from each specimen the right eye was used to make cross sections for sampling receptor densities. The left eye was used to make whole eye cross sections, thin sections and sections of other features such as pectens. After fixation was completed, the retina was cut away from the anterior portion of the eye along its border near the ora. This boundary is very evident. The retina then resembled a shallow bowl and was ready for slicing and embedding.

In order to compare receptor densities between eyes which varied in size, it was necessary to devise a method of sampling that assured the measurements were taken at corresponding points in eyes of different species. Figure 9 shows how the retina was prepared for sectioning and sampling. This sampling method enabled detection of the various possible arrangements of foveae and areas and made the pattern of sampling comparable for different sizes of eyes. If central foveae were present they were removed and their characteristics studied separately

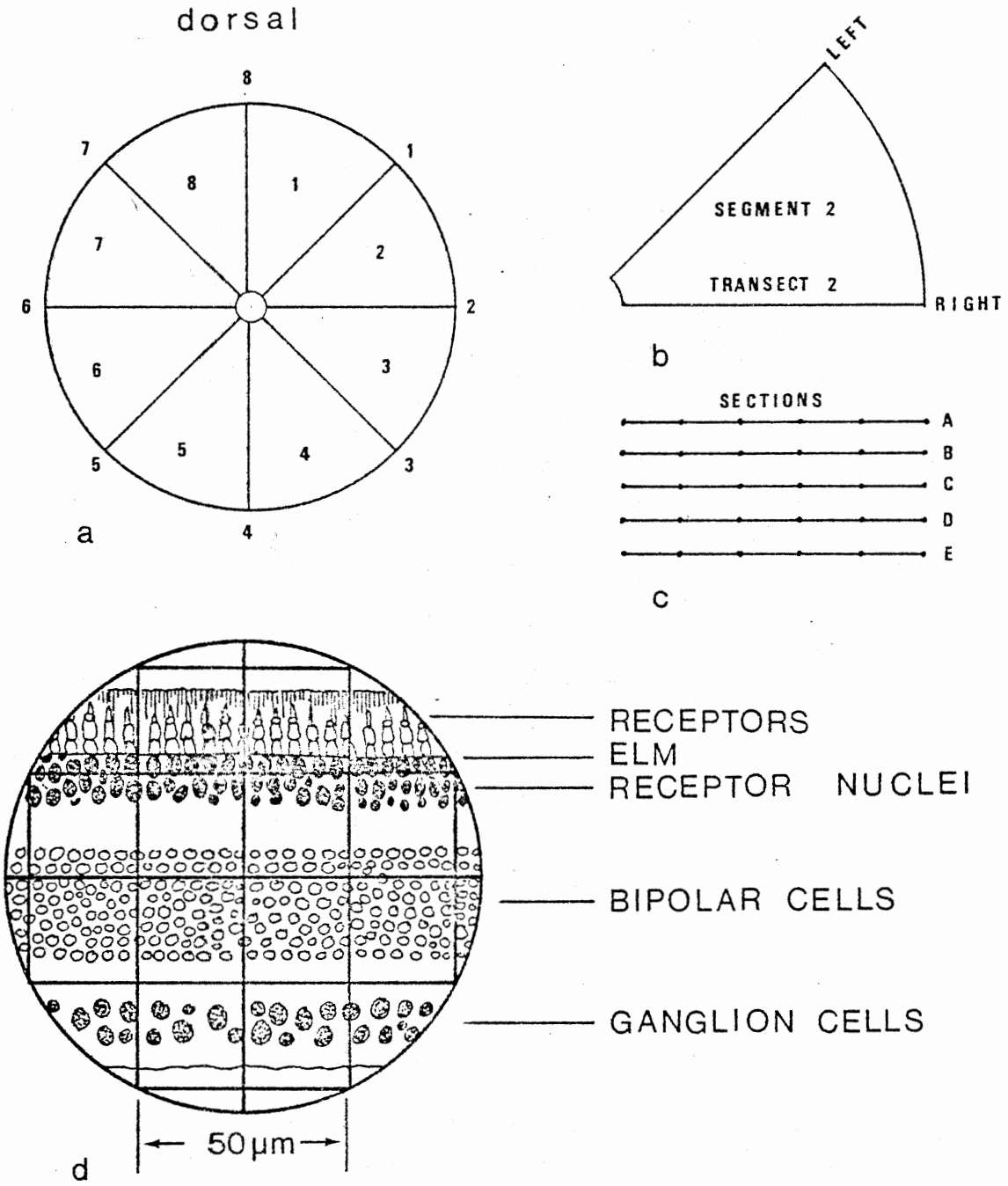

Figure 9. Preparation of the retina for sampling retinal cell densities. a, After fixation the fovea is removed using a 3 mm skin punch. The cup-shaped fundus of the eye is then cut into 8 pie-shaped segments labeled 1-8. b, Each segment is then embedded in celloidin and oriented so that sections can be cut from its right edge (edges = transects labeled 1-8). c, Five sections 6 µ thick are then cut from the segment, stained, and mounted on microscope slides. Six equidistant sample points are then marked on the coverglass with small dots of ink. d, Various retinal cells are counted at 100X using a whipple disc placed in the ocular lens of the microscope. All retinal cells within a 50 µ wide swath are counted.

to avoid damage. The eye was then cut into pie-shaped segments labeled 1 through 8 (Figure 9a). These segments were embedded in celloidin and prepared for light microscopy. Segments were then oriented in celloidin so that the right edge (Figure 9b) formed a block which could be sectioned on a microtome. The sections cut from these blocks form transects from the foveal region to the ora. Rods, cones, bipolar and ganglion cells were counted along these transects at six equidistant sample points labeled 1-6 (Figure 9c). Amacrine, horizontal and Müller cells were not sampled. Bipolar cells were differentiated from these cells by their size, position in the inner nuclear layer and staining properties. Five sections from each transect were sampled. Each sample point on the retina was identified using a species code and three numbers. For example, Great Egret 1, transect 1, sample point 1 identifies one of four Great Egret eyes, one of eight transects within this eye and the first sample point along this transect.

With this sampling scheme the estimated mean value of a particular retinal element was calculated from an n-value of 20 (five repetitions at each sample point, corresponding sample points from four eyes thus 4 x 5 = 20). Retinal cells were sampled along a vertical axis at each of these sample points (Figure 9d). Counts were made using a whipple disc. All elements within a two increment wide band of the whipple were counted. At 100 x magnification these bands were approximately 25 μm wide. Thicknesses of retinal layers and dimensions of the elements were measured using a Philar micrometer. All counts were made on a Zeiss 600 research microscope.

Measuring Retinal Area

The area of each retina was determined by measuring the altitude and base of each pie shaped-section of the eye before embedding, then calculating the area of the triangle and adding these areas together (Figure 10). This method only measured relative areas because these triangular sections were not flat triangles from one plane but were actually somewhat concave. Also, the bases of the triangles were curved but they were measured as if they were straight. Thus, this method tended to underestimate the actual area but was still useful for comparison when only relative areas were needed.

Measuring the Relative Sizes of Pectens

Relative size of pectens was not considered to be an important aspect of this study and therefore an elaborate method of determination was not devised. However, a crude measure of relative size was desirable. Longitudinal sections of the pecten were stained in hematoxylin and eosin and mounted on a microscope slide. A grid composed of 1 mm squares was superimposed over the section and the number of squares covering the pecten were counted.

Measuring Relative Sizes and Shapes of Eyes

The relative sizes of the heron eyes were compared using eye volume to head volume ratios. Conjunctiva and muscles were carefully trimmed off freshly enucleated eyes. The volume was measured by immersing the eye in water in a graduated cylinder and measuring water displacement. Head volumes were also measured by displacement before the eyes were removed. The head was always severed at the atlas. A surfactant was

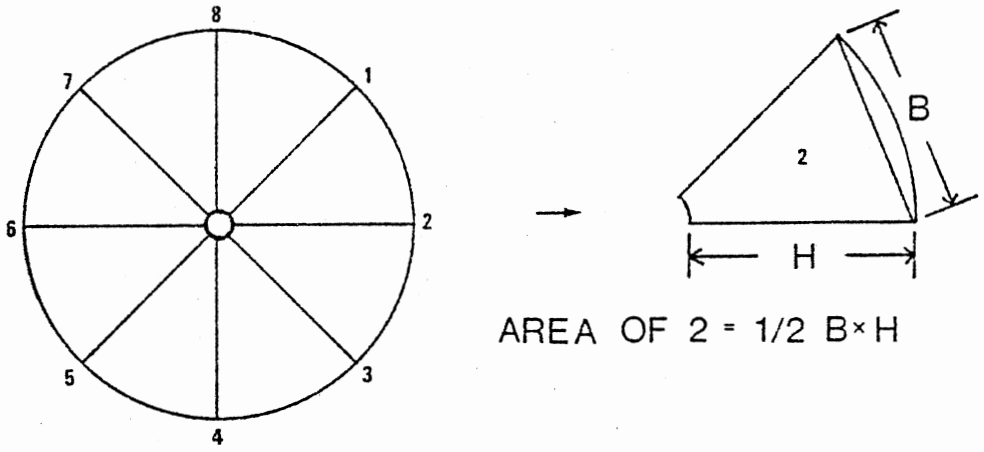

Figure 10. A figure illustrating how relative retinal areas were determined. The approximate retinal areas were determined by calculating the area of each segment using the formula for the area of an isosceles triangle. The total of all 8 segments yields an approximate retinal area.

used to help reduce error due to trapped air. Eye and head weights were also measured and used as an index to relative eye proportions.

The ratio of axial length to width is an index to eye shape (Figure 1). Axial length and width were measured on freshly enucleated eyes after trimming away excess tissue. Calipers were used to make these measurements. Axial length was also measured from slides of whole eyes. The width was always measured along the nasal temporal axis.

Determining the Position of the Eyes in the Head

The degree to which the eyes face forward with respect to their position in the head was determined by calculating the angle between the sagittal axis of the head and the visual axis of the eyes (Figure 11). The resulting angle is termed the angle of eye position and was used as an index of binocularity. To determine this angle a horizontal section forming a plane through the foveae of both eyes and the tip of the beak was prepared using whole heron heads fixed in Perenyi fixative. The bones of the skull and beak were completely decalcified by the nitric and chromic acid of the Perenyi fixative, making it possible to section them using a microtome knife attached to a handle. On good sections the visual axis and the sagittal axis were easily determined.

The visual axis was defined as a straight line starting from the fovea, passing through the pupil and crossing the cornea at its symmetrical apex. This method has been used by other investigators Wood 1917; Rochon-Duvigneaud 1943). The sagittal axis was defined as a line starting at the tip of the beak which passed posteriorly through the center of the foramen magnum. Thus, the sagittal axis perfectly bisected the head (Figure 11). The visual angle was defined as the

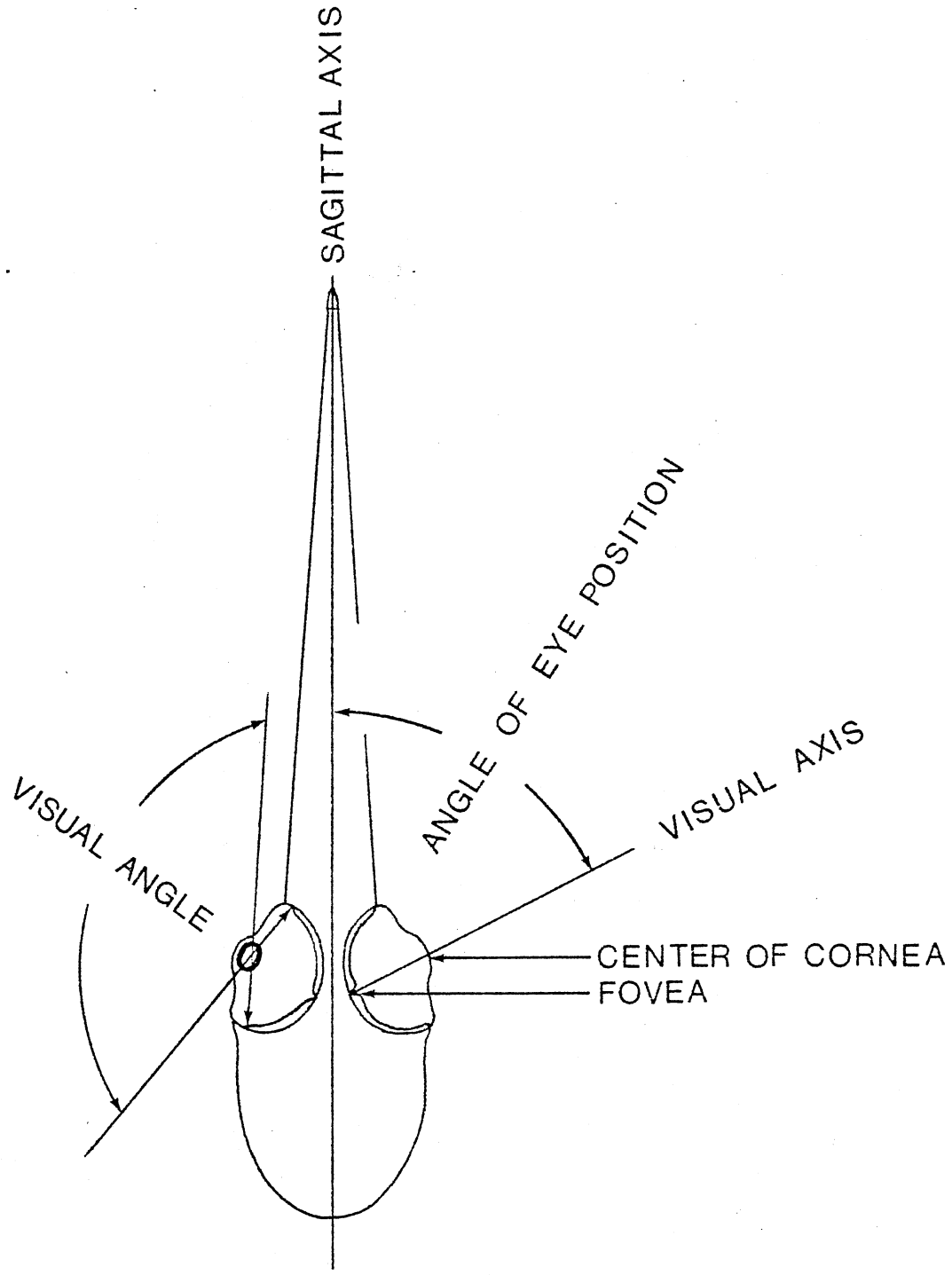

Figure 11. Horizontal section through a heron's head showing how the angle of eye position was measured.

angle formed by two lines in the same plane passing through the center of the lens, one going to the ora at the temporal edge of the retina and the other going to the ora at the nasal edge of the retina (Figure 11).

Some error in this method results from the fact that the eyes of herons are partially movable within their orbits. However, the degree of mobility is thought to be slight as in most other birds (Welty 1975).

Determining the Dorsal Aspect of the Eye

Determining the dorsal aspect of the eye was extremely important and had to be done consistently to make the sample points between eyes of the various species correspond. Also, this dorsal aspect should accurately reflect the true dorsal aspect of the eye. Determining the true dorsal aspect is always arbitary without knowing what the bird actually sees. However, some logical assumptions can be employed to help determine true dorsal. For example, most herons capture prey by impaling or biting the prey item with their beaks. This is analogous to an archer or a rifleman who must have his eyes directly behind the arrow or rifle barrel to shoot accurately. Thus herons must have their eyes directly behind the beak to accurately impale prey. The relationship between the tip of the beak and the line of sight was evident in the herons that were examined during this study. In all four species examined the eyes were perfectly bisected when a plane is drawn through the tip of the beak and the fovea (Figure 12a). This is probably not accidental in view of the hunting habits of herons. Dorsal was considered to be exactly 90° dorsal to this line. Thus in all eyes dorsal exactly corresponded to transect 8. This method of determining

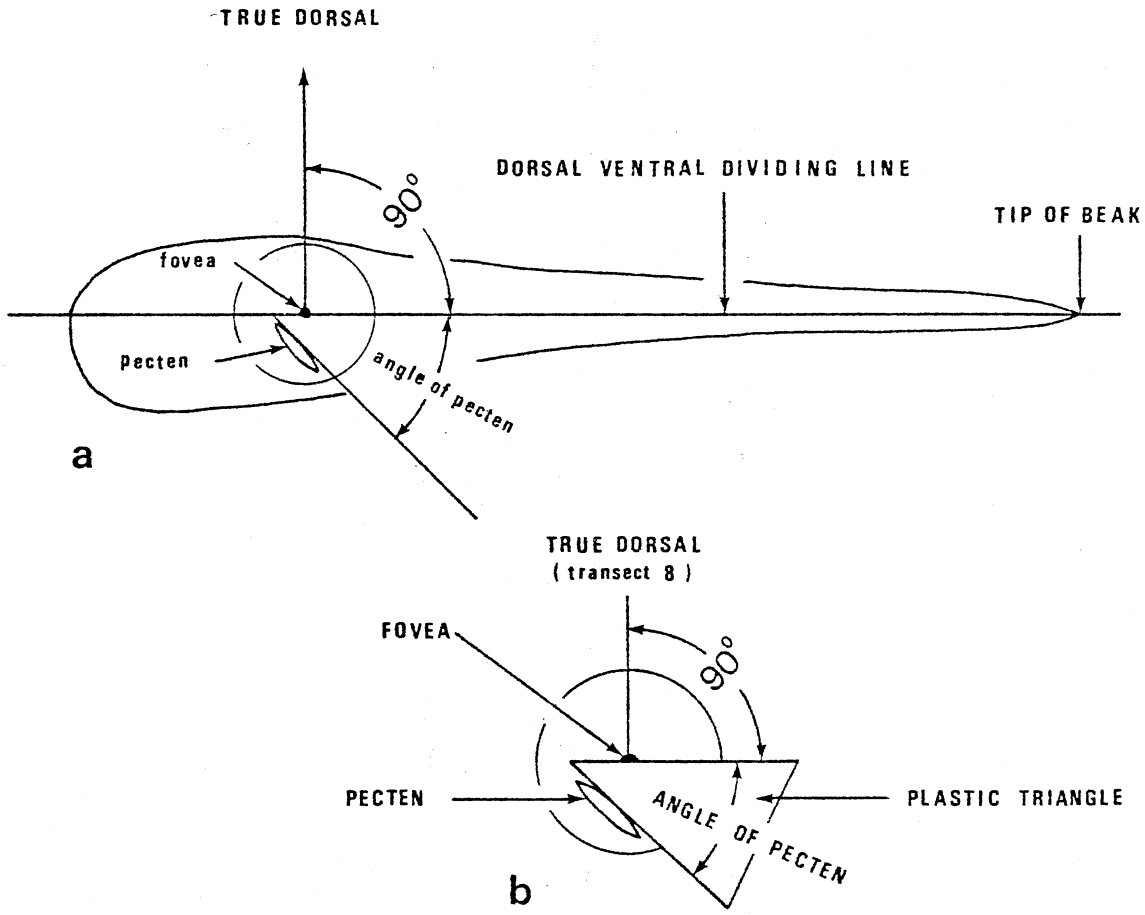

Figure 12. Diagram illustrating how the dorsal aspect of the retina was determined. True dorsal was determined by measuring an angle exactly 90^{o} dorsal to a line passing from the fovea to the tip of the beak (a). The angle formed by the pecten and the dorsal ventral line was used to mark true dorsal on enucleated eyes (b), by using a plastic triangle cut at the appropriate angle.

dorsal can only be done while the eyes are in the head. Because the eyes used to sample the retina were removed in the field, a method to mark dorsal in enucleated eyes had to be developed. This was accomplished by measuring the angle formed between the dorso-ventral dividing line and the angle of the pecten. A thin piece of plastic cut with scissors to this exact angle was used to mark dorsal in ennucleated eyes as shown in Figure 12b.

Statistical Analysis

The data obtained from sampling retinal elements were analyzed using the statistical analysis system (Bar et al. 1979). Analyses of variance were used to test for differences between eyes within species and between species. Student's t-test was used to test for differences within eyes.

To visualize the spatial distribution of various retinal cells I employed a computer mapping program called Symap. This program interpolates and prints symbols for values between several sample points. The results are lines of density (usually referred to as iso-density lines) similar to contour lines on a topographic map. This program not only provided a way to visualize the data but also provided estimates of values between the 48 sample points.

CHAPTER III

RESULTS

General Appearance of the Retinae

General microscopic examination of the retinae of these four species of herons shows no major differences in organization. The average thickness of each layer at six different points between the fundus and ora is shown in Figure 13. The data used to construct this figure represent mean values of thickness for all eight transects combined. Thus, these data do not give any indication of differences in thickness of the retinae along different transects. In all four species all layers tend to decrease in thickness from fundus to ora. The large species of herons (Great Blue Heron and Great Egret) have the thickest retinae although these differences are not significant. The receptor, inner nuclear layer and inner plexiform layers are the thickest layers of the retina in all four species. There is not much variation between species in the thickness of the various layers except in the ganglion cell layer which shows great variation, particularly for the Great Egret (Figure 13).

Relative Sizes of Retinal Areas

The total retinal area is correlated with the size of the bird. The average retinal area for the four species collected is shown in Table 5. The two small species (Snowy and Cattle Egret) have much

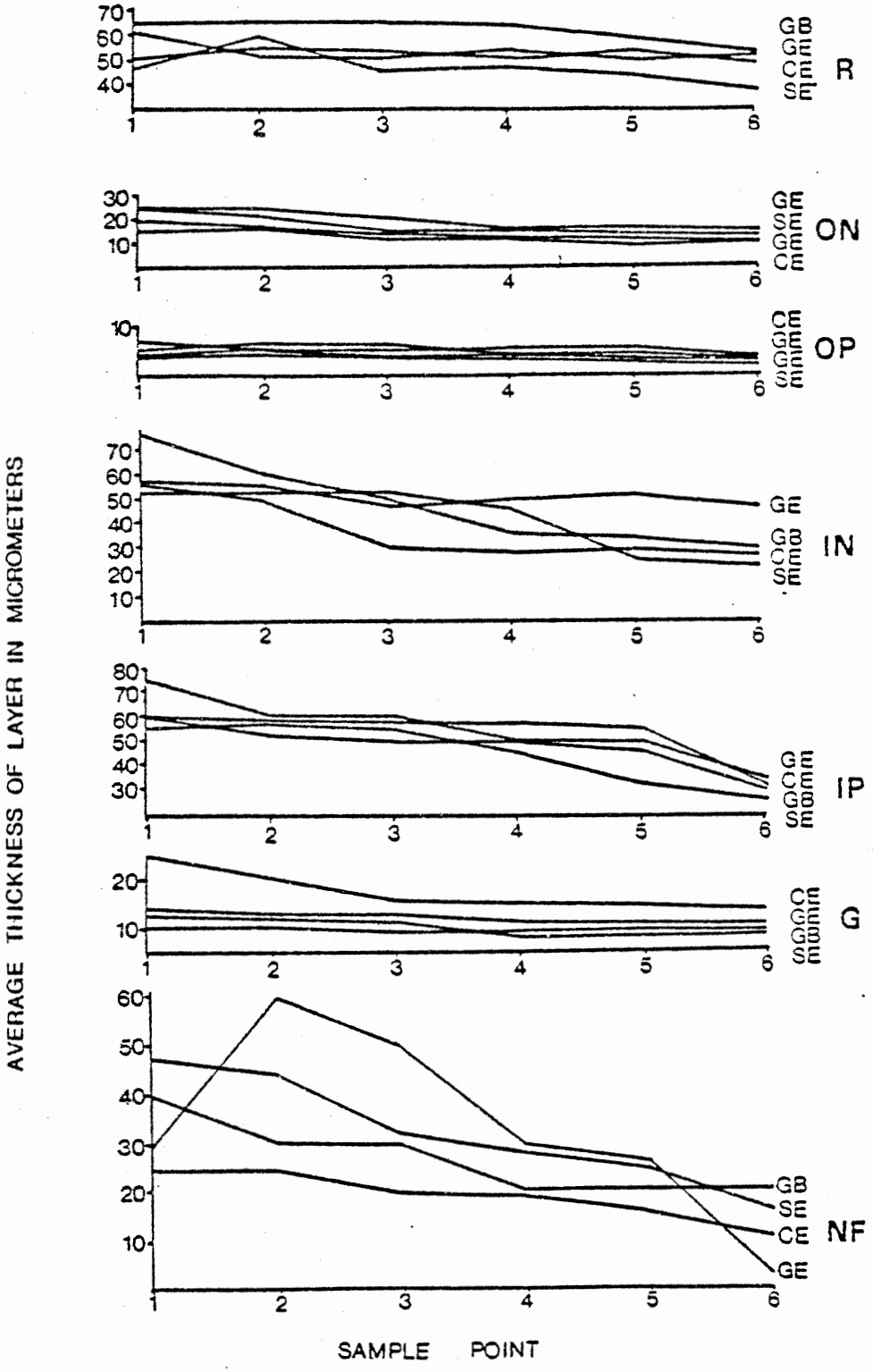

Figure 13. Figure showing the average thickness of each layer of the retinae of four species of herons. ON, Outer Nuclear. OP, Outer Plexiform. IN, Inner Nuclear. IP, Inner Plexiform. G, Ganglion Cell Layer. R, Receptor Layer, NF, Nerve Fiber Layer. GE, Great Egret. CE, Cattle Egret. SE, Snowy Egret. GB, Great Blue Heron.

Table 5. Retinal areas of four species of herons collected in Oklahoma during 1980-1981.

	Cattle Egret n=8		Snowy Egret n=5		Great Egret n=6		Great Blue Heron n=5	
	Mean area in sq. mm	Percent of total area	Mean area in sq. mm	Percent of total area	Mean area in sq. mm	Percent of total area	Mean area in sq. mm	Percent of total area
SECTION 1	45*	14.8	39	13.7	49	12.8	88	15.9
SECTION 2	55	17.9	49	17.2	63	16.5	102	18.5
SECTION 3	58	18.9	46	16.1	58	15.2	98	17.8
SECTION 4	41	13.4	35	12.3	53	13.8	85	15.5
SECTION 5	33	10.7	35	12.3	43	11.2	55	9.9
SECTION 6	23	7.6	21	7.3	31	8.2	33	6.0
SECTION 7	21	6.8	24	8.5	36	9.5	39	7.1
SECTION 8	30	9.9	36	12.6	49	12.8	51	9.3
SECTIONS 1-4	199	65.0	169	59.3	223	58.3	373	67.7
SECTIONS 5-8	107	35.0	116	40.7	159	41.7	178	32.3
TOTAL	306	100	285	100	382	100	551	100

*Rounded to nearest mm.

smaller retinal areas than the two larger species. The data shown in Table 5 also demonstrate the asymmetry of the eyeball which is typical of many vertebrates (Rochon-Duvigneaud 1943). In all species examined the half of the retina representing the nasal side of the eye (Segments 1-4) had an area much larger than that of the temporal side. Conversely, the dorsal and ventral halves of each eye were roughly symetrical with regard to area as shown by comparing the areas of segments 7, 8, 1, 2 (dorsal) with the areas of segments 3, 4, 5, 6 (ventral). The optical center of the eye of all four species is the central fovea. However, as stated above these are not in the geometric center of the cup shaped retinae.

The Position of the Eyes in the Head

The angle of eye position in these four species of heron is shown in Table 6. There is little variation in the average angles with a range of 64.5° in the Great Blue Heron to 69.7° in the Snowy Egret. Average visual angles (n=4) were 146° for Great Egrets, 145° for Great Blue Herons, and 150° and 146° respectively for Cattle and Snowy Egrets.

Relative Sizes of Pectens

Table 7 gives the average size of the pecten in each species and the ratio of pecten size to retinal area. The absolute size of the pecten of these four species is correlated with the size of the bird. Great Blue Herons had the largest pectens followed by Great Egrets, Snowy Egrets and Cattle Egrets. Great Egrets have the largest pecten relative to retinal area. Snowy Egrets also have relatively large

Table 6. Measurements of eye position angles for four species of herons collected in Oklahoma during 1980-1981.

Sample No.	Cattle Egret n=8	Snowy Egret n=5	Great Egret n=6	Great Blue Heron n=5
1	64.5°	69.0°	67.5°	63.6°
2	66.0°	69.5°	68.0°	64.0°
3	66.5°	69.5°	68.0°	64.5°
4	66.5°	70.0°	68.5°	65.0°
5	67.0°	70.5°	69.0°	65.5°
6	67.5°	-	69.5°	-
7	67.5°	-	-	-
8	67.5°	-	-	-
$\overline{X}$	66.6°	69.7°	68.4°	64.5

Table 7. Pecten sizes of four species of herons.

Species	n	Mean pecten size (sq. mm)	Retinal area (sq. mm)	Ratio of pecten size to retinal area
Snowy Egret	14	20.2	285.0	.070
Cattle Egret	20	18.6	306.0	.060
Great Egret	16	28.6	382.0	.074
Great Blue Heron	12	31.2	551.0	.056

pectens but Great Blue Herons and Cattle Egrets have relatively small pectens.

Relative Sizes and Shapes of Eyes

Shape

The average axial length and width of the eyes of these four species is given in Table 8. Although there is variation in the absolute parameters, there is little variation in the ratio of axial length to width which is a measure of the shape of the eye. The eyes of these birds are very spherical as indicated by the axial length/width ratios in Table 8. There is little variation between species; however, the larger herons have slightly more spherical eyes.

Size

There is a direct relationship between the size of the bird and the size of the eyes (Table 8). More meaningful information is obtained when relative eye sizes rather than absolute eye sizes are compared. The ratio used here to obtain relative eye sizes is expressed as the percent of the total head volume devoted to eyes. Some interesting differences appear in these data. In larger herons less of the total head volume consists of eyes (Table 8). Smaller herons have up to twice as much head volume allocated to eyes as in the larger birds.

Table 8. Relative sizes and shapes of eyes in four species of herons.

Species	Axial length (mm)		Width (mm)		Axial length/width ratio	$\bar{x}$ volume (ml) of 2 single eyes		Head volume (ml)		Percent eyes
	n	$\bar{x}$	n	$\bar{x}$		n	$\bar{x}$	n	$\bar{x}$	
Cattle Egret	24	12.78	24	16.28	.7850	24	2.10	12	21.60	19.44
Snowy Egret	18	10.21	18	13.10	.7793	18	1.85	9	18.32	20.19
Great Blue Heron	18	20.81	18	24.80	.8391	18	3.91	9	71.41	10.95
Great Egret	20	15.13	20	19.05	.7942	20	2.90	10	42.30	13.71

Descriptions of Photoreceptors

General Information

Three types of visual cells have been described in avian retinae (Walls 1942). These include single cones, uneven double cones and rods. These three types of photoreceptors were found in different proportions in the retinae of the herons examined. In general, the visual cells of all four species were thin and long near the fundus but much wider and shorter at the ora. The ellipsoids and outer segments of all species examined were very eosionphilic while other parts of these cells stained very faintly pink or not at all. Examination of tangential sections of heron retinae revealed no mosaic pattern for visual cell distribution as has been reported for other vertebrates, particularly fish (Lythgoe 1979). Various cell types appear to be distributed evenly but not in any particular pattern. A comparison of photoreceptors for the four species is shown in Table 9. Photoreceptors from these birds are illustrated in Figures 14-21. No single cones were found in the retinae of Great Blue Herons or Snowy Egrets. This may be because they closely resemble accessory cones or perhaps because they are actually absent.

Uneven double cones are a conspicuous feature of the receptor layer in the retinae of these four species and consist of two members here referred to as chief and accessory cells. Chief cells have a large oval paraboloid and a faintly staining ellipsoid; oil droplets are absent. The inner segments of these cells are 2-3 times wider (across the paraboloid) than are those of the accessory cells. Like all visual cells. the uneven double cones appear much larger in the fundus than near the ora.

Table 9. Morphological characteristics of photoreceptors of four species of herons. All measurements are in micrometers. GB, Great Blue Heron. GE, Great Egret. CE, Cattle Egret. SE, Snowy Egret. elm, external limiting membrane.

Species	Length of outer segment	Length of* cell	Paraboloid	Shape of Nucleus	Distance of** nucleus from elm	Width of*** cell at fundus	Width of*** cell at ora	Ellipsoid	Oil droplet
				Rods					
GB	15-37	40-85	Present distinct	Elliptical	4-8	3-4	4-5	Present distinct	Absent
GE	18-32	42-72	Present not distinct	Elliptical	3-8	3-4	4-6	Present distinct	Absent
CE	10-15	25-35	Present distinct	Elliptical	6-8	2-3	4-6	Present distinct	Absent
SE	10-20	26-37	Present distinct	Elliptical	3-7	2-3	3-4	Present distinct	Absent
				Single Cones					
GB	-	-	-	-	-	-	-	-	-
GE	3-8	17-28	Present distinct	Spherical to elliptical	0-3	3-4	5-8	Present distinct	Present

Table 9. Continued.

Species	Length of outer segment	Length of* cell	Paraboloid	Shape of Nucleus	Distance of** nucleus from elm	Width of*** cell at fundus	Width of*** cell at ora	Ellipsoid	Oil droplet
				Single cones					
CE	8-18	3-24	Present but small	Elliptical	2-4	2-3	2-3	Present distinct	Present
SE	-	-	-	-	-	-	-	-	-
				Chief cones					
GB	3-22	13-42	Present distinct	Elliptical	0-2	3-4	6-8	Present faint	Absent
GE	3-20	17-35	Present distinct	Elliptical	0 to -2	3-4	6-8	Present distinct	Absent
CE	3-17	20-36	Present distinct	Elliptical	0-2	4-6	6-8	Present distinct	Absent
SE	3-20	18-37	Present distinct	Elliptical	1-3	2-4	5-8	Present distinct	Absent

Table 9. Continued.

Species	Length of outer segment	length of* cell	Paraboloid	Shape of Nucleus	Distance of** nucleus from elm	Width of*** cell at fundus	Width of*** cell at ora	Ellipsoid	Oil droplet
				Accessory cones					
GB	2-8	15-33	Absent	Elliptical	2-4	3-4	3-4	Present distinct	Present
GE	2-7	14-22	Absent	Elliptical	0 to -2	3-4	4-7	Present distinct	Present
CE	3-17	18-34	Absent	Elliptical	2-3	3-5	4-6	Present distinct	Present
SE	3-12	17-30	Absent	Elliptical	Elm	3-5	4-6	Present distinct	Present

*Measured from the elm to the tip of the outer segment.

**Negative numbers indicate the distance the nuclei protrudes above the elm.

***Measured across widest part of receptor.

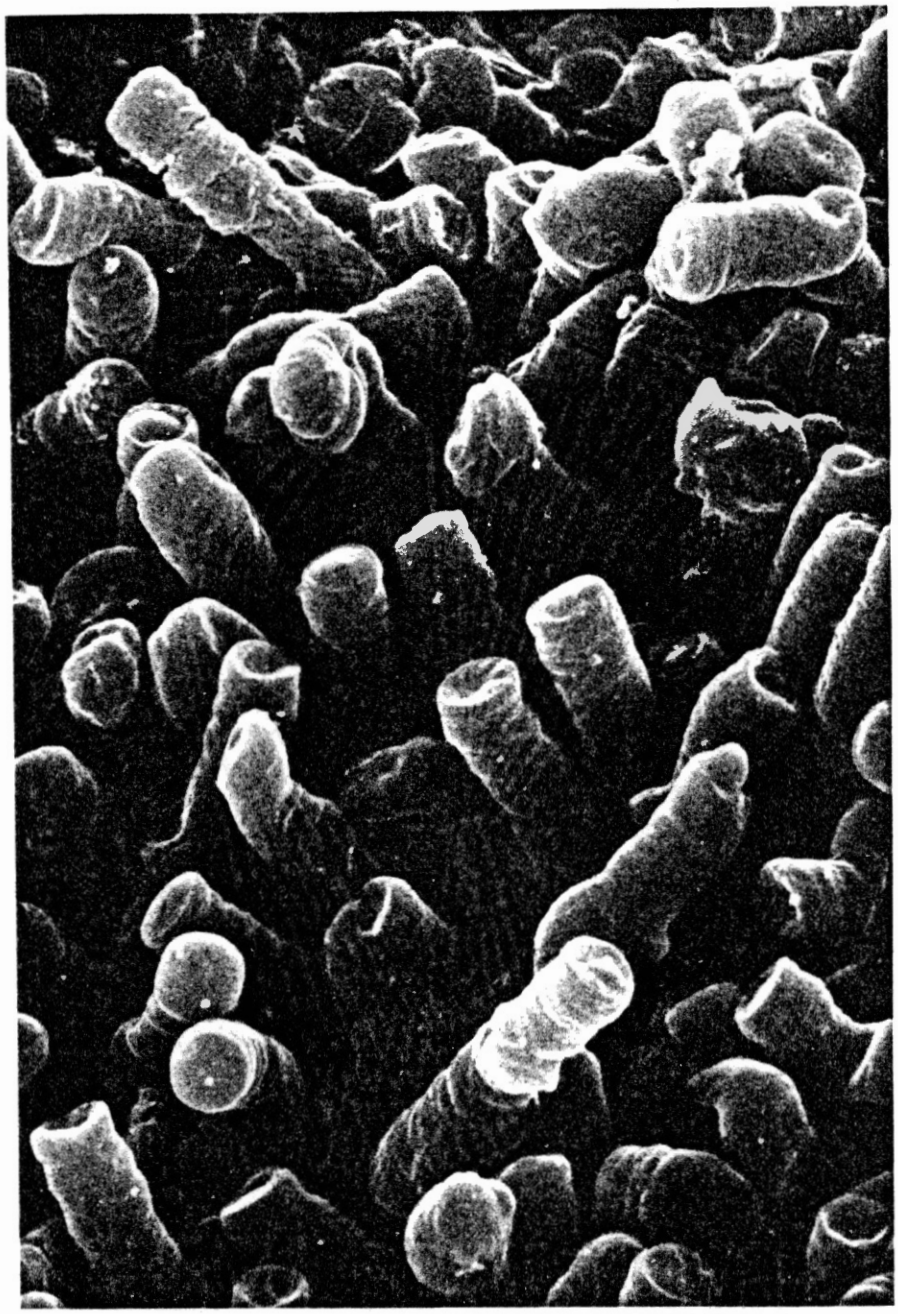

Figure 14. Scanning electron micrograph of outer segments of Cattle Egret rod cells (202X).

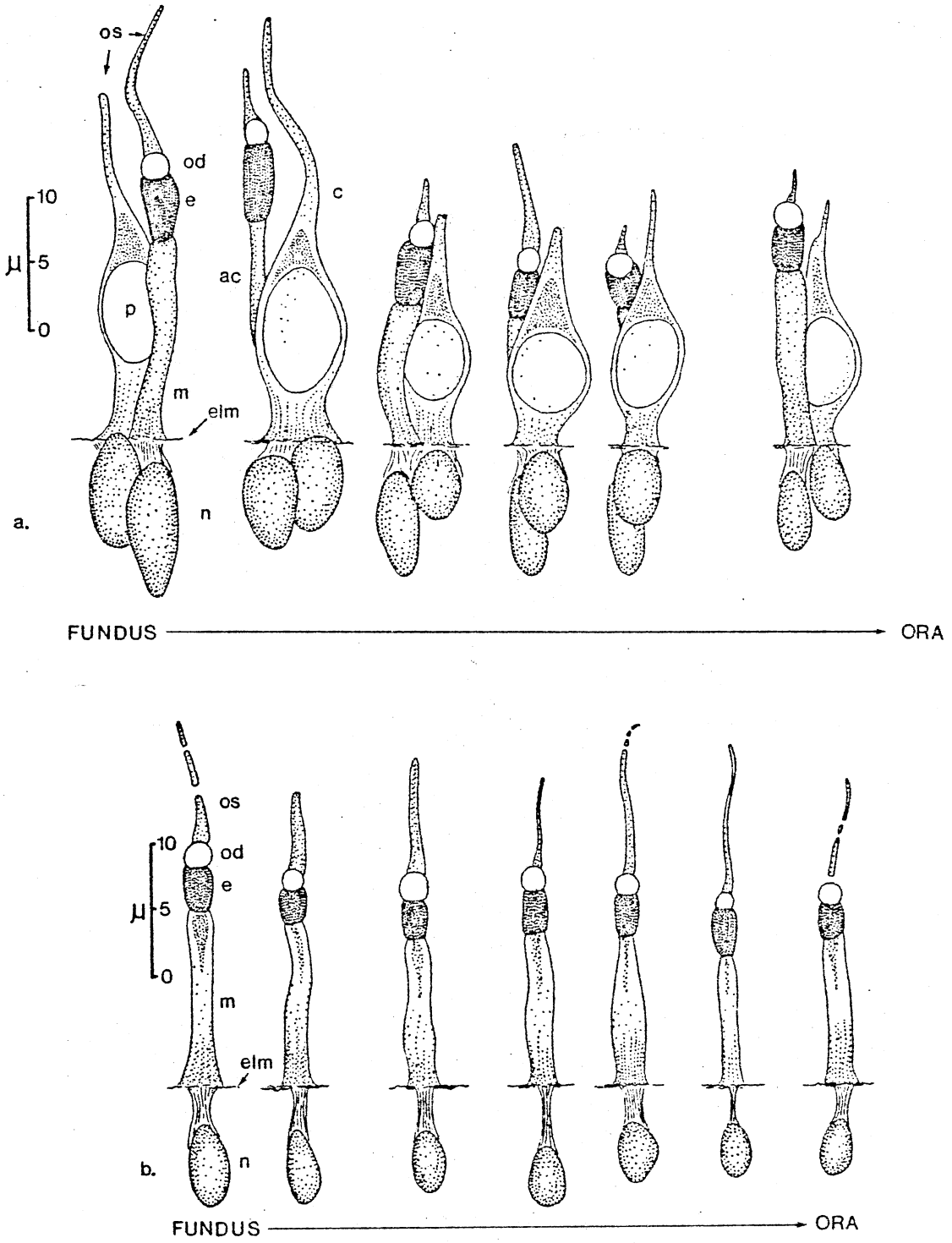

Figure 15. Visual cells from the retina of the Cattle Egret. a, double cone cells showing chief (c) and accessory (ac) cells. b, single cones. e, ellipsoid. elm, external limiting membrane. m, myoid. os, outer segment. od, oil droplet. p, paraboloid. n, nucleus. Diagram illustrates how cell size changes from near the center of the retina (fundus) to the edge of the retina (ora).

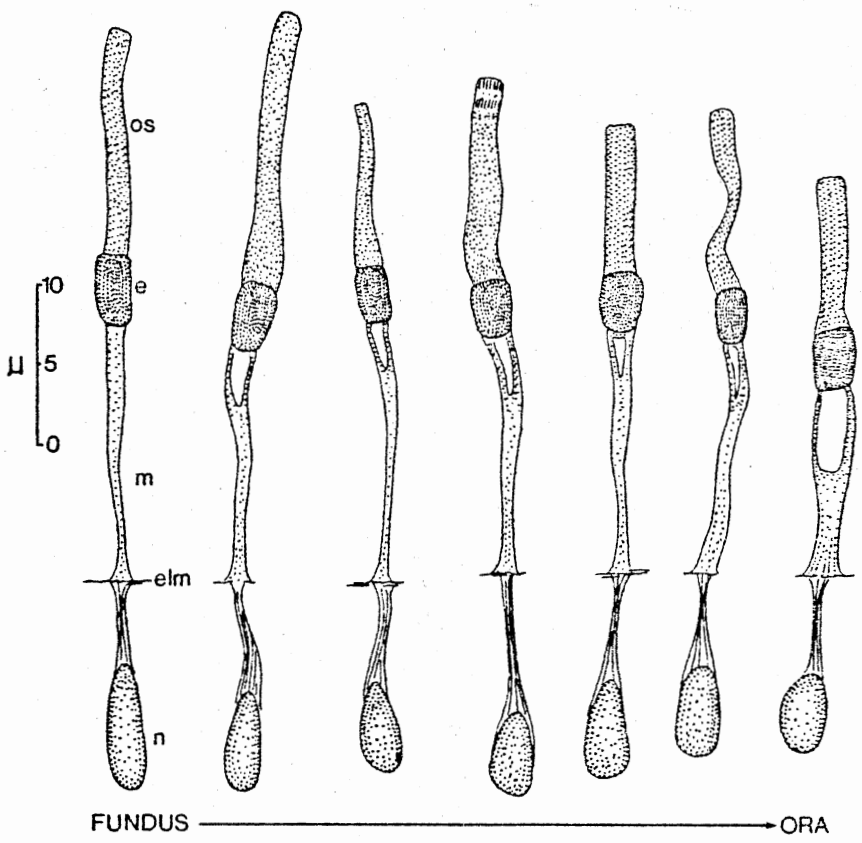

Figure 16. Rod cells from the retina of the Cattle Egret. e, ellipsoid. elm, external limiting membrane. m, myoid. n, nucleus. os, outer segment. Diagram illustrates how cell size changes from near the center of the retina (fundus) to the edge of the retina (ora).

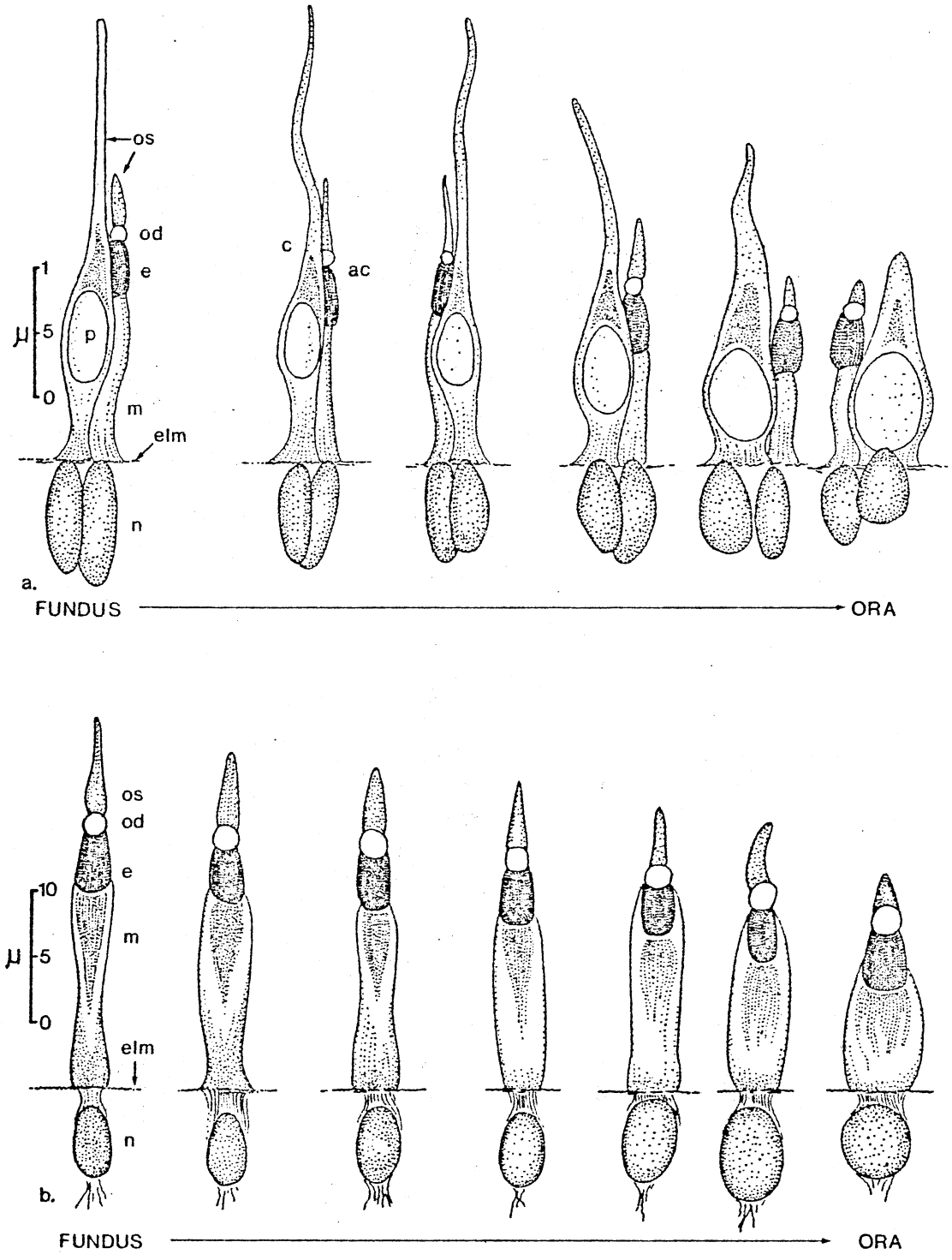

Figure 17. Visual cells from the retina of the Great Egret. a, double cones showing chief cells (c) and accessory cells (ac). b, single cone cells. e, ellipsoid. elm, external limiting membrane. m, myoid. n, nucleus. os, outer segment. od, oil droplet. p, paraboloid. Diagram illustrates how cell size changes from near the center of the retina (fundus) to the edge of the retina (ora).

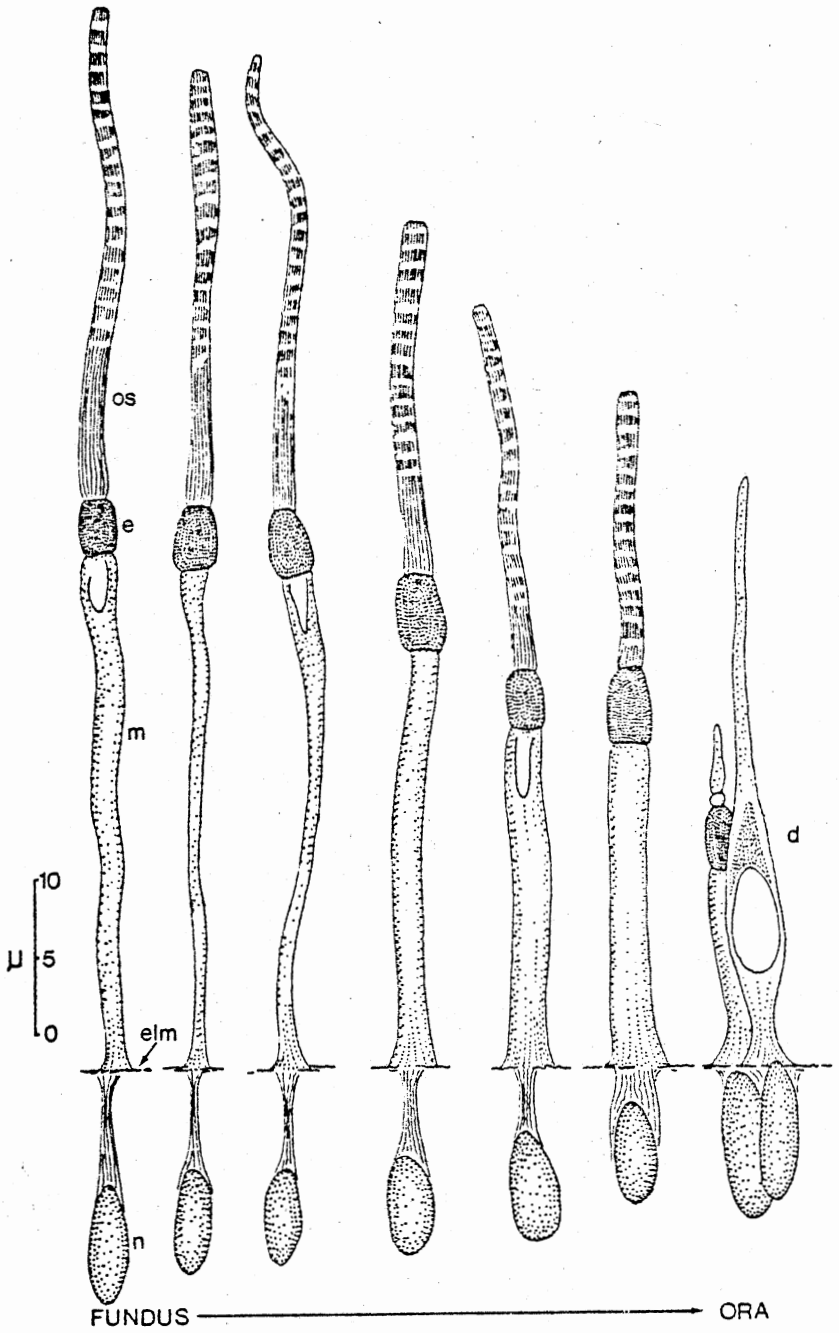

Figure 18. Rod cells from the retina of the Great Egret. e, ellipsoids. m, myoid. n, nucleus. os, outer segment. p, paraboloid. d, fundic double cone for comparison. Diagram illustrates how cell size changes from near the center of the retina (fundus) to the edge of the retina (ora).

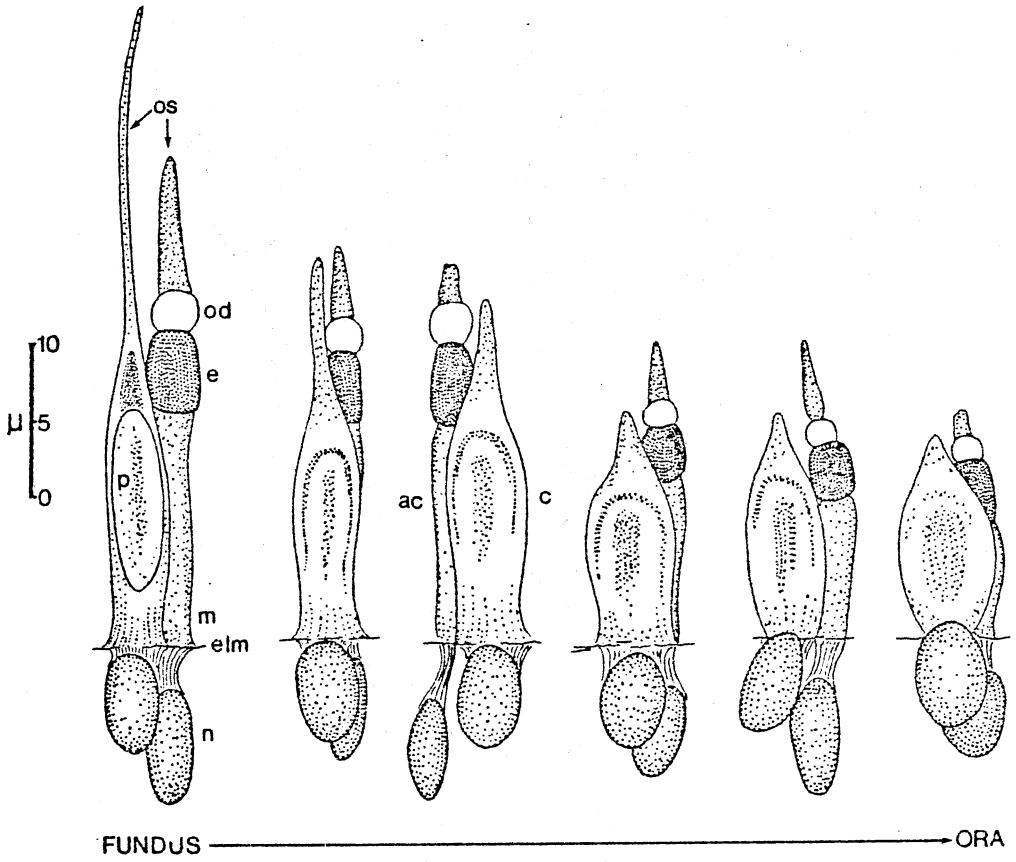

Figure 19. Double cones from the retina of the Great Blue Heron. Chief cells (c) and accessory cone cells (ac). e, ellipsoid. elm, external limiting membrane. m, myoid. n, nucleus. os, outer segment. od, oil droplet. p, paraboloid. Diagram illustrates how cell size changes from near the center of the retina (fundus) to the edge of the retina (ora).

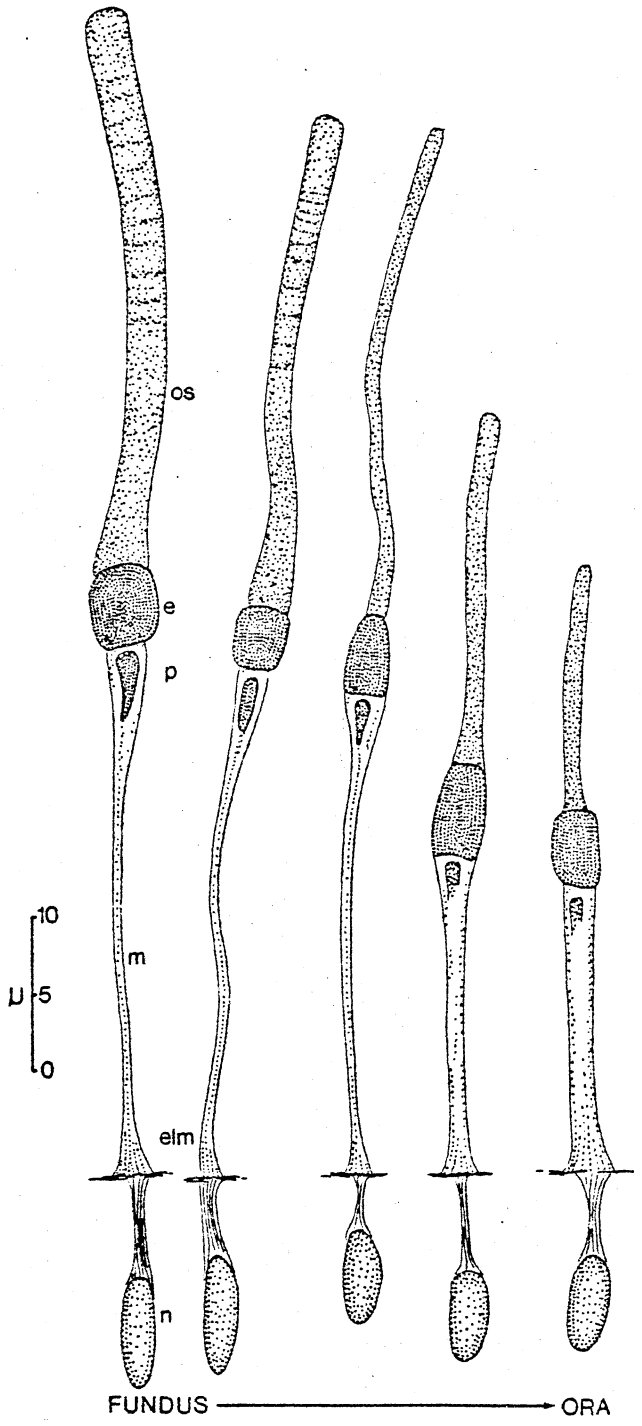

Figure 20. Rod cells from the retina of the Great Blue Heron. e, ellipsoid. elm, external limiting membrane. m, myoid. n, nucleus. os, outer segment. p, paraboloid. Diagram illustrates how cell size changes from near the center of the retina (fundus) to the edge of the retina (ora).

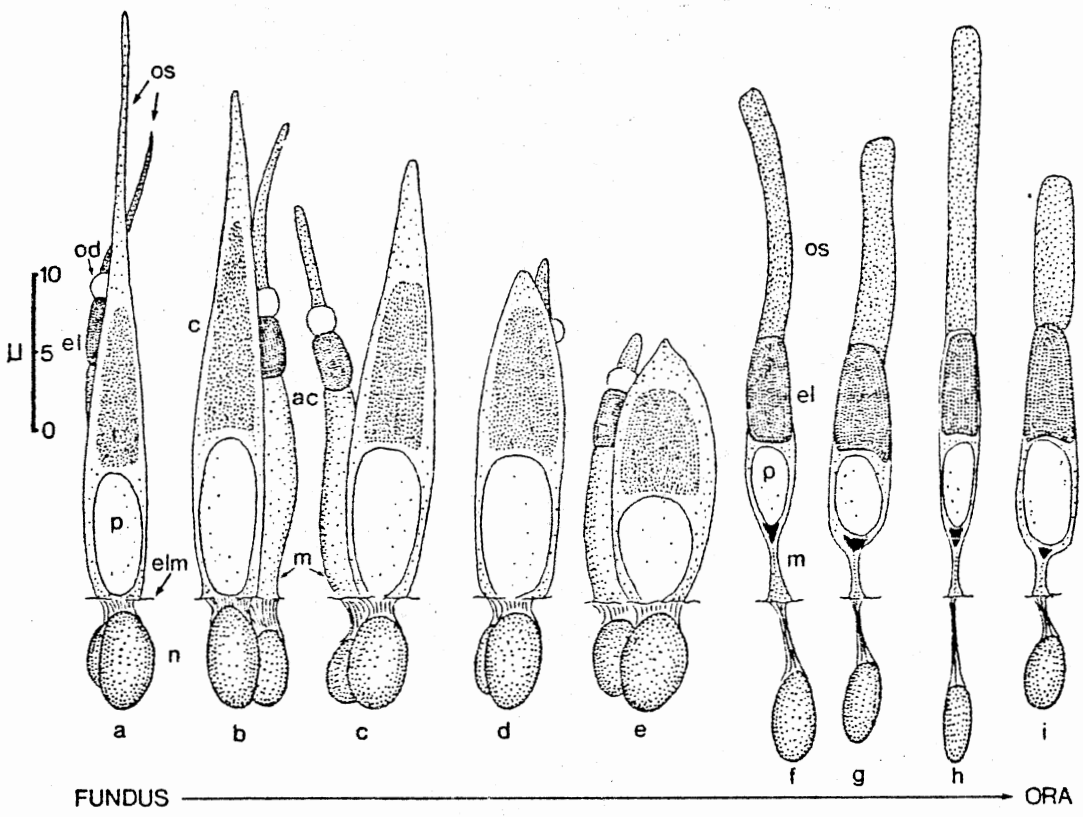

Figure 21. Visual cells from the retina of the Snowy Egret. a-e, double cones showing chief cells (c) and accessory cells (ac). f-i, rod cells. el, ellipsoid. elm, external limiting membrane. m, myoid. n, nucleus. os, outer segment. od, oil droplet. p, paraboloid. Diagram illustrates how cell size changes from near the center of the retina (fundus) to the edge of the retina (ora).

Chief cells are always associated with accessory cells. In sections they often appear to be superimposed on one another. Also, they appear to be attached at the myoid region. Accessory cells are more elongated and have no visible paraboloid. They do have a well defined ellipsoid and an oil droplet near the base of the outer segment. Accessory cells are usually slightly shorter than the chief cells and are much narrower. The cytoplasm of the accessory cell is generally less granular than that of chief cells.

The nuclei of these two cell types are elliptical in the fundic region but much more spherical near the ora. The nucleus of a chief cell is positioned directly adjacent to, touching or protruding through the external limiting membrane while that of an accessory cell is well below (1-3 μm vitread) this membrane. There does not appear to be any difference in the distribution of chromatin in the nuclei of these two cells.

The single cones are sometimes difficult to distinguish from chief cells. However, there are some subtle differences. The nucleus of the single cone is generally much smaller than that of the accessory cell. Also, below the base of the ellipsoid there is sometimes a granular dark staining area (possibly a paraboloid) which is a consistent feature of single cones. The outer segment of a single cone is very delicate and attenuated as is that of the accessory cones. An oil droplet is always present in single cones.

The rod cells of these herons are morphologically very different from the cone cells. In general, the myoid section is much thinner than that of the cone cell. The outer segment and the ellipsoid of a rod is usually located above those of the cone cells because the myoid of a rod

is much longer than that of a cone. The ellipsoids usually form a distinct layer above the cone cells (in light adapted retinae) which makes them very easy to count in cross sections. The outer segments of the rods (Figure 14) stain very darkly with eosin and are always truncated (never attenuated as in cones). They sometimes appear to be banded towards the tip. These horizontal striations are typical of many vertebrate rod cells. The paraboloids are very evident on most rod cells but are difficult to see on others. The nuclei of rod cells usually form a distinct layer and are farther (5-10 μm) below the external limiting membrane than are the nuclei of cone cells. Also, these nuclei are distinctly egg-shaped.

The Distribution of Retinal Cells

Overall Differences Between Species

All four species have similar proportions of retinal cells (Figure 22). The two large herons (Great Blue Heron and Great Egret) are very similar in the number of bipolar cells in the sample area but differ greatly in the numbers of rods, cones and ganglion cells. Likewise, the two small species (Cattle and Snowy Egrets) have similar numbers of bipolar cells and ganglion cells but differ greatly in the number of rods and cones (Figure 22). Table 10 shows how the four species rank with respect to the various retinal cells. In general, the abundance of retinal cells is correlated with the size of the bird and the size of the eye except for the fact that the Cattle Egret, a relatively small species, has a higher average number of rods than the much larger Great Egret. When the total number of rods at all 48 sample points is calculated as a percentage of the total number of receptors (excluding

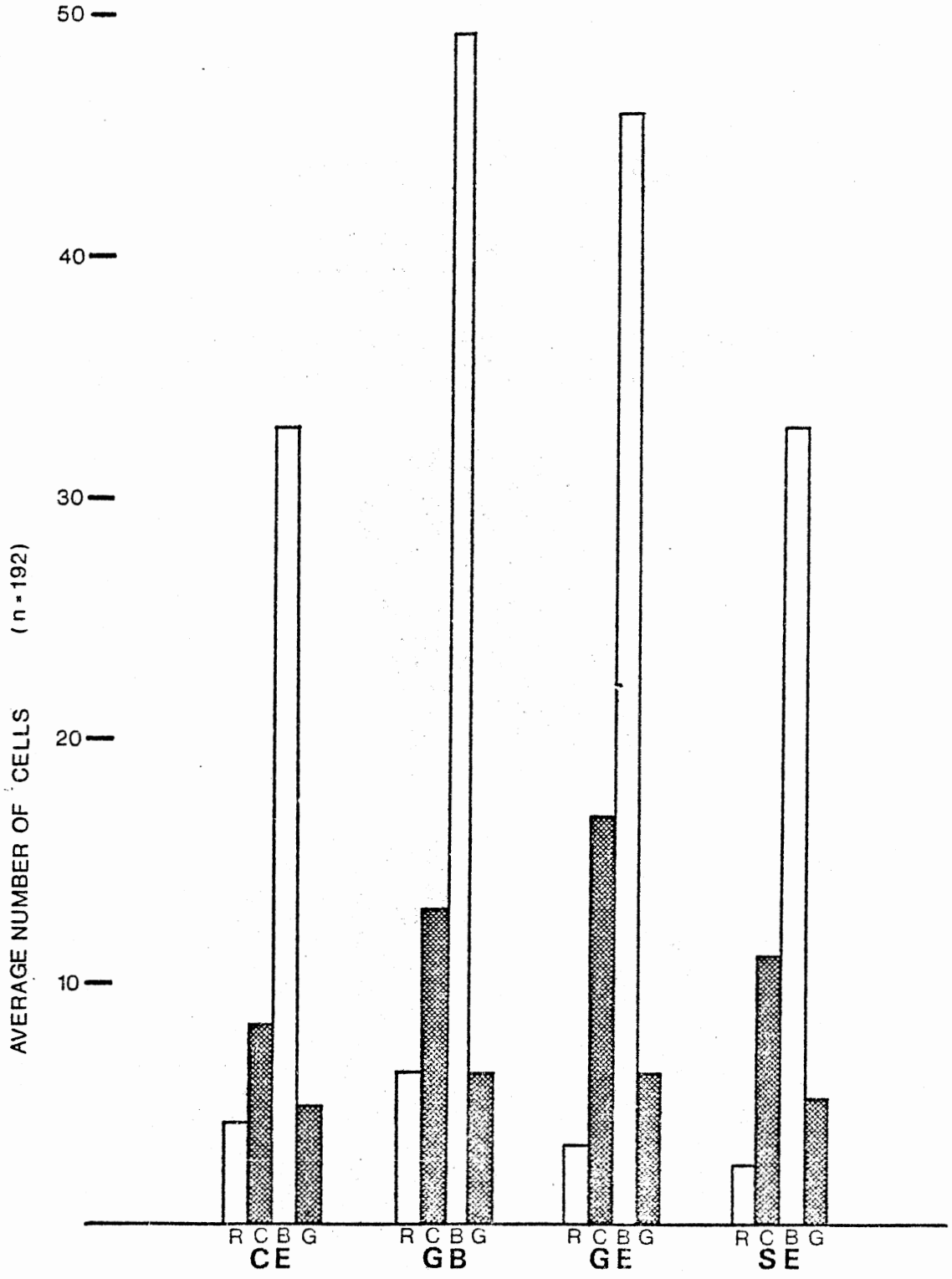

Figure 22. Average number of each cell type calculated from counts taken at all 48 sample points in four species of herons. CE, Cattle Egret. GB, Great Blue Heron. GE, Great Egret. SE, Snowy Egret. R, Rods. C, Cones. B, Bipolar cells. G, Ganglion cells.

Table 10. Ranking of species by number of cells.

variable	Highest ---------->			Lowest
Rod	GB	CE	GE	SE
Cone	GE	GB	SE	CE
Bipolar cell	GB	GE	CE	SE
Ganglion cell	GB	GE	SE	CE

GB = Great Blue Heron
GE = Great Egret
SE = Snowy Egret
CE = Cattle Egret

the cells of the fovea) both Cattle Egrets and Great Blue Herons have retinae composed of approximately one-third rods (33% and 33.2%, respectively). Great and Snowy Egrets have a smaller percentage of rods in their retinae (15.8% and 18.3%, respectively). These percentages reflect the relative duplicity found in the retinae of these birds.

Variation of Retinal Cells Along Transects

Species differ significantly with respect to the variation in mean numbers of rods, cones, bipolar and ganglion cells along each transect (Table 11 and Figures 23-26). Species are least different with respect to mean numbers of ganglion cells and rods (Figures 23 and 26). The four species are most different with respect to the mean numbers of cones (Figure 24). With respect to the mean numbers of bipolar cells Great Egrets and Great Blue Herons are very similar as are Cattle and Snowy Egrets (Figure 25). Great Blue Herons and Great Egrets have the highest mean values for all retinal cells except rods.

Differences Between Sample Points

When the four species are compared, there are significant differences in mean values for retinal cells with respect to sample points (Table 12). The sample points of species vary most with respect to the mean values of rod cells (Figure 27). Great Blue Herons have the highest mean value of rod cells for all six sample points followed by Cattle, Great and Snowy Egrets. Species are also very different with respect to the mean values of cones at the six sample points, although there is some overlap between Snowy Egrets and Great Blue Herons near sample point 1 (Figure 28). With respect to mean numbers of bipolar and

Table 11. Analysis of variance comparing species by transect. df, degrees of freedom. ANOVA SS, analysis of variance sum of squares. Mean SS, mean sum of squares. P, probability. there are significant differences in transects between species if P > F is less than .05.

Variable	df	ANOVA SS	Mean SS	F-Value	P > F
Rod	21	78.62		8.08	.0001
Rod error	576	266.86	.46		
Cone	21	914.55		13.49	.0001
Cone error	576	1859.65	3.22		
Bipolar cell	21	3627.45		11.55	.0001
Bipolar cell error	576	8613.70	1458.08		
Ganglion cell	21	283.96		9.70	.0001
Ganglion cell error	576	802.91	1.39		

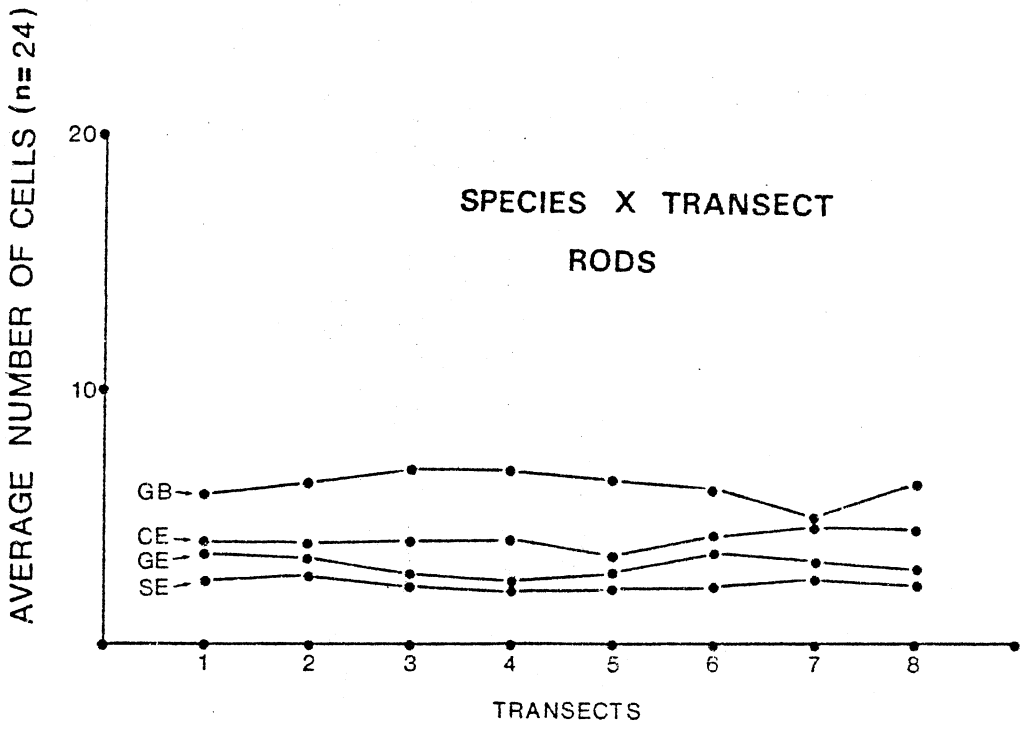

Figure 23. Variation in average number of rods between transects by species. Each value represents the average obtained by combining all sample points along each transect. GB, Great Blue Heron. CE, Cattle Egret. GE, Great Egret. SE, Snowy Egret.

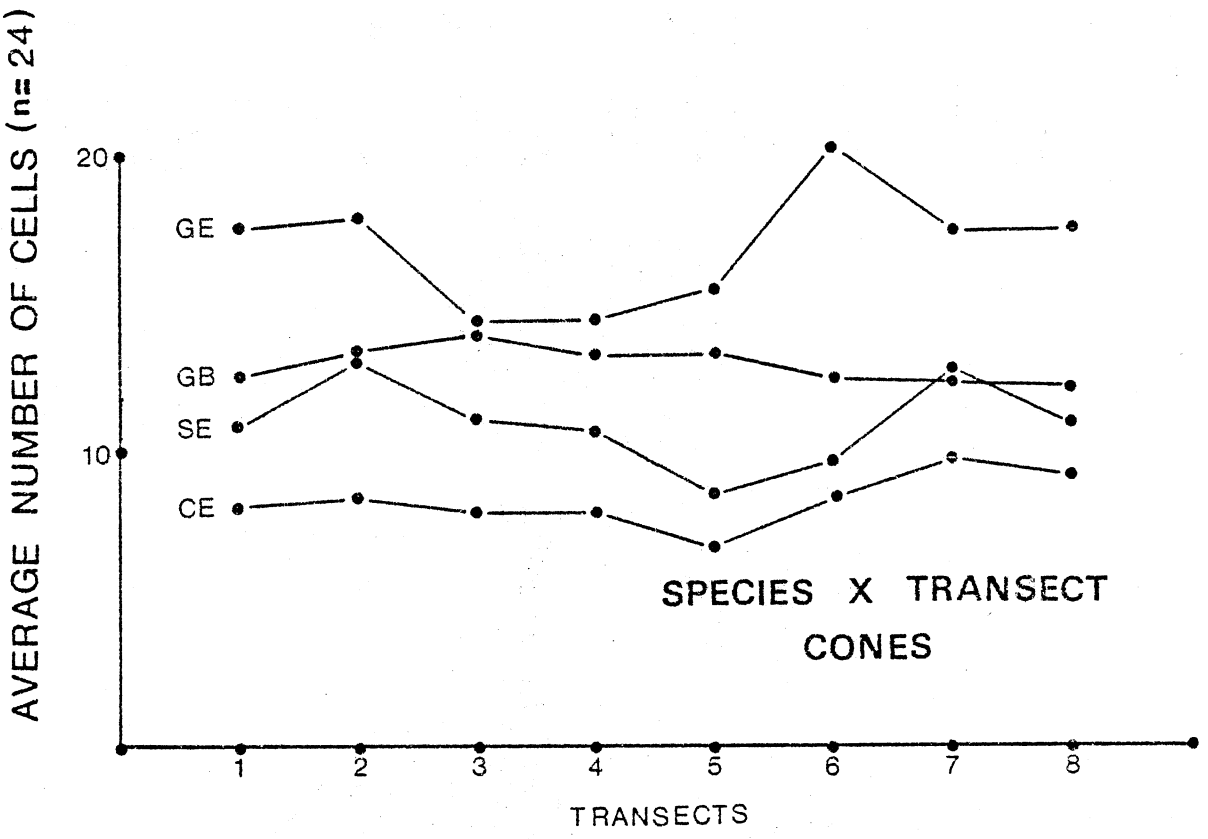

Figure 24. Variation in average number of cones between transects by species. Each value represents the average obtained by combining all sample points along each transect. GE, Great Egret. GB, Great Blue Heron. SE, Snowy Egret. CE, Cattle Egret.

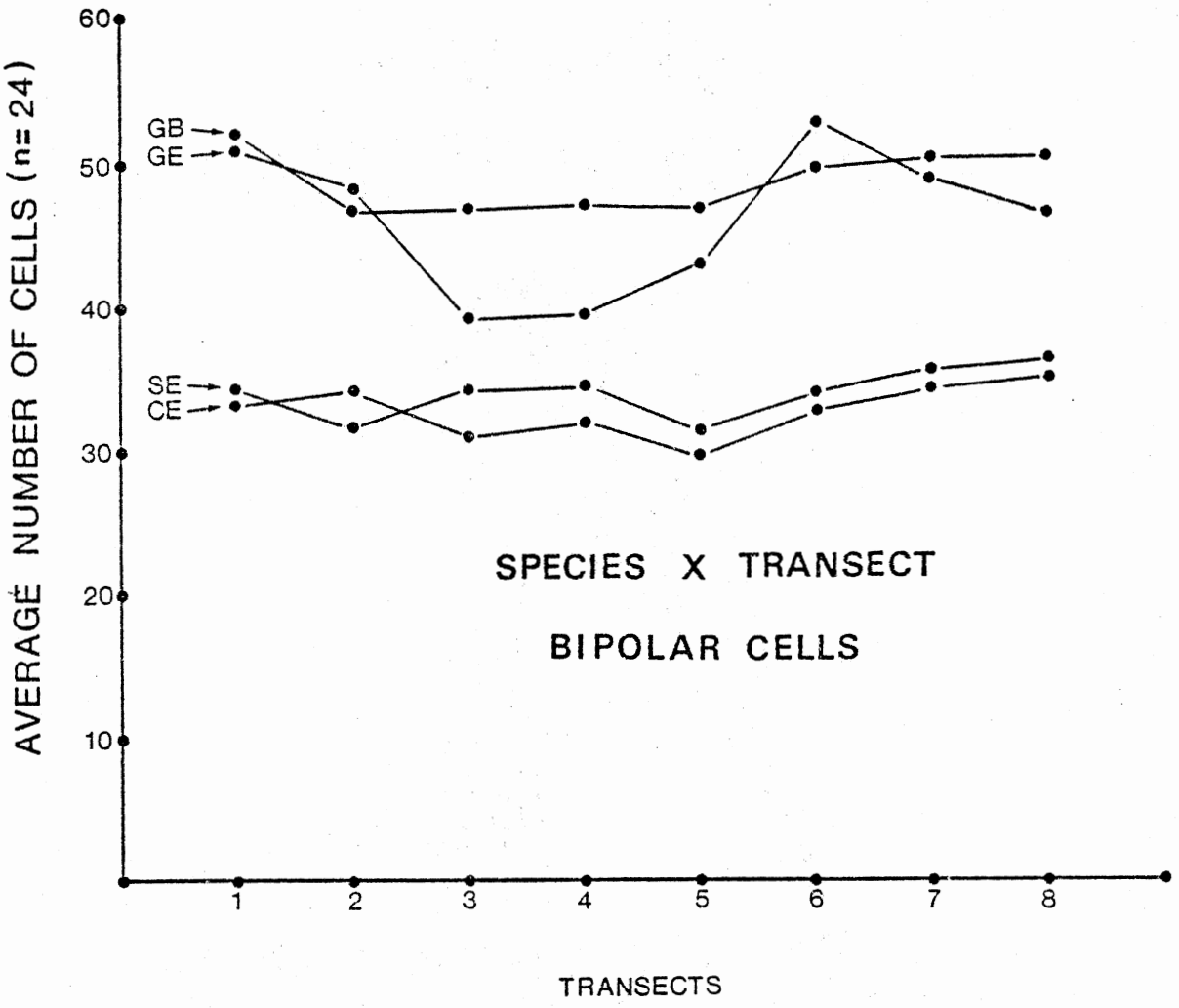

Figure 25. Variation in average number of bipolar cells between transects by species. Each value represents the average obtained by combining all sample points along each transect. GB, Great Blue Heron. GE, Great Egret. SE, Snowy Egret. CE, Cattle Egret.

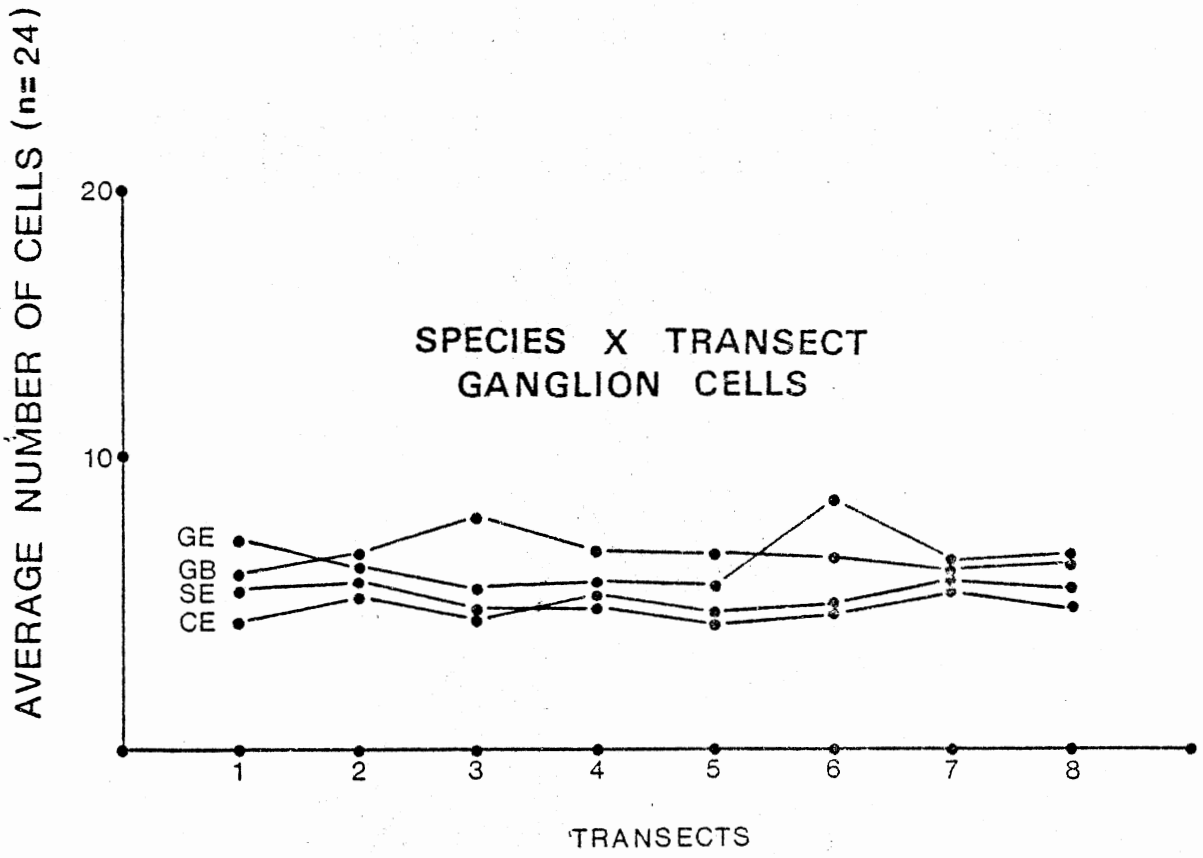

Figure 26. Variation in average number of ganglion cells between transects by species. Each value represents the average obtained by combining all sample points along each transect. GE, Great Egret. GB, Great Blue Heron. SE, Snowy Egret. CE, Cattle Egret.

Table 12. Analysis of variance comparing species by sample point. df, degrees of freedom, ANOVA SS, analysis of variance sum of equares. Mean SS, mean sum of squares. P, probability. There are significant differences in points between species if P > F is less than .05.

Variable	df	ANOVA SS	Mean SS	F-Value	P > F
Rod	15	334.92		48.19	.0001
Rod error	576	266.86	.46		
Cone	15	864.68		17.85	.0001
Cone error	576	1859.65	3.22		
Bipolar cell	15	10592.19		47.22	.0001
Bipolar cell error	576	8613.70	14.95		
Ganglion cell	15	223.19		10.67	.0001
Ganglion cell error	576	802.91	1.39		

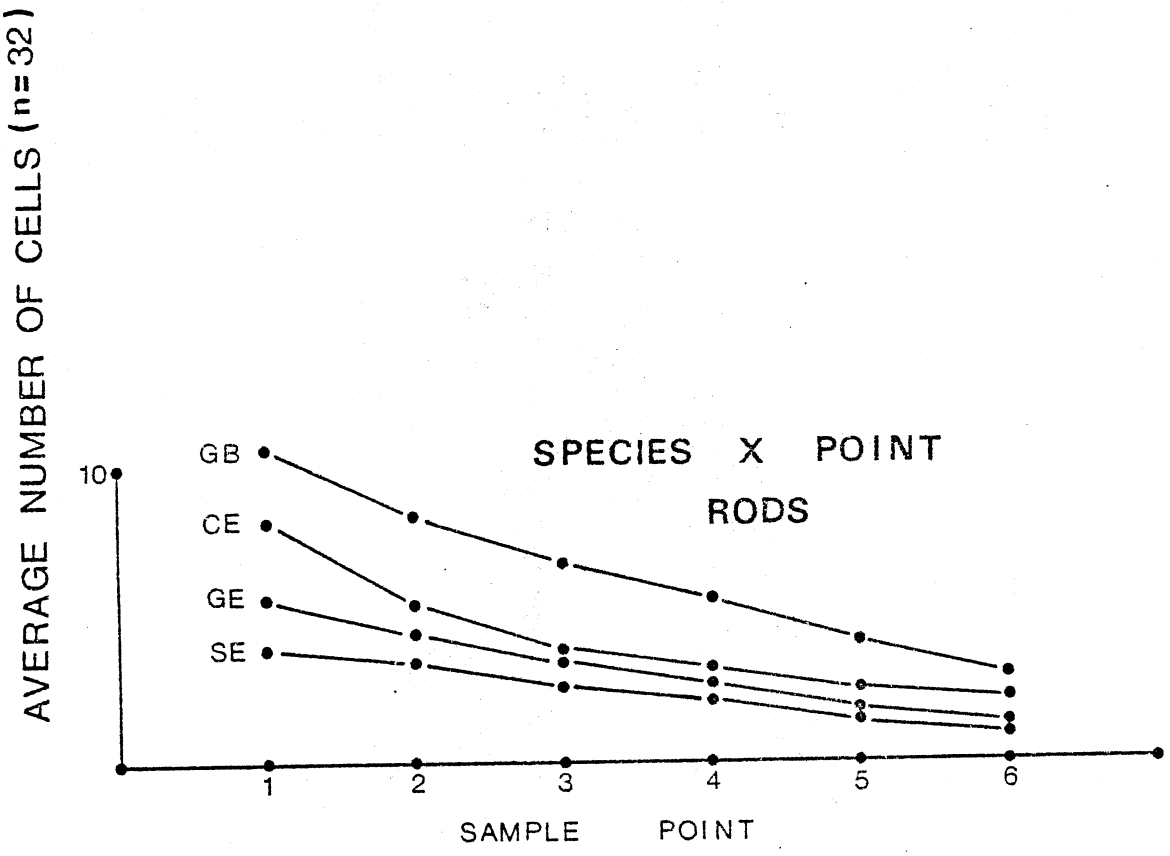

Figure 27. Variation in the average number of rods by sample point. GB, Great Blue Heron. CE, Cattle Egret. GE, Great Egret. SE, Snowy Egret.

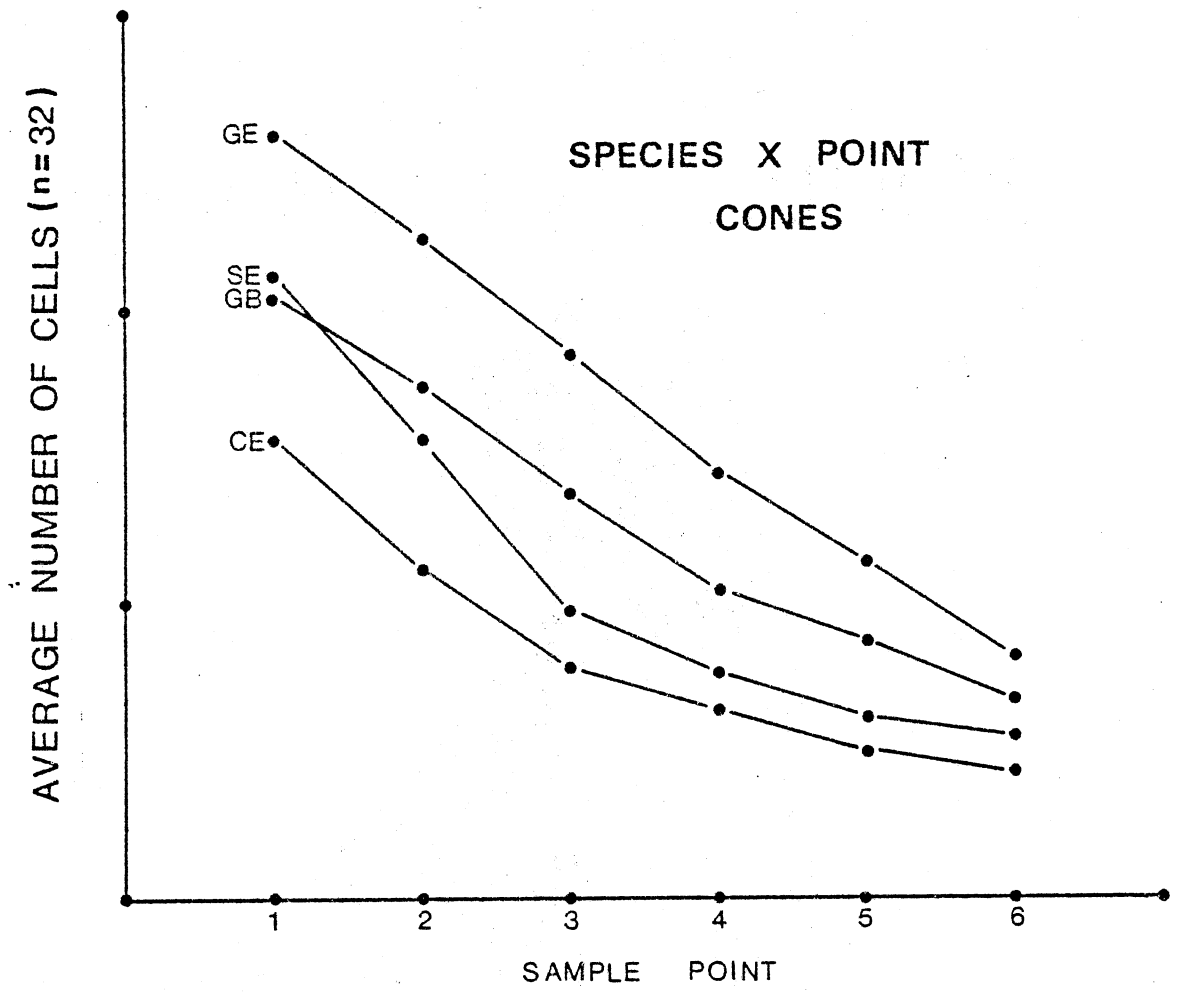

Figure 28. Variation in the average number of cones by sample point. GE, Great Egret. SE, Snowy Egret. GB, Great Blue Heron. CE, Cattle Egret.

ganglion cells, Great Blue Herons and Great Egrets are very similar to each other but different from the smaller Snowy and Cattle Egrets. These two small egrets are also very similar with respect to this variable (Figures 29 and 30).

Differences Between Dorsal and Ventral Aspects of the Retina

To determine how different dorsal and ventral aspects of the retinae are within species I conducted a series of t-tests. The results of these tests are shown in Table 13. Almost all species showed significantly higher mean values of retinal cells on the dorsal half of the retinae. Curiously, Great Blue Herons showed significantly fewer rods, cones, and ganglion cells on the dorsal half of the retinae (Table 13).

Differences Between Nasal and Temporal Aspects of the Retinae

To determine if there were nasal and temporal differences in mean numbers of retinal cells within species I conducted a series of t-tests (Table 14). Great Egret bipolar and Snowy Egret rod and cone cells were significantly higher on the nasal half of the retina although at only a 90% probability level.

Visual Representation of Retinal Cell Distribution

The computer mapping program produced the iso-density maps of rod, cone, bipolar and ganglion cell distributions shown in Figures 31-46.

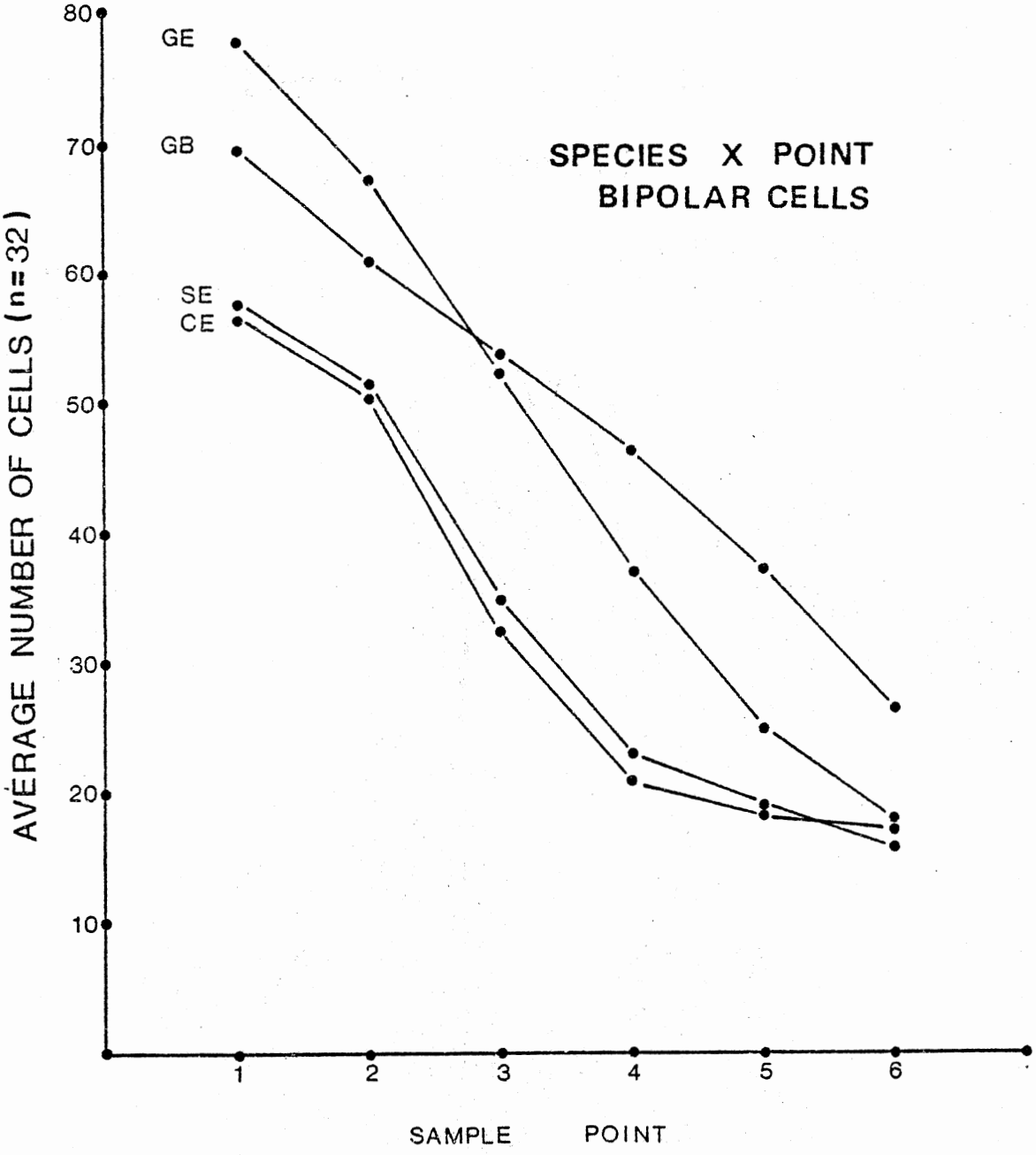

Figure 29. Variation in the average number of bipolar cells by sample point. GE, Great Egret. GB, Great Blue Heron. SE, Snowy Egret. CE, Cattle Egret.

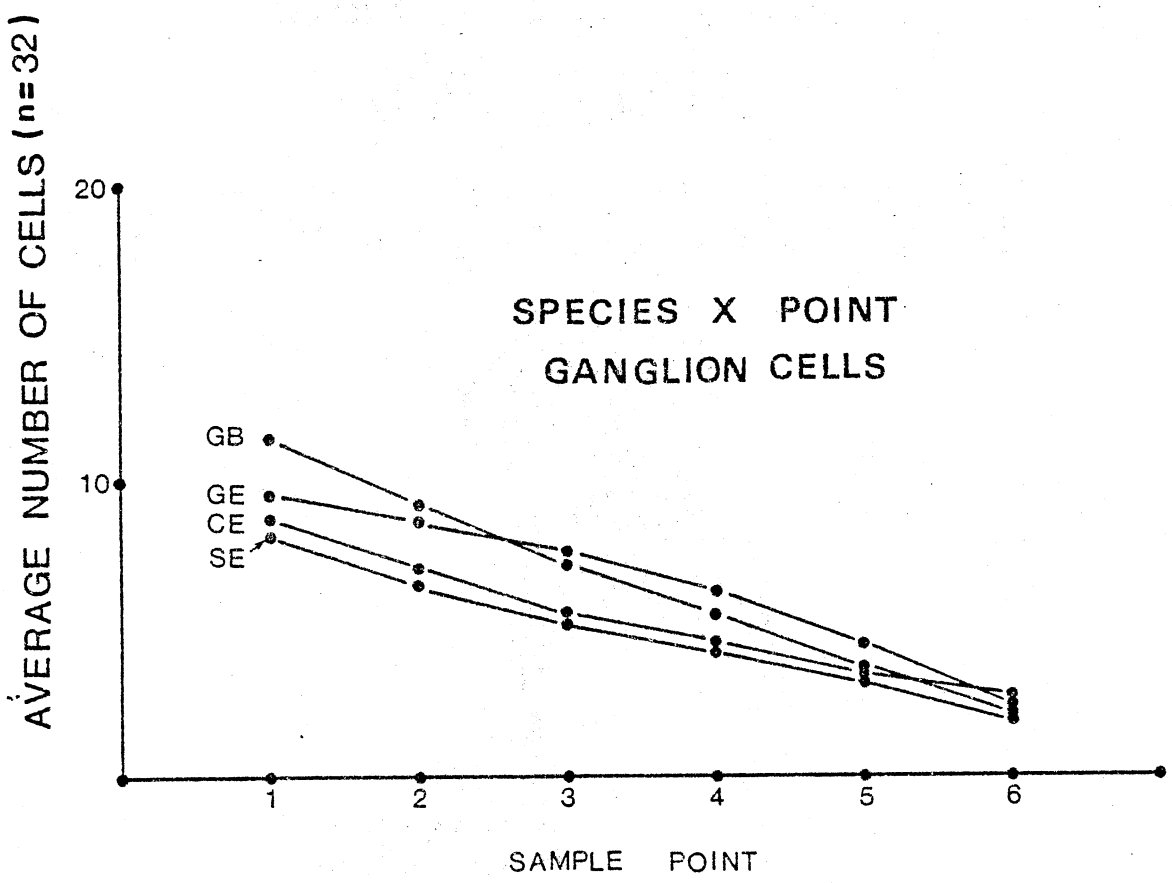

Figure 30. Variation in the average number of ganglion cells by sample point. GB, Great Blue Heron. GE, Great Egret. CE, Cattle Egret. SE, Snowy Egret.

Table 13. T-tests comparing the distribution of retinal cells on dorsal versus ventral halves of the retinae.

Species	Variable	Dorsal x	Ventral x	T	df	P
Cattle Egret	Rods	4.46	3.95	1.66	6.0	.1473**
	Cones	9.10	7.68	2.86	3.3	.0579**
	Bipolar cells	34.52	31.00	4.46	6.0	.0043*
	Ganglion cells	4.97	4.86	.44	3.6	.6793
Great Egret	Rods	3.40	2.73	5.20	6.0	.0020*
	Cones	17.63	14.73	3.94	6.0	.0076*
	Bipolar cells	48.79	40.06	5.28	6.0	.0032*
	Ganglion cells	6.58	5.35	4.74	6.0	.0032*
Great Blue Heron	Rods	6.06	6.81	-3.31	6.0	.0173*
	Cones	12.42	13.66	-2.78	3.3	.0229*
	Bipolar cells	51.62	47.23	4.95	6.0	.0026*
	Ganglion cells	6.11	7.12	-6.33	6.0	.0007*
Snowy Egret	Rods	2.61	2.31	5.32	6.0	.0018*
	Cones	11.53	10.14	3.31	6.0	.0161*
	Bipolar cells	34.85	33.04	2.06	6.0	.0839**
	Ganglion cells	5.5	4.7	2.41	6.0	.0521**

*Significant at 95% level.

**Significant at 90% level.

Table 14. T-tests comparing the distribution of retinal cells on nasal versus halves of the retina.

Species	Variable	Nasal	Temporal	t	df	P
Cattle Egret	Rods	4.12	4.18	.2300	6.0	.8236
	Cones	3.25	8.47	.6200	6.0	.5542
	Bipolar cells	32.84	32.42	1.9400	6.0	.0998*
	Ganglion cells	4.79	5.04	-1.0100	6.0	.3510
Great Egret	Rods	3.33	3.34	.0130	6.0	.9900
	Cones	16.65	17.79	-1.8490	6.0	.1138
	Bipolar cells	45.91	48.35	-2.1600	6.0	.0733*
	Ganglion cells	6.33	6.70	-1.2800	6.0	.2446
Great Blue Heron	Rods	6.40	6.27	.4400	6.0	.6735
	Cones	13.08	12.80	1.3800	6.0	.2167
	Bipolar cells	48.94	49.46	.5592	6.0	.6512
	Ganglion cells	6.79	6.48	.8809	6.0	.4123
Snowy Egret	Rods	2.58	2.36	2.2800	6.0	.0622*
	Cones	11.76	10.30	2.0500	6.0	.0852*
	Bipolar cells	33.29	32.76	.7695	6.0	.4708
	Ganglion cells	5.28	5.06	1.0000	6.0	.3536

*Significant at 90% probability level.

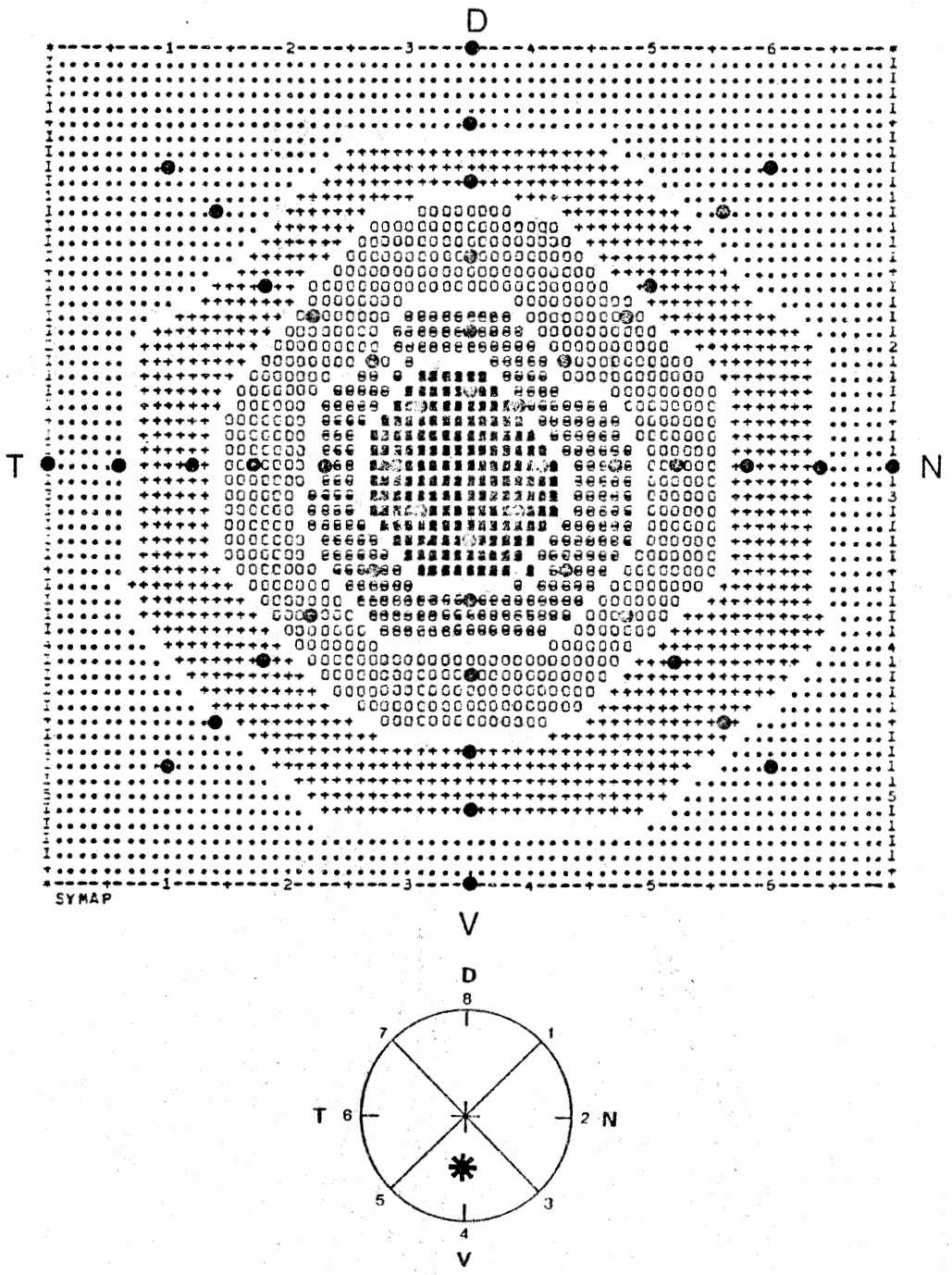

Figure 31. Iso-density map of rod cell distribution in the Great Blue Heron. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at the 95% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

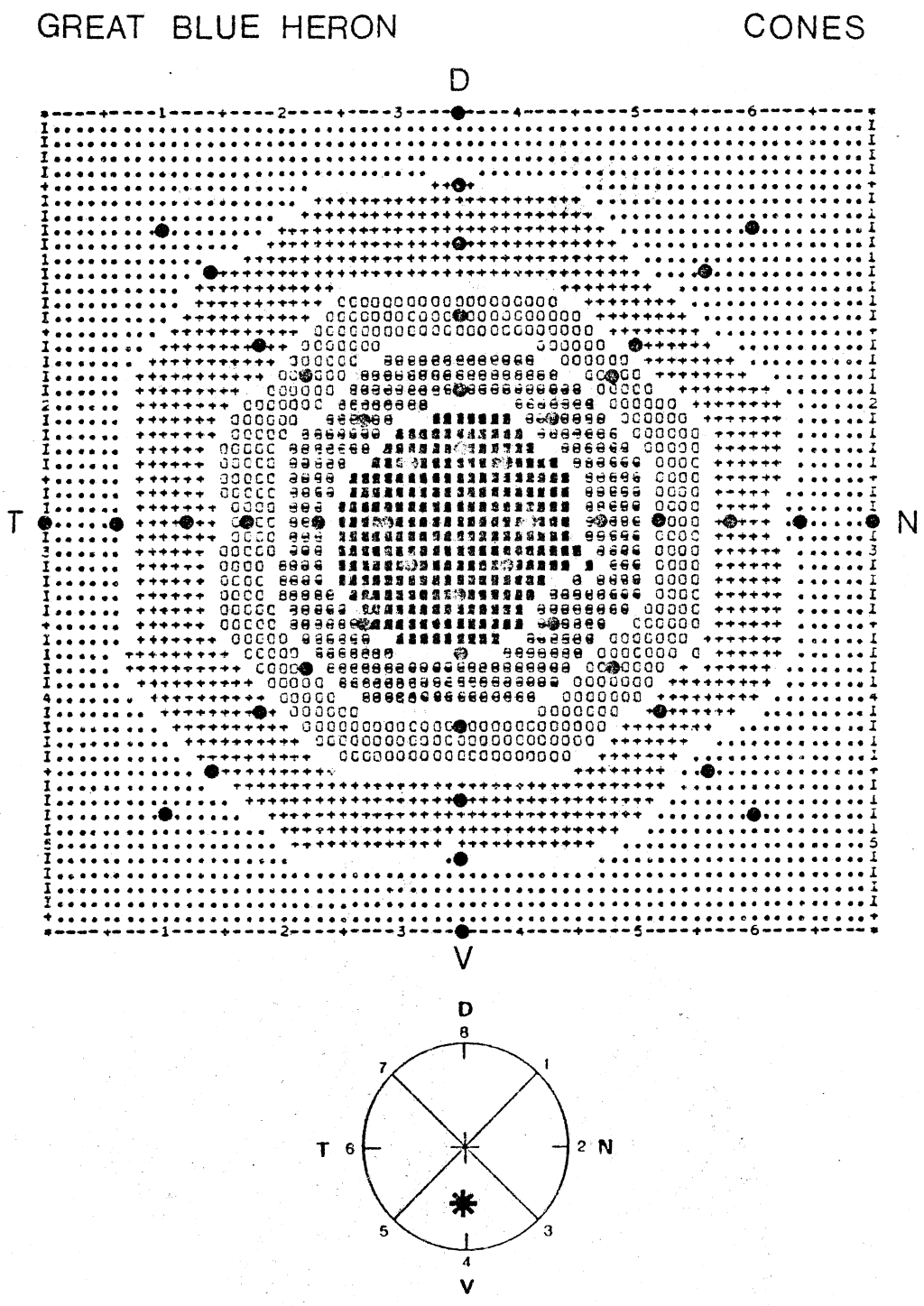

Figure 32. Iso-density map of cone cell distribution in the Great Blue Heron. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at the 95% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

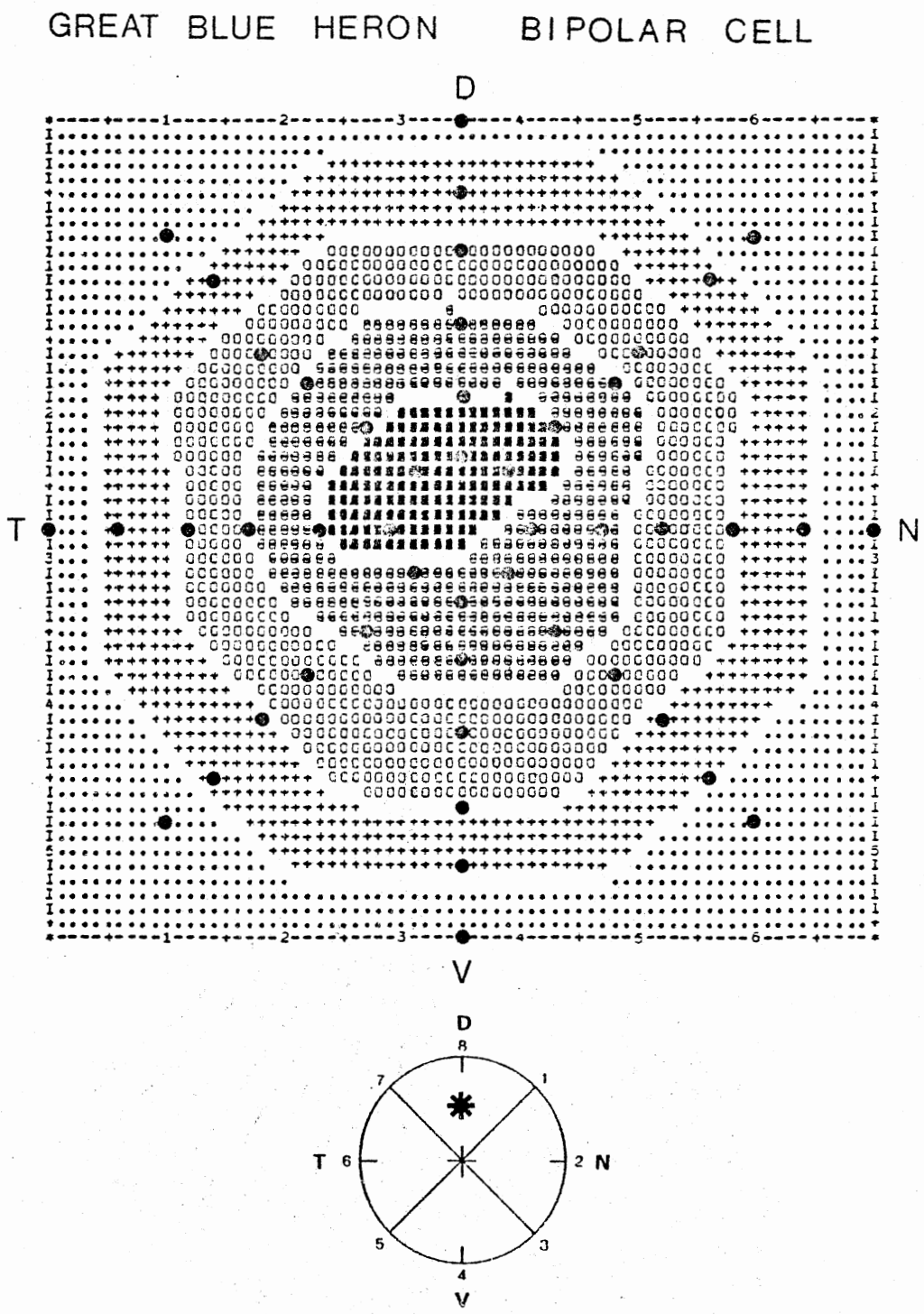

Figure 33. Iso-density map of bipolar cell distribution in the Great Blue Heron. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at 95% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

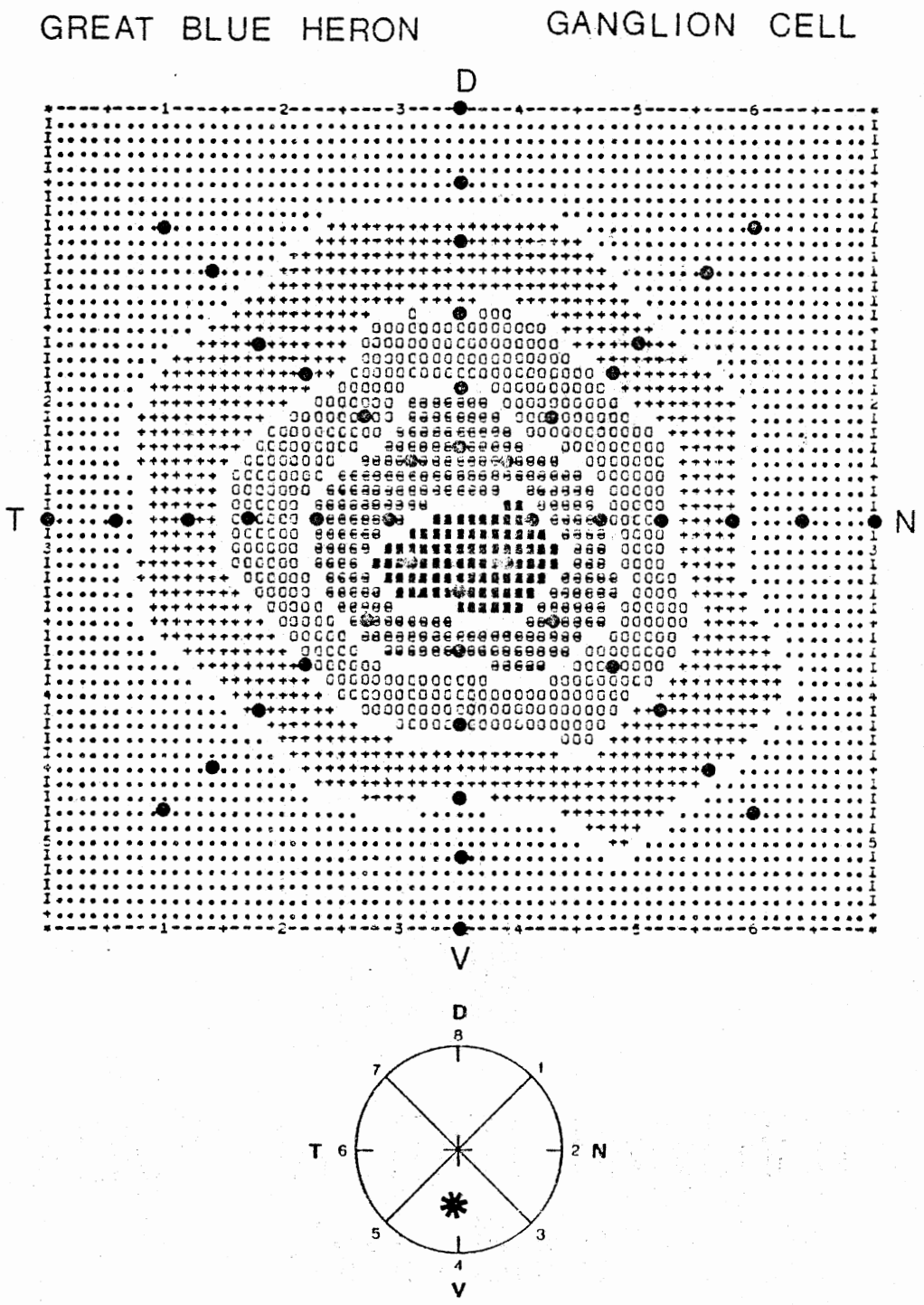

Figure 34. Iso-density map of ganglion cell distribution in the Great Blue Heron. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at 95% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

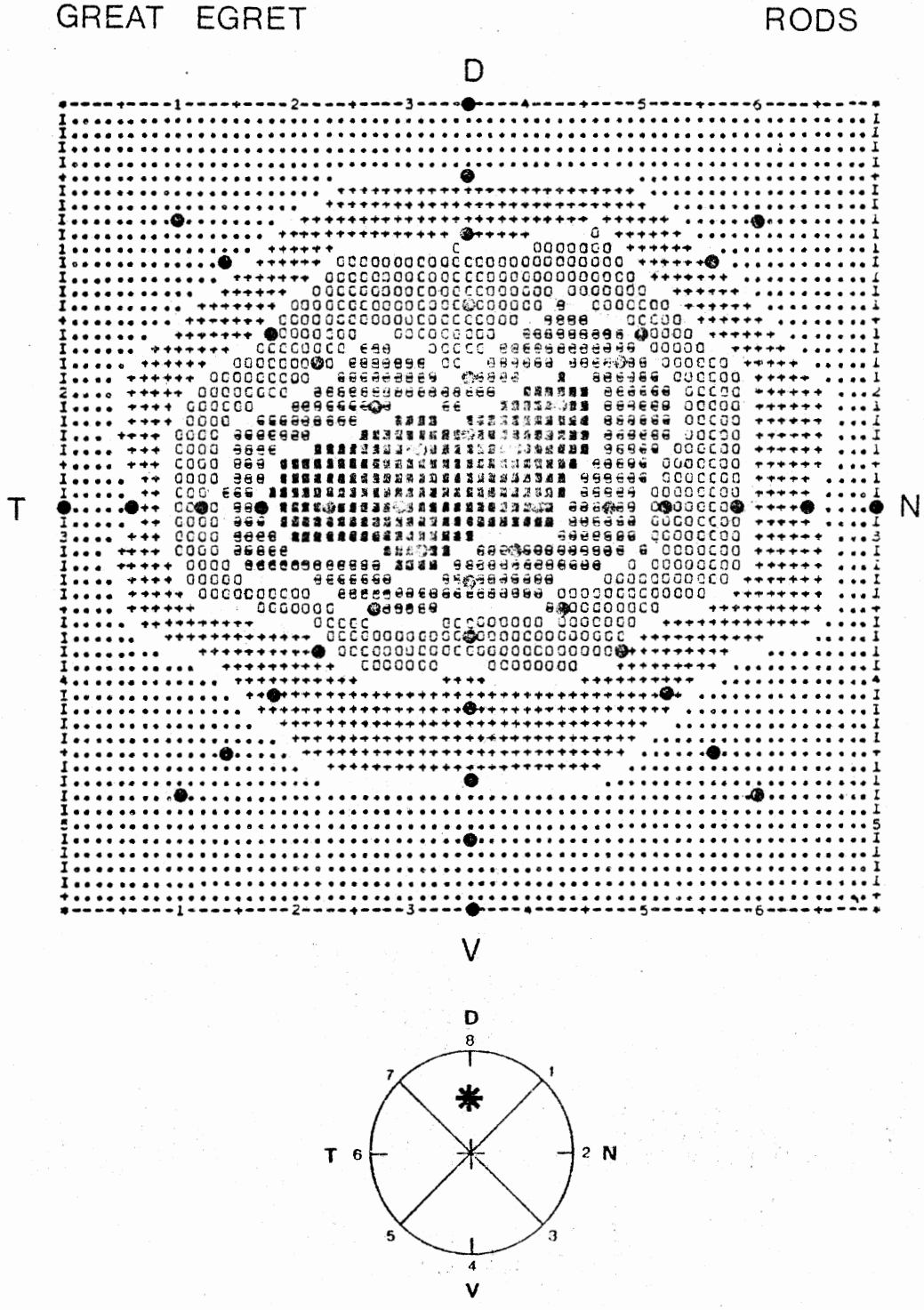

Figure 35. Iso-density map of rod cell distribution in the Great Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at 95% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

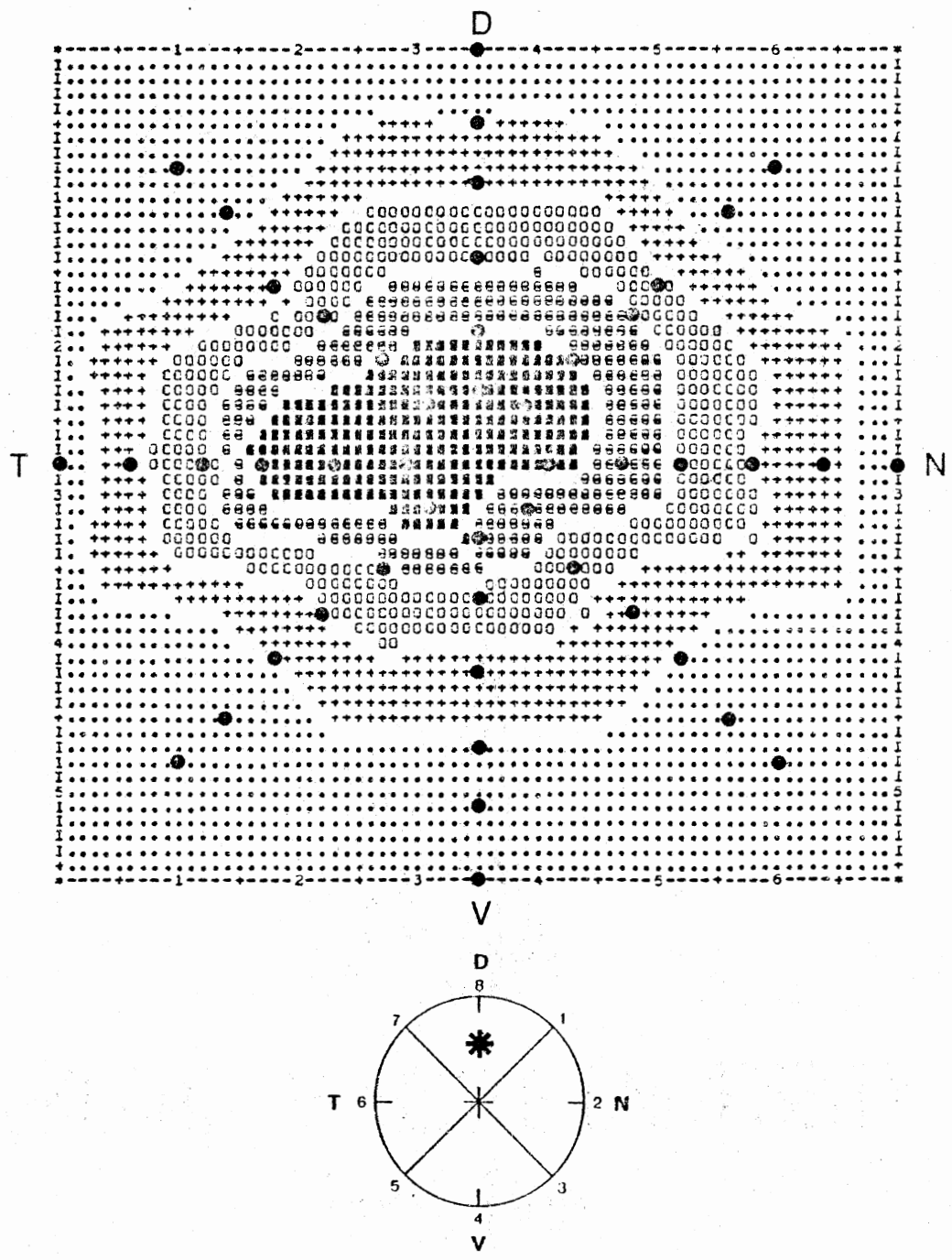

Figure 36. Iso-density map of cone cell distribution in the Great Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at 95% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

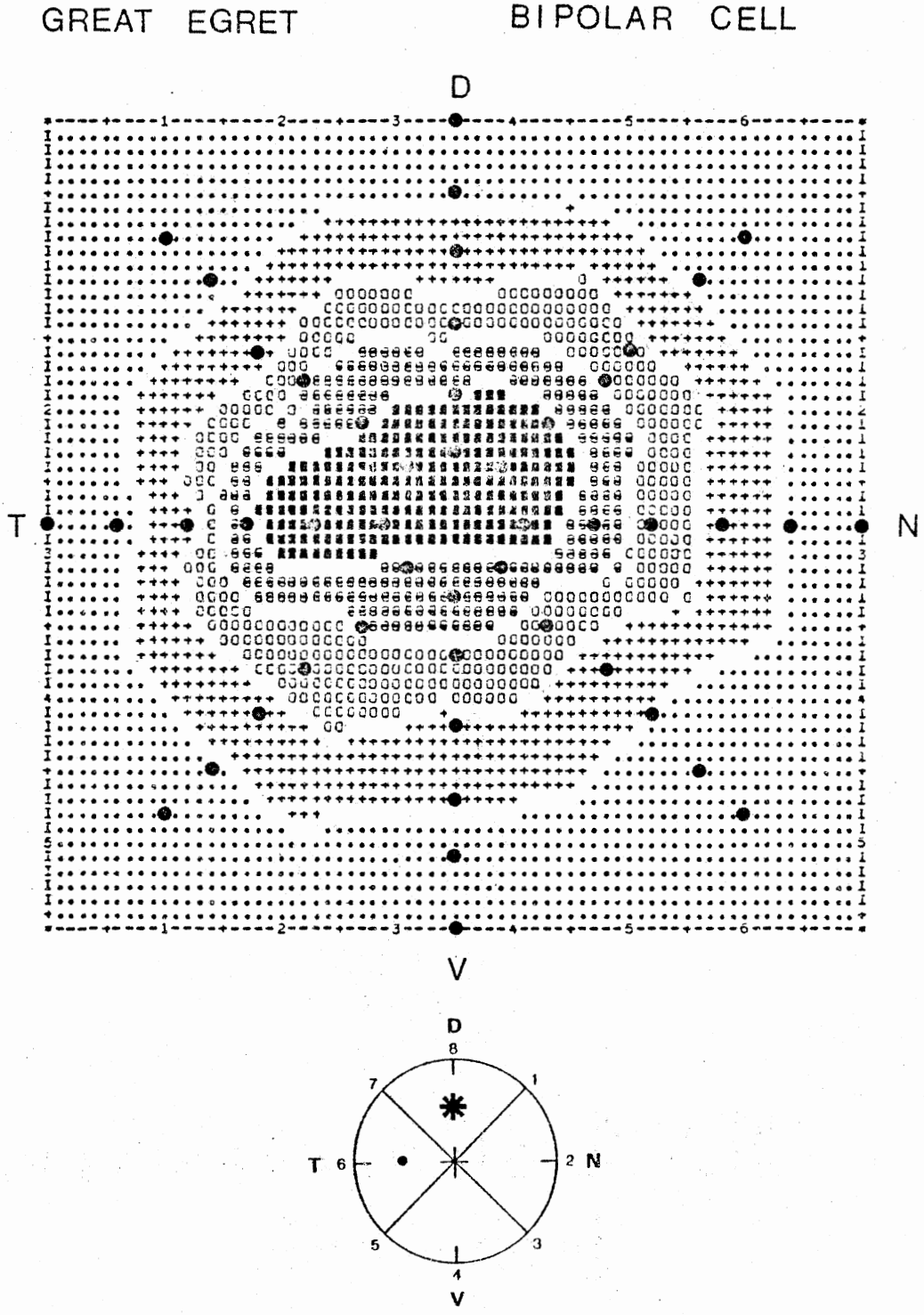

Figure 37. Iso-density map of bipolar cell distribution in the Great Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at 95% probability level. The small dot indicates differences at the 90% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

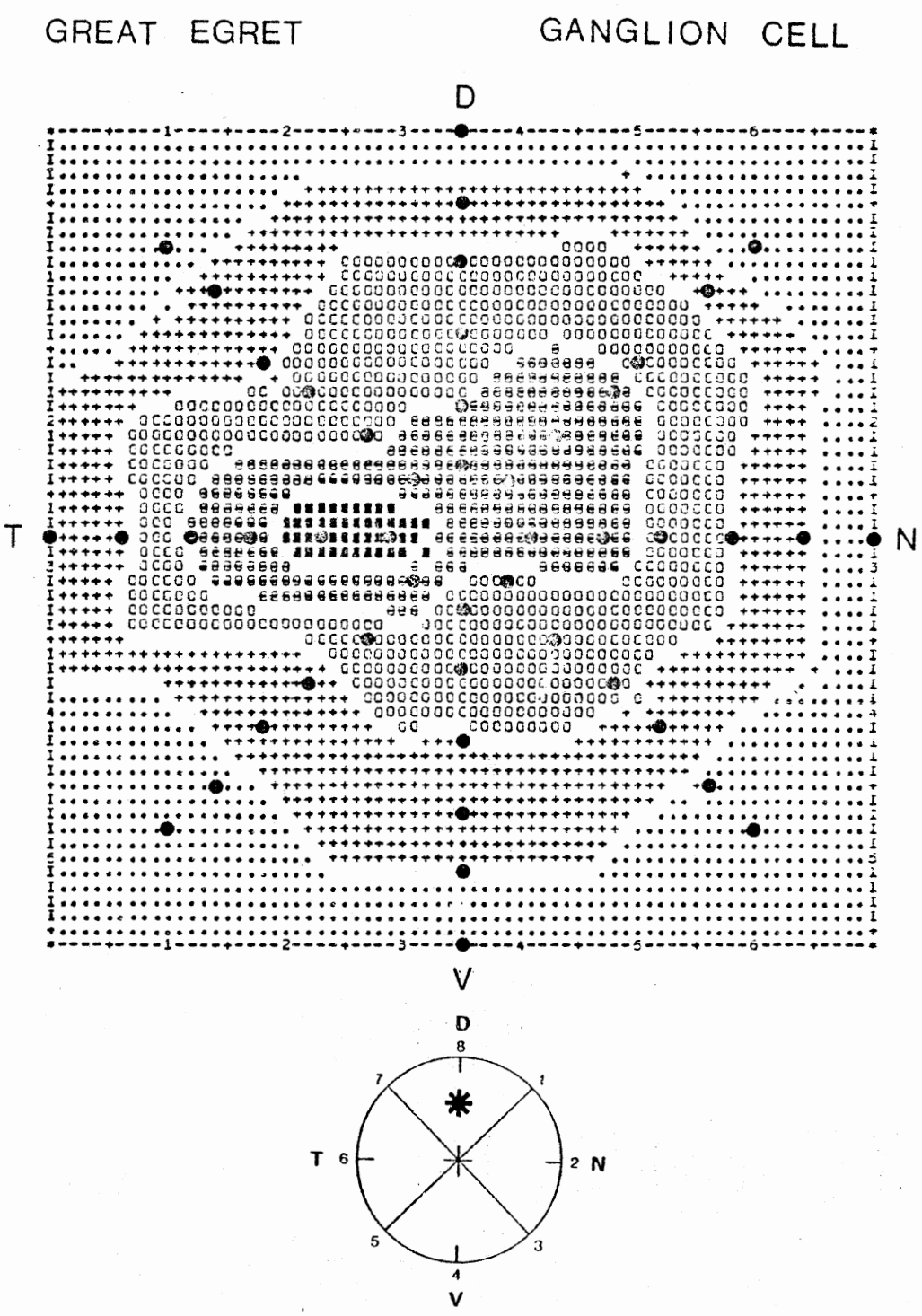

Figure 38. Iso-density map of ganglion cell distribution in the Great Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at 95% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

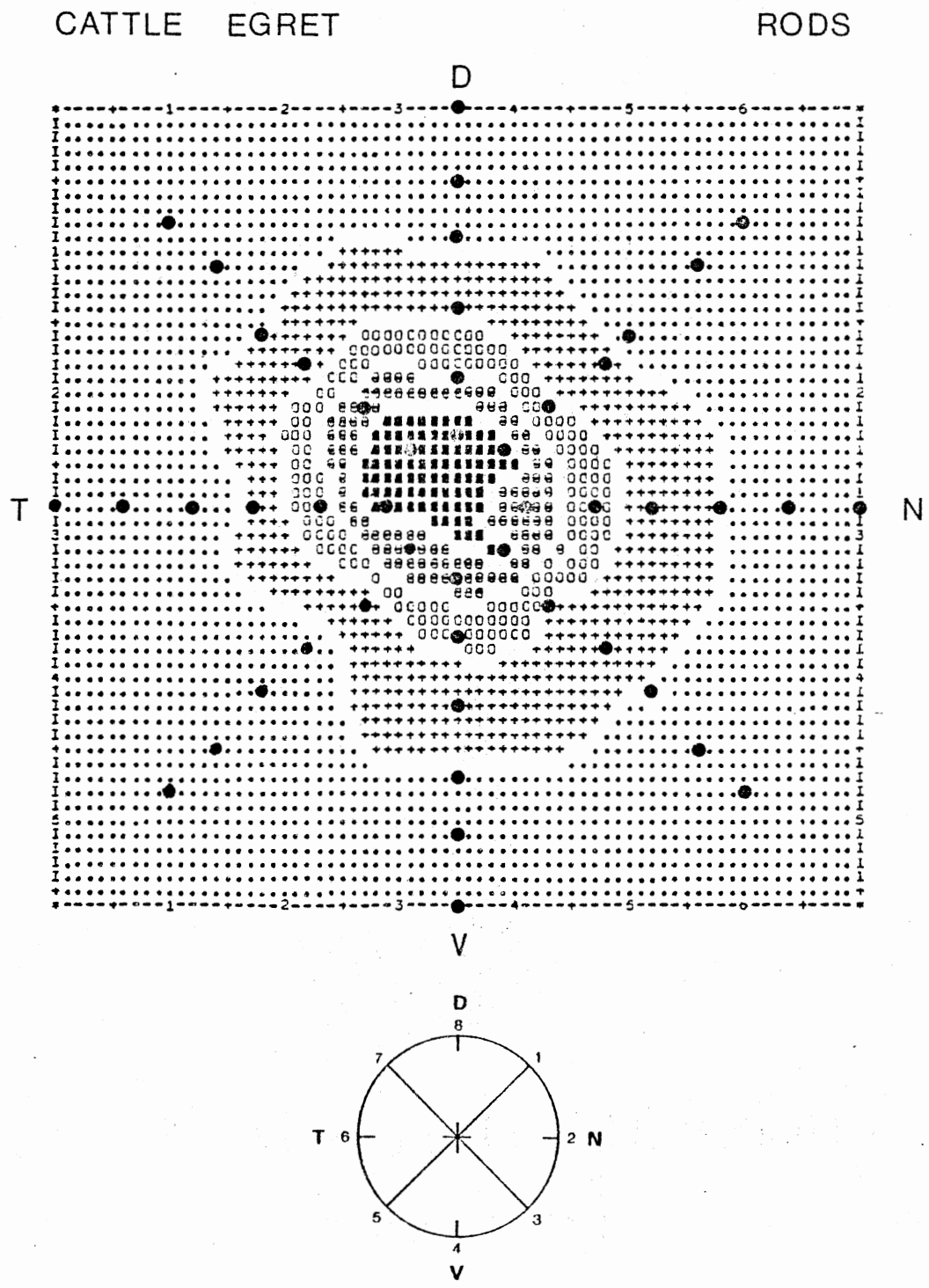

Figure 39. Iso-density map of rod cell distribution in Cattle Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. There were no significant differences between opposite quadrants. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

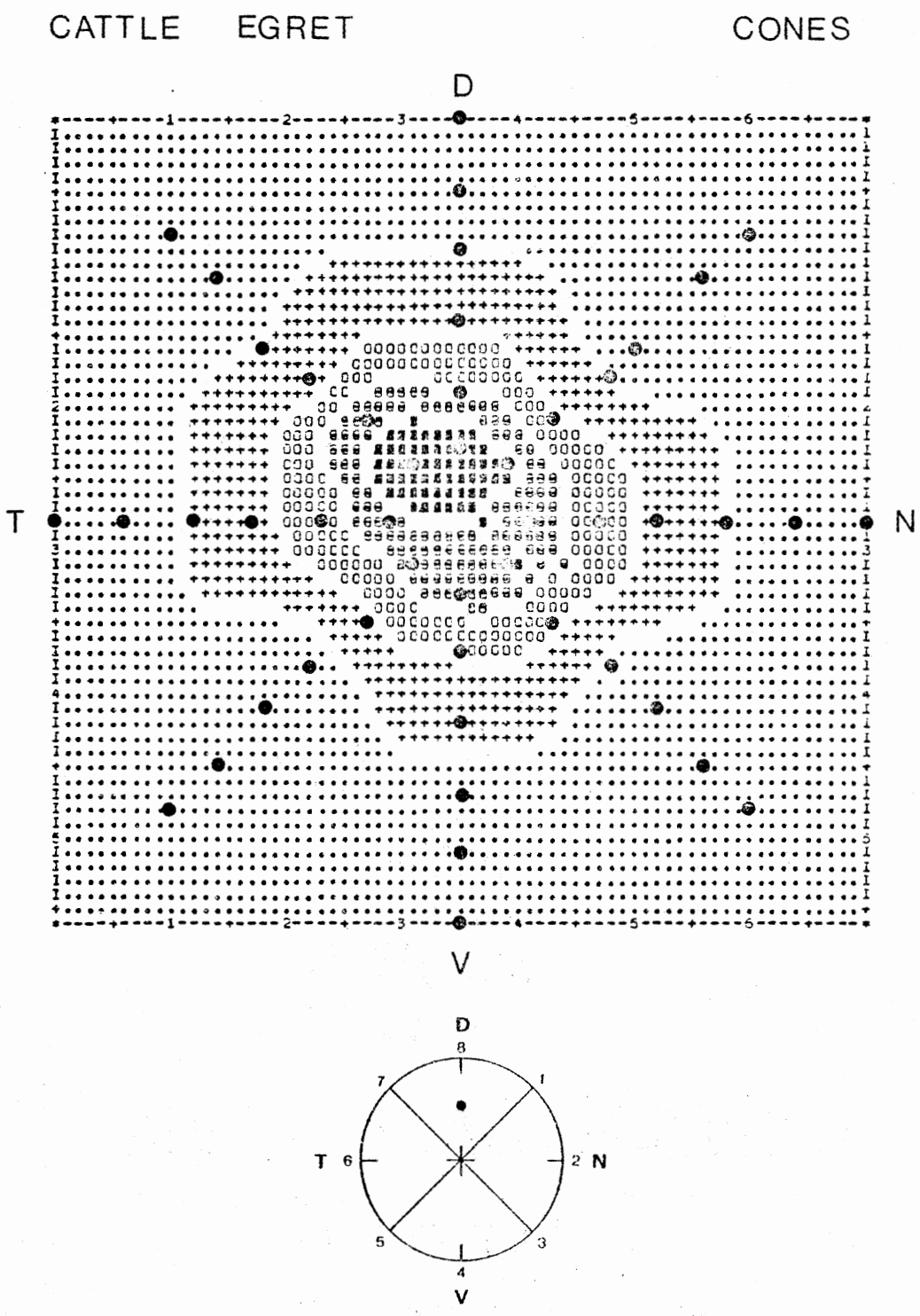

Figure 40. Iso-density map of cone cell distribution in Cattle Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The small dot in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at the 90% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

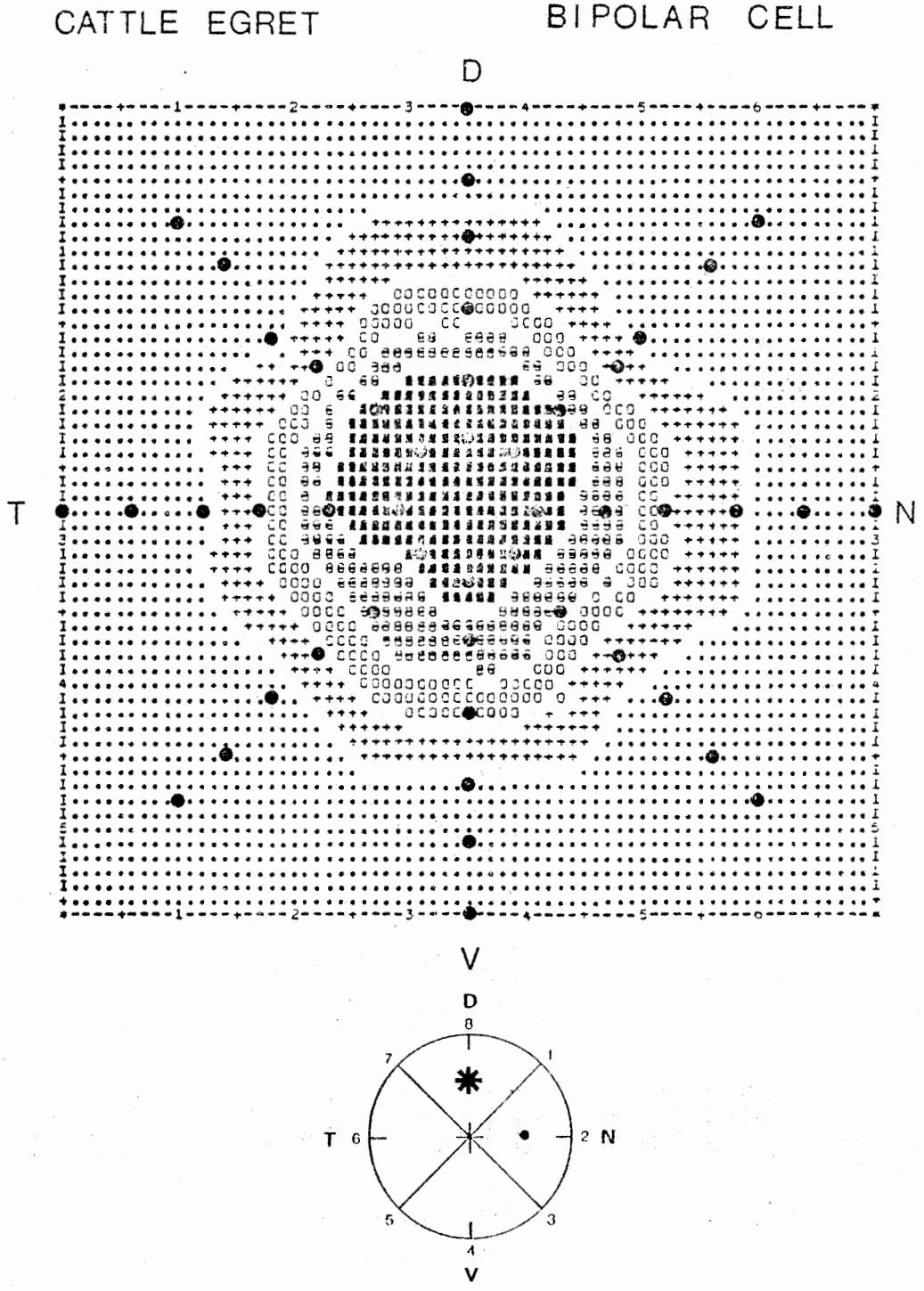

Figure 41. Iso-density map of bipolar cell distribution in Cattle Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at 95% probability level. The small dot indicates differences at the 90% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

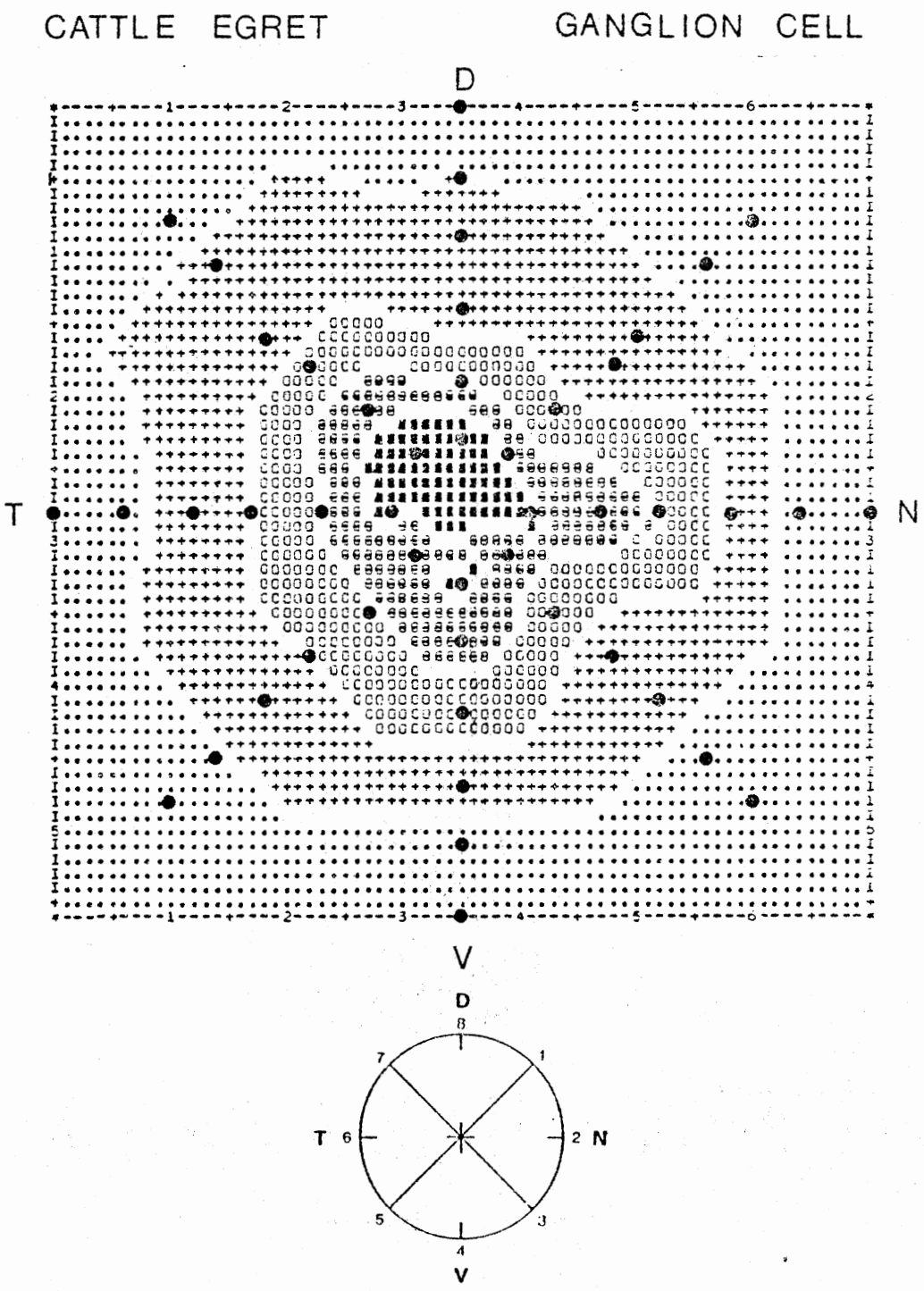

Figure 42. Iso-density map of ganglion cell distribution in Cattle Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. There were no significant differences between opposite quadrants. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

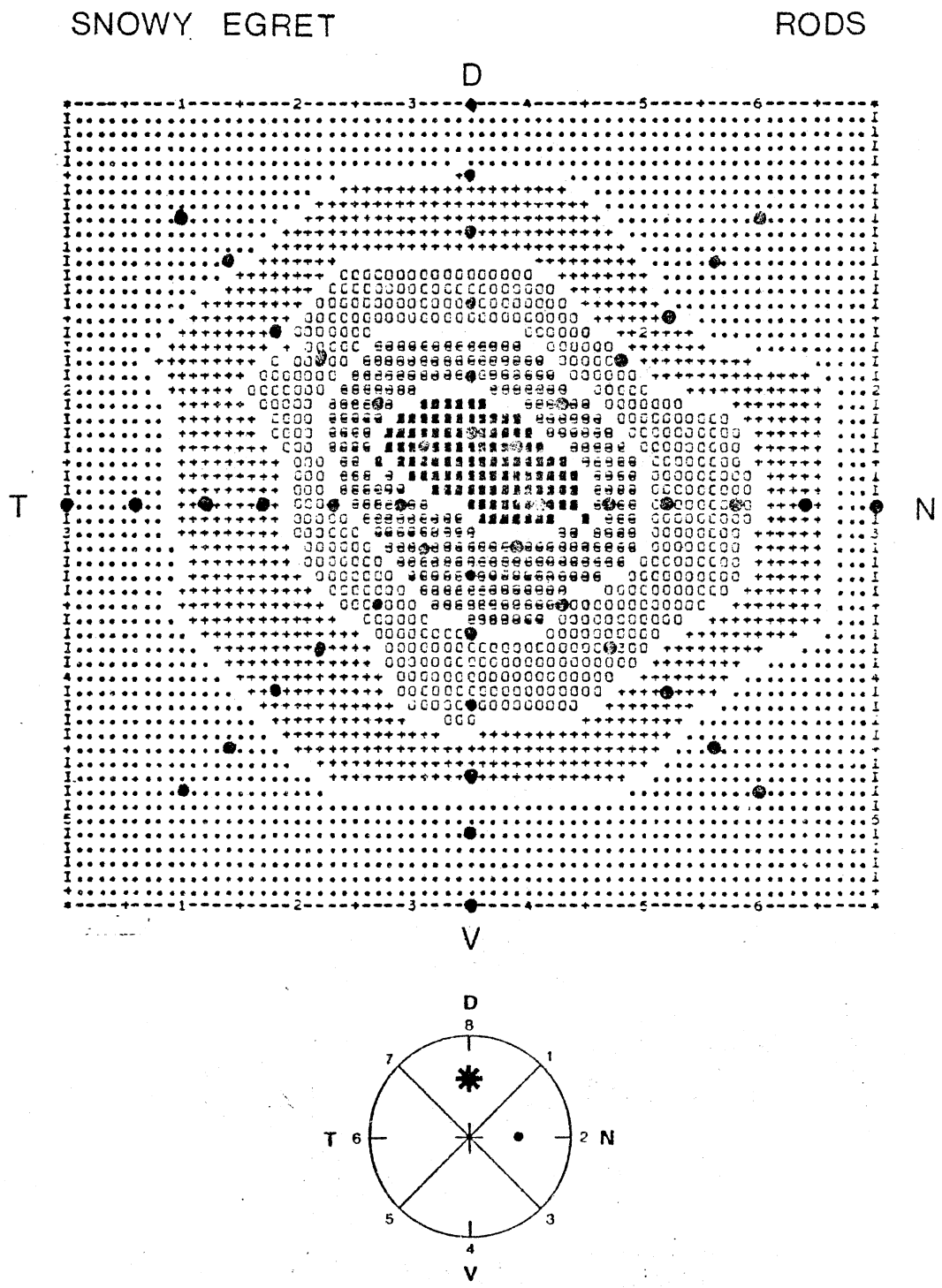

Figure 43. Iso-density map of rod cell distribution in Snowy Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at 95% probability level. The small dot indicates differences at the 90% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

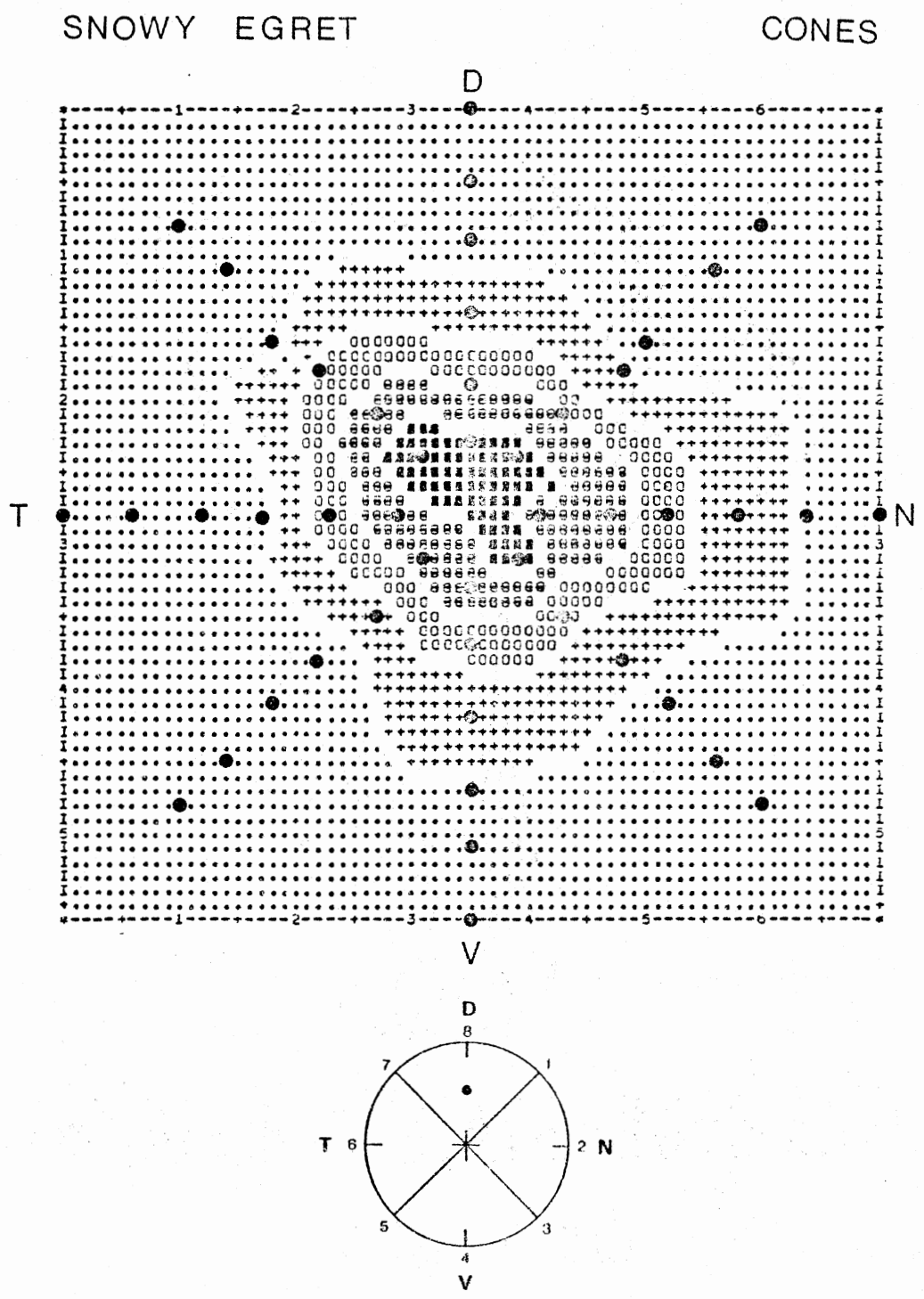

Figure 44. Iso-density map of cone cell distribution in Snowy Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The small dot in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at the 90% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

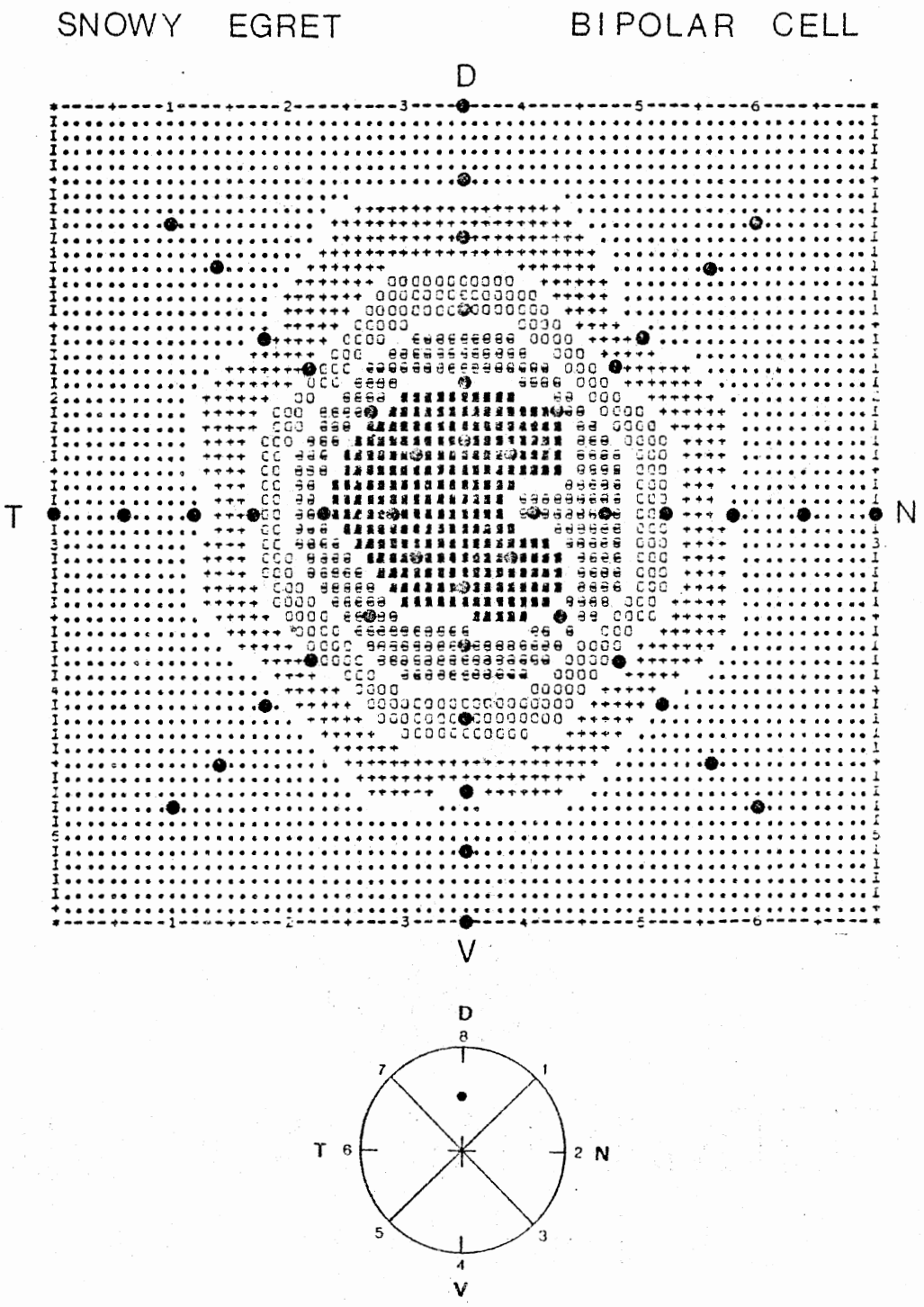

Figure 45. Iso-density map of bipolar cell distribution in Snowy Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The small dot in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at the 90% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

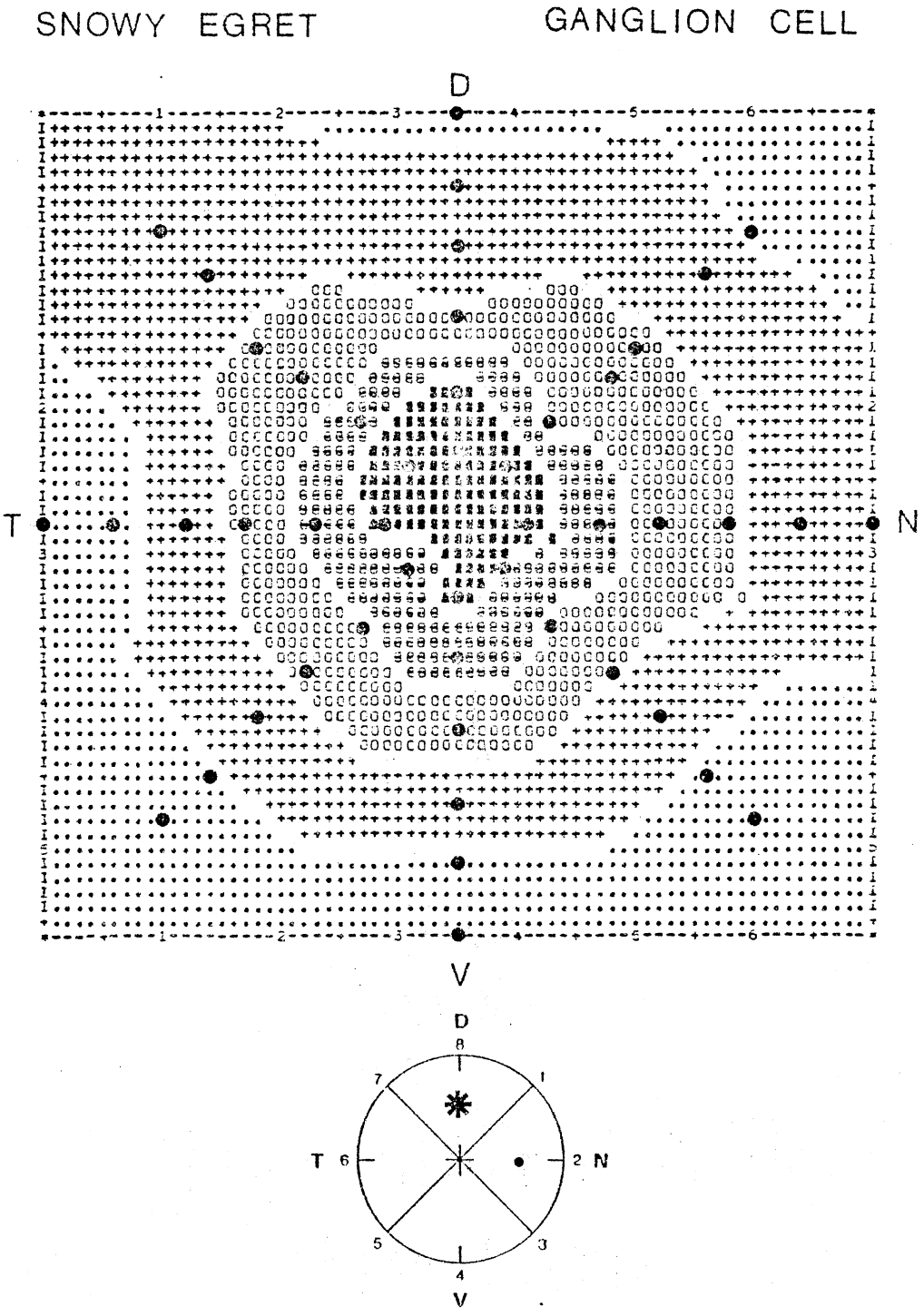

Figure 46. Iso-density map of ganglion cell distribution in Snowy Egret. Dark shading indicates areas of high cell densities. Dots within map represent sample points. The asterisk in a quadrant in the lower figure indicates a significantly larger number of cells compared to the opposite quadrant at 95% probability level. The small dot indicates differences at the 90% probability level. D, Dorsal. V, Ventral. T, Temporal. N, Nasal.

In conjunction with the information provided by the statistical analysis of retinal cell distribution the iso-density maps provide interpolated values for all points between the 48 radially arranged sample points.

Because the statistical and visual methods provide two views of the same data, the results of each are included in these figures to help aid in comparison. In dorsal versus ventral statistical comparisons the three dorsal transects (7, 8, 1) were compared with those of the three ventral transects (3, 4, 5). This eliminates some bias due to dorso-ventral orientational error. Also, transects 6 and 2 could be classified as belonging to either dorsal or ventral since they are on the boundary between the two halves. Likewise, only transects 1, 2 and 3 were compared with 5, 6 and 7 for nasal versus temporal comparisons.

The retinal cells of all four species are distributed in the same general pattern of iso-density (Figures 31-46). Concentric circles of density are characteristic of the distribution of all four species and all four retinal cell types. These concentric patterns are not simply the result of the radially arranged sample pattern. This pattern should have enough sample points distributed evenly across the eye to detect ellipses, radical dorsal/ventral or nasal/temporal differences, or visual streaks. Therefore, these concentric patterns seem to indicate gradual decreases in concentrations of retinal cells from the fovea to the ora. Also, this rate of decrease does not change radically from transect to transect.

Another aspect of these data is that only cones are found in the foveal area. The other retinal cells are moved away from the central fovea (Figure 47); therefore, high concentrations of rod, bipolar and ganglion cells may form rings of high density near the fovea rather than

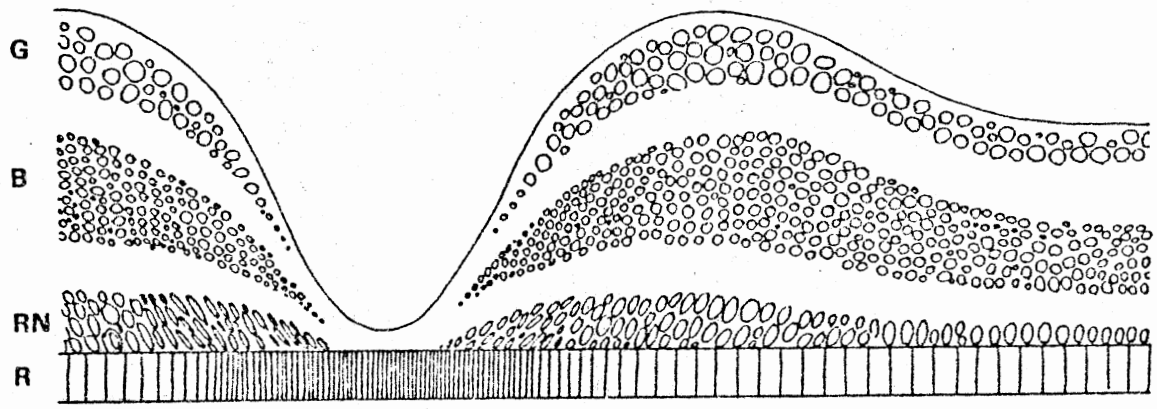

Figure 47. Hypothetical avian fovea showing how bipolar and ganglion cells accumulate near the periphery. G, Ganglion cells. B, Bipolar cells. RN, Receptor Nuclei. R, Receptors.

discs.

In spite of the overall similarities there are subtle but consistent differences in retinal cell distribution. For example, all four retinal cell types in the Great Egret form definite temporally oriented elongated central areas (Figures 35-38). However, when comparing transects 7, 8, 1 with 3, 4, and 5, they are generally not significantly different (except for bipolar cells at the 90% probability level). This trend is particularly noticeable in the distribution of ganglion cells (Figure 38). This temporal area of high visual cell concentration can also be detected when plotting these data graphically (Figure 48). This temporal area is consistent and is not shown by any other species. Great Blue Herons have consistently large and circular areas of iso-density and also show the most perfect concentric patterns.

Statistical analysis revealed tendencies for various retinal cells to be more numerous on the dorsal half of the retina. This tendency is also evident upon visual examination of these maps. These differences are, however, very slight. Nasal temporal differences are only visually evident in the Great Egret but statistical analysis did show some nasal-temporal differences for Snowy Egret rods and cones (Figures 43 and 44).

Foveal Characteristics

All four species examined exhibited well developed single and slightly temporally located foveae which are typical of many avian species. Counts were made of foveal cone densities and are shown in Table 15. These cone counts were corrected using the Abercrombie correction factor (Abercrombie 1946). Uncorrected counts were approximately 20% higher than corrected cone counts. These densities

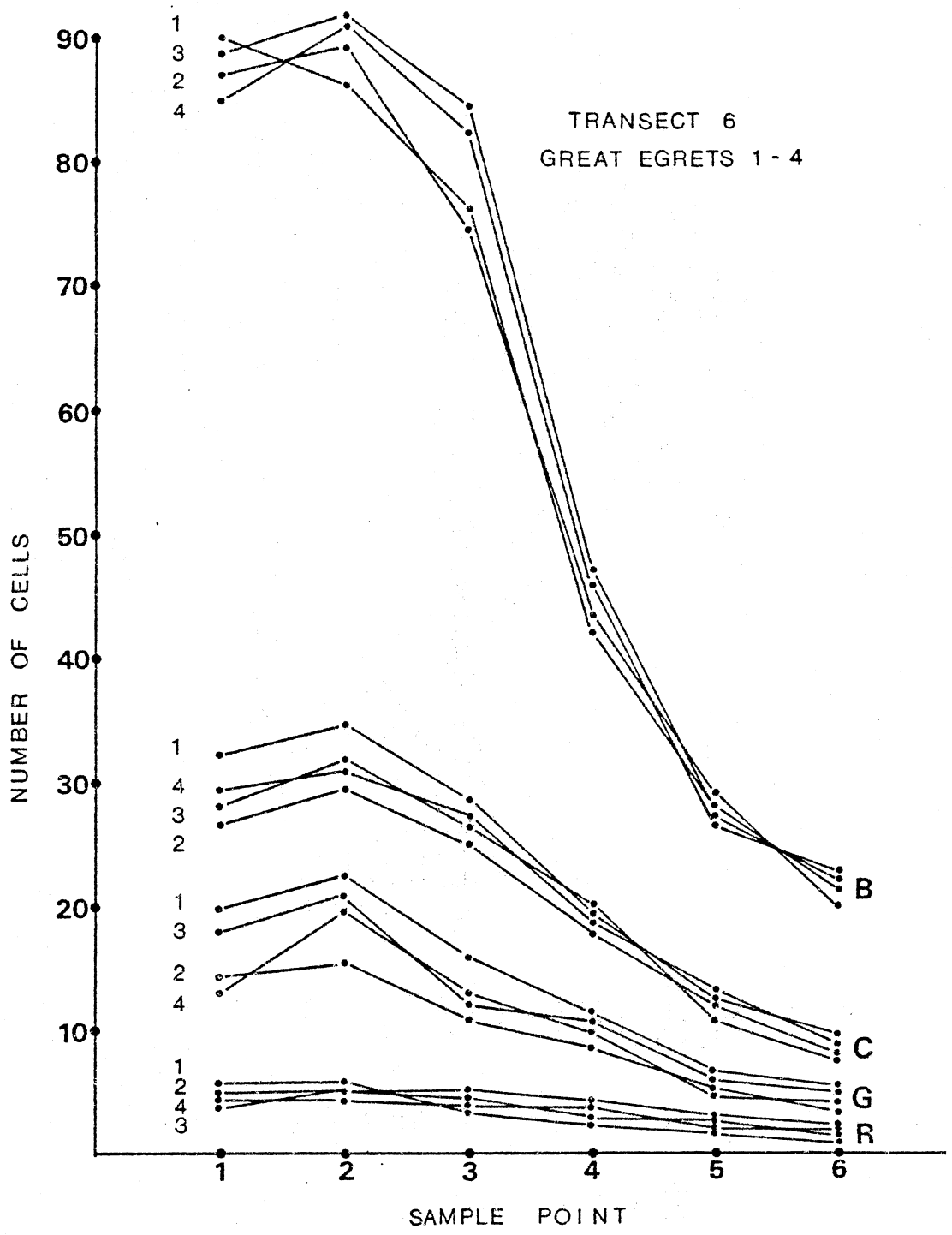

Figure 48. Variation in the average number of retinal cells for Great Egrets 1-4 at transect 6. High values of all cell types occur at sample points 1 and 2. B, Bipolar cells. R, Rod cells. C, Cone cells. G, Ganglion cells.

Table 15. Retinal planimetric densities of cone cells from the central foveae of four species of herons.

Species	Number of foveal cones per sq. mm
Great Egret	632,000
Great Blue Heron	449,230
Cattle Egret	424,615
Snowy Egret	472,000

are referred to as retinal planimetric densities and are reported as the number of cones per sq. mm since this is the typical unit of measurement for reporting avian foveal cone counts.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Sampling Accuracy

The accuracy of estimating cell populations from cross sections has been discussed by many investigators notably, Abercrombie (1946) and Agduhr (1941). Error results from the fact that although nuclei and other cell parts are easily counted, not all objects thus counted are whole. Some must be fragments because some lie partially within an adjacent section. This error is increased when sections become thinner in relation to the cell diameter or when cells have a wide range of sizes. Thus, estimates of cell densities are usually larger in uncorrected counts.

Abercrombies correction factor was not applied to the data in this study except for foveal cone counts. This was thought to be justified since relative rather than absolute values of cell populations could be just as useful for comparisons if sampling error between species is constant. To determine the extent of this error I applied the correction factor to part of the data. I compared corrected and uncorrected counts for bipolar and ganglion cells at sample point 1, transect 2 (fundus) and sample point 6, transect 2 (ora) in all four species. Uncorrected counts of bipolar cells at the fundic sample points were 15-18% higher than corrected counts. At the ora, uncorrected bipolar cell counts were 17-19% higher than corrected

values. Thus, there was only a 2-3% variation in bipolar cell counts between species. Uncorrected counts overestimated ganglion cells by 18-22% and 25-27% in the fundus and ora, respectively. The range of variation between species was 3-4%. Therefore ganglion cell counts are subject to the greatest amount of error. This is because of the larger size of the cells and the greater variation in the size of these cells.

I estimated my counting error by counting the number of cells at one sample point (as described in the methods section) and without moving the microscope stage counting them again the next day; then compared the two counts. I did this at several points and determined that my counts varied by less than 8%. I also compared my counts with those of other persons and found that the two counts varied by less than 10%. Counting error was highest for bipolar cells and lowest for ganglion cells.

The Relationship Between Ocular Morphology and Behavior

Results of this study confirm what other investigators have previously discovered about avian eye morphology. These results include: (1) the decrease in retinal thickness from fundus to ora, (2) the asymmetry in the eyes, (3) the relatively larger pectens in diurnal herons, and (4) general morphology of photoreceptors. These aspects of avian eye morphology have been reported for other species of birds (Walls 1942; Prince 1956; Polyak 1957) but not specifically for herons. Even though this is not necessarily new information, these results deserve a more detailed discussion.

The nasal asymmetry of the eyeball and the visual angles of these four species (Table 6) indicate that they probably have good peripheral vision even though the visual axis is more oriented forward. The degree of this nasal asymmetry does not, however, seem to be related to feeding behavior or body size, since the Cattle Egret (a small active feeder) has a similar percentage of the retinal area on the nasal side as does the Great Blue Heron (a large passive feeder).

The relative pecten sizes correlate well with circadian activity patterns (Table 8). Results show that species with retinae composed of a high percentage of rods (Great Blue Herons and Cattle Egrets) have relatively small pectens while those with more cones (Snowy and Great Egrets) have relatively large pectens. These data support the theories of Dunn (1968) and Wigstrand and Munk (1965) that larger pectens are correlated with a relatively higher percentage of cones in the retinae and thus a higher metabolic rate.

The foveae of these four species show no variation with respect to circadian activities or feeding patterns. All four species have a well developed, single, slightly temporally located fovea. Even the relatively nocturnal Great Blue Heron has a fovea, but it is slightly shallower than the other herons. This may indicate that although Great Blue Herons do exploit a nocturnal niche at certain times of the year, they cannot be considered to be highly specialized for a nocturnal lifestyle. Woods (1917) examined the eyes of a captive Great White Heron (Ardea occidentalis) with an ophthalmoscope yet reported that it had no fovea. This bird is now considered to be a subspecies of the Great Blue Heron and is referred to as Ardea herodius occidentalis. It is surprising that the Great Blue Heron would have such a well developed

fovea and yet this subspecies would not.

The relative ocular size data (Table 9) may indicate that there is a lower limit to eye size in these birds. Apparently, the particular life style of Herons dictates that eyes and corresponding retinal area cannot go below a certain minimum size. This hypothesis is convincing when one considers the reliance birds place on their visual systems. Walls (1942) stated that aquatic birds have relatively small eyes, but the herons examined in this study have relatively large eyes.

The overall ratio of cone to ganglion cells (Figure 22) appears to be quite unusual when compared to ratios reported for other birds. Prince (1956) stated that in most birds the ratio of cone to ganglion cells is approximately 1:1; however, the ratio of cone to ganglion cells is not frequently reported. In the herons I examined the overall ratio of cone to ganglion cells ranged from approximately 1.3:1 in the Cattle Egret to as high as 1.5:1 in the Great Egret. An extremely large error in differentiating between rod and cone cells would be necessary to account for this unusual ratio. I feel that the ganglion cell to cone cell ratios are probably accurate. Rod and cone cells are morphologically very different and their various parts are often separated spatially. Thus, an error of up to 50% would not be expected, nor would an error this high be expected in ganglion cell counts.

It is surprising that Cattle Egrets have such a high (33.2) percentage of rods in the retina. Ecological studies have not reported nocturnal behavior in this species yet they have the same proportion of rods as does the relatively nocturnal Great Blue Heron.

The computer maps of visual cell distribution are perhaps the most valuable information obtained from this study. Analysis of these maps

forces one to conclude that in general, the distribution of retinal cells in these birds is much more similar than different, with the exception that the central area of Great Egrets is more oval and extends more temporally than that of the other species. Also, the patterns of concentric isodensity lines which vary gradually and evenly in all directions from the fovea are patterns typical of nocturnal or relatively unspecialized eyes (Walls 1942). The only conspicuous retinal specialization is the deep fovea found in the eyes of these herons; otherwise, the eyes are very generalized. This is certainly not true for all Ciconiiformes since Wood (1917) has shown that other species may have multiple foveae or visual streaks. It is not known if the herons used for this study could be considered typical for the majority of Ciconiiformes.

When viewing the maps of retinal cell distribution, it is also evident that although there are statistical differences between mean numbers of retinal cells on dorsal versus ventral aspects of the retina, the differences between these two halves appear slight when viewed visually, and therefore biologically they may not be significantly different even though probability values for statistical differences in means do seem very high for some retinal cells.

The retinal planimetric densities (Table 15) of these herons appear to be intermediate when compared to those reported for other birds. For example, the house sparrow (Passer domesticus) has about 500,000 cones per mm^2 in its fovea while the common European Buzzard (Buteo buteo) is reported to have over 1,000,000 per mm^2 (Rochon-Duvigneaud 1943). Other vertebrates such as the Chamaeleon (Chamaeleo sp.) have approximately 750,000 and humans about 200,000 cones per mm^2 in the central fovea

(Walls 1942). Birds and reptiles typically have higher planimetric densities than do mammals, fish or amphibians (Prince 1956).

These densities in themselves do not reveal much about the visual acuity of an animal. The size of the eye, the distance from the lens to the retina, and neurological connections can also affect the number of photoreceptors involved when the lens casts an image on the retina. All these factors thus affect visual acuity.

A better estimate of potential visual acuity is obtained from a quantitative descriptor called visual planimetric density (Fisher and Easter 1979). Visual planimetric density is defined as the number of elements per unit of retinal surface as viewed from the lens and measured in visual degrees. This descriptor is obtained by multiplying the retinal planimetric density by the retinal magnification factor (squared). The retinal magnification factor takes into account the size of the retina and the axial length of the eye and is obtained by dividing the retinal length by the degrees of visual angle subtended by the eye.

When visual planimetric densities are calculated for these four species of herons, the values shown in Table 16 are obtained. Great Egrets have the highest visual planimetric density, followed by the Great Blue Heron, Cattle Egret and Snowy Egret, in order of descending densities. The two larger herons (Great Egret and Great Blue Heron) have approximately twice the visual planimetric densities as the smaller herons. Also, the two large herons have densities which are very similar to each other but are obtained in very different ways. The Great Egret obtains a high visual planimetric density by having very densely packed foveal cones (nearly 200,000 more per mm^2 than the Great

Table 16. Visual planimetric densities of four species of herons. All values are means (n=4).

Species	Foveal cone diameter	Retinal length (mm)	Visual angle	Retinal magnification factor (mm/°)	Visual planimetric density
Great Egret	1.2 m	27	146°	.184	21,396 cones/°visual angle
Great Blue Heron	1.5 m	31	145°	.213	20,215 cones/°visual angle
Cattle Egret	1.5 m	23	150°	.153	9,766 cones/°visual angle
Snowy Egret	1.3 m	20	146°	.136	8,496 cones/°visual angle

Blue Heron). The Great Blue Heron has less densely packed foveal cones but still attains a high potential acuity by having a generally larger eye and correspondingly longer retinal length. An important question arises from these results; why do the larger passive feeders (Great Egret and Great Blue Herons) have nearly twice the potential visual acuity as the smaller active feeders (Snowy and Cattle Egrets)? It seems that the smaller active feeders should have higher potential acuity since they feed on smaller prey. I speculate that not only the size of the prey, but the distance at which the prey is detected and seized is a factor which may influence the extent to which high visual acuity is selected for in these birds. In general, the smaller active feeders can move up close to discriminate between various potential prey items, and they seize these prey items at a relatively close distance from the eye. In contrast, the passive feeders do not usually move towards prey but rather wait until prey moves within striking distance. Because they are larger (and therefore taller), they may employ increased visual acuity to discriminate and accurately seize this prey at a greater distance from the eye than do the smaller active feeders. Both active and passive feeders frequently feed on insects (Hancock and Elliot 1978) and other small prey for which high visual acuity would be an asset, especially for the larger birds which must catch them at a greater distance.

Another factor that must be considered is that mechanisms other than high visual planimetric densities may be employed by these herons to detect small prey. For example, there may be physiological adaptations in the eyes, such as flat edge detectors which make the eye very sensitive to small moving objects. These have been reported in

other vertebrates (Lythgoe 1979) but require physiological and behavioral analysis to be detected.

Douglas Mock (personal communication) has recently discovered that Great Blue Herons and Great Egrets, although similar in body size, feed on prey of significantly different size ranges. Great Egrets feed on prey items (usually small fish) which are about 80% smaller than those utilized by Great Blue Herons. This may account for the fact that although the Great Egret is slightly smaller, it has a higher potential visual acuity due to its densely packed foveal cones. These high cone densities in conjunction with the temporal area in this species may be adaptations which help this large passive feeder to utilize relatively small prey items.

In conclusion, it is evident that the species selected for this study are most different with respect to the size of the pectens, visual planimetric densities, and proportions of rod to cone cells in the retinae. These differences are correlated with circadian activity patterns and passive vs. active feeding behavior. The distribution of retinal cells is less variable, but the Great Egret does show differences in that it has a temporal area which may be an adaptation for feeding on small prey. Therefore, the hypothesis that the eyes of closely related birds that have diverse ecological lifestyles and behaviors will be morphologically different, particularly with respect to the spatial distribution of retinal cells is valid, except that, the range of variation in retinal cell distribution is not as wide as expected. Since the proportion, type, density and distribution of retinal cells is best correlated with circadian activity patterns and passive vs. active feeding. Future investigations of this type could

help substantiate these correlations by using both active and passive feeders. These may include; Reddish Egret (Dichromanassa rufescens), American Bittern, Louisiana Heron (Hydranassa tricolor). Also, a strongly nocturnal species such as the Yellow or Black-Crowned Night Heron would be desirable.

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Education: Graduated from Miami High School, Miami, Oklahoma, in May, 1965; received the Bachelor of Science degree in Wildlife Ecology from Oklahoma State University in 1973; received Master of Science degree in Wildlife Ecology from Oklahoma State University in 1975; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in July, 1982.

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