Double cone mosaic pattern in the retina of larval and adult piranha, *Serrasalmus spilopleura*

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Abstract. The mosaic pattern of double cones of retinas in larval and adult piranha, *Serrasalmus spilopleura,* was examined. Samples from two habitats in the same reservoir, characterized by different light conditions, were examined. The same square pattern was found in all sampled retinas indicating that, for this species, there is no apparent relation between the patterns of double cone arrangement and environmental luminosity.
Key words. Retinal organization; ontogenetic changes; *Serrasalmus* sp.; characiform fish.

Physiological and morphological characteristics of the retinas of fish have been related to their environment, since for many teleost species vision is very important for the search for food, for territorial defence and for the detection of predators. Changes in their active periods, migrations, or alterations of life habits are common during the ontogenesis of many fish species; and thus variations in available light may occur during their development. As a consequence, there may be structural and functional changes in the eyes of fish, especially in the retinas, in order to adapt to new light situations[1-4].

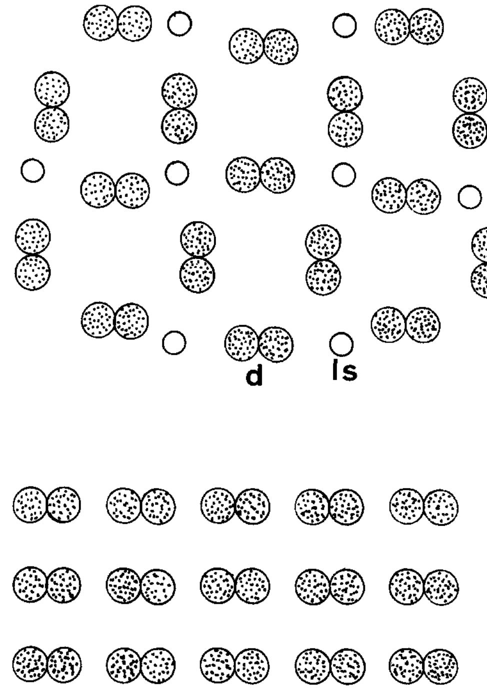
A response to light changes which is found frequently in teleost retinas is called the retinomotor response[1, 5-8]. This process enables the best use of the available light to be made by the appropriate photoreceptor cells, cones or rods, which change their position in relation to the pigment epithelium according to the luminosity[5]. In well-lit conditions, the rods expand into the pigment epithelium and are protected by this pigment, which spreads throughout epithelial projections and surrounds the outer segments of the rods. The cones remain close to the external limiting membrane of the retinas. In the dark, the rods contract and move towards the external limiting membrane and are thus better exposed to the incoming light, while the cones expand into the pigment epithelium[5].

Kunz[3] relates the double cone organization in regular mosaic patterns in teleost retinas to environmental light intensity through the retinomotor response. Two mosaic patterns are known: 1) Square mosaic (fig. 1 A) in which double cones display a zig-zag appearance, forming an angle of 60°–90° between adjacent double cones, and 2) row mosaic (fig. 1 B) which shows parallel lines of double cones. According to Kunz[3], when double cones expand into the pigment epithelium to adapt retinas to darkness through the retinomotor response, the double cones rotate in relation to their long axis forming parallel lines (row pattern). This interpretation correlates the square mosaic to luminous environments, and the row mosaic to dark environments. On the other hand, Fernald[9] disagrees with the interpretation of Kunz and ascribes it to methodological errors.

Serrasalmus spilopleura Kner, 1859 (Osteichthyes, Characidae) is a piranha species whose larvae live in association with floating aquatic vegetation, such as the water hyacinth[10]. The larval environment is characterized by low luminosity and close proximity between this predator and its prey (microcrustaceans, larval and juvenile insects and nematodes)[10]. During ontogenesis juvenile piranhas move progressively away from vegetation[10], and adults feed on the fins of other fish species in open water, mainly during the daytime[11-15]. Characteristics of this change of environment are the increase in luminosity and in the relative distance between predator and prey, in comparison with the larval stage and habitat[10, 15].

This study was undertaken to describe the retinal structure of larvae and adults of *Serrasalmus spilopleura*, and to check the supposed ontogenetic retinal changes related

1A



d ls

1B

Figure 1. Schematic representation of the organizational patterns of the double cones observed in a cross-section in the retina of fish. *A* Square mosaic. *B* Row pattern. d, double cone; ls, long single cone.

to alterations of light intensity, caused by photoreceptor migration in response to a change of the micro-habitat during ontogeny. The occurrence of these retinal changes was questioned for fish in general[16] and for *S. spilopleura* in particular[10].

Material and methods

Specimens of *S. spilopleura* were caught in the Rio Atibaia Reservoir near Americana, São Paulo, southeastern Brazil (ca. 22°43′ S, 47°14′ W). Larvae were netted within floating aquatic vegetation ($\bar{X} = 8.00 \pm 3.34$ lux) and adults in open water ($\bar{X} = 9{,}300.00 \pm 6{,}454.37$ lux). Larvae and juveniles used to study the influence of environmental luminosity on the double cone mosaic pattern were kept as follows. Larvae were kept in a diurnally well-illuminated aquarium ($\bar{X} = 245.00 \pm 83.85$ lux), and juveniles in a darkened aquarium ($\bar{X} = 4.50 \pm 1.51$ lux), for five days before being processed as described below. Light intensity in the dark aquaria was even lower than that measured within the floating aquatic vegetation on the reservoir ($t = 4.764$; $df = 48$; $p < 0.001$).

The light intensities given above were measured with a Weston Master photometer; in all cases, 25 measurements were made and the underwater readings were carried out at a depth of 25 cm.

The fish specimens were decapitated immediately after being caught. The eyes were enucleated, perforated behind the iris and fixed in 1% paraformaldehyde–2.5% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.2–7.4 with 3% sucrose, in an ice bath. In the laboratory, retinas were isolated and randomly sectioned in pieces of 1 mm^2. After 20-h fixation the blocks were washed for 6 h in phosphate buffer, postfixed in 1% osmium tetroxide (OsO_4) in the same buffer, dehydrated in a series of ethanols and embedded in epon resin. Semithin sections (about 0.5 μm) were made with the LKB ultramicrotome, Ultratome III, and observed with a light microscope after staining with 1% Toluidine blue in 0.5% Na_2CO_3, pH 12.

For all groups, sequential sections were cut, and sections at the level of the inner pigmented epithelial layer were used to compare the patterns of the double cone mosaic in the different experimental groups. Random areas of the retinas were used to compare the experimental groups, because only one pattern of cone mosaic arrangement was found in each retina.

Results

The same pattern of double cone mosaic occurs in different areas of each retina. All sections of the retinas from each experimental group studied showed the same square mosaic pattern. In most cases, the entire retina was sectioned and no different cone arrangement was found.

Fish of different ages and from different light intensities showed the same square mosaic pattern of the double cones; larvae (23 mm total length) collected within aquatic vegetation roots, regarded as a dark environment (fig. 2A); larvae kept in a well-lit aquarium (fig. 2B); juveniles (65 mm) kept in a darkened aquarium (fig. 2C) and adults (250 mm) caught in open waters regarded as a luminous environment (fig. 2D).

A greater density of double cones in retinas of larvae when compared to the retinas of adults is shown in figures 2A and 2B (larvae) versus 2C and 2D (adults).

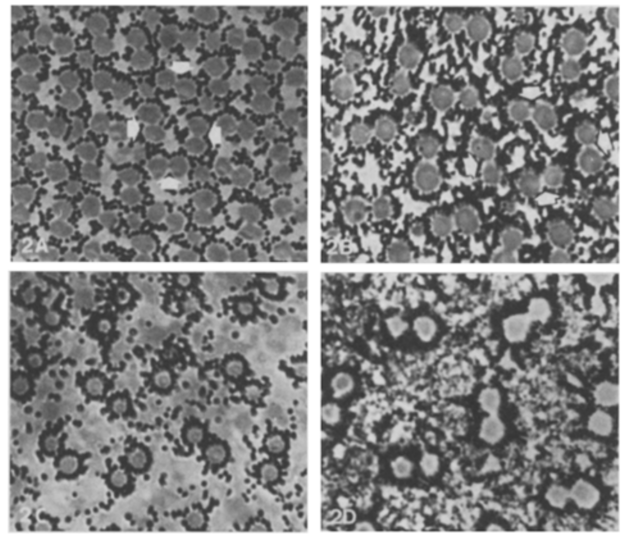


Figure 2. Cross-section of the photoreceptor cell layer showing the square mosaic in retinas of *S. spilopleura*: *A* Larva in a dark environment, *B* larva in a luminous environment, *C* juvenile in a dark environment and *D* adult in a luminous environment. Arrows emphasize the square pattern in A and B. Note greater density of double cones in the larvae when compared to adult fish retina. 2,200 ×.

Discussion

Despite the fact that we found no difference in the double cone mosaic pattern within the same retina, there are other regional, structural, and functional specializations in the retina of *Serrasalmus spilopleura*. For example, there are differences between the dorsal and ventral parts of the retina (which we did not study in detail), related to the nature of the pigment in the epithelial cells[5].

The fact that the double cone mosaic pattern was similar in the retinas of individuals of *Serrasalmus spilopleura* living under different light intensities at various stages of their biological cycle disagrees with the suggestion of Kunz[3]. This author regards the square mosaic pattern as indicative of retinal adaptation to well-lit environments and the row mosaic of adaptation to dark conditions, because of the rotation of photoreceptor cells during the retinomotor response. Another fact which seems to contradict Kunz's concept of dependence of the mosaic pattern on environmental luminosity is that many other fish species have both mosaic patterns (square and row) in different regions of the same retina[5]. We found this double pattern in the retinas of *S. spilopleura* adults caught in the Pantanal region of Mato Grosso, western Brazil. The retinas of these specimens have one small region showing the row pattern while the rest of the same retina shows the square pattern (E. S. Rossetto et al., unpublished data).

The square mosaic pattern maintained in the retinas of fish kept in darkened aquaria for five days raises the question of the persistent dark rhythms of retinomotor activity[17]. These rhythms have been reported for several marine and freshwater teleosts, persisting for 2–8 days in general. Nevertheless, the persistent dark rhythms of retinomotor activity are not found in every fish species and, for many of those in which these rhythms were present, there was also a decrease in motility after the first or second day in darkness (Ali[17], apud Besharse[18]). Even if the square mosaic found in the retinas of piranhas kept in darkened aquaria for five days is related to this persistent retinomotor activity, this kind of response cannot be regarded as very efficient, since the persistent dark rhythms would delay adaptation of vision to new light conditions.

We do not regard the greater density of double cones found in the retinas of larval *S. spilopleura* (fig. 2A) as an improvement which would compensate for the low luminosity of the habitat in which larvae live. Our opinion is based on the decrease of cone density observed in *Sebastes diploproa* during its ontogenetic migration from the surface (light) to deep (dark) waters[2]. Both this, and our results, suggest that the decrease in cone densities is due to ontogenetic increase of retinal area and cone diameter without an accompanying increase in cone number. Therefore, in some fish species cone density should not be regarded as a characteristic associated with environmental luminosity.

Finally, the square mosaic has also been regarded as a morphological characteristic that may facilitate perception of rapid movements[3, 19–22]. If this is indeed the case, square mosaics may be expected in the retinas of most visually oriented predatory fish. Thus the predatory larval and adult *S. spilopleura* may be regarded as well adapted to their hunting activity[14, 15] since both stages present the square mosaic. We think that the unchanged square pattern of double cones in the retina of *S. spilopleura* may indicate that the mosaic pattern is not necessarily directly related to light intensity (as suggested for *Serrasalmus* sp.[4]), and to the retinomotor response (as suggested for fish in general[3]).

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