

Developmental changes and specialisations in ocular anatomy and retinal morphology of the bighead tubeshoulder *Holtbyrnia anomala* Krefft, 1980 (Platyroctidae; Teleostei)

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Abstract

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The present study examines retinal morphology of the alepocephaloid deep-sea teleost *Holtbyrnia anomala* KREFFT, 1980 using light, and electron microscopy. Only few changes in retinal architecture are recorded through ontogeny. The development and morphology of specialisations related to vision are discussed. The convexiclivate fovea located in the temporal retina contains thin rod photoreceptor outer segments (OS) elongated to more than double the length found extrafoveally. Only rods in a single bank were present. Novel application of retinal wholemount technique reveals topographic densities of both photoreceptor (PR), and ganglion cell (GC) populations, presented in topographic maps of retinae from juvenile and adult specimens. Significant alterations in retinal topography through ontogeny are recorded and compared to

similar specialised regions of both diurnal teleosts and other Alepocephaloids. Peak densities of PR and GC populations are found in the fovea ($53 \times 10^4 \text{ mm}^{-2}$ and $42 \times 10^3 \text{ mm}^{-2}$ respectively). A vertical band shaped region with moderate cell density fluctuations is present in the dorsal fundus retina of juveniles, and vanishes through ontogeny, while a **possible** horizontal visual streak with increase in particularly PR density develops, the first occurrence of such a region reported from deep-sea fish. A dorso-rostral specialised region with high GC densities ($12.5\text{-}17 \times 10^3 \text{ cells mm}^{-2}$) is found in both juvenile and adult specimens. Distinct annuli like patterns of ganglion cell proliferation are related to asymmetrical long-term retinal growth. Resolving power in cycles per degree is calculated for PR and GC populations separately, and shows regional variance across the retina. The mean resolving power of the fundus retina more than doubles from juvenile to adult. Values derived from both cell populations are comparable in the fundus retina, while a peculiar divergence is observed for peripheral and foveal regions. Resolving powers of retinal regions in *H. anomala* are compared with those found in other teleost species. The consistency in foveal morphology through ontogeny suggests foveal vision to be important to all ontogenetic stages examined. Comparisons of foveal morphology are made with other alepocephaloids, and morphological implications of the convexiclvate foveal function as a movement detecting region with high resolving power are proposed and discussed. Findings and implied theories on feeding behaviour are related to what little is known of the life strategy of *H. anomala*.

Introduction

The bighead tubeshoulder *Holtbyrnia anomala* KREFFT, 1980 (Fig. 1) is the dominating platytroctid species (family Platytroctidae ROULE, 1919, previously Searsiidae PARR, 1951; Sazonov 1980) in the deep water basins around Southern Greenland (Nielsen and Bertelsen 1992). Although it has been recorded from epipelagic depths (from 200 m) most reports are from bathy, to benthopelagic depths (to 2700 m) (Quero *et al.* 1984) as in the present study. Like in most other deep-sea teleosts which are generally collected moribound, little or no data on feeding biology is available platytroctids (Quéro *et al.* 1984)

Fig. 1 approximately here.

One of the aims of the present study was to determine if ocular, and in particular retinal morphology is specialised to the extent commonly found in teleosteans inhabiting the meso, to bathypelagic zone (200 to 1000 m depth, and below) where ambient light intensity rapidly decrease towards attenuation and visual cues result from static or flashing point-sources of bioluminescence (Herring 1989). Many species of the superfamily Alepocephaloidea (including genus *Holtbyrnia*) are attracted to light, as demonstrated by increased capture rates of these species in trawls fitted with artificial light sources (Swinney *et al.* 1986).

Only three of 37 known platytroctid species (Matsui and Rosenblatt 1987) have received attention in histological studies of visual morphology: Brauer (1908) examined *Platytroctes procerus* BRAUER, 1906, a junior synonym of *Platytroctes apus* GÜNTHER, 1878. *P. apus* was also examined in an electron microscopy study by Locket (1971), an account in overall consistency with the present study.

Brauer (1908) also examined specimens referred to as *Bathytroctes rostratus* GÜNTHER, 1878 while Parr (1960) argued that at least some of these specimens were *Searsia koefoedii* PARR, 1937. The remainder of Brauer's *Bathytroctes* specimens were proposed by Munk (1975) to be *Mentodus rostratus* GÜNTHER, 1878, which is now placed in synonymy with *H. anomala* (Kreffft 1980). Munk (1966) examined *Platytroctegen mirus* LLOYD, 1909. These studies all described retinae with a single bank of elongated rod photoreceptors and a temporal convexiclvate fovea, with an increased density of elongated rod outer-segments (OSs). In the present study, retinae from juvenile and adult specimens of *H. anomala* were examined with LM and TEM with emphasis on the morphology and ultrastructure of the fovea.

Previous examinations of retinae from around 20 alepocephalid species have illustrated marked varieties in morphology, contrasting the uniformity of platytroctid retinae. Some alepocephalid retinae are, however, virtually identical to the platytroctid retinae examined previously and here e.g. *Conocara macroptera* (VALLIANT 1888) (Locket 1992 and Collin 1997). Munk (1968) found three banks of rod OSs in the fundus retina, and one bank in the temporal fovea of *Bathylaco nigricans* GOODE & BEAN, 1896 and so far the complexity of the alepocephalid retina culminates in *Bajacalifornia megalops* (LÜTKEN 1898) studied by Locket (1992) under the common synonym *B. drakei* (BEEBE, 1929). Locket recorded one or two poorly defined banks of rods in the retina proper, while the fovea housed banks of rod OSs accumulating through post-metamorphic life in a pronounced fovea externae with 28 banks recorded in a 39 mm specimen.

The long-term growth related aspects of ocular and retinal morphology in the post-metamorphic ontogeny of *H. anomala* are described. Parr (1960) encouraged clear separation between pre, and postmetamorphic specimens of any teleost species when conducting structural research.

Platytroctids are *c* 30 mm around metamorphosis (Parr 1960) and with a minimum specimen length of 60 mm (Table 1) this study exclusively addresses postmetamorphic specimens.

Table 1 approximately here.

The vertical distribution of both pre, and postmetamorphic specimens of the platytroctids *Holtbyrnia bauchoti* MEYER & NALBANT, 1972 (as *H. latifrons*) and *Sagamichthyes abei* PARR, 1953 has been shown to coincide (Matsui 1991), and thus the retinal morphology of premetamorphic larvae may be comparable to that of recently settled juveniles in terms of sensitivity and spatial resolving power. However, for most shallow water teleost species with pelagic larvae, the ocular morphology exhibits drastic changes prior to, and during metamorphosis (Fernald 1990; Munk 1990; Shand 1997).

In this study, retinal wholemounts of photoreceptor (PR) and ganglion cell (GC) densities from juvenile and adult *H. anomala* yielded topographic maps of the cell populations. Calculations of resolving power from PR and GC population densities provide evidence of regional specialisations of this retina.

Recent years have seen a marked increase in these types of retinal studies (Stone 1981; Collin 1997).

The ‘dual-cell survey’ (using a single wholemount for successive studies of both PR and GC populations) was suggested by Curcio *et al.* (1987) as an option for comparative studies of man and Pigtail Macaque.

This method allows quantitative description of morphologically identifiable retinal cell populations due to minimisation of tissue distortion (swelling and shrinkage).

Additionally the wholemount technique has made it possible to reveal the topography of retinal regions of high cell density (retinal RHCDs) with respect to both PR and GC populations. Prior to quantitative wholemount studies, the criterion for arguing a RHCD was solely increased PR density, since the layering of GCs otherwise complicated counting procedures (Collin 1997).

The wholemount technique was only recently applied to the retina of members of the Alepocephaloidea by Collin and Partridge (1996), who determined the GC topography and calculated spatial resolving powers for two mesopelagic species (*S. koefoedii* and the alepocephalid *Xenodermichthyes copei* (GILL, 1884) the bathypelagic *P. apus* and four benthopelagic alepocephalids (*Roulenia attrita* (VALLIANT, 1888); *Conocara murrayi* (KOEFOED, 1927); *Bathytroctes microlepis* GÜNTHER, 1878 & *Alepocephalus rostratus* RISSO, 1820).

In the present study both PR, and GC topographies and the resolving power of various retinal regions in *H. anomala* are compared with the findings in other members of the Alepocephaloidea and with shallow water teleosts. However useful, certain limitations of the wholemount technique to the study of deep-sea fish retinæ occur due to several retinal specialisations, such as OS elongation and foveal displacement of nuclei.

The convexiculate or deep fovea is a retinal region of high cell density (RHCD) occurring in the eye of many deep-sea teleosts, where it is commonly located in the temporal region of the retina. Many authors believe the fovea to be a specialisation with either increased resolving power or high sensitivity (Munk 1975, 1977; Locket 1985, 1992; Collin and Partridge 1996; Collin 1997; Wagner *et al.* 1998).

The fovea found in deep-sea teleosts is likely to have evolved convergently with the foveae found in a range of photic zone vertebrates (e.g. in reptiles: Walls 1942; Harkness and Bennet-Clark

1978, and in raptorial birds: Fite and Rosenfield-Welles 1975; Snyder and Miller 1978; Locket 1992).

A common denominator of convexiclivate foveal occurrence in these widely different environments may be the need for high acuity vision and improved range-finding abilities in a visual space often devoid of fix points (Pumphrey 1948; Locket 1992).

The functional significance of the convexiclivate fovea has previously been discussed by several authors (Steenstrup and Munk 1980; Locket 1992; Collin 1997, who all reviewed and elaborated on the theories of Walls 1942; Pumphrey 1948; Harkness and Bennet-Clarke 1978; Snyder and Miller 1978).

Possible aspects of the functional foveal morphology of *H. anomala* are discussed and compared to the morphology of other alepocephaloid foveae.

Materials and Methods

Fixation procedure

The specimens were caught in a bottom trawl (Alfredo III) during the annual Greenland Halibut surveys of the Greenland Institute of Natural Resources with *R/V Paamiut* in the Denmark Strait during Sept., Oct. 1997.

As soon after trawl retrieval as capture-handling permitted, the specimens were keyed to species aided by Quero *et al.* (1984) and measured (standard length (SL) or total length (TL) depending on operator). Prior to fixation corneas were pierced on as many specimens as possible by triangular razorblade incisions, to ease fixative access to the retina. Specimens were decapitated

and heads immersed in a mixed aldehyde fixative (0.2M, partly adopted from Glauert 1975) consisting of 2% paraformaldehyde and 2.5% glutaraldehyde used in equal amounts with 0.2M mixed phosphate buffer, pH. 7.3. The resulting fixative (0.1M) was adjusted near-hyperosmotic to the expected osmolarity of oceanic fishes with 3% sucrose. The resulting solution has been used by various authors on deep-sea fish eyes, often producing good fixations (Collin and Partridge 1996; Collin *et al.* 1998). All material was kept cold storage on board to minimize aldehyde polymerisation. After minimum 24 hours in fixative the specimens were transferred to 0.1M sucrose adjusted phosphate buffer. Prolonged fixation should theoretically buffer for post-fixational changes during prolonged storage in buffer (N. A. Locket, pers. comm. 1997). Crystalline thymol was used as mould protection in addition to the residual aldehydes in the specimens.

Later the specimens were examined in dissection microscope to determine the quality of individual fixation through observation of the retina *in situ* through the pupils apertures. This procedure proved to be inadequate for determining the quality of fixation at the level of light, and electron microscopy. Further, the quality of fixation appeared to be random with respect to piercing of corneae (Table 1). However, random quality of fixation is a common characteristic of fixation schemes in general and of ocular fixation in particular (N. A. Locket pers. comm. 1997).

Specimens

A summary of collection data and meristics for the specimens examined is presented in Table 1. Some specimens with poorly fixed retinæ and were only included in the gross anatomical study of ocular development, while some blocks of retina were used for trial processing. Three specimens (total lengths of 60 mm, 120 mm and 190 mm; Table 1) were fully processed. An eye of each

specimen was used for wholemound surveys while blocks of retina with known orientation from the other eyes were embedded in Araldite. After conclusion of the wholemound surveys, which revealed the specialised retinal regions to be further examined, sectioning of the embedded retinal region of the other eye was done.

Retinal tissue preparation

Eyeballs were enucleated in buffer solution using microscissors and watchmakers forceps. Conjunctiva, episcleral connective tissue, extraocular muscles and optic nerve was carefully severed. Dissection was performed to leave an intact peripheral retina (Stone 1981) while the musculus retractor lentis and the annular ligament was kept in association with the anterior segment and lens allowing future studies of possible accommodation patterns. Eyecup orientation was marked with nasal and dorsal incisions and vitreous humour was carefully removed. If the retinal pigment epithelium (RPE) was detachable (possibly because the specimen died at an early stage of the haul) the retina was either processed further as a wholemound specimen or an attempt was made to maintain what little apposition remained. Often the RPE maintained apposition, especially in the fundus and the foveal regions. These specimens could have experienced some degree of light adaptation if they perished late during the haul. RPE apposition impedes wholemound studies (see below), and only parts of the RPE in the 120 mm specimen (Table 1) could be removed.

Retina preparations for embedding.

Slices of retina with known orientation were post-fixed in a 1:1 mixture of 2% OsO₄ and 0.2M storage buffer without sucrose for one hour, rinsed in 0.1M buffer and dehydrated in ascending

concentrations of alcohol and finally in either HCl activated 2.5 di-methyl oxy-propane or propylene oxid. Specimens were embedded in Araldite.

Both radial and tangential semi-thin sections of various retinal regions were cut on wet glass knives. Sections were stained with 1% aqueous Toluidin Blue on a 90°C hotplate for *c* 15 seconds, and flame dehydrated.

Light microscopy.

Digital light micrographs were frame-grabbed with Leica LIDA - LITE software ver. 1.52 from a Leica DMRXA compound microscope via a Sony Power HAD ccd camera to a PC as tagged image file format (.TIF) and arranged in Corel Photo-Paint and Corel Draw (versions 8.0).

Electron microscopy.

Gold to silver range sections were cut on a glass- or a diamond knife, stretched in a vapour of chloroform and ether (Reid 1975), and collected on 50 mesh grids. After contrasting with uranyl acetate and lead citrate (Lewis and Knight 1977) grids were examined in a JEOL, JEM 100sx. transmission electron microscope (TEM). Electron micrographs were produced on Kodak Estar 4489 film.

Correction of cell counts and retinal measures from sectioned material.

Tissue shrinkage due to Araldite embedding is known to be highly variable (10% linear shrinkage is estimated by Glauert (1975)). However, all embedded specimens received similar treatment so

the counts and measures from sections could be corrected from wholemount counts in similar sized specimens (the opposing eye).

Wholemount preparation and photoreceptor survey

The technique applied in this study was a combination of protocols from Stone (1981); Curcio *et al.* (1987); Collin and Pettigrew (1988a, b, 1989). Dissections were carried out according to Stone (1981). After the time consuming task of clotting away vitreous humour with filter paper the posterior part of the eyeball could be flattened using peripheral incisions with a 0.1 mm razorblade. These incisions allowed a uniform flattening of the retina onto a standard microscope slide, receptor side up. The retina was cleaned and dried with filter paper and cleared in 100% dimethyl sulpho-oxid (DMSO) under constant surveillance. Excessive clearing with DMSO caused brittleness of the wholemount with retinal ruptures occurring during successive steps. The specimen was transferred through 2 steps of 100% glycerol to remove residual DMSO and orientated with the horizontal and vertical meridians parallel to the slide edges. Since the photoreceptor wholemount technique relies on uniform visualisation at a level close to the outer-inner segment junction, a uniform flattening of the retina is crucial for correct density counts. Hence, care was taken to flatten the retina thoroughly and air bubbles were carefully removed between slide and specimen preceding mounting in 100% glycerol and coverslipping. Wholemounts were sealed with Enthallan. The praxis of removing the fovea of juveniles was adopted, since it was squeezed in wholemount preparations due to its relative thickness. Wholemount will last approximately 10 days after processing if kept in cold storage.

Counting photoreceptors in wholemounts.

The counting protocol was modified from Stone (1981) and Collin and Pettigrew (1989).

Preliminary experiments established that the selected sample area provided an accurate estimate of the number of cells present in the material. Counts were plotted on a camera lucida tracing of the wholemount with a superimposed size adjusted (Xerox magnified millimetre) grid. All recognisable topographic features in the wholemount were plotted onto the tracing as surface fix-points. In wholemounts of juvenile retinae with the fovea removed, counting along the incision margin was intensified in order to calculate a correction factor for the standard tissue preparation for sectioning described below. Counting was also intensified in RHCDs. Wholemount preparations were viewed with differential interference contrast (DIC) optics on a Nikon Microptex - FX compound microscope with a ccd camera linked to a monitor. Counts from a 100×100 mm. field on the monitor were converted to photoreceptors $\times 10^4 \text{ mm}^{-2}$.

Correction for distortion; photoreceptor survey.

Wholemounts were rinsed in the mounting medium (DMSO) and since no dehydration occurs in this procedure, correction was omitted. Water rinsing deteres phosphate precipitation, but causes swelling, estimated to be *c* 3% linear (Curcio *et al.* 1987).

Remounting for ganglion cell survey

Wholemounts were opened using a No. 3 scalpel blade, and inverted onto a slide coated with Fohls mounting medium (Stone 1981). Distilled water was applied to simultaneously detach the retina from the top slide and ridding it from residual glycerol. After 12 hours of desiccation at

30°C the specimens were thoroughly attached to the slide and staining for Nissl substance was done using cold 0.05% aqueous Cresyl Violet in acetate buffer at pH 4.3. Every 1½ minute during staining the specimen was monitored in a compound microscope to ensure an optimal degree of staining to the ganglion cell layer only. Small and large retinas appeared to require similar staining times (c 11-12 min.) but stain affinity is known to vary both intra, and inter specifically (Stone 1981). Dehydration in ascending alcohol from 70% to 90% (½ min. each) and finally 100% (1 min) preceded mounting and coverslipping in Enthallan.

During staining trials the alcoholic dehydration was observed to intensify the staining somewhat, and shortening of staining time was thus necessary.

Viewing of Ganglion cells in Retinal wholemounts.

The ganglion cell survey was done on a Leica DMRXA compound microscope with standard compound microscope optics, oil immersion and excessive aperture diaphragm opening for visualising the ganglion cell nuclei at various depths in the ganglion cell layer. Digital image files were obtained as described above. Cell counts were done with an eyepiece square graticule and counts recorded on a tracing of the retina (as in the photoreceptor survey). Counting was intensified in regions of high cell density and along the incision margin on juveniles. Cell counts were converted to ganglion cells $\times 10^3 \text{ mm}^{-2}$.

Correction for distortion; ganglion cell survey.

The re-tracing of the inverted retina revealed shrinkage to be of negligible dimensions occurring in the rare case where the retina had not attached properly to the gelatinized slide. Thus correction for shrinkage was omitted in the ganglion cell survey.

Calculations

Calculations of resolving power were done from both PR densities and GC densities. For the GC counts the visual angle (α) was determined using the Posterior Nodal Distance (PND) derived from lens radius (r) and Matthiessens ratio (2.55) (Collin and Pettigrew 1989). Matthiessens ratio is the focal length (f) divided by lens radius. This value is known to vary interspecifically (Fernald 1990) and possibly through ontogeny (Shand 1994). The value 2,55 is the mean value of ratios calculated by Matthiessen (1880).

α was used to calculate the spatial resolving power in cycles per degree (CPD_{GC}) for extrafoveal retinal regions:

$$CPD_{GC} = (Linear\ GC\ density / \alpha) / 2 \quad (1)$$

where α equals the denominator.

Calculation of resolving power from PR densities (CPD_{PR}) was adopted from Shand (1997), who determined visual acuity as the minimum separable angle, or:

$$MSA = 2p / (2.55 * r); \text{ (radians)} \quad (2)$$

where p is the centre-to-centre separation of photoreceptors. The inverse value of MSA, or CPD_{PR} , is converted into degrees (S. Steenstrup, pers. comm. 1998):

$$CPD_{pr} = (1 / MSA) (180/\pi); \text{ (degrees)} \quad (3)$$

After correcting CPD_{PR} with the summation ration (PR density divided by GC density), CPD_{PR} and CPD_{GC} could be compared in plots.

The information value of CPD_{PR} corrected with SUM is however questionable. The effects on SUM caused by any neural population intermediary of the PR and GC populations is not incorporated, and further, at least a proportion of the small nuclei in the ganglion cell layer (GCL) may belong to a population of ectopically migrated (displaced) amacrine cells (DACs) (Collin and Pettigrew 1989; Wagner *et al.* 1998). Both CPD_{PR} and CPD_{GC} will probably require adjustment when future studies reveal the true size of GCL populations. Since GC density is related to the CPD_{GC} by a square function, correction will alter less on CPD_{GC} than on CPD_{PR} corrected with SUM, which is related to area density. However, CPD_{PR} is the only value applicable to the foveola, from where GCs are displaced, and to parafoveal and peripheral regions, where GC counts are possibly influenced by shrinkage.

Results

Gross anatomy of head and eye

H. anomala has the biggest head relative to body length of the platytroctids (Parr 1960), with large eyes placed laterally on the head. Allometric growth of the eye cup changes the eye shape through ontogeny from oval and latero-medially flattened in juveniles to an almost hemispherical eye cup with a deepened caudal fundus in adults (Fig. 2). This eye shape alteration through development is consistent with that observed for other platytroctids (Parr 1960; Munk 1966; Locket 1971).

Fig. 2 A - D approximately here.

The pupil is horizontally oval with a rostro-ventral tilt of the maximal pupil axis in relation to the horizontal meridian of the eye. This orientation aligns the maximal pupil axis with the deeply cut

rostral visual grooves. The lens protrudes through the pupil aperture into the anterior chamber and is displaced temporal to the pupil centre creating a rostral aphakic aperture. Dislocation of the lens position relative to the pupil aperture was a frequent *post mortem* disturbance along with ruptures in the pupil margin due to the corneal piercing procedure. However the ventro-caudal location of the prominent musculus retractor lentis indicates that the lens is moved temporally during accommodation, thus increasing the aperture area. In combination these morphological specialisations, common to members of the Alepocephaloidea, effects a widening of the rostral binocular visual field.

Both displacement and increase of the inter-orbital width is produced by broad triangular wings of the orbitosphenoids, which is also seen in adults of several other platytroctids (Parr 1960). The flaring of the dorso-lateral orbital margins are further emphasised by delicately ossified wide subvertical prefrontals, found to occur from approx. 40-43 mm TL, and conspicuous from 60 mm TL, when their invasion over the dorsal palperal fold accelerates. These bones are likely to interfere with the dorsal visual field, and in some species e.g. *Maulisia mauli* (Maul 1957; Parr 1960) the entire dorso-rostral quadrant of the eye is 'hooded' (Parr 1960). Parr did not recognise subvertical prefrontals in *H. anomala* (referred to as *M. rostratus*) where they are in closer association with the orbitosphenoid, but the widening and displacement of inter-orbital width is apparent and potentially the dorso-rostral visual field is effected.

The paired premaxillary tusks (Fig. 2) pointing posteriorly in several platytroctids were recorded by Maul (1957; p. 7, Fig. 1), Parr (1960) and Quéro *et al.* (1984). Compared to the oral dentition, the tusks are disproportionally large and, due to orientation, seemingly useless for feeding activity. In platytroctid species with proposed specialisations for rostral binocular vision the tusk align as a continuum of the rostro-ventral visual groove margin, as seen in *S. kofoedii* (Maul 1957), *H.*

schnakenbeckii (KREFFT, 1953), *H. macrops* MAUL, 1957 (Parr 1960) and *Paraholtbyrnia cyanocephala* KREFFT, 1967 (Pers. obs. 1997).

Retinal Pigment Epithelium.

The retinal pigment epithelium (RPE) is composed of a single layer of closely associated cells with the nuclei located in the basal and sclerad-most part of the soma. Radial sections of adult retinae show the RPE cells as ovoid shaped with few and short projections along the vitread surface (Fig. 3A).

Fig. 3 A - D approximately here.

Viewed from sclerad in wholemounts or in tangential sections the cells are penta, to hexagonal in shape (Fig. 3B). Processes were observed in adult specimens with all pigment granules retracted to the basal region of the cell, while the pigment granules were evenly distributed throughout the cytoplasm of foveal RPE cells in juveniles (Fig. 3C). Tips of OSs enclosed in phagocytotic vesicles (Fig. 3D) were observed in nearly all foveal RPE processes from the adult, while no such observations were made in RPE cells of juvenile specimens.

Multiple papilla.

The optic disc or papilla is well elongated and macroscopically seen as a dark line traversing the fundus retina ventral to the horizontal meridian from temporal, and immediately inferior of the fovea, across the fundus reaching the naso-ventral peripheral retina. Along the entire horizontal meridian, multiple papillae composed of fascicles of retinal GC axons permeate the inner retina (Fig. 4) and rejoin extra-retinally in a band shaped ridge eventually forming a typical cylindrical

optic nerve. The RPE and choroid elaborately ensheath this elongated optic nerve head. No distinct choroid fissure was observed here, while Munk (1966) reported a short fissure nasally in *Platyroctegen mirus*.

Fig. 4 - 6 approximately here.

The trajectory of the elongated optic disc is sub-meridionaleal in the genera *Roulenia* and *Alepocephalus*, as illustrated by Collin and Partridge (1996) and Wagner *et al.* (1998). In the genus *Conocara*, the papilla slopes steeply from the dorso-temporal fovea to the ventral peripheral retina (Collin 1997). In all alepocephalids examined by the author and by Munk (O. Munk, pers. comm. 1998) no choroid fissure was associated with the dark line.

Choroid gland.

A choroid gland (consisting of a choroid rete-mirabile) is located in the choroid of the eye fundus as a bi-lobed structure macroscopically resembling retina. Each lobe was *c* 8 mm long in specimen 3683. The lobes are attached to the optic nerve stalk as observed in *Alepocephalus agazzisii* GOODE AND BEAN, 1883, (Pers. obs. 1998). Brauer (1908) recorded and illustrated the choroid gland in his platyroctid specimen (possibly *S. kofuedii*), merely labelling it 'blood-vessel' (Plate XXXVIII, fig 7; Munk 1966).

The retina of *H. anomala* is avascular as reported for many other teleosts (Munk 1966; Nicol 1989).

Ontogenetic changes in retinal architecture and topography

The changes in retinal architecture of *H. anomala* through post metamorphic ontogeny and across temporalis, fundus, and RHCD retinal regions are limited to variations in, *i*) packing efficiency of

the OSs (i.e. centre-to-centre distance between PRs), *ii*) OS dimensions and, *iii*) relative thickness of inner plexiform layer (IPL) and banking of nuclei of inner and outer nuclear layer.

There is no evidence of ontogenetic specialisations like multibank formation or regional changes of PR mosaics and hence the morphological retinal description provided here, unless otherwise stated, applies equally well to the entire retina in a post-metamorphic size range of *H. anomala*.

The ultrastructural retinal morphology of *H. anomala* is very similar to that found by Locket (1971) in *P. apus* thus, emphasis is made here on the ultrastructural foveal morphology since embedding difficulties prevented Locket from describing it in detail.

The fovea.

The foveal region is located in the temporo-ventral retina, *c* 1 mm from ora terminalis and inferior to the horizontal meridian. The foveal visual field is thus orientated towards the rostral to dorso-rostral and alignment of the foveal, and lens axis and the aphakic aperture is present (Fig. 2). The foveal morphology of *H. anomala* changes little through ontogeny as observed in many foveated species (Walls 1942; Locket 1992; S. P. Collin, pers. comm. 1998). In the juveniles the foveal region appears as a macroscopically recognisable thickness in the temporal peripheral retina while its prominence is masked during ontogeny with thickening of the fundus retina. This development accounts for the presence of a fovea externa in juveniles (up to *c* 70 mm TL). The fovea externa does not bulge the sclera to the extent observed in the alepocephalid genus *Bajacalifornia* (Locket 1985; pers. obs. 1997, both on *B. megalops*) but a distinct protuberance of the retina, sclerad into the choroid marks the fovea externa.

General description of the retina

The following description of the retinal morphology travels from the sclerad-most OSs, vitread to the GC layer finally covering the distribution of the Müller cell radial fibres in the neuroretina (Fig. 5 and 6).

PR morphology.

The photoreceptive component of the retina is a single bank of rod receptors with long slender OSs (Fig. 6). The retina proper may be divided into two morphologically discrete regions on the basis of PR morphology, *viz.* *a*) the extrafoveal retina with *c* 80 μm long slender OSs (2-3 μm . in cross sectional diameter) and *b*) the foveal region, of which the centre (foveola) possess OSs that are thin (1.1 μm) and elongated to more than double the length (180 μm) of OSs in the fundus retina of the juvenile specimen (Fig. 5). This elongation causes a tendency of vitreo-scleral compression during processing for histology, hereby bending the thin OSs (Fig. 5).

Photoreceptor outer segments.

The cross sectional diameter of OSs in both foveal and extrafoveal retina shows localised variance. In the parafoveal region of the juvenile specimen (Fig. 7) some OSs are seen attaining only 1.2 μm in diameter, *c* 40% of the mean OS diameter observed. These may be newly proliferated OSs. In the peripheral retina OSs are shorter (40-60 μm) and attain higher diameter (*c* 4 μm).

Irregularly occurring lobulations, a common feature of vertebrate rod OSs are well observable in EM tangential sections (Fig. 7). Towards the vitreal zone of outer-inner segment junction calycal processes encircle OSs.

Fig. 7 – 13 approximately here.

Photoreceptor inner segments and the external limiting membrane.

The short and conical inner segment is broadest at the inner-outer segment junction and tapers off vitreally towards the external limiting membrane (ELM). From the sclerad part and throughout the inner segment are large and tightly packed mitochondria (Fig. 8). The largest are just vitread to the junction, attaining lengths of $c 2 \mu\text{m}$ or nearly 50% of the entire inner segment diameter. The connecting cilium, responsible for the process of OS disc formation (Fröhlich and Wagner 1996) is seen eccentrically in the sclerad inner segment where it is partly surrounded by calycal processes. From the inner segment membrane located further vitreal, calyx walls are formed which ensheath the inner segment to the sclerad. Apically the wall extends calycal processes further sclerad in the ventricular space, as mentioned above (Fig. 8 and 21A-C).

Wagner (1990) suggested, that the calycal processes may represent structural support to the OSs. Neither the glycogenous paraboloid nor an oil droplet were observed, as normal in teleost rod PRs.

The foveolar inner segments are gradually elongated towards the foveal centre (Fig. 5 and 10), the longest foveolar inner segments are $c 35 \mu\text{m}$. A vitreally concave displacement of the boundary between outer and inner segments is created by the elongation pattern, and emphasised by the fibre of Henle layer (see below). Further vitreal, still in the ventricular space and surrounding the

inner segment region corresponding to the myoid, are villous fibre prolongations from the Müller cell projections of the inner retina. These villi create fibre baskets around the basal parts of the inner segments (Fig. 9), possibly facilitating exchange of metabolites between inner retina and the ventricular fluid (Locket 1971, 1990).

Immediately vitreal, the basal-most parts of inner segments and the scleral terminations of Müller cell projections form lateral junctions (apparently as zonulae adherentes) defining the external limiting membrane (Fig. 5 and 6).

Outer nuclear layer and fibres of Henle.

When projecting through the ELM the inner segment is continued by the outer conducting fibre. In the extrafoveal retina the outer conducting fibre travels directly vitread to the PR nucleus (Fig. 6). Foveal outer conducting fibres (here named fibres of Henle) are extremely elongated to allow maintained connection between the foveolar PRs and their displaced nuclei (Fig. 5). These fibres of Henle radiate horizontally, away from the foveola in an array of outer conducting fibres lengths, the longest attain lengths of c 150 μm . (Fig. 10). Consequences of this elongation are marked thickening of this foveal layer and a vitreally concave displacement of the ELM (Fig. 5). In radial sections, the PR nuclei occupying the outer nuclear layer (ONL) were readily identified due to the continuity of conducting fibres from inner segment to nucleus. PR nuclei are of non-spherical shape and often elongated radially when stacked in the foveal shoulder in distinct bands. They stain intensely with Toluidin Blue and their nucleoplasm is mottled by heterochromatin. Spherical to ovoid nuclei which stain lighter are also observed in the ONL (Fig. 11A).

Photoreceptor synapses and the outer plexiform layer.

From the PR nuclei the inner conducting fibres proceed vitreally entering the sclerad-most region of the outer plexiform layer where they are terminated as PR synapses. The terminal is spherical (hence termed spherule), and of the single, or oligosynaptic type (Pedler 1969; Locket 1971, 1977 and in press). They are 3.5-4.0 μm across at the terminal and conspicuous by light microscopy (Fig. 11A). High synapse densities in the foveal shoulder region corresponds well with the PR density increase of this region.

A single elongated density, the synaptic ribbon, is present within the synaptic terminal (Fig. 12A), rarely two ribbons are observed in the same spherule. Synaptic ribbons are static structures in rods as opposed to the ribbons of cone photoreceptors which disorganise during synaptic activity (Pedler 1969). The ribbon, here with an ultrastructure of 3 lamellar components (in other species 5 lamellae are present) created by plate-like structures indenting the spherule cavity, is believed to guide synaptic vesicles from golgi apparatus to the synaptic junction where the glutamate contents of the vesicles is released (Wagner 1990; Sarantis and Mobbs 1992). Synaptic vesicles are abundant and mottle the spherule cytoplasm proximal to the ribbon (Fig. 12B). Glutamate is the primary neurotransmitter of the neuroretina (Sarantis and Mobbs 1992) only to be replaced by an electrical synapse potential in the GCL which is transmitted to the CNS (Wagner 1990). Second order cell processes are in close association with the synaptic cleft (Fig. 12C). These are known to originate both from horizontal, and bipolar cell bodies. The spherules are closely enveloped in lamellar tissue of Müller cell origin (see below).

The vitread-most region of the outer plexiform layer (OPL) is composed by bipolar and amacrine cell projections and of radial fibre material of Müller cell origin.

Inner nuclear layer.

Morphology of the diverse range of cell populations in the inner nuclear layer (INL, Fig. 11B) is addressed shortly. It is noted, that the INL - compartmentalisation of the foveal shoulder is defined by radial fibre material of Müller cell origin in a honeycomb meshwork. The sclerad-most compartment houses nuclei tentatively labelled horizontal cells by Wagner (1990). In the extrafoveal region these nuclei exhibit a characteristic alignment parallel to the limiting membranes. The vitread-most nuclei compartment houses either amacrine, and/ or interplexiform cells. They stain lighter and possess ample cytoplasm in their spherical to ovoid pericarya and have mottled nuclei (Wagner 1990). Apart from defining the compartmentalisation, the radial fibre material is arborised as horizontal fibres in the intercellular space of the INL, where the nuclei of these cells and the bipolar cell nuclei also are located.

Inner plexiform layer.

The inner plexiform layer (IPL) is primarily composed by the branchings of 2. order neurones with nuclei in either INL, or GCL. For an extensive review of INL and IPL morphology, Wagner *et al.* (1998).

Ganglion cell layer.

Morphology of the GCL was studied in retinal wholemounts and is further addressed below in the wholemount results section.

Internal limiting membrane and radial fibres of Müller.

The internal limiting membrane (ILM) is formed by the basal membrane of the vitread Müller cell terminals or ‘endfeet’ (Wagner 1990) in close conjunction with collagenous fibres of the vitreous. From the Müller cell pericaryon in the INL (fig 11A) radial fibre processes are distributed towards the limiting membranes and in the nuclear layers they ensheath the nuclei as a honeycomb meshwork. In the plexiform layers the radial fibres arborise to a finer network of horizontal fibres, villiform processes of which interdigitate with the synaptic terminals of PRs, and second order neurones (Fig. 12C). Like the glial cell processes the Müller cell horizontal fibres also form myelin-like sheaths around the dendritic fields of second order neurones (Fig. 14C).

Fig. 14 A –D approximately here.

The Müller cell ‘endfeet’ of the extrafoveal retina are not prominent and only observed in TEM but in the fovea the situation is different. Here at least some of the Müller cells constitute a palisade like layer of cells lining the foveal pit (Fig. 5). Using glutamine synthetase and vimentin immunocytochemistry it has been established that the ‘palisade’ cells are of Müller cell origin (S. P. Collin, unpublished results). Both macroscopically and in LM of whole mounts the ‘palisade’ cell insert in the foveal pit is observable without staining, suggesting altered refractive properties of this tissue. The ‘palisade’ cell soma appear distinct from the numerous radial fibre fascicles or ‘trunks’ (Locket 1971) extended towards the INL (Fig. 5 and 13), but the continuity between these is evident in radial EM sections of the foveola (Fig. 14B), where the ‘palisade’ structure also appears very electron dense as opposed to the inner plexiform layer.

Entering the INL, the radial fibre trunks arborise and maintain the ensheathment of the nuclei as horizontal fibres. Additionally a compartmentalisation of the nuclei is produced (see above). In the extrafoveal retina however, the compartmentalisation is ill defined and radial fibre trunks are

largely absent (Fig. 5). All intercellular space of the foveola, devoid of nuclei, due to their displacement, is occupied by radial fibre material (Fig. 5). The ultrastructure of palisade like cell type and radial fibres is different. The ‘palisade’ cell cytoplasm (Fig. 14A) is darker and mottled with abundant proteinaceous microfibrils (resembling collagen, according to Snyder and Miller (1978)) of regular thickness (c 100 Å), along with vesicles and granules, possibly of glycogen. Travelling sclerad, towards the periphery of the palisade cell pericaryon the fibrils become more closely aligned and in the region where radial fibre trunks are extended (Fig. 14B), the multi-directional lamellar structure is initially observed along with constellations of neurotubuli (Fig. 14C), which may be involved in the formation of radial fibre lamellae, possibly a process similar to the OS disc formation from the connecting cilium. Entering the radial fibre, the lamellar structure is highly organised and the cytoplasm is largely homogenous (Fig. 14D). The interdigitations of the horizontal fibres with the PR spherules are shown in Fig. 12. Sarantis and Mobbs (1992) determined an important function of the horizontal fibres to be the absorption of glutamate from the PR spherule vicinity, hereby terminating the synaptic output. Also the ONL nuclei are closely ensheathed in horizontal fibres (Fig. 11A). Finally, sclerad-most in the inner retina the Müller cell ‘endfeet’ participate in the formation of the ELM as described above.

Wholemout results

Iso-density contour maps illustrating retinal specialisation of PR, and GC distribution in the right eye of a juvenile (#3382, 60 mm TL) and an adult (#3683, 190 mm TL) *H. anomala* are presented in Fig. 15.

Fig. 15 approximately here.

PR densities, GC densities and summation ratio (SUM) for the horizontal, and vertical meridian and the meridian passing through the fovea (foveal meridian, all indicated by arrowheads in Fig. 15) are presented in Fig. 16.

Fig. 16 approximately here.

The resolution in cycles pr. degree (CPD) derived from GC densities (CPD_{GC}) and from PR densities (CPD_{PR}) corrected with the summation ratio (i.e. CPD_{PR} / SUM) for the same trajectories are presented in Fig. 17.

Fig. 17 approximately here.

Morphology of the ganglion cell layer.

The GCL is known to house the nuclei of at least four functionally different cell populations; large ganglion cells (GC), small, or dwarf ganglion cells (sGC), displaced amacrine cells (DAC) and glial cells (Fig. 18). The GCs are *c* 10-12 μm in diameter with large spherical to ovoid nuclei, mottled granular soma and abundant cytoplasm. Smaller irregularly shaped nuclei which stain darker can belong to sGCs. This nucleus type is particularly abundant in the dorso-rostral RHCD (both in juveniles and adults) and in the annuli-like proliferation pattern from this zone in adults. (Fig. 19A and below).

Some, if not all of the so-called sGCs may be DACs. Future examination with retrograde labelling from the optic nerve head will undoubtedly reveal the proportion and signature of true GCs with axons extended in the optic nerve (Collin and Partridge 1996; Wagner *et al.* 1998, see below).

Nuclei belonging to opticus fibre layer glial cells were readily distinguished from other nuclei due to their cigar shaped soma and dark Nissl staining properties (Fig. 19A, B). The processes of these

glial cells form loose myelin-like sheaths around the GC axons (Wagner 1990). The GCL glial cells are thus distinguished from the other glial component of the retina, the Müller cells.

Fig. 18 - 21 approximately here.

Correction for DACs.

It is emphasised, that CPD_{PR} is the absolute upper limit of resolving power in a given eye. CPD_{PR} determines the spatial resolving power of a retina only if summation is absent (e.g. in the human foveal centre). Summation is probably present in any retinal region at any stage of development in *H. anomala*.

In Fig. 17 the comparison of CPD_{GC} (tentatively regarded as the neural upper limit to resolution) compares well, at least in the non-specialised fundus retina, with CPD_{PR} corrected for summation. Future correction for DACs will undoubtedly increase the differences between CPD_{PR} and CPD_{GC} , a pattern that is not explainable. As GCs are re-labelled DACs summation will increase in peripheral retinal regions.

Recently, correction values for DACs between 20 and 25% have been applied in studies of other deep-sea species (Collin and Pettigrew 1989; Collin *et al.* 1998) This is significantly less than the variance inherent in behaviourally determined visual acuity (c 40% according to Collin and Pettigrew (1989)).

Dorso-rostral specialised region.

The dorso-rostral region exhibits a GC density peak increasing from 12 to 17×10^3 cells \times mm⁻² through this ontogenetic range, densities which are only surpassed in the foveal retina (Fig. 15 and

16). Accordingly CPD_{GC} increases to 6.5 cycles pr degree in the adult specimen. Following this, CPD_{PR} is higher than CPD_{GC} because PR density is slightly lower in this region than in the non-specialised fundus retina. The PRs of the dorso-rostral area are shorter ($c 50 \mu\text{m}$) than elsewhere but of normal thickness ($c 3 \mu\text{m}$) measured in a belt $c 200 \mu\text{m}$ from, and parallel with the dorso-rostral ora terminalis.

In the adult, a ribbon-like proliferation pattern of GCs with a distinct orientation in annuli concentric to the central retina is observed (Fig. 19A). The annuli are most prominent here, but not exclusive to the dorso-rostral retina, and in the juvenile which does not exhibit the dorso-rostral annuli pattern, there is a dorso-ventral orientated band of nuclei aligned in ribbons crossing the central fundus (Fig. 19B). The elongated glial cell nuclei are aligned with the ribbon pattern rendering it readily detectable.

Vertical specialisation and fundus retina in juveniles.

A region similar to the vertical specialisation in the dorsal fundus of the juvenile *H. anomala* (Fig. 15A, Cand Fig. 16B) has not been recorded previously. Although the density gradients of this retinal region are moderate (GC: 1.6:1, PR: 1.2:1) the region shows a summation gradient of 1.7:1 with a central region of lower summation (6.6:1) flanked dorso-ventrally by regions of higher summation (10.3-11:1). The vertical pattern was not observed in the intermediary specimen (#3396). The average summation ratio of the juvenile non-specialised fundus retina is $c 8:1$ (Fig. 16A-C). Notable is also the GC density increase to $7 \times 10^3 \text{ cells} \times \text{mm}^{-2}$ along the fundus trajectory of the multiple papilla (Fig. 15A).

Horizontal visual streak and fundus retina in adults.

The horizontal visual streak found in the adult retina has three distinctive centres along the naso-temporal trajectory (Fig. 15C-D). The central region has low PR density ($5.6 \times 10^4 \text{ mm}^{-2}$) while the PR densities of the peripheral centres exceed the density of the non-specialised fundus retina ($7.6 - 8.3 \times 10^4 \text{ mm}^{-2}$).

A GC density of $8.5 \times 10^3 \text{ cells} \times \text{mm}^{-2}$ and summation of 7.8:1 was found in the central region, with summation ratios of 13.5:1 in the two peripheral regions.

An average GC density of $5.4 \times 10^3 \text{ mm}^{-2}$ and summation averaging c 11:1 is found in the non-specialised adult fundus retina.(Fig. 16D).

CPD_{GC} in the central region is 4.61 cycles pr. degree, the highest value recorded in the fundus retina.

The summation ratio of the adult visual streak approaches 10:1 in the nasal and caudal regions (Fig. 16D), while the fundus area, with low PR densities exhibit a low-peak at c 6.5:1 matching the lowest summation ratio in the juvenile fundus retina, found in the meridional region of the vertical streak and in the meridional rostral region (Fig. 16A-B).

Fovea.

Centro-peripheral asymmetries in PR and GC densities are observed along the retinal meridians, and found to be most pronounced along the temporo-rostral meridian, with the peak GC density in the foveal shoulders found to increase from 28 to $42 \times 10^3 \text{ cells} \times \text{mm}^{-2}$, while the peak PR density increases from 49-53 $\times 10^4 \text{ cells} \times \text{mm}^{-2}$ from juvenile to adult. The GC peak values are contained in the foveal shoulder apex while PR peak density is located in the temporal foveolar region (Fig.

15D and Fig. 16C,F). The temporal foveal shoulder of the adult show a low summation of 4.2:1, comparable to what is found in the non-specialised peripheral retina (2.9-4.1:1). The summation in the nasal shoulder is slightly higher (6:1), but again significantly lower than the values observed in the non-specialised fundus retina (10-16:1). The density isoclines of the foveal cell populations are asymmetrically distributed with an elongation of the density isocline towards the fundus, and with the steepest gradient located in the temporal shoulder. The PR population within the peak density isocline of the adult is c 350 receptors occupying c $360 \mu\text{m}^2$ in the ventro-temporal foveola (Fig. 20A). These are also the longest and thinnest OSs, attaining an estimated length of c $210 \mu\text{m}$ (Fig. 5). With a cross sectional diameter of only c $1.1 \mu\text{m}$ (Fig. 21A). In the adult OSs located in the foveola, c $2 \mu\text{m}$ towards the fundus, attain lengths of c $110 \mu\text{m}$ (Fig. 5) and diameter of c $1.2 \mu\text{m}$ (Fig. 21B). The variations in PR densities across the non-specialised fundus retina (Fig. 15B,D) are not readily accounted to variance in OS diameter but predominantly to the degree of packing efficiency. In the fundus retina (e.g. c $10 \mu\text{m}$ nasal to the foveal centre, Fig. 20B and 21C) the OSs are 3.5 - $4.0 \mu\text{m}$ in diameter, and c $80 \mu\text{m}$ long (Fig. 6). Few variations in appearance of the nuclei in the foveal GCL are noted. Temporo-ventrally, c $1.5 \mu\text{m}$, from the fovea is a peripheral region with marked increase in large GCs. Otherwise, only general trends in relative abundance in the GCL cell populations were noted. The density of GCs and glial nuclei appears to increase more through ontogeny than the density of sGCs (putative DACs) but no further attempts were made to map this pattern.

Discussion

Some indication towards the life strategy of *H. anomala* has become available to the author.

The *Alfredo III* trawl samples almost exclusively on the bottom (P. R. Møller, pers. comm. 1998).

This corresponds well with the presumed bathy, to benthopelagic rather than mesopelagic distribution of this species (Quéro *et al.* 1984; Weitzman 1997). Further *H. anomala* was the only platytroctid caught of 8 species known to occur in the David Strait (Nielsen and Bertelsen 1992).

The only other alepocephaloid representative was *Alepocephalus agazzisii*, which is commonly classified as benthopelagic and occurs at depths from 600 to 2400 m (Markle and Quéro 1984).

It is suggested that at least post-metamorphic *H. anomala* exhibit some degree of bottom affinity and generally occur deeper than daylight penetrates (i.e. below approx. 900 m; Herring 1989; Nicol 1989).

Morphological evidence suggest, that *H. anomala* searches for luminous cues. The large eyes with large rostral aphakic gaps and lens (Munk 1980; Nicol 1989) are well adapted to scotopic vision.

Further the binocular overlap, increased by the rostral visual grooves significantly increases the sensitivity of the visual apparatus (Munk 1980). The adults may be better adapted than the juveniles for monocular detection of a faint visual cue in the lateral monocular field of vision. An

indication of this was previously recorded by Swinney *et al.* (1986) for the platytroctid

Sagamichthyes schnackenbecki (KREFFT, 1953), manifested by a five-fold increase in mean specimen weight captured when trawls were fitted with artificial light (70 W). Although this

capture increase may also be linked with the increased cruising speed of adults, the importance of visual specialisations cannot be neglected.

Retinal Architecture

No significant alterations in retinal architecture through ontogeny were observed in this study in contrast to several other deep-sea teleosts where significant changes are known to occur, e.g. restructuring of PR mosaics (Munk 1990) and PR bank-formation (Locket 1980, 1985; Munk 1990). Apart from being an adaptation to the drastic alterations in life-style commonly occurring with metamorphosis (Shand 1997) such architectural changes have been suggested to occur in deep-sea teleosts as a response to altered light regimes through life. The lack of retinal reorganisation in *H. anomala* and other platytroctids studied (Munk 1966; Locket 1971) may suggest that juveniles and adults share vertical distribution pattern and possibly diet. However, juveniles were not caught as deep as adults (Table 1) and, since the depth range is limited in the Davids Strait differences in the vertical distribution of juveniles and adults may exist in deeper oceanic regions. Matsui (1991) observed the distribution of juveniles and adults of other platytroctids (*H. bauchoti* and *Sagamichthyes abei*) to coincide. Although many deep-sea teleosts are known to have pelagic larval stages with duplex retinae i.e. both cone and rod PRs (Munk 1990), some species maintain a constant retinal architecture through ontogeny. This constancy could be associated with a coinciding vertical distribution pattern of larvae and post-metamorphic stages. Cone PRs were certainly not observed in the size range studied here, but retinal morphology of larvae must be examined to confirm the theory of ontogenetically constant pure-rod retinae as indication of common lifestyles in juvenile and adult *H. anomala*.

Radial thickening of the fundus retina through ontogeny is mainly a product of plexiform layer widening and increased PR density resulting in more PR nuclei in the ONL. OS length does not appear to increase significantly.

Previous histological examinations.

Brauer (1908) found 1.0×10^6 rod nuclei pr. mm^2 and 1.6×10^5 ganglion cells pr. mm^{-2} in a 17 mm specimen he referred to as *Bathytroctes rostratus*, but which may belong to either of the two species *S. kofoedii* or *H. anomala* (Parr 1960, Munk 1975; Krefft 1980). A drawing provided by Brauer from a horizontal meridional section of the examined eye indicate that this specimen was subject to severe shrinkage. According to Munk (1980) *c* 20 % linear shrinkage is common with paraffin embedded material but using as high correction as 35% shrinkage of retinal area on Brauers counts still result in high numbers (*c* 6.2×10^5 rod nuclei pr. mm^2 and 9.9×10^4 ganglion cells pr. mm^2) compared to densities found in the retina of *H. anomala* (Fig. 15 and Fig. 16). Correction for split cell error does not apply to the counts of Brauer, due to a section thickness far exceeding the maximal diameter of cellular structures surveyed (Abercrombie 1946).

Munk (1966) found 7.1×10^4 rods pr. mm^2 and 7.6×10^3 ganglion cells pr. mm^2 (neither with split cell error correction) in a 78 mm specimen of *Platytroctegen mirus*, but no lens diameter was reported. However, the counts of Munk (1966) compare well with counts in several retinal regions of a similar sized *H. anomala*. The account of Locket (1971) on *Platytroctes apus* is purely qualitative with respect to retinal architecture.

Observations from the histological examinations of *H. anomala* are discussed below with related findings in wholemount material.

Optics through ontogeny

The small relative size of juvenile eyes makes accommodation less important than it supposedly is to the adult. PR size is relatively unaltered through ontogeny, and if the criterion level of focus is

the total length of OSs (as suggested by Walls 1942) and not confined to the ELM, then the eye only requires accommodation when a stimuli comes within close proximity. Further the close proximity of the lens to the retina provides greater depth of focus (Walls 1942).

Resolving power, however is limited if enlarged OS diameter increase the centre to centre distance between PRs, and certainly by the short focal length of a juvenile eye (Fernald 1988; Nicol 1989). Thus resolving power is limited in a small and latero-medially compressed eye, a pattern which is readily observable in juvenile *H. anomala* (Fig. 17). It is noted that despite the potential non-accommodative facilitation of long-range vision inherent to a small eye, the short focal distance of such an eye may impede discrimination of a distant point source luminescent visual cue (Nicol 1989; Walls 1942). This relation may contribute to the pattern of capture reported by Swinney *et al.* (1986).

During growth an increased resolving power of the eye can theoretically be accomplished in different ways. Through increase in photoreceptor density (increasing the degree of summation, since the GC density of a given retinal region should not increase significantly through ontogeny) or through an increase in focal length or both (Fernald 1989).

With the growth pattern of a typical lateral (i.e. non-tubular) teleost eye the focal length increases through ontogeny yielding increased resolving power and, provided PR spacing and image quality is maintained constant, maintained sensitivity of the retina (Fernald 1990).

In *H. anomala* both PR densities and focal length increase through ontogeny while GC densities decrease. Overall an improved resolution is thus achieved with age (Fig. 17). The GC density decrease is certainly limiting to the resolving power increase, but it is more than compensated for by the increase in focal length and the implicit increase in summation ratio (Fig. 16) ensures maintained retinal sensitivity.

Diffraction is not dependent on aperture if the full lens diameter is available, and thus not limiting in the teleost eye since pupil aperture is larger than lens diameter (simultaneously yielding low f-number; Fig. 2), but the minimal OS diameter of 1.1 μm found in the adult foveola closely approach the theoretical lower threshold of PR functionality as photon capturing devices. Due to their behaviour, photons are likely to miss PRs less than 1 μm in diameter and travel along their exterior in the ventricular space (Land 1981).

Under the prevalent low illumination conditions in the deep-sea environment depth contrast is maintained while scotopic specialisations such as a large lens, which optimises sensitivity to point source stimuli and a large aphakic aperture which increases the relative illumination of the retina would compromise depth contrast in the photic zone (Munk and Frederiksen 1974; Land 1981; Collin *et al.* 1997).

As illustrated above, both resolving power and sensitivity of the eye is furthered (Walls 1942), provided the rate of photon capture is high enough to exceed the scotopic threshold. Without loss of PR determined resolving power (CPD_{PR}) a maximisation of photon capture (i.e. sensitivity) can be obtained either through increased PR diameter, with intensified PR packing efficiency, or by elongation of OSs. All strategies are apparent in different retinal regions of *H. anomala* through ontogeny (see below).

The PR mosaic with hexagonal arrays found in *H. anomala*, as in other deep-sea teleosts (Lockett 1977) is potentially the most efficient packing of cylindrical elements. With a uniform OS diameter all receptors in a given region are equally spaced and when the packing efficiency is optimised, only 9.3% of available space is unoccupied (Munk 1980). In *H. anomala* the packing efficiency is higher in adults, and overall higher in the fundus retina, decreasing eccentrically

towards the retinal margin (except for the foveal region). Simultaneously the OS length increases eccentrically towards the peripheral retina, only to drop abruptly in the terminalis retina, a pattern previously recorded by Fröhlich *et al.* (1995).

On the contrary resolving power is lost if light gathering exclusively is pursued through increase of summation to a point where the pooled neural outputs from a receptive field is sufficient to trigger a GC synapse potential.

The high resolving power of a low summation retina allows the detection of minute movements, e.g. the human foveal centre, with acuity of one minute of arc and no summation can discriminate a 0.5-1.7 μm relocation across the visual cell layer (Munk 1980). Implicit for high resolving power under low illumination conditions is a high contrast sensitivity (Nicol 1989) which aids the discrimination of weak luminous point sources like photophores of potential prey in the deep oceanic darkness.

As demonstrated, the alterations of eye morphology through ontogeny of *H. anomala* results in an optical system where balance between resolving power and sensitivity of the retina is furthered.

While the fundus retina may not receive a focussed image at all in juveniles, the changes in relative proportions of optics and accommodation (eye cup shape, pupil aperture and lens size, Table 1) may improve lateral monocular vision through ontogeny as a complement to temporal, and in particular foveal vision.

Retinal growth

It has not been attempted to determine the short scale aspects of retinal growth and PR turn-over, which has been extensively covered for many teleost species by several authors (e.g. Johns 1981;

Johns and Fernald 1981; Easter 1992; Fröhlich *et al.* 1995; Fröhlich and Wagner 1996; Kwan *et al.* 1996; Wagner *et al.* 1998). However, factors controlling the long-term patterns in ocular and retinal growth of deep-sea teleost retinae remains obscure.

Reasons for the observed differences in RPE pigment retraction and the variable apposition of the RPE to the OS tips cannot be determined conclusively. A diurnal cycle of RPE adaptation is essentially ruled but even though most specimens were moribund upon trawl retrieval a capture dependent adaptation cannot be excluded since surface light exposure occurred. The presence of phagocytised OS tips in the adult RPE, and predominantly in the fundus and foveal regions concurs with the observations of Fröhlich and Wagner (1996) and also with the increased adhesion of RPE to OSs observed (adhesion during phagocytosis was also reported by Matsumoto *et al.* 1987).

This suggest that the light controlling function of the RPE common to diurnal species has been suppressed through evolution in the non-diurnal *H. anomala*, while the PR-turnover function is still increasing with age. Phagocytosis of rod OS tips has been proposed to occur when the RPE of diurnal species is dark-adapted (Matsumoto *et al.* 1987) and in *H. anomala* the observed pigment granule retraction may simply facilitate phagocytotic activity.

The wholemount investigation established that regional PR and GC densities changes through ontogeny and although some specialised regions (e.g. foveal, and rostro-ventral fundus) are maintained through ontogeny, others vanish (e.g. the vertical dorsal-temporal fundus region in juveniles) and yet others appear (the punctuated visual streak in adults). These ontogenetic changes are complex (Fig. 15) and an intrinsic pattern of regional retinal stretching, or compression may occur through ontogeny. This pattern may concur with macrophage phagocytosis in the neuro-retina, OS renewal from the connecting cilium, as indicated by

periciliary vesicles (Fröhlich and Wagner 1996) and interstitial renewal of PRs (Fröhlich *et al.* 1995). Neither mitotic structures nor identifiable macrophages were observed in the retina, and the only direct momentary evidence of growth is RPE turn-over of OSs. Some retinal regions (Fig. 7) shows significant variance in PR cross sectional diameter, and the thin PRs may in fact be newly proliferated ones.

The retinal neurogenesis in the cichlid *Haplochromis burtonii* GÜNTHER, 1894 was found to be asymmetrical in both space and time by Kwan *et al.* (1996). The arguments for the space-related (naso-temporal) asymmetry applies well to *H. anomala* since both species possess RHCDs as well as other regional retinal specialisations which have to remain in a functionally determined position (Easter 1992).

Interstitial neurogenesis of rod PRs has been demonstrated in both shallow water (Kwan *et al.* 1996) and deep-sea teleosts (Fröhlich *et al.* 1995; Wagner and Fröhlich 1996; Wagner *et al.* 1998). Identification of retinal proliferation zones require labelling techniques which were not applied here, but the pattern of PCNA-ir. cells disseminating through the ONL of *Alepocephalus agassizii* and *Conocara macroptera*, as recorded by Wagner *et al.* (1998), corresponds well with the distribution of the light staining nuclei in the ONL of *H. anomala*. Therefore, these nuclei can with some certainty be labelled rod precursors (Fig. 11A).

This type of prevalent PR proliferation pattern is peculiar to deep-sea teleosts (Wagner *et al.* 1998) contrasting shallow water teleosts where the peripheral growth is dominating (Mack and Fernald 1995; Kwan *et al.* 1996). This occurrence aids the explanation of the local increase in PR density observed here with growth.

Kwan *et al.* (1996) determined a time dependency of the inner-retina proliferation activity on a diurnal scale, possibly linked to the periods of low photopigment activity. The GC density was

high throughout the peripheral retina, proving the peripheral proliferation pattern to exist, but the proliferation was observed to be non-uniform. The annuli-like pattern of GC distribution from the dorso-rostral region suggests that proliferation should occur on a significantly prolonged scale compared to that of diurnal species like *H. burtoni* (Kwan *et al.* 1996) for the annuli pattern to be evident. Alternatively the annuli may originate due to irregular stretching of the anterior retina. Regardless of what pattern is correct, the dorso-rostral region can be interpreted as the most active centre of proliferation in the peripheral retina from where all types of retinal cells are added appositonally.

The presence of a multiple papilla may possibly yield an alternative explanation to occurrence of the annuli pattern of GC density. The interrupted pattern of both GC annuli and papilla fascicles across the rostro-ventral retina can reflect an adaptation providing local GC axon outlets along almost the entire horizontal retinal meridian. If GC axons could leave the retina at the papilla fascicle closest to the GC nucleus it would decrease the radial thickness of the opticus fibre layer, and hence the retinal thickness. This would in turn decrease the optically interrupted travel distance of light through the inner retina.

Species with pronounced papillary elongation and normal papillae (optic discs) must be examined for the presence of annuli patterns, and retrograde labelling studies of GC dendritic fields and axons may reveal if the GC axons actually leave the retina at the point of the nearest fascicle.

The population of α -like GCs in the temporo-ventral retina recembles the “area gigante cellularis” reported from some tubular-eyed scopelarchids (Collin *et al.* 1997, 1998; Wagner *et al.* 1998) but in *H. anomala* this designation does not apply, since small nuclei (sGCs or DACs) are also present in this region. Still, it is interesting to speculate on the possible physical limitation of retinal

stretching caused by α -like GCs, which are known to possess large dendritic fields with extensive arborisation (Wagner 1990).

Retinal Topography

While retinal architecture and cell morphology appears relatively constant through the ontogeny of *H. anomala* investigated, substantial alterations occur with respect to topographic density changes during post-metamorphic growth.

Dorso-rostral region of high cell density.

The higher resolving power of the dorso-rostral retina (CPD_{GC} increases to 6.5 in the adult) compares well with the same region observed by Collin and Partridge (1996) in a range of alepocephalids, including *A. rostratus* (16.2×10^3 cells mm^{-2} and $CPD_{GC} = 4,27$) and *R. attrita* (7.8×10^3 cells mm^{-2} ; $CPD_{GC} = 3,43$; see also Wagner *et al.* 1998).

The region was not found in neither *S. kofuedii* (Collin and Partridge 1996) nor in the genus *Conocara* (Wagner *et al.* 1998). However *C. macroptera*, an alepocephalid with similar retinal morphology to *H. anomala* does have a PR density maximum in this region with 1.46×10^5 PRs mm^{-2} and $CPD_{RP} = 119$ (not SUM-corrected). This PR density nearly match its foveal PR density of $c 1.7 \times 10^5$ PRs mm^{-2} .

Interestingly, when CPD_{PR} is corrected for the high summation ratio (11:1) in the rostral retinal region of *C. macroptera*, a much lower CPD_{PR} value than in the dorso-rostral region of the adult *H. anomala* is found (3.68 vs. 11.2), and similar with CPD_{GC} values (Fig. 17F).

A rostral region of high resolving power has also been observed in shallow water teleosts (i.e. the serranid *Cephalopholis miniatus* FORSSKÅL, 1775, with 22×10^3 GCs mm^{-2} and $\text{CPD}_{\text{GC}} = 10$, Collin and Pettigrew 1988a). The spatial resolving power in the dorso-rostral region of a 190 mm *H. anomala* is doubled compared to that of a 310 mm *R. atrita*, and is comparable to that of a 140 mm *A. rostratus* (relative size taken into account through lens size), but none of these deep-sea alepocephaloids rivals a 350 mm *C. miniatus*.

High retinal sensitivity and a resolving power increase compared to that of the fundus retina suggest that the dorso-rostral region may be an advantage when aiming to detect visual cues entering the lateral visual field from posterior (Brauer 1908 ; Locket 1977).

Vertical specialised region in the juvenile.

The varying levels of summation in the vertical region (Fig. 16B) may allow the juvenile to optically ‘flick’ between resolution and sensitivity perception of a visual cue through muscular rotation of the eye (the usual 6 extra-ocular muscles are prominent, and the eyeball rests loosely in the eye socket), but further investigations of this region is needed to determine its functional significance. Also the horizontal retinal meridian of the juvenile exhibits fluctuating summation (Fig. 17A). However, the overall variation in summation gradients of the fundus retina does not vary from juvenile to adult when the high mean GC-density of juveniles is incorporated (see above).

Horizontal visual streak and fundus retina in the adult.

In shallow water species with visual streaks the resolving power of this retinal region is often as high as 14 cycles pr. degree (*Choerops albigena* DE VIS, 1885) and 22 cycles pr degree was recorded in *Lethrinus chryosostomus* RICHARDSON, 1848 (Collin and Pettigrew 1989). The maximal resolving power found for *H. anomala* in this region ($CPD_{GC} = 4.61$) is far from these values but it approaches the weak visual streak of *Parapercis cylindrica* OKAMURA, 1984 with a $CPD_{GC} = 5-6$. and lens diameter of 3.2 mm (Collin and Pettigrew 1988a).

P. cylindrica demonstrate the same unusual pattern of punctuated regions with increased GC density along the horizontal retinal meridian observed in *H. anomala*.

The resolving power along the visual streak of *H. anomala* exceeds that of the cave dwelling reef antennerid teleost *Halophryne diemensis* (LESUEUR, 1824) ($CPD_{GC} = 3.55$ and lens diameter of 4 mm (Collin and Pettigrew 1989)).

This is the first report of a horizontal visual streak in the retina of a deep-sea teleost. Visual streak presence is somewhat surprising since a strong correlation between the presence of horizon in the visual field and the presence of retinal visual streaks was predicted by Hughes (1977) in 'The Terrain Theory'. It is stated that the distribution of GCs across the retina is dependent of the symmetry of the perceived world. However, the visual streak of *H. anomala* can potentially be of functional significance, but evolutionary it is probably a reminiscent structure rather than a novel development (as probably the case with *P. cylindrica*).

The visual streak with increased resolving power can thus be particularly important for perception of luminous point-sources present in the lateral field of vision. A relatively high summation ratio (maintaining retinal sensitivity) may also prevent the region from being rendered blind by near-threshold illumination levels (Munk 1980).

From juvenile to adult *H. anomala*, an approximate doubling of the resolving power of the non-specialised fundus retina is developed primarily as a result of increased GC densities (Fig. 15A,C). With ocular growth the lens has simultaneously been moved away from the fundus retina allowing a focussed image further improving lateral monocular vision.

In the rostro-ventral retina the low-peak of both PR and GC density is consistently observed through ontogeny, and the region is gradually displaced along a rostro-ventral oblique trajectory readily illustrating the naso-temporal asymmetry of retinal proliferation (see above and Fig. 15B,D). However summation is not altered compared to the fundus retina values and is not discussed further.

The low summation in the retinal region close to the optic nerve head (or where it would be located in a species with single papilla) is a result of low peak cell densities (Fig. 15A-D), and the quality of visual perception in this region is probably inferior.

The high GC density of the dorso-temporal region in juveniles decreases during ontogeny with retinal stretching and this region illustrates no improvement of resolving power, and is not discussed further. Also the GC density increase along the multiple papilla in the juvenile disappears with age (Fig. 15A,C).

The Fovea.

The peripheral and far temporo-ventral location of the foveal region in the retinal eye cup physically limits the foveal binocular visual field to subtending the rostral to dorso-rostral visual field when the eye is not rotated in the orbit. The marked frontal directionality of the visual field in *H. anomala* may be emphasised by the following specialisations of the head morphology.

The premaxillary tusks, that may not be employed as foresights *sensu stricto* due to the low intensity of the prevalent ambient illumination, still define an extension of the ventral part of the visual groove and may possibly aid in maintaining binocular focus, by indicating when the luminous visual cue is moving sideways or downwards out of the binocular visual field.

Similarly, the rostral displacement of the maximum interorbital width produced by flaring of the prefrontals may functionally be considered a dorsal limitation of the rostral binocular visual field of the adult. Hypothetically this platytroctid specialisation could limit the risk of blinding ie. assist in maintaining image quality and directionality of vision when occasionally entering the upper and more illuminated mesopelagic zone.

Binocular disparity, which emphasises skew of an image caused by simultaneous observation by the two eyes, may provide at least some of the depth vision that the absent parallax effect accounts for in a photic zone environment (Walls 1942; Locket 1992; Collin 1997). The foveal region is well developed in juveniles and it alters relatively little in size through ontogeny. Apart from the dorso-rostral RHCD the fovea is the only regional specialisation maintaining its position in the eye-cup during growth.

The shoulder displacement of foveal nuclei coupled with the changing PR densities of the fovea prevents correct estimates of the presumeable array of summation rates from foveal centre to foveal periphery. The lack of exact summation values impedes correction of CPD_{PR} in the fovea and this value cannot be compared with values from other retinal regions. When CPD_{GC} is calculated from the peak foveal GC density, the value obtained is more representative of the mean resolving power of the entire foveal region than of the local shoulder region, or foveal centre.

The foveolar summation might be lower than summation in the surrounding foveal regions. For comparison, both behavioural and morphological studies indicate that summation is absent in the

human foveal centre. However with summation absent the foveola could be rendered blind at low levels of illumination.

When the marked asymmetry of the PR topography within the fovea is considered, it is likely that the resolving power is far from uniform across the foveal region. Thus the calculated foveal CPD_{GC} of 10 in the adult *H. anomala* (Fig. 17F) is probably underestimating the peak resolving power of the foveola.

For comparison, the foveal CPD_{PR} (without correction for summation) and CPD_{GC} was calculated from data in Wagner *et al.* (1998) on a 300 mm long *Conocara macroptera* (lens diameter; 8.5 mm.) and compared with the adult *H. anomala* (Fig.17F).

The foveal morphology of *C. macroptera* (Locket 1992; Collin 1997; Wagner *et al.* 1998) appears very similar to that of *H. anomala*, and with a peak foveal PR density of $c 170 \times 10^3$ pr. mm^2 , a non-corrected $CPD_{PR} = 127$ is found, which corresponds well to the value 119 calculated for the adult *H. anomala*.

Additionally, CPD_{GC} from the foveal shoulder of *C. macroptera* is lower than the value for *H. anomala*.

The minimum OS diameter in the foveola of *C. macroptera* is 2.8 μm which is 85% of the diameter found for non-foveal OSs. In contrast the foveolar OS diameter of adult *H. anomala* is 1.1 μm i.e. only 35% of the non-foveal average diameter.

This comparison, though not providing unequivocal evidence of the actual relations between resolution and sensitivity of foveal vision in these two species, still indicates that the foveae may perform in different ways. The fovea of *H. anomala* may emphasise resolving power over sensitivity, indicated by the extremely thin and densely organised foveolar OSs in the foveola of

H. anomala. Meanwhile the fovea of *C. macroptera* may emphasise sensitivity. This makes for the assumption that *C. macroptera* has adopted a feeding strategy where accuracy is of less importance. The fovea of this species is located dorso-temporally subtending the rostral to rostro-ventral visual field (Locket 1992). Golovan and Pakhorukov (1975) established the diet of *Alepocephalus bairdii* GOODE & BEAN, 1879 to be largely consisting of slow moving species of benthic-pelagic coelenterates and the possibility of a similar diet of *C. macroptera* finds support here.

Contrarily, juveniles of *H. anomala* are believed to feed on crustaceans (N. Merrett, pers. comm. 1998), a diet that would require good resolving power for recognition of point source stimuli in motion, and there is no morphological evidence (e.g. alteration of dentition pattern through ontogeny) suggesting that adults turn to other diets.

Possible functional aspects of the fovea

Although the foveae of *H. anomala* and *C. macroptera* appear similar, it is suggested here that *H. anomala* emphasises resolving power as suggested for other platytroctids and alepocephalids with nuclear displacement in the inner retina (Wagner *et al.* 1998), while the fovea of *C. macroptera* may be emphasise sensitivity to a greater extent. Previous attempts of determining functional properties of convexiculate foveae suggested that the extreme case of sensitivity dependent fovea was the multiple bank type as seen in *Bajacalifornia drakei*, where summation is significantly higher than in the fovea of *C. macroptera* (Wagner *et al.* 1998).

The asymmetrical PR density gradient of the foveola found in *H. anomala* is aligned meridionally, with the centro-peripheral foveal density gradient steeper in the temporal shoulder and the PR density peak located in the temporal region of foveola. The density gradient of the

inferior shoulder is less marked. Thus the symmetry axis of the fovea is parallel to the horizontal meridian of the eye, where a visual stimulus is most likely to be located prior to engagement of feeding activity. Hence, a stimulus moving horizontally along the foveal symmetry axis will traverse all the local specialisations (mentioned above) in the foveal retina.

The characteristic displacement of nuclei from the foveolar inner retina removes a potentially significant source of refraction that could otherwise scatter lens focused rays, but in the convexiclvate fovea of *H. anomala*, *C. macroptera* and other alepocephaloids (Locket 1992; Collin 1997) and also in raptorial birds (Snyder and Miller 1978) photons instead have to pass through the thick radial fibre lining of the foveal pit. Further, this layer defines a smooth curve of the foveal vitreo-retinal boundary.

Radial fibre material is also the most prominent cellular component of the foveolar inner retina, which is devoid of nuclei. The ultrastructure of radial fibre material suggest a relatively high refraction index of this retinal component. This assumption was made previously by Snyder and Miller (1978) who used interference microscopy and determined the relative refractive index of a falconiform foveal retina to be 1.369, and suggested that the refractive index of radial fibre material may be as high as that of OSs (1.44), which are also of lamellar ultrastructure. However, no specific refractive index for the radial fibre material was determined. Steenstrup and Munk (1980) and Locket (1992) also assumed a high relative refractive index of the radial fibre component.

Further sclerad in the fovea the elongations of the foveolar PR inner segments produce a more pronounced displacement of the level of inner-outer segment boundaries in the foveola. Walls (1942) proposed that focus could be obtained along the entire OS length, while Pugh and Lamb (1990) stated that lens refracted light has to be focused at the ELM in order to produce sharp

images. As well as the OSs, the inner segments are thought to possess light gathering properties, and the elongation of inner segments in the foveola may thus maximise photon capture here, where OS cross sectional diameter is approaching the lower threshold for photon capture ability. If the theory of Walls (1942) is correct, the foveal OS displacement to the sclerad, which was also observed in *C. macroptera* (Locket 1992; Collin 1997) hypothetically effects the degree of accommodation needed to focus an image and it may correct for hypermetropia by displacing the level of focus sclerad.

Locket (1992) traced a 40° cone of rays centred in the foveola (Fig. 2A) to illustrate that the focus level of the foveal retina of *C. macroptera* may be vitread to the ELM. The solid foveal visual angle was calculated from the posterior nodal distance on fixed material, and the true angle may be different (no attempts were made to determine the focus length of the lens directly, and fixed tissue was used despite the effects of shrinkage inherent to the process of fixation). Measurements on *H. anomala* (still fixed material) suggest that the solid visual angle of the fovea may be closer to $c. 30^\circ$ (α in Fig. 2A). Also, the foveal tracing used by Locket was a product of interpolation of the foveal curvatures from both eyes, thus any asymmetries of the foveae were masked.

In teleosts, the large solid angles are products of the large lens and pupil aperture. In falconiform birds this angle is presumed to be $c. 17^\circ$ and a cone of rays centred in the foveola hardly impinge on the foveal walls (Snyder and Miller 1978). With a wider solid angle even a centred cone will extend over the foveal shoulders and undergo various degrees of refraction at the vitreo-retinal boundary. This refraction will be enhanced if the radial fibre lining of the fovea has a refractive index higher than that of the remaining inner retina. Since refraction additionally relates to angle of entrance into the refractive medium the central-most rays crossing the vitreo-retinal boundary

perpendicularly to the surface are not refracted, while peripheral rays of the cone experience various degrees of refraction. This pattern impedes a sharp image formation in any given point, and Locket (1992) determined a blurred circle with a diameter of 20 μ m as the probable foveal image of *C. macroptera*.

Prior to the study of Locket (1992), Steenstrup and Munk (1980) conducted a mathematical study of foveal imaging of the notosudid *Scopelosaurus hoedti* BLEEKER, 1860 and determined, that the symmetrical convexiclvate fovea of *S. hoedti* distorts a de-centred point source image into an elongated or double point image which is brighter away from the foveal centre. Locket (1992) also found a skewed image of a de-centred cone which was brighter away from the foveola in *C. macroptera*, and his tracings additionally demonstrated that a de-centred image is magnified, as previously suggested by Walls (1942), and recently again by Wagner *et al.*(1998). It is possible that a focused foveal image is not attained, and that a blur circle may be the foveal image of *H. anomala* as well.

The foveolar resolving power of *H. anomala* may be high, due to the abrupt PR density increase of the foveola. The sclerad boundary between the radial fibre foveal lining against the GCL is highly irregular (Fig. 5 and 13) and Locket (1992) proposed, that such irregularity could reduce the potentially complex array of refractive effects at this boundary. The present work shows that almost the entire foveolar inner retina of *H. anomala* is occupied by radial fibre material, and this composition may instead optically homogenise at least the foveolar centre.

In this respect, attention is drawn to the 'theory of the fovea' by Pumphrey (1948), who suggested that the continuously altered refraction of incident rays along the curved walls of the convexiclvate fovea emphasises the movement of a point source stimuli across the foveal retina.

Considering parallel rays, Pumphrey proposed that the image of a point source moving across the fovea would lag as it moved towards the foveal pit, then lead as it moved away, and only in the bottom of the foveal pit would the image be perceived at the virtual acceleration.

Considering a three dimensional cone of rays will complicate the appreciation of this theory. A higher refractive index of the radial fibre foveal lining may emphasise both relative acceleration and image skew, thus rendering the relative changes easily detectable to a fovea with high resolving power.

The foveolar asymmetry, displacing the maximum PR density laterally and the skew of the receptive field produced by lateral de-centring along the foveal symmetry axis may enhance the perception of movement in the foveola. Additionally a higher degree of refraction occurs as the cone moves to the medial, and out of the foveal pit (and inversely if the image moves in reverse direction). Additionally the vitread concavity of the foveolar ELM may magnify the foveolar image, as the tracings of Locket (1992) suggested.

The author believes that the abrupt changes in foveolar photoreceptor morphology observed in *H. anomala* (increase in density along with decreasing cross sectional diameter) contributes to the perception of relative change in acceleration of a point stimulus and this effect is emphasised by the high resolving power believed to be present in the foveola.

A sudden increase in PR synaptic output will occur as the stimulus crosses this confined region.

With the stimuli moving away from the foveal pit (with rays undergoing rapidly increased refraction) an accelerating displacement of the image across the parafoveal receptive field of the medial shoulder will be registered. With these effects mirrored in the fovea of the other eye the perception in either eye of abrupt change in relative acceleration would define the outer borders of a horizontal region of binocular foveal visual field in which an image has to be observed for a

subsequent feeding strike to be efficient. Simultaneously, binocular disparity of the foveal binocular vision provides means of depth perception (Walls 1942; Locket 1985).

Interference microscopy studies on fresh (cryo-preserved) retina determining the individual refraction of various retinal layers and components is one of many studies needed for quantifying this presumed array of effects on visual perception caused by the fovea, and for obtaining conclusive evidence of the refractive index of radial fibre material (Locket 1992; S. P. Collin, pers. comm. 1998). Meanwhile, measuring the posterior nodal distance on the matching fresh lenses (e.g. using an argon laser as done by Fernald 1988), will provide increased applicability of visual angle tracing experiments in 3D on reconstructed foveal profiles.

In order to obtain a certain knowledge of the specific resolving power of the foveolar retina, studies of the location of foveal shoulder GCs in respect to their foveolar receptive fields are needed. Using retrograde labelling techniques, correction coefficients for DACs can also be established.

Conclusion

In both young and adults of *H. anomala*, the primary region of visual significance is believed to be the temporo-ventral fovea, which is specialised towards high resolving power. For visual mediation of feeding activity the fovea must be important. It is argued that the fovea maintain both discrimination of, and range-finding to a potential prey item. The regional specialisations of the extrafoveal retina varies through ontogeny but at no stage does any other region approach the resolving power and movement detection capability assumed to characterise the foveal vision. The binocular foveal vision subtends the rostral visual field where a prey has to be located for an

efficient strike. The non-foveal specialised regions of the retina may be used when aiming to detect visual stimuli in most of the broad lateral visual field subtended by the eyes. Although no significant behavioural, and feeding data on either platytroctids or most other deep-sea teleosts has been published so far (N. Merrett and Y. Sazonov, pers. comm. 1998), it has been shown here, that the non-foveal resolving power of an adult specimen is increased compared to that of a juvenile *H. anomala*. The relative importance of lateral monocular vision may thus increase with age.

The functional patterns of the convexiculate fovea, and its application in the visual system of deep-sea teleosts is difficult to appreciate, due to the structural complexity of this region. The above mentioned future research approaches may well reveal the refractive consequences of the radial fibre lining in the foveal pit, as well as the true summation rates in the fovea.

Applicability of the 'dual wholemount survey' technique on deep-sea fish retinae

The 'optical sectioning' of the retina available in wholemounts is a convenient method for determining both density, packaging efficiency and tangential measures of structures like the cross sectional diameter of OSs. Regional variations and delicate fluctuations in both GC and PR populations are readily detected, and the topographic overview resolves growth patterns better than histological sectioning.

The gentle processing minimises technique-related distortion of the retina and allows for accurate estimates of cell densities. Further, the method provides a correction standard for results obtained using standard histological techniques, provided the material is comparable and the initial processing is identical (Frederiksen 1976). Applied to PR populations, the technique provides excellent appreciation of photoreceptor mosaics and during trial mountings of syngnatid retinae it

was noted, that cone PRs are readily distinguishable from rod PRs. The method is limited to single-bank PR layers, and it is not optimal when the differences in retinal thickness vary between foveal and non-foveal retina as in the juvenile specimens of *H. anomala*. Elongated OSs bend under the coverslip, and at the level of focus required (basal-most of the OSs) oblique ‘optical sectioning’ tends to complicate the survey.

Removal of the retinal pigment epithelium is necessary, and this is not always possible with deep-sea teleost retinae as illustrated in the present study. With specimens collected and stored alive, and under more convenient circumstances than at sea, the RPE can be rendered dark adapted and dissection carried out in a dark room under a safe light. The layers situated between photoreceptor, and ganglion cell layer can be visualised, but they are not properly available and thus, the wholemount method does not replace histological sections for light microscopy in retinal studies. It is likely, that the retina can be demounted and embedded for sectioning preceding the dual wholemount survey, but the technique remains to be tested.

The GC annuli revealed in the wholemount material of both juvenile and particularly in the adult *H. anomala* could seriously skew ganglion cell counting results obtained from radial sections. If the plane of radial sectioning was perpendicular to the orientation of the annuli, it would cause a density count with marked irregularity in the cell spreading along the section plane. But even worse, with a plane of sectioning parallel to the annuli, some of the resulting sections could be devoid of nuclei while adjacent sections would show a fully packed GCL, and highly irregular cell counts would be obtained.

As illustrated in this study the exclusive wholemount monitoring of GC populations alone, however useful information it may provide, fails to reveal some photoreceptor density-dependent specialisations, as well as certain photoreceptor related architectural specialisations, like the

asymmetrical foveola of *H. anomala*. Inversely the sole monitoring of PR populations would not provide a complete image of the retinal specialisations with out, for instance the accompanying GC density needed to correct CPD_{PR} .

In the foveal shoulder, the GCL accommodates a double layer of nuclei complicating counting, and this aspect could be significant in species with multiple-layered GCs. Increased sampling rates should buffer for counting errors in a RHCD (Curcio *et al.* 1987), but not to the extent, where layers are hidden beneath several others. Determining the specific resolution of the foveolar retina has still not been achieved.

The foveal displacement of GCs impedes determination of CPD_{GC} , while CPD_{PR} needs correcting with summation ratio to be comparable with CPD_{GC} . No reliable information on foveolar summation is available (Fig. 16 C, F) without the designation of shoulder displaced GCs to their receptive field which may be located anywhere in the fovea. It follows, that the GC density alone can not readily be used for estimating resolving power of a fovea with nuclei displacement.

Without applying retrograde labelling, a technique which was not applied here due to inherent problems associated with *in vitro* preparation, it is impossible to determine the percentage of small nuclei in the GCL belonging to either sGC or DAC populations. Previous retrograde labelling studies of *Lampanyctus macdonaldii* (GOODE & BEAN, 1896) (Wagner *et al.* 1998 and S. P. Collin and R. V. Hoskins, in prep.) revealed, that over 80% of the putative sGCs or DACs belonged to the latter population. However, in the tubular-eyed *Scopelarchus michaelsarsi* KOEFOED, 1955 86% of the GCL nuclei possessed an axon in the optic nerve (Collin *et al.* 1998).

Cell counts conducted in this GC wholemount survey did not distinguish between these two populations, since their respective morphological characteristics has not been determined conclusively, and future studies are needed for correction of the GC densities counted here. Still,

the techniques applied here for estimating resolving power are not apparently inferior to behavioural measurements, which are impossible with deep-sea material.

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Abbreviations used in text

CPD: spatial resolution in cycles pr. degree

CPD_{GR}: CPD derived from GC density

CPD_{PR}: CPD derived from PR density

DAC: displaced amacrine cell

MSA: visual acuity, or minimum seperable angle in minutes of arch

RHCD: Region of High Cell Density

SUM: summation, or PR density / GC density

Figure Legends

Fig. 1. Drawing of *H. anomala*. From Nielsen and Berthelsen (1992).

Fig. 2. Developmental head morphology.

- A. Dorsal semi-schematic presentation of a 60 mm *H. anomala*. Noteable latero-medial flattening of the eye, lens (l) in proximity to fundus (fu) retina (re) and far temporally located fovea (f).

Alignment of foveal visual axis (vax) and premaxillary tusks (pmt). Foveal visual angle, α (*c* 30°) is indicated. Additional labelling, ch: choroidea. co: cornea. i: iris. ap: aperture or pupil. s: sclera.

- *B.* Lateral view of specimen in *A.* Elongated eye cup. Horizontally oval pupil with rostral aphakic aperture (ap), aligned with deeply cut rostral visual grooves, see also *D.*
- *C.* Lateral view of 190 mm *H. anomala.* Deeply cut visual grooves (vg) and flaring prefrontals (pf). Proportional changes in head morphology comparable with *A.*
- *D.* Frontal view of specimen in *C.* Aphakic aperture widenes rostral binocular visual field. Bar: 10 mm.

Fig. 3. Retinal pigment epithelium (RPE).

- *A.* Radial section of foveal RPE in 60 mm *H. anomala.* RPE cells are ovoid, few short projections (ep) of cell membrane further vitread (Vd) into ventricular space, which also contains photoreceptor outer segments (os). Pigment granules (pg) are abundant in entire RPE cell pericarya. Nuclei (nu) to the sclerad (Sd). Light micrograph Toluidine Blue.
- *B.* Wholemout view (tangential) of RPE in 190 mm *H. anomala.* RPE-cells (RPE) are penta, or hexagonal (DIC optics).
- *C.* Radial section of RPE in 190 mm *H. anomala.* Epithelial process (ep) with phagocytotic vesicle (phv) projects vitread in ventricular space. Basal, or sclerad nucleus (nu). Electron micrograph.
- *D.* Magnification of (ep) in *C.* Photoreceptor outer segment tip (os) is seen in phagocytotic vesicle (phv). lp: lipid droplet. Electron micrograph. Bars: *A* - 10 μm ; *B* - 5 μm ; *C* - 5 μm ; *D* - 1 μm .

Fig. 4. Radial section through multiple papilla fascicle (mp) in nasal retina of 190 mm *H. anomala*. Fascicles composed of ganglion cell axons permeate from ganglion cell layer (GCL) through inner retina and RPE to optic nerve head (on) as a streak inferior to nearly the entire horizontal eye meridian. Labelling, see *Fig. 5*. Light micrograph. Bar: 50 μm .

Fig. 5. Radial section of foveal retina in 60 mm *H. anomala*. Note elongated photoreceptor outer (os), and inner (is) segments in foveola (fv), and marked displacement of retinal nuclear layers to foveal shoulders. Artificial rupture at *. Labelling: ILM: inner limiting membrane. pa: palisade cell of Müller. rft: radial fibre trunk. GCL: ganglion cell layer. IPL: inner plexiform layer. INL: inner nuclear layer. OPL: outer plexiform layer. ONL: outer nuclear layer. sp: photoreceptor synapses. ocf: outer conducting fibres. hl: layer of Henle. ELM: external limiting membrane. RPE: retinal pigment epithelium. Orientation; Vd: vitread. Sd: sclerad. Tmp: temporal. Fu: Fundus. Light micrograph, DIC optics, Toluidin Blue. Bar: 50 μm .

Fig. 6. Radial section of nasal fundus retina in 190 mm *H. anomala*. Light micrograph, DIC optics, Toluidin Blue. Labelling, se *Fig. 5*. Bar: 20 μm .

Fig. 7. Tangential section sclerad to inner-outer segment junction of parafoveal photoreceptors in 60 mm *H. anomala*. Significant localised variance in cross sectional diameter of the outer segments is seen. Outer segments (os) have various degrees of lobulisation (arrowheads) of outer segment inner membrane (im) separating lobules (lo) along length axis of outer segment. Ruptures in outer segment outer membrane (om) are preparation artifacts. Electron micrograph. Bar: 1 μm .

Fig. 8. Tangential section from foveola in 190 mm *H. anomala*. Photoreceptor inner segment immediately sclerad to level of inner-outer segment junction. Large mitochondria (mc) are abundant. Connecting cilium (cci, see also insert) is located eccentrically in inner segment, is of 9×2 microtubule (mi) structure and is surrounded by 6 calycal processes (ca). Abundance of periciliary vesicles (pv) in cytoplasm proximal to cci. Calycal processes (cp) in ventricular space, surrounds inner segments, see also *Fig. 21*. Electron micrograph. Bar: 0.5 μm.

Fig. 9. Tangential section of parafoveal retina, just vitread to connecting cilium basal section in 190 mm *H. anomala*. Photoreceptor inner segments (is) immediately sclerad to ELM. Inner segments are tightly packed in hexagonal array and containing mitochondria (mc) with radial orientation in inner segment cytoplasm. Ventricular space is also occupied by villous projections (vp) from sclerad Müller cell terminals of the inner retina. Electron micrograph. Bar: 0.5 μm.

Fig. 10. Foveal, and inner-retinal morphology. Elongated photoreceptor inner segments (is) and Henle layer (hl) of foveola in 190 mm *H. anomala*. Radially elongated inner segments approach 35 μm. in length. Henle fibres, the longest attaining lengths of *c* 150 μm. radiate horizontally from foveola to reach photoreceptor nuclei (prn) in foveal shoulder. Arrow points vitread (Vd). Additional labelling as *Fig. 5*. Light micrograph, DIC optics. Toluidin Blue. Bar: 25 μm.

Fig. 11. Nuclear layers of the inner retina.

- *A.* Radial section of scleradmost inner-retina and nuclear layers of temporal foveal shoulder in 190 mm *H. anomala*. Outer nuclear layer (ONL). Some outer conducting fibres (ocf) can be traced from Henle layer (hl) to nuclei (prn), and inner conducting fibres (icf) joining these nuclei with synaptic terminals, or spherules (sp). Lighter staining nuclei (rpn) may be those of proliferative, or photoreceptor precursor cells (see discussion). Synaptic terminals, a main constituent of the outer plexiform layer (OPL), are readily distinguishable in a horizontal ribbon-like layer.

- *B.* Vitread to *A.* Inner nuclear layer (INL) with compartmentised nuclear populations confined in honeycomb meshwork of radial fibre material (rfl) of Müller cell origin, of which some nuclei are present (mcn). Sclerad-most are horizontal cell nuclei (hcn) in horizontal alignment, vitread-most are interplexiform- and or amacrine cell nuclei (acn). Light micrograph, Toluidin Blue. Bars: 10 μm .

Fig. 12. Ultrastructure of photoreceptor spherules.

- *A.* Tangential section of foveal shoulder photoreceptor synaptic layer in 190 mm *H. anomala*. Spherules (sp) of 3-4 μm in tangential diameter are abundant in a single layer vitread to ONL. A single synaptic ribbon (sr) is seen in each spherule but some spherules are radially displaced and ribbon is not shown (y). Second order cell process (x) surround the terminals, which are elaborately ensheathed in horizontal fibres of Müller cell origin (hf). Electron micrograph. Bar: 1 μm .

- *B.* Higher magnification of *A.* Five electron dense lamellar components (la) compose the synaptic ribbon structure. Synaptic vesicles (sve) are abundant in spherule cytoplasm, especially

in proximity of the lamella. Some of the horizontal fibres (hf) are cut radially. Electron micrograph. Bar: 1 μm .

- C. Golgi apparatus (go) in close proximity of synaptic ribbon. Tangentially cut horizontal fibres (hf) are seen. Electron micrograph. Bar: 1 μm .

Fig. 13. Palisade cells and radial fibre trunks.

Radial section of temporal foveal shoulder retina in 190 mm *H. anomala*. Continuity is apparent (black arrow) at extension of radial fibre trunks (rft) from palisade cells bodies (pa). Rtf and pa cytoplasm appear different in composition to the intermediar radial fibre cytoplasm (rfc). Radial fibre trunks project sclerad through ganglion cell layer (GCL), (gc): ganglion cell nuclei.

Additional labelling as *Fig. 5*. Light micrograph, DIC optics, Toluidin Blue. Bar: 10 μm .

Fig. 14. Radial fibre of Müller cell ultrastructure. Black arrows point vitread.

- A. Radial section of palisade cells most vitread showing close apposition and interdigitations of cell membranes (arrowhead depicts intercellular space). Palisade cell cytoplasm is electron dense and mottled with granular material (gr), possibly glycogen, and abundant microfibrils (mf), approx. 100 Å in cross sectional diameter. Other palisade cell process at (x). Electron micrograph.

Bar: 1 μm .

- B. Radial section showing region of radial fibre trunk (rft) extension (trunk seen in *Fig. 13*).

Cytoplasm of palisade cell is lighter than in A and additionally showing few vesicles (ve) and no microfibrils. The lamellar composition of the radial fibre trunk (rfl) gradually occurs from

continuities between palisade cell and radial fibre trunk cytoplasm. Electron micrograph. Bar: 1

μm .

- C. Radial section further sclerad. Radial fibre trunk (rft) extends sclerad from radial fibre cell cytoplasm (rfc). Neurotubuli (nt) are ensheathed in radial fibre lamellae. Electron micrograph.

Bar: 1 μm .

- D. Radial section further sclerad. Radial fibre trunk (rft) with highly organised lamellae and homogenous cytoplasm. Electron micrograph. Bar: 0.5 μm .

Fig. 15. Iso-density contour maps of ganglion cell, and photoreceptor populations.

Retinal wholemounts (right eye) from juvenile (60 mm), and adult (190 mm) *H. anomala*.

Arrowheads indicate the trajectories (the horizontal meridian, vertical meridian and foveal meridian), plotted in *Fig. 16* and *Fig. 17*. The fovea was removed in juvenile due to risk of

damage, see text. Ganglion cell (GC) densities refer to all neuronal nuclei within the ganglion cell layer except glial cells. D: dorsal. N: nasal. A - Ganglion cell densities ($\times 10^3$ cells $\times \text{mm}^{-2}$) in juvenile, C - in adult. B - Photoreceptor densities ($\times 10^4$ cells $\times \text{mm}^{-2}$) in juvenile, D - in adult.

Fig. 16. Density profiles of photoreceptor, and ganglion cell populations and summation ratios along the horizontal, vertical, and foveal meridians (arrowheads on wholemounts in *Fig. 15*).

Right eyes in juvenile (60 mm), and adult (190 mm) *H. anomala*. Orientation: A - and D -

Horizontal meridian, caudal (C) to nasal (N). B - and E - Vertical meridian, dorsal (D) to ventral

(V). C - and F - Foveal meridian, temporal (T) to dorso-rostral (R). Black: GC density ($\times 10^3$ cells mm^{-2}). Light gray: summation ratio (PR density / GC density). Dark gray: PR density ($\times 10^4$ cells mm^{-2}).

Fig. 17. Resolving power profiles along the horizontal, vertical, and foveal meridian (arrowheads on wholemounts in *Fig. 15*). Right eyes in juvenile (60 mm), adult (190 mm) *H. anomala*, and 300 mm *Conocara macroptera*. Orientation as *Fig. 16*. Graph labels: □, Resolving power derived from photoreceptor density corrected with summation ratio. (CPD_{PR}). Δ, Resolving power derived from ganglion cell density (CPD_{GC}). Black column; CPD from PR density. Striped column; CPD from GC density of 300 mm specimen of *C. macroptera*; (Wagner *et al.*, 1998).

Fig. 18. Wholemount preparations showing cell populations in ganglion cell layer in 190 mm. *H. anomala*.

- A. High densities of ganglion cell layer nuclei, dorso-rostral proliferative region. GC: large ganglion cells. sGC: small ganglion cells (or displaced amacrine cells). Light micrograph. Cresyl-violet. Bar: 10 μm.
- B. Temporo-ventral peripheral region with high densities of large ganglion cells (GC). Labelling as *Fig. 18a*. Light micrograph. Cresyl-violet. Bar: 10 μm.

Fig. 19.

- A. Dorso-rostral annuli pattern in 190 mm *H. anomala*. Annuli are concentric with fundus centre (Left). Few cigar shaped glial cell nuclei (glc). Labelling as *Fig. 18a*. Light micrograph. Cresyl-violet. Bar: 25 μm.
- B. Fundus ribbon pattern with dorso-ventral distribution in 60 mm *H. anomala*. Light micrograph. Cresyl-violet. Bar: 25 μm.

Fig. 20. Wholemout preparations of photoreceptor layer.

- *A.* Foveola in 190 mm *H. anomala*. Sclerad view. Total foveolar outer segment (os) population is visible, surrounded by superior (sup) and inferior (inf) foveal shoulders. A marked temporo-fundal (Tmp-fu) elongation of the foveola (fv) is noted. Light micrograph, DIC optics. Bar: 10 μm .

- *B.* Extrafoveal fundus rod mosaic in 190 mm *H. anomala*. Outer segments (os) are packed in hexagonal array. Light micrograph, DIC optics. Bar: 10 μm .

Fig. 21. Comparable photoreceptor outer segment diameter in 190 mm *H. anomala*.

- *A.* Foveolar outer segments (os) sclerad to outer-inner segment junction, surrounded by calycal processes (ca). Additional labelling see *figs 7 and 8*. Electron micrograph. Bar: 1 μm .

- *B.* Non-foveolar outer segments, *c* 2 μm rostral to *A*. Noteable increase in outer segment cross sectional diameter, and decrease in packing efficiency. Electron micrograph. Bar: 1 μm .

- *C.* Further 5 μm rostrally. Outer segments now with average cross sectional diameter of fundus retinal population. Electron micrograph. Bar: 1 μm .

Table 1. Summary of collection data and meristics for the *H. anomala* specimens examined.

Fixation (+) indicates proper quality of fixation, and further processing of retinal tissue.