Acuity and contrast sensitivity of the bluegill sunfish and how they change during optic nerve regeneration

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Abstract
Spatial vision was studied in the bluegill sunfish, Lepomis macrochirus (9.5–14 cm standard length) to assess the limitations imposed by the optics of the eye, the retinal receptor spacing and the retinotectal projection during regeneration. Examination of images formed by the dioptric elements of the eye showed that spatial frequencies up to 29 c/° could be imaged on the retina. Cone spacing was measured in the retina of fresh, intact eyes. The spacing of rows of double cones predicted 3.4 c/° as the cutoff spatial frequency; the spacing between rows of single and double cones predicted 6.7 c/°. Contrast sensitivity functions were obtained psychophysically in normals and fish with one regenerating optic nerve. Fish were trained to orient to gratings (mean luminance = 25 cd/m²) presented to either eye. In normals, contrast sensitivity functions were similar in shape and bandwidth to those of other species, peaking at 0.4 c/° with a minimum contrast threshold of 0.03 and a cutoff at about 5 c/°, which was within the range predicted by cone spacing. Given that the optical cutoff frequency exceeds that predicted by cone spacing, it is possible that gratings could be detected by aliasing with the bluegill’s regular cone mosaic. However, tests with high contrast gratings up to 15 c/° found no evidence of such detection. After crushing one optic nerve in three trained sunfish, recovery of visual avoidance, dorsal light reflex and orienting to gratings, were monitored over 315 days. At 64–69 days postcrush, responses to gratings reappeared, and within 2–5 days contrast sensitivity at low (0.15 c/°) and medium (1.0 c/°) spatial frequencies had returned to normal. At a high spatial frequency (2.93 c/°) recovery was much slower, and complete only in one fish.

Keywords: Contrast sensitivity function, Fish vision, Fish visual acuity, Optic nerve regeneration

Introduction
The dozen or so species of Lepomis, members of the perciform family Centrarchidae, are indigenous to eastern North America but have been introduced widely to other areas of the continent. One of the commonest is the bluegill sunfish (Lepomis macrochirus) which is easily caught in the shallows of warm-water ponds and lakes, often around weeds and logs, which they use for cover. They bite voraciously on any small natural bait or artificial lure. These fish adapt and survive well in the laboratory. They are quickly tamed and become cooperative subjects for behavioral experiments. Lepomis, and related centrarchids have been used in a number of visual psychophysical studies, including determinations of spectral sensitivity (Grundfest, 1931), flicker fusion frequency (Wolf & Zerrahn-Wolf, 1936), color discrimination (Hurst, 1954), critical duration and contrast thresholds (Kawamura & Shimowada, 1993), polarization detection (Cameron & Pugh, 1991), and texture discrimination (Bando, 1991). The bluegill sunfish is attractive as a subject because of its attentiveness to visual stimuli and its trainability. It readily holds a position in space until something attracts its attention when it turns swiftly to align its body axis precisely with the stimulus. This ballistic response can be reinforced with food rewards and was used in behavioral perimetry experiments to study the effects of partial ablations of the tectum, eye rotation, and retinotectal compression and expansion (Northmore, 1981; Northmore et al., 1981).

Our main objective was to follow up on a study of visual recovery in bluegills during optic nerve regeneration following optic nerve crush (Northmore & Masino, 1984). It was found that orienting to flashed lights using the regenerated nerve did not appear until nearly 60 days post crush, but once responding started to return, most of the visual field on the crushed side recovered in about three days. This delayed but sudden recovery correlated with the reappearance of ON-OFF activity recorded electrophysiologically in the superficial layers of the tectum. Whereas optic nerve regeneration studies in various fish species have tended to show that behavioral responses to luminance changes appear early in regeneration around the time that optic fibers first reinnervate the tectum, responses that depend on stimulus pattern or location recover later (Weiler, 1966; Hodos & Yolen, 1976; Springer et al.,...
In the process of measuring contrast sensitivity at different spatial frequencies, we derive a full contrast sensitivity function (CSF) for the bluegill sunfish to compare with the spatial resolution data of other aquatic animals that had been surveyed by Muntz (1974). We also compare the cone spacing in the bluegill retina with the high spatial frequency cutoff to see whether the optics of the eye impose a limit. In goldfish and octopus (Northmore & Dvorak, 1979; Sroczynski & Muntz, 1985), the optical cutoff is substantially higher than the behavioral cutoff frequency. Given the likelihood of this mismatch occurring also in bluegills, and given the regularity of their cone mosaic, we wondered whether spatial frequencies above the Nyquist limit might be detectable by aliasing.

Materials and methods

Subjects
Bluegill sunfish (Lepomis macrochirus), ranging from 9.5–14 cm were obtained from Kurtz Fish Hatchery (Elverson, PA). They were fed Purina Trout Chow daily and maintained at 21°C to 25°C and kept on a lighting regime of 14 h of light and 10 h of dark. All procedures were approved by the University of Delaware IACUC.

Optical resolution
Six bluegills, which had not been involved in behavioral experiments, were sacrificed by immersion in a pH neutralized solution of tricaine methanesulfonate (Sigma, St. Louis, MO) and the left and right eyes were removed. The eye that was not immediately used was stored in teleost Ringer solution. The posterior half of the eye was removed by making an equatorial cut with fine scissors. The anterior half with the cornea and lens facing upward was positioned centered over a 6-mm diameter hole in a black Nylon disc. The disc was inserted into a black cylindrical chamber (height 1.5 cm, inner diameter 1.5 cm) with the axes of the cylinder and the eye’s optics aligned. The chamber was filled with teleost Ringer’s solution, and sealed top and bottom with glass coverslips. The chamber was placed on the stage of an inverted microscope with a 10× objective lens (N.A. = 0.25) to view images formed by the anterior half of the eye within the fluid of the chamber. The test object pattern was a high contrast square wave-grating pattern (40 cycles/cm on Kodalith Ortho Film) back illuminated with a tungsten halogen light source. The effective spatial frequency presented to the eye was varied by moving the grating up and down above the eye.

The theoretical resolution of the microscope is given by \( d = \frac{\lambda}{2N_A} \), where \( d \) is the resolution, \( \lambda \) is the wavelength of light and \( N_A \) is the numerical aperture of the objective. For \( \lambda = 560 \text{ nm} \) the microscope objective should theoretically resolve 1.12 \( \mu \text{m} \), which is less than 10% of the cone row spacing (12 \( \mu \text{m} \), see Table 2).

The resolution of the bluegill’s optics was determined by finding the distance of the test grating at which the image viewed through the microscope could be just resolved as bars. Two observers used a method of adjustment of the height of the test grating above the eye in ascending and descending series. The cutoff spatial frequency was calculated from the average of the two series, taking into account the planar air-water interface in front of the cornea. Finally, the lens diameter was measured with calipers.

Receptor mosaic
We examined the photoreceptor mosaic and measured the intercone spacing in fixed histological sections and in unfixed, fresh retina. Four fish were sacrificed as described earlier and the right and left eyes were removed. The eye was measured with calipers and sketched to provide a guide for the orientation of the retina. The anterior part of the eye was carefully cut away from the eyecup with fine scissors and the lens diameter was measured. The eyecups were placed in Bouin fixative for 24 h, embedded in paraffin, sectioned at 7 \( \mu \text{m} \) and stained with Mallory’s trichrome stain.

The receptor mosaic was also studied in the fresh retinas of four bluegills. Fish were dark-adapted for 15–20 h and then light-adapted for 5 h. This procedure facilitated the removal of the pigment epithelium during dissection by producing a partial photomechanical contraction of the cones without excessive return of the pigment epithelium. Fish were then sacrificed by immersion in a solution of tricaine methanesulfonate, and either the right or left eye was removed. Connective tissue was cleaned from the eye with forceps and scissors. The eye was placed, corneal side down, on a wax cup covered by moist Kimwipe tissue at room temperature. Using fine forceps and scissors, the sclera was cut away and the pigmented layer removed by gently brushing it away with a fragment of a Kimwipe held by forceps. Ringer’s solution was used to wash the back of the eye from time to time to aid in this process. The retina was illuminated by light from a fiber optics and photographed with a photomicroscope at 1.25× magnification. The photoreceptor mosaic of the central retina that views laterally was photographed for cone spacing measurement (see Fig. 2).

Behavioral measurement of resolution and contrast sensitivity

Fig. 1 shows the training/testing apparatus. The walls and floor of a 10-gallon aquarium (50 × 29 × 31 cm) were lined with matte green contact paper to eliminate internal reflections except for 9-cm diameter circular windows at both ends of the tank through which the stimuli were presented. High water clarity was ensured by constant filtration between sessions and partial daily renewal. The average water temperature (24°C) and pH (7.2) matched those of the tanks in which the bluegills were housed. Two fluorescent strip lights (15 watts each), positioned outside the tank provided diffuse ambient illumination through the contact paper on the walls to give a mean luminance of 25 cd m² as viewed from within the tank. The luminance of the tank sides was relatively uniform and comparable to that of the oscilloscope screens used to display the grating pattern.

Two oscilloscopes (Tektronix 620) with 10 cm × 12 cm rectangular screens, and a P31 (green) phosphor, were placed one at each end of the tank. Their screens were uniformly illuminated by a raster produced by sawtooth waveforms generated by two function generators (Tektronix, FG 301, FG 503). A stationary, vertical grating pattern with a sinusoidal luminance profile was generated by modulating the beam intensity (Z-axis) of the oscilloscope with a third function generator (Tektronix, FG 503). The space-
The averaged luminance of the screens was determined prior to training using a photometric probe (Alphametrics Model 1010 photometer). These measurements were used to calibrate two fixed photometer probes (see Fig. 1) that continuously monitored the average luminance of each oscilloscope screen during training and testing. Grating contrast \( C \) was determined according to the formula:

\[
C = \frac{(L_{\text{max}} - L_{\text{min}})}{(L_{\text{max}} + L_{\text{min}})} + 2A
\]

where \( L_{\text{max}} \) and \( L_{\text{min}} \) are the luminances of the light and dark bars respectively, and \( A \) is the screen luminance caused by ambient light. From photometer measurements of these three luminances a calibration table was calculated and included in the computer program that collected the data.

A computer controlled (1) the grating contrast via the Z-axis modulation of the displays, (2) rewards from the two worm dispensers, and (3) the fixation light for controlling the fish’s initial orientation. The fixation light consisted of a green LED encased in a white Plexiglas housing of trapezoidal cross section, positioned in the center of either side A or side B of the tank. When the fish was correctly oriented to the fixation light as shown in Fig. 1B, its viewing distance was 24 cm from either screen. Rewards of tubifex worms were delivered by one of the two worm dispensers (Northmore, 1968) positioned over each end of the tank. Water in the dispensers was continually stirred to keep the worms from clumping. A video camera looking down into the tank permitted a top view of the fish on a video monitor. The entire testing apparatus was enclosed to exclude room light.

During training and testing, the experimenter operated a panel with three push switches connected to the computer. Pressing the center switch kept the fixation light on; releasing it caused it to dim out over the course of 3–5 s. The direction of the fish’s turn was signaled to the computer by pressing the right or left switches.

**Training**

Initially fish were trained to swim towards a stationary, vertical, high-contrast sinusoidal grating pattern (spatial frequency = 0.44 c/°, contrast = 0.2 to 0.5, mean luminance = 25.0 cd/m²) presented at either end of the tank. This was accomplished by manually shaping each fish’s behavior by dispensing tubifex worms when it turned towards a grating (Northmore et al., 1981). The appropriate dispenser then dropped a few worms in front of the oscilloscope displaying the grating. Both the intertrial interval and the oscilloscope on which the grating pattern was presented were determined at the discretion of the experimenter.
The second phase, training fish to orient to the fixation light, was begun when fish were turning fully towards gratings and swimming up to the oscilloscope screen. The position of the fixation light at either side A or side B of the tank was randomly changed from session to session. Each fish was shaped by operant conditioning to orient to the fixation LED by aligning its body parallel to the two screens, thereby ensuring that each oscilloscope was seen by only one eye. When the fish held the correct orientation for 5 s, the fixation LED was dimmed out and a high-contrast stimulus grating was presented randomly on one of the oscilloscopes, giving the fish the opportunity to approach the grating for reward. If the fish failed to maintain orientation for 5 s, the fixation LED would be left on until it oriented correctly.

Training continued until the fish reliably oriented to the fixation light and responded promptly to a grating pattern by turning and swimming towards it. A single training session contained 40–60 trials, depending on the behavior of the fish. The training session was terminated if the fish refused to eat or swam erratically. Training continued until a criterion of 90% correct responding was achieved.

*Testing—normal contrast sensitivity*

Once trained, each fish was tested over a range of spatial frequencies (0.1–4.4 c/°) at a mean luminance of 25.0 cd/m². Sine-wave gratings of different contrast or spatial frequency were randomly presented on either the right or the left side of the fish using a Gellerman-type sequence such that no more than three consecutive stimulus presentations occurred on the same side. The location of the fixation light was randomly changed for each testing session. Immediately after the fixation light was turned off, the stimulus grating was presented. To score a correct response and receive reward, the fish had to turn its body parallel to the long axis of the tank and cross a criterion line 3 cm from the window in front of the grating. A turn to the opposite, blank screen, or a partial turn toward the grating was counted as an incorrect response and was not rewarded. The fish’s response was signaled to the computer by the experimenter who did not know the side of the stimulus presentation. After the fish responded correctly, the grating remained on for 2 s; after an incorrect response the grating was turned off immediately. The intertrial interval was determined by how long the fish took to feed and varied from 10–30 s.

To obtain a complete contrast sensitivity function, 12 spatial frequencies ranging from 0.1 to 4.4 c/° were presented. For each spatial frequency the fish underwent four testing sessions consisting of 90 trials. For the first two testing sessions testing began at the lowest spatial frequency (0.1 c/°) and proceeded to the highest spatial frequency (4.4 c/°). Spatial frequencies were presented in the reverse order for the last two testing sessions. Each test session consisted of 60 stimulus grating trials, interleaved with 30 blank trials in which no gratings or rewards were presented. The number of responses on blank trials was used to calculate a chance or false-alarm rate.

In a given test session, gratings at three contrast levels differing by 0.3 log units were presented in random order. The starting contrast level in the first test session was relatively high (0.2–0.5). The lowest contrast level on which the fish obtained the highest percentage correct was used as the highest contrast level for the following three test sessions. Adjustments in the starting contrast level for the first test session for each new spatial frequency were made based on performance at a neighboring spatial frequency.

*Testing—normal cutoff spatial frequency*

To obtain another estimate of the cutoff spatial frequency and to test for aliasing, two additional fish were trained and tested on mainly high spatial frequencies at a fixed contrast of 0.5 and mean luminance of 55.0 cd/m². Because many of the high spatial frequencies were undetectable, trials at lower, detectable spatial frequencies were interposed to prevent extinction of responding. The grating stimuli were presented on either the right or the left side of the fish in a Gellerman-type sequence. The screen not containing the stimulus grating pattern remained illuminated but blank. The fish were tested at spatial frequencies ranging from 0.6–15.1 c/°. Each of a fish’s five testing sessions consisted of 120 trials, involving 8 different spatial frequencies, each presented five times.

*Testing—optic nerve regenerated fish*

In order to generate a longitudinal time course of recovery of contrast sensitivity, three additional fish were trained as mentioned earlier and then underwent right optic nerve crushes. These fish were anesthetized in a solution (0.05%) of tricaine methanesulfonate (Sigma) buffered to neutral pH. After cessation of opcular movements the conjunctival membrane surrounding the dorsal half of the eye was cut. The eyeball was rotated ventrally to expose the optic nerve which then was crushed with fine forceps close to the back of the eye. Interruption of the nerve within the dural sheath was verified visually. Fish were revived by passing fresh water over the gills, and then placed in a tank treated with tetracycline to prevent infection.

The postcrush testing procedure remained as already described with the following exceptions. Three test sessions contained randomly ordered trials at a low (0.1 c/°), medium (1.0 c/°), and high (2.9 c/°) spatial frequency. Thirty test trials per eye and 30 blank trials were randomly presented at each spatial frequency. Thus each session consisted of a total of 270 trials. Fish were tested every other day from 1 day postcrush to 86 days postcrush for two of the fish, and to 85 days postcrush for a third fish. Subsequently, fish were tested on a variable schedule up to 315 days postcrush for two of the fish, and 337 days postcrush for the third fish.

In addition to measuring contrast sensitivity, two informal behavioral tests, the dorsal light reflex (DLR), and the object avoidance response were performed to check that fish were indeed blinded on one side, and also to monitor recovery over time. The DLR test relies on the tendency of fish to tilt toward the more luminous side of an asymmetric radiance field. Following the crush of one optic nerve, fish tilt toward the sighted side, reflecting the imbalance of the two optic nerve signals (Springer et al., 1977). This test was given the day immediately following surgery and before each spatial frequency testing period until tilting was no longer present. It was done in a darkened room after 10 h of dark adaptation by shining a flashlight in front of the fish so as to illuminate both eyes equally. The experimenter drew a line on the tank to indicate the angle of tilt. In the object avoidance test, a 3-cm diameter black plastic cylinder, attached to a rod was presented to the fish. The cylinder was lowered into the fish tank, successively beside the right and left eye. When the cylinder was moved towards the normal eye the fish darted away; when moved toward the operated eye, prior to the return of vision, the fish ignored it.

*Data analysis*

For each spatial frequency the percent correct response was tabulated for each contrast level. The percent correct data was cor-
rected by Abbott’s formula (Finney, 1952) for the rate of chance responding on blank trials. Psychometric functions (i.e., graphs of percent detection vs. log contrast) were generated for each spatial frequency and fitted with normal ogives using probit analysis (Finney, 1952), from which contrast thresholds were determined using a 50% detection criterion, together with 95% confidence intervals.

Results

Optical

Table 1 shows the measurements on the eyes of six bluegills and the resolution of their optics, given as averages of both eyes. The highest spatial frequencies of gratings that could be detected in the images formed by the optics of the eyes ranged from 25.2 c/° to 30.7 c/°, giving an average cutoff frequency of 28.53 c/°. In order to compare these figures with cone spacing, Table 1 also shows minimum separable angles in minutes of arc, given by 60/(cutoff freq. × 2).

Retinal receptor mosaic

Tangential sections of fixed retina confirmed what others (Hirston et al., 1982; Williamson & Keast, 1988; Cameron & Easter, 1993) have described for *Lepomis*: a rosette pattern of single cones surrounded by double cones, as diagrammed on the photomicrograph of the fresh receptor mosaic in Fig. 2. Double cones exhibit a cleft oriented radially with respect to the single cone where the two identical halves are fused along their inner segments. In the bluegill sunfish mosaic double cones form the sides of a rhombus with a single cone at the center.

In the photographs of the receptor mosaic in the unfixed eye (Fig. 2), the most obvious periodic pattern was formed by neighboring rows of double cones as shown by the vertical lines. The mean spacing of such vertical rows was measured in one eye of each of four fish, ranging in standard length from 9.5 to 11.0 cm. and are shown in Table 2. Calculation of morphological acuity from receptor spacing requires an estimate of lens focal length, which is obtainable from Matthiesen’s ratio, the focal length divided by the lens radius. In various teleost fishes Matthiesen ratio ranges from 2.3–2.4 (Charman & Tucker, 1973; Sivak, 1973). Measurements of ocular dimensions made from photographs taken while sectioning frozen eyes of bluegills yielded a Matthiesen ratio of 2.4 (Northmore, unpublished data), which is the value used in Table 2. Minimum separable angle (MSA) in minutes of arc was determined by the arc tan of the double cone row spacing divided by the focal length, and the cutoff spatial frequency predicted from this was calculated as 60/(2 × MSA). The mean MSA was 8.96 min of arc, giving a mean predicted cutoff frequency of 3.36 c/°. If the operative spacing of vertical receptor rows were that of double cones to single cones, the predicted MSA would be 4.48 min of arc with a cutoff spatial frequency of 6.72 c/°.

Psychophysics—normal CSF

Two normal bluegill sunfish (BG25 and BG28) of 12 cm standard length were trained and extensively tested to obtain complete CSFs. Thirteen different spatial frequencies, ranging from 0.1–4.4 c/°, were used to obtain contrast thresholds. There were no significant differences between the thresholds of the left and right eyes (p = 0.55) or between fish (p = 0.17) as determined by a two-way ANOVA (Fish × Eye). The CSFs for the two fish shown in Fig. 3 were obtained by combining the data from the left and right eyes. The bars around the data points show 95% confidence

### Table 1. Optical resolution. Data from combined measurements of both eyes of six bluegills

<table>
<thead>
<tr>
<th></th>
<th>Standard length: head-base of tail (cm)</th>
<th>Eye dia. (mm)</th>
<th>Lens dia. (mm)</th>
<th>Cutoff freq. (c/°)</th>
<th>Minimum separable angle (min of arc)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td>11.3–14.0</td>
<td>10.5–12.3</td>
<td>4.6–7.2</td>
<td>25.2–30.7</td>
<td>0.98–1.19</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>13.10</td>
<td>11.78</td>
<td>5.52</td>
<td>28.53</td>
<td>1.06</td>
</tr>
<tr>
<td><strong>S.E.M.</strong></td>
<td>0.44</td>
<td>0.28</td>
<td>0.50</td>
<td>0.76</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### Table 2. Acuity predicted from morphology. Data from one eye of four bluegills. Assuming Matthiesen ratio = 2.4

<table>
<thead>
<tr>
<th></th>
<th>Lens diameter (mm)</th>
<th>Focal length (mm)</th>
<th>Double cone row spacing (μm)</th>
<th>Min. separable angle (min of arc)</th>
<th>Predicted cutoff (c/°)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td>3.60–4.20</td>
<td>4.32–5.04</td>
<td>11.60–13.00</td>
<td>8.25–9.80</td>
<td>3.06–3.64</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>3.93</td>
<td>4.71</td>
<td>12.24</td>
<td>8.96</td>
<td>3.36</td>
</tr>
<tr>
<td><strong>S.E.M.</strong></td>
<td>0.14</td>
<td>0.17</td>
<td>0.29</td>
<td>0.34</td>
<td>0.13</td>
</tr>
</tbody>
</table>
using a least-squares criterion by adjusting the constants $\alpha$, $\beta$ and $k$, where $s(\nu)$ is contrast sensitivity as a function of spatial frequency, $\nu$ (Campbell et al., 1969). At a mean luminance of 25 cd/m$^2$, contrast sensitivity peaked at 0.37 c/$\circ$ for BG25 and 0.5 c/$\circ$ for BG28. Minimum contrast thresholds were 0.032 for BG25 and 0.030 for BG28. Acuity, in terms of MSA, is the half-period of a just-resolvable grating of unity contrast. It was estimated for each fish by extrapolating its fitted double exponential function to the just resolvable grating of unity contrast. It was estimated for each fish by extrapolating its fitted double exponential function to the just resolvable grating of unity contrast. It was estimated for each fish by extrapolating its fitted double exponential function to the just resolvable grating of unity contrast. It was estimated for each fish by extrapolating its fitted double exponential function to the just resolvable grating of unity contrast.

Contrast sensitivity functions for two normal bluegill sunfish (BG 25, BG 28). Data points show mean contrast sensitivity with 95% confidence intervals. The curve was fitted to the data as described in the text.

Fig. 3. Contrast sensitivity functions for two normal bluegill sunfish (BG 25, BG 28). Data points show mean contrast sensitivity with 95% confidence intervals. The curve was fitted to the data as described in the text. intervals. The data points were fitted to a double exponential function of the form

$$s(\nu) = k(e^{-2\pi\alpha\nu} - e^{-2\pi\beta\nu})$$

using a least-squares criterion by adjusting the constants $\alpha$, $\beta$ and $k$, where $s(\nu)$ is contrast sensitivity as a function of spatial frequency, $\nu$ (Campbell et al., 1969). At a mean luminance of 25 cd/m$^2$, contrast sensitivity peaked at 0.37 c/$\circ$ for BG25 and 0.5 c/$\circ$ for BG28. Minimum contrast thresholds were 0.032 for BG25 and 0.030 for BG28. Acuity, in terms of MSA, is the half-period of a just-resolvable grating of unity contrast. It was estimated for each fish by extrapolating its fitted double exponential function to the spatial frequency axis. The cutoff spatial frequencies for BG25 and BG28 respectively were 5.5 and 5.0 c/$\circ$, yielding MSAs of 5.45 and 5.0 min of arc.

Psychophysics—normal cutoff spatial frequency

Two additional fish (BG1 and BG2) of 13 and 11 cm standard length were trained to orient to gratings of a constant high contrast (=0.5) that varied in spatial frequency from trial to trial. Fig. 4 shows their performance in terms of percent correct as spatial frequency varied from 0.6–15.1 c/$\circ$, three times the cutoff frequency of the fish that yielded complete CSFs. The highest spatial frequency meeting a 75% correct responding criterion was 3–4 c/$\circ$ for these two fish, which is close to the values predicted from the fitted curves of Fig. 3 (See also Fig. 9).

Because the optics of the bluegill sunfish eye pass spatial frequencies up to at least 32 c/$\circ$, and because these fish possess a regular retinal mosaic, it is possible that gratings above the Nyquist limit might be detected by aliasing. If this were so, high spatial frequency gratings would appear as beat frequencies in a range of 4.0–12.5 c/$\circ$ (see Fig. 4). However, there was no significant detection of high contrast gratings in that frequency band. A Z-test showed that the level of responding below 63% was attributable to chance ($p = 0.01$).

Psychophysics—optic nerve regeneration

Prior to optic nerve crush, three trained fish (BG24, BG30, and BG31) (10–12 cm standard length) were tested for five days at a low (0.15 c/$\circ$), a medium (1.0 c/$\circ$), and a high (2.93 c/$\circ$) spatial frequency to determine contrast thresholds for the left and right eyes. Contrast sensitivities at the three spatial frequencies tested were comparable to the values obtained for BG25 and BG28. A two-way ANOVA (Fish X Eye) on the data from BG24, BG30, and BG31 at the three spatial frequencies tested showed that preoperatively, there were no significant differences in contrast sensitivity between fish ($p = 0.99$) and none between left and right eyes ($p = 0.61$). The highest contrast sensitivity was again found at the middle spatial frequency of 1.0 c/$\circ$.

Following a right optic nerve crush in the three fish, visual recovery was monitored by noting (1) the degrees of tilt of the dorsal light reflex (DLR), (2) the reappearance of object avoidance, and (3) the first orientations to gratings in the testing apparatus. Fig. 5 shows that in all three fish the DLR diminished before the first grating orientations using the right eye. The DLR, as measured by the tilt away from 90$\circ$, decreased over time from 10$\circ$ to 0$\circ$, starting from 40–66 days postcrush. In all three fish,
object avoidance returned on the same day that the first grating orientations occurred: Day 63 for BG24 and Day 69 for BG30 and BG31. As seen in Fig. 5, responding to gratings first reappeared at the low and middle spatial frequencies, and later for the high spatial frequency. The time from optic nerve crush to the return of vision ranged from 63 days for one fish at the lowest spatial frequency to 70 days for another fish at the highest spatial frequency.

The longitudinal plots of Figs. 6, 7, and 8 shows the recovery of contrast sensitivity at the low, middle and high spatial frequencies for each fish and for each eye separately. A moving average of three consecutive contrast sensitivity values smoothed the data. In order to compare sensitivities with the regenerating right optic nerve to the normal side, the mean and standard deviation of the log contrast sensitivity of the left eye during the regeneration period is also shown in the longitudinal plots as horizontal lines. The onset of visual orienting with the operated eye after a long period of no responding was sudden rather than gradual, and contrast sensitivity was not measurable prior to day 64 in BG24 and day 69 in BG30 and BG31. Full recovery was considered to have been achieved if contrast sensitivity rose above one standard deviation below mean contrast sensitivity.

At the low spatial frequency (0.15 c/°) all fish recovered full sensitivity with the regenerate right eye very quickly and maintained stable values for that eye. At the middle spatial frequency (1.0 c/°) full recovery of contrast sensitivity was sudden for BG31 and more gradual for BG24 and BG30. At the high spatial frequency (2.93 c/°) full recovery of contrast sensitivity occurred for only one fish (BG24) and at later time (day 82).

**Discussion**

**Contrast sensitivity functions**

Fig. 9 shows the bluegill CSF together with those of other species. Compared to the goldfish curve (Northmore & Dvorak, 1979), which was obtained by a classical conditioning method, the bluegill curve is an almost exact replica shifted to higher spatial frequencies by a factor of 1.51. It is also a good match to the shape of the CSFs of the other species shown (see Uhlrich et al., 1981). The relatively low contrast sensitivity of the two fish curves are likely the result of the particular psychophysical methods and criteria employed. Bilotta and Powers, (1991) using an observer-based, two-alternative forced choice technique on goldfish obtained a minimum contrast threshold of 0.01 compared to approximately 0.05 for our goldfish and bluegill results. Hawryshyn et al. (1988) also using classical conditioning obtained contrast thresholds in bluegills of less than 0.01 with stimuli presented frontally. They noted that lateral stimulation required higher contrasts for conditioning to occur. The location of the bluegill CSF at the low end of the spatial frequency axis, peaking as it does at about 0.4 c/°, probably represents an adaptation to typical freshwater environments where images of underwater objects are blurred, especially at a distance (Guthrie & Muntz, 1993). Given a limited spatial frequency bandwidth that all animal visual systems are subject to (Uhlrich et al., 1981), the bluegill and goldfish visual systems have specialized in detecting low spatial frequencies where they presumably obtain most of the important visual information for their activities.

**Optics and acuity**

A direct examination of the images formed by the optics of bluegill eyes gave cutoff spatial frequencies ranging from 25 to 31 c/°, corresponding to MSAs of 0.98’ to 1.19’. This could be a high estimate of MSA because the theoretical resolution of the microscope was 0.77’ at 560 nm (see Materials and methods), and its actual resolution with white light would have been worse. Nevertheless, these results show that the optics of the bluegill eye is capable of higher resolution than that expected of the retinal cone mosaic (4.5–9’) or of the animal as a whole (~5’). The same was found to be true of goldfish whose optical MSA is 3–4’ (Charman & Tucker, 1973), its retinal morphological MSA is 10–16’, whereas its behavioral MSA ranges from 9–18’ in different studies (Northmore & Dvorak, 1979; Bilotta & Powers, 1991; Neumeyer, 2003). In octopuses, too, lens resolution (~1’”) (Sroczynski & Muntz, 1985) tend to be better than behavioral resolution (~10’), but not as good as receptor spacing (~12’”) (Muntz & Gwyther, 1988).

**Receptor mosaic and acuity**

To estimate morphological acuity, measurements were made on the retinas of fresh, essentially intact bluegill eyes (Fig. 2). The regular retinal mosaic pattern of a single cone surrounded by double cones is typical of retinas in highly visual teleosts (Wagner, 1990) and the same as that previously described for two species of *Lepomis* (Williamson & Keast, 1988; Cameron & Easter, 1993). Because the double cones contain a long-wavelength pigment ($\lambda_{\text{max}} = 620 \text{ nm}$) and the single cones a mid-wavelength pigment ($\lambda_{\text{max}} = 536 \text{ nm}$) (Hawryshyn et al., 1988), our acuity measurements using the yellow-green P31 phosphor (510–590 nm half max) might be expected to reflect mainly single cone spacing. However, at the luminance levels studied, the double cones are almost certainly engaged too. Indeed, Neumeyer (2003) found that acuity in goldfish was unaffected by grating wavelength over mid spectral regions (446–683 nm) and was no worse than with white-light gratings. We would not, therefore, expect that the acuity we obtained with the P31 phosphor to be significantly different from that obtained with stimuli generated with other mid-spectrum, broad-band lights.

To our eyes, the most salient periodicity was formed by the vertical rows of double cones and this spacing is what we measured (average 12.24 µm) (Table 2). If these rows were detecting the stimulus gratings on the Helmholtzian principle of one unstimulated cone between two stimulated cones, as discussed by Muntz
Fig. 6. Recovery of contrast sensitivity of BG24 at spatial frequencies of 0.15, 1.00 and 2.93 c/°, as a function of days postcrush, using a moving average of 3 consecutive threshold determinations. Precrush data are shown on the left; postcrush on the right, for the normal, left eye (filled squares) and the operated right eye (open triangles). Horizontal solid lines show the mean normal eye sensitivity averaged over the postcrush period; dashed lines ± 1 SD.
Fig. 7. Recovery of contrast sensitivity of BG30. See legend of Fig. 6.
Fig. 8. Recovery of contrast sensitivity of BG31. See legend of Fig. 6.
(1974), they would predict an MSA of 8.96 min. of arc and a cutoff spatial frequency of 3.36 c/°; if the alternate rows of double and single cones were responsible for detection, half this MSA and twice the spatial frequency cutoff would be predicted. The psychophysically measured values for the two normal bluegills (BG25 and BG28) gave an averaged MSA of 5.23 min of arc and a spatial frequency cutoff of 5.25 c/°—values between the double-to-double and double-to-single predictions. Reviewing several studies on various fish species, Douglas and Hawryshyn (1990) conclude that behavioral and morphological acuities are close, although in many cases behavioral acuities are poorer than morphological acuities. However adjustments in procedure, lowering threshold criteria, and increasing adaptation level can push behavioral acuities beyond that predicted by the cone spacing (Penzlin & Stubbe, 1977; Bilotta & Powers, 1991). As Muntz (1974) pointed out, the Helmholtzian principle need not necessarily hold, as processing subsequent to the receptors could have an important bearing on acuity.

The functional significance of regular cone mosaics is not understood, although they have been hypothesized to assist the detection of motion (Lyall, 1957; Wagner, 1990) and polarized light (Cameron & Pugh, 1991; Cameron & Easter, 1993). A possible drawback to the possession of regular cone mosaics in conjunction with optics capable of imaging spatial frequencies considerably finer than the cone mosaic is that fish like bluegills might be prone to nonveridical perception by aliasing (Wagner, 1990). High spatial frequencies imaged onto the regular mosaic will generate a beat pattern of receptor illumination. In Fig. 4, we indicate ranges of high spatial frequencies that when imaged onto a regular square mosaic of 12 microns pitch would produce beat spatial frequencies that would be detectable below the behavioral cutoff of about 5 c/°. However, bluegills performed at chance level at spatial frequencies in the range of 4.0–12.5 c/° (Fig. 4). It is probable that the pooling of receptor signals within the retina acts as a low-pass, antialiasing filter.

**Contrast sensitivity during optic nerve regeneration**

Previous studies of the recovery of vision during optic nerve regeneration in fish have observed that different visual functions return at different times (Springer & Agranoff, 1977; Edwards et al., 1981). In the present experiments, three different tests of visual recovery were given at regular intervals after the right optic nerve was crushed. The exaggerated DLR started to recover first between 40 and 66 days (Fig. 5). Object avoidance and grating orientation appeared at the same time between 63 and 69 days postcrush, after which recovery at low and mid spatial frequencies occurred rapidly. Recovery of sensitivity to the high spatial frequency (2.93 c/°) occurred more slowly in all three fish, and incompletely in one.

From various electrophysiological studies of the regenerating retinotectal system in goldfish and sunfish, it is possible to discern two distinct phases of visual recovery (for reviews see Northmore & Celenza, 1992; Northmore & Oh, 2001). The first phase involves the reconnection of a dimming or OFF system that begins as soon as regenerating optic fibers invade the optic tectum, characterized by a gradual refinement of topography and a progressive strengthening of OFF visual responses recorded in the optic tectum. The second, phase occurs rather suddenly as both ON and OFF responding reappear in the superficial layers of tectum with nearly normal retinotopography and rapidly sharpening re-

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**Fig. 9.** Cross-species comparisons of contrast sensitivity functions. Goldfish: Northmore and Dvorak (1979); cat: Bisti and Maffei (1974); macaque & human: DeValois et al. (1974). The Xs are the averaged data of BG25 and BG28. The open circles are averaged from the normal eye data of BG24, BG30 and BG31 which received unilateral optic nerve crush. The open square is the averaged data from BG1 and BG2 (75% correct criterion. See Fig. 4).
ceptive fields. A behavioral correlate of first-phase recovery seen in this experiment is the early recovery of the DLR, a behavior which does not depend directly on tectum (Springer et al., 1977) but involves a photometric input from the retina, possibly served by the dimming pathways (Gibbs & Northmore, 1996) that recover early in regeneration (Northmore & Oh, 2001). The delayed but sudden recovery of visual avoidance and orienting to gratings found here is very likely associated with the second phase. Long delayed (32–56 days) but sudden recovery of visual orienting was found by Northmore and Masino (1984) in Lepomis after unilateral optic nerve crush. In those experiments visual orienting was correlated with the return of ON/OFF responding in superficial tectum. We presume that it is only in the second stage that the retinotopic mapping is precise enough, and the multiunit receptive fields sharp enough to allow the discrimination of grating patterns, at least for low and mid spatial frequencies.

The delayed and incomplete recovery of sensitivity to high spatial frequencies in the bluegill echoes the results of Weiler’s (1966) study on the cichlid, Astronotus ocellatus. She found that at longer times after optic nerve section than we studied, visual acuity recovered only to 78% of normal. It may be that resolution of fine detail is critically dependent on the fine-scale integrity of the retinotectal map. In goldfish, disorder in the map and sub-normal densities of retinotectal synapses persist for months after optic nerve crush (Murray & Edwards, 1982; Cook & Rankin, 1986). Interestingly, the detection of low and medium spatial frequencies is not susceptible to these abnormalities, perhaps because it depends upon triggering activity in the appropriate types of retinal ganglion cells whose activity can be effectively transmitted by suboptimal retinotectal pathways.

Conclusion

The results show that the psychophysically-determined high spatial frequency cutoff of the bluegill sunfish is (1) not limited by the optics; (2) is comparable to the grain of the retinal mosaic; and (3) depends on the precision and reliability of retinotectal connections, the latter having much less influence on contrast sensitivity at low to medium spatial frequencies.

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References


