

## OPTOKINETIC TESTING OF CYCLOPEAN AND SYNOPHTHALMIC FISH HATCHLINGS<sup>1</sup>

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Previous work on *Fundulus heteroclitus* embryos that were perfect cyclopeans, owing to treatment with ethyl alcohol, indicated that there is a strong tendency for the right side of the eye to function developmentally as a right eye would, in sending optic fibers to the left side of the brain, and for the left side of the eye to send fibers to the right side of the brain (Rogers, 1952). It seemed of interest to determine whether the right side of the cyclopean eye functions physiologically as a right eye would, and the left side, as a left eye. Optokinetic drum testing of optically abnormal amphibia (Sperry, 1944) suggested a means of making such tests. Even if positive optokinetic drum results were obtained with perfect cyclopean fish, it would be impossible to eliminate only one side of the retina surgically without damage to the other, but it might be possible to ablate one optic tectum to learn something further of the pathways involved. Nerve-stained sections could then be used to correlate the microscopic anatomy with the physiological results.

Pearcy and Koppanyi (1924) moved the left eye of a large goldfish to an artificial orbit in the top of the cranium, just to the left of the midline, without severing the nerve. The effect on equilibrium was noted and vision tested during four weeks. They stated that they had produced a real "experimental cyclops." This eye, however, has few of the characteristics of the cyclopean eye. It is not formed from the medullary plate material on both sides of the midline, it is not in the typical ventral position, and it did not develop its nerve connections while it was in close relation to the diencephalic floor on both sides of the midline.

Stockard (1909, p. 285) says in regard to cyclopean *Fundulus heteroclitus* treated with magnesium chloride solutions in the early stages: "Many embryos, showing the cyclopean defect in various degrees, hatched normally and were capable of swimming in a manner indistinguishable from ordinary two-eyed fish. These monsters gave many indications of ability to see. They went to the more brilliantly lighted side of the dish with the normal ones. They darted away in normal fashion when any object was placed in front of the eye, while similar objects put at equal distances from their tails caused no excitement." There appear to be no other reports in the literature of attempts to test cyclopean individuals for vision.

### MATERIALS AND METHODS

In the 1955 season 16,987 *Fundulus heteroclitus* eggs that successfully passed the early cleavage stages were treated with magnesium chloride solutions. Of

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11,912 embryos surviving at least until the eyes were well formed, 0.3% were perfect cyclopeans with an eye of close to normal size, and only three such embryos hatched (Rogers, 1956). In the 1956 season 190 bowls containing somewhat larger numbers of eggs than in the 1955 work were treated in the same manner with magnesium chloride. An estimate was made that about 25,000 embryos survived until the eyes were well formed, 76 embryos (about 0.3%) were perfect cyclopeans with an eye close to normal size, and again only three such embryos hatched and swam. In 1955 the effect of temperatures between 11° and 16° C. was not tested. In 1956 water in bowls kept on the water table between June 6th and 12th was never below 15° or above 16° C. At this temperature seven bowls of 19/60 M MgCl<sub>2</sub> solution, with a total of 1,020 eggs that were living when they reached the blastula stage, produced 7% optic abnormalities among the 560 embryos that survived until the eyes were formed. All 12 cyclopean embryos among them had reduced eyes and stunted bodies. Although the mortality rate rose to 45%, as compared with 20% in the 1955 series at 18° C., production of optic abnormalities was increased by only 1%.

In the material just described, three cyclopeans, three synophthalmics, and one anophthalmic in the 1955 series, and three cyclopeans and one synophthalmic in the 1956 series hatched and swam well enough to be tested in the optokinetic drum. In addition, from batches of *Lucania parva* eggs fertilized with *Fundulus heteroclitus* sperm, one perfect cyclopean in 1955, and another in 1956, hatched and were tested. At first, a very large striped drum was tried, mounted on a horizontally-placed bicycle wheel revolving around a stationary central platform to hold the dishes of fish. Normal adults responded only moderately well, and hatchlings, not at all. Feeling that the hatchlings might not be affected by movement at such a distance, Mr. Michael Baron, helping with the technical work in 1955, lined an oatmeal carton only 10.5 cm. in diameter with vertical black and white stripes of one-half centimeter to one centimeter in width. When this drum was turned smoothly by hand around a small beaker containing normal hatchlings, the fish readily turned with the drum and quickly reversed directions when the drum was reversed. The fish were kept 6 to 10 days after hatching, and tested a number of times. When most of their yolk was used up, selected ones were drawn with camera lucida while under urethane anaesthesia, and all were fixed, sectioned, and stained with a modification of the Bodian protargol stain in the manner previously described (Rogers, 1952).

#### RESULTS OF OPTOKINETIC TESTS

Synophthalmic fish 89-1 (bowl number—individual fish number) and 89-2 (Fig. 4) responded directionally to drum rotation in the same manner as normal control hatchlings. Fish 89-1 did not start to turn with the drum as readily, and did not turn as rapidly as control fish, but otherwise exhibited normal optokinetic reflexes. Fish 89-2 responded nearly as well as controls. Synophthalmic fish 341-1, similar in appearance to the preceding two, did not respond to the drum, although it frequently swam at times when there were no apparent external stimuli. The unusual synophthalmic fish with right eye much reduced, 76-5 (Fig. 5), was slightly but permanently flexed toward its left. It did not orient well and often lay on its back on the bottom of the dish. Nevertheless, in any orientation, it



could always be made to flex vigorously and repeatedly toward its own left with drum rotation in the *opposite* direction, whereas no movement could be elicited by drum rotation to its left. If nerve connections of such an eye are contralateral, as they normally are, the animal ought to flex or turn readily in the same direction as the drum when the drum is rotated toward the blind side, and turn hesitantly or not at all when the drum is rotated toward the seeing side.

The cyclopean "magnesium" fish 89-3, 105-1 (Fig. 1), 105-2, 340-2, 340-3, and 360-1, the cyclopean *Lucania-Fundulus* hybrids 263-1 (Figs. 2 and 3) and 478-1, and the anophthalmic "magnesium" fish 109-1 all failed to respond to any drum stimulation tried. Various tests were made on all these fish, but hatchling 105-1 was tested more extensively than the others, over a period of five days. Drums of various sizes with various widths of striping and turned at various speeds under various lighting conditions were used. The fish were sometimes positioned very close to the beaker side and the drum rotated so that its surface passed just outside the glass and very near the fish. Black-and-white, and red-and-white stripes, striped cones revolving under as well as around the beaker, and flat discs with alternate opaque cardboard and cut-away strips radiating from a central hub and rotated over a light bulb with the fish just above the disc, were also tried with uniformly negative results. Any movements on the part of the fish appeared to be fortuitous and unrelated to the drum rotation. When testing was not being carried on, the anophthalmic 109-1 appeared to swim around the dish at more frequent intervals than normal control fish.

The failure of the perfect cyclopean fish to respond to the drum in 1955 led to the second season's work with the hope of obtaining positive results or of making the negative results more certain. The lack of response also made of prime importance the question as to whether the perfect cyclopean fish can see at all. Unfortunately, all tests so far tried have failed to give rigorous proof. "Baiting" the fish from outside a glass container (Sperry, 1949) appears to be impossible with these hatchlings. Numerous attempts to observe the effect of a differentially lighted container (Stockard, 1909) failed to yield unequivocal results in the present work. The chromatophore response to visually perceived light in adult *Fundulus* (Butcher, 1938) was considered, but light does not have an obvious effect on the chromatophores of normal hatchlings. Potential-recording methods seem impractical with these small fish. Fish 89-3 was placed in a glass container within the large drum mounted on the bicycle wheel, and the drum rotated for five-minute periods with three- to four-inch-wide black-and-white stripes visible, and then for similar periods with the stripes covered by a blank white paper. The number of times the fish swam, and the total length of time in seconds it was swimming, were recorded. A number of tests made it clear that these data were too variable to afford rigorous proof of ability to see. The only evidence obtained consisted of the apparent ability of perfect cyclopeans 89-3, 105-1, and 105-2 to localize the tip of a fine forceps when it was placed in front of them. These observations were not considered conclusive and therefore no attempt was made to repeat them on the perfect cyclopeans of the second season. Fish 105-1 responded 5 or 6 times in succession to a forceps placed close in front of it, by jerking quickly away. On the two succeeding tries, however, the fish slowly and deliberately came off the bottom and moved forward, accurately placing its tubular mouth against the forceps.



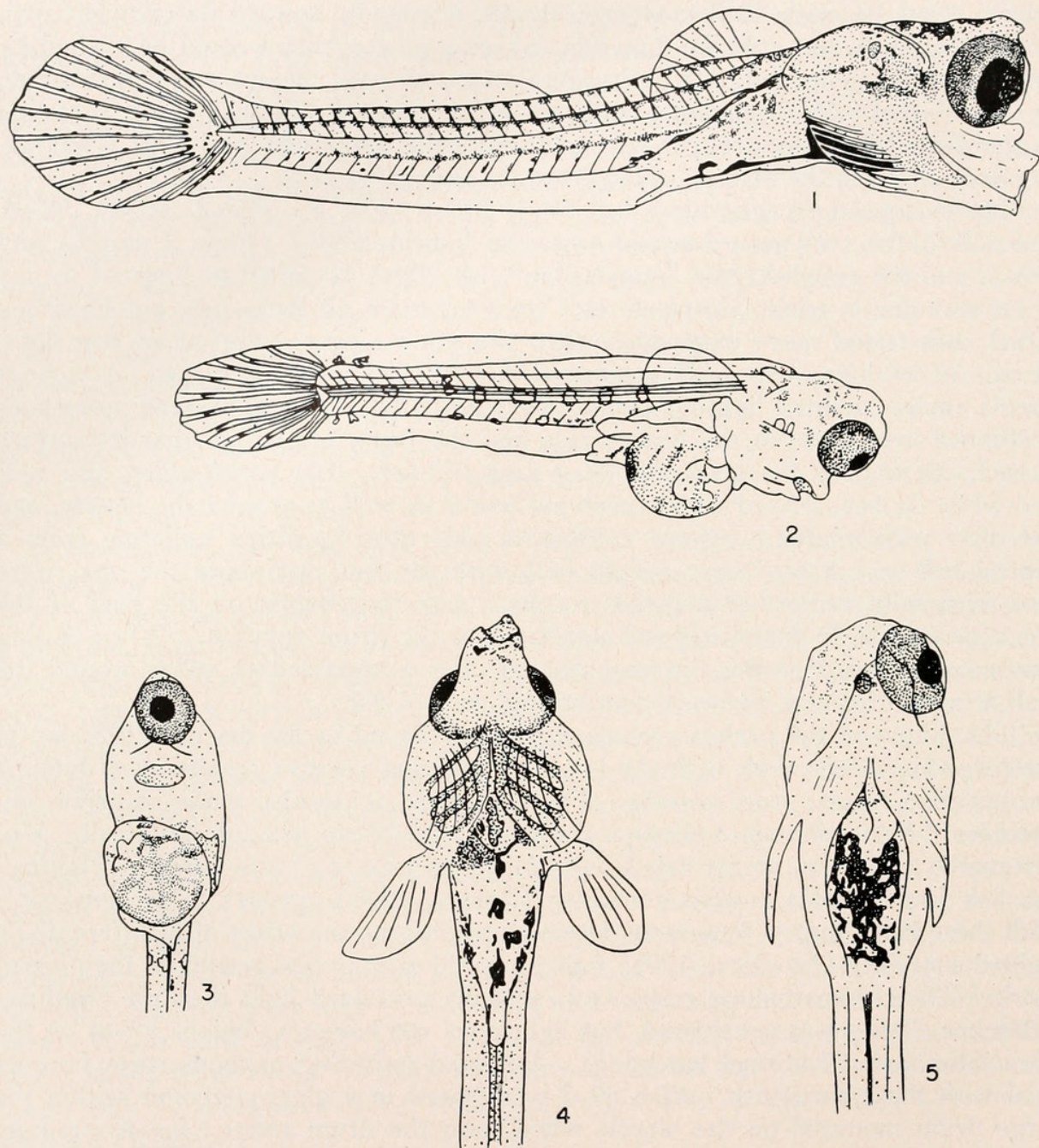


FIGURE 1. View of right side of cyclopoean *Fundulus heteroclitus* hatchling 105-1. The anterior part of the body is flexed slightly to the right to show the eye. Drawn with camera lucida, urethane anaesthesia,  $\times 20$ .

FIGURE 2. View of right side of cyclopoean *Lucania parva*—*Fundulus heteroclitus* 263-1. Chromatophores are opaquely white rather than black as in *Fundulus*.  $\times 20$ .

FIGURE 3. Ventral view of anterior part of 263-1,  $\times 20$ .

FIGURE 4. Ventral view of anterior part of synophthalmic *Fundulus heteroclitus* 89-2,  $\times 20$ .

FIGURE 5. Ventral view of anterior part of *Fundulus heteroclitus* 76-5,  $\times 20$ .

It ignored the forceps when the latter was placed the same distance from its tail. When fish 105-2 had exhausted its yolk and become fairly inactive, it repeatedly exhibited following eye movements in the proper direction as a forceps tip was



moved across its front. Fish 89-3 responded to the forceps in front of it by moving away. All of these responses could conceivably be initiated by movement of water created by the forceps, but particularly in the case of the immobile forceps that was approached and accurately localized by fish 105-1, visual cues would seem to be a more likely explanation.

#### HISTOLOGICAL CORRELATIONS

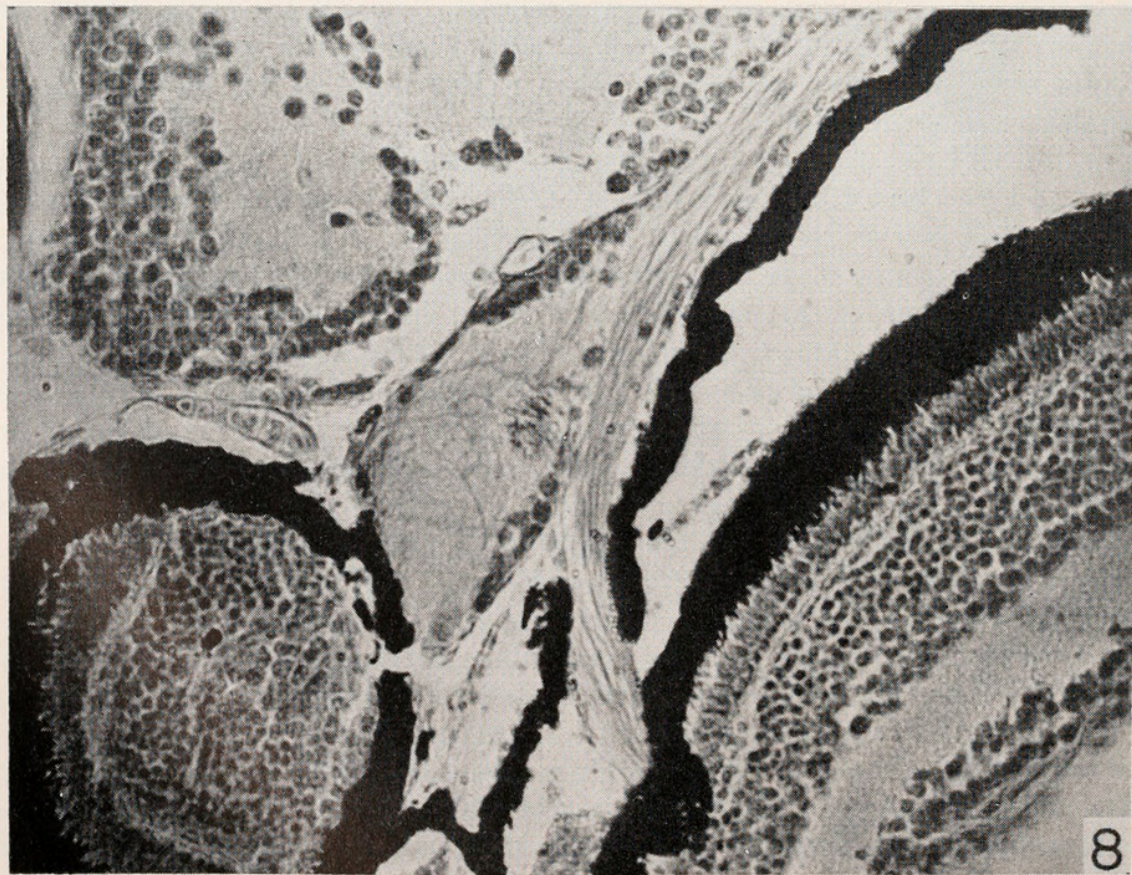
Synophthalmic fish 89-1, 89-2, and 341-1 each have two pupils and lenses, two arcs of sensory retina and pigment epithelium, and nerve bundles collecting from each retinal arc and passing out from the retina medially between the two arcs. Fish 341-1 has two choroid sacs and lacks an extensive fused area between the eyes, but 89-1 and 89-2 each have a single choroid sac enclosing the retinas, and a ventro-medial continuity of the sensory retinas (Fig. 6). The optic nerves in 89-1 and 89-2 pass posteriorly through the choroid coat in a single bundle and, possibly with a complete decussation, enter the floor of the diencephalon and proceed dorsally in two fairly equal bundles to distribute to the two optic tecta. Nerve fibers are so numerous, and they run parallel so far before turning up into the brain, that it is difficult to follow specific groups of fibers certainly enough to be sure there is complete decussation. The optic nerve in 341-1 collects from the retinal arcs and leaves the eyes in much the same manner, but after entering the diencephalic floor, it turns abruptly anteriorly and distributes to the telencephalon on the right side only. Fish 76-5 has a left eye that is fairly normal in form but that is displaced antero-medially over a cyclopean-type tubular mouth. The right eye consists of a small mass of sensory retinal cells, completely surrounded by a layer of pigment epithelium, and lacking a nerve. The left eye sends a large nerve entirely to the left side of the brain (Fig. 8). Part of this nerve passes into the left optic tectum and part passes anteriorly to distribute in the dorsal part of the diencephalon on the left side.

The form of the cyclopean eye is well known (Fig. 7). The eyes of perfect cyclopeans 105-1 and 105-2 each have a stalk similar to that of a normal eye. In both these fish, nerve fibers collect from both sides of the retina, leave the eye posteriorly, just behind a ventral retinal gap interpreted as the remains of a choroid fissure, run together in the optic nerve, sort out in the base of the diencephalon with a possible decussation, and distribute about equally to the two tecta.

Cyclopean fish 89-3 has no optic stalk, but instead the eye is continuous with the diencephalic floor over a wide area, the pigment epithelium appearing as a reflection of the diencephalic surface. The optic nerve collects from both sides of the retina but goes only to the right tectum. The eyes of the two hybrid cyclopeans, 263-1 and 478-1, have the same close relation to the diencephalon, with the stalk lacking, but in these cases the nerve distributes to both tecta. In 263-1 three-fourths of the optic fibers go to the left tectum and the remainder to the right. As they enter the diencephalon, some of these fibers certainly decussate but others do not. In 478-1 the fibers collect in a bundle and run parallel to each other for some distance before leaving the eye so that it is hard to tell whether they decussate, but about half go to each tectum after they sort out in the base of the brain.

The cyclopean eyes of 340-2, 340-3, and 360-1 lack optic stalks and are even more closely related to the diencephalon than the preceding cases, in that the sensory







retina projects posteriorly well into the third ventricle, instead of simply being continuous with the diencephalic wall. The pigment epithelium, however, is again a reflection of the diencephalic wall. In 340-2 the optic fibers clearly decussate as they leave the retinal tissue, with a large bundle from the right side of the eye going to the left tectum and a bundle half as large from the left side of the eye going to the right tectum. Fish 340-3 also has a large fiber bundle going to the left tectum and a small bundle to the right tectum, but fiber paths as they leave the eye cannot be followed clearly enough to see whether they decussate. All of the optic fibers of 360-1 go to the left tectum.

Anophthalmic fish 109-1 has no eye tissue identifiable in section.

### DISCUSSION

The two synophthalmic fish that responded directionally to the optokinetic drum in the same manner as control fish were found to have optic nerves that distribute equally to the two optic tecta after decussating at least in part, and possibly entirely. The synophthalmic fish of similar external appearance that failed to respond to the drum was found to have optic fibers distributing solely to the telencephalon on one side, and was probably blind.

The failure of the perfect cyclopean fish to respond to the drum is harder to explain. Until the histological sections of the first season were studied, it was thought possible that in the four cases tested the optic nerve fibers from each side of the eye just happened to distribute about equally to both optic tecta. Horizontal optokinetic reflexes might then be cancelled out. This cannot be the explanation, however, because two of the total of eight perfect cyclopean fish tested have optic nerve fibers distributing to the optic tectum of one side only. No other explanation for the failure to respond is apparent. A right or left eye, even when displaced far toward the midline (fish 76-5), will send impulses that will initiate reflexes in response to the drum. Therefore, the two pupils or two separate retinal arcs of the typical synophthalmic condition are not requisite for the response. There is slight evidence that the cyclopean fish can see objects placed close in front of them. Comparison of their behavior with that of anophthalmics when objects are introduced near them, leads to the belief that the cyclopeans can see, but does not afford rigorous proof. If they can see, the failure to respond to the drum remains perplexing.

The individual with only one functional eye in the synophthalmic position and nerve distributing only homolaterally, responded directionally to a rotating drum in the same way as one-eyed anurans in which the regenerating optic nerve had been forced to grow to the homolateral optic centers (Sperry, 1945). Thus, Sperry's results in a regenerative situation are extended to a primary developmental situation.

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All figures are photomicrographs of Bodian preparations.

FIGURE 6. Transverse section through synophthalmic eyes of fish 89-2. Note ventral fusion of sensory retinal arcs.  $\times 115$ .

FIGURE 7. Transverse section through perfect cyclopean eye of fish 105-1. Nasal pits are above, tubular mouth below.  $\times 115$ .

FIGURE 8. Transverse section of portion of eyes of fish 76-5. The large left eye sends a normal-sized nerve to the left side of the brain.  $\times 800$ .



## SUMMARY

1. Perfect cyclopean, closely synophthalmic, and anophthalmic fish hatchlings were obtained by magnesium chloride treatment or by hybridization.
2. Synophthalmic fish, with distribution of optic nerve fibers generally similar to controls, responded essentially normally to a horizontally rotating optokinetic drum.
3. A synophthalmic fish, with optic fibers distributing to the telencephalon of one side, failed to respond to the drum.
4. A fish with only one functional eye in the synophthalmic position, and optic fibers distributing entirely homolaterally, responded in the *opposite* direction when the drum was rotated toward the blind side.
5. Although there was other, somewhat inconclusive, evidence that they could see, eight perfect cyclopean fish failed to respond to a horizontally rotating drum.

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