Ontogenetic shift in spectral sensitivity of Pacific snapper *Chrysophrys auratus*

Esme Robinson[1,2*], Alistair Jerrett[2], Suzanne Black[2] & William Davison[1]

[1] School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
[2] The New Zealand Institute for Plant & Food Research Limited, Seafood Production, PO Box 5114, Port Nelson, Nelson, New Zealand
[*] Corresponding author: esme.robinson@canterbury.ac.nz

(Received November 2016, revised and accepted May 2017)

Abstract

Electroretinogram (ERG) experiments on the sparid fish *Chrysophrys auratus* (Forster 1801) demonstrated an ontogenetic shift in spectral sensitivity, from long wavelengths (yellow-orange) in small (60 g) fish, to shorter wavelengths (blue) in larger (800 g) fish. Changes in spectral sensitivity are consistent with the life history of the species, since juveniles migrate from predominantly long wavelength estuarine nursery habitats to more open coastal waters, in which shorter wavelengths dominate. These results strengthen conclusions from earlier studies on *C. auratus*, in which spectral sensitivity of the optomotor response was tested, and confirms the value of combining physiological and behavioural measures of visual function in fish.

Keywords: electroretinogram, fish, light, vision, wavelength.

 © New Zealand Natural Sciences

Introduction

Chrysophrys auratus (Perciformes, Sparidae) is a temperate marine fish that sustains important recreational and commercial fisheries in New Zealand and other areas of the Pacific, including Australia and Japan. Previous work has investigated spectral sensitivity in different sized juveniles using the optomotor response (Robinson et al., 2011). It was shown that visual acuity in small fish (about 100 g) is optimal in long wavelengths (red) and impaired in short wavelengths (blue). In contrast, in larger (about 800 g) fish, acuity in short wavelengths (blue) is better than in long wavelengths (red). It was hypothesised that variation in visual sensitivity is related to differences in habitat use by this species. Small, juvenile *C. auratus* inhabit estuarine environments (Parsons et al., 2016), where light is predominantly of longer wavelengths, but fish later move to coastal habitats where short wavelength light prevails (Hartill et al., 2003).

Although the optomotor technique provides a strong indication of visual sensitivities, it is beneficial to compare findings from this behavioural response with results from investigations of a physiological response. This can be achieved using the electroretinogram (ERG) technique. The ERG technique is a commonly used method for measuring changes in electrical activity in the eye, caused by functioning of the retinal cells, in response to light (Makhanov et al., 2004). Therefore, the primary aim of the current research was to determine whether spectral sensitivity results obtained using the optomotor response (Robinson et al., 2011) could be verified by the ERG technique. In a related species, the red sea bream (*Pagrus major*), results from the optomotor technique and the ERG technique did not show similar tendencies (Hasegawa, 2005).

Methods

C. auratus were reared at the Plant & Food Research aquarium facilities (Nelson, New Zealand), and transported to the University of Canterbury (Christchurch, New Zealand). At the University of Canterbury, fish were held in 1000 l recirculating tanks for at least 3 weeks prior to experimental use (16°C, 11:11: h light cycle with 1 h sunrise and 1 h sunset between light and dark periods). Fish were fed to satiation on cubed fish pieces (mostly gurnard *Chelidonichthys kumu*, and hoki *Macruronus novaezelandiae*).

Electroretinograms (ERGs) were recorded from two groups of fish – small (mean ± S.E. mass, M, 61.6 ± 9.0 g; mean ± S.E. total length, LT, 148 ± 9 mm; n = 6); and large (M 831.6 ± 25.7 g; LT 293 ± 6 mm; n = 6). Animals were anaesthetised using tricaine methanesulphonate (MS222). Dosage of MS222 was specific to the size group (70 mg l-1 for small fish; 75 mg l-1 for large fish). After animals reached Stage 4 anaesthesia (no response to caudal peduncle squeeze, approximately 10 – 15 min), they were placed onto a surgical sling and fitted with an oral ventilation tube that supplied a maintenance dose of MS222 (60 mg l-1 small fish; 70 mg l-1 large fish). Anaesthetic was pumped from an aerated reservoir in which temperature was maintained at 16 ± 2°C. Animals were fitted with two silver wire electrodes (0.3 mm diameter). A recording electrode was positioned in the vitreous humour of the eye, and a sub-cutaneous reference electrode was positioned dorsal to the eye. ERGs were recorded from the right eye in all fish. One earth wire was placed on the body of the animal, and a second

was placed in the anaesthetic reservoir. All experiments were commenced at least 3 h after the time of 'lights on' in the home aquarium, and ceased at least 3 h before 'lights off' in order to minimise potential circadian influences on ERG responses. The experimental set up was housed in a light-tight, grounded Faraday cage.

ERG responses were stimulated by flashing a 500 ms pulse of wavelength-specific light onto the pupil of the eye every 30 sec. Light was generated by a xenon fibre-optic light source (Spectral Products ASB-XE-175) and split into appropriate wavelengths with a monochromator (Spectral Products CM110 1/8 Monochromator), and pulsed using an optical shutter (Uniblitz DSS10). The light source was positioned about 5 cm in front of the eye, in such a way that light projected onto the entire pupil of the eye. Irradiance affects the magnitude of the ERG response (Bilotta et al., 2005), so lights were adjusted to ensure irradiance was constant irrespective of wavelength. Irradiance was measured using an Ocean Optics USB2000+ Spectrometer and optical probe connected to a laptop running OceanView software (Ocean Optics, version 1.5.2).

ERG signals were amplified, filtered (low pass 1 kHz; high pass 0.3 Hz) and collected (sample rate 200 s-1) using a Power Lab data acquisition system (AD Instruments) and stored using Chart software (AD Instruments, LabChart 7).

A typical ERG trace consists of an initial negative 'a' wave, followed by a positive 'b' wave and then a later 'd' wave (Fig. 1). The b wave amplitude (the change in amplitude between the trough of the a wave and the peak of the b wave, McComb et al., 2010) of three ERG responses at each wavelength was averaged for each fish. ERG results for each individual fish were normalised and then pooled within a size group to generate a mean spectral sensitivity curve for each size group.

Results

The magnitude of the ERG response varied with wavelength, and the wavelength that produced the greatest magnitude response varied with fish size (Fig. 2). In small fish, (M 61.6 g), a sensitivity peak was apparent in the yellow-orange (around 570 nm) region of the spectrum. Responses in the blue-green region were reduced (around 420nm). In contrast, large fish, (M 831.6 g), demonstrated peak sensitivities in blue-green wavelengths (470nm). However, responses in the yellow, orange and red region of the spectrum were reduced.

Figure 1. A typical *C. auratus* electroretinogram (ERG) waveform. This example was generated in a small fish in response to yellow (590 nm) light. An initial negative 'a' wave is followed by a positive 'b' wave, and a later 'd' wave.

Discussion

The electroretinogram results obtained in this study confirm the findings of previous behavioural investigations using the optomotor response (Robinson et al., 2011). Small *C. auratus*, which inhabit estuarine environments (Hartill et al., 2003), demonstrated sensitivity peaks in the longer wavelength region of the spectrum. In contrast, large fish, which inhabit coastal environments, demonstrated sensitivity peaks at shorter wavelengths. These peak sensitivities are suited to the habitats occupied by these animals, as light in estuarine waters tends to be chiefly comprised of red-yellow wavelengths, whereas coastal waters are predominantly blue-green (Jerlov, 1968). The age and size at which visual sensitivity changes is not entirely certain. Unpublished results from estuary surveys conducted by New Zealand's National Institute of Water and Atmospheric Research (NIWA) suggest that *C. auratus* aged around 3 - 4 years, and measuring approximately 25 cm in length (around 360g) begin to leave the estuarine environment. It is currently unknown precisely when visual changes occur, or whether further changes to the visual systems of these fish occur later in development. During spawning, adult fish may return to the estuary for a brief period (Hartill et al., 2003), but it is unknown whether there is a concomitant change in the visual sensitivity of these fish. Such questions may prove an interesting avenue for future research. Seasonal variation in spectral sensitivity has been reported for other species, for example adult Nile tilapia (*Oreochromis niloticus*) (Lisney et al., 2010).

Combining physiological and behavioural approaches to investigating fish visual function is beneficial, providing a greater depth of information than the use of individual techniques in isolation. In the current study, results from the ERG and the optomotor response were in agreement. However, in an earlier study on red sea bream (*P. major*), sensitivity curves obtained using the optomotor response differed from those obtained from ERG experiments. The author of that study attributed the difference to possible variations in the optical adaptation state of the tested fish, which was not the case in the current study as fish were similarly adapted to ambient light conditions.

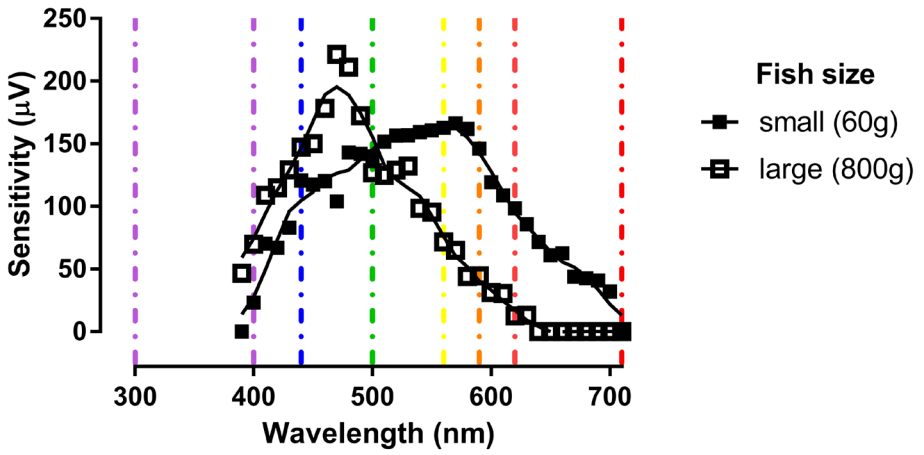

Figure 2. Mean relative sensitivity of *C. auratus* to a range of light wavelengths, determined using normalised ERG data.

In conclusion, results from this study demonstrate that snapper undergo an ontogenetic shift in spectral sensitivity that is probably related to change in habitat use. Results also support the importance of combining physiological and behavioural techniques when investigating fish vision.

Acknowledgements

The authors thank S. Ford and the staff of Plant & Food Research Nelson for *C. auratus* culture and transport, and Alasdair Noble for statistical analysis. The authors also thank G. Robinson, A. Woods, N. Etheridge and D. Leonard for technical expertise and construction of the equipment. The constructive revision by two anonymous reviewers was greatly appreciated. The experimental procedures were approved by the University of Canterbury Animal Ethics Committee.

References

Bilotta, J., Trace, S.E., Vukmanic, E.V. & Risner, M.L. 2005. Ultraviolet- and short-wavelength cone contributions alter the early components of the ERG of young zebrafish. *International Journal of Developmental Neuroscience*: 23, 15-25.

Hartill, B. W., Morrison, M. A., Smith, M. D., Boubée, J. & Parsons, D. M. 2003. Diurnal and tidal movements of snapper (*Pagrus auratus*, Sparidae) in an estuarine environment. *New Zealand Journal of Marine and Freshwater Research*: 54, 931-940.

Hasegawa, E. 2005. Comparison of the spectral sensitivity of juvenile red sea bream investigated by the physiological technique and by the behavioural technique. *Fisheries Science*: 71, 79-85.

Jerlov, N. G. 1968. *Optical Oceanography*. New York, NY: Elsevier.

Lisney, T. S., Studd, E., & Hawshryn, C. W. 2010. Electrophysiological assessment of spectral sensitivity in adult Nile tilapia *Oreochromis niloticus*: evidence for violet sensitivity. *Journal of Experimental Biology*: 213, 1453-1463.

Makhanov, Y. V., Rinner, O. & Neuhauss, S. C. F. 2004. An inexpensive device for non-invasive electroretinography in small aquatic vertebrates. *Journal of Neuroscience Methods*: 135, 205-210.

McComb, D. M., Frank, T. M., Hueler, R. E. & Kajiura, S. M. 2010. Temporal resolution and spectral sensitivity of the visual system of three coastal shark species from different light environments. *Physiological and Biochemical Zoology*: 83, 299-307.

Parsons, D., Buckthought, D., Middleton, C. & Mackay G 2016. Relative abundance of snapper (*Chrysophrys auratus*) across habitats within an estuarine system. *New Zealand Journal of Marine and Freshwater Research*: 50, 358-370.

Robinson, E., Jerrett, A. R., Black, S. E. & Davison, W. 2011. Visual acuity of snapper (*Pagrus auratus*): effect of age and spectral composition. *Journal of Fish Biology*: 79, 1883-1894.

Tovée, M. J. 1995. Ultra-violet photoreceptors in the animal kingdom: their distribution and function. *Trends in Ecology and Evolution*: 10, 455-460.

White, E. M., Goncalves, D. M., Partridge, J. C. & Oliveira, R. F. 2004. Vision and visual variation in the peacock blenny. *Journal of Fish Biology*: 65, 227–250.