Fisheries Research and Development Corporation Report
FRDC Project No. 1998/329

Visual Development of the West Australian Dhufish (*Glaucosoma hebraicum*)

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March 2001
ISBN: 1 74052 040 8

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Objectives:

1. To establish the timing and sequence of development of photoreceptors in the retina of the dhufish.
2. Relate photoreceptor changes to possible lifestyle requirements during the larval period of the WA dhufish to provide advice on a suitable light environment to the Fremantle Maritime Centre's WA dhufish culture program.

Non-technical summary

Vision is the primary sensory system used by larval fish for feeding during a critical time in their life history. Small developmental changes may result in large changes in visual function and knowledge of such changes could translate to an increase in survival and subsequent fitness of hatchery-reared fish. This is particularly important if the fish are to be used for restocking programmes. The aim of this project was to ascertain whether or not morphological changes during the development of the eye of the West Australian dhufish could be used to infer the light environment for which larval dhufish are adapted at different stages of their early life history. Such information could then be used to suggest possible lighting conditions for rearing dhufish. Details of the sequence and timing of the development of different photoreceptor types is presented and changes in the lighting conditions at specific times during larval development are suggested.

The natural habitat and ecology of larval and juvenile West Australian dhufish is unknown and as a result, the lighting conditions in which dhufish are hatchery-reared has not been based on knowledge of the light environment in which they occur in the wild. However, different species of fish have evolved specific adaptations to their visual system that can give clues to their natural habitat and behaviour. Thus, the eyes

of deep-sea or nocturnal species are very different, in their complement of cells, to those of shallow living coral reef fish. Likewise, the timing of development of the eyes of different species is known to show specific adaptations that can be related to their habitat and behaviour. This project was initiated to investigate structural changes in the eyes of dhufish during development and to infer lighting conditions that would enhance rearing success and reduce stress in aquaculture-reared larvae.

The development of the light-sensitive cells (photoreceptors) in the eye of the W.A. dhufish was investigated using light and electron microscopy. The larvae were spawned at Fremantle Aquaculture Development Unit and sampled every three days during their early development. The investigation revealed that at hatching the eye was undifferentiated but by day 3 post-hatch, photoreceptors known as cones, which operate in bright light conditions, were present and the larvae were able to use vision to feed. The photoreceptors were arranged in tightly packed rows, a strategy known to improve resolution of fine detail and hence increase visual acuity for feeding on plankton. In addition, many cones appeared to be linked to neighbours to form double or triple cone units. The size of the cones increased as the eye grew, thus increasing the sensitivity of the eye to lower levels of light. At approximately 3 weeks, a new type of photoreceptor, that is responsible for vision at night, known as a rod, was added to the retina. The addition of rods proceeded rapidly and, by day 50 post-hatch, they were present in high densities. The rapid addition of rods further increased sensitivity to low levels of light. At the time the rods began to be added the spatial arrangement of the cones changed. By 3 months the row arrangement of the cones had been replaced by the arrangement of a single cone surrounded by four double cones. Such an arrangement, known as a square mosaic, was also found to be the arrangement in the eyes of adult dhufish.

During the first 2 weeks, when small cones were present in the retina, bright lighting conditions would be required to enhance feeding success on small prey items. However, as the cones increase in size and the rods are added to the eyes from 3 weeks onwards, lower light levels are necessary. The changes in retinal structure coincided with observed behavioural changes as the larvae tried to avoid the bright regions of the rearing tank. In the wild the colour of the light would also change as the fish move to deeper water, which is more prevalent in blue/green regions of the spectrum. Further experiments are required to investigate the possibility that the colours to which the dhufish are sensitive may change as the eye grows and the fish move to deeper water.

This study has given insight to the structural changes in the developing dhufish eye and has contributed to the currently very limited knowledge of different lighting requirements for different species. It is recommended that aquaculture of dhufish should involve reduction in the intensity of the light, or provision of shaded regions in the rearing tanks from 2 weeks of age. It is postulated that, in the wild, the larvae would be found at progressively increasing depths during development.

Key words: Dhufish, retina, photoreceptors, development, aquaculture.

Background

This project was initiated to look at the development of the visual system of the WA dhufish, specifically the timing of the incorporation of different photoreceptor types into the retina. Marine teleosts typically hatch with one photoreceptor type, with others being added at species-specific times during larval development (Shand et al. 1999). Such changes can result in an increase in sensitivity or the ability to see new colours. Thus the visual capabilities of larvae are changing rapidly during development and knowledge of such changes could assist in the design of the most beneficial light environment, in terms of both intensity and spectral composition, in the aquaculture situation (Pankhurst and Hilder, 1998). Although the photoreceptor complement is usually complete following metamorphosis, the addition of new cells continues as the eye grows throughout the lifespan of the fish.

The photoreceptors of teleosts are similar in basic plan to those of other vertebrates, composed of a nucleus, an inner segment containing high densities of mitochondria and an outer segment that contains the light sensitive visual pigment. Two types of photoreceptor are classified as rods and cones due to the shape of their outer segment. Rods have a low threshold of stimulation and are sensitive to low levels of light (scotopic). Cones, with a higher threshold for stimulation, are active in brighter light conditions (photopic) and, depending on their size can provide higher resolution of detail. Teleosts possess up to four sub-types of cones, classified by their size, association with neighbours (double cones), and the region of the spectrum to which their visual pigments are maximally sensitive. The majority of marine teleosts possess a single, blue-sensitive cone and double cones that are sensitive to green or red regions of the spectrum. Shallow living species may contain a second smaller type of single cone sensitive to ultra-violet wavelengths. The relative densities and sizes of teleost photoreceptors can be related to the habitat or period of activity of the species. For example, nocturnal or deep-water species have a rod-dominated retina and any cones that are present are large in size to increase the possibility of stimulation in low levels of light (Lythgoe, 1979; Pankhurst, 1989).

During retinal differentiation the larval fish eye usually has only cones, and in a number of species these are single cones that would require relatively bright light conditions to function. At around the time of metamorphosis the rods develop and double cones are formed. These developmental events increase the sensitivity of the eye and allow exploitation of deeper water, where the light intensity is reduced. However, not all species follow the same sequence of photoreceptor differentiation and interspecific differences can be related to the ecology and behaviour of the species (Shand, 1997). The retinal structure of relatively few temperate Australian species have been investigated, and nothing is known about retinal development in any local West Australian species, with the exception of the black bream, *Acanthopagrus butcheri* (Shand et al., 1999).

Need

Dhufish are a valued recreational and commercial table fish in Western Australia. Rearing of artificially spawned larvae began in W.A. in the 1990's (Pironet and Neira, 1998). However, because larval dhufish have never been caught from the wild, almost

nothing is known about their natural light environment in terms of intensity or spectral composition. Marine teleosts hatch with eyes rudimentary in structure. Investigation of the development of eye and the light sensitive cells gives information about the timing of significant developmental events as the eye grows and can be indicative of changes in the lighting regime in which an animal is living. It is important to know this because feeding in larval fish is mediated primarily by the visual system and the detection of prey items could then be optimised by tailoring the light environment to that most suited to the stage of development of the species being reared.

The project will assist the Fremantle Maritime Centre (FMC) to establish the light regimes required for the WA dhufish larvae and will provide support for larval rearing. The information provided should enable FMC to avoid long-term trial and error light regime experimentation and increase larval survival. The information obtained will also benefit fisheries biologists to assist in identifying areas which may act as nursery grounds for the WA dhufish larvae (which are currently unknown).

Objectives

1. To establish the timing and sequence of development of photoreceptors in the retina of the dhufish.
2. Relate photoreceptor changes to possible lifestyle requirements during the larval period of the WA dhufish to provide advice on a suitable light environment to the Fremantle Maritime Centre's WA dhufish culture program.

Methods

Rearing conditions
Dhufish eggs were collected from captive female dhufish (treated with LHRHa) and fertilised *in vitro* with sperm from captive male dhufish (Pironet and Neira, 1998). Larvae were sampled from four different batches of fish undergoing rearing trials at the Aquaculture Development Unit, Fremantle Maritime Centre during the summer of 1998/1999. Sampling became opportunistic during the course of the study due to differing survival rates and hence availability of fish from tanks undergoing different treatments. Larvae were reared according to procedures outlined for black bream (Jenkins et al., 1999) with modifications for dhufish (Cleary and Jenkins, 2001). From 9th December 1998 to 9th May 1999 a total of 150 larval and juvenile dhufish were sampled, ranging in age from day 0 (hatching) to day 50. In addition, 3 juvenile fish (14 weeks, SL, 30 mm) and the eyes of an adult (SL, 50 cm) were sampled.

The differences in the rearing conditions of the four batches were due primarily to early differences in the microalgal cultures for feeding the rotifers, upon which the dhufish larvae fed during the first 3 weeks post-hatch. A summary of the rearing conditions for the different batches is shown in Table1.

Table 1. Rearing conditions for dhufish larvae during the first 24 days post hatch.

	Batch 1	Batch 2	Batch 3	Batch 4
Water exchange	1l/min (green flow-through)	Static	Static	Static
Water colour	Clear	Green	Green	Brown
Microalgal species	*Nannochloropsis oculata*	*N. oculata*	*N. oculata*	*Isochrysis galbana*
Lighting	Florescent (Philips Coolwhite, 36W)	Natural daylight	Metal halide (Silvana metalarc, 400W)	Natural daylight
Intensity (Lux) centre of tank	700	variable	16,000 – 18,000	variable
Photoperiod (light/dark)	14 hr/ 10 hr	Approx. 14 hr/10 hr	14 hr/10 hr	Approx. 14 hr/ 10 hr
Secchi depth (cm)	80 - 90	30- 70	30 – 70	30 - 70

All trials had copepods introduced at approximately 17 days post hatch and they were concomitantly weaned off rotifers by 24 days post-hatch. Batches 1, 3, 4 were weaned onto cultured *Artemia*, whereas batch 2 was fed natural bloom copepods (Payne et al., 2000). Weaning to artificial pellets (0.2-0.4 mm diameter, Nippai ML) began at approximately 55 days post-hatch.

All fish were reared in cylindro-conical 4000 l tanks with blue sides and a white base. The water temperature was between 22 – 24^{o} C throughout the sampling period. The tanks that were initially static were topped up daily to balance pH and ammonia levels. However, on the introduction of copepods, the water was gradually cleared of algal cultures (by day 30) and flow rates gradually increased to 20 l/min by day 50 in all tanks.

The lights were situated over the centre of the tanks, with intensity dropping towards the edges. Under the metal halide lamp the reduction from centre to edge was 18,000 to 700 Lux and under the florescent lamps the reduction was from 700 to 50 Lux. In the green and brown water cultures the secchi depth records varied between 30 – 70 cm, depending upon the daily variation in the concentration of the algal cultures. The turbidity resulted in a rapid reduction in transmission of light with depth of water. Following the clearing of algal cultures from the tanks the secchi depth readings were in excess of 110 cm. The lighting was changed from metal halide to 36W fluorescent bulbs on day 30. A shaded area of the tank in which batch 3 was held was provided as the water was cleared. At all stages, the treatments in artificial light were provided with a “dawn” and “dusk” where the light levels were gradually increased or reduced respectively.

Formal behavioural experiments were not attempted, however, daily notes were made of general behavioural characteristics within the rearing tanks, such as avoidance of light at any particular stage.

Histology

To ensure representation of a range of sizes, at least 3 fish for each sample were obtained at regular intervals. Whole fish were anaesthetised in a lethal dose of methanesulfonate (MS222, Sigma-Aldrich Pty, 1:2000 in seawater) and placed in 2% paraformaldehyde, 2% glutaraldehyde in cacodylate buffer (pH 7.2) for at least 24 hr. Larger larvae had their eyes pierced to allow rapid penetration of the fixative. Following fixation the notochord length (TL, prior to flexion) or standard length (SL, following flexion) was recorded, as was the horizontal eye diameter, using a dissecting microscope with a calibrated eyepiece graticule.

Representative samples of fish, of different sizes, were selected for further processing. Fish were post-fixed in 2% osmium tetroxide, dehydrated in an ethanol series and infiltrated with araldite resin. To maintain the orientation of the eyes during subsequent sectioning, small larvae were embedded whole, and the heads of the larger specimens were embedded.

Semi-thin radial and tangential sections of a size range of fish were obtained using a glass knife. Sections were mounted on microscope slides and stained with toluidine blue. Ultra-thin sections were obtained using a diamond knife, stained with uranyl acetate and viewed on a transmission electron microscope.

Results

Morphology

The general change in body shape and pigmentation during development is shown in Fig. 1. Notochord flexion occurred by approximately day 15 - 20 when the larvae were between 4.5 – 5.5 mm. The size/age distribution of larvae from the 4 tanks is shown in Fig. 2. Growth rates appeared to be similar in the fish at early stages but differed at later stages. Fish in batch 1 did not survive beyond day 30. The size/eye diameter distribution is shown in Fig. 3. Note that the growth of the eye followed the same trajectory in fish from the different rearing treatments.

Behaviour

Behavioural observations indicated that during early stages (up to day 12 post hatch) the larvae were frequently found near the surface of the tanks and showed no reaction to the lamps being switched on. However, from days 13 onwards, the fish were less likely to be seen near the surface and from day 20 an avoidance reaction when the lights were switched on was often observed.

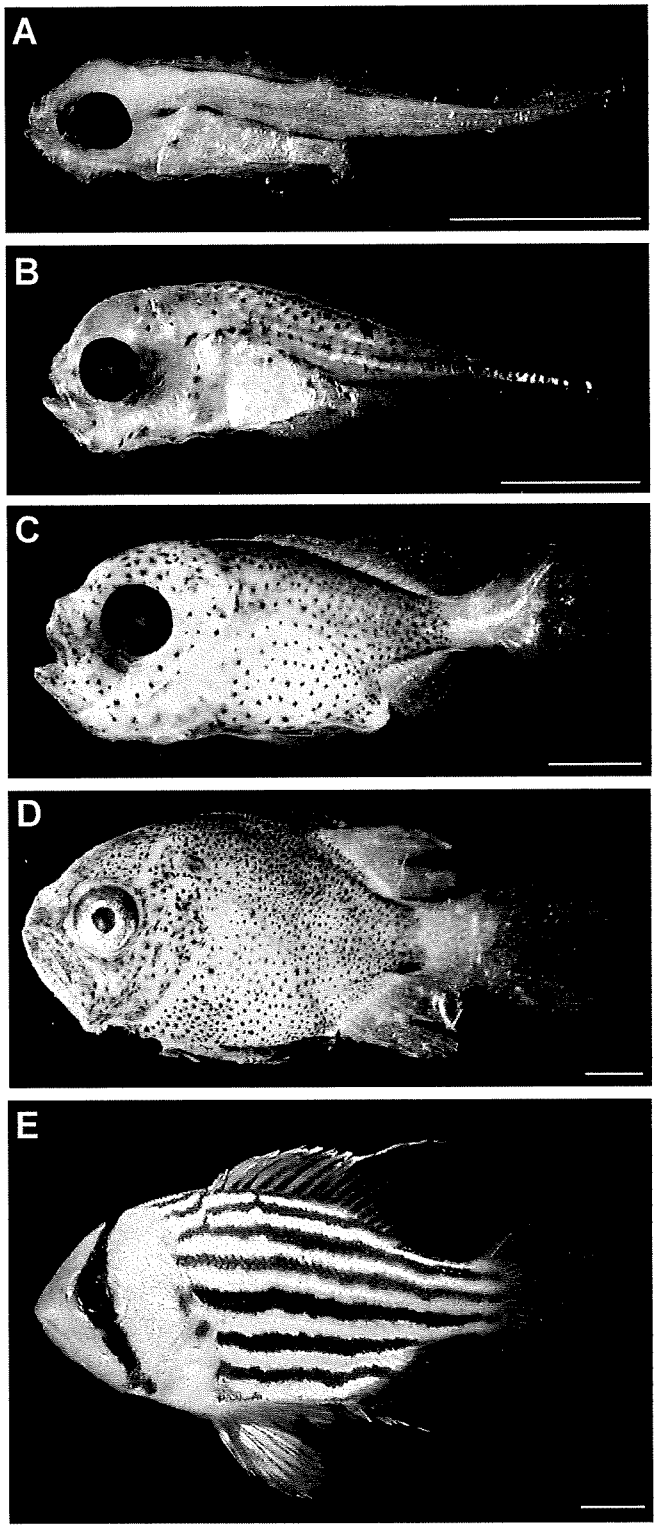

Fig. 1. Photographs of dhufish showing developmental changes in eye, head and body morphology of larval and juvenile dhufish. **A**: preflexion (day 3, TL 3 mm). **B**: flexion (day 13, TL 4 mm). **C**: early postflexion (day 25, SL 6 mm). **D**: late postflexion (day 33, SL 9 mm). **E**: juvenile (3 months, SL 30 mm). Scale bars: **A-D**: 1 mm; **E**: 4 mm.

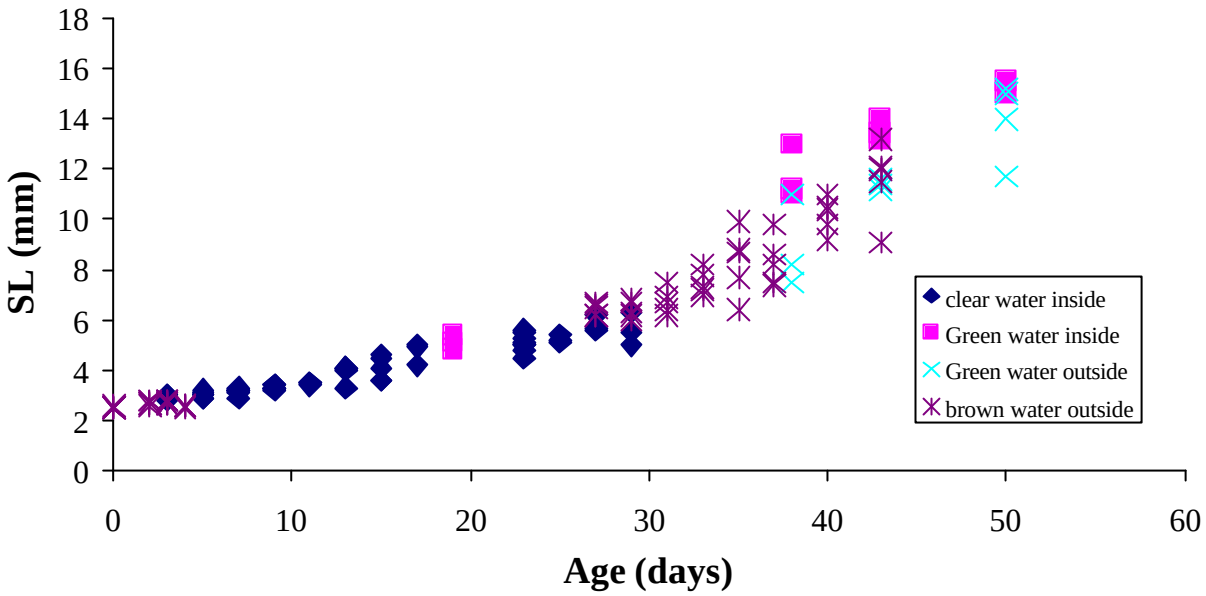

Fig. 2. The size of larval dhufish sampled from 4 different experimental tanks, in relation to age.

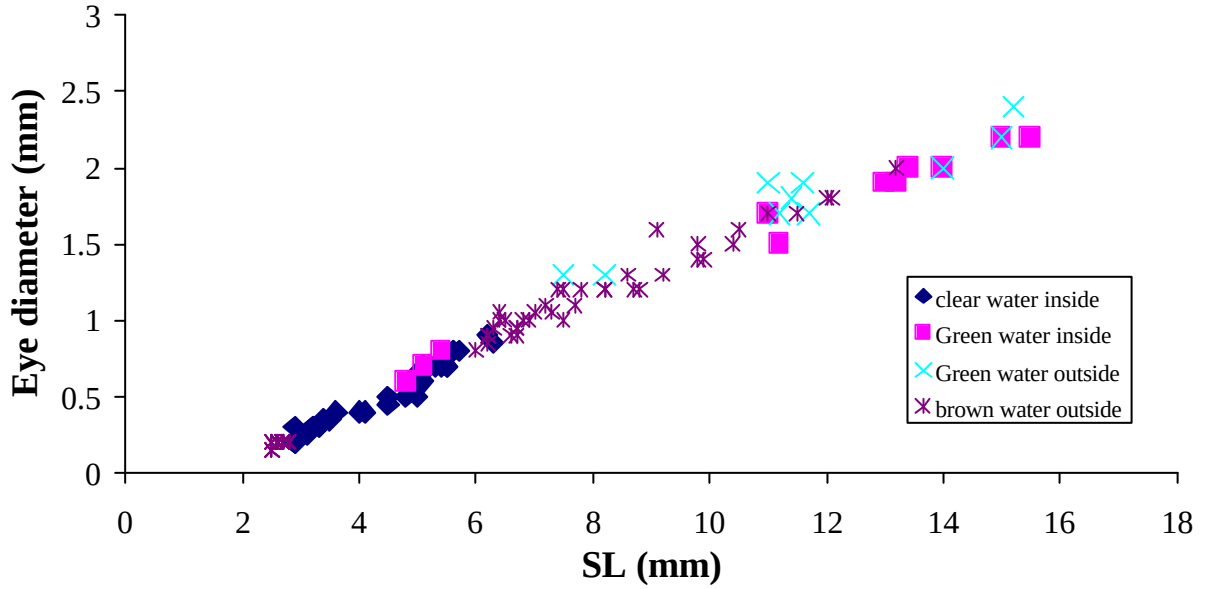

Fig. 3. The eye diameter of larval dhufish sampled from the 4 experimental tanks, in relation to standard length.

Retinal morphology

Low power photomicrographs of transverse sections through the whole eye at 4 different stages are shown in Figure 4. At hatching the retina and lens were undifferentiated (Fig. 4A). However, by day 3, a layer of melanin screening pigment was present behind the eye and differentiation of the ganglion cell layer, inner plexiform layer, inner nuclear layer and photoreceptors had taken place (Fig. 4B). The optic nerve carrying axons from the ganglion cells in the eye to their terminals in the visual centres of the brain was also visible. Pre-flexion larvae at day 13 had an enlarged eye and further differentiation of the photoreceptor layer in which the nuclei of the photoreceptors could be distinguished in the outer nuclear layer (Fig. 4C). By day 29, the fish had both cone and rod photoreceptors in a retina that was well differentiated exhibiting all the structural features of the teleost eye (Fig. 4D).

Examination of the retina using electron microscopy revealed further detail of the photoreceptor differentiation (Fig. 5). At day 2, differentiation had proceeded from the ganglion layer to the outer nuclear layer, containing the nuclei of the photoreceptors. The inner segments of the photoreceptors, containing mitochondria were visible, however, the outer segments that contain the light-sensitive visual pigment were as yet undifferentiated, nor was there any screening pigment present (Fig. 5A, B). By day 3, differentiation of the photoreceptors was complete with the nuclei, inner segments and outer segments clearly visible, as were the melanin granules that form the screening pigment in the pigment epithelium (Fig. 5C). At day 3 the photoreceptors appeared to be single cone cells, but by day 4, when the inner segments had expanded to include high densities of mitochondria, some of the cones appeared to be associated with neighbours and formed double cones (Fig. 5D). However, the membranes were not consistently joined along the whole length of the inner segments, as is characteristic in the double cones of adults (see below for further detail). At this stage the photoreceptors were closely packed and the outer segment diameter of approximately 1 μm was the minimum size that could trap and absorb light (due to it's wave form). For the following 3 weeks the retina continued to grow by the addition of cells at the retinal margins and during this time only cone photoreceptors were observed. There was however an increase in the diameter and length of the cone outer segments to approximately 4 μm by 10 μm.

From approximately day 21 when the larvae were about 5.5 mm in length, the first rod photoreceptor nuclei became visible in the retina. The rod nuclei were differentiated within the existing retina in a position vitread to the cone nuclei and the rod outer segments were first seen adjacent to the cone outer segments. (Fig. 6A). As rod differentiation proceeded, the outer segments become displaced towards the back of the eye (Fig. 6B). The differentiation of rods was observed to be more rapid and the rods became more numerous in dorsal regions of the eyecup.

Examination of high power electron micrographs of the photoreceptors showed the association of the cone inner segments, with regions of the common membrane possessing a thickening known as subsurface cisternae (Fig. 7A, B). The thickening between the cone inner segments became consolidated at the time the rods began to appear (Fig. 7B) and eventually extended the whole length of the inner segment. Confirmation of the presence of rods could be obtained by examining the differences between the arrangements of the outer segment lamellae of the photoreceptor types

(Fig. 7C, D). Cone outer segments were composed of sheets of membrane continuously folded back over one another, whereas rod outer segments were composed of discs or "packets of membranes stacked upon each another.

The densities of rods increased rapidly from the time of their first appearance. At day 35 the rod and cone layers were of similar thickness (Fig. 8A) but by day 50, rod layer was at least twice the cone layer in thickness (Fig. 8B). A juvenile dhufish, examined at Day 96 showed a further increase in rod density, facilitated by a decrease in rod diameter without a noticeable increase in the thickness of the rod outer segment layer (Fig. 8C). However, examination of the adult retina showed that the rod layer continued to increase during growth of the eye, attaining a thickness of approximately 5 times that of the cone outer segment layer (Fig. 8E). Figure 8 also shows how the cone inner and outer segments increased in size during growth to reach a length of approximately 25 µm.

Examination of tangential sections of the retina, at the level of the cone inner segments during development showed the changes that occurred in the arrangement of cones in relation to each other (Fig. 9). The packing of the cones in the larvae is shown in Figure 9A. The hexagonal shape allowed close packing in a row arrangement. Examination of the borders between cones shows that many were associated as groups of 2 or more, but, as observed in the radial sections, the membrane thickening was not always complete along the entire cell-cell margin (Fig. 9B). This situation continued until flexion, when a gradual rearrangement of the cones began. By day 35 (SL, 8 mm) only single, double and triple cones were observed (Fig. 9C). However, it was not until about day 50 (SL, 12 mm) that a square mosaic of four double cones surrounding a single cone began to be obvious (Fig. 9D). Even at day 50 a number of triple cones could still be observed. By day 96 and in the adult, the mosaic consisted of a well-defined square mosaic arrangement and no sign of any triple cones (Fig. 9E).

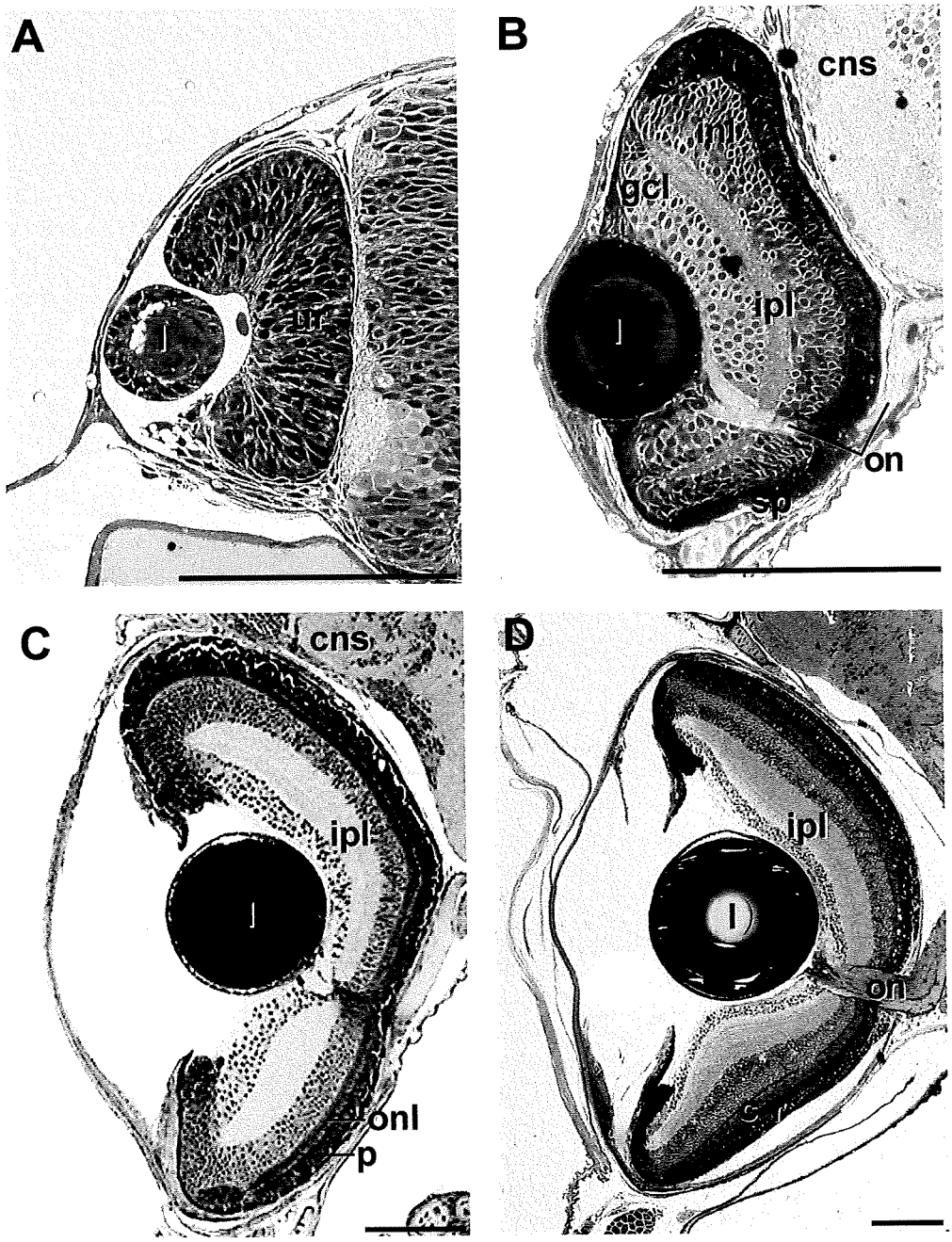

Fig. 4. Series of photomicrographs showing radial sections of the eye, at the level of the insertion of the optic nerve, of the dhufish in four stages of development. **A:** At hatching, showing the undifferentiated retina (ur) and lens (l). **B:** At day 3, showing the eyecup now backed by screening pigment (sp) and differentiation of the retina, including the presence of photoreceptors (p) and differentiated outer nuclear layer (onl). **C:** At day 13, showing an increase in both retinal size and neuronal density. **D:** At day 27, showing an increase in the thickness of the photoreceptor layer following rod (r) differentiation. c, layer of cones; cns, central nervous system; gcl, ganglion cell layer; ipl, inner plexiform layer; inl, inner nuclear layer; on, optic nerve.
Scale bars=50μm.

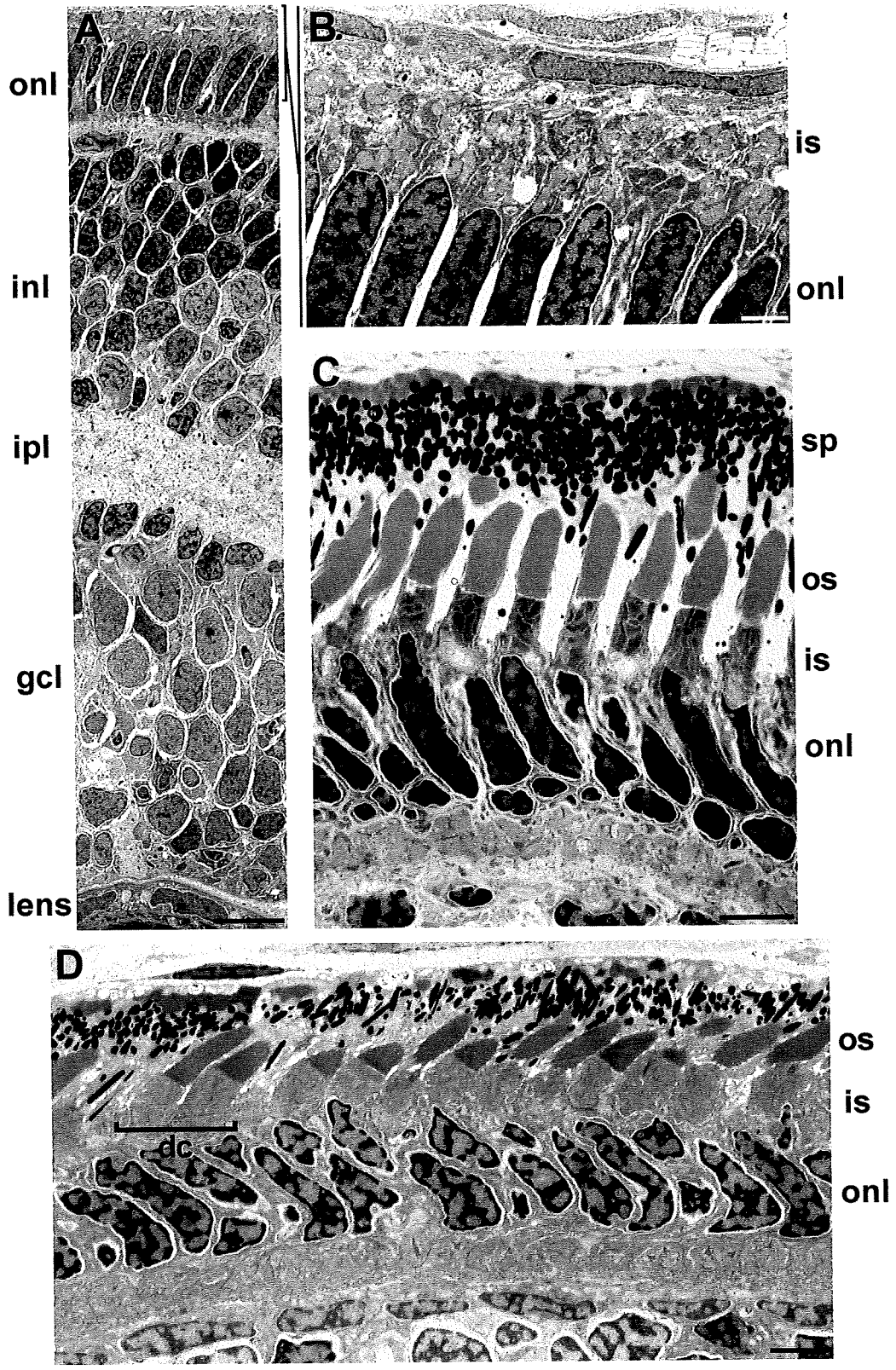

Fig. 5. Early development of photoreceptors. **A, B:** Day 2, cone nuclear layers (onl) are differentiated and formation of inner segments (is) is underway, however, outer segments (os) and screening pigment are absent. **C:** Day 3, outer segments of cones and screening pigment (sp) are present. **D:** Day 4, a number of single cones have associated with neighbours to form double cones (dc). inl, inner nuclear layer; ipl, inner plexiform layer; gcl, ganglion cell layer. Scale bars: A: 5μm, B: 1μm, C, D: 2μm.

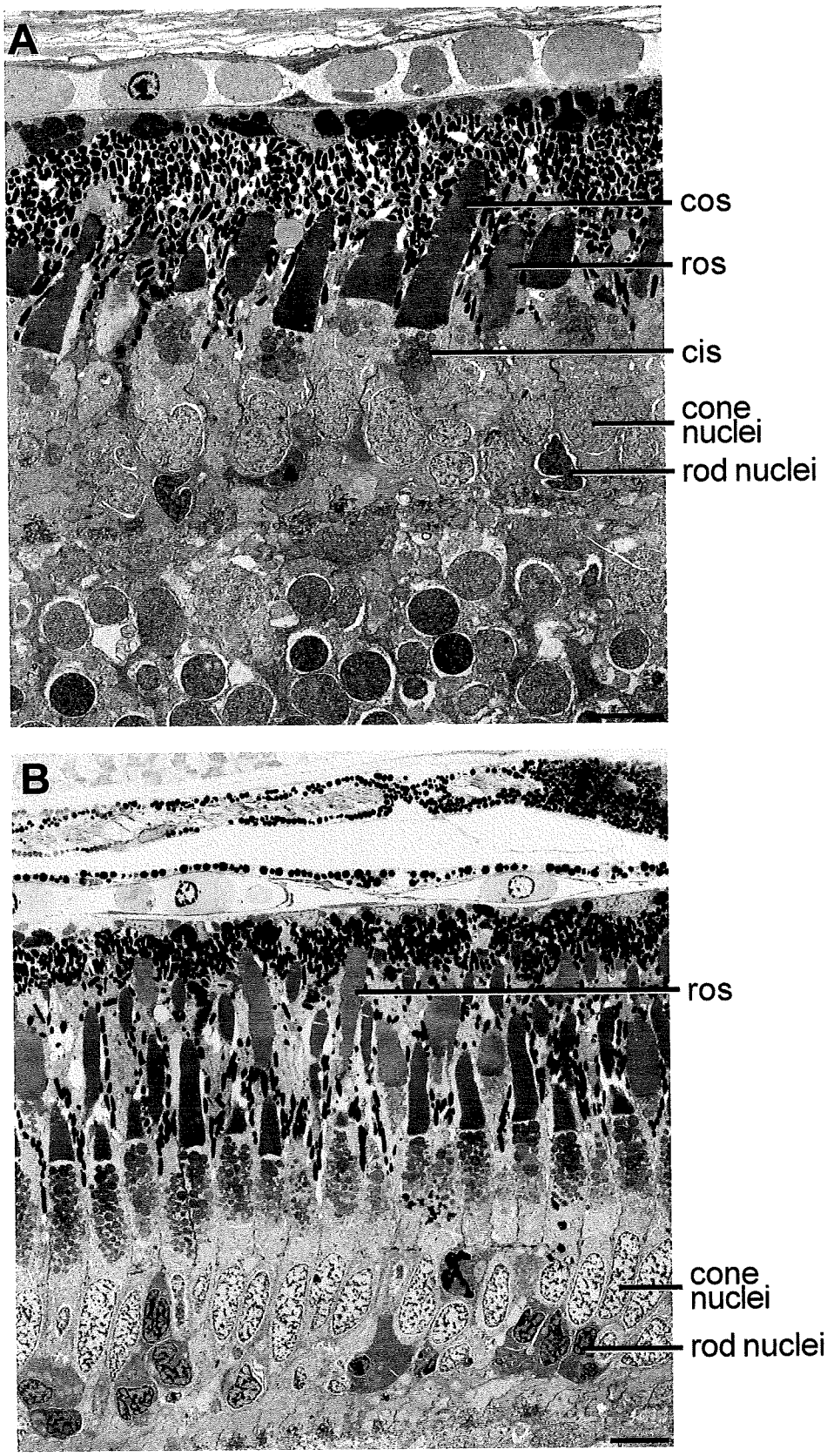

Fig. 6. Radial sections of retinae at **A:** 22 days, showing rod and cone nuclei and the first appearance of outer segments; **B:** 28 days, showing rod nuclei plus outer segments displaced to a scleral position relative to the cone outer segments (cos). cis, inner segment; ros, rod outer segment. Scale bars: 5μm.

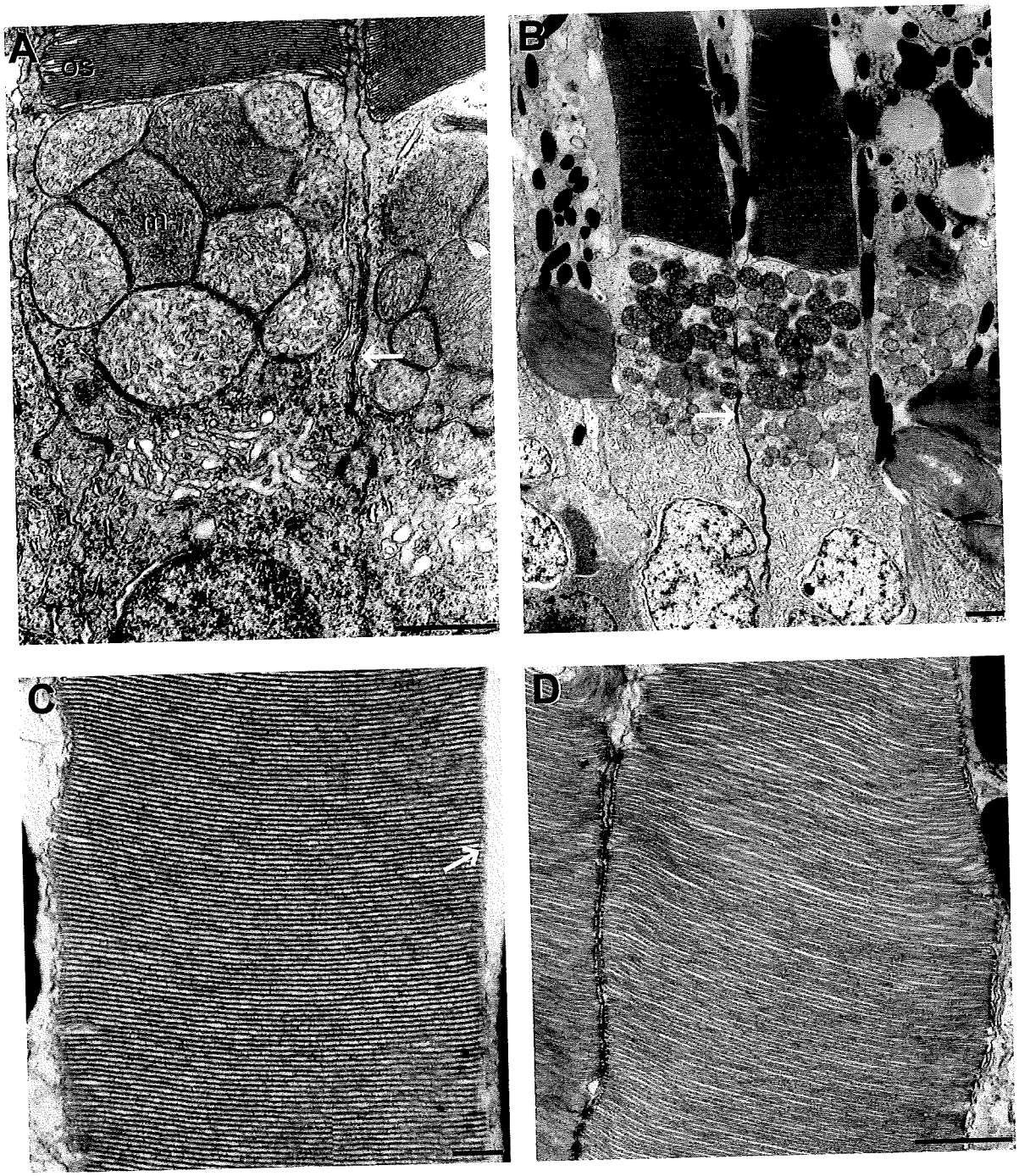

Fig. 7. Electron micrographs showing membranes of rods and cones. **A:** Day 15, cones are joined at the level of the inner segments by subsurface cisternae along part of their length (arrow). **B:** Day 31, double cone with subsurface cisternae showing thickening and lengthening along the border of the inner segments (arrow). **C:** Magnification of a cone outer segment showing folded lamellae membranes with one side open to the cytoplasm (arrow). **D:** Rod outer segment composed of stacked disks of lamellae that are separated from each other. m, mitochondria; os, outer segment. Scale bars: A, B: 1μm; C: 200nm; D: 500nm.

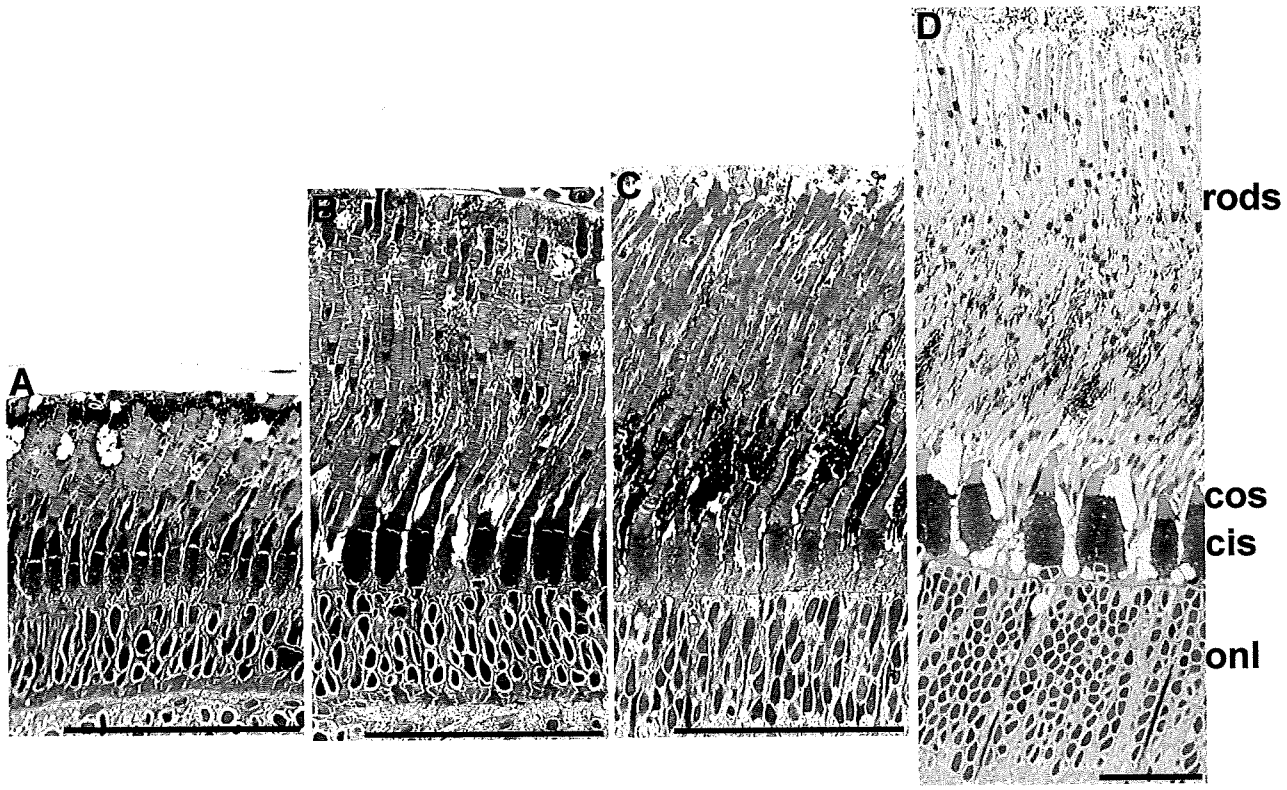

Fig.8. Comparison of rod differentiation in dhufish of four ages. **A:** Day 35, **B:** Day 50, **C:** Day 96, **D:** Adult. All sections are aligned along the level of the external limiting membrane. Note the increase in rod numbers, both at the outer segment and nuclear region. Note also the increase in the size of the cones. cis, cone inner segment; cos, cone outer segment, onl, outer nuclear layer. Scale bars = 50μm.

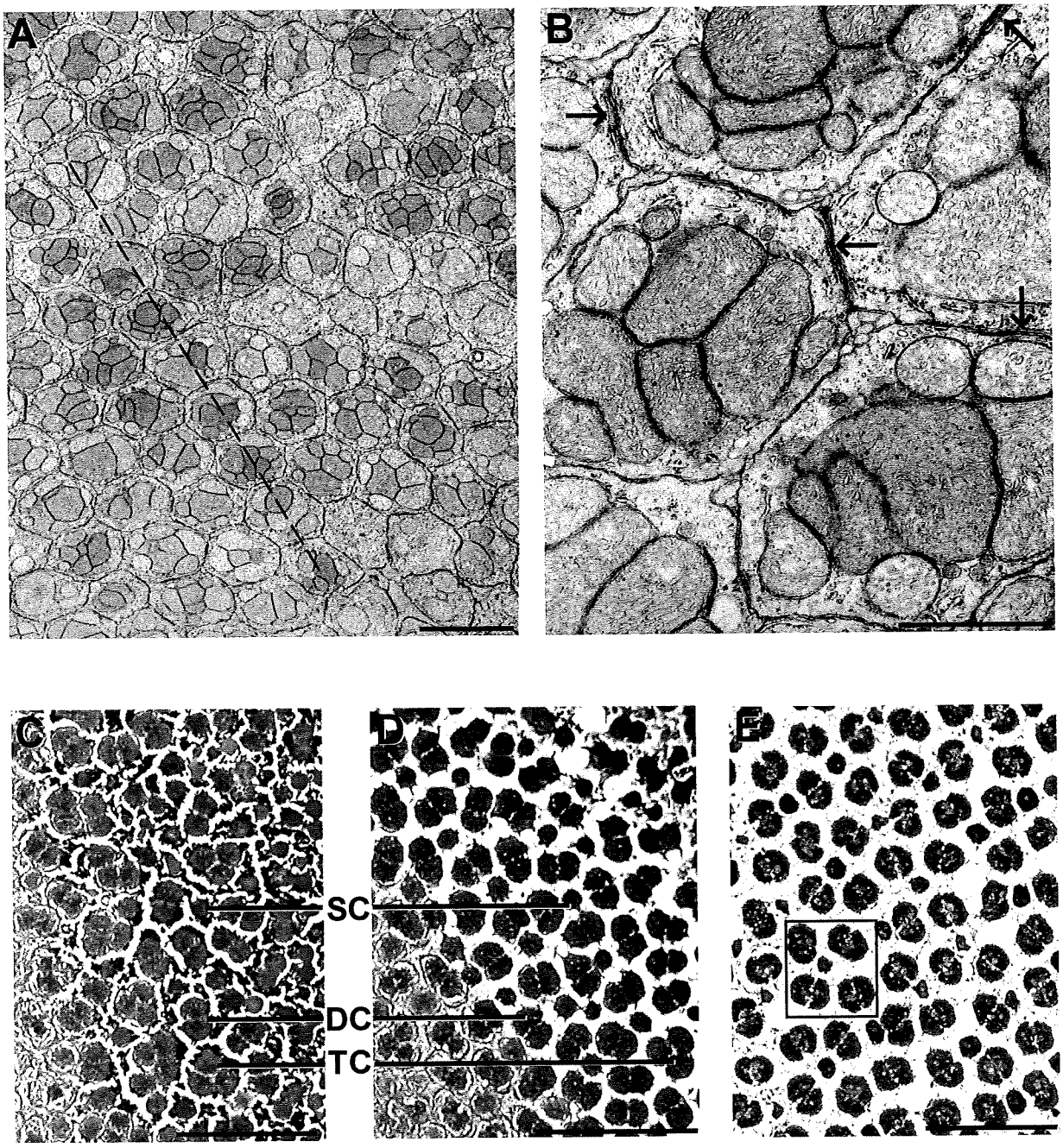

Fig. 9. Tangential sections at the level of the inner segments. **A, B**: Day 13, showing tight packing of cells in rows (broken line). **B** shows thickening between some cells though not always along entire borders (arrows). **C:** Day 35, showing single, double and triple cones but with no clear arrangement. **D:** Day 50, showing double cones surrounding single cones, although a few triples remain. **E:** Adult, only double cones surrounding single cones in a square mosaic (outlined). SC, single cone; DC, double cone; TC, triple cone. Scale bars: A: 5μm; B: 1μm; C, D: 25μm, E: 50μm.

Summary of the developmental stages and corresponding changes in retinal structure are presented below.

Day	Approx. SL (mm)	Stage/ Food	Eye development/behaviour
0	2.5	Hatching / yolk sac	No photoreceptors
3 – 5	3	First feeding / rotifers	• Pigment epithelium present • Cones present in row mosaic
?	?		• Coupling of cones • Cone outer segment length (2 µm) increases
15	4.5	Flexion begins	
17	5	Copepods introduced Water begins to clear	• Avoidance of light first observed
21	5.5	Flexion complete	• First rod nuclei observed
?			• Rod outer segments formed
25	6	Weaned off rotifers Water clear	• Double cones membranes consolidated
?			• Row mosaic breaks down
			?
35	9	Increase in growth rate Body pigmentation/ stripes develop	• Rod densities increase rapidly • Triple cones present
?			?
45	10	Metamorphosis complete	
55	12	Weaned onto pellets	• Square cone mosaic forms • Cones up to 25 µm long
96	30		All characteristics of adult retina present.

Discussion

During the first three weeks post-hatch the retina of the dhufish larvae is composed entirely of cones. Although it is not known what the threshold of the cone stimulation is in the larval dhufish, a pure cone retina is likely to restrict larvae to bright surface waters for feeding. The presence of a tightly packed row arrangement of cones in the first weeks post-hatch is a strategy for increasing the resolution, and is significant in a small eye that can does not have a large lens for increasing magnification of the image. The constraints of small jaw size in larval fish necessitate feeding on zooplankton, which are in turn difficult to detect. As the eye grows, the optics allow increased resolution and the need to maintain photoreceptors of small diameter are reduced (Shand, 1997). Thus many larval fish appear to have eyes disproportional in relation to body size. However, increase in the size of the cones after the first week post-hatch will increase the sensitivity of the eye and it is possible the larvae could move into deeper water. It is known the larvae of the nocturnal coral reef cardinal fishes (Apogonidae) can feed at substantial depths (to at least 200 m in clear oceanic waters) prior to the development of rods (see Job and Bellwood, 2000; Job and Shand, 2001). A behavioural increase in sensitivity during development has also been observed in the temperate striped trumpeter, *Latris lineata* (Pankhurst and Hilder, 1998), the red drum, *Sciaenops ocellatus* (Fuiman and Delbos, 1998) and three coral reef species (Job and Bellwood, 2000). In the case of the striped trumpeter and red drum the increase in sensitivity was correlated with the appearance of double cones. However, few other studies have tried to correlate structural changes with increased sensitivity in feeding behaviour.

The appearance of connections between three or more cones is unusual. Double cones are thought to increase sensitivity due to their larger sampling of image space and there is evidence to show this in the cichlid (van der Meer, 1995) however, the significance of cones being joined to several neighbours is unclear. When comparing the developmental schedule with that of the black bream, *Acanthopagrus butcheri*, differences can be noted. In the black bream there was no record of cones being associated with one another until after day 20, following which the rods first appear. The size of the cones in dhufish is greater than in larval black bream at all stages after the first week post-hatch, and the densities of rods in dhufish are much greater than in the black bream (Shand et al, 1999). The presence of multiple cones with large outer segments indicates dhufish larvae are adapted for lower light conditions than those of black bream, from one week post-hatch. The rapid addition of rods from 3 weeks post-hatch implies the retina is progressively increasing in sensitivity from an early stage, in preparation for the nocturnal and deep-water behaviour patterns known to be exhibited by the adult dhufish (Hutchins and Thompson, 1983).

It was noted from the behavioural observations from day 20, as the water was being cleared of algae, that the fish often reacted to the light being switched on. It is at this time that the rods were first forming and the eye becoming rapidly more sensitive to light. The increase in transmission of light through the water column as the algae is cleared from the tanks means the larvae are unable to retreat to the bottom of the tank to escape excess light and are thus subjected to brighter light just at the time their retinae are becoming more sensitive. Thus it is recommended that the light intensity be progressively decreased from the time the weaning from rotifers begins.

It should be noted that light levels inappropriate to those experienced by the fish in the natural environment can cause damage to the photoreceptor outer segments and lead to impairment of visual function, although no evidence of retinal damage was observed in any of the specimens examined in this study. However, long-term studies comparing hatchery-reared dhufish with wild-caught juveniles are needed to ascertain whether or not larvae reared in artificial light develop differences in retinal structure. If such changes were observed, the consequences for restocking programmes would need to be considered because survival rates of released fish may be affected.

Benefits

This study highlights the need to consider the sensory development of individual species when designing lighting conditions for rearing larval fish. The timing of retinal development in dhufish larvae differs from that in of other temperate species whose retinae have been investigated. The early development of the double cones and the large size of these photoreceptors indicate that from early stages the larvae are increasing their sensitivity. The rapid addition of rods, from their first appearance at day 21, further increases the sensitivity of the eye. The corresponding increase in water clarity as the larvae are being weaned to copepods is a time when the larvae are most likely to be stressed by the lighting conditions. This information will be of benefit to the Fremantle Maritime Centre and other parties interested in the rearing of dhufish for aquaculture or restocking purposes. Consideration of light intensity should be included in hatchery design, with progressively lower light levels required following the second week post-hatch.

The rapid increase in sensitivity, observed in dhufish implies that in the natural environment the larvae will be found at increasing depth during development. It is highly likely that following the first week post-hatch they will leave surface waters and be found in deeper waters, associated with reefs and overhangs. Such information will be made available to any Fisheries researchers investigating the ecology of the early life history of dhufish.

Further Development

A component not addressed in this study is the contribution of colour vision to the ability of larval fish to discriminate prey against the background space-light. Knowledge of the absorption characteristics of the visual pigments in the outer segments of the photoreceptors would give an indication of the colours of lights most suitable for rearing larval fish. As new photoreceptors develop, visual pigments could change the colours they absorb, as has been found in the juvenile goatfish, *Upeneus tragula,* at settlement (Shand, 1993) and the black bream, *Acanthopagrus butcheri,* when they migrate to deeper water (Shand et al., 2001). The role that environmental cues play in such developmental events is unknown. Thus, the long-term effects of rearing larvae in artificial lighting are not clearly understood. The effects of rearing larvae in different light intensities and spectral composition should be considered, especially in species to be used in restocking programmes.

Conclusions

This study highlights the inter-specific differences in the development of the retina in larval fish. In contrast to the larvae of black bream, dhufish appear to be adapted to low levels of light from early stages and thus consideration of the intensity of lights in the aquaculture situation should be given careful attention. This is particularly important at the time the water is cleared of algal cultures because this time corresponds to that when the retina begins a rapid increase in sensitivity due to the differentiation of rods.

Although larval dhufish have never been captured from the natural environment (J. St. John, WA Fisheries, personal communication), the features of the retina that indicate the eye is designed to increase retinal sensitivity imply the dhufish is likely to be found at increasing depth within the water column as they develop. Following metamorphosis it is likely that juveniles will be found in the vicinity of reefs and ledges, in minimum depths of 20 – 30 m.

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Appendix

Staff

Greg Jenkins of the Fremantle Maritime Centre has provided administrative support at all stages of the project. The skills and dedication of Jennifer Cleary, Anthony Aris, Gavin Partridge and hatchery staff of Fremantle Maritime Centre have made this project possible by successfully rearing dhufish. Similarly, I wish to acknowledge Michael Archer, of the University of Western Australia, for preparing and sectioning the retinal samples. Nichole Thomas assisted with the preparation of the figures.