

THE SPECTRAL SENSITIVITY OF THE GOLDFISH AND THE CLAWED TOAD TADPOLE UNDER PHOTOPIC CONDITIONS*

BY J. R. CRONLY-DILLON AND W. R. A. MUNTZ

Institute of Experimental Psychology, Oxford University

(Received 23 September 1964)

The visual pigments of the vertebrate retina fall into two major classes, depending on whether they are based on vitamin A₁ or vitamin A₂. The rod pigment in the former case is rhodopsin, and in the latter case porphyropsin, and the scotopic sensitivities of the animals agree well with the absorption spectra of the relevant pigment. Later work led to the view that the photopic sensitivities of animals also fall into the same two classes: those animals with visual pigments based on vitamin A₁ are maximally sensitive at about 560 m μ , and those with pigments based on vitamin A₂ at about 620 m μ (Granit, 1941, 1947). The first type of photopic sensitivity can be roughly fitted by the absorption spectrum of the pigment iodopsin, the second by the absorption spectrum of the pigment cyanopsin (Wald, Brown & Smith, 1953, 1955). The difference between the rods and cones within each group is due to the opsin part of the molecule.

As far as the photopic sensitivity is concerned, the position outlined above, which will be referred to as the classical position, has mainly arisen from electrophysiological work on comparatively few species. Cone pigments are difficult to isolate, so that although a large amount of data is available for rod pigments, the data on cone pigments are equivocal (Dartnall, 1960); the only cone pigment that has been extracted is iodopsin, and the fit between photopic sensitivity data and its absorption spectrum is not perfect. Similarly, Granit's photopic data were obtained from only a few species, and some physiological data do not agree with the classical view (Tansley, Copenhagen & Gunkel, 1961, for squirrel).

Behavioural studies of photopic sensitivity are found to give widely varying results. Sometimes a more or less classical curve is obtained (Brown, 1937, on rabbits; Blough, 1957, on pigeons; Wald, 1945, on humans; Jacobs, 1963, on monkeys), but the curve may be unexpectedly narrow (Grundfest, 1932*b*, on sunfish), or may show secondary rises and inflexions (Stiles & Crawford, 1933, on humans; Weale, 1953, on humans). On other occasions the photopic curve bears no apparent relation to the classical curve (Gunter, 1954, on cats; Muntz, 1962, on *Rana*).

From the above brief review it is clear that further data are needed on the photopic sensitivity of different animals, especially as data obtained by electrophysiological and biochemical means do not necessarily correlate with the animal's behaviour. In

* This work forms part of a project on stimulus-analysing mechanisms jointly financed by the American Office of Naval Research (Contract Number N62558-2453) and the Nuffield Foundation. Support was also received from the U.S. National Institutes of Health (Contract No. NB 0442201).

humans, for example, the electroretinogram resulting from a single light flash under photopic conditions yields a curve agreeing approximately with the absorption spectrum of rhodopsin, but the subjects' report of the relative brightness of the different colours agrees with the classical photopic sensitivity curve (Riggs, Berry & Wagner, 1943). In the present paper photopic curves obtained by means of the optomotor response, are presented for two cold-blooded vertebrates: the goldfish (*Carassius auratus*) and the clawed toad (*Xenopus laevis*).

Spectral sensitivity curves under photopic or scotopic conditions may be obtained by determining the energy of various spectral lights necessary to produce a constant response. Where a behavioural response is used as the index of sensitivity, it is desirable to employ a method that does not depend on previous learning. A suitable response is the optomotor response, where the experimenter exploits the natural tendency of animals to follow a movement in the visual field. *Xenopus* was selected because behavioural data using the phototactic response are available for this animal (Denton & Pirenne, 1954). These data do not agree with the classical curve for either rod or cone sensitivity, and almost certainly results from interaction between receptors of more than one type (Dartnall, 1956). It is therefore important to determine the spectral sensitivity using another type of behaviour, especially as in *Rana* the spectral curve using the optomotor response agrees with the classical position (Birukow, 1949), whereas the curve obtained using the phototactic response is entirely different and similar to that obtained with *Xenopus* (Muntz, 1962). A further reason for using *Xenopus* was that the photopic sensitivity revealed by means of the electroretinogram in this animal is unusual in form, and unlike both the phototactic curve and the classical curve (Muntz, 1965). It is therefore of interest to see whether the curve obtained using the optomotor response agrees with the curve obtained in the phototactic situation, or with the curve obtained using the electroretinogram, or with the classical curve, or with none of these. *Xenopus* tadpoles were used, as opposed to adults, since they proved to give much more reliable optomotor responses.

Goldfish were selected as a second animal because a considerable amount of work has been done on colour vision in goldfish, both behaviourally (McCleary & Bernstein, 1959), and physiologically (MacNichol, Wolbarsht & Wagner, 1961). The photopic sensitivity is a necessary datum to be considered if colour vision is to be explained.

METHODS

(a) Apparatus

The apparatus is shown schematically in Fig. 1. The fish or tadpole was placed in a cylindrical glass container 5 in. in diameter. This was filled with water to a depth of 3 in., and kept at room temperature (approximately 20° C.). The container itself rested at the bottom of a bucket lined with white paper; the distance between the walls of the bucket and the walls of the glass container was about 1 in. at the bottom of the bucket, and approximately 2½ in. at water level.

The image of a small transparent Perspex wheel with black stripes radiating from the centre was projected into the bucket. The image of the centre of the wheel was made to coincide with the axis of the container, and the image of the wheel itself cast an alternating pattern of light and dark stripes on the inner walls of the bucket.

The thickness of the stripes at the bottom of the bucket was 1 in., and the angle subtended between adjacent stripes was 45° . The Perspex wheel was coupled to a reversible electric motor which rotated the stripes at an angular velocity of $19.50^\circ/\text{sec}$. A lens system collimated the projector beam before passing it through a narrow-band interference filter. A further lens system then focused a monochromatic image of the stripes on to the inner surface of the bucket. The image of the wheel could only be focused accurately at one level on the walls of the bucket, but the extent of blurring elsewhere was small.

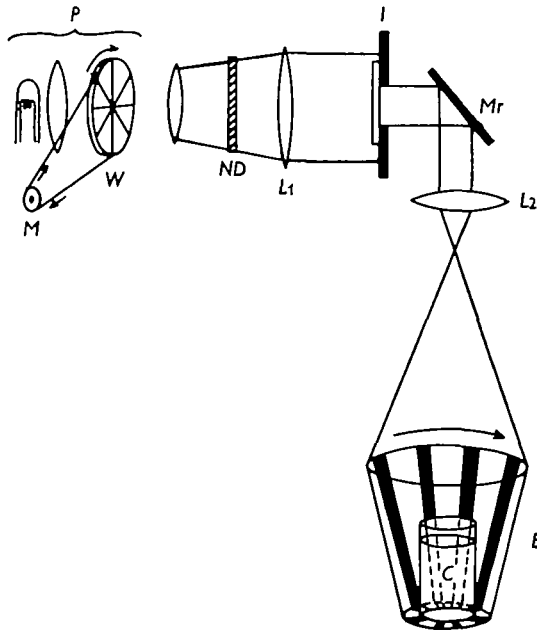


Fig. 1. Schematic diagram of apparatus. *P*, projector. *W*, striped wheel in position of slide holder, driven by reversible motor *M*. *ND*, neutral density filter. *L*₁, collimating lens. *I*, interference filter mounted on wheel. *Mr*, mirror. *L*₂, lens focusing image of wheel *W* upon walls of bucket, *B*. *C*, container for animal.

(b) Light sources

Spectral bands were obtained from nine interference filters. The light source was provided by a 500 W. projector lamp with a colour temperature of 3100°K . During a threshold determination the intensity was controlled with Kodak Wratten neutral density (N.D.) filters whose density values increased in steps of 0.2 log. units. The relative energy of the different colours was calculated from the colour temperature of the source, and the spectral transmission curves of the filters provided by the makers (Balzars, Liechtenstein). The absolute energy of the light reflected from the walls of the bucket was obtained by measuring the luminosity with an S.E.I. photometer when no filters were in the beam, and then calculating the radiosity in W./cm.^2 from the spectrophotometric curves of the interference filters. For this calculation it was assumed that the light source had a colour temperature of 3100°K ., and also that, at $530\text{ m}\mu$, one lumen is produced by 0.0019 W .

The results of these calculations are shown in Table 1, which gives for each filter

the wavelength at which transmission was maximal, the half-maximum bandwidth, the energy obtained through each filter when no N.D. filters were placed in the beam, and the amount, in log. units, by which each colour of light must be adjusted in order to obtain an equal quantum spectrum (the correction factor).

Background illumination was obtained by indirect lighting from a 60 W. tungsten bulb, and by stray light from the projector. The level of background illumination was measured by an S.E.I. photometer. In one experiment on goldfish a coloured background light was used; this was obtained from a second projector illuminating the walls of the bucket by means of a second mirror.

Table 1. *Characteristics of the interference filters used in the experiments*

λ_{max} in m μ	426	462	494	528	585	615	635	670	700
Half max. bandwidth (m μ)	12	8	11	0.8	9	9	10	10	10
Max. available energy ($\mu\text{W./cm.}^2$)	9.5	13.5	25.7	30.1	44.6	51.1	63.0	84.6	89.8
Correction factor	+0.34	+0.29	-0.09	-0.16	-0.33	-0.39	-0.48	-0.61	-0.64

(c) *Threshold criteria*

(i) *Goldfish*. Not all fish responded in exactly the same way to the moving stripes. Some fish would attempt to follow a particular stripe and swim around the bowl at approximately the same speed as the stripes, others would start to follow a stripe, overtake it, then double back, then attempt to follow another stripe, and so on, repeating the cycle indefinitely. Initially this behaviour caused some difficulty, especially at those intensities where the experimenter could not see the stripes himself and did not know beforehand which way they were moving. However, it soon became apparent that the movements which a fish makes when it starts to follow a stripe are different from those which it makes when it starts to double back. In addition we noted whether the swimming pattern was altered when the motor was reversed.

Sometimes a fish would lie in contact either with the side or bottom of the container, and would show no response to the moving stripes, while other fish would sometimes dart about wildly every time the stripes started to move. In both cases the fish were either discarded or tested at some other time when they were more disposed to give a recognizable optomotor response.

During a threshold determination at a particular wavelength the intensity was reduced with the aid of N.D. filters whose intensity values increased in steps of 0.2. At each intensity the fish was given ten trials, and the direction of rotation of the stripes was varied randomly during the test sequence. A time limit of 2 min. was set for each trial. A particular intensity was considered above threshold if the fish gave a clear optomotor response in 80% of the trials.

The different colours of light were tested in a random order. In general, we attempted to select a presentation sequence where the spectral separation between succeeding test wavelengths was as great as possible. This procedure was adopted as a precaution against the possibility that the thresholds might be affected as a result of selective adaptation introduced by tests for the colour previously used.

(ii) *Clawed toad*. When undisturbed, tadpoles of the clawed toad normally remain more or less motionless with their heads pointing downwards at an angle of approxi-

mately 45° . This posture is maintained by rapid vibrations of the end of the tail. Under these conditions the optomotor response is easy to observe; the only change in behaviour is that the tail bends slightly with the result that the animal rotates about its own axis in the same direction as the movement of the stripes. When the stripes are well above threshold the rotation of the animal may be equal to the rotation of the stripes, but as the intensity of the light is reduced the animal lags progressively farther behind. The threshold was taken as that intensity of light at which no rotation of the animal or bending of the tail could be seen within 2 min of the motor being switched on. Sometimes an animal would dash violently around the tank; under these conditions the optomotor response could not be observed and the trial was discontinued.

During a threshold determination at a particular wavelength the intensity of the light was reduced by steps of 0.2 log. units until the response could no longer be seen. The different colours of light were presented in a random order, and two threshold determinations were made at each wavelength.

RESULTS

(a) *Goldfish*

Five $2\frac{1}{2}$ in. goldfish were used in these experiments. In order to get the fish fully light-adapted before starting an experiment they were all subjected for 2 hr. to a bright light provided by a 100 W. tungsten bulb.

The data obtained with a background illumination on the walls of the bucket of 0.41 ft. lamberts are presented in Table 2. This background illumination was provided by the indirect light from a 60 W. tungsten lamp, together with stray light from the projector. In Table 2 the measurements for any given wavelength are presented in terms of the N.D. filter at which the response was just above the threshold. In the last column on the right the correction factor given in Table 1 has been added to the mean of the measurements for the five fish. The results are shown plotted against wavelength in the lower curve of Fig. 2.

Table 2. *Results for individual goldfish with background luminosity of 0.41 ft. lamberts*

Wavelength	Fish					Mean	Corrected mean
	I	II	III	IV	V		
426	0.2	—	0.2	0.2	0.4	0.25	0.59
462	0.6	0.6	0.8	0.8	0.8	0.72	1.03
494	1.0	1.0	1.2	1.4	1.4	1.20	1.11
528	1.4	1.4	1.6	1.4	1.4	1.44	1.28
585	1.4	1.6	1.8	1.6	1.6	1.60	1.27
615	1.6	1.8	2.0	1.8	2.0	1.84	1.45
635	2.0	2.0	2.0	1.8	2.0	1.96	1.49
670	1.0	1.0	0.8	0.8	1.0	0.92	0.33
700	0.6	0.6	0.2	0.2	0.4	0.40	-0.21

This curve appears to have two maxima. One peak occurs in the region of 615–630 $m\mu$, and is probably due to cone activity. The other less distinct peak occurs in the region 520–535 $m\mu$. The fact that the rod pigment of the related species *Carassius*

carassius has its absorption maximum at $523\text{ m}\mu$ (Dartnall, 1961) suggests that this secondary peak in the photopic sensitivity curve of the goldfish might be due to activity of rods that are still functioning under photopic conditions. In order to test this hypothesis a second experiment was run using a lower adapting light.

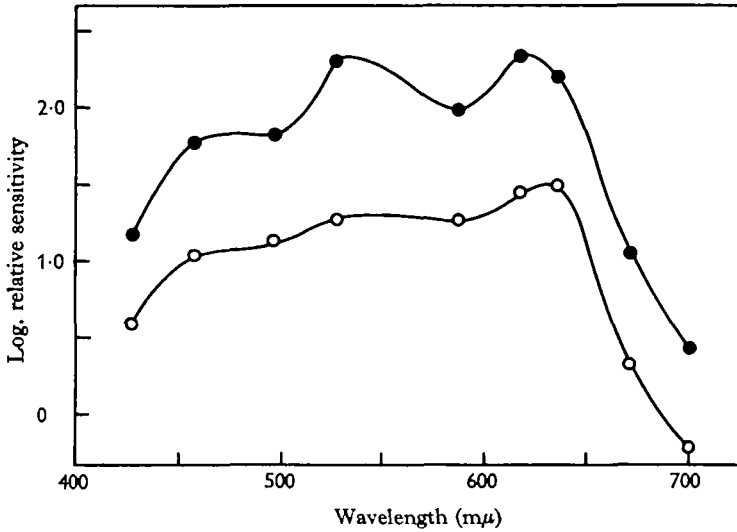


Fig. 2. Spectral sensitivity of goldfish under two conditions of background illumination. Open circles, background illumination of 0.41 ft. lamberts; closed circles, background illumination of 0.052 ft. lamberts.

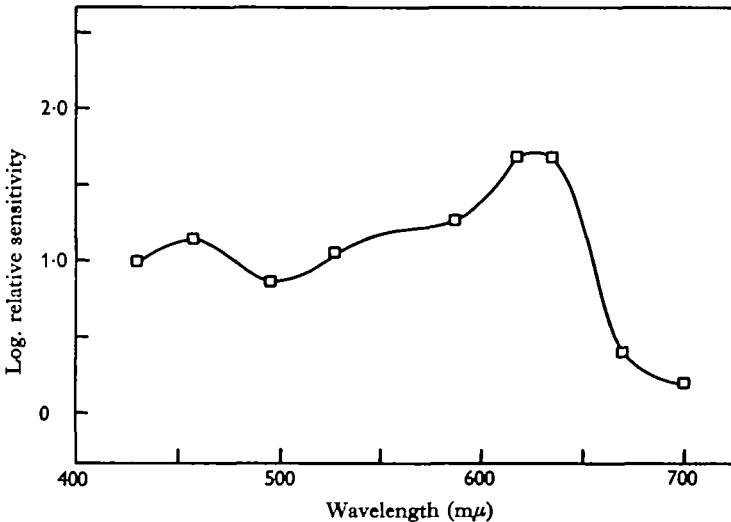


Fig. 3. Spectral sensitivity of goldfish with green background illumination.

Table 2 presents the data when the adapting light was only 0.052 ft. lamberts. The background illumination in this case was provided by stray light from the projector only. The uppermost curve in Fig. 2 shows the photopic sensitivity under these

conditions and represents the results averaged for five fish. In this curve the peak in the region 520–535 $m\mu$ is greatly enhanced. Otherwise the overall shape of the curve on either side of this peak remains unchanged. This result strengthens our belief that some rods are operating under photopic conditions.

An additional feature which appears in both curves is the inflexion which occurs in the short wavelength region 460–470 $m\mu$. This suggested the existence of a short wavelength blue receptor whose activity was largely masked by activity of the rods. We decided to test this possibility by selectively bleaching the rods. To do this we used a Kodak Wratten No. 74 filter in combination with a 0.8 N.D. filter to produce a narrow-band 'green'-adapting background. The maximum transmission of this green filter is at 538 $m\mu$, and the half-maximum bandwidth is 30 $m\mu$. The energy

Table 3. Results for individual goldfish with background luminosity of 0.052 ft. lamberts

Wavelength	Fish					Mean	Corrected mean
	I	II	III	IV	V		
426	0.8	1.0	0.8	0.8	0.8	0.84	1.18
462	1.4	1.6	1.4	1.4	1.6	1.48	1.79
494	2.0	2.0	2.0	1.6	2.0	1.92	1.83
528	2.6	2.4	2.4	2.4	2.6	2.48	2.32
585	2.4	2.2	2.2	2.4	2.4	2.32	1.99
615	2.4	2.6	2.8	2.8	2.6	2.64	2.33
635	2.4	2.8	2.8	2.8	2.6	2.68	2.21
670	1.6	1.8	1.6	1.6	1.6	1.64	1.05
700	—	1.2	1.0	1.2	0.8	1.05	0.44

Table 4. Results for individual goldfish with green background illumination

Wavelength	Fish					Mean	Corrected mean
	I	II	III	IV	V		
426	0.6	0.6	0.6	0.8	0.6	0.64	0.98
462	0.8	0.8	0.8	1.0	0.8	0.84	1.15
494	0.8	0.8	1.0	1.0	1.2	0.96	0.86
528	1.0	1.2	1.2	1.2	1.4	1.20	1.04
585	1.6	1.6	1.6	1.6	1.6	1.6	1.27
615	2.2	2.2	2.0	2.0	2.0	2.08	1.69
634	2.4	2.2	2.2	2.0	2.0	2.16	1.69
670	1.2	1.4	1.2	1.2	1.0	1.20	0.61
700	0.8	1.0	0.8	0.8	0.6	0.80	0.19

reflected from the walls of the bucket was 4.2 $\mu W./cm^2$. Additional background light was provided by stray light from the two projectors, and this amounted to 0.10 ft. lamberts. The light source for the 'green'-adapting background was provided by a second 500 in. tungsten projector lamp (colour temp. 3100° K.). Table 4 presents the data for five fish in this partial bleaching experiment.

The average curve, which is shown in Fig. 3, shows a peak in the short wavelength region 455–465 $m\mu$; the sensitivity in the region 520–535 $m\mu$ was greatly reduced.

(b) *Clawed toad*

Ten *Xenopus* tadpoles were used in these experiments, all of which were between the stages 56 and 58 of Nieuwkoop & Faber's (1956) table of normal development. All the animals had been light-adapted before the experiments by means of a 100 W. tungsten lamp. The same two levels of background illumination were used as for the

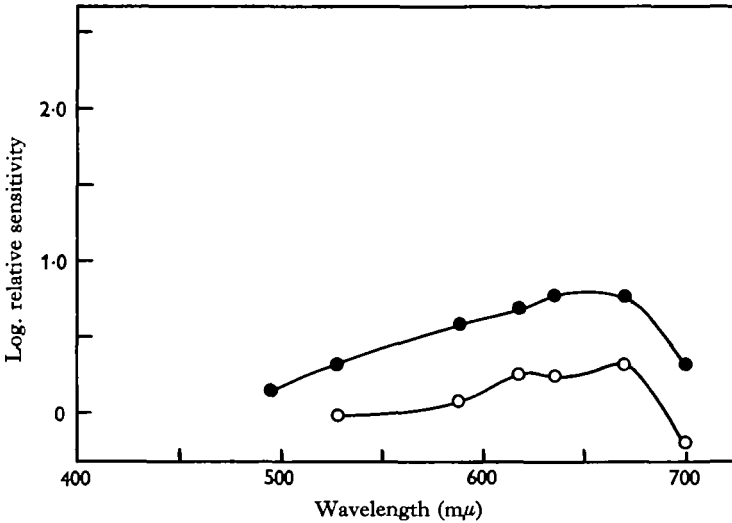


Fig. 4. Spectral sensitivity of clawed toad tadpole under two conditions of background illumination. Open circles, background illumination of 0.41 ft. lamberts; closed circles, background illumination of 0.052 ft. lamberts.

Table 5. Results for individual clawed toad tadpoles with background luminosity of 0.41 ft. lamberts

Wavelength	Tadpole					Mean	Corrected mean
	I	II	III	IV	V		
528	0.2	0.1	0.3	0.1	0.0	0.14	-0.02
585	0.6	0.5	0.6	0.1	0.3	0.42	0.09
615	0.8	0.6	0.8	0.7	0.3	0.64	0.25
635	0.6	0.7	0.7	0.8	0.7	0.70	0.23
670	0.9	1.1	1.0	0.8	0.7	0.90	0.31
700	0.5	0.4	0.5	0.2	0.4	0.40	-0.21

Table 6. Results for individual clawed toad tadpoles with background luminosity of 0.052 ft. lamberts

Wavelength	Tadpole					Mean	Corrected mean
	I	II	III	IV	V		
494	0.2	0.2	0.2	0.4	0.2	0.24	0.14
528	0.6	0.2	0.6	0.6	0.4	0.48	0.32
585	0.8	1.0	1.0	1.0	0.8	0.92	0.59
615	1.0	1.0	1.0	1.2	1.2	1.08	0.69
635	1.0	1.4	1.4	1.2	1.2	1.24	0.77
670	1.2	1.4	1.4	1.4	1.4	1.36	0.77
700	0.8	0.8	0.8	1.2	1.0	0.82	0.31

goldfish, 0.41 and 0.052 ft. lamberts, and five animals were tested at each level. The results for the individual animals are shown in Tables 5 and 6, and summarized in Fig. 4. At the lower level of background illumination the threshold was lower, as would be expected, but the shape of the spectral sensitivity curve was not greatly altered. It can be seen that under both conditions of background illumination the sensitivity in the red was very marked. This red sensitivity was very noticeable during the course of an experiment; thus stimuli which were above 650 m μ and that were invisible to the experimenter, could still elicit strong responses from the tadpoles, while on the other hand the tadpoles failed to respond to blue-green and green lights that were greatly above the experimenter's threshold.

DISCUSSION

The results obtained with *Xenopus* do not agree with the classical position; they agree well, however, with results previously obtained with the electroretinogram (Muntz, 1965). The sensitivity curve is very broad compared with Dartnall's nomogram, and extends far into the red; there is also a slight indication of two maxima on the curve suggesting that two receptors, with distinct spectral properties, may be contributing to the animals' photopic sensitivity. The results obtained with the electroretinogram provided stronger evidence on this point. Thus in very young tadpoles the maximal sensitivity was at about 630 m μ , but as the animals developed the position of the maximum shifted to about 610 m μ ; the sensitivity curve remained very broad throughout. It was also found that, in adults, changing the level of the background illumination changed the position of maximal sensitivity; under low background illuminations the maximum was at about 610 m μ , but under high illuminations the position of the maximum shifted to about 630 m μ . In the present experiment changes in the background illumination had little or no effect. This is presumably because the changes in the background illumination used were small (0.9 log. units) compared to the range of background illuminations used in the previous experiment (2.4 log. units).

Two quite distinct behavioural curves can thus be obtained with *Xenopus* under photopic conditions, depending on the type of behaviour that the animal is showing. The first is that reported by Denton & Pirenne (1954) for phototactic behaviour. This curve has two peaks at about 560 and 460 m μ , with a pronounced dip in between (the phototactic results of Denton & Pirenne were in fact obtained under scotopic conditions, but they obtained the same curve in light-adapted animals using changes in the colour of the skin instead of phototactic behaviour, and similar curves have been obtained in this laboratory under photopic conditions using the phototactic response). The second curve is the one presented here for the optomotor response.

These two photopic curves have their counterparts in two photopic curves that have been reported for *Rana*. Thus using the phototactic response a bimodal curve is obtained, with a maximum in the blue, a minimum at about 510 m μ , and a second maximum at about 560 m μ (Muntz, 1962); this curve is very similar to that obtained by Denton & Pirenne on *Xenopus*, except that the maximum in the blue is accentuated. Using the optomotor response, however, a typical smooth function maximal in the yellow-green is obtained (Birukow, 1949), agreeing with the electroretinogram results

of Granit (1947). The two spectral curves of *Rana* are adapted to the two types of behaviour. The optomotor response yields a sensitivity curve of the classical form, so that the animal will respond maximally to the brightness of the stimulus, irrespective of colour. On the other hand, in the phototactic situation the response is specific to wavelength, and relatively independent of brightness; it has been suggested that this is a specialization helping the animal to escape towards the water when frightened (Muntz, 1962). Inhibitory effects have been shown to be involved in the phototactic curve, so that some sensitivity is sacrificed in obtaining the wavelength specificity.

In the case of *Rana* there is evidence as to the neural basis of these differences as well. Thus it is probable that the sensitivity in the phototactic situation is mediated by a system of *on* fibres projecting to the diencephalon from the retina (Muntz, 1962) and the optomotor response is presumably mediated by fibres passing to the optic tectum, probably *on-off* fibres.

The optomotor response shows that *Xenopus* possess a marked sensitivity in the red part of the spectrum, and the question therefore arises as to whether the animal can respond to red as a colour, irrespective of brightness and saturation. An experiment reported by Burgers (1952), using the optomotor response, suggests that it can. Burgers showed that stripes of blue or of green could be matched by stripes of a suitable shade of grey so that no response was obtained; no shade of grey was found, however, which the animals were unable to distinguish from red. A second question concerns the usefulness of this marked red sensitivity to the animal in its natural environment, but at present it is not possible to suggest any hypothesis on this point.

The results obtained from *Xenopus* suggest that more than one receptor contributes to the photopic sensitivity of this animal. The same is true of the photopic sensitivity of the goldfish, where at least three receptors appear to be involved. These three receptors can be separated out to some extent by varying the background illumination. Thus decreasing the background illumination causes a marked increase in sensitivity in the region of $530\text{ m}\mu$. It is probable therefore that the sensitivity in this region is due to the rods, since these would be expected to have their maximal sensitivity at about this point, and it would also be expected that their sensitivity would be enhanced, relative to that of the cones, by decreasing the background illumination. A difficulty with this view is that the hump at this point cannot be fitted by Dartnall's nomogram, being too narrow, particularly on the blue side of the maximum. Grundfest (1932*a*), using the optomotor response and working on the scotopic sensitivity of the sunfish, also found his spectral sensitivity curve to be too narrow: the narrowness in his experiment was much more extreme than it was in the case of the $530\text{ m}\mu$ hump in the goldfish. He suggested that the effect was due to photo-insensitive pigments which were acting as filters and absorbing light on either side of the maximum and therefore sharpening the curve, and he discusses what the pigments might be. If this explanation applies to the goldfish only one pigment need be postulated, absorbing towards the blue end of the spectrum. Although no definite evidence is available, such a situation is very probable. Granit (1947), for example, has presented several illustrations of the fact that spectral curves obtained on intact eyes suggest little sensitivity in the blue, but that if the lens and optic media are removed, so that the light falls directly on to the retina itself, the spectral sensitivity approaches much more nearly to that expected from the absorption spectrum of the appropriate visual pigment.

It thus appears that, in the goldfish, the rods contribute to the spectral sensitivity curve under photopic conditions as well as under scotopic conditions. This situation was also found by Granit (1941) on a related species, *Cyprinus*, using the electroretinogram; irrespective of the state of light-adaptation two humps were always apparent one at the characteristic position for rods and one at the characteristic position for cones. The effect of dark-adaptation or light-adaptation was to alter the relative heights of the two humps, but Granit never succeeded in abolishing either hump entirely. The result is interesting since it shows that in certain circumstances the rods are functioning under photopic conditions. Other work also suggests that this may sometimes occur. In humans, for example, the photopic sensitivity curve does not appear to contain any contribution from the rods, but the electroretinogram indicates that the rods may still be functioning (Riggs *et al.* 1943), and calculations show that only a small amount of the rhodopsin need have been bleached away (Rushton, 1960). Although therefore the rods do not appear to contribute to brightness perception under photopic conditions in most vertebrates, they may well still be functioning, and could be involved as one of the receptors of colour vision, as has been suggested, for example, by Willmer (1946). An hypothesis to explain one particular type of colour vision, in which the rods are involved as a receptor having inhibitory properties, has previously been put forward for *Rana* (Muntz, 1963).

The present experiments demonstrate two other receptors contributing to the photopic sensitivity of the goldfish, apart from the rods. The first of these is maximally sensitive at about 610 m μ , in the classical position for cone sensitivity. The curve is again rather narrow, and does not, for example, give a good fit with cyanopsin, as would be required by the classical position. Such narrowness is, however, common for photopic sensitivity curves, and was again found by Grundfest (1932*a*) for the sunfish. The third receptor appears to be maximal at about 450 m μ , in the blue. Blue receptors are common in other animals. Thus blue-sensitive pigments have been extracted from the retina of the tench (Dartnall, 1952) and the frog (Dartnall, 1957) and blue receptors are strongly suggested by experiments on several other animals (for example, Granit, 1947, guinea-pig, Ingvaar, 1959, cat; Blackwell & Blackwell, 1961, man).

The results suggest that the goldfish has three receptors, in the blue, the green, and the orange, and that all three are capable of functioning under photopic conditions. These three receptors could clearly form the basis for a system of colour vision according to the trichromatic scheme. The work of MacNichol *et al.* (1961), however, shows that colour is coded in goldfish according to an opponents scheme of the type put forward by Hering, and not according to a trichromatic scheme. The recordings were made at the level of the ganglion cells, however, so that it is possible that the receptors function according to a trichromatic scheme, but that, after analysis by the retina, the information is coded in the optic nerve according to opponent principles of the type put forward by Hering. Several 'zone theories' of this sort have been worked out in some detail, and have been reviewed by Judd (1951). The data from the present experiments are not detailed enough to warrant working out such a scheme in detail for the goldfish, but it is easy to see in a general way how the analysis could be performed. For example, consider a retinal ganglion cell receiving inputs from an equal number of rods and cones, and connected in such a way that the input from

the rods is excitatory and causes the ganglion cell to fire at the onset of illumination, whereas the input from the cones is inhibitory and causes the ganglion cell to fire by 'rebound' at the end of illumination. Such a ganglion cell will have precisely the characteristics described for one class of optic nerve fibre by MacNichol *et al.* (1961): it will give an *off* response to red, and an *on* response to blue. Different retinal interconnexions of this sort between receptors and ganglion cells could similarly give rise to the other characteristic types of spectral response described for the optic nerve fibres.

SUMMARY

1. Photopic spectral sensitivity curves have been obtained by means of the optomotor response for the tadpole of the clawed toad (*Xenopus laevis*), and for the goldfish (*Carassius auratus*). Both these animals have visual pigments based on vitamin A₂, and would be expected therefore to have photopic sensitivity curves maximal at about 615 m μ and fitting, at any rate approximately, the absorption spectrum of cyanopsin.

2. The results with *Xenopus* show a broad curve extending far into the red, and having its maximal sensitivity at about 630 m μ . It is probable that this curve reflects the summated activity of two receptors, maximally sensitive at 610 and 630 m μ .

3. The results are discussed in relation to other behavioural work with *Xenopus*, using the phototactic response, in which an entirely different form of spectral curve was obtained, and in relation to behavioural and physiological data which are available for *Rana*.

4. The photopic curve of the goldfish shows three humps, which can be separated out to a large extent by using different background illuminations. One hump is maximal at about 610 m μ , the classical position for cone sensitivity in freshwater fish. A second hump is maximal at about 530 m μ , and is probably due to the rods, and the third hump is maximal at about 450 m μ in the blue. The relation of these three receptors to colour vision in this animal are discussed.

REFERENCES

- BIRUKOW, G. (1949). Die Entwicklung des Tages—und des Dämmerungsehen im Auge des Grasfrosches (*Rana temporaria* L.). *Z. vergl. Physiol.* **31**, 322-47.
- BLACKWELL, H. R. & BLACKWELL, O. M. (1961). Rod and cone mechanisms in typical and atypical congenital achromatopsia. *Vision Res.* **1**, 62-107.
- BLOUGH, D. S. (1957). Spectral sensitivity of the pigeon. *J. Opt. Soc. Amer.* **47**, 827-33.
- BROWN, R. H. (1937). The bright sensitivity curve of the rabbit. *J. Gen. Psychol.* **17**, 323-8.
- BURGERS, A. C. J. (1952). Optomotor reactions of *Xenopus laevis*. I. *Physiol. comp.* **2**, 272-81.
- DARTNALL, H. J. A. (1952). Visual pigment 467, a photosensitive pigment present in tench retinae. *J. Physiol.* **116**, 257-89.
- DARTNALL, H. J. A. (1956). Further observations on the visual pigments of the clawed toad, *Xenopus laevis*. *J. Physiol.* **134**, 327-38.
- DARTNALL, H. J. A. (1957). *The Visual Pigments*. London: Methuen and Co. Ltd.
- DARTNALL, H. J. A. (1960). Visual pigments of colour vision. In: *Mechanisms of Colour Discrimination*, ed. Y. Galifret. Oxford: Pergamon Press.
- DARTNALL, H. J. A. (1961). The identity and distribution of visual pigments in the animal kingdom. In: *The Eye*, vol. 11, ed. H. Davson. New York and London: Academic Press.
- DENTON, E. J. & PIRENNE, M. H. (1954). The visual sensitivity of the toad, *Xenopus laevis*. *J. Physiol.* **125**, 181-207.
- GRANIT, R. A. (1941). A relation between rod and cone substances based on scotopic and photopic spectra of *Cyprinus*, *Tinca*, *Anguilla* and *Testudo*. *Acta physiol. scand.* **3**, 334-46.

- GRANIT, R. (1947). *Sensory Mechanisms of the Retina*. Oxford University Press.
- GRUNDFEST, H. (1932*a*). The sensibility of the sunfish, *Lepomis*, to monochromatic radiation of low intensities. *J. Gen. Physiol.* **15**, 307-28.
- GRUNDFEST, H. (1932*b*). The spectral sensitivity of the sunfish as evidence for a double visual system. *J. Gen. Physiol.* **15**, 507-24.
- GUNTER, R. (1954). The spectral sensitivity of light-adapted cats. *J. Physiol.* **123**, 409-15.
- INGVAAR, D. H. (1959). Spectral sensitivity as measured in cerebral visual centres. *Acta Physiol. scand.* **46**, suppl. 159.
- JACOBS, G. H. (1963). Spectral sensitivity and colour vision of the squirrel monkey. *J. Comp. Physiol. Psychol.* **56**, 616-21.
- JUDD, D. B. (1961). Basic correlates of the visual stimulus. In: *Handbook of Experimental Psychology*, ed. S. S. Stevens. New York: John Wiley and Sons, Inc.
- MACNICHOL, E. F., JR., WOLBARSH, M. L. & WAGNER, H. G. (1961). Electrophysiological evidence for mechanism of colour vision in the goldfish. In *Light and Life*, W. D. McElroy and H. B. Glass (eds.). Baltimore, Maryland: Johns Hopkins Press.
- MCCLEARY, R. A. & BERNSTEIN, J. J. (1959). A unique method for control of brightness cues in study of colour vision in fish. *Physiol. Zool.* **32**, 284-92.
- MUNTZ, W. R. A. (1962). Effectiveness of different colours of light in releasing the positive phototactic behaviour of frogs, and a possible function of the retinal projection to the diencephalon. *J. Neurophysiol.* **25**, 712-20.
- MUNTZ, W. R. A. (1963). The development of phototaxis in the frog (*Rana temporaria*). *J. Exp. Biol.* **40**, 371-9.
- MUNTZ, W. R. A. (1965). The spectral sensitivity of *Xenopus laevis* as determined by the electroretinogram threshold (in preparation).
- NIEUWKOOP, P. D. & FABER, J. (1956). *Normal table of Xenopus laevis Daudin*. Amsterdam: North-Holland Publishing Company.
- RIGGS, L. A., BERRY, R. N. & WAYNER, M. (1943). A comparison of electrical and psychophysical determinations of the spectral sensitivity of the human eye. *J. Opt. Soc. Amer.* **39**, 427-36.
- RUSHTON, W. A. H. (1960). At the retinal level. In: *Mechanisms of Colour Vision*, ed. Y. Galifret. Oxford: Pergamon Press.
- STILES, W. S. & CRAWFORD, B. H. (1933). The luminal brightness increment as a function of wavelength for different conditions of the foreal and paraforeal retina. *Proc. Roy. Soc. B*, **113**, 496-530.
- TANSLEY, K., COPENHAVER, R. M. & GUNKEL, R. D. (1961). Spectral sensitivity curves of diurnal squirrels. *Vision Res.* **1**, 154-65.
- WALD, G. (1945). Human vision and the spectrum. *Science*, **101**, 653-8.
- WALD, G., BROWN, P. K. & SMITH, P. H. (1953). Cyanopsin, a new pigment of cone vision. *Science*, **118**, 505-8.
- WALD, G., BROWN, P. K. & SMITH, P. H. (1955). Iodopsin. *J. Gen. Physiol.* **38**, 623-81.
- WEALE, R. A. (1953). Colour vision in the peripheral retina. *Brit. Med. Bull.* **9**, 55-60.
- WILLMER, E. N. (1946). *Retinal Structure and Colour Vision: a Restatement and an Hypothesis*. Cambridge University Press.