ly three peaks of light sensitivity in an insect system. In *Megoura viciae* (*7*) there was no evidence of a circadian rhythm, and in *Carpocapsa pomonella* maintained in an LD 8 : 64 cycle there was evidence of a complex timing system that involved two conceptually different clocks: an hour-glass timer associated with "dusk" and a 24-hour rhythm associated with "dawn" (*10*). The 72-hour experiment also removes the objection that each peak represents the result of a direct interaction between the pulse and the main light component. In other words, the middle peak is too far removed from either the preceding or following light periods to be caused by a direct interaction; the nearest effective light signals are those at the other peaks, both 24 hours away.

Since it was possible that the apparent 24-hour rhythm was generated in some way by the choice of light : dark cycles, which both included a 12-hour main component, two further experiments were carried out with 48-hour cycles that contained a 10-hour and a 14-hour main light component, respectively (Fig. 1). In these experiments the females were exposed to LD 10 : 14 and LD 14 : 10 for four cycles before being released into the longer regimens. Both of these experiments revealed maximums of photoperiodic effect at Zt 19 and, in the LD 10 : 38 experiment, a second maximum at Zt 43. This showed that the positions of the peaks were unaffected by the duration of the main light component. It is not known why the amplitude of the first peak is greater in the experiment with the 14-hour main light component.

In a 24-hour light : dark cycle two peaks of diapause inhibition are observed in *N. vitripennis* (*11*) and other insects (*12*) when the night is scanned by light interruptions. In these insects the first peak (A) is always a certain number of hours after lights-on ("dawn") and the second peak (B) always the same number of hours before lights-off (dusk). These observations form the basis of the coincidence model for the photoperiodic clock (*13*). This model is essentially a rhythm of sensitivity to light which is itself phase-set by the light : dark cycle, and has the same properties as overt circadian rhythms such as pupal eclosion in *Drosophila* or oviposition in the moth *Pectinophora gossypiella* (*13, 14*). The crux of this model is the dual action of light: (i) as an entraining agent and (ii) as an inducer when the light coincides with the photoperiodically inducible phase. According to Pittendrigh (*14*), pulses of light placed at both A and B act as entraining agents, but only one (at either A or B) coincides with the inducible phase.

In the present experiments the first maximum of photoperiodic effect had the same relationship to the beginning of the main light component in each of the regimens tested. For this reason it must be equivalent to a peak A in a 24-hour cycle (Fig. 3). The later peaks must therefore represent this phase of the rhythm repeating itself in the night with circadian frequency. In *N. vitripennis* maintained in 24-hour night interruption experiments, peak A occurs 15 to 16 hours after lights-on (*11*). The fact that it is 19 hours after lights-on in 48- and 72-hour cycles must indicate that the photoperiodic rhythm assumes a different phase relationship with a 48-hour or 72-hour Zeitgeber. The last peak in the night is not considered to be equivalent to peak B because it is 15 hours before lights-off in the LD 10 : 38 cycle and 17 hours before lights-off in the LD 12 : 36 and LD 12 : 60 cycles.

In none of the experiments was there any indication of two peaks of diapause inhibition in each "subjective night." This was so even in the LD 14 : 34 experiment in which the first 10 hours of the night were scanned by 1-hour pulses, and the two peaks, if present, would be expected to be at their most divergent (Fig. 1). It appears, therefore, that peak B or its equivalent does not appear in 48- or 72-hour cycles. Although these experiments are consequently difficult to interpret in terms of the coincidence model, they do provide strong evidence for the circadian nature of the seasonal photoperiodic clock.

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15. I thank Mrs. M. H. Downie and Miss H. Graham for technical assistance and the Science Research Council for financial support.

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Terrestrial and Aquatic Orientation in the Starhead Topminnow, Fundulus Notti

Abstract. *Starhead topminnows from various shores of a small woodland pond were displaced to unfamiliar surroundings, and their orientation was tested in aquatic and terrestrial arenas. These fish used a sun compass to move in a direction which, at the location of their capture, would have returned them to the land-water interface. The fish accomplished directional terrestrial locomotion by using the position of the sun to align its body for each jump. On heavily overcast days many fish were unable to orient their bodies in a consistent direction from jump to jump; this inability to orient resulted in random rather than linear movement. There was considerable individual variation in terrestrial locomotor ability.*

Terrestrial locomotion and orientation of fishes have been investigated in tidewater *Fundulus* (*1*) and in *Bathygobius soporator* (*2*). *Bathygobius soporator* use local landmarks for orientation and do not rely on sun cues; however, the cues used for orientation by *Fundulus* were not understood. The mosquito fish, *Gambusia affinis* (*3*), and several species of amphibians (*4,*

5) use sun cues for y-axis orientation, that is, movement in a direction perpendicular to the shore of their capture. I here report that the starhead topminnow, *Fundulus notti*, displaced to unfamiliar surroundings and tested on land, or in water, orients by means of a sun compass. The fish traveled in a direction that would have returned them to the land-water interface in the area of their capture.

A total of 534 *F. notti* (25 to 41 mm in standard length) were captured along various shorelines of a small woodland pond located in Aiken County, South Carolina, 13 km southeast of the test facility. Fish were taken from areas of the pond selected so that the expected orientation included all cardinal directions (Table 1). The fish were placed in covered Styrofoam containers, returned to the laboratory, and held in phase with local time 3 to 24 hours before being tested. Tests were conducted outdoors between 9 a.m. and 3 p.m.

A total of 116 fish were tested in the aquatic arena which consisted of a wading pool (250 cm in diameter) containing water to a depth of 25 cm and located within a structure which obscured the horizon. Individual fish were placed in a clear plastic cylinder (15 cm in diameter) located in the center of the pool. After a period of 15 to 30 seconds, a nylon string was used to lift the cylinder and free the fish. Observations were made through any one of four periscopes located at 90° intervals around the pool. The directional choice of the fish was recorded as the direction that the fish first approached to within 15 cm of the arena wall.

I tested a total of 418 fish for terrestrial orientation by placing five to ten individuals at a time on the center of a level polyethylene-covered circular table (125 cm in diameter). The direction of movement recorded for each fish was the direction from the center of the table to the point where the fish left the table surface. In the event that a fish moved 45 cm or more from the release point but was unable to leave the table surface, its directional choice was determined by its final position. Directional choices of fish moving less than 45 cm were not recorded. Fish were allowed to remain on the table surface until they were unable to jump farther.

Table 1. Number of starhead topminnows from y-axes ± 45° of cardinal compass directions for which directional preferences were obtained.

Direction	Aquatic		Terrestrial	
	Sunny	Cloudy	Sunny	Cloudy
North	13	10	51	
East	15		71	30
South	13	15	78	21
West	25	25	58	
Total	66	50	258	51

In aquatic tests conducted under sunny skies most of the fish tested swam on a course that was perpendicular to the shore of their capture (Fig. 1A). When tested on heavily overcast days (100 percent cloud cover), the fish appeared disoriented (Fig. 1B). These tests indicate that *F. notti*, in a manner similar to that found in mosquito fish (*5*), are able to use the sun to swim in a direction perpendicular to the shore of their capture.

In terrestrial tests conducted under sunny skies most fish oriented in a direction that would have led them back to water at the location of their capture (Fig. 1C). Fish tested on land under overcast skies appeared disoriented (Fig. 1D). Locomotion on land was accomplished by successive jumps with the fish orienting between jumps by turning until the long axis of the body was in line with the direction of travel. Fish varied greatly in their ability to move on land with some reaching the edge of the table in less than 5 seconds with four or five jumps; others required 15 or more jumps and often took 5 minutes or more. In tests conducted on sunny days 84.6 percent moved 45 cm or more, but on overcast days only 45.1 percent moved the same distance. These results are consistent with the observation that most fish tested on cloudy days appeared unable to orient in a constant direction from jump to jump. This inability resulted in random rather than linear movement. These data indicate that the fish accomplishes oriented terrestrial locomotion by using the position of the sun to align its body on successive jumps.

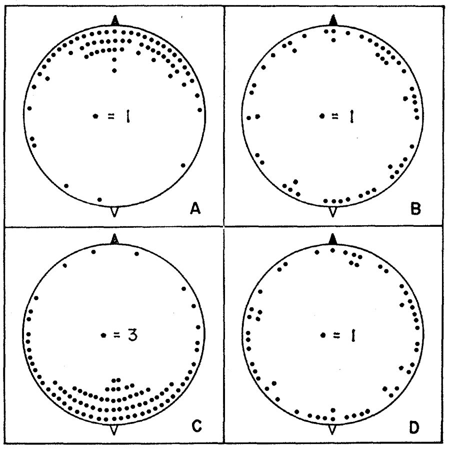

Fig. 1. Directional preferences of starhead topminnows tested (A) in water under sunny skies; (B) in water under overcast skies; (C) on land under sunny skies; and (D) on land under overcast skies. ▲, Land; ▽, water; •, score. The directional preferences were pooled into groups of three for presentation of the results of tests conducted on land under sunny skies. According to a chi square test for randomness (*7*) at three degrees of freedom, the orientations depicted in (A) ($\chi^2 = 64.2$) and in (C) ($\chi^2 = 249.4$) were nonrandom at the .99 level of probability. According to the same test, the directional preferences depicted in (B) ($\chi^2 = 1.36$) and in (D) ($\chi^2 = 1.78$) were random.

After being tested, many of the fish were released into a small pond containing several largemouth bass (*Micropterus salmoides*). The starhead topminnows could escape predation only at one end of the pond where water was shallow. Along all other margins of the pond the water depth was insufficient to afford protection from predation. In five instances *F. notti*, which were being chased by bass, were seen to jump onto the bank and remain there for several minutes before jumping back into the pond. The bass remained only a few seconds after the *F. notti* left the water and were gone when the starhead topminnow returned.

These observations indicate that the fish learn the direction to the land-water interface as a response to predation in much the same manner as mosquito fish do (*5*). The great variation in terrestrial performance that I observed may reflect previous terrestrial exposure caused by predation. Fish failing to escape or return to water during intermittent periods of active predation would be eliminated from the sample. In addition, most collections were made where cover was relatively sparse, although there were more fish in areas of dense vegetation. The fish population studied possibly consisted of highly experienced residents and less experienced immigrants from nearby areas with extensive cover.

This study indicates that the sun is an important cue for orientation in *Fundulus notti*. However, the study does not exclude an ability on the part of the fish to orient on the basis of local landmarks or olfaction, both of which appear important in aquatic orientation of other fish species (*6*). It also seems likely that the tidewater species of *Fundulus* studied by Mast (*1*) used the sun to orient on land, especially since his investigations demonstrated that neither the slope of the beach nor local landmarks were used.

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Retinoscopy and Eye Size

Abstract. *Retinoscopy was performed on animals with different sized eyes, all of whom appeared hypermetropic. The data were well fitted by an equation of the form* $y = kx^{-2}$ *where* y *is refractive error in diopters, and* x *is the corneo-retinal length of the eye. Apparent hypermetropia may be due to the reflection from the inner surface of the retina.*

The retinoscope is widely used for objective measurement of the refractive state of the eye (*1*). Light is projected into the eye by an inclined mirror and reflected back from the retina to emerge from the pupil. Movement of the retinoscope produces movement of the reflected light. Dependent on the observer's position relative to the anterior focal plane of the eye, the movement appears to be with or against the direction of movement of the instrument.

A basic problem which arises when analyzing retinoscopic measurements is the question of the layer in the retina from which the observed light is reflected. A number of authors have studied retinal reflection for various purposes and have ascribed such reflections to different layers within the eye, for example, choroid (*2*), Bruch's membrane (*3*), and pigment epithelium (*4*).

If the light used in retinoscopy were reflected from some retinal layer in a plane other than that of the receptors themselves, an error would be introduced in determination of the focal plane of the eye; hence the estimate of refractive state would be incorrect. The error introduced by a difference in reflective layers can be determined by reference to the equation defining the refractive power of a simplified eye:

$$D = \mu f^{-1} \qquad (1)$$

where D is refractive power in diopters, μ is the refractive index of the ocular media, and f the posterior focal length of the eye. Reflection from some layer other than that of the receptors would constitute an error in the measurement of the posterior focal length of the eye; hence an error would be introduced in determining the optical power of the eye. The error introduced by a difference between the receptor and reflective layers can be determined by differentiating Eq. 1, that is,

$$\frac{dD}{df} = -\mu f^{-2} \qquad (2)$$

In other words, the error of retinoscopy should be proportional to the inverse square of the focal length of the eye. If we assume that the refractive index of the media is approximately constant for all eyes, from Eq. 2 it is also clear that the size of the error would be determined by the distance from the reflective layer to the layer of the outer segments of the receptors, and the sign of the error would indicate the position of the reflective layer. If the reflection were from a plane behind the rods and cones, the eye would appear spuriously myopic; if the reflection were from a plane in front of the receptors, the eye would appear spuriously hypermetropic.

The retina of mammals is of a rather constant thickness. For example, in our own experience, the corneo-retinal length of the elephant eye is about 17

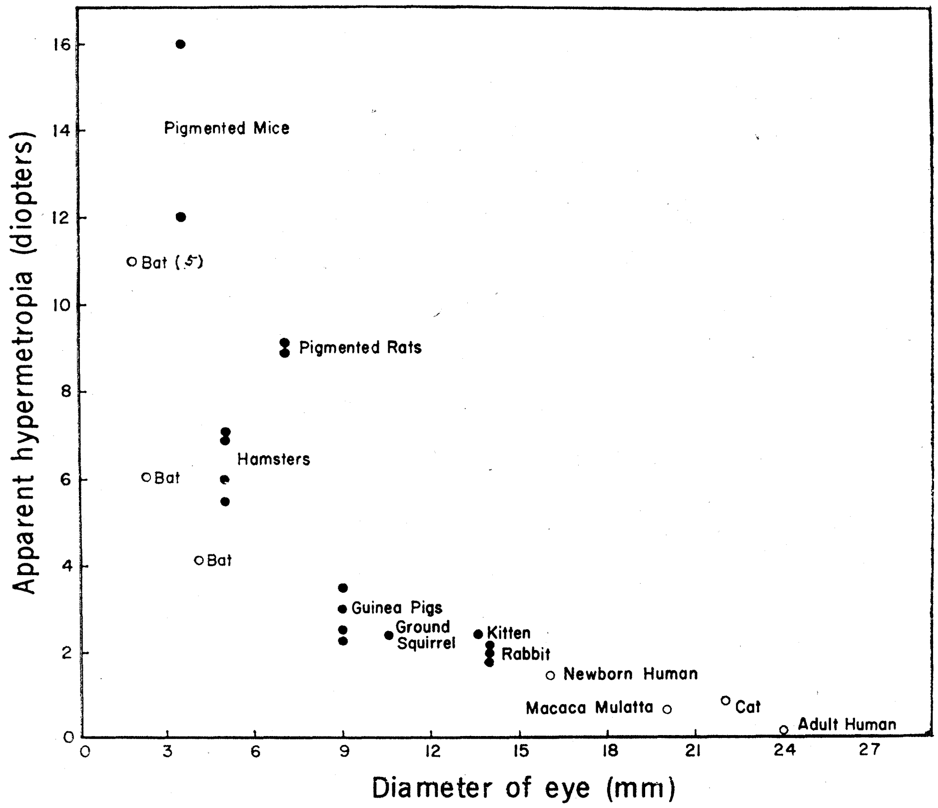

Fig. 1. Measured refractive error and eye diameter in millimeters in several mammalian species. Filled circles are our own measurements. Three of the open circles for bat eyes are from measurements on three species of bat furnished by Suthers and Wallis (*5*). The open circle for newborn human is from Cook and Glasscock (*5*); that for *Macaca mulatta* is from Young (*5*); that for cat is from Vakkur *et al.* (*5*); and that for adult human is from Fry (*4*).