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Spectral sensitivity of the two-spotted goby *Gobiusculus flavescens* (Fabricius): a physiological and behavioural study

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Summary

Microspectrophotometry of *Gobiusculus flavescens* photoreceptors revealed a single rod visual pigment (λ_{max} at 508 nm) and the three cone pigments (λ_{max} 456, 531 and 553 nm). The cone population was dominated by identical double cones containing the middle-wave-sensitive (MWS) pigment, but with a small number of non-identical MWS/LWS (long-wave-sensitive) and identical LWS double cones. Small populations of large single cones also contained either the MWS or LWS pigment. The short-wave-sensitive (SWS) pigment was found in small single cones. Lens transmission was great reduced below 410 nm.

The spectral sensitivity of the behaviourally determined reaction distance (RD) to prey at a high irradiance level (0.5 μmol m^{-2} s^{-1}) correlated with the maximum sensitivity of the MWS cones, both peaking around 530 nm. However, at a lower irradiance level (0.015 μmol m^{-2} s^{-1}) such a correlation was not so apparent. The RD was greatly reduced, though still maintaining a peak around 530–550 nm, but with a relatively smaller reduction in RD at shorter wavelengths. Optomotor behaviour displayed a somewhat similar spectral sensitivity to the RD responses at the higher light intensity. However, the peak was at slightly longer wavelengths at 550 nm, suggesting a greater input from LWS cones to the optomotor response.

Key words: visual pigment, reaction distance, optomotor response, *Gobiusculus flavescens*.

Introduction

The visual performance of fish can be studied by physiological, histological or behavioural studies. Absorbance spectra of the visual pigments of cones and rods can be measured directly by microspectrophotometry (Bowmaker, 1995) or inferred from electrophysiology (Parkyn and Hawryshyn, 2000; Whitmore and Bowmaker, 1989) and can give indications of spectral sensitivity and the potential for colour vision (Barry and Hawryshyn, 1999; Cameron, 2002). Histological studies can reveal the distribution of the various photoreceptor classes (Reckel et al., 2002; Van der Meer et al., 1995), complementing the physiological measurements of spectral sensitivity and providing estimations of visual acuity (Fritsches et al., 2003; Shand, 1997). However, none of these methods can define visual performance, which can only be determined by behavioural experiments (Douglas and Hawryshyn, 1990; White et al., 2004) involving either innate behaviours such as the optomotor response (Krauss and Neumeyer, 2003; Schaerer and Neumeyer, 1996) or behavioural activities such as feeding strategies (Job and Shand, 2001; Utne-Palm, 2002). Correlations of these methods of investigation can give insights into the retinal mechanisms underlying visual performance, but can produce divergent results (Neave, 1984; Browman et al., 1990; Miller et al., 1993; Pankhurst et al., 1993; Van der Meer, 1994; Van der Meer, 1995). In the present study of the two-spotted goby, *Gobiusculus flavescens* (Fabricius), we have used microspectrophotometry (MSP) to determine the complement of its visual pigments. In addition, behavioural studies were carried out to investigate the visual ability of the goby, by studying its reaction distance (RD) to prey and its optomotor response, under light conditions that both matched and mismatched the peak sensitivities of its visual pigments. The contributions of the different photoreceptor types to prey or motion detection were studied by comparing the action spectra of the RD and optomotor response studies with the spectral sensitivities of the visual pigments.

Increasing illumination causes a significant increase in RD in *G. falvescens*, with an asymptotic log-linear increase in RD with increasing illumination (Utne, 1997). Furthermore a change in wavelength composition seems to have the same significant effect on RD as a change in illumination level (Utne-Palm, 1999). *G. flavescens* was found to have a significantly longer RD at 450–550 nm compared to 630–730 nm or white light from a halogen source (Utne-Palm, 1999), indicating a higher sensitivity in the blue-green. Our predictions are that gobies will be more sensitive to light that

matches the peak absorbance of its visual pigments than to light that falls between these maximum sensitivities. In other words, RD will be longer and a small change in illumination level at the peak absorbance will have a much greater effect on RD than the same change in illumination at a wavelength away from the pigment maxima. Accordingly, we predict that the optomotor response will persist at lower illumination levels within wavelengths close to pigment maxima compared to wavelengths away from the maxima. Furthermore, we predict that the relative numbers of the different cone classes will have an effect, so that the dominant spectral class of cone will have a positive effect on RD and optomotor response.

Materials and methods

Animals

Gobiusculus flavescens (Fabricius) were collected in May, on a rocky substrate covered with *Fucus* spp., by the use of a beach seine in Raunefjorden close to Bergen (western Norway). Fish of similar size (40–45 mm) and age (1 year) and of both sexes were used, since visual ability is known to be size dependent (Blaxter and Staines, 1970; Hairston, Jr et al., 1982; Breck and Gitter, 1983; Walton et al., 1992) and can also be sex dependent (Douglas and Hawryshyn, 1990).

Microspectrophotometry

Retinas from ten gobies, five of each sex, were used in the MSP study. Fish were dark adapted overnight, then sacrificed by cervical transection. All procedures were carried out under dim red light. Eyes were enucleated, hemisected and the anterior portion discarded. The retina was then separated from the pigment epithelium and one or two small samples were prepared immediately for measurement. The remaining tissue and the eyecup from the other eye were lightly fixed in 2% glutaraldehyde solution for about 15–30 s, washed in saline and then stored at 4°C in saline. Retinal samples from this fixed tissue were used on subsequent days for up to 2 weeks after preparation. Retinal samples were teased apart on a coverslip with razor blades and the dispersed tissue mounted in saline containing 5% or 10% dextran, then squashed with a second coverslip, which was sealed with wax. Since MSP can only sample photoreceptors randomly, at least two pieces of retina were analysed from different regions of each eye, in an attempt to overcome any regional distribution of different classes of cone.

Microspectrophotometric recordings were made in the conventional manner using a Liebman dual-beam microspectrophotometer (Bowmaker et al., 1991; Liebman and Entine, 1964; Mollon et al., 1984). Spectra were recorded at 2-nm intervals from 750 to 350 nm and from 351 to 749 nm on the return scan. The outward and return scans were averaged. A baseline spectrum was measured for each cell, with both beams in an unoccupied area close to the cell, and this was subtracted from the intracellular scan to derive the final spectrum. Two baseline scans were recorded for each cell and averaged. All cells were fully bleached with white light and post-bleach spectra recorded. The maximum absorption wavelength (λ_{max}) of both the absorbance spectra and difference spectra were determined by a standard computer programme that best fits a visual pigment template to the right hand limb of the spectra (Bowmaker et al., 1991; Mollon et al., 1984). Selection criteria were used to discard records that either had low absorbance or were clearly distorted. In all cases, the spectra were best fitted with a pure rhodopsin, vitamin A_1-based template (Govardovskii et al., 2000). Three estimates of the λ_{max} were made from the selected records for each class of pigment; the λ_{max} of the mean absorbance spectrum, the λ_{max} of the mean difference spectrum and the mean of the λ_{max} of the individual cells. The λ_{max} of the mean absorbance spectrum is taken as the most reliable estimate of the peak sensitivity (Mollon et al., 1984).

Lens transmission

The transmission spectra of the lenses from a single fish were recorded, courtesy of R. H. Douglas (City University, London, UK). The lenses, about 1 mm in diameter, were mounted in a lens holder and spectra measured using a Shimadzu-UV240 spectrophotometer fitted with an integrating sphere, as detailed elsewhere (Douglas and McGuigan, 1989; Thorpe et al., 1993).

Studies of behavioural visual capacity

About 200 wild-caught adult gobies were acclimatised in two 80 l aquaria for at least 7 days, before being moved to the experimental aquarium. The temperature was maintained at 8–10°C. Artificial white light was used (Osram Lumilux de lux daylight LF 12-950, wavelength 400 to 750 nm), which simulated (with exception of the UV part of the spectrum) the outside light conditions of the actual time of the year, with full 'daylight' (32 μmol m^{-2} s^{-1}) lasting for 10 h (autumn) to 8 h (winter), and a daily dusk and dawn period of 45 min. All experiments began after 3 h of full light and lasted for up to 3 h. Live copepods were offered daily.

Two different experimental paradigms were used to resolve the behavioural visual capacity of the goby: reaction distance (RD) to live prey, and the optomotor response. Both studies were preformed under different wavelengths conditions and illumination levels.

The light source used in the behavioural experiments was a 1000 W quartz tungsten halogen lamp, with narrow band filters (half-bandwidth 10 nm) peaking at 460, 510, 532, 550 and 560 nm, and a 660-nm cut-on filter. The peaks of four of the narrow band filters (460, 510, 532 and 550 nm) matched the λ_{max} of the cones and rods of the goby, as determined by MSP. The 560-nm narrow band filter, slightly offset from the long-wave-sensitive (LWS) cone peak sensitivity (553 nm), and the 660-nm cut on filter, at a significantly longer wavelength, were chosen to determine how the sensitivity of the goby changed at wavelengths longer than the cone peak sensitivities.

To ensure that the observed differences in RD were due to wavelength sensitivity and not brightness, all the RD studies

were performed at two controlled irradiance levels (0.5 and 0.015 μmol m^{-2} s^{-1}). The irradiance level in the experimental arena was controlled by adjusting the energy/W of the lamp and/or by adding layers of plankton cloth (a loosely woven white cloth made of polypropylene) between the light source and the interference filter. A spectroradiometer (LI-COR, LI-1800UW; Lincoln, New England, USA) was used to establish that no change in wavelength composition was caused by these dimming methods. The irradiance was measured over the range of 380–760 nm, using a Biospherical Instruments QSP-170B with a QSR 240 sensor (Lincoln, New England, USA).

G. flavescens uses a saltatory search (SS) or 'pause-travel' search (Utne, 1997). The SS strategy is a punctuated repositioning movement, in which the predator only scans for prey during a brief stationary period (search pause) (Browman and O'Brien, 1992). If the goby locates prey during the search pause, the pause is followed by an attack. If prey is not located, the goby moves a little and stops again for a new scanning pause. The distance between the last search pause, where the reaction occurred, and the location of the attacked prey is defined as the reaction distance (RD) (Vinyard and O'Brien, 1976; Gregory and Northcote, 1993). The reaction is characterised by a rapid tail beat ('fast-start') (Webb, 1978; see also Gordon, 1983), which gives the fish an acceleration towards the prey.

Reaction distance

Experimental design

The experimental apparatus consisted of a 200×30×30 cm glass aquarium that was divided into three compartments; two conditioning compartments (50×30×30 cm) at each end of the aquarium, and a central experimental compartment (100×30×30 cm). Sliding doors connected the compartments (for details, see Utne, 1997). Water was turned off during the observation period, and water depth was maintained at 5 cm. The experimental compartment had a 2×2 cm grid on the bottom. A remotely operated video camera placed over the aquarium was used to record observations. Live prey were introduced to the experimental arena in two glass cylinders (2 cm in diameter and 10 cm tall). By placing the prey in a glass cylinder, senses other than vision were eliminated. The cylinders were placed randomly in the experimental compartment to minimise the influence of spatial memory (Benhamou, 1994; Noda et al., 1994).

In order to differentiate between the sensitivity of gobies to changes in overall light intensity and changes at specific wavelengths, RD studies were performed at two irradiance levels of 0.5 and 0.015 μmol m^{-2} s^{-1} at the five different spectral locations given above. Both of the chosen light intensities are below the white light saturation level of *G. flavescens* (8 μmol m^{-2} s^{-1}) (Utne, 1997). *Calanus* spp. (length 3.0–4.0 mm) was used as prey, with transparent individuals being selected in order to reduce the influence of contrast variability due to wavelength. For further details of the methods, see Utne (Utne, 1997) and Utne-Palm (Utne-Palm, 1999).

Conditioning and observation

Ten gobies at a time (from a total of 20) were introduced into the experimental aquarium for acclimatisation. The gobies were trained daily (1–2 h), over a 2-week period, to enter the experimental compartment and search for prey. During the training, copepods were introduced to the compartment, which taught the gobies to search the arena for food. Before each trial, gobies were starved for 24 h, since hunger is known to influence (increase) RD (Confer et al., 1978).

At the beginning of the experiment, the 10 gobies were kept in one of the two conditioning compartments, from which two fish at the time were introduced to the experimental compartment by opening one of the slide-doors (when needed, a net was used to hold back the rest of the fish from rushing in). Two fish were used, since the fish appeared stressed when isolated. During 10 randomly chosen observation periods an empty glass cylinder was placed in the experimental arena. No attacks or reactions to these empty tubes were ever observed. The gobies were conditioned to the experimental light conditions for 1.5 to 2 h before observation. Light conditions were randomised, with only one wavelength condition being tested on a single day. Between 15 and 20 fish were tested at each wavelength condition. Using only the longest observed RD of each tested fish, this yielded between 15 and 20 RD observations for each tested wavelength condition.

Optomotor response

Experimental design

The optomotor response was measured in an apparatus similar to the one used by Krauss and Neumeyer (Krauss and Neumeyer, 2003), consisting of a stationary glass cylinder/experimental arena (9.15 cm diameter, 15 cm high) in which the tested fish could swim freely. This glass cylinder was concentrically surrounded by a cylinder (diameter 14.4 cm) consisting of 1 mm wide stripes made of white cardboard and equally wide slits. The striped cylinder was placed on a clear Plexiglas disk that could be rotated by a motor (Multifix, Constant, Germany) at a velocity ranging from four rotations per min to one rotation per 2 min, in both directions. The light source (described earlier) illuminated the test tank and the white stripes of the rotating cylinder from above. The behaviour of the fish was monitored from below by a video camera (Panasonic WV bp550) with infrared filter (Optolite 50% IR) connected to a monitor (Panasonic, WV-5340). The experimental setup was surrounded by black cloth to eliminate stray light and to provide high contrast between the slits and white cardboard stripes. At the lowest illumination levels, infra red (Derwent, MF100, 950 nm; Birmingham, UK) was added to enhance camera visibility.

Conditioning and observation

The experimental fish was placed in the test tank to acclimatise to the cylinder and the wavelength, 15 min prior to starting the motor rotating the pattern. The recording of the optomotor behaviour started 30 s later, to avoid recording an

initial startle reaction shown by many fish. During a threshold determination at each particular wavelength, the irradiance level (measured in the glass cylinder/experimental arena) was reduced in steps of <0.05 μmol m^{-2} s^{-1}. At each illumination level the fish was given ten trials in which the direction of the rotating stripes varied randomly during the test sequence. An illumination level was considered above threshold if the fish followed the stripes in eight of the ten trials. Twenty fish were used to test the six wavelengths, with five different fish tested at each wavelength. Only five fish were used to test more than one wavelength, since most of the fish became inactive after being tested for a while. When one fish was used for more than one wavelength, the wavelengths were tested in random order.

The gobies did not follow the stripes by swimming at a constant pace, but instead jumped along with the stripes (in small steps). Their inability to follow a visual stimulus at a constant pace is probably the result of their saltatory search (SS) behaviour in which they only scan for prey or other visual stimuli during brief stationary periods (search pause). Furthermore, some fish jumped along only for a short distance and then doubled back, started to follow another stripe, and so on, repeating the cycle. This behaviour is similar to the optomotor behaviour of the goldfish described (Cronly-Dillon and Muntz, 1965). Initially this last behaviour caused some difficulty, but it became apparent that the movements the goby makes when it starts to follow a stripe are different (short jumps with the eyes close to the glass wall) from those that it makes when it starts to double back (longer jumps and eyes not in close contact with the glass wall). Sometimes the goby placed itself in the centre of the test tank, and simply rotated around its own axis following the stripes, or occasionally some fish were inactive, lying on the bottom of the test tank and following the stripes with their eyes. Data from fish showing these types of behaviour were not used.

Data analysis

G. flavescens uses a SS strategy, searching for prey only during stationary pauses. Some of the measured RDs will therefore be underestimates because the prey items might be relatively close to the predator when the predator stops for a pause. Maximum RD has therefore been used to describe the visual ability of this (Utne, 1997) and other SS foraging planktivorous fish (O'Brien and Evans, 1991). Furthermore, the two-spotted goby is known to decrease its activity and feeding rate with decreasing light, and in the dark they are inactive, 'sleeping' on the kelp or rocks (Gordon, 1983; Costello, 1992). Thus, in the present study their feeding motivation should be quite low, since very low illumination levels were used. At the lowest irradiance level (0.015 μmol m^{-2} s^{-1}) there were a large number of very short RDs, independent of wavelength, which was not the case in the higher irradiance treatment (0.5 μmol m^{-2} s^{-1}). Therefore, to make sure that only the most motivated fish was used in the analysis, we chose to use only the five longest RDs measured (from five different fish) at each wavelength and illumination level.

Two-way analysis of variance (ANOVA) was used to resolve any significant relations between RD, wavelength and illumination conditions. An ANOVA, *post hoc* test (Neuman–Keuls) was used to test for differences in RD between wavelengths within each illumination level. In addition, an ANOVA, *post hoc* test (Neuman–Keuls) was used to test for differences in RD between illumination levels within each wavelength. RD raw data were log transformed to achieve a normal distribution.

The optomotor response results were also treated with an ANOVA, *post hoc* test, to test for differences in illumination threshold between the different wavelengths.

Results

Visual pigments

G. flavescens has a photoreceptor complement similar to many teleosts, with rods, double cones and single cones. In all cases, spectra were best fitted with a pure retinal$_1$ (rhodopsin) template with no indication of any 3-dehydroretinal$_2$ (porphyropsin) pigment present. The λ_{max} of the rods was at about 508 nm and there was a population of small short-wave-sensitive (SWS) single cones with λ_{max} close to 456 nm. The majority of double cones were identical middle-wave-sensitive (MWS) cones with both members containing a 531-nm pigment, though a small number of identical long-wave-sensitive (LWS) double cones with λ_{max} at about 553 nm and non-identical double cones, with the 553-nm and 531-nm pigments, were also identified. In addition, there were small populations of large single cones, the majority of which contained the MWS 531-nm pigment, but with a small number of cones containing the LWS 553-nm pigment. We cannot exclude the possibility that these represent double cones in which the two members have become separated. For details of the λ_{max}, transverse absorbance and the number of cells analysed, see Table 1 and Fig. 1. There was no difference between the sexes in numbers and types of visual pigments.

Lens transmission

The lens (Fig. 1F) shows maximum transmission from 700 nm through into the shorter wavelengths, with a clear cut-off at about 410 nm. Transmission was therefore greatly reduced in the ultraviolet below 410 nm.

Reaction distance

A two-way ANOVA revealed that both wavelength ($F_{5,160}$=62.8, P<0.00001) and illumination ($F_{1,160}$=219.2, P<0.00001) had a significant effect on RD, and that there was an interaction between wavelength and illumination ($F_{5,160}$=6.6, P=0.00001). Owing to the interaction between wavelength and illumination level, an ANOVA *post hoc* test (Newman–Keuls) was conducted for each illumination level. This test revealed that RD at 0.5 μmol m^{-2} s^{-1} was longest at 532 nm (MWS matching light), but not significantly longer than at 550 nm (LWS matching light). The RD was significantly longer at 510 nm (rod matching light) than at

Table 1. *Visual pigments of rods and cones from G. flavescens*

	LWS	MWS	SWS	Rods
λ_{max} of mean spectrum	553.5±2.6	531.4±0.5	455.8±3.1	508.1±1.0
λ_{max} of mean difference	552.7±3.5	532.0±0.6	457.5±2.3	509.5±0.9
Mean of individual λ_{max}	557.2±8.8	531.3±2.7	453.8±4.0	508.1±1.2
Maximum absorbance	0.015	0.025	0.012	0.032
Number of cells	9	25	13	9

LWS, long-wave sensitive cones; MWS, middle-wave sensitive cones; SWS, short-wave sensitive cones.

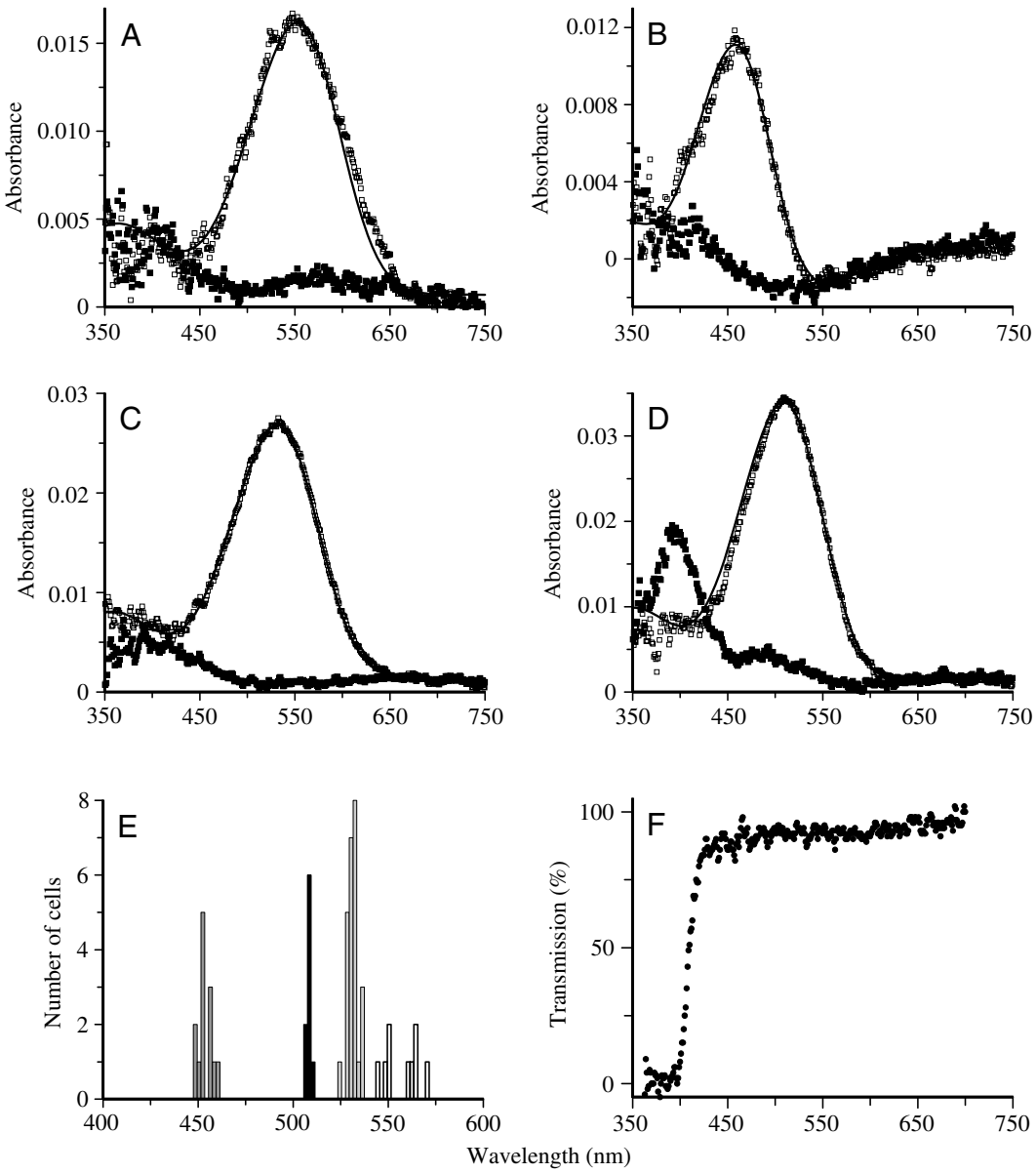

Fig. 1. Absorbance spectra of rods and cones, and lens transmission. (A) Long-wave-sensitive (LWS) cones, λ_{max}=553 nm; (B) short-wave-sensitive (SWS) cones, λ_{max}=456 nm; (C) middle-wave-sensitive (MWS) cones, λ_{max}=531 nm; (D) rods, λ_{max}=508 nm. Open symbols, pre bleach spectrum; closed symbols, after a white light bleach. Solid lines are visual pigment templates (Govardovskii et al., 2000). (E) Distribution histogram of the λ_{max} of individual cells. Cones, open bars; rods, black and shaded bars (bin size 2 nm). (F) Mean lens transmission of both lenses from a single fish.

460 nm (SWS matching light) and >660 nm, but was significantly shorter than at 532 and 550 nm (ANOVA, $P<0.01$). For the same wavelengths, but at the lower irradiance of 0.015 μmol m^{-2} s^{-1}, there was no difference in RD found between the λ_{max} matching wavelengths (460, 510, 530 and 550 nm) (ANOVA, $P>0.01$), with the exception of a shorter RD at 460 nm compared to 530 nm ($P=0.026$). In addition, RD was significantly shorter at the longer, non-pigment matching wavelengths (>660 nm and 560 nm), compared to the pigment matching wavelengths (ANOVA, $P<0.05$).

An ANOVA *post hoc* test (Newman–Keuls) was conducted to look for significant change in RD when changing the illumination within wavelength. Within all tested wavelengths, an increase in illumination level led to a significant increase in RD (ANOVA, $P<0.01$), with the exception of 460 nm (SWS matching light) (ANOVA, $P=0.11$) (Fig. 2A). The positive effect of an increase in illumination was most pronounced at 530 nm (MWS matching light) and 550 nm (LWS matching light) (Fig. 2A).

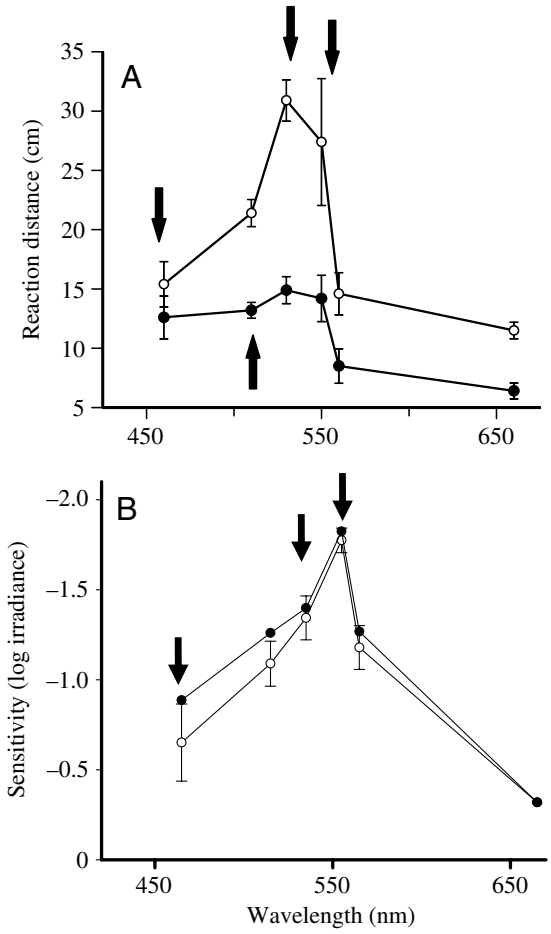

Fig. 2. (A) Reaction distance at irradiances of 0.5 μmol m^{-2} s^{-1} (open symbols) and 0.015 μmol m^{-2} s^{-1} (closed symbols). Values are means ± s.d. Downward pointing arrows indicate the λ_{max} of the three cone pigments; upward pointing arrow, that of the rods. (B) Optomotor responses. The maximum responses of the fish are indicated by filled symbols and the average responses by open symbols. Values are means ± s.d. Downward pointing arrows indicate the λ_{max} of the three cone pigments.

Optomotor response

Wavelength composition had a significant effect on the sensitivity of the goby (one-way ANOVA, $F_{5,4}=143$, $P<0.0001$). The ANOVA *post hoc* test (Newman–Keuls) revealed that there was a significant difference in sensitivity at all wavelengths, with the exception of 510 nm (rod matching light) and 560 nm, and between 530 nm (MWS matching light) and 550 nm (LWS matching light) (Fig. 2B).

Discussion

The spectral range of light maximally transmitted by natural waters is generally characteristic of its geographical location. Oceanic water transmits mainly short wavelengths, whereas coastal and inland waters contain relatively more long wavelength light, as a consequence of both the amount of decaying organic material washed from the land and suspended particles of silt. As a general observation, fish living in the upper layers of clear oceanic waters possess cones that are more sensitive to shorter wavelengths, whereas those living in coastal waters are more long-wave sensitive, and it has been argued that double cones contain visual pigments that tend to match the spectral distribution of the light they receive (Bowmaker, 1995; Loew and Lythgoe, 1978; Lythgoe, 1979).

The visual pigments reported here for *G. falvescens* fit comfortably into this pattern with the retina dominated by MWS double cones. However, the pigments in the double cones are somewhat different from those published previously for the two-spotted goby (Partridge, 1990). The data for the rods and SWS small cones are similar, but the MWS and LWS pigments are noticeably different; 525 and 570 nm (Partridge, 1990), but 531 and 553 nm in the present study. This discrepancy is difficult to explain, but could suggest differences between individuals from the southwest of England (Partridge, 1990) and those from Norway. Nevertheless, this combination of cones, which form a square mosaic of four double cones, maximally sensitive at longer wavelengths, surrounding a central small single SWS cone is typical of shallow coastal water species (Loew and Lythgoe, 1978; Lythgoe, 1979). The lack of UV-sensitive cones is also a common feature of inshore fish and, in the goby, correlates with the lens transmission that effectively cuts off light below about 410 nm (Losey et al., 2003; Thorpe et al., 1993).

The aim of the present investigation was to explore the spectral sensitivity of the two-spotted goby, from a physiological and behavioural perspective, and to test the hypothesis that the goby would be more sensitive to a change in illumination level at its pigment maxima than away from the pigment maxima. *G. flavescens* is a shallow water (0–10 m depth) species, which, therefore, experiences a great range of illumination levels (1000–20 μmol m^{-2} s^{-1} at the surface on a sunny day or on a cloudy winter day, respectively) as well as wavelength. An illumination level similar to the one chosen in

the present study is representative of the top 3–10 m of the water column during a spring or autumn algal bloom (Utne-Palm, 2004).

The RD data at the higher irradiance of 0.5 μmol m^{-2} s^{-1} (~26 lux) shows a spectral sensitivity function with a maximum around 530–550 nm. The maximum RD observed at 550 nm in the present study was 35 cm, which is much greater than the 22 cm measured in an earlier study using white light of 8 μmol m^{-2} s^{-1} (~400 lux) from a halogen light source (Utne, 1997). [In the same study (Utne, 1997) 8 μmol m^{-2} s^{-1} was found to be the light saturation level for the two-spotted goby, and 20 μmol m^{-2} s^{-1} was found to be the level of increasing dazzle effect.] The fact that lower illumination was needed to obtain a long RD when light was composed of wavelengths close to the maximum sensitivity of the majority of cones, than when light was composed of wavelengths away from this peak, clearly demonstrates the importance of the spectral composition of the available light. Increasing the irradiance level from 0.015 μmol m^{-2} s^{-1} (~0.8 lux) to 0.5 μmol m^{-2} s^{-1} (~26 lux) led to a significant increase in RD for all wavelengths, with the exception of 460 nm (SWS matching light) (Fig. 2A). The positive effect of an increase in illumination was most pronounced at 530 nm (MWS matching light) and 550 nm (LWS matching light) (Fig. 2A). Thus, at the higher illumination, there is a clear correlation between the spectral sensitivity of the RD and the peak sensitivity of the double cones. This is the most straightforward comparison, since the majority of the double cones are identical doubles with a 531-nm pigment, but a small contribution from the LWS pigment of some double cones to a luminosity sensitivity function cannot be excluded. However, at the lower illumination, which is clearly scotopic to the human eye, such a correlation is not so clear. At this low light level, the RD is greatly reduced, though still maintaining a peak around 530–550 nm. The relatively smaller reduction in RD at shorter wavelengths may represent either a greater input from SWS cones, or more probably, evidence of a rod intrusion at these low light levels (Fig. 2A).

The optomotor results identify a somewhat similar spectral sensitivity to the RD responses at the higher light intensity (Fig. 2B). However, the peak is at slightly longer wavelengths at 550 nm, which could indicate that the optomotor response is driven not only by the dominant MWS identical double cones, but also by input from the LWS cones of the minority population of non-identical double cones. This would be in agreement with findings in goldfish (Schaerer and Neumeyer, 1996) and zebrafish (Krauss and Neumeyer, 2003) where motion detection appears to be driven primarily by LWS cones. However, the narrow sensitivity function obtained in the present study suggests that the optomotor response may be driven by a more complex chromatically opponent input. This is supported by monochromatic rearing studies in the cichlid, *Aequidens pulcher* (Kröger et al., 2003), which showed significant changes in optomotor responses to chromatic stimuli.

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