Fine structure of the retina and
pigment epithelium in the creek chub,
Semotilus atromaculatus (Cyprinidae, Teleostei)

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Summary. The structure of the light- and dark-adapted
retina, the pigment epithelium and the choroid of the
creek chub, *Semotilus atromaculatus* (Cyprinidae,
Teleostei) is examined by light and electron microscopy.
An extensive network of vitreal blood vessels emanating
from the hyaloid artery enters the eye with the optic
nerve and overlies the inner limiting membrane. This
membrane closely apposes the fine protrusions of the
Müller cell processes which traverse the entire retina,
dividing the inner retina into alternating fascicles of
ganglion cells and optic axons. The inner nuclear layer
consists of bipolar, amacrine, Müller cell soma and two
layers of horizontal cells. The outer plexiform layer
possesses both rod spherules and cone pedicles. Each
rod spherule consists of a single synaptic ribbon in either
a triad or quadrad junctional arrangement within the
invaginating terminal endings of the bipolar and
horizontal cell processes. In contrast, cone pedicles
possess multiple synaptic ribbons within their junctional
complexes and, in the light-adapted state, the horizontal
cell processes show spinule formation. Four
photoreceptor types are identified on morphological
criteria; unequal double cones, large single cones, small
single cones and rods. All but the small single cones are
capable of retinomotor responses. The rod to cone ratio
is approximately 5:1 and the rods form two ill-defined
rows in the light-adapted condition. The retinal pigment
epithelium possesses two types of osmiophilic granules.
These are bound within slender microvilli and migrate
vitread to surround the photoreceptors in response to
light. Bruch's membrane is trilaminar and the
vascularised choroid consists of up to three layers of
melanocytes. The endothelial borders of the choroidal
blood vessels abutting the outer lamina of Bruch's
membrane are fenestrated.

Key words: Retina, Fish, Photoreceptors, Retinal
pigment epithelium, Electron microscopy

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Introduction

Previous ultrastructural studies of the eyes of
teleosts (Dunn, 1973; Fineran and Nicol, 1974; Munk,
1977, 1985; Kunz et al., 1985; Collin and Collin, 1988,
1993; Braekevelt, 1992a,b) have revealed a number of
unique features. These include the retinomotor
responses, equal and unequal double cones which are
often arranged in a regular mosaic, and the rich diversity
of filtering structures (corneal, lenticular and tapetal)
that permit only specific wavelengths of light to reach
the photoreceptor array. These visual adaptations have
no doubt evolved in response to the varied visual
environments. The effectiveness of these adaptations and
the organism's ability to successfully feed and reproduce
can therefore be reflected in the abundance of a
particular species.

One example of an abundant species which has
become important as a baitfish and has received
extensive attention by fisheries ecologists is the creek
chub, *Semotilus atromaculatus* (Cyprinidae, Teleostei).
This species is one of the most common stream minnows
in North America and has been the subject of a large
number of studies on body form (Scott and Crossman,
1973), life history (Magnan and Fitzgerald, 1984a), nest
building (Reighard, 1910), reproductive behaviour
(Dobie et al., 1956; Copes, 1978), growth (Dinsmore,
1962; Magnan and Fitzgerald, 1982) and feeding
strategies (Barber and Minckley, 1971).

A clear water habitat, a feeding preference for live
insect larvae and the construction of intricate underwater
nests, suggests that vision plays a crucial role in the
survival of this species. However, the only studies
related to vision in this species have concentrated on the
effects of light intensity on visual behaviour (Magnan
and Fitzgerald, 1982, 1984b; Cerri, 1983), the scotopic
visual pigments within the photoreceptors (Yoon et al.,
1988; Heinermann and Ali, 1989) and the topography of
cells within the retinal ganglion cell layer (Collin and
Ali, 1994). These few studies show that the creek chub
feeds as efficiently in both low and high light levels but
that adults are more nocturnal than juveniles (Magnan

and Fitzgerald, 1984b). The retina of the creek chub also possesses three "areae centrales" defined as regions of higher retinal ganglion cell density indicating that some degree of active fixation of the eyes may take place to align these acute zones for higher spatial resolution in specific regions of the visual field (Collin and Ali, 1994).

Although this study does not examine the onto-genetic changes in retinal structure of *S. atromaculatus*, it aims to provide morphological evidence for either a diurnal or nocturnal feeding preference providing the basis for a comparison with the few other studies of nocturnally active species (Locket, 1977; Lythgoe, 1979, 1988; Shapley and Gordon, 1980; Pankhurst, 1989).

Materials and methods

Five adult individuals of the creek chub, *Semotilus atromaculatus* (Mitchill, 92-117 mm total length) were collected from Lake Cromwell, at the Biological Research Station of the Université de Montréal, near St. Hippolyte, Quebec, Canada. Collections were made between December and February when the Lake was covered by approximately 1 metre of ice. To immerse the fish traps, a number of holes were bored (0.3 m in diameter) using a diesel powered auger and a steel crowbar. Fish traps, each consisting of a plastic basket (0.25 m long and 0.15 m wide) with a funnel-shaped opening at each end, were filled with bread and suspended in midwater (at 2 m) overnight. The following day, accumulated ice formation was removed and the traps retrieved. Each specimen caught was then transferred into a well aerated tank and transported via snowmobile to the laboratories of the Biological Station.

Fish were either light-adapted for two hours (two animals) or dark-adapted for three to four hours (three animals), before being sacrificed with an overdose of tricaine methane sulphonate (MS222, 1:2,000). Sampling was carried out according to the ethical guidelines of the National Health and Medical Research Council of Australia. The eyes were excised and following the removal of the cornea, lens and vitreous, each eyecup was fixed in either 4% paraformaldehyde in 0.1M phosphate buffer (for light microscopy) or 4% glutaraldehyde in 0.067M sodium cacodylate buffer (for electron microscopy) overnight. For light microscopic examination, small retinal pieces cut from various regions of the fundus were washed in phosphate buffer and embedded in Historesin (Reichert-Jung). One to two micron sections were cut using an American Optical microtome and a steel knife. Transverse and tangential sections were stained with either toluidine blue or Richardson's stain, dehydrated and coverslipped for analysis with a compound light microscope (Olympus, BH-2). For electron microscopic examination, retinal pieces were post-fixed in 2% osmium tetroxide with 1.5% potassium ferrocyanide in 0.1M sodium cacodylate buffer (reduced osmium method of Collin and Allansmith, 1977, which is a slight modification of the osmium potassium ferricyanide method of Dvorak et al., 1972). Tissue was then dehydrated in acetone and embedded in resin (Polybed/812, Polysciences Inc). Thick (1 μm) sections were stained with Richardson's stain and examined by light microscopy or stained with paraphenylenediamine and examined using phase-contrast microscopy. Selected thin sections were then prepared for transmission electron microscopy, stained with lead citrate and uranyl acetate and examined on either a Siemens Elmiskop 1A (School of Optometry, University of New South Wales) or an Hitachi H500 (Department of Marine Biology, University of California San Diego) electron microscope.

Measurements were made on enlargements of light and electron micrographs using a magnifier and graticule. Photographs were taken on either 35 mm Kodak Technical Pan film (rated at 50 ASA) (light microscopy) or Kodak 4489 electron microscope film.

Results

The retina of the creek chub, *Semotilus atromaculatus* is approximately 310 μm in thickness with an extensive system of vitreal blood vessels overlying its inner surface (Fig. 1). From the hyaloid artery which enters the fundus with the circular optic nerve, small calibre vessels branch and traverse the retina towards the periphery, attached to the inner limiting membrane (ILM) via collagen fibrils (0.02 μm in diameter). Large collecting veins, which anastomose with small veins, follow the retinal perimeter at the ora terminalis. Pericytes of various sizes surround both arterioles and venules (Fig. 1b).

The ILM consists of a thin membrane (0.02-0.03 μm thick), which is covered completely by Müller cell processes. Small convolutions of the Müller cell endfeet lie in register with the ILM and the walls of the apposing vitreal blood vessels (Fig. 1b), making contacts with the ILM at the apex of each protrusion (Fig. 1c). Numerous mitochondria lie within the Müller cell processes which divide the nerve fibre and ganglion cell layers into alternating columns or fascicles (Fig. 1a,b). Ganglion cell axons are predominantly unmyelinated but rare myelinated profiles are sometimes observed. Ganglion cell somata are of various sizes (4-10 μm in diameter) and stain differentially in some cases. The ganglion cells are not confined to a single lamina but lie at various levels within aggregations between the fascicles of nerve axons (Fig. 1a). The layer in which ganglion cells lie is approximately 22 μm in thickness, while the maximum thickness of the axon fascicles is 37 μm.

The inner plexiform layer is approximately 38 μm in thickness and consists of a complex meshwork of ganglion and inner nuclear layer cell dendrites traversed by Müller cell processes. Müller cells, amacrine cells (5-12 μm in diameter), bipolar cells (3-8 μm in diameter) and two rows of horizontal cells (10-15 μm in diameter), identified by size, shape and staining, form a multilayered inner nuclear layer approximately 30 μm in

thickness (Fig. 1a).

The outer plexiform layer (OPL) is 23 µm thick and consists of a mixture of bipolar, horizontal and photoreceptor terminals. The photoreceptor terminals are surrounded by Müller cell processes (0.02-0.05 µm in thickness) at this level and typically make contacts with

the bipolar and horizontal cell processes via synaptic ribbons. In a complex arrangement of invaginations, rod spherules, when viewed in transverse section, possess a single synaptic ribbon (1.00±0.2 µm in length, n=21, Fig. 2a,b) while cone pedicles possess either three or four synaptic ribbons (0.70±0.2 µm in length, n=20, Fig.
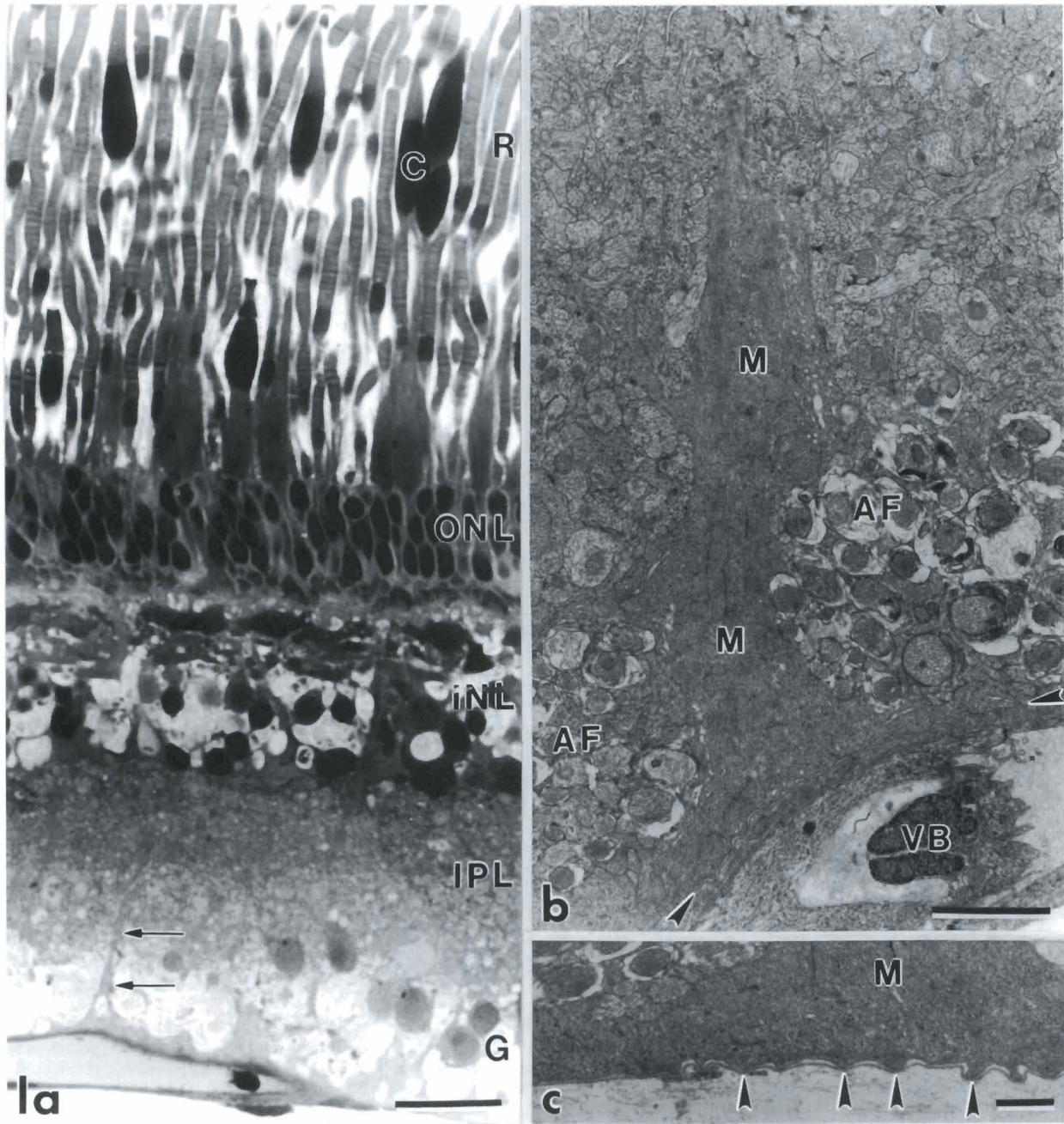

Fig. 1. a. Light micrograph of a transverse section (1 µm) of the light-adapted retina of *Semotilus atromaculatus*. Arrows denote the long process of a Müller cell, the soma of which lies in the inner nuclear layer (INL). **b.** Electron micrograph of the thick Müller cell processes (M) dividing two axon fascicles (AF). The arrowheads mark the boundary between the inner limiting membrane and the vitreal blood vessel (VB). **c.** Higher magnification of an inner process of a Müller cell (M) showing its fine protrusions (arrowheads) abutting the inner limiting membrane. C: cone photoreceptors; G: ganglion cell layer; IPL: inner plexiform layer; ONL: outer nuclear layer; R: rod photoreceptors; RPE: retinal pigment epithelium. Scale bars: 20 µm (a), 3 µm (b), 1µm (c).

2c,d). Serial sections were not attempted to confirm the absolute number of synaptic ribbons in each receptor terminal. In both types of terminals, the synaptic ribbon projects from the juncture of two lateral processes and either a single or double central process forming a triad or quadrad, respectively (Fig. 2). All synaptic ribbons are surrounded by an amorphous substance and synaptic vesicles (39.0±7.0 nm in diameter, n=64) aligned along the length of the ribbon. In addition to the synaptic contacts formed by the synaptic ribbon, basal arciform body and the invaginating processes, surface contacts are also common and appear as an increased density of both
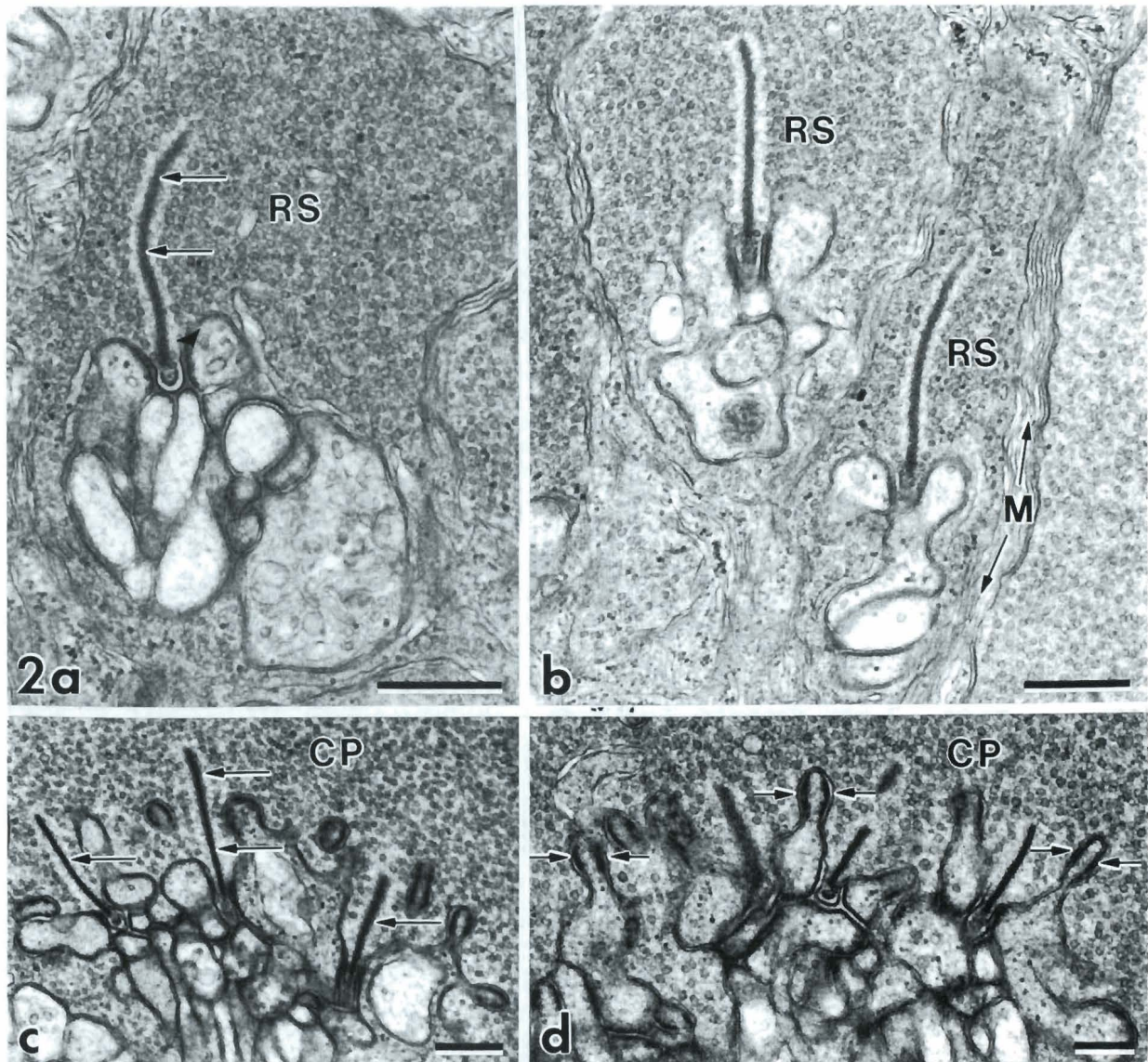
Fig. 2. Fine structure of the photoreceptor terminals. **a.** Synaptic arrangement (quadrad) of the sclerad processes of the inner nuclear layer cells and a rod spherule (RS). Each rod possesses a single synaptic ribbon (arrows). **b.** Two other examples of rod spherules showing the two and three terminal arrangements. **c.** Complex synaptic arrangement of a light-adapted cone pedicle (CP) consisting of a number of synaptic ribbons (arrows). **d.** Three synaptic terminals of a light-adapted cone pedicle showing a number of spinules (arrows) located on the inner surface of the horizontal cell processes. M: microvilli of the Müller cells. Scale bars: 0.2 μm (a), 0.5 μm (b), 0.4 μm (c), 0.3 μm (d).

Fig. 3. Cone morphology. **a.** Light micrograph of a transverse section (1 μm) of the light-adapted retina at the level of the photoreceptors showing the three types of cone cells. The large single cones (lsc) and double cones (dc) both display retinomotor responses in contrast to the small single cones (ssc). **b.** Fine structure of the double cone which consists of a closely apposed large principal cone (PC) and an accessory cone (AC). **c.** Two large single cones in the light-adapted state. The structure lacking discs (arrowed) abutting the outer segment (OS) is an accessory outer segment. **d.** An enlargement of the disc structure of a single cone. Note membranes may surround more than one disc (arrowheads). cn: cone nucleus; IS: inner segment; m: mitochondria; R: rods; rn: rod nuclei; *: calycal process. Scale bars:15 μm (a), 3 μm (b), 3μm (c), 1 μm (d).
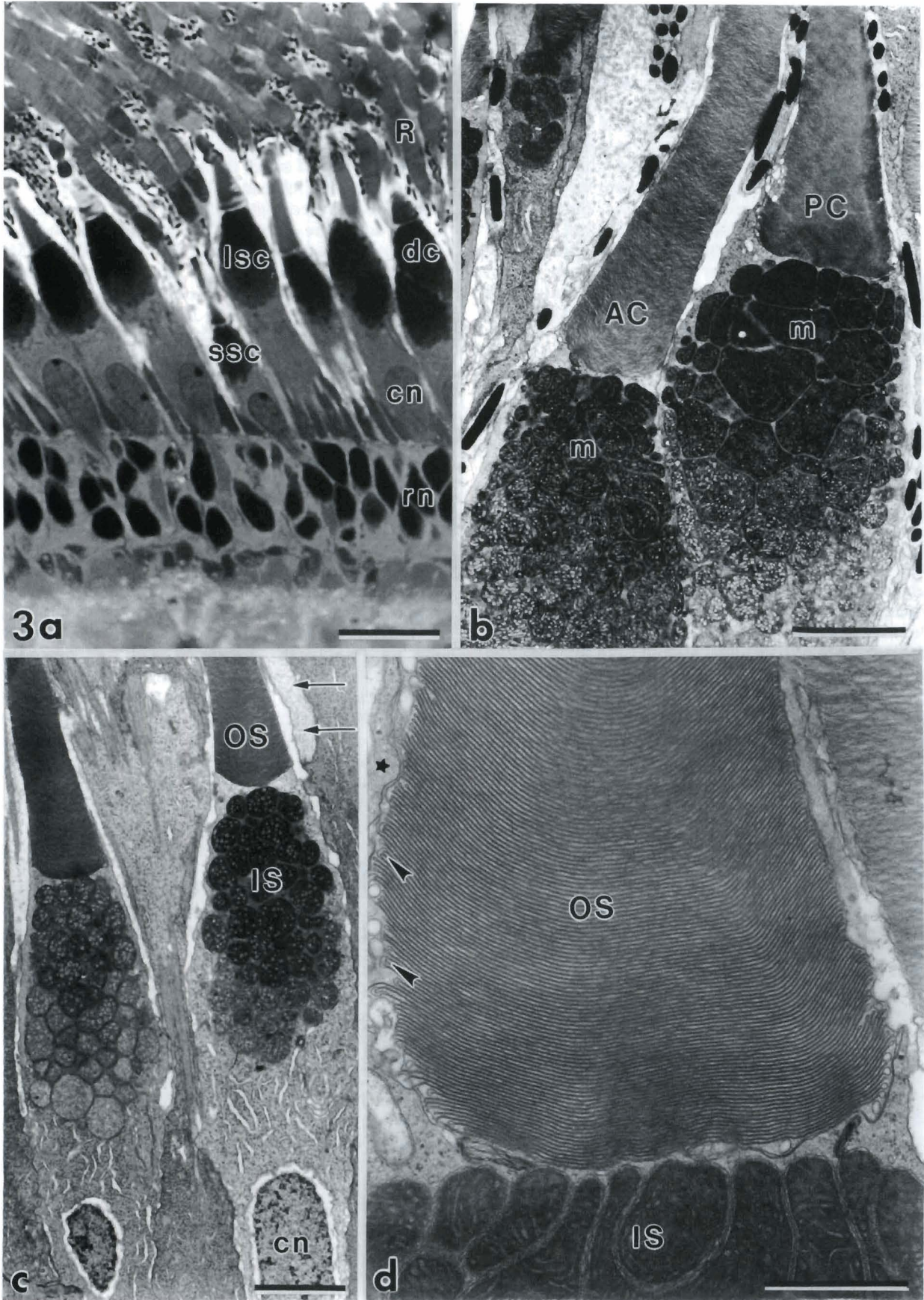
pre- and post-synaptic cytoplasm lying on each side of an interspace containing osmiophilic material (Fig. 2a).

In the light-adapted retina, numerous spinules appear as membrane densities at the tips of protruding horizontal cell processes which surround the synaptic ribbons in cone pedicles. The membrane densities are either continuous or form interrupted clumps of an osmiophilic substance along the inner membrane surface of the horizontal cell process (Fig. 2c,d). Spinules are not observed in dark-adapted retinae and are not associated with the terminal endings of rod spherules.

The outer nuclear layer (ONL) (20 µm thick) consists of three or four layers of darkly staining rod nuclei which lie vitread to the cone nuclei that typically straddle the outer limiting membrane (OLM) (Fig. 3a). The ascending finger-like processes or microvilli of the Müller cells (Fig. 2b) continue sclerad from the OPL to surround the photoreceptor nuclei and form desmosome-like junctions with the myoids of the photoreceptors at the OLM. The Müller cell microvilli extend as far as the bases of the photoreceptor outer segments.

The photoreceptors are classified into four types; unequal double cones, large and small single cones and rods (Fig. 3a). None of the photoreceptor types contains either a paraboloid or an oil droplet. Each unequal double cone comprises a principal cone (40 µm in length in light-adapted and 66 µm in length in dark-adapted condition) which is longer than a closely apposing smaller accessory cone (36 µm in length in light-adapted and 50 µm in length in dark-adapted condition). The mitochondria within the inner segments of both components of the unequal double cone vary in size. Larger mitochondria situated in the centre of the inner segment gradually become smaller towards the edges of the ellipsoid and towards the myoid region (Fig. 3b). The large (43 µm in length in light-adapted and 57 µm in length in dark-adapted condition) and small (23 µm in length) single cones constitute approximately a quarter of the cone population (Fig. 3c). The small single cones remain stationary and contracted in the dark-adapted condition and are lacking a myoid region (Fig. 3a). All the cone outer segments are short, tapered and consist of a series of closely packed membranous discs comprising a double unit membrane (14.5 nm thick) maintaining an interdisc space of approximately 16.6 nm. Parts of the cone outer segments are surrounded by a thin membranous sheath which may surround either one or more discs (Fig. 3d). Accessory outer segments (AOS) were observed in both single and unequal double cones but not in rods. The AOS appear as elongated protrusions of the inner segment which lie along the outside of the disc-containing outer segment and are joined by a connecting cilium. The contents of the AOS are homogeneously granular, completely devoid of discs and other organelles (Fig. 3c).

Rods constitute the bulk of the photoreceptors in the adult retina (rod:cone ratio is approximately 5:1) and two irregular rows of rods could be distinguished. The vitread row (68 µm in length in light-adapted and 42 µm

in length in dark-adapted condition) and the sclerad row (107 µm in length in light-adapted and 87 µm in length in dark-adapted condition) of rods are both approximately 74% longer in the retinal periphery than in central retina with the length of the myoid accounting for most of the variation in size between the two rows. In the light-adapted state, the two rows of rods lie sclerad to the layer of double and single cones (Fig. 4a) whilst in the dark-adapted state, rods, double cones and large single cones are distributed throughout this layer with a predominance of rods lying at the level of the OLM.

The rod outer segments are long and cylindrical. Their membranous discs (16.6 nm thick) maintain an average interdisc space of 23.5 nm. In contrast to the cone outer segments, the outer segments of the rods possess a number of incisures which can either run the length of the outer segment or occur as a series of isolated spaces (arrows in Fig. 4b,c). In tangential section, the incisures appear as deep invaginating grooves oriented toward the centre of each disc from the extracellular matrix. However, some incisures lie in the central region of the outer segment where a stack of separated discs lie in register. Calycal processes surround the vitread portion of both rod (7-10) and cone (8-11) outer segments.

Although an extensive study of the photoreceptor mosaic was not carried out for different regions of the retina, a regular pattern was observed throughout most of the retina. In tangential section at the level of the inner segments, the double cones lie in a row mosaic (Fig. 5a) with the junction of each unequal double cone lying parallel (Fig. 5b). Four double cones border either a large or small single cone. The large and small single cones form a square mosaic. Rods are interspersed within the cone mosaic and do not appear to form a regular pattern.

The retinal pigment epithelium (RPE) is vitread and adjacent to the photoreceptors, where osmiophilic granules within slender microvilli invade the extracellular matrix surrounding the outer segments of each photoreceptor in the light-adapted condition. The RPE consists of a single layer of cells joined by junctions of either the zonula adhaerens, zonula occludens or desmosome types (Fig. 6b,c). These mononucleate cells are rich in both smooth endoplasmic reticulum (especially within the apical processes of the microvilli) and mitochondria (of various shapes) but contain only a few profiles of rough endoplasmic reticulum (Fig. 6e). Aggregations of ribosomes (polysomes) are scattered throughout the RPE, particularly concentrated in the region abutting Bruch's membrane. Phagosomes containing shed outer segment discs, myeloid bodies, lysosome-like bodies, lipid droplets and Golgi apparatus are also common features of the RPE cells in this species (Fig. 6e).

Bruch's membrane (1.4 µm in thickness) consists of two zones; the inner zone consists of a series of layers (up to 10) of collagen fibrils below a lighter staining outer zone of finer fibrils. The inner layer of collagen
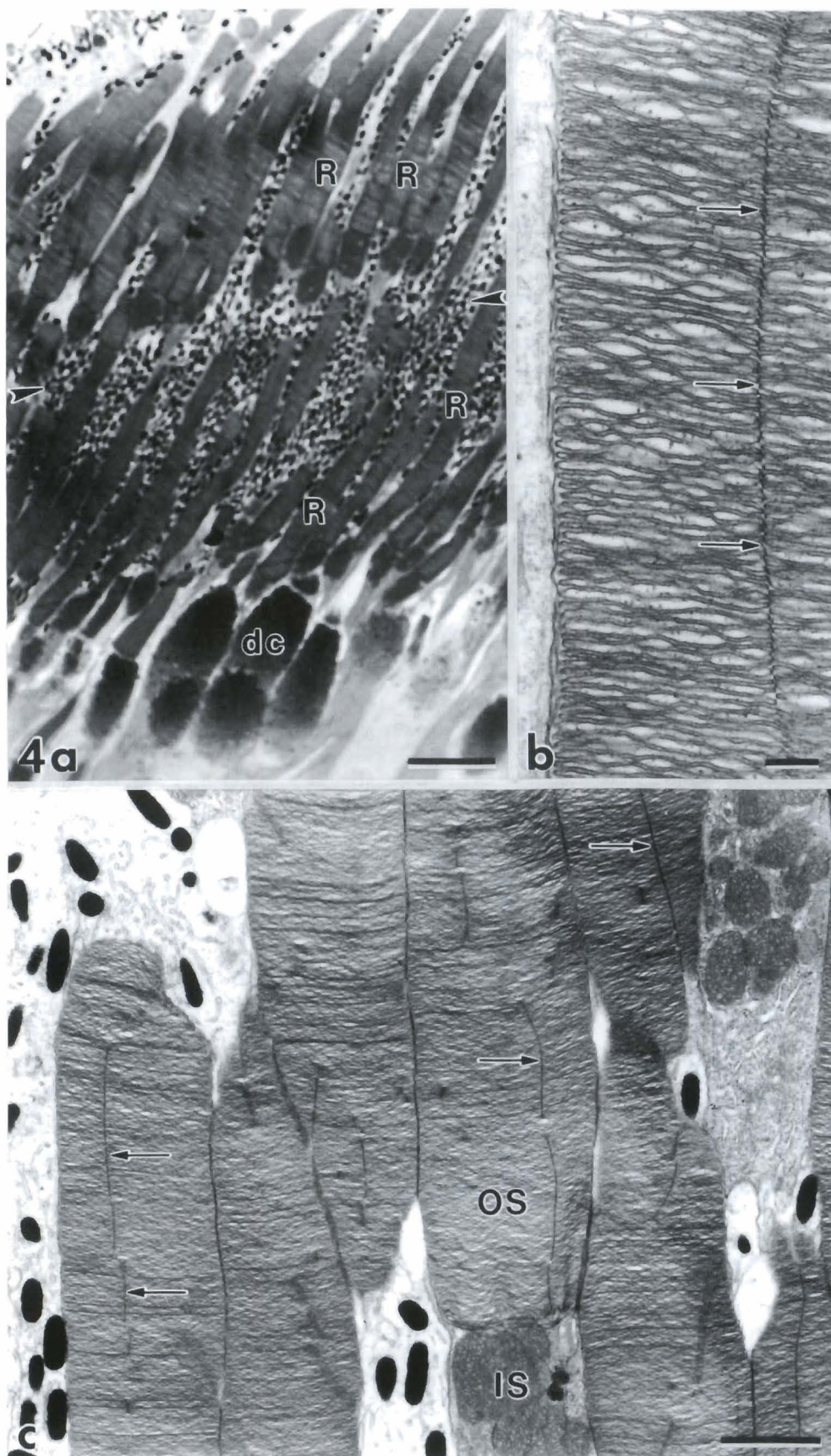
Fig. 4. Rod morphology. **a.** Light micrograph of a transverse section (1 µm) of the light-adapted retina showing two banks of rods (R, demarcated by the two arrowheads) sclerad of the cone photoreceptors. **b.** A longitudinal registration of many incisures (arrows) which are gaps in individual discs of a rod outer segment. **c.** Rod photoreceptor outer segments (OS) showing incisures (arrows) of various lengths and orientations. dc: double cones; IS: inner segment. Scale bars: 10 µm (a), 0.2 µm (b), 2 µm (c).

fibrils are 0.03 μm in diameter with a periodicity of 20 nm while the outer collagen fibrils are thinner in the range of 0.01-0.02 μm in diameter. The basement membranes of both the RPE (0.02 μm thick) and vascular endothelium are ill-defined and incomplete (Fig. 6a,e). There are few basal infoldings of the RPE. Yellow elastic fibres were not found within Bruch's membrane. The endothelial cells of the choriocapillaris have numerous fenestrations (Fig. 6d), with "knobs" of Rhodin occasionally observed. Behind Bruch's membrane, the choroid consists of three layers of melanocytes containing two to three layers of melanosomes oriented perpendicular to the incident light path (Fig. 6a). A complex arrangement of both arteries and veins form a horseshoe-shaped choroidal gland overlying the choroid and surrounding the optic nerve.

Discussion

An extensive pattern of vitreal blood vessels overlying the ILM of the retina in *Semotilus atromaculatus* has previously been found for several different teleosts including the carp, *Cyprinus carpio* (Kohbara et al., 1987), the rippled blenny, *Istiblennius edentulus*, the blue tusk fish, *Choerodon albigena* (Collin, 1989) and a number of other marine teleosts (Hanyu, 1962). Although the vitreal vessels are known to be loosely attached to the ILM by collagen fibrils in the Florida garfish, *Lepisosteus platyrhincus* (Collin and Collin, 1993) and the southern pacific rattlesnake, *Crotalus viridis helleri* (Dunn, 1973), this is the first

report in teleosts of convolutions of the ILM and Müller cell endfeet. In *S. atromaculatus* the increased surface area provided by the extensive system of Müller cell endfeet and the ILM would ensure a more effective transport mechanism for the exchange of nutritive substances throughout the retina. The oxygen supply to the inner retina mediated via the vitreal vessels, together with the choriocapillaris and choroidal body, therefore provide the entire retina with the nutrition it requires.

The fasciculation of the nerve fibre layer and the ganglion cell layer is widespread among primitive fishes, including the bowfin, *Calamoichthyes calabaricus* (Munk, 1964) and the Florida garfish, *Lepisosteus platyrhincus* (Collin and Collin, 1993), the shovel-nosed ray, *Rhinobatos batillum* (Collin, 1988) and other teleosts (i.e. the frogfish, *Halophryne diemensis*, Collin and Pettigrew, 1988) but is also found in amphibians (Graydon and Giorgi, 1984) and mammals including some primates (Stone and Johnstone, 1981). The function of this neuronal arrangement is unknown but may act to reduce the thickness of the retina and assist in providing adequate retinal nutrition. Alternatively, the fasciculation of ganglion cell axons may provide some advantage (developmentally or physiologically) as they grow towards the optic nerve.
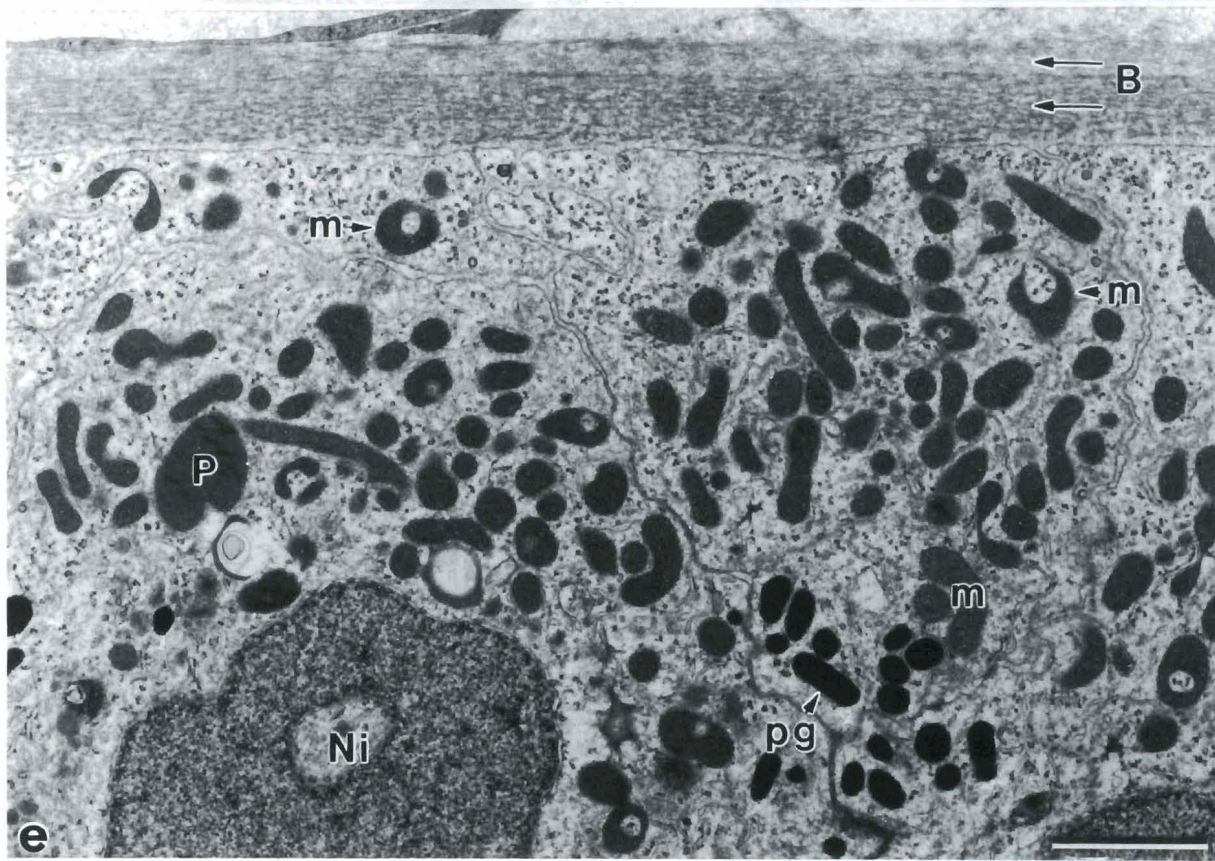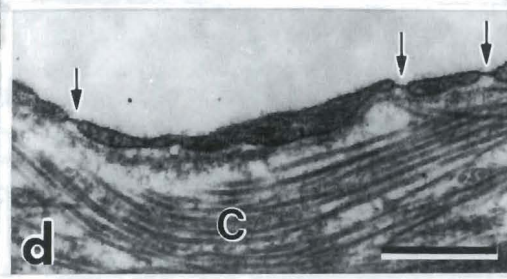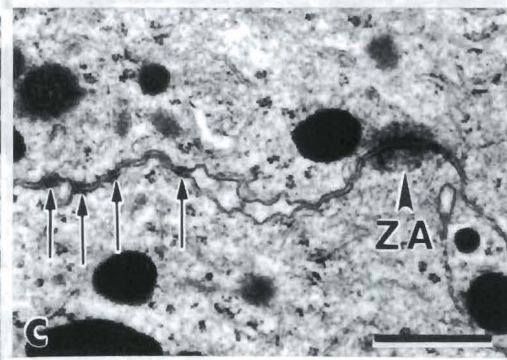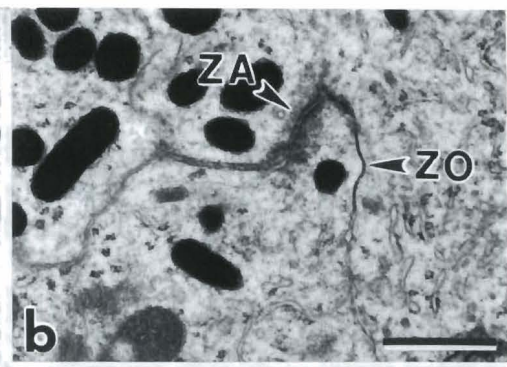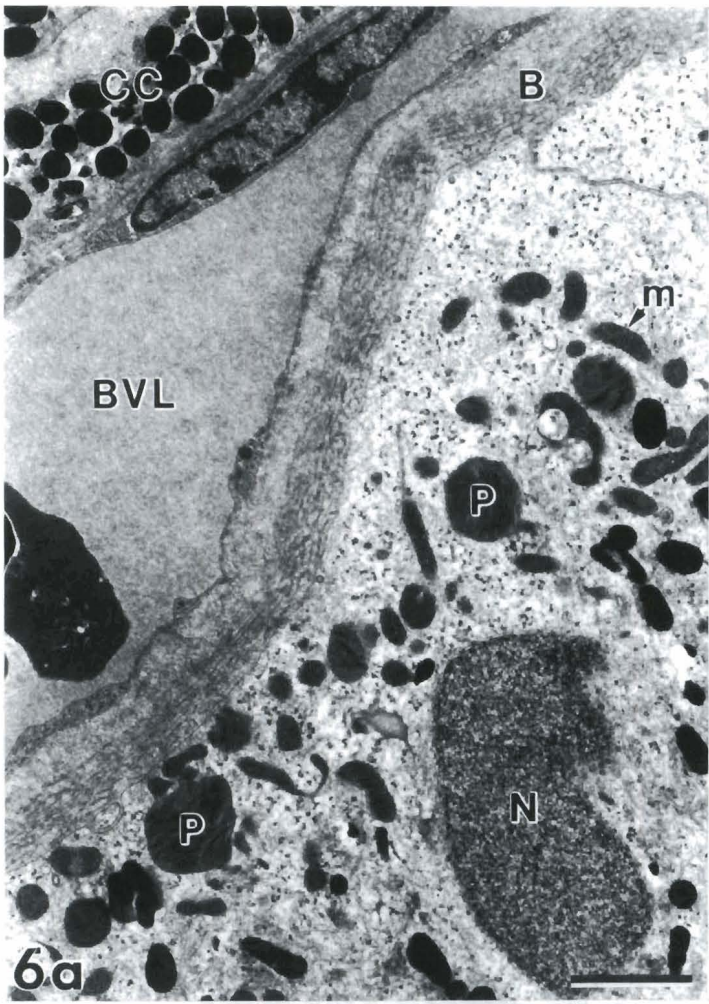
Rod and cone terminals make single and multiple synaptic ribbon connections with the cells of the inner nuclear layer, when viewed in transverse section, although surface contacts were also observed. This suggests that the cone terminals of the creek chub may be similar to the catfish (Hidaka et al., 1986) but simpler
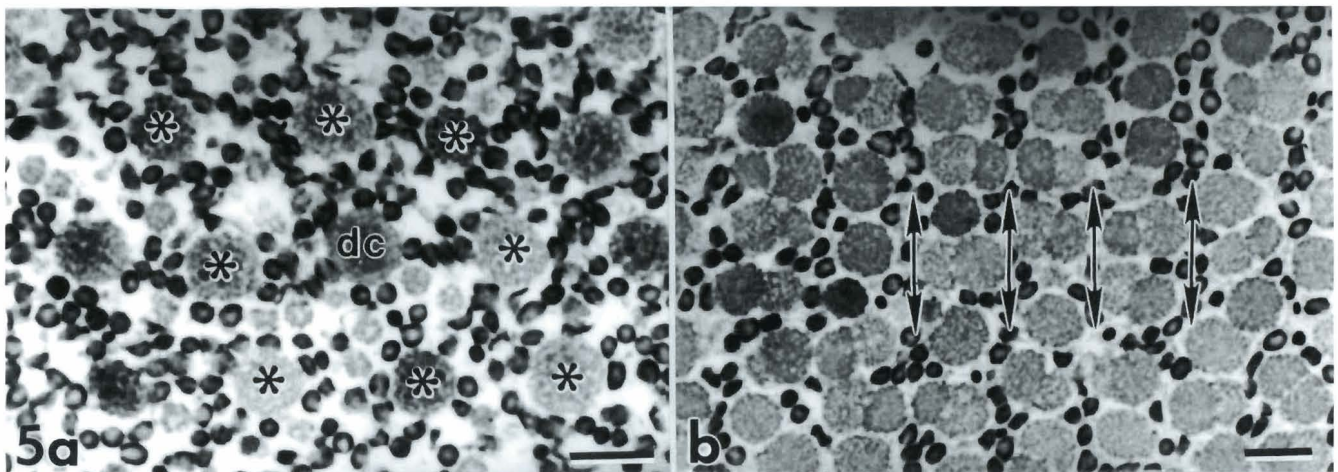
Fig. 5. a. Light micrograph of a tangential section of the retina at the level of the inner segments of the principal cones (asterisks) of the unequal double cones (dc) showing the regular row mosaic. **b.** The row mosaic at a more vitread level showing the orientation of the rows of double cones. The darkly-staining profiles are the rod outer segments. Scale bars: 10 μm (a), 10 μm (b).

Fig. 6. Structure of the retinal pigment epithelium and Bruch's membrane. **a.** Electron micrograph of the RPE, Bruch's membrane (B) and the choriocapillaris (CC). Large thin-walled blood vessels line the back of the eye surrounded by layers of pigment granules. **b.** Zonula adhaerens (ZA) and zonula occludens (ZO) between the membranes of adjoining retinal epithelial cells. **c.** Zonula adhaerens and desmosome-like junctions (arrows) between the membranes of adjoining retinal epithelial cells. **d.** Fenestrations in the endothelium of a blood vessel of the choriocapillaris. **e.** The RPE containing numerous mitochondria (m) of heterogeneous shape, phagosomes (P), pigment granules (pg) and a large number of polysomes along its sclerad border. Note the two distinct layers of collagen (arrowed) in Bruch's membrane. BVL: blood vessel lumen; C: collagen fibrils; N: nucleus; Ni: nucleus with invagination. Scale bars: 2 μm (a), 1 μm (b), 1 μm (c), 0.5 μm (d), 2 μm (e).

Figure 6. Electron micrographs showing cellular ultrastructure. (a) overview with CC, B, BVL, m, P, N labeled; (b) detail showing ZA and ZO; (c) detail showing ZA and arrows; (d) detail showing C and arrows; (e) overview showing B, m, P, Ni, pg labeled.

than those found in other cyprinids (Saito et al., 1985). Hidaka et al. (1986) using serial electron microscopy and synaptic reconstruction revealed that the triad of the rod spherule terminal is composed of a central bipolar process and two outer horizontal cell processes originating from a single horizontal cell which lies vitread in the ONL. However, the triad or quadrad arrangement of the cone pedicle is composed of two or three lateral processes of different horizontal cells, all originating in the sclerad region of the ONL. The synaptic terminals in the OPL of *S. atromaculatus* appear very similar to those described for the catfish by Hidaka et al. (1986) and this dimorphic arrangement of synaptic ribbons in rods and cones enables terminals from each cell type to be readily identified.

The spinules observed in the light-adapted retina of this study have been described previously by Wagner (1980) and their formation and disappearance have been induced by dopamine (in the dark-adapted retina) and glutamate (in the light-adapted retina), respectively suggesting the plasticity of spinule formation may be under the control of two different neurotransmitter systems (Weiler et al., 1988; Kohler et al., 1990).

The presence of small and large single cones, unequal double cones and rods in *S. atromaculatus* suggests a diversity of photoreceptor function. A rod to cone ratio of 5:1 and the presence of two rows of rods may indicate a lower scotopic threshold in this species. The arrangement of two rows of rods enables a greater number of rods to be packed into the photoreceptor array as reported in a number of deep sea teleosts (Locket, 1977). In addition, double cones are thought to be either optically or neurally coupled together and may function as a single cone (Walls, 1942) thereby possessing an enlarged cross-sectional area to increase sensitivity and mediate vision at low light intensities (Boehlert, 1979; Lythgoe, 1979).

The existence of different types of cones has previously been recognised for cyprinids by Marc and Sperling (1976) who recorded up to four cone pigments, the peak of spectral sensitivities correlated with the dimensions of the outer segments of the different cone types. The finding that the small single cones of *S. atromaculatus* are not capable of retinomotor responses, remaining at the level of the outer limiting membrane in the light and the dark, is only common to a few teleost species. Previously reported in two other species of cyprinids, *Leuciscus rutilus* (Engström and Rosstorp, 1963) and *Ericymba buccata* (Moore et al., 1950), the lack of retinomotor responses of small single cones in these species seems simply due to the lack of a myoid. The retinomotor movements shown to exist in *S. atromaculatus* ensure either the rods (scotopic vision) or cones (photopic vision) maximise light absorption and increase the sensitivity of light adaptation during crepuscular vision. This may be particularly true for the stationary small cones. In *Nannacara anomala* (Nicol, 1989) and *Trachinus vipera* (Kunz et al., 1985), the double cones fail to move while in the dorsal retina of

the plaice, *Pleuronectes platessa* (Nicol, 1989) and in two species of goldeyes (*Hiodon alosodes* and *H. tergisus*, Wagner and Ali, 1978), all the cones remain stationary.

Generally, retinomotor activity allows the rods and cones to alternately occupy a maximal area adjacent to the ONL for light absorption. This response is aided by the sclerad migration of pigment granules within the pigment epithelium in the dark. Since retinomotor movements are known to occur at low light intensities (Nicol, 1989), this suggests that the marked changes in the positions of the photoreceptors in *E. atromaculatus* may reflect a high sensitivity in dark and light adaptation between the photopic and scotopic states.

AOS were observed only in cones and not rods. These structures, which are completely devoid of organelles, have also been termed accessory elements by Engström (1963) and lateral sacs by Fineran and Nicol (1974). Originating from the sclerad end of the ellipsoid (Yacob et al., 1977), it is suggested that due to their paucity of organelles they are deformed to fill the extracellular spaces left after the rods have migrated sclerad in the light, ensuring more effective light capture by the cones. Alternatively, although not found in *S. atromaculatus*, the AOS of the rods could fill the spaces occupied by the cones in some species in the dark-adapted condition (Engström, 1960). In addition, the AOS may act as a reservoir for high energy metabolites to aid in nutrition (Fineran and Nicol, 1974).

The incisures of the rod outer segment discs in *S. atromaculatus* are a common feature of teleosts (Cohen, 1972). Their function is unknown but it is thought that the diffusion of substances to and from the disc membrane may be more efficient in this configuration and that the incisures may have a supportive role in preventing the outer segment from rotating about the eccentrically situated connecting cilium (Rodieck, 1973).

The regular row mosaic of the photoreceptors in *S. atromaculatus* is similar to the salmon, *Salmo salar* (Ahlbert, 1976), the minnow, *Phoxinus laevis* (Lyall, 1957), the roach, *Rutilus rutilus*, the bream, *Abramis brama* (Engström, 1960; Zaunreiter et al., 1991) and the cutlips minnow, *Exoglossum maxillingua* (Collin et al., 1996). In other studies of cyprinids, the carp, *Cyprinus carpio*, the asp, *Aspius aspius*, the sabre carp, *Pelecus cultratus* and the rudd, *Scardinius erythrophthalmus* are reported to have no regular mosaic (Lyall, 1957; Zaunreiter et al., 1991). The arrangement of unequal double cones in the goldfish, *Carassius auratus*, however, is a square pattern with four double cones surrounding a single cone (Engström, 1960). Based on extensive microspectrophotometric analyses and observations of differential staining and morphological differences in the two components of the double cones, each cone is known to possess a different photopigment (Loew and Lythgoe, 1978; Levine and MacNicol, 1979). The "square pattern" is thought to be associated with highly visual fish (Fernald, 1982) although this pattern

may change to a "row pattern" during ontogeny or be different in various regions of the retina.

The RPE of the creek chub is similar to that of other teleosts (Braekevelt, 1985, 1992b). The apical processes of the RPE cells and their pigment granules migrate sclerad in the dark returning vitread in response to light. The RPE cells help to isolate the two rows of rods in the light-adapted condition and support and orient the photoreceptor outer segments to the incident light (Enoch, 1979). The various types of junctions described between the RPE cells may serve as a medium for the exchange of material from one cell to another (Zinn and Benjamin-Henkind, 1979). The lack of infoldings of the RPE cells at Bruch's membrane, in contrast to most other vertebrate species, including elasmobranchs (Braekevelt, 1994) may be due to the presence of a choroidal gland in *S. atromaculatus* which may provide a high oxygen tension (Wittenberg and Wittenberg, 1974), negating the necessity to increase the surface area of the RPE.

The large number of fenestrations in the endothelial cells of the blood vessels in the choriocapillaris have been described in mammals (Collin, 1969) and are indicative of selective nutritive exchange with the RPE cells (Moyer, 1969). These fenestrations are thought to allow much larger than normal amounts of fluid and macromolecules to enter or be removed from the tissues (Casley-Smith, 1981).

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