Intraspecific Evidence for the Function of Single and Double Cones in the Teleost Retina

Abstract. *Retinal growth in young* Sebastes diploproa *involves the succession of three distinct cone patterns. Development of the final pattern with the loss of single cones occurs in close temporal association with a permanent migration from the surface to deep water. The results suggest that loss of single cones depends upon the change in environment and that the loss occurs through fusion to double elements.*

Teleost retinas contain several morphologically distinct cone types, most often single or double but occasionally triple or quadruple (*1*). Some species have only one type of cone, and others have a mixture of cone types (Fig. 1). Interspecific comparisons show that active species living in shallow water have well-developed mosaics of single and double cones, whereas deeper-living species have double cones only (*1, 2*). Degeneration of cones with increasing habitat depth has been suggested since cones in the retina of *Sebastes marinus mentella* captured in deep water were small in relation to those of a specimen from shallow water (*3*). Within the retina of a few teleost species, cone types change quantitatively or qualitatively during ontogeny. For example, some species have only single cones as larvae but acquire a mixture of single and double cones when older (*4, 5*); similarly, the trout has an equal mixture of single and double cones when young, but twice as many double as single cones when older (*2*).

Because the correlation of cone type and morphology with habitat and environmental light is based primarily on interspecific comparisons, a study of the rockfish *Sebastes diploproa* was of particular interest. These fish spend their first year of life at the sea surface (as larvae and prejuveniles), and then migrate to depths averaging several hundred meters where the rest of the life is spent near the bottom (as juveniles and adults) (*6, 7*). I have studied the retinas of freshly caught surface prejuveniles and benthic juveniles and adults (*8*) as well as those of prejuveniles held in the laboratory and prevented from migrating to depth.

Tangential sections through the visual cell layer of the retina in a series of *S. diploproa* reveal three types of cone mosaics. The first pattern (Figs. 1A and 2A), present in relatively small prejuveniles, consists of rows of alternating single cones and equal double cones in a ratio of 1:1. Two types of single cones are present; these are classified as additional and central single cones on the basis of position in the pattern (no morphological difference is apparent). The second pattern (Figs. 1B and 2B) is first seen in the peripheral regions of the retina of surface prejuveniles. Here, most or all of the additional single cones are missing, and only central single cones remain. The third pattern (Figs. 1C and 2C) is present over the entire retina of larger benthic juveniles; this pattern consists of rows of double cones. With the changing cone pattern, two other phenomena are also apparent; cone density decreases and cone diameter increases. Cone diameter may differ within the retina of a single individual in many species, being generally greater in areas of lower cone density (*5, 9*). Within the retina of individual *S. diploproa*, however, cone density is relatively uniform compared with the variation apparent in other species, but it decreases with increasing eye diameter (*10*). Relative eye diameter is greater in benthic than in surface specimens (analysis of covariance, $P < .005$), so that retinal changes associated with eye growth are accelerated after migration.

Comparison of the ratios of single to double cones in the central and peripheral parts of the retina in surface prejuveniles and benthic juveniles indicates that the loss of single cones begins near the periphery and proceeds inward. The value of this ratio is close to 1.0 in the central part of the retina for surface prejuveniles (Fig. 3A), indicating the presence of the first pattern (Fig. 1A). In the peripheral retina (Fig. 3B), values of the ratio are near 1.0 for small eye diameters, but decrease with growth to near 0.5, indicating the second pattern (Fig. 1B). The ratio in the central part of the retina is less than 0.5 in the smallest benthic juveniles and decreases with growth to 0, whereas the ratio near the retinal periphery is near 0 for all benthic juveniles. Since new cones are formed in the peripheral ora serrata (*2*), these data suggest that peripheral addition of cones undergoes a succession of the three patterns during growth. The change from pattern 1 to pattern 2 occurs in surface prejuveniles with eye diameters of 3 to 5 mm (Fig. 3B), whereas the change from pattern 2 to pattern 3 occurs after migration. Thus the change from pattern 1 to 2 may not result from the loss of single cones, but rather from the cessation in the formation of additional single cones in the peripheral ora serrata; with growth of the retina, peripheral cones become more central. Examining the retina of larger surface prejuveniles shows this to be the case; patterns 1 and 2 occur in the central and peripheral retina, respectively, of the same fish. Although not observed, it is therefore conceivable that all three patterns could exist in the retina of an early benthic juvenile prior to loss of the single cones.

To determine the relationship of the changes in pattern formation and in the ratio of single to double cones with the timing of the migration, surface prejuveniles were held in the laboratory under surface light conditions beyond the time and size at which migration normally occurs. Although several of these specimens were larger than small benthic juveniles, the value of the ratio was intermediate between surface and benthic values in both central and peripheral regions (Fig. 3, A and B). Thus although the change from pattern 1 to pattern 2 occurs in the retinas of laboratory-held and surface prejuveniles, the change to pat-

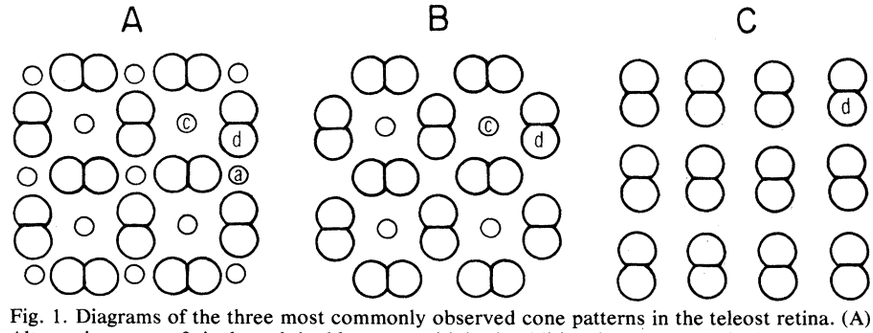

Fig. 1. Diagrams of the three most commonly observed cone patterns in the teleost retina. (A) Alternating rows of single and double cones with both additional and central single cones. (B) Pattern with double and central single cones. (C) Rows of double cones only. Patterns (A) and (B) are observed in retinas of active, shallow-living teleosts and in surface prejuvenile *S. diploproa*; pattern (C) is observed in deeper-living teleosts and in benthic *S. diploproa*. Abbreviations: *a*, additional single cone; *c*, central single cone; *d*, double cone.

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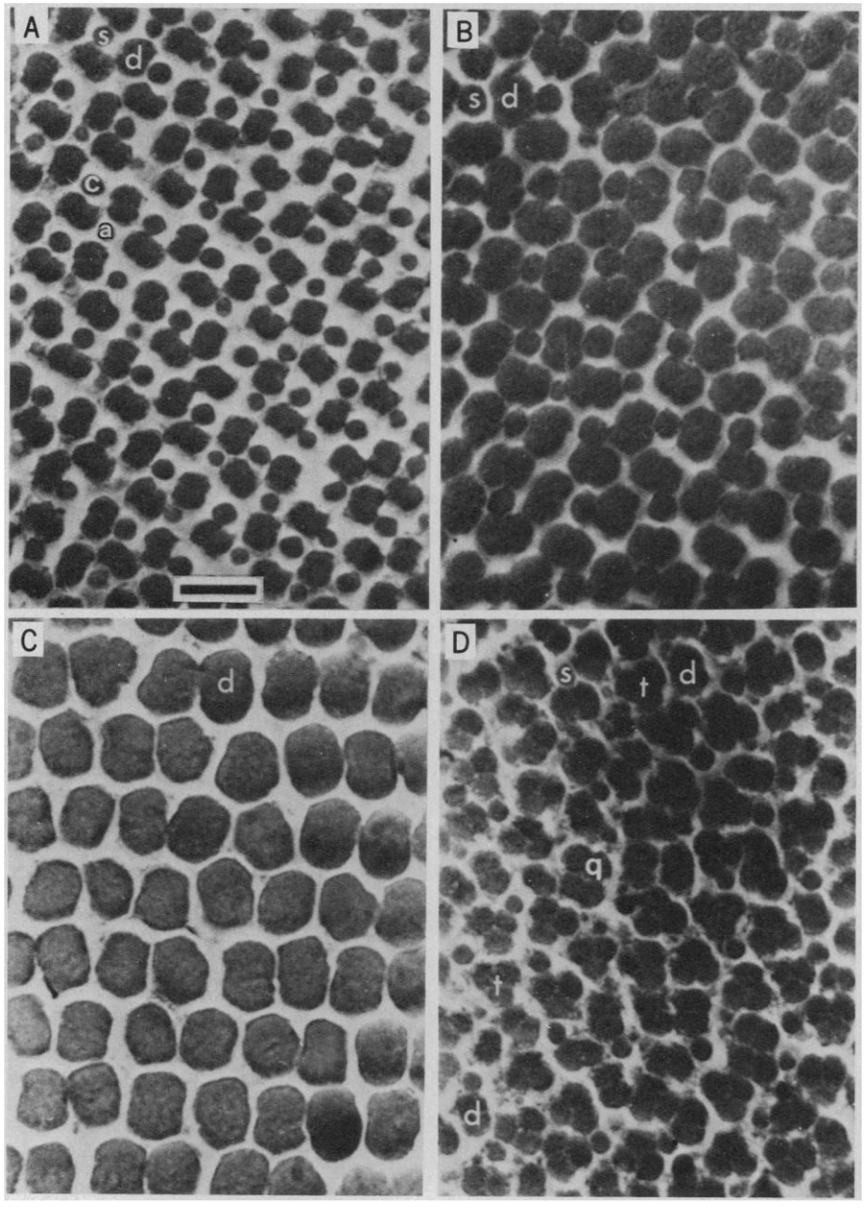

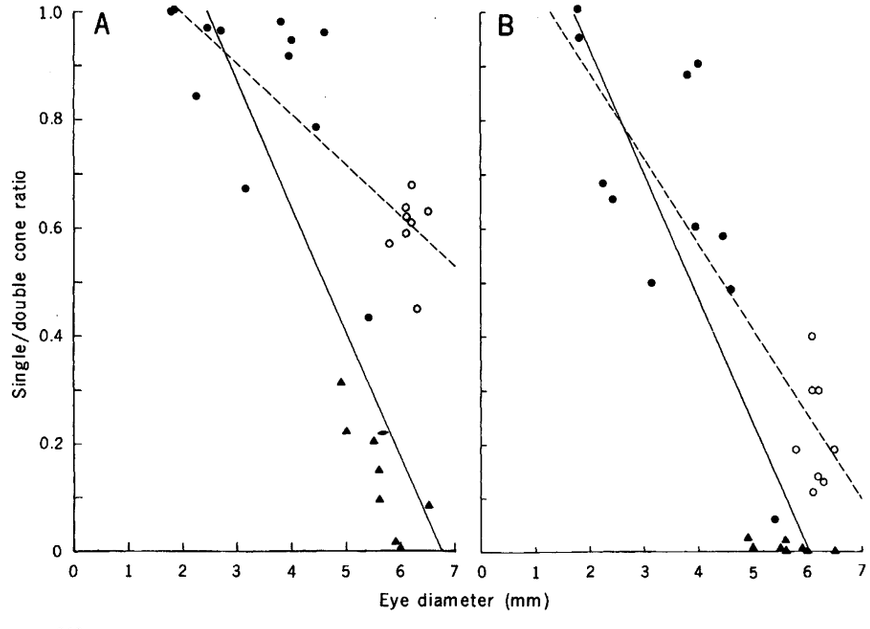

tern 3 and the complete loss of single cones is accelerated only after migration, presumably by the changes in intensity or spectral composition of light associated with increasing depth.

One can speculate on the fate of the remaining single cones. The change from cone pattern 1 to 2 (loss of additional single cones) was reported with retinal growth in *Salmo trutta*. Since she saw no degenerating single cones in the retina, Lyall suggested that the single cones transmuted into rods and showed putative intermediates (*2*). The presence of similar cells in the rod-free area temporalis of the herring, however, cast doubt on this conclusion (*5*). Neither degenerating single cones nor cone-rod intermediates were apparent in the central retina of early benthic juvenile *S. diploproa*. Loss of single cones in this species proceeds from the peripheral to the central retina, opposite from the direction in *S. trutta* (*2*) but similar in pattern to the development of double cones in the retina of larval perch and pike-perch (*4*). Ontogenetic formation of double cones occurs through fusion of two single cones (*4*, *11*). Triple and occasional quadruple cones, which are associated with cone pattern disorganization (*1*), were found in moderate abundance in

Fig. 2 (top). Tangential sections (paraffin-embedded, 5 μm) of the visual cell layer of *S. diploproa* taken at the level of the cone ellipsoid in light-adapted specimens. Retinas were divided by the method of O'Connell (*9*); all photomicrographs are from the centralmost segment of the dorsotemporal quarter of the retina. (A) Surface prejuvenile [30.3 mm standard length (SL), eye diameter 3.2 mm]. (B) Laboratory-held prejuvenile (56.6 mm SL, eye diameter 6.1 mm). (C) Benthic juvenile (58.3 mm SL, eye diameter 6.5 mm). (D) Largest surface prejuvenile captured (58.7 mm SL, eye diameter 5.4 mm). Abbreviations: *s*, single cones; *a*, additional single cone; *c*, central single cone; *d*, double cones; *t*, triple cones; *q*, quadruple cone. Bar represents 10 μm. Fig. 3 (bottom). Ratio of single to double cones as a function of eye diameter of surface prejuvenile (●), laboratory-held (○), and benthic juvenile *S. diploproa* (▲). Surface prejuveniles (13.4 to 58.7 mm SL) were captured by dip net, and benthic juveniles (41.9 to 58.3 mm SL) were taken in trawls at depths of 180 to 275 m off the coast of southern California. Laboratory-held specimens (49.0 to 60.6 mm SL) were maintained in running seawater for periods from 1 to 2 months (*10*). Ratios are an average determined from two photomicrographs of tangential sections from each specimen. (A) Centralmost segment of the dorsotemporal quarter of the retina. The solid line ($r = -0.86$, $y = -0.23x + 1.56$) is based on ratio values for surface and benthic fish. The dashed line ($r = -0.80$, $y = -0.09x + 1.18$) is based on values for surface and laboratory-held fish. (B) Peripheral segment of the dorsotemporal quarter of the retina. Solid line: $r = -0.87$, $y = -0.23x + 1.40$. Dashed line: $r = -0.86$, $y = -0.16x + 1.22$.

the central retina of the largest surface prejuvenile captured (Fig. 2D) but were not observed in older juveniles or adults. Thus the loss of single cones in this species may occur through fusion of two single cones to form a double cone or by fusion of two single cones and a double cone which might then divide to form two double cones. This latter process may explain the disorganization of the cone pattern and the presence of intermediate triple and quadruple cones in transitional specimens (Fig. 2D).

Interspecific comparisons correlating cone type and pattern with behavior and ecology of teleosts have suggested that species with well-developed patterns of single and double cones feed on fast-moving prey and that these patterns may improve perception of movement (*2*), possibly providing a structural basis for high temporal and spatial resolution (*12*). Double cones and poorly developed patterns, on the other hand, are associated with less acute vision in deep water. The intraspecific changes in cone type and pattern observed in *S. diploproa* in association with changing habitat and environmental light confirm past observations on the function of single and double cones and cone patterns based on interspecific comparisons.

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7. Surface prejuveniles may grow to 45 to 55 mm standard length (SL), although specimens as large as 58.7 mm SL have been captured. The smallest observed benthic juvenile is 41.9 mm SL, but the smallest specimens normally encountered are 45 to 50 mm SL. The migratory season for this species lasts about 4 months and may include an intermediate midwater habitat.
8. Although I examined the retinas of benthic adult *S. diploproa* (eye diameters up to 21.3 mm), they were not included in this analysis. Determination of the density of single and double cones requires analysis of tangential sections of retina, since a section of one member of a double cone might be mistaken for a single cone in retinal cross section. Density determinations further require that the retinas be fully light-adapted, so that all cones are on the same level within the visual cell layer and consequently appear in the same tangential section. Cross sections of adult retinas revealed incomplete and variable states of light-adaptation. I examined serial tangential sections from adults, however, and determined that single cones were not present.
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Prolactin Synthesis by Human Chorion-Decidual Tissue: A Possible Source of Prolactin in the Amniotic Fluid

Abstract. *Explants of human chorion-decidual tissue obtained at delivery from normal, full-term pregnancies synthesize and secrete prolactin. This hormone is indistinguishable from pituitary prolactin by chromatographic, electrophoretic, immunologic, and receptor assay techniques. These results suggest that chorion-decidua may be the source of the large quantities of prolactin in amniotic fluid.*

Prolactin (PRL), a polypeptide pituitary hormone, influences lactation, water and electrolyte transport across membranes, fertility, and growth in a variety of species (*1*, *2*). Human PRL concentrations in the maternal circulation rise steadily during pregnancy becoming tenfold higher than those in the nonpregnant state; concentrations in the amniotic fluid exceed those in the maternal or fetal circulation by 100-fold early in pregnancy and by five- to tenfold at term (*3*, *4*). Biologically, chemically, and immunologically, amniotic fluid human PRL is similar or identical to the pituitary hormone (*5–7*), yet its origin and physiologic function are unknown. An extra-pituitary source of PRL in amniotic fluid is suggested by experiments with pregnant rhesus monkeys in which neither maternal hypophysectomy nor fetal death decreased amniotic fluid PRL concentration (*8*). Friesen *et al.* (*9*) demonstrated that explants of chorionic tissue released small amounts of human PRL during a 24-hour culture, and suggested that chorion might be the source of amniotic fluid PRL. However, Riddick and Kusmik (*10*) reported that tissue composed predominantly of decidual cells secreted human PRL during 18 hours of culture. We report here studies of the synthesis and secretion of human PRL in vitro by placental trophoblast, amnion, chorion, and decidua.

Human placentas and the associated membranes from uncomplicated, full-term pregnancies were obtained within minutes of delivery. Membrane pieces were dissected from several locations peripheral to the placenta and rinsed with Earle's balanced salt solution. The amnion was carefully removed from the closely attached chorion-decidua, but attempts to separate the chorion manually from decidua were unsuccessful. The tissues were then cut into explants (5 by 5 mm) and pooled. Placental tissue was prepared for culture as described elsewhere (*11*). Explants of placenta, amnion, or chorion-decidua with an average wet weight of 100 mg each were cultured in separate incubation flasks with 5 ml of minimal essential medium containg 15 percent fetal calf serum, penicillin-G (25 unit/ml), and streptomycin at 25 μg/ml at 37°C in an atmosphere of 95 percent O_2 and 5 percent CO_2 for periods of up to 6 days with medium changes every 24 hours. The media removed at the end of each day were centrifuged at 1000*g* for 10 minutes at 4°C and stored at −20°C. At the end of the culture period, tissues were homogenized in 3 ml of ice-cold 0.1*M* ammonium bicarbonate, *p*H 9.2, with 1 m*M* α-toluenesulfonyl fluoride and 0.5 percent Triton X-100. Each homogenate was rapidly frozen and thawed five times to disrupt cell membranes, and then centrifuged at 500*g* for 10 minutes at 4°C. The resulting pellet was washed with 2 ml of homogenizing solution, and the combined supernatants were centrifuged at 12,000*g* for 30 minutes at 4°C. The clear supernatant was stored at −20°C until assayed. Concentration of human PRL in the incubation media and tissue homogenates were measured by homologous radioimmunoassay (*12*). [The human PRL used as the standard and for iodination (VLS-2) and the rabbit antiserum to human PRL were provided by the Hormone Distribution Program, NIAMDD.] Iodination of PRL was performed by the lactoperoxidase method (*13*), and the reaction was stopped by addition of sodium azide (*14*). Iodine-125-labeled human PRL was separated from the free ^{125}I and damaged hormone by chromatography on Sephadex G-150.

Cultures of the amniotic layer or placental tissue released less than 10 ng of prolactin during the first 24 hours of incubation and none thereafter. However, the explants of chorion-decidua secreted human PRL over the entire 6-day culture period at a relatively constant rate (Table 1). The average daily secretion was 294 ± 34 ng/100 mg (wet weight of tissue) and the total amount of human PRL released over the 6-day period exceeded by 1800 percent the amount in the tissue before culture. Addition of cycloheximide in concentrations of 0.05 m*M* to chorion-decidual explants for 24

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