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Developmental changes in the cone visual pigments of black bream *Acanthopagrus butcheri*

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Summary

The spectral absorption characteristics of the visual pigments in the photoreceptors of the black bream *Acanthopagrus butcheri* Munro (Sparidae, Teleostei), were measured using microspectrophotometry. A single cohort of fish aged 5–172 days post-hatch (dph), aquarium-reared adults and wild-caught juveniles were investigated. During the larval stage and in juveniles younger than 100 dph, two classes of visual pigment were found, with wavelengths of maximum absorbance (λ_{max}) at approximately 425 nm and 535 nm. Following double cone formation, from 40 dph onwards, the short wavelength-sensitive pigment was recorded in single cones and the longer wavelength-sensitive pigment in double cones. From 100 dph, a gradual shift in the λ_{max} towards longer wavelengths was observed in both cone types. By 160 dph, and in adults, all single cones had a λ_{max} at approximately 475 nm while the λ_{max} in double cones ranged from 545 to 575 nm. The relationships between the λ_{max} and the ratio of bandwidth:λ_{max}, for changes in either chromophore or opsin, were modelled mathematically for the long-wavelength-sensitive visual pigments. Comparing our data with the models indicated that changes in λ_{max} were not mediated by a switch from an A_1 to A_2 chromophore, rather a change in opsin expression was most likely. The shifts in the λ_{max} of the visual pigments occur at a stage when the juvenile fish begin feeding in deeper, tannin-stained estuarine waters, which transmit predominantly longer wavelengths, so the spectral sensitivity changes may represent an adaptation by the fish to the changing light environment.

Key words: vision, retina, cones, opsin, microspectrophotometry, fish, black bream, *Acanthopagrus butcheri*.

Introduction

The adults of most species of shallow-living diurnal teleost fish have well-developed visual systems, with both rod and cone photoreceptors. Cones, which subserve vision at higher light intensities than rods, can be characterised both morphologically and by the spectral absorption characteristics of their visual pigments. The spectral absorption of the long wavelength-sensitive cones, the double cones (so called because they are intimately associated along their inner segment regions), can be related to the colour of the ambient light in which the fish are living (Lythgoe, 1984; Lythgoe and Partridge, 1989; Bowmaker, 1995). Most diurnal teleosts have also been found to possess at least one class of short wavelength-absorbing single cone, thought to facilitate contrast detection of objects against the prevailing background light (Lythgoe and Partridge, 1989, 1991).

The life history of many fish involves changes in spectral environment during migration from one body of water to another. In association with such migrations, the spectral sensitivity of the fish may also change. The changes can be the result of the loss of a cone class from the retina; alternatively, there may be physiological changes in the visual pigments within a cell type (for a review, see Beaudet and Hawryshyn, 1999). Visual pigment absorption characteristics are governed in two main ways: by the amino acid sequence of the transmembrane protein (the opsin), and/or whether the chromophore that binds with the opsin is retinal (vitamin A_1-based) or 3,4-didehydroretinal (vitamin A_2-based), and changes in either moiety affect visual pigment spectral sensitivity (for a review, see Loew, 1995). For example, in the eel *Anguilla anguilla*, shorter wavelength sensitivity is facilitated by the rod chromophore changing from to 3,4-didehydroretinal to retinal as the adults begin their breeding migration from rivers to the deep ocean (Carlisle and Denton, 1959; Wood et al., 1992), whereas a switch in opsin expression has been implied in the short wavelength-sensitive single cones of pollack *Pollachius pollachius* (Shand et al., 1988), and the long wavelength-sensitive double cones of the goatfish *Upeneus tragula*, as the habitat and feeding behaviour of the fish change during development (Shand, 1993).

In contrast to the retinae of adults, the pre-flexion larval

stages of many species of teleost possess only one morphological cell type, single cones, and it is not until a later stage of development, often the time of metamorphosis from larval to juvenile stages (the time of fin ray formation and body pigmentation), that double cones and rods are observed (Blaxter and Jones, 1967; Blaxter and Staines, 1970; Ahlbert, 1973; Evans and Fernald, 1993; Pankhurst and Eagar, 1996; Shand et al., 1999). The possession of only one morphological cell type in the larval retina raises the question of whether only one visual pigment is expressed at this stage, and what the spectral absorption characteristics might be. In addition, it is not known whether there are visual pigment changes at the time that single cones begin to associate with neighbours and form double cones.

We performed a microspectrophotometric study of the photoreceptors of black bream, *Acanthopagrus butcheri*, during their early life history to determine whether changes in visual pigments in juvenile fish are initiated during structural changes in the retina associated with metamorphosis, or correlated with the timing of ontogenetic changes in habitat and behaviour. In addition, we analysed the visual pigment measurements to determine the possible mechanisms by which the spectral characteristics are being altered in black bream.

Materials and methods

Animals

Retinal tissue was obtained from a single cohort of aquarium-reared black bream *Acanthopagrus butcheri* Munro between larval and juvenile stages [5–172 days post-hatch (dph); 2–34 mm standard length (*SL*)] and adults (*SL*>100 mm). As an adjunct to the experimentally reared fish, juvenile bream (ages unknown; *SL* 15–40 mm) were obtained from the Swan River, Western Australia.

Larvae were reared according to procedures outlined by Jenkins et al. (1999). From hatching to 25 dph they were held in a semi-intensive green water system, so called because the microalgae, *Nannochloropsis oculata*, which are present to provide a food source for rotifers upon which the larval bream feed, colour the water green. Between 22 and 25 dph the larvae were transferred to clear water and fed a diet of cultured branchiopod brine shrimps (*Artemia* spp.). From 55 dph the juveniles were gradually shifted onto a diet of dried pellets (0.2–0.4 mm diameter, Nippai ML®, Fuki and Co. Ltd). The fish were reared under fluorescent room light (Philips Coolwhite, 36W tubes) on a 14 h:10 h light:dark regime.

Preparation for microspectrophotometry

Experimental procedures were approved by the University of Western Australia Ethics Committee and followed the guidelines of the National Health and Medical Research Council of Australia. Fish were dark-adapted for at least 2 h prior to immersion in a lethal dose of methanesulphonate (MS 222; Sigma-Aldrich Pty, 1:2000 w/v in seawater). For examination by microspectrophotometry, preparations of unfixed retinal tissue were teased apart in teleost phosphate-buffered saline, containing 10% dextran (Sigma 250k RMM), on a rectangular 50×22 mm No. 1 coverslip (Marienfeld, Germany). During the early larval stages (*SL* 2–4 mm), whole fish were placed on the coverslip and teased apart with forceps. With larger fish it was possible to first remove the eyes and dissect the retina on the coverslip. The retinae of juveniles (*SL* <20 mm) could be dissected prior to transferring small pieces (1–2 mm^2) to the coverslip. In all cases the preparation was covered with a smaller (19 mm^2) No. 1 coverslip and sealed with nail varnish to prevent dehydration of the sample. All preparations were carried out in infrared illumination provided by a bank of 28 infrared emitting diodes and visualised using an infrared image converter (FJW Industries, USA).

Measurement of visual pigment absorbance spectra

A single-beam wavelength-scanning microspectrophotometer (MSP) was used to measure the absorption characteristics of the photoreceptor outer segments. The MSP described previously (Partridge et al., 1992), has been modified recently to improve the optics and hence transmission of short wavelengths to the specimen. Briefly, light from a quartz-halogen bulb was focused onto a holographic grating monochromator (Jobin Yvon). The output from the monochromator was linearly polarised, using a calcite crystal, and used to illuminate a variable rectangular aperture that controlled the dimensions of the measuring beam (typically 1–2×3–5 μm, depending on photoreceptor dimensions). The aperture plane was then focused by a series of fused-silica biconvex lenses (Oriel) and a Zeiss Ultrafluar (×32, NA 0.4) objective into the plane of the specimen on a micrometer-manipulated microscope stage. Above the stage, a Zeiss Neofluar objective (×100, NA 1.3) imaged the measuring beam onto the photocathode of a photomultiplier (R3896, Hamamatsu, Japan). The signal from the photomultiplier was digitised and recorded by a CTM05 counter timer board in a personal computer, which also controlled the scanning process. To view the sample and align the measuring beam, the specimen was illuminated with infrared wavelengths and the image was directed to an infrared-sensitive video camera, the image being viewed on a video monitor. Spectral absorbance measurements were made by placing the outer segment in the path of the measuring beam and scanning over the wavelength range 350–750 nm. Data were recorded at each odd wavelength on the ‘downward’ (long-wavelength to short-wavelength) spectral pass and at each interleaved even wavelength on the ‘upward’ (short-wavelength to long-wavelength) spectral pass. Only one sample scan was made of each outer segment, but this was combined with two separate baseline scans from an area adjacent to the outer segment being scanned. The two absorbance spectra thus obtained were averaged to improve the signal-to-noise ratio of the absorbance spectra used to determine the λ_{max} values. Following these ‘pre-bleach’ scans, outer segments were bleached with white light from the monochromator for 2–4 min and an identical number of sample and baseline scans made subsequently. The post-bleach average spectrum thus obtained was deducted from the pre-bleach average to produce a

difference spectrum for each outer segment and confirm the presence of visual pigment. Photoreceptor dimensions were measured for each cell scanned.

Analysis

Baseline and sample data were converted to absorbance values at 1 nm intervals and the upward and downward scans were averaged together by fitting a weighted three-point running average to the absorbance data (Hart et al., 2000). Absorbance spectra were normalized to the peak and long-wavelength offset absorbances, obtained by fitting a variable-point unweighted running average. Following the method of MacNichol (1986) a regression line was fitted to the normalized absorbance data between 30% and 70% of the normalized maximum absorbance at wavelengths longer than that of the absorbance peak. The regression equation was used to predict the wavelength of maximum sensitivity (λ_{max}) and fit the visual pigment template following the methods of Govardovskii et al. (2000). Acceptable pre-bleach spectra (Levine and MacNichol, 1985; see Partridge et al., 1992) had a characteristic 'bell-shaped' curve with a clear alpha peak, low noise and flat long wavelength tail above the wavelength at which the absorbance had fallen to less than 0.5% normalized maximum absorbance, and those of similar λ_{max} were averaged and reanalysed. For display, averaged spectra were overlain with an A_1 visual pigment template of the same λ_{max}, generated using the equations of Govardovskii et al. (2000).

To investigate how the shift in long wavelength-sensitive cone λ_{max} occurs, and specifically to determine whether chromophore and/or opsin exchanges take place during development and underlie the observed sensitivity changes, we examined the relationship between the running average λ_{max}, referred to as the over-the-top (OTT) λ_{max}, and the full width at half maximum (FWHM) bandwidth of the visual pigment curves at the 50% normalized absorbance point. OTT λ_{max} was used in preference to values derived from fitting visual pigment templates so that λ_{max} values were not biased by the goodness-of-fit of the template. Although absorbance spectra were all best-fitted by an A_1 rather than A_2 visual pigment template, some deviations (notably increased FWHM bandwidth) from the mathematical template were noted in measured spectra that did not appear to be due to classical MSP artefacts such as photoproduct build up, bypassing light or scatter at short wavelengths. In view of the possibility of visual pigment co-expression, selection of absorbance spectra that are good fits to mathematical template spectra might lead to a bias for the selection of spectra that represent only one opsin rather than a mixture. Using the OTT λ_{max} is a more objective way of classifying spectra, although the chance of including spectra that contain artefacts not due to spectral absorbance by one or more visual pigments is necessarily increased. MSP absorbance spectra from both aquarium-reared and wild-caught fish were included in this analysis. Computer models were constructed using the rhodopsin (A_1) and porphyropsin (A_2) visual pigment templates of Govardovskii et al. (2000) to calculate the bandwidth of mixtures of visual pigment, simulating an exchange of chromophore and/or a change in the underlying opsin. In all cases the ratio of bandwidth:OTT λ_{max} was calculated for the range of observed OTT λ_{max} values (approx. 520–575 nm), and plotted with the empirical data. Similar modelling methods were not attempted for the short wavelength-sensitive cones, as there were too few data.

Results

Visual pigment absorbance spectra meeting selection criteria (see above) were obtained from 210 individual cone photoreceptors in laboratory-reared black bream at stages of development between 5 and 172 dph and from adults. An additional 71 records were obtained from wild-caught fish. Photoreceptor dimensions were 2 μm diameter × 8 μm length for rods and between 2× 5–10 μm for cones, depending on the age of the fish. All individual scans were best-fitted by an A_1 rather than A_2 visual pigment template. To show the range of visual pigments recorded at different stages of development, the averaged visual pigment absorbance scans of short- and long-wavelength-sensitive cone records for larval and early juveniles prior to the formation of the square mosaic (5–40 dph) and juveniles with adult-like retinal structure (160–172 dph) are shown in Fig. 1A and Fig. 1B, respectively. Rods were detected from 25 dph onwards. The average absorption curve for all rod records gave a λ_{max} of 508.5 nm (Fig. 1C).

To show the changes in visual pigments with age, the mean λ_{max} for each cone class in each individual aquarium-reared fish is shown is shown in Fig. 2. The variance in the rod data is typical for MSP measurements of cells with the dimensions measured in this study (Shand, 1993) and no change in the λ_{max} of rod pigments was observed. In the cones, there is a change in the $\lambda_{max,}$ in both the short- and long-wavelength absorbing cones, from approximately 100 days, with a wide spread in the λ_{max} of the cone records, up to 160 days. Cones of the size we measured would not give the variance in λ_{max} values observed unless there were differences in the opsin mixtures between cells, indicating the possibility that a number of different pigments are expressed in both cone classes during this time.

Between 5 and 20 dph, when the fish are in their larval stage and all records were from single cones, two classes of visual pigment were recorded with λ_{max} at approximately 425 nm or 534 nm. Between 21 and 40 dph, as the fish undergo metamorphosis, double cones were first observed. At this time all records close to 425 nm were recorded from single cones; records close to 534 nm were from both double (62.5%) and single (37.5%) cones. From 41 dph onwards, all long wavelength records were from double cones. Between 108 and 154 dph, all single cones were found to have a λ_{max} displaced to between 450 nm and 482 nm and the double cones had a mean λ_{max} at 539 nm. Fish aged between 160–172 dph and adult were found to have single cones with a mean λ_{max} at 476 nm and long-wavelength-sensitive double cones with λ_{max} between 545 and 575 nm.

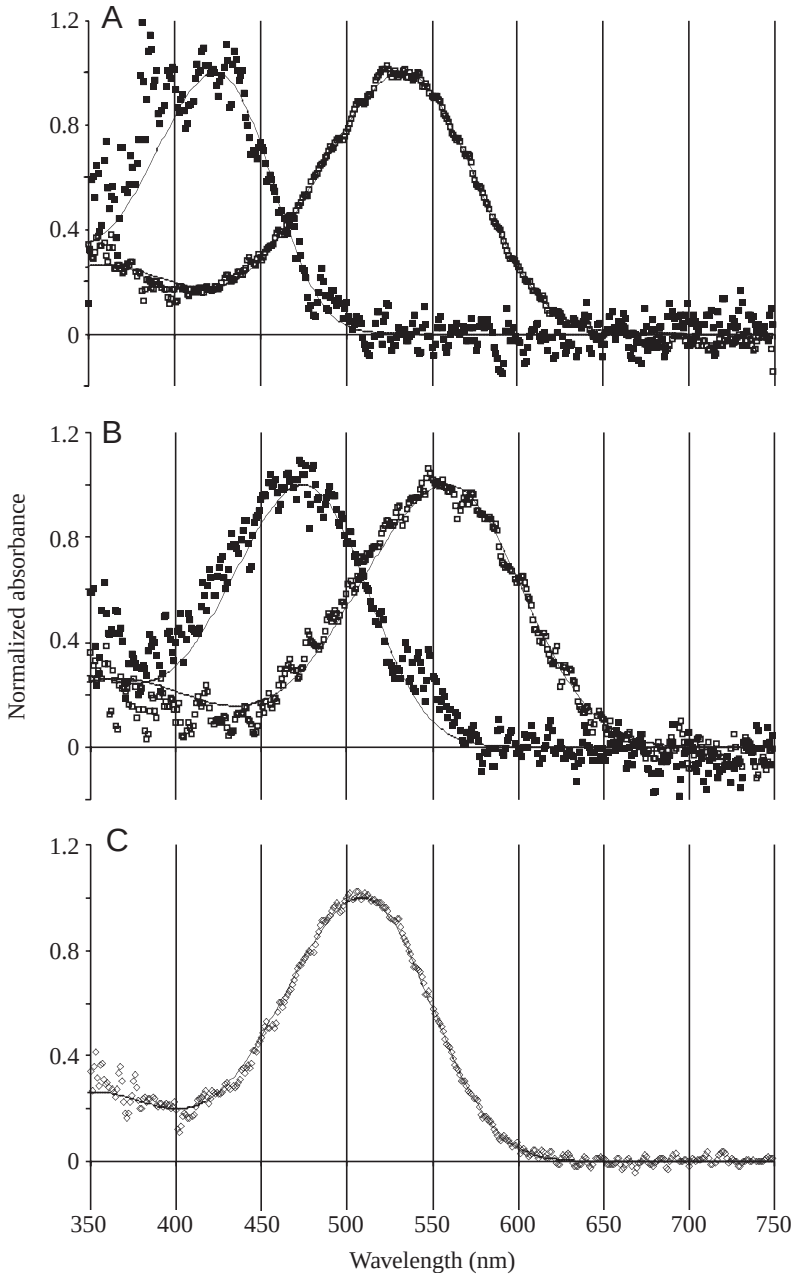

Fig. 1. Normalized average absorbance spectra of the range of visual pigments found in aquarium-reared black bream at two different developmental stages. (A) Cones, 5–40 dph. The short wavelength visual pigment has a λ_{max} at 425 nm (filled squares; N=5); maximum transverse absorbance (optical density, OD) =0.0148. The long wavelength pigment has a λ_{max} at 533 nm (open squares; N=65, OD=0.0178). (B) Cones, 160–172 dph. The short wavelength visual pigment now has a λ_{max} at 475 nm (filled squares; N=5, OD=0.0178) and the long wavelength pigment has a λ_{max} at 558 nm (open squares; N=13, OD=0.0188). (C) Rods, from 25–172 dph. The curve has a λ_{max} at 508 nm (N=34, OD=0.0267). All curves are fitted with an A_1 visual pigment template (solid line), calculated using the equations of Govardovskii et al. (2000).

From the time of their formation, long-wavelength-sensitive double cones were observed with the visual pigments in the two outer segments differing by up to 5 nm. The difference in the λ_{max} of the double cones was variable during the time of change in visual pigments but showed a significant increase as the fish grew (regression analysis; y=0.038x+2.7404, P=0.017, d.f.=33, r^2=0.166). The maximum difference in λ_{max} observed between visual pigments in the two outer segments of the double cones was 19 nm.

A frequency histogram showing the λ_{max} of all long wavelength-sensitive cone absorbance spectra from all fish, calculated using the running average λ_{max} (OTT), is shown in Fig. 3A. The range in observed OTT λ_{max} values runs from approximately 520 to 575 nm. These records were used to investigate the relationship between bandwidth and λ_{max} shown in Fig. 3B. Also plotted in Fig. 3B is the modelled relationship between bandwidth:OTT λ_{max} and the OTT λ_{max} for changes in chromophore and opsins. The data are expressed as bandwidth:OTT λ_{max} because this ratio is invariant with λ_{max} for any pure visual pigment. Thus all A_1 pigments have a ratio close to 0.20 (A_1-boundary on the graph), and all porphyropsins have a ratio close to 0.24 (A_2-boundary on the graph). The data fall within these boundaries, indicative of visual pigment mixtures. As shown in Fig. 3B, for λ_{max} values greater than approx. 525 nm the bandwidth of the empirical measurements is generally less than that calculated for mixtures of an A_1 pigment with a λ_{max} of 520 nm ($P520_1$) and an A_2 pigment with a λ_{max} of 575 nm ($P575_2$). Fig. 3B also shows the corresponding relationship between bandwidth and λ_{max} for mixtures of two A_1 pigments, $P520_1$ and $P575_1$, and three A_1 pigments expressed sequentially, in mixtures of $P520_1$ with $P550_1$, and $P550_1$ with $P575_1$. In order to compare how well these various simple models fit the data, the sums-of-squares differences between the data and the five models were calculated. Division by the degrees of freedom (229) provided estimates of the variances, the error between the models and the data. In order of goodness of fit these models, with variances, are: $P520_1$–$P550_1$–$P575_1$ (2.67×10^{-4}); A_1-boundary (3.79×10^{-4}); $P520_1$–$P575_1$ (1.01×10^{-3}); A_2-boundary (1.04×0^{-3}); $P520_1$–$P575_2$ (2.88×10^{-3}). The best-fitting model is therefore that involving the expression of three opsins, and an F_{max} test (Sokal and Rohlf, 1995) was conducted to compare the variance of this model with that of the next-best-fitting model [A_1-boundary; which implies an (implausible) multiplicity of rhodopsin pigments of varying λ_{max}]. This insensitive test shows that the three-opsin model provides a significantly better fit (F_{max}=1.417; d.f.=2, 228; P<0.01) than the A_1 boundary, and therefore than all other models.

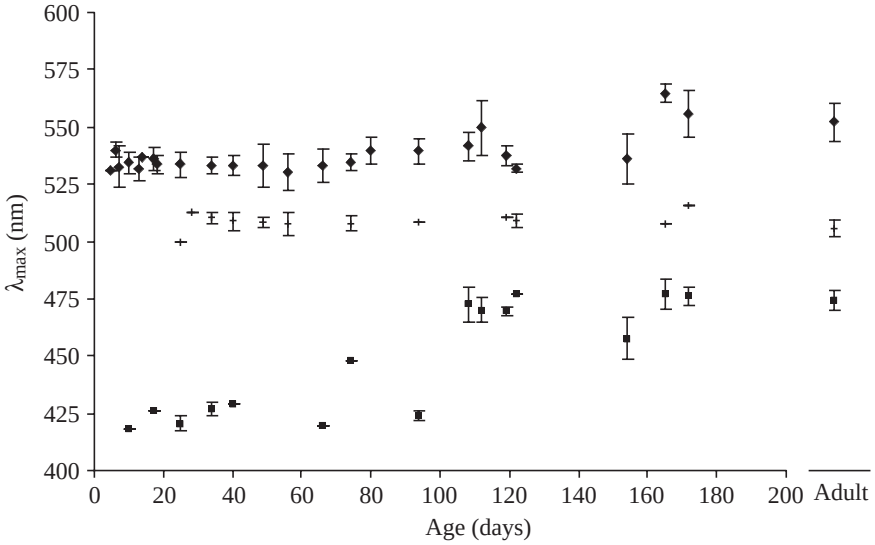

Fig. 2. Graph illustrating the change in visual pigment absorption characteristics during growth of aquarium-reared black bream. The mean λ_{max} (±S.D.) for individual fish for each photoreceptor class as a function of age is presented. Note the displacement to longer wavelengths from day 100 in both cone classes and the low standard deviation for the rod records. Squares, short wavelength-absorbing cones; horizontal bars, rods; diamonds, long wavelength-absorbing cones.

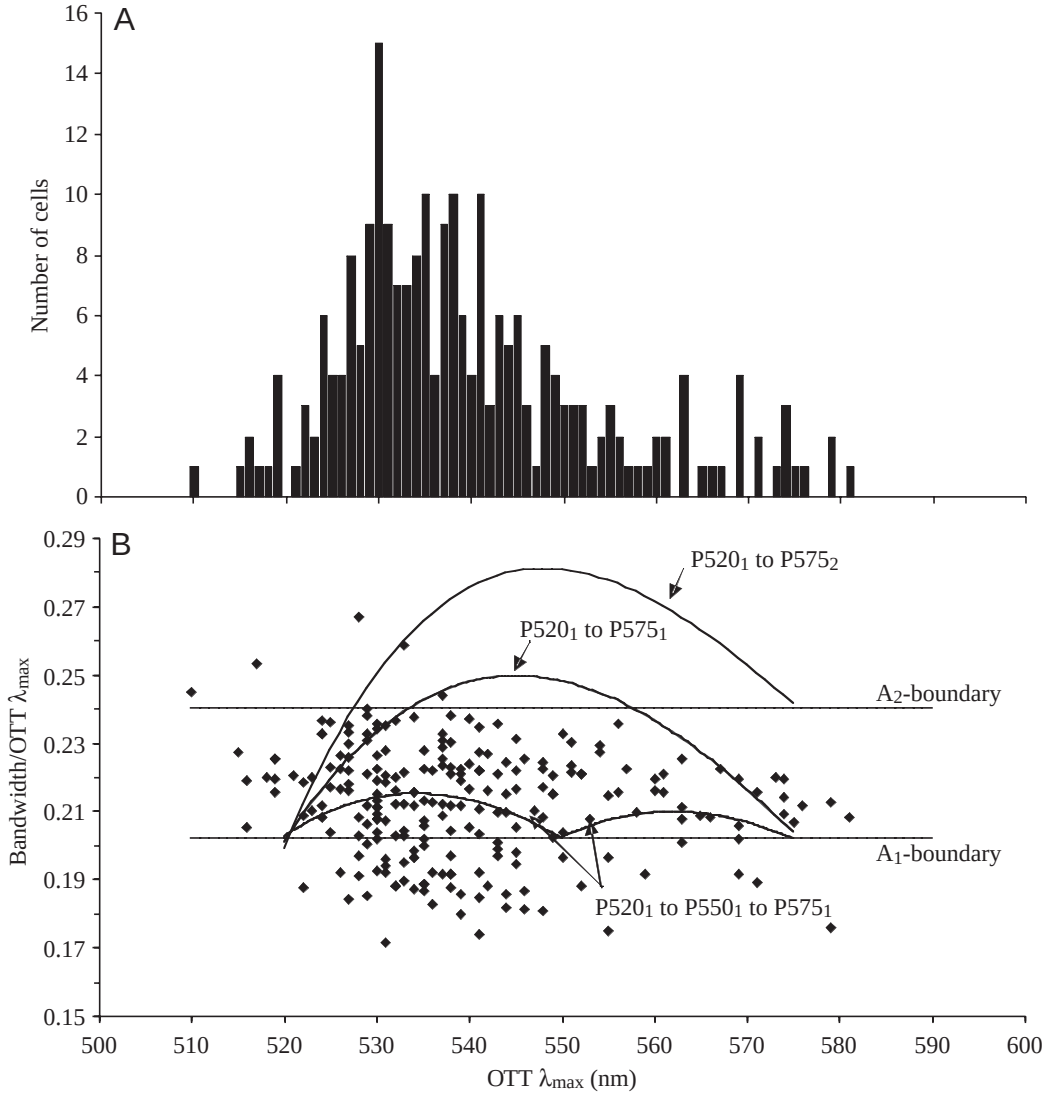

Fig. 3. (A) A frequency histogram for the λ_{max} of all long wavelength cone scans, obtained from both aquarium-reared and wild-caught fish, calculated using the running average ('over-the-top' OTT) λ_{max}, but only presented if bandwidth data was also available from the individual scan. These data were used to calculate the ratio of bandwidth:OTT λ_{max} shown in (B). (B) Plot of OTT λ_{max} *versus* bandwidth:OTT λ_{max}, calculated for long-wavelength-sensitive visual pigment records, from black bream of different stages of development (diamonds). The lines labelled 'A_1-boundary' and 'A_2-boundary' model the bandwidth ratios of pure rhodopsin and porphyropsin pigments, respectively. The lines labelled '$P520_1$ to $P575_1$' and '$P520_1$ to $P575_2$' show the modelled bandwidth ratios of visual pigment mixtures formed firstly by two rhodopsins, and secondly by a hypothetical rhodopsin and porphyropsin. The best-fitting, two-peaked, curve (labelled '$P520_1$ to $P550_1$ to $P575_1$') is provided by the model involving the mixture of these rhodopsins in sequentially expressed pairs

Discussion

Retinal structure and visual pigments

During the larval stage, the photoreceptors of the black bream retina consist entirely of single cones arranged in a hexagonal mosaic (Shand et al., 1999). At this time both violet- and green-sensitive visual pigments were found within the single cone population. Green-sensitive visual pigments, together with either blue- or UV-sensitive pigments, have recently been reported in the single cone population of larvae of a range of marine fishes (Britt et al., 2001). In addition, using *in situ* hybridisation, four different cone visual pigment opsins have been shown to be expressed in the single cone retina of larval halibut (Helvik et al., 2001). In the halibut the majority of cells expressed green-sensitive opsin, with the remaining 10% of cones expressing red-, blue- or UV-sensitive opsins. The UV-sensitive opsin-expressing cones were very few in number and restricted to ventral retina. The possibility of regional variation in visual pigments in the black bream retina was not addressed in this study, mainly due to the inability to maintain orientation of retinae in very young fish.

At the time of double cone formation in black bream the green-sensitive cones are those that become associated with one another to form double cones, the violet-sensitive cells remaining single cones. The ability to identify neighbouring cells with similar opsin expression is likely to take place by a cell signalling mechanism, possibly the same mechanisms involved in formation of the photoreceptor mosaic during retinal growth (Cameron and Easter, 1995; Raymond et al., 1995; Stenkamp et al., 1996).

Behavioural significance of visual pigment changes

During the larval and early juvenile stages, black bream are found in shallow estuarine waters feeding on plankton (Sarre, 1999). The presence of short wavelength-absorbing cones during the early stages may aid the detection of plankton by increasing the contrast of short wavelength-absorbing or reflecting zooplankton, as has been suggested for other shallow-water planktivorous teleosts with similar visual pigments (Bowmaker and Kunz, 1987; Browman et al., 1994; McFarland and Loew, 1994; Britt et al., 2001). In black bream, the shift in the absorption of both cone types to longer wavelengths begins when they move to deeper, lower light intensity, tannin-stained water, with reduced proportions of short wavelength light. Combined with the move to deeper water, black bream also begin to change their feeding strategy from planktivory to feeding from the substrate, a change in behaviour that is correlated with a shift in the main visual axis and relocation of the ganglion cell *area centralis* to more dorsal regions of the retina (Shand et al., 2000). Thus, a combination of changes in both feeding strategy and spectral qualities of the water need to be considered when rationalising the visual pigment changes.

The factors that initiate the changes in the visual pigments and control wavelength specificity are unknown. The variance in the λ_{max} of the long-wavelength-sensitive cones is unusual and will have consequences for wavelength discrimination and hence the behaviour of the fish. We have also noted a degree of variability in the timing of the changes between different individuals. In addition, animals obtained from the wild appear to initiate the changes at a smaller size, and possess longer-wavelength sensitivity following the changes, than fish reared in clear-water aquarium conditions under the artificial lights used in this study (J. Shand and N. Thomas, personal observations). Experiments to determine whether it is colour or intensity of the environmental light that initiates changes in the visual pigments are underway. Nevertheless, the ability to respond to environmental factors by regulation of wavelength specificity during development would be an appropriate adaptation for fish living in a variable light environment, such as the temperate estuaries in which black bream are found.

Mechanism of visual pigment changes

From our data and the modelling, we propose that the shifts in spectral sensitivity in black bream are due to changes in opsin gene expression, rather than to a switch in chromophore from retinal (A_1) to 3,4-didehydroretinal (A_2). The observed range of change cannot be due to a single opsin in association with A_1 shifting by chromophore exchange to A_2 *via* intermediates with mixed chromophores, because the λ_{max} range is too great. For instance, the A_2 analogue of a 520 nm λ_{max} A_1 would have a λ_{max} of approximately 545 nm (Parry and Bowmaker, 2000). Given the fact that neither the rod pigments, nor any cone data, are better-fitted by A_2 templates, it is likely that there is little or no A_2 chromophore in the retina of black bream. Although our modelling suggests that the shift can best be explained by the expression of at least three opsin genes, forming visual pigment mixtures in the double cone outer segments, this conclusion is necessarily tentative. Indeed, the exact mechanism underlying the λ_{max} shift must remain a matter of conjecture until molecular biological studies are completed.

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References

Ahlbert, I.-B. (1973). Ontogeny of double cones in the retina of perch fry (*Perca fluviatus*, Teleostei). *Acta Zool. (Stockh.)* **54**, 241-254.

Beaudet, L. and Hawryshyn, C. W. (1999). Ecological aspects of vertebrate visual ontogeny. In *Adaptive Mechanisms in the Ecology of Vision* (ed. S. N. Archer, M. B. A. Djamgoz, E. R. Loew, J. C. Partridge and S. Vallerga), pp. 413-437. Dordrecht: Kluwer Academic.

Blaxter, J. H. S. and Jones, M. (1967). The development of the retina and retinomotor responses in the herring. *J. Mar. Biol.* **47**, 677-697.

Blaxter, J. H. S. and Staines, M. (1970). Pure-cone retinae and retinomotor responses in larval teleosts. *J. Mar. Biol. Assn. UK* **50**, 449-460.

Bowmaker, J. K. (1995). The visual pigments of fish. *Prog. Ret. Eye Res.* **15**, 1-31.

Bowmaker, J. K. and Kunz, Y. W. (1987). Ultraviolet receptors, tetrachromatic colour vision and retinal mosaics in brown trout (*Salmo trutta*): age dependent changes. *Vision Res*. **27**, 21201-2108.

Britt, L. L., Loew, E. R. and McFarland, W. N. (2001). Visual pigments in the early life history stages of Pacific northwest marine fishes. *J. Exp. Biol*. **204**, 2581-2587.

Browman, H. I., Novales Flamarique, I. and Hawryshyn, C. W. (1994). Ultraviolet photoreception contributes to the foraging performance of two species of zooplanktivorous fishes. *J. Exp. Biol.* **186**, 187-198.

Cameron, D. A. and Easter, S. S. (1995). Cone photoreceptor regeneration in adult fish retina: phenotypic determination and mosaic pattern formation. *J. Neurosci.* **15**, 2255-2271.

Carlisle, D. B. and Denton, E. J. (1959). On the metamorphosis of the visual pigments of *Anguilla anguilla* L. *J. Mar. Biol. Assn. UK* **38**, 97-102.

Evans, B. I. and Fernald, R. D. (1993). Retinal transformation at metamorphosis in the winter flounder (*Pseudopleuronectes americanus*). *Visual Neurosci.* **10**, 1055-1064.

Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G. and Donner, K. (2000). In search of the visual pigment template. *Visual Neurosci.* **17**, 509-528.

Hart, N. S., Partridge, J. C., Bennett, A. T. D. and Cuthill, I. C. (2000). Visual pigments, cone oil droplets and ocular media in four species of estrildid finch. *J. Comp. Physiol. A* **186**, 681-694.

Helvick, J. V., Drivenes, O., Harboe, T. and Seo, H.-C. (2001). Topography of different photoreceptor cell types in the larval retinae of Atlantic halibut (*Hippoglossus hippoglossus*). *J. Exp. Biol.* **204**, 2553-2559.

Jenkins, G. I., Frankish, K. R. and Partridge, G. J. (1999). *Manual for the Hatchery Production of Black Bream (*Acanthopagrus butcheri*)*. Fremantle: Aquaculture Development Unit, Fremantle Maritime Centre, Australia.

Levine, J. S. and MacNichol, E. F. J. (1985). Microspectrophotometry of primate photoreceptors: art, artifact, and analysis. In *The Visual System* (ed. A. Fein and J. S. Levine), pp. 73-87. New York: Liss.

Loew, E. L. (1995). Determinants of visual pigment spectral location and photoreceptor cell spectral sensitivity. In *Neurobiology and Clinical Aspects of the Outer Retina* (ed. M. B. A Djamgoz, S. N. Archer and S. Vallerga), pp. 57-77. London: Chapman and Hall.

Lythgoe, J. N. (1984). Visual pigments and environmental light. *Vision Res.* **24**, 1539-1550.

Lythgoe, J. N. and Partridge, J. C. (1989). Visual pigments and the acquisition of visual information. *J. Exp. Biol.* **146**, 1-20.

Lythgoe, J. N. and Partridge, J. C. (1991). The modelling of optimal visual pigments of dichromatic teleosts in green coastal waters. *Vision Res*. **31**, 361-371.

McFarland, W. N. and Loew, E. R. (1994). Ultraviolet visual pigments in marine fishes of the family Pomacentridae. *Vision Res*. **34**, 1393-1396.

MacNichol, E. F. J. (1986). A unifying presentation of photopigment spectra. *Vision Res*. **26**, 1543-1556.

Pankhurst, P. M. and Eager, R. (1996). Changes in visual morphology through life history stages of the New Zealand snapper, *Pagrus auratus*. *NZ J. Mar. Fresh. Res.* **30**, 79-90.

Parry, J. W. L. and Bowmaker, J. K. (2000). Visual pigment reconstitution in intact goldfish retina using synthetic retinaldehyde isomers. *Vision Res.* **40**, 2241-2247.

Partridge, J. C., Speare, P., Shand, J., Muntz, W. R. A. and Williams, D. McB. (1992). Microspectrophotometric determinations of rod visual pigments in some adult and larval Australian amphibians. *Visual Neurosci.* **9**, 137-142.

Raymond, P. A., Barthel, L. K. and Curran, G. A. (1995). Developmental patterning of rod and cone photoreceptors in embryonic zebrafish. *J. Comp. Neurol.* **395**, 537-550.

Sarre, G. A. (1999). Age compositions, growth rates, reproductive biology and diets of black bream *Acanthopagrus butcheri* in four estuaries and a coastal lake in south-western Australia. PhD thesis, Murdoch University, Western Australia.

Shand, J. (1993). Changes in the spectral absorption of cone visual pigments during settlement of the goatfish *Upeneus tragula:* the loss of red sensitivity as a benthic existence begins. *J. Comp. Physiol. A* **173**, 115-121.

Shand, J., Archer, M. A. and Collin, S. P. (1999). Ontogenetic changes in the retinal photoreceptor mosaic in a fish, the black bream, *Acanthopagrus butcheri*. *J. Comp. Neurol.* **412**, 203-217.

Shand, J., Chin, S. M., Harman. A. M., Moore, S. and Collin, S. P. (2000). Variability in the location of the retinal ganglion cell area centralis is correlated with ontogenetic changes in feeding behaviour in the black bream, *Acanthopagrus butcheri* (Sparidae, Teleostei). *Brain Behav. Evol.* **55**, 176-190.

Shand. J., Partridge, J. C., Archer, S. N., Potts, G. W. and Lythgoe, J. N. (1988). Spectral absorbance changes in the violet/blue sensitive cones of the juvenile pollack, *Pollachius pollachius*. *J. Comp. Physiol. A* **163**, 699-703.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*. New York: Freeman.

Stenkamp, D. L., Hisatomi, O., Barthel, L. K., Tokunaga, F. and Raymond, P. A. (1996). Temporal expression of rod and cone opsins in embryonic goldfish retina predicts the spatial organisation of the cone mosaic. *Invest. Ophthalmol. Vis. Sci.* **37**, 363-376.

Wood, P., Partridge, J. C. and De Grip, W. J. (1992). Rod visual pigment changes in the elver of the eel *Anguilla anguilla* L. measured by microspectrophotometry. *J. Fish Biol.* **41**, 601-611.